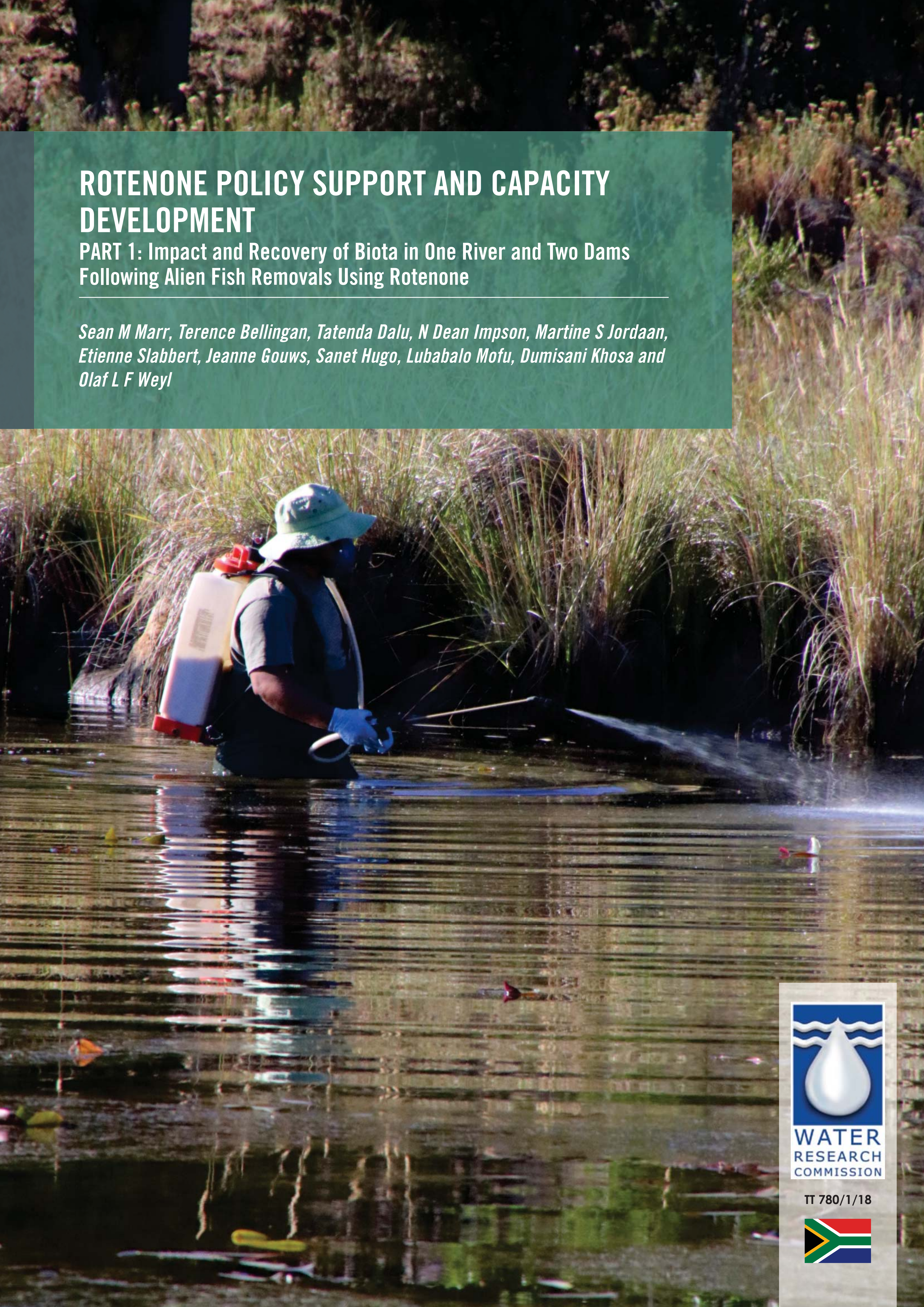

ROTENONE POLICY SUPPORT AND CAPACITY DEVELOPMENT

PART 1: Impact and Recovery of Biota in One River and Two Dams Following Alien Fish Removals Using Rotenone

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WATER RESEARCH COMMISSION
TT 780/1/18

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PART 1: Impact and Recovery of Biota in One River and Two Dams Following Alien Fish Removals Using Rotenone

Report to the
WATER RESEARCH COMMISSION

by

Sean M Marr[1], Terence Bellingan[2], Tatenda Dalu[3], N Dean Impson[4], Martine S Jordaan[4], Etienne Slabbert[5], Jeanne Gouws[4], Sanet Hugo[1], Lubabalo Mofu[1], Dumisani Khosa[1] and Olaf L F Weyl[1]

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EXECUTIVE SUMMARY

A South African perspective to Piscicide Treatments

Fish invasions are a major threat to imperilled South African fishes and other aquatic fauna. Many introduced fishes are, however, also valuable in inland fisheries which are important contributors to food security, livelihoods and the economy. To facilitate management of alien species, South Africa has promulgated the National Environmental Biodiversity Act (NEMBA): Alien Species Regulations and the NEMBA: Alien Invasive Species Lists. These legislative instruments recognise the need to balance biodiversity impacts with economic benefits and allow for the use of non-native fishes in areas that are of low conservation concern because they are already invaded. It is, however, recognised that in some areas, and particularly those of high conservation priority (e.g. protected areas, the Freshwater Ecosystem Priority Area “fish sanctuaries”), the removal of non-native fish is necessary to restore natural processes. From a river rehabilitation perspective, eradicating non-native fish using piscicides such as Rotenone, allows for the rehabilitation of several kilometres of river.

The value of the chemical Rotenone in conducting fish eradication projects is globally recognised and is regarded by South African fish conservation experts as the best, fastest and safest way of eliminating unwanted fishes from a dam or confined stretch of river, thereby improving the conservation status of highly threatened fishes or the health of the dam for a variety of uses (irrigation, conservation, recreational angling). While non-native fish removal using Rotenone has been demonstrated to be an effective management tool, its collateral effects on non-target aquatic organisms is a cause for concern. Developing National Policy on the use of Rotenone for river and dam rehabilitation, therefore, requires knowledge on the potential impacts of Rotenone treatments in South Africa.

The primary objective of the research reported in this Technical Report were to:

- Provide data on ecosystem responses of one river and two dams following Rotenone treatment to guide national policy on the use of Rotenone for non-native fish removals,
- Monitor rates of recovery of fish communities in the Rondegat River continuously to determine when complete recovery has occurred by testing the hypothesis that native fish communities rebuild to approximate those in the non-invaded zone of the river within 5 years after the first treatment,
- Assess the recruitment and recovery rates of invertebrate communities to the removal of alien fishes using Rotenone in two off-channel dams.

Rondegat River Long-term Monitoring

Following the two successful WRC Projects monitoring the treatments of the Rondegat River with Rotenone to remove non-native fish, and subsequent recovery following the treatments, (project K9/822: Monitoring of the impact and recovery of the biota of the Rondegat river after the removal of alien fishes [Woodford et al., 2012] and project K5/2261: Evaluating fish and macro-invertebrates recovery rates in the Rondegat river, Western Cape, after river rehabilitation by alien fish removal using Rotenone [Weyl et al., 2016]), further monitoring of the Rondegat River continued as part of the current WRC project. The findings of the long-term monitoring included:

- No smallmouth bass have been detected following the Rotenone treatment and the Rotenone treatment is considered to have been successful.
- Native fish rapidly colonised the reach where smallmouth bass had been eradicated. The densities of the three cyprinid species in the rehabilitated area are beginning to resemble those in the control area.
- A catastrophic invertebrate-drift event occurred during the application of Rotenone to the Rondegat River. The effect was immediate, with the number of invertebrates in the drift climbing two orders of magnitude above natural background drift levels, which remained constant at the monitoring site in the control area throughout the Rotenone treatment. Following the end of Rotenone treatment, drift rapidly declined to near-pre-treatment levels.
- Stone and kick sampling of aquatic invertebrates were conducted to quantitatively sample invertebrates such that the impact of the Rotenone treatment on invertebrates could be made based on quantitative assessments of species numbers and diversity.
- Results from assessments based on the most sensitive taxa, commonly referred to as EPT (Ephemeroptera, Plecoptera and Tricoptera) demonstrated a rapid recovery following treatments.

Treatment of two farm dams

The ecosystem responses of two farm dams in the Olifants-Doring catchment to the removal of non-native fishes through Rotenone treatments were evaluated. The Chalet Dam on Krom River Farm was treated with the piscicide Rotenone on the 26th of January 2017 by CapeNature to remove the non-native bluegill *Lepomis macrochirus.* The Kranskloof Dam near Nieuwoudtville in the Northern Cape was treated on the 29th of March 2017 by CapeNature and the Northern Cape Department of Environment and Nature Conservation to remove non-native common carp *Cyprinus carpio*.

- There were no statistically significant changes in physico-chemical water parameters over the treatment of both dams, although seasonal changes were noted.
- The target fish species were successfully removed from both treatment dams and was not recorded in the sampling one year post treatment.
- The Chalet Dam was subsequently colonised by rainbow trout *Oncorhynchus mykiss*, which are present in the upstream reaches of the river.
- The time required before the Rotenone concentrations were no longer toxic to fish in the Chalet Dam (Krom River) was determined to be less than 14 days.

- The turbidity in both treatment dams decreased following treatment, i.e. improved water clarity. The Kranskloof Dam displayed the greater decrease in turbidity with the water being particularly turbid prior to treatment at 32 NTU, clearing up to 20 NTU the day after the treatment and having 15 NTU six months post treatment.
- As expected, a dramatic decrease in the abundance and species composition of zooplankton was detected in both dams immediately after Rotenone treatment (elevated Rotenone levels are typically used in the treatment of artificial water bodies like farm dams). Zooplankton communities showed considerable recovery 6 and 12 months post treatment at both treatment dams. There was a turnover of species but this could not be attributed to the impact of Rotenone.
- Following fish removals, the phytoplankton community of both dams changed to a community that is typically representative of waters with a lower nutrient status. The Kranskloof Dam was dominated by blooms of blue-green and green algae prior to the treatment. The blue-green algae were not present six months after the treatment. At Chalet Dam, the algal community changed from green algae dominated community to a green algae and diatom community.
- The macroinvertebrate communities of both treatment dams were reduced to two thirds of their pre-treatment densities immediately after the treatment. However, the macroinvertebrate densities had recovered to their pre-treatment levels six to 12 months following the treatment.
- The macroinvertebrate communities of the Chalet and Kranskloof dams were very different largely because of habitat differences between the two dams. The Chalet Dam had substantial aquatic vegetation whereas the Kranskloof Dam had no aquatic vegetation and a thick layer of silty substrate. The invertebrate community of the Kranskloof Dam was dominated by Diptera while that of the Chalet Dam was dominated by Odonata, Diptera and Trichoptera prior to treatment.
- At the Chalet Dam, only Odonata and Diptera were recorded immediately after the treatment with Trichoptera and Ephemeroptera being eliminated. Twelve months later, the Odonata still dominated the Chalet Dam invertebrate community, the Diptera are still present, and the Ephemeroptera and Trichoptera have returned. Hemiptera, not present in the pre-treatment samples, have colonised the dam.
- At the Kranskloof Dam, the pre-treatment community was dominated by Diptera, with minor contributions from Annelida and Hemiptera. Most invertebrate taxa survived the Rotenone treatment as there was no change in the species richness over the treatment. Six months post treatment the community structure was dominated by Annelida and Diptera, with a substantial increase in the density of Hemiptera. As a result of a severe drought, Kranskloof Dam dried up in January 2018 so the collection of the 12 month post treatment sample was not possible.

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Ms D Muir	DEA: Natural Resource Management Programmes
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LIST OF ABBREVIATIONS

ASPT	Average score per taxon
CAPE	Cape Action Plan for People and the Environment
CFE	Cape Fold Ecoregion
CFR	Cape Floristic Region
Chl-a	Chlorophyll-a
DAFF	South Africa Department of Agriculture, Forestry and Fisheries
DEA	South African Department of Environmental Affairs
DENC	Northern Cape Department of Environment and Nature Conservation
DWS	South African Department of Water and Sanitation
EIA	Environmental Impact Assessment
EPT	Ephemeroptera-Plecoptera-Trichoptera
GEF	Global Environmental Facility
HDPE	high density poly-ethylene
HEI	Higher Education Institute
NEMBA	National Environmental Management: Biodiversity Act
NMDS	Non-metric multidimensional scaling
NTU	Nephelometric Turbidity Unit
SAIAB	South African Institute for Aquatic Biodiversity
SASS	South African Scoring System
TDS	Total dissolve solutes
UWVA	Underwater video analysis
WRC	Water Research Commission

CHAPTER 1. INTRODUCTION

1.1 Introduction

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 Rondegat River in the Cederberg region of the Western Cape (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 in the river 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, 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 the Krom River and treatment of two off channel dams (Chalet Dam in the Krom River and Kranskloof Dam in the Oorlogskloof River catchment, in the Northern Cape) in 2017. These treatments provided an ideal opportunity to expand the Rondegat experiment to four off-channel dams (two treatment, two control). The current project also provided the opportunity to incorporate capacity building in aquatic ecosystem monitoring into South Africa's higher education institutions (HEIs), including the University of Fort Hare, University of Venda, University of the Western Cape and the University of Stellenbosch. By partnering with these HEIs, this project offered opportunities for aquatic research to students whom might not otherwise have access to similar opportunities at their home institutions by linking the monitoring of aquatic ecosystems to BSc. Honours research projects and using the experiences gained to identify similar opportunities close to the HEIs. This partnership between The South African Institute for Aquatic Biodiversity (SAIAB) and 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 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 (this report) and PART 2: KROM RIVER BASE-LINE MONITORING AND CAPACITY DEVELOPMENT.

1.2 Rotenone

Ground-up roots of many tropical plants of the Leguminosae family containing Rotenone, most commonly the derris plant *Derris eliptica*, have been used for centuries by the indigenous peoples of Africa, South America and South-East Asia to capture fish for human consumption (Ling, 2002). Rotenone has been extensively used as an organic pesticide for food crops (Ling, 2002) in South Africa (as Expellar), the United States and elsewhere. Freshwater and marine scientists also use Rotenone as a fish-sampling tool to capture cryptic species (McClay, 2005). However, the major use of Rotenone is as a fisheries management tool (Rowe, 2003; Kolar et al., 2010) for which it has been used in the United States since the 1930s (Kolar et al., 2010).

1.2.1 Rotenone for Fisheries Management

Rotenone is the most commonly used piscicide for controlling or eradicating invasive fish internationally (Cailteux et al., 2001; Rowe, 2003). In the United States, Rotenone has been used from 1988 to 2002 for the quantification of fish populations (34% of the waters treated), manipulation of fish populations to maintain sport fisheries (27%), treatment of rearing ponds (17%), the eradication of introduced species (10%), and in the restoration of threatened species (7%) (McClay, 2005). Elsewhere in the world, Rotenone has been used to remove invasive fish from reservoirs and streams in Britain (Britton and Brazier, 2006; Britton et al., 2008), Spain (Maceda-Veiga, 2013), Australia (Lintermans, 2000; Rayner and Creese, 2006) and New Zealand (Chadderton et al., 2003; Pham et al., 2013). In all of these cases, alien fish were successfully eradicated from the treated water body. For example, native fish were successfully re-introduced after a British reservoir was treated with Rotenone to remove introduced species (Britton and Brazier, 2006), and the Rotenone treatment of a stream section between two barriers in Australia resulted in the natural re-colonisation by native fish from upstream after the introduced fish had been removed (Lintermans, 2000).

Since the introduction of the Atlantic salmon parasite *Gyrodactylus salaris* (Malmberg, 1957) in the 1970s, Norwegian Environmental Authorities have applied a Rotenone based eradication strategy to eradicate the host species, Atlantic salmon *Salmo salar* Linnaeus, 1758, and the parasite from infected rivers because these pose a serious and continuous risk of spreading the parasite to neighbouring river systems (Sandodden et al., 2018). The Atlantic salmon parasite has resulted in an average mortality among juvenile Atlantic salmon of 86%, driving the locally adapted Atlantic salmon stocks to the brink of extinction (Sandodden et al., 2018). This has led to severe negative impacts for local fishing tourism, recreation and business. Native fish stocks affected by the Rotenone treatment are re-introduced through a five year reintroduction programme; including planting of eyed eggs and releases of one year old yolk sack juveniles and smolt to the treated rivers (Sandodden et al., 2018). The two other fish species present in the treated rivers, threespined stickleback *Gasterosteus aculeatus* Linnaeus, 1758 and European eel *Anguilla* Linnaeus, 1758, are both expected to recolonize the treated rivers from the fjords (Sandodden et al., 2018).

However, the use of Rotenone or other piscicides to control invasive fish may also be mismanaged. Possibly the most controversial use of Rotenone in the USA occurred in 1962 when 715 km of the Green River and its tributaries in south-western Wyoming and north-eastern Utah were treated with Rotenone to depress populations of undesirable fish species, primarily common carp *Cyprinus carpio* Linnaeus, 1758, before closure of Flaming Gorge Dam (1962), and upstream Fontenelle Dam (1964) (Holden, 1991; Wiley, 2008). Because of an inadequate supply of the neutralising agent, potassium permanganate, the Rotenone remained active in the river at concentrations toxic to fish and continued killing fish downstream of the project area, resulting in heavy losses of native fishes, including some threatened species. The project was widely criticised at the time leading to a senate level investigation (Wiley, 2008). However, the closure of the two dams and associated dam operations resulted in the Green River changing from a warm, turbid, free-flowing river to a clear, cold, regulated river and flooded prime canyon habitat for the native big-river fish populations (Wiley, 2008). The Rotenone treatment depressed undesirable non-native fishes without eliminating the native big-river fish populations. In retrospect, the native fish populations were impacted to a greater extent by the closure and operation of the impoundments than by the Rotenone treatment (Wiley, 2008).

Recognizing the need for reasonable environmental protection and controlled use of Rotenone and other piscicides, the American Fisheries Society developed a training course to standardise piscicide application training, implemented a Rotenone stewardship program, including the registration of all trained piscicide applicators (Schnick, 2001), and prepared a Rotenone standard operating procedure manual (Finlayson et al., 2010) to ensure that all piscicide applications contain adequate due diligence and environmental protection measures (Kolar et al., 2010).

1.2.2 Impacts on non-target species

Although piscicides, such as Rotenone, are reliable and cost-effective tools for the removal of non-native fishes (Pham et al., 2013), their non-specificity may reduce the abundance of non-target species directly via toxicity-induced mortality, e.g. non-native and native fishes and aquatic invertebrates (Kolar et al., 2010), or indirectly via loss of prey, e.g. restocked native fish (Pham et al., 2013) and riparian birds (Donnelly, 2018). The impact of Rotenone on stream invertebrates varies greatly in its toxicity across species, families and orders of insects (Ling, 2002; Vinson et al., 2010). However, laboratory tests have shown that smaller invertebrates appear to be more sensitive than larger invertebrates and invertebrates respiring with gills are more sensitive than those respiring through other means (Vinson et al., 2010).

In field studies of the effect of Rotenone treatment of invertebrates in streams (lotic systems), some studies have reported negligible impacts, e.g. Pham et al. (2018), while others have reported significant alterations in invertebrate density and diversity, e.g. Mangum and Madrigal (1999). This may be dependent on the Rotenone concentration used and the ambient conditions (e.g. water temperature) during the treatment. Aquatic insects appear to be more sensitive to Rotenone than molluscs and decapod crustaceans (Dalu et al., 2015), and the insect orders Ephemeroptera, Plecoptera, and Trichoptera appear to be more sensitive than Coleoptera and Diptera (Vinson et al., 2010). The immediate and short-term responses of aquatic invertebrates to Rotenone treatments in streams have been large reductions in invertebrate abundance and taxonomic richness (Woodford et al., 2013; Bellingan et al., 2015). Recovery time of invertebrate communities to pre-treatment status is highly variable and studies have reported recovery times ranging from months (Pham et al., 2018) to years (Mangum and Madrigal, 1999), with rare species, in particular, often not returning to the site during the post-treatment monitoring (Hamilton et al., 2009; Vinson et al., 2010; Woodford et al., 2013). Rotenone treatments in streams often trigger "catastrophic drift" in many insect species, resulting in large numbers of animals exiting the treated area on contact with Rotenone and drifting downstream (Dudgeon, 1990; Arnekleiv et al., 2001; Lintermans and Raadik, 2003; Kjærstad and Arnekleiv, 2011). Catastrophic drift is seen as a behavioural response to contact with Rotenone and, depending on the Rotenone concentration and the water temperature, the majority of insects in the drift survive (Dudgeon, 1990) or expire (Arnekleiv et al., 2001; Kjærstad and Arnekleiv, 2011).

In lentic systems (still waters, lakes, impoundments and ponds), Rotenone effects on zooplankton are greater than on benthic organisms, and zooplankton assemblages are significantly reduced in both abundance, species composition and diversity (Vinson et al., 2010). Studies on the effects of Rotenone on benthic organisms reported small differences in total benthic invertebrate abundance or biomass between pre- and post-treatment samples, with effects on Chironomidae, likely the most dominant organism, being greatest. Recovery of zooplankton to pre-treatment abundances ranged from 1 month to 3 years. Rotifer and Copepod assemblages appeared to recover quicker than Cladoceran assemblages. Benthic invertebrate assemblages generally recovered to be similar to control pond assemblages within 6 months, with no differences between pre- and post-treatment samples found within 1 year of treatment (Vinson et al., 2010).

A number of factors influence the impact and recovery of aquatic invertebrate assemblages following piscicide treatments. These include factors related to the nature and extent of the piscicide treatment (concentration, duration and timing (Dalu et al., 2015, Kjærstad et al., 2016), environmental variables during treatment (e.g. water temperature) (Booth et al., 2015; Kjærstad et al., 2016) and the type of water body (lotic or lentic) being treated. Biological factors include invertebrate morphology and life history characteristics, including surface area to volume ratios, size e.g. zooplankton or benthic (Dalu et al., 2015), type of respiration organs e.g. gill or plastron (Booth et al., 2015), generation time, and propensity to disperse; presence of refugia; and distance from colonization sources (Vinson et al., 2010).

1.2.3 Rotenone and human health

One of the concerns expressed regarding the use of Rotenone during the public participation process during the Environmental Impact Assessment (EIA) for the Cape Action for People and the Environment (CAPE) Alien Fish Removal Project (Marr et al., 2012) was the putative links between exposure to Rotenone and Parkinson's disease (Enviro-Fish Africa, 2009). Parkinson's disease is a "*long-term degenerative disorder of the central nervous system that mainly affects the motor system"* (Johnson and Bobrovskaya, 2015). Currently, Parkinson's disease is the second most common neurodegenerative disease and most common movement disorder worldwide (Cannon et al., 2009). There is, at present, no cure for Parkinson's disease, with treatment directed mainly at treating symptoms and improving the quality of life of the patient.

The causes of Parkinson's disease are generally unknown, but are believed to involve both genetic and environmental factors (Betarbet et al., 2000; Cannon et al., 2009).

Pesticide exposure, specifically Rotenone and paraquat, have been correlated with Parkinson's disease, with people with prolonged exposure to either chemical being 2.5 times more likely to develop Parkinson's disease than non-exposed subjects (Tanner et al., 2011).

Rotenone has been reported to result in Parkinson's disease-like symptoms in rats exposed to intravenous and intraperitoneal doses of 2-3 mg/kg (Betarbet et al., 2000; Johnson and Bobrovskaya, 2015). No measurable effects were observed for inhalation while oral or intragastric administration resulted in decreases in tyrosine hydroxylase neurons in substantia nigra and striatum, decrease in striatal dopamine, increase in α-Synuclein expression/inclusions in substantia nigra, no change in substantia nigra and striatum and motor deficits detected (Johnson and Bobrovskaya, 2015). Doses for the oral and intragastric tests varied between 0.25 and 100 mg Rotenone per kg body mass (Johnson and Bobrovskaya, 2015). However, it is not physically possible for mammals to consume sufficient water to achieve these doses at the concentrations of Rotenone used in the removal of fish from freshwater systems.

The animal exposure to Rotenone was believed to be a suitable model for explaining the development of Parkinson's disease (Sherer et al., 2003), however, a recent review has concluded that "*none of the existing animal models of experimental Parkinson's disease completely mimics the aetiology, progression, and pathology of human Parkinson's disease*" (Martinez and Greenamyre, 2012).

1.2.4 Rotenone use in South Africa

Contrary to public perception, there is a long history of the use of Rotenone in South Africa. Not only is Rotenone used in organic pesticides (Robertson and Smith-Vaniz, 2008; Dalu et al., 2015), Rotenone has also been extensively used by freshwater, estuarine, inter-tidal fish and marine researchers to collect fish since the 1970s. Robertson and Smith-Vaniz (2008) describe Rotenone as essential for assessing marine fish diversity because it is strongly selective for fishes, its use for sampling does not remove all fishes or destroy fish habitat, Rotenone disperses quickly and degrades rapidly, shore-fish population are resilient and recover rapidly from local disturbances, and Rotenone based surveys are few, far between and very small in scale. A number of universities and research institutes have used Rotenone for fish collection including the Universities of Natal (Blaber, 1977; Blaber and Whitfield, 1977; Whitfield and Blaber, 1978a; Whitfield and Blaber, 1978b), Port Elizabeth (Beckley, 1985), Rhodes (Wood et al., 2000) and Cape Town (Beckley, 1988), the Oceanographic Research Institute (Beckley, 2000), Port Elizabeth Museum (Smale, 1986; Smale and Buxton, 1989) and the JLB Smith Institute, now the South African Institute for Aquatic Biodiversity, (Winterbottom, 1976; Skelton et al., 1989; Whitfield, 1993). Laboratory trials were conducted by the Natal Parks Board to determine the tolerances (Rowe-Rowe, 1971), reaction time (Rowe-Rowe, 1979c), and revival (Rowe-Rowe, 1979b) of selected freshwater fish species in Natal. The neutralization of Rotenone in fresh water was also investigated in these laboratory trials (Rowe-Rowe, 1979a). There is no evidence that Natal Parks Board used Rotenone as a fisheries tool to remove fish species, however, Rotenone was used to sample fish in the Natal midlands for a study to determine the diet of otters (Rowe-Rowe, 1977). Perhaps the earliest use of

Rotenone in South Africa to control fish was in 1956, when the Cape Department of Nature Conservation imported Noxfish for experimental use (Harrison 1956). This Rotenone was then used in a water supply of 9 million gallons in East Griqualand to control common carp. The common carp had increased turbidity in the dam making it unfit for use, and Noxfish was applied to the dam on 17 April 1956 by Inland Fisheries Officials at 1ppm by means of stirrup pumps. The Rotenone killed all common carp, the water quality of the dam improved substantially, and Provincial Inland Fishery Officer, A.C. Harrison noted that “It would thus appear that this is an extremely efficient method of fish eradication, which could be widely used in the Cape Province under legal control”. (Harrison, 1956). In 1995, the Grahamstown Municipal Dam, Grey Dam, in the upper Kowie River catchment was treated with Rotenone by Eastern Cape Nature Conservation, Department of Ichthyology and Fisheries Science (Rhodes University), Albany Museum and the City of Grahamstown to remove sharptooth catfish *Clarias gariepinus* (Burchell, 1822), illegally introduced by anglers, in order to establish a sanctuary for the threatened *Sandelia bainsii* Castelnau, 1861 (Cambray, 1995; Cambray, 2003). Unfortunately, this benefit was short lived as anglers reintroduced predatory non-native fishes soon after the intervention.

In April and May 2005, two water bodies were treated with Rotenone in the Greater Cape Town area (Impson et al., 2005); Die Oog, a small bird sanctuary in the upper Sand River catchment, Bergvliet, Cape Town and Paardevlei on the former De Beers/AECI explosives factory site in Somerset West, respectively. The two treatments were completed for very different reasons. Industrial activities at the Paardevlei site had ceased in the 1990s and the land had been rezoned for a housing development. Paardevlei was originally a small open body wetland characterised by heavily weeded clear water containing a large population of Cape kurper *Sandelia capensis* (Cuvier, 1831). From 1910 to 1970, this waterbody was stocked with a variety of fish species including largemouth bass *Micropterus salmoides* (Lacepède, 1802), perch *Perca fluviatilis* Linnaeus, 1758, carp *Cyprinus carpio* Linnaeus, 1758, tench *Tinca* (Linnaeus, 1758), rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), and bluegill *Lepomis macrochirus* Rafinesque, 1819 (Impson et al., 2005). Soon after the introduction of largemouth bass in 1930 (de Moor and Bruton, 1988), the Cape kurper population was extirpated (Impson et al., 2005). The proliferation of carp during the 1980s resulted in other species declining and degradation of water quality due to sediments suspended by carp feeding activities and the elimination of aquatic plants. Paardevlei was treated using a Russian Mi8P helicopter that had been converted to a crop-sprayer (Impson et al., 2005; Williams, 2007). The disturbance caused by the helicopter to the 20 species of waterbirds, including large numbers of Great White Pelicans *Pelecanus onocrotalus*, White-breasted cormorants *Phalacrocorax lucidus*, Darters *Anhinga melanogaste*r and Egyptian geese *Alopochen aegytiacus*, present during the Rotenone spraying of Paardevlei was less than that caused by an African fish eagle *Haliaeetus vocifer* flying over the water body (Williams, 2007). Piscivorous birds were observed feeding extensively on the dead or dying fish (Williams, 2007). The dead fish, 99.9% carp, were collected and sent to the Vissershoek waste disposal site. The water body was subsequently drained and the sludge removed to reduce the risk of contaminated residues from the De Beers/AECI factory remaining on the site. On the morning after the treatment, the shoreline was littered with large (> 1 kg) carp, too big for the pelicans to consume, with very few 4-12 cm dead fish indicating that the birds had consumed a large portion of the fish killed (Williams, 2007). There was no indication that any birds were affected by the Rotenone treatment, or the consumption of dying fish, and no bird carcasses were found during the fish clearing operation that continued seven days after the Rotenone treatment (Williams, 2007).

Die Oog is a 1 ha pond in the Bergvliet suburb of Cape Town is primarily a bird sanctuary and green space managed by the City of Cape Town. The pond lies in the upper Sand River catchment and would naturally have contained Cape kurper and Cape galaxias *zebratus* Castelnau, 1861. Over time, common carp, banded tilapia *sparrmanii* A. Smith, 1840, Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), and mosquitofish *Gambusia affinis* (Baird & Girard, 1853) colonised Die Oog (Impson et al., 2005). No angling is permitted in Die Oog, therefore, the fish present were only a source of food for the birds roosting at Die Oog. Water quality deterioration resulted in an algal scum covering the water surface and a local firm of freshwater ecologists undertook several water quality remediation trials. In order to reduce the nutrient input into the water column, draining the water body and removing the sludge was recommended, but this proved difficult to achieve. The decision was then taken to remove the fish using Rotenone and the water body was treated in April 2005. The fish community composition by numbers was roughly 30% carp, 30% banded tilapia, 30% Mozambique tilapia and 10% mosquitofish (Impson et al., 2005). Subsequent fish surveys using gill nets and seine nets failed to find any fish and the treatment was deemed a success. City of Cape Town and Friends of Die Oog released Cape kurper and Cape galaxias from the Sand River into Die Oog in 2006.

The Helderberg Nature Reserve is a 403 ha reserve owned and managed by the City of Cape Town situated on the slopes of the Helderberg Mountain in Somerset West. It was established 50 years ago and has been nurtured by the Helderberg community from its inception. The reserve falls within the Lourens River catchment. In 2005, the City of Cape Town approached CapeNature to remove non-native fish from a small off-stream dam on the reserve to create a refuge for the local genetically unique populations of Cape kurper and Cape galaxias, both of which are extremely rare in the Lourens River due to non-native fish predation (rainbow trout and largemouth bass) and pollution. CapeNature conducted the Rotenone treatment in 2005, with the assistance of staff from the reserve, using powdered Rotenone donated by the University of Cape Town. Largemouth bass, banded tilapia and carp were successfully removed from the off-stream dam and Cape kurper from the Lourens River were stocked by CapeNature in 2005. These are now well established in the dam.

Century City is a 200 ha mixed-use development about 10km from Cape Town. It includes a shopping mall, theme park, retirement village, office parks and several residential complexes. The development incorporates the 16 ha Intaka Island Nature Reserve, comprised of an 8 ha natural seasonal wetland and an 8 ha artificial wetlands complex whose prime function is to clean the water of the development’s 6 km of navigable canals. The artificial wetland consists of two shallow-water reed beds of ~2 ha each; a 1.5 ha deeper-water lake; and a small <1 ha shallow marsh (Harrison et al., 2010). The closed system is fed from a nearby wastewater treatment works, via groundwater infiltration and run-off from the surrounding developed areas. The construction of these wetlands was mandated to provide some compensation for the loss of the Blouvlei wetland, which was drained and destroyed during property development, and to provide a "green lung" within the Century City complex (Harrison et al., 2010). Blouvlei was an important water fowl breeding site and a series of attempts were made to attract colonial water birds (e.g. cormorants, herons, darters, etc.) to use the reserve as a roost and breeding area including the construction of several artificial roosting structures; see Harrison et al. (2010). Native water plants and fish species Cape kurper and Cape galaxias were introduced to create a sanctuary for these species. In addition, Mozambique tilapia and sterile triploid grass carp *Ctenopharyngodon idella* were legally stocked to control water weeds. However, non-native common carp were then illegally introduced, and the rapidly increasing carp population resulted in a dramatic deterioration in water quality throughout the system, including an unpleasant odour and presence of blue-green algae. In order to reduce the nutrient input into the canal network, the number of artificial roosts was reduced and a decision taken to eradicate the carp using Rotenone. In March 2008, the entire canal and wetland system was treated with Rotenone by the Nature Conservation Corporation, under the leadership of an accredited piscicide applicator, with the objective of removing the carp. Prior to treatment a number of Cape kurper were captured for restocking of the canal system after the treatment. The treatment was successful at removing all carp, but a population of Mozambique tilapia survived the treatment.

1.3 CAPE Alien Fish Eradication Project

The Cape Fold Ecoregion (CFE), *sensu* Abell et al. (2008), contains the highest concentration of threatened fishes in South Africa (Tweddle et al., 2009). The freshwater fishes of the CFE are predominantly threatened by the presence of non-native fishes and habitat degradation (Tweddle et al., 2009) highlighting the need for river rehabilitation projects, particularly the eradication of non-native fishes (Impson et al., 2002). The Cape Action for People and the Environment (CAPE) Project, a comprehensive conservation plan for the Cape Floristic Region (Younge and Fowkes, 2003), recognised the need for intervention to conserve the freshwater fishes of the CFE. Subsequently the CAPE Alien Fish Eradication Project was established to identify conservation-priority rivers for non-native fish eradication (Impson, 2007). The project was funded by the World Bank and managed by CapeNature. The pilot project aimed to evaluate the eradication of non-native fish from four streams in the CFE using the piscicide Rotenone and to monitor the subsequent recovery of the treated reaches, specifically the recovery of threatened native fishes. A series of workshops established criteria for the selection of appropriate rivers including biological, land-use, social, financial and logistical considerations (see Impson (2007) for discussion). Field surveys resulted in the selection of four rivers for the pilot project: the Rondegat, Krom and Twee Rivers in the Cederberg; and the Krom River in the Eastern Cape (Marr et al., 2012). Further field surveys, using a combination of fyke netting, seine netting, electric fishing and snorkel surveys, were conducted between August 2005 and October 2006 to delineate the distribution ranges of native and non-native fish in these four rivers and to identify potential barriers that could be used as the upper or lower barriers for the eradication of non-native fish using piscicides (Marr et al., 2012).

The Rondegat River was selected as the first river for treatment because it drains a moderately transformed catchment, flows in a single shallow channel through the proposed intervention area, did not require the construction of barriers (waterfall and existing weir), and holds healthy populations of five native fish taxa upstream of the treatment area. Furthermore, the Rondegat River is not an important river for anglers, and the landowners supported the treatment (Marr et al., 2012).

1.3.1 Environmental Impact Assessment

In 2008, CapeNature appointed Enviro-Fish Africa (Pty) Ltd to carry out an Environmental Impact Assessment (EIA) on its behalf to assess the use of piscicides for the treatment of the four rivers included in the CAPE Alien Fish Eradication Project, including to removal rainbow trout, bluegill and largemouth bass from the Krom River catchment; see Marr et al. (2012) for the reasoning behind conducting the EIA and Enviro-Fish Africa (2009) for the full EIA report. The EIA concluded that the treatment would have some negative initial impacts on the aquatic invertebrate fauna but that the majority of organisms could be expected to survive the treatments. In addition, rapid recovery of the stream faunas was predicted, following colonisation from reaches up- and down-stream of the treatment reaches. Further, the project was unlikely to have any significant impacts, either positive or negative, on the regional conservation status of non-fish vertebrate fauna, because mammals, reptiles, amphibians and birds would not be affected by the concentrations of Rotenone required to kill fish. The project was endorsed as vital for the survival of endangered fishes. Systematic monitoring was recommended for each of the four rivers before, during and after the treatments. The legal assessment component of the EIA concluded that Section 28 of NEMBA placed an obligation on CapeNature to remedy the degradation of environments harmed by non-native and/or invasive species and that the proposed project was in accordance with international best practices for managing invasive non-native species (Enviro-Fish Africa 2009).

