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Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage: a lesser of two evils?

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Abstract The piscicide rotenone is used as a conservation tool to remove alien fishes from rivers, though there is controversy over its effects on aquatic insects. An alien fish removal operation in the Rondegat River, Cape Floristic Region, South Africa, allowed the immediate impact of rotenone on an aquatic insect community in a region with high conservation values to be quantified. The insect community within the treated river was sampled in February 2011 (1 year before rotenone operations), February 2012 (1 week before) and March 2012 (1 week after). Insects were collected using kick sampling across multiple biotopes, together with samples from individual stones. We considered rotenone-precipitated losses to be those taxa captured a week before treatment but absent after, and assessed the endemism of lost species to determine the

conservation impact of the rotenone. Species richness decreased significantly following treatment, even though many rare taxa were not recorded immediately prior to treatment. Of the 85 taxa identified, 18 were lost including five endemic to the mountain range which the river drains. Ephemeroptera were most severely affected, with a significant loss of density on stones post-rotenone and six out of 20 species missing. Since half the missing taxa were recorded upstream of the treatment area, recovery of diversity is likely to be relatively rapid. Given that alien invasive fish negatively affect both fish and aquatic insect communities in South Africa, the long-term positive conservation impact of removing these fish is likely to outweigh the short-term negative effects of the piscicide.

Keywords Rotenone · Collateral impacts · Species diversity · Endemism · Conservation intervention

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Introduction

Introduced predatory freshwater fish have had profound negative effects on native species across the globe (Cox and Lima 2006), affecting aquatic invertebrate community structure and ultimately ecosystem functioning (Simon and Townsend 2003). Conservation management of these introduced species is seen as a priority where their continued presence and on-going expansion threatens native ecosystems (Britton et al. 2008; Vander Zanden and Olden 2008). One solution to this problem is the eradication of the introduced predator from freshwater ecosystems with high conservation significance. Eradication using the piscicide rotenone has been successfully carried out in the United States (Demong 2001), the United Kingdom (Britton and Brazier 2006), and Australia (Lintermans 2000), all with

the objective of improving native fish conservation status in the treated river and lake systems.

Rotenone nonetheless poses a challenge to conservation managers, in that it does have detrimental impacts to other freshwater organisms such as amphibians (Billman et al. 2011) and invertebrates (Vinson et al. 2010). The impacts on aquatic invertebrates tend to be highly variable and taxon-specific, making the environmental impacts of proposed rotenone operations difficult to predict (Vinson et al. 2010). For example, aquatic macroinvertebrates of the order Ephemeroptera have been shown to be highly susceptible to rotenone exposure (Arnekleiv et al. 2001; Lintermans and Raadik 2003). Published impact assessments also tend to lack adequate pre-treatment sampling to establish a taxonomic diversity baseline, needed to properly assess impacts on macroinvertebrate diversity (Vinson et al. 2010). These problems highlight the need for pre- and post-treatment monitoring of invertebrate diversity during rehabilitation operations using rotenone.

In South Africa, invasive fish species are recognised as the largest threat to endangered native fishes, particularly in the Cape Floristic Region (CFR; Tweddle et al. 2009). This region, covering most of the Western Cape Province and parts of the Northern and Eastern Cape Provinces, holds one of the world's six floral kingdoms, as well as highly endemic fish and amphibian faunas (Skelton et al. 1995; Giliomee 2003). Endemism is also relatively high among the aquatic invertebrate fauna, where 54 % of invertebrate species assessed occurred only in the CFR (Wishart and Day 2002; de Moor and Day 2013). While the invertebrate group with the highest endemism (Amphipoda, 96 % species endemic) tends to occur in fishless streams, there are also groups that co-occur with fish in CFR streams, and that display particularly high endemism. These include the caddisflies (Order Trichoptera: 71 % endemic), mayflies (Order Ephemeroptera: 54 % endemic) and blackflies (Order Diptera, Family Simuliidae: 41 % endemic), all of which are preyed on by both native and introduced fishes (de Moor and Day 2013; Woodford and Impson 2004; Lowe et al. 2008). The CFR thus represents a region where introduced fish may pose a significant risk to aquatic insect conservation as well as to fish conservation.

