


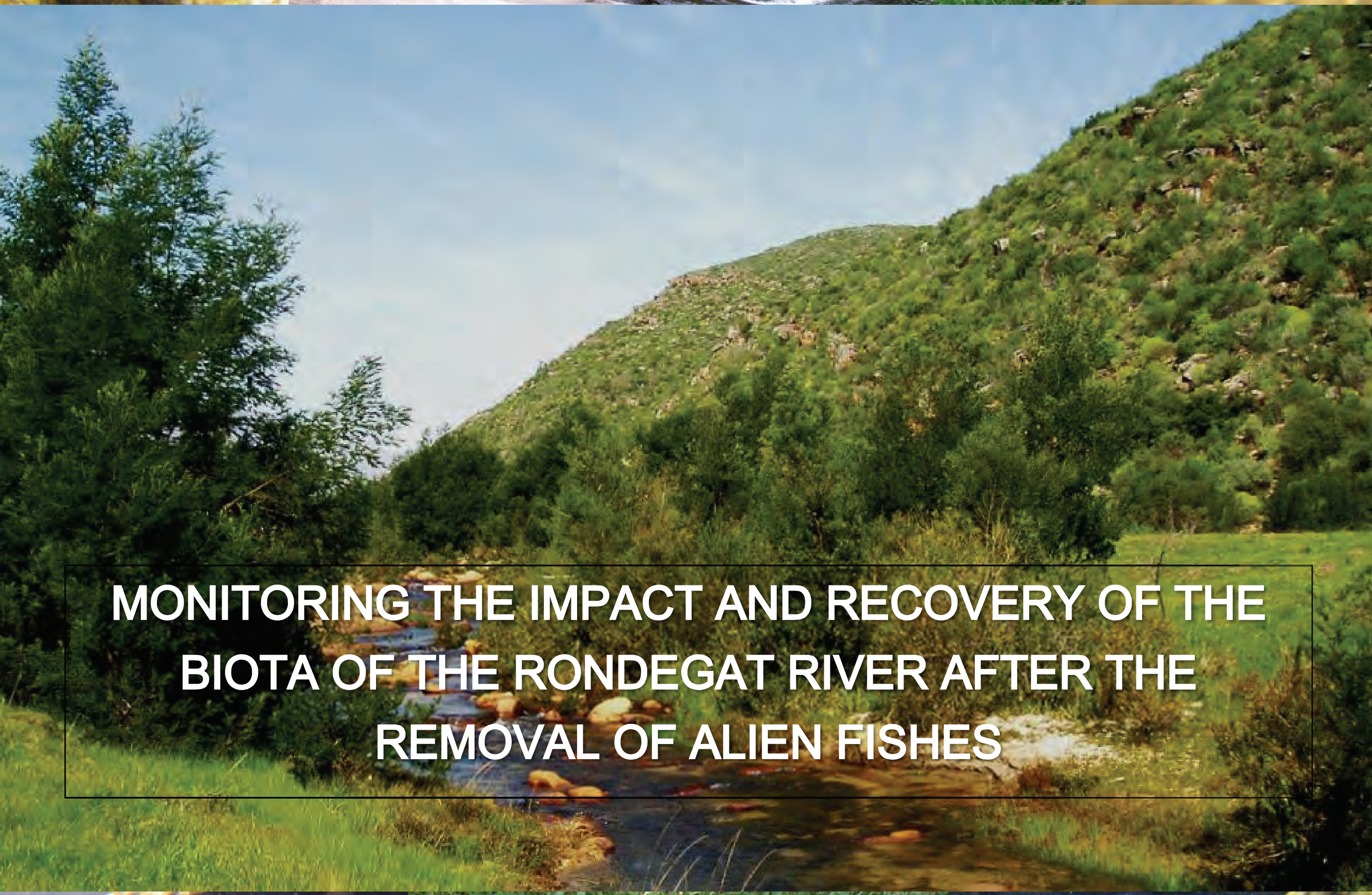

MONITORING THE IMPACT AND RECOVERY OF THE BIOTA OF THE RONDEGAT RIVER AFTER THE REMOVAL OF ALIEN FISHES



Monitoring the Impact and Recovery of the Biota of the Rondegat River after the Removal of Alien Fishes

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

Alien invasive fishes pose the greatest threat to the survival of native fishes in the Cape Floristic Region of South Africa. While the majority of invasive fish are now too widely spread to be eradicated, targeted removal of these fishes from key reaches where re-invasion can be prevented offers a near-term way to improve the survival of some threatened fish populations. The CAPE project, a joint venture between Western Cape government and civil society organisations, began a process in 2003 to identify priority streams where alien invasive fish could be targeted for removal. The Rondegat River in the Cederberg was identified as an ideal candidate for a pilot project whereby invasive smallmouth bass (*Micropterus dolomieu*) would be removed from the stream using the piscicide rotenone. The implementing agency for this project, CapeNature, commissioned a comprehensive Environmental Impact Assessment (EIA) to assess the feasibility and justifiability of using the piscicide rotenone on a 4 km reach of the Rondegat River. The EIA found the project to be justified, and recommended a comprehensive environmental monitoring programme be set up to assess the impacts of fish eradication operations on the ecosystem of the Rondegat River. This report summarises the findings of that monitoring programme.

RATIONALE

The Rondegat River rehabilitation pilot study, in which CapeNature applied rotenone to 4 km of stream to eradicate smallmouth bass in February 2012, represented an important opportunity to quantify the collateral impacts of rotenone as a river rehabilitation method on the invertebrate and amphibian fauna of the river. The project, which sought to remove invasive fish and promote the security of native fish populations upstream of the treated river sections, is part of CapeNature’s ongoing strategy to preserve the biodiversity of CFR rivers as part of its obligations under the National Environmental Management: Biodiversity Act (NEMBA, Act 10 of 2004). The Rondegat, together with the Krom (Cederberg) Suurvlei (Cederberg) and Krom (Eastern Cape) were chosen as pilot study locations through a lengthy expert consultation process to identify rivers that were both logistically feasible rivers for rotenone and stood to improve their conservation status significantly through alien fish removal. There nonetheless was considerable concern voiced in the angling community both in the lead up to the EIA and in the early stages of the public consultation process conducted during the EIA. This controversy, fuelled for the most part by the lack of knowledge of rotenone effects on South African rivers, highlighted the importance of these relatively small-scale pilot studies to empirically assess the pros and cons of rotenone. By quantifying the effect of the treatment on the aquatic invertebrates, as well as assessing its effect on other non-target organisms like tadpoles in the Rondegat River, this monitoring programme contributes knowledge that will allow conservation managers to determine whether rotenone is a feasible and environmentally sound fish conservation tool in South Africa going forward.

OBJECTIVES

Aim 1: Establish a baseline for invertebrate, frog and fish diversity

The first aim of the monitoring programme was to confirm the pre-treatment distribution of native and non-native fishes in the Rondegat River and to confirm the early findings of the environmental impact assessment that no threatened frog species occurred in the vicinity of the treated reach. The initial aim of the invertebrate monitoring programme was to assess the taxonomic richness of the Rondegat river aquatic insect community, using identification to genus or species when possible.

Aim 2: Assess the impact of rotenone on fish, amphibians and invertebrates

The research team assessed the efficacy of rotenone in removing all fish from the treated river reach. Mortality effects on adult frogs and tadpoles would also be assessed during and immediately following the rotenone treatment. During treatment, the effect of rotenone on drift behaviour would be assessed, while its effect on the presence and abundance of key taxa in the treatment zone would be assessed both one week after and two months after the operation.

Aim 3: Incorporate lessons learned into a revised protocol for future rotenone monitoring

Following the completion of initial monitoring in the months following the rotenone treatment, the efficacy and practicality of the monitoring protocol would be critiqued, and recommendations for an improved future protocol for monitoring rotenone operations on streams would be made.

METHODOLOGY – INITIAL MONITORING PROTOCOL

Fish surveys were conducted in February 2011 and February 2012. The latter survey comprised an immediately before and immediately after treatment survey. To ensure all species present in the river were detected, a combination of electrofishing, mask-and-snorkel surveys and underwater video analysis was employed in all zones of the river. A specialist amphibian survey was also conducted in the week before treatment, on the day of treatment, and shortly after treatment. Visual surveys including walked and snorkelling transects were employed together with aural encounter searches to detect frog species. Invertebrate surveys were conducted seasonally, beginning in May 2010, and culminating in a survey in May 2012, two months after the rotenone treatment. The invertebrate surveys comprised kick sampling and individual stone surveys to assess diversity and relative densities of key invertebrate taxa. Fieldwork also included rapid assessment of river health using the SASS5 bioassessment method, while food-web effects were measured by assessing algal production on stone surfaces.

RESULTS AND DISCUSSION

Assessing the impact of rotenone on fish

The river is known to support five species of native fish including two small cyprinid minnows (*Pseudobarbus phlegethon* and *Barbus calidus*), a large cyprinid yellowfish (*Labeobarbus capensis*) a small austroglanidid catfish (*Austroglanis gilli*) and a currently undescribed galaxiid *(Galaxias* cf. *zebratus*). Two other species, the Clanwilliam sawfin (*Barbus serra*) and Clanwilliam sandfish (*Labeo seeberi*) have not been recorded in the river since the 1960s and are locally extinct in the Rondegat. Surveys conducted in 1998 and 2004 showed two alien fish species, the smallmouth bass (*Micropterus dolomieu*) and the bluegill sunfish (*Lepomis macrochirus*) to occupy the lower reaches of the river, although *L. macrochirus* only occurred in the last 500 m of river before it flows into Clanwilliam dam.

Pre-treatment surveys showed fish distributions in the river to be nearly identical to the last major survey of the river's fish fauna, conducted in 2004. This result demonstrated that the barrier to upstream invasion by *M. dolomieu* was stable, and that they were unlikely to invade further without human intervention. The treatment area supported only one native species, the yellowfish *L. capensis*, whereas the other species had been extirpated by *M. dolomieu*. The *L. capensis* population appeared to be an adult-dominated sink population with no local juvenile recruitment. A baseline dataset of fish densities and distributions is provided in Appendix A. Prior to the rotenone operations, a fish rescue operation was conducted, which removed significant proportions of the *L. capensis* and *M. dolomieu* populations from the stream. The remaining fish were all apparently killed by the rotenone operations, and no live *M. dolomieu* were found during fish surveys conducted during the following week. Juvenile *L. capensis* were recorded in the first pool of the treatment area three days after the operation, and two months later both *L. capensis* and *B. calidus* were recorded as far as 200 m downstream of the bass barrier, indicating recolonisation of the treatment area by native fish was underway.

Assessing the impact of rotenone on amphibians

The EIA conducted prior to this project found that three common species of frog occur in the vicinity of river reach earmarked for rehabilitation: the Cape river frog (*Amietia fuscigula*), the Clicking stream frog (*Strongylopus grayii*) and FitzSimons' ghost frog (*Heliophryne depressa*). These species, as well as the Raucous toad (*Amietophrynus rangeri*) and the Cape sand frog (*Tomopterna delalandii*) were found in the vicinity of the treatment area in February 2012. Only the tadpoles of *A. fuscigula* and *T. delalandii* were recorded in waters that were treated with rotenone, which included the many irrigation furrows that run parallel to the river in the treatment area. While many tadpoles of these two species were killed in the operation, these likely represent a small fraction of the total population for either species. 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, before recolonisation by native fishes restores these populations to near-pre-treatment levels.

Assessing initial response and recovery of invertebrates

Seasonal surveys conducted between May 2010 and February 2012 revealed a highly dynamic invertebrate community, which varied significantly in species diversity from year to year. This meant that the invertebrate community was represented by "common" species which were consistently detected in the treatment area, and "rare" species that were only occasionally encountered. As these rare taxa are likely seasonal or incidental residents of the sample sites within the treatment area, they are less informative in demonstrating the impact of rotenone operations than the more common resident species. A baseline dataset of all identified invertebrate taxa is provided in Appendix C. Rotenone operations resulted in the apparent loss of

10 common species, as well as significant declines in the abundance of mayfly families Baetidae and Heptageneidae and the dipteran family Tipulidae. The treatment also precipitated a catastrophic drift event, in which numbers of invertebrates drifting in the treatment zone rose to 100 times natural levels during rotenone application. The treatment did not however significantly alter the community composition of the treatment area, and 5 of the 10 missing common species had returned by May 2012. SASS5 rapid bioassessment scores and Chlorophyll-a analyses revealed no significant changes in ecosystem health that could be attributed to the rotenone operations.

CONCLUSIONS AND RECOMMENDED FUTURE MONITORING PROTOCOL

The fundamental conclusion of the monitoring programme is that CapeNature's river rehabilitation has been a success, in that all bass appear to have been removed from the treatment area without significant long-term damage being accrued by the other faunas of the Rondegat River. The major findings of the research are:

- Rotenone successfully killed all fish present in the treatment area, the majority of which were the alien smallmouth bass.

- While many tadpoles were also killed by the rotenone, this did not appear to represent a significant proportion of the frog populations in the treatment area.

- Although some apparently common dominant species of invertebrate were lost as a result of rotenone operations and had not yet returned to the stream by May 2012, it is highly likely these species will return in the coming years.

- By examining the invertebrate community at the species level over several seasons, we were able to place the effect of rotenone on local diversity in context, and found it to be a minor negative effect in relation to natural seasonal fluctuations in the presence and absence of individual taxa.

- The indirect impact of rotenone on overall ecosystem health was not detectable either through the use of the SASS5 rapid bioassessment scoring system or by monitoring fluctuations in algal production via chlorophyll-a analysis. While we suspect that SASS scoring may indicate ecosystem health effects of rotenone on pristine mountain stream communities, it is unlikely that most rivers earmarked for alien fish removal in the future will contain such communities (with the possible exception of the Krom River in the Cederberg). Neither of these techniques is therefore recommended future monitoring programmes.

- While this project has indicated rotenone to have limited negative effects on the treated ecosystem, it is strongly recommended that on-going monitoring be conducted to assess both fish and invertebrate community recovery following the removal of smallmouth bass from the Rondegat River.

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List of Acronyms

CAPE	Cape Action for People and the Environment
CFR	Cape Floristic Region
EIA	Environmental Impact Assessment
FL	Fork Length
GSM	Gravel-sand-mud
IUCN	International Union for Conservation of Nature
MV	Marginal Vegetation
SAIAB	South African Institute for Aquatic Biodiversity
SASS	South African Scoring System
SIC	Stones-in-current

1 THE ALIEN FISH PROBLEM IN THE CAPE FLORISTIC REGION AND THE REASON FOR THE CAPE PROJECT

1.1 Introduction

The Cape Floristic Region (CFR), which encapsulates the Cape Floral Kingdom, is one of the world's most unique and endangered bioregions (CEPF 2002). It contains a relatively small, but highly endemic fish fauna, the majority of species within this fauna are severely threatened (Skelton 2001). The major threats to most of these species are habitat loss due to poorly managed water abstraction, and alien invasive fishes (Impson et al. 2000; Tweddle et al. 2009). Alien fish species introduced to South Africa for sport fishing, particularly smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*Micropterus salmoides*) have had significant negative impacts on native fish species and aquatic ecosystems in general (Cambray 2003; Tweddle et al. 2009). As an example, the invasion of the Rondegat River in the Cederberg, Western Cape, by smallmouth bass has resulted in the local extinction of three native fishes (Woodford et al. 2005), leading to significantly altered invertebrate communities in the invaded river (Lowe et al. 2008). Alien invasive fish, through direct predation pressure, can completely override the effects of habitat quality on native fish population integrity (Woodford and McIntosh 2010).

1.2 The CAPE Project

In order to address the invasion issues summarised in the previous paragraph, CapeNature – the provincial conservation authority for the Western Cape – has begun a targeted alien fish eradication programme in four pilot areas. This programme has been manage as part the Cape Action for People and the Environment (CAPE) project, a joint venture between government and civil society organisations in the Western Cape, aimed at conserving the unique diversity of the CFR. There are very few effective ways to control the impact of invasive predatory fish on native fish. Mechanical removal through electric fishing is a method of controlling alien fish abundance, but it is highly labour intensive (Moore et al. 1986), and is only a short term solution to the problem of alien predators, as eradication using this method is extremely difficult (Thompson and Rahel 1996). One method that has shown significant recent success in eradicating pest fish from streams where they threatened native fish is the use of the piscicide rotenone.

The CAPE project seeks to remove invasive fish and promote the security of native fish populations upstream of the treated river sections and is part of CapeNature's ongoing strategy to preserve the biodiversity of CFR rivers as part of its obligations under the National Environmental Management: Biodiversity Act (NEMBA, Act 10 of 2004). The Rondegat River, together with the Krom River (Cederberg) Suurvlei River (Cederberg) and Krom River (Eastern Cape) were chosen as pilot study locations through a lengthy expert consultation process to identify rivers that were both logistically feasible rivers for rotenone and stood to improve their conservation status significantly through alien fish removal (Enviro-Fish Africa 2009). A comprehensive Environmental Impact Assessment (EIA), which included a lengthy consultation process with scientists, landowners and other affected parties, concluded that the pilot study on the Rondegat River was both appropriate and necessary, provided the impact of the piscicide on non-target organisms was properly monitored to inform future rehabilitation efforts using rotenone in South Africa (Enviro-Fish Africa 2009).

The Rondegat River rehabilitation pilot study, in which CapeNature applied rotenone to 4 km of stream to eradicate smallmouth bass in February 2012, represented an important opportunity to quantify the impacts of rotenone as a river rehabilitation method on native vertebrates and invertebrates. This was seen as an opportunity to use this relatively small-scale pilot study to empirically assess the pros and cons of rotenone. By accurately quantifying the effect of the treatment on target and non-target organisms, it is possible to determine whether or not rotenone is a feasible and environmentally sound fish conservation tool in South Africa.

1.3 The Need for a Robustly Monitored Alien Removal Operation in the Rondegat River

The primary goal of CapeNature's pilot rehabilitation programme on the Rondegat River is to rehabilitate the stream's native fish fauna through the removal of invasive alien fish. The programme also has the additional objective to assess the feasibility of using rotenone to rehabilitate other rivers in the Cape Floristic Region that are threatened by invasive alien fish (Enviro-Fish Africa 2009). To achieve this secondary objective, quantitative monitoring of the immediate and long-term effects of alien eradication by rotenone on the fish, amphibian and invertebrate communities is critical, so that the effectiveness of the treatment method can be ascertained. The success of the project will depend firstly on the ability of the piscicide to completely eradicate alien species, and secondly on the ability of invertebrates, native fish and amphibians to re-colonise the river after treatment. Such success should result in at least a partial restoration of the ecosystem integrity of the treated river section, which has been significantly altered by the introduced fish (Lowe et al. 2008).

2 LITERATURE REVIEW ON THE USE OF ERADICATION TREATMENTS IN RIVERS FOR CONSERVATION PURPOSES

2.1 Use of Rotenone as a Tool for Alien Fish Control in Rivers

Rotenone (see Box 2.1 for a detailed description) has been used in the United States as a fisheries tool since the 1930s for fisheries management, being used to eradicate pest fishes in order to promote commercially important species and protect native species threatened by introduced fishes (Ling 2003). The emphasis on its usage has shifted over time from “renovation” of water bodies to support the introduction of sport fishes to an emphasis on eradication of invasive species from sensitive waterways, although the maintenance of sport fisheries is still listed as the most common reason for its use in America (Ling 2003; McClay 2000). 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), Australia (Lintermans 2000; Rayner and Creese 2006) and New Zealand (Chadderton et al. 2003). In all of these cases, alien fish were successfully eradicated from the treated water body. In one case, native fish were successfully re-introduced to a treated reservoir after the removal of the aliens (Britton and Brazier 2006). In another case, a stream section between two barriers to upstream movement saw the natural re-colonisation of native fish from upstream after the invasive fish had been removed (Lintermans 2000). This example indicates that rotenone can be an effective conservation tool in sensitive river areas threatened by invasive fish, provided adequate barriers exist in the stream to prevent re-invasion by the alien fish.

While alien fish removal by rotenone has been demonstrated to be an effective management tool, it has been surrounded by controversy in recent years due to its known and unknown collateral effects on aquatic organisms (McClay 2000). Native fish are generally as susceptible to the toxin as the target introduced species, such that rotenone is preferably used in situations where the invasive fish has severely depleted or completely eradicated native fish in the water bodies marked for treatment. As such, the short-term drawback of killing low numbers of native fish in the rotenone treatment is eventually outweighed by the successful re-colonisation of that reach by native fishes over time (Lintermans 2000).

The major controversy surrounding rotenone treatment in recent times has resulted from the limited and conflicting data on the effects of the toxin on invertebrate communities (Vinson et al. 2010). This controversy has led to public opposition to piscicide use in fisheries management in America, and even resulted in some states placing a moratorium on the use of rotenone (McClay 2000). However, the severity of rotenone impacts on invertebrate communities has proved to be highly variable, ranging from minimal to severe, thus making broad conclusions about the danger posed by rotenone to the aquatic environment difficult. An overview of previous research into this problem follows.