The EIA concluded that the justification for the project and the choice of rivers was sound. In most of the rivers proposed for the project, the use of piscicides, specifically those containing Rotenone, was recommended. The Rondegat River was recommended as the first river to be treated. In the upper part of the Krom River (Cederberg), the trial of physical eradication methods was recommended, to minimise impacts on native Clanwilliam rock catfish *Austroglanis gilli* and macroinvertebrates. Should the physical methods prove ineffective, Rotenone treatment should proceed, with rescue populations of aquatic fauna being kept in holding facilities for the duration of the treatment.

1.3.2 Angler Concerns regarding Rotenone

Through the EIA process, anglers and angling groups expressed a number of concerns regarding the use of Rotenone to remove alien fish from the rivers of the CFE; summarised from Enviro-Fish Africa (2009). The most vociferous group were the trout anglers whose underlying concern was that rainbow trout *Oncorhynchus mykiss* was specifically being targeted for removal.

Another major concern expressed was the impact of Rotenone treatments on aquatic invertebrates. Some declared that the treatment would turn the treated rivers into aquatic deserts, devoid of life. Health risks to humans, including the risk of Parkinson's disease were also noted. In addition, the anglers believed that monitoring of the treatments by an independent organisation was required to provide an unbiased assessment of the outcome of each river treated.

1.3.3 Rationale for the Project

Together with habitat modification and pollution, non-native invasive fishes are one of the greatest threats to South Africa's aquatic biodiversity (Tweddle et al., 2009). Non-native fishes impact invaded ecosystems primarily by predation and competition which can result in altered plant, invertebrate, fish and plant communities (Ellender and Weyl, 2014). Native fishes, many of which are now red-listed as Critically Endangered, Endangered or Vulnerable (Tweddle et al., 2009), are highly affected by the presence of non-native invasive fishes. It is also noteworthy that some non-native fishes such as common carp are able to negatively affect water quality as their bottom-grubbing feeding behaviour increases turbidity and suspends nutrients which can result in eutrophication and increased water treatment costs (de Moor and Bruton, 1988). Management of non-native invasive fishes is, therefore, important not only from a biodiversity and ecosystem conservation perspective but also from a socio-economic one.

To facilitate the management of non-native species, South Africa has promulgated the National Environmental Management Biodiversity Act (NEMBA): Alien and Invasive Species Regulations (Republic of South Africa 2014a) and the NEMBA: Alien and Invasive Species Lists, 2016 (Republic of South Africa, 2016a; b). These legislative instruments recognise the need to balance biodiversity impacts with economic benefits and allow for the use of non-native fishes in areas that are of low conservation concern because they are already invaded. It is however recognised that in some areas, and particularly those of high conservation priority (e.g. Protected areas, FEPA "fish sanctuaries"), the removal of non-native fish is necessary to restore natural processes.

To help managers to decide what course of action to take when faced with a non-native invasive fish species, the WRC supported a project on DEVELOPING A DECISION SUPPORT TOOL FOR MANAGING INVASIVE FISH IN SOUTH AFRICA (K5:2039) (Kimberg et al., 2014). This project used case studies from throughout South Africa to develop a framework with which to decide under what conditions the removal of non-native fishes was desirable and feasible (from an implementation point of view). This framework demonstrated that eradication of non-native fishes is only feasible under conditions where re-invasion was unlikely, i.e. where the area to be treated is isolated from source populations of potential re-invasion. As a result, projects aiming at removing non-native fishes need to consider potential upstream and downstream source populations of non-native fishes but also those in off-channel dams that might invade during periods of high flow which result in dam breaching. In addition, non-native fish eradications are only appropriate using methods that are able to completely, efficiently and safely eradicate the target organism in the area under consideration.

The value of the use of Rotenone in conducting fish eradication projects is globally recognised and is regarded by South African fish conservation experts as the best and fastest way of improving the conservation status of the highly threatened fishes (Marr et al., 2012; Weyl et al., 2013; Woodford et al., 2013; Weyl et al., 2014). The first river rehabilitation using Rotenone in South Africa was conducted by CapeNature when the Rondegat River in the Cedarberg was treated on the 27th February 2012 (Impson et al., 2013). While the primary goal of the CapeNature rehabilitation project was to rehabilitate the Rondegat river's native fish fauna through the removal of the non-native smallmouth bass, the program also has the additional objective to assess the feasibility of using Rotenone to rehabilitate other rivers in the CFE that are threatened by invasive non-native fish (Marr et al., 2012). This feasibility assessment was also considered to be a valuable case study to guide national policy on the use of Rotenone for river rehabilitation.

1.3.4 Rotenone Policy Development

For the policy development process, the WRC supported two independent research projects with the objectives of evaluating the efficacy of the Rotenone treatment and assessing the impact of the treatment on native amphibians, fish and invertebrates. The two concurrent projects were coordinated by SAIAB and implemented in collaboration with the University of Cape Town, the Albany Museum and Cape Nature.

The first project K9/822: MONITORING OF THE IMPACT AND RECOVERY OF THE BIOTA OF THE RONDEGAT RIVER AFTER THE REMOVAL OF ALIEN FISHES (Woodford et al., 2012) was implemented from 2010-2012 to include comprehensive assessments of aquatic macro-invertebrate, fish and amphibian distributions in treatment and control areas before and after the treatment. This project established a baseline of macroinvertebrate, amphibian and native and non-native fish distribution and relative abundance and determined the efficacy of the Rotenone treatment on removing non-native fish from the treatment zone (Weyl et al., 2013; Woodford et al., 2013; Weyl et al., 2014). In addition, the K9/822 project resulted in the development of a standardized methodology for determining baseline invertebrate, amphibian and fish distributions and abundance (Woodford et al., 2012).

The second project (K5/2261: EVALUATING FISH AND MACRO-INVERTEBRATE RECOVERY RATES IN THE RONDEGAT RIVER, WESTERN CAPE, AFTER RIVER REHABILITATION BY ALIEN FISH REMOVAL USING ROTENONE), was initiated in 2013 and completed in April 2016 (Weyl et al., 2016). This project provided comprehensive species level assessments of invertebrate and vertebrate distributions both prior to and for three years after the Rotenone treatment (Weyl et al., 2014; Bellingan et al., 2015; 2019). Overall these research projects demonstrated that the two Rotenone treatments were effective at removing smallmouth bass from the treatment zone (Weyl et al., 2013) and that native invertebrate and fish communities are recovering to resemble the control reach densities after the treatments (Weyl et al., 2014; Bellingan et al., 2015). The most recent fish surveys demonstrate that native fish populations are recovering and that aquatic insect abundances are now similar to those before treatment (Bellingan et al., 2015).

Although project results from the two Rondegat treatments have demonstrated the recovery of native biota in a lotic system, CapeNature was advised that the Registrar at the Department of Agriculture, Forestry and Fisheries (DAFF) required additional case studies, particularly of the effects of Rotenone on lentic environments (dams and/or wetlands) before considering its registration as a national tool for river rehabilitation. The opportunity to address this concern was provided in 2017 by CapeNature's plans to treat two off channel farm dams in the Krom and Oorlogskloof catchments, within the Olifants-Doring River System.

1.4 **Aims and Objectives**

The project strongly contributes towards Informing Policy and Decision Making by assessing ecosystem responses to the removal of fishes using the piscicide Rotenone in both rivers and dams. This was requested as necessary by several regulatory (DAFF, Department of Water and Sanitation) and funding (Department of Environmental Affairs: National Resource Management Planning) authorities that took an interest in this project and the proposed registration of Rotenone for use in alien fish eradication. The primary objective of this report was therefore to:

- Provide data on ecosystem responses of one river (two treatments) and two dams following Rotenone treatment to guide national policy on the use of Rotenone for non-native fish removals,
- Monitor rates of recovery of fish communities in the Rondegat River to determine when complete recovery has occurred by testing the hypothesis that native fish communities rebuild to approximate those in the non-invaded zone of the river within 5 years after the first treatment,
- Assess the recruitment and recovery rates of invertebrate communities to the removal of alien fishes using Rotenone in two off-channel dams,
- Develop a Policy Brief to justify Rotenone as the chemical of choice for alien fish eradication. This Policy Brief will facilitate national policy support and buy in,

CHAPTER 2. RONDEGAT RIVER TREATMENT

2.1 Introduction

The Rondegat River is a small perennial tributary of the Olifants River (Olifants-Doring catchment) that flows into Clanwilliam Dam (Figure 2.1). Historically, the Rondegat River contained six native species, including Clanwilliam sawfin *Cheilobarbus serra* (Peters, 1864), Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist & Thompson, 1913), Clanwilliam sandfish *Labeo seeberi* Gilchrist & Thompson, 1911, fiery redfin *Pseudobarbus phlegethon* (Barnard, 1938), Clanwilliam redfin *Sedercypris calidus* (Barnard, 1938), Clanwilliam rock catfish *Austroglanis gilli* (Barnard, 1943) and Cape galaxias *zebratus* Castelnau, 1861 (Woodford et al., 2005). Fish surveys conducted in 1998 and 2004-2006 showed that smallmouth bass *Micropterus dolomieu* (Lacepède 1802) had invaded the lower sections of the Rondegat River up to a small waterfall about 5 km from its inflow into Clanwilliam Dam (Bills, 1999; Woodford et al., 2005; Marr et al., 2012). In the invaded section, predation by smallmouth bass had extirpated both the native redfin species (Woodford et al., 2005; Marr et al., 2012) and had altered the invertebrate community structure (Lowe et al., 2008). In the invaded area, only sub-adult and adult Clanwilliam yellowfish that were too large to be consumed were able to co-exist with smallmouth bass while native fishes occurred at high densities in the non-invaded reaches above the Rooidraai Waterfall (Woodford et al., 2005).

2.2 Rondegat River Treatment

The treatment of the Rondegat River was the first treatment in a CapeNature managed pilot project to evaluate the efficacy and ecological impact of the piscicide Rotenone as a conservation tool in the management of non-native fishes (Impson et al., 2013; Weyl et al., 2014). The objective of the treatment was to remove non-native smallmouth bass from about 5 km of the Rondegat River such that native fish from above the waterfall barrier could recolonise the treated area, thereby increasing the population range for the native fish species (Weyl et al., 2013). In addition, the riparian zone in the middle and lower reaches of the Rondegat River, previously heavily invaded by non-native trees, mainly black wattle (*Acacia mearnsii* De Wild.), blackwood (*Acacia melanoxylon* R.Br.) and red river gum (*Eucalyptus camaldulensis* Dehnhardt), was cleared of the invasive trees from July 2010 to June 2012 (Impson et al., 2013). In total, 437 ha of the riparian zone along the lower Rondegat River was cleared and natural native plant regeneration from isolated and previously suppressed plants, and seed banks in the soil, occurred such that by January 2013 the riparian zone was returning to a more natural state (Impson et al., 2013).

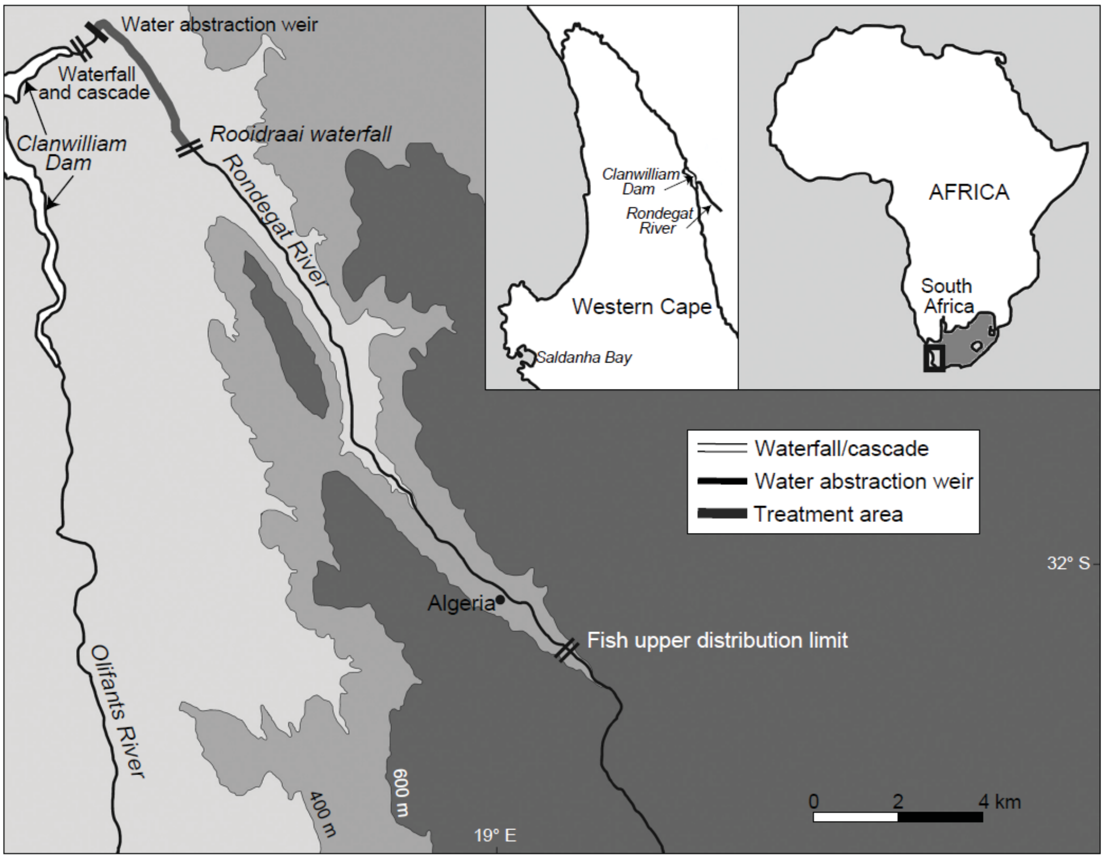


Figure 2.1: Map of the Rondegat River, showing treatment area in relation to natural and artificial barriers; from Weyl et al. (2013)

The project employed international best practice in piscicide treatments (Finlayson et al., 2010) and was further guided by on-site advice from experts from the USA and Norway (Weyl et al., 2014). Two weeks before the planned treatment, volunteers caught live fish from the targeted stretch of river using fyke nets or by angling, which were either released into the Clanwilliam Dam (45 Clanwilliam yellowfish), or used in ecotoxicological studies (85 smallmouth bass) (Weyl et al., 2013) to determine the concentration of Rotenone that would be needed to ensure complete mortality of smallmouth bass (Jordaan and Weyl, 2013). The flow rates were then determined to estimate the appropriate dosage rates for the piscicide dosing stations (Impson et al., 2013).

The target area was first treated on 29 February 2012 using seven treatment stations to maintain a target concentration of 50 μg L^{-1} Rotenone. Eight backpack sprayers treated side channels and pools to ensure adequate coverage of the treatment in all back waters. At the end of the treatment area, Rotenone was deactivated using potassium permanganate (at a concentration of 2.5%). The effectiveness of the Rotenone, and of the deactivation, was monitored through the use of sentinel fish (smallmouth bass) in keep-nets. All sentinel fish in the treatment area died within 2 hours of treatment commencing, whereas sentinel fish below the deactivation station survived the treatment, indicating adequate deactivation of the Rotenone downstream of the treatment area (Impson et al., 2013). During the Rotenone treatment, all dead fish were collected by volunteers who patrolled the entire 4 km treated reach of the river. During the 2012 Rotenone operation, 385 smallmouth bass and 94 Clanwilliam yellowfish were collected (Weyl et al., 2013).

According to standard operating procedure (Finlayson et al., 2010), a second treatment was conducted almost a year later on 13 March 2013. This treatment used fewer treatment sites (four) and a lower target concentration of 37.5 µg L^{-1} Rotenone as recommended by the international experts (Dr B. Finlayson, Dr J. Steinkjer). Following the treatment, no further bass were collected, although one bass was observed during pre-treatment monitoring just above the barrier weir and was presumably killed during the treatment. During the 2013 treatment, the Rotenone concentration in the river was measured at selected points along the treatment zone to determine whether Rotenone was building-up in the treatment zone and whether the deactivation stations were effectively deactivating the Rotenone (Slabbert et al., 2014). Slabbert et al. (2014) found no evidence of Rotenone building-up in the treatment zone and reported that the Rotenone concentration fell below the minimum effective dose for smallmouth bass of 12.5 µg L^{-1} Rotenone, determined by Jordaan and Weyl (2013), at certain points in the treatment zone. The deactivation station was found to be effective in deactivating the Rotenone, however, the Rotenone residence time in the treatment zone was longer than expected and Rotenone was detected in the river below the deactivation station after deactivation operations had ceased (Slabbert et al., 2014).

During the 2013 treatment, ~3000 young-of-year (<10 cm) native fishes were collected from the treatment area, including Clanwilliam yellowfish, fiery redfin, Clanwilliam redfin and Clanwilliam rock catfish. These fish were absent from the treatment area prior to bass removal and their presence one year later suggests that a large number of native fishes were previously being consumed by bass and that rapid recolonization by native fishes of the treated reaches from upstream populations areas would take place (Weyl et al., 2014).

2.3 Pre- and Post-treatment Monitoring

The immediate impact of the Rotenone treatments and subsequent monitoring of the recovery post treatments were evaluated during the WRC K9/822 and K5/2261 projects (Woodford et al., 2012; Weyl et al., 2016) and published in Impson et al. (2013), Weyl et al. (2013), Woodford et al. (2013), Weyl et al. (2014), and Bellingan et al. (2015). The following discussion is synthesised from these references.

A Before-After-Control-Impact (Green, 1979) monitoring strategy was used to evaluate the recovery of aquatic taxa (macroinvertebrates, fish and frogs) following the treatment of the Rondegat River (Weyl et al., 2013). The river was divided into the control (upstream of the Rooidraai Waterfall) and treatment zones. Pre-treatment surveys of the control and treatment zones were conducted in February 2011, 2012 and 2013 during the low-flow period at the end of summer (Weyl et al., 2013). In addition, the treatment zone was surveyed immediately post-treatment in March 2012 and 2013. Additional post-treatment surveys of the control and treatment zones were conducted in March 2014 and 2015 for macroinvertebrates and in March 2014 to 2017 for fish (Weyl et al., 2016).

2.3.1 Fish Monitoring

Three sampling methods, including backpack electrofishing, snorkelling transects and underwater video analysis (UWV), were used to assess the fish community for species composition, population structure and relative abundance. Habitat type and site characteristics determined the sampling method employed at each site. Electrofishing was limited to 30 shallower sites, <1 m deep while snorkelling (40 sites) and UWV (37 sites) were used in a wide range of habitats (Weyl et al., 2013).

Monitoring demonstrated that in the treatment area, estimates of fish density (snorkel survey) and relative abundance (UWV) varied in the two years prior to the first treatment in February 2012. Shortly after the 2012 Rotenone treatment no smallmouth bass were detected (Woodford et al., 2012) and Rotenone treatment was considered successful (Figure 2.2). Native fish rapidly colonised the reach where smallmouth bass had been eradicated (Weyl et al., 2016). The results demonstrated that densities of the three cyprinid species in the rehabilitated area are beginning to resemble those in the control area (Figure 2.2).

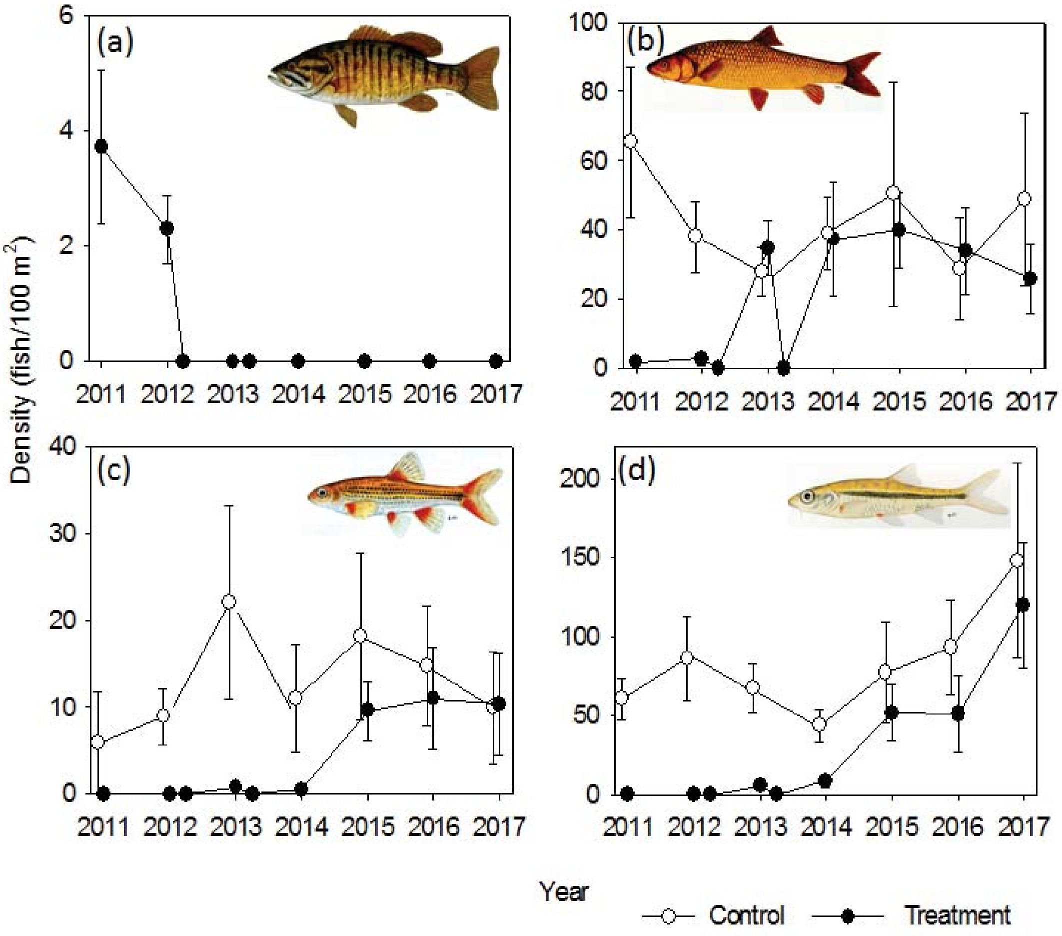


Figure 2.2: Density estimates of native and non-native fishes in the Rondegat River (2011-2017) in the reach previously invaded by smallmouth bass compared to a control reach above the treatment reach; (a) Smallmouth bass *Micropterus dolomieu* (b) Clanwilliam yellowfish *Labeobarbus seeberi*, (c) Fiery redfin *Pseudobarbus phlegethon,* and (d) Clanwilliam redfin *Sedercypris calidus*.

2.3.2 Frogs

Five common species of frog were recorded from the treatment reach of the Rondegat River: the Cape river frog *Amietia fuscigula*, the Clicking stream frog *Strongylopus grayii*, FitzSimons’ ghost frog *Heliophryne depressa*, the Raucous toad *Amietophrynus rangeri* and the Cape sand frog *Tomopterna delalandii*. (Woodford et al., 2012) Only the tadpoles of *A. fuscigula* and *T. delalandii* were recorded in the treatment zone, which included several irrigation furrows that ran parallel to the river. While many tadpoles of these two species were killed during the treatment, especially in the furrows, these likely represent a small fraction of the total population for either species in the Rondegat River. Post-treatment surveys indicated no difference in the numbers of adult frogs, and the removal of fish from the treatment area is expected to result in a short-term increase in amphibian densities (Woodford et al., 2012).

2.3.3 Macroinvertebrates

Sampling of macroinvertebrates was conducted at seven monitoring sites, three monitoring sites in the control zone upstream of the treatment zone, three sites within the treatment zone and one site downstream of the treatment zone. Macroinvertebrates were collected by kick sampling using the SASS5 rapid bioassessment protocol (Dickens and Graham, 2002). In addition, four stones from the stones-in-current biotope c.f. Dickens and Graham (2002) were collected from runs 20-40 cm deep to ensure biotope standardisation, with a 200 ųm mesh net held downstream to capture escaping invertebrates (Woodford et al., 2012). Each stone was inspected by hand, large invertebrates were removed with forceps, and the stones were then scrubbed and all collected invertebrates preserved in ethanol. Because Rotenone is known to cause catastrophic drift events of insects, insect drift levels were recorded at the central monitoring site of the control and treatment zone before, during and after the 2012 Rotenone treatment, to determine the immediate effect of Rotenone on the major insect groups within the stream.

As was expected, a catastrophic drift event occurred during the 2012 treatment of the Rondegat River. The effect was immediate, with the number of invertebrates in the drift increasing by two orders of magnitude above natural background drift levels, which remained constant at the monitoring site in the control area throughout the Rotenone treatment (Figure 2.3). Following the end of Rotenone treatment, drift rapidly declined to near-pre-treatment levels. Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) were more vulnerable to Rotenone than other groups of invertebrates, e.g. Coleoptera and Diptera (Woodford et al., 2013). The proportional abundance of macroinvertebrate orders also shifted over the course of the treatment, initially dominated by Ephemeroptera but moving to Coleoptera at peak drift before returning to near-pre-treatment drift levels dominated by Diptera; see Weyl et al. (2016).

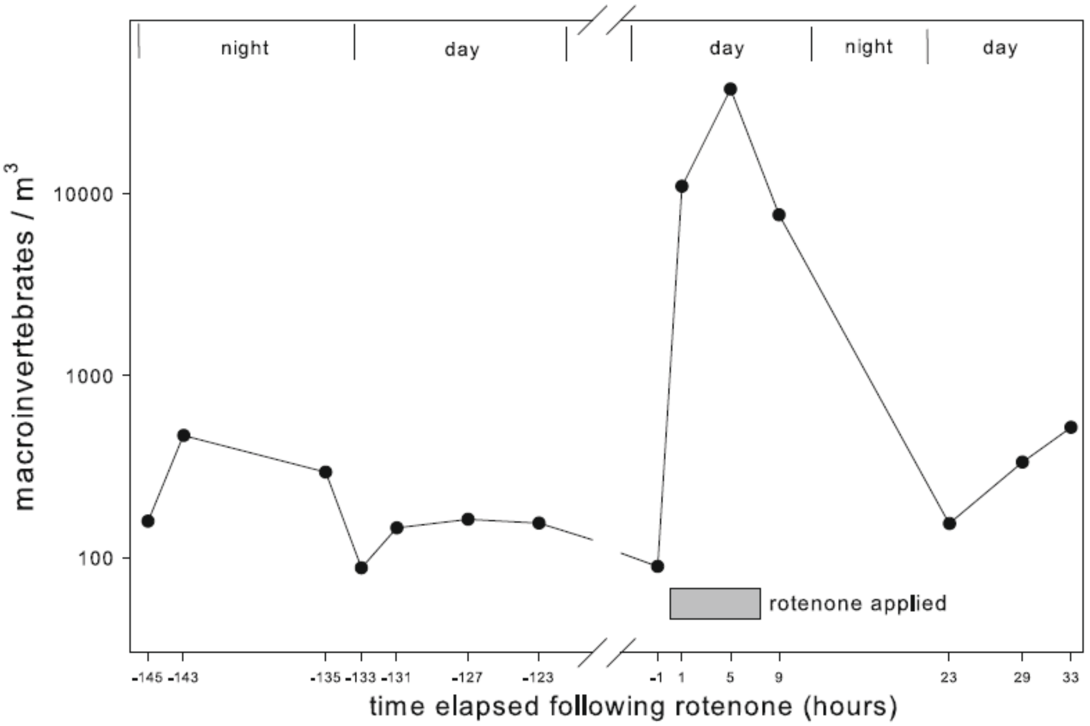


Figure 2.3: Total invertebrate drift abundance at on the day and the day following the 2012 treatment of the Rondegat River. The period of Rotenone treatment is denoted by the grey area above the x-axis; after Woodford et al. (2013).

There was a significant decline in average score per taxon (ASPT) following the 2012 treatment, however, the ASPT had recovered to pre-treatment ASPT scores by May 2012. In contrast, there was no significant decline in mean overall SASS5 score from the pre- to post-treatment scores. The ASPT scores recorded before and after Rotenone treatments were considered "below reference" relative to reference communities for Western Cape streams (Dallas and Day, 2007); see Figure 2.4. This is most likely a consequence of the moderate levels of agricultural development of the riparian zone in the middle and lower Rondegat. However, considerable seasonal and inter-annual variation in ASPT and SASS scores were found. Considering these findings, Weyl et al. (2016) concluded that ecosystem health as estimated by the SASS5 scoring system was not significantly altered by the Rotenone treatment.

Species richness decreased significantly following treatment, even though many rare taxa were not recorded immediately prior to treatment. A comparison of species-level taxonomic diversity revealed that ten common invertebrate species were not recorded in the treatment zone immediately following the 2012 Rotenone treatment. Overall, 82% of the common species were recorded in the treatment zone after just two months of recovery and by the end of the monitoring program all common species had returned to the treatment area. Rare species were not considered good indicators of impact as their presence, or absence, is likely to be incidental because such species have a random chance of being detected irrespective of time or area. Of the rare taxa, 36 species were not recorded in May 2012 and 27 species were not recorded by the end of the monitoring. However, 19 new species were recorded in the treatment zone two months after the 2012 treatment. While this "wave" of previously undetected species could represent colonisation of the treatment area as a result of predatory release due to the removal of fish or competitive release due to the removal of dominant macroinvertebrates, it could also be an artefact of sampling efficiency. As a result, taxon specific assessments were unable to adequately demonstrate impact (see Woodford et al., 2013).

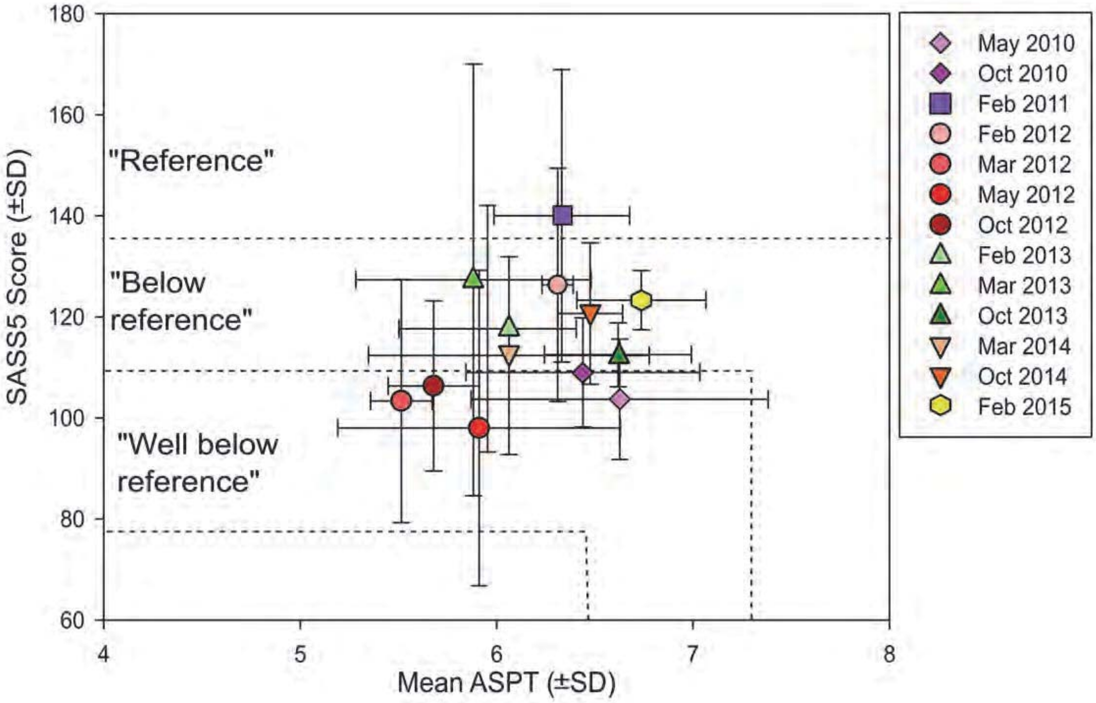


Figure 2.4: Mean ASPT and SASS scores at monitoring sites in the treatment area on the Rondegat River. This includes all surveys conducted between May 2010 and February 2015; after Weyl et al. (2016).

Bellingan et al. (2019) evaluated the long-term data collected by kick sampling and determined that there was no long-term difference in the Shannon diversity of the invertebrate communities and Ephemeroptera, Plecoptera and Tricoptera (EPT) taxa between the control and treatment reaches of the Rondegat River before and following the two treatment with Rotenone (Figure 2.5). A similar result was found for the invertebrate abundances (Figure 2.6).

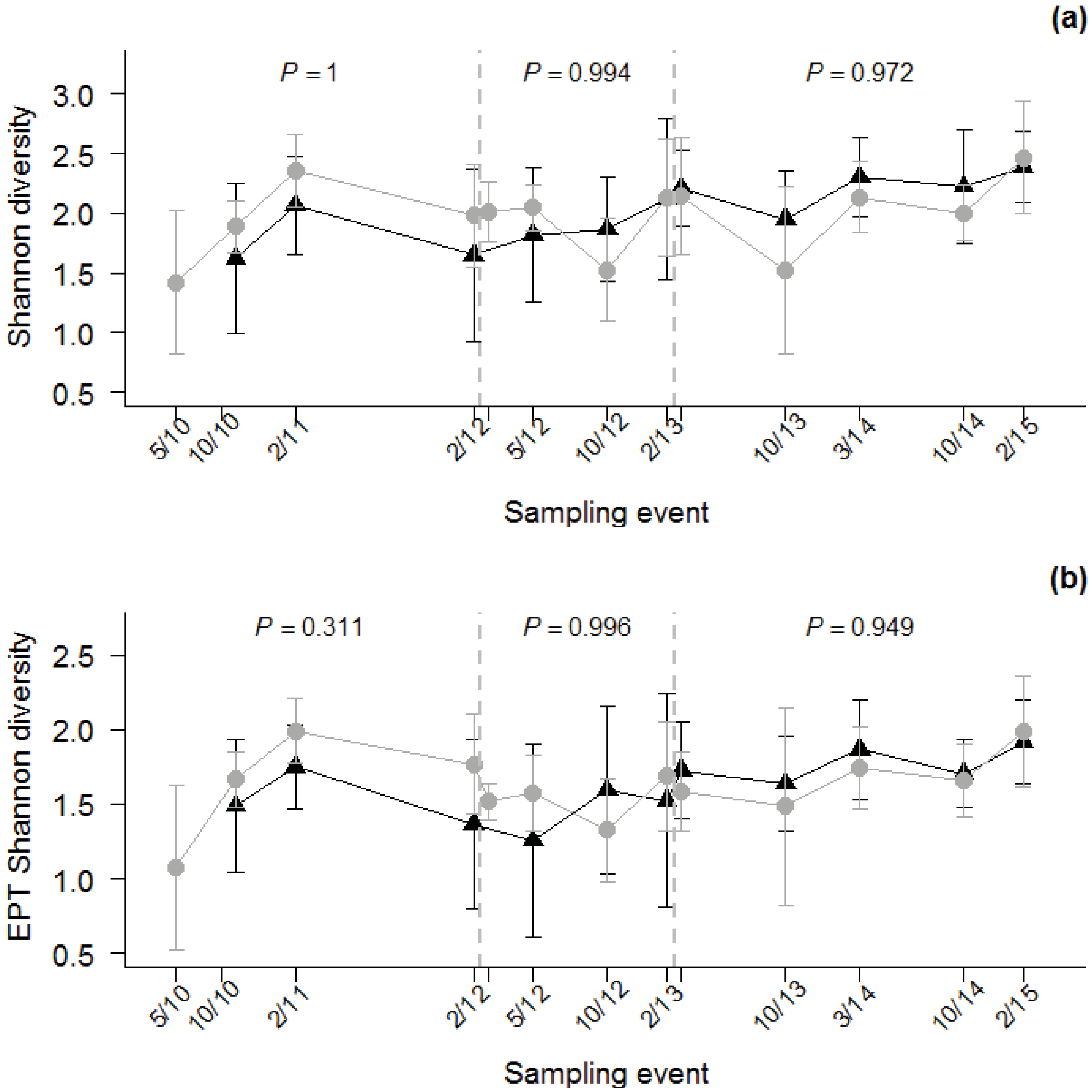


Figure 2.5: Variation in Shannon diversity of invertebrates collected through the kick sampling method across the five-year sampling period. Mean and standard deviation for each sampling event (month/year) from the control reach (black) and treatment (grey), for (a) invertebrate diversity, and (b) diversity of EPT taxa are presented. The dashed lines represent Rotenone application events. *P*-values represent pairwise post hoc tests, comparing treatment and control sites within each sampling phase. From Bellingan et al. (2019).

