Monitoring of invertebrate and fish recovery following river rehabilitation using rotenone in the Rondegat River

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

A South African perspective to Piscicide Treatments

Fish invasions have been cited as a primary threat to imperilled South African fishes and other aquatic fauna. As a result, the management and control of alien invasive species is a legislated priority in South Africa. From a river rehabilitation perspective, eradicating alien fish allows for the rehabilitation of several kilometres of river, with very significant benefits for the endangered fish species present and for the associated aquatic biota. In South Africa, the piscicide rotenone is one of the preferred methods for achieving eradication.

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 non-target aquatic organisms. As a result, monitoring is an essential component of eradication projects. The primary objective of the research reported in this Technical Report was to use the Rondegat River as a case study to (1) first assess the efficacy of South Africa's first alien fish eradication project, and (2) provide information of how the ecosystem in a river recovers following treatment with rotenone. The specific aims of the project were to:

- Determine how the Rondegat River ecosystem responds to the removal of alien fishes over a three year period (2012-2015).
- Assess rates of recovery of the invertebrate community following rotenone treatment.
- Assess the recovery rates of native fish communities following rotenone treatment.
- Test the hypothesis that native invertebrate and fish communities rebuild to approximate those in the non-invaded zone of the river.
- Develop recommendations for monitoring fish and invertebrate populations for future river rehabilitation projects.
- Develop human capacity in fish and invertebrate monitoring.

Study site

The Rondegat River is typical of many invaded streams in the Cape Floristic Region. The 28 km-long single-channel river is shallow (<1 m deep) and relatively narrow (2-4 m wide). The river receives most of its flow in winter and early spring (May to September), and the groundwater-dependent summer discharge is very low (0.07-0.08 m^3/s). The river flows into a 1124-ha warm-water impoundment, Clanwilliam Dam, where alien black bass populations have been established since 1948. Historically these fish had invaded the lower Rondegat River up to a waterfall located 5 km upstream of the dam. The subsequent construction of a weir some 4 km below the waterfall (sometime in the 1960s) effectively isolated the smallmouth bass in the 4 km stretch of river between the waterfall and weir. This section of river was treated using rotenone in February 2012 and March 2013 based on the assumption that the removal of Smallmouth bass from the bounded section of river (i.e. between the weir and Rooidraai) would result in the recovery of the native fish.

Monitoring methods

River surveys were conducted to document the short term impact and efficacy of the first rotenone and second rotenone treatments and assessing recovery rates of native fishes and invertebrates following treatment.

Fish populations were monitored at 42 sites between February 2011 and March 2015. Fish abundance at each site was estimated using two independent methods: underwater video analysis (UWVA) and snorkel surveys. Underwater Video Analysis was carried out using GoPro® HD Hero® cameras. Cameras were placed in each site and recorded footage for a minimum of 30 minutes and viewed to obtain estimates of fish abundance and diversity. Snorkel surveys used the two pass method where all fish encountered by the observer were counted on each pass and an estimate of abundance is derived from the mean of the two counts. To assess for size structure, all fishes that died during the rotenone operations were collected and measured. For comparisons in the control area and in the treatment area during the recovery period (2014 and 2015), fish were sampled using seine nets and fyke nets. All fish that were caught were identified to species level, measured to the nearest 1 mm fork length (FL) and released at the site of collection.

Invertebrate monitoring was conducted seasonally at three monitoring sites within the treatment area, three monitoring sites in the control area upstream of the treatment section, as well as at a monitoring site downstream of the treatment area. Monitoring comprised a total of 13 sampling events between May 2010 and February 2015. The sampling techniques included drift, stone and kick sampling. These are described in detail in Woodford et al. (2013) "*Monitoring the impact and recovery of the biota of the Rondegat River after the removal of alien fishes*" (Water Research Commission Report No. KV 304/12) and involved both SASS5 methods as well as species specific estimates of abundance.

Rondegat River monitoring results

Monitoring of fish and invertebrate communities in the Rondegat River demonstrated that:

- The treatment was successful with no alien smallmouth bass *Micropterus dolomieu* detected in the rehabilitated reach of river following treatment.
- Native fishes rapidly re-colonised the reach where smallmouth bass had been eradicated with fish densities approximating those in control sites after 3 years.
- Assessments of invertebrate communities demonstrated that communities quickly recovered following the short-term impacts of rotenone treatment including “catastrophic drift” and decrease in abundance of EPT taxa (Ephemeroptera, Plecoptera and Tricoptera).
- In the long-term, ecosystem health as estimated by the SASS5 scoring system was not significantly altered by the rotenone treatment and densities of EPT taxa recovered to pre-treatment levels within one year following treatment.

Guidelines for future monitoring projects

Guidelines for future monitoring projects to assess for the efficacy of rotenone treatments with regard to removing alien fish, and for monitoring the responses by macro-invertebrates and fish to these treatments. In summary, recommendations include:

- Sampling recommendations including: appropriate sampling periods, site selection and number of sampling sites, water quality parameters and site descriptions.
- Recommendations for assessing fish communities using multiple sampling methods (snorkel survey, underwater video and electrofishing) to assess fish diversity and abundance.
- Invertebrate sampling methods including the use of the SASS5 scoring system and stone samples to provide the best quantitative data for monitoring.
- Choice of appropriate taxa, which in the case of the Rondegat Project the most useful were EPT taxa i.e. Ephemeroptera (Mayflies), Plecoptera (Stoneflies) and Trichoptera (Caddisflies).
- It is our suggestion that future monitoring uses a 2-part process: (1) SASS5 scores and (2) stone sampling; identification efforts should be directed at the EPT taxa but full samples should be retained for potential future work on other invertebrate groups.

Practical guidance for implementing monitoring projects is provided together with example datasheets.

Capacity Development

The Rondegat Project has been used as a training platform for Interns, BSc Honours, MSc and PhD students since its inception in 2011. Impacts include:

- BSc Honours Students: Annual participation of Rhodes University Honours students in monitoring activities. This has resulted in increased awareness on the impacts of alien species on aquatic environments and an interest in students pursuing careers in the aquatic sciences. In total 45 BSc Honours students participated in annual monitoring trips.
- MSc Student: Stuart Barrow earned an MSc degree for from the University of Stellenbosch in November 2014. His thesis was titled, "Contrasting impact of alien invasive sport fish in the Cape Floristic Region: a focus on *Micropterus dolomieu.*
- PhD student: Terrence Bellingan is making progress towards completing his PhD thesis which includes samples collected during this project and will be submitted in 2016.
- DST/NRF Interns: In total, four DST/NRF Interns were trained using project samples. All of these interns went on to undertake further studies in aquatic ecology.
    - Lubabalo Mofu was trained in 2013 and registered for an MSc in invasion biology in 2014.
    - Phumsa Ndaleni and Bosupeng Motshegoa were trained in 2014 registered for higher degrees in 2015.
    - Ann Wu was trained in 2015 has registered for a PhD in 2016.

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1. A SOUTH AFRICAN PERSPECTIVE TO PISCICIDE TREATMENTS

1.1. Alien invasive fishes, impacts and the need for management

The introduction and spread of non-native species is one of the least reversible human-induced global changes. In South Africa, 55 fish species have been introduced into novel environments over the last two and a half centuries (Ellender and Weyl, 2014). Only 11 introduced species failed to establish and of the 44 species that have established, 37% are considered fully invasive. Impacts on native biota include increased competition, direct predation, habitat alterations, hybridisation and the transfer of parasites and diseases. As a result, fish invasions have been cited as a primary threat to imperilled South African fishes and other aquatic fauna (Tweddle et al., 2009).

A recent catchment-scale assessment of the distribution and impacts of black bass (*Micropterus* spp.) on the Olifants-Doorn River system by van der Walt et al. (2016) illustrates the extent of the problem. Data from 41 tributaries demonstrated that black bass had invaded 81% of stream habitat in the basin where they had extirpated small-bodied cyprinid minnows (e.g. Clanwilliam Redfin Minnow *Pseudobarbus calidus* and Fiery Redfin Minnow *Pseudobarbus phlegethon*), while larger cyprinid species (e.g. Clanwilliam Yellowfish *Labeobarbus capensis*) co-occurred with black bass only at sizes too large to be preyed upon by the bass. These findings were similar to observations on trout and bass impacts in the Breede River system (Shelton et al., 2015), largemouth bass impacts in the Groot Marico River (Kimberg et al., 2014) and Swartkops River systems (Ellender et al., 2011). These findings also demonstrate the severe habitat loss to native fishes as a result of invasive predatory fish invasions. The prevention of the further spread and removal of alien predatory fishes from conservation priority areas such as Fish Sanctuaries identified in the National Freshwater Ecosystem Priority Areas (NFEPA, Nel et al., 2011) is therefore a high priority (Impson et al., 2013).

In recognition of the impacts of these and other alien invasive organisms on native environments, the management and control of alien invasive species is a legislated priority in South Africa (Republic of South Africa, 2014a; 2014b). To help managers to decide what course of action to take when faced with an alien invasive fish species, the WRC-supported K5/2039 Project: "**Developing a decision support tool for managing invasive fish in South Africa"** used results from case studies from throughout South Africa to develop a framework with which to decide under what conditions the removal of alien fishes was desirable and feasible from an implementation point of view (Kimberg et al., 2015). This framework is illustrated in Figure 1. As eradication of alien fishes is only feasible under conditions where re-invasion is unlikely (i.e. where the area to be treated is isolated from source populations of potential re-invasion), projects aiming at removing alien fishes need to consider potential upstream and downstream source populations of alien fishes . An additional consideration are the fish in off-channel dams that might invade if dams are breached during periods of high flow. Furthermore, it is important to note that eradications are only appropriate using methods that are able to completely eradicate the target organism in the area under consideration.

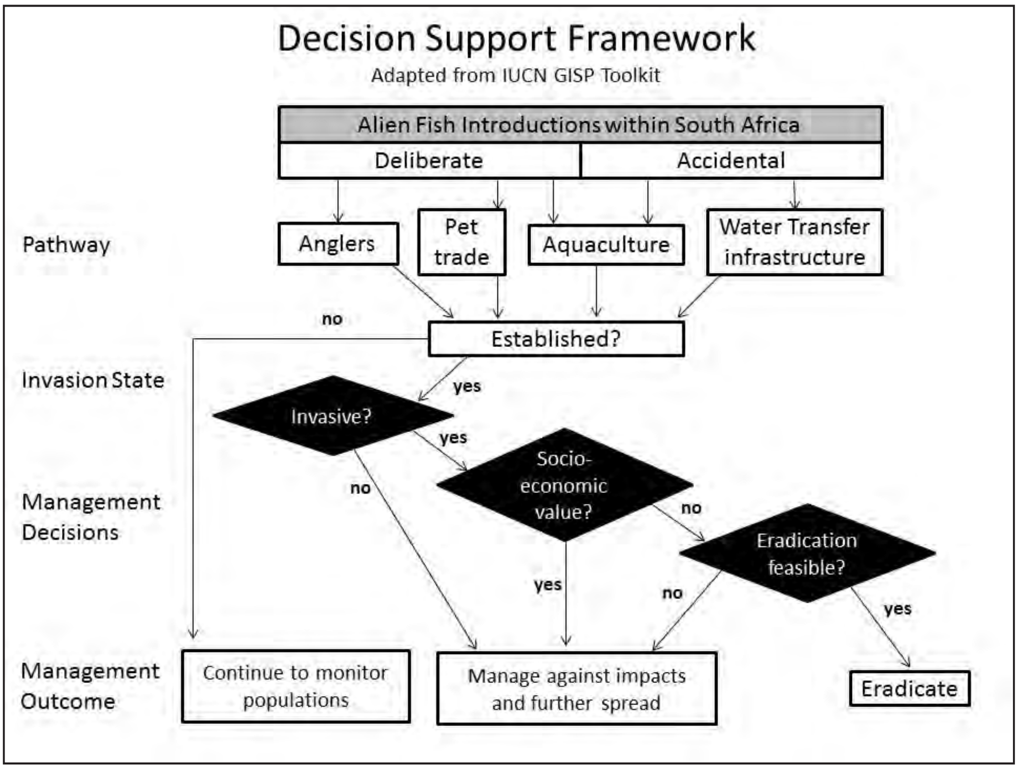


Figure 1

Umbrella decision support framework for managing alien freshwater fish species within South Africa developed during the WRC K5/2039 Project. Key questions are whether the species under consideration is invasive, has a socio-economic value (e.g. in recreational fisheries) and if eradication is feasible (Kimberg et al., 2015).

1.2. Use of piscicides in river rehabilitation

A process for managing alien invasive fishes has been initiated by several provincial conservation authorities (see Marr et al., 2012). CapeNature in particular, has consulted with key conservation stakeholders, including the South African Institute of Aquatic Biodiversity

(SAIAB) and the American Fisheries Society's (AFS) Fish Management Chemicals Subcommittee, to determine realistic fish eradication strategies and priorities for a total of 18 Rivers in the Cape Floristic Region (Weyl et al., 2014). From a river rehabilitation perspective, eradicating alien fish allows for the rehabilitation of several kilometres of river, with very significant benefits for the endangered fish species present and for the associated aquatic biota. This strategy is regarded by South African fish conservation experts as the best and fastest way of improving the conservation status of the highly threatened fishes.

