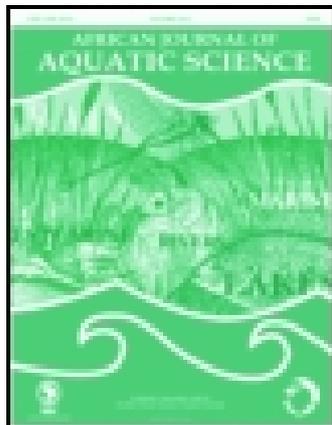


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Rapid bioassessment of the effects of repeated rotenone treatments on invertebrate assemblages in the Rondegat River, South Africa

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The potential collateral effects of eradicating invasive fishes in streams necessitate the monitoring of invertebrate communities during treatment. In an environmental rehabilitation programme, non-native smallmouth bass were removed from the lower reaches of the Rondegat River, Western Cape, South Africa, in 2012 and again in 2013 using the piscicide rotenone. A monitoring programme tracked the ecological response of organisms to these activities using quantitative sampling of macroinvertebrates on stones and the ISO-certified SASS5 rapid bioassessment method for assessing macroinvertebrate community integrity. We recorded a significant decrease in macroinvertebrate densities from the stones-in-current biotope following both rotenone treatments. The average score per taxon (ASPT) declined after the first treatment, indicating a loss of taxa sensitive to diminished water quality, then recovered prior to the second treatment, and subsequently no decline was detected after the lower dose used in the 2013 treatment. The SASS values were too variable to reveal trends. The ASPTs indicated that the community may have been resistant to low dose and resilient to high dose, due to inter-treatment recovery following the 2012 treatment, suggesting that the invertebrate assemblage is resilient to the conservative use of rotenone for localised river rehabilitation when upstream sources of recruitment exist.

Keywords: freshwater, invasion, macroinvertebrates, piscicide, rehabilitation, SASS5

Introduction

Biological invasions pose a significant threat to freshwater ecosystems, contributing to biodiversity losses that are more severe than those in terrestrial or marine environments (Dudgeon et al. 2006). In particular, invasive predatory fish have been responsible for disproportionately high losses of aquatic diversity (Cox and Lima 2006). Management of such invasions often involves the use of piscicides (e.g. Demong 2001; Pham et al. 2013; Weyl et al. 2014) but the collateral ecological effects require further quantification to optimise the technique (Vinson et al. 2010).

Rivers in South Africa's Cape Floristic Region (CFR) are of special conservation concern because of the high levels of endemism of their vertebrate and invertebrate faunas, with individual species often being restricted to specific river systems (Linder et al. 2010). In this region the local nature conservation authority, CapeNature, has begun a process of removing alien fishes from selected rivers using the piscicide rotenone (Marr et al. 2012) and the Rondegat River was the first river to be so treated (Weyl et al. 2014). In this river, smallmouth bass *Micropterus dolomieu* Lacepède, 1802 had invaded the lower reaches, where they had extirpated several native fishes and had had a demonstrable impact on invertebrate communities (Woodford et al. 2005; Lowe et al. 2008). In accordance with standard operating procedure (Finlayson et al. 2010), two rotenone treatments

were conducted on this river, the first in February 2012 at a concentration of 50 µg l⁻¹, and the second a year later in early March 2013 at a concentration of 37.5 µg l⁻¹ (Slabbert et al. 2014; Weyl et al. 2014). These treatments not only resulted in the successful removal of smallmouth bass from the river but also provided an opportunity to assess the collateral effect of the fish removal activities on the aquatic insect fauna of the Rondegat River.

The analysis of macroinvertebrate assemblages to detect water quality degradation over time and for assessing the ecological integrity of aquatic systems is a widely accepted practice (Reynoldson and Metcalfe-Smith 1992; Resh and Jackson 1993; Ollis et al. 2006). In particular, the insect orders Ephemeroptera, Plecoptera and Trichoptera play crucial roles as they are sensitive to perturbations in water quality (Madikizela and Dye 2003; Dallas 2004; Vinson et al. 2010). The ISO-certified SASS rapid bioassessment protocol has been used throughout South Africa (Chutter 1995; Dallas 1997; Dickens and Graham 2002; Vos et al. 2002) and within the CFR for this purpose (Dallas 2004, 2013). The SASS5 [South African Scoring System, version 5] method (Dickens and Graham 2002) is similar to many other rapid bioassessment techniques such as RIVPACS (Wright et al. 1984), IBMWP (Alba-Tercedor et al. 2002) and SIGNAL (Chessman 1995) in that it assesses