Introduced centrarchid sport fishes in particular, including the smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*Micropterus salmoides*), pose significant threats to CFR fish conservation (Marr et al. 2012). *M. salmoides* has been shown to also alter invertebrate assemblages, eliminating large conspicuous aquatic insect taxa from an invaded stream reach (Weyl et al. 2010). In the Rondegat River, a CFR mountain stream where *M. dolomieu* has been present in the lower reaches of the river for approximately 60 years, the invasion has caused a loss of fish diversity, as well as shifts in invertebrate community

structure (Woodford et al. 2005; Lowe et al. 2008). There have however been no species-level assessments made of the impact on introduced fish on entire insect communities in the CFR to date.

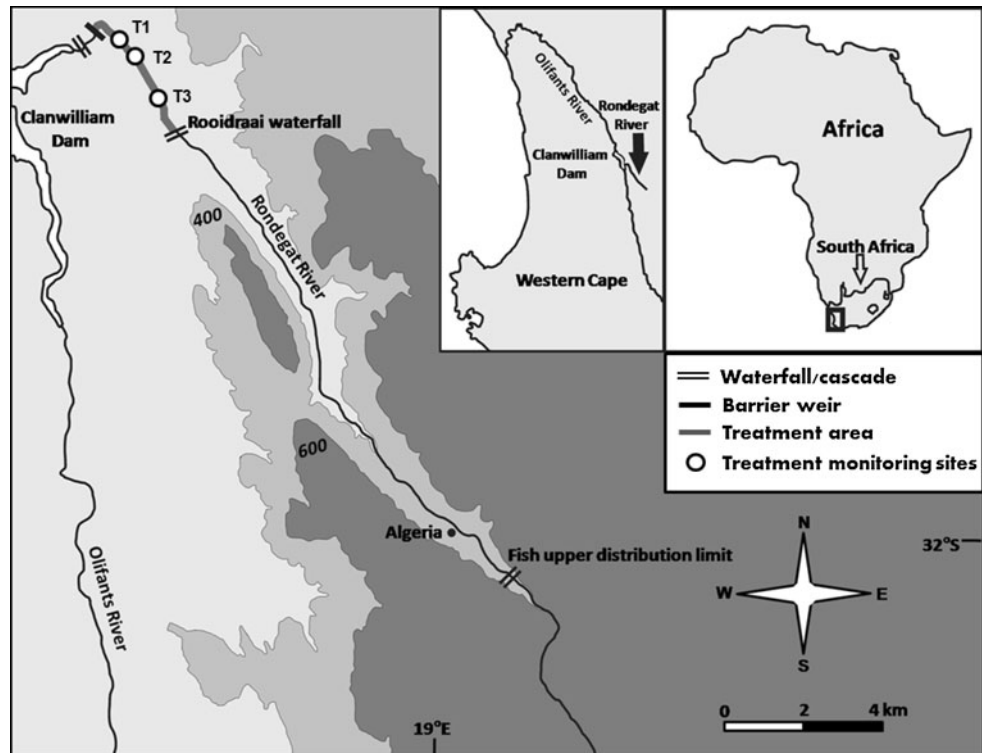
The small geographic extent of smallmouth bass in the lower Rondegat River, together with the relatively good accessibility of the invaded reach, prompted the local conservation authority CapeNature to initiate a pilot project to eradicate smallmouth bass from the river (Marr et al. 2012). The project generated substantial controversy in the years preceding the operation, particularly from anglers concerned that the use of piscicides in a mountain stream would cause an unacceptable loss of aquatic insect diversity (Flemming 2007). An environmental monitoring programme was set up to assess the aquatic invertebrate community before and after the operation, which took place in February 2012, with apparent success (Weyl et al. 2013). The monitoring programme provided a unique opportunity for assessing whether rotenone-based fish eradication operations posed a significant risk to the conservation of aquatic invertebrates within the treated area. In this paper we assess the immediate impacts of the rotenone on invertebrate densities and diversity, and discuss whether the predicted long-term positive impacts of the alien fish removal are justifiable relative to the operation's collateral community impacts.

