ROTENONE POLICY SUPPORT AND CAPACITY DEVELOPMENT

PART 2: Krom River Base-Line Monitoring and Capacity Development

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N Dean Impson, Jeanne Gouws, and Olaf L F Weyl*

WATER RESEARCH COMMISSION

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PART 2: Krom River Base-Line Monitoring and Capacity Development

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Capacity Development in the Water Science Sector

It is recognised that there is a need for Transformation and Redress, as well as Capacity Development, in the Water Science Sector in South Africa. However, students from some universities are currently not being offered sufficient opportunities to develop skills in aquatic (freshwater) research that are desperately needed for the protection of South Africa's freshwater ecosystems and the sustainable long-term utilisation of freshwater resources. The majority of the emphasis in the development of water scientists is currently on the utilisation of water as a resource for agricultural, industrial and domestic use in the water and sanitation sector. The ecological and biological components of water science are less emphasised and opportunities for research in these areas may not be offered to students at higher education institutions (HEIs). Therefore, exploring opportunities for linking monitoring of aquatic ecosystems to University science programmes (such as BSc. Honours research projects) could create an interest in ecological based research of freshwater ecosystems and the experiences gained could equip (and inspire) students involved to pursue careers in ecological research of freshwater ecosystems.

The primary objective of this Technical Report is to explore how a baseline assessment of the Krom River, Olifants-Doring River System, Western Cape, prior to the river being treated with the piscicide Rotenone could be used to integrate human capacity development into an ecological project with the intention of developing a BSc Honours level programme to integrate aquatic research and monitoring techniques into post-graduate and supervisor training at HEIs. The specific objectives were to:

- Generate baseline data for the Krom River that CapeNature plans to treat with Rotenone in the near future,
- Integrate post-graduate student projects into a long-term monitoring framework to develop national capacity in freshwater fish and invertebrate monitoring.

Krom River Baseline Monitoring

The Krom River rises in the Cederberg Mountains at an elevation of ~1500 m and flows in an easterly direction for approximately 20 km before joining the Matjies River which then flows into the Doring River. Surveys were conducted to document the baseline condition of the Krom River before the planned treatment with Rotenone to remove non-native fishes. Fish populations, water quality and habitat parameters were recorded at 29 sites in the Trout, Rock catfish, Treatment and Sawfin zones on the Krom River over two field trips: January-February 2017 and January-February 2018. Fish abundance at each site was estimated using three independent methods: fyke nets, underwater video analysis (UWVA) and snorkel surveys. Underwater Video Analysis was carried out using GoPro® HD Hero® cameras. Cameras were placed in each site and recorded footage for a minimum of 15 minutes and then viewed to obtain estimates of fish abundance and diversity. Snorkel surveys used the two pass method where all fish encountered by the observer were counted on each pass and an

estimate of abundance is derived from the mean of the two counts. Fyke nets were set overnight and cleared the next morning.

Invertebrate monitoring was conducted at 20 monitoring sites within the Fishless, Trout, Rock Catfish, and Treatment zones of the Krom River over two field trips: January-February 2017 and January-February 2018 by kick sampling following the guidelines set out by Weyl et al. (2016) and involved both SASS5 methods as well as species specific estimates of abundance.

Baseline monitoring of fish and invertebrate communities in the Krom River demonstrated that:

- The distribution of fish in the Krom River has not changed from earlier studies in 2006 and 2007. The only native fish in the upper catchment is the Clanwilliam rock catfish *Austroglanis gilli,* while sawfin *Cheilobarbus serra* and Clanwilliam yellowfish *Labeobarbus seeberi* were found in the lower reaches in the Matjies River floodplain. Clanwilliam yellowfish were not recorded from the Krom River in previous studies.
- Three non-native fish species were identified from the Krom River catchment: rainbow trout *Oncorhynchus mykiss* in the whole river, bluegill *Lepomis macrochirus* in the river from just above the barrier weir at Krom River Farm to the confluence with the Matjies River as well as the farm dams, and largemouth bass *Micropterus salmoides* in at least one of the farm dams.
- Assessments of invertebrate communities demonstrated that communities were distinct for each of the zones delineated for the Krom River likely responding to gradient change along the river length in keeping with the River Continuum Concept. There was also inter-annual variation in the invertebrate assemblages between the two years of the study.
- There were variations in water quality between the zones of the Krom River. The upper reaches differed from the lower reaches below the Krom River Farm complex by increased turbidity and electrical conductivity.

Capacity Development

The Krom River Project was used as a training platform for BSc Honours level students from selected universities

- Eight BSc. Honours Level Students through annual participation in monitoring activities during the 2017 and 2018 field trips to the Cederberg. This has resulted in training of these students in freshwater research techniques and afforded the students the opportunity to collect data for their research projects. In addition, one BSc. Honours student and one Diploma student from the Universities of Fort Hare and Venda, respectively, also participated in the 2018 field trip. One BSc. Honours Student from the University of the Western Cape also completed a project funded by the project in 2016.
- One peer reviewed paper has been published to date from the BSc Honours projects with four more in preparation.
- One MSc. Student supported by the project earned a MSc. degree from Rhodes University in March 2018.

- MSc and PhD students from Prof Weyl's DST/NRF Research Chair in Inland Fisheries and Freshwater Ecology at the South African Institute for Aquatic Biodiversity acted as field trip leaders for the 2017 and 2018 field trips, participating in all activities to become more familiar with freshwater research techniques.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASPT	Average score per taxon
CFE	Cape Fold Ecoregion
CFR	Cape Floristic Region
DEA	South African Department of Environmental Affairs
EIA	Environmental Impact Assessment
GEF	Global Environmental Facility
HEI	Higher Education Institute
HSD	Honest Significant Difference
NEMBA	National Environmental Management: Biodiversity Act
NMDS	Non-metric multidimensional scaling
PCA	Principal Component Analysis
PDP	Personal Development Programme
SASAQS	South African Society for Aquatic Scientists
SASS	South African Scoring System
UWC	University of the Western Cape
UWVA	Underwater video analysis
WRC	Water Research Commission

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CHAPTER 1. INTRODUCTION

1.1 Introduction

It is recognised that there is a need for Transformation and Redress, as well as Capacity Development, in the Water Science Sector in South Africa. However, students are often unable to access sufficient opportunities to develop skills in aquatic (freshwater) research desperately needed for the protection of South Africa's freshwater ecosystems and the sustainable long-term utilisation of freshwater resources. Therefore, exploring opportunities for linking monitoring of aquatic ecosystems to BSc. Honours research projects could create an interest in ecological based research of freshwater ecosystems and the experiences gained could equip students involved to pursue careers in this field

The current K5/2538 project follows two previous projects (K9/822 and K5/2261) that monitored the impact of river rehabilitation using the piscicide Rotenone on the nearby Rondegat River (Woodford et al., 2012; Weyl et al., 2016). These projects provided comprehensive species level assessments of invertebrate and vertebrate distributions both prior to, and for three years after, the Rotenone treatment. Overall these research projects demonstrated that Rotenone treatment was effective at removing smallmouth bass from the treatment zone (Weyl et al., 2013) and that native invertebrate and fish communities are recovering after treatment (Weyl et al., 2014; Bellingan et al., 2015). Despite this evidence, the development of National Policy requires additional information, particularly on the effects of Rotenone on lentic environments, such as dams and wetlands, before considering the registration of Rotenone as a national tool for river rehabilitation. The opportunity to address these knowledge gaps was provided by CapeNature's plan to treat two off channel dams (Chalet Dam in Krom River and Kranskloof Dam in the Northern Cape) in 2017 in addition to collecting base-line data in anticipation for the treatment of the Krom River as part of the CAPE Alien Fish Eradication pilot project. However, delays in receiving the General Authorisation from the Department of Water and Sanitation for the treatment of the Krom River and in receiving funding from DEA: NRMP during the 2018/19 financial year prevented the treatment of the Krom River from occurring at the time of completing this project and the scope of the project was thus reduced to collecting base-line data for the Krom River and monitoring of the treatment and recovery of the two off-channel dams.

The current project also provided the opportunity to incorporate capacity building in aquatic ecosystem monitoring by including some of South Africa's higher education institutions (HEIs), including the Universities of Fort Hare, Venda, the Western Cape and Stellenbosch. By partnering with these HEIs, the project offered opportunities for exposure to aquatic research and experts in this field to students whom might not otherwise have access to similar opportunities at their home institutions by linking the monitoring of aquatic ecosystems to B Sc. Honours research projects. These partnerships between SAIAB and the HEIs contributes to the achievement of national transformation goals and increased interest and opportunities for students at HEIs to pursue careers in aquatic ecology.

The greater aims of the current project were therefore two-fold: (1) to support policy development through robust monitoring of ecosystem responses to management interventions (e.g. conservation, rehabilitation and monitoring) and (2) developing appropriate methods to integrate post-graduate students in longer term monitoring projects. These two components are reported separately as PART 1: IMPACT AND RECOVERY OF NATIVE BIOTA IN ONE RIVER AND TWO DAMS FOLLOWING ALIEN FISH REMOVALS USING ROTENONE and PART 2: KROM RIVER BASE-LINE MONITORING AND CAPACITY DEVELOPMENT (this report).

1.2 Aims and Objective

The primary objective of this Technical Report is to explore using baseline monitoring of the Krom River to integrate human capacity development into an ecological project. The Krom River is scheduled for alien fish eradication by CapeNature in the near future. As is standard practise, a pre-treatment monitoring baseline assessment prior to Rotenone treatment is necessary (Woodford et al., 2012; Weyl et al., 2016). The techniques used in the baseline monitoring could be used to develop a B Sc. Honours level programme to integrate aquatic research and monitoring techniques into post-graduate and supervisor training at HEIs. This baseline assessment presented an opportunity to train B Sc. Honours level students in freshwater research techniques over two three-week field trips held in January-February 2017 and 2018. The specific objectives were to:

- Generate baseline data for the Krom River that CapeNature plan to treat with Rotenone in the near future,
- Integrate post-graduate student projects into a long-term monitoring framework to develop national capacity in fish and invertebrate monitoring.

CHAPTER 2. KROM RIVER BASE-LINE MONITORING

2.1 Introduction

The Krom River, a tributary of the Matjies River, tributary of the Doring River of the Olifants-Doring River system in the Western Cape, South Africa, was included as one of the four rivers selected for the pilot project to evaluate the effectiveness and ecological impact of using Rotenone as a non-native fish removal tool under the Cape Action for People and the Environment (CAPE) Alien Fish Eradication Project (Marr et al., 2012). The Rondegat River was selected as the first river to be treated with the Krom River (Cederberg) planned to be the second. Following the successful treatments of the Rondegat River in 2012 and 2013, CapeNature turned their attention to the Krom River. In preparation for the treatment of the Krom River, a base-line monitoring programme was established comprising of two field trips in January/February 2017 and 2018.

2.1.1 Krom River Catchment

The Krom River rises in the Cederberg Mountains at an elevation of ~1500 m and flows in an easterly direction for approximately 20 km before joining the Matjies River which then flows into the Doring River (Figure 2.1). The upper river has a narrow (generally <5 m wide), shallow (generally <1 m deep) wetted channel and comprises pools, chutes and bedrock steps interspersed by occasional cobble-bed riffles. The lower river becomes more seasonal and continues the template of the upper river but the pools are considerably deeper, > than 3 m deep in some areas, and isolated at periods of low flow in mid to late summer. The area has a Mediterranean climate with warm, dry summers and cold, wet winters (Cowling and Holmes, 1992). The Krom River flows through the Cederberg-Tanqua tension zone (Low et al., 2004; Low and Pond, 2005); a distinct rainfall gradient from the source, which receives about 1000 mm rainfall per annum, through to the Krom River farm complex, which receives about 500 mm, down to the confluence with the Matjies River, which receives about 250 mm, largely as a result of the rain shadow of the Olifants River mountains at the west of the Cederberg Mountains. The river channel lies in geology that is comprised of layered quartzitic

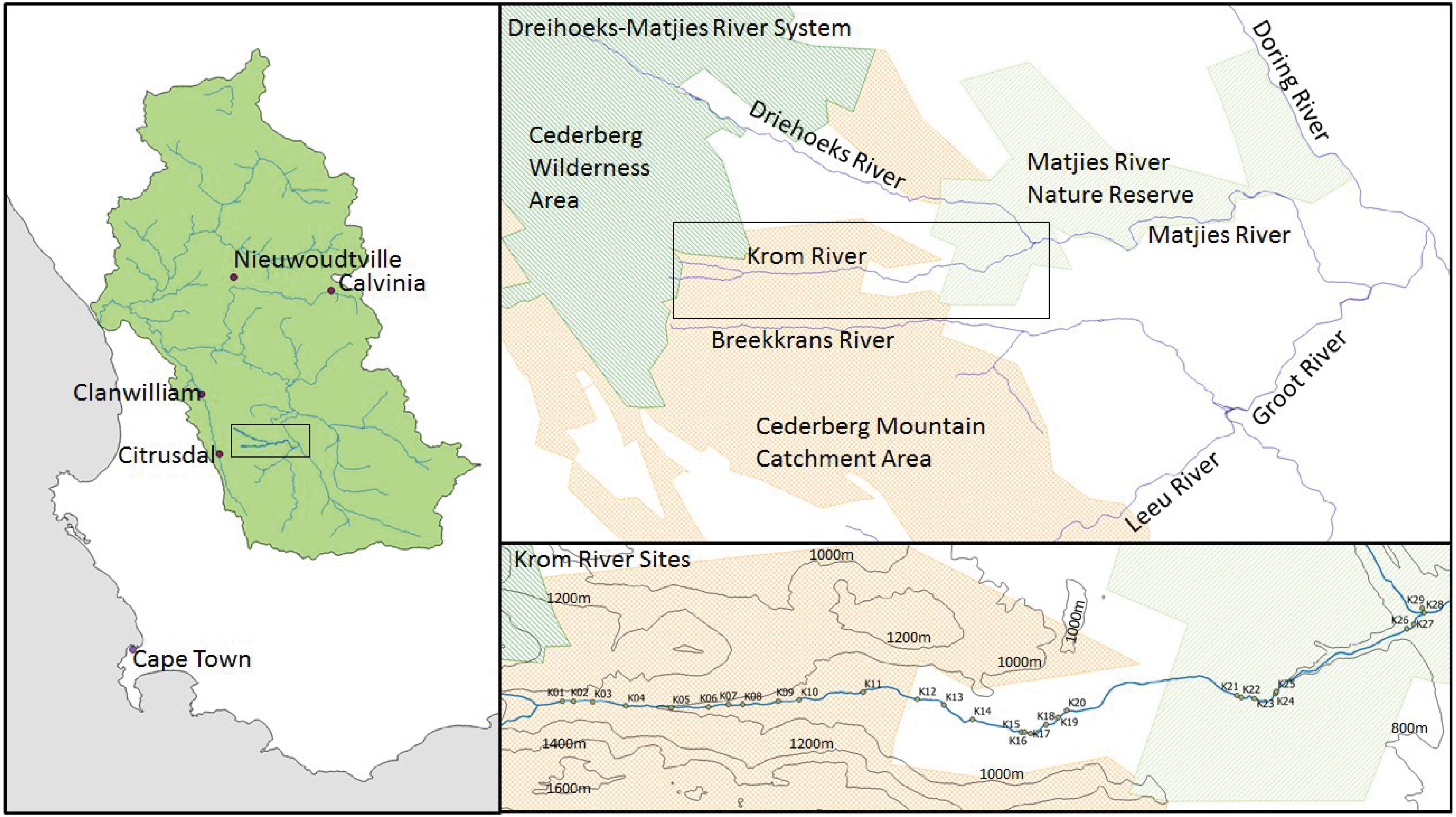


Figure 2.1: Location of the Krom River and a map showing the study sites and its position relative to the Olifants-Doring River System

sandstones with minor shale bands of the Nardouw subgroup, Table Mountain Group, Cape Supergroup (Thamm and Johnson, 2006). Near-pristine Cederberg Sandstone Fynbos dominates the catchment above the farm complex. The river passes through a thin band of Northern inland shale band vegetation just east of the farm complex, before passing through the arid extreme of Cederberg Sandstone Fynbos in the Matjies River Nature Reserve (Mucina and Rutherford, 2006).

The 3 m Disa Pool waterfall marks the upper limit of fish distribution in the Krom River, and the 9 km of river down to the Krom River chalets is largely unaffected by human impacts (Marr et al., 2012). Below the Disa Pool waterfall, the river flows through a gorge of bedrock steps, pools and chutes. The valley opens up below the gorge and the low-gradient river, with sandy runs and pools, flows through a near-pristine valley before entering the resort and chalet area on the highly transformed Krom River Farm. There is major abstraction from the Krom River just above the resort chalet area, with water diverted to several dams for recreational and irrigation uses. Below the farm, the river becomes more seasonal and flows through the near-pristine Matjies River Nature Reserve to its confluence with the Matjies River.

2.1.2 Fish Distributions

Only one native fish species, the Clanwilliam rock catfish *Austroglanis gilli* (Barnard, 1943), has been confirmed to occur in the river above Krom River Farm (Bills, 1999; Marr et al., 2012; Bills and Impson, 2013). Conflicting anecdotal reports have been recorded of redfin, possibly Clanwilliam redfin *Sedercypris calidus* (Barnard. 1938) and/or Doring fiery redfin *Pseudobarbus* sp. 'phlegethon Doring', which occur in the Driehoeks-Matjies River, occurring in pools near the campsite before the introduction of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in the 1950s (Enviro-Fish Africa, 2009; Marr et al., 2012). The Krom River is unique among the Cederberg streams in that it is the only stream where rock catfish occur without the two redfin species and the reasons for this have been debated. Some researchers feel that the introduction of trout resulted in the extirpation of the redfin, as rainbow trout have had severe effects on native species in several rivers elsewhere in the CFE (Shelton et al., 2015), including redfin extirpations in the Eerste (De Moor and Bruton 1988), while others argue that environmental drivers may have resulted in the current isolated rock catfish population because there are no formal records of redfin occurring in the Krom River, only conflicting anecdotes from the same source (Enviro-Fish Africa, 2009; Marr et al., 2012). The presence of a bedrock step in the Matjies River Nature Reserve and the presence of bluegill and trout in pools have been proposed as a possible barrier for common Cederberg fish species not recolonised the Krom River. Below the bedrock step, in the flood plain of the Matjies River, both Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist & Thompson, 1913) and sawfin *Cheilobarbus serra* (Peters, 1864) have been recorded from the Krom River and Clanwilliam rock catfish have been recorded in the Matjies River just above the confluence with the Krom River.

Clanwilliam rock catfish *Austroglanis gilli* from the Krom River (Photo Sean Marr)

Three non-native species have been introduced: rainbow trout in 1957 (Weaver, 2008), and bluegill *Lepomis macrochirus* Rafinesque 1819 and largemouth bass *Micropterus salmoides* (Lacepède, 1802) in the 1980s. Rainbow trout occur from the Disa Pool waterfall to just above the Matjies River confluence, while bluegill and largemouth bass occur in the two farm dams fed from the river via a furrow system. Largemouth bass have been occasionally observed in the Krom River below the road bridge in the chalets, but have not been captured in any fish survey of the river and may have been flushed from the river during the winter floods. Largemouth bass were present in both farm dams but were extirpated from the "Chalet" Dam in the chalet area when the dam was drained and allowed to dry-up in 2014 to reduce the leech population, however, they persist in the "House" Dam. Bluegill are found in both farm dams, in the Krom River from the chalets downstream to the Krom-Matjies River confluence and have been observed in the furrow to the House Dam.