Because the control of alien fish to benefit native fish is difficult, the direct intervention through the use of piscicides is the most appropriate method because complete removal of non-native fish from a particular area is usually required to recover the ecosystem's ability to support native species (Marr et al., 2012). The piscicide rotenone was chosen because it had recently been approved for reregistration (U.S. Environmental Protection Agency 2007), and a Rotenone Standard Operating Procedures Manual (Rotenone SOP Manual) to guide safe and effective use has been recently published by AFS (Finlayson et al., 2010). The value of the use of the chemical rotenone in conducting fish eradication projects has been extensively reviewed in the previous WRC K8/922 Project entitled "***Monitoring of the impact and recovery of the biota of the Rondegat River after the removal of alien fishes"*** (see Woodford et al., 2013).

Rotenone has been used to remove invasive fish from reservoirs and streams in the USA (Finlayson et al., 2005), Britain (Britton and Brazier, 2006; Britton et al., 2008), Australia (Lintermans, 2000) 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). These examples indicated 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.

1.3. Public perception, accountability and the importance of monitoring impacts of rehabilitation measures

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 non-target aquatic organisms (Dalu et al., 2015). 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; Weyl et al., 2014).

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 (e.g. 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). In South Africa, the responses of angling sectors to conservation projects that involve the control of alien fish species have varied, and appear to be mainly driven by vested interests of recreational anglers whom expressed concerns about the necessity of removing alien game fish and the risks of using rotenone on non-target taxa such as aquatic insects, native fishes, amphibians, and humans (see Ellender et al., 2014; Weyl et al., 2015). In addition, the Department of Water and Sanitation (DWS) have expressed concern on the potential impact of river treatments on non-target organisms which has resulted in recent delays in the approval of CapeNature's planned rotenone treatments of several rivers and off-channel impoundments (Dalu et al., 2015). As a result of good stakeholder involvement,

BOX 1

An overview of the piscicide rotenone

(after Woodford et al., 2013)

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 understanding the impacts of treatments and monitoring the response of native biota to interventions such as rotenone applications is an important part of river rehabilitation projects.

1.4. Purpose of monitoring

While rotenone treatments and alien fish eradication projects provide excellent opportunities to conduct research into better understanding impacts of alien fishes on invaded ecosystems, the primary objective of monitoring fish eradication projects is to determine the efficacy of the intervention and to determine how the ecosystem in a river impacted by alien fishes. Monitoring therefore focusses on four key questions:

1. Was the eradication successful?
2. Did the removal of alien fishes result in the desired objective (e.g. recovery of native fishes)?
3. Did the rotenone treatment have adverse impacts on non-target taxa?
4. What are the long term impacts on non-target taxa?

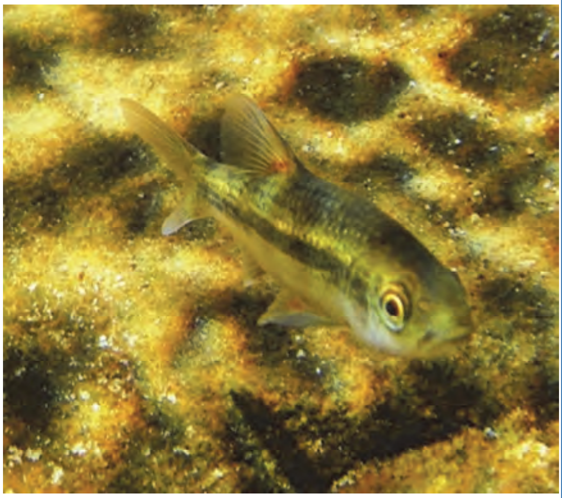
To provide guidance for the use of rotenone for future interventions native fish restoration projects and for the development of a National Policy, the current report uses the Rondegat River as a case study to assess these four key questions and make recommendations for monitoring of future projects.

BOX 2

Fishes of the Rondegat River

Smallmouth Bass *Micropterus dolomieu*

Fiery Redfin Minnow *Pseudobarbus phlegethon*



Clanwilliam Redfin Minnow *Pseudobarbus calidus*

2. RONDEGAT RIVER PILOT PROJECT

2.1. Background

The Rondegat River (Figure 2) is typical of many invaded CFR streams. The 28-km-long single-channel river is shallow (<1 m deep) and relatively narrow (2-4 m wide). The river receives most of its flow in winter and early spring (May to September), and the groundwater-dependent summer discharge is very low (0.07-0.08 m^3/s). The geology of the catchment is primarily sandstone resulting in river water of great clarity (summer turbidity 0.5-2.8 NTU), moderate acidity (pH 5.4-6.3), and relatively low conductivity (14-120 µS/cm). Water temperature varies from about 8°C in winter (June-August) to 27°C in summer (December-February). The river flows into a 1,124-ha warm-water impoundment, Clanwilliam Dam, where alien black bass populations have been established since 1948 (Weyl et al., 2013). The lower river has three barriers to fish invasions from the impoundment: (1) a 1-m-high waterfall and bedrock cascade located 0.6 km above the high water mark of the impoundment; (2) a 2-m-high weir 0.4 km upstream of the bedrock cascade, and (3) the 1.3-m-high Rooidraai waterfall located 4 km upstream of the weir (see Figure 3).

BOX 2 (continued)

Fishes of the Rondegat River

Clanwilliam yellowfish *Labeobarbus capensis*

Clanwilliam rock catlet *Austroglanis gilli*

Pre-treatment electrofishing and snorkel surveys demonstrated that alien Smallmouth Bass had invaded to the Rooidraai waterfall (Weyl et al., 2013). In the invaded reach, adult Clanwilliam Yellowfish were the only native fish able to coexist with Smallmouth Bass but native Fiery Redfin, Clanwilliam Redfin minnows and juvenile Yellowfish were abundant above Rooidraai. The project was implemented based on the assumption that the removal of Smallmouth bass from the bounded section of river (i.e. between the weir and Rooidraai) would result in the recovery of the native fish (see Marr et al., 2012).

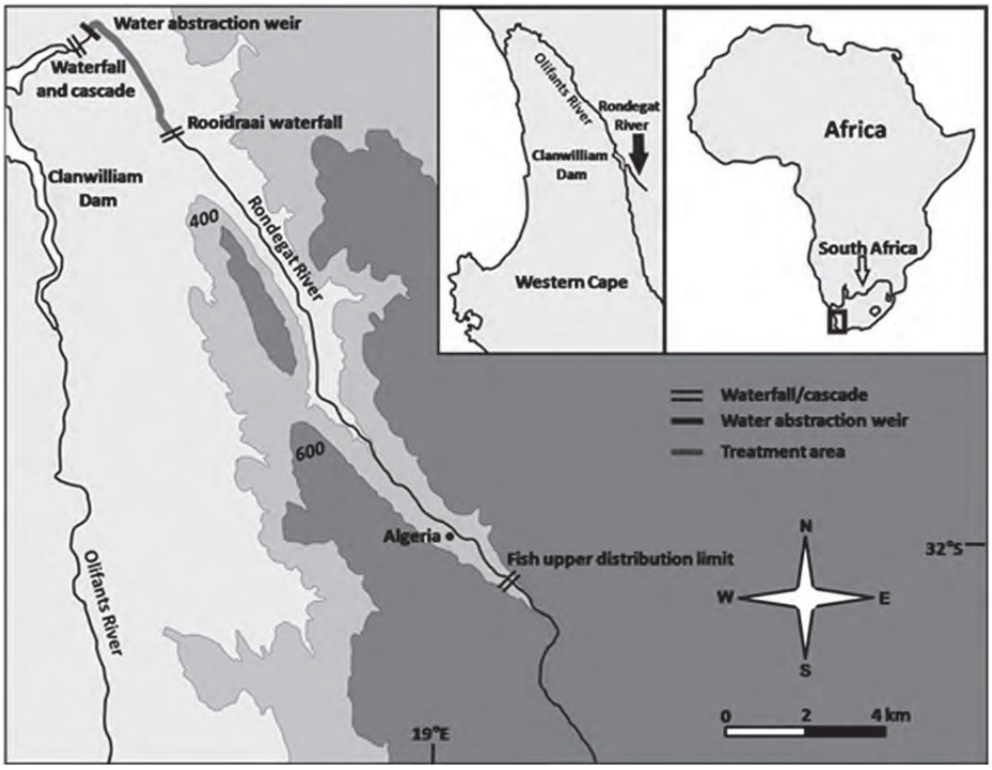


Figure 2

The Rondegat River, Western Cape South Africa showing the reach where treatment occurred as well the reaches up- and downstream of the barriers to invasion.

Figure 3

Two physical barriers to smallmouth bass invasion on the Rondegat River, Western Cape, South Africa. ***Left:*** *the Rooidraai waterfall in February 2013.* ***Right:*** *the raised in-stream weir in September 2013. (after Barrow 2014).*

2.2. Rotenone treatment

The Rondegat River was first treated on 29 February 2012, when water temperatures were between 23°C and 27°C and stream discharge (0.07 m^3/s) and velocity (0.5 km/h) were low. Treatment was conducted according to the guidelines in the AFS Rotenone SOP Manual (Finlayson et al., 2010). Rotenone was applied to the river using a series of drip cans sited at seven locations spaced approximately at 1-h water travel time intervals to maintain the recommended treatment concentration of 50 µg/L rotenone (Jordaan and Weyl, 2013) during a 6-h treatment. Six backpack sprayers were used to treat the backwater, seep, and spring areas with a 1% v/v CFT Legumine solution. To minimize off-target effects, deactivation of rotenone downstream of the water diversion weir was accomplished using a 2.5% w/v solution of potassium permanganate (KMnO4). Deactivation began at the same time as the rotenone treatment and lasted until 2 March 2012. To monitor the effectiveness of the treatment and deactivation, sentinel Smallmouth Bass were placed in net enclosures upstream of the emitters and at the 30-min travel time location downstream of the deactivation point. A second treatment was conducted one year after the first treatment on 13 March 2013 using 4 treatment stations and a lower concentration of 37.5 µg/L (see Slabbert et al., 2013, Figure 4).

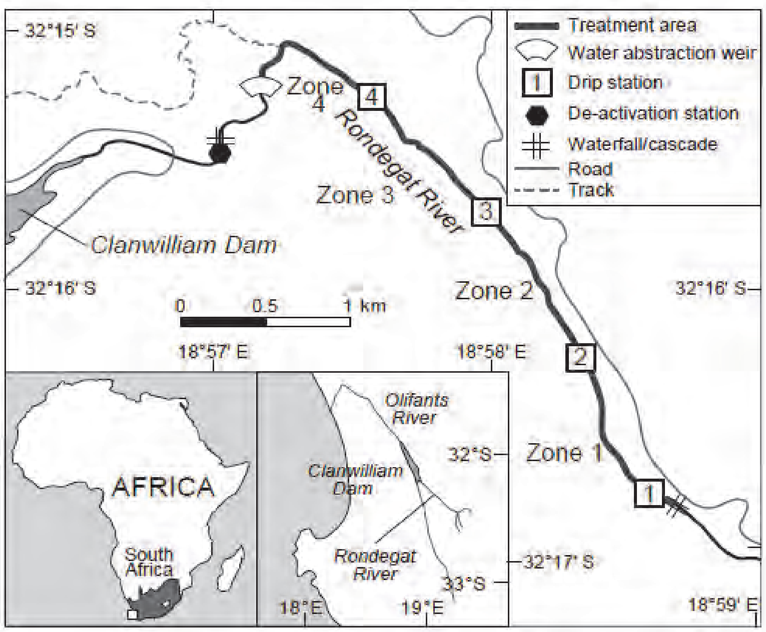


Figure 4

Map of treated section of the Rondegat River, indicating locations of rotenone drip stations and deactivation station. Treatment Zones 1-4 were the river segments between respective drip stations (after Slabbert et al., 2014).

2.3. Fish monitoring

The primary reason for intervention was to restore habitat for native fishes, which were therefore the primary focus of monitoring. River surveys were conducted to document the short term impact and efficacy of the first rotenone and second rotenone treatments (Weyl et al., 2013; 2014); and assessing recovery rates of native fishes following the removal of alien smallmouth bass. To do this, sampling trips were made between February 2011 and March 2015 (see Table 1) during which 42 sites were monitored using snorkel transects, underwater video analysis UWVA, fyke netting, seine netting and electrofishing.

TABLE 1
The two treatments and monitoring trips made to the Rondegat River, Western Cape, South Africa, as well as the sites monitored on those trips.

Date	Sites monitored/treated	Monitoring methods
February 2011	1-42	UWVA, snorkel transects, fyke netting, seine netting and electrofishing
February 2012	1-42	UWVA, snorkel transects, fyke netting, seine netting and electrofishing
February 2012	9-28	First rotenone treatment
February 2012	1-28	UWVA, snorkel transects, fyke netting, seine netting and electrofishing
October 2012	9-28	UWVA and snorkel transects
February 2013	1-42	UWVA, snorkel transects, fyke netting, seine netting and electrofishing
March 2013	1-28	UWVA and snorkel transects
March 2013	9-28	2nd rotenone treatment
March 2013	1-28	UWVA and snorkel transects
October 2013	1-28	UWVA and snorkel transects
March 2014	1-42	UWVA, snorkel transects, fyke netting, seine netting and electrofishing
October 2014	1-28	UWVA and snorkel transects
March 2015	1-42	Electrofishing, UWVA and snorkel transects

To provide baseline habitat data; water temperature, conductivity and pH were measured using a Hanna HI98129 Combo pH and electrical conductivity meter and turbidity (NTU) was measured using a Hanna HI 98703 turbidimeter (HANNA Instruments Inc. Woonsocket, USA). The dimensions of each site were also measured. One length transect, three to ten width transects and three depth readings per width transect were recorded per site. These dimensions were used to estimate each site’s surface area which is summarised in Table 2.