Methods

Sample sites and rotenone treatment

The Rondegat River flows 28 km from its source in the Cederberg mountain range to its confluence with the Olifants River at the Clanwilliam Dam reservoir (Fig. 1). The river is a small second order stream, which flows through the fynbos biome in its upper reaches and the succulent karoo biome in its lower reaches. Sampling on the Rondegat River was conducted at three monitoring sites within the 4 km long reach earmarked by CapeNature for treatment with rotenone (Fig. 1). The sites were located in easily accessible areas that were at least 200 m downstream of the nearest rotenone application point, to ensure that the amount of rotenone passing through each site was as even and well-mixed as possible. Each monitoring site was 20 m long and incorporated riffle-run-pool sections where marginal vegetation was present. Each monitoring site was surveyed in February 2011 (1 year before treatment), in February 2012 (1 week before treatment) and in March 2012 (1 week after treatment). Replicated sampling was also performed prior to treatment at three sites located 1–2.5 km upstream of the treatment zone (within the succulent karoo biome as are the treatment sites) to establish

Fig. 1 Map of the Rondegat River, a tributary of the Olifants River system draining the Cederberg mountain range in the Cape Floristic Region, South Africa. The three monitoring sites are shown located within 4 km treatment area of the lower Rondegat River



which taxa occurred both in the treatment zone and in reaches that could serve as post-treatment sources of recolonisation.

The rotenone treatment was conducted on 29 February 2012, following standard operating procedures (Finlayson et al. 2000). Rotenone was applied to the river using a series of seven drip stations, spaced at approximately 1-h water travel time intervals to maintain the treatment concentration of 1 mg/L CFT Legumine (5 % rotenone) for a 6-h treatment at 50 ppb active toxicant (Weyl et al. 2013).

Kick sampling

At each monitoring site, kick samples were conducted following the SASS5 rapid bioassessment methodology (Dickens and Graham 2002). This methodology was used to sample variation in insect diversity over time using a rapid, standardised method that could be used by researchers not necessarily specialised in aquatic invertebrate taxonomy. While SASS5 does not sample specialist biotopes like the hyporheos or hygropterid seeps, it covers all biotopes directly exposed to flowing water, which are most vulnerable to toxicants. 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 areas of the sampling reach where SIC and GSM biotopes were available in turn, while marginal vegetation was sampled along

two metres of bank during a site visit. Marginal vegetation was sampled with the net below and along the water's edge by repeatedly pushing the net into the vegetation and scooping through the water column collecting fleeing/dislodged invertebrates. All sampling was performed moving from downstream to upstream, using a standardised SASS5 kick net (30 cm × 30 cm wide × 50 cm deep with a 1 mm mesh). All samples were preserved in 95 % ethanol for later sorting and identification.

Stone sampling

Prior to kick samples being taken at a site, four stones-in-current were collected and the invertebrates on them removed. Individual stone sampling assessed the relative abundance of key invertebrate taxa over time by collecting from a consistently available biotope and comparing densities standardised for the surface area of the substrate sampled. Stones were all sampled from shallow run biotopes (20–40 cm deep) with a rippled surface flow type (after Wadson and Rowntree 1998) to control for variation in water velocity. All stones were partially (5–25 %) embedded in sand, were not associated with leaf packs, and were selected to be roughly fist sized. Once a stone was selected, a kick-net with a mesh size of 200 μm was placed immediately downstream, and the stone was then placed in the kick net, allowing any organisms underneath the stone to be washed into the net before it was retrieved. Each

stone was brushed to remove all attached invertebrates, and together with the invertebrates captured by the net the sample was preserved in 95 % ethanol. Stones were then measured across their three longest axes to estimate surface area, using the following equation (Graham et al. 1988):

$$\text{Surface Area} = 1.15 * (X * Y + Y * Z + Z * X) \quad (1)$$

where X, Y and Z refer to the three longest axes of length, width and height of stones. Stone surface area provided an estimate of available substrate sampled with which to standardise counts of invertebrates collected from the stones. Neither mean stone depth (one-way ANOVA: $F_{(2,33)} = 2.3$; $p = 0.12$) nor stone surface area (one-way ANOVA: $F_{(2,33)} = 0.74$; $p = 0.48$) varied significantly among the three sampling events.