2.2 Previous Studies in the Krom River

While a number of studies have been conducted on the tributaries of the Olifants River, studies of the tributaries of the Doring River are less numerous, e.g. studies of the Twee River (Marriott, 1998; Impson et al., 2007; Marr et al., 2009), and the Matjies/Driehoeks (Shelton et al., 2008; Paxton and King, 2009). There have, however, been a few studies that have included the Krom River.

2.2.1 WWF *Austroglanis* Surveys

The siluriform catfish family Austroglanididae has been described by taxonomists as unusual, problematic and difficult to place in phylogenetic trees (Sullivan et al., 2006). The origin of catfishes probably occurred before the breakup of Gondwana in the late Mesozoic (Teugels, 1996). The Austroglanididae is an ancient family with the closest relatives amongst African catfish (Paul Skelton, SAIAB, pers. comm. 2018). Austroglanididae, known only from the westward flowing Orange and Olifants Rivers Systems in South Africa, comprise one genus, *Austroglanis* Skelton, Risch & De Vos, 1984, of three species: the rock catfish *Austroglanis sclateri* (Boulinger, 1901) from the Orange River and the spotted rock catfish *Austroglanis barnardi* (Skelton, 1981) and Clanwilliam rock catfish *Austroglanis gilli* (Barnard, 1934) from the Olifants-Doring River system (Skelton, 2001). The Near Threatened *A. gilli* is known from a number of populations in tributaries of both the Olifants and Doring Rivers (Van der Walt et al., 2017a) whereas the Endangered *A. barnardi* is only known from three rivers in the Olifants River (Van der Walt et al., 2017b).

The first SAIAB record of native fish in the Krom River was noted in the WWF survey of *Austroglanis* distributions in the Olifants-Doring River system (Bills, 1999). Prior to this, *Austroglanis gilli* was only known from two localities in the Doring River catchment, the Matjies-Driehoeks and the Tra-Tra rivers (Skelton, 1987). The WWF survey identified *A. gilli* populations in the Breekkrans, Krom, Matjies-Driehoeks, Eselbank, Tra-Tra and Biedouw rivers in the Doring catchment (Bills, 1999). Interestingly, *A. gilli* from tributaries of the Olifants River are more closely related genetically to *A. barnardi* than to the *A. gilli* populations from the tributaries of the Doring River (Swartz, 2013). Loss of the Doring populations of *A. gilli* would constitute the loss of a unique lineage and unique morphological forms (Swartz, 2013).

2.2.2 CAPE Project Surveys

The Krom River was one of the rivers selected for the CAPE Alien Fish Eradication Project. Within the evaluation of the potential rivers for this project, fish surveys were conducted of the Krom River to determine the distribution of the native and non-native fishes, the locations of barriers to fish distributions and potential locations for the construction of barriers to prevent the reinvasion by the non-native fishes. The fish surveys of the Krom River, summarised in Marr (2006) and Marr (2007), confirmed that the distribution range of the native Clanwilliam rock catfish was primarily above the Krom River Farm complex in about a 5km reach of river. The upper barrier to fish distribution in the Krom River was confirmed to be the large waterfall at Disa Pool. To date, no fish, native or non-native, have been detected above the Disa Pool Waterfall. Non-native rainbow trout were found to occur from Disa pool downstream to a series of large pools in the Matjies River Nature Reserve. Two farm dams, fed by a furrow system from the Krom River, were found to contain bluegill and largemouth bass. Bluegill were detected in the river from the chalets downstream to the large pools in the Matjies River Nature Reserve. Largemouth bass were known to occur in the river downstream of the campsite but were not detected in the river during the sampling effort. Below the farm complex, water abstraction reduces the river in the Matjies River Nature Reserve to a series of large deep isolated pools in late summer and autumn.

Only rainbow trout and bluegill were recorded from the pools sampled. Where the Krom River enters the Matjies River flood plain, sawfin were recorded in small pool. It was uncertain whether sawfin only utilised the flood plain reaches of the Krom River or whether they moved upstream to the large permanent pools in the Matjies River Nature Reserve. These reaches of the Krom River were not surveyed before CapeNature terminated the surveys in October 2006. The distribution of fish within the Krom River as determined from the data collected during these field surveys is presented in Figure 2.2.

2.2.1 Environmental Impact Assessment

Field surveys were conducted to inform the Environmental Impact Assessment (EIA) carried out by Enviro-Fish Africa (Pty) Ltd to assess the use of piscicides for the treatment of the four rivers included in the CAPE Alien Fish Eradication Project; see Marr et al. (2012) for the reasoning behind conducting the EIA and Enviro-Fish Africa (2009) for the full EIA report. An overview of the EIA recommendations is presented in Section 1.3. During the EIA, field data was collected for the aquatic invertebrate and amphibian specialist reports but the fish distribution data from Marr (2006) and Marr (2007) collected during the CAPE Project surveys were used for the fish specialist reports, in conjunction with discussions with researchers knowledgeable on the fishes of the Cape Fold Ecoregion.

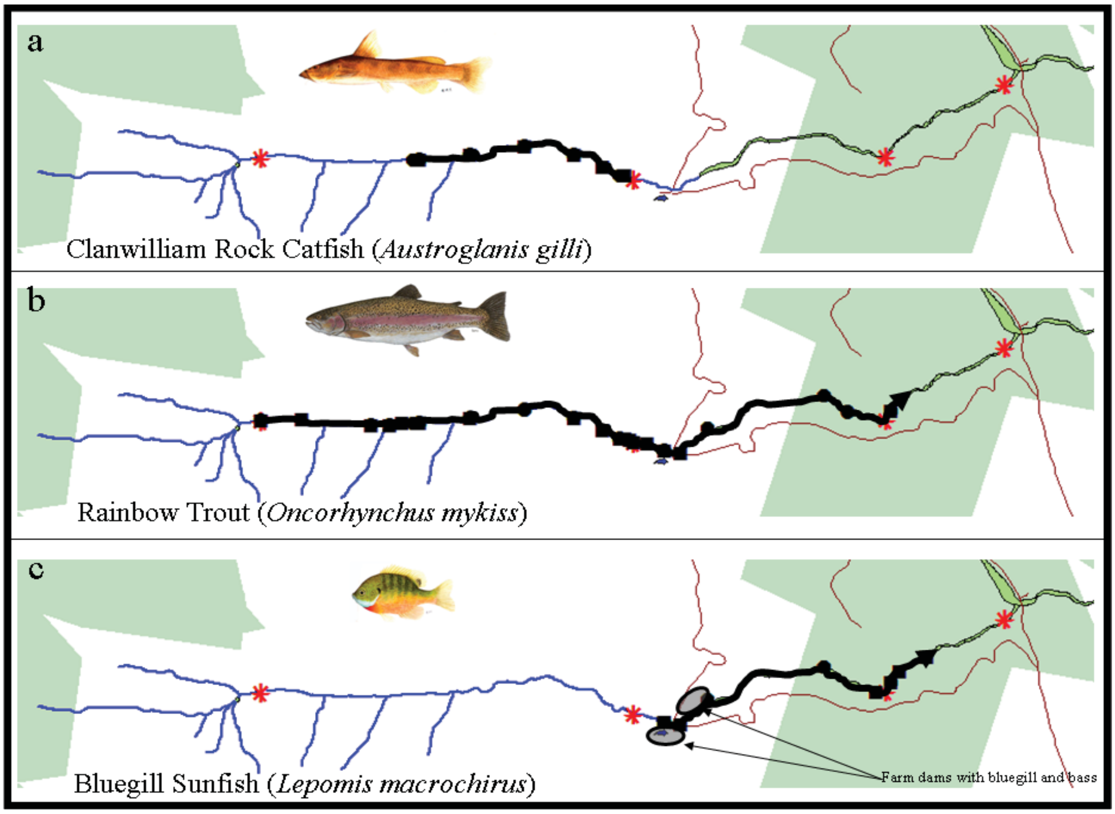
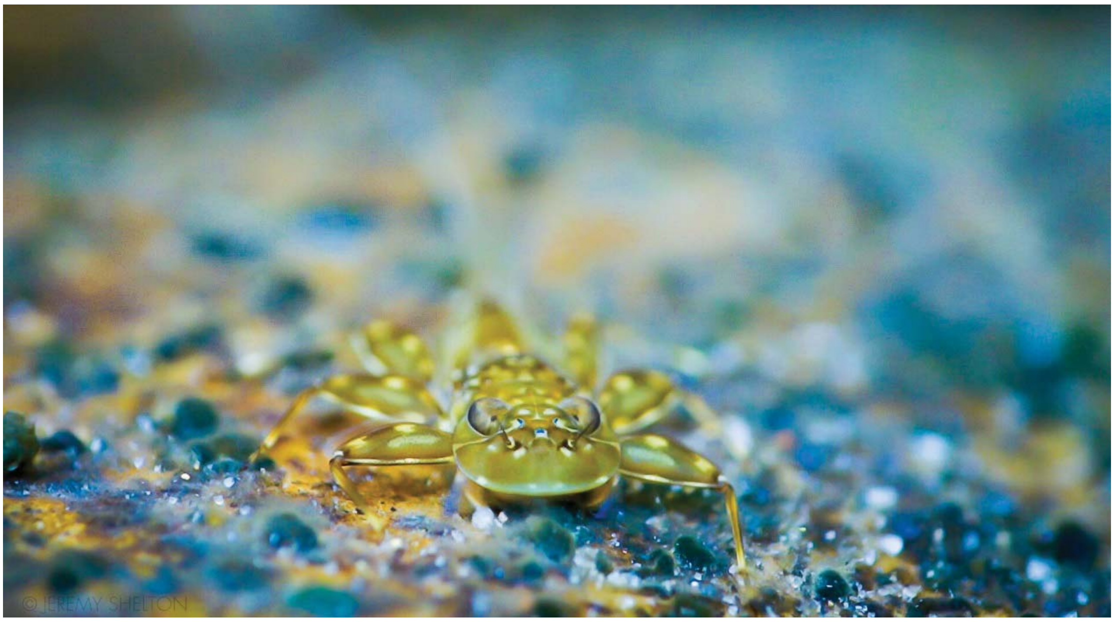


Figure 2.2: Distribution of fish in the Krom River from CAPE surveys a) Clanwilliam rock catfish *Austroglanis gilli*, b) rainbow trout *Oncorhynchus mykiss* and c) bluegill *Lepomis macrochirus*; adapted from Marr et al. (2012). The red asterisk indicates barriers within the Krom River catchment

The amphibian specialist report concluded that although the Cape Floristic Region is a globally important centre of diversity and endemism for amphibians, however, the Cederberg, lying on the periphery of the Cape Floristic Region in terms of amphibian diversity, harbours relatively few of regionally endemic or threatened species. Six species of amphibians were predicted to occur around the Krom River. Further, only one frog species of the Krom River, FitzSimon's Ghost Frog *Heleophryne depressa*, was likely to be impacted significantly by the presence or absence of non-native fishes. This species lives and breeds within streams and shows partial co-occurrence with some native fishes but is almost always absent in the presence of predatory fishes (both native and non-native).



Heptageniid mayfly larvae from the Krom River (Photo Jeremy Shelton)

Samples of aquatic invertebrates from the Krom River were collected using kick sampling (marginal vegetation) or individual stone sampling (benthos) in order to better quantify invertebrate densities. Samples were stored in 98% ethanol and identified mostly to family level in the laboratory. The aquatic invertebrate community between Disa Pool waterfall and the mouth of the gorge was found to be different to that above the waterfall, therefore colonization from upstream by many species was not possible. Further, the aquatic invertebrate community from the reach between the mouth of the gorge and the farm were distinct those upstream. Re-colonisation from adjacent streams (e.g. upper Driehoeks River) was considered to have a low probability due to the high mountain ridges separating the streams. The aquatic invertebrate community below the farm was found to have low diversity and abundance due to the impact of the water abstraction, non-native fishes and invaded riparian vegetation zone, and increased nutrient input from the farm complex. The invertebrate specialist rated the upper catchment a medium to high concern and recommended attempting the mechanical removal of non-native fishes in these reaches and, if this proved ineffective, piscicide application to remove the non-native fishes with appropriate conservation actions for aquatic invertebrates and native Clanwilliam rock catfish, e.g.

storage in port-a-pools beside the river during the treatment for restocking after the treatment. The invertebrate specialist rated the lower catchment a low concern.

2.2.2 Trout Removal Project

In order to implement the aforementioned EIA recommendations, CapeNature contracted a work team to remove rainbow trout from the upper Krom River using a combination of angling, fyke nets and gill nets (mesh size 25–50 mm) between October 2013 and February 2014 (Shelton et al., 2017). While mechanical removal projects generally receive higher levels of public support than piscicidal treatments, the success of mechanical removal can be influenced by habitat complexity, gear choice, species or size-class specific responses to treatment, and budgetary constraints (Shepard et al., 2014; Propst et al., 2015). The effectiveness of mechanical removal is density-dependent, and declines rapidly as non-native fish densities decrease towards zero (Bomford and Tilzey, 1997). Complete eradication is challenging because of the high cost and labour effort required to capture every individual, particularly small size-classes (Thompson and Rahel, 1996) or due to overcompensation by the remaining adult fish to the harvesting effort (Zipkin et al., 2008). Furthermore, the benefits of reducing the density of a non-native fishes will only be short-term unless the removal effort is sustained to extirpation of the target species (Finlayson et al., 2000).

From October 2013 to February 2014, 354 rainbow trout were removed by angling (58%), fyke netting (28%) and gill netting (14%); see Shelton et al. (2017). This resulted in a marked reduction, but not eradication, of the rainbow trout population (fish relative abundance decreased from 0.53 ± 0.09 fish per net per night in October 2013 to 0.21 ± 0.09 fish per net per night in February 2014). Following the cessation of manual removals, the relative abundance of rainbow trout had increased to 0.56 ± 0.18 fish per net per night by March 2016, suggesting that without sustained removal effort, the population will rapidly return to its pre-removal abundance level.

2.2.3 Scope of the Krom River Rotenone Treatment

The original proposal for the CAPE Alien Fish Eradication Project was to totally eradicate all non-native fish (rainbow trout, bluegill and largemouth bass) from the Krom River to enable a conservation translocation of native fishes from the Matjies River System to take place. This required treating the river from the Disa Pool waterfall downstream to a point upstream of the confluence with the Matjies River. For this proposal, the construction of a lower barrier was required to prevent the Krom River being re-colonised by non-native fish from the Matjies River. In anticipation that the Rotenone treatment of the Krom River would be completed in two stages, a fish barrier (~ 2 m vertical concrete weir, funded by Kromrivier Cederberg Park) was constructed in the chalet area in 2013 to prevent recolonization of the upper reaches by non-native fish following the treatment to remove rainbow trout. However, the barrier was constructed below the offtakes of the two furrows that fed the two farm dams and reach between the weir and the furrow offtakes contained and/or has subsequently been colonised by bluegill. During surveys of the Krom River in 2017 in planning for the Rotenone treatment, CapeNature scientists identified a substantial bedrock step in the

Matjies River Nature Reserve that met the criteria established by van der Walt et al. (2016) for effective natural barriers to upstream movement of bass.

Barrier weir on the Krom River constructed by the Cederberg Tourist Park within the Resort area in support of the proposed fish conservation project (Photo Sean Marr).

The original CapeNature proposal of how to ensure that the Clanwilliam rock catfish population, whose distribution range in the Krom River fell within the rainbow trout distribution between Disa Pool waterfall and the barrier weir, could be secured was to catch as many rock catfish as possible prior to the Rotenone application and keep them in aerated pools alongside the river, returning the fish to the river once the Rotenone had worked through the system. This would be repeated the following year when the river was treated a second time as per the Rondegat River. A number of freshwater fish researchers, particularly from SAIAB, expressed their concerns regarding this plan for the rock catfish citing that the losses of individuals from this unique population of *A. gilli* would be unacceptable, especially since the population would pass through two severe bottlenecks and could be at risk of being extirpated. As a result, CapeNature in 2017 reviewed its treatment plans and adjusted the treatment area to focus on controlling invasive bass and bluegill in the middle and lower sections of the river below the water offtake point.

CapeNature successfully applied for a General Authorisation from the Department of Water and Sanitation to treat the Krom River farm dams to remove source populations of bluegill and largemouth bass. The Rotenone treatment of the Chalet Dam to remove bluegill was successfully completed in January 2017 as a component of this project and is discussed in detail in Chapter 3 of this report. The House Dam was used as the control for the treatment of the Chalet Dam and CapeNature plans to treat the House Dam when the Krom River is treated.

CapeNature reconsidered the treatment of the Krom River and, in their application for a General Authorisation from the Department of Water and Sanitation to treat the Krom River, applied to treat the Krom River downstream of the barrier weir to a bedrock step in the Matjies River Nature Reserve, effectively removing the rainbow trout eradication from the scope of the Rotenone treatment. The focus on this species in this river will thus be on control and encouraging visiting anglers to practice catch and kill on this species. This is supported by the landowners of the Resort.

2.3 Methodology

To fully evaluate the use of Rotenone as a non-native fish removal and river rehabilitation tool it is important that both the immediate and long-term impact of Rotenone on community composition and recovery is evaluated. Such research is critical as it would contribute to the successful outcome of the project through helping determine whether native and translocated fish communities recover after the removal of non-native fishes or if the system moves towards an alternative state. As a result, the current project continued the monitoring of the Rondegat River as well as initiated monitoring on the Krom River.

The methodology for monitoring fish and aquatic macro-invertebrates followed that developed during the previous K8/922 and K5/2261 projects in order to allow for comparisons with results from these projects. Permits from CapeNature (0028-AAA008-00260 and 0056-AAA008-00067) and Ethics Approval from SAIAB (25/4/1/7/5_2018-03) were obtained for the project. Two field trips with students were held from the 15th of January to the 3rd of February 2017 and from the 15th of January to the 3rd of February 2018. Base-line pre-treatment data for habitat and water quality, were also collected.

2.3.1 Selection of Study Sites

The Krom River was divided into five reaches based on the fish present: Fishless, Trout, Rock Catfish, Treatment and Sawfin based on the fish distributions previously determined (Marr et al., 2012); see Figure 2.2. Fish and habitat sampling was conducted at 28 sites in the Krom River and one in the Matjies River (Figure 2.3a). Four of the five reaches were sampled for fish and habitat, the Fishless reach being excluded for obvious reasons. Habitat and fish sampling was conducted in the Trout, Rock Catfish, Treatment, and Sawfin reaches (Figure 2.3a) while SASS5 (Dickens and Graham, 2002) was conducted in the Fishless, Trout, Rock Catfish, and Treatment reaches (Figure 2.3b). Stone samples for aquatic invertebrates were also collected and stored for comparative assessment when the river is ultimately treated.

2.3.2 Water Sampling

At each site, temperature, conductivity, pH and turbidity (NTU) were measured using a Hanna HI98129 Combo pH and electrical conductivity meter and a Hanna HI 98703 turbidimeter (HANNA Instruments, Woonsocket, USA). For the 2017 field trip, the water parameters were collected when the habitat data was collected. For the 2018 field trip, one transect across all sites was conducted in a single day for the water parameters. A Hobo temperature logger (Onset Electronics, Onset, MA, USA) was deployed in the Treatment reach at the barrier weir in the Krom River campsite in March 2016. A further seven Hobo loggers were deployed in the Krom River (Figure 2.4) during the 2017 field trip to provide a more comprehensive coverage of the temperature profile of the Krom River. One logger was deployed in the Fishless reach, two in the Trout reach, two in the Rock catfish reach, one in the Treatment reach (in addition to the one deployed in 2016), and one in the Sawfin reach.