TABLE 2
The morphological and edaphic characteristics of sites within the control and treatment reaches of the Rondegat River, Western Cape, South Africa, recorded in March 2014. C = control, T = treatment.

Character	Min.		Max.		Average	
	C	T	C	T	C	T
Length (m)	5.1	7.2	30.0	49.0	15.8	14.3
Width (m)	1.7	1.7	7.9	14.5	4.5	5.2
Depth (m)	0	0	1.1	1.2	0.3	0.4
Surface area (m^2)	15.5	34.7	146.9	478.3	74.8	81.2
Volume (m^3)	4.9	3.3	78.9	271.0	25.9	34.0
pH	5.9	5.9	6.3	6.5	6.1	6.2
Temperature (ºC)	18.3	19.7	23.6	24.5	21.5	20.7
Turbidity (NTU)	0.7	1.3	1.8	3.3	1.2	2.5
Conductivity (µS/cm)	11	45	45	64	23.2	53.2

To assess for recovery, fish abundance at each site was estimated using two independent methods: underwater video analysis (UWVA) and snorkel surveys (Figure 5). These methods follow those described by Ellender et al. (2012) and Weyl et al. (2013). In addition, downstream electrofishing was used to determine the presence or absence of Clanwilliam Rock Catfish *Austroglanis gilli,* that due to their nocturnal nature were not adequately sampled using visual census methods.

For snorkel surveys, each pool was snorkelled in two consecutive passes. On each pass, fish were counted and the abundance of fish at the site was recorded as the average of the two counts. The size of each fish was also estimated during snorkel surveys. The estimated surface area of each site and the number of fish observed per site during the snorkelling was used to estimate the fish density per species at each site.

Electrofishing was conducted using a Samus© 725G backpack electrofisher connected to a 12V battery and the settings standardized at a duration of 0.3 ms and a frequency of 80 Hz. Block nets were deployed to avoid fishes escaping the sampling area.

Underwater filming was carried out using GoPro® HD Hero® cameras as described by Ellender et al. (2012) and Weyl et al. (2013). Cameras were placed in each site and recorded footage for a minimum of 30 minutes. The underwater video footage was analysed as described by Ellender et al. (2012). The highest number of fish of a given species observed at the same time (in the same frame) was determined for each 30 minute video. This number is the MaxN; an estimate of relative abundance of the given species at that site. This is done for every species observed at the site. One hundred and eighty videos were filmed from October 2012 till March 2014. A total of 97 hours and 48 minutes of recorded footage was watched. In order to enable accurate counting of the fish in the videos the footage was watched at varying playback speeds (from 20% to 100%).

To assess for size structure, all fishes that died during the rotenone operations were collected and measured. For comparisons in the control area and in the treatment area during the recovery period (2014 and 2015), fish were sampled using seine nets (3 mm stretched mesh size) and fyke nets. All fish that were caught were identified to species level, measured to the nearest 1 mm fork length (FL) and released at the site of collection.

Figure 5

Different survey methods used in this study to monitor fish within the Rondegat River, Western Cape, South Africa. ***A.*** *Snorkelling.* ***B.*** *Underwater video analysis using a GoPro® HD Hero® camera.* ***C.*** *Measuring the fork length of fish caught by electrofishing.*

(Photos by S. Barrow)

2.3.1. Was the eradication successful?

The successful eradication of smallmouth bass was reported in the Weyl et al. (2013) paper entitled "*Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of rotenone treatment in an invaded reach" (African Journal of Aquatic Science 38: 201-209)*. In summary, monitoring demonstrated that in the treatment area, estimates of fish density (snorkel survey) and relative abundance (UWV) varied in the two years (2011 & 2012) prior to the first treatment in 2012. After the 2012 rotenone treatment no fish were detected in the treatment area (Figure 6). One smallmouth bass was detected in a snorkel survey and on UWV prior to the second treatment. During the second treatment, this fish was not recovered and is suspected to have swam down an irrigation pipe as a large fish was recovered from the irrigation pump. The subsequent absence of Smallmouth Bass in all surveys following the second treatment has demonstrated that the rotenone treatment was successful at eradicating smallmouth bass from the Rondegat River. The presence of a smallmouth bass following the first treatment demonstrates the importance of repeated treatments.

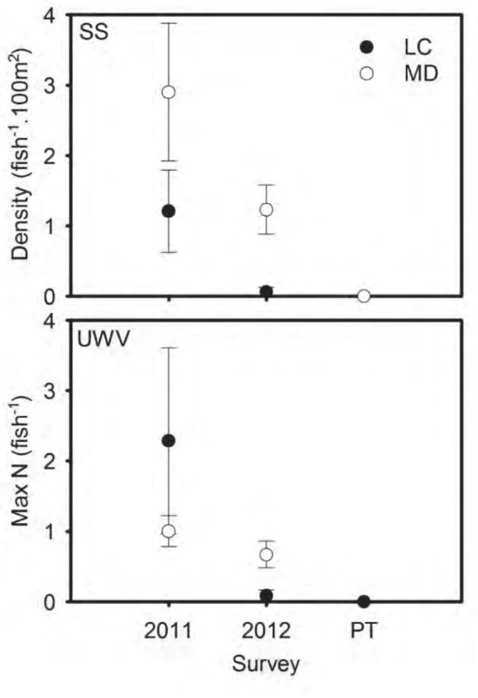


Figure 6

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). LC = Labeobarbus capensis, MD = Micropterus dolomieu. (after Weyl et al., 2013).

2.3.2. Did the removal of alien fishes result in the desired objective?

Following the first treatment, the Rondegat River was surveyed annually to assess for (1) the potential re-invasion by smallmouth bass and (2) the recovery of native fishes. The initial recovery of native fishes following the first treatment was reported in the Weyl et al. (2014) paper *"Threatened Endemic Fishes in South Africa's Cape Floristic Region: A New Beginning for the Rondegat River"* (Fisheries 39: 270-279). This and subsequent 2013-2015 surveys demonstrated that native fish rapidly colonised the reach where smallmouth bass had been eradicated. A summary of the distribution of alien Smallmouth Bass and native fishes in the monitoring area over the assessment period is shown in Figure 7.

Before treatment, snorkel survey estimates (mean ± SE) of smallmouth bass densities in the treatment area were 2.29 ± 0.56 fish/100 m^2. Native fish density estimates in the treatment reach were 0.29 ± 6.52 fish/100 m^2 prior to intervention. In comparison, native fish density in the control reach was 41.78 ± 7.24 fish/100 m^2 in February 2012, prior to intervention (Figure 7).

One year after the first treatment, native fish density had increased to 23.81 ± 5.56 fish/100 m^2. A single smallmouth bass was observed in the treatment reach in February 2013. But this fish was removed during the

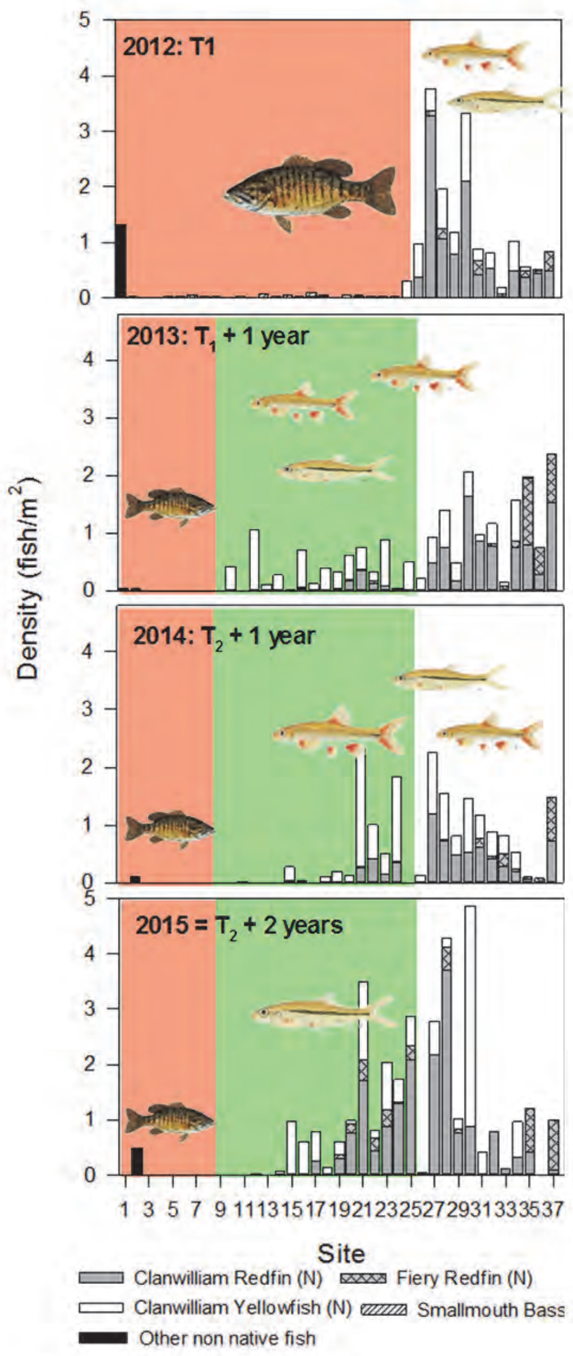


Figure 7

Density estimates from snorkel surveys of the Rondegat River, Western Cape, South Africa, showing the change in fish community structure brought about by the eradication of smallmouth bass. Red = invaded zone, green = treatment area (adapted from Weyl et al., 2014). N = native second treatment. The density of native fish in the control reach in February 2013 was 23.71 ± 7.24 fish/100 m^2.

To assess for the recovery of native fish densities, a Before-After-Control-Impact BACI type experiment was used (see Figure 8). In such experiments the response of the treatment population is directly compared to the population in the control area. The results indicate that densities of the three cyprinid species in the rehabilitated area are beginning to resemble those in the treatment area.

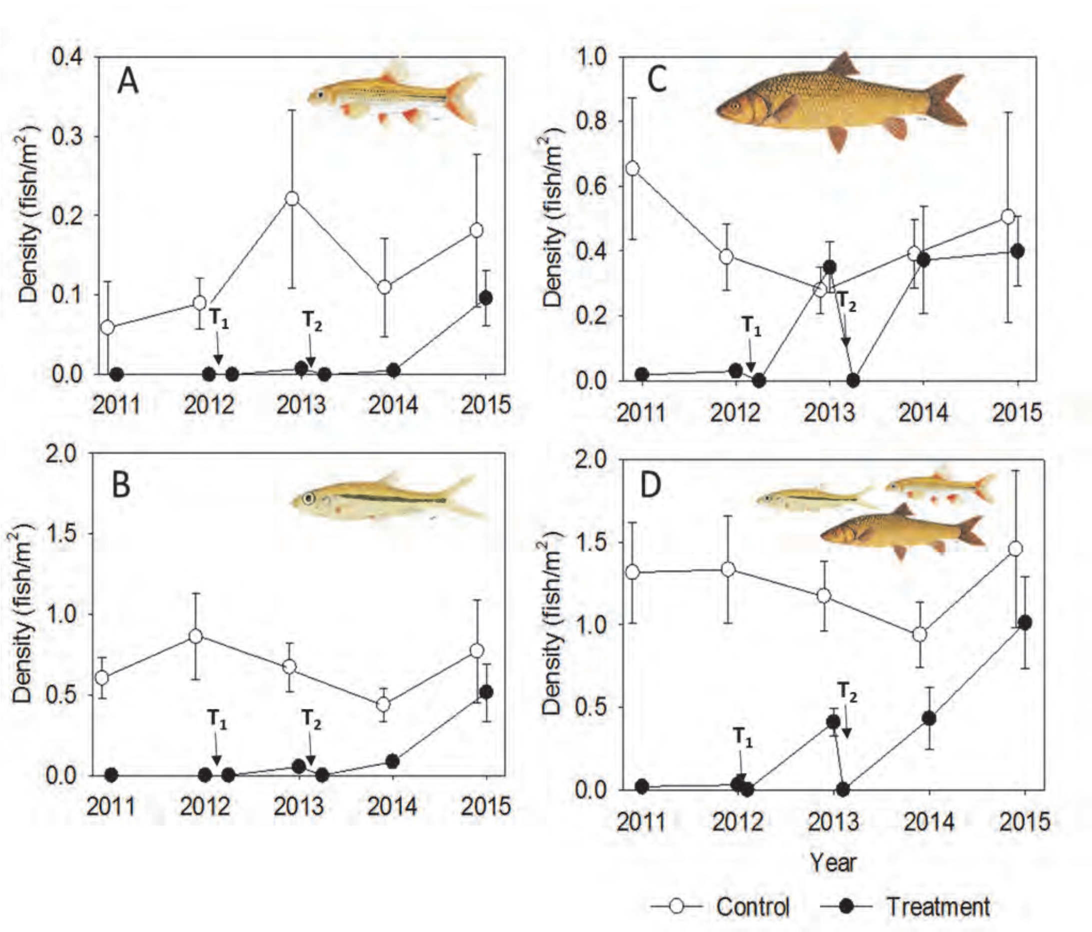


Figure 8

***T**he mean density of native fishes before and after CapeNature interventions in control and treatment reaches of the Rondegat River, Western Cape, South Africa. **A**. Clanwilliam redfin **B.** fiery redfin, **C**. Clanwilliam yellowfish and D. all native fishes. Error bars denote one standard error. T_1 and T_2 indicate the two rotenone treatments.*

Comparisons of the length structure of the native fishes was based on multiple methods. Initially, all fishes that died during the rotenone operation were collected and measured (Figure 9A, 9B) and subsequent length structure was obtained from seine and fyke nets deployed in the treatment area (Figure 9C, 9D). The resultant length frequencies show that the removal of the large Clanwilliam yellowfish and bass was followed by a recruitment pulse of young-of-year native cyprinids. These were again removed during the second treatment. Subsequently, the process was repeated and after 2 years, distinct year classes were visible.