Drift sampling

Because rotenone is known to precipitate catastrophic insect drift events (Arnekleiv et al. 2001; Lintermans and Raadik 2003), drift levels were recorded at the central monitoring site before, during and after the rotenone operations, to ascertain its immediate effect on the major insect groups within the stream. A 250 μm mesh drift net with a square 400 \times 400 mm mouth was placed in a run within site T2. A total of 13 drift samples were taken, including seven samples over a 24-h period 5 days prior to treatment, four on treatment day, and three on the day following treatment. Pre-treatment drift included day, night and twilight samples, to record natural baseline drift levels. On the day of rotenone application operations samples were collected 1 h before the rotenone plume reached the site (0700 hours) and then 1, 5 and 9 h after it reached the site (0900, 1300 and 1700 hours). The 1700 hours sample occurred 2 h after rotenone operations had ceased. Samples were then collected at 0700, 1300 and 1700 hours on the day after rotenone treatment, to ascertain whether drift had returned to natural levels. Each drift sample was performed for 30 min, with depth and water velocity at the mouth of the net measured with a flow meter. These measurements allowed drift samples to be quantified per the volume of water filtered by the net. Drift samples were preserved in 95 % ethanol.

Data analysis

Aquatic insects were identified to genus and morphospecies, or species where taxonomic authority was available. This was possible for the orders Ephemeroptera, Plecoptera, Odonata, Hemiptera, Trichoptera and Coleoptera. Most families of Diptera were also sorted to morphospecies with the exception of the Chironomidae, where identification beyond the family level was considered impractical.

We sorted non-insect invertebrates to order only. See “Appendix” for the full list of taxa sampled.

Once species lists were completed for the kick and stone samples, the immediate impact of the rotenone operations on invertebrate diversity was assessed. To attain an overall estimate of how species richness changed from year to year, and from before to after rotenone operations, individual-based rarefaction curves (Gotelli and Colwell 2001) were produced for each site and each sampling event, using the software package EstimateS (Version 8, Colwell 2009). We combined stone and kick sample data to create site-specific curves, using all taxa identified to species or genus (the latter counted as morphospecies). The species count for each site was then rarefied by standardising for the smallest total number of individual insects sampled per site per year (Gotelli and Colwell 2001). Rarefied richness estimates were log transformed to meet assumptions of normality and homoscedasticity, and changes across the three sampling events were compared using repeated measures ANOVA. To assess whether key taxa of conservation importance had been removed from the river by rotenone, the species lists for the kick and stones samples were compared and species that were present in the week-before-treatment sample but absent in the post-treatment sample were noted. The endemism and known ecology of each of these missing taxa was examined.

To assess how abundances of key invertebrate groups varied annually, and as a result of rotenone operations, mean numbers sampled per stone surface area were compared. The abundances of Ephemeroptera, Odonata, Hemiptera, Trichoptera, Coleoptera, Diptera and Oligochaeta were evaluated. Mean abundances were compared between February 2011 and February 2012, as well as mean abundances between samples collected in the week preceding and following the rotenone treatment. Since stone density numbers were not normally distributed even after transformation, the untransformed densities were compared using non-parametric Mann–Whitney U tests (MWU). All statistical tests were performed using Statistica 10 (Statsoft 2011).

The effect of rotenone operations on key groups was further assessed by analysing the overall abundance of invertebrate drift, as well as the proportional abundance of key insect orders within the drift before, during and after the treatment. In the case of drift samples taken during the rotenone treatment, which contained very large numbers of invertebrates, a subsample of one quarter volume was sorted and the numbers per taxon recorded multiplied up to match the fully sorted non-treatment samples.