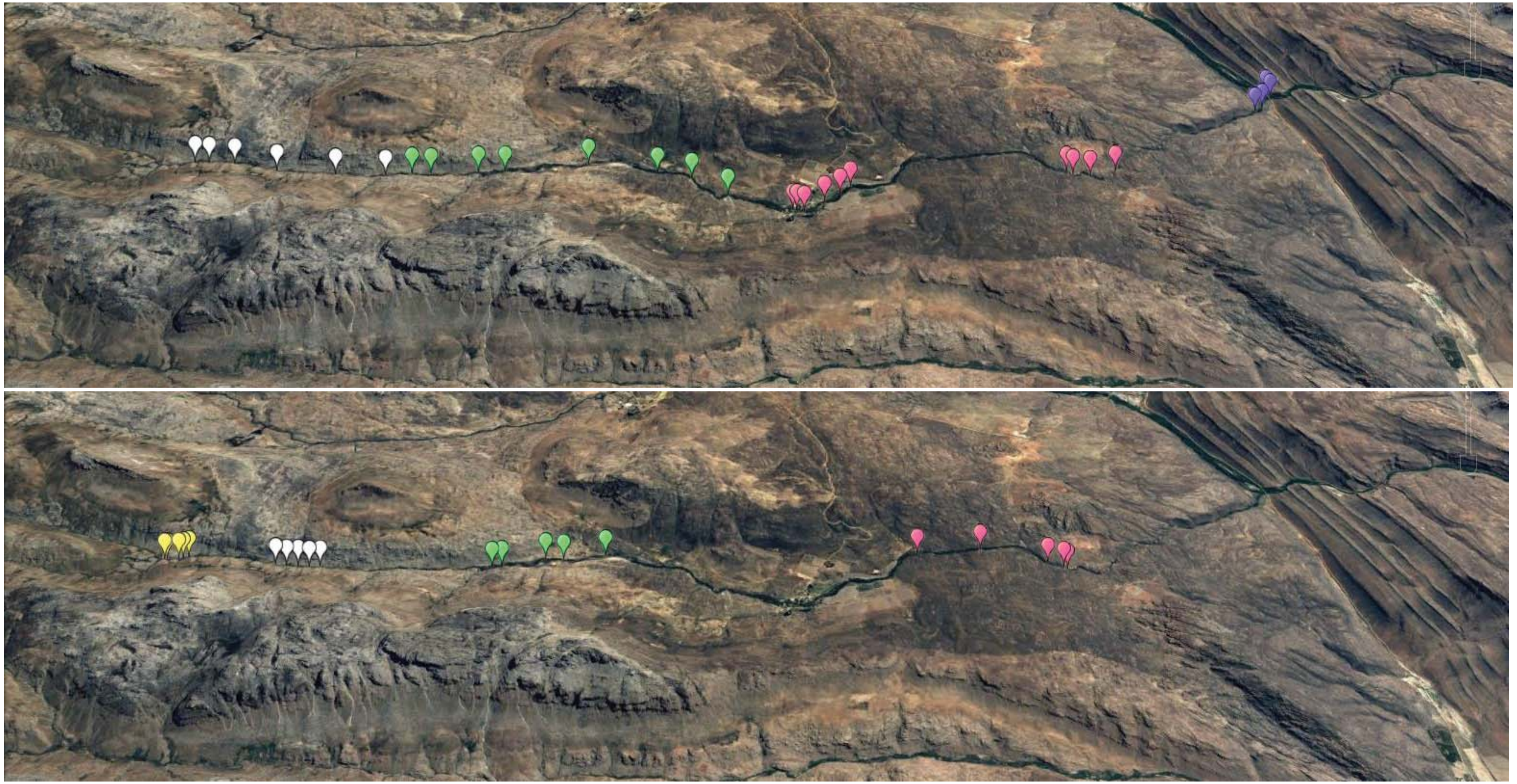


Figure 2.3: Location of sampling sites on the Krom River for a) fish and habitat and b) SASS and invertebrates. The reach is depicted by the colour of the markers: Fishless – yellow, Trout – white, Rock catfish – green, Treatment − pink, and Sawfin − magenta

Figure 2.4: Location of the Hobo temperature loggers installed in the Krom River. The white icon indicates the location of the logger installed in 2016 at the chalet weir in the Krom River farm whereas the yellow icons indicate the location of the loggers installed during the 2017 field trip.

2.3.3 Habitat Mapping

Physical characteristics of each site were collected in 2017. To estimate pool volume, the length of each pool was measured followed by between three and five (depending on habitat), equally spaced, width measurements. On each width transect, five depths were measured and the substrate and vegetation, if any, at each depth measurement recorded. The site was sketched noting riparian and instream vegetation, with samples of vegetation collected for species identification.

2.3.4 Macroinvertebrate monitoring

Twenty sites, five in each of the four reaches, were sampled using the SASS5 methodology (Dickens and Graham, 2002; Weyl et al., 2016). The SASS score and number of taxa present were calculated for each site, and summarised as the average score per taxon (ASPT). The relationship between the SASS score and the ASPT was plotted using the biological bands for the Western Fold Mountains from Dallas & Day (2007).No samples were collected from the Sawfin reach because this reach lacked the required habitat for the SASS5 protocol and there was no flow at sites within the reach in mid-summer. At each site, the SASS5 protocol (Dickens and Graham, 2002) was carried out in all mandatory biotopes by a trained SASS5 practitioner. In addition, water physico-chemical parameters, selected habitat requirements and flow parameters were recorded.

2.3.5 Fish

Fish surveys were conducted in summer (January-March) each year. The timing of the surveys at the end of summer falls within a low flow period, during which sampling was considered most effective, allowing meaningful comparisons with subsequent surveys conducted during the same season. Fish were sampled at each site using three methods. Three sampling methods; fyke netting, snorkel transects and underwater video analysis, were used to assess for species composition, population structure and relative abundance in the fish assemblage. A standard protocol for the fish surveys were used: underwater video analysis, snorkel transects followed by fyke netting overnight (Ellender et al., 2012).

Underwater video analysis was conducted using GoPro HD Hero high-definition cameras. Camera settings were standardised at; Field of view = 127, Resolution (Full HD) = 1080p (1920 to 1080), Frames per second = 30 NTSC, 25 PAL. Methods for placement, observation time and analysis followed those recommended by Ellender et al. (2012). Three cameras were deployed at each site on tripods for 18 minutes, including a 3 minute acclimation time.

Snorkel transects were conducted following the method described by Ellender et al. (2011) whereby the number of fish were enumerated during two consecutive snorkel passes and averaged to give an estimate for the number of fish present in the pool.

Fish were sampled using double-ended fyke nets (8 m guiding net, first-ring diameter of 55 cm, 10 mm mesh size at the cod end). One to two fyke nets (depending on the size of the pool) were then set overnight and recovered the following morning, recording the species and length (TL, mm) of each fish captured. All fyke nets were fitted with an “otter guard” comprising plastic mesh with openings no larger than 10 × 10 cm to prevent non-target species, such as the Cape clawless otters *Aonyx capensis,* entering the nets. Although the use of these otter guards influenced the maximum size of fish that could enter the nets, their use was considered critical to avoid air breathing bycatch. All fyke nets were set in the evening (between 16:00 and 18:00) and retrieved the next morning (between 06:00 and 08:00) with an average soak time of 16 hours. All the fyke nets were set and collected in the same sequence as to minimize variance in soak time. Other taxa captured in the fyke nets were also recorded.

2.4 Base-Line Results

The data collected during the 2017 and 2018 field trips is summarised here. Comparisons are made between the study reaches and sampling years. It should be noted that the two sampling events took place during a protracted drought in the Western Cape and that the 2017 winter did not deliver the rainfall required to break the drought. Consequently, the flow during the 2018 field trip was lower than the flow of the 2017 field trip. This is concerning because the 2017 field trips took place when the river had exceedingly low flow for this time of the year. It is recommended that further base-line data be collected before the Rotenone treatment of the Krom River at higher flow conditions following the good rains of the 2018 winter rainfall season.

2.4.1 Water Quality

Box plots and longitudinal profiles were prepared to summarise the temperature, pH, conductivity and turbidity for each reach by year. In addition a two-way ANOVA was conducted to determine whether there a difference between the temperature, pH, conductivity and turbidity in the respective reaches and between the respective years. The significance level for the two-way ANOVA was set at $p<0.05$. If a significant result was returned for the two-way ANOVA, Tukey's Honest Significant Difference test (Tukey HSD) was performed post-hoc to determine where the significant differences lay. All tests were conducted using the R statistical software (R Development Core Team, 2018).

Temperature

The box plots and longitudinal profiles for the temperature measurement are presented in Figure 2.5. A significant result was returned for the two-way ANOVA for the zones and the interaction between the years and zones (Zone F=12.615 df =3 p<0.001; Year F=2.894 df=1, p=0.095; and interaction F=6.580 df=3 p<0.001). The Tukey HSD showed that the significant result for the zones was a result of significant differences for the pair-wise comparisons between the Trout-Treatment, Rock Catfish-Treatment and Rock Catfish-Sawfin zones. There was no statistical significance between the years for any of the zones.

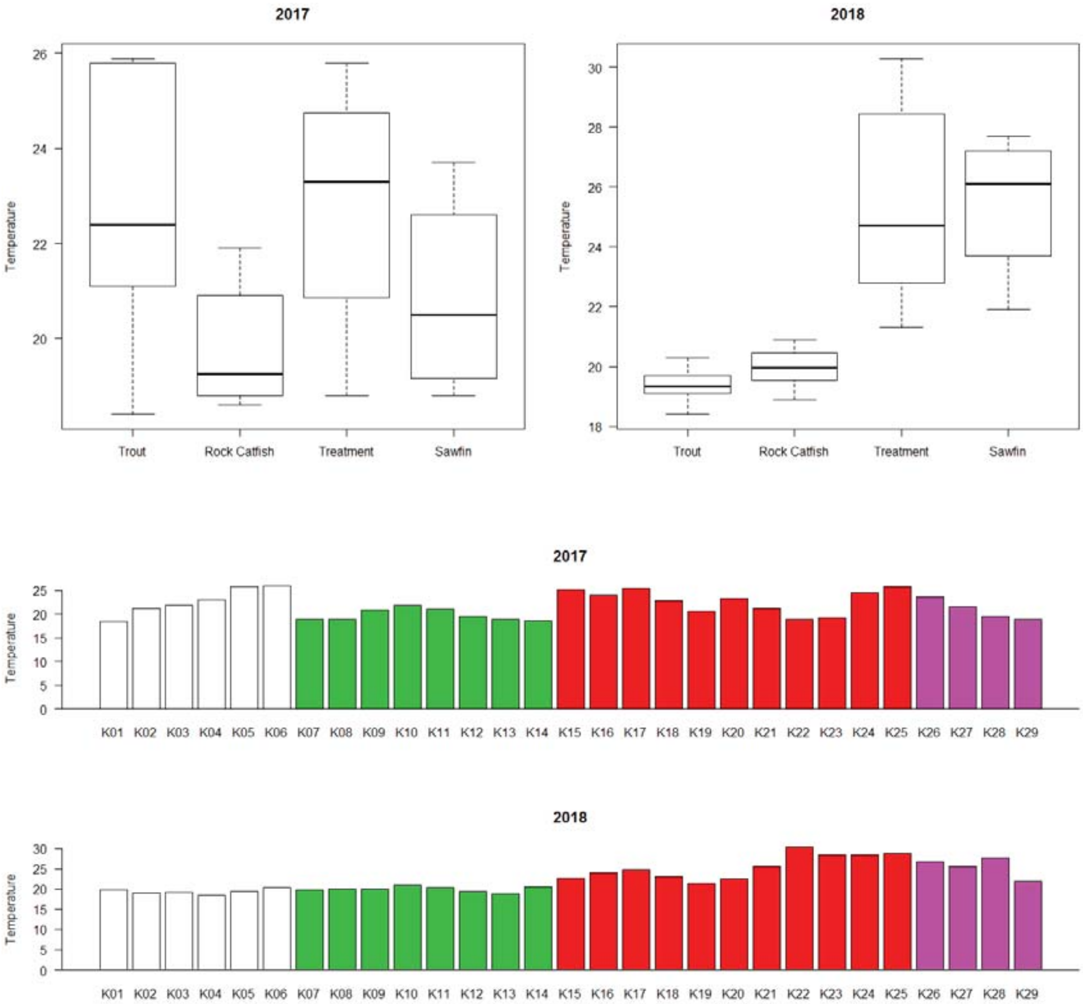


Figure 2.5: Box plots and longitudinal profiles summarising the water temperature by zone and year in the Krom River. The zone is depicted by the colour of the bar in the longitudinal plot: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

pH

The box plots and longitudinal profiles for the pH measurement are presented in Figure 2.6. A significant result was returned for the two-way ANOVA for the zones, year and the interaction between the years and zones (Zone F=3.835 df=3 p=0.015; Year F=18.043 df=1, p<0.001; and interaction F=12.033 df=3 p<0.001). The Tukey HSD showed that the significant result for the zones was a result of significant differences for the pair-wise comparisons between the Rock Catfish-Treatment zones. There was also a statistical significance between the years for the Trout zone.

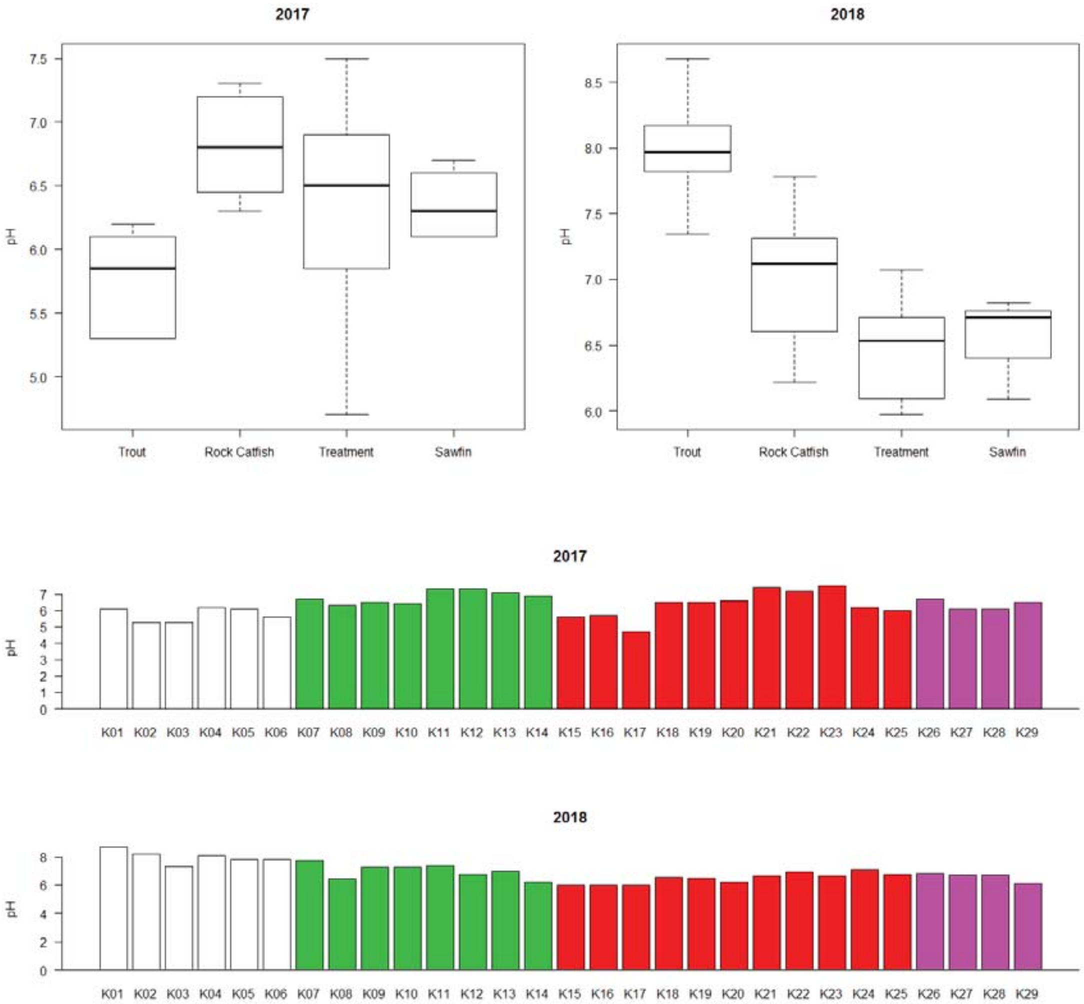


Figure 2.6: Box plots and longitudinal profiles summarising the pH by zone and year of the Krom River. The zone is depicted by the colour of the bar in the longitudinal plot: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

Conductivity

The box plots and longitudinal profiles for the conductivity measurement are presented in Figure 2.7. A significant result was returned for the two-way ANOVA for the zones, years and the interaction between the years and zones (Zone F=58.990 df =3 p<0.001; Year F=63.440 df=1, p<0.001; and interaction F=8.890 df=3 p<0.001). The Tukey HSD showed that the significant result for the zones was a result of significant differences for the pair-wise comparisons between the Trout-Treatment, Trout-Sawfin, Rock Catfish-Treatment and Rock Catfish-Sawfin zones. There was a statistical significance between the years for the Treatment and Sawfin zones.