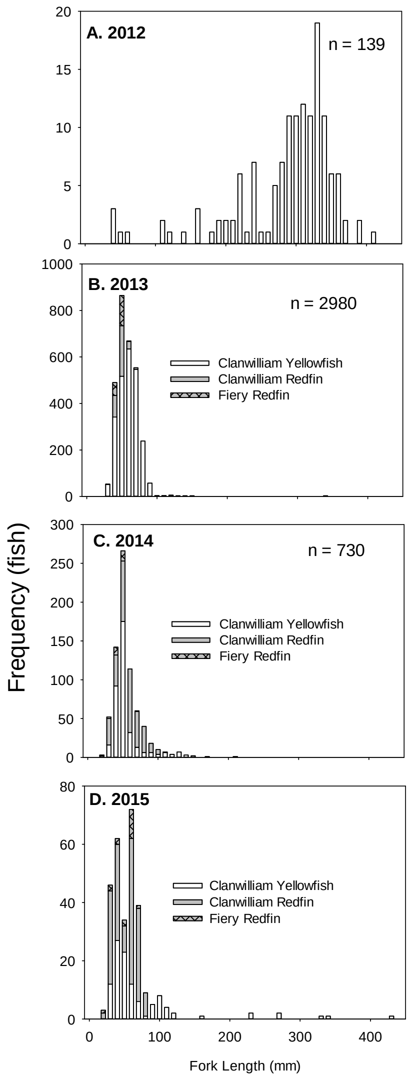


Figure 9

*The mean fork length of native fishes in the treatment reach of the Rondegat River, Western Cape, South Africa. **A**. before any rotenone treatments **B**. a year after the first treatment and **C.** a year after the second treatment and **D.** 2 years after the second treatment.*

For comparison, native fish populations were sampled in the control region using the same gears as in the treatment area. When the length structure of native fishes in the treatment area is compared to that in the control region (see Figure 10) it is evident that length structure of the native fishes is also beginning to approximate that in the control region (Figure 10). It must however be noted that there has been no recovery of Clanwilliam catfish and only 2 individuals were seen evading the gear during electrodishing.

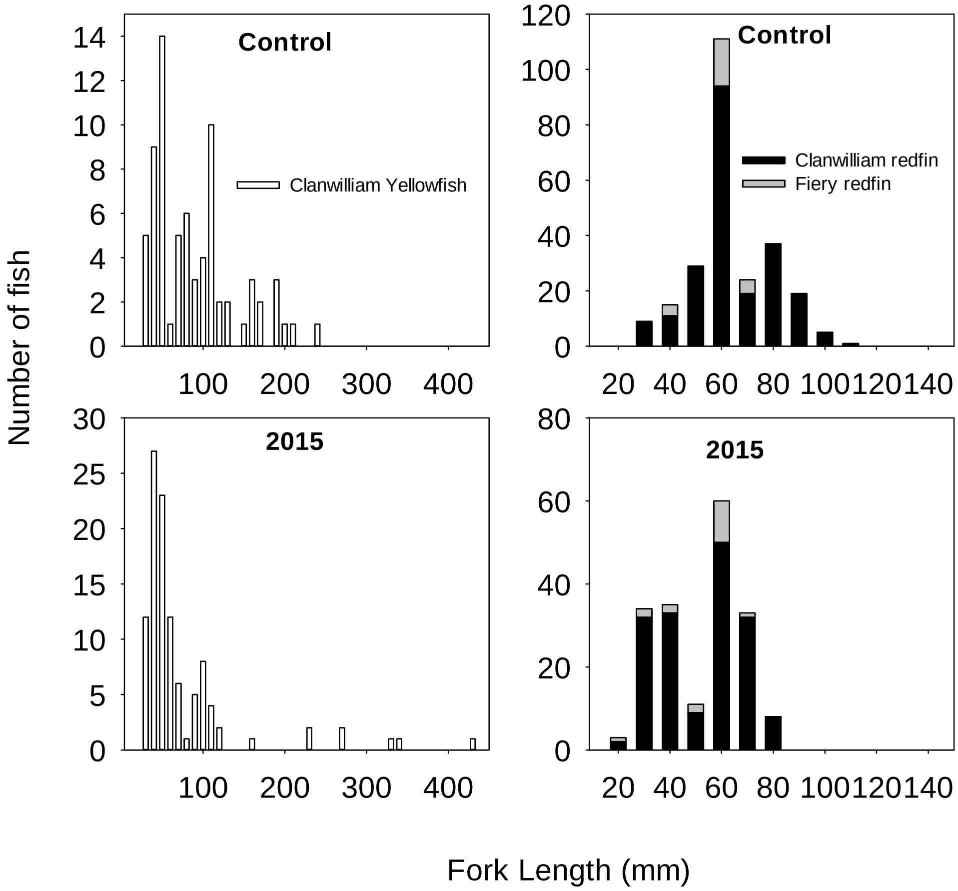


Figure 10

Length frequency histograms of native cyprinids in the control (top) and treatment (bottom) regions of the Rondegat River in 2015.

2.4. Invertebrate monitoring

The reason for comprehensive invertebrate monitoring was to determine what the impact of rotenone treatments would be on the aquatic invertebrate communities in the Rondegat River. Surveys were conducted to address the questions:

1. Did the rotenone treatment have adverse impacts on non-target taxa?
2. What are the long term impacts on non-target taxa?

As a result, invertebrate monitoring was conducted seasonally at three monitoring sites within the treatment area, three monitoring sites in the control area upstream of the treatment section, as well as at a monitoring site downstream of the treatment area. Monitoring comprised a total of 13 sampling events i.e. May 2010, October 2010, February 2011, February 2012 (before and after treatment), May 2012, October 2012, February/March 2013 (before and after treatment), October 2013, February 2014; October 2014 and February 2015. The sampling techniques included drift, stone and kick sampling. These are described in detail in Woodford et al. (2013) "*Monitoring the impact and recovery of the biota of the Rondegat River after the removal of alien fishes*" (Water Research Commission Report No. KV 304/12) and a summary is provided in BOX 3.

BOX 3.1
Invertebrate sampling techniques

Drift
A key impact of previous rotenone treatments in rivers has been to precipitate catastrophic drift in aquatic invertebrates. One week before the rotenone treatment, drift nets at one treatment monitoring site and one control monitoring site captured natural drift levels in the stream at one treatment-monitoring site. In order to quantify this effect, 250 ɥm drift nets were set up 200 m downstream of a drift station, as well as in the control zone. Invertebrate 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.

Drift net set up in the Rondegat River to collect invertebrates

BOX 3.2

Invertebrate sampling techniques

Kick sampling

Kick sampling is a sampling method used in rapid bio-assessment protocols to assess river health in terms of invertebrate community structure. This technique, while considered a semi-quantitative assessment of 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. 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. Kick sampling allowed for the use of the South African Scoring System (SASS) for assessing river health using macroinvertebrates to be performed on all kick samples collected at each monitoring site. A comparison of SASS scores and our quantitative estimates of invertebrate community change allowed us to assess the appropriateness of employing the SASS methodology in part or in full to assess the impacts of rotenone operations.

Terrence Bellingan and Jeanne Gouws doing a SASS Assessment

(after Woodford et al., 2013, WRC Report No. KV 304/12)

BOX 3.3
Invertebrate sampling techniques

Stone sampling
Stone sampling is a technique that assesses the density of invertebrates associated with individual stones in the riverbed, and through measuring the stone size can give a quantitative estimate of individual species density per surface area. At each site, four stones in current were collected from runs 20-40 cm deep to ensure biotope standardisation. With a 200 ɥm 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 ɥm sieve to capture all other insects. Each stone was measured across three axes before being replaced in the stream.

Researcher Darragh Woodford collecting stones into a 20 µm mesh net

2.5. Did the rotenone treatment have adverse impacts on non-target taxa?

To assess for whether there were long term changes in insect communities, both experimental and field observations were used. Sensitivity of several invertebrate taxa to rotenone concentrations that impact on fish were tested experimentally by Dalu et al. (2015) “An Assessment of the Effect of Rotenone on Selected Non-Target Aquatic Fauna” (PLoS ONE 10(11): e0142140), while field observations have been published in the peer reviewed literature. The immediate impact of treatments was assessed by Woodford et al. (2013) “*Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage – a lesser of two evils*?” (*Journal of Insect Conservation* 17, 959-973) and in Bellingan et al. (2015) “*Rapid bioassessment of repeated rotenone treatments on a stream invertebrate assemblage in the Rondegat River, South Africa*” (African Journal of Aquatic Science 40, 89-94). Together, these studies provide strong evidence that observed impacts on non-target taxa were limited to short-term effects.

2.5.1. Catastrophic drift and other immediate impacts

A 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 (Woodford et al., 2013). 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). Although generally considered a sub-lethal impact, catastrophic drift can result in significant short-term changes to community structure in the aftermath of rotenone treatment. In order to quantify this effect, 250 ɥm drift nets were set up 200 m downstream of a drift station, as well as in the control zone. 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. 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.

As was expected, a catastrophic drift event occurred during the application of rotenone to the Rondegat River (Figure 11). 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 11). 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.

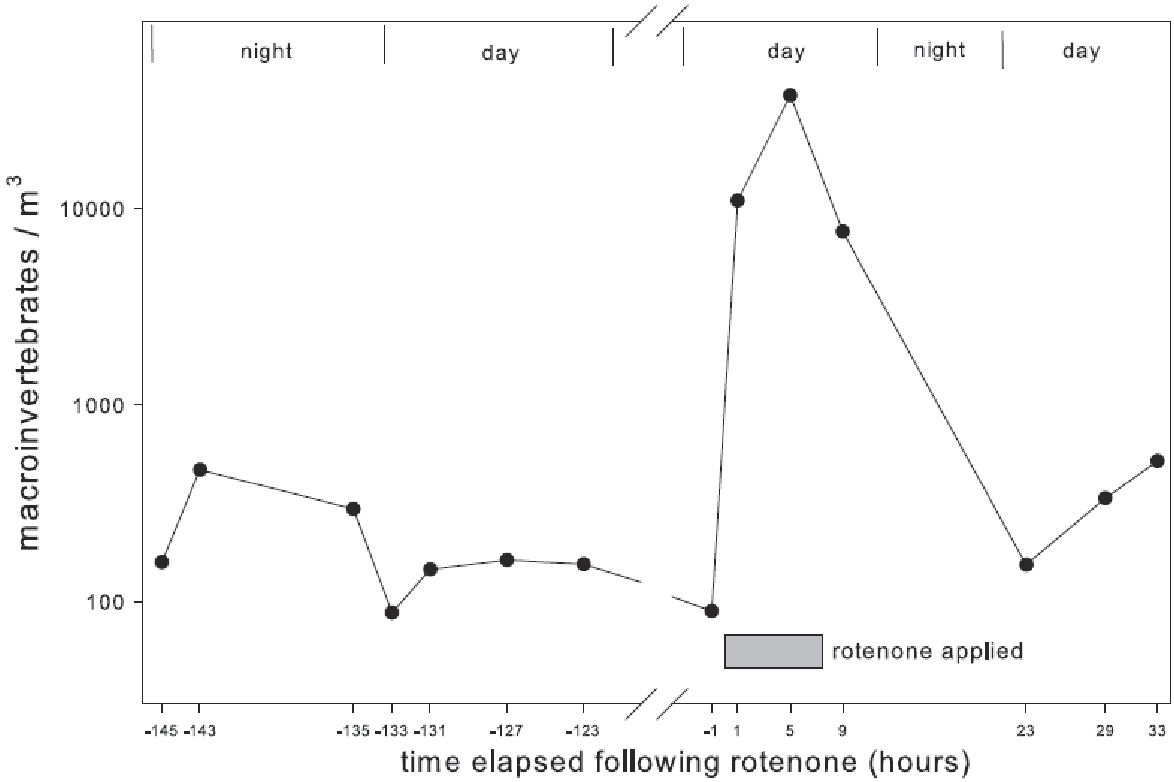


Figure 11

Total invertebrate drift abundance at 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 (after Woodford et al., 2013).

Observations on drift demonstrated that Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) are more vulnerable to rotenone than other groups of invertebrates (e.g. Coleoptera and Diptera, Woodford et al., 2013). The proportional abundance of macroinvertebrate orders also shifted over the course of the treatment (Figure 12). 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 12). By 1pm, just over halfway through the treatment and the time of peak drift (Figure 12), Coleoptera were numerically dominant, comprising 52% of all macroinvertebrates captured (Figure 12). 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 11 and 12).