Box 2.1: An overview of the piscicide rotenone

Rotenone is a natural toxic chemical (Empirical formula: $C_{23}H_{22}O_6$) found in the roots of many tropical plants of the Leguminosae family. The most common commercial source is the derris plant (*Derris eliptica*), the roots of which contain on average 5% rotenone. The chemical acts as an inhibiter of cell metabolism, resulting in the failure of respiratory functions and death by tissue anoxia. While highly toxic at sufficient doses to many organisms, lethal concentrations vary greatly among different animal groups, although it is extremely toxic to fish. The chemical does not have any endocrine disrupting properties, does not appear to be carcinogenic, and breaks down rapidly under natural conditions. While it has been shown to produce Parkinson's Disease-like symptoms when injected at high concentrations into lab rats, subsequent research indicates that people exposed to piscicides containing rotenone are unlikely to develop Parkinson's Disease. Rotenone is highly sensitive to light and air, and quickly breaks down when exposed to sunlight. It has a half-life in water of 1 to 3 days, losing its toxicity faster in warm water than in cold water. Rotenone does not leach easily into the soil, thus limiting the threat to ground water. Its toxicity can be quickly neutralised by exposure to potassium permanganate ($KMnO_4$).

Ground-up roots containing rotenone have been used for centuries by the indigenous peoples of South America and South-East Asia to narcotise (render unconscious) fish for human consumption. It has been used extensively as a pesticide on food crops, particularly in the United States, and as a piscicide for fisheries management. Freshwater and marine scientists also use rotenone as a fish-sampling tool, where it is used to capture cryptic species. Rotenone is considered to be the most environmentally benign fish toxicant in use today.

Information adapted from Ling (2003) and Muller (2009)

2.2 Known Impacts of Rotenone on Invertebrates in Streams

2.2.1 Laboratory tests

Recent reviews on the impact of rotenone on stream invertebrates (Ling 2003; Vinson et al. 2010) have shown that the chemical varies greatly in its toxicity across species, families and orders of insects. The majority of laboratory data that have been obtained for rotenone toxicity are measurements of acute toxicity, exposing animals to the chemical for 96 hours or less, and recording the dosage which is lethal to 50% of the test population (LC50). The results of toxicity tests for riverine invertebrates and selected fish species are summarised in Table 2.1 (where LC50 concentration is listed) and Table 2.2 (where only concentrations at invertebrate mortality, with no population-proportion information, were available).

Lethal concentrations of rotenone range from 0.003 mg/l over 3 hours to 0.2 mg/l over 48 hours, depending on the species being tested (Tables 2.1 and 2.2). Two major trends emerging from the laboratory tests are that:1) smaller invertebrates appear to be more sensitive than larger invertebrates, and 2) invertebrates with gills are more sensitive than invertebrates that derive oxygen using other means (Vinson et al. 2010). The driving factor in this variation appears to be surface area-volume ratio of the animal and its respiratory organs, with small planktonic invertebrates and animals with gills (which have a high sa:v ratio) absorbing the most rotenone. Regarding the relative vulnerability of invertebrates to fish, it is interesting to note that the centrarchid fishes, represented by sunfish (*Lepomis macrochirus*) in Table 2.1, but which include smallmouth bass, the target species on the Rondegat River, have an LC50 an order of magnitude lower than that recorded for the majority of invertebrate taxa (0.007; Table 2.1), while lethal concentrations for invertebrate taxa in Table 2.2 are generally above 0.01 (Table 2.2).

Table 2.1: Toxicity data for fish and riverine invertebrates sourced from Ling (2003), Vinson and Vinson (2007) and the US EPA AQUIRE database [adapted from Muller (2009)].

Taxonomic group	Family	Common name	Species	Test end point	Exposure duration (h)	Concentration (mg/L)
Turbellaria		Flatworm	*Catenula* sp	LC50	3	8.9
				LC50	6	6.4
				LC50	24	5.1
				LC50	96	1.7
		Flatworm	*Planaria* sp.	LC50	24	<0.5
Hirudinea		Leech		LC50	48	<0.1
Hydracarina	Hydrachnidae	Water mite		LC50	96	0.05
Plecoptera		Stonefly	*Pteronarcys californica*	LC50	24	2.9
				LC50	48	1.9
				LC50	96	0.32
Ephemeroptera	Baetidae	Mayfly	*Cloeon dipterum*	LC50	3	1.6
				LC50	6	0.18
				LC50	24	0.075
				LC50	48	0.056
Odonata	Macromiidae	Dragonfly	*Macromia* sp.	LC50	3	275
				LC50	6	34
				LC50	24	4.7
				LC50	96	1
			Basiaeschna janata	LC50	96	0.22
Hemiptera	Notonectidae	Backswimmer	*Notonecta* sp	LC50	1	105
				LC50	3	21
				LC50	6	9
				LC50	24	3.4

				LC50	96	1.6
			Notonecta sp.	LC50	24	0.1
Trichoptera	Hydropsychidae	Caddisfly	*Hydropsyche* sp.	LC50	1	10.7
				LC50	3	8
				LC50	6	3.6
				LC50	96	0.6
Coleoptera	Gyrinidae	Whirligig beetle	*Gyrinus* sp.	LC50	1	47.5
				LC50	3	8.8
				LC50	6	8
				LC50	24	3.6
Coleoptera	Gyrinidae	Whirligig beetle	*Gyrinus* sp.	LC50	48	0.7
Diptera		Midge	*Tanytarsus dissimilis*	LC50	48	0.04
Fish	Galaxidae	Black mudfish	*Neochanna diversus*	EC50	1	<0.001
	Cyprinidae	Common carp	*Cyprinus carpio*	LC50	6	0.014
				LC50	24	0.015
				LC50	96	0.003
		rudd	*Scardinius erythropthalmus*	LC50	1	0.025
		roach	*Rutilus rutilis*	LC50	1	0.085
				LC50	6	0.038
				LC50	24	0.025
	Centrarchidae	sunfish	*Lepomis macrochirus*	LC50	24	0.007
	Salmonidae	Rainbow trout	*Oncorhynchus mykiss*	LC50	3	0.006
				LC50		
				LC50	48	0.002
				LC50	96	0.002

Table 2.2: Invertebrate toxicity data for which test endpoints (lethal concentration for percentage of test population) were not recorded [US EPA AQUIRE database – adapted from Muller (2009)].

Taxonomic group	Common name	Species	Test endpoint	Exposure duration (hrs)	Concentration (mg/L)
Plecoptera	Stonefly	*Agnetina sp.*	Mortality	14	0.016
	Stonefly	Chloroperlidae sp.	Mortality	48	0.048
	Stonefly	*Isogenus sp.*	Mortality	6	0.006
	Stonefly	*Pteronarcys sp.*	Mortality	48	0.072

Ephemeroptera	Mayfly	*Paraleptophlebia sp.*	Mortality	48	0.144
	Mayfly	Heptageniidae sp.	Mortality	14	0.016
Odonata	Dragonfly	*Aeshna sp.*	Mortality	3	0.003
Trichoptera	Caddisfly	*Glossosoma sp.*	Mortality	72	0.072
	Caddisfly	*Cheumatopsyche sp.*	Mortality	48	0.144
	Caddisfly	Philopotamidae sp.	Mortality	9	0.009
	Caddisfly	*Limnephilus sp.*	Mortality	24	0.024
	Caddisfly	*Brachycentrus sp.*	Mortality	48	0.144
	Caddisfly	*Psychomyia sp.*	Mortality	6	0.006
	Caddisfly	*Phryganea sp.*	Mortality	48	0.2
	Caddisfly	*Psilotreta sp.*	Mortality	24	0.024
	Caddisfly	*Pycnopsyche sp.*	Mortality	24	0.024
	Caddisfly	*Rhyacophila sp.*	Mortality	14	0.016
Coleoptera	Riffle beetle	*Limnius sp.*	Mortality	48	0.048
Diptera	Cranefly	*Antocha sp.*	Mortality	48	0.009
	Crane fly	*Eriocera sp*	Mortality	48	0.072
	Midge	*Pentaneura sp.*	Mortality	24	0.024
	Snipefly	*Atherix sp.*	Mortality	48	0.048
	Alderfly	*Chauliodes sp.*	Mortality	48	0.048
	Blackfly	Simuliidae sp.	Mortality	6	0.006
	Mosquito	*Aedes aegypti*	Mortality	24	100

2.2.2 Field studies

There have been several field studies that have investigated the effect of rotenone treatment of invertebrates in streams, which were summarised by Vinson et al. (2010). While some studied have reported negligible impacts of rotenone, others have reported significant alterations in invertebrate density and diversity. Part of this variation is likely the result of variation in rotenone concentrations and treatment duration, variation in sampling effort and length of time monitoring after treatment, as well as varying vulnerability to rotenone among the invertebrate taxa present (Vinson et al. 2010).

General trends to emerge have been that aquatic insects are more vulnerable to rotenone than other aquatic invertebrates, and that Ephemeroptera, Plecoptera and Trichoptera are more vulnerable than Coleoptera and Diptera.

Perhaps the most striking effect of rotenone treatment in streams is that it often triggers “catastrophic drift” in many species, resulting in large numbers of animals exiting the treated area on contact with the rotenone and drifting downstream (Arnekleiv et al. 2001; Dudgeon 1990; Lintermans and Raadik 2003). Catastrophic drift is generally an immediate and short-lived response of the invertebrate community when rotenone is applied, although raised levels in drift has been recorded for several days after treatment in some instances, presumably due to residual amounts of rotenone leaching into the water column (Lintermans and Raadik

2003). Catastrophic drift is seen as a behavioural response to contact with rotenone, and the majority of insects in the drift have been found to be alive, with few moribund individuals (Dudgeon 1990). Thus rotenone treatment may not be lethal to the majority of invertebrates with high drift propensity, though it could have severe impacts on less mobile species.

Recovery time of invertebrate communities to pre-treatment status is highly variable and previous studies have reported times ranging from months to years, with rare species in particular often not returning to the site during the period of monitoring (Vinson et al. 2010). Recovery rates are dependent on multiple factors, including the proportion of sensitive, rare and immobile species in the invertebrate community, the length of the stream being treated and the availability of nearby untreated habitats. Re-colonisation rates of individual taxa will also be affected by generation time and dispersal behaviour of individual taxa, with the amount of stream habitat upstream of the treatment area being extremely important for recovery of drifting invertebrates (Niemi et al. 1990).

3 THE VERTEBRATE BIOTA OF THE RONDEGAT RIVER

3.1 Introduction

The Rondegat river, a small (average width 5 m), clear, perennial river, flows 25 km from its source into the 1043 ha Clanwilliam Dam. The river flows through relatively pristine fynbos in its upper reaches and through agricultural pasture in its lower reaches. The dense alien riparian vegetation that dominated the middle reaches of the river in 2004 (Woodford et al. 2005, Lowe et al. 2008) was cleared during catchment rehabilitation in 2011. Three potential barriers to fish movement are present in the system. The first, a small one meter waterfall followed by a long bedrock cascade (32 15.365 S, 18 57.135E) is located 625 m from the Clanwilliam Dam and a 3 m high water abstraction weir (32 15.536S, 18 57.812E) is located another 365 m upstream. Four km after this weir the Rooidraai waterfall marks the upstream barrier to alien fish invasion (Woodford et al. 2005).

Figure 3.1 shows the Rondegat River from its headwaters to its confluence with the Clanwilliam Dam. Bass are present in the river from the dam up to the Rooidraai waterfall barrier, and CapeNature treated the four kilometres between this barrier and the weir downstream with rotenone in February 2012 (area shown in red on Figure 3.1). Monitoring was undertaken in all the different colour zones as indicated in Figure 3.1. The treatment area (red) and below treatment area (yellow) are referred to as invaded zones, while the control area (green) and pristine area (blue) are referred to as non-invaded zones.

This chapter briefly reviews the current vertebrate fauna of the Rondegat River, including an assessment of the likely recovery rate of each species following alien fish removal.

3.1.1 The fish of the Rondegat River

The fish of the Rondegat River are part of the Olifants-Doring fauna, which is notable for its extremely high proportion of both endemic and threatened species (Skelton et al. 1995; Tweddle et al. 2009). The Rondegat River fish fauna is dominated by cyprinid species, while also including a galaxiid species that is currently undergoing taxonomic revision, and an austroglanidid catfish. Like many other rivers in the Olifants-Doring catchment, the native species are restricted to the upper reaches of the river, while the lower reaches are dominated by two introduced North American centrarchid species. Two native cyprinid species, the Clanwilliam sandfish (*Labeo seeberi*) and the Clanwilliam sawfin (*Barbus serra*), were recorded in the lower reaches of the Rondegat River in the 1960s (Van Rensberg 1966), but have not been confirmed in the river in subsequent surveys. It is likely a combination of predation by the introduced centrarchid fishes and the fragmenting effect of Clanwilliam dam on migrations have led to the local extinction of these species in the Rondegat.

Table 3.1 below outlines the main characteristics of the native fish species recently recorded in the Rondegat River, and their likely recovery rate following an eradication treatment. All native species are endemic to the Clanwilliam-Olifants River system. Table 3.2 details the alien fish in the Rondegat River, the way in which these species are introduced and spread, their effect on native species, and their likely recovery rate. Plates 3.1 to 3.7 show photographs of each species.

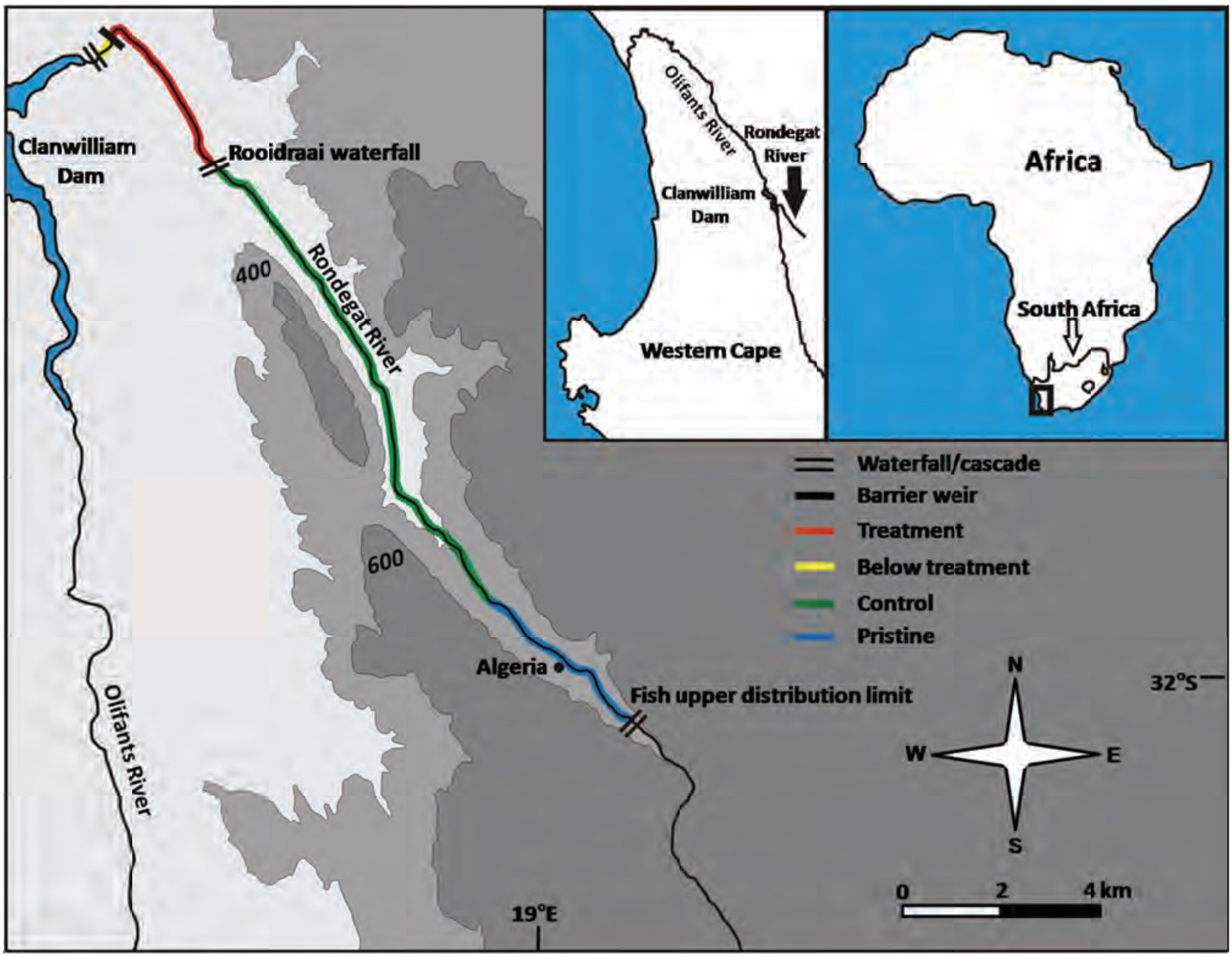

Figure 3.1: Map of the Rondegat River indicating the four areas: treatment (red) and below treatment (yellow) areas in the invaded zone and the control (green) and pristine (blue) areas in the non-invaded zone.

Table 3.1: The main threats and estimated recovery rates of the native fish species found in the Rondegat River

Common Name	Species Name	IUCN Red-list status	Main Threats	Estimated Recovery Rate
Clanwilliam yellowfish	*Labeobarbus capensis* (Smith, 1841)	Vulnerable	Inappropriate agricultural practices leading to habitat destruction and impacts of alien invasive fish species (Cambray 1999). While adults are able to inhabit sections of river that are invaded by piscivores, their juveniles are susceptible to predation which is likely to affect recruitment. Healthy numbers of recruiting fishes of *L. capensis* are found only in areas free of alien fishes (Impson 2007).	Due to its large size, the high age to maturity, longevity and slow growth rate, *L. capensis* is likely to have longer recovery rates than the smaller cyprinids in the Rondegat River. It currently survives as an adult-dominated sink population in the lower reaches of the Rondegat River, where a lack of juvenile recruitment makes the species dependent on immigration from the bass-free upper reaches (Woodford et al. 2005). As a result of the long recovery time of this population, the removal of individual fish prior to the rotenone treatment and subsequent restocking is recommended. Rapid reintroduction should ensure a quicker recovery to pre-invasion population numbers than the other native species in the river.
Clanwilliam redfin	*Barbus calidus* (Barnard, 1938)	Vulnerable	Inappropriate agricultural practices leading to habitat destruction and impacts of alien invasive fish species (Impson and Swartz 2002).	Neither the growth rate nor population structure of this fish has been evaluated. Given the biology of other small barbs, however, it is expected that populations will rebuild in shorter time spans than more k-selected species.
Fiery redfin	*Pseudobarbus phlegethon* (Barnard, 1938)	Endangered	Directly threatened by predation from alien predators (Swartz et al. 2004), its reproductive and feeding biology make it vulnerable to habitat degradation.	Neither the growth rate nor population structure of this fish has been evaluated. Given the biology of other small barbs, however, it is expected that populations will rebuild in shorter time spans than more k-selected species.

Clanwilliam rock catfish	*Austroglanis gilli* (Barnard, 1943)	Vulnerable	Inappropriate agricultural practices leading to habitat destruction and impacts of alien invasive fish species (Skelton 2001).	The life-history of *A. gilli* is characterised by slow growth, long life span and low relative fecundity suggesting that the species relatively precocial and k-selected. The population of a precocial species is relatively stable, and when the population numbers were to be greatly reduced, they would require a long time to rebuild. Re-colonisation from upstream habitats will also be slow as there are indications of territoriality.
Cederberg galaxias	*Galaxias nov. sp*. [currently *Galaxias zebratus* Castelnau (1861)]	Data deficient	This species has only recently been discovered and threats have never been assessed. Likely threats, however, include inappropriate agricultural practices leading to habitat destruction and impacts of alien invasive fish species (Skelton 2001).	It is hard to know if the Cederberg galaxias ever occurred in the lower reaches of the river, or if these reaches represent appropriate habitat for the species (Woodford et al. 2005). Thus it is unclear if Galaxias will colonise the reach after alien fish are removed.

Table 3.2: The alien fish species found in the Rondegat River.

Common Name	Species Name	Introduction and spread	Main Threats to Native species	Estimated Recovery Rate
Smallmouth bass	*Micropterus dolomieu* (Lacepede, 1802)	An angling species imported from the USA in 1937 to fill the gap between high altitude trout waters and slow flowing low lying largemouth bass zone (De Moor and Bruton 1988). Subsequently spread by anglers into various river systems. It is widespread in temperate regions of South Africa. In the Olifants system it was stocked in Clanwilliam Dam which where a major sport fishery occurs for this species. Eradication from the dam is not feasible.	Predation and extirpation by this species on native fishes and invertebrates in South African rivers has been linked to ecosystem effects that include changes in invertebrate community structure that resulted in differences in grazing and concomitant differences in algal biomass between invaded and non-invaded sections in the Rondegat River (Lowe et al. 2008).	Unless restocking and reinvasion by migration from downstream sources can be stopped recovery in the Rondegat River is unlikely.
Bluegill sunfish	*Lepomis macrochirus* (Rafinesque, 1819)	This angling and fodder fish was imported from the USA in 1938 primarily as a forage fish for bass (De Moor and Bruton 1988). Has been stocked widely through formal stocking initiatives and illegally by anglers and has established populations in parts of most major South African river systems. Prefers slow flowing or still waters. It was stocked in Clanwilliam Dam from where it has invaded the Rondegat River.	Bluegill is a prolific invader that dominates the fish fauna in many localities. Because it is a predator, it exerts considerable predation pressure on invertebrates and on juveniles of indigenous fishes. Examples include the decline of indigenous cyprinid populations after the stocking of bluegill in the Krom and Kouga River systems (De Moor and Bruton 1988).	Unless restocking and reinvasion by migration from downstream sources can be stopped recovery in the Rondegat River is unlikely.