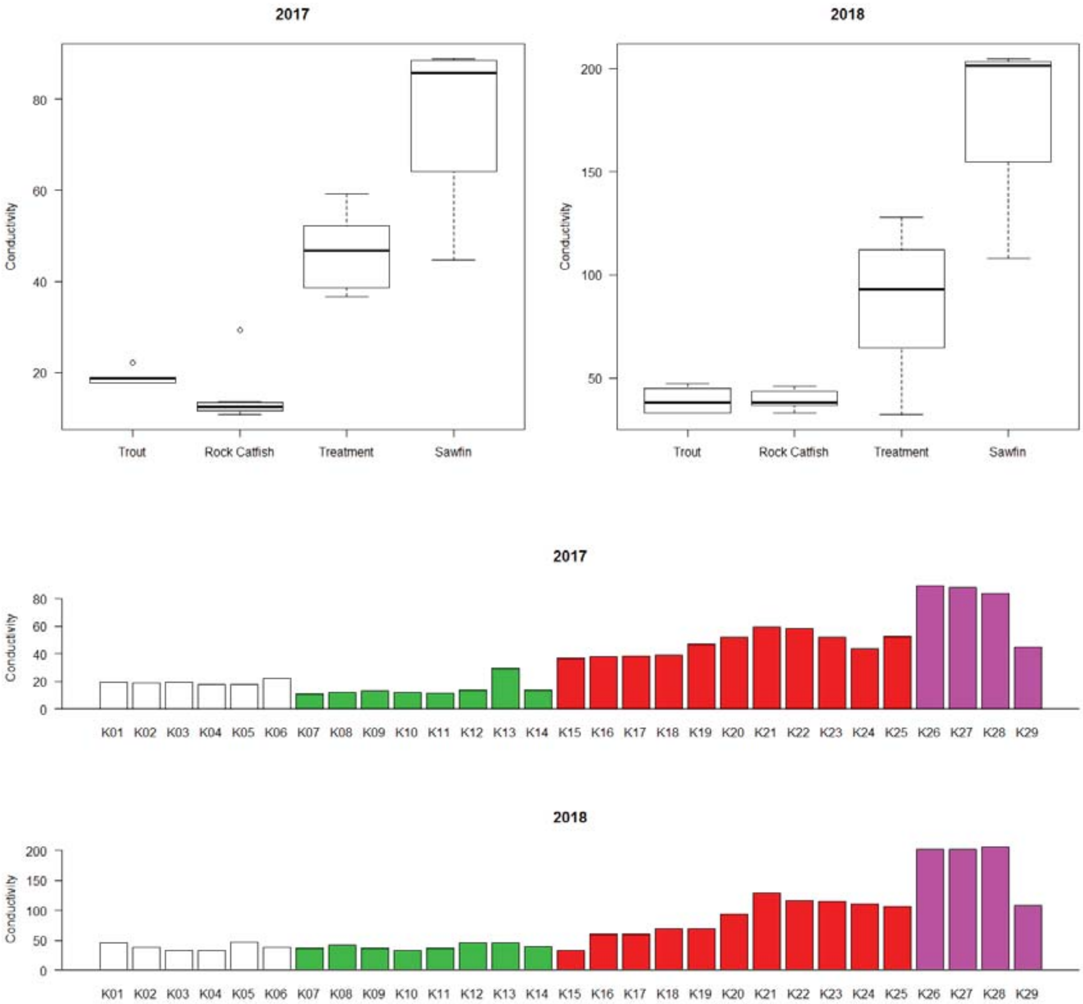


Figure 2.7: Box plots and longitudinal profiles summarising the conductivity by zone and year of the Krom River. The zone is depicted by the colour of the bar in the longitudinal plot: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

Turbidity

The box plots and longitudinal profiles for the turbidity measurement are presented in Figure 2.8. A significant result was returned for the two-way ANOVA for the zones (Zone F=37.127 df =3 p<0.001; Year F=0.003 df=1, p=0.955; and interaction F=2.309 df=3 p=0.082). The Tukey HSD showed that the significant result for the zones was a result of significant differences for the pair-wise comparisons between the Trout-Treatment, Trout-Sawfin, Rock Catfish-Treatment and Rock Catfish-Sawfin zones. There was no statistical significance between the years for any of the zones.

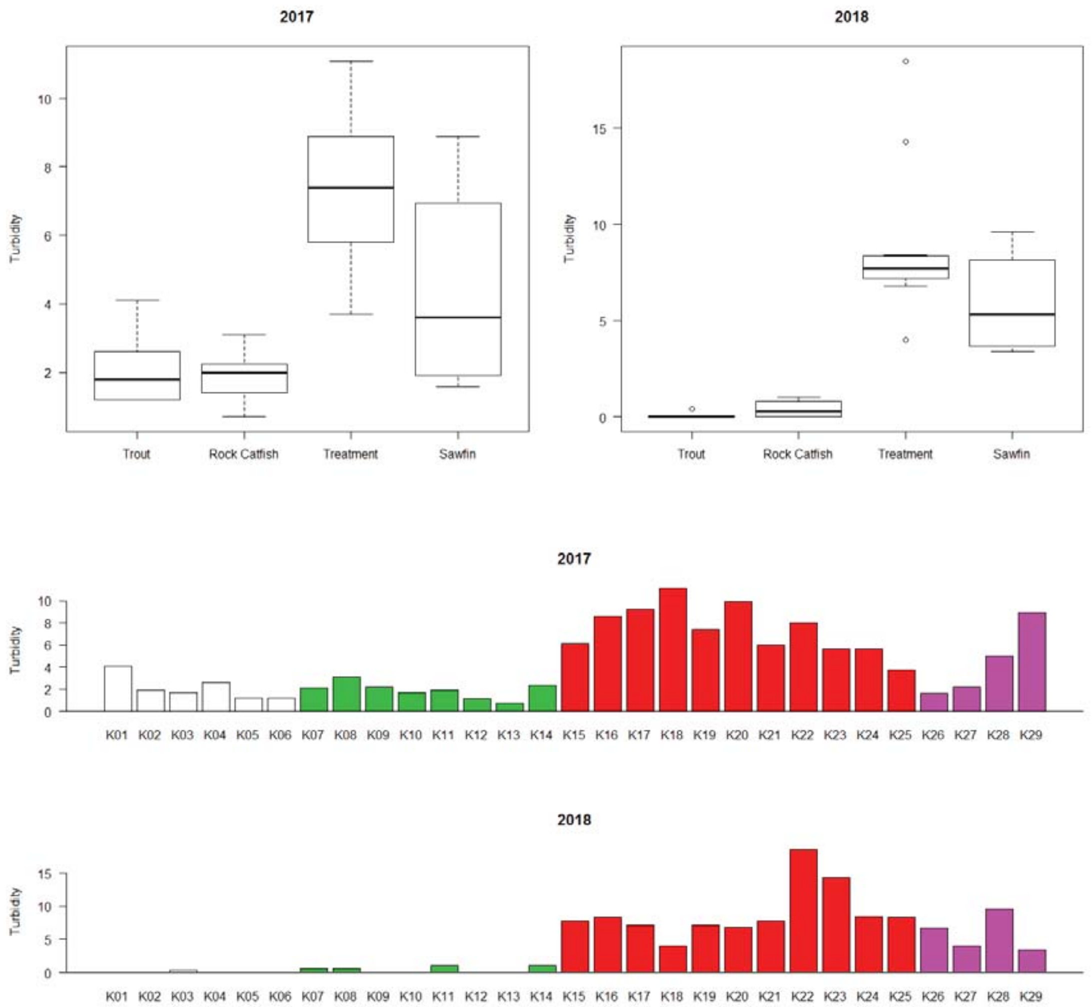


Figure 2.8: Box plots and longitudinal profiles summarising the turbidity by zone and year of the Krom River. The zone is depicted by the colour of the bar in the longitudinal plot: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

Dissolved Oxygen

Dissolved oxygen was only collected during the 2018 field trip due to the failure of the dissolved oxygen meter during the 2017 field trip. The box plots and longitudinal profiles for the dissolved oxygen measurement are presented in Figure 2.9. A significant result was returned for the one-way ANOVA for the zones for dissolved oxygen (F=5.654 df =3 p=0.004) but not for % oxygen saturation (F=2.284 df =3 p=0.093). The Tukey HSD showed that the significant result for the zones was a result of significant differences for the pair-wise comparisons between the Trout-Treatment and Rock Catfish-Treatment zones.

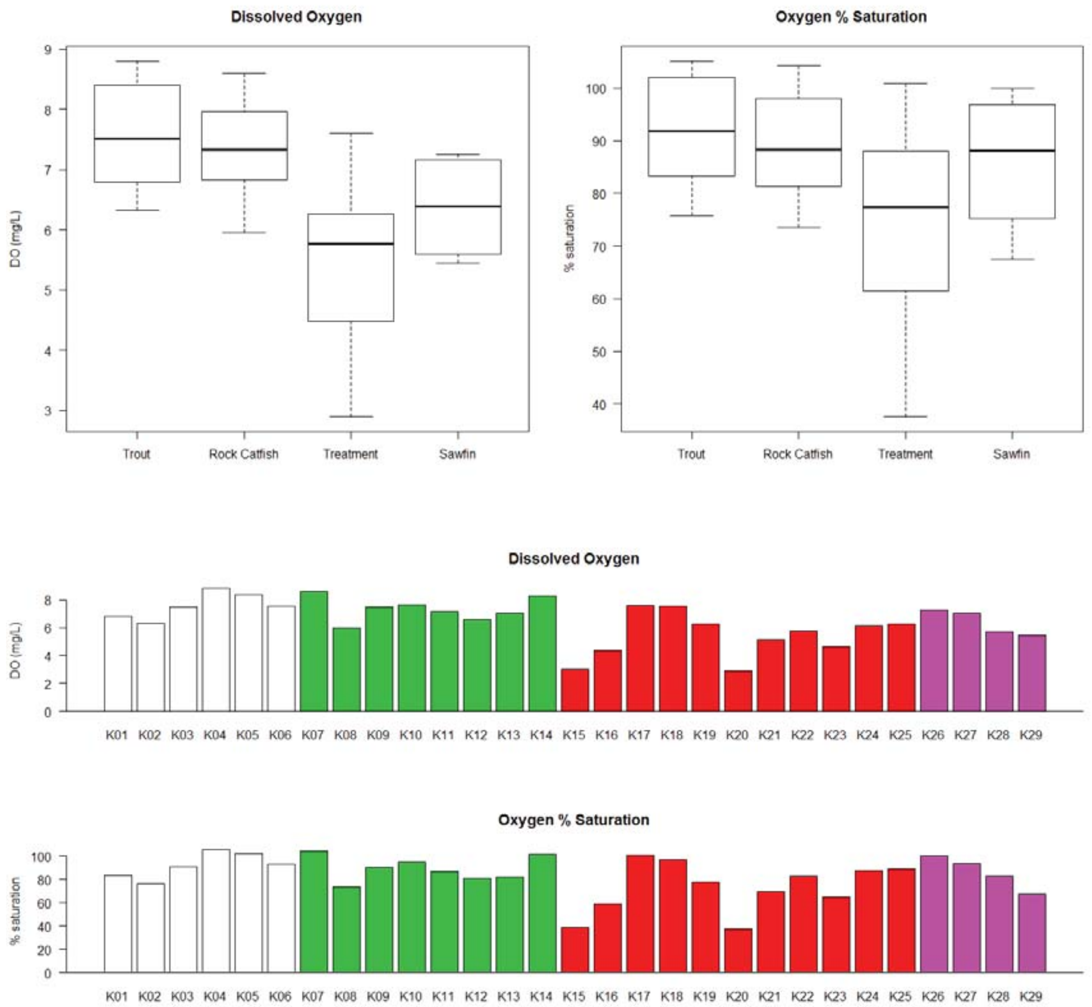


Figure 2.9: Box plots and longitudinal profiles summarising the dissolved oxygen by zone of the Krom River. The zone is depicted by the colour of the bar in the longitudinal plot: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

Multivariate Analysis

A Principal Component Analysis (PCA) was conducted to summarise patterns in the physico-chemical variables among sites down the length of the Krom River using R statistical software (R Development Core Team, 2018). The data from the two years were analysed separately. The data were first normalised before the PCA was performed. The results are presented in Figure 2.10.

The 2017 data showed that the river can be divided into two reaches, with a major change in water quality taking place in the chalet areas. This corresponds to the boundary between the Rock Catfish and Treatment zones. Above the Treatment zone, the sites were arranged along a pH and temperature gradient, while the sites from the Treatment zone downstream were also along a similar gradient. The Trout zone had a higher temperature and lower pH than the Rock Catfish zone during the time of measurement. The two reaches were separated on a conductivity and turbidity gradient with the lower reach having higher values for both variables.

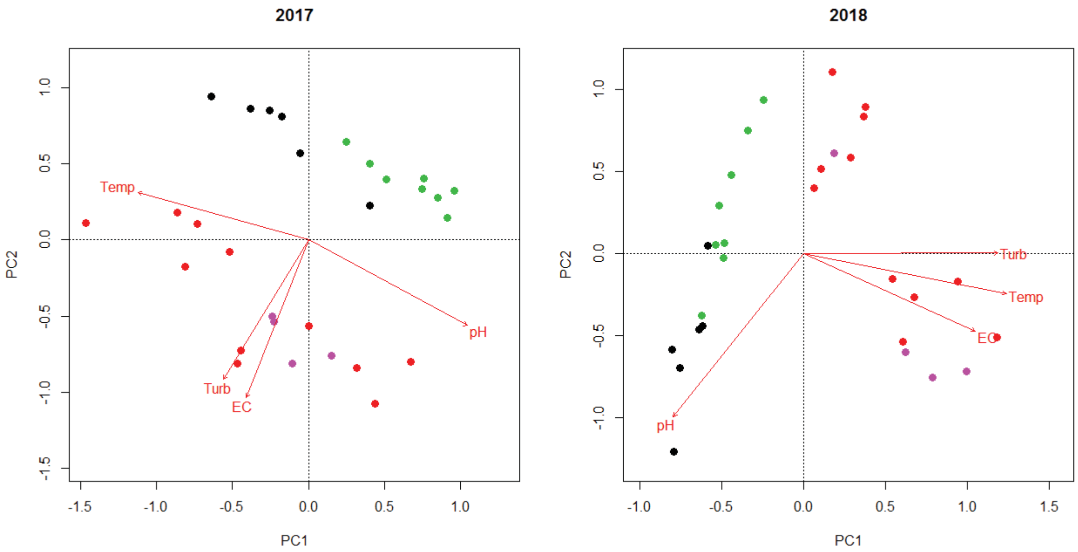


Figure 2.10: Principal Component Analyses summarising the physico-chemical data of the Krom River for 2017 and 2018. The zone is depicted by the colour of the point in the plot: Trout black, Rock catfish green, Treatment red, and Sawfin magenta

A similar pattern was observed for the 2018 data with some subtle variations. The impact of water temperature was less important for the upper reach and pH was the major variable distinguishing the sites of the Trout and Rock Catfish zones. However, this year the Trout zone had the higher pH in comparison to the Rock Catfish zone and temperature was not a factor separating these two zones. The lower reach was divided into two distinct groups, the sites on Krom River Farm and those downstream in the Matjies River Nature Reserve. The Matjies River site actually grouped with the sites within the Farm, rather than with lower Krom River sites. The three groups were separated on the temperature, turbidity and conductivity gradient with all three parameters increasing between the Farm and the Nature Reserve. This could be as a result of the reduced flow in 2018 with the Farm sites being closer to the upstream sites with the temporary connectivity of the lower sites resulting in higher temperature, turbidity and conductivity as a result of the increased evaporation.

2.4.2 Habitat Mapping

The box plots and longitudinal profiles for the depth, width and length of pools surveyed are presented in Figure 2.11. A significant result was returned for the one-way ANOVA for the zones for width (F=4.265 df =3 p=0.015) but not for depth and length (F=1.525 df =3 p=0.233 and F=2.136 df =3 p=0.121, respectively). The Tukey HSD showed that the significant result for width was a result of significant differences for the pair-wise comparisons between the Rock Catfish and Treatment zones.

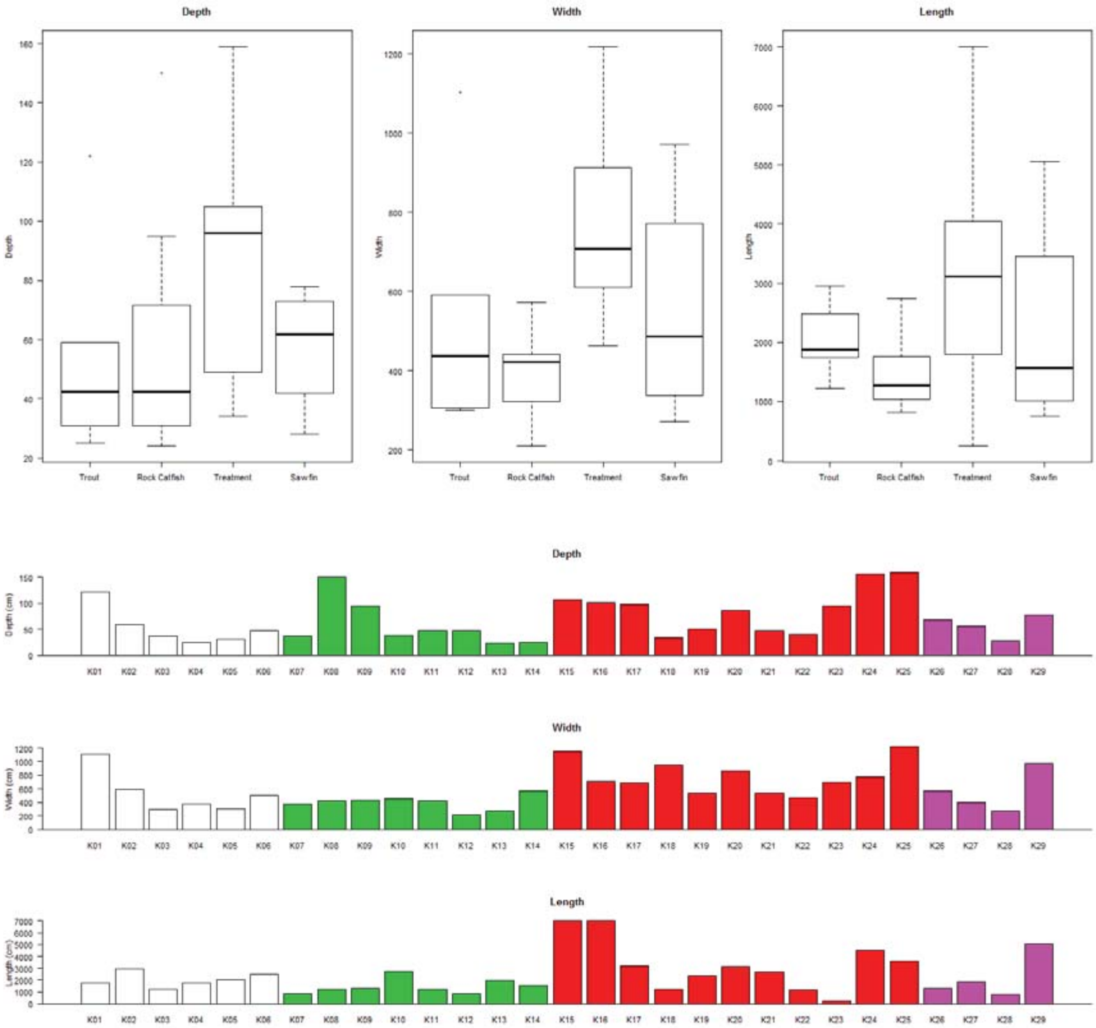


Figure 2.11: Box plots and longitudinal profiles summarising the depth, width and length of the pools surveyed by zone of the Krom River. The zone is depicted by the colour of the bar in the longitudinal plot: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

Substrate

Because the geology of the Krom River catchment is comprised of layered quartzitic sandstones with minor shale bands of the Nardouw subgroup (Thamm and Johnson, 2006), the substrate of the river cycles between bedrock, boulders and cobbles, and gravel and sand down the length of the river. A PCA was conducted on the substrate data to determine whether there were any substrate variables that distinguished between the four zones of the Krom River. The PCA showed that there were three major vectors among the variables, bedrock, boulders and cobbles, and gravel and sand, acting in three equally spaced directions (Figure 2.12). The Trout zone sites appear to be spread mainly along the cobble and boulder axis (PC2) whereas the Treatment zone are spread along the bedrock-gravel and sand axis (PC1). The Rock Catfish zone lies on a similar axis but has a greater cobble and boulder component.