invertebrate diversity at the familial or ordinal rank and assigns sensitivity scores to each family based on an understanding of each group's sensitivity to perturbations in habitat or water quality. The river is then assessed by using its overall SASS5 score and its average score per taxon sampled (ASPT), which provide complementary indicators of the richness and sensitivity of the invertebrate community. Use of the SASS5 score and ASPT enabled macroinvertebrate assemblages in Western Cape rivers to be classified relative to those in pristine 'reference' rivers, using a 'biological banding' system developed by Dallas and Day (2007) that ranges from A (reference) to D (impoverished). The biological banding system thus provides a means of classifying the reach of river sampled based on its water quality as indicated by the macroinvertebrate fauna. The Western Cape is particularly interesting as a result of high levels of endemism within the aquatic biota that are found in its streams (Harrison and Agnew 1962; Wishart and Day 2002).

The SASS5 method was used to assess the nature and magnitude of collateral impacts of fish removal activities on the Rondegat River ecosystem. The sampling of the surfaces of individual stones was also carried out to quantify densities of key taxa for a known area across a consistently-occurring biotope. Woodford et al. (2013) used kick samples and measured the density of invertebrates collected from stones to detect the immediate impact of rotenone operations on the invertebrate community within the Rondegat River in 2012. They determined that, while the rotenone had an immediate short-term detrimental impact on density and diversity of invertebrates, recovery ought to be swift from source populations upstream.

We hypothesised that the application of the piscicide rotenone would have a short-term, dose-dependent negative effect on SASS5 and ASPT scores, coupled with a decrease in the density of the Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa from the stones-in-current biotope, because of the known negative immediate impact of rotenone on insect diversity (Woodford et al. 2013).

Materials and methods

Sample sites

Three invertebrate monitoring sites were chosen within the smallmouth bass zone where rotenone was applied (Figure 1). Sites were selected after a visual assessment of the proposed treatment zone, ensuring that similar proportions of the necessary biotopes were available for SASS5 sampling within a 20 m reach. Sampling took place both before and after CapeNature's rotenone operations on 29 February 2012 and 13 March 2013. Post-treatment sampling took place within two days of each treatment in both years, avoiding any confounding factors attributable to seasonal variation or weather events. Pre-treatment samples were, however, also taken seasonally in 2010 and 2011 to gauge natural variability prior to this study.

The rotenone treatment followed standard operating procedures (Finlayson et al. 2010). In each year, rotenone was applied to the river from drip stations at a consistent rate for six hours, allowing for a steady concentration of rotenone over the whole length of the treatment zone.

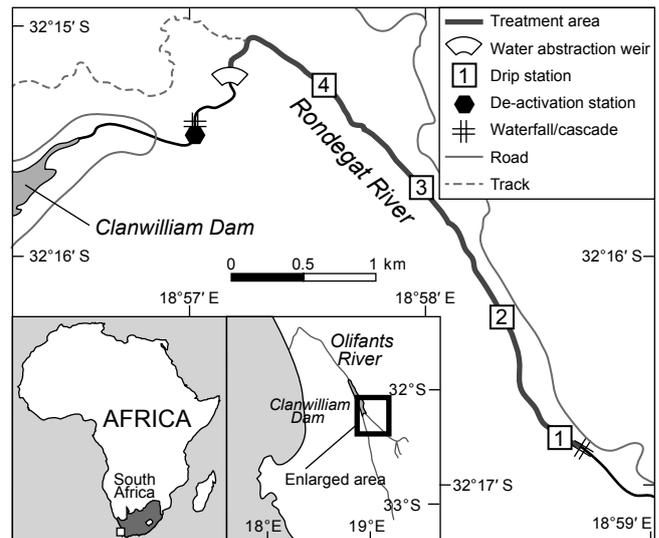


Figure 1: Map of lower reaches of the Rondegat River showing locations of four drip stations used for the 2013 rotenone treatment. Invertebrate samples were taken approximately 100 m downstream of Stations 1, 2 and 3

Concentrations differed between the treatments. In 2012, the river was treated using a rotenone concentration of $50 \mu\text{g l}^{-1}$ (Jordaan and Weyl 2013) but, because a significant short-term impact on macroinvertebrate diversity and abundance was noted (Woodford et al. 2013), for the second treatment the rotenone concentration was reduced to $37.5 \mu\text{g l}^{-1}$ (Slabbert et al. 2014).