Plate 3.1: Clanwilliam yellowfish (SAIAB/Weyl)

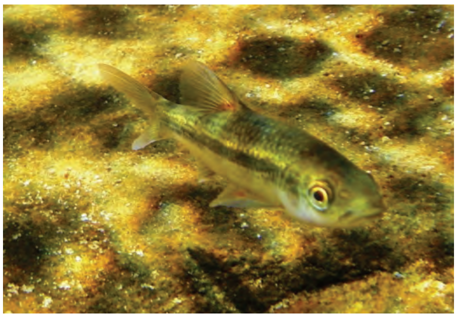

Plate 3.2: Clanwilliam redfin (SAIAB/Weyl)

Plate 3.3: Fiery redfin (SAIAB/Weyl)

Plate 3.4: Clanwilliam rock catfish (SAIAB/Weyl)

Plate 3.5: Cederberg galaxias (SAIAB/Woodford)

Plate 3.6: Smallmouth bass (SAIAB/Woodford)

Plate 3.7: Bluegill sunfish (SAIAB/Skelton)

3.1.2 The frogs of the Rondegat River

In contrast to the fish fauna, the Cederberg contains a relatively high diversity of frogs but few endemic species (Cunningham 2009). The fynbos and succulent karoo biomes interdigitate across the Greater Cederberg, resulting in relatively high regional amphibian species diversity (frogs are the only class of amphibian occurring in South Africa). At a local scale, however, the transition zone shows relatively low species diversity and few biome endemics. The Rondegat Valley, and in particular the treatment zone, traverses a strong local gradient from fynbos to succulent karoo habitats. An earlier compilation of amphibian records from across the Cederberg recorded only three species within the Rondegat catchment with no records within the treatment zone at the lower end of the valley (Cunningham, 2011). Bioclimatic modelling of potential species occurrence predicted 6-7 frog species in the treatment zone with a further 2-3 species restricted to montane fynbos of the upper catchment (Cunningham, 2011).

The three species of amphibians occurring in the vicinity of the treatment area are the FitzSimons' ghost frog (*Heliophryne depressa*), the Cape river frog (*Amietia fuscigula*) and the clicking stream frog (*Strongylopus grayii*). All three species are primarily aquatic in their larval phase, and are thus most vulnerable to rotenone as tadpoles. Thus monitoring of the amphibian community in this programme focused on tadpoles rather than adults, the biology of which are described in Table 3.3 below. Plate 3.8 shows live photographs of the Cape river frog.

Plate 3.8: Live pictures of Cape river frog tadpoles (SAIAB/Weyl)

Table 3.3: The three frogs occurring in the vicinity of the treatment area of the Rondegat River

Common Name	Species Name	IUCN Red-list status	Main Threats	Estimated Recovery Rate
FitzSimons' ghost frog	*Heliophryne depressa* (FitzSimons, 1946)	Least Concern	This species occurs primarily in mountain stream reaches upstream of alien fish and the majority of freshwater fish, in what are generally near-pristine fynbos streams. As such they face no significant natural or anthropogenic threats at present.	Due to its complementary distributions with large predatory fish, tadpoles of *H. depressa* are not expected to occur within the treatment area and should not be affected by the rotenone treatment.
Cape river frog	*Amietia fuscigula* (Dumeril & Bibron, 1841)	Least Concern	Due to its wide range and flexible habitat preferences, *A. fuscigula* does not face any serious threats in the Cederberg or elsewhere.	Because the species is present in the treatment area and tadpoles occur at all times of the year, it is likely that some tadpoles will be vulnerable to the rotenone treatment. However, because adult *A. fuscigula* will be largely unaffected by the treatment, and will begin breeding in the months directly following implementation, it is likely that tadpoles will re-appear within the treatment area far quicker than any of the native fish species (apart from Clanwilliam yellowfish which will be re-introduced from holding pens at the conclusion of the rotenone treatment).
Clicking stream frog	*Strongylopus grayii* (Smith, 1849)	Endangered	Due to its wide range and flexible habitat preferences, *S. grayii* does not face any serious threats in the Cederberg or elsewhere.	A single record of adult *S. grayii* near the top of the treatment area in the Rondegat River (Cunningham 2009) suggests tadpoles may be present there at the time of rotenone treatment. Because adult *S. grayii* will be largely unaffected by the treatment, and will begin breeding in the winter months following implementation, recovery of tadpole populations in the treatment area is expected to be rapid.

4 SAMPLING METHODS

4.1 Introduction

The purpose of the fish and amphibian component of the research was to quantify the initial impact of the eradication treatment, and enable further research into the nature and extent of ecological recovery. This was be achieved by undertaking fish and amphibian surveys of the upper and lower Rondegat River prior to and immediately after the treatment. The invertebrate survey techniques used are described after the vertebrates.

4.2 Methods for Monitoring the Fish Fauna of the Rondegat River

4.2.1 Field methods

Forty three sites were sampled, 17 sites in the non-invaded zone upstream of the Rooidraai waterfall and 26 sites in the invaded zone downstream of the waterfall. Within the invaded zone, 17 sites were sampled in the 4 km treatment area between the Rooidraai waterfall and the weir (treatment area – Figure 3.1). Sampled habitats included riffle, run and pool habitats. At each site, temperature, conductivity and pH were measured using a Hanna HI98129 Combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, USA). Turbidity (NTU) was measured using a Hanna HI 98703 turbidimeter (HANNA Instruments Inc.). To estimate pool volume, the length (± 0.1 m) of each pool was measured followed by between three and five (depending on habitat), equally spaced, width measurements (± 0.1 m). On each width transect, three depths (± 0.1 m) were measured, the outer two were each 0.2 m from the left- and right-hand river bank and the third measurement taken midstream.

Pre-treatment fish surveys were conducted in February 2011 and February 2012. The timing of the surveys at the end of summer falls within a low flow period, during which sampling was considered most effective, allowing for better replicability on subsequent surveys. In the treatment area, a post treatment survey was conducted 24 hours after the rotenone treatment. Three sampling methods, snorkel transects (Plate 4.1), underwater video analysis (Plate 4.2), and backpack electrofishing (Plate 4.3), were used to assess for species composition, population structure and relative abundance in the fish community. Habitat type and site characteristics determined the sampling method employed at each site. While electrofishing (30 sites) was limited to shallower sites (<1 m deep), snorkelling (40 sites) and underwater video analysis (37 sites) were used in a wide range of habitats.

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

As a result of the low conductivity in the river (11-70 $\mu S.s^{-2}$) electrofishing was conducted downstream into a fine meshed block net. A Samus© 725G backpack electrofisher was used and the settings for the electrofisher were standardised at a duration of 0.3 ms and a frequency of 90 Hz. At each sample site, three-passes were conducted with the electrofisher. Fish captured during each pass were placed in a separate bucket where after they were identified to species level, counted, measured to the nearest mm fork length (FL) and released. The total number of fish sampled during three passes was taken as the representative of the number of fish in the sampling site.

Underwater video analysis was conducted using a GoPro® HD Hero® high definition camera fitted with a corrective lens for full use underwater. Camera settings were standardised at; Field of view = 127º, Resolution (Full HD) = 1080p (1920×1080), Frames per second = 30 NTSC, 25 PAL. Methods for placement, observation time and analysis followed those recommended by Ellender et al. (2012). The camera was deployed at each site for 30 minutes; the first five minutes were then excluded from analyses as an acclimation period for conditions to return to normal in the sample pool following camera deployment.

4.2.2 Collection of fishes during eradication exercise

The eradication exercise had two phases: (1) a fish rescue operation conducted in January 2012 when fish were caught in the treatment area using fyke nets and by angling and (2) the rotenone treatment in February 2012. The rotenone treatment consisted of a $50 \mu g.l^{-1}$ (active ingredient) treatment for a duration of six hours. All fish caught during both phases were identified, enumerated, measured and weighed (nearest 0.1 g). During the rotenone treatment, all dead fish were collected with the help of 15 volunteers whom patrolled the entire river.

4.2.3 Analysis

In all analyses each site sampled was treated as a replicate and all tests were conducted at a significance level of $p<0.05$. To compare the efficacy of different sampling methods, a detection rate (sample sites with positive records/all sample sites) was calculated for each species. Differences between methods were assessed by 2×3 methods χ^2 contingency analysis (MS Excel 2007, Microsoft®). To test for differences in fish abundance between surveys and between invaded and non-invaded sites for *L. capensis* (the only species that occurred in both reaches), snorkel survey and electrofishing fish counts were converted to density of fish per m^2 of habitat sampled and compared using the non-parametric Mann-Whitney U Test (Statistica 8.0, StatSoft®). Underwater video analysis 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). Estimates of fish density obtained in the treatment area using snorkel transects were compared to the estimated fish density (from fish rescue and rotenone treatments) using a 2 tailed t-test.

Plate 4.1: Snorkel surveying (SAIAB/Weyl).

Plate 4.2: Underwater video survey images (SAIAB/Weyl).

Plate 4.3: Electrofishing with nets placed at the pool entrance and exits (SAIAB/Weyl).

4.3 Methods for Monitoring the Amphibian Fauna of the Rondegat River

Visual and aural encounter searches for frogs were made along the stream banks, in side pools, and around isolated ponds, seepages and drainage ditches away from the stream (Figure 4.1 and Table 4.1). The full length of the treatment area was searched twice before treatment, once during the release of rotenone, and once after treatment. Similar searches were conducted of approximately 1 km stream sections above and below the treatment area before and after treatment, and of a 3 km stretch above Algeria in the pristine area, ±15 km upstream of the treatment area, which includes the upper limit of indigenous fishes. Night-time spotlighting searches were made along half the stream length within the treatment area and around irrigation wetlands at Rietvlei and Keurbos farms. A further spotlighting search was made at Algeria, covering around 400 m of stream, including a section of the Rondegat River with indigenous fishes as well as a fish-free side stream. The aim of these searches was to determine which frog species occur in the area and to provide a rough gauge of frog activity and abundance. GPS point localities were recorded wherever frogs were encountered. Tadpoles were captured with an aquarium net or collected as drift by participants at fish monitoring stations during the rotenone release. These collections were preserved in 10% formalin as vouchers for identification, to be registered at the South African Institute for Aquatic Biodiversity (SAIAB). Photographs were taken of frogs and typical biotypes, and calls were digitally recorded as audio vouchers. Plate 4.4 shows Frog Transect 1 (Rg1) at the lower end of the treatment area (refer to Figure 4.1 for the location of Rg 1).

Table 4.1: Amphibian abundance transects (above and below water).

Transect	Area	Position (km)	Length (m)	% Snorkelled
Rg0	Treatment area	-0.30-0.00	300	60%
Rg1	Treatment area	0.22-0.62	400	50%
Rg2 – Rietvlei	Treatment area	1.38-1.68	300	80%
Rg3 – Keurbos	Treatment area	2.49-2.79	300	80%
Rg4 – Rooidraai	Treatment area	3.93-4.23	300	70%
Rg5	Control area	5.67-5.97	300	70%
Rg6 – Grootfontein	Control area	6.38-6.76	380	10%
Rg7 – Algeria above weir	Pristine area	19.73-20.33	600	0%
Rg8 – above fish barrier	Above pristine area	22.25-22.65	400	60%

Area refers to the areas shown in Figure 3.1.

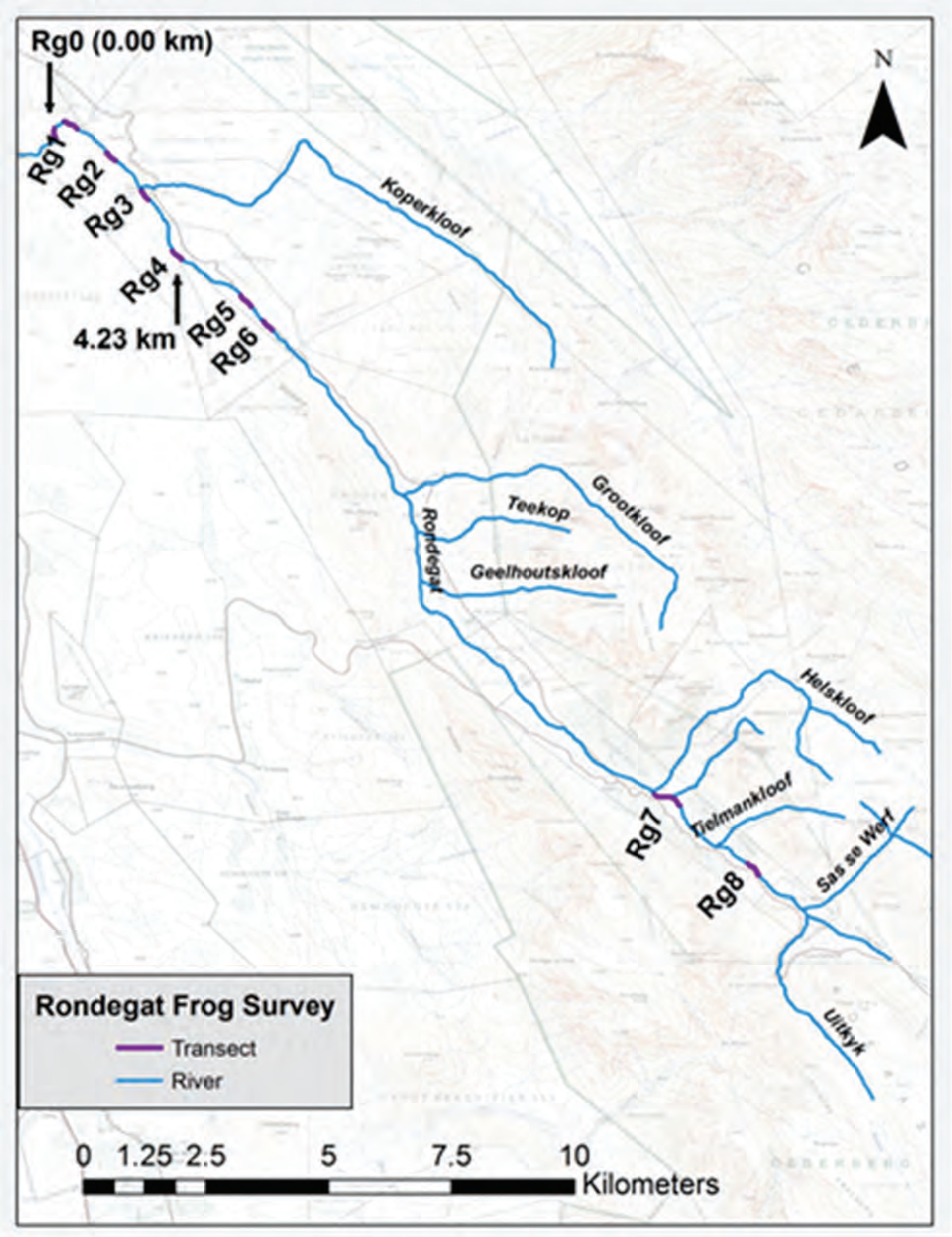

Figure 4.1: The Rondegat Catchment showing the treatment area and the nine frog survey transects. The 0 km marker indicates the location of the weir and the 4.23 km marker indicates the position of the Rooidraai waterfall (the treatment area).

Plate 4.4: Frog transect Rg1 at the lower end of the treatment zone, with disturbance to banks caused by clearing of Black wattle *Acacia mearnsii*

4.4 Methods for Monitoring the Invertebrate Fauna of the Rondegat River

4.4.1 Location of monitoring sites and frequency of sampling

The invertebrate monitoring programme conducted seasonal sampling of aquatic invertebrates at monitoring sites in the Rondegat River. Sampling was conducted at three monitoring sites within the treatment area, three monitoring sites in the control area upstream of the treatment area, as well as at a monitoring site downstream of the treatment area in the below treatment area (Figure 3.1).

An autumn sample was conducted at treatment sites in May 2010, although flooding during the field trip prevented sampling of the sites in the control area. Successful sampling of all sites was achieved in October 2010 (spring), February 2011 (summer) and again in February 2012, one week before treatment. The immediate impacts of rotenone treatment on the invertebrate community were assessed by resampling the sites one week after treatment, while short-term recovery of the invertebrate community was assessed by resampling two months later, in May 2012.

4.4.2 Collection of invertebrates

Stone sampling

To successfully interpret the effect of field application of rotenone on invertebrates, it was important to capture invertebrates in such a way that quantitative assessments of species numbers could be made. Stone sampling is a technique that assesses the density of invertebrates associated with individual substratum particles in the riverbed, and through measuring the stone size can give a quantitative estimate of individual species density per surface area (Wrona et al. 1986). By collecting four replicate stones from a single biotope, stones-in-current (SIC), a quantitative measure of invertebrate biomass variability over time that is robust to the spatial variability inherent in samples from different biotopes was obtained (Dallas 2007). This method allowed the focussed assessment of the impacts of rotenone treatment on invertebrate densities.

Four stones-in-current were collected from runs 20-40 cm deep to ensure biotope standardisation. With a 200 ųm mesh net held downstream to capture escaping invertebrates, each stone was picked up and then placed in the net. Large invertebrates were visually removed from the stones and placed in 96% ethanol. Following this, the algae on each stone was scrubbed for 2 minutes in a basin, and each algal sample was checked for missed invertebrates. The algal slurry was filtered through a 200 ųm sieve to capture all other insects. Each stone was measured across three axes before being replaced in the stream.

Kick sampling

Kick sampling is a sampling method used in rapid bioasessment protocols to assess river health in terms of invertebrate community structure (Dickens and Graham 2002). This technique, while only semi-quantitative in its assessment on individual species density, can provide an assessment of overall community composition, allowing major changes in diversity to be tracked across multiple biotopes. This method, in combination with quantitative assessment of the stones-in-current biotope, allowed a logistically feasible assessment of both broad-scale and fine-scale invertebrate response to rotenone treatment. A kick sample was conducted at each site within the available biotopes. Biotopes included stones-in-current (SIC), gravel-sand-mud (GSM) and marginal vegetation (MV). Each sample was collected using a standard 1 mm “SASS net”, with sampling limited to 2 minutes per kick (SIC and GSM) or 2 m of marginal vegetation within the monitoring site.

Drift

A key impact of previous rotenone treatments in rivers has been to precipitate catastrophic drift in aquatic invertebrates (Lintermans and Raadik 2003). While this is generally a sub-lethal impact, it can result in significant short-term changes to community structure in the aftermath of rotenone treatment. In order to quantify this effect, 250 µm mesh drift nets were set up in the treatment and control areas. Drift was collected at both sites four times on the day of treatment: one hour before the commencement, one hour into the treatment, five hours into the treatment, and two hours after the completion of treatment. Drift was taken again at the same times of day on the day after treatment, to assess whether drift had returned to pre-treatment levels.

4.4.3 SASS

The South African Scoring System version 5 (SASS5) for assessing river health using macroinvertebrates (Dickens and Graham 2002) was performed on all kick samples collected at each monitoring site. A comparison of the SASS scores and the quantitative estimates of invertebrate community change was used to determine the appropriateness of employing the SASS5 methodology in part or in full to assess the impacts of rotenone operations.

4.4.4 Periphyton

The difference in periphyton biomass before and after rotenone treatment provided a proxy assessment of food-web effects of fish and invertebrate community change. Periphyton was collected from each of the four stone-in-current samples taken at each monitoring site, using an adaptation of the quantitative stone sampling protocol of Biggs and Kilroy (2000).

In order to do this, four stone-in-current samples were collected (the same as those collected for invertebrates). After invertebrates were removed, each stone was scrubbed in a deep-walled basin with 500 ml of water for 2 minutes. After checking for invertebrates, the removed periphyton slurry was homogenised and a 200 ml sub-sample was taken and frozen. These sub-samples were transported to the University of Cape Town Zoology department, where they were analysed for concentrations of chlorophyll-a as well as phaeopigments using spectrophotometry. These concentrations were compared relative to the surface area of the stones from which the samples were taken.

4.4.5 Taxon identification and analysis of impacts

Invertebrate taxa were identified to genus or species where possible in the case of aquatic insects, while other invertebrate groups were recorded to order. Voucher specimens were sent to the Albany Museum for taxonomic confirmation.

To assess the impact of rotenone treatment of invertebrate community structure, a null hypothesis (Ho) that there was no difference in invertebrates among treatment sites or dates was tested using two primary methods. Firstly, community structure among sites was compared using Bray-Curtiss similarity matrices in the statistical package PRIMER (Clarke and Gorley 2001). These tests allowed the impact of rotenone on invertebrate community structure to be assessed relative to natural seasonal and spatial variation among treatment and control sites. Second, abundance of individual taxa and overall invertebrate densities from stone-in-current samples before and after treatment was compared using parametric univariate general linear models, using seasonal and spatial variation as factors. Total densities and proportions of living invertebrates were assessed for drift samples and compared between sites and collection times. Variation in algal chlorophyll-a concentration per stone surface area was also assessed statistically using general linear models. Monitoring seasonal concentrations of chlorophyll-a enabled the impact of rotenone on broad-scale ecological processes, such as food-web interactions, to be assessed.

5 EFFECTS OF ERADICATION TREATMENT ON FRESHWATER FISHES IN THE RONDEGAT RIVER

5.1 Introduction

The rotenone treatment of the Rondegat River was delayed by one year until the 29th February 2012, meaning that two pre-treatment surveys in February 2011 and in February 2012 were undertaken. This chapter therefore presents two sets of pre-treatment fish community data (February 2011 and February 2012) and one set of post-treatment fish community data (March 2012).