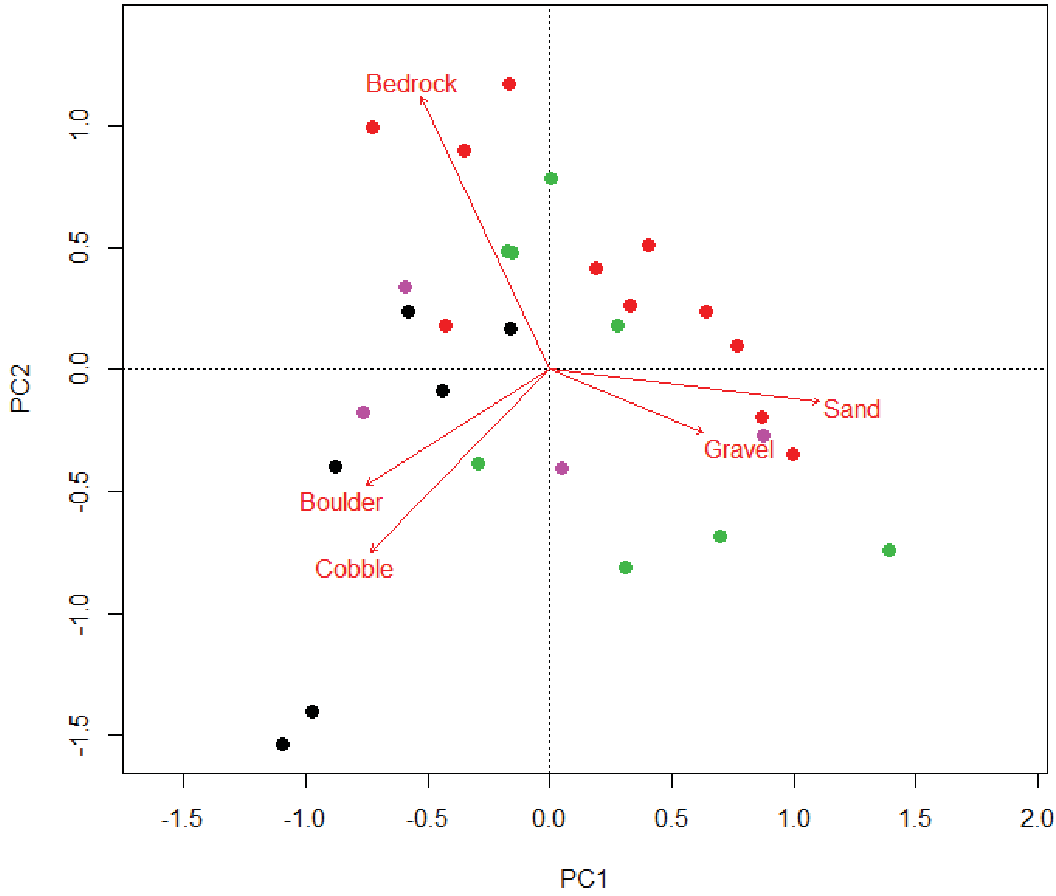


Figure 2.12: Principal Component Analyses summarising the substrate data of the Krom River. The zone is depicted by the colour of the point in the plot: Trout black, Rock catfish green, Treatment red, and Sawfin magenta

A multivariate analysis of the substrate data was conducted using the PRIMER 6 and PERMANOVA+ software (Clarke and Warwick, 2001; Anderson et al., 2008). A resemblance matrix was constructed using Euclidean Distance of the untransformed average percentage of each substrate per site. Non-metric multi-dimensional scaling (NMDS) ordination (Clarke and Warwick, 2001) was then used to visualise the data as 2-dimension ordination plots. The ordination plot shows some separation between the Trout and the Rock Catfish and Treatment Zones (Figure 2.13).

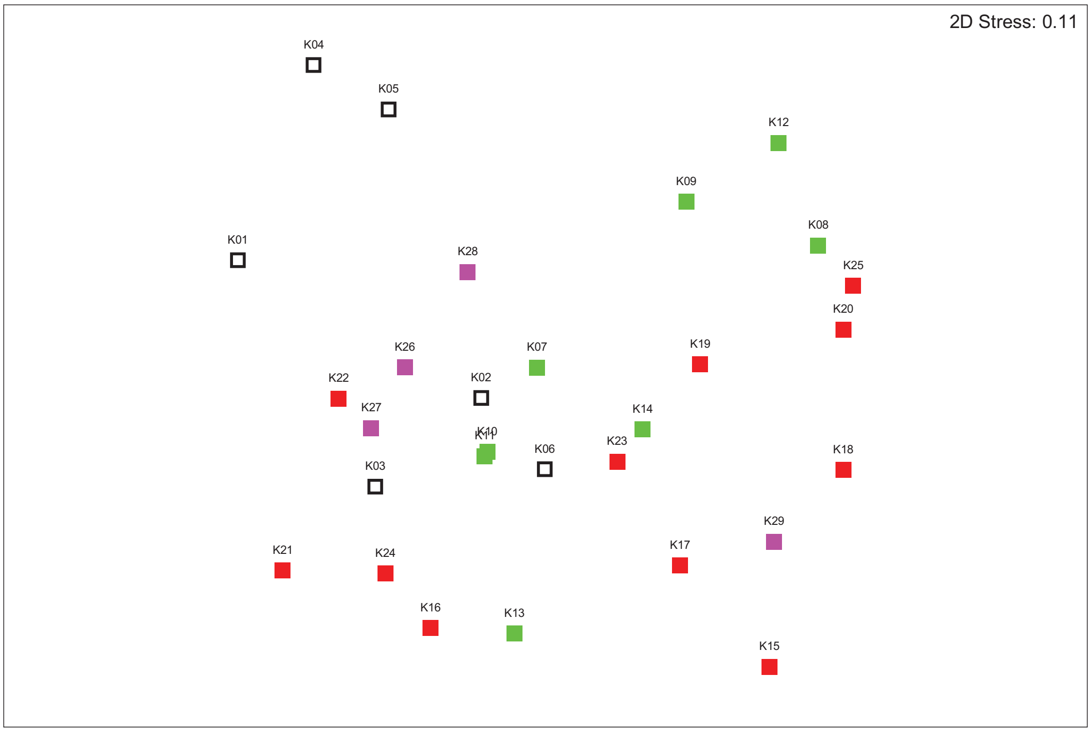


Figure 2.13: Non-Metric Multidimensional Scaling Ordination summarising the substrate data by zone of the Krom River. The zone is depicted by the colour of the marker: Trout white, Rock catfish green, Treatment red, and Sawfin magenta

A distance-based test of homogeneity of multivariate dispersion and a permutational multiple analysis of variance (Anderson, 2001a; b; Anderson and Ter Braak, 2003; Anderson, 2006) were performed to determine whether there was a statistically significant difference in substrates composition by zone using the PERMDISP and PERMANOVA routines of the PERMANOVA+ statistical software. The PERMDISP routine determines whether the multivariate dispersion about the group centroid differed between the impoundments, whereas the PERMANOVA routine determines whether the position of the group centroids in multivariate space and/or the multivariate dispersion about the group centroid differed between the impoundments (Anderson, 2001a; b; Anderson and Ter Braak, 2003; Anderson, 2006). The dispersion about the centroid relates to the within group variation and the group centroids relates to the between group variation. A SIMPER analysis (Clarke and Warwick, 2001) was performed to determine the substrates contributing most to the differences between zones using the SIMPER routine in PRIMER 6 statistical software.

The PERMDISP returned a non-significant result (F=0.548, df=3, p(perm)=0.712) indicating that there was homogeneity in multivariate dispersion between the four zones of the Krom River. The PERMANOVA returned a significant result (Pseudo F=2.144, df=3, p(perm)=0.025) indicating that there was a difference in the position of the centroids of one or more of the zones. A pair-wise PERMANOVA was then performed to determine which of the pair-wise comparisons between the zones were contributing to the significant result of the PERMANOVA. Only the Trout-Rock Catfish and Trout-Treatment pair-wise comparisons returned significant results (t=1.802

p(perm)=0.013 and t=2.205 p(perm)=0.005, respectively). The SIMPER analysis showed that the differences between the Trout and Rock Catfish zones were as a result of sand (31%), cobble (21%) and boulder (12%) with sand being more prominent in the Rock Catfish zone and cobble and boulder being more prominent in the Trout zone. The differences between the Trout and Treatment zones were as a result of sand (38%), cobble (27%), bedrock (21%) and boulder (13%) with sand and bedrock being more prominent in the Treatment zone and cobble and boulder being more prominent in the Trout zone.

2.4.3 Temperature Logger Data

The Hobo data loggers were down-loaded during the 2017 (weir logger at Site K15) and 2018 (Sites K02 near the top of the Trout Zone, K14 near the bottom of the Rock Catfish Zone, K15 (weir) in the Treatment Zone within the Krom River farm complex, and K24 in the Treatment Zone within the Matjies River Nature Reserve) field trips. The data file from each logger was opened in HOBOware version 3.7.13 software (Onset Computer Corporation) and averaged over five-day intervals. The resultant data file was exported as a Microsoft Excel spreadsheet and plotted using Microsoft Excel. Two years of data was plotted for the Weir site K15 (Figure 2.14) as this logger was installed in 2016. One year of data was plotted for sites K02, K14 and K24 (Figure 2.15).

Figure 2.14: Seasonal water temperatures from a logger in the chalet area (Site K15, Treatment Zone) of the Krom River in 2016 and 2017.

Figure 2.15: Seasonal water temperatures from loggers installed at three sites in the Krom River: K02 Trout Zone (black), K14 Catfish Zone (green) and K24 Treatment Zone (red)

2.4.4 Macroinvertebrates – SASS

The SASS5 results suggest that the majority of the sites sampled along the Krom River are in a healthy condition with three sites within the Fishless zone and one in the Trout zone in a Reference Condition (Figure 2.16). One site, the most downstream site in the Treatment zone, was shown to be on the border between Fair and Poor condition. This site had a very low flow and was sub-optimal for the SASS5 protocol.

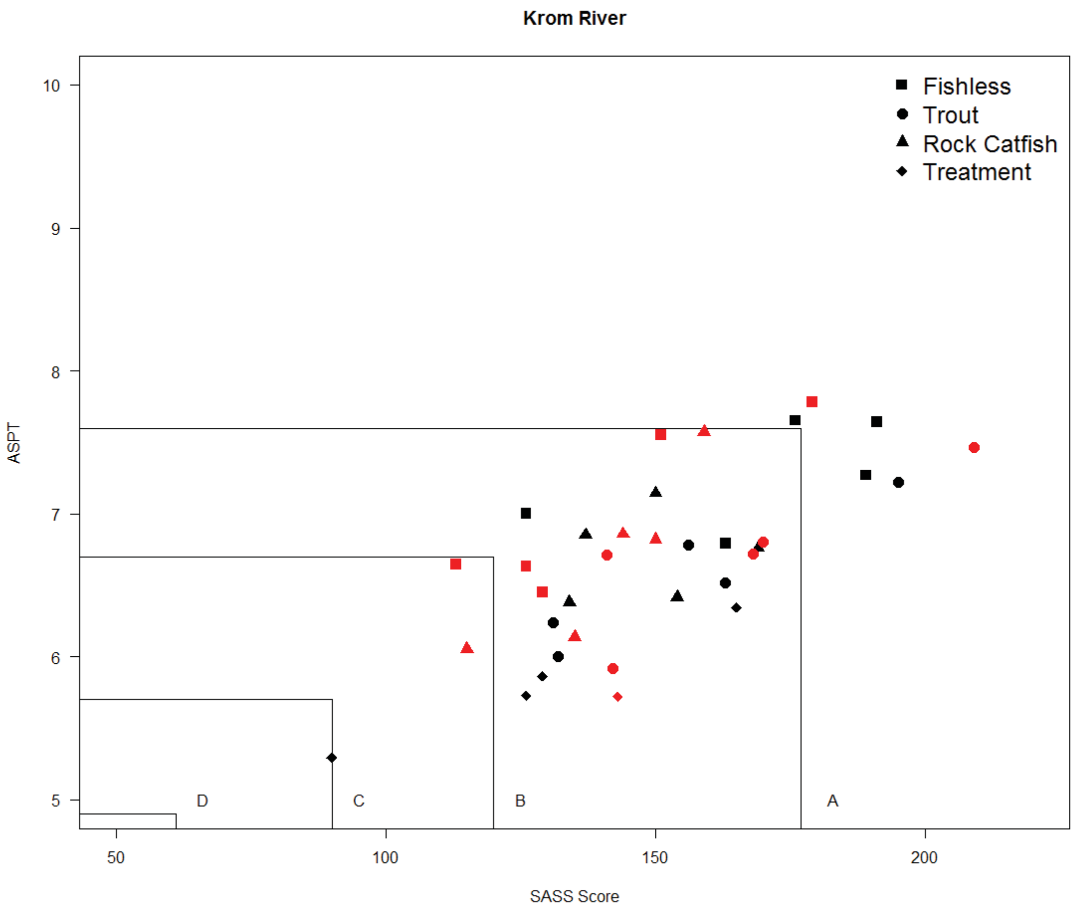


Figure 2.16: Relationship between SASS5 score and average score per taxon (ASPT) recorded for four zones of the Krom River. The 2017 data are represented by the black markers and the 2018 by the red markers. The biological bands of the Western Fold Mountains representing the invertebrate community condition of A – REFERENCE, B – GOOD, C – FAIR and D – POOR; adapted from Dallas and Day (2007).

Box plots were prepared to summarise the ASPT, SASS Score and number of Taxa each reach. In addition, a two-way ANOVA was conducted to determine whether there a difference between the ASPT, SASS Score or number of Taxa in the respective zones over the two years. The significance level for the ANOVA was set at $p<0.05$. If a significant result was returned for the ANOVA, Tukey's Honest Significant Difference test (Tukey HSD) was performed post-hoc to determine where the significant differences lay. All tests were conducted using the R statistical software (R Development Core Team, 2018).

A significant result was returned for the two-way ANOVA of the ASPT for the zone, but not for the year or the interaction between the year and the zone (Zone F=7.723 df =3 p<0.001; Year F=0.100 df=1, p=0.754; and interaction F=0.339 df=3 p=0.797) with the Tukey HSD returning a significant result for the Fishless-Treatment, Trout-Treatment and Rock catfish-Treatment zones (p<0.001, p=0.038 and p=0.022, respectively); see Figure 2.17. The ANOVA returned non-significant results for the SASS Score (Zone F=2.274 df =3 p=0.102; Year F=0.628 df=1, p=0.435; and interaction F=1.417 df=3 p=0.259) and number of taxa (Zone F=2.371 df =3 p=0.092; Year F=0.695 df=1, p=0.412; and interaction F=2.225 df=3 p=0.107).

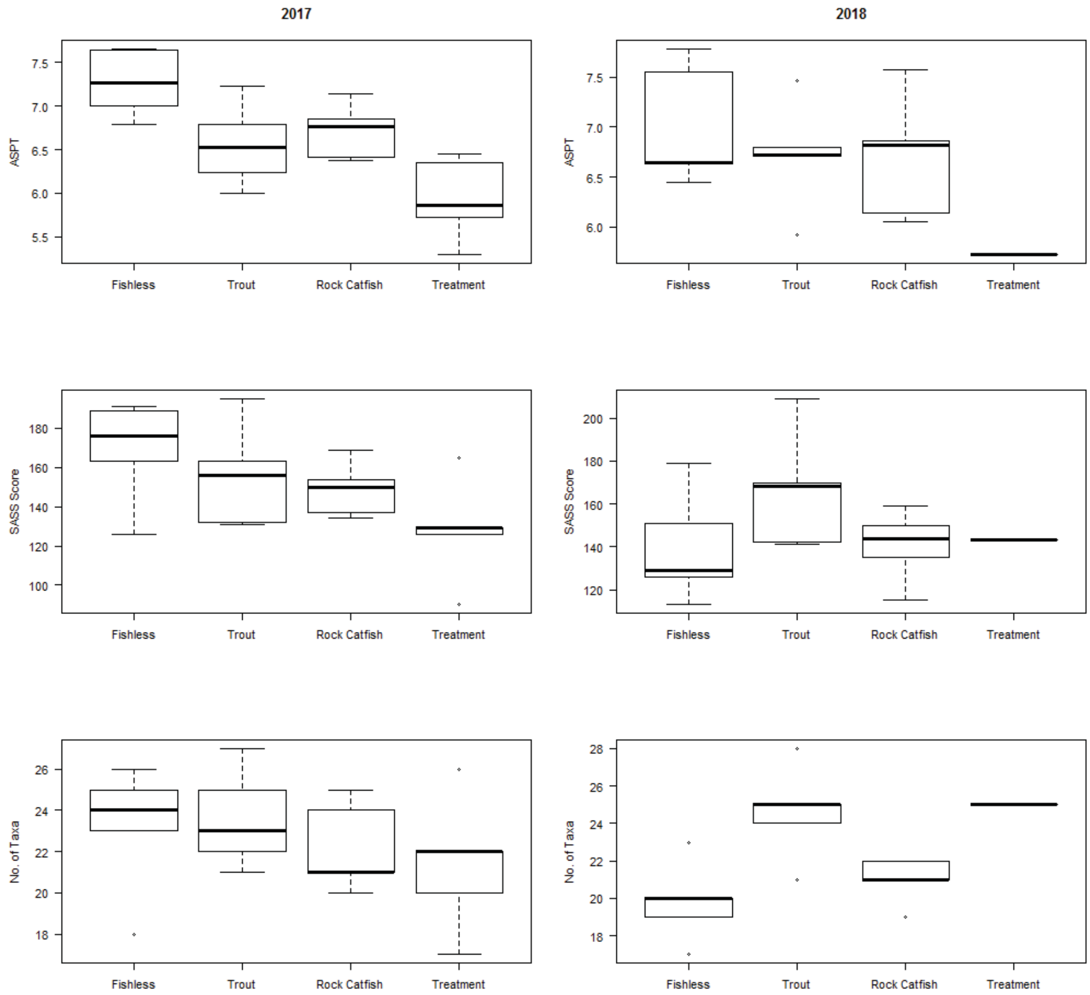


Figure 2.17: Box plots summarising the average score per taxon (ASPT), SASS score and the number of taxa recorded by zone sampled in 2017 and 2018 in the Krom River.

A multivariate analysis of the SASS invertebrate presence-absence data was conducted using the PRIMER 6 and PERMANOVA+ software (Clarke and Warwick, 2001; Anderson et al., 2008). A resemblance matrix constructed using Bray-Curtis Similarity of the presence-absence data for each site. Non-metric multi-dimensional scaling (NMDS) ordination (Clarke and Warwick, 2001) was then used to visualise the data as 2-dimension ordination plots. The ordination plot shows some separation between the Trout and the Rock Catfish and Treatment Zones (Figure 2.18).