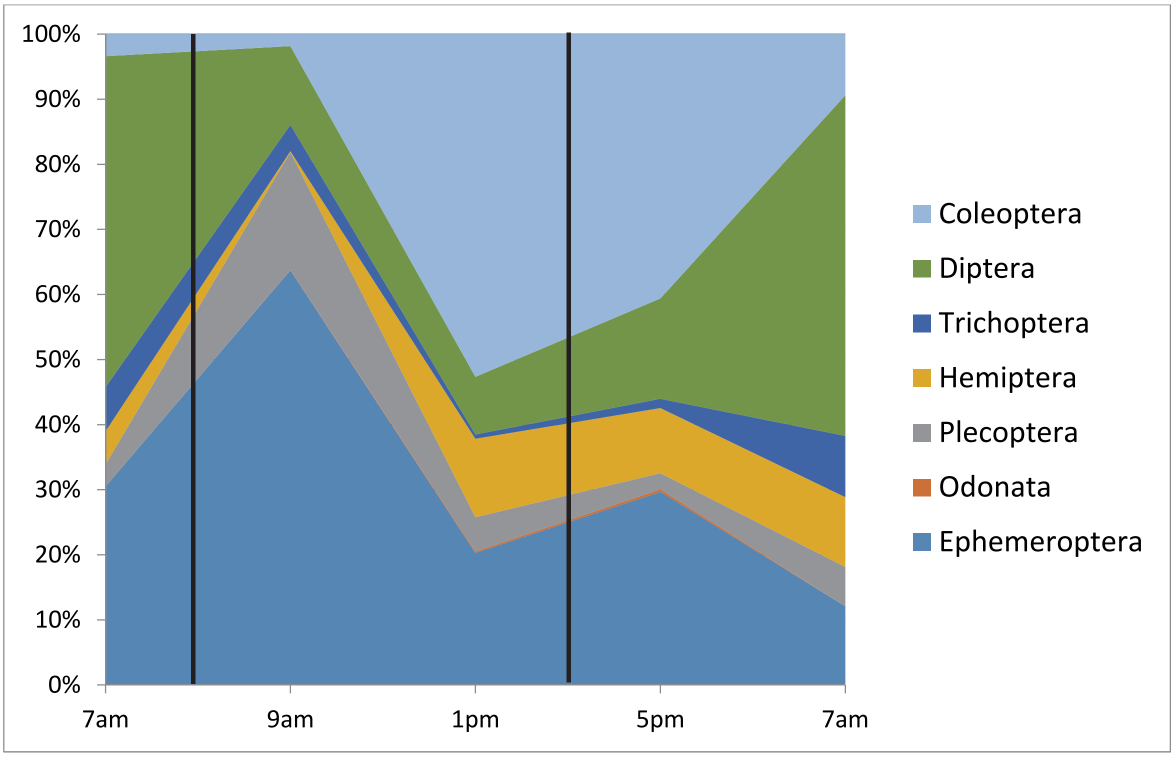


Figure 12

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.

2.6. Recovery of invertebrate communities

2.6.1. Results from SASS assessments

The SASS5 scoring system was applied to kick samples collected before and after treatments to assess for changes in overall macroinvertebrate community health. The results of the SASS assessment are published in Bellingan et al. (2015). In summary, there was a significant decline in average score per taxon (ASPT) following the treatment, whereas there was no difference between the Post-Treatment May 2012 and pre-treatment ASPT scores (post-hoc Tukey test, $p > 0.05$). In contrast, there was 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 13). 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, 2006). 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, 2006). This is most likely a consequence of the moderate levels of agricultural development of the riparian zone in the middle and lower Rondegat. 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.*** As a result, assessments of impact require the use of alternative metrices.

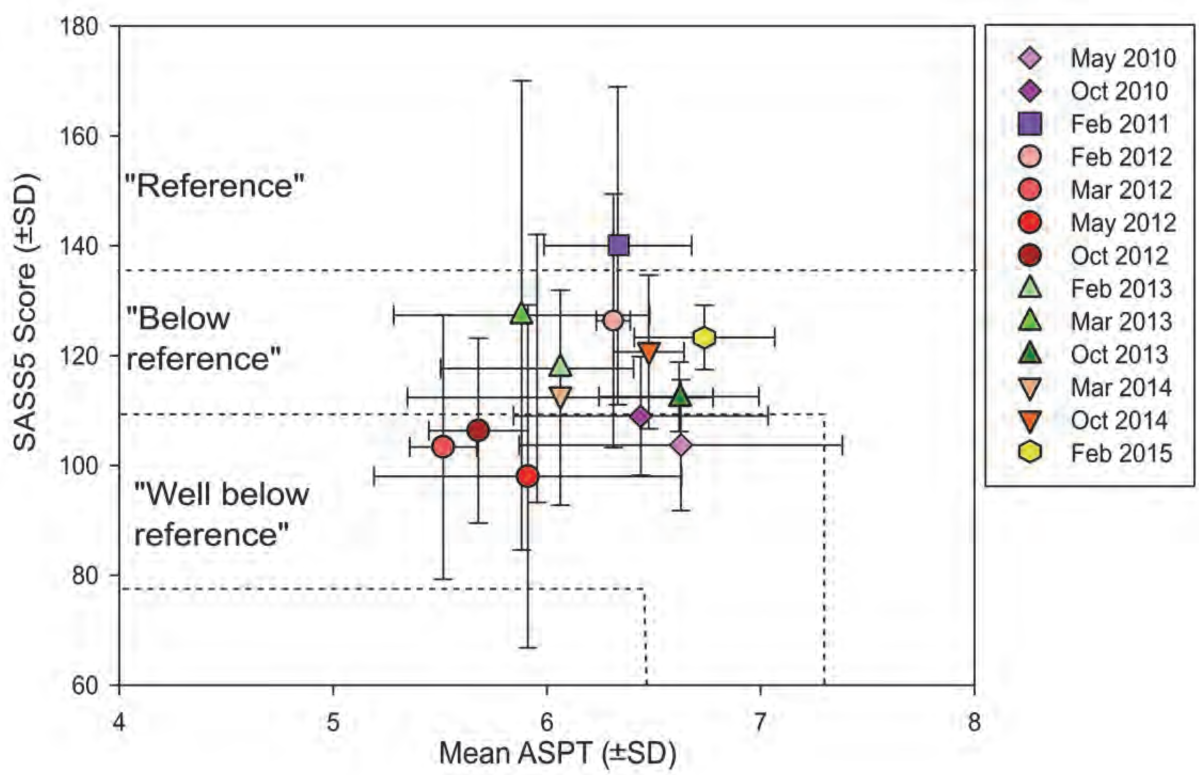


Figure 13

Mean ASPT and SASS scores at monitoring sites in the treatment area on the Rondegat River. This includes all surveys conducted between May 2010 and February 2015.

2.6.2. Stone sampling

Stone sampling was conducted because for the successful interpretation of 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. 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 (Figure 14). The group did however appear to have recovered to near pre-treatment densities in May 2012 (Figure 14). Within the Ephemeroptera, two families were significantly affected (Baetidae and Heptageniidae). Whereas Baetidae had recovered to the point of not being significantly less abundant than pre-treatment levels by May 2012.

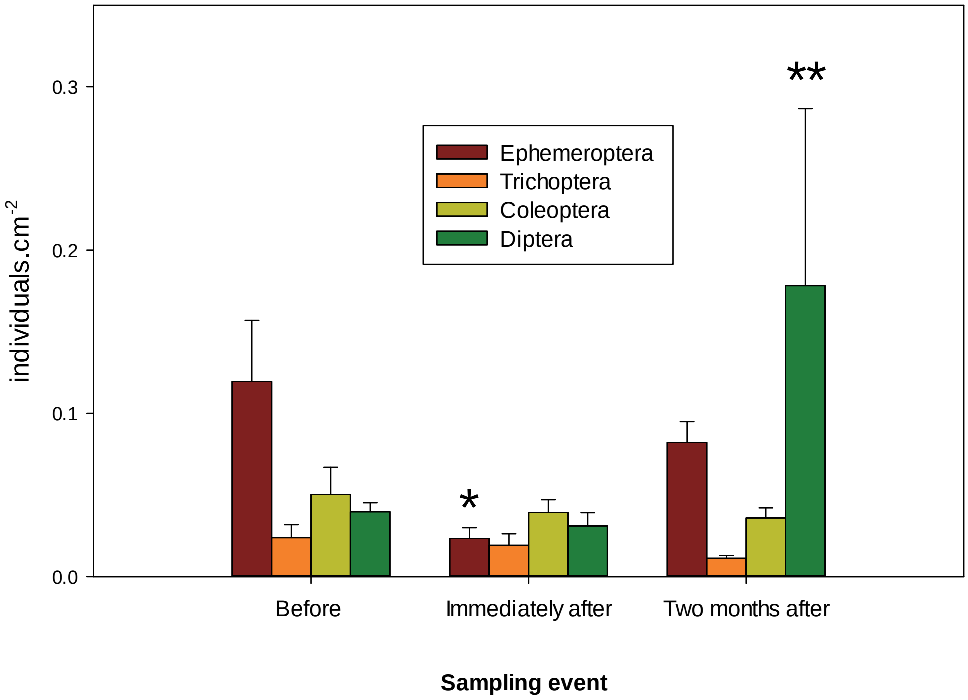


Figure 14

Short term responses of invertebrates to rotenone treatments illustrated by 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 (after Woodford et al., 2013)

Over the longer term, Bellingan et al. (2015) quantitatively assessed the abundance of EPT taxa on the rocks and demonstrated not only a consistent decline in EPT taxa following treatment but also a rapid recovery in biomass in the year between the 2012 and 2013 treatments (Figure 15). This recovery of EPT taxa in the years following the two treatments is shown in Figure 16. The data illustrate a rapid recovery of EPT taxa following treatments and from a density perspective, rotenone had little long-term impact on macroinvertebrate communities.

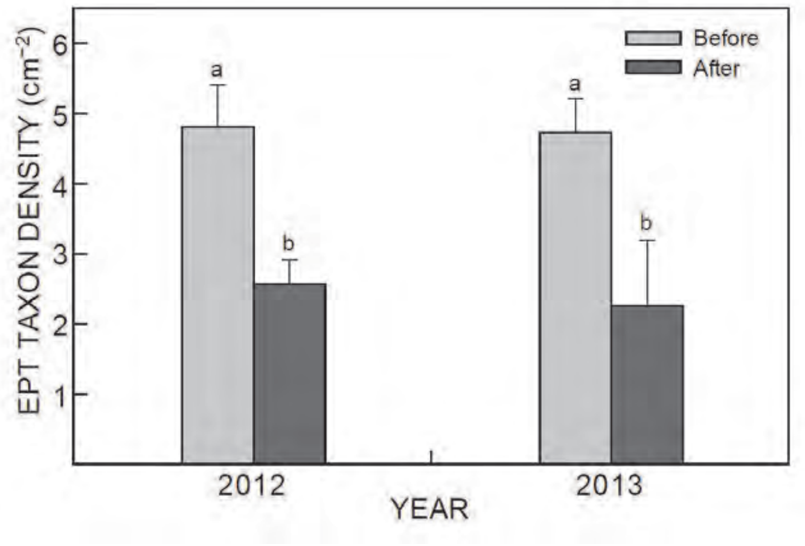


Figure 15
Mean densities of Ephemeropteran, Plecopteran and Trichopteran (EPT) taxa recorded on stones collected one week before and two days after rotenone treatments in 2012 and 2013. Differing letters above bars indicate significant differences in EPT taxa density obtained from t-tests ($p < 0.05$), attributable to the application of rotenone. Error bars denote SE. (after Bellingan et al., 2015)

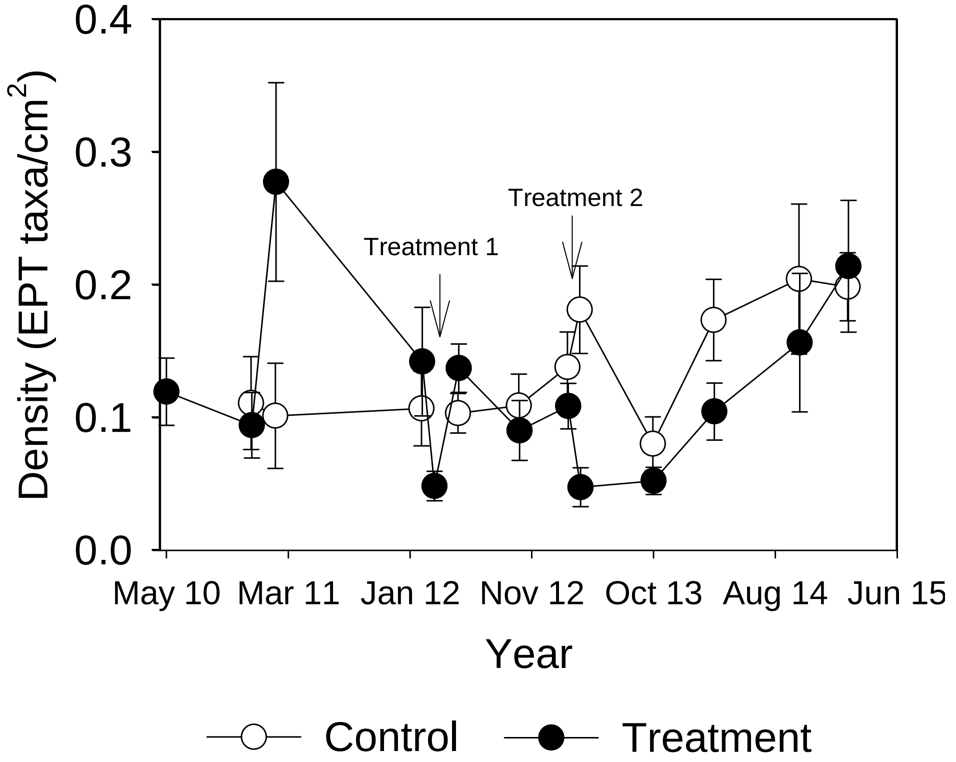


Figure 16
The mean density of EPT taxa on stones in current expressed as individuals per m^2 in control and treatment reaches of the Rondegat River. Error bars denote one standard error.