The pre-treatment data was used to assess the distributions of native and alien fish in the river, and to confirm the status of the bass invasion prior to the treatment. During the treatment, a group of volunteers collected all dead fish within the treatment area. These data were used to definitively quantify the fish community in the invaded zone of the Rondegat River. Two days after treatment, the survey team re-assessed the fish populations in the invaded zone of the river.

5.2 Results of the Fish Monitoring

5.2.1 Species composition

All fish species recently recorded in the Rondegat River (Woodford et al., 2005) except for the undescribed Cederberg galaxias (pf *Galaxias zebratus*) were detected during surveys.

Figure 5.1 below shows the maximum number of fish recorded using either electrofishing, snorkel survey or underwater video analysis at all 43 sites surveyed during the pre-treatment data collection on the Rondegat River. The fish presences and absences shown in Figure 5.1 indicate that *M. dolomieu* were collected at most sites in the treatment area as well as most sites in the below treatment area. The absence of *M. dolomieu* in the control and pristine areas confirms that bass have not moved upstream of the bass barrier first identified by Bills (1999) over a decade ago. These data suggest that the bass invasion within the Rondegat River is stable and unlikely to expand without future human intervention. Distribution data for the three sampling techniques are individually shown in Appendix A.1.

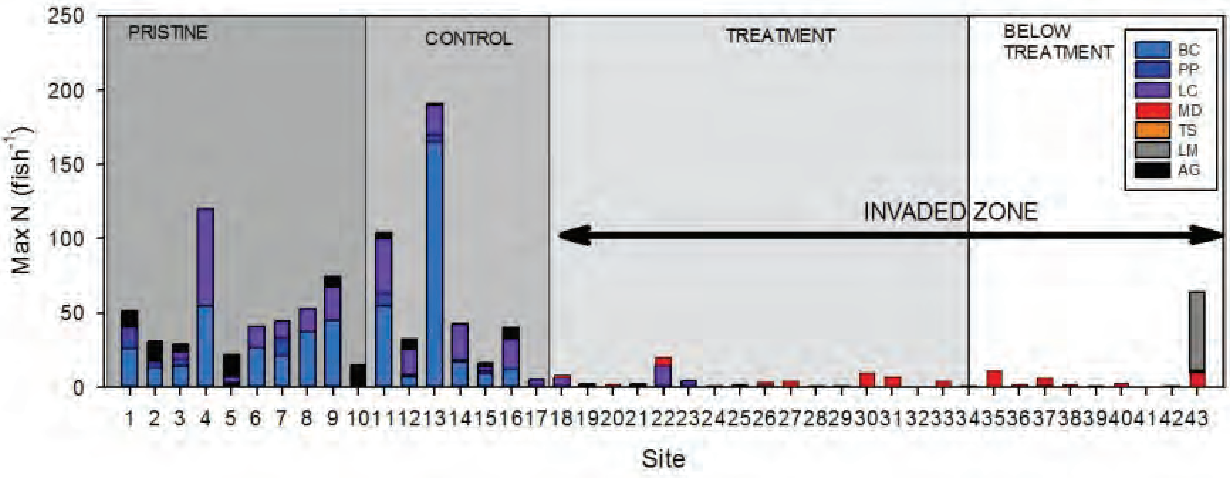

Figure 5.1: Maximum number of fish recorded using either electrofishing, snorkel survey or under water video analysis at 43 sites on the Rondegat River, Western Cape prior to rotenone treatment. BC = *Barbus calidus*, PP = *Pseudobarbus phlegethon*, LC = *Labeobarbus capensis*, MD = *Micropterus dolomieu*, TS = *Tilapia sparrmanii*, LM = *Lepomis macrochirus*, AG = *Austroglanis gilli*.

Native fish distributions were consistent between 2011 and 2012 surveys as well as with the results of the previous major survey of the Rondegat River (Woodford et al., 2005), which found that only one native species, *Labeobarbus capensis,* co-occurred with non-native fish in the invaded zone. *L. capensis* was absent from the pristine area upstream of Algeria campsite (sites 1-3 in Figure 5.1), although three individuals were observed in a deep pool that occurs in the river where the Algeria campsite is located. The pristine area upstream of Algeria campsite may fall outside the species' natural distribution on the Rondegat River, as the stream becomes narrower and has fewer deep pools than occur downstream of the campsite, which the species is known to favour (Skelton, 2001).

The remaining three native species, *Barbus calidus, Pseudobarbus phlegethon* and *Austroglanis gilli*, show no longitudinal trend in their occurrence in the un-invaded zone. Of these species, *B. calidus* was the most widespread in the un-invaded zone, occurring in all but one of the control and pristine sites (Figure 5.1). The data also indicate that the downstream distribution of *Lepomis marcochirus* has not changed since the Woodford et al. (2005) surveys and that *Tilapia sparrmanii* has now also invaded the lower reaches of the Rondegat stream. Further, the recent collection of an African sharptooth catfish *Clarias gariepinus* by CapeNature staff in the lower Rondegat is cause for concern and indicates the need for continued monitoring of this river (M. Jordaan, CapeNature, pers. comm.).

5.2.2 Size structure

Size structure of alien and native fishes sampled using electrofishing in the non-invaded zone and those collected following the rotenone treatment in the invaded zone are shown in Figure 5.2. The invasive *M. dolomieu* population comprised four recognisable cohorts (modal length 8, 16, 21 and 25 cm FL) but was dominated by fish smaller than 20 cm FL. The *L. capensis* population differed between invaded and non-invaded zones with the population in the non-invaded zones comprising both juvenile and adult fish (5-35 cm

FL) while in the invaded zone the population was comprised almost entirely of adults larger than 20 cm FL (Figure 5.2).

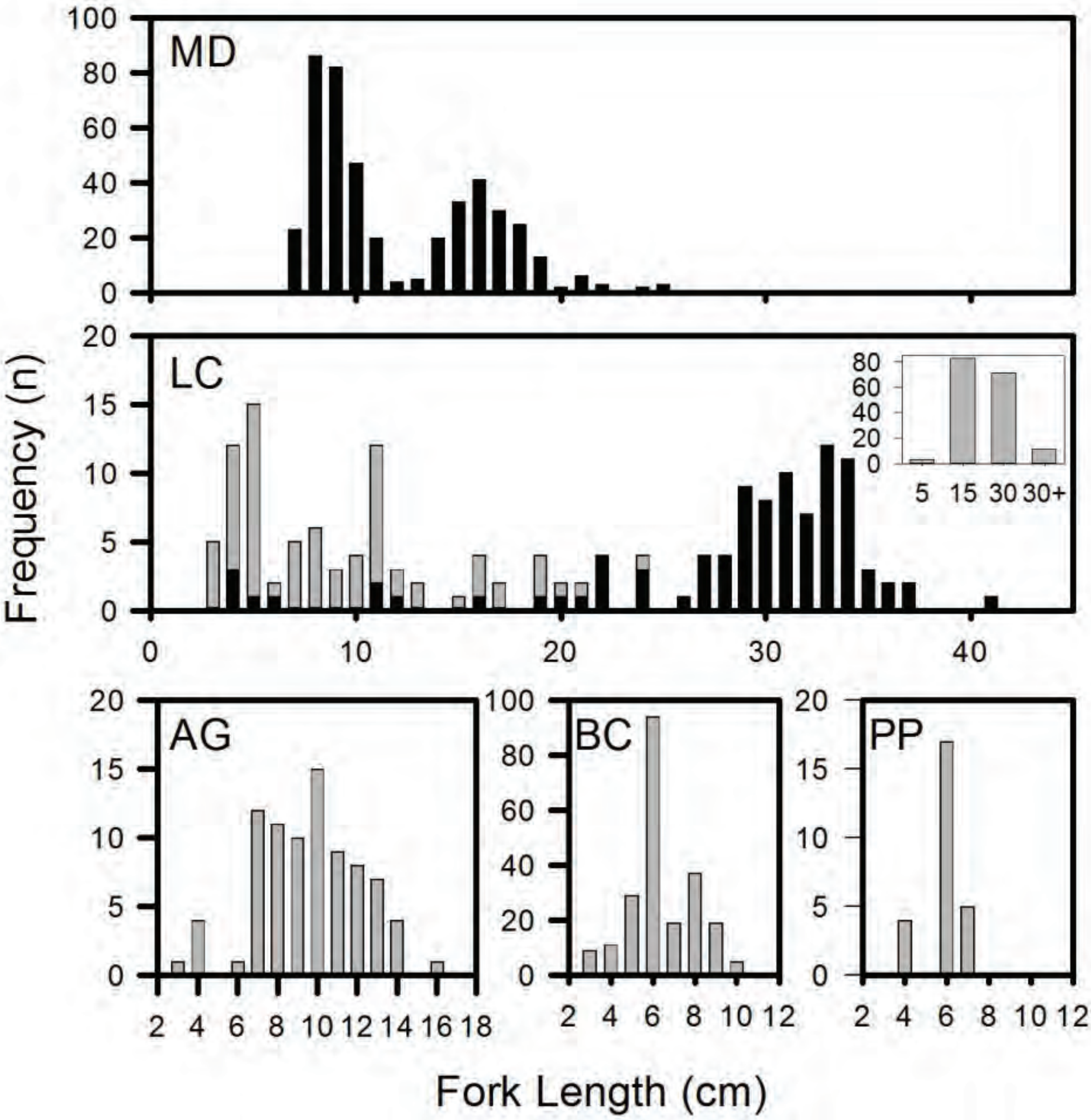

Figure 5.2: Fork length (cm) frequency of non-native *Micropterus dolomieu* (MD) and native *Labeobarbus capensis* (LC), *Austroglanis gilli* (AG), *Barbus calidus* (BC) and *Pseudobarbus phlegethon* (PP) in invaded (black bars) and non-invaded (grey bars) zones in the Rondegat River, South Africa. In the invaded zone length frequencies are based on the total population of fish removed during a 2012 rotenone treatment. In the non-invaded zone length structure was estimated from fish measured during 2011 and 2012 electrofishing surveys. The insert in the *L. capensis* length frequency distribution are length structure estimates from 2011/12 snorkel surveys that are included to demonstrate that fish larger than the sizes sampled using electrofishing were present in this region.

5.2.3 Density and abundance

In the treatment area, estimates of fish density (snorkel survey) and relative abundance (UWV) decreased between 2011 and 2012 and after the rotenone treatment no fish were detected in the treatment area (Figure 5.3). Density did not differ significantly between 2011 and 2012 pre-treatment surveys for *M. dolomieu* but differed significantly for *L. capensis* (Mann-Whitney-U, $p<0.05$).

Snorkel survey estimates of fish density did not differ from densities estimated from fish removal (rescue and rotenone treatment) for *M. dolomieu* during either the 2011 (t-test, $t = 0.149$, $df = 14$, $p = 0.88$) or 2012 pre-treatment survey ($t = 1.01$, $df = 16$, $p = 0.38$). *Labeobarbus capensis* 2011 density estimates did not differ from fish removal estimates (t-test: 0.399, $df = 14$, $p = 0.69$) but 2012 estimates of were significantly lower (t-test: $t = 10.4$, $df = 16$, $p<0.001$).

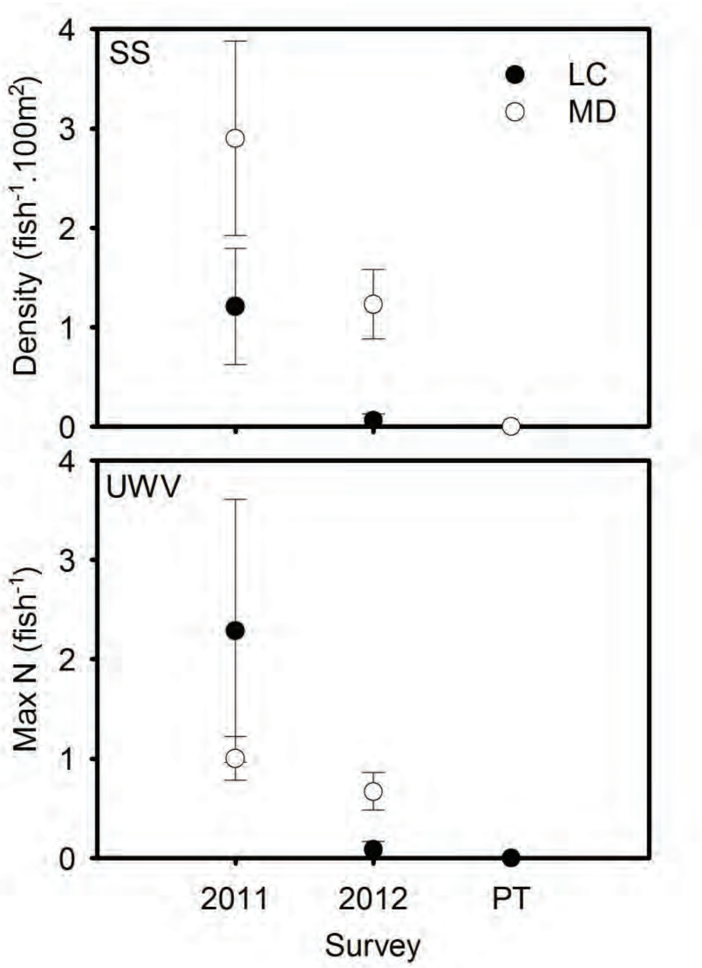

Figure 5.3: Estimates of fish density from snorkel surveys (SS) and relative abundance from underwater video analysis (UWV) in the rotenone treatment area of the Rondegat River, South Africa pre-rotenone treatment in February 2011 and 2012 and 24 hours after the rotenone treatment in February 2012 (PT)

Juvenile *L. capensis* were detected as having recruited into a cascade pool at the site where rotenone was applied pool immediately after treatment but no fish were observed in any of the 12 treatment sites that were monitored suggesting that the rotenone treatment was successful.

Two sites below the treatment area were also sampled and fish abundance there was similar to that recorded before treatment. This indicates that the neutralisation of the rotenone was also effective.

In May 2012, Dr Woodford undertook a snorkel survey of selected sites of the river. Visibility was impaired by increased river flow. Nonetheless, the survey confirmed that some *B. calidus* had already colonised the uppermost pool in the treatment area and two large *L. capensis* were recorded in a pool 200 m downstream of the beginning of the treatment area.

5.3 Discussion

The preliminary results of the two summer fish surveys indicate that the bass invasion is stable and unlikely to expand without human assistance. The fish distribution data collected in the summer surveys provide an up-to-date baseline of the fish community with which to monitor immediate after-effects and long-term recovery following rotenone treatment. The wide distribution of *L. capensis* within the treatment zone indicates that significant mortality of this species was to be expected during rotenone treatment, even after fish were actively removed from accessible pools prior to rotenone treatment. These native fish kills, while unfortunate, nonetheless provide valuable scientific insight. For example, recording ages and numbers of all live and dead fish reveals the complete structure of the *L. capensis* population co-occurring with bass, and can be used to confirm whether or not the population was a demographic sink as was postulated by Woodford et al. (2005). It is also evident that the rotenone treatment was effective at removing smallmouth bass from the Rondegat River. A follow up treatment is planned for October 2012. This will provide a unique opportunity to determine whether smallmouth bass were able to recolonize the river after treatment and to assess how native fishes responded to the removal of smallmouth bass from the treatment zone. Close monitoring of the fish community in the river is recommended.

6 EFFECTS OF ERADICATION TREATMENT ON AMPHIBIANS IN THE RONDEGAT RIVER

6.1 Introduction

This chapter details the findings of an amphibian survey on the Rondegat River which was conducted over five days before, during and after rotenone piscicide treatment. The methods used to collect the amphibian samples are detailed in section 4.3.

6.2 Results of the Amphibian Monitoring

Six amphibian species were found in the Rondegat catchment during this survey (Table 6.1; Figure 6.1, which combines 69 point locality records from this survey, covering the lower Rondegat valley and Algeria, with 82 previous records from Algeria, Uitkyk Pass and Grootkloof). Four species were found in the treatment area (Plate 6.1, Figure 6.2).

Table 6.1: Frog species of the Rondegat Valley and their habitats

Species	Treatment area?	Breeding biotypes
Cape river frog *Amietia fuscigula*	Yes	Sheltered, shallow river edges with grass or sedge cover in the presence of fish; throughout streams without fish, off-stream wetlands, semi-permanent ponds and irrigation channels with standing water
Cape sand frog *Tomopterna delalandii*	Yes	Open sandy areas along irrigation ditches, ponds, shallow stream pools and temporary water bodies
Clicking stream frog *Strongylopus grayii*	Yes	Semi-permanent grassy or sedgy seepage areas, wetlands and ditches with thick ground cover
Raucous toad *Amietophrynus rangeri*	Yes	Open areas along slow flowing river pools and permanent ponds
Common platanna *Xenopus laevis*	No	Permanent or semi-permanent ponds and river pools
FitzSimons' ghost frog *Heleophryne depressa*	No	Montane torrent streams on cobble substrates without fish, or with very limited overlap. Generally with thick riparian fynbos. Particularly congregated around cascades

By far the most common species encountered was the Cape river frog, *Amietia fuscigula* (Plate 6.1). In the lower Rondegat adult *A. fuscigula* were occasionally encountered along the stream bank but were far more common at off-stream water bodies such as the irrigation ponds at Rietvlei and Keurbos, and the extensive north-east bank wetland between Keurbos and Rooidraai. Tadpoles of this species were not observed during snorkelling surveys in the transect zone or in the adjacent transects (Table 6.2). However, at least 8 moribund tadpoles and 2 frogs were collected from the stream as drift during the rotenone treatment. The distinctive, large tadpoles of this species were abundant in persistent puddles along the irrigation furrows, with over 20 individuals per metre dispersed along the trench in some stretches. In the upper Rondegat several very large tadpoles of this species were seen in the pool at the Algeria campsite, along with indigenous fishes, and several large frogs were observed at night in the riffle below the causeway. River frogs were most obvious, however, in the uppermost transect, above the fish barrier, where several frogs and numerous tadpoles, across the full size range, were observed in daytime out on stream rocks both when walking and snorkelling this transect (Table 6.2).

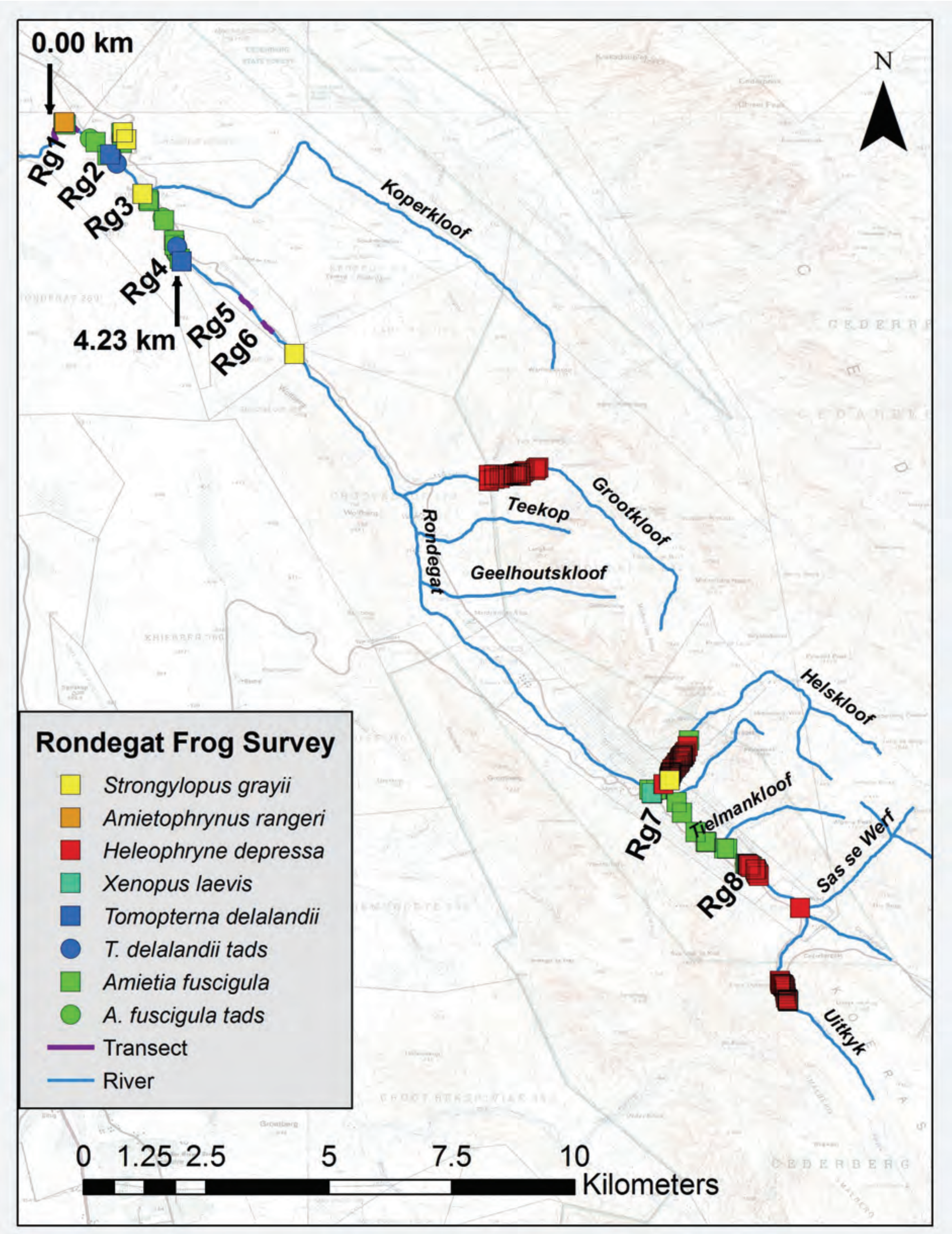

Figure 6.1: Frog records from the Rondegat catchment. The 0 km marker indicates the location of the weir and the 4.23 km marker indicates the position of the Rooidraai waterfall (the treatment area).