Data collection and analysis

At each monitoring site, kick sampling was conducted following the SASS5 method (Dickens and Graham 2002). Three main biotopes, stones-in-current (SIC), marginal vegetation (MV), and gravel/sand/mud (GSM), were targeted within the 20 m reach. Kick sampling was performed for 2 min in SIC biotopes, and for 1 min in GSM biotopes, while marginal vegetation was sampled along 2 m of bank. All sampling was performed moving from downstream to upstream, using a standard SASS5 kick net (30 cm \times 30 cm wide \times 50 cm deep with a 1 mm mesh).

Once collected, each sample was visually assessed in a 50 cm \times 30 cm white tray for up to 15 min, using the SASS5 datasheet to catalogue the indicator taxa that were present and to obtain their sensitivity scores. Total SASS5 score, total number of taxa and ASPT were calculated for each sample. These data were graphed and placed within the biological bands of Dallas and Day (2007) to assess overall trends in the integrity of the macroinvertebrate assemblages.

Individual stones were sampled following the method described by Woodford et al. (2013). Briefly, four stones of approximately equal size, depth and degree of embeddedness within the substrate were chosen from the stones-in-current biotope and the macroinvertebrates were collected from them. The surface area of each stone was calculated and the density of Ephemeroptera, Plecoptera and Trichoptera per unit of surface area of substrate was compared between sites.

The invertebrate density data were square-root-transformed prior to parametric analysis (Zar 1999). Student's *t*-test for paired samples was used to test for significant differences between EPT densities before and after rotenone treatment within the same year. Invertebrate densities before and after rotenone treatment were then compared across years using a nested-design, repeated-measures ANOVA run as a general linear model, because the sampled stones were nested within sites that were sampled before and after the intervention. A Levene's test for homogeneity of variances showed no significant difference between variances, indicating that the data fitted the assumptions of ANOVA. The ASPT scores were calculated per site (and not per stone) and compared within years using paired *t*-tests. *F*-tests for homogeneity of variance were carried out and the ASPT scores were not found to differ significantly. The statistical package Statistica (v. 12.0; Statsoft Inc) was used for all statistical analyses.

Results

The sites yielded a wide range of SASS5 and ASPT scores over the period of monitoring (Figure 2), placing the macroinvertebrate assemblage's integrity status at various points between 'reference' and 'impoverished'. Variability was also evident in pre-study seasonal monitoring samples, where SASS5 and ASPT 95% confidence intervals ranged from 7 to 32 and from 0.39 to 0.82, respectively. The majority of the scores placed the community within the 'below reference' band, with the highest ASPT and SASS5 scores being recorded before the first rotenone treatment in 2012. This was followed by a decline in ASPT, although not a change in overall biological band position, after the 2012 treatment. Both pre- and post-treatment samples in 2013 displayed high variability in SASS5 and ASPT scores and remained within the 'below reference' biological band (Figure 2). These data indicated no permanent decline in community integrity over the course of the study.

Rotenone treatment significantly altered the EPT densities in 2012 ($t = 4.11$; $df = 11$; $p < 0.002$) and 2013 ($t = 6.85$; $df = 11$; $p < 0.002$) (Figure 3). No significant difference was observed between the 2012 and 2013 pre-treatment samples, or between 2012 and 2013 post-treatment samples (Figure 2). The nested repeated-measures ANOVA demonstrated that rotenone treatment had a significant negative effect on EPT densities in both treatment events ($F = 64.35$; $df = 1$; $p < 0.0001$), and that pre-treatment EPT densities were equal in both years (Table 1). Where significant differences were found the power of the tests equalled 1.00, indicating reliable results.