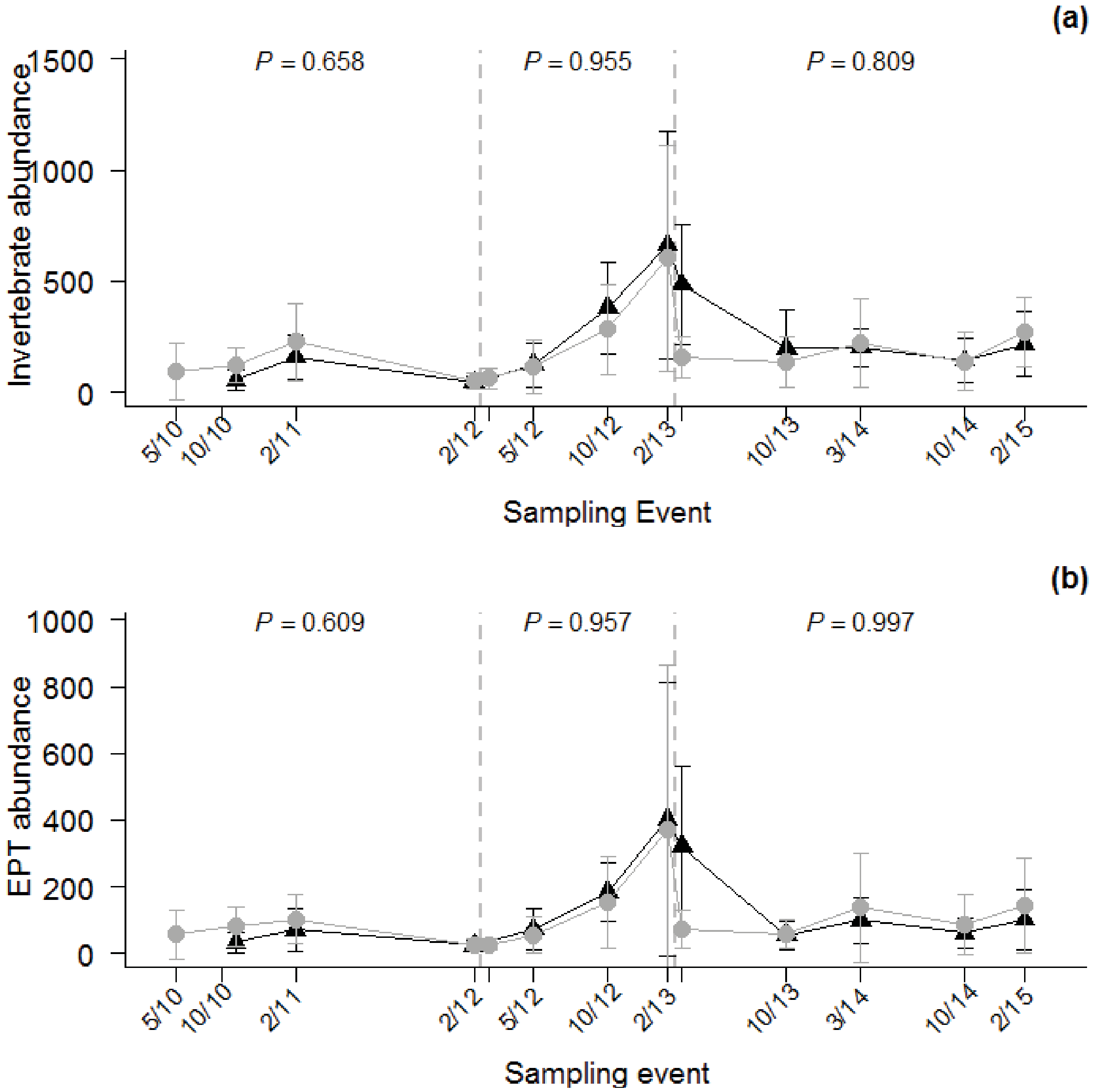


Figure 2.6: Variation in invertebrate abundance (density) collected through the kick sampling method across the five-year sampling period. Mean and standard deviation are given for each sampling event (month/year) from the control reach (black) and treatment (grey), for (a) invertebrate abundance, and (b) abundance of EPT taxa. The dashed lines represent Rotenone application events. *P*-values represent pairwise post hoc tests, comparing treatment and control sites within each sampling phase. From Bellingan et al. (2019)

Bellingan et al. (2015) and Bellingan et al. (2019) quantitatively assessed the long-term abundance of macroinvertebrates (excluding larval chironomids), larval chironomids, and Ephemeroptera, Plecoptera and Tricoptera (EPT) taxa on the surface of stones finding that macroinvertebrate abundance varied widely among sampling events for control and treatment sites, and sampling event is a significant predictor in all models.

After controlling for the influence of sampling site and event, no significant differences were found between the control and treatment reach for macroinvertebrate abundance excluding larval chironomids (Figure 2.7). Larval chironomid abundance was significantly lower along the treatment reach compared to the control reach throughout the sampling period (Figure 2.7). Treatment phase was a significant but weak predictor of EPT taxon abundance from stone samples; and there was no significant difference when the treatment and control reaches were compared within each particular treatment phase (Figure 2.7).

2.3.1 Algal Production

The food-web effects were also measured by assessing algal production on stone surfaces (Woodford et al., 2012). Each stone was scrubbed for 2 minutes in a basin, the algal slurry was then filtered and the algal residue enclosed in aluminium foil. Each stone was measured across three axes to estimate the surface area before being replaced in the stream. Seasonal concentrations of chlorophyll-a across all treatment zone sites showed significant variation among seasonal samples with the February 2012 stones having more algae production than in any of the other months (Woodford et al., 2012). It is therefore difficult to attribute changes in algal abundance on the stones to the Rotenone treatment, as the May 2012 concentrations (the only samples taken post-treatment) were in line with previous samples for that month. The significant difference between the February 2012 samples and the others may have been driven by fluctuations in summer grazing pressure unrelated to the Rotenone treatment, as the Ephemeroptera (a key grazer group) were significantly less abundant in the February 2012 samples than in the February 2011 samples. This confounding factor further suggests that natural variation in grazing pressure over time may make distinguishing long-term impacts of the Rotenone treatment on food web processes using chlorophyll-a analysis extremely challenging.

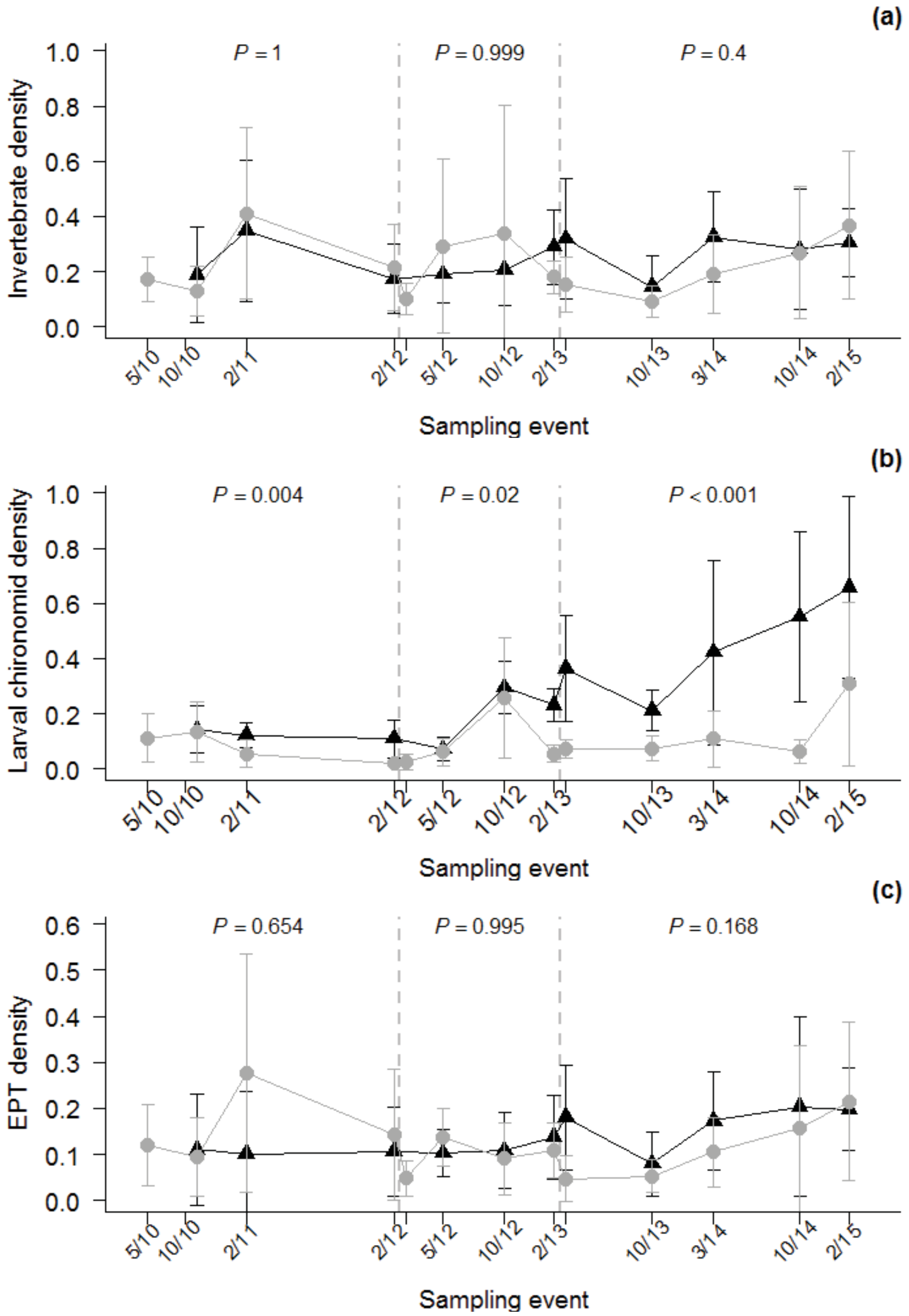


Figure 2.7: Variation in invertebrate abundance per unit area of stone surface (species density) collected through the stone sampling method across the five-year sampling period. Mean and standard deviation is given for each sampling event (month/year) from the control reach (black) and treatment reach (grey), for (a) invertebrate density without larval chironomids, (b) larval chironomid abundance only, and (c) abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT). The dashed lines represent Rotenone application events. *P*-values represent pairwise post hoc tests, comparing treatment and control sites within each sampling phase. From Bellingan et al. (2019)

2.4 Associated Research

A number of research projects related to Rotenone were initiated in preparation for the treatment of the Rondegat River. These studies have increased scientific knowledge of the toxicity of Rotenone to various fish and aquatic invertebrates and of the persistence of Rotenone in the treatment area. Edited versions of the abstracts from these studies are presented here.

Prior to the treatment of the Rondegat River in March 2012, the target concentration for the treatment to remove smallmouth bass needed to be determined for the Rondegat River. Jordaan and Weyl (2013) determined the sensitivity of smallmouth bass to various concentrations of the Rotenone formulation CFT Legumine (5% active Rotenone) using standard toxicity tests to determine the minimum effective dose for 100% mortality after an exposure of four hours. The minimum effective dose was found to be 12.5 μg L^{-1} Rotenone. Standard operating procedures for Rotenone treatment recommend a minimum of twice the calculated MED. Due to uncertainty related to Rotenone losses under field conditions, the Rondegat River was treated at twice the recommended dose (50 μg L^{-1} Rotenone) for six hours in March 2012.

After the initial Rotenone treatment of the Rondegat River in March 2012, a second treatment in March 2013 was executed. Concerns were expressed following the 2012 treatment regarding the potential build-up of Rotenone between the treatment stations, therefore, Slabbert et al. (2014) monitored the Rotenone concentrations during the 2013 treatment. Measured concentrations were consistently below the 37.5 μg L^{-1} target Rotenone concentration and dropped below the tested 12.5 μg L^{-1} effective Rotenone concentration at some sampling points. No build-up of Rotenone within the treatment zones was found, but Rotenone took longer than expected to be flushed out of the treatment area. The neutralisation station effectively neutralised the Rotenone when operational, but Rotenone was still present at detectable concentrations after neutralisation was terminated.

The sharptooth catfish *Clarias gariepinus,* native to northern and eastern South Africa but extensively translocated in southern and western South Africa, is also an emerging global invader for which control strategies might include the use of piscicides such as Rotenone. Jordaan et al. (2017) demonstrated that *C. gariepinus* was less susceptible to Rotenone than most other fish species, with unexpected survival recorded at Rotenone concentrations of 87.5 and 100 μg L^{-1}. Sharptooth catfish exhibited avoidance behaviour to Rotenone treated water and were capable of recovering from Rotenone exposure. Effective eradication of sharptooth catfish might not be attainable even at a doses exceeding 100 μg L^{-1} for longer than 24 hours, doses that pose an unacceptable risk to non-target fauna. The potential use of other piscicides, such as Antimycin A, that are less detectible to fish and do not elicit avoidance behaviour (Finlayson et al., 2002) could be considered for future studies involving sharptooth catfish.

Using laboratory studies, Dalu et al. (2015) investigated the effects of different Rotenone concentrations (0, 12.5, 25, 37.5, 50, 100 μg L^{-1}) on selected invertebrate groups; Ephemeroptera, Odonata (Aeshnidae), Hemiptera (Belostomatidae), Gastropods (Pulmonata), Decapods, and zooplankton (Ostracoda, Copepoda and Cladocera) over a period of 18 hours. Based on field observations and body size, it was hypothesized that Ephemeroptera and zooplankton would be more susceptible to Rotenone than Decapods, Hemiptera and Gastropoda. Experimental results supported this hypothesis and mortality and behaviour effects varied considerably between taxa, ranging from no effect (crab *Potamonuates sidneyi*) to 100% mortality (Cladoceran *Daphnia pulex* and Copepod *Paradiaptomus lamellatus*). Planktonic invertebrates were particularly sensitive to Rotenone even at very low concentrations.

While dose-response relationships have been developed for fish, there are limited comparative data available on aquatic insects that respire either with tracheal gills or with a plastron – a thin layer of air trapped by hairs on the exterior of the body. Booth et al. (2015) assessed the temperature-dependent toxicity of Rotenone to gill-respiring damselflies, order Odonata family Coenagrionidae, and plastron respiring water boatmen, order Hemiptera family Corixidae, at concentrations lethal to Mozambique tilapia *Oreochromis mossambicus*. Both groups of insects were found to be differentially susceptible to Rotenone, with survival decreasing as functions of both increased concentration and temperature. The dose-response relationship of Mozambique tilapia was found to be similar to that of other fishes, with 100% mortality achieved at 25 µg L^{-1} at both 20 °C and 28 °C. At this concentration, mortality in gill-respiring insects after 48 hours was 10% at 20 °C and 28% at 28 °C, which was higher than that of plastron-respiring insects, being 2% and 7% at the same temperatures. At higher concentrations (50-100 µg L^{-1}), however, mortality of both gill- (>50%) and plastron-respiring (>10%) insects became substantial.

CHAPTER 3. TREATMENT OF TWO FARM DAMS

3.1 Introduction

To inform National Policy on the registration and use of Rotenone for ecosystem rehabilitation with regard to the removal of invasive fishes, information on the effects of Rotenone in both lentic and lotic environments is required. As these environments are often inter-connected, any successful Rotenone eradication program for a river needs to consider the treatment of all potential invasion sources which might include reservoirs, farm dams or wetland areas, to reduce the risk of non-native fish re-colonising the river system after treatment. Because lentic environments hold different aquatic communities to river systems, and because non-target taxa may not be able to recolonise a lentic environment from upstream sources as is the case in river systems; the rates of recovery or re-colonisation of a lentic environment following a Rotenone treatment may differ. It is therefore important that the potential impact of Rotenone treatments on aquatic communities in both environments be fully understood. The lack of such information was considered a major gap in the knowledge required for informing guidelines for the use of Rotenone to rehabilitate aquatic ecosystems in South Africa.

The opportunity to address these knowledge gaps was provided by CapeNature's treatment of two off channel dams in the Olifants-Doring River catchment. Two dams were treated, the Chalet Dam (32.541563°S 19.281056°E) on the Krom River was treated on the 26th of January 2017 by CapeNature to remove non-native bluegill and the Kranskloof Dam (31.509452°S 19.134511°E) in the Oorlogskloof catchment near Nieuwoudtville in the Northern Cape was treated on the 29th of March 2017 by CapeNature, in conjunction with the Northern Cape Department of Environment and Nature Conservation (DENC), to remove non-native common carp.

3.2 Study Sites

The sites used in this study are two farm dams on Krom River Farm in the Western Cape and two farm dams on Driefontein and Kranskloof farms near Nieuwoudtville in the Northern Cape (Figure 3.1). Permits for the project were obtained from CapeNature (0028-AAA008-00260 and 0056-AAA008-00067) and the Northern Cape DENC (FAUNA 0032/2017 and FAUNA 0031/2016) and Ethics Approval was obtained from SAIAB

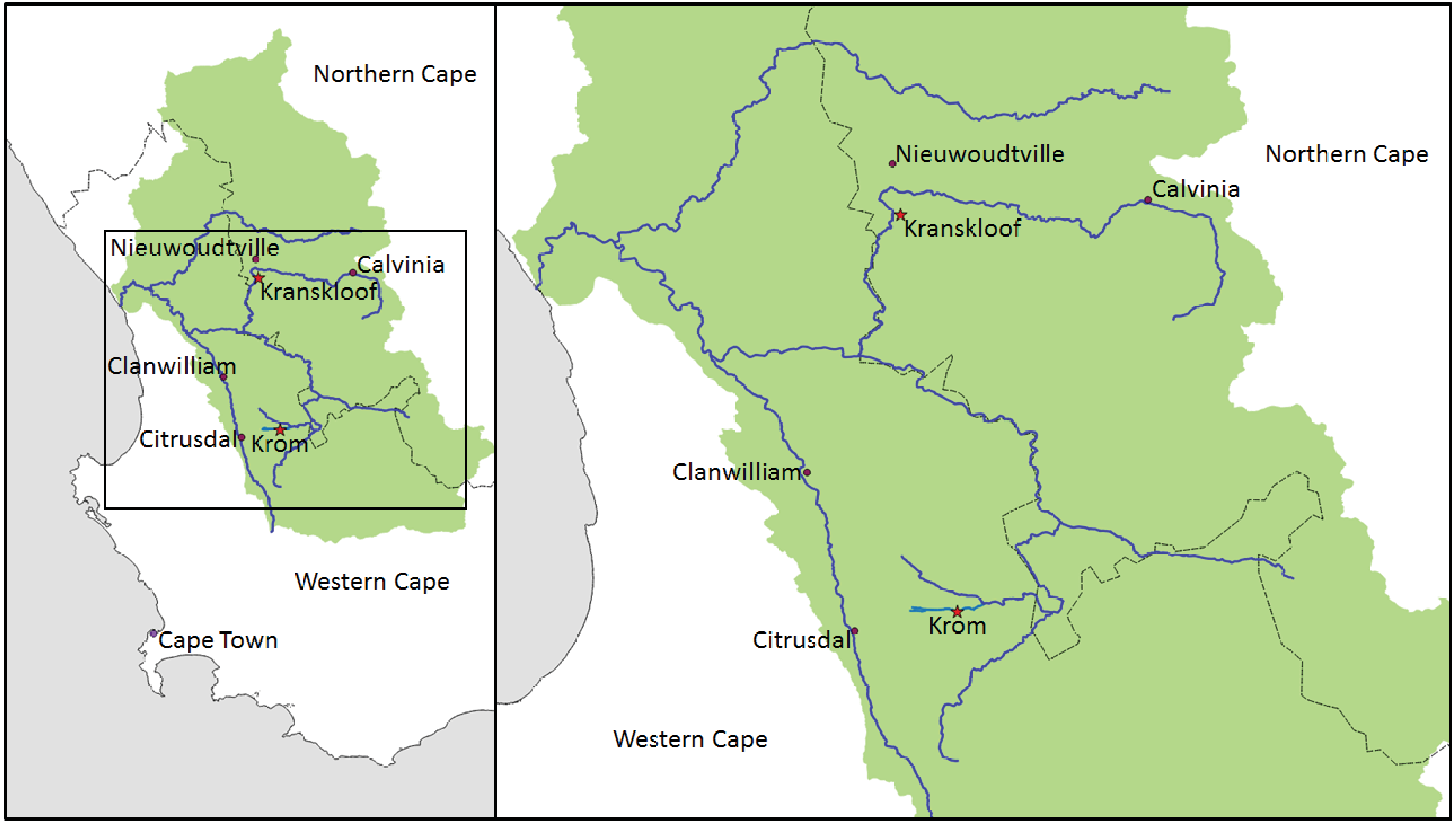


Figure 3.1: Map indicating the location of major towns and the study two sites (stars) in the Western and Northern Cape provinces. The area shaded green depicts the extent of the Olifants Doring catchment and the blue lines the major rivers

(25/4/1/7/5_2018-03) for the project. General Authorisations for the treatment of the Chalet Dam (Krom River) and Kranskloof Dam (Nieuwoudtville) (27/2/1/E224/214/1 and 27/2/1E340/215/1, respectively) were granted by the Department of Water and Sanitation to CapeNature and the Northern Cape Department of Environment and Nature Conservation (DENC).

3.2.1 Dams near Nieuwoudtville

Both the treatment and control dams are situated on a plateau on the eastern boundary of the Oorlogskloof Nature Reserve, just south of Nieuwoudtville in the Northern Cape (Figure 3.2A). Both dams are located in ephemeral water courses that form part of the Oorlogskloof River. The Oorlogskloof River contains one of the last recruiting populations of the endangered Clanwilliam sandfish *Labeo seeberi* and the river is a FEPA “fish sanctuary” and thus of high conservation value. Common carp are present in both dams and the treatment of both dams was planned to reduce the threat of carp entering the Oorlogskloof River. The dams are situated on two separate farms; Driefontein and Kranskloof. The Kranskloof dam was selected as the treatment while the Driefontein dam was the control dam for this study. Treatment of the Driefontein dam is planned following the conclusion of this project.

Both dams were illegally stocked (no permits were issued by DENC) with carp and the water in both dams was found to be eutrophic and turbid, a common feature of inland waters dominated by this species. Frequent algal blooms have been recorded in these dams. . Aquatic vegetation is absent from both dams and the substrate is covered with a layer of fine sediment that is suspended by the feeding activities of carp.

3.2.2 Krom River Dams

The two dams situated on Krom River farm in the Western Cape were selected for the project because the land owners are supportive of conservation projects for native fishes of the Matjies River catchment, including the removal of non-native fish from their property. The Krom River was identified as one of four rivers to be treated with Rotenone in the CAPE Alien Fish Removal Project (Marr et al. 2012). The treatment of the dams are a prerequisite for the treatment of Krom River for which the Department of Water and Sanitation granted the General Authorisation 27/2/1E1121/389/1 for in January 2018.

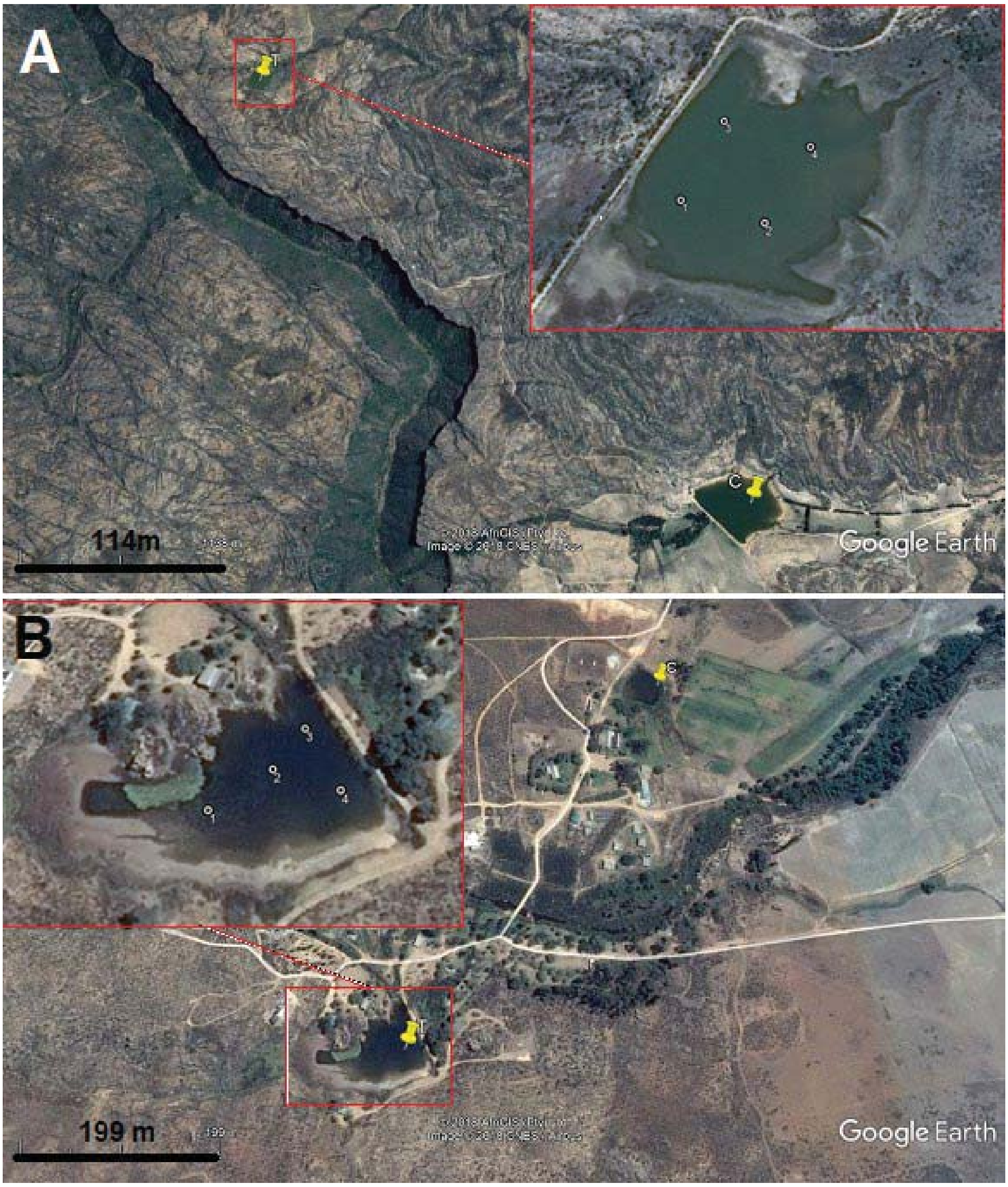


Figure 3.2: Google Earth images showing (**A**) Kranskloof treatment (T) and Driefontein control (C) dams in the Northern Cape and (**B**) Krom River treatment (T) and control (C)) dams, Chalet and House dams, respectively, in the Western Cape. The location of the plankton study sites are indicated by the grey dots.

The treatment dam (Chalet Dam) is located amongst the Cederberg Tourist Park chalets and is fed via a furrow system from the Krom River (Figure 3.2B). A grid in the furrow just upstream of the discharge into the dam prevents fish from entering or leaving the dam. The dam was drained in 2013 and allowed to dry to reduce the population of leeches in the dam about which visitors to the Cederberg Tourist Park had complained. When the dam was refilled, a population of non-native bluegill established in the dam. The origin of the bluegill, and the mechanism for establishment, are not known, but it is suspected that they either colonised the dam through the furrow network or were stocked by visitors from fish caught in the Krom River. The House Dam was selected as the control dam for the Krom River site. Both dams have considerable aquatic vegetation biomass and hold clear water with low turbidity.

3.3 Monitoring Techniques

CapeNature was wholly responsible for the application of Rotenone in both systems and the disposal of the fish killed during the treatments. SAIAB's role was strictly to establish a baseline assessment and to evaluate changes from this baseline following the completion of the treatment and over a specified recovery time. The aims of this study were to sample the treatment and control dams for water parameters, phytoplankton and zooplankton, macroinvertebrates and fish prior to, and at intervals following, the treatment of the dams with the piscicide Rotenone to gain an understanding of the ecological impact of the treatment and the recovery post treatment.

3.3.1 Rotenone Treatments

Krom River Dams

On the 26th of January 2017, CapeNature treated the Chalet Dam with Rotenone (CFT Legumine) to remove non-native bluegill sunfish. The initial treatment dose of 1.75 ppm CFT Legumine was increased to 2 ppm, and subsequently raised to 2.5 ppm. This resulted in a nominal Rotenone concentration of 125 µg/L of active Rotenone. The increased treatment dose was to compensate for the biological demand from a considerable amount of aquatic vegetation in the treatment dam. Rotenone was dispensed from a boat using the wash of the propeller to disperse the Rotenone through the water column. In addition, back-pack sprayers were used to treat the shallow areas on the perimeter of the dam and the wetland area in the southwest corner of the dam.

Nieuwoudtville Dams

On the 29th of March 2017, CapeNature, in conjunction with the Northern Cape Department of Environment and Nature Conservation (DENC), treated Kranskloof Dam with Rotenone (CFT Legumine) to remove non-native common carp. The target treatment dose was 1.5 ppm CFT Legumine. This resulted in a nominal Rotenone concentration of 75 µg/L of active Rotenone. Rotenone was dispensed from a boat using the wash of the propeller to disperse the Rotenone through the water column (Figure 3.3B). In addition, back-pack sprayers were used to treat the shallow areas on the perimeter of the dam (Figure 3.3A). Sampling of the Nieuwoudtville dams one year after Rotenone treatment was not possible as the treatment dam dried up completely in January 2018 during a severe drought in the region (Figure 3.4). The sampling of the Nieuwoudtville dams in October 2017 allowed for a post treatment assessment of the Kranskloof Dam before it dried up.

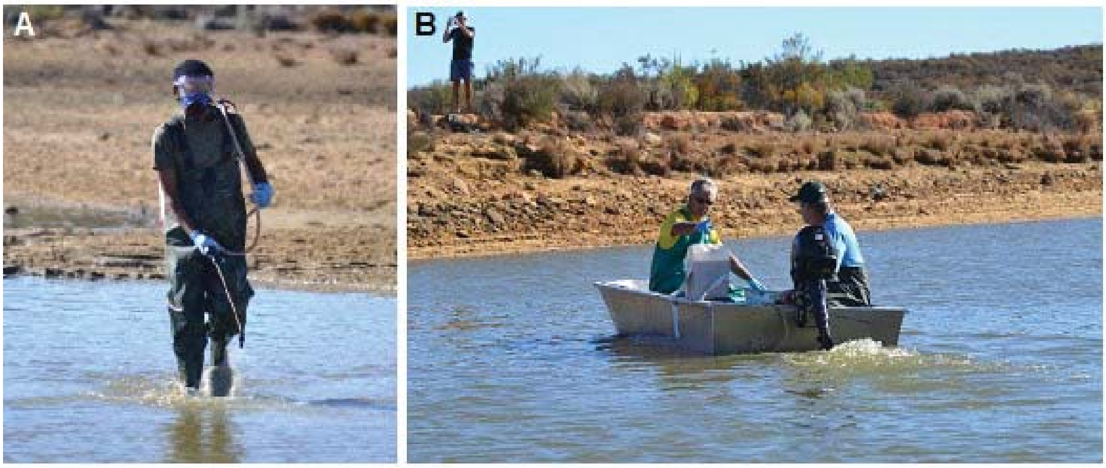


Figure 3.3: Northern Cape DENC conservator (**A**) and CapeNature scientists, Dean Impson and Riaan van der Walt (**B**) applying Rotenone in the shallows and deep areas of Kranskloof Dam, Nieuwoudtville, Northern Cape, using a backpack unit and a boat. Photo Tatenda Dalu.

Following the Rotenone treatments, all dead fish were collected by volunteers who patrolled the lakeshore. All fish caught or collected during both phases of the process were identified to species, enumerated and measured, and then buried on the farm away from any watercourses.

Figure 3.4: Kranskloof dam taken on the 23rd of January 2018 showing the system almost dry due to the prevailing drought conditions. Photo Mandy Schumann

3.3.2 Rotenone Breakdown

For rivers, the treated water is constantly moving through the treatment zone and the Rotenone remaining in the water can be deactivated at the end of the treatment zone using potassium permanganate or ozone. In still waters, Rotenone breaks down naturally over time to a concentration where it is no longer lethal to fish or other aquatic organisms. Although the breakdown of Rotenone is well understood (Cailteux et al. 2001), the Rotenone treatments allowed for a demonstration of the breakdown of Rotenone in a South African small offstream farm dam. Only Chalet Dam on Krom River farm was monitored for the breakdown of Rotenone as the high turbidity of Kranskloof Dam complicated sample extraction.

The aim of the analyses was two-fold: (1) to validate the actual treatment concentration present in the dam and (2) to determine the breakdown pattern of Rotenone post treatment. Water samples were collected in triplicate at 12 points in the dam (edge, middle and bottom) for 10 time intervals (2 hours to 15 days post treatment). Samples were collected in food grade high-density polyethylene (HDPE) plastic bottles and stored at -20 °C prior to analysis.

Samples were processed prior to analysis by extracting Rotenone with C18 columns (Waters) using the standardized methods of Dawson et al. (1983). The samples were analysed by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Waters API Xevo coupled to a Waters Ultra-Performance Liquid Chromatography (UPLC). Samples were quantified against a serial dilution of the Rotenone analytical standard (Pestanal, Sigma-Aldrich).

3.3.3 Water Chemistry and Plankton

Samples of plankton were collected at four sites (pre- (n = 4) and post-treatment (n = 4) per sampling event (1 day, 6 and 12 months)) by drawing plankton nets (20 µm mesh for phytoplankton and 63 µm mesh for zooplankton) vertically through the water column at midday. Water samples for Chlorophyll-*a* (chl-*a*) were taken at each site. Physico-chemical parameters (turbidity, water clarity, temperature, pH, salinity, total dissolved solids and conductivity) were recorded at each sampling event (n = 12 per sampling event (4 sites with 3 replicates)). Both treatment and control dams were sampled pre-treatment but only the treatment dam was sampled immediately post treatment.

The chl-*a* concentration was used to determine pelagic phytoplankton biomass. In the laboratory, water samples (n = 4 per dam, pre- and post-treatment) were used for the determination of chl-*a* measurements by filtering aliquots (100-250 mL, vacuum <5 cm Hg) through a 0.7 µm Whatman GF/F filter. After filtration, the chl-*a* was extracted by placing filters in separate labelled vials containing 10 mL of 90 % acetone for 24 hrs in the dark. A Turner Designs 10-AU fluorometer fitted with a narrow-band, non-acidification system was used to determine chl-*a* concentration through fluorescence measurements (Welschmeyer, 1994).

3.3.4 Macroinvertebrates

Macroinvertebrates were sampled by sweeping a SASS net (300x300mm frame with 1mm mesh) in a 1 × 1 m quadrat at 20 locations on the perimeter of the dams (Figure 3.5). The samples were transferred to 150ml plastic honey jars and preserved in 96% ethanol. The aquatic macroinvertebrate taxa were picked from the contents of the sample and identified to family or species level, as appropriate, using the WRC aquatic invertebrate guides for South Africa (Day et al., 1999; Day et al., 2001a; b; Day and De Moor, 2002a; b; Day et al., 2003; de Moor et al., 2003a; b; Stals and De Moor, 2007).

Figure 3.5: Terence Bellingan (holding SASS net) leading a group to sample for macroinvertebrates along the littoral zones, Chalet Dam (Krom River). Photo Sean Marr

3.3.5 Fish

Fish were sampled using a multi-method approach including fyke nets, gillnets, seine nets and underwater video analysis (Figure 3.6). 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) set in both the control and treatment dams. Fyke nets are considered a passive gear type and were set in water approximately 1-2.5 m deep (Figure 3.6A). 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 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 lifted 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.

Gill nets each measuring 35 m × 2.75 m with stretch meshes of 35, 45, 57, 73, 93, 118 and 150 mm (5 m per mesh size) were set over night in different positions in both the treatment and control dams (Figure 3.6B). Gill nets were deployed at sunset and had an average soak time of twelve hours. Several mesh sizes were used to eliminate size and species selectivity which could cause potential problems when investigating population and size frequency analyses (Prchalová et al., 2009).

A pursed seine net (20 m long × 2 m deep with purse; 5 mm stretched mesh) was used to capture fish from the littoral zone of the dams (Figure 3.6C). Seine nets were only used for the dams near Nieuwoudtville because they were free from vegetation. Both Krom River dams had dense aquatic vegetation which constrained the efficient use of seine nets. The seine net was drawn for a distance of 20m before being closed towards the bank. All fish captured were identified to species level using Skelton (2001) and returned to the water.