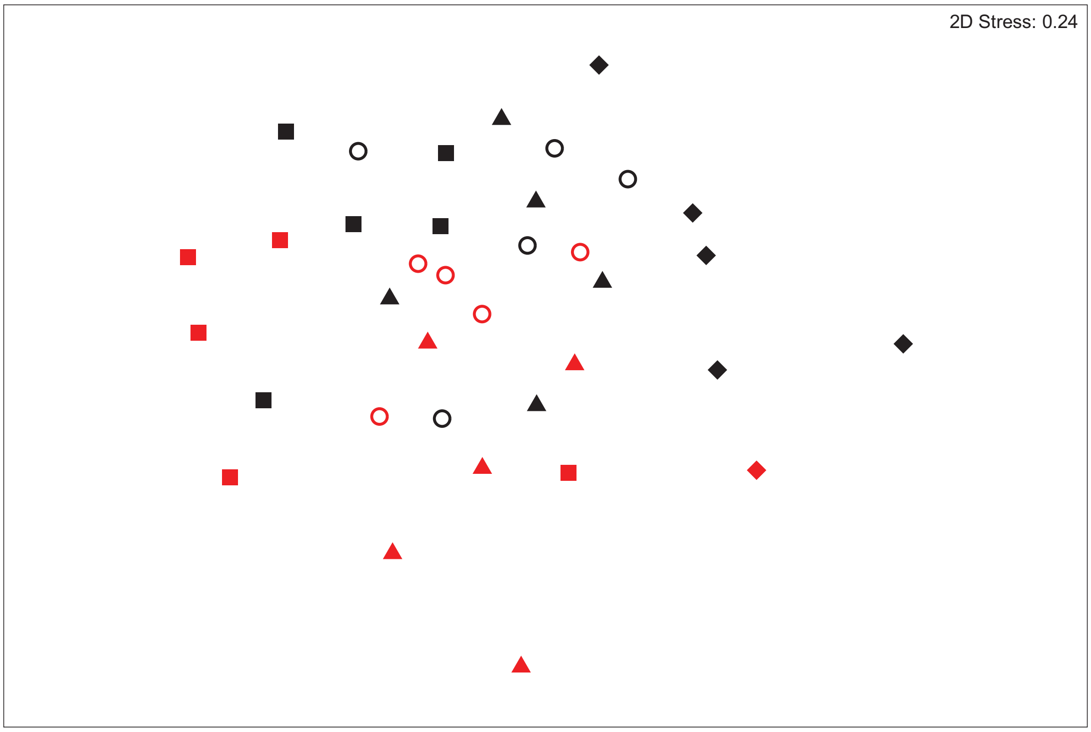


Figure 2.18: Non-Metric Multidimensional Scaling Ordination summarising the SASS invertebrate presence-absence data by zone of the Krom River. The zone is depicted by the marker: Fishless ▲, Trout ○, Rock catfish ■, and Treatment ♦. The 2017 data are represented by the black markers and the 2018 by the red markers.

A distance-based test of homogeneity of multivariate dispersion and two-way permutational multiple analysis of variance were performed to determine whether there was a statistically significant difference in the SASS invertebrate presence-absence data by year and by zone using the PERMDISP and PERMANOVA routines of the PERMANOVA+ statistical software. A SIMPER analysis was performed to determine the taxa contributing most to the differences between zones using the SIMPER routine in PRIMER 6 statistical software.

The PERMDISP returned a non-significant result for both year ($F=0.734$, $df=1$, $p(perm)=0.434$) and zone ($F=0.557$, $df=3$, $p(perm)=0.706$) indicating that there was homogeneity in multivariate dispersion between the two years and four zones of the Krom River. The PERMANOVA returned significant results for both zone (Pseudo $F=4.060$, $df=3$, $p(perm)=0.001$) and year (Pseudo $F=3.228$, $df=1$, $p(perm)=0.001$), but not the interaction between zone and year (Pseudo $F=0.812$, $df=3$, $p(perm)=0.726$) indicating that there was a difference in the position of the centroids of one or more of the zones and between the years. A pair-wise PERMANOVA was then performed to determine which of the pair-wise comparisons between the zones were contributing to the significant result of the PERMANOVA. All pairwise comparisons returned significant results (all $p<0.002$) indicating that each zone had a distinct assemblage of aquatic invertebrates.

The SIMPER analysis showed that the sites within the respective zones shared between 65 and 75% similarity with respect to their invertebrate communities based on presence-absence data. Between zones, the average similarity varied between 53% between the Fishless and Treatment zones and 65% between the Trout and Rock Catfish zones (Table 2.1).

Table 2.1: Average similarity within and between the invertebrate assemblages (presence-absence) per zone of the Krom River. The within group similarities are on the diagonal in bold.

	Fishless	Trout	Rock Catfish	Treatment
Fishless	**67.27**			
Trout	64.15	**70.23**		
Rock Catfish	62.06	65.44	**66.06**	
Treatment	53.09	61.03	59.37	**65.62**

The taxa that contributed most to the similarity within the respective zones are presented in Table 2.2. A number of taxa were common between the zones but there were differences in the frequencies at which the taxa were recorded within each zone of the Krom River. A number of taxa were recorded at each site within a zone both years and these are indicated in bold in Table 2.2.

Table 2.2: Summary of the invertebrate assemblages per zone of the Krom River. The taxa in bold were found at all the sites sampled in both years.

Fishless	Trout	Rock Catfish	Treatment
Leptophlebiidae	**Baetidae**	**Baetidae**	**Baetidae**
Synlestidae	**Leptophlebiidae**	**Libellulidae**	**Caenidae**
Naucoridae	**Libellulidae**	**Naucoridae**	**Leptophlebiidae**
Elmidae	**Leptoceridae**	**Veliidae**	**Libellulidae**
Gyrinidae	**Elmidae**	**Leptoceridae**	**Notonectidae**
Chironimidae	**Hydraenidae**	**Elmidae**	**Elmidae**
Veliidae	**Chironimidae**	Leptophlebiidae	**Chironimidae**
Baetidae	**Simuliidae**	Gomphidae	Gomphidae
Leptoceridae	Corydalidae	Simuliidae	Simuliidae
Hydraenidae	Veliidae	Hydraenidae	Coenagrionidae
Petrothrincidae	Culicidae	Aeshnidae	Aeshnidae
Hydrophilidae	Synlestidae	Chironimidae	Leptoceridae
Corydalidae	Coenagrionidae	Gyrinidae	Dytiscidae
Hydropsychidae	Hydrophilidae	Coenagrionidae	Gyrinidae
Simuliidae	Gomphidae	Potamonautidae	Hydraenidae
Ecnomidae	Aeshnidae	Ceratopogonidae	Ceratopogonidae
Notonemouridae	Notonectidae	Scirtidae	Culicidae
Ceratopogonidae	Hydropsychidae	Hydrophilidae	Potamonautidae
Notonectidae	Ceratopogonidae	Caenidae	
Libellulidae	Petrothrincidae	Culicidae	
	Potamonautidae	Oligochaeta	
	Philopotamidae		

2.4.5 Fish

The results of the fish distribution analyses confirmed the results of previous studies in the Krom River, e.g. Marr et al. (2012), confirming the validity of the zones delineated for this report. Three native species and three non-native species were recorded during the surveys. The only native species in the upper catchment, Clanwilliam rock catfish, is distributed from just above the chalets to about 1 km above the car park for hikers visiting the Disa Pool waterfall (Figure 2.19). Clanwilliam rock catfish were also recorded in the Matjies River but were not recorded from the Krom River below the barrier weir in the chalet area. They may use the lower Krom River in the Matjies River flood plain, but the low flow conditions during the surveys probably eliminated suitable habitat for this species in this zone of the catchment. The only difference in the distribution of the rock catfish was the first record of the species in the barrier weir pool in the chalet area in 2018. This is the lowest downstream the species has been recorded in the Krom River. The number of rock catfish recorded in the 2018 was also lower than the number recorded in 2017 (Table 2.3). However, a survey of rock catfish habitat revealed that the population size of the rock catfish was substantial in the upper Krom River; > 3000 individuals (B Müller, unpublished data).

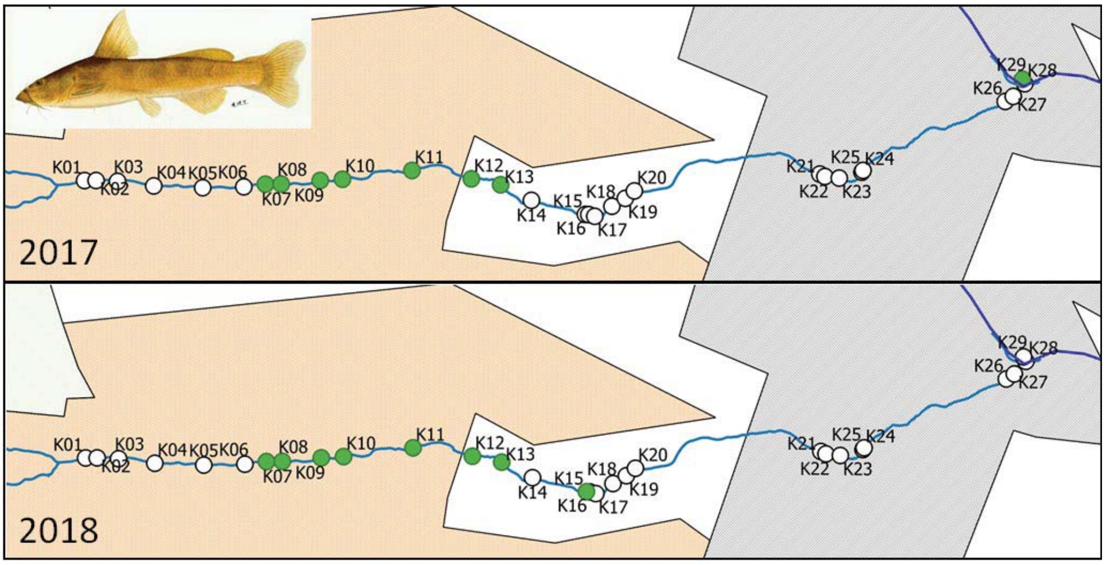


Figure 2.19: Distribution of Clanwilliam rock catfish in the Krom River, Western Cape in 2017 and 2018. Markers indicate sites sampled. Green markers indicate the presence of the species at the site.

Sawfin were restricted to the lower reach of the Krom River within the flood plain of the Matjies River. They were recorded in permanent pools where the Krom River enters the Matjies River flood plain. It is expected that these fish would migrate to the Matjies River main stem during periods of connectivity between their pools and the main stem. During the 2018 survey, the population of sawfin in these pools all had rotting tail fins, however, Clanwilliam yellowfish cohabiting the pool with them did not show signs of fin rot. A sample was collected by CapeNature and inspected by a fish parasite expert of the Department of Agriculture, Forestry and Fisheries in Cape Town, Dr Kevin Christison, who confirmed the presence of fin rot in these sawfin.

Clanwilliam rock catfish *Austroglanis gilli* from the Krom River, Western Cape

Table 2.3: Summary of the fish species' abundance in the Krom River by survey method and year

	Rainbow Trout		Rock catfish		Bluegill		Sawfin	
Technique	2017	2018	2017	2018	2017	2018	2017	2018
Fyke	24	9	61	23	74	56	21	14
Snorkel	9.5	6.5	0	2.75	15	30.75	6.5	2.5
Video	15	20	3	3	37	114	9	49
Photo	13		3		24		6	

Clanwilliam yellowfish were not recorded in the Krom River or Matjies River during the 2017 survey. However, yellowfish were recorded in both pools in the lower Krom River and the Matjies River main stem in the 2018 survey. It is known that Clanwilliam sandfish *Labeo seeberi* Gilchrist and Thompson, 1911 also occur in the Matjies River and this endangered fish may also enter the pools of the lower Krom River during periods of connectivity to the Matjies River main stem.

Three non-native fish species have been recorded in the Krom River catchment: rainbow trout, bluegill and largemouth bass. Rainbow trout are distributed from the Disa Pool waterfall down to the Matjies River. Bluegill were present in both farm dams and the river from the chalets down to the Matjies River confluence (Figure 2.20). A surprising record was that trout were detected in the Matjies River and the lower reaches of the Krom River in 2017. Previous surveys have recorded rainbow trout in the large deep pools in the Matjies River Nature Reserve with water temperatures of 26°C (SMM pers. obs.). The Matjies River is cooler than Krom River in the Matjies River Nature Reserve and likely falls within the thermal tolerance envelope of rainbow trout. The number of sites at which trout were recorded decreased from 2017 to 2018. The number of trout recorded in the 2018 was also lower than the number recorded in 2017 (Table 2.3).

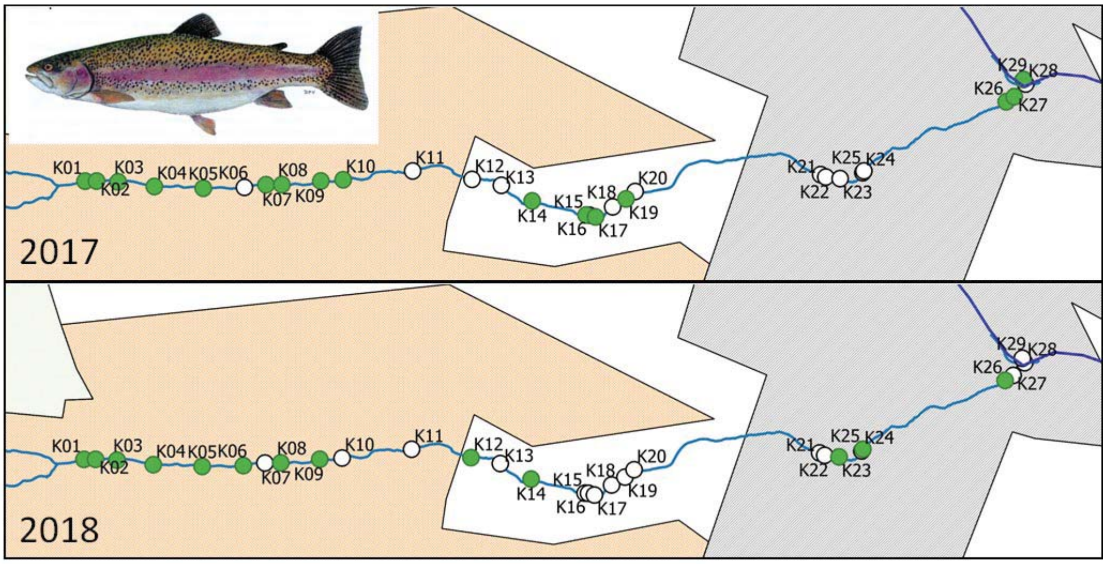


Figure 2.20: Distribution of rainbow trout in the Krom River, Western Cape in 2018. Markers indicate sites sampled. Green markers indicate the presence of the species at the site.

Bluegill were present in both farm dams on Krom River Farm but were removed from the chalet dam following the treatment of the dam with Rotenone by CapeNature in 2017 (see Part 1 of this report). The majority of the river population of bluegill is in the large permanent pools within the Matjies River Nature Reserve. However, it is concerning that bluegill were recorded from above the barrier weir in the campsite and were observed in the furrow to the House Dam near the Restaurant in the Farm Area in 2017 (SMM Pers. Obs.). Colonisation of the Krom River by bluegill from the House Dam via the furrow system is likely and CapeNature should consider including the furrows and the river between the furrow offtakes and the barrier weir in their treatment of the Krom River. The number of sites at which bluegill were recorded increased from 2017 to 2018 (Figure 2.21). The number of bluegill recorded in 2018 was also higher than the number recorded in 2017 (Table 3.3). The increase in bluegill may be related to the lack of strong winter floods in the 2017 winter during the prolonged drought in the Western Cape.

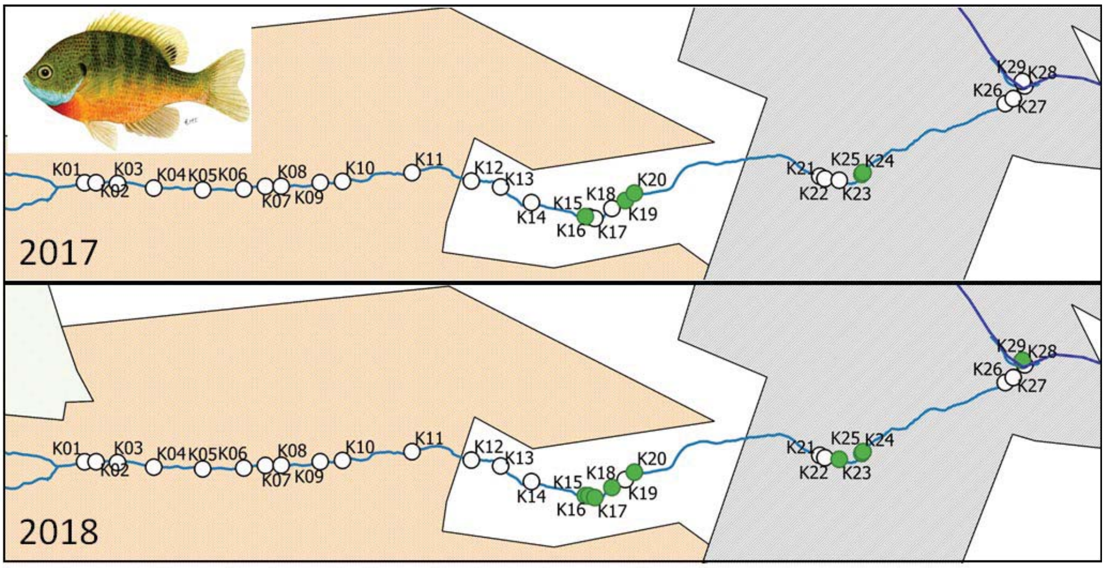


Figure 2.21: Distribution of bluegill in the Krom River, Western Cape in 2018. Markers indicate sites sampled. Green markers indicate the presence of the species at the site.

Previous surveys have recorded largemouth bass in both farm dams on Krom River Farm and infrequently in the river within the chalet area (Marr, 2006; 2007; Marr et al., 2012; Shelton et al., 2017). The Chalet Dam was drained and allowed to dry in 2014 removing the largemouth bass and bluegill from the Chalet Dam. A population of largemouth bass remains in the House Dam and could be a source population for largemouth bass colonisation of the Krom River. This species is likely also present in the dam downstream of House Dam. During both years, no largemouth bass were recorded from the Krom River but they were recorded in the House Dam. Largemouth bass were also recorded from the Matjies River main stem. This was not unexpected as largemouth bass are common in pools of the Driehoeks River which becomes the Matjies River lower down (Paxton and King, 2009).

Three different survey methods were used to determine the distributions of the respective fish species in the Krom River: Fyke nets, snorkel surveys and underwater video analysis (both video and photo in 2017 and only video in 2018). The detection probability of these methods was calculated as the ratio of the number of sites where the method detected the species and the number of sites where the species was detected (expressed as a percentage). Snorkelling had the lowest detection probability of the three survey techniques (Table 2.4). It was particularly poor in detecting the predominantly nocturnal rock catfish and trout but was more successful in detecting sawfin and bluegill. Overall, fyke nets had the highest detection probability, especially for rock catfish, trout and sawfin. Underwater video analysis had the highest detection probability for bluegill, and was a little higher than the fyke net detection probability for this species.

Table 2.4: Detection probability (%) for the fish species in the Krom River by survey method and year

	Rainbow Trout		Rock catfish		Bluegill		Sawfin	
Technique	2017	2018	2017	2018	2017	2018	2017	2018
Fyke	68.42	53.85	100.00	88.89	71.43	55.56	100.00	100.00
Snorkel	21.05	38.46	0.00	55.56	28.57	44.44	33.33	100.00
Video	52.63	61.54	37.50	33.33	71.43	77.78	33.33	100.00
Photo	47.37		37.50		100.00		66.67	

The abundances for each of the survey methods per year and zone of river are presented in Table 2.5.