Plate 6.1: Cape river frog, *Amietia fuscigula*, Transect Rg1 (12 hrs after treatment) (Cunningham).

Table 6.2: Frog abundance along transects (see the text for interpretation)

Transect	Area	Frogs (walk/snorkel)	Tadpoles (walk/snorkel)	Fish (walk/snorkel)
Rg0	Treatment area	0/0	0/0	Md 1/1
Rg1	Treatment area	0/0	0/0	Md 9+3/10+2
Rg2	Treatment area	Af 1/0	0/0	Md 9+1/14+1
Rg3	Treatment area	0/0	0/0	Md 9+0+0/38+4+5
Rg4	Treatment area	0/0	0/0	Md 5+1/8+2
Rg5	Control area	0/0	0/0	Bc & Lc >80/>600
Rg6	Control area	0/0	0/0	Bc & Lc 30+210/>30
Rg7	Pristine area	Af 1/-	Af 10/-	Bc, Pp & Lc >200
Rg8	Above pristine area	Af 7/0; Hd 0/0	Af >50/20; Hd 1/7	0/0

Af = *Amietia fuscigula,* Hd = *Heleophryne depressa;* Md = *Macropterus dolomieu,* Bc = *Barbus calidus,* Lc = *Labeobarbus capensis,* Pp = *Pseudobarbus phlegethon* (fish counts are given for very small + small (flagtail) + medium to large size classes, or as pooled counts. These are separate counts to those undertaken for the fish results presented in Chapter 5). Area refers to the areas shown in Figure 3.1.

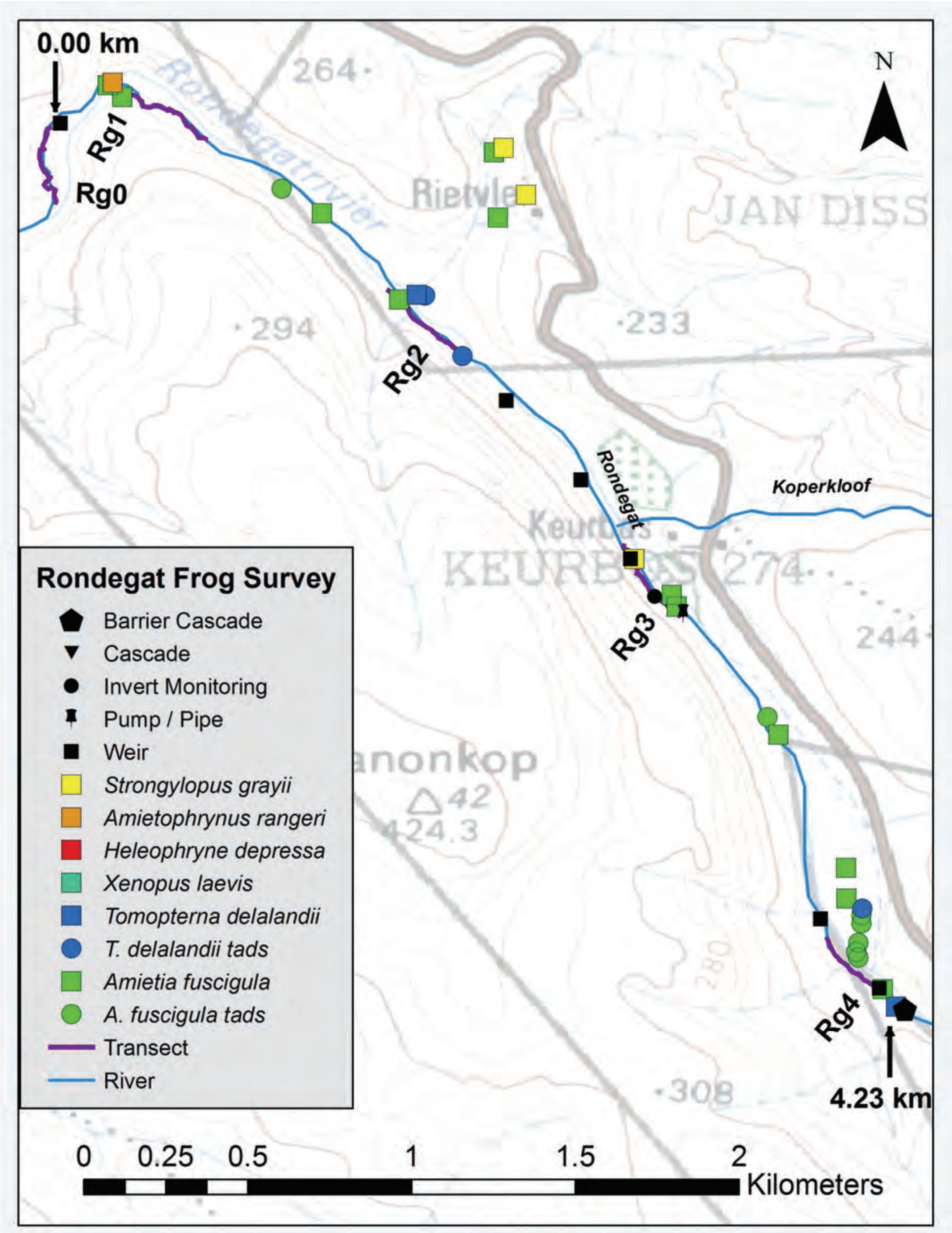

Figure 6.2: Frog species detected in the lower Rondegat river treatment area. The 0 km marker indicates the location of the weir and the 4.23 km marker indicates the position of the Rooidraai waterfall (the treatment area).

The metallic ringing call of the Cape sand frog, *Tomopterna delalandii*, was heard at night from the seepage wetlands at Rietvlei and Keurbos, and tadpoles were abundant along the irrigation ditches from Rooidraai to Rietvlei. Within 20 min of the commencement of the rotenone treatment a single adult frog was observed leaving the stream from the sandy pool at Rooidraai (this individual, which escaped into vegetation on the far bank, may have been flushed into the water by the commotion of the treatment and monitoring crews in this area). No other individuals were seen or heard along the river nor were tadpoles encountered along any of the transects. It is likely that this is a common species throughout the Rondegat valley below Algeria, wherever there is standing water on sand. Around 2 hours after spraying of rotenone from backpacks, several large clusters of dead tadpoles (>200 individuals) were found in an irrigation furrow near Rooidraai. Several *A. fuscigula* tadpoles were observed feeding on these without obvious ill effects. Further along this furrow abundant living *T. delalandii* tadpoles were observed at least 8 hours after the rotenone treatment. The dead tadpoles were already decaying and it is likely that this mortality occurred shortly after spraying. This patch may have received a more concentrated dose of rotenone, as it is near the start point for backpack spraying.

The Clicking stream frog, *Strongylopus grayii* (Plate 6.2), was seen and heard around semi-permanent seepage areas associated with irrigation infrastructure at Rietvlei and Keurbos. Although this species can breed in fringing vegetation along streams, none were found along the river in this survey. No tadpoles were found although it is likely that these were present in shallow water bodies around seepages.

Plate 6.2: Clicking stream frog, *Strongylopus grayii*, Rietvlei farm (Cunningham).

One apparently healthy Raucous toad, *Amietophrynus rangeri* (Plate 6.3) was encountered while spotlighting along Rg1 on the evening of the rotenone treatment day (±12 hrs after treatment). This is a common and easily detected species in the region, but the breeding season for this species ends in February and no breading calls were heard during the survey. This species breeds in long-lasting or permanent ponds and in slow flowing river sections. The tadpoles of this species are also easily detected and often abundant, including in this season, but no toad tadpoles were found during this survey.

Plate 6.3: Raucous toad, *Amietophrynus rangeri*, Transect Rg1, lower treatment section (Cunningham).

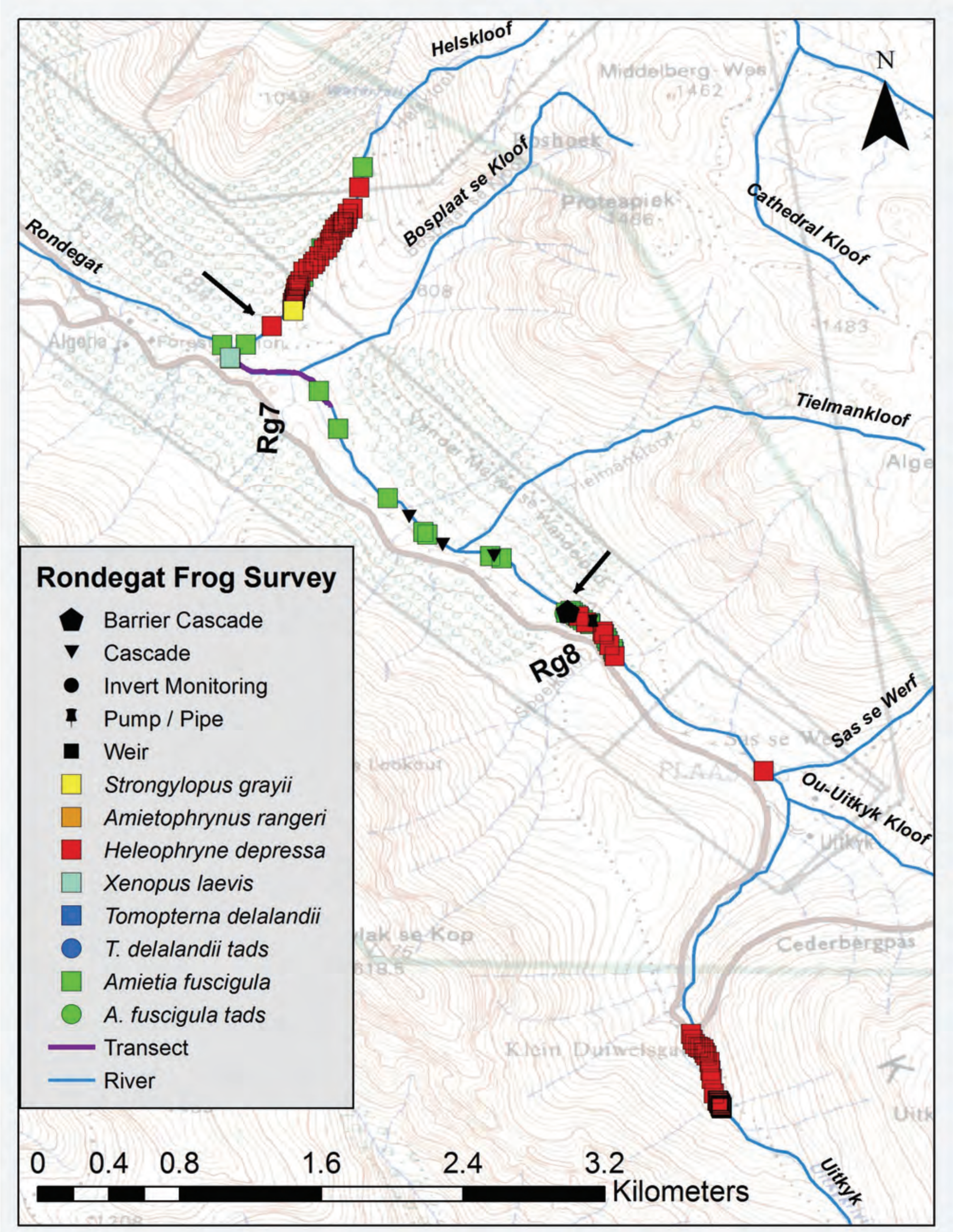

Figure 6.3: Frog species occurrence in the upper Rondegat catchment. Arrows show the limits of fish occurrence in the catchment

6.3 Other Frog Species in the Rondegat Catchment

A single large Common platanna, *Xenopus laevis*, was observed co-existing with abundant indigenous fishes in the pool above the causeway at Algeria. This species is widespread and common in this region but tends to be under-reported in surveys due to its subdued (underwater) call and obligate aquatic lifestyle. It can occur in virtually any long-lasting water body and its absence in the snorkelling surveys is surprising. It is likely to be found elsewhere in the Rondegat valley, particularly in irrigation ponds.

FitzSimons' ghost frog, *Heleophryne depressa*, is a stream-dwelling species endemic to montane fynbos in the north-western Cape Floristic Region. This ghost frog is abundant along streams in the southern and central Cederberg but reaches its northern limit at Taaiboschkraal near Clanwilliam. The adult *H. depressa* is secretive and rarely encountered (generally found by rolling rocks in the stream, or by spotlighting in suitable sites). *H. depressa* tadpoles were completely absent from the lower Rondegat, including the treatment area, and were only encountered in fish free headwater torrents such as the mainstream along transect Rg8, immediately above the fish barrier, and in the Helskloof tributary at Algeria (Figure 6.3). Previously this species has been found in similar sites within the Rondegat catchment, in Grootkloof and at the top of the catchment, in Uitkyk Pass (Figure 6.3). There are few other permanent streams in the Rondegat catchment that are suitable for this species.

The only other species likely to occur in the lower Rondegat valley, including the treatment area, is the Cape sand toad, *Vandijkophrynus amatolicus*, which has previously been found at Clanwilliam. Although bioclimatic modelling in Cunningham (2011) predicted that the Karoo toad, *Vandijkophrynus gariepensis*, may also occur here, this species is largely restricted to the eastern versant of the Cederberg and is rarely found together with the Cape sand toad. Three montane fynbos endemic species, the Cape mountain rain frog, *Breviceps montanus*, Tradouw's mountain toad, *Capensibufo tradouwi*, and the Banded stream frog, *Strongylopus bonaespei*, may potentially occur at the upper periphery of the catchment such as in seepages around the Uitkyk River headwater, above Uitkyk Pass. This area is at the edge of these species bioclimatic tolerance and their northern or western distributional limits.

6.4 Conclusion

The occurrence of frogs within the Rondegat catchment is limited by the presence of fishes. Several lines of evidence point to this conclusion. Stream breeding frogs, and particularly tadpoles, are scarce and difficult to detect along streams with fish. These same species are more common in adjacent off-stream water bodies where there are few or no fish. In the presence of fish, widespread generalist species that are common in the district and easily detected, such as *Xenopus laevis* and *Amietophrynus rangeri*, are rare, absent or only encountered as large adults capable of dispersing large distances over land, and do not appear to be breeding successfully in the stream. Results from the snorkelling survey, which provides a rough index of abundance show inverse patterns for fish and frogs (Table 6.2). This is particularly clear for the ghost frog *Heliophryne depressa*, which here, as throughout its distribution, reaches an abrupt end-point of occurrence at natural barriers preventing upstream movement of fishes. There are other environmental changes downstream, in particular disturbance by cattle, but these are insufficient explanation for the observed pattern of occurrence. Frogs breed successfully away from the stream despite these disturbances, and in other sites, frogs such as *Amietia fuscigula* and *Amietophrynus rangeri* breed in fish-free streams which are similarly affected by these disturbances. The mechanism by which fish limit the occurrence of frogs is probably predation of eggs and tadpoles. Frog eggs are a rich source of energy and are avidly consumed by many invertebrate and vertebrate species, including other frog tadpoles. Although some frog species, especially toads, have distasteful or toxic eggs and larvae, these appear to be incomplete protection from fish predation. Only large fish can catch and eat adult frogs, and small fish are unable to eat larger tadpoles. The complimentary occurrence of fish and frogs is not absolute as some fish species, such as *Pseudobarbus phlegethon*, are inefficient frog predators, and some frog species have behavioural and other adaptations, such as preferences for sheltered biotypes, that allow limited co-occurrence with fish.

The inverse abundances recorded between fish and frogs are also distorted by other factors such as species differences in detectability among frog species and variation among fish species in effectiveness as predators. An extremely low abundance of tadpoles was recorded in the lower Rondegat treatment area despite low numbers of the invasive and highly effective predator, Smallmouth bass, *Micropterus dolomieu*. The control area above the treatment area shows a near absence of frogs and a super-abundance of indigenous *Labeobarbus capensis* and *Barbus calidus*. Above Algeria in the pristine area, larger tadpoles of *A. fuscigula* are able to survive as there are fewer large fish and abundant populations of frogs in adjacent fish free tributaries, providing a source of dispersing tadpoles. The *H. depressa* tadpoles, with their sucker-mouths, tend to remain attached under stones during the day and are only observed in the stream when they are present at high densities. The near absence of both fish and frogs below the treatment area, in transect Rg0, is an anomaly that suggests either that this segment has previously been invaded periodically by bass or other invasive species (both *Tilapia sparmanii* and *Lepomis macrochirus* were observed above the bridge downstream of this section), or that this section is only capable of maintaining a very small population of invasive fish, perhaps washed over the weir at the end of the treatment area, sufficient to restrict the occurrence of frogs in what is otherwise suitable habitat.

Due to the scarcity of frogs in the stream, the lower toxicity of rotenone for frogs than fish, and the difficulty in accessing and treating wetland habitats on the river flats, where frogs are abundant, the immediate impacts of rotenone treatment on frogs of the lower Rondegat were modest. Although some frogs were affected and many tadpoles killed, many more frogs and tadpoles of the same species survived the initial stages of treatment. Follow up monitoring should be conducted in September-October, which is the peak season for frog breeding activity here. It is possible that there will be a lag of several years during which there is low fish abundance and few large fish in the treatment area. In this case, it is predicted that an explosive increase in frog and tadpole numbers along the stream will take place, as has been seen after rotenone treatments in the USA and elsewhere. Given the absence of frogs in control sites and the presence of pools accommodating very large yellowfish within the treatment area, this reproductive boom would be followed by a relaxation to something close to the current state as with increasing indigenous fish populations and the growth of larger fish in the treatment area. It is unlikely that rehabilitation of the lower Rondegat River will have any lasting influence on the abundance and diversity of frogs in the stream, with the caveat that large tadpoles of *A. fuscigula* may be better able to survive in rocky stretches of the stream, as at Algeria.

7 EFFECTS OF ERADICATION TREATMENT ON INVERTERATES IN THE RONDEGAT RIVER

7.1 Pre-Treatment Assessment of Macroinvertebrate Diversity

Assessment of the species-level taxonomic diversity and relative abundance of taxa from the SASS kick samples revealed a surprisingly diverse invertebrate fauna within the treatment area of the Rondegat River. The mayfly family Baetidae was particularly diverse, with 17 distinct taxa being identified. Of these, two taxa*,* *Afroptilum sp*. and *Peuhlella sp*., are believed to be undescribed species (Figure 7.1). The discovery of *Peuhlella* is of particular interest, as this is an Afrotropical genus that has previously only been recorded in Guinea (Lugo-Ortz and McCafferty, 1998). Three other taxa were also identified as being potentially new to science. These were the caenid mayflies *Afrocaenis sp*. and *Caenis sp*. and the polycentropodid caddisfly, *Paranyctiophylax sp.* (Figure 7.1).