Underwater video analysis (UWVA) was conducted using a GoPro® HD Hero3 high-definition camera. Camera settings were standardised: field of view = 127º, resolution (Full HD) = 1080p (1920 × 1080), frames per second = 30 NTSC, 25 PAL. Methods for camera placement, time of observation and analysis followed those recommended by Ellender et al. (2012). The cameras were deployed from an inflatable boat (Figure 3.5) at each site for 30 minutes, with the first five minutes regarded as an acclimation period for conditions to return to normal in the sample pool following camera deployment, and therefore excluded from the subsequent analysis. Underwater videoing lacks a spatial dimension and therefore the MaxN index, which is the maximum number of individuals for each species visible in the field of view simultaneously during a 25 minute filming session, was used as a measure of relative abundance (Ellender et al., 2012). At the Kranskloof and Driefontein Dam, UWVA was not effective due to the high turbidity and thus this gear was not used. Sampling was conducted prior to the Rotenone application, immediately after the application, six months after treatment (all dams) and again one year later in the Chalet Dam, Krom River only.

Figure 3.6: Different fish sampling gear used to monitor fish community structure within the dams in the Western and Northern Cape: (**A**) fyke nets, (**B**) gill nets and (**C**) seine netting. Photos Tatenda Dalu

3.4 Results

The results of the Rotenone breakdown experiment are presented first, then the results of the environmental variables, plankton, macroinvertebrates and fish are presented for each locality, Krom River (i.e. Chalet Dam) and near Nieuwoudtville (i.e. Kranskloof Dam).

3.4.1 Rotenone Breakdown

Rotenone breaks down in water due to a number of factors including ultra-violet light, dissolved oxygen and absorption into plant material or humic acids. The higher the organic content of a waterbody, the faster the reactive Rotenone concentration in the water column reduces. This continual degradation of the active Rotenone results in the

Rotenone concentration in the water having a half-life, which is the time taken to reduce the concentration in the environment to 50% of the original concentration.

A maximum mean Rotenone concentration of 87.26 μg/L (SD = 31.28) was measured in samples taken 2 hours post treatment. The mean concentration of measured Rotenone decreased at each subsequent time point (Figure 3.7). The data was used to fit a number of trend-lines, including power, functional, piecewise and exponential models, using R statistical software package. The logarithmic model ($y = -17.19 \ln(x) + 104.43$) provided the best fit for the Rotenone concentration over time inferring a logarithmic reduction in the Rotenone concentration (adjusted $R^2 = 0.7389$; $p < 0.001$) with a theoretical maximum concentration of 104.43 μg/L (SE 4.53 μg/L). The half-life of the Rotenone concentration for the Chalet Dam treatment was calculated to be 53.42 hours according to the aforementioned logarithmic model.

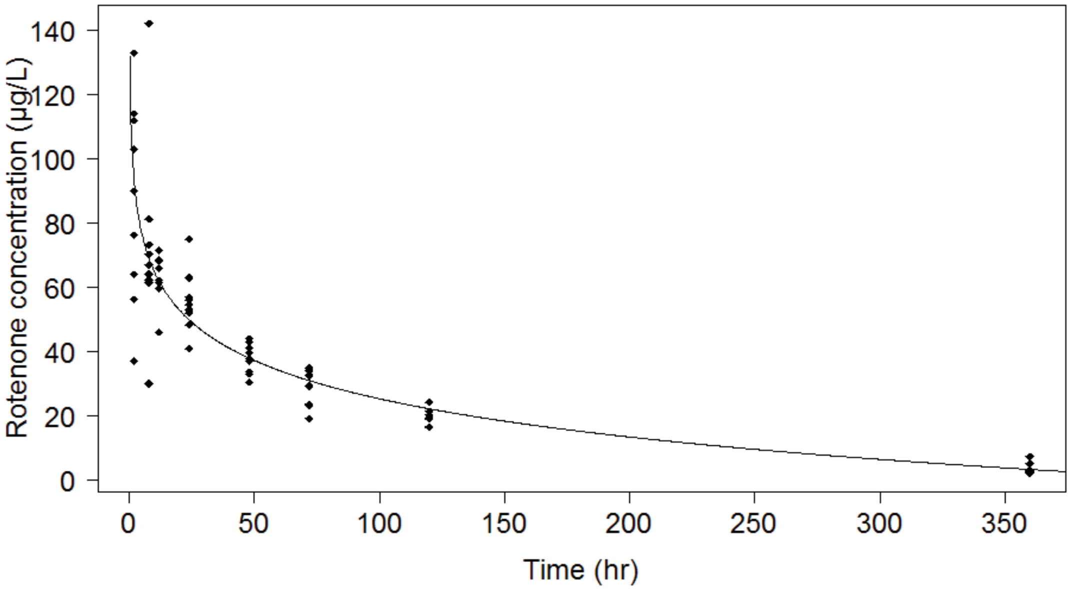


Figure 3.7: The breakdown of Rotenone in the water column of the Chalet Dam, Krom River catchment. The black line represents the logarithmic regression model fitted to the data.

Throughout, no significant differences in Rotenone concentration were observed between the surface, edge and bottom samples for all sampling times (Kruskal-Wallis test $p > 0.05$). Bioassays using the target species, bluegill sunfish, indicated that Rotenone was still present at concentrations lethal to fish seven days post treatment but had broken down to below lethal concentration levels 15 days post treatment.

3.4.2 Krom River Dams

The Chalet Dam at Krom River was treated on the 26th of January 2017. Pre-treatment samples were collected on the 15th of January 2017 for both the treatment and control dams. A follow-up survey was conducted in January 2018.

Environmental variables

Physico-chemical data are presented in Table 3.1. Significant variation (ANOVA, $p = 0.05$) in physico-chemical variables (i.e. temperature, total dissolved solids (TDS), conductivity, salinity) were observed over the year, whereas similarities (ANOVA, $p > 0.05$) were observed across the study sites. Physico-chemical variables pre- (1 day before) and post-treatment (1 day after) were similar (Tukey's post-hoc, $p > 0.05$).

The water clarity or transparency (the depth to which light penetrates the water) of the Chalet Dam, Krom River, was measured using a Secchi disk and the change in water clarity was highly significant over the treatment (ANOVA, F = 102.811, $p < 0.01$). The water clarity was ~72 % relative to the water depth before the treatment but dropped to ~50 % one day after the treatment. This could be attributed to suspension of sediment benthic algae and sediment attached on macrophytes during Rotenone application (mainly applied in the propeller wash of a small boat). However, after six and twelve months, the system had 100 % water clarity. The turbidity (cloudiness or haziness of water caused by particles or organisms not visible to the naked eye) was generally elevated for this system before (~7 NTU) and one day (~9 NTU) after Rotenone application in comparison to the ~2 NTU twelve months after the treatment (Figure 3.8A). The observed changes resulted in significant treatment differences (ANOVA, F = 69.762, $p < 0.01$) being observed for turbidity within the dam.

Table 3.1. Basic physico-chemical variables recorded in the Chalet treatment dam (Krom River catchment) over a one year period. Abbreviations: TDS – total dissolved solids. Water clarity is relative to water depth

Parameter	Pre-treatment				Post-treatment			
	1 day		0 day		1 day		12 month	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Chalet Dam								
Temperature (°C)	23.5±1.0	21.7-25.5	22.8±0.6	22.1-23.4	23.5±1.1	21.8-25.5	24.5±1.4	21.7-26.9
TDS (mg L^{-1})	22.3±0.5	21.7-23.9	23.8±0.3	23.6-24.1	23.5±0.2	23.2-23.7	35.7±2.6	32.0-52.0
Conductivity (ppt)	31.2±0.4	30.7-32.6	34.1±0.1	34.0-34.2	33.0±0.3	32.2-33.5	55.6±4.2	52.0-83.0
Salinity (ppt)	0.02±0.01	0.02-0.02	0.02±0.01	0.02-0.02	0.02±0.01	0.02-0.02	0.02±0.01	0.02-0.03
pH	6.9±0.5	6.4-8.4	7.4±0.04	7.4-7.5	7.7±0.5	7.1-8.7	7.0±0.4	6.4-8.1
Turbidity (NTU)	7.2±2.1	6.5-8.7	8.4±1.9	7.8-10.1	9.2±2.0	7.0-11.5	1.9±1.7	0.3-4.3
Water clarity (%)	72.3±4.2	45.5-81.7	65.6±2.5	59.5-71.9	51.1±6.6	32.9-91.7	99.8±0.2	94.1-100.0

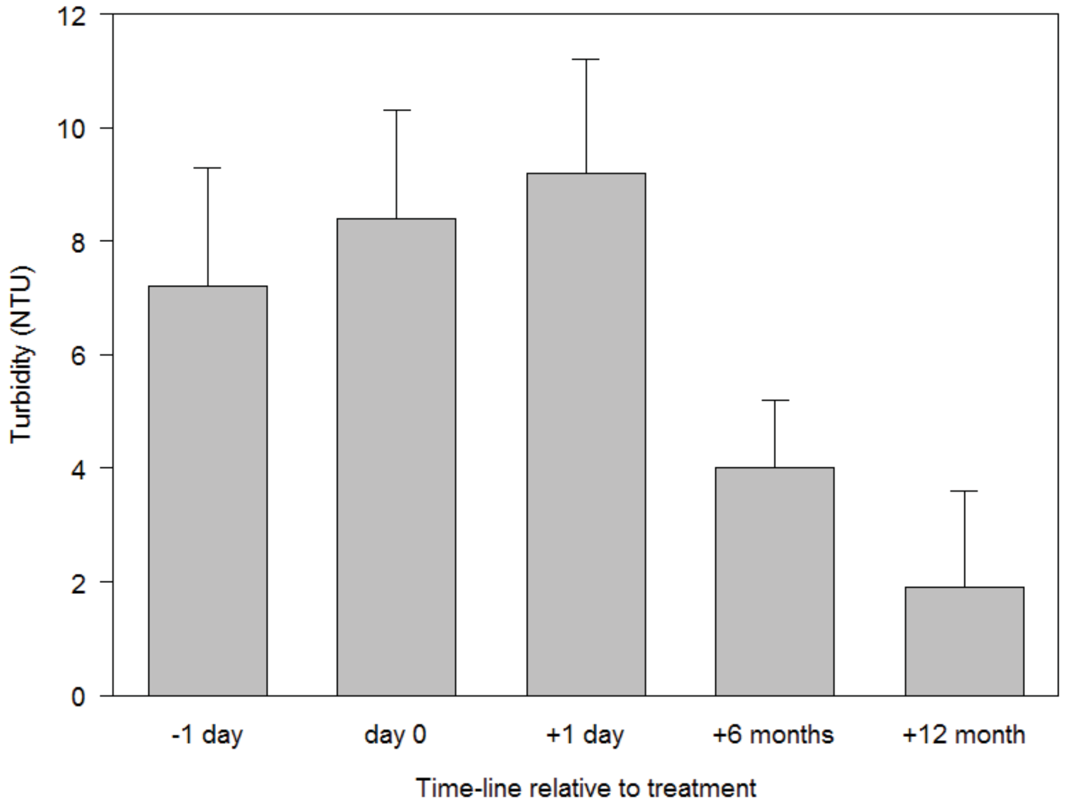


Figure 3.8: Turbidity levels recorded at the Krom River Chalet Dam

Fish community structure

During the Rotenone treatment 4305 bluegill were collected from the Chalet Dam, of which 2342 were measured (Figure 3.9). The fish collected comprised a biomass of 17.7 kg and an estimate of 23.4 kg/ha (reservoir size 7544 m^2). As numerous fish were observed entangled in the macrophyte beds, the total biomass presented here should be considered a minimum estimate. All gears detected the presence of bluegill prior to treatment and no bluegill were sampled after treatment in any gear in the treatment dam (Figure 3.10). Prior to treatment mean (± SE) CPUE was 15 ± 4 fish/net.night for fyke nets and 0.3±0.3 fish/net.night for gill nets. Although no bluegill were sampled post treatment, nine rainbow trout were sampled in gill nets set one year after the treatment, the resultant CPUE was 1.5±0.7 fish/net.night. This demonstrates that the treatment was successful in eradicating bluegill, but rainbow trout have colonised the dam from the river suggesting that the fish grids in the furrow may not be effective. Depletion sampling using gill nets removed 7 trout from the Chalet Dam in January 2018.

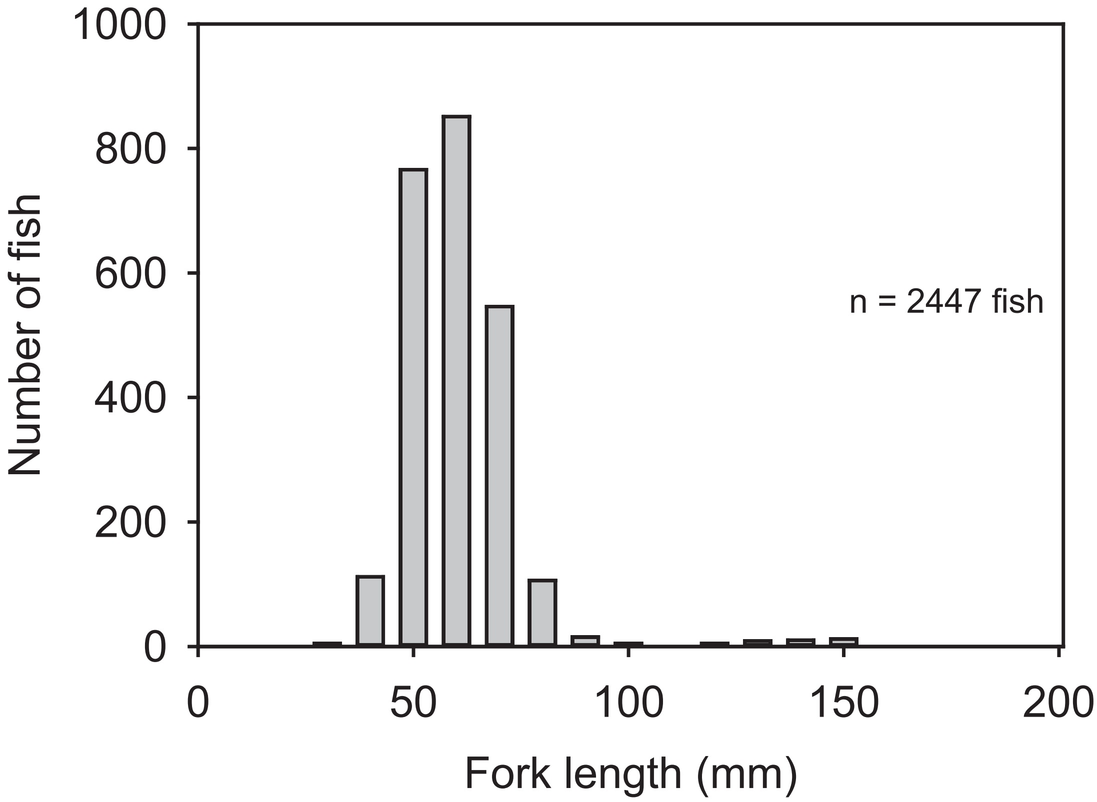


Figure 3.9: Length frequency of bluegill *Lepomis macrochirus* collected from Chalet Dam, Krom River, Olifants River system, South Africa

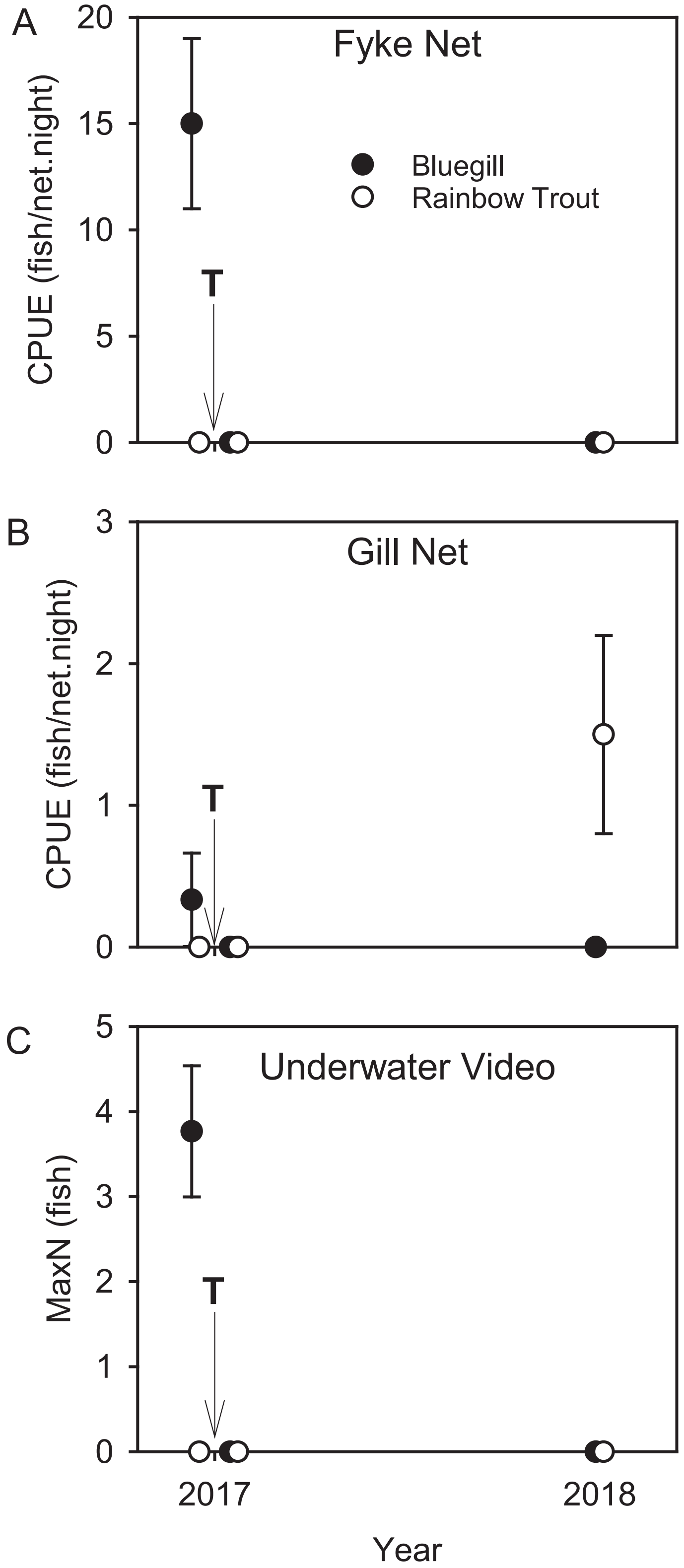


Figure 3.10: CPUE in fyke nets and gill nets and underwater video MaxN for bluegill *Lepomis macrochirus* and rainbow trout *Oncorhynchus mykiss* in Chalet Dam, Krom River, immediately before, immediately after and one year after Rotenone treatment.

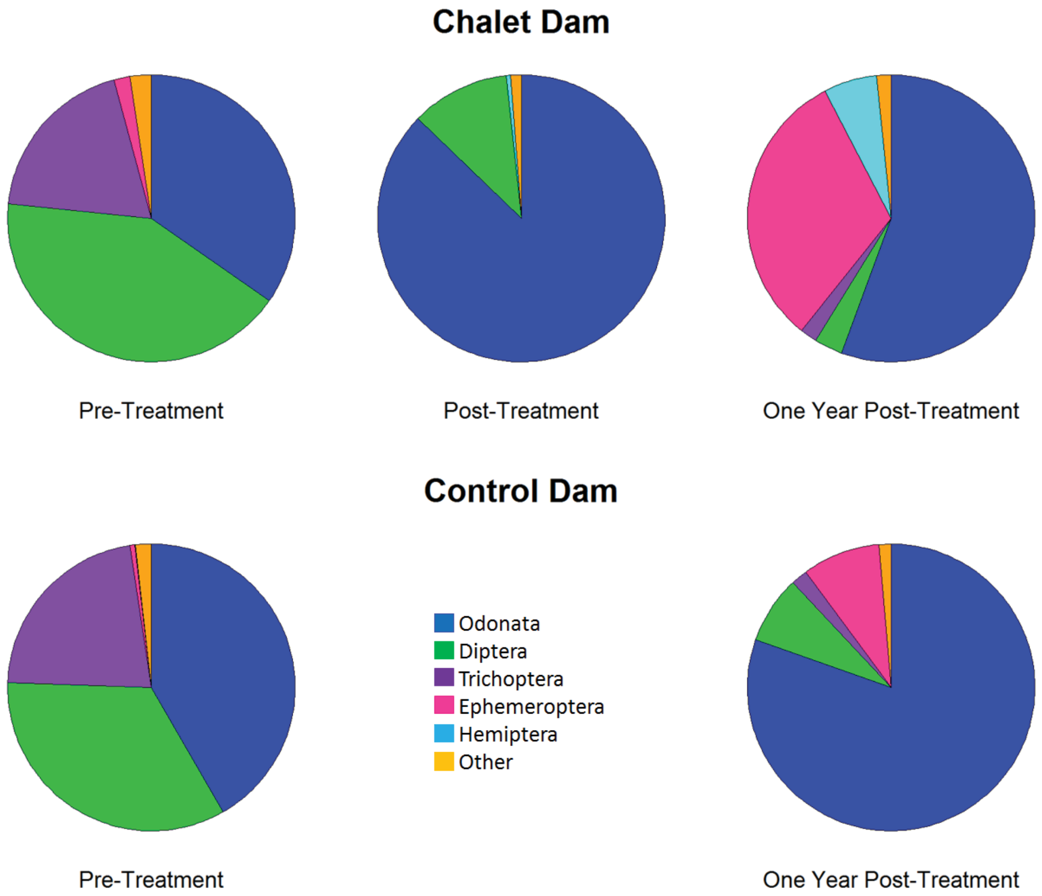
Macroinvertebrate community structure

A total of 20 samples were taken at both the treatment and control dams pre-treatment, immediately post-treatment (treatment dam only), and a year after the treatment. All samples were identified to family level, however, for the treatment dam, 20 pre- and post-treatment and 10 1-year post-treatment samples were identified to species level. In addition, 20 pre-treatment samples from the control dam were identified to species level. The chironomid larvae were not identified beyond family level due to time constraints and the magnitude of the number of chironomid larvae present in some of the samples, particularly those from the dams near Nieuwoudtville.

The species richness of the Chalet Dam (treatment) was 18 taxa prior to the treatment, in comparison to the 19 taxa recorded from the control dam. The species richness in the treatment dam dropped to 12 taxa immediately following the treatment. One year after the treatment, 19 taxa were recorded from the treatment dam. Five taxa survived the Rotenone treatment and were also recorded one year later (4 Odonata and the Chironomidae larvae), although the density of the chironomid larvae were severely reduced. Five of the species not recorded immediately after the treatment were recorded one year after the treatment including 3 Ephemeroptera, 1 Gomphidae and 1 Trichoptera (Hydroptilidae) taxa. Five taxa comprising 1 Ostracoda, 1 Cladocera, 2 Trichoptera and a Coleoptera (Dytiscidae) were not recorded after the treatment. However, 9 taxa not recorded pre-treatment were recorded one year after the treatment.

The Jaccard Index (Jaccard, 1908), based on species level presence-absence data, was used to determine the change in *Beta*-diversity of the invertebrate taxa over the Rotenone treatment and the year following the treatment. There was a 36.4% similarity between the macroinvertebrate assemblages pre- and post-treatment confirming the substantial changes in the assemblage. Even though some recovery is intimated by increase in species richness, there was only a 37% similarity between the macroinvertebrate assemblages pre- and one year post-treatment, showing a large turn over in taxa over the treatment and recovery. The assemblage one year post-treatment only shared a 24% similarity with the assemblage immediately post treatment.

Prior to Rotenone treatment, the treatment and control dams were dominated by Odonata (mostly Libellulidae and Coenagrionidae), Diptera (Chironomidae), and Trichoptera (mostly Hydroptilidae: *Oxyethira velocipes*); see Figure 3.11. Following the Rotenone treatment, dramatic declines were observed in the Diptera, Ephemeroptera and Trichoptera, confirming that these groups were sensitive to Rotenone application; see Vinson et al. (2010), Booth et al. (2015) and Dalu et al. (2015). However, a 30% decline was observed for the Odonata (from 26 to 18 individuals per m^2), suggesting a resilience to Rotenone for this non-air-breathing group.



Chalet Dam

Pre-Treatment

Post-Treatment

One Year Post-Treatment

Control Dam

Pre-Treatment

One Year Post-Treatment

Figure 3.11: Pie charts representing the change in composition of the order level invertebrate community in the treatment and control dams from the Krom Dam as a result of use of Rotenone to remove fish from the Chalet Dam; pre-treatment, post-treatment, one year post-treatment

The drop in species richness was mirrored in a drop in invertebrate density in the treatment dam (Figure 3.12). The pre-treatment average invertebrate density in the treatment dam was 86.6 ± 74.4 individual per m^2, almost half of the invertebrate density in the control dam (147.5 ± 93.5 individuals per m^2). Immediately after the treatment, the invertebrate density dropped to 31.7 ± 24.7 individuals per m^2, one third of the pre-treatment density, but had recovered to 124.5 ± 80.2 individuals per m^2 over the year following the treatment. The control dam invertebrate density, however, dropped to 32.7 ± 15.7 individuals per m^2 one year after the treatment, possibly as a result of the prolonged drought and the density of largemouth bass and bluegill in the dam.

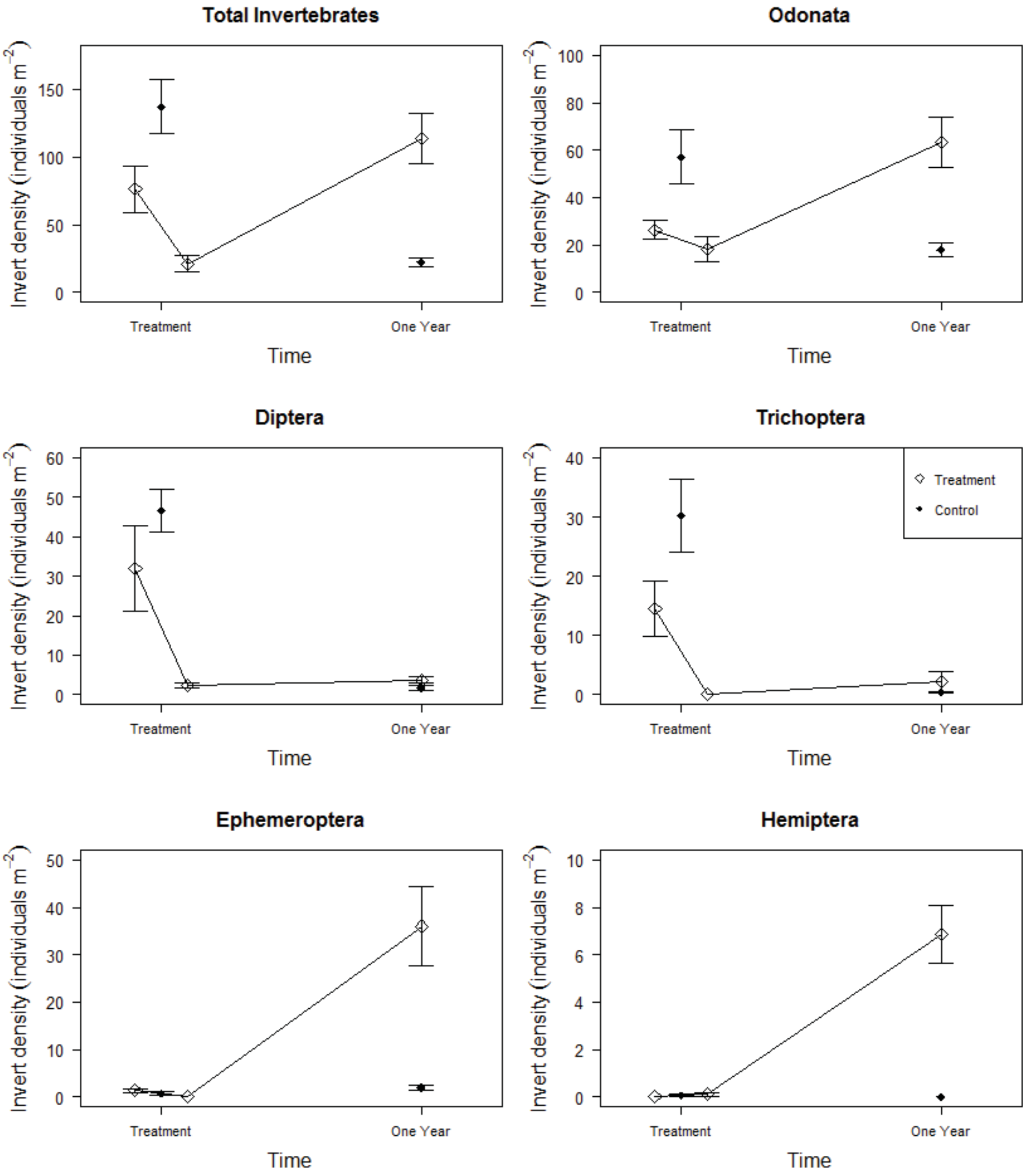


Figure 3.12: Mean density of invertebrate orders in terms of total and five most abundant orders sampled from the Krom River treatment dam. The open circle represents the treatment dam and the filled circle represents the control dam.

A year after the Rotenone treatment there was an increase in the mean density of Odonata from 18.5 ± 5.9 to 63.3 ± 10.6 individuals per m^2, and an increase in mean Ephemeroptera abundance from 1.4 ± 0.4 to 36.1 ± 8.3 per m^2, after being reduced to zero immediately after the Rotenone treatment. Similarly, the Trichoptera were also reduced to zero, from 14.6 ± 4.7 individuals per m^2, following the Rotenone treatment but rebounded to 2.2 ± 1 individuals per m^2 a year after the treatment, suggesting a recovery for this group with time, although only one of the three taxa present prior to the treatment were recorded one year post treatment. Interestingly, no Hemiptera were detected pre-treatment in either the treatment or control dams, however, after the fish were removed from the treatment dam, they were detected immediately post treatment at 0.1 ± 0.31 individuals per m^2, increasing to 6.9 ± 5.5 individuals per m^2 one year after Rotenone treatment. The Diptera, predominantly Chironomidae, however, did not recover to pre- treatment densities persisting at about 10% of their pre-treatment densities. (Figures 3.12).

The presence of abundant aquatic vegetation in the Krom dams provided habitat for many taxa. Aquatic invertebrates, like mayflies, use submerged vegetation as a refuge from predators, while the *Africallagma* damselflies (Odonata: Coenagrionidae) are camouflaged to hide in aquatic vegetation where they can ambush prey. Furthermore, aquatic vegetation in the form of macrophytes and filamentous algae form an important component in the diet of microcaddisflies (Trichoptera: Hydroptilidae) which feed on and imbibe the cellular contents of these plants (de Moor and Scott, 2003), accounting for the abundance of *Oxyethira velocipes* in the Krom dams. The Krom dams contained a wide diversity of commonly occurring dragonfly nymphs, at least five species from both Anisopteran and Zygopteran suborders. Water boatman (Corixidae), saucer bugs (Naucoridae) and backswimmers (Notonectidae) were only represented from the Krom treatment dam post Rotenone treatment.

The insect order Megaloptera, represented by a single species *Leptosialis africana* (Sialidae), was sampled from the Krom control dam pre-treatment but was not detected pre-treatment in the treatment dam. However, this insect was sampled one year post-treatment from the treatment dam. This suggests that it was either very rare pre-treatment, or that colonisation from the nearby control dam took place, through winged adult females ovipositing in the treatment dam. These insects are thought to be extremely rare (Price et al., 2012), and thus represent a very interesting entity within these dams.

A multivariate analysis of the Order level data was conducted using the PRIMER 6 and PERMANOVA+ software (Clarke and Warwick, 2001; Anderson et al., 2008). The Order level abundance data was first transformed, using the log(x+1) transformation, and a resemblance matrix constructed using the Bray-Curtis similarity. Non-metric multi-dimensional scaling (NMDS) ordination (Clarke and Warwick, 2001) was then used to visualise the data as 2-dimension ordination plots. 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 the macroinvertebrate assemblages pre-, post- and one year post-treatment 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 invertebrate orders contributing most to the differences between assemblages pre-, post- and one year post-treatment using the SIMPER routine in PRIMER 6 statistical software.

The NMDS ordination plot (Figure 3.13), the PERDISP-PERMANOVA results (Table 3.2) and the results of the SIMPER analysis together provide valuable insights regarding the changes in the macroinvertebrate assemblages at an order level. The change in community structure in the treatment dam over the Rotenone application resulted in a significant change in the position of the centroid of the macroinvertebrate assemblage as a result of the significant decrease in abundance of Odonata, Diptera and Annelida and the disappearance of Ephemeroptera and Trichoptera from the treatment dam. The average dissimilarity between the pre- and post-treatment assemblages was 50%. When the pre- and post-treatment assemblages of the treatment dam were compared to that one year post-treatment, the PERMDISP-PERMANOVA results (Table 3.2) and the NMDS plot clearly show that both the dispersion about the group centroid and the position of the group centroid have changed significantly as a result of the Rotenone treatment. The SIMPER analysis showed that, in the year post treatment, the differences between the macroinvertebrate assemblages in the treatment dam could be explained by the recolonization by Ephemeroptera and increases in abundances of Hemiptera, Odonata, Diptera and Coleoptera whereas comparing the pre-treatment to the year post-treatment assemblages, the differences could be explained by the colonisation by Hemiptera.

Table 3.2. Results of the Order level PERMDISP-PERMANOVA analysis of the macroinvertebrate assemblages of the Treatment and control dams at Krom River

	PERMDISP		PERMANOVA		
Pair-wise Comparisons	t	P(perm)	t	P(perm)	Change
Pre-treat, Post-treat	0.5305	0.6642	4.0717	0.0001	Centroid
Pre-treat, 1 Year Post-treat	2.7664	0.0158	5.6903	0.0001	Both
Post-treat, 1 Year Post-treat	2.8388	0.0213	6.4771	0.0001	Both
Pre-treat, Control	3.4742	0.0068	2.3133	0.0048	Both
Control, 1 Year Control	3.5081	0.0041	6.0476	0.0001	Both

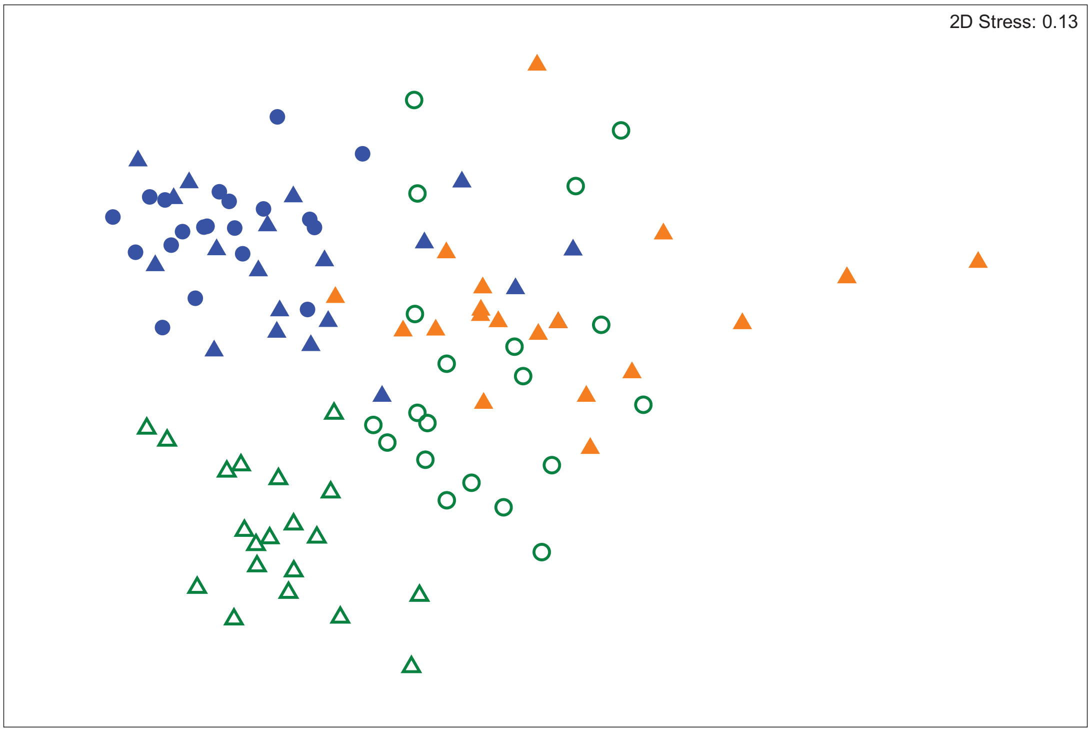
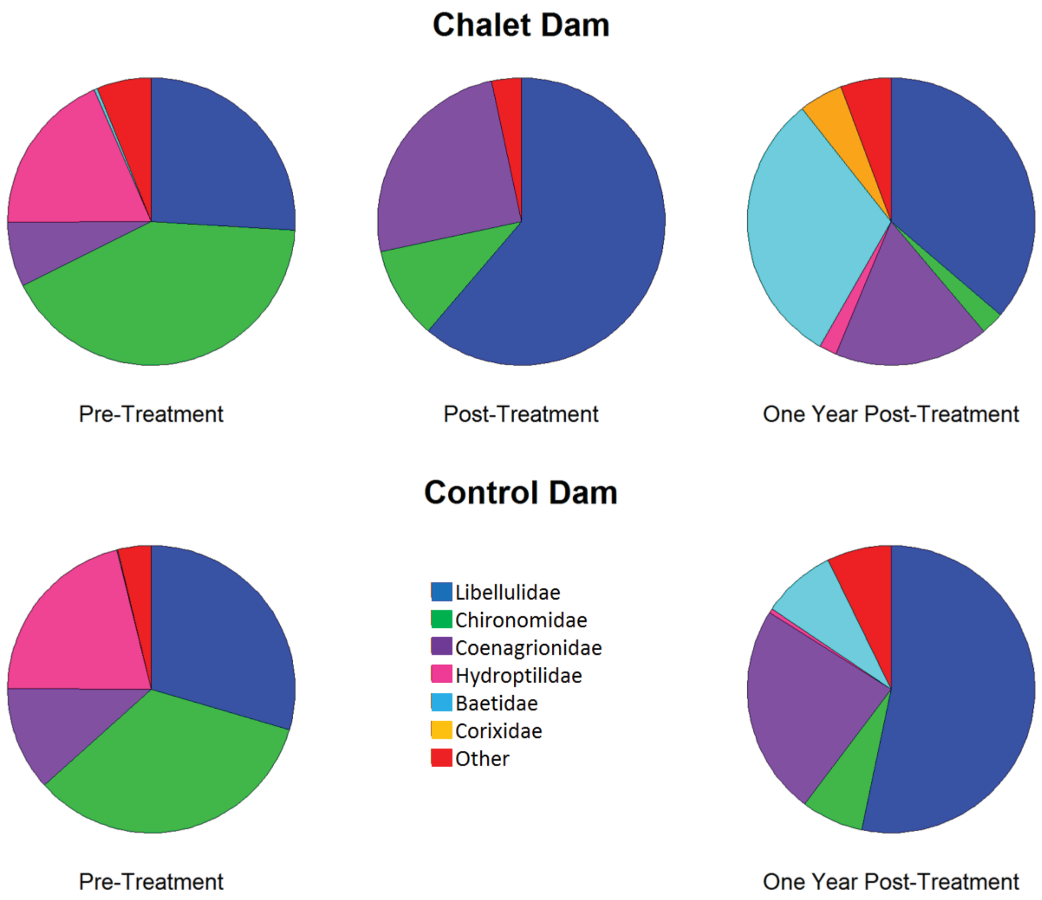


Figure 3.13: A Non-Metric Dimensions Scaling Ordination plot at the Order level for the samples from the Treatment and control dams at Krom River. The treatment dam is represented by triangles and the control dam by circles. The pre-treatment samples are represented by filled blue markers, the post-treatment by filled orange markers while the one-year post-treatment samples are represented by open green markers decreases in abundances of Diptera and Trichoptera, and increases in abundances of Ephemeroptera, Odonata and Coleoptera. The average dissimilarity in invertebrate assemblages between the pre-treatment and one year post-treatment assemblages was 51.4% whereas that between the pre-treatment and one year post-treatment assemblages was 59.8%.