Results

A total of 85 individual morphospecies were identified from the samples collected from the Rondegat River

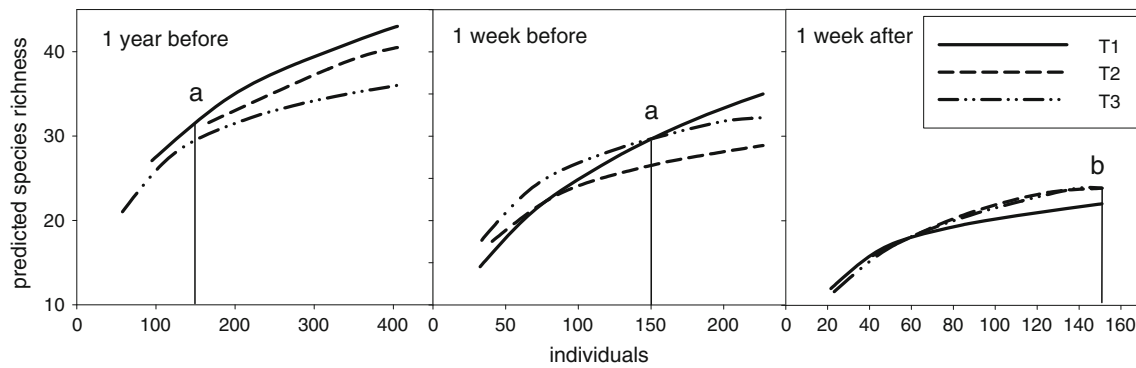


Fig. 2 Individual-based rarefaction curves for the three monitoring sites (T1, T2, T3), indicating changes in species richness between the three sampling events **a** 1 year before rotenone treatment, **b** 1 week before treatment and **c** 1 week after treatment. Comparison between sampling events was standardised by rarefying richness to the lowest

number of individuals captured per site per year (150), indicated by vertical bars on the figure. Differing letters indicate significant differences in mean rarefied richness between sampling events (repeated measures ANOVA; post hoc Tukey test $p < 0.05$)

(“Appendix”). In February 2011, 70 unique taxa were recorded from 21 accumulated samples across the three sampling sites, while 49 and 41 taxa were recorded in the weeks before and after rotenone treatment respectively. Taxonomic groups that revealed extensive diversity included the mayfly family Baetidae (15 taxa), caddisflies of the family Leptoceridae (6 taxa), the beetle family Elmidae (5 taxa) and blackflies (Simuliidae, 6 taxa). Five taxa which appeared to be previously undescribed species were identified, including two baetid mayflies (*Afroptilum* sp., *Peulhella* sp.), two caenid mayflies (*Caenis* sp., *Afrocaenis* sp.), and a polycentropodid caddisfly (*Paranyctiophylax* sp.). Individual-based rarefaction curves showed only post-treatment taxonomic samples reaching a species diversity asymptote, and that more than twice as many individual invertebrates were collected in 2011 than in either 2012 survey (Fig. 2). These data indicate that most pre-treatment samples may not have captured total diversity within the three surveyed biotopes, with some rarer taxa being missed particularly in 2011.

Comparing overall diversity between sampling events using rarefied species richness revealed a significant difference by repeated measures ANOVA ($F_{(2,4)} = 14.480$, $p = 0.01$), with the post-treatment sample having significantly lower species richness than both the one-year-before-treatment sample and the one-week-before-treatment sample (Fig. 2). In a comparison of samples from 1 week before with 1 week after rotenone treatment, 16 taxa found in pre-treatment kick samples, and 13 in pre-treatment stone samples, were missing from respective post-treatment samples (Table 1). Of these missing kick and stone sample taxa, only five and three respectively were not recorded in the river upstream of the treatment zone (Table 1, “Appendix”). The taxa absent following rotenone treatment included six mayflies, three odonates, four caddisflies, two beetles and two blackflies, of which