Table 2.5: Summary of the fish abundances in the Krom River for each survey method by Zone and Year

		2017				2018			
	Zone	Trout	Rock catfish	Bluegill	Sawfin	Trout	Rock Catfish	Bluegill	Sawfin
Fyke net	Trout	15	0	0	0	6	0	0	0
	Rock Catfish	4	51	0	0	2	19	0	0
	Treatment	3	0	74	0	0	1	56	0
	Sawfin	2	10	0	21	1	3	0	14
	Total	**24**	**61**	**74**	**21**	**9**	**23**	**56**	**14**
Snorkel	Trout	8	0	0	0	5.5	0	0	0
	Rock Catfish	1.5	0	0	0	0.75	2.75	0	0
	Treatment	0	0	15	0	0.25	0	30.75	0
	Sawfin	0	0	0	6.5	0	0	0	2.5
	Total	**9.5**	**0**	**15**	**6.5**	**6.5**	**2.75**	**30.75**	**2.5**
Video	Trout	4	0	0	0	15	0	0	0
	Rock Catfish	4	3	0	0	2	3	0	0
	Treatment	4	0	37	0	3	0	104	0
	Sawfin	3	0	0	9	0	0	10	49
	Total	**15**	**3**	**37**	**9**	**20**	**3**	**114**	**49**
Photo	Trout	4	0	0	0				
	Rock Catfish	3	3	0	0				
	Treatment	3	0	24	0				
	Sawfin	3	0	0	6				
	Total	**13**	**3**	**24**	**6**				

2.5 Discussion

The baseline data confirmed that the fish distributions determined in previous studies, e.g. Marr et al. (2012), changed slightly in the intervening period and that the mechanical removal programme (Shelton et al., 2017) had not had the desired impact on the rainbow trout population. Clanwilliam yellowfish were recorded in the lower reaches of the Krom River for the first time and it is possible that other native species utilised these lower reaches under the right conditions; e.g. Clanwilliam rock catfish and Clanwilliam sandfish, as these are known to occur in the Matjies River near the confluence with the Krom River.

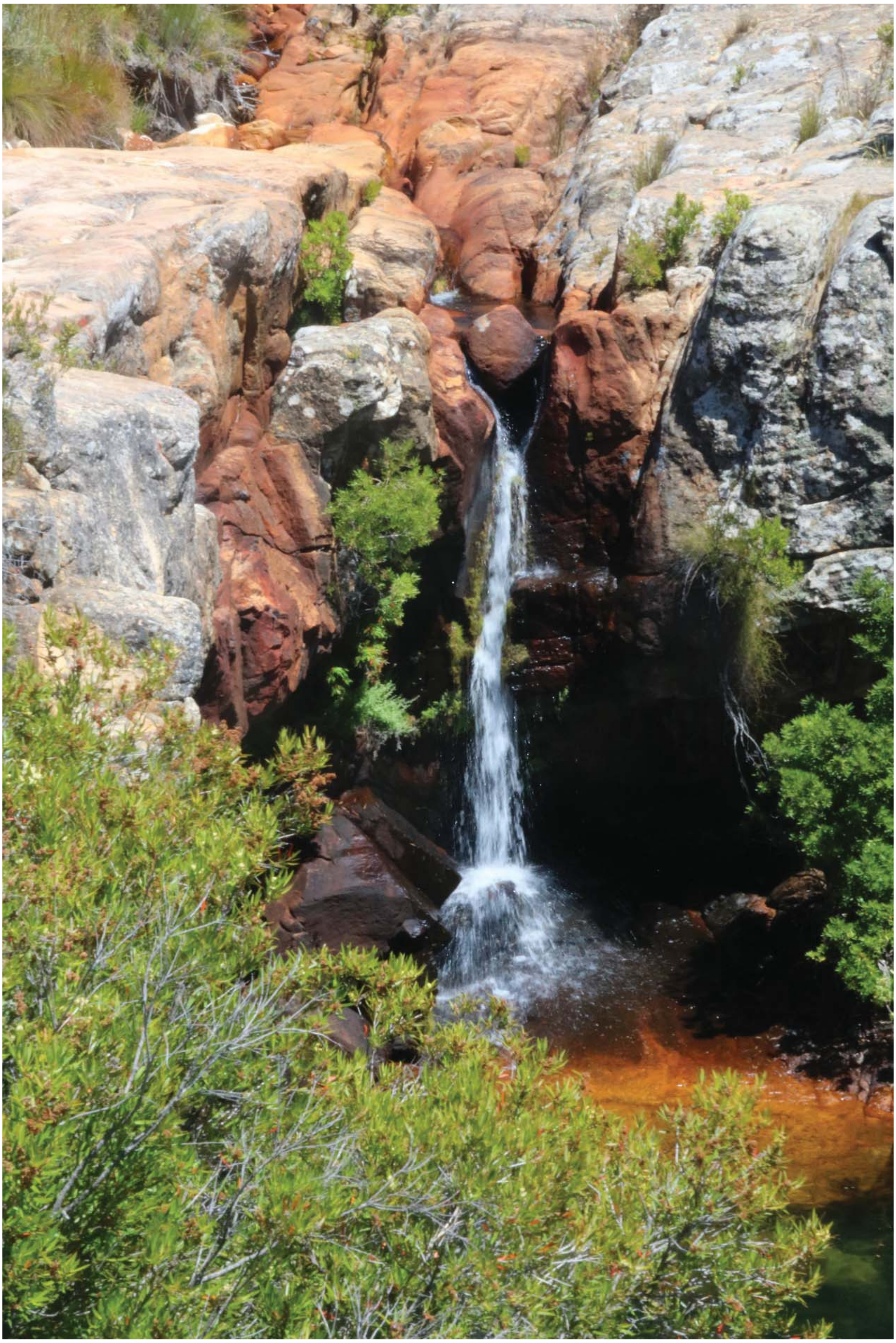
Largemouth bass were historically in both farm dams (Marr et al., 2012) and infrequently in the river from the farm weir downstream (Marr et al., 2012, Shelton et al. 2017). One farm dam was drained and allowed to dry in 2014 removing the largemouth bass and bluegill. During both years, no largemouth bass were recorded from the Krom River but they were recorded in one farm dam. Largemouth bass were also recorded from the Matjies River main stem. This was expected as largemouth bass have been recorded in the Driehoeks-Matjies River (van der Walt 2016).

The analysis of the physico-chemical data identified that the farm complex had a dramatic change on water quality with temperature, conductivity and turbidity increasing and dissolved oxygen decreasing through and downstream of the farm complex. The most severe impact is likely the substantial water abstraction for domestic, irrigation and recreational purposes, but resort development (chalets, camping) and the craft beer brewery are having an impact on the water quality of the Krom River, although the nature of the impact was not investigated in this monitoring project. Future studies should evaluate the nutrient profile and other contaminants of the river as it passes through the farm complex to help mitigate these impacts as the success of any fish translocation will be dependent on the river having good water quality. On a positive note, the Cederberg Tourist Park, owners of the farm complex, are in the process of upgrading the chalets and installing waste water treatment facilities to replace the septic tanks, which should over time reduce inputs into the Krom River.

The Krom River is perennial above the farm complex but abstraction and low summer flows result in the river becoming a series of isolated pools below the farm complex. The pools below the farm complex are deep and large and hold water even in the most severe droughts. The substrate of the catchment is driven by the underlying geology, a layered series of sandstone and shales. This results in a large degree of overlap between the substrates between the respective fish zones.

The Macroinvertebrate data showed that the river was in a good to pristine condition above the farm complex but deteriorated to a poor to fair condition below the farm complex. The ASPT in the Bluegill zone was significantly different to that of the Fishless, Trout and Rock catfish zones in terms of the macroinvertebrate assemblage composition. The results of this study confirmed that the macroinvertebrate communities in each zone were spatially distinctive confirming the findings from the EIA (Enviro-Fish Africa 2009). It is known that the easterly flowing tributaries of the Doring River have different macroinvertebrate species to those of the westerly flowing tributaries of the Olifants River; e.g. Trichoptera (de Moor 2011), therefore conservation of the easterly flowing rivers of the Cederberg is important for maintaining the aquatic biodiversity of the region. Inter-annual variation on the macroinvertebrate assemblages was identified, a pattern consistent with other studies of Cederberg streams, e.g. Woodford et al. (2013), Bellingan et al. (2015) and Bellingan et al. (2019), however, the prolonged drought may have contributed to the inter-annual variation between the 2017 and 2018 data.

The Krom River is proposed to be the next river to be treated with Rotenone for the removal of non-native fishes (Marr et al., 2012). The original proposal to remove all non-native fish from the Krom River has evolved over time as the complexities of removing trout from the upper reaches, and the conservation of the unique rock catfish population and unique assemblages of aquatic invertebrates in the respective zones of the river have become apparent. The current scope of the treatment of the Krom River, for which the General Authorisation has been granted, is the removal of bass and bluegill from the river below the barrier weir at the farm. Of concern is that this upper limit of the treatment is below a known population of bluegill and that the treatment does not include the furrow network feeding the two off-stream dams, a known pathway for bluegill to colonise the Krom River (SMM pers. obs.). As a result of this CapeNature revised their treatment limits to begin at the furrow off-takes for the off-stream dams in order to remove the bluegill population above the barrier weir.



Waterfall above Disa Pool during the exceptionally low flow of the 2017 field trip.

CHAPTER 3. CAPACITY DEVELOPMENT

3.1 Introduction

One of the aims of the project was to develop freshwater research and monitoring capacity in young South African researchers, specifically in students from some universities. Conceptually, the project team proposed to incorporate BSc Honours level students in the field data collection activities as a means to expose the students to freshwater research and monitoring techniques, allow them to interact with leaders in South Africa's freshwater research and provide an opportunity for Honours project data to be collected and supervised by experienced freshwater researchers who also assisted in the supervision of the projects. The students selected for the field trips were also afforded the opportunity to attend the annual SASAQS conference and present their work as a poster presentation. The exposure of the BSc Honours level students to a local conference also allowed the students to gain a greater understanding of aquatic research in South Africa.

One MSc student and nine BSc Honours level students have been supervised and mentored through this project (Table 3.1). Of these, all have completed their degrees. Five universities have provided students that have participated in the project: Rhodes University, University of Venda, University of Fort Hare, University of the Western Cape, and the University of Stellenbosch. Two students won best poster awards – one at the 2017 and one at the 2018 SASAQS Conferences and three of the five BSc Honours level students won best project awards at their respective universities in the 2017 academic year. One peer reviewed publication has been published from one of the 2017 BSc Honours projects and four more are in preparation from the 2018 projects.

In addition to the capacity development of the students, the field team led by SAIAB's PDP Post-Doctoral Fellow Dr Sean Marr offered the opportunity for capacity development as field leaders taking responsibility for aspects of the field data collection for the long-term monitoring project and the students BSc Honours projects for Post-Docs, PhD and MSc students aligned with Prof Olaf Weyl's DST/NRF Research Chair in Inland Fisheries and Freshwater Ecology at SAIAB. The field team included SAIAB PDP Post-Doctoral Fellow Dr Sanet Hugo, University of Stellenbosch and Centre for Invasion Biology Post-Doctoral Fellow Dr Jeremy Shelton (now at the Freshwater Research Centre in Cape Town), Albany Museum's Dr Terence Bellingan (formerly a

Table 3.1: List of students developed and mentored during the current project.

Student	University	Degree	Year	Gender	Race	Project
Previn Pillay	Western Cape	BSc Hons	2016	Male	Indian	A comparison of the aquatic macrophyte communities and their abundance in reservoirs under different invasive pressures in the Cederberg − Krom catchment area
Bianca Hannweg	Rhodes	MSC	2016-7	Female	White	Using action cameras to assess habitat use by *Pseudobarbus afer* and *Sandelia capensis* in the Swartkops River, Eastern Cape, South Africa
Lorraine Ramotjiki	Venda	BSc Hons	2017	Female	Black	The effectiveness of Rotenone treatment by comparing the detection probability of the three non-destructive fish survey methods in the two farm dams at Krom River Cederberg Mountain, Western Cape
Kanie Chauke	Venda	BSc Hons	2017	Female	Black	Comparative analysis on aspects of bluegill biology (sexual maturity, diet and growth) sampled from lentic and lotic environments in the Krom river catchment, Western Cape
Peter Mochechela	Fort Hare	BSc Hons	2017	Male	Black	Detecting the presence of native and non-native fishes in the Krom River, Western Cape, South Africa: A comparison between four non-destructive fish monitoring techniques
Emiline Miller	Western Cape	BSc Hons	2017	Female	Coloured	Occurrence of submerged macrophytes in the Krom River (Cederberg), with respect to the changes in water quality and habitat requirements
Stephen Avidon	Stellenbosch	BSc Hons	2017	Male	White	Do non-native rainbow trout *Oncorhynchus mykiss* pose a threat to the Cederberg ghost frog *Heleophryne depressa* in the upper Krom River, Cape Fold Eco-Region?
Gcinikaya Nkele	Fort Hare	BSc Hons	2018	Male	Black	Detecting the presence of native and non-native fishes in the Krom River, Western Cape, South Africa: A comparison between three non-destructive fish monitoring techniques
Kylen Brown	Western Cape	BSc Hons	2018	Female	Coloured	Understanding the functional and ecological role of *Isolepis digitata* Schrad. along the Krom River in the Cederberg, Western Cape
Bethel Muller	Stellenbosch	BSc Hons	2018	Male	White	Estimating the population size and habitat association of the Clanwilliam rock catfish *Austroglanis gilli* in the Krom River, Olifants-Doring River Catchment, Cape Fold Ecoregion

Rhodes University PhD candidate), Rhodes University PhD candidates Mr Dumisani Khosa, Mr Casey Broom and Mr Lubabalo Mofu, North-West University PhD candidate Ms Marliese Truter, Rhodes University MSc students Ms Lesley Bloy, Ms Bianca Hannweg, Mr Harold Kaebeb, Mr Angus van Wyk and Ms Ncumisa Matam, University of Western Cape MSc candidate Ms Emiline Miller, University of Fort Hare MSc candidate Mr Peter Mochechela, University of Fort Hare BSc Honours student Ms Sharone Bajaba and University of Venda Fisheries Diploma student Mt Rhidela Sithole. Further, Rhodes University Post-Doctoral Fellow Dr Tatenda Dalu (now a Lecturer at the University of Venda) took responsibility for the collection of the plankton samples for the treatment and control dams.

The field team was augmented for a short period by Prof Olaf Weyl of SAIAB, Prof Stefan Foord and Dr Hermien Roux of the University of Venda, Prof Niall Vine of the University of Fort Hare, Dr John Measy of the University of Stellenbosch, Dr Marian Wong of the University of Wollongong (Australia) and Dr Anusha Rajkaran of the University of the Western Cape. The combined experience of these academics afforded the students and field team an opportunity to gain from their knowledge and expertise. The students and field team also had the opportunity to engage with the CapeNature team led of Mr Dean Impson, Ms Jeanne Gouws, Dr Martine Jordaan and Mr Riaan van der Walt, who joined the team at Krom River in both years.

3.2 Student Projects

In total, nine student projects were completed by BSc Honours level students who participated in the Cederberg field trips. Three studies were supervised by Dr Anusha Rajkaran of the University of Cape Town (Previn Pillay 2016, Emiline Miller 2017; and Kylen Brown 2018), two by Prof Stefan Foord of the University of Venda (Lorraine Ramotjiki and Kanie Chauke, both 2017), Prof Niall Vine of the University of Fort Hare (Peter Mochechela 2017 and Gcinikaya Nkele 2018) and Prof Karen Esler of the University of Stellenbosch (Stephen Avidon 2017 and Bethel Müller 2018). All projects, with the exception of that of Previn Pillay in 2016, were co-supervised by Dr Sean Marr of SAIAB. Al BSc Honours students participating in the Cederberg field trips successfully completed their degrees, some receiving awards as the best student projects in their respective classes. The title of the projects and the abstract from the project report are presented hereafter.

Previn Pillay, University of the Western Cape, 2016 – *A comparison of the aquatic macrophyte communities and their abundance in reservoirs under different invasive pressures in the Cederberg – Krom catchment area*

A comparison of the freshwater aquatic macrophyte communities, their abundance and the effects of the invasive freshwater fish species that inhabit the in two catchment areas situated in the Olifants/Doring Krom river catchment was done. Non-native freshwater fish species are currently causing severe environmental pressures in South Africa’s freshwater ecosystems. The study looked at how the macrophyte communities were affected by the invasive pressures caused by *Micropterus salmodies* (bass) and *Lepomis macrochirus* (blue gill) and how they both are affected by the difference in the physical parameters. Macrophyte percentage cover was determine from 5 random quadrants along a line transect. Along these same transects the physical parameters of both dams were also measured and recorded. GoPro footage was used to determine the presence and absence of fish species in the two dams and the source river. A Pearson's correlation was performed mainly to compare the relationship between chlorophyl-α and all the other physical parameters and the results revealed that only the significantly strongly positive relationship occurred between TDS and salinity ($R=0.988$, $p=0.00$), however a negative correlation between chlorophyll-α and sediment organic content was also observed ($p<0.05$) between the two ($R=-0.897$, $p=0.015$). The results obtained from various methods such as line transects, sediment sampling and chlorophyll-α spectrophotometry provides a baseline of information on the distribution and function of macrophytes in these systems with regard to the effects of the ecological pressures caused by the invasive fish species by observing the difference in macrophyte and fish species abundances.

Kanie Chauke, University of Venda, 2017 – *The effectiveness of Rotenone treatment by comparing the detection probability of the three non-destructive fish survey methods in the two farm dams at Krom River Cederberg Mountain, Western Cape*

Alien fish are one of the greatest threats to the native fish communities of the Cape Fold Ecoregion (CFE). As a result, the conservation authority, CapeNature, has begun evaluating the effectiveness of the piscicide Rotenone to remove alien fish populations from rivers of high conservation value. Rotenone was used with great success to remove smallmouth bass from the Rondegat River in the Cederberg, Western Cape. Following the treatment, the aquatic invertebrate communities recovered, and the native fish population has colonized the treatment area. Further successful treatments are required before Rotenone can be registered for use as a piscicide for the conservation of imperiled fishes. However, detection of the presence of fish in aquatic environments is challenging. This study compares detection probability of the three non-destructive techniques (fyke net and underwater video and photos) to determine the probability at which they detect the presence of the invasive bluegill sunfish *Lepomis macrochirus* (Rafinesque, 1819) in an off-stream dam following treatment with Rotenone. Fish surveys using all three techniques were conducted in two off-stream farm dams at Krom River Cederberg; before and after the treatment. The detection probability for bluegill was determined for all three techniques from the control dam and treatment dam (prior to treatment) and used to determine whether the piscicide treatment had successfully eradicated all bluegill in the treatment dam. Fyke nets recorded the highest detection probability (80%) while the underwater photos recorded the lowest (51.85%). No fish were detected in the treatment dam after the treatment therefore Rotenone treatment was estimated to be 99.4% effective in eradicating the alien bluegill sunfish from the dam.

Lorraine Ramotjiki, University of the Venda, 2017 – *Comparative analysis on aspects of bluegill biology (sexual maturity, diet and growth) sampled from lentic and lotic environments in the Krom river catchment, Western Cape*

The introduction of non-native fish species often precipitate change in the structure and functioning of natural ecosystems. Bluegill *Lepomis macrochirus* are a freshwater fish from the United States and were introduced into South Africa in 1939 for angling and as a fodder fish for black bass *Micropterus spp.* Little is known about the impact of bluegill in South African water bodies. The objective of this study is to compare biological parameters, including sexual maturity, diet and growth, for bluegill in lentic and lotic environments of the Krom River catchment, Olifants-Doring catchment, Western Cape, in order to determine their invasiveness. A total of 107 male and 46 female bluegill were collected, measured, sexed and dissected. Their stomachs and otoliths were examined and analysed for diet and age, respectively. The diet of bluegill in the dam comprises mainly of Diptera, Fish and Hemipteran whereas in the river it comprises mainly on Diptera, Coleopteran and Ephemeroptera. Bluegill in the river were smaller, weigh less and reach sexual maturity at smaller size than those in the dam. Sex ratios did not differ between the two habitats (p = 0.57) and many of the bluegill were not much older than two years. It is difficult to eradicate non-native species and control is often the best solution. Understanding aspects of bluegill biology will aid in control further spread.