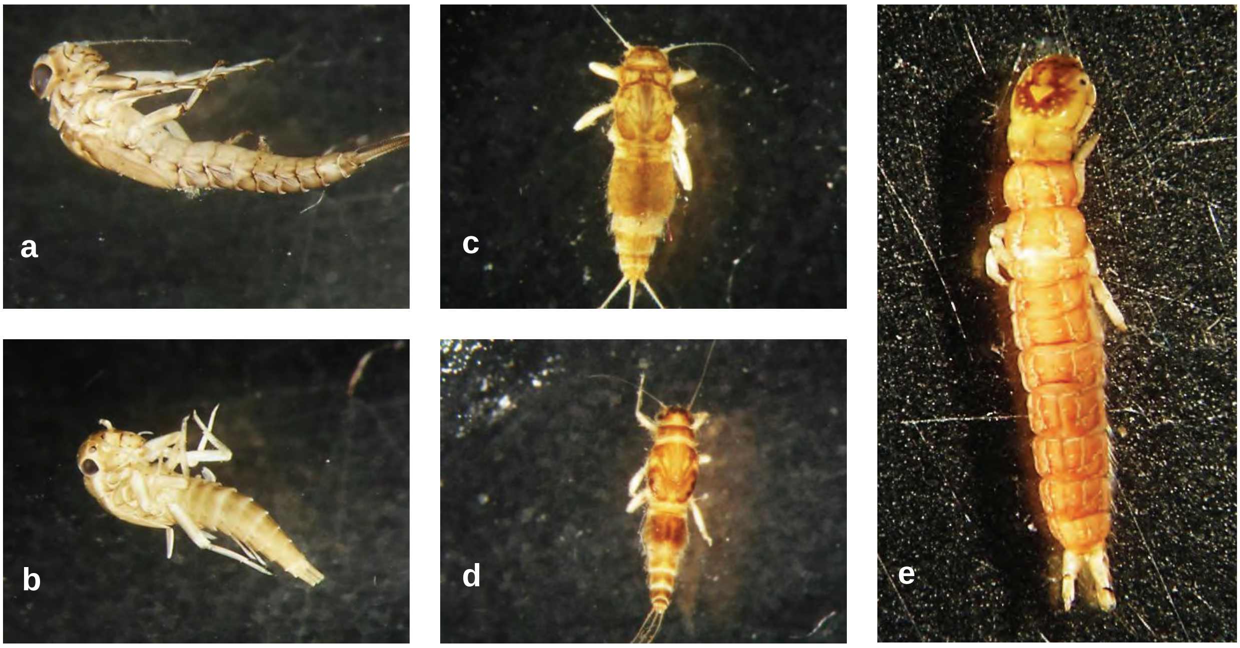

Figure 7.1: Images of five invertebrates collected in the Rondegat River that are believed to be new species. These are a) *Afroptilum sp* (Ephemeroptera: Baetidae); b) *Peuhlella sp* (Ephemeroptera: Baetidae); c) *Afrocaenis sp* (Ephemeroptera: Caenidae); d) *Caenis sp* (Ephemeroptera: Caenidae); e) *Paranyctiophylax sp* (Trichoptera: Polycentropodidae). (Images: T. Bellingan)

Peuhlella sp. was only recorded at treatment monitoring sites that fall within the treatment area (Figure 3.1). This made it a potentially important indicator species for monitoring invertebrate community recovery following rotenone treatment, as it may need to re-colonise the river from nearby streams rather than drifting down from the control area. Other species found only in the treatment area included the large caddisfly *Macrostemum capense* (Family Hydropsychidae), the water beetles *Parhydraena sp*. and *Prosthetops sp.* (Family Hydraenidae) and the blackflies *Simulium pomeroyellum sp., Simulium unicornutum*, and S*imulium vorax* (Family Simuliidae). It is important to note that these species may still occur upstream of the bass barrier despite not being sampled from control sites in the control area. Furthermore, some species, such as

Potamonautes sp. crabs, *Afronurus sp.* mayflies (Family Heptageniidae) and scirtid beetle larvae, were also only detected in the treatment area but were previously recorded in the control area in surveys documented by Lowe et al. (2008) and Lowe (2009).

To assess how this level of taxonomic detail affected the patterns of similarity among sites, they were compared using a Bray-Curtis similarity matrix. Analysis was performed using the stones-in-current (SIC), sand (GSM) and marginal vegetation (MV) biotopes separately, as well as using data from all biotopes combined. The percentage similarities between sites are presented in cluster diagrams (Figure 7.2).

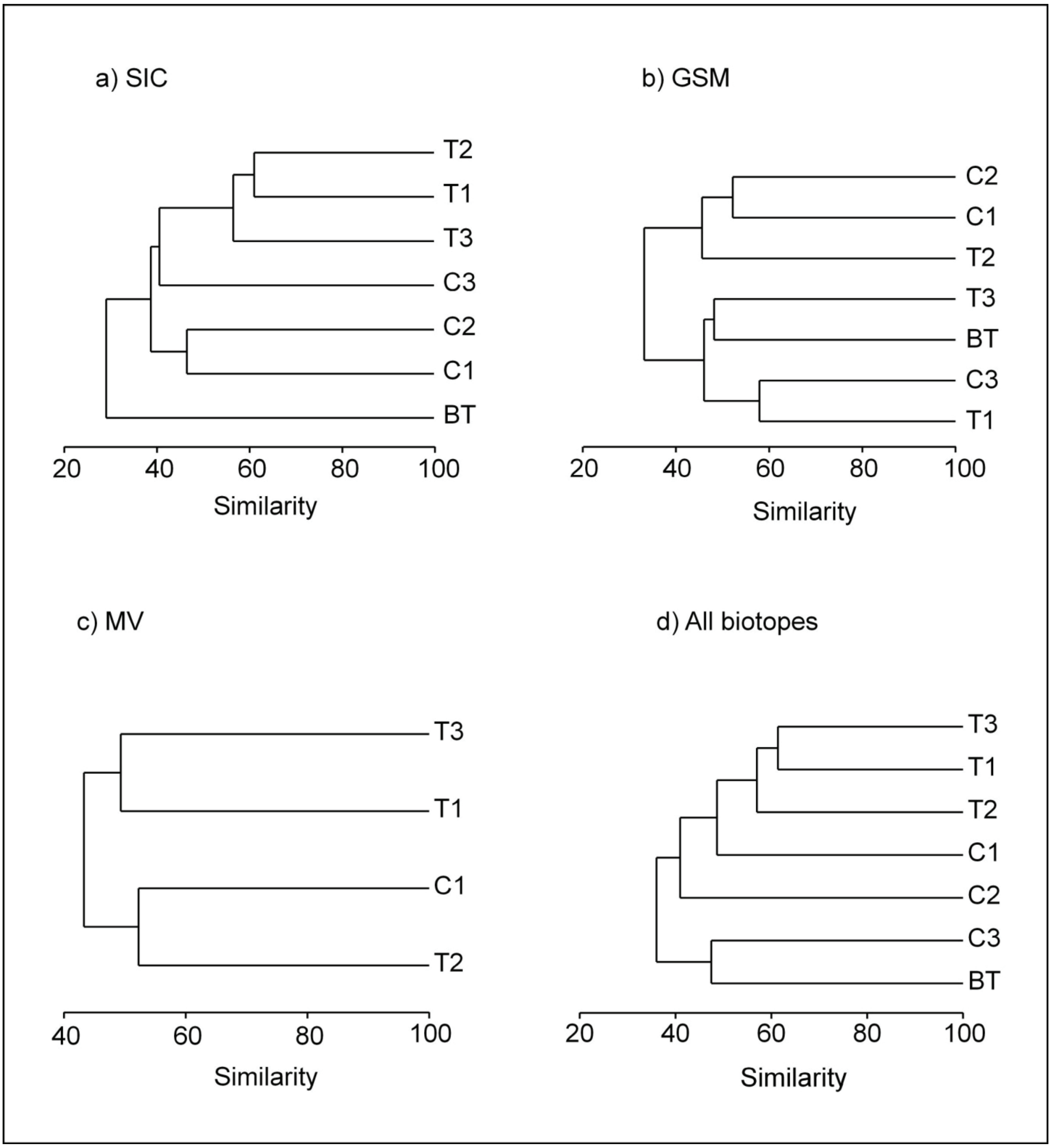

Figure 7.2: Cluster plots illustrating Bray-Curtis similarity between invertebrate communities within cobble (a), sand (b), marginal vegetation (c) and combined biotopes (d) at treatment (T), control (C) and below treatment (BT) monitoring sites on the Rondegat River in February 2011.

Species data from the SIC and combined biotopes (Figures 7,2 a and d) showed treatment area sites clustered together well, suggesting stone-dwelling invertebrate communities shifted in structure between areas, and that these species drove overall community shifts between the areas. In comparison, sand and marginal vegetation communities were not well distinguished (Figures 7.2 b and c), indicating a mixed fauna that did not vary much between treatment and control sites. These results suggest that the majority of species that define the treatment area community are stone-dwelling species. The findings concur with the family-level analyses of Lowe et al (2008), who found significant differences in the SIC communities of the treatment and control zones, suggesting bass-driven shifts in stone-dwelling community structure.

7.2 Impacts of Rotenone Treatment on Diversity and Community Structure

Comparison of species-level taxonomic diversity collected in February 2011, February 2012 (immediately before rotenone treatment), March 2012 (one week after treatment) and May 2012 (two months after treatment) revealed a number of common species (recorded in both February 2011 and February 2012) and rare species (recorded in only one February sample) that were not found in the river immediately following rotenone treatment. Of the common species apparently lost as a result of the treatment, five were found again in May 2012, while a further five species remained unaccounted for (Table 7.1). Of the rare species, 36 were still missing in May (Table 7.1). It should be pointed out that many of these species may have either returned by May or have never disappeared, but were simply present in such naturally low densities as to be undetectable by the kick sampling. Thus, the common species found in both summer pre-treatment surveys likely offer a more accurate indication on the effects of rotenone treatment on the macroinvertebrates. Overall, 82% of these common species were present in the river after just two months of recovery.

Table 7.1: Effects of the rotenone treatment on presence/absence of invertebrate taxa identified from the SASS5 kick samples. Common species refers to species recorded in the treatment zone in both February 2011 and February 2012 pre-treatment surveys. Rare species refers to species only recorded in one of the pre-treatment February surveys.

Taxon type	All taxa	Taxa only recorded in treatment area
Common species not affected	19	
Common species lost but recovered	5	
Common species still missing	5	2
Rare species not affected	13	
Rare species that may have been affected	36	21
Species only detected post treatment	19	
Total species detected in treatment zone	**107**	**26**

Perhaps more startling, was the detection of 19 new species in the treatment area in May 2012 that had never been recorded there prior to the rotenone treatment. This wave of new 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 however also simply be an artefact of sampling efficiency, where many species have an equal random chance of being detected by the sampling methods in any given season. Future monitoring would be needed to assess whether these new records represent actual colonisation events.

To assess overall shifts in community structure following the rotenone treatment, the macroinvertebrate community was compared across four different seasonal samples using Bray-Curtiss similarity analysis on both family-level and species-level abundance data from the kick samples. A comparison between the February 2011, February 2012, May 2012 and May 2010 data was made to assess whether the community structure shifted significantly as a result of the treatment. The family-level data (Figure 7.3) showed no clear separation among the pre- and post-rotenone treatment samples, with immediately pre- and post-treatment site samples being intermingled in terms of their relative similarity. There was a distinctive grouping together of May 2012 site samples (Figure 7.3), although the overall community recorded during this site visit was not significantly different from any of the other samples (ANOSIM, $p > 0.05$).

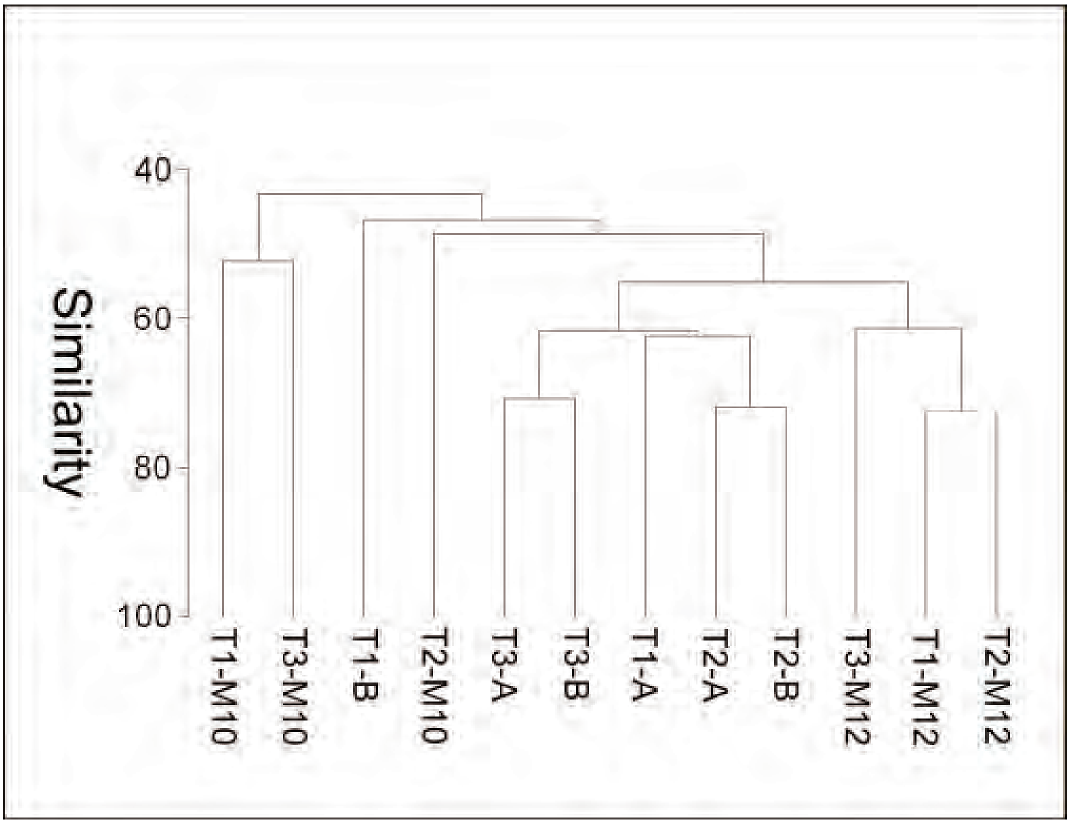

Figure 7.3: Cluster analysis based on Family level data. Three sampling sites (T1-T3) in the treatment area were compared across four seasonal samples. These are May 2010 (M10), February 2012 before treatment (B), March 2012 after treatment (A) and May 2012 (M12).

Assessment of community similarity using species-level data showed the May 2010 samples group together separately from the other samples (Figure 7.4), although these community samples were not significantly different from the other seasonal samples (ANOSIM, $p > 0.05$). Once again there was little distinction between sites sampled immediately before and one week after treatment, while the May 2012 samples were more similar to each other than the other pre- or post-treatment samples. The relative lack of separation between immediately before and after treatment samples suggests that even though both common and rare species appeared to be removed by the rotenone, their loss did not perceptibly alter the overall macroinvertebrate community structure at treatment sites. The clear clustering together of the May 2012 and May 2010 sites based on species-specific abundance (Figure 7.4) further suggests that both annual (2010 vs. 2012) and seasonal (May vs. February) variation had a far bigger effect on species-level community structure than the rotenone treatment, and implies that the presence or absence of rare species in May 2012 may have just as much to do with natural seasonal shifts in community structure as the effects of the rotenone treatment.

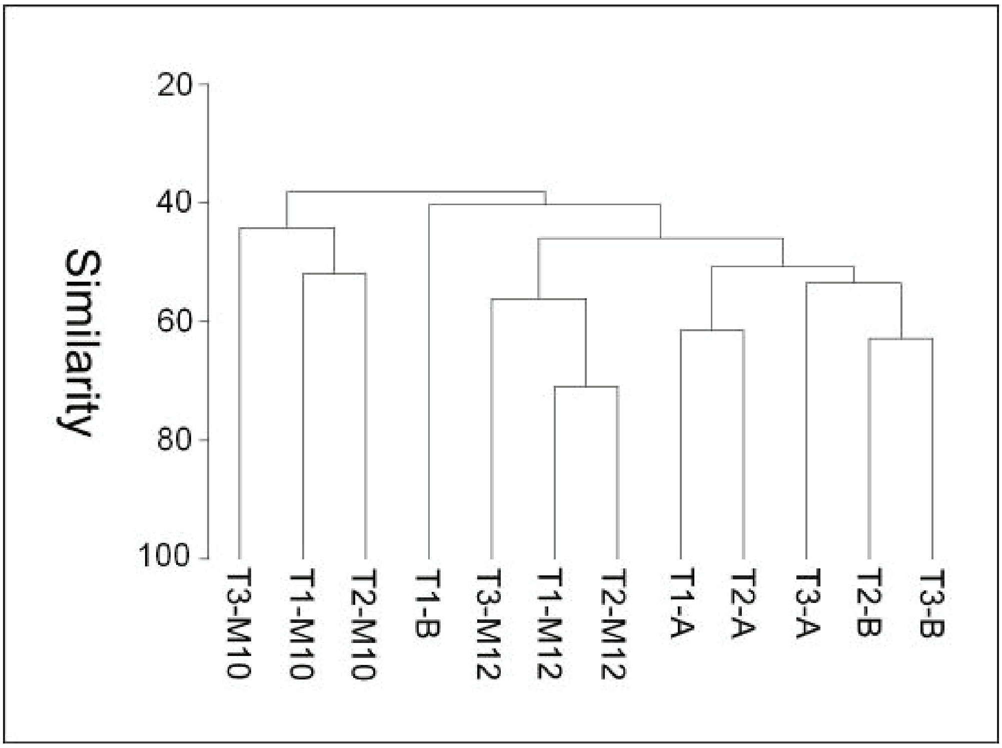

Figure 7.4: Cluster analysis based on species level data. Three sampling sites (T1-T3) in the treatment area were compared across four seasonal samples. These are May 2010 (M10), February 2012 before treatment (B), March 2012 after treatment (A) and May 2012 (M12).

7.3 Ecosystem Health Changes as Detected by SASS5 Scoring

The SASS5 scoring system was applied to kick samples from the treatment area before, one week after, and two months after rotenone treatment, to see if this rapid bioassessment technique could be used to detect changes in overall river ecosystem health. There was a significant decline in average score per taxon (ASPT) following the treatment (1-way ANOVA: $F_{(2,6)} = 7.76$, $p < 0.03$), whereas there was no difference between the May 2012 and pre-treatment ASPT scores (post-hoc Tukey test, $p > 0.05$). There was in contrast no significant decline in mean overall SASS5 score from the pre- to post-treatment scores (1-way ANOVA: $F_{(2,6)} = 0.94$, $p > 0.4$; Figure 7.5). While Chutter (1998) suggests ASPT is a better indicator of river health in “good quality rivers” than in poor quality rivers, the ASPT recorded before and after rotenone treatment fell within a band of scores (ASPT <6.6) that is considered to be impoverished relative to reference communities in Western Cape streams (Dallas and Day 2007). The SASS scores, in comparison, place the Rondegat treatment zone in either a “below reference” or a “well below reference” biological band (Dallas and Day, 2007). The macroinvertebrate fauna collected before and after rotenone treatment could thus be characterised as that of a "poor quality river" for which total SASS score ought to be as informative as ASPT in describing changes in ecosystem health. Considering these findings, it can be concluded that ecosystem health as estimated by the SASS5 scoring system was not significantly altered by the rotenone treatment.

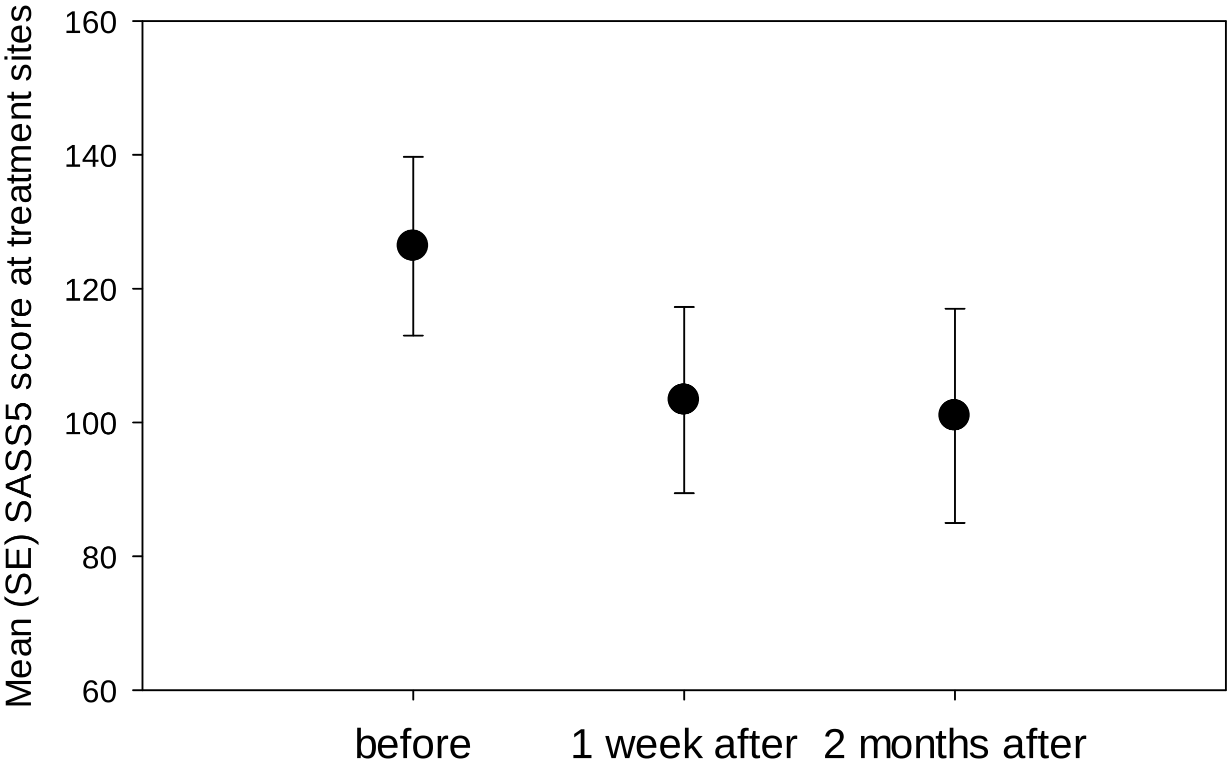

Figure 7.5: Mean (SE) SASS5 scores recorded at treatment sites one week before, one week after and two months after rotenone treatment.

7.4 Loss and Recovery of Invertebrate Densities on Stones

Unlike the community-level assessments, individual stone sampling did reveal some significant negative impacts on the abundance of specific macroinvertebrate groups. Ephemeroptera was the insect order most severely affected by the rotenone treatment, showing significant decreases in density immediately after treatment (Table 7.2, Figure 7.6). The group did however appear to have recovered to near pre-treatment densities in May 2012 (Figure 7.6). Within the Ephemeroptera, two families were significantly affected (Baetidae and Heptageneiidae, Table 7.2). Whereas Baetidae had recovered to the point of not being significantly less abundant than pre-treatment levels by May 2012, Heptageneidae were missing entirely from the stones following treatment and had not returned by May 2012.