In contrast to the consistent decline in EPT densities following both rotenone treatments, ASPT declined significantly only following the first treatment in 2012 ($t = 11.05$; $df = 2$; $p < 0.05$). The overall pattern of changes in ASPT and SASS5 scores recorded before and after rotenone treatments was inconsistent between years (Figure 2): rotenone treatment did not impact significantly on the total SASS5 scores in either year, and affected ASPT only during the first year. Examples of EPT taxa with high sensitivity values that were not found, or were found in

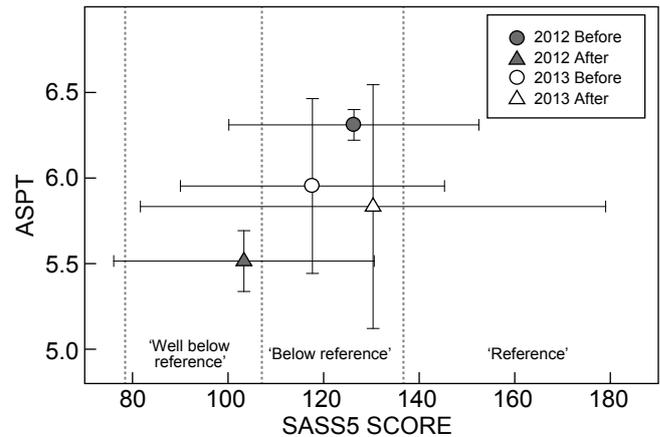


Figure 2: Means of total SASS5 score and average score per taxon (ASPT) for invertebrate monitoring sites one week before and two days after rotenone treatment in 2012 and 2013 ($n = 3$ per sample date). Interpretive biological bands for bioassessment in south-western Cape streams (after Dallas and Day 2007) indicated by dotted lines. Bars denote the 95% confidence intervals

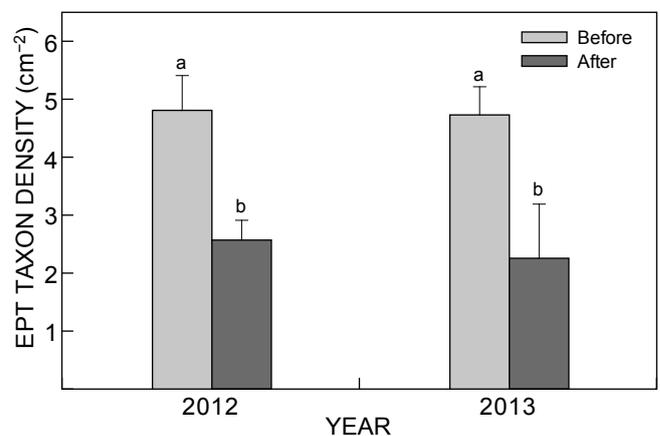


Figure 3: Mean densities of ephemeropteran, plecopteran and trichopteran (EPT) taxa recorded on stones collected one week before and two days after rotenone treatments in 2012 and 2013. Differing letters above bars indicate significant differences in EPT taxa density obtained from *t*-tests ($p < 0.05$), attributable to the application of rotenone. Error bars denote SE

lower densities, during post-rotenone sampling included the mayfly family Heptageniidae and the caddisfly families Barbarochthonidae and Polycentropodidae. The diversity of baetid mayfly genera also declined.

Discussion

The SASS5 sensitivity/tolerance score attributable to each invertebrate taxon is a reflection of that group's sensitivity to environmental perturbation, normally in the form of water pollution and habitat change (Dickens and Graham 2002). The ASPT is generally seen as a more consistent indicator of local stressors, as it assesses the relative proportion of highly sensitive taxa within the overall sampled assemblage.

Table 1: Nested repeated-measures ANOVA results comparing the densities of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa per unit surface area of stone sampled pre- and post-rotenone application in 2012 and 2013. EPT density before and after the application of rotenone differed significantly ($p < 0.05$; emphasised in bold) for both years, and these differences outweighed the significant natural variation amongst sites