The expectation that the treatment dam would recover to the pre-treatment assemblages is unfounded (Cowx and van Zyll de Jong, 2004) because the removal of bluegill has resulted in a significant change in the food web structure through the removal of an abundant apex predator from the system. The SIMPER analysis showed a 32% dissimilarity between the invertebrate assemblages of the pre-treatment and control dams. The dissimilarity originates from differences in abundances of Trichoptera, Diptera, Odonata, Annelida and Ephemeroptera, of which only Ephemeroptera were more abundant in the treatment dam. The dissimilarity between the control dam at the time of the treatment and one year later was 54.1%, again as a result of changes in abundances of Trichoptera, Diptera, Odonata, Annelida and Ephemeroptera, of which only Ephemeroptera were more abundant on year post treatment. The PERMDISP-PERMANOVA and NMDS plot clearly show that both the dispersion about the group centroid and the position of the group centroid were significantly different between the treatment and the control dam before the Rotenone treatment and that the assemblage in the control dam had changed significantly over the year following the Rotenone treatment. This could be as a result of the prolonged drought experienced over large portions of the Western Cape or warmer and drier conditions in the month preceding the 2018 field trip resulting in earlier emergence of some of the taxa.

Prior to Rotenone treatment, the treatment and control dams were dominated by Libellulidae, Chironomidae and Hydroptilidae; see Figure 3.14. Following the Rotenone treatment, dramatic declines were observed in the Chironomidae and Hydroptilidae, and less severe declines in the Odonata Libellulidae and Coenagrionidae, resulting in the treatment dam being dominated by these two Odonata post-treatment. In the year following the treatment, the Baetidae population rebounded to dominate the assemblage structure with the Libellulidae, and the hemipteran Corixidae colonising the dam. The chironomid population, however, did not recover to its former abundances. The formerly dominant Hydroptilidae were recorded one year post treatment but at only 15% of its former abundance.

In the control dam, changes in the assemblage composition were also noted including an increase in the Baetidae and severe reductions in the abundances of chironomids and Hydroptilidae, the latter becoming rare in the control dam.



Chalet Dam

Pre-Treatment

Post-Treatment

One Year Post-Treatment

Control Dam

Pre-Treatment

One Year Post-Treatment

Figure 3.14: Pie charts representing the change in composition of the Family level invertebrate community in the treatment and control dams from the Krom Dam as a result of use of Rotenone to remove fish from the Chalet Dam; pre-treatment, post-treatment, one year post-treatment

Three patterns can be identified in the macroinvertebrate abundances as a result of the Rotenone treatment of the Chalet Dam at Krom River (Figure 3.15). The Odonata Lubellulidae and Coenagrionidae showed a slight decrease in abundances over the treatment and then rebound to abundances similar to those of the control dam at the time of the treatment. The Chironomidae and Hydroptilidae were almost eliminated as a result of the Rotenone treatment and have failed to recover to their former abundance. And finally, the Baetidae and Corixidae were rare (Baetidae) or unrecorded (Corixidae) prior to the Rotenone treatment but had rebounded (or colonised) to become dominant in the assemblage one year post treatment. Interestingly, the six most abundant families all show a dramatic decline in abundances in the control dam, with the exception of Baetidae, indicating that environmental factors may have played a substantial part in the changes in invertebrate assemblages at Krom River.

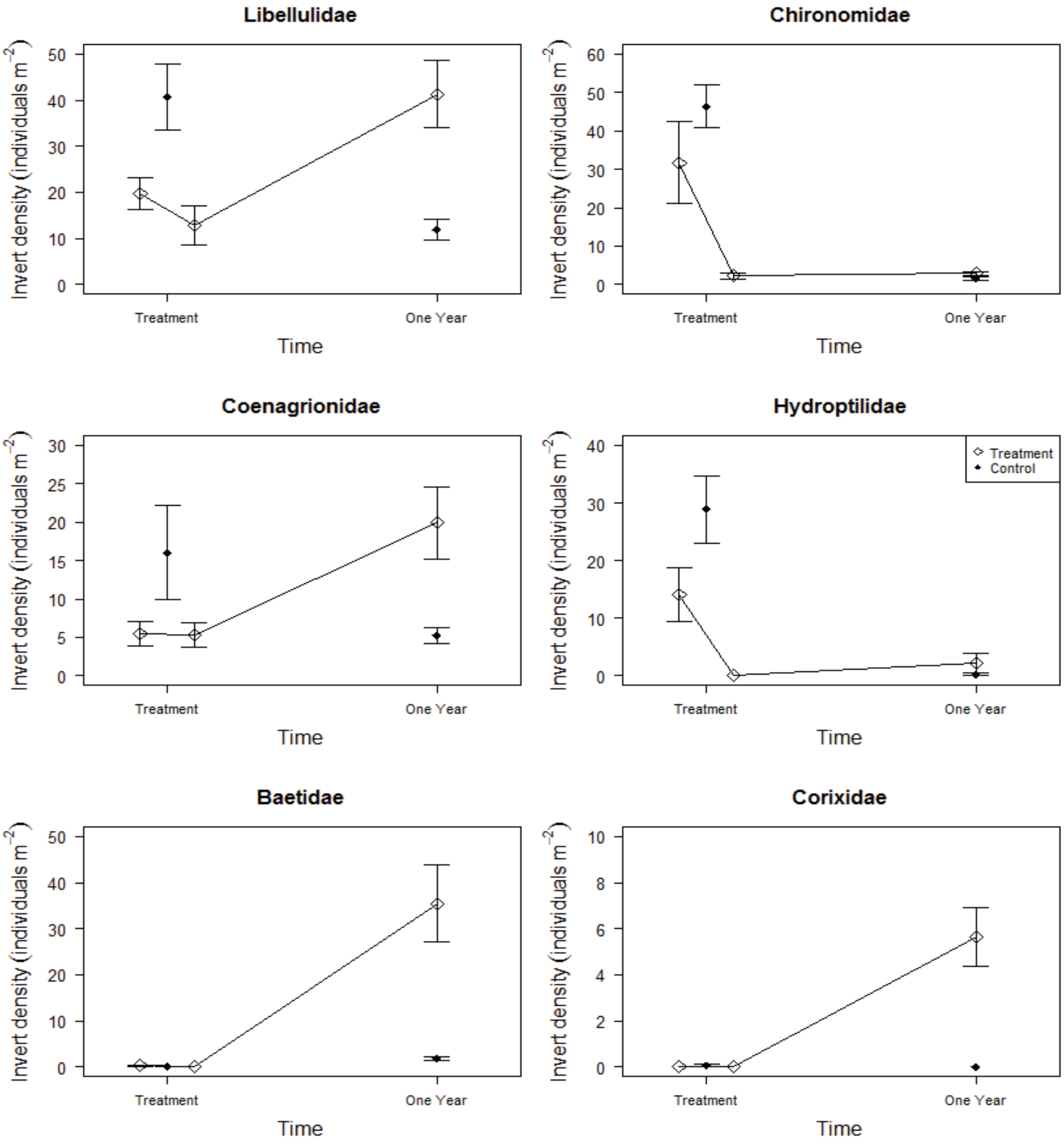


Figure 3.15: Mean density of the six most abundant invertebrate families sampled from the Krom River treatment and control dams. The open marker represents the treatment dam and the filled marker represents the control dam

The NMDS ordination plot (Figure 3.16) and the PERDISP-PERMANOVA results (Table 3.3) confirm the changes in the macroinvertebrate assemblages at a Family level. The change in community structure in the treatment dam over the Rotenone application resulted in a significant change in the position of the centroid of the macroinvertebrate assemblage as a result of the significant decrease in abundance of Chironomidae, Libellulidae, Coenagrionidae, Oligachaeta and Aeshnidae and the disappearance of Hydroptilidae, Caenidae and Gomphidae. The average dissimilarity between the pre- and post-treatment assemblages is 56.9%.

Table 3.3. Results of the Family level PERMDISP-PERMANOVA analysis of the macroinvertebrate assemblages of the treatment and control dams at Krom River

	PERMDISP		PERMANOVA		
Pair-wise Comparisons	t	P(perm)	t	P(perm)	Change
Pre-treat, Post-treat	0.1213	0.9177	3.3785	0.0001	Centroid
Pre-treat, 1 Yr Post-treat	2.8256	0.837	4.7784	0.0001	Centroid
Post-treat, 1 Yr Post-treat	1.7856	0.1213	4.8411	0.0001	Centroid
Pre-treat, Control	3.9141	0.022	2.0109	0.0067	Both
Control, 1 Yr Control	3.3368	0.0092	4.9605	0.0001	Both

When the pre- and post-treatment assemblages of the treatment dam are compared to those one year post-treatment, the PERMDISP-PERMANOVA (Table 3.3) and NMDS plot clearly show a significant change in the position of the group centroid as a result of the Rotenone treatment and subsequent recovery. The SIMPER analysis showed that, in the year post treatment, the differences between the macroinvertebrate assemblages could be explained by the recolonization by Baetidae, Caenidae and Corixidae, and increases in abundances of Libellulidae, Coenagrionidae, Chironomidae, Aeshnidae, Dytiscidae and Notonectidae, whereas comparing the pre-treatment to the year post-treatment, the differences in invertebrate assemblages could be explained by the colonisation by Corixidae and Notonectidae, decreases in abundances of Chironomidae, Hydroptilidae, Caenidae and Gomphidae, the loss of Oligachaeta and increases in abundances of Baetidae, Coenagrionidae, Libellulidae, Aeshnidae, and Dytiscidae. The average dissimilarity in invertebrate assemblages between the pre-treatment and one year post-treatment assemblages was 62.7%, whereas that between the pre-treatment and one year post-treatment assemblages was 59.4%, both highlighting the substantial turnover in invertebrate taxa as a result of the Rotenone treatment and the subsequent recovery.

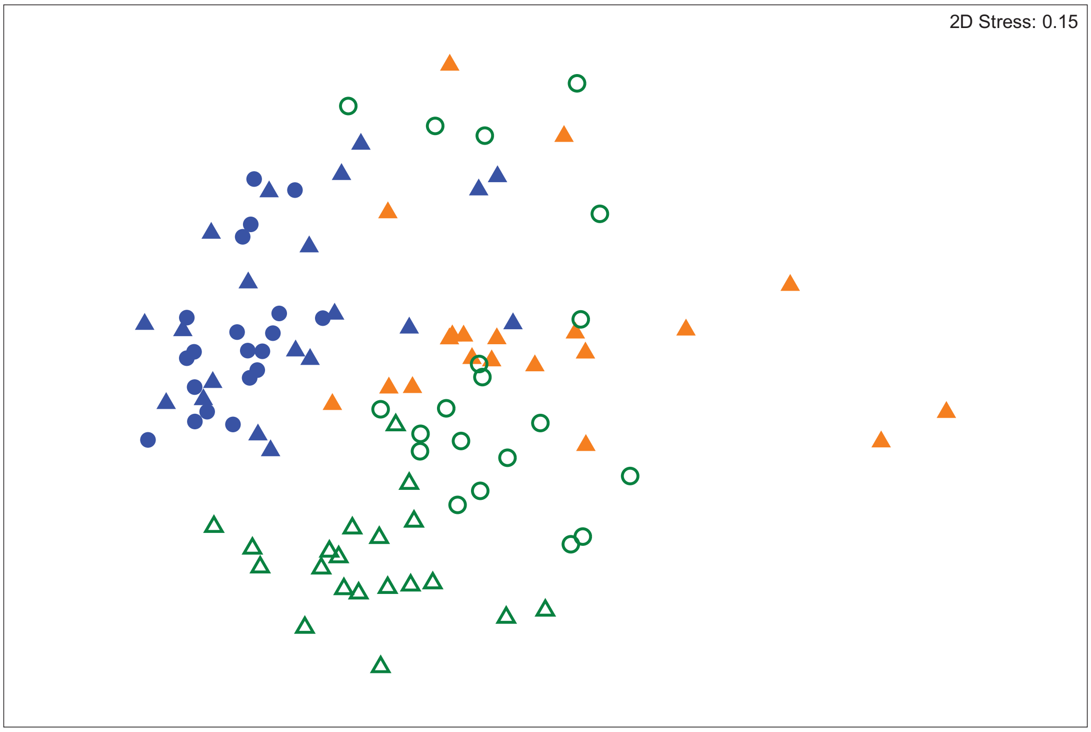


Figure 3.16: A Non-Metric Dimensions Scaling Ordination plot at the Family level for the samples from the treatment and control dams at Krom River. The treatment dam is represented by triangles and the control dam by circles. The pre-treatment samples are represented by filled blue markers, the post-treatment by filled orange markers while the one-year post-treatment samples are represented by open green markers

The SIMPER analysis showed a 40.2% dissimilarity between the invertebrate assemblages of the pre-treatment and control dams. The dissimilarity originates in differences in abundances of Hydroptilidae, Chironomidae, Coenagrionidae, Libellulidae, Caenidae, Huridinae, Oligachaeta, Leptocerridae, Gomphidae and Aeshnidae, of which Caenidae, Oligachaeta, Gomphidae and Aeshnidae were more abundant in the treatment dam. The dissimilarity between the control dam at the time of the treatment and one year later was 60.3%, as a result of changes in abundances of Chironomidae, Hydroptilidae, Libellulidae, Coenagrionidae, Baetidae, Hirudinae, Aeshnidae, Ecnomidae and Caenidae, of which only Baetidae, Aeshnidae and Gomphidae were more abundant on year post treatment. The PERMDISP-PERMANOVA and NMDS plot clearly show that both the dispersion about the group centroid and the position of the group centroid were significantly different between the treatment and the control dam before the Rotenone treatment and that the assemblage in the control dam had changed significantly over the year following the Rotenone treatment. This provides some support to the hypothesis that environmental factors had played a role in the changes in macroinvertebrate assemblages at in the year following the Rotenone treatment.

A Sample suspended in ethanol from the Kranskloof Dam near Nieuwoudtville after sorting from debris, prior to identification of the insect groups. Chironomidae and Ceratopogonidae are represented in the top left and bottom left of the petri dish respectively, while Hemiptera and Oligochaeta are represented at the top and middle right, respectively. Coleoptera are grouped in the middle.

Plankton community structure

The zooplankton taxa of the Chalet Dam, Krom River, before the Rotenone treatment consisted of 16 species belonging to three main groups: Rotifera (7 spp.), Cladocera (6 spp.), Copepoda (3 spp.); see Tables 3.4 and 3.5. Significant differences in abundances across pre- and post-Rotenone treatment were observed for Rotifera (PERMANOVA, Pseudo-F = 7.437, $p < 0.001$), Cladocera (PERMANOVA, Pseudo-F = 9.078, $p < 0.001$) and Copepoda (PERMANOVA, Pseudo-F = 6.039, $p < 0.001$). Using PERMANOVA pairwise comparisons, similarities were observed in abundances for Cladocera ($t = 2.965$, $p = 0.053$) and Copepoda ($t = 2.202$, $p = 0.062$) but a weak significant difference in abundance was observed for Rotifera ($t = 2.190$, $p = 0.043$). The results suggest that the zooplankton communities were almost similar pre- (1 day) and post-Rotenone (12 months) treatments.

Table 3.4. Micro-crustacean species (presence/absence) identified before and after Rotenone treatment from the Chalet Dam, Krom River catchment. Abbreviation: ++ indicate dominant species

Taxa	**Before**	**After**	**6 months**	**12 months**
CLADOCERA				
Alonella sp.	+		++	+
Bosmina longirostris	++		++	++
Ceriodaphnia sp.	+		++	
Diaphanosoma excisum	+		+	+
Eurycercus sp.	+			+
Kurzia sp.	+		+	+
Moina micrura			+	
COPEPODA				
Mesocyclops major	+		+	
Microcyclops sp.	+			+
Nauplii	++		++	++
Species Richness	**9**	**0**	**8**	**7**

Bosmina longirostris and copepod nauplii were the most dominant taxa prior to the treatment. A day after treatment all the zooplankton species had died out due to the toxic effects of Rotenone (Table 3.4, Figures 3.17 and 3.18A): Dalu et al. (2015) and Van Ginkel et al. (2015) observed similar findings. Six months after the treatment, most of the species had returned to the system, with species richness similar to that before treatment (13 spp.) with both Cladocera and Copepoda showing 100% recovery. Even though 100 % recovery was observed, different taxa were observed at 6 and 12 months post-treatment (Table 3.4). A year after the Rotenone treatment, 14 spp. were recorded (7 Rotifera, 5 Cladocera, 2 Copepoda), with only Rotifera *Asplancha priodonta* and *Filinia longiseta,* which were rare before treatment, not being recorded. New Rotifera taxa, *Asplanchna sieboldi*, *Keratella lenzi*, *Hexarthra mira* and *Trichocera navicular*, were recorded for the first time in the system (Table 3.5).

Table 3.5. Rotifer species (presence/absence) identified before and after Rotenone treatment from the Chalet Dam, Krom River catchment. Abbreviation: ++ indicate dominant species

Taxa	**Before**	**After**	**6 months**	**12 months**
Acroperus harpae			+	
Asplancha sieboldi				+
Asplancha priodonta	+			
Filinia longiseta	+			
Graptoleberis gr. *Testudinaria*			+	
Keratella tecta	+		++	
Keratella lenzi				+
Keratella cohlearis			++	
Hexarthra mira				+
Lecane clasterocerca	+			
Polyarthra sp.	+			
Polyarthra vulgaris			+	+
Trichocera navicular				+
Trichocera similis	+			++
Trichocera tropis	+			+
Species Richness	**7**	**0**	**5**	**7**

The densities of zooplankton within the Krom Dam before treatment were ~75 individuals per Litre (ind./L) before dropping to 0 ind./L after Rotenone application. The post-treatment densities increased to higher levels compared to the pre- treatment levels after six (~90 ind./L) and twelve (~82 ind./L) months (Figure 3.19A). No significant differences (ANOVA, $p > 0.05$) were observed for zooplankton densities pre- and six to 12 months post-treatment.

The chlorophyll-*a* concentration was generally low pre- and post-treatment, ranging between 0.5 and 0.9 mg/L, with the exception of one day post-treatment which recorded ~1.6 mg/L (Figure 3.19). The high values recorded one day post-treatment could be attributed to phytoplankton bursting after Rotenone treatment and releasing chlorophyll-*a* into the water column (Finlayson et al., 2014). Pre-treatment, the phytoplankton community was dominated by green algae such as *Cosmarium* spp., *Groenbladia undulata*, *Nitzschia linearis* var. *subtilis*, *Staurastrum chaetoceros* and *Peridinium* spp. before being dominated diatoms (e.g. *Nitzschia ambigua*, *N. amphibia* and *Cyclotella* spp.) and green algae *Coenococcus* spp. twelve months post-treatment; see Sanni and Wærvågen (1990).

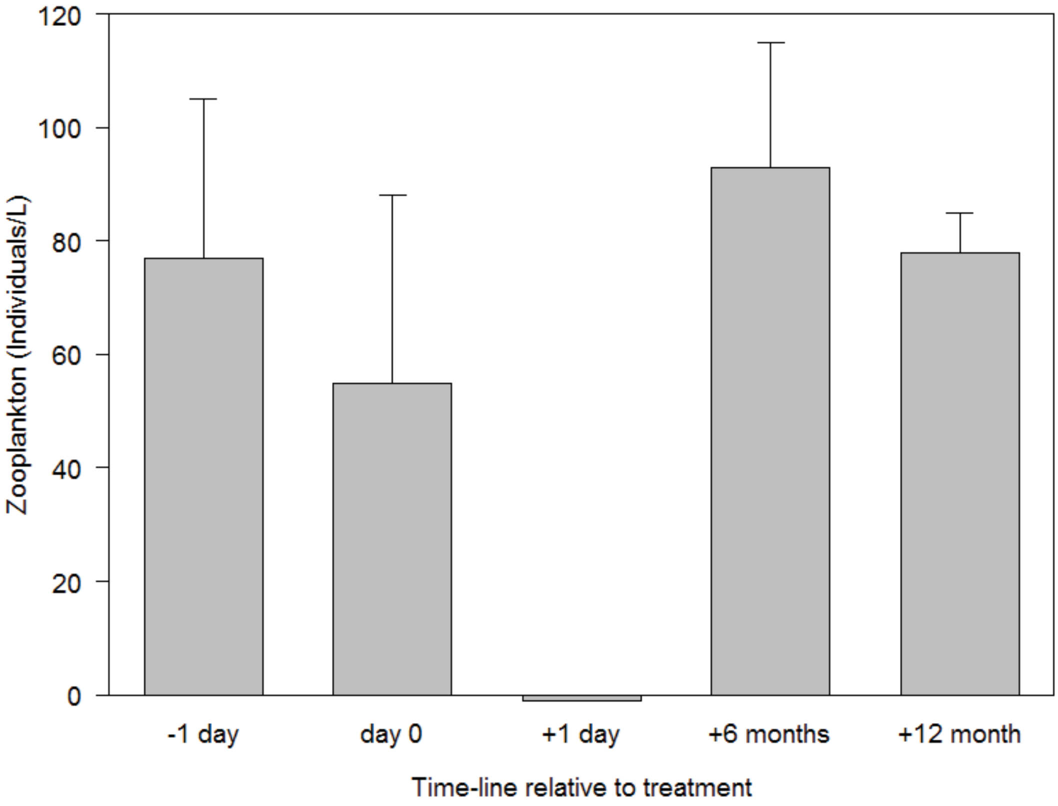


Figure 3.17. Zooplankton densities (individual per Litre (L)) recorded in the Krom River Chalet Dam

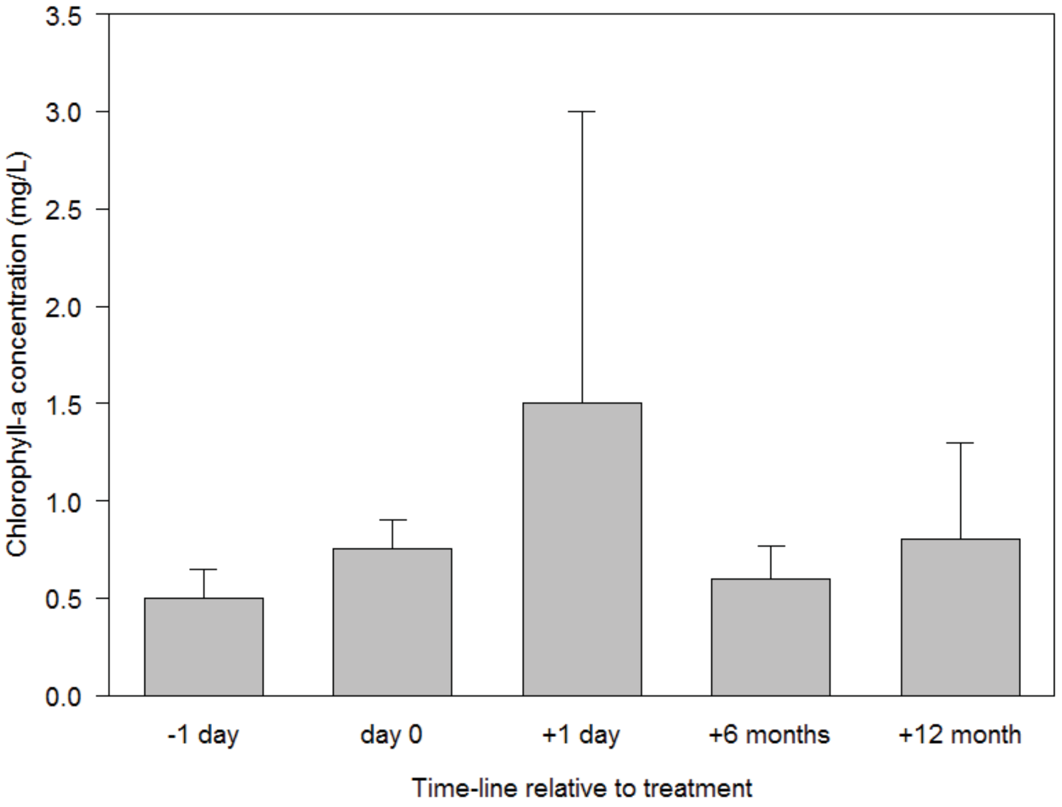


Figure 3.18: Chlorophyll-*a* concentration recorded before, on the day of Rotenone application and after treatment from the Krom River Chalet Dam

3.4.3 Dams near Nieuwoudtville

The Kranskloof Dam near Nieuwoudtville was treated on the 29th of March 2017. Pre-treatment samples were collected on the 27th and 28th of March 2017 for both the treatment and control dams. The treatment dam was sampled post treatment on 30th of March 2017 and both treatment and control dams sampled on the 14th of October 2017.

Environmental variables

Physico-chemical data variations are presented in Table 3.6 for the Kranskloof Dam. Similar to the Krom Dams, significant variation (ANOVA, $p < 0.05$) in physico-chemical variables were observed over six months before the dam dried, whereas similarities (ANOVA, $p > 0.05$) were observed across the study sites. Variations in physico-chemical variables pre- (1 day before) and post-treatment (1 day after) were similar (Tukey's post-hoc, $p > 0.05$) suggesting no statistically significant differences were observed for temperature, salinity, conductivity, TDS and pH.

The treatment dam, Kranskloof Dam, was eutrophic pre-Rotenone treatment, with a very low water clarity and high turbidity (Figures 3.19 and 3.20). The water clarity was ~50 % pre-Rotenone before increasing immediately after Rotenone application to ~80 % and 100 % after 6 months. Similarly, turbidity levels were the opposite of water clarity, very high pre-Rotenone treatment (~32 NTU) and dropped significantly after Rotenone application to ~20 NTU before falling further to ~15 NTU (Figures 3.19 and 3.20). The increase in water clarity, and the decrease in turbidity, were a result of phytoplankton cells dying and sinking to the bottom and the settling of the fine sediment suspended by the feeding behaviour of carp, which cause significant sediment resuspension.

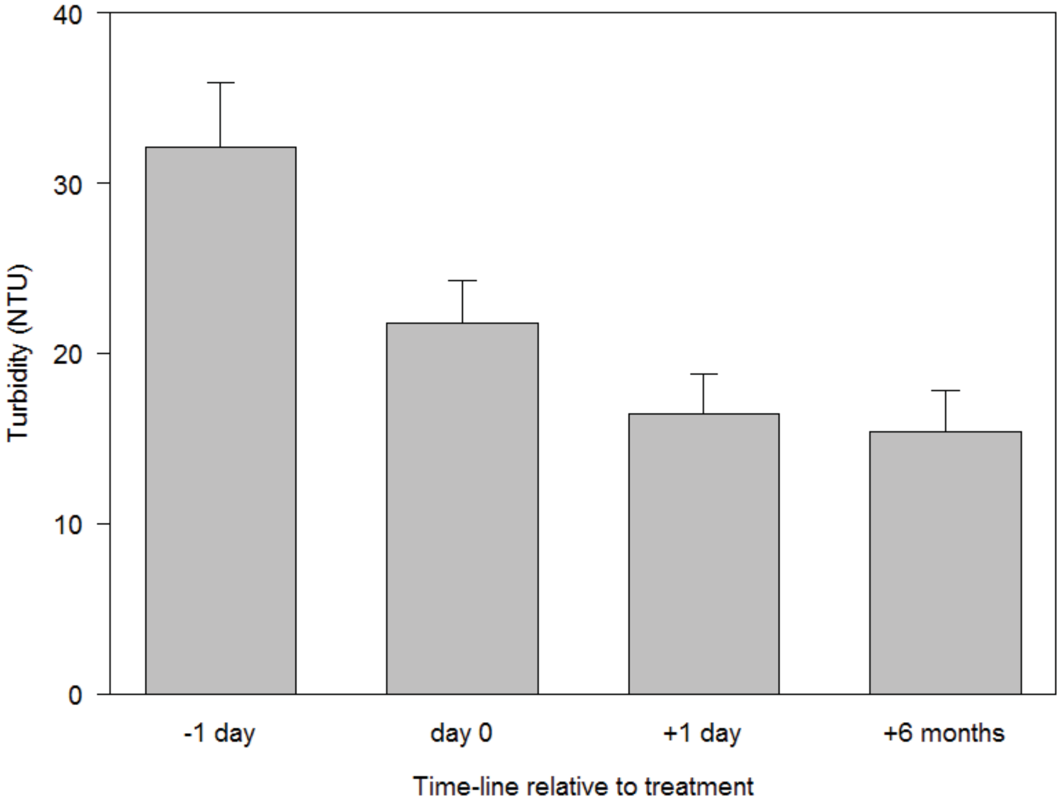


Figure 3.19: Turbidity levels recorded at the Kranskloof Dam near Nieuwoudtville

Table 3.6: Basic physico-chemical variables recorded in the Kranskloof treatment dam near Nieuwoudtville (Northern Cape) over a one year period. Abbreviations: TDS – total dissolved solids. Water clarity is relative to water depth

Parameter	**Pre-treatment**				**Post-treatment**			
	1 day		**0 day**		**1 day**		**6 month**	
	Mean±SD	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**
Kranskloof Dam								
Temperature (°C)	20.8±2.2	17.1-24.0	20.9±2.7	17.2-27.1	21.2±1.8	18.4-24.4	16.5±2.0	13.5-19.2
TDS (mg L^{-1})	792.8±7.1	743.0-802.0	782.9±109.9	768.1-810.0	805.2±5.5	778-810	805.5±3.2	795-811
Conductivity (ppt)	1118±10.4	1043-1139	1129.1±5.1	1109-1139	1136.6±4.1	1119-1144	1135.8±2.8	1127-1140
Salinity (ppt)	550.9±2.1	542-557	555.4±3.7	538-560	559.0±4.2	542-567	560.5±3.2	549-566
pH	8.7±0.5	7.8-9.6	8.8±0.7	7.7-9.8	8.4±0.6	7.2-9.7	9.8±0.2	9.4-10.1
Turbidity (NTU)	31.2±3.8	25.1-35.6	21.8±2.5	17.6-25.8	16.4±2.4	14.6-19.5	15.4±2.4	11.6-23.7
Water Clarity (%)	49.2±5.4	40.1-66.7	50.5±6.7	39.1-70.3	78.9±5.9	49.3-97.4	100.0±0.0	100.0-100.0

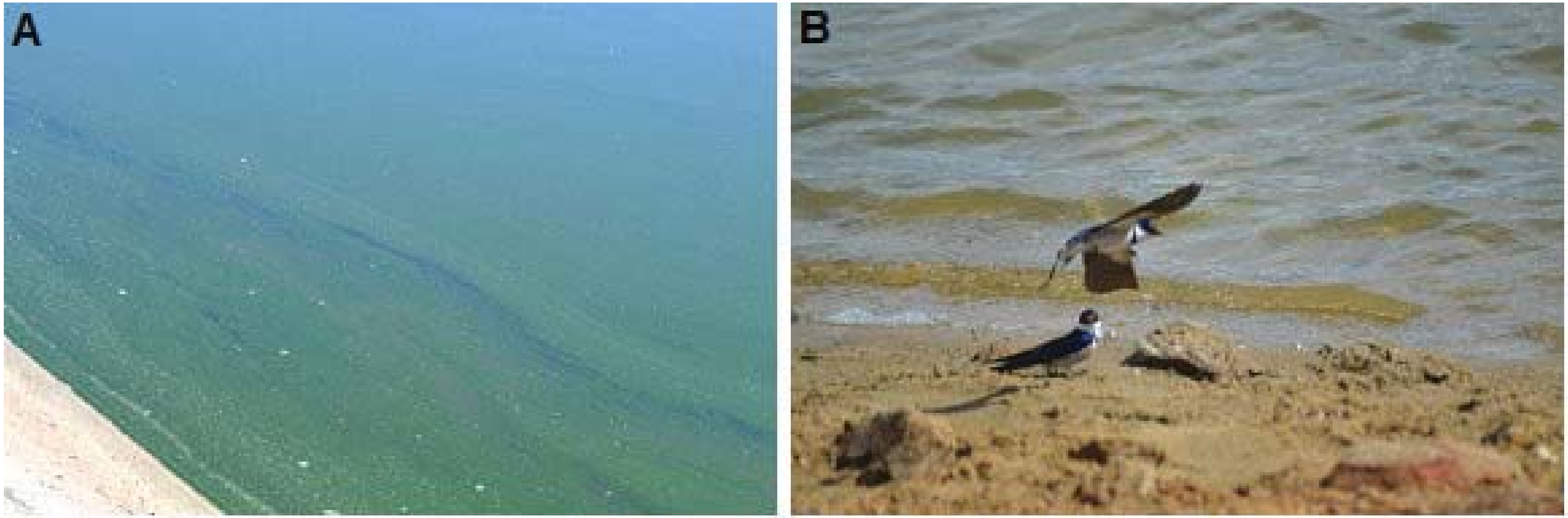


Figure 3.20: Algal blooms observed before application of Rotenone for the Kranskloof Dam (**A**) and after (5 hrs) Rotenone application (**B**). Note the change in water clarity and disappearance of algal blooms. Photos Tatenda Dalu

Fish community structure

During the Rotenone treatment 1020 Common carp were collected from the dam (Figure 3.21). The fish collected comprised a biomass of 291 kg and this made an estimate of 338.5 kg/ha (reservoir size 8598 m^2). All sampling gears detected the presence of common carp prior to treatment and no common carp were sampled after treatment in any gear (Figure 3.22) demonstrating that the treatment was successful. Prior to treatment mean (± SE) CPUE was 1.2 ± 1.0 fish/net.night for fyke nets, 8.5±4.5 fish/net.night for gill nets and 4.8±1.4 fish/haul for seine nets (Figure 3.22). No Common carp were sampled subsequent to the treatment.