Table 1 Variability in diversity effects of rotenone treatment detected using kick and stone samples

Taxon type	Kick samples	Stones samples
Species missing (absent upstream)	5 (6 %)	3 (7 %)
Species missing (present upstream)	11 (14 %)	10 (22 %)
Species unaffected	24 (31 %)	16 (36 %)
Absent species (only detected in 2011)	24 (31 %)	13 (28 %)
Species only detected post-treatment	14 (18 %)	3 (7 %)
Total taxa	78	45

Species numbers and percentage of total diversity are shown for kick and stone samples. Missing species are divided into those that were and were not found in surveys upstream of the treatment zone. Unaffected species are those detected both 1 week before and 1 week after rotenone treatment. Species only detected 1 year before the treatment are listed separately, as are those found for the first time 1 week after rotenone treatment

five were Cederberg endemics (Table 2). A relatively large number of taxa (24 and 13 from kick and stone samples respectively) were only recorded in 2011 (Table 1, “Appendix”). These species generally each comprised less than 1 % of all individuals collected, and were often a single individual record within a sample (“Appendix”). As these taxa may have been too sparsely distributed within the sites to be consistently detected, we considered them an “incidental” component of the assemblage, on which the effect of rotenone could not be appropriately assessed.

The Ephemeroptera (MWU = 37.0, replicates = 12, $p < 0.05$) and Trichoptera (MWU = 30.0, replicates = 12, $p < 0.02$) both showed significant declines from the 2011 pre-treatment stone samples to the 2012 pre-treatment samples (Fig. 3). A significant decline in densities of Ephemeroptera was also detected when the one week pre- and post-treatment samples were compared (MWU = 15.0, replicates = 12, $p < 0.002$; Fig. 3).

Table 2 List of taxa recorded in either kick or stones samples pre-treatment, but missing from all post-treatment samples

Kick losses	Stone losses	Endemism	Present upstream
<i>Afroptilum</i> sp.	<i>Afroptilum</i> sp.	Cederberg ^a	Y
<i>Afroptilum sudafricanum</i>	Not present pre-treatment	Southern Africa	Y
<i>Baetis cf harrisoni</i>	<i>Baetis cf harrisoni</i>	Cederberg ^b	Y
<i>Peuhlella</i> sp.	<i>Peuhlella</i> sp.	Cederberg ^a	Y
<i>Pseudocloeon vinosum</i>	Not present pre-treatment	Southern Africa	Y
<i>Afronurus</i> sp.	<i>Afronurus</i> sp.	Cederberg ^c	Y
<i>Platycypha</i> sp.	Not present pre-treatment	Unknown ^d	N
<i>Ictinogomphus</i> sp.	Not present pre-treatment	Unknown ^e	N
<i>Sympetrum fonscolombii</i>	Not present pre-treatment	Southern Africa	N
<i>H. cruciata</i>	Not present pre-treatment	Widespread in Africa	Y
Not present pre-treatment	<i>Cheumatopsyche thomasseti</i>	Widespread in Africa	Y
Not present pre-treatment	<i>O. modesta</i>	South Africa	Y
Not present pre-treatment	<i>Paranyctiophylax</i> sp.	Cederberg ^c	Y
<i>Uvarus</i> sp.	Not present pre-treatment	Unknown ^d	N
Not present pre-treatment	<i>Tropidelmis hintoni</i>	Cape Floristic Region	Y
Tabanidae sp.	Not present pre-treatment	Unknown ^f	Y
<i>Simulium impukane</i>	<i>S. impukane</i>	Widespread in Africa	Y
<i>S. unicornutum</i>	<i>S. unicornutum</i>	Widespread in Africa	N

These taxa are believed to represent genuine losses as a result of the rotenone operations. The endemism of each species provides an indicator of the conservation significance of the apparent loss

^a Possible undescribed Cederberg endemic species

^b Believed to be endemic member of widespread species complex

^c Believed to be Cederberg endemic species of widespread Genus

^d Only identified to Genus, species endemism unknown

^e New extra-limital record for Genus, not previously known from Cape Floristic Region