Peter Mochechela, University of Fort Hare, 2017 – *Detecting the presence of native and non-native fishes in the Krom River, Western Cape, South Africa: A comparison between four non-destructive fish monitoring techniques*

Monitoring the remaining populations of imperilled native fishes and detecting the presence of non-native fishes is fundamental for the conservation of native fishes. However, the techniques available to monitor freshwater fish populations differ in their ability to detect the presence of native and non-native species. It is therefore important to know the probability that a specific technique would detect the presence of a specific fish species. This study compared four non-destructive fish monitoring technique (fyke netting, snorkelling, underwater video and photos) to determine the detection probability of each technique and to report on the distribution of native and non-native fish species in the Krom River, Cederberg, South Africa. Twenty-eight sites along the Krom River, in four zones (trout, rock catfish, bluegill and sawfin), were surveyed to detect fish species present. Trout were detected in all four zones while the other species were only detected in the respective zones. Overall, fyke nets consistently had the highest detection probability while snorkelling had the lowest detection probability across all species. The video and photo detection probabilities were the same for all species except bluegill, which had detection probabilities of 1 and 0.71 for videos and photos, respectively. On both occasions, the fish was visible for less than 3 seconds. No relationship was found between detection probability and turbidity for the visual methods.

Emiline Miller, University of the Western Cape, 2017 – *Occurrence of submerged macrophytes in the Krom River (Cederberg), with respect to the changes in water quality and habitat requirements*

Submerged macrophytes (SM) fulfil important roles within habitats by absorbing nutrients, generating oxygen, modifying physio-chemical conditions including flow patterns and providing habitat for fish, invertebrates and periphyton. Due to their sensitivity to both short- and long-term changes in environmental factors, SM can be useful indicators of aquatic ecosystem health. The Krom River in the Cederberg has been severely modified by invasion of alien fish, dense stands of riparian alien vegetation, river bank clearing, intensive water abstraction and dam construction. This study evaluated the distribution of SM along the river in relation to physio-chemical characteristics and investigated the changes in biomass of SM in the river and dams. The role of *Stuckenia pectinata* in altering water characteristics over an 18-hour cycle in relation to biological characteristics was also evaluated. *Isolepis digitata* and *S. pectinata* were the only SM present in the Krom River and its presence was strongly associated with changes in water chemistry and habitat associated with anthropogenic changes. Furthermore, the results of this study indicate *S. pectinata* plays no role in altering the pH, temperature, and conductivity of the water column and that its biomass is limited by increased depth and turbidity in the dams.

Stephen Avidon, University of Stellenbosch, 2017 – *Do non-native rainbow trout Oncorhynchus mykiss pose a threat to the Cederberg ghost frog Heleophryne depressa in the upper Krom River, Cape Fold Eco-Region?*

The impact of non-native rainbow trout *Oncorhynchus mykiss* on a population of endemic Cedarberg ghost frog *Heleophryne depressa* in the upper Krom River (Olifants-Doring River Catchment, Cape Fold Ecoregion) was evaluated. *Heleophryne depressa* abundance (using kick-sampling and underwater video analysis) and environmental conditions between sites above and below a waterfall that marks the upper distribution limit of *O. mykiss* was compared. *Heleophryne depressa* abundance was significantly greater above the waterfall than that below it, and, because there was no significant difference in measured environmental variables, *O. mykiss* presence is identified as the most likely explanation for the observed decrease in *H. depressa* abundance. This project was published in the African Journal of Aquatic Sciences as Avidon et al. (2018).

Kylen-Leigh Brown, University of the Western Cape, 2018 – *Understanding the functional and ecological role of Isolepis digitata Schrad along the Krom River in the Cederberg, Western Cape*

Submerged macrophytes are integral to the functioning of freshwater ecosystems due to their complex ecological role in structuring freshwater communities and providing sites for feeding and shelter. The submerged macrophyte, *Isolepis digitata* (Schrad, 1832) is found in fast flowing streams where it forms dense clusters attached to rock surfaces. Little is known about the function, ecology and distribution of this species. This study measured the changes in morphology of *Isolepis digitata* in relation to the physical environment as well as identified the ecosystem role of this species through comparing the abundance of aquatic invertebrates within *Isolepis digitata* to that in the surrounding bedrock environment. The biological variables measured for *Isolepis digitata* showed a negative correlation with depth with no strong correlations being observed between the biological and water physico-chemical parameters. The abundance of aquatic invertebrates was significantly greater within *Isolepis digitata* than in the surrounding bedrock habitat with it being greater within *Isolepis digitata* at shallow that at deep depths. This species was also shown to play a crucial role in carbon storage with a greater percentage of organic carbon being stored in the culms than in the roots.

Gcinikaya Nkele, University of Fort Hare, 2018 – *Detecting the presence of native and non-native fishes in the Krom River, Western Cape, South Africa: A comparison between four non-destructive fish monitoring techniques*

The Cape Fold Ecoregion is a hotspot for aquatic diversity and endemism that has the highest concentration of endemic and threatened freshwater fishes in South Africa. The greatest threats to the freshwater fishes of the CFE are habitat destruction and the presence of non-native fishes, especially salmonids Salmonidae (G.Cuvier 1816) and black bass Micropterus (Lacepède, 1802) species. Monitoring the populations of these threatened fishes in the CFE is essential for providing information for their management of conservation. Due to the highly threatened status of CFE fishes, non-harmful survey techniques are required for the monitoring of the remaining populations. In this study, three non-harmful techniques (fyke nets set overnight, snorkelling, and underwater video cameras – GoPro) were used to estimate fish relative abundance in the Krom River, a tributary of the Doring River in the Olifants-Doring catchment, Western Cape, South Africa. A total of 25 sites were sampled in the Krom River using all three methods. The distribution map of both native and invasive fish species is clearly documented. From our results fyke nets detected high 87,5% abundances of Clanwilliam rock catfish followed by underwater video camera 66,7% detection probabilities. The combination of underwater video camera and fyke net are recommended as effective and non-distractive sampling methods. Fisher’s exact test (2X3) was used calculate probability of dependence between the results of 2017 and 2018 for each of the three sampling techniques (fyke net; sum of fish species 247, no of tables evaluated 2455, P = 0.0370, Snorkel; sum of fish species 64.5, no of tables evaluated 51, PA = 0.0025, PB = 0.0034, video; sum of fish species 192, no of tables evaluated 252, PA = 0.0370).

Bethel Muller, University of Stellenbosch, 2018 – *Estimating the population size and habitat association of the Clanwilliam rock catfish Austroglanis gilli in the Krom River, Olifants-Doring River Catchment, Cape Fold Ecoregion*

Freshwater ecosystems are considered to be one of the most threatened systems, suffering the highest biodiversity loss comparing to other habitats. Numerous drivers are responsible for the global freshwater fish declines which are categorized under five interacting groups, including: invasion of non-native species; overexploitation; habitat degradation or destruction; and flow modification. The Cape Fold Ecoregion (CFE) contains the highest concentration of endemic and threatened freshwater fishes in South Africa. The region is considered a hotspot of endemic freshwater fishes in South Africa of which several are near extinction, largely due to the drastic spread of non-native fish species throughout the river systems. An isolated population of the CFE endemic Clanwilliam rock catfish *Austroglanis gilli* (Barnard, 1943) is found in the Krom River tributary of the Matjies River, Doring River Catchment. This unique population occurs in a 6 km stretch of the Krom River, all of which has been invaded by non-native rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Concern has been expressed regarding the security of this rock catfish population due to long-term monitoring programmes which has only detected low numbers. The aim of this study is to provide further understanding on the population size and habitat requirements (in terms of substrate) of the *A. gilli* population in the Krom River. A survey of the upper reach of the river was conducted in order to identify reaches dominated by distinct habitat classes followed by three pass depletion electrofishing within each habitat type. It was seen that *A. gilli* showed a strong preference to increased habitat complexity, while a population size of 3121 was estimated.

3.3 Benefits of the involvement of the Students

While the completion of degrees, publication of peer reviewed papers and attending and presenting at conferences are tangible outcomes that can be enumerated, the long-term benefit of the project to the students involved is more difficult to quantify. At present, three of the 2017 cohort of students and one of the 2018 cohort have continued their studies to MSc level, two of which have continued in freshwater ecosystem research (Emiline Miller is currently reading towards a MSc in ephemeral wetland vegetation at the University of the Western Cape and Peter Mochechela is reading towards a MSc in evaluating the potential of long-line fisheries in the Eastern Cape at the University of Fort Hare).

Rhidela Sithole, a Fisheries Diploma student from the University of Venda, described the 2018 field trip experience as "*quite incredible*" because researchers from different universities participated in the field trip including BSc Honours, MSc and PhD students "*and they even invited other students, like me, …. a diploma student.*" Emiline Miller a BSc Honours student from the University of the Western Cape, felt that interacting with professionals in their fields on a field trip allowed for the transfer of knowledge and useful tips that students would not learn in lecture halls, concluding "*… because what I have learned this past 3 weeks, I don't think I could have learned in six months [in lectures]. It has helped me for my future*." Stephen Avidon, a BSc Honours student from the University of Stellenbosch, descried the field trip as "… *a really great and a unique opportunity to be around people who know what to look at these things [freshwater ecosystems] and can direct you in the right way [to study them]. It is really valuable…".* All the students interviewed after the 2017 field trip expressed gratitude for the experience and commented that they "*learned a lot*" on the field trips.

Many of the students learned new skills on the field trips. One skill considered to be essential in working in aquatic systems is the ability to swim and snorkel. For some, it was their first experience swimming and snorkelling and, while some found the experience challenging, others revelled in the exposure to a new domain. Gcinikaya

Nkele, BSc Honours student from the University of Fort Hare, excitedly exclaimed "*Ai, it is fantastic, especially in this pool, that you are able to see a lot of fish, especially bluegill. They come and look at you in big numbers, and all of a sudden they run away.*"

Dean Impson of CapeNature summarised the benefits of the field trips as follows:

"The involvement of the students in the Krom River Rehabilitation Project has been fantastic because they have been confronted by a real project on the ground, not a project in a book, and it is a very challenging project. They have been exposed to a range of senior scientists who have given valuable information about the project. This is also a beautiful part of South Africa, the Cederberg, with a lot of interesting conservation issues. By participating in the monitoring programme, catching fish with gill nets, seine nets, fyke nets, snorkelling, they have been exposed to different methodologies and, I think, benefitted tremendously."

CHAPTER 4. SUMMARY OF OUTPUTS

4.1 Dissemination of Results

The research team has published five peer-reviewed papers from the data collected during the previous two projects K9/822 and K5/2261 and one peer-reviewed paper from the current project. In addition, one manuscript is in the peer-review process from this body of work. Further peer-reviewed publications are planned from the current project and should be submitted for publication in the near future. The peer reviewed publications generated to date are listed in Table 4.1.

The project team and BSc Honours level students gave oral and poster presentations related to the project at the 2017 and 2018 annual SASAQS conferences. At the 2017 SASAQS Conference Ms Emiline Miller won the best poster award and at the 2018 SASAQS Conference Mr Gcinikaya Nkele won a best poster award. A total of 14 conference presentations have been delivered to date; one international conference oral presentation, four national conference oral presentations and nine national conference poster presentations. A list of the conference presentations made from the project is presented in Table 4.2.

4.2 Film Media

In addition to the peer reviewed publications and conference presentations, two short films were produced by Dr Jeremy Shelton of the Freshwater Research Centre. The first short film summarises the experiences of the student group during the 2017 Cederberg Field trip while the second short film highlights some of the techniques used during the Krom River monitoring as articulated by students and field leaders of the 2018 Cederberg Field trip.

The videos can be viewed online from the following repositories:

Krom video 2017 (129 plays)	https://www.vimeo.com/224102742
Krom video 2018 preview (312 plays)	https://www.vimeo.com/276527084
Krom video 2018 (250 plays)	https://www.vimeo.com/278514046

Number of plays as of 30/01/2019

Table 4.1: List of peer-reviewed papers containing outputs from the current project.

No.	Authors	Title	Type (Audience)	Journal	Year
1	Beatty, S., Allen, M., Lymbery, A., Jordaan, M. S., Morgan, D., Impson, D., Marr, S., Ebner, B. & Weyl, OLF.	Rethinking refuges: implications of climate change for dam busting	Peer reviewed paper (academics)	*Biological Conservation*	2017
2	Jordaan, M. S., Dalu, T., Wasserman, R. J., Slabbert, E. & Weyl, O. L. F.	Unexpected survival of sharptooth catfish *Clarias gariepinus* (Burchell 1822) during acute Rotenone toxicity trials will complicate management of invasions	Peer reviewed paper (academics)	*Biological Invasions*	2017
3	Woodford DJ, Ivey P, Jordaan M, Kimberg P, Zengeya TA, Weyl OLF	Optimising invasive fish management in the context of invasive species legislation in South Africa	Peer reviewed paper (academics)	*Bothalia: African Biodiversity and Conservation*	2017
4	Fill, J. M., Kritzinger-Klopper, S. & van Wilgen, B. W.	Short-term vegetation recovery after alien plant clearing along the Rondegat River, South Africa	Peer reviewed paper (academics)	*Restoration Ecology*	2017
5	Shelton, J. M., Wey, O. L. F., Van Der Walt, J., Marr, S. M., Impson, D., Maciejewski, K., Tye, D., Dallas, H. & Esler, K.	Effect of an intensive mechanical removal effort on a population of non-native rainbow trout Oncorhynchus mykiss in a South African headwater stream	Peer reviewed paper (academics)	*Aquatic Conservation: Marine and Freshwater Ecosystems*	2017
6	Avidon, S., Shelton, J. M., Weyl, O. L. F., Marr, S. M., Bellingan, T. A. & Esler, K. J.	Preliminary evaluation of rainbow trout (Oncorhynchus mykiss) impact on the Cedarberg ghost frog (*Heleophryne depressa*) in South Africa's Cape Fold Ecoregion	Peer reviewed paper (academics)	*African Journal of Aquatic Science*	2018
7	Bellingan, T. A., Hugo, S., Woodford, D. J., Gouws, J., Villet, M. H. & Wey, O. L. F	Macroinvertebrates in a vulnerable South African Cape Fold Ecoregion stream recover swiftly after Rotenone treatments	Peer reviewed paper (academics)	*Hydrobiologia*	2019

Table 4.2: List of conference presentations and public lectures containing outputs from the current project.

No.	Authors	Title	Conference	Location	Year
1	Weyl, OLF, Van der Walt, JA, Impson, ND, Jordaan, MS, Woodford, DJ.	Non-native fish eradication facilitates the rapid recovery of native stream fishes in the Cape Fold Ecoregion, South Africa	Symposium of the Fisheries Society of the British Isles	University of Exeter	2017
2	Weyl, OLF, Van der Walt, JA, Jordaan, MS, Impson, ND, Woodford, DJ.	Is native fish conservation in headwater streams as easy as removing alien fishes?	2017 SASAQS Conference	Boksburg, South Africa	2017
3	Marr, SM, Bellingan, TA, Bloy, L, Dalu, T, Esler, KJ, Foord, SH, Hannweg, B, Hugo, S, Mofu, L, Rajkaran, A, Roux, H, Shelton, JM, Vine, NG, Weyl, OLF.	Training the next generation of aquatic scientists through full immersion field exposure to river research and monitoring methods	2017 SASAQS Conference	Boksburg, South Africa	2017
4	Impson, ND, , Van der Walt, JA, Jordaan, MS, Gouws, J, Marr, SM, Weyl, OLF	Rehabilitation projects to conserve threatened Cape fynbos fishes: getting invasive alien fishes out of selected priority inland waters	Freshwater Ecosystem Network (FEN) workshop	Boksburg, South Africa	2017
5	Miller, E, Marr, SM, Rajkaran, A.	The impact of water quality and habitat availability on submerged macrophytes in the Krom River (Cedarberg)	2017 SASAQS Conference	Boksburg, South Africa	2017
6	Mochechela, P, Hannweg, B, Marr, SM, Vine, N.	Detecting the presence of native and non-native fishes in the Krom River, Western Cape, South Africa: A comparison between four non-destructive fish monitoring techniques	2017 SASAQS Conference	Boksburg, South Africa	2017
7	Chauke, IK, Foord, SH, Roux, H, Mofu, L, Marr, SM.	A comparison of the biology of bluegill sunfish *Lepomis macrochirus* from lentic and lotic environments in the Krom River catchment, Western Cape	2017 SASAQS Conference	Boksburg, South Africa	2017
8	Ramotjiki, ML, Foord, SH, Roux, H, Mofu, L, Marr, SM.	An evaluation of Rotenone treatment by comparing the detection probability of three fish survey methods (fyke nets, snorkeling and camera) in the rivers and dams of Cederberg Krom River.	2017 SASAQS Conference	Boksburg, South Africa	2017

Table 4.2 (cont.): List of conference presentations and public lectures containing outputs from the current project.

No.	Authors	Title	Conference	Location	Year
9	Avidon, S, Shelton, JS, Marr, SM Esler, KJ.	The impact of rainbow trout Oncorhynchus mykiss on Cederberg ghost frog *Heleophryne depressa* tadpole abundance above and below a waterfall barrier, Krom River, Olifants-Dooring River catchment, Cape Fold Eco-region	2017 SASAQS Conference	Boksburg, South Africa	2017
10	Impson, D, Jordaan, M, Gouws, J, van der Walt, R, Marr, S, Weyl, O. 2017	Moving forward with Rotenone projects: rapid removal of non-native fishes from two farm dams and strong recovery of threatened fishes in a priority river for conservation	2017 Fynbos Forum		2017
11	Marr, S.M., Dalu, T., Bellingan, T., Jordaan, M., Slabbert, E, & Weyl, O.L.F.	Ecosystem responses to Rotenone treatment in two dams in the Western and Northern Cape provinces.	2018 SASAQS Conference	St Francis, South Africa	2018
12	Bajaba, S., Vine, N., van Wyk, A., Marr, S.M., Hugo, S., & Weyl, O.L.F.	The use of Baited Remote Underwater Video (BRUV) as a monitoring tool for freshwater fishes	2018 SASAQS Conference	St Francis, South Africa	2018
13	Brown, K.L., Miller, E, Marr, S.M., Rajkaran, A.	The role and distribution of freshwater macrophytes along the Krom River	2018 SASAQS Conference	St Francis, South Africa	2018
14	Müller, B., Shelton, J., Marr, S.M., Weyl, O.L.F., & Esler, K.J.	Estimating population size and habitat association of the Clanwilliam rock catfish *Austroglanis gilli* (Barnard, 1943) in the Krom River, Olifants-Doring River catchment, Cape Fold Eco-region	2018 SASAQS Conference	St Francis, South Africa	2018
15	Nkele, G., Mochechela, P. Vine, N., Marr, S.M., & Weyl, O.L.F.	Detecting the presence of native and non-native fishes in the Krom River, Western Cape, South Africa: A comparison between three non-destructive fish monitoring techniques	2018 SASAQS Conference	St Francis, South Africa	2018
16	Impson, D., Muir, D., Madikizela, B., Jordaan, M. & Weyl, O.	Invasive fish projects in the Cape: progress so far and plans for the next three years	2018 SASAQS conference	St Francis, South Africa	2018
17	Impson, D., Madikizela, B., Muir, D., Weyl, O.	Control of invasive fishes in South Africa's Cape Fold Ecoregion: partner agencies collaborate to ensure successful use of the piscicide Rotenone	2018 African Fish and Fisheries conference in Malawi.	Mangochi, Malawi	2018

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