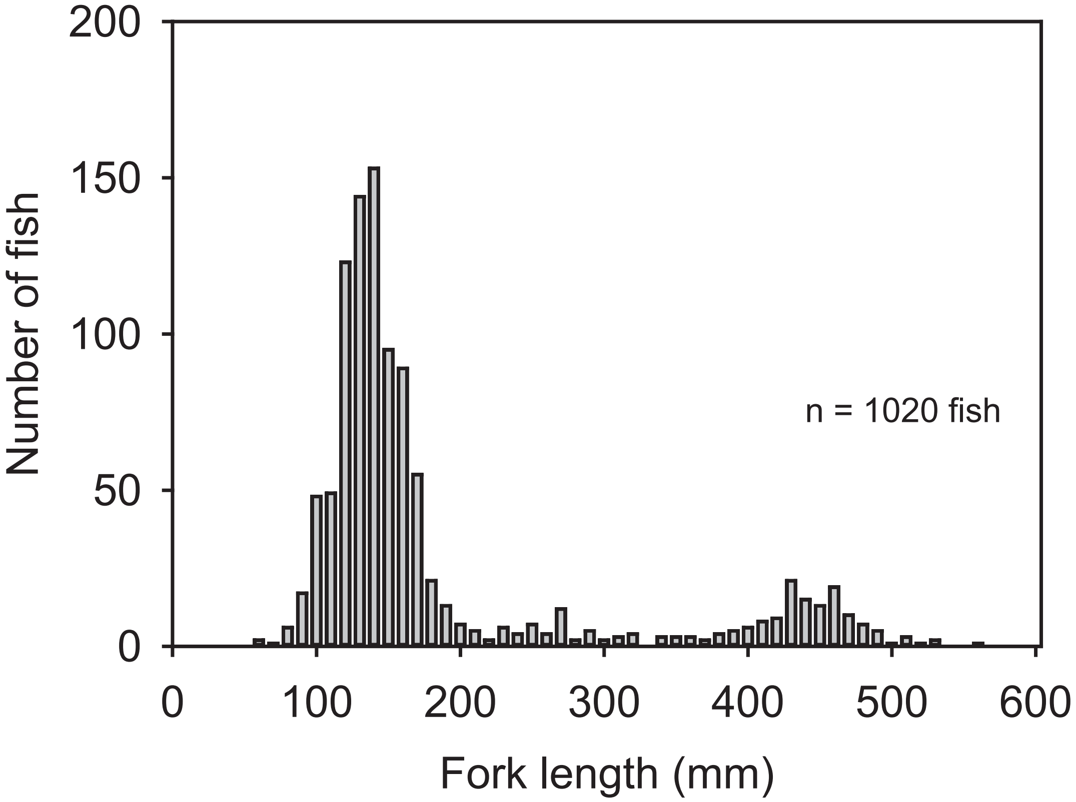


Figure 3.21: Length frequency of common carp *Cyprinus carpio* collected from the Kranskloof Dam, near Nieuwoudtville, Northern Cape, South Africa

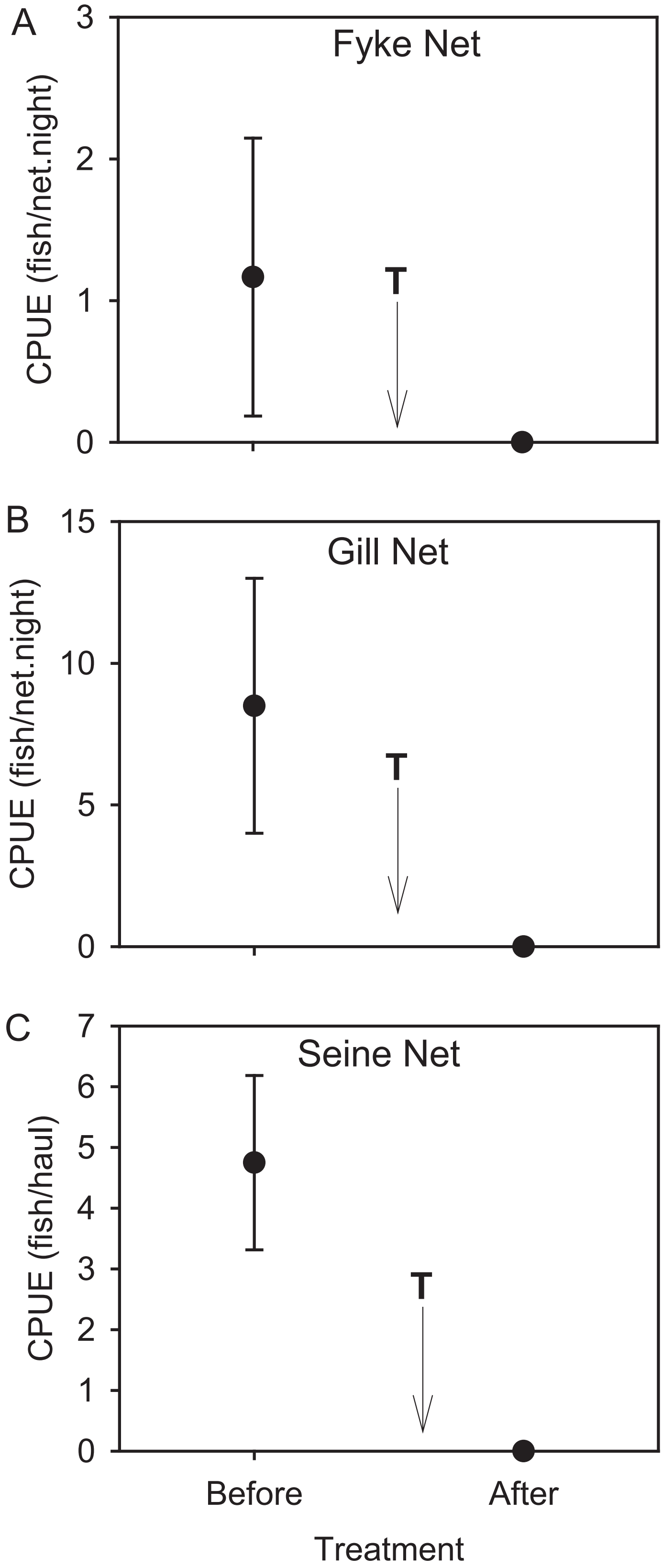


Figure 3.22: CPUE in fyke nets and gill nets and seine nets for common carp *Cyprinus carpio*, collected from the Kranskloof Dam, near Nieuwoudtville, Northern Cape, immediately before and immediately after Rotenone treatment.

Macroinvertebrate community structure

A total of 20 samples were taken at both the treatment and control dams pre-treatment, immediately post-treatment, and about 6 months after the treatment. All samples were identified to family level, however, for the treatment dam samples, 10 pre- and 6-months post-treatment and 14 post-treatment samples were identified to species level. In addition, 10 pre-treatment samples from the control dam were identified to species level. As with the Krom River dams, the chiromonid larvae were not identified beyond family level. The taxon richness of the Kranskloof Dam (treatment) was 19 taxa prior to the treatment, in comparison to the 9 taxa recorded from the control dam (Driefontein). The species richness of macroinvertebrates increased to 22 taxa in the treatment dam immediately following the treatment but decreased to 20 taxa six months after the treatment.

All taxa recorded prior to the Rotenone treatment survived the treatment but 5 of these taxa were not recorded six months later; namely Hirudinea, 2 Lubellulidae, Ecnomidae and the Corethrellidae. Three taxa not recorded prior to the treatment were recorded immediately post treatment, an Ostracod and 2 Baetidae, although only the Ostracod was recorded six months later. An additional five taxa not recorded pre-treatment were recorded one year after the treatment.

The Jaccard Index, based on species level presence-absence data, was used to determine the change in *Beta*-diversity of the invertebrate taxa over the Rotenone treatment and the year following the treatment. There was an 86.4% similarity between the macroinvertebrate assemblages pre- and post-treatment as a result of the three additional taxa recorded post treatment. Even though some recovery is intimated by the species richness, there was only a 56% similarity between the macroinvertebrate assemblages pre- and one year post-treatment, showing a substantial turn over in taxa over the treatment. The assemblage one year post-treatment only shared a 55.6% similarity with the assemblage immediately post treatment based on the taxa present at each time period.

Figures 3.23 illustrates the extent to which the Diptera dominated the macroinvertebrate community from the Kranskloof Dam, pre- and post-Rotenone treatment and that 6 months post-treatment the Annelida had increased in density. The control dam invertebrate community was completely dominated by Diptera at the time of the treatment. Hemiptera were present in both the treatment and control dams and appear to be unaffected by Rotenone, in keeping with our understanding that plastron respiring insects are not as adversely affected by the piscicide as are gill respiring invertebrates (Booth et al., 2015).

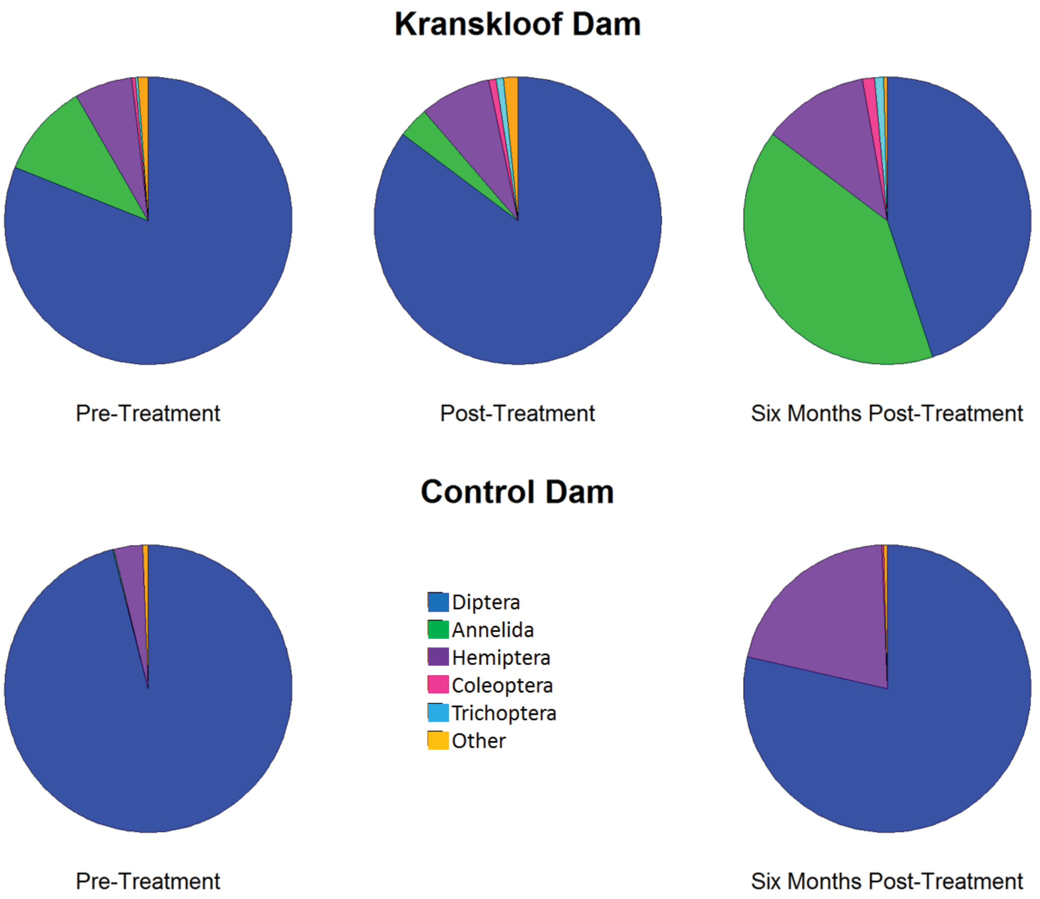


Figure 3.23: Pie charts representing the change in composition of the Order level invertebrate community in the treatment and control dams, near Nieuwoudtville, Northern Cape, as a result of use of Rotenone to remove fish from the Kranskloof Dam; pre-treatment, post-treatment, one year post-treatment

There was, however, a drop in invertebrate density in the treatment dam following the treatment. The pre-treatment average invertebrate density in the treatment dam was 523.4 ± 932.1 individuals per m^2, almost the same invertebrate density as the control dam (689.5 ± 956.3 individuals per m^2). Immediately after the treatment, the invertebrate density dropped to 272.9 ± 379.8 individuals per m^2, half of the pre-treatment density, but had recovered to 543.0 ± 712.56 individuals per m^2 six months after the treatment (Figure 3.24).

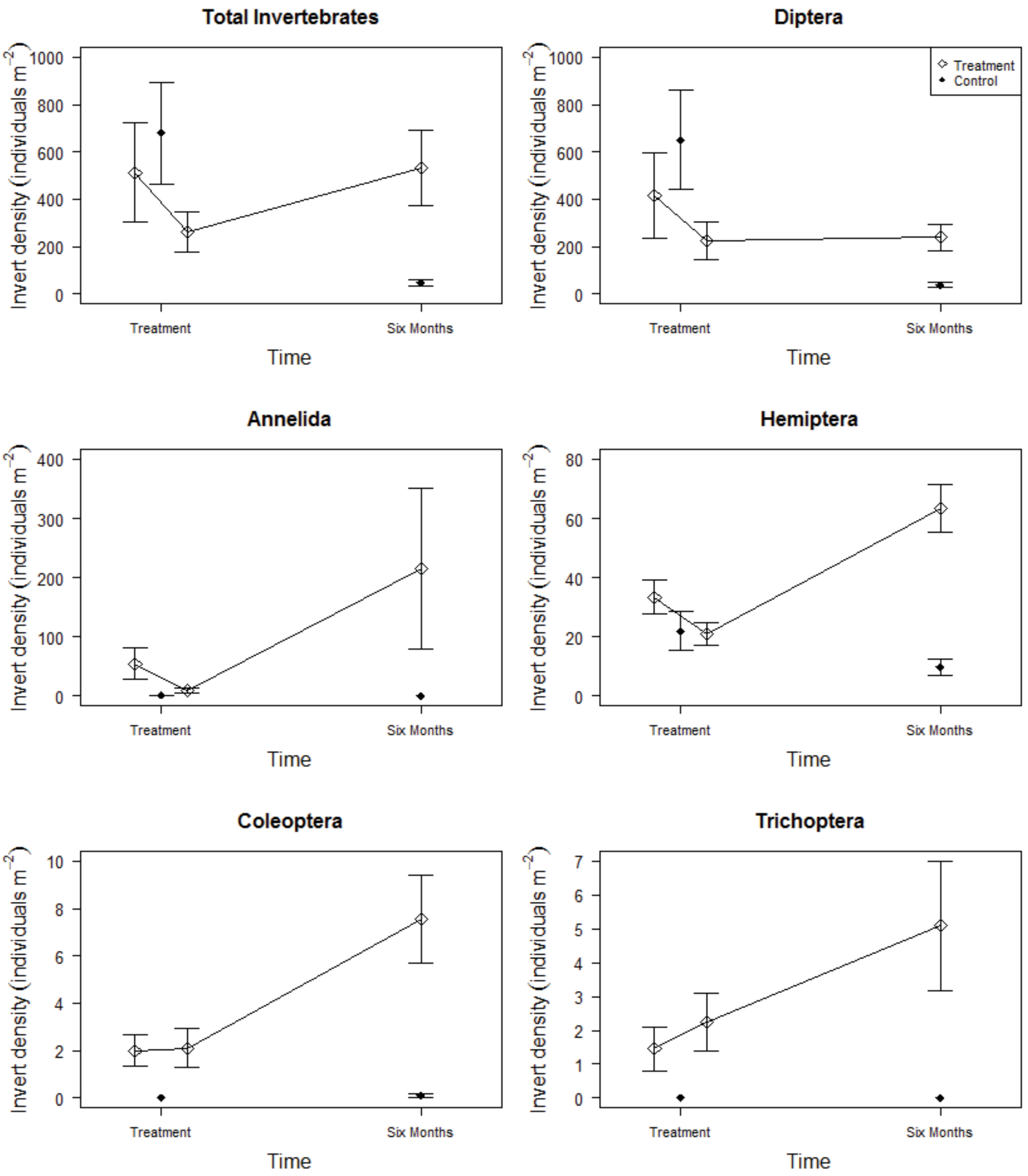


Figure 3.24: Mean density of invertebrate orders, total and five most abundant orders, sampled from the Kranskloof Dam (treatment) and Driefontein Dam (control), near Nieuwoudtville, Northern Cape. The open circle represents the treatment dam and the filled circle represents the control dam.

Kranskloof Dam and the control dam were void of any vegetation and highly turbid prior to the treatment, providing a possible explanation for the vastly different invertebrate faunas to the Krom Dams (Western Cape). The high turbidity and lack of vegetation of the Kranskloof Dam may excluded many of the predatory Odonata, with the exception of the Gomphidae, which are ambush predators. The density of macroinvertebrates were approximately an order of magnitude higher from the Northern Cape impoundments compared to the Western Cape impoundment; however similar trends are still visible over the sampling period. A decrease in overall density of macroinvertebrates was observed following Rotenone treatment, followed by an increase to above or approximately equal to pre-treatment densities. However, there is an extremely large variance between a few highly abundant groups and the remainder. Kranskloof Dam in particular was dominated by Annelid segmented worms (Oligochaeta) and larval Diptera. Some taxa, while recorded as present, were extremely rare including Nematodes and Hydrozoan taxa, which represented less than 5 individuals from both dams, out of a total of over 40 000 invertebrates catalogued.

The Kranskloof Dam also contained higher densities of aquatic Hemiptera, in particular the genera *Micronecta* and *Sigara*, both predatory water boatmen (Corixidae). The most abundant mayflies from the Krom River dams were both minnow mayflies from the genera *Cheleocloen* and *Cloen*, both stillwater forms common throughout the South African region (Barber-James and Lugo-Ortiz, 2003). In comparison, mayflies were less common from the Kranskloof Dam with the most common species being the cainfly *Caenis* sp (Caenidae: Ephemeroptera) known to be tolerant of brackish conditions. The Kranskloof Dam Odonata was dominated by members of the burrowing club-tail genus *Paragomphus* (Gomphidae: Odonata). This is indicative of the muddy substrate characteristic of this water body. Water boatman (Corixidae) were extremely abundant from the Kranskloof Dam, along with saucer bugs (Naucoridae) and backswimmers (Notonectidae). Caddisflies, Trichoptera, were uncommon. A single specimen of the Trichopteran family Ecnomidae was sampled pre- Rotenone treatment, while more than an order of magnitude more were sampled 6 months after treatment.

The NMDS ordination plot (Figure 3.25), the PERDISP-PERMANOVA results (Table 3.7) and the results of the SIMPER analysis show that the change in community structure in the treatment dam over the Rotenone application was not significant. The average dissimilarity between the pre- and post-treatment assemblages was 33.2%. When the pre- and post-treatment assemblages of the treatment dam are compared to that one year post-treatment, the PERMDISP-PERMANOVA (Table 3.7) and NMDS plot clearly show that both the dispersion about the group centroid and the position of the group centroid have changed significantly as a result of the Rotenone treatment for the comparison between pre-treatment and one year post treatment but only for the position of the centroid for the year following the treatment. The SIMPER analysis showed that for between pre- and post-treatment and the year post-treatment, the differences between the macroinvertebrate assemblages could be explained by increases in abundances of Annelida, Diptera, Coleoptera, Hemiptera and Trichoptera and decreases in abundance of Ephemeroptera and Odonata. The average dissimilarity in invertebrate assemblages between the pre-treatment and one year post-treatment assemblages was 35.7% whereas that between the pre-treatment and one year post-treatment assemblages was 36.4%.

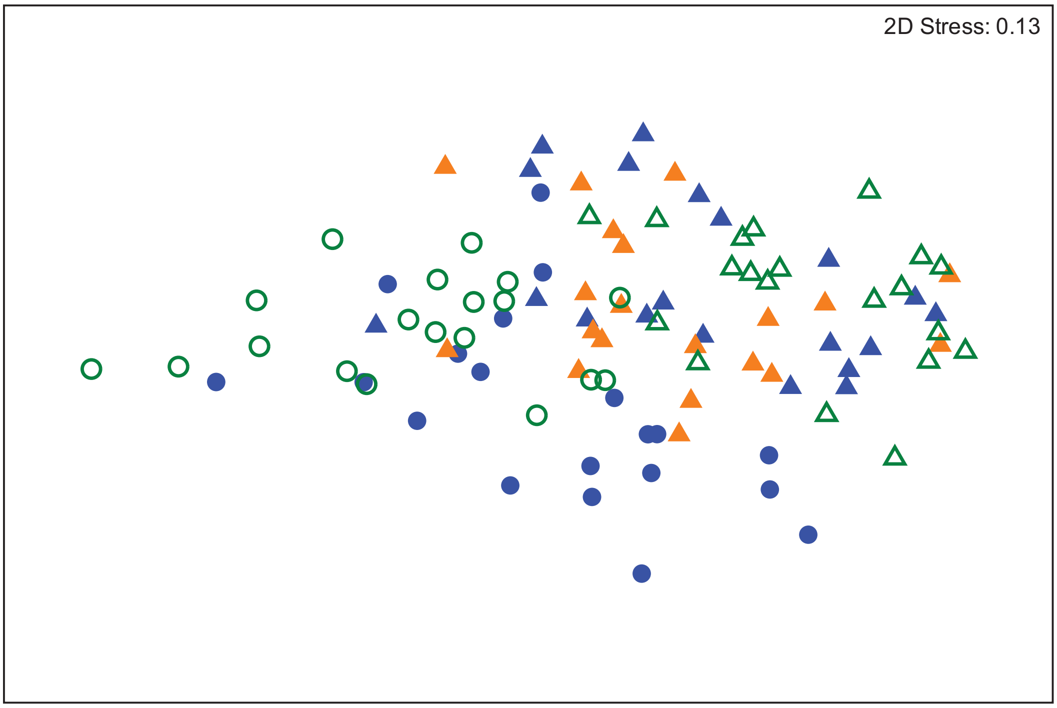


Figure 3.25: A Non-Metric Dimensions Scaling Ordination plot at the Order level for the samples from the Kranskloof and Driefontrein dams, near Nieuwoudtville, Northern Cape. The Kranskloof treatment dam is represented by triangles and the Driefontein control dam by circles. The pre-treatment samples are represented by filled blue markers, the post-treatment filled orange markers while the one-year post-treatment samples are represented by open green markers

The SIMPER analysis showed a 42% dissimilarity between the invertebrate assemblages of the pre-treatment and control dams. The dissimilarity originates in differences in abundances of Diptera, Hemiptera, Annelida, Ephemeroptera, Coleoptera, Odonata and Hydrozoa. Diptera were more abundant at the control dam whereas all the other taxa were more abundant at the treatment dam, with the exceptions of Hydrozoa which were absent from the treatment dam and Coleoptera which were absent from the control dam. The dissimilarity between the control dam at the time of the treatment and one year later was 36.5%, again as a result of changes in abundances of Diptera, Odonata, Annelida, Hemiptera, Coleoptera and Ephemeroptera, all of which were more abundant at the time of the treatment.

Table 3.7: Results of the Order level PERMDISP-PERMANOVA analysis of the macroinvertebrate assemblages of the Kranskloof and Driefontein dams, near Nieuwoudtville, Northern Cape

	PERMDISP		PERMANOVA		
Pair-wise Comparisons	t	P(perm)	t	P(perm)	Change
Pre-treat, Post-treat	0.707	0.5063	1.0229	0.3632	None
Pre-treat, 1 Year Post-treat	2.2484	0.0355	2.7401	0.0003	Both
Post-treat, 1 Year Post-treat	1.51	0.1439	3.2426	0.0001	Centroid
Pre-treat, Control	0.1812	0.869	3.089	0.0001	Centroid
Control, 1 Year Control	1.5625	0.1588	2.7252	0.0015	Centroid

A Sample from the control dam near Nieuwoudtville, partially sorted. Macroinvertebrates are removed as the sample is sorted from left to right, one fragment at a time.

Prior to Rotenone treatment, the treatment and control dams were dominated by Chironomidae; see Figure 3.26. Following the Rotenone treatment, dramatic declines were observed in the Chironomidae and Oligochaeta, but there was an increase in Notonectidae. In the six months following the treatment, the Chironomidae rebounded to dominate the assemblage structure with Corixidae the other substantial component of the invertebrate fauna.

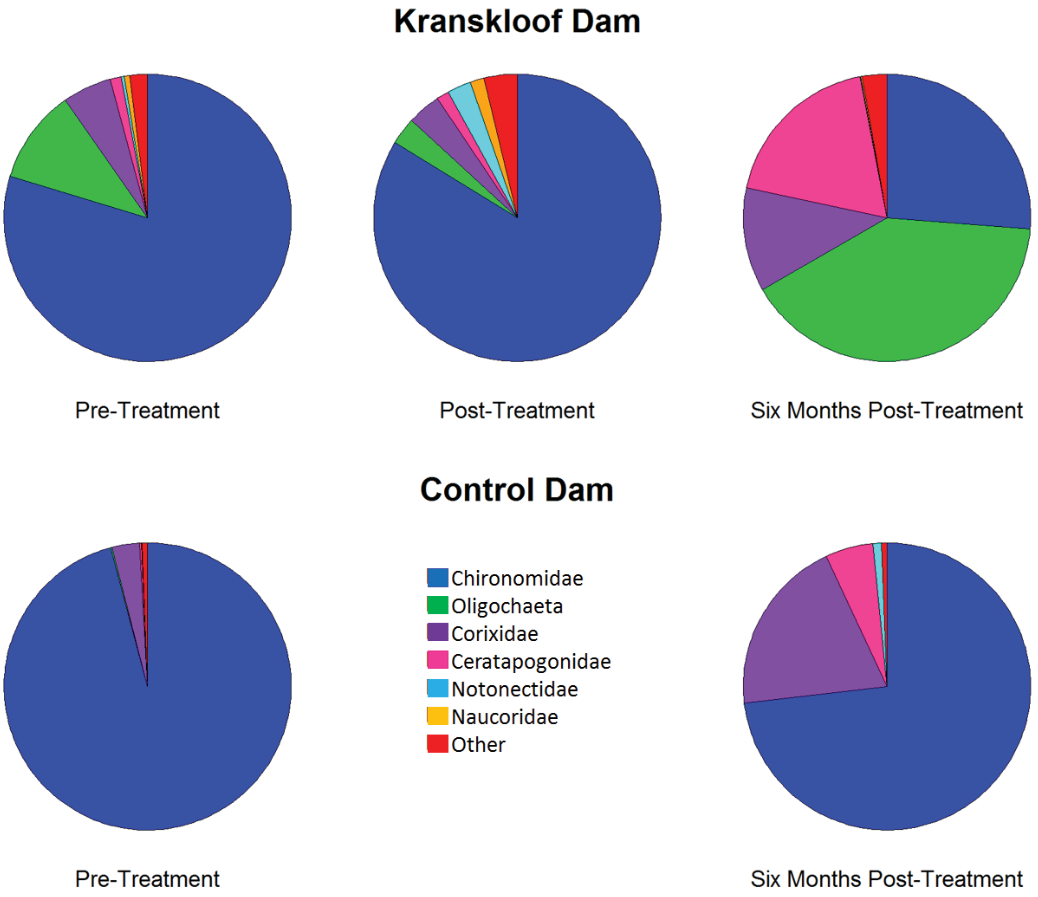


Figure 3.26: Pie charts representing the change in composition of the Family level invertebrate community in the treatment and control dams, near Nieuwoudtville, Northern Cape, as a result of use of Rotenone to remove fish from the Kranskloof Dam; pre-treatment, post-treatment, one year post-treatment.

Three patterns can be identified the macroinvertebrate abundances as a result of the Rotenone treatment of the Kranskloof Dam near Nieuwoudtville (Figure 3.27). Oligochaeta, Corixidae and Ceratopogonidae showed a slight decrease in abundances over the treatment but rebounded to abundances higher than those at the time of the treatment. The Chironomidae declined as a result of the Rotenone treatment and continued to decline in abundance over the following six months. And finally, the Notonectidae and Naucoridae increased over the Rotenone treatment but decreased in abundance over the subsequent six months. Interestingly, the six most abundant families all show a dramatic decline in abundances in the control dam, which is likely related to the severe drought and very low water levels at the time.

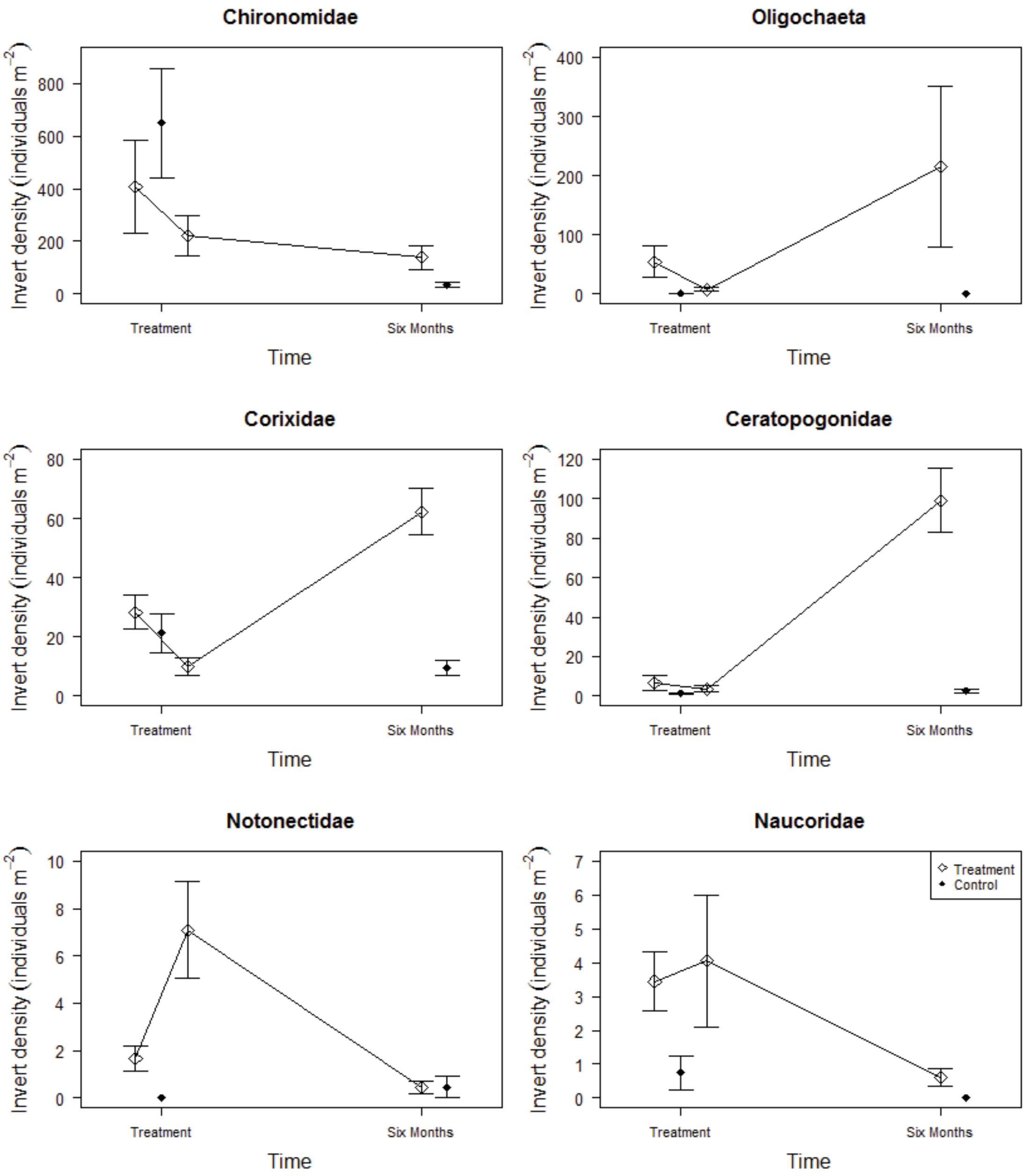


Figure 3.27: Mean density of the six most abundant per invertebrate families sampled from the Kranskloof Dam (treatment) and Driefontein Dam (control), near Nieuwoudtville, Northern Cape. The open marker represents the treatment dam and the filled marker represents the control dam.

The NMDS ordination plot (Figure 4.38), the PERDISP-PERMANOVA results (Table 3.8) confirm the changes in the macroinvertebrate assemblages at a Family level. The change in community structure in the treatment dam over the Rotenone application resulted in a significant change in the position of the centroid of the macroinvertebrate assemblage as a result of the significant decrease in abundance of Oligachaeta, Corixidae, Chironomidae, Naucoridae, Ceratopogonidae, Caenidae, Leptoceridae, Gomphidae, Hydrophilidae and Gyrinidae and an increase of Notonectidae, Dytiscidae and Ecnomidae. The average dissimilarity between the pre- and post-treatment assemblages is 44.8%.

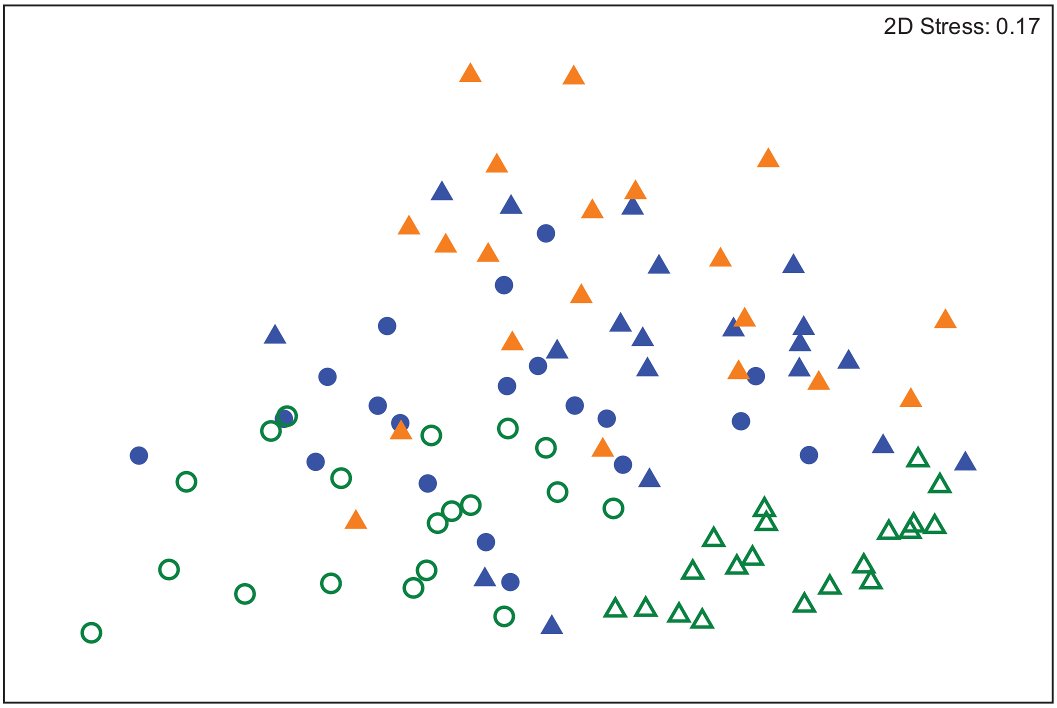


Figure 3.28: A Non-Metric Dimensions Scaling Ordination plot at the Family level for the samples from the Kranskloof treatment and Driefontein control dams near Nieuwoudtville, Northern Cape. The treatment dam is represented by triangles and the control dam by circles. The pre-treatment samples are represented by filled blue markers, the post-treatment filled orange markers while the one-year post-treatment samples are represented by open green markers

When the pre- and post-treatment assemblages of the treatment dam are compared to that six months post-treatment, the PERMDISP-PERMANOVA (Table 3.8) and NMDS plot clearly show significant changes in the dispersion about the group centroid and the position of the group centroid as a result of the Rotenone treatment and subsequent recovery. The SIMPER analysis showed that, in the six months post treatment, the differences between the macroinvertebrate assemblages pre- and post-treatment could be explained by increases in abundances of Ceratopogonidae, Oligochaeta, Corixidae, Dytiscidae, Leptoceridae, Dytiscidae and Hydrophilidae and decreases in the abundances of Notonectidae, Chironomidae, Naucoridae, Caenidae, and Ecnomidae The average dissimilarity in invertebrate assemblages between the pre-treatment and one year post-treatment assemblages was 47.9% whereas that between the pre-treatment and one year post-treatment assemblages was 53.5%.