^f Single morphospecies identified to Family, species endemism unknown

An analysis of invertebrate abundances in the drift revealed a catastrophic drift event where peak invertebrate densities increased by nearly two orders of magnitude over natural pre-treatment levels (Fig. 4), peaking at 37,507 invertebrates per cubic meter of water filtered midway through the treatment. Commencement of treatment saw a rapid increase in the relative abundance of Ephemeroptera in the drift, which were the second most abundant order in pre-treatment drift samples after Diptera, but became the most abundant order 1 h into the treatment (Fig. 5). By midday, when overall invertebrate abundances were peaking, Coleoptera (both adults and larvae) became the most abundant order in the drift, and were also recorded at elevated levels relative to pre-treatment abundances in samples from the day after the rotenone treatment (Fig. 5).

Discussion

Rotenone operations conducted in the Rondegat River had a significant effect on both the density and diversity of macroinvertebrates, although the true impact of the

piscicide was somewhat obscured by inter-annual variation in community density and diversity. This finding highlights the challenge of accurately assessing the threat posed by rotenone to aquatic insect conservation.

The immediate impact of rotenone operations on the Rondegat River appears to have been most severe on the Ephemeroptera, the most diverse order of insects found in the treated reach of the stream. The group was among the quickest to respond to rotenone in the water through mass drift, and was the only order of aquatic insects in which significant declines in densities were recorded on stones in the week following rotenone operations. These findings are not surprising, as previous assessments both in the field and through laboratory toxicity trials have found ephemeropterans to be particularly vulnerable to rotenone (Arnekleiv et al. 2001; Lintermans and Raadik 2003; Vinson et al. 2010). The direct conservation implications of this impact for the Rondegat River can best be assessed by examining the six species of Ephemeroptera that were not recorded in any samples in the week following the rotenone operation.

Three missing morphospecies of baetids, including two undescribed species (*Afroptilum* sp., *Peuhlella* sp.) and one

member of a putative species complex (*Baetis cf harrisoni*) (Pereira da Conceicao et al. 2012), are likely to be endemic to the Olifants-Doring catchment that drains the Cederberg

mountain range. The discovery of the undescribed taxa points to our relatively poor knowledge of aquatic invertebrate diversity in the succulent karoo biome, through which the lower Rondegat River flows. *Peuhlella* sp. and *Afroptilum* sp. may be endemic to the succulent karoo rather than mountain fynbos, given their absence from previous surveys of the Cederberg which have generally focussed on rivers in the mountain fynbos biome (de Moor and Barber-James 2007). The remaining two missing baetid taxa were widespread species (Lugo-Ortiz and McCafferty 1997; Gillies 1990), thus arguably of lower conservation value. The remaining ephemeropteran taxon, *Afronurus* sp., is probably endemic to the Cederberg, but further research including the collection of adults is needed to substantiate this. The same applies for the polycen-tropodid caddisfly *Paranycetiophylax* sp.

In contrast to the Ephemeroptera, the Odonata, Trichoptera, Coleoptera and Diptera appeared largely unaffected by the rotenone operations. Of the species from these groups apparently extirpated, only the caddisflies *Hydroptila cruciata* and *Oecetis modesta* comprised more than 1 % of collected individuals within samples. *O. modesta* is a fairly common taxon in the region (Barnard 1934; Harrison and Elsworth 1958) and *H. cruciata* is common throughout Africa. *Simulium unicornutum* and *Simulium medusaeforme* were also missing after the rotenone treatment; these are both widespread common species throughout much of Africa (Palmer and de Moor 1998; de Moor 2003).