2.6.3. Species level responses

The immediate responses to rotenone treatment were evaluated during the K8/922 Project and published in Woodford et al. (2013). The 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 incidental 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 3). By the end of the monitoring program, these five species had however returned to the treatment area. Of the incidental species, 36 were still missing in May 2012 and 27 by the end of the monitoring (Table 3). It should be pointed out that species that are present at naturally low densities may simply be undetectable by the kick sampling. It is therefore impossible to use these species as indicators of impact. This is illustrated by the fact that 6 incidental species were also lost in the untreated control reaches. 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 3

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 at all sites for two consecutive pre-treatment monitoring events prior to rotenone application. Incidental species refers to species only recorded once during all surveys.

Taxon type	Immediate impact 2012	Long-term impact 2015	Taxa only recorded from treatment area
Common species not affected	19	31	
Common species initially lost but recovered	10	4	
Common species still missing	5	0	1
Incidental species not affected	13	10	0
Incidental species lost	36	27	13
Incidental species sample	5	19	5
Species only detected post treatment	19	10	6
Total species detected	**107**	**132**	**24**

Woodford et al. (2013) also report on the detection of 19 new species in the treatment area two months after treatment. While 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 also be an artefact of sampling efficiency, where many species have an equal random chance of being detected by our sampling methods in any given season. ***As a result, taxon specific assessments were unable to adequately demonstrate impact (see Woodford et al., 2013).***

In addition, taxon-specific assessments took considerable time and expertise. For example, high-level technical expertise was available from the Albany Museum; a dedicated PhD candidate (Terrence Bellingan) was attached to the project; and 4 DST/NRF Interns were required to sort through the samples. While this resulted in an impressive checklist of species (Table 4) their utility in determining impacts of rotenone treatments was limited. This is because, much of the diversity is made up of species that occur at such low densities that their presence and absence in samples is by chance. In addition, experimental work has shown that some taxa, e.g. the Coleoptera, are not susceptible to rotenone. For this reason the inclusion of high level identification in future treatments is neither practical, nor logistically feasible. It is, therefore, recommended that future monitoring focusses on EPT taxa as these orders are most affected by rotenone. Experimental support for the use of

EPT-taxa is provided by Dalu et al. (2015) who investigated the effects of different rotenone concentrations (0, 12.5, 25, 37.5, 50, 100 μg L^{-1}) on selected invertebrate groups; Aeshnidae, Belostomatids, Decapods, Ephemeroptera, Pulmonata and zooplankton over a period of 18 hours. They demonstrated that Ephemeropterans and zooplankton were more susceptible to rotenone than Decapods, Belostomatids and snails. Mortality and behaviour effects varied considerably between taxa, ranging from no effect (crab *Potamonuates sidneyi*) to 100% mortality (*Daphnia pulex* and *Paradiaptomus lamellatus*). Planktonic invertebrates were particularly sensitive to rotenone even at very low concentrations and they suggested that these should be included in monitoring where they occur (e.g. in dams).

TABLE 4

Checklist of invertebrate taxa sampled from the Rondegat River during the current Project. n = number.

Taxon	n	Taxon	n	Taxon	n
Ephemeroptera	6442	Odonata	33	Diptera	12730
Baetidae	5549	Aeshnidae	7	Athericidae	1
Small baetids	4623	Aeshna sp	6	Athericid sp	1
Afroptilum sp CED120AE	67	Anax speratus	1	Dixidae	1
Afroptilum sudafricanum	15	Gomphidae	14	Dixid sp	1
Baetis harrisoni	475	Paragomphus sp	14	Ceratopogonidae	21
Cheleocloeon excisum	97	Libellulidae	12	Bezzia sp	18
Dabulamanizia media	2	Tiny Libellulidae	1	Atrichopogon sp	1
Peuhlella sp CED120AF	151	Trithemis sp	3	Forcipomyia sp 1	2
Pseudopannota maculosa	61	Zygonyx sp	8	Empididae	36
Pseudocloeon piscis	32			Empidid sp	36
Pseudocloeon vinosum	10	Hemiptera	4	Culicidae	8
Cloeodes sp	11	Corixidae	4	Anopheles sp	4
Demoreptus monticola	5	Mirconecta sp	4	Culex sp	4
Caenidae	191			Chironimidae	10990
Afrocaenis sp CED 104A	63	Lepidoptera	1	Chironimid larvae	10990
Caenis sp CED 104B	128	Pyralidae	1	Tabanidae	1
Heptageniidae	57	Pyralid sp	1	Tabanid sp 1	1
Afronurus sp	57	Coleoptera	2084	Tipulidae	305
Leptophlebiidae	510	Dytiscidae	2	Antocha sp	295
Euthraulus elegans	510	Larval Dytiscid	2	Tipula sp	1
Teloganodidae	1	Elmidae	1636	Limnophila sp	9
Lestegella pennicillata	1	Tropidelmis hintoni	1	Simuliidae	1367
		Helminthopsis sp	2	Small simuliid	1243
Plecoptera	102	Elpidelmis capensis	57	Simulium impukane	12
Notonemouridae	102	Elmid oval morph	474	Simulium medusaeforme	80
Small nontonemourid	89	Elmid semi-oval morph	357	Simulium unicornatum	20
Aphanicercopsis sp	9	Elmid elongate morph	445	Simulium adersi	1
Aphanicercella sp	4	Peloriolus sp	298	Simulium ruficorne	1
		Protelmis chutteri	2	Simulium bequaerti	10
Trichoptera	839	Gyrinidae	35		
Ecnomidae	123	Orectogyrus	15	Oligochaeta	715
Ecnomus sp CED105L	123	Aulonogyrus sp	20	Oligochaete sp 1	715
Hydropsychidae	377	Hydraenidae	154		0
Small hydropsychids	206	Mesoceration sp	146	Malacostraca	6
Cheumatopsyche afra	27	Discozantaene sp	3	Potamonautes sp	6
Cheumatopsyche sp CED42AA	128	Aulacochthebius sp	1		
Cheumatopsyche thomasseti	10	Parhydraena sp	4	Acari	1056
Macrostemum capense	6	Hydrophilidae	5	Hydrocarinidae	1056
Hydroptilidae	78	Berosus sp	4	Hydrocarina sp	1056
Oxyethira velocipes	1	Hydrophilid larvae	1		
Hydroptila cruciata	74	Psephenidae	2	Cladocera	15
Othotrichia sp	3	Afropsephenoides sp	2	Daphnia	15
Leptoceridae	228	Ptylodactylidae	34		
Athripsodes sp	147	Ptylodactylid sp	34		
Leptecho helicotheca	5	Scirtidae	193		
Leptecho sp	22	Scirtid sp	193		
Oecetis modesta	54	Nitidulidae	23		
Polycentropodidae	27	Nitidulida sp	23		
Paranyctiophylax sp	27				
Sericostomatidae	6				
Petroplax sp	6				

3. GUIDELINES FOR FUTURE MONITORING PROJECTS

As a result of the severe impact that alien fishes are having on native fish communities, CapeNature has identified 16 rivers for rehabilitation. It is anticipated that rotenone will be an important tool in this process. To guide future monitoring programmes it was considered useful to undertake a retrospective analysis of the approaches employed. It is also important to note that the following guidelines are developed for small, clear streams. Here, the recommendations arising from the previous (K8/922) project as well as from this current project are summarised.

3.1. Recommendations for future monitoring projects

3.1.1. General sampling

- In Cape Floristic Region mountain streams, sampling from late autumn (May) to spring (October) is not recommended for monitoring, as the variable timing of autumn floods could severely confound any assessment of invertebrate community structure (see K8/922).
- In both fish and invertebrate assessments the inclusion of control, and below treatment reaches is essential for monitoring. Sites should be distributed equally between these three reaches so that effects can be tested using a Before-After-Control Impact (BACI) methodology.
- The inclusion of the capability for constant logging of water temperature in the river should be considered as such data allow for later interpretation of observed trends in species recovery rates.
- Water quality parameters such as clarity, conductivity and pH are important environmental variables that should be included.
- Site dimensions and descriptions of habitat are essential for interpreting data and providing a background to the system being monitored.

3.1.2. Fish abundance, diversity and length structure

- 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.
- 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 area will dictate the relative efficacy of snorkel surveys, underwater video analysis (UWVA) and electrofishing, and early assessment of

these environmental variables should be undertaken to guide the fish monitoring strategy.

- When possible use snorkel surveys as they tend to give immediate results which can be integrated into a BACI analysis.
- UWVA is an excellent method for assessing presence/absence, especially of rare and "shy" species. There are, however, considerable costs associated with equipment, data storage and analysis. Most important is analysis time which is at least 5 X the recording time. A good compromise is to initially use UWVA for presence/absence and the snorkel surveys for abundance.
- Catfishes are nocturnal and are inadequately detected using UWVA and snorkel surveys. Here electrofishing is necessary. However, due to the possibility of impacts associated with this method, we suggest that this be focussed at detection of catfishes only.
- It is important that handling of fishes in the treatment zone needs to be kept to a minimum. As a result, length frequency surveys should be once a year and then be conducted using either fyke nets or seine nets. Sample sizes should be small and care must be taken not to injure fish, as mortality associated with sampling will affect densities and potentially confound monitoring results. This is especially important when population size is small.
- It is important to separate between species because different species may recover at different rates. In addition, the detectability and vulnerability of individual species to different sampling gear will differ.
- Adequate sample sites need to be allocated. During the current programmes we increased sample sites to 42. While this appears adequate given the small length of the treatment area, increased sample sites would have been beneficial. We suggest that in future studies, an attempt is made to have at least 30 sites in the treatment and 30 sites in the control area. If feasible, a similar number of sites should be sampled below the treatment area. This off course depends on the availability of suitable habitats for sampling.

3.1.3. Invertebrate assessments

- Drift sampling was useful to demonstrate "catastrophic drift" during the rotenone treatment. Its utility as a monitoring tool was limited due to the high level of expertise required for invertebrate assessments. As a result, sampling Drift is not considered an essential component of long-term monitoring.

- The SASS5 system was useful in applying a well-known method to demonstrate that ***ecosystem health as estimated by the SASS5 scoring system was not significantly altered by the rotenone treatment.*** As such, SASS assessments should be integrated into monitoring. It must however be noted that the SASS5 scoring system is unlikely to be a reliable tool for assessing fine-scale responses of invertebrates to rotenone impacts.
- 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 (Riffle beetles for example).
- The utility of SASS5 as a monitoring system would be strengthened by increasing the number of sites to at least 6 in each reach.
- Stone sampling provided the best quantitative data for monitoring and is considered an essential component of any monitoring programme. The collection of all invertebrates on stones allows for assessments at several levels of complexity. Given the need for efficiency, assessments should focus on sensitive taxa only. In the case of the Rondegat Project the most useful were EPT taxa i.e. Ephemeroptera (Mayflies), Plecoptera (Stoneflies) and Trichoptera (Caddisflies).
- It is our suggestion that future monitoring uses a 2-part process: (1) SASS5 scores and (2) stone sampling; identification efforts should be directed at the EPT taxa but full samples should be retained for potential future work on other invertebrate groups.

3.2. Practical guidelines

This section of the report is intended to provide practical guidance for implementing monitoring projects to assess for the efficacy of rotenone treatments with regard to removing alien fish, and for monitoring the responses by macro-invertebrates and fish to these treatments. The intention is to use the experience from the monitoring of the Rondegat River to develop this protocol which will allow for the determination of:

- Whether the treatment was effective at removing alien fish
- What fishes colonise the river where alien fish were removed.
- What impacts the treatment has on macro-invertebrates.
- How long it takes for the macro-invertebrate community to recover.

3.2.1. Before you start

It is important to prepare adequately for monitoring. Here we list some important first steps.

- **Do your homework. C**onsult topographic maps and view the area using satellite imagery (e.g. using google earth), compile expected species lists and choose your monitoring methods accordingly. Also consult with local experts during planning as this can provide valuable insights into local conditions.
- **Get land owners permission to access the river**. Permission from the landowner is a legal requirement and needs to be obtained prior to monitoring. Access should be negotiated at the outset of the project, and should be enquired for at least one week before the commencement of any visit to a monitoring site.
- **Develop and maintain good relationships with all stakeholders.** It is critical that researchers build up and maintain a good relationship with landowners, conservation staff and other stakeholders (e.g. Department of Water Affairs Staff, local anglers etc.). Not only does this ensure good information flow that is useful for the monitoring team but can also result in logistic support when needed.
- **Where possible, use local facilities.** If suitable accommodation is available on site it is good practise to use, and pay for, such facilities. This has the advantage that land owners have vested interests in monitoring and often support the monitoring through facilitating access both on their farms and on neighbouring properties.
- **Walk the entire study area.** The whole extent of the river that is to be rehabilitated should be walked or snorkelled. This allows for a full picture before choosing monitoring sites.
- **Planning is important.** Once the survey team is familiar with the area is important that a research plan is formulated and discussed with all stakeholders to determine feasibility. This survey plan should include site selection, sampling methods and intensity and reporting.

3.2.2. Sampling intensity

Sampling intensity is largely determined by budget and availability of personnel. The following is a recommendation for inclusion in budgets and/or monitoring proposals.

- **Fish should be monitored annually** as community structure is unlikely to vary dramatically from year to year. Sampling should thus be conducted immediately before the operation, immediately after and then annually during the same season as the treatment.
- **Sample as many sites as possible**. Based on our experience in the Rondegat River we suggest that in future studies, an attempt is made to have at least 30 sites in the treatment area and 30 sites in the control area. The area below the treatment zone

should also be sampled if suitable habitat is available. Here the number of sites will depend on the availability of habitat.