Effect	SS	df	MS	F	p	Partial eta-squared	Non-centrality	Observed power ($\alpha = 0.05$)
Intercept	602.350	1	602.375	472.172	0.000	0.981	472.172	1.000
Site	13.536	2	6.768	5.305	0.030	0.541	10.610	0.688
Error	11.482	9	1.276					
Year	1.068	1	1.068	0.515	0.491	0.054	0.515	0.099
Year \times Site	5.601	2	2.801	1.352	0.307	0.231	2.704	0.221
Error	18.644	9	2.072					
Pre/post	64.356	1	64.356	37.751	0.000	0.807	37.751	1.000
Pre/post \times Site	0.093	2	0.046	0.027	0.973	0.006	0.054	0.053
Error	15.343	9	1.705					
Year \times Pre/post	0.145	1	0.145	0.092	0.769	0.010	0.092	0.059
Year \times Pre/post \times Site	0.163	2	0.082	0.052	0.950	0.011	0.103	0.056
Error	14.194	9	1.577					

The ASPT scores recorded in the present study show a significant decrease in sensitive taxa after the first rotenone treatment. This apparent decline in community integrity was attributable to the Ephemeroptera, which displayed significant declines in density and diversity immediately following the treatments (see Woodford et al. 2013). Ephemeroptera are widely used as indicator taxa due to the group's consistently high sensitivity to water quality, and they are known to be highly susceptible to rotenone (Vinson et al. 2010). However, the SASS5 scores showed high variability across the monitoring period, which probably made any effects of rotenone on overall community integrity hard to detect. Comparison of SASS5 and EPT density data across the alien fish removal operations indicated notable differences in impact and subsequent recovery in the macroinvertebrate assemblages. Therefore ASPT appeared to be a more accurate measure of impacts on sensitive taxa by the rotenone treatment, in that it tracked declines in densities of the EPT taxa.

The significant changes in the density of sensitive EPT taxa indicate that the rotenone had an immediately measurable influence on the invertebrate assemblage (Woodford et al. 2013). The results are thus inconsistent, and support only one of our hypotheses, in as much as there were immediate and significant declines in EPT taxon densities. Given the significant decline in densities during both treatments, we expected the SASS5 score to decrease repeatedly following a detected loss in densities of sensitive Ephemeroptera, Plecoptera and Trichoptera, the majority of which contain taxa that score highly in the SASS5 method (Dickens and Graham 2002). This was true for the ASPT score, but not for the SASS5 score. We suggest that the lower concentration of rotenone used in the 2013 treatment, while still 100% efficient at removing fish (Weyl et al. 2014), had less impact on the invertebrate assemblage, since no significant declines in ASPT or the SASS5 score were recorded. Nonetheless, the recovery of the ASPT values to pre-treatment levels prior to the second treatment suggests that the community was *resilient* to the higher rotenone concentration used in the first treatment

because the invertebrate community recovered after losing sensitive taxa, and was *resistant* to the lower concentration used in the second treatment because it did not deplete the sensitive taxa to below detectable levels, and the ASPT score remained comparable to the pre-treatment value.

When comparing the community integrity status obtained from the SASS5 bioassessment method after differing doses of rotenone and the immediate impacts of rotenone on the sensitive EPT taxa, contrasting effects are revealed. The resistance of key sensitive taxa to the lower dose of rotenone appeared to drive these effects. While their numbers were depleted by both treatments, the sensitive EPT taxa appeared to be temporarily extirpated (or depleted below detectable levels) by the higher rotenone concentration, only to recolonise later. No such extirpation was recorded in the second treatment. That these changes were detectable using SASS5 suggests that it is a good generalised method for long-term monitoring of river restoration programmes, though the monitoring of species-specific changes in community structure is needed to assess the conservation impacts of these activities (Samways et al. 2011; Woodford et al. 2013). The stable position of the treatment sites within the biological banding system of Dallas and Day (2007) pre- and post-rotenone treatment in 2013 indicates no permanent decline in ecological integrity. This finding suggests that the use of rotenone at a conservatively low level that still achieves its intended purpose of fish eradication (i.e. no higher than the recommended lethal concentration of $50.0 \mu\text{g l}^{-1}$), causes only a temporary perturbation to invertebrate community integrity.

Rapid bioassessment techniques should be precursors to detailed and quantitative studies of macroinvertebrate community health (Ollis et al. 2006). While SASS5 is useful as a cost-effective technique for assessing the integrity of riverine ecological health (de Moor 2002; Bonada et al. 2006), it is suggested that detailed biological surveys should be carried out when assessing the efficacy and collateral conservation impacts of river rehabilitation using piscicides.

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