Table 3.8: Results of the Family level PERMDISP-PERMANOVA analysis of the macroinvertebrate assemblages of the Treatment and control dams near Nieuwoudtville, Northern Cape

	PERMDISP		PERMANOVA		
Pair-wise Comparisons	t	P(perm)	t	P(perm)	Change
Pre-treat, Post-treat	0.1862	0.8613	1.5428	0.039	Centroid
Pre-treat, 1 Yr Post-treat	4.179	0.0003	3.6339	0.0001	Both
Post-treat, 1 Yr Post-treat	4.6297	0.0001	4.5235	0.0001	Both
Pre-treat, Control	1.2379	0.2415	3.093	0.0001	Centroid
Control, 1 Yr Control	1.0869	0.3258	2.6228	0.0004	Centroid

The SIMPER analysis showed a 49.5% dissimilarity between the invertebrate assemblages of the pre-treatment and control dams. The dissimilarity originates in differences in abundances of Chironomidae, Corixidae, Oligochaeta, Naucoridae, Ceratopogonidae, Notonectidae, Gomphidae, Hydrozoa, Leptoceridae, and Dytiscidae, of which only Chironomidae were more abundant in the control dam and Notonectidae, Leptoceridae, and Dytiscidae were absent from the control dam. The dissimilarity between the control dam at the time of the treatment and one year later was 41.5%, again as a result of decreases in abundances of Chironomidae, Corixidae, Ceratopogonidae, Gomphidae and Dytiscidae and the disappearance of Oligochaeta, Caenidae, Naucoridae and Leptoceridae. The PERMDISP-PERMANOVA and NMDS plot clearly show that both the dispersion about the group centroid and the position of the group centroid were significantly different between the treatment and the control dam before the Rotenone treatment and that the assemblage in the control dam had changed significantly over the year following the Rotenone treatment.

Plankton community structure

Pre-Rotenone treatment, the zooplankton community structure consisted of 10 Rotifera, 2 Cladocera and 3 Copepoda species (Tables 3.9 and 3.10). Significant differences in abundances across pre- and post-Rotenone treatment were observed for Rotifera (PERMANOVA, Pseudo-F = 3.268, $p = 0.044$), Cladocera (PERMANOVA, Pseudo-F = 17.766, $p = 0.004$) and Copepoda (PERMANOVA, Pseudo-F = 8.412, $p = 0.019$). Using PERMANOVA pairwise comparisons, similarities was observed in abundances for Copepoda ($t = 1.347$, $p = 0.211$) but significant differences in abundance were observed for Cladocera ($t = 4.348$, $p = 0.010$) and Rotifera ($t = 1.978$, $p = 0.035$). The results suggest that the zooplankton communities were almost similar pre- (1 day) and post-Rotenone (6 months) treatments for mostly the copepods, with some slight differences observed for Cladocera and Rotifera abundances.

Table 3.9: Macro-crustaceans species (presence/absence) identified before and after Rotenone treatment from the dams near Nieuwoudtville, Northern Cape. Abbreviation: ++ indicate dominant species

Taxa	Before	After	6 months
CLADOCERA			
Bosmina longirostris	++		++
Daphnia laevis			++
Diphanosoma excisum	++		++
COPEPODA			
Mesocyclops major	++		++
Metadiaptomus sp.			++
Microcyclops sp.	+		++
Nauplii	++		++
Species richness	**5**	**0**	**7**

Four Rotifera taxa (i.e. *Ascomorpha ecaudis*, *Keratella tecta*, *Polyarthra vulgaris*, *Pompholyx sulcata*), two Cladocera (i.e. *Bosmina longirostris*, *Diphanosoma excisum*) and Copepoda (*Mesocyclops major*, Nauplii) were the most dominant taxa pre-Rotenone treatment (Tables 3.9 and 3.10). After Rotenone treatment, five rotifer species (i.e. *Anuraeopsis fissa*, *Brachionus dimidiatus inermis*, *Keratella cohlearis*, *K. lenzi*, *Trichocera similis*) survived the treatment, but in very low abundances and densities (Table 3.10). The laboratory studies of Dalu et al. (2015) and van Ginkel et al. (2015) highlighted severe impacts on zooplankton communities due to Rotenone, which were corroborated by our field studies. After 6 months, most of the zooplankton taxa had rebounded with the exceptions of *Keratella lenzi*, *Keratella cohlearis*, *Ascomorpha ecaudis* and *Anuraeopsis fissa*, which were not detected after the Rotenone treatment. However, new taxa were recorded during the same period (i.e. *Filinia peljeri*, *Daphnia laevis*, *Metadiaptomus* sp.). Thus, six months post-Rotenone treatment 5 Rotifera, 3 Cladocera, and 4 Copepoda species were recorded from Kranskloof Dam (Tables 3.9 and 3.10).

Table 3.10: Rotifer species (presence/absence) identified before and after Rotenone treatment from the dams near Nieuwoudtville, Northern Cape. Abbreviation: ++ indicate dominant species

Taxa	Before	After	6 months
ROTIFERA			
Anuraeopsis fissa	+	+	
Ascomorpha ecaudis	++		
Brachionus dimidiatus inermis		+	
Brachionus plicatilis	+		+
Filinia peljeri			+
Keratella cohlearis	+	+	
Keratella lenzi	+	+	
Keratella tecta	++		++
Horaella thomassoni	+		++
Polyarthra vulgaris	++		
Pompholyx sulcate	++		
Trichocerca similis	+	+	+
Species richness	**10**	**5**	**5**

The zooplankton density was generally very high (~200 to ~350 individuals per L) pre-Rotenone treatment and significantly dropped to about 4 ind./L due to the survival of a few rotifer species. The densities increased 6 months post-treatment to pre-Rotenone treatment levels of ~195 ind./L (Figure 3.29). The high densities were a result of the Copepoda, Cladocera and Rotifera (i.e. *Horaella thomassoni*, *Keratella tecta*) (Table 3.5).

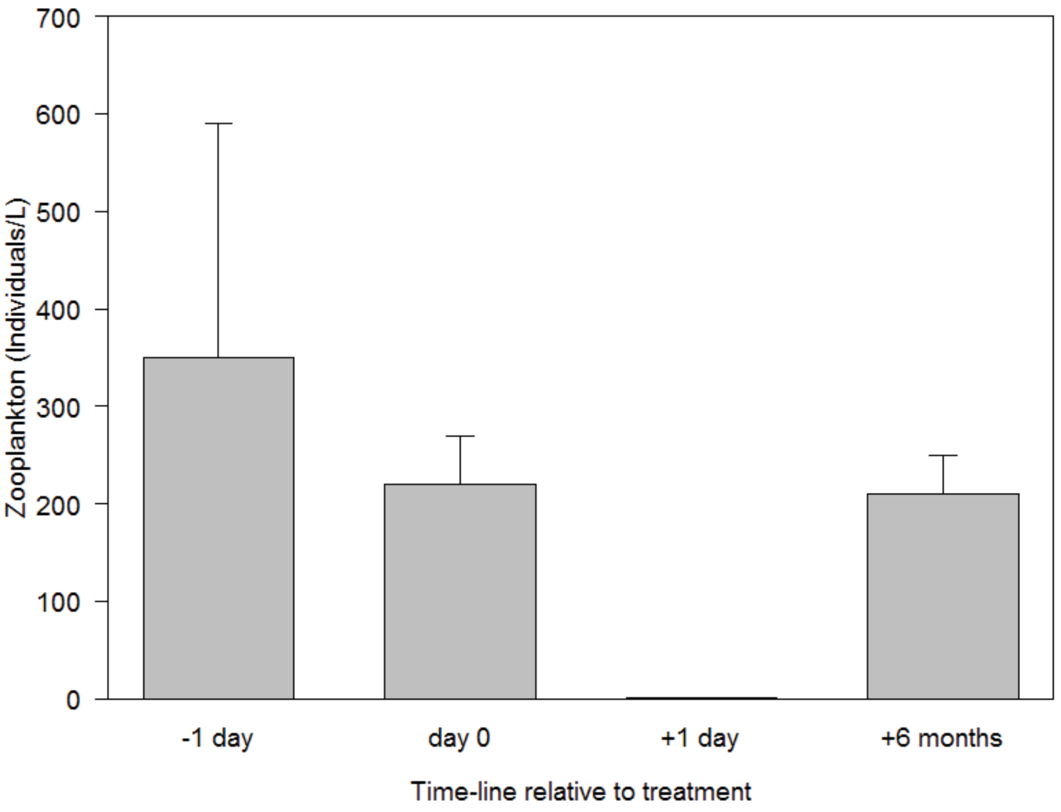


Figure 3.29. Zooplankton densities (individual per Litre (L)) recorded in the Kranskloof Dam near Nieuwoudtville before and after Rotenone treatment

The phytoplankton biomass was high (~7 to ~9 mg/L) pre-Rotenone treatment and it remained the same one day post-treatment (Figure 3.19B). The phytoplankton was dominated by blooms of blue-green algae (i.e. *Microcystis* sp., *Merismopedia glauca*) and green algae (i.e. *Scenedesmus communis*, *Scenedesmus ellipticus*, *Scenedesmus planctonicus*) pre-Rotenone treatment (Figure 3.20A). However, algal blooms were not observed six months post-treatment (Figure 3.20B) and the chlorophyll-*a* concentrations had significantly dropped to ~2 mg/L (Figure 3.30).

3.5 Discussion

The treatments of both farm dams were successful in that they successfully removed the target fish species (bluegill – Chalet Dam (Krom River), common carp – Kranskloof Dam). The Chalet Dam in the Krom River catchment was subsequently colonised by rainbow trout, which are present in the river that feeds the dam but bluegill have not been recorded one year post treatment.

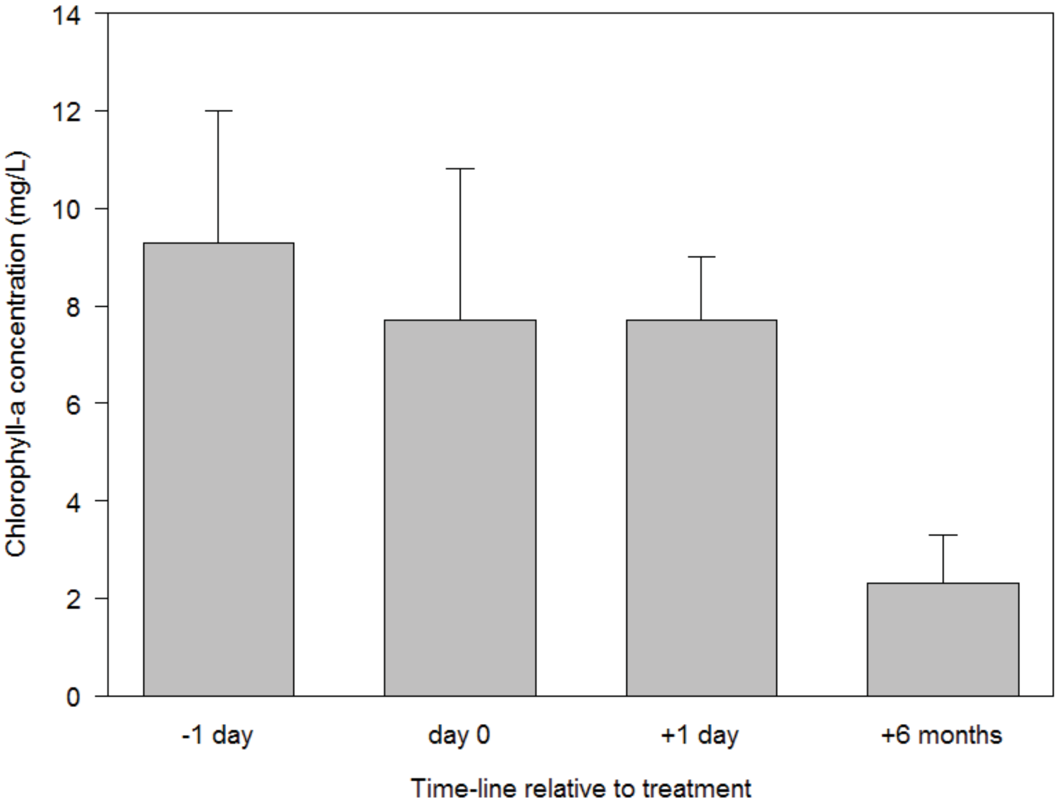


Figure 3.30: Chlorophyll-*a* concentration recorded before, on the day of Rotenone application and after treatment from the Kranskloof Dam near Nieuwoudtville, Northern Cape,

In both treatment dams, the water clarity improved following Rotenone treatment. The Kranskloof Dam displayed the greater decrease in turbidity with the water being particularly turbid prior to treatment at 32 NTU, clearing up to 20 NTU the day after the treatment and having 15 NTU six months post treatment. The impact of carp on water clarity was evident by their removal resulting in a dramatic increase in water clarity within a day of their removal. Carp are renowned for their negative impacts on water clarity in dams (Roberts and Tilzey, 1997). Carp feeding activities re-suspend sediments increasing the suspended solids load and releasing nutrients in the water column (Vilizzi, 2012; Vilizzi and Tarkan, 2015; Vilizzi et al., 2015). The algal bloom in the Kranskloof dam prior to treatment disappeared as soon as the treatment had been completed and the carp began to perish.

As expected, the zooplankton communities at both treatment dams were severely impacted by the treatment with dramatic decreases in density and loss of taxa. However, the zooplankton communities showed considerable recovery 6 and 12 months post treatment at both treatment dams. This is not surprising as off-stream dams are artificial water bodies which can be colonized by invertebrates in a variety of ways. There was a turnover of species with portions of the communities being replaced by other taxa. At the Krom River treatment dam, there was a turnover of zooplankton communities between 6 and 12 months post treatment indicating that changes in the zooplankton community may be a natural process driven by seasonal factors not addressed in the study.

The data presented here demonstrate that Rotenone treatment had a significant immediate impact on the zooplankton communities but these quickly recovered but with a turnover of species composition. The removal of the fish and the change in nutrient status of the dams as a result of the removal of the fish must play a substantial role in determining the composition of the zooplankton communities and comparisons to communities prior to treatment should be explored with this in mind.

Figure 3.31: Dead common carp *Cyprinus carpio* along the edge of Kranskloof Dam near Nieuwoudtville, Northern Cape, following the Rotenone treatment. Photo Tatenda Dalu

The phytoplankton community of the Kranskloof Dam was dominated by blooms of blue-green and green algae prior to the treatment. The blue-green algae were not present six months after the treatment indicating a change in the nutrient status of the dam to lower nutrient concentrations in the water column. At Krom River, the algal community changed from a green algae dominated community to a green algae and diatom community, again indicating a change in the nutrient status of the dam to lower nutrient concentrations.

The macroinvertebrate communities of the Krom River and Nieuwoudville dams were very different largely because of the differences between the two dams as habitats. The Krom River dams had substantial aquatic vegetation whereas the Nieuwoudtville dams had no aquatic vegetation and a thick layer of silt as substrate. The Rotenone treatment resulted in a two thirds reduction in the density of aquatic invertebrates at both treatment sites. The invertebrate densities had recovered to their pre-treatment densities six to 12 months following the treatment. The invertebrate communities of the Nieuwoudtville dams were dominated by Diptera while the Krom River invertebrate communities contained Odonata, Diptera and Trichoptera prior to treatment.

At Krom River, only Odonata and Diptera were recorded immediately post treatment with Trichoptera and Ephemeroptera being eliminated. Twelve months post treatment, the Odonata dominated the Krom River invertebrate community but the Diptera, although still present, had not reached their pre-treatment levels. The Ephemeroptera, and to lesser extent Trichoptera, had returned and Hemiptera, not present in the pre-treatment samples, colonised the dam since the removal of bluegill. These changes in the Krom River invertebrate community were likely strongly influenced by the removal of the fish from the dam. Bluegill are predators on aquatic invertebrates and control invertebrate community structure through predation on Ephemeroptera and Odonata while the Diptera were spared strong invertebrate predation pressure. Removal of the fish resulted in the Odonata becoming the top predators and the prey palate changed with greater predation pressure on the Diptera and Trichoptera. Data from the control dam one year post treatment indicates that environmental factors could have contributed to the change in the invertebrate assemblages in the year post treatment due to the prolonged drought in the area and increased ambient temperatures preceding the 2018 field trip possibly providing an earlier cue for the invertebrates to emerge.

At the dams near Nieuwoudtville, the pre-treatment community was dominated by Diptera, with minor contributions from Annelida and Hemiptera. Most invertebrate taxa survived the Rotenone treatment and there was an increase in the species richness over the treatment due to three additional taxa being recorded post treatment. Six months post treatment the species richness had increased by a single taxon but the community structure had changed significantly and was dominated by Annelida and Diptera, with a substantial increase in the density of Hemiptera. Interestingly, the Ephemeroptera were rare six months post treatment. This may have been as a result of the Ephemeroptera hatching in the early spring. Unfortunately, this may remain unknown because the treatment dam dried up in January 2018, so the collection of the 12 month post treatment sample was not possible. At the time of the treatment, both dams were less than 10% of their capacity. The control dam was substantially larger than the treatment dam (full capacity) and had a higher density of carp with a number of carp carcasses littered around the remaining water. Larger carp captured in the control dam at the time of the treatment were visibly emaciated indicating a lack of food in the dam. Samples from the control dam six months post treatment contained very few invertebrates, less than 10% of the densities at the time of the treatment. In addition, Cladocera ephippia (resting stage eggs in eggs cases) were found in the control dam samples six months post treatment indicating that the conditions within the dam were approaching conditions where Cladocera were moving to the dormant stage of their life cycle. As with the Krom River dams, the removal of the fish has had a major impact on the ecological functioning of the treatment dam.

A dead mirror carp following Rotenone treatment of Kranskloof Dam. Note turbidity of water.

A dead mirror carp following Rotenone treatment of Kranskloof Dam. Note turbidity of water.

CHAPTER 4. POLICY SUPPORT

4.1 Policy Brief

A Policy Brief dialogue was held in Pretoria on World Biodiversity Day, 22nd May 2018 to inform government decision makers from the Departments of Water and Sanitation (DWS), Environmental Affairs (DEA) and Agriculture, Forestry and Fisheries (DAFF) of the findings of the recent WRC funded projects investigating the ecological impacts of Rotenone treatments to remove non-native fish. A report summarising the data and findings from the three WRC projects was circulated to the delegates invited to the Policy Brief prior to the workshop (See Appendix 1).

The Policy Brief was attended by 30 representatives from 20 organisations. These included representatives from the following organisations with the number of delegates in brackets: Water Research Commission (1), South African Institute for Aquatic Biodiversity (2), the national and provincial departments of Water and Sanitation (4), Environmental Affairs (3) and Agriculture, Forestry and Fisheries (3), the universities of Venda (1), Limpopo (1), Fort Hare (1), Johannesburg (1), and Witwatersrand (1), South African National Biodiversity Institute (1), South African National Parks (1), CapeNature (2), Mpumalanga Parks and Tourism (2), Limpopo Department of Economic Development, Environment and Tourism (1), Endangered Wildlife Trust (2), Council for Scientific and Industrial Research (1), Inkomati-Usuthu Catchment Management Agency (1) and independent consultants (2). Apologies were received from 6 representatives of a further 3 organisations.

After presentations on "*Aquatic Biodiversity and the management options in rivers and lakes*"; "*The CapeNature Rotenone project and public concerns*" and "*Results of the monitoring project on the impacts on, and subsequent recovery of native biota in a River and two Dams*" a discussion session on the use of Rotenone for inland water rehabilitation was conducted. Discussions arising from questions raised during these presentations are summarised in the "*Notes from discussions at the Rotenone Policy Brief Meeting: 22.05.2018*", included as Appendix 3.

In summary, it was proposed that a National Policy on Rotenone use could be developed based on the draft policy for the use of Rotenone in the Western Cape developed by CapeNature. However, a number of issues still need to be resolved including the registration of Rotenone as a piscicide for fish conservation projects; guidelines for the accreditation of Rotenone practitioners; development of training courses for accreditation of practitioners; guidelines for the approval of Rotenone projects; and the development of Norms and Standards.

The registration documentation for restricted use of Rotenone as a piscicide with CapeNature as registration holder was submitted to DAFF in February 2018. The current status of this application was not known at the time of the meeting. There was agreement that Rotenone should receive restricted use registration only, but the potential to use Rotenone as a fisheries management, water quality (irrigation dams) and aquaculture tool should be taken into account during the registration process. Clear guidelines are needed to define the criteria for registered users, e.g. only registered and accredited Rotenone applicators would be permitted to submit applications for Rotenone treatments and undertake any treatments of rivers and dams.

Public engagement should only commence once all research findings have undergone peer review and have been published in ISI-rated Journals so that a holistic picture can be presented to the public. The peer reviewed science needs to be distilled into information sheets suitable for public consumption.

REFERENCES

ABELL R, THEIME ML, REVENGA C, BRYER M, KOTTELAT M, BOGUTSKAYA N, COAD B, MANDRAK N, CONTRERAS-BALDERAS S, BUSSING W, STIASSNY MLJ, SKELTON P, ALLEN GR, UNMACK P, NASEKA A, NG R, SINDORF N, ROBERTSON J, ARMIJO E, HIGGINS JV, HEIBEL TJ, WIKRAMANAYAKE E, OLSON D, LOPEZ HL, REIS RE, LUNDBERG JG, SABAJ PEREZ MH and PETRY P (2008) Freshwater Ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience* **58**: 403-414.

ANDERSON M and TER BRAAK C (2003) Permutation tests for multi-factorial analysis of variance. *Journal of statistical computation and simulation* **73**: 85-113.

ANDERSON MJ (2001a) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32-46.

ANDERSON MJ (2001b) Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 626-639.

ANDERSON MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**: 245-253.

ANDERSON MJ, GORLEY RN and CLARKE KR (2008) *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*, PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK.

ARNEKLEIV JV, DOLMEN D and RØNNING L (2001) Effects of Rotenone treatment on mayfly drift and standing stocks in two Norwegian rivers. in *Trends in Research in Ephemeroptera and Plecoptera,* Springer, pp. 77-88.

BARBER-JAMES HM and LUGO-ORTIZ CR (2003) Ephemeroptera. in DE MOOR IJ, DAY JA and DE MOOR FC (eds), *Guides to the Freshwater Invertebrates of Southern*

Africa. Volume 7: Insecta I. Ephemeroptera, Odonata & Plecoptera, Water Research Commission, WRC Repoort TT207/03, Pretoria, pp. 16-159.

BECKLEY LE (1985) The fish community of East Cape tidal pools and an assessment of the nursery function of this habitat. *African Zoology* **20**: 21-27.

BECKLEY LE (1988) Notes on the biology of juvenile Sparodon durbanensis (Pisces : Sparidae) from tidal pools in the Eastern Cape, South Africa *South African Journal of Zoology* **23**: 66-68.

BECKLEY LE (2000) Species composition and recruitment of tidal pool fishes in KwaZulu-Natal, South Africa. *African Zoology* **35**: 29-34.

BELLINGAN TA, HUGO S, WOODFORD DJ, GOUWS J, VILLET MH and WEY OLF (2019) Macroinvertebrates in a vulnerable South African Cape Fold Ecoregion stream recover swiftly after Rotenone treatments. *Hydrobiologia*.

BELLINGAN TA, WOODFORD DJ, GOUWS J, VILLET MH and WEYL OLF (2015) Rapid bioassessment of the effects of repeated Rotenone treatments on invertebrate assemblages in the Rondegat River, South Africa. *African Journal of Aquatic Science* **40**: 89-94.

BETARBET R, SHERER TB, MACKENZIE G, GARCIA-OSUNA M, PANOV AV and GREENAMYRE JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience* **3**: 1301.

BILLS IR (1999) *Biology and conservation status of the Clanwilliam rock catfish and spotted rock catfish*, World Wide Fund for Nature, Grahamstown.

BLABER SJM (1977) The feeding ecology and relative abundance of mullet (Mugilidae) in Natal and Pondoland estuaries. *Biological Journal of the Linnean Society* **9**: 259-275.

BLABER SJM and WHITFIELD AK (1977) The feeding ecology of juvenile mullet (Mugilidae) in south-east African estuaries. *Biological Journal of the Linnean Society* **9**: 277-284.

BOOTH AJ, MOSS S and WEYL OLF (2015) Effect of Rotenone on gill-respiring and plastron-respiring insects. *African Journal of Aquatic Science* **40**: 95-100.

BRITTON JR and BRAZIER M (2006) Eradicating the invasive topmouth gudgeon, *Pseudorasbora parva*, from a recreational fishery in northern England. *Fisheries Management and Ecology* **13**: 329-335.

BRITTON JR, BRAZIER M, DAVIES GD and CHARE SI (2008) Case studies on eradicating the Asiatic cyprinid *Pseudorasbora parva* from fishing lakes in England to prevent their riverine dispersal. *Aquatic Conservation: Marine and Freshwater Ecosystems* **18**: 867-876.

CAILTEUX RL, DEMONG L, FINLAYSON BJ, HORTON WD, MCCLAY W, SCHNICK RA and THOMPSON C (2001) *Rotenone in Fisheries: Are the rewards worth the risks?*, American Fisheries Society, Bethesda, MD.

CAMBRAY JA (1995) *Sandelia bainsii* will it be next? *Ichthos* **45**: 2-3.

CAMBRAY JA (2003) The need for research and monitoring of the impacts of translocated sharptooth catfish, *Clarias gariepinus*, in South Africa. *African Journal of Aquatic Science* **28**: 191-195.

CANNON JR, TAPIAS V, NA HM, HONICK AS, DROLET RE and GREENAMYRE JT (2009) A highly reproducible Rotenone model of Parkinson's disease. *Neurobiology of Disease* **34**: 279-290.

CHADDERTON WL, GRAINGER N and DEAN T (2003) Appendix 1 – Prioritising control of invasive freshwater fish. in DEPARTMENT OF CONSERVATION (ed) *Managing invasive freshwater fish in New Zealand. Proceedings of a workshop hosted by Department of Conservation, 10-12 May, 2001, Hamilton, New Zealand,* Department of Conservation, Wellington, New Zealand pp. 171-174.

CLARKE KR and WARWICK RM (2001) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd edition*, PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK.

COWX IG and VAN ZYLL DE JONG M (2004) Rehabilitation of freshwater fisheries: Tales of the unexpected? *Fisheries Management and Ecology* **11**: 243-249.

DALLAS HF and DAY JA (2007) Natural variation in macroinvertebrate assemblages and the development of a biological banding system for interpreting bioassessment data – a preliminary evaluation using data from upland sites in the south-western Cape, South Africa. *Hydrobiologia* **575**: 231-244.

DALU T, WASSERMAN RJ, JORDAAN M, FRONEMAN WP and WEYL OLF (2015) An assessment of the effect of Rotenone on selected non-target aquatic fauna. *PloS one* **10**: e0142140.

DAWSON VK, HARMAN PD, SCHULTZ DP and ALLEN JL (1983) Rapid method for measuring Rotenone in water at piscicidal concentrations. *Transactions of the American Fisheries Society* **112**: 725-727.

DAY JA and DE MOOR IJ (2002a) Guides to the Freshwater Invertebrates of Southern Africa – Volume 5: Non-Arthropods. in, Water Research Commission, WRC Report No. TT 167/02, Pretoria, pp. 293.

DAY JA and DE MOOR IJ (2002b) Guides to the Freshwater Invertebrates of Southern Africa – Volume 6: Arachnida and Mollusca. in, Water Research Commission, WRC Report No. TT 182/02, Pretoria, pp. 141.

DAY JA, HARRISON AD and DE MOOR IJ (2003) Guides to the Freshwater Invertebrates of Southern Africa – Volume 9: Diptera. in*,* Water Research Commission, WRC Report No. TT 201/02, Pretoria, pp. 200.

DAY JA, STEWART BA, DE MOOR FJ and LOUW AE (1999) Guides to the Freshwater Invertebrates of Southern Africa – Volume 2: Crustacea I – Notostraca, Conchostraca and Cladocera. in*,* Water Research Commission, WRC Report No. TT 121/00, Pretoria, pp. 126.

DAY JA, STEWART BA, DE MOOR IJ and LOUW AE (2001a) Guides to the Freshwater Invertebrates of Southern Africa – Volume 3: Crustacea II – Ostracoda, Copepoda and Branchiura. in*,* Water Research Commission, WRC Report TT148/01, Pretoria, pp. 177.

DAY JA, STEWART BA, DE MOOR IJ and LOUW AE (2001b) Guides to the Freshwater Invertebrates of Southern Africa – Volume 4: Crustacea III – Bathynellacea, Amphipoda, Isopoda, Spelaeogriphacea, Tanaidacea and Decapoda. in*,* Water Research Commission, WRC Report TT141/01, Pretoria, pp. 141.

DE MOOR FC and SCOTT M (2003) Trichoptera. in DE MOOR IJ, DAY JA and DE MOOR FC (eds), *Guides to the Freshwater Invertebrates of Southern Africa. Volume 8: Insecta II: Hemiptera, Megaloptera, Neuroptera, Trichoptera and Lepidoptera,* Water Research Commission, WRC Repoort TT214/03, Pretoria, pp. 84-181.

DE MOOR IJ and BRUTON MN (1988) *Atlas of alien and translocated indigenous aquatic animals in Southern Africa*, South African National Scientific Programmes Report No. 144. CSIR, Pretoria.

DE MOOR IJ, DAY JA and DE MOOR FC (2003a) Guides to the Freshwater Invertebrates of Southern Africa – Volume 7: Insecta I – Ephemeroptera, Odonata and Plecoptera. in*,* Water Research Commission, WRC Repoort TT207/03, Pretoria, pp. 288.

DE MOOR IJ, DAY JA and DE MOOR FC (2003b) Guides to the Freshwater Invertebrates of Southern Africa – Volume 8: Insecta II – Hemiptera, Megaloptera, Neuroptera,Trichoptera and Lepidoptera. in*,* Water Research Commission, WRC Repoort TT214/03, Pretoria, pp. 209.

DICKENS CWS and GRAHAM PM (2002) The South African Scoring System (SASS) Version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science* **27**: 1-10.

DUDGEON D (1990) Benthic community structure and the effect of Rotenone piscicide on invertebrate drift and standing stocks in two Papua New Guinea streams. *Archiv für Hydrobiologie* **119**: 35-53.

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

ELLENDER BR and WEYL OLF (2014) A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions* **9**: 117-132.

ENVIRO-FISH AFRICA (2009) *Environmental impact assessment of the proposed eradication of invasive alien fishes from four river sections in the Cape Floristic Region*, Enviro-Fish Africa (Pty) Ltd, Grahamstown.

FINLAYSON BJ, EILERS JM and HUCHKO HA (2014) Fate and behavior of Rotenone in Diamond Lake, Oregon, USA following invasive tui chub eradication. *Environmental toxicology and chemistry* **33**: 1650-1655.

FINLAYSON BJ, SCHNICK RA, CAILTEUX RL, DEMONG L, HORTON WD, MCCLAY W and THOMPSON CW (2002) Assessment of Antimycin A use in fisheries and its potential for registration. *Fisheries* **27**: 10-20.

FINLAYSON BJ, SCHNICK RA, SKAAR D, ANDERSON J, DEMONG L, DUFFIELD D, HORTON WD and STEINKJER J (2010) *Planning and standard operating procedures for the use of Rotenone in fish management – Rotenone SOP Manual.*, American Fisheries Society, Bethesda, Maryland.

GREEN RH (1979) *Sampling design and statistical methods for environmental biologists*, Wiley Interscience, Chichester, England.

HAMILTON BT, MOORE SE, WILLIAMS TB, DARBY N and VINSON MR (2009) Comparative effects of Rotenone and antimycin on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. *North American Journal of Fisheries Management* **29**: 1620-1635.

HARRISON AC. (1956). Carp from the nature conservation angle. Department of Nature Conservation, Cape Province. Annual Report No. 13: 75-80.

HARRISON JA, WILLIAMS AJ and MACIVER M (2010) Breeding site selection by colonial waterbirds given various combinations of constructed or natural alternatives over a 10-year period. *Ostrich* **81**: 197-203.

HOLDEN PB (1991) Ghosts of Green River: Impacts of Green River poisoning on management of native fishes. in MINKLEY WL and DEACON JE (eds), *Battle Against Extinction – Native Fish Management in the American West,* The University of Arizona Press, Tucson & London, pp. 43-54.

IMPSON ND (2007) Freshwater fishes. in CAPE NATURE (ed) *Western Cape Province State of Biodiversity 2007,* Cape Nature Scientific Services, Stellenbosch, pp. 18-36.

IMPSON ND, BILLS IR and CAMBRAY JA (2002) Freshwater fishes. in WESTERN CAPE NATURE CONSERVATION BOARD (ed) *Western Cape State of Biodiversity 2000,* Western Cape Nature Conservation Board, Stellenbosch, pp. 1-20.

IMPSON ND, HARDING B and STEYN G (2005) The use of Rotenone as a tool for restructuring fish populations in inland waters of the fynbos biome. *Piscator* **137**: 63-69.

IMPSON ND, VAN WILGEN BW and WEYL OLF (2013) Coordinated approaches to rehabilitating a river ecosystem invaded by alien plants and fish. *South African Journal of Science* **109**: 1-4.

JACCARD P (1908) Nouvelles recherches sur la distribution florale. *Bulletin Société Vaudoise des Sciences Naturelles* **44**: 223-270.

JOHNSON ME and BOBROVSKAYA L (2015) An update on the Rotenone models of Parkinson's disease: their ability to reproduce the features of clinical disease and model gene-environment interactions. *Neurotoxicology* **46**: 101-116.

JORDAAN MS, DALU T, WASSERMAN RJ, SLABBERT E and WEYL OLF (2017) Unexpected survival of sharptooth catfish *Clarias gariepinus* (Burchell 1822) during acute Rotenone toxicity trials will complicate management of invasions. *Biological Invasions* **19**: 1739-1744.

JORDAAN MS and WEYL OLF (2013) Determining the minimum effective dose of Rotenone for eradication of alien smallmouth bass *Micropterus dolomieu* from a South African river. *African Journal of Aquatic Science* **38**: 91-95.

KIMBERG PK, WOODFORD D, WEYL O, HUI C, RICHARDSON D, MSEZANE T, VAN DER WALT KA, SWARTZ ER, CHIMIMBA CT and ZENGEYA T (2014) *Understanding the Unintended Spread and Impact of Alien and Invasive Fish Species-Development of Management Guidelines for South African Inland Waters*, Water Research Commission, WRC Report No. 2038-1-14, Pretoria.

KJÆRSTAD G and ARNEKLEIV JV (2011) Effects of Rotenone treatment on lotic invertebrates. *International Review of Hydrobiology* **96**: 58-71.

KJÆRSTAD G, ARNEKLEIV JV and SPEED JDM (2016) Effects of three consecutive Rotenone treatments on the benthic macroinvertebrate fauna of the River Ogna, Central Norway. *River Research and Applications* **32**: 572-582.

KOLAR CS, COURTENAY JR WR, NICO LG and HUBERT W (2010) Managing undesired and invading fishes. in HUBERT W and QUIST M (eds), *Inland fisheries management in North America, 3rd edition,* American Fisheries Society, Bethesda, Maryland, pp. 213-259.

LING N (2002) *Rotenone – a review of its toxicity and use for fisheries management*, Science for Conservation 211. Department of Conservation. Wellington. New Zealand.

LINTERMANS M (2000) Recolonization by the mountain galaxias *Galaxias olidus* of a montane stream after the eradication of rainbow trout *Oncorhynchus mykiss*. *Marine and Freshwater Research* **51**: 799-804.

LINTERMANS M and RAADIK T (2003) Local eradication of trout from streams using Rotenone: the Australian experience. in DEPARTMENT OF CONSERVATION (ed) *Managing invasive freshwater fish in New Zealand. Proceedings of a workshop hosted by Department of Conservation, 10-12 May, 2001, Hamilton, New Zealand,* Department of Conservation, Wellington, New Zealand pp. 95-111.

LOWE SR, WOODFORD DJ, IMPSON ND and DAY JA (2008) The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science* **33**: 51-62.

MACEDA-VEIGA A (2013) Towards the conservation of freshwater fish: Iberian Rivers as an example of threats and management practices. *Reviews in Fish Biology and Fisheries* **23**: 1-22.

MANGUM FA and MADRIGAL JL (1999) Rotenone effects of aquatic macroinvertebrates of the Strawberry River, Utah: a five-year summary. *Journal of Freshwater Ecology* **14**: 125-135.

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

MARTINEZ TN and GREENAMYRE JT (2012) Toxin models of mitochondrial dysfunction in Parkinson's disease. *Antioxidants & Redox Signaling* **16**: 920-934.

MCCLAY W (2005) Rotenone use in North America (1988-2002). *Fisheries* **30**: 29-31.

PHAM L, JARVIS MG, WEST D and CLOSS GP (2018) Rotenone treatment has a short-term effect on New Zealand stream macroinvertebrate communities. *New Zealand Journal of Marine and Freshwater Research* **52**: 42-54.

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

PRCHALOVÁ M, KUBEČKA J, ŘÍHA M, MRKVIČKA T, VAŠEK M, JŮZA T, KRATOCHVÍL M, PETERKA J, DRAŠTÍK V and KŘÍŽEK J (2009) Size selectivity of standardized multimesh gillnets in sampling coarse European species. *Fisheries Research* **96**: 51-57.

PRICE BW, LIU X, DE MOOR FC and VILLET MH (2012) A review of the alderfly genus Leptosialis Esben-Petersen (Megaloptera, Sialidae) with description of a new species from South Africa. *ZooKeys*: 27.