- **Invertebrates should be sampled seasonally**. As treatments generally occur during summer, we suggest sampling in spring and then, immediately before and after the treatment. Pending on funding, post-treatment assessment of invertebrate community structure should continue for at least two years after treatment.

3.2.3. Site selection

Selecting appropriate monitoring sites is fundamental to the success of the monitoring programme. The monitoring sites must be:

- **Easily accessible** such that equipment can be effectively deployed. Ideally, there should be road access to within 500 m.
- **Contain appropriate habitat** for the species being monitored. In an ideal set of circumstances, the same stretch of instream habitat should be sampled in the same way, in order to make the samples collected comparable between monitoring sites as well as between monitoring events. This is important as the purpose of monitoring is to detect a response to rehabilitation from selected taxa. Thus sampling sites need to be chosen such that the appropriate taxa are represented.
- **Approximate the "natural state" of the river** and free of human induced disturbance such that confounding effects that may hamper the detection of impacts due to the rehabilitation process are excluded.
- **Distributed throughout the research area** such that comparisons can be made between treatment and control reaches both below and above the treatment area.
- **Small enough to be adequately sampled but large enough to resist short-term changes** (e.g. should not be dry at low water levels).

3.2.4. Fishes

Typically fish monitoring approaches in small streams include snorkel surveys, underwater video analysis, electrofishing, seine netting and fyke netting. The use of multiple approaches is important for three reasons: (1) different species are best detected using different gears; (2) detection at low densities can be challenging and (3) different approaches allow for different measurements.

Examples here include Clanwilliam rock catfish that are not generally detected during snorkel surveys or UWV, but respond well to electrofishing and fyke netting.

Sampling sites for electrofishing are generally shallow riffles (Figure 17) while ideal sampling sites for snorkel surveys and UWV assessments are >50 cm deep and include runs and pools with variable habitat such as woody debris, boulders and aquatic vegetation (Figure 18). A good start is to snorkel the river and gain experience of the habitat preferences of the target species and then sample accordingly.

It is important to remember that the purpose of monitoring is to determine if the treatment has been effective. For this reason sampling sites must be selected such that they not only have good spatial coverage of the treatment area but also include sites where the target species are likely to occur. If native fish recovery is to be monitored, then appropriate habitats need to be selected. As methods for monitoring fish abundance are generally less time consuming than invertebrate monitoring methods, larger sample sizes are possible. In the Rondegat River project we used 43 sample sites that were monitored.

Figure 17

A typical riffle suitable for electrofishing to determine the presence of catfishes. Such sites, while not suitable for snorkel surveys may be sampled using underwater video analysis and/or electrofishing.

Figure 18

A typical pool surveyed using snorkel surveys and underwater video analysis.

3.2.5. Aquatic Invertebrates

Typical macro-invertebrate monitoring approaches include kick-netting and stone sampling. As these techniques require wading, reaches of river that contain deep stretches should be avoided. Ideal sampling areas are: shallow, swift-flowing riffles and runs with a stony or cobbled substrate with a low degree of embeddedness not deeper than 60 cm (Figure 19 and 20). This is because aquatic invertebrates are selective about the specific hydraulic region within a river where they occur. Different genera within the same family have modifications for dealing with areas of quicker and slower water flow. These different habitat types are referred to as biotopes and are specifically targeted in the SASS5 methodology for this reason, in order to optimize the collection of the maximum possible variety of invertebrates (Dickens & Graham 2002). In addition, the presence of stones and cobbles allows for quantitative measurements of macroinvertebrate densities made during stone sampling.

Figure 19

A desirable invertebrate monitoring site characterized by a stretch of shallow riffle habitat ideal for sampling aquatic macroinvertebrates. The presence of marginal vegetation at the site is also useful if "out of current" taxa are to be sampled. Rondegat River, Western Cape.

Figure 20

An illustration of an undesirable monitoring site, where alien vegetation removal has altered the riparian zone, and where stony riffle biotope is limited. Rondegat River, Western Cape.

3.2.6. Site descriptions

Good site descriptions are essential. There are many guidelines for undertaking habitat assessments. A good guide is the rapid habitat assessment method developed for South African rivers (DWAF, 2009). This includes detailed methods for describing the riparian zone. A datasheet for site descriptions is included in Table 5. Good site descriptions should include the following:

1. **A good photograph and sketch.** Photographs and site sketches are important to have for each site.
2. **GPS coordinates** are essential and must be taken at the head (upstream section) of the site such that the site can be located easily.
3. **Dimensions.** Length of the site should be measured using a tape measure. Maximum and mean width are obtained by taking four to seven (depending on the size if the site) equally spaced width measurements across the sample site (see Figure 21).
4. **Depth.** Maximum and mean depth are obtained from measurements taken on width transects. A minimum of three depth measurements should be obtained. The outer two, 0.2 m from the left- and right-hand stream bank and the third measurement taken midstream.
5. **Habitat.** At each depth measurement, the habitat type should be recorded. In addition, a schematic diagram of the sampling site is useful for reference.
6. **Vegetation.** Canopy cover should be estimated as a percentage of total cover and bankside vegetation type recorded, with particular reference to the presence of alien vegetation.
7. **Substrate** type can be calculated by demarcating a transect down the length of the site and measuring the contribution of different substrate types and working out a percentage of overall site length.
8. **Physical** variables that should be collected include discharge across the width of the stream using a flow meter; this can be used to calculate the average flow rate at each site for each monitoring event.
9. **Water quality.** While rotenone treatments do not have long-term impacts on water quality, the collection of data on temperature, pH, total dissolved solids, dissolved oxygen (both as a percentage value and milligrams per litre), electrical conductivity, turbidity and temperature, is advised. This is because these descriptors of the aquatic environment may later help to explain variability in the abundance and composition of the faunal communities that are to be monitored. As a result, temperature, conductivity, pH and turbidity should be measured.

Figure 21

Nkosinathi Mazungula and Geraldine Taylor measuring a monitoring site.

TABLE 5 Example of a datasheet for sampling site descriptions							
Date: / Site ID:	Site name:			Coordinates:		Elevation:	
Photo/Image number:	pH:	TDS:	EC:	DO (g/L)	DO (%)	NTU	Temp ^{o}C:
Qualitative Site Description							
Length:		Mean width:			Flow condition:		
Bank condition:		Plant Cover:			Vegetation type:		
% Bedrock	% Pebbles		% Sand		% Mud		
Site sketch:							
Width & Depth transects							
Length:	Depth						
	1	2	3	4	5	6	7
Transect 1 / Width:							
Substrate							
Velocity (m/s)							
Transect 2 / Width:							
Substrate							
Velocity (m/s)							

3.3. Sampling fish

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

3.3.1. Electrofishing

Although the efficacy of electrofishing can vary depending on conductivity, the Rondegat experience was that the inclusion of this technique was essential. Clanwilliam rock catfish for example, were inadequately sampled using UWVA and snorkel surveys but were detected at all sites in pristine environments using electrofishing.

Sites for electrofishing should have an average depth of <1 m. There are a number of electrofishers available in South Africa. In the Rondegat River, electrofishing was conducted using a Samus 725G backpack electrofisher, attached to a 12 V battery with settings standardized at the duration of 0.3 ms and a frequency of 80 Hz. Due to concerns about the potential long-term effects of electrofishing on native fishes, this technique was used exclusively to determine the presence and absence of Clanwilliam Rock Catlets.

To obtain quantitative results using electrofishing, sites need to be isolated using block nets to prevent escape of fish. Subsequently, three passes should be made. The first pass should be conducted from the downstream side (tail) of the pool in an upstream direction, covering the entire length of each pool. All fish captured during each pass should be placed in separate buckets. Upon completion, fish are to be identified to species level, measured, counted, and released.

3.3.2. Snorkel surveys

Snorkel surveys are only effective in pools or runs where depths exceed 0.5 m. In pools with extensive shallow areas (<0.5 m deep), snorkelling was ineffective and backpack electrofishing (SAMUS-725GN) should be performed as a supplementary sampling method.

Snorkel surveys were conducted using a modified zigzag method. Pass one was initiated at the tail end of the pool, with the observer swimming upstream, zigzagging to cover as much of the pool as possible. Pass two was a repeat of pass one but in a downstream direction. During each pass, all fish seen were identified to species level and counted. The estimate of abundance is then the average of the two counts. In some cases, silt kicked up during the first pass, makes a second pass impractical. In this case only results from one pass are used. See Table 6 for datasheet example.

TABLE 6 Example of a datasheet for snorkel and electrofishing surveys								
Date:	Site ID:	Site name:			Coordinates:		Elevation:	
Photo/Image number:		pH:	TDS:	EC:	DO (g/L)	DO (%)	NTU	Temp ^{o}C:
Sampling description								
Method used:			Mean width:			Flow condition:		
Notes:								
Fish counts								
Species		Number of fish			Length (TL or FL)			
		Pass 1	Pass 2	Pass 3				

3.3.3. Under Water Video

Throughout the Rondegat project, underwater video analysis was employed 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. 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). In addition, UWVA proved extremely useful for the detection of fishes at low abundance.

UWVA is both expensive and time consuming, especially with regard to analysis. It does however provide a verifiable record of abundance and provides verification for snorkel surveys. As a result, it is suggested that this method be included and used, at the very least, for presence/absence.

3.3.4. Collection of fishes during rotenone treatments

The collection of fish during eradication events is essential because (1) it allows for the quantification of fishes in the treatment area and; (2) if conducted twice (as is a SOP requirement), allows for an assessment of the efficacy of the first treatment. It is also an excellent opportunity to collect biological data. It is important that adequate manpower is allocated to collect all fish. During the rotenone treatments in the Rondegat River for example, all dead fish were collected with the help of 15 volunteers whom patrolled the entire river.

3.4. Sampling aquatic invertebrates

It is our suggestion that future monitoring uses a 2-part process: (1) SASS5 scores and (2) stone sampling; identification efforts should be directed at the Ephemeroptera, Plecoptera and Trichoptera. The recent publications by Dalu *et al.* (2015) and Booth *et al.* (2015) experimentally demonstrated that selected taxa occurring within South African rivers respond differently to the application of rotenone. Therefore, the selection of specific taxa for monitoring the impacts of river rehabilitation make most sense, as this has the potential to eliminate "ecological noise" within a data set. The Ephemeroptera, Plecoptera and Trichoptera (EPT) represent the most diverse and numerically abundant invertebrate groups within lotic freshwater systems in South Africa. They are sensitive to water quality perturbations (Dickens and Graham, 2002) and because of their high densities make ideal candidates for monitoring impacts of river rehabilitation. Furthermore, it is possible to target members of these groups specifically by sampling specific hydraulic habitats within the river. Stony and cobbled riffles and runs are ideal for sampling EPT taxa, furthermore they are relatively easy to identify and count which speeds up the turnover of data.

3.4.1. Qualitative vs. quantitative responses.

Monitoring the responses of macroinvertebrates can be done in two ways: either by taking repeated and replicated samples in a manner where the effort for each sample is quantifiable and consistent or, samples can be taken in order to optimize the estimation of the diversity of invertebrates. There are shortcomings and advantages to both approaches, depending on what the monitoring in attempting to achieve. The former approach facilitates the collection of data in a straightforward and simple manner; requires little expertise and provides data that is suited to robust statistical analysis. This approach however precludes aspects of biodiversity which may be valuable in particular ecosystems. The latter qualitative approach is laborious, time consuming, and requires expertise which are not always available drawing out the realisation of quality data. Furthermore the data can be difficult to interpret without extensive analysis and descriptive statistics. Qualitative "biodiversity data" can be advantageous if rare, endemic or flagship species are present in the river which may afford better conservation status outcome post treatment.

With the above considered, we recommend the quantitative approach as it produces sound, replicable data. Monitoring the density and diversity of EPT taxa in a quantitative manner, for example number of individuals per surface area of substratum, is the simplest and most cost effective manner of detecting the impacts of rehabilitation in rivers.

3.4.2. Kick sampling

In order to target and collect a wide variety of macroinvertebrates, kick sampling can be employed. The method used should follow the standardised SASS5 methodology (see https://www.dwa.gov.za/iwqs/rhp/methods/rhp%20site%20charactersation%20manual%202005.pdf) such that a SASS score can be assigned to each site prior to quantitative assessment. Kick sampling is one of the simplest and most effective ways of collecting invertebrates. Starting at the downstream end of the monitoring site, a long handled water net with a frame dimension preferably larger than 30x30 cm is held down current of the collector and the substratum is disturbed with the feet. This will require gum boots or waders, as a lot of force must be exerted to disturb vegetation, cobbles and stones onto which the macroinvertebrates cling. Safety is also a concern as rivers often contain glass, thorns or other sharp objects and protective gear should be worn. Once the sample has been collected, the net can be emptied into a tray filled with water at which point unnecessary debris can be removed, taking care not to cast away any macroinvertebrates such as simulid larvae that may be either holding on or attached to the material.

Kick sampling is a qualitative method of collecting a sample, though, the collection time can be recorded in order to make the method more consistent between sites, per biotope. This does not overcome sampling bias between different monitoring teams or projects and coupled with the patchy distribution of macroinvertebrates along a stream bed, making comparisons between samples collected using this method should therefore be done with care.