Table7.2: Effects of the rotenone treatment on invertebrate densities assessed by comparing taxon-specific density immediately before, 3 days after, and 2 months after treatment, using 1-way general linear models. Significant effects are displayed in bold. After

Taxon	F	df	p	After 3 days	After 2 months
Ephemeroptera	**11.72**	**2, 32**	**0.001**	**Lower**	**n/s**
Baetidae	**22.48**	**2, 32**	**<0.001**	**Lower**	**n/s**
Heptageneidae	**5.12**	**2, 32**	**0.01**	**Lower**	**Lower**
Odonata	1.92	2, 32	0.16	n/s	n/s
Trichoptera	1.08	2, 32	0.35	n/s	n/s
Coleoptera	0.15	2, 32	0.86	n/s	n/s
Hydraenidae	**5.41**	**2, 32**	**0.009**	**Higher**	**n/s**
Diptera	**6.31**	**2, 32**	**0.004**	**n/s**	**Higher**
Chironomidae	**6.28**	**2, 32**	**0.004**	**n/s**	**Higher**
Tipulidae	**11.36**	**2, 32**	**<0.001**	**Lower**	**Lower**
Gastropoda	**278.39**	**2, 32**	**<0.001**	**n/s**	**Higher**

Note: Lower indicates significant decrease relative to pre-treatment density; Higher indicates significant increase relative to pre-treatment density; n/s indicates no significant deviation from pre-treatment density.

While the Coleoptera overall showed no significant change in abundance, densities of one family (Hydraenidae) actually increased on the stones immediately following the treatment (Table 7.2). This may be a result of increased mobility and activity in some beetle groups, as Coleoptera were significantly more abundant in the drift on the day following rotenone treatment compared to natural drift levels captured before the treatment (Student t test: $t = 2.98$, $df = 5$, $p < 0.05$). Densities of hydraenid beetles were nonetheless back to pre-treatment abundances on the stones by May.

Among the Diptera, Tipulidae were severely impacted by the rotenone treatment and had not recovered to pre-treatment densities on the stones by May. In sharp contrast, the Chironomidae and Simuliidae significantly increased in abundance on the stones in May, significantly boosting overall Diptera densities on the stones (Figure 7.6). The significant increase in Diptera densities suggests a community-level shift driven by rotenone. Overall, Diptera were naturally less abundant on treatment area stones than control area stones in February 2011 ($t = 3.03$, $df = 22$, $p < 0.01$) and February 2012 ($t = 3.72$, $df = 22$, $p < 0.01$), whereas they were not significantly different from control area densities in May 2012 ($t = 0.74$, $df = 22$, $p > 0.05$). It is possible that rotenone allowed the rapid colonisation of the stones by opportunistic dipteran taxa by removing key predators or competitors. Future follow-up surveys would be needed to assess whether pre-treatment community compositions will reassert themselves as these key taxa recover.

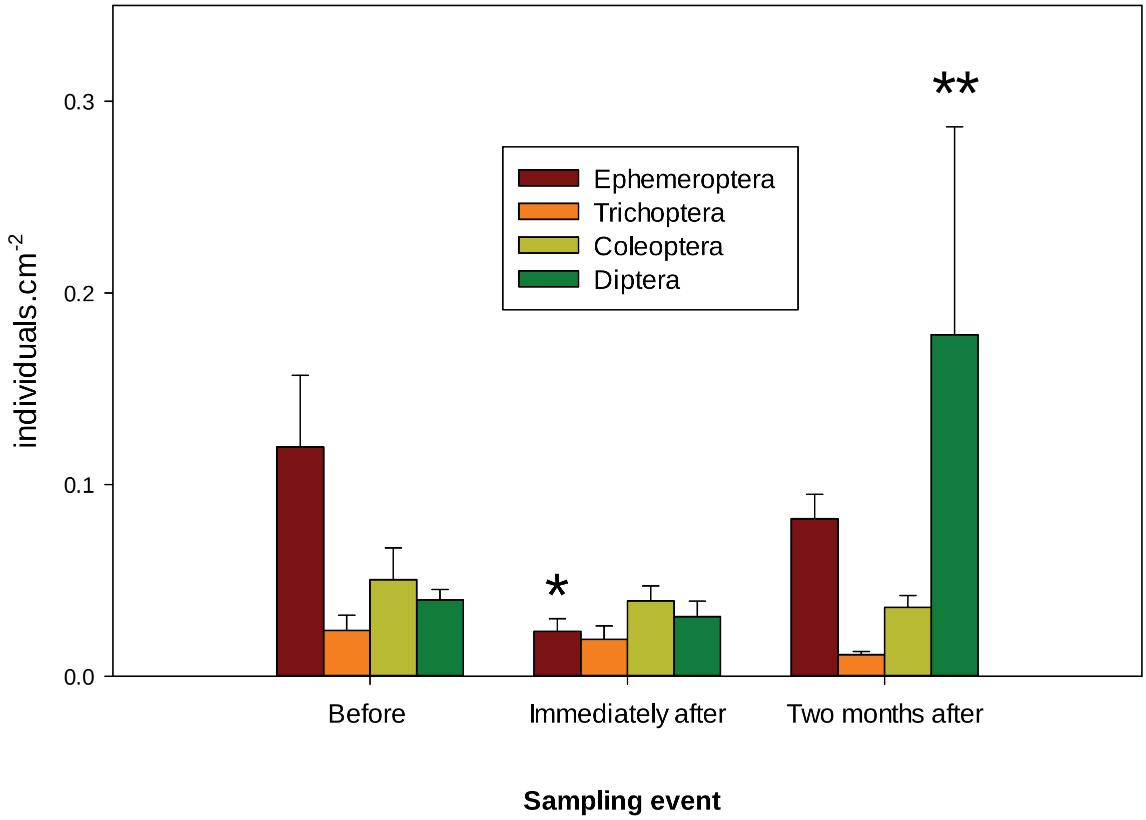

Figure 7.6: Densities of key common insect orders on sampled stones collected 1 week before, 3 days after (March 2012) and 2 months after (May 2012) rotenone treatment. Single asterisk (*) indicates a significant decline relative to pre-treatment levels. Double asterisk (**) indicates a significant increase relative to pre-treatment levels.

7.5 Rotenone Treatment Effects on Drift

As was expected given the historical data on rotenone treatments in rivers, a catastrophic drift event occurred during the application of rotenone to the Rondegat River. The effect was immediate, with total 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 (Figure 7.7). Following the end of rotenone treatment, drift rapidly declined to near-pre-treatment levels, although the Coleoptera continued to drift at significantly higher-than-baseline levels for at least 48 hours. The proportional abundance of macroinvertebrate orders also shifted over the course of the treatment (Figure 7.8). Ephemeroptera, which were the second most abundant group after Diptera in the drift two hours before treatment commenced, rose to 60% of all macroinvertebrates captured in the first hour of treatment (Figure 7.7). By 1pm, just over halfway through the treatment and the time of peak drift (Figure 7.7), Coleoptera were numerically dominant, comprising 52% of all macroinvertebrates captured (Figure 7.8). By 7am the following morning, 16 hours after rotenone treatment ceased, Diptera had become numerically dominant once again, and the drift had returned to near-pre-treatment levels (Figures 7.7 and 7.8).

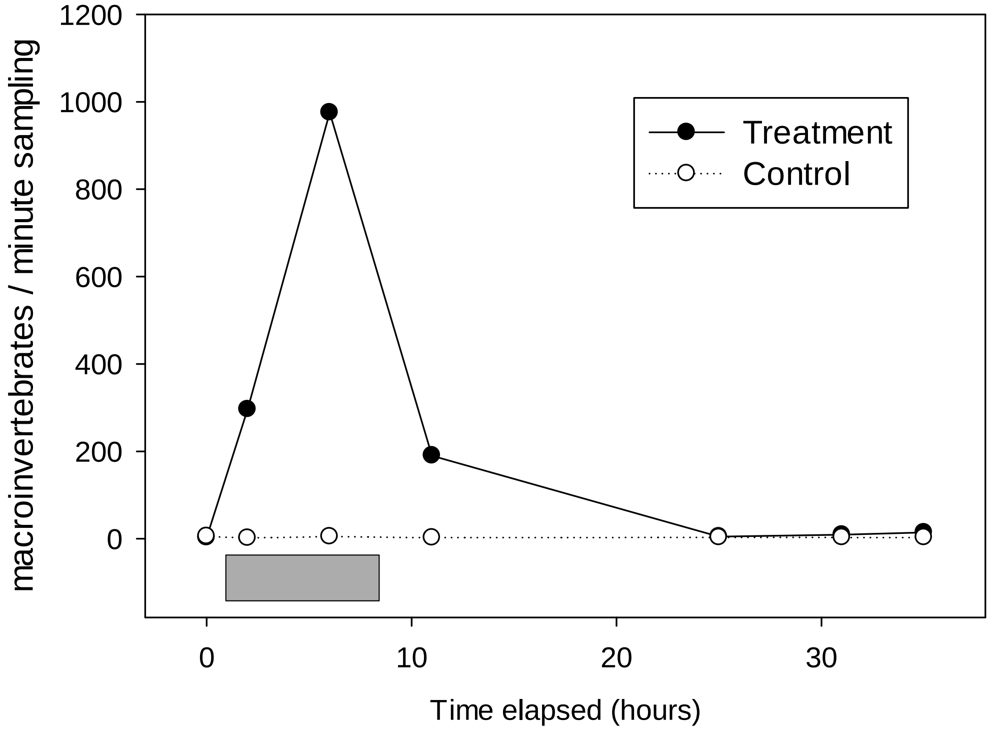

Figure 7.7: Total invertebrate drift abundance at control and treatment sites on the day of treatment (29 February) and on the following day (1 March). The period of rotenone treatment is denoted by the grey area above the x-axis.

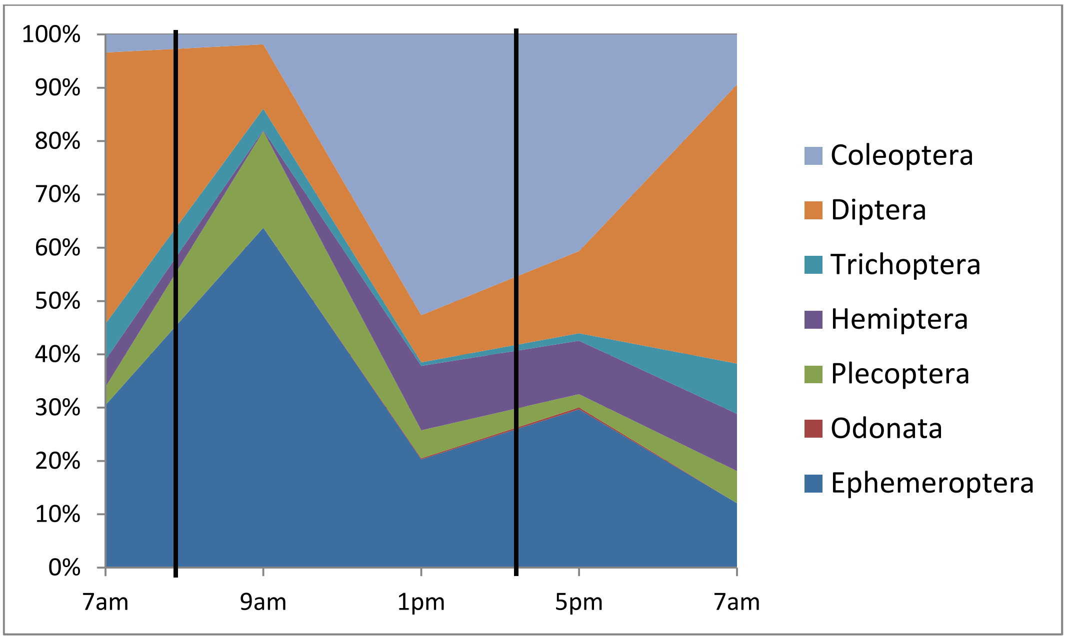

Figure 7.8: Proportional abundances of invertebrate orders in drift before, during and after treatment. Samples taken during rotenone treatment fall within the two vertical bars on the graph.

7.6 Ecosystem Health Effects as Detected by Chlorophyll-a Analyses

Seasonal concentrations of cholrophyll-a across all treatment zone sites showed significant variation among seasonal samples (ANOVA: $F_{(4, 70)} = 12.41$, $p < 0.0001$), with the February 2012 stones having more algae production than in any of the other months (Post-hoc Tukey, $p < 0.05$). This finding makes it difficult to attribute changes in algal abundance on the stones to the rotenone treatment, as the May concentrations (the only samples taken post-treatment) were in line with previous samples for that month (Figure 7.9). 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 ($t = 2.48$, $df = 22$, $p < 0.02$). 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.

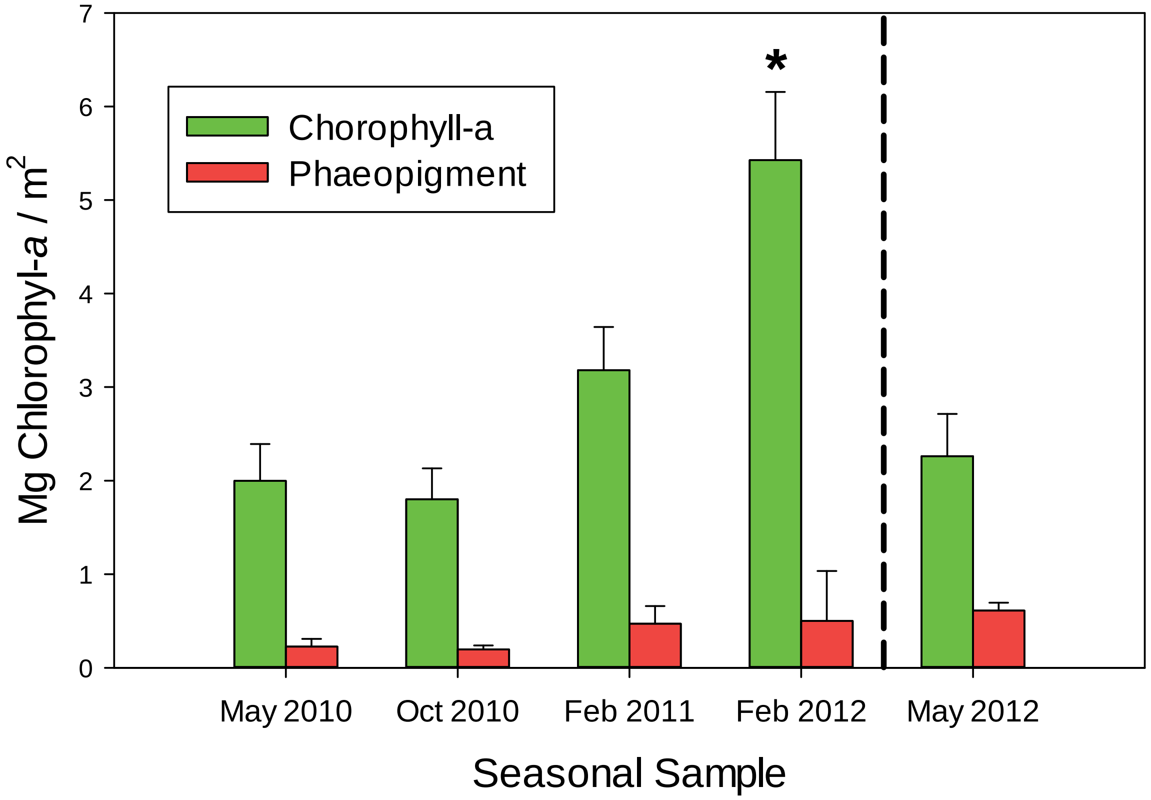

Figure 7.9: Mean (+SE) Chlorophyll-a concentrations recorded on sampled stones in the treatment zone in three pre-treatment samples and one post-treatment sample. Asterisk denotes a significantly higher concentration than in other samples. The division between pre- and post-treatment samples is illustrated by a dashed vertical line.

7.7 Conclusions

The fundamental conclusion that can be gathered from comparing pre- and post-rotenone treatment samples of the macroinvertebrate community is that the rotenone treatment had a relatively minor and short-term negative impact on community composition. An assessment of common taxa demonstrates the overall robustness of the Rondegat aquatic community: by May 2012, the reduction in diversity that could be linked to the rotenone treatment stood at 18%. This represents a relatively low proportion when compared to previous studies summarised in Vinson (2010), where species losses ranged from 10 to 54% in the first year following treatment. The missing common species included only two taxa that did not occur upstream of the treatment zone, indicating further recovery of the dominant invertebrate fauna should continue rapidly. Moreover, the extreme variability in species presences and relative abundances detected in seasonal monitoring before and after demonstrates that the Rondegat River hosts a highly dynamic macroinvertebrate community that experiences significant seasonal variation, which appeared to eclipse even the short-term negative impacts of the rotenone treatment.

The monitoring data demonstrated that South African macroinvertebrate taxa react to rotenone in much the same way as their American and European relatives. Ephemeroptera from the families Baetidae and Heptageneidae, as well as Diptera from the family Tipulidae appeared the most severely affected in terms of abundance on the Rondegat River. All of these taxonomic groups have displayed high or moderate sensitivity to rotenone both in laboratory studies (Vinson et al. 2010) and, in the case of Baetidae, in previous field studies (Arnekleiv et al. 2001; Lintermans and Raadik, 2003). The catastrophic drift event recorded during the rotenone treatment further supported previous studies of this phenomenon, which found it to be a behavioural response to rotenone rather than a mass mortality event (Dudgeon 1990). For example, Coleoptera drift was significantly elevated both during, and on the day following, the rotenone treatment, but Coleoptera were not significantly less abundant on stones sampled in the week following the treatment.

Assessment of ecosystem-scale changes resulting from rotenone treatment failed to detect significant shifts, either in ecosystem health as determined by SASS scoring, or in secondary food-web effects as determined by Chlorophyll-a analysis. Given the relatively minor, short-term effects of rotenone on the macroinvertebrate community, these results are not surprising. In the case of SASS, the lack of clear evidence of ecosystem health effects may be due in part to the nature of the treatment area. The bass-invaded reach was a low-gradient foothill stream with evidence of long-term riparian alteration from cattle farming, and thus had a relatively degraded macroinvertebrate community before the rotenone was applied. It is thus likely that highly sensitive species that might have shown equally negative responses to the rotenone as they would to other impacts on water quality (such as increased oxygen demand emanating from agricultural impacts on the stream) were already missing from the treatment area, and that the remaining macroinvertebrate fauna was better able to cope with the rotenone than a pristine mountain stream community may have been.

8 LESSONS LEARNT AND PROTOCOL FOR FUTURE TREATMENTS

The combined evidence of the monitoring programme indicate that the Rondegat River rehabilitation project has been a success, in that it achieved its goal of removing invasive alien fish from the treatment area without causing a significant negative impact on the macroinvertebrate or amphibian communities. The fish surveys produced no evidence of bass surviving the treatment, and while a few common invertebrate species were apparently lost, it is highly likely that these species will return to the stream in the coming months and years. The long-term prognosis for the stream community, as well as the ultimate ecosystem-scale effect of the treatment (including the eventual replacement of the bass by native insectivorous fishes), will only become clear with continued monitoring in the coming years. CapeNature is committed to performing a second treatment during the summer of 2012/2013 and will require monitoring of the immediate and long term impacts of this follow-on operation (see Box 8.1 for a statement by CapeNature's Rondegat Project manager regarding completed and planned impact monitoring). It is thus recommended that this monitoring programme be continued for as long as is logistically feasible.

The amphibian monitoring data confirmed that while tadpoles and some frogs were negatively impacted by the rotenone treatment, the long term effects are likely to be negligible, while the removal of bass may result in short-term positive effects on the abundance and diversity of frogs in the treatment zone. The invertebrate monitoring data revealed a highly variable community of macroinvertebrates, with many species that appeared and disappeared on a seasonal basis. The long-term pre-treatment monitoring, which spanned two years of species-level assessment prior to the operation, proved to be most valuable in placing the immediate effects of the rotenone in context. In particular, the comparison of 2011 and 2012 pre-treatment data demonstrated that many apparent species losses recorded post-treatment were likely the result of high variability in seasonal abundance, as well as low detectability. The high proportion of apparent losses attributed to rare and incidental species within the greater macroinvertebrate community demonstrated the overall robustness of this community to the rotenone.

Given the relatively minor immediate effects of rotenone on overall community composition, it is not surprising that SASS5 field scoring of ecosystem health did not reveal a significant impact of the treatment. It should nonetheless be noted that had the operation been conducted in a higher-altitude mountain stream with a more intact riparian zone than the lower Rondegat River, it is quite possible that the macroinvertebrate community may have had a more noticeable negative response, which in turn would have been reflected in the total SASS and ASPT metrics. For example, another candidate river for rotenone treatment, the Krom River in the Cederberg, does flow through relatively pristine fynbos and may therefore support a more diverse "reference" community that is consequently more vulnerable to rotenone. Regardless of scoring results, the SASS5 sampling methodology did provide a robust collection strategy by which the dominant biotopes were adequately and consistently sampled each season. This method allowed major seasonal shifts in community structure to be detected, and the common species that dominate the macroinvertebrate community to be identified. Thus, while the SASS5 scoring system may not be a particularly useful measure of the impact of rotenone in agriculturally impacted streams like the Rondegat, the field methodology associated with the technique provides a good strategy for the collection of species-level monitoring data.