RAYNER TS and CREESE RG (2006) A review of Rotenone use for the control of non-indigenous fish in Australian freshwaters, and an attempted eradication of the noxious fish, *Phalloceros caudimaculatus*. *New Zealand Journal of Marine and Freshwater Research* **40**: 477-486.

REPUBLIC OF SOUTH AFRICA (2016a) List 7: National List of Invasive Freshwater Fish Species. *Government Gazette* **613 no 40166**.

REPUBLIC OF SOUTH AFRICA (2016b) List 7: Prohibited Freshwater Fishes. *Government Gazette* **613 no 40166**: 93-96.

ROBERTS J and TILZEY R (1997) *Controlling carp: Exploring options for Australia. Proceedings of a workshop 22-24 October 1996, Albury*, CSIRO and Murray-Darling Basin Commission.

ROBERTSON DR and SMITH-VANIZ WF (2008) Rotenone: an essential but demonized tool for assessing marine fish diversity. *BioScience* **58**: 165-170.

ROWE DK (2003) Rotenone-based approaches to pest fish control in New Zealand. in DEPARTMENT OF CONSERVATION (ed) *Managing invasive freshwater fish in New Zealand. Proceedings of a workshop hosted by Department of Conservation, 10-12 May, 2001, Hamilton, New Zealand,* Department of Conservation, Wellington, New Zealand, pp. 131-142.

ROWE-ROWE DT (1971) Rotenone tolerances of some freshwater fishes of Natal. *The Progressive Fish-Culturist* **33**: 206-209.

ROWE-ROWE DT (1977) Food ecology of otters in Natal, South Africa. *Oikos*: 210-219.

ROWE-ROWE DT (1979a) The detoxification of two Rotenone-containing piscicides in fresh water. *South African Journal of Wildlife Research* **3**: 22-23.

ROWE-ROWE DT (1979b) Laboratory and field tests in the revival of fish affected by Rotenone. *South African Journal of Wildlife Research-24-month delayed open access* **3**: 19-21.

ROWE-ROWE DT (1979c) Reaction times of certain freshwater fishes to a Rotenone-containing piscicide. *South African Journal of Wildlife Research-24-month delayed open access* **3**: 16-18.

SANDODDEN R, BRAZIER M, SANDVIK M, MOEN A, WIST AN and ADOLFSEN P (2018) Eradication of Gyrodactylus salaris infested Atlantic salmon (Salmo salar) in the Rauma River, Norway, using Rotenone. *Management of Biological Invasions* **9**: 67-77.

SANNI S and WÆRVÅGEN SB (1990) Oligotrophication as a result of planktivorous fish removal with Rotenone in the small, eutrophic, Lake Mosvatn, Norway. *Hydrobiologia* **200**: 263-274.

SCHNICK RA (2001) AFS Rotenone Stewardship Program. in CAILTEUX RL, DEMONG L, FINLAYSON BJ, HORTON WD, MCCLAY W, SCHNICK RA and THOMPSON C (eds), *Rotenone in Fisheries: Are the rewards worth the risks?,* American Fisheries Society, Bethesda, MD, pp. 81-85.

SHERER TB, BETARBET R, TESTA CM, SEO BB, RICHARDSON JR, KIM JH, MILLER GW, YAGI T, MATSUNO-YAGI A and GREENAMYRE JT (2003) Mechanism of toxicity in Rotenone models of Parkinson's disease. *Journal of Neuroscience* **23**: 10756-10764.

SKELTON PH, WHITFIELD AK and JAMES NPE (1989) Distribution and diversity of Mkuze swamp fishes during a summer flood. *Southern African Journal of Aquatic Science* **15**: 50-66.

SLABBERT E, JORDAAN MS and WEYL OLF (2014) Analysis of active Rotenone concentration during treatment of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science* **39**: 467-472.

SMALE MJ (1986) The feeding biology of four predatory reef fishes off the south-eastern Cape coast, South Africa. *African Zoology* **21**: 111-130.

SMALE MJ and BUXTON CD (1989) The subtidal gully fish community of the Eastern Cape and the role of this habitat as a nursery area. *African Zoology* **24**: 58-67.

STALS R and DE MOOR IJ (2007) Guides to the Freshwater Invertebrates of Southern Africa – Volume 10: Coleoptera. in*,* Water Research Commission, WRC Report No. TT 320/07, Pretoria, pp. 263.

TANNER CM, KAMEL F, ROSS GW, HOPPIN JA, GOLDMAN SM, KORELL M, MARRAS C, BHUDHIKANOK GS, KASTEN M and CHADE AR (2011) Rotenone, paraquat, and Parkinson's disease. *Environmental Health Perspectives* **119**: 866-872.

TWEDDLE D, BILLS R, SWARTZ E, COETZER W, DA COSTA L, ENGELBRECHT J, CAMBRAY J, MARSHALL B, IMPSON D, SKELTON P, DARWALL WRT and SMITH KG (2009) The status and distribution of freshwater fishes. in DARWALL WRT, SMITH KG, TWEDDLE D and SKELTON P (eds), *The status and distribution of freshwater biodiversity in Southern Africa*, Gland, Switzerland: IUCN and Grahamstown, South Africa: SAIAB, pp. 21-37.

VAN GINKEL SW, IGOU T, HU Z, NARODE A, CHERUVU S, DOI S, JOHNSTON R, SNELL T and CHEN Y (2015) Taking advantage of rotifer sensitivity to Rotenone to prevent pond crashes for algal-biofuel production. *Algal Research* **10**: 100-103.

VILIZZI L (2012) The common carp, *Cyprinus carpio*, in the Mediterranean region: origin, distribution, economic benefits, impacts and management. *Fisheries Management and Ecology* **19**: 93-110.

VILIZZI L and TARKAN AS (2015) Experimental evidence for the effects of common carp (Cyprinus carpio L., 1758) on freshwater ecosystems: a narrative review with management directions for Turkish inland waters. *Journal of Limnology and Freshwater Fisheries Research* **1**: 123-149.

VILIZZI L, TARKAN AS and COPP GH (2015) Experimental evidence from causal criteria analysis for the effects of common carp Cyprinus carpio on freshwater ecosystems: a global perspective. *Reviews in Fisheries Science & Aquaculture* **23**: 253-290.

VINSON MR, DINGER EC and VINSON DK (2010) Piscicides and invertebrates: after 70 years, does anyone really know? *Fisheries* **35**: 61-71.

WELSCHMEYER NA (1994) Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnology and Oceanography* **39**: 1985-1992.

WEYL OLF, BARROW S, BELLINGAN T, DALU T, ELLENDER BR, ESLER K, IMPSON D, GOUWS J, JORDAAN M and VILLET M (2016) *Monitoring of invertebrate and fish recovery following river rehabilitation using Rotenone in the Rondegat River*, Water Research Commission, WRC Report No. 2261/1/16, Pretoria.

WEYL OLF, ELLENDER BR, WOODFORD DJ and JORDAAN MS (2013) Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of Rotenone treatment in an invaded reach. *African Journal of Aquatic Science* **38**: 201-209.

WEYL OLF, FINLAYSON B, IMPSON ND, WOODFORD DJ and STEINKJER J (2014) Threatened endemic fishes in South Africa's Cape Floristic Region: A new beginning for the Rondegat River. *Fisheries* **39**: 270-279.

WHITFIELD AK (1993) Fish biomass estimates from the littoral zone of an estuarine coastal lake. *Estuaries* **16**: 280-289.

WHITFIELD AK and BLABER SJM (1978a) Food and feeding ecology of piscivorous fishes at Lake St Lucia, Zululand. *Journal of Fish Biology* **13**: 675-691.

WHITFIELD AK and BLABER SJM (1978b) Resource segregation among iliophagous fish in Lake St. Lucia, Zululand. *Environmental Biology of Fishes* **3**: 293-296.

WILEY RW (2008) The 1962 Rotenone treatment of the Green River, Wyoming and Utah, revisited: lessons learned. *Fisheries* **33**: 611-617.

WILLIAMS TJ (2007) Responses of waterbirds to helicopter disturbance and fish poisoning by Rotenone at Paardevlei, South Africa. *Waterbirds* **30**: 429-432.

WINTERBOTTOM R (1976) Additions to, and range extensions of, the South African marine ichthyofauna. *African Zoology* **11**: 59-73.

WOOD AD, BROUWER SL, COWLEY PD and HARRISON TD (2000) An updated check list of the ichthyofaunal species assemblage of the Tsitsikamma National Park, South Africa. *Koedoe* **43**: 83-95.

WOODFORD DJ, BARBER-JAMES HM, BELLINGAN TA, DAY JA, DE MOOR FC, GOUWS J and WEYL OLF (2013) Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage: a lesser of two evils? *Journal of insect conservation* **17**: 959-973.

WOODFORD DJ, IMPSON ND, DAY JA and BILLS IR (2005) The predatory impact of invasive alien smallmouth bass, *Micropterus dolomieu* (Teleostei: Centrarchidae), on indigenous fishes in a Cape Floristic Kingdom mountain stream. *African Journal of Aquatic Science* **30**: 167-173.

WOODFORD DJ, WEYL OLF, CUNNINGHAM M, BELLINGAN TA, DE MOOR FC, BARBER-JAMES HM, DAY JA, ELLENDER BR and RICHARDSON NK (2012) *Monitoring the impact and recovery of the biota of the Rondegat River after the removal of alien fishes*, Water Research Commission, WRC Report No. KV 304/12, Pretoria.

YOUNGE A and FOWKES S (2003) The Cape Action Plan for the Environment: overview of an ecoregional planning process. *Biological Conservation* **112**: 15-28.

APPENDICES

K5/2538: ROTENONE POLICY SUPPORT AND CAPACITY DEVELOPMENT THROUGH INTEGRATING AQUATIC-ECOSYSTEM MONITORING IN POST GRADUATE RESEARCH PROJECTS

DELIVERABLE 8

Policy Brief to gain support for the registration of Rotenone as a River Rehabilitation Tool

Progress report to the

Water Research Commission

By

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July 2018

Cover Photo: Back-pack application of Rotenone in the vegetated margins during the treatment of the Chalet Dam, Krom River Farm, Western Cape Photo Sean Marr

EXECUTIVE SUMMARY

This report summarises the Policy Brief dialogue held to inform government decision makers of the findings of the recent WRC funded projects investigating the ecological impacts of Rotenone treatments to remove alien fish. The data from the three WRC projects shows that Rotenone treatments of rivers and farm dams remove the alien fish species without demonstrable impacts on water quality or macroinvertebrate communities. For rivers, natural colonisation of the treated reaches by native fish from upstream populations is possible. As all of the current knowledge is derived from sites in the Cape Fold ecoregion data from case studies undertaken in different environments will be required to guide national policy development on Rotenone.

The development of a National Policy on Rotenone, could be based on the draft policy for the use of Rotenone in the Western Cape developed by CapeNature. However, a number of issues still need to be resolved including the registrations of Rotenone as a piscicide for fish conservation projects; guidelines for the accreditation of Rotenone practitioners; development of training courses for accreditation of practitioners; guidelines for the registration of Rotenone projects; and the development of Norms and Standards.

The registration documentation for restricted use of Rotenone as a piscicide by CapeNature was submitted to DAFF in February 2018. The current status of this application was not known at the time of the meeting. There was agreement that Rotenone should receive restricted use registration only, but potential to use Rotenone for fisheries management and aquaculture tool should be taken into account during the registration process. Clear guidelines are needed to define the criteria for registered users, e.g. only registered and accredited Rotenone applicators would be permitted to compile applications for Rotenone treatments.

Public engagement should only commence once all research findings have undergone peer review and are published in ISI-rated Journals so that a holistic picture can be presented to the public. The peer reviewed science needs to be distilled into information sheets suitable for public consumption.

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INTRODUCTION

The current K5/2538 project follows two previous projects (K9/822 and K5/2261) on monitoring the impact of river rehabilitation using the piscicide Rotenone. These projects provided comprehensive species level assessments of invertebrate and vertebrate distributions both prior to and for three years after the Rotenone treatment in the Rondegat River. Overall, the research demonstrated that Rotenone treatment was effective at removing smallmouth bass from the treatment zone and that native invertebrate and fish communities recovered following the treatment (Woodford et al., 2012; Weyl et al., 2014). The current K5/2538 project has advanced our understanding of the ecological impact of Rotenone through the monitoring of the treatment of two farm dams in the Olifants-Doring catchment. The ecosystem responses to the Rotenone treatment of the Krom (i.e. Chalet) and Kranskloof Dams in the Western and Northern Cape provinces is detailed in Deliverable 7 of the project (Dalu et al., 2018). In summary, the key findings/effects were that: (1) the non-native fish were successfully removed; (2) Rotenone took less than two weeks to dissipate; (3) there was no change in the physico-chemical properties of the water over the treatment; (4) water clarity improved following the treatment due to the removal of fish; (5) insect (i.e. macroinvertebrate) communities had recovered within a year; (6) larger zooplankton (Copedods and Cladocerans) had also returned within a year and (7) small zooplankton dynamics were hard to predict but rotifer abundances had returned to pre-treatment levels within a year of treatment. Within each group, there were species changes, but these are likely to be part of the altered predator/prey dynamics resulting from fish removals.

One of the outcomes of the current K5/2538 project was a Policy Brief to relevant decision makers in the departments of Water and Sanitation, Environmental Affairs and Agriculture, Forestry and Fisheries to inform National Policy on the registration of Rotenone as a tool for habitat rehabilitation with regard to the removal of invasive fishes. The purpose of the Policy Brief was to provide the relevant parties scientifically defensible data regarding the ecological impact of Rotenone on aquatic biota based on the findings of the treatment of the Rondegat River and the two farm dams in the Western and Northern Cape. The objective was not to develop a policy on the use of Rotenone, rather to provide the data which could be used to guide the development of National Policy on the use of Rotenone as a river rehabilitation tool.

PREPARATIONS FOR THE POLICY BRIEF

The Policy Brief is one of the most important deliverable of the current K5/2538 project and a great deal of planning was invested to make sure that the relevant decision makers were present.

Invitation of Potential Delegates

A list of potential delegates was established in consultation with the Water Research Commission and the project partners including CapeNature. The decision was taken to invite the Director Generals of the departments of Water and Sanitation, Environmental Affairs and Agriculture, Forestry and Fisheries to the Policy Brief and ask that they send the relevant personnel to the Brief. A letter was prepared for each of the Director Generals and emailed to the office of the three Director Generals. The office of each of the Director Generals confirmed that their departments would be represented at the Policy Brief.

In addition, the conservation authorities of selected provinces, South African National Parks, other conservation organisations, e.g. Endangered Wildlife Trust, South African National Biodiversity Institute, the Reference Group for the WRC Project K5/2538, and academics from selected South African Universities were invited to the Policy Brief.

Background Material

The WRC Reports for the previous WRC projects on the treatment of the Rondegat River (K9/822 and K5/2261) and a draft of Deliverable 7 from the K5/2538 project (Dalu et al., 2018) were circulated to the delegates who had confirmed their attendance at the Policy Brief. In addition, a document summarising the findings of the two WRC projects on the Rondegat River and preliminary results of the treatment of the two dams was prepared as a handout for the Policy Brief. The document concludes that data demonstrates that the Rotenone treatment in the Rondegat River removed the alien target species and allowed for the recovery of native fishes without demonstrable impacts on macroinvertebrate communities and that the Rotenone treatment in dams removed the alien target species with no long term impacts on either macroinvertebrate or zooplankton communities.

POLICY BRIEF

The Policy Brief was held at Milkplum Café Conference Centre in the South African National Biodiversity Institute in Pretoria (Pretoria Botanical Gardens) on International Biodiversity Day the 22nd of May 2018, commencing at 10:30. The Policy Brief was chaired by Mr. Madikizela of the Water Research Commission.

Participation

The Policy Brief was attended by 30 representatives from 20 organisations. These included representatives from the Water Research Commission (1), South African Institute for Aquatic Biodiversity (2), the national and provincial departments of Water and Sanitation (4), Environmental Affairs (3) and Agriculture, Forestry and Fisheries (3), the universities of Venda (1), Fort Hare (1), Johannesburg (1), and Witwatersrand (1), South African National Biodiversity Institute (1), South African National Parks (1), CapeNature (2), Mpumalanga Parks and Tourism (2), Limpopo Department of Economic Development, Environment and Tourism (1), Endangered Wildlife Trust (2), Council for Scientific and Industrial Research (1), Inkomati-Usuthu Catchment Management Agency (1) and independent consultants (2). Apologies were received from 6 representative of a further 3 organisations. A list of the representatives who attended the Policy Brief and their contact email addresses are included as Appendix 2.

Presentations

Dr Marr of SAIAB opened the meeting, thanked everyone present for attending, and invited the delegates to introduce themselves. Following this, the Chairman provided a brief explanation of the purpose of the Policy Brief dialogue.

Prof Weyl of SAIAB delivered a presentation entitled “*Aquatic Biodiversity and the management options in rivers and lakes*” to provide a biodiversity context to the piscicide projects in the Western Cape. Mr Impson of CapeNature then delivered a presentation titled “*The CapeNature Rotenone project and public concerns*” to explain the CapeNature Rotenone project and provide details of the concerns around the use of Rotenone. Dr Roets of DWS delivered a short presentation clarifying the roles and responsibilities of DWS within the broader context of the National Water Act. Finally, Prof Weyl delivered a presentation titled “*Results of the monitoring project on the impacts on, and subsequent recovery of native biota in a River and two Dams*” that present the results of WRC-funded monitoring projects to assess the impact on, and recovery of, native biota following CapeNature Rotenone treatments. Discussions arising from questions raised during these presentations are summarised in the “*Notes from discussions at the Rotenone Policy Brief Meeting: 22.05.2018*”, included as Appendix 3.

Discussion

Following the presentations, the Chairman opened the floor providing representatives from the departments of Water and Sanitation, Environmental Affairs and Agriculture, Forestry and Fisheries to respond to the information presented by Prof Weyl and Mr Impson. These discussions are summarised in the “*Notes from discussions at the Rotenone Policy Brief Meeting: 22.05.2018*”, included as Appendix 7.

GENERAL SYNTHESIS

The data from the three WRC projects has provided scientifically defensible evidence that, if conducted with due diligence, Rotenone treatments in rivers and farm dams removed the target alien fish species without demonstrable impacts on water quality or macroinvertebrate communities and, in the case of rivers, allows for natural colonisation of the treated reaches by native fish from upstream populations. Key topics from the discussions generated by the presentations are summarised below.

Alternatives to Rotenone. At present, only Rotenone was considered suitable as a long-term tool river rehabilitation projects targeting alien fish removal. It is widely available and has a shelf life of 15 years. Mechanical removal has been successful in very specific conditions and is not considered a feasible alternative to achieve complete removal of the target species. Alternative piscicides, such as Antimycin A, are produced in small batches and are not freely available, but may be considered for the removal of species that have been found to have low susceptibility to Rotenone, e.g. sharptooth catfish *Clarias gariepinus*.

Broader concerns. The projects completed concentrated on the ecological impacts within the aquatic environments. The broader concern of impact of Rotenone, such as the consumption of fish killed using Rotenone by predatory and scavenging animals, e.g. birds and mammals, was raised. At concentrations used for fish eradication, Rotenone toxicity to non-gill-breathing animals was low and there is negligible risk to birds and other taxa feeding of fish killed by Rotenone. The impact of using of treated water for irrigation was raised but dismissed as a negligible risk to crops as it was a registered organic pesticide in South Africa. Rotenone was not detectable in the river three days after the treatment and had reduced to below lethal levels for fish with 15 days of the dam treatments.

More than a conservation tool. The use of Rotenone as a freshwater fish conservation tool has been the focus of recent studies. However, Rotenone can be applied as a tool for broader fisheries management and aquaculture and registration for the use of Rotenone should not be limited to conservation goals alone.

Delays in awarding General Authorisations. Currently, a General Authorisation (GA) from the Department of Water and Sanitation is required before the treatment of a water body with Rotenone can commence. Delays in awarding GAs has delayed the implementation of treatments after Environmental Impact Assessments have been completed and the treatment authorised by the Department of Environmental Affairs. The treatment of water bodies to remove alien species in in alignment with the improvement of ecological structure defined in the National Water Act. The treatment of water bodies with Rotenone is considered a low risk activity and therefore there should be no further delays in the awarding of GAs because of the alignment with the National Water Act. The General Authorization 509, which is required for Rotenone use, is currently in the process of being amended and can included the management of invasive species for conservation purposes without the necessity of completing a risk matrix.

National Policy. The applicability of Rotenone as a national tool was discussed. It is currently not know whether the efficacy of Rotenone demonstrated in waters of the Western Cape would be replicated in the varied water conditions in the other provinces of South Africa. Therefore, research regarding the efficacy of Rotenone in other provinces is required before discussion of Rotenone as a national tool can be engaged in. However, should Rotenone be found to be effective across the spectrum of water conditions found in the country, consideration needs to be given to the development of a National Policy on Rotenone. CapeNature have developed a draft policy for the use of Rotenone in the Western Cape and offered this draft policy as a basis from which a National Policy can be developed. However, a number of issues still need to be resolved before work towards a National Policy on Rotenone can be developed including, but not limited to: the registrations of Rotenone as a piscicide for fish conservation projects; guidelines for the accreditation of Rotenone practitioners; the development of training courses for the accreditations of practitioners based on the use of Rotenone compliant with South African legislation; guidelines for the registration of Rotenone projects to monitor the use of Rotenone and evaluate the outcomes; and the development of Norms and Standards. CapeNature is currently the only registered user of Rotenone in South Africa and a monopoly on the use of Rotenone should be avoided.

Registration of Rotenone. The Department of Agriculture Forestry and Fisheries (DAFF) is the registration authority for pesticides in South Africa. Several registration categories exist and for the proposed use for Rotenone, the correct category must be determined. The existing Rotenone registration is limited to insecticidal use for citrus production. The registration documentation for restricted use of Rotenone as a piscicide by CapeNature had been submitted to DAFF in February 2018. **The current status of this application was not known at the time of the meeting.** Approval for the import of Rotenone has already been granted. There was agreement that Rotenone should receive restricted use registration only, which means that registered users can only procure it. Currently, CapeNature is the only registered user for Rotenone use under experimental conditions. Rotenone has the potential to be a useful fisheries management and aquaculture tool and this should be taken into account during the registration process. While clear guidelines and processes exist for registration of Rotenone, a similar process is needed to define the criteria for registered users, e.g. only registered and accredited Rotenone applicators would be permitted to compile applications for Rotenone treatments.

Public engagement. Given that initial media and public perception of Rotenone projects was negative, media engagement to change public perceptions following the success of the current projects was raised. While CapeNature appointed a communications specialist for the Rondegat treatments which contributed to the change in public perception, the bulk of the current research has been published in peer-reviewed literature and engagement with the public should only commence once all research findings have undergone peer review and are published in ISI-rated Journals so that a holistic picture can be presented to the public. The peer reviewed science needs to be distilled into information sheets suitable for public consumption. It is also critical to have the input and support of all regulatory agencies before the public engagement process is initiated.

REFERENCES

Dalu, T., S.M. Marr, T. Bellingan, M. Jordaan, E. Slabbert and O.L.F. Weyl. (2018). Deliverable 7 – Ecosystem responses to Rotenone treatment in two dams in the Western and Northern Cape provinces. Unpublished Water Research Commission Project Progress Report for WRC Project K5/2538.

Weyl, O.L.F., B. Finlayson, N.D. Impson, D.J. Woodford and J. Steinkjer. (2014) Threatened endemic fishes in South Africa's Cape Floristic Region: A new beginning for the Rondegat River. *Fisheries* **39**, 270-279.

Woodford D.J., O.L.F. Weyl, M. Cunningham, T.A. Bellingan, F.C. de Moor, H.M. Barber-James, J.A. Day, B.R. Ellender, and N.K. Richardson. (2012) Monitoring the impact and recovery of the biota of the Rondegat River after the removal of alien fishes. Water Research Commission Report No. KV 304/12, Pretoria, SA. 72p.

Appendix 2 – Delegate List for the Rotenone Policy Brief Workshop 22nd of May 2018

WRC Project K5-2538 Meeting held at Milkplum Café, SANBI, National Botanical Gardens, Pretoria

Delegate	Organization	Email
Mr Bonani Madikizela	Water Research Commission	bonanim@wrc.org.za
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Apologies

Dr Bruce Paxton	Freshwater Research Centre
Dr Helen Dallas	Freshwater Research Centre
Dr Terence Bellingan	Albany Museum
Dr Leon Barkhuizen	Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs
Mr Thilivhali Nepfumbada	Department of Agriculture, Forestry and Fisheries
Ms Nancy Job	South African National Biodiversity Institute

Did not arrive

Ms Shashika Maharaj	Department of Environment Affairs and Tourism
Mr Michael Braack	Department of Environment Affairs and Tourism
Prof Nico Smit	University of North West

Appendix 3 -Notes from discussions at the Rotenone Policy Brief meeting: 22.05.2018

Held at Milkplum Café, National Botanical Gardens, SANBI, Pretoria

Compiled by Dr Martine Jordaan and Dr Sean Marr

1. General questions and discussion following presentations

- **Mr Madikizela** (WRC) stated that going forward the Rotenone policy must be relevant to the NEMBA AIS regulations and stated that the WRC funded work is contributing to the scientific basis needed for the Rotenone registration process. He also referred to International Biodiversity Day and the need for pro-active actions for biodiversity conservation, such as plastic pollution. It was stated that the WRC currently has a funding call open and that proposals must link to the current WRC strategy plan for 2018-2023, which focus on impact and innovation. The call is open until the end of June 2018.
- **Mr Mills** enquired whether the focus of Rotenone will mainly be on the management of NEMBA listed species and conservation initiatives and whether it will have application for fisheries management and/or the management of non NEMBA listed species. The example was made of the trout sector and their concern around the management of Category 2 invasive species. It was reiterated that the large-scale eradication of trout, or other Category 2 species, from the country is neither economically desirable nor logistically feasible. **Mr Mills** further stated that conservation is often thought of in a narrow definition but is actually a protection-utilization continuum. **Prof Weyl** (SAIAB) agreed and stated the Rondegat River was an example of an integrated management approach where one could support recreational fisheries for alien species in the Dam while reducing impacts in the river. He also highlighted the need for stakeholder support to ensure successful project implementation and long-term success.
- **Dr Greenfield** (University of Johannesburg) enquired about species-specific sensitivity. Prof Weyl responded that in general fish are more sensitive that aquatic invertebrates and that within both groups, significant differences existed in species-specific sensitivity. **Dr Jordaan** (CapeNature) added that based on current USA labelling requirements there is a permitted dosage range which has to be adhered to and that most fish species are within this range. She added that bio-assays are conducted using target species in water from the treatment waterbody to determine appropriate treatment concentrations.
- **Mr Impson** (CapeNature) stated that CapeNature had prepared a draft Rotenone use policy and suggested that the draft WC policy could be a template from which a National Policy could be developed. **Mr Madikizela** enquired how the draft WC policy could be elevated to National policy level and raised the question of accreditation for implementers of Rotenone treatments. **Mr Impson** indicated that there plans were being developed for accreditation training in South Africa with **Ms Muir** (DEA-NRM) but that there are still a

number of issues to be resolved. **Dr Jordaan**, whom recently participated in training in the USA, stated that roughly a third of the US Rotenone training course constituted guidelines on how to be compliant with US legislation and that a similar section would be required for local training. The need for registered SA Rotenone practitioners was stressed. Currently, CapeNature is the only registered user of Rotenone in South Africa.

- **Dr Roets** (DWS) responded to concerns raised by CapeNature on the long waiting periods for the approval of a General Authorization (GA) for the use of Rotenone. He delivered a short presentation on the roles and responsibilities of DWS within the broader context of the National Water Act. He stated that given that one of the aims of Rotenone treatments is the improvement of ecological infrastructure, there should not be significant delays in the approval of GA applications for this purpose as the activity is low risk if implemented correctly. He also added that the internal expertise and capacity for reviewing Rotenone use applications is available in the WC DWS office.

- **Dr Roets** questioned whether Rotenone would be effective on species and ecosystems outside the Cape Fold Ecoregion (CFE) where most of the current research was based. **Prof Weyl** responded that the efficacy of Rotenone is strongly influenced by water quality and environmental parameters and that further research outside the CFE is warranted. The issue around the efficacy of Rotenone on fishes such as the African sharptooth catfish *Clarias gariepinus* was raised. **Prof Weyl** stated that this species was not very susceptible to Rotenone and that it is likely that there will be limited success in managing it using Rotenone. **Dr Jordaan** added that based on experimental work, *C. gariepinus* showed avoidance response, size specific sensitivity and unexpected survival following Rotenone exposure. **Prof Weyl** stated that concerns around the efficacy of Rotenone treatment in a specific system should be addressed as part of the viability analysis of that specific project.

- **Dr Ntshotsho** (CSIR) raised the concerns about the effects of Rotenone on fish eating animals such as piscivorous birds. **Mr Impson** responded that toxicity of Rotenone to non gill-breathing animals was low and there was negligible risk to birds and other taxa feeding of fish killed by Rotenone. **Prof Weyl** added that Rotenone induced a toxic effect by blocking cellular respiration and thus the affected fish died quickly, thereby not allowing Rotenone to build up in the tissue of affected fish. **Mr Impson** added that there is a long history of humans using Rotenone-containing compounds to harvest fish for consumption purposes.

- **Dr Roets** raised the issue of the breakdown of Rotenone in field conditions, and whether there was engagement with downstream water users during the experimental treatments. **Prof Weyl** responded that there was detailed chemical analysis done during both treatments. For the Rondegat River, Rotenone was neutralised and remaining Rotenone had broken down to just above detectable levels within three days (Slabbert E, Jordaan MS, Weyl OLF. 2014. Analysis of active Rotenone concentration during treatment of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic*

Science, 39: 467-472). For the Krom Dam, (no neutralization) Rotenone remained at piscicidal concentrations for a week and was detected at very low levels by 15 days post treatment. **Mr Impson** also added that Rotenone has current registration in SA as an organic pesticide so there is negligible risk to using water for irrigation purposes.

- **Dr Roets** enquired about the efficacy of Rotenone treatment to remove snails in order to manage Bilharzia. **Dr Dalu** (Uni. Ven) responded that this is unlikely to be feasible based on current research results that indicated that snails showed an avoidance response and high survival rate following Rotenone exposure.
- **Dr Zengeya** (SANBI) inquired why alternative piscicides, such as Antimycin A, had not been considered. Mr Impson responded that Rotenone has experimental use registration in SA and that it was a globally preferred piscicide. Furthermore, Antimycin A has low production volumes and is not readily available for use. It was agreed that there is merit to evaluating this, and other piscicides for *C. gariepinus*.
- **Mr Hoffman** (Mpumalanga Tourism and Parks Agency) inquired regarding the timing of Rotenone treatments and whether flooding during treatments posed a risk to Rotenone impacts extending beyond the treatment area. **Mr Impson** responded that treatments are only implemented during desirable weather and flow conditions to avoid complications from variable flows.
- **Mr Madikizela** enquired if there was media engagement around the success of the current projects, and what the current media and public perception was, given that initially it was negative. **Mr Impson** responded that for the Rondegat treatments a communications specialist was appointed which contributed to the change in public perception. **Prof Weyl** added that the bulk of the current research has been published in peer-reviewed literature but that engagement with the public should only be done once all research findings have undergone peer review and are published in ISI-rated Journals.
- **Dr Roets** enquired whether the level of organic load in the water could result in Rotenone no longer being effective. **Dr Jordaan** responded that there are guidelines in the AFS operating procedures and treatments of waterbodies including compensation for organic loads and an absolute maximum concentrations for fish removals.
- **Dr Rivers-Moore** (Freshwater Research Centre) enquired whether mechanical removal and clove oil had been considered. Issues around the use of clove oil were discussed as was the efficacy of manual removals. Neither was considered a feasible alternative to achieve complete removals of target species.
- **Dr Rivers-Moore** warned that the lessons of the blackfly control project, where the chemical was no longer produced shortly after the commencement of the project because there was no market, should be heeded. He inquired about the shelf-life of Rotenone. **Ms Muir** stated that the shelf-life was 15 years and **Prof**

Weyl pointed out that Rotenone was widely used in fisheries management in the USA and Norway and it is therefore expected that production of Rotenone will continue for the foreseeable future.

- **Mr Madikizela** enquired about the level of conviction that Rotenone could be a national tool for invasive fish control. **Mr Impson** responded that there are a number of factors that need to be considered prior to a Rotenone application, e.g. the species being targeted and water parameters. He stated that there was a need to determine the efficacy of Rotenone in a variety of water types around the country and that this was required for a national policy on Rotenone.

2. Inputs from registration committee

Mr Madikizela requested inputs from the various departments involved in the registration process.

<u>DAFF</u>: Mr Sigube (DAFF) stated that DAFF is the registration authority for pesticides in South Africa. Several registration categories exist and for the proposed use for Rotenone, the correct category must be determined. The existing Rotenone registration is limited to insecticidal use for citrus production. **Ms Muir** stated that the registration document for restricted use of Rotenone as a piscicide by CapeNature had been submitted to DAFF 3-4 months ago. Rotenone should receive restricted use registration only, which will be limited to conservation agencies and have a significant focus on conservation and ecological restoration. It was stated that there is a significant R&D component to registration, which is expensive for the company applying to have a compound registered. It was also stated that Rotenone should be registered as a restricted use chemical, which means that registered users can only procure it. Approval for the import of Rotenone has already been granted. Currently CapeNature is the only registered user for Rotenone use under experimental conditions, similar to the City of Cape Town who has experimental use approval for starlicide.

Mr Sankar (DAFF) raised the point that even though Rotenone should be a restricted use compound, it has the potential to be a useful fisheries management/aquaculture tool so this should be taken into account during the registration process. He indicated that the policy on Inland fisheries is about to go out for public comment (June or July), therefore Rotenone use will not be included in this policy, but should form part of the regulations promulgated from the policy. He also added, that the use of Rotenone as a fisheries management tool be considered.

<u>DEA: MRM</u>: Ms Muir stated that in 2011 the process was initiated to find a tool for controlling invasive fish, which led to the experimental registration of Rotenone and the subsequent pilot treatments, as well as monitoring of the impacts. The question now is how to enable the current knowledge to inform policy development and it was suggested that the development of a policy document could form part of the DEA APO as a deliverable. It was also noted that the current Rotenone work is centred in the Western Cape and the question was asked how it would work in other provinces. Can a single operational approach work in all provinces or should different criteria apply in different provinces?

In terms of public engagement and information, **Ms Muir** supported waiting until all experimental work is completed so that a holistic picture can be presented to the public. In terms of a communication strategy, the peer reviewed science needs to be distilled into information sheets for on the ground users as scientific publications is not easily accessible and often not read by all stakeholders. **Ms Muir** further stated it is critical to have the input and support of all regulatory agencies before any public engagement process is initiated. The current provincial draft policy that was developed for the Western Cape should for now be management guidelines as it is too soon to embark on the development of a national policy.

<u>**DWS**</u>**: Dr Roets** stated that DWS fully supports the use of Rotenone for biodiversity restoration purposes and agreed with **Mr Sankar** on the need to explore a wider range of uses. He added that the criteria for General Authorization 509, which will be required for Rotenone use, is in the process of being amended and can included the management of invasive species for conservation purposes without the necessity of completing a risk matrix.

3. General discussion

Dr Greenfield raised concerns around a possible monopoly of service providers and requested that going forward, there needs to be clarity on who can be an accredited implementer and stated the need for clear criteria on this.

Mr Sigube responded that while clear guidelines and processes exist for registration of Rotenone, a similar process is needed to define the criteria for registered users. **Dr Roets** indicated that only SACNASP registered professional are permitted to compile applications to DWS and a similar criteria such as only registered and accredited Rotenone applicators would be permitted to compile applications for Rotenone treatments.

Mr Mills also enquired about the scope of use for the registration. **Prof Weyl** stated that while the current mandate is limited to biodiversity conservation and restoration, it defines the framework for expanding the registered used in future.

Mr Impson added that the current provincial draft policy makes provision for a range of uses, as well as requiring that all projects must be registered to allow the monitoring of Rotenone use patterns.

Mrs Jonker (Endangered Wildlife Trust) enquired about the possibility of setting Norms and Standards within DEA for the use of Rotenone and **Ms Muir** agreed that this will likely be part of the way forward.

Prof Weyl stated that a summary document from the meeting will be circulated as soon as possible and that everyone present at the meeting would be invited to comment. Once all comments from the existing group has been included and any concerns addressed, the document will be circulated to a wider stakeholder group. **Prof Weyl** thanked all stakeholders for their contributions and concluded the meeting.

Corrections

Mr Impson asked that the following change be made to the notes circulated following the Policy Brief Dialogue

Mr Impson requested that the sentence:

Mr Impson also added that Rotenone has current registration in SA as an organic pesticide so there is **no** risk to using water for irrigation purposes.

Be change to:

Mr Impson also added that Rotenone has current registration in SA as an organic pesticide so there is **negligible** risk to using water for irrigation purposes.