The remaining missing species did not form a significant component of the overall fauna. While these taxa were “rare” within the river, they all represent widespread taxa of little conservation interest. Their incidental status does

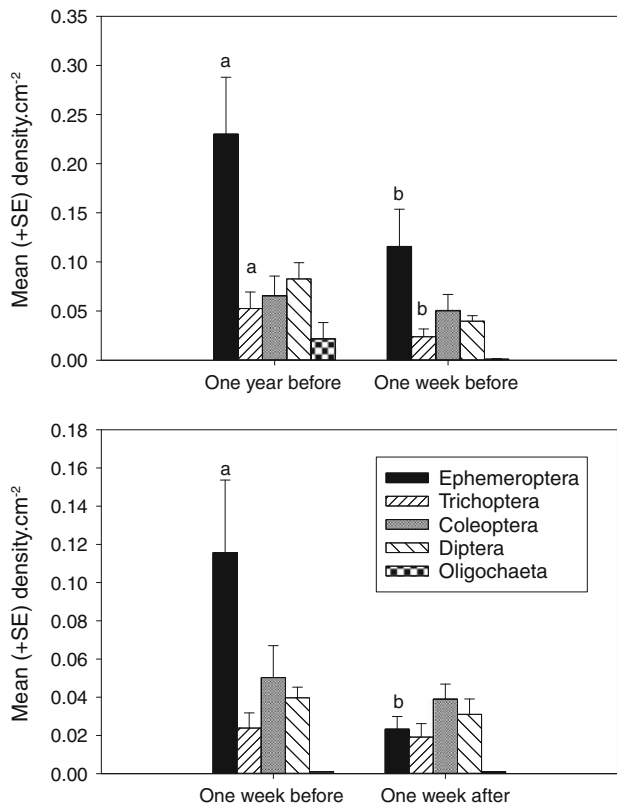


Fig. 3 Mean (+1 SE) densities of key invertebrate orders on stones collected in February 2011 (1 year before treatment), February 2012 (1 week before treatment) and March 2012 (1 week after treatment). Differing letters above bars represent significant differences within an order (Mann–Whitney *U* test, *p* < 0.05)

Fig. 4 Densities of invertebrates captured in a drift net before, during and after rotenone was applied to the stream. Densities are represented on a log scale due to the order of magnitude increase in drift during treatment. The rotenone was first applied at 0800 hours, 2 h after sunrise, and was discontinued at 1500 hours, 4 h before sunset. The period of rotenone application is demarcated by the horizontal grey bar

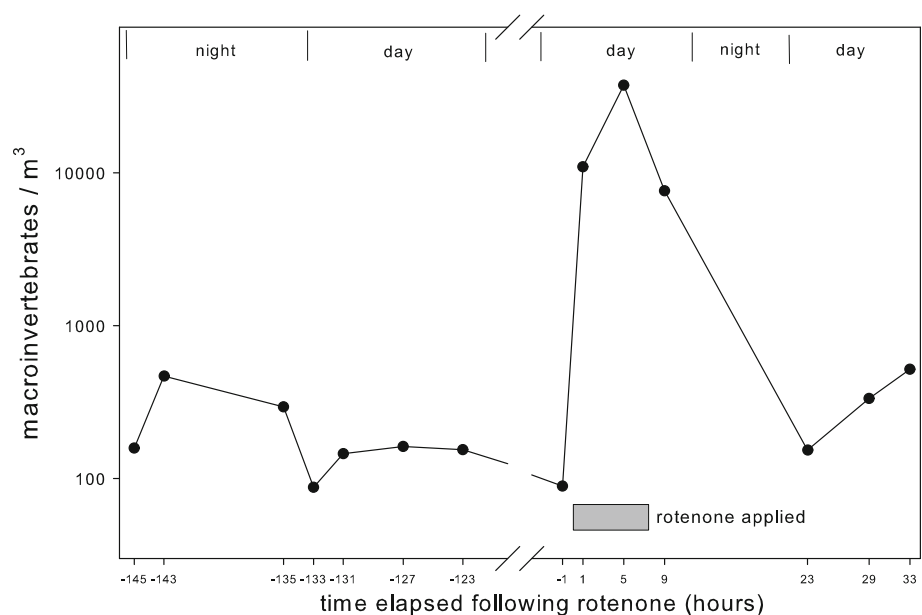