3.4.3. Stone sampling

Stone sampling is a quantitative method designed to collect macroinvertebrates from the surface of stones from the stones-in-current biotope (Dickens and Graham, 2002). Once the stretch of riffle habitat has been selected, a set number of stones are selected from the stream bed one at a time advancing in the opposite direction to the flow of the river. First, the large and conspicuous specimens are picked off the stone using fine forceps, which may include prong-gilled mayflies or flat-headed mayflies, thereafter the stone is gently scoured with an appropriately sized brush to remove the smaller individuals like the minnow mayflies. The surface area of the stone can be calculated on a standardised way using the formula from (Graham et al., 1988):

Surface Area = 1.15*(X*Y + Y*Z + X*Z)

Once the surface area is obtained, the number of individuals or the number of taxa can be divided by the surface area of each stone to obtain a density measure which can be compared across all of the monitoring sites and tested statistically. This method proved the most useful during the current project.

TABLE 7
Example of a datasheet for stone sampling

Date:	Site ID:	Site name:		Coordinates:		Elevation:		
Notes:								
Stone sample details								
Length:	Stone dimensions (mm)			Habitat		Sample Details		
	X	Y	Z	Depth (cm)	Flow (m/S)	Habitat	Biotope	Details
Stone 1								
Stone 2								
Stone 3								
Stone 4								

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Appendix 1 International conference contributions		
Year	**Title**	**Location**
2015/16	Weyl, O.L.F., Barrow S, Impson ND, Esler KJ, Finlayson B, Jordaan M, Woodford DJ. 2015. Response of Endemic Species in South Africa's Rondegat River to Removal of Smallmouth Bass. Watershed Scale Piscicide-Driven Restoration Efforts: Challenges and Successes in Endemic Species Recovery, American Fisheries Society, 145th Annual Meeting, 16-20 August 2015, Portland, Oregon, USA. https://afs.confex.com/afs/2015/webprogram/Paper18792.html	American Fisheries Society, 145th Annual Meeting, 16-20 August 2015, Portland, Oregon, USA.
2015/16	Impson ND, Barrow S, van der Walt R. Weyl OLF. 2015. Challenges and Successes Facing South Africa's Endemic Species Recovery in the Future: The Rondegat River Experience. Watershed Scale Piscicide-Driven Restoration Efforts: Challenges and Successes in Endemic Species Recovery, American Fisheries Society, 145th Annual Meeting, 16-20 August 2015, Portland, Oregon, USA. https://afs.confex.com/afs/2015/webprogram/Paper21015.html	American Fisheries Society, 145th Annual Meeting, 16-20 August 2015, Portland, Oregon, USA.
2014/15	Woodford DJ, Barrow S, Esler KJ, Finlayson B, Impson ND, Weyl OLF. 2014. Control of invasive smallmouth bass in South African River systems: overcoming practical and socio-economic factors. 144th Meeting of the American Fisheries Society, Québec City, Canada. 17-21 October 2014. https://afs.confex.com/afs/2014/webprogram/Paper14311.html	American Fisheries Society, Québec City, Canada.
2014/15	Weyl OLF. 2014. History, status and management of Black bass (Micropterus) species in South Africa. Southern Division of the American Fisheries Society, Spring Meeting, 22-26 January 2014, Charleston, USA.	American Fisheries Society, Spring Meeting, Charleston, USA
2014/15	Ellender, B.R., Woodford, D.J., Weyl, O.L.F., Cowx, I.G. Managing conflicts arising from fisheries enhancements based on non-native fishes in Southern Africa, The Fisheries Society of the British Isles, Annual International Symposium, University of Hull, United Kingdom	Fisheries Society of the British Isles, University of Hull, United Kingdom
2013/14	Weyl OLF (2013) Managing alien fishes in South Africa. Freshwater Invasives: Networking for Strategy conference, Galway, Ireland. 9-10 April, 2013.	Galway, Ireland
2013/14	Impson D, Weyl OLF, Woodford D. (2013) Rehabilitation of a Freshwater Ecosystem Priority River in South Africa using a piscicide to kill alien smallmouth bass. Pan African Fish and Fisheries Association (PAFFA 5) Bujumbura, Burundi 16-20 September 2013	Pan African Fish and Fisheries Association (PAFFA 5) Bujumbura, Burundi.
2013/14	Madikizela B, Woodford D, Weyl O, Impson D. 2013. Restoration of native fish communities through the eradication of alien fish in a South African river. 5th World Conference on Ecological restoration, 6-11 October, Madison, Wisconsin, USA.	World Conference on Ecological restoration, Madison, Wisconsin, USA.
2013/14	Jordaan MS, Weyl OLF (2013) Evaluating the minimum effective dose of rotenone for the eradication of alien smallmouth bass *Micropterus dolomieu* from a South African River. Poster presentation at the 23rd annual European meeting of the Society of Environmental Toxicology and Chemistry (SETAC), 12-16 May, Glasgow, United Kingdom.	European meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Glasgow, United Kingdom.

Appendix 2 National Conference Contributions

Year	Title	Location
2015/16	Weyl OLF, Barrow S, Ellender BR, Impson ND, Jordaan M, Woodford DJ. 2015. Native fish responses to the removal of an alien predator in the Rondegat River, South Africa. Joint Entomological Society of Southern Africa (ESSA)/ Zoological Society of Southern Africa (ZSSA) Conference: Invasions Day. Grahamstown 15 July 2015.	Zoological Society of Southern Africa (ZSSA) Conference: Invasions Day. Grahamstown
2015/16	Jordaan M, Weyl OLF. 2015. Evaluation of the acute toxicity of the piscicide rotenone to the sharptooth catfish Clarias gariepinus. Joint Entomological Society of Southern Africa (ESSA)/ Zoological Society of Southern Africa (ZSSA) Conference: Invasions Day. Grahamstown 15 July 2015.	Zoological Society of Southern Africa (ZSSA) Conference: Invasions Day. Grahamstown
2014/15	Weyl OLF. 2015. Rotenone use to control alien invasive species: a South African case study. Water Research Commission, Ecosystems Research & Innovation Symposium: 17-18 February 2015. Broederstroom, Hartbeespoort	Water Research Commission, Broederstroom, Hartbeespoort
2014/15	Weyl, O.L.F, Woodford, D.J. & Ellender, B.R. 2014. Present status and future management of black bass (Micropterus spp.) in South Africa. Southern African Society of Aquatic Scientists Conference, 22-26 June 2014, Thaba Nchu, South Africa.	Southern African Society of Aquatic Scientists Conference, Thaba Nchu, South Africa.
2014/15	Jordaan MS, Slabbert E, Weyl OLF. 2014. Analysis of active rotenone concentration during treatment of the Rondegat River, Cape Floristic Region, South Africa: evaluation of the Minimum Effective Dose (MED). Poster presentation at the South African Society for Aquatic Scientists Conference, 22-26 June, BlackMountain Leisure and Conference Hotel, Thaba Nchu, Free State.	Southern African Society of Aquatic Scientists Conference, Thaba Nchu, South Africa.
2014/15	Bellingan, T.A., Jackson, M., Woodford, D.J., Villet, M.H.V. & Weyl, O.L.F. 2014. Community and food web structures in the Keiskamma River System, Eastern Cape, South Africa. Southern African Society of Aquatic Scientists Conference, 22-26 June 2014, Thaba Nchu, South Africa.	Southern African Society of Aquatic Scientists Conference, Thaba Nchu, South Africa.
2014/15	Barrow, S., Weyl, O.L.F., Esler, K. & Jordaan, M. 2014. Economic impact of smallmouth bass sport fishing provides invasive species management insight. Southern African Society of Aquatic Scientists Conference, 22-26 June 2014, Thaba Nchu, South Africa.	Southern African Society of Aquatic Scientists Conference, Thaba Nchu, South Africa.
2014/15	Jackson, M.C., Woodford, D.J., Bellingan, T., Chimimba, C. & Weyl, O.L.F. 2014. Do invasive trout alter aquatic energy flux to riparian consumers? Southern African Society of Aquatic Scientists Conference, Thaba Nchu, South Africa.	Southern African Society of Aquatic Scientists Conference, Thaba Nchu, South Africa.
2013/14	Weyl OLF. 2013. Managing alien fishes in South Africa.	Southern African Society of Aquatic Scientists Conference, Arniston, Western Cape, South
2013/14	Woodford DJ and Weyl OLF (2013) Restoring indigenous fish biodiversity by managing alien fishes: implication s for NEM:BA.	Water Research Commission Symposium, CSIR, Pretoria, South Africa.
2013/14	Jordaan M, Weyl OLF (2013) Evaluating the minimum effective dose of rotenone for the eradication of alien smallmouth bass Micropterus dolomieu from a South African River. Poster presentation. Southern African Society of Aquatic Scientists Conference, 30 June-4 July, Arniston, Western Cape, South Africa	Southern African Society of Aquatic Scientists Conference, Arniston, Western Cape, South Africa

Appendix 3 List of peer-reviewed papers, conference presentations and public lectures containing outputs from the current project.

1. Dalu T, Wasserman RJ, Jordaan M, Froneman WP, Weyl OLF (2015) An Assessment of the Effect of Rotenone on Selected Non-Target Aquatic Fauna. PLoS ONE 10(11): e0142140. doi: 10.1371/journal.pone.0142140

2. Bellingan T, Woodford DJ, Villet M, Weyl OLF. (2015) Rapid bioassessment of repeated rotenone treatments on a stream invertebrate assemblage in the Rondegat River, South Africa. African Journal of Aquatic Science 40(1): 89-94.

3. Impson ND, Van Wilgen BW, Weyl OLF. 2013. Coordinated approaches to rehabilitating a river ecosystem invaded by alien plants and fish. S Afr J Sci. 2013;109(11/12), Art. #a0041, 4 pages. http://dx.doi. org/10.1590/sajs.2013/a0041

4. Jordaan MS, Weyl OLF (2013) Determining the minimum effective dose of rotenone for eradication of alien smallmouth bass *Micropterus dolomieu* from a South African river. *African Journal of Aquatic Science* , African Journal of Aquatic Science, 38:sup1, 91-95, DOI: 10.2989/16085914.2013.784699

5. Slabbert E, Jordaan MS, Weyl OLF (2014) Analysis of active rotenone concentration during treatment of the Rondegat River, Cape Floristic Region, South Africa: evaluation of the Minimum Effective Dose (MED). African Journal of Aquatic Science 39: 467-472. **DOI:** 10.2989/16085914.2014.981144

6. Weyl OLF, Ellender BR, Woodford DJ, Jordaan M (2013) Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of rotenone treatment in an invaded reach. African Journal of Aquatic Science 38(2): 201-209. doi: 10.2989/16085914.2012.753401

7. Weyl OLF, Finlayson B, Impson ND, Woodford DJ, Steinkjer J. 2014. Threatened Endemic Fishes in South Africa's Cape Floristic Region: A New Beginning for the Rondegat River. Fisheries 39: 270-279.

8. Woodford DJ, Barber-James HM, Bellingan TA, Day JA, de Moor FC, Gouws J, Weyl OLF. (2013) Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage – a lesser of two evils? Journal of Insect Conservation 17: 959-973.

Appendix 4 Capacity Building

The Rondegat Project has been used as a training platform for Interns, BSc Honours, MSc and PhD students since its inception in 2011. Impacts include:

- **BSc Honours Students**: Annual participation of Rhodes University Honours students in monitoring activities. This has resulted in increased awareness on the impacts of alien species on aquatic environments and an interest in students pursuing careers in the aquatic sciences.
  - Honours 2012 (8 Students): Melissa Mayo, Anne Wu, Brendon Dredge, Christopher Gornall, Sarah Halse, Brittany Oliver, Aron Simmons, Albert Snyman
  - Honours 2013 (13 Students): Elethu Duna, Jessica Joyner, Richard Taylor, Willem Malherbe, Simon Leigh, Tia Jordan, Mathew Farthing, Leigh De Necker, Paul Denckwerts, Kyle Lloyd, Ndangisa Nomonde, Dylan Howell, Bruce Mcclure
  - Honours 2014 (12 Students): Adrian Astier, Mike Dames, Steven Dünser, Bernard Erasmus, Judge Inglis, Roxy Juby, Mathew Machell-Cox, Emily Moxham, Rachel Mullins, Nicolas Schmidt, Yonela Sithole, Timothy Smith
  - Honours 2015 (12 Students): Lesley Bloy, Edward Butler, Andreas Cross, Bianca Hannweg, Manda Kambikambi, Pule Mpopetsi, Martinus Scheepers, Sheena Talma, Jefferson Van Staden, Nicholas Van Wyk, Shannon Wilsnagh, Sibusiso Yokwana

- **MSc Student**: Stuart Barrow earned an MSc degree for from the University of Stellenbosch in November 2014. His thesis was titled, "Contrasting impact of alien invasive sport fish in the Cape Floristic Region: a focus on *Micropterus dolomieu"* and contrasted the impacts of the alien bass on native fishes in the Rondegat River with the positive economic impact of sport-fishing in Clanwilliam Dam and the costs involved in rehabilitating the river.

- **PhD student**: Terrence Bellingan is making progress towards completing his PhD thesis which includes samples collected during this project and will be submitted in 2016.

- **DST/NRF Interns**:
  - Lubabalo Mofu was trained in 2013 using project samples and registered for an MSc in invasion biology in 2014.
  - Phumsa Ndaleni was trained in 2014 using project samples and registered for an MSc in invasion biology in 2015.
  - Bosupeng Motshegoa was trained in 2014 using project samples and registered for a PhD in fish taxonomy in 2015.
  - Ann Wu was trained in 2014 using project samples and registered for a PhD in Aquaculture in 2016