Box 8.1: A CapeNature perspective on the Rondegat River monitoring programme

CapeNature's project to eradicate invasive alien smallmouth bass in the Rondegat River of the Cederberg region, Western Cape Province, has been at least 10 years in the making. Invasive alien fishes are the prime reason for the highly threatened status of the freshwater fishes of the Fynbos region, most of which are found nowhere else. Fish conservation experts agree that eradication of alien fishes from National Freshwater Ecosystem Priority Areas and Fish Critical Biodiversity Areas, to recover areas of critical habitat, will play a major role in helping to down-list the conservation status of many species, several of which are endangered or critically endangered. During the projects development, especially during the EIA phase in 2008, there was considerable criticism of aspects of the project, especially from flyfishermen who primarily target trout. There was also considerable criticism in the media at that time. Most of the antagonism and criticism was focused on the use of rotenone and its impact on non-target fauna such as the aquatic macroinvertebrates. Sensational statements were made in the media that the use of rotenone would "create aquatic deserts". Rotenone projects worldwide, especially in the USA, are usually carefully planned and use "best practice" methodologies to obtain legislative approval. The American Fisheries Society has developed a manual to ensure the safe and responsible use of pisicicides containing rotenone by USA environmental agencies. A key requirement in the manual is the development of a series of plans to guide a pisicicide project, one of which is a Monitoring plan that includes a biological monitoring component. This is to ensure that the impact of a rotenone operation on the biological communities inhabiting an inland water body is properly quantified by appropriate experts. The EIA for the project also addressed this issue by developing an Environmental Management Plan and Research Monitoring Plan.

CapeNature is pleased that Phase 1 of the biological monitoring for the Rondegat River Fish Rehabilitation Project has been timeously completed with the compilation of this report. We have stated to our project stakeholders repeatedly over the last four years that the success of this conservation project will be determined by the eradication of smallmouth bass from the treatment section and by an ecologically healthier river afterwards. The stakeholders, including the angling community, agree that these are the two pillars that will determine the project's success, thus allowing further projects to be undertaken across the Fynbos biome. This report suggests that the first task has been accomplished, although it is Standard Operating Procedure in the USA and Norway when undertaking piscicide projects involving rotenone to do two river treatments one year apart to maximise the likelihood of a 100% kill of the target species. The report also suggests that the aquatic invertebrates are recovering well post-treatment, but noted that five common species found pre-treatment have not yet re-appeared in the treatment section three months post-treatment. This is of concern to CapeNature and highlights the urgent need for biological monitoring to continue for at least another 2-3 years, so that we have a clearer understanding of the recovery of the system.

Statement by Dean Impson (Project leader: Rondegat River Rehabilitation Project), August 2012

Chlorophyll-a analysis showed more variation attributable to seasonal patterns than the indirect impacts of rotenone. It is unclear whether future monitoring of algae in the Rondegat will represent a useful activity, given that the natural processes driving algal production in the Rondegat River are not well understood. As algal spectrophotometry represents a significant investment in time and money, its continued use in on-going monitoring does not appear justified. A better way to assess ecosystem-scale impacts may be to assess the invertebrate community already captured in terms of the presence and relative importance of functional feeding groups, as these data will provide a more direct assessment of shifts in food-web structure than trying to monitor impacts at lower trophic levels.

8.1 Recommended protocol for future rotenone monitoring on rivers

Given these overall lessons learned, it is recommended that future ecosystem monitoring efforts that accompany rotenone treatments adhere to the following recommendations:

- Assessment of fish communities prior to treatment is important, but need only be done once, as fish community structure is unlikely to vary dramatically from year to year if the barrier to fish invasion is stable, as was the case in the Rondegat River.

- Multiple sampling methods should be applied to assess fish abundances, and compared to ensure accurate assessments of fish diversity and abundance. The turbidity and conductivity of water in the monitoring reaches will dictate the relative efficacy of snorkel surveys, underwater video analysis and electrofishing, and early assessment of these environmental variables should be undertaken to guide the fish monitoring strategy.

- The vulnerability of amphibians to both native and non-native fishes means that amphibians are unlikely to occur in significant numbers in any river reach targeted for alien fish removal, and it is thus highly unlikely that on-going monitoring of amphibians will be required in most situations. Nonetheless, a one-off assessment of amphibian diversity and abundance within the planned treatment area should always be undertaken early in the monitoring programme to assess whether any threatened frog species occur in the proposed treatment area. If none are detected, then further monitoring of the herpetofauna will be unnecessary.

- Amphibians are generally not easily detected using the fish survey methods utilised in this study. It is thus recommended that a once-off specialist survey by a trained herpetologist be conducted to assess the proposed treatment area, and that this is done at a time when the majority of species predicted to occur in the treatment area are breeding and thus detectable by aural encounter surveys.

- Sampling of invertebrates prior to the operation should be conducted seasonally and include a minimum of one survey a year before the proposed treatment during the season in which the operation is planned (either spring or late-summer), as well as a survey immediately prior to the operation. This will ensure annual variation in community structure is taken into account. It is further recommended that one more pre-treatment survey in the season where treatment is not planned (either spring or late-summer) be conducted to assess seasonal variation in community structure.

- Depending on funding, post-treatment assessment of invertebrate community structure should continue in these seasons to assess how the removal of alien fishes affects the ecosystem.

- In Cape Floristic Region mountain streams, late autumn (May) is not recommended as a monitoring season for future pre- or post-treatment assessment, as the variable timing of autumn floods could severely confound any assessment of invertebrate community structure.

- The natural variability detected in stone-surface chlorophyll/phaeopigment analysis, coupled with the logistical and financial investment required to generate these data, means that algal production is not a practical indicator for monitoring ecosystem-scale effects of rotenone treatment. It is therefore not recommend for use in future monitoring efforts.

- While SASS sampling methods proved to be a highly efficient method of sampling the instream invertebrate community, the SASS5 scoring system should only be used as an indicator of rotenone impacts if the treated reach is in an otherwise pristine environment (upstream of urban or agricultural development) where a "reference condition" community is likely to occur. However, given the fact that most alien invaded reaches in the CFR are unlikely to contain such communities, due to the combined impacts of the alien fish, riparian zone impacts and the effect of lower gradient on instream habitat, it is unlikely SASS scoring will provide a meaningful measure of ecosystem response to rotenone treatment should it be employed in rehabilitation efforts.

- One drawback of the SASS sampling methodology is the use of a 1 mm mesh kick net, which allows smaller invertebrates to sometimes evade capture. It is recommended that kick-sampling be conducted with a 500 µm mesh net to ensure the entire macroinvertebrate community within a biotope is captured.

- While this study assessed both the presence and abundance of invertebrate taxa through the use of individual stone samples, this extra step is not necessary in determining the overall impact of rotenone on invertebrate community structure. Given that this report does not recommend algal surveys for future monitoring efforts, the use of stone samples as an optional extra to kick sampling, should be utilised for its academic value and if funding permits.

- Similar to stone sampling, the sampling of drift is not considered to provide a critical indicator of the long-term impacts of rotenone treatment. Given the time-intensive nature of processing drift samples, especially those collected during rotenone applications, collecting drift should only be part of the monitoring strategy if the produced data's research value can be justified.

- At a minimum, it is recommended that annual follow-up surveys of fish and invertebrate communities be conducted for the first two years after treatment, to confirm the long term absence of the eradicated species and to monitor the recovery of the ecosystem.

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10 APPENDIX A: RESULTS FROM VARIOUS METHODS OF ASSESSING FISH SPECIES COMPOSITION AND ABUNDANCE IN THE RONDEGAT RIVER

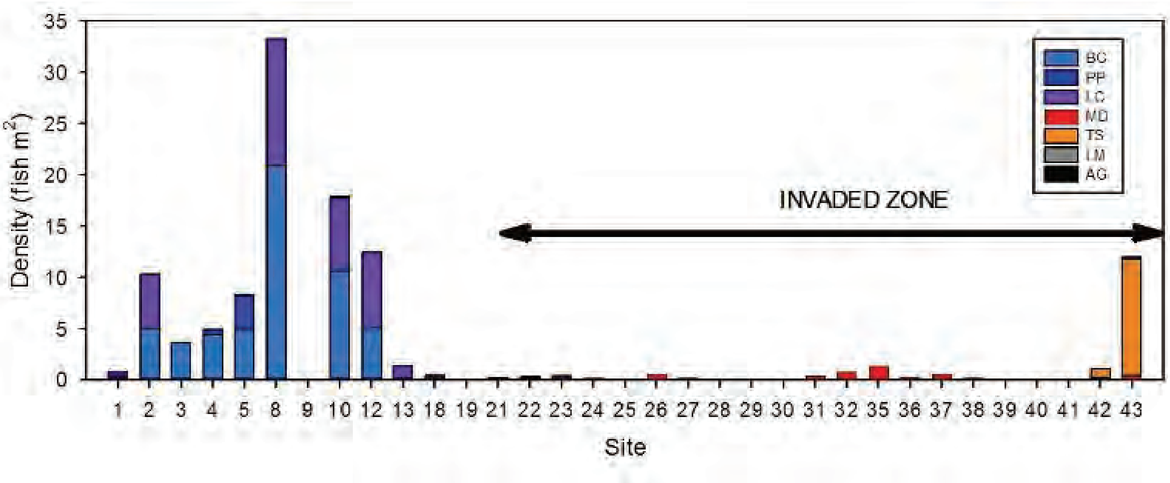

Figure A.1. Underwater video analysis max N for native and alien fish species in sampled sections of the Rondegat River, Western Cape. BC = *Barbus calidus*, PP = *Pseudobarbus phlegethon*, LC = *Labeobarbus capensis*, MD = *Micropterus dolomieuii*, TS = *Tilapia sparrmanii*, LM = *Lepomis macrochirus*, AG = *Austroglanis gilli*.

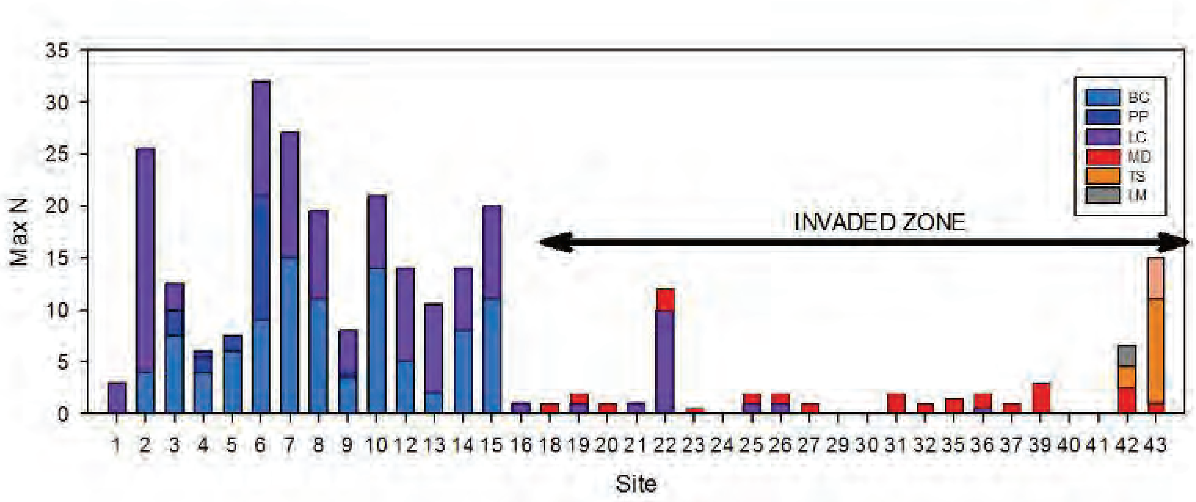

Figure A.2. Fish density determined from snorkelling surveys for native and alien fish species in sampled sections of the Rondegat River, Western Cape. BC = *Barbus calidus*, PP = *Pseudobarbus phlegethon*, LC = *Labeobarbus capensis*, MD = *Micropterus dolomieuii*, TS = *Tilapia sparrmanii*, LM = *Lepomis macrochirus*, AG = *Austroglanis gilli*.

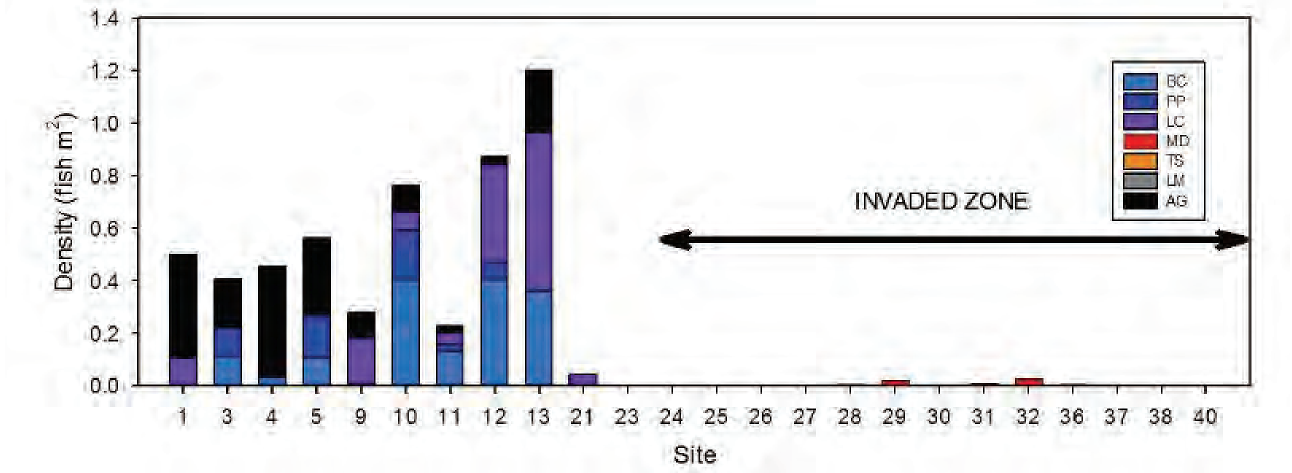

Figure A.3. Fish density determined from electrofishing surveys for native and alien fish species in sampled sections of the Rondegat River, Western Cape. BC = *Barbus calidus*, PP = *Pseudobarbus phlegethon*, LC = *Labeobarbus capensis*, MD = *Micropterus dolomieuii*, TS = *Tilapia sparrmanii*, LM = *Lepomis macrochirus*, AG = *Austroglanis gilli*.

11 APPENDIX B: SOME FROG HABITAT FEATURES IN THE RONDEGAT VALLEY

Indigenous Palmiet, *Prionium serratum* (left) with introduced Madumbe, *Colocasia esculenta* (centre) and Bramble, *Rubus sp*. (right), tangles at Keurbos. This patch of Colocasia, which extended across the stream, may be the first record of this invasive and potentially stream transforming species in the Cederberg (Cunningham).

Pond and wetland at Rietvlei, with *Amietia vertebralis* and *Strongylopus grayii* (Cunningham).

Irrigation furrow on the river flats at Rietvlei, looking SE towards Keurbos (Cunningham).

Rietvlei transect Rg2; shallow side pools with grassy edges and *Amietia fuscigula* (Cunningham).

Felled wattle obstructing access to the stream immediately above the treatment zone (Cunningham).

Large sandy pool at Rooidraai just below the barrier to upstream movement of bass (Cunningham).

Neutralisation of rotenone in Rg0 with non-toxic potassium permanganate (Cunningham).

Site in the control area Rg5 (above the treatment zone) in the early morning (Cunningham).

Fish barrier on the Rondegat, Rg8 above Algeria, 23.75 km upstream from the confluence. *Pseudobarbus phlegethon* was present in the pool at centre (Cunningham).

Amietia fuscigula in aquatic moss, transect Rg8 above Algeria (Cunningham).

The Rondegat River exits the mountains at the confluence with Clanwilliam Dam on the Olifants River at Rondegat Farm (late summer, low water level). The bridge over the Rondegat, in the kloof at centre-right, is 1.16 km below the neutralisation station (Cunningham).

12 APPENDIX C: INVERTEBRATE BIODIVERSITY BASELINE CHECKLIST FOR THE RONDEGAT RIVER TREATMENT ZONE

Table C.1: Abundances of identified macroinvertebrate taxa collected from the treatment zone prior to rotenone operations in February 2011 and February 2012.

Taxon	February 2011 Kick samples	February 2012 Kick samples	February 2011 Stone samples	February 2012 Stone samples
Ephemeroptera				
Baetidae				
Afroptilum sp	24	3	31	18
Afroptilum sudafricanum	21	1	1	0
Baetis harrisoni	93	31	29	12
Cheleocloeon excisum	40	15	16	5
Cloeon sp 1	10	0	0	0
Dabulamanizia media	1	0	0	0
Demoulinia crassi	2	0	0	0
Glossidion sp	1	0	0	0
Peuhlella sp	15	3	25	16
Pseudopannota maculosa	35	20	7	12
Pseudopannota sp	1	0	0	0
Pseudocloeon glaucum	11	0	0	0
Pseudocloeon piscis	169	30	3	0
Pseudocloeon vinosum	13	9	0	0
Securiops macaffertorum	1	0	0	23
Caenidae				
Afrocaenis sp	78	47	0	2
Caenis sp	109	9	56	2
Heptageniidae				
Afronurus sp	11	1	1	4
Leptophlebiidae				
Euthraulus elegans	23	13	76	20
Teloganodidae				
Lestegella pennicillata	0	0	1	0
Odonata				
Aeshnidae				
Aeshna sp	10	0	2	0
Anax sp	0	0	0	0
Chlorocyphidae				
Platycypha sp	1	1	0	0
Coenagrionidae				
Pseudagrion sp	22	46	0	0
Gomphidae				
"Ictinogomphus sp"	0	1	0	0

Paragomphus sp	54	36	5	0
Libellulidae				
Sympetrum fonscolombii	1	1		
Trithemis sp	29	5	2	0
Zygonyx sp	0	1	1	0
Protoneuridae				
Ellatoneura sp	1	0	0	0
Plecoptera				
Notonemouridae				
Aphanicercopsis sp	0	0	0	0
Aphanicercella sp	0	0	3	0
Hemiptera				
Veliidae				
Rhagovelia sp	76	4	0	0
Naucoridae				
Neomacrocoris sp	1	5	0	0
Nepidae				
Borborophilus afzelii	2	0	0	0
Notonectidae				
Anisops sp	2	0	0	0
Corixidae				
Mirconecta sp	0	1	0	1
Enithares sp	0	0	0	0
Trichoptera				
Ecnomidae				
Ecnomus sp	5	4	10	12
Hydropsychidae				
Cheumatopsyche afra	4	3	0	0
Cheumatopsyche sp	101	32	31	20
Cheumatopsyche thomasseti	0	0	1	1
Macrostemum capense	8	0	0	1
Hydroptilidae				
Oxyethira velocipes	6	0	0	0
Hydroptila cruciata	94	1	44	0
Leptoceridae				
Athripsodes harrisoni	12	17	0	0
Athripsodes prionii	12	0	0	0
Athripsodes sp	0	0	0	0
Leptecho sp	3	0	3	0
Leptecho helicotheca	1	0	0	0
Oecetis modesta	15	0	18	2

Polycentropodidae				
Paranyctiophylax sp	0	2	10	3
Sericostomatidae				
Petroplax sp	0	0	2	0
Coleoptera				
Dytiscidae				
Uvarus sp	0	1	0	0
Sharphydrus sp	0	0	0	0
Elmidae				
Tropidelmis hintoni	0	0	0	1
Elpidelmis capensis	3	0	0	2
Helminthopsis sp	0	0	0	1
Microdinodes sp	2	0		
Elmid oval morph	37	0	74	40
Elmid semi-oval morph	0	0	13	32
Elmid elongate morph	0	5	64	29
Peloriolus sp	5	0	22	19
Gyrinidae				
Aulonogyrus sp	0	0	0	0
Orectogyrus sp	0	0	0	2
Hydraenidae				
Mesoceration sp	0	2	1	1
Parhydraena	2	0	0	0
Prosthetops sp	2	0	0	0
Hydrophilidae				
Hydrophilid larvae	1	0	0	0
Ptylodactylidae				
Ptylodactylid sp	6	0	6	4
Scirtidae				
Scirtid sp	10	0	15	5
Diptera				
Blephariceridae				
Elporia sp	1	0	0	0
Ceratopogonidae				
Bezzia sp	8	0	1	0
Atrichopogon sp	0	1	0	0
Culicidae				
Anopheles sp	24	11	0	0
Culex sp	199	0	0	0
Tabanidae				
Tabanid sp 1	0	1	0	0

Tipulidae				
Antocha sp	5	4	46	30
Limnophila sp	2	0	0	0
Simuliidae				
Simulium impukane	17	1	1	2
Simulium medusaeforme	139	45	5	4
Simulium unicornutum	10	0	5	1
Simulium pomeroyellum sp	3	0	0	0
Simulium ruficorne	0	0	0	0
Simulium bequarti	0	0	1	0
Malacostraca				
Potamonautes sp	9	6	1	1
Gastropoda				
Ancylidae				
Ferrissia sp	1	0	0	0
Burnupia sp	0	0	0	0
Unidentified Taxa				
Diptera				
Chironimidae				
Chironimid larvae	421	48	173	61
Oligochaeta				
Various ologochaets	3	2	66	3
Acari				
Hydrocarinidae				
Various hydracarinids	5	0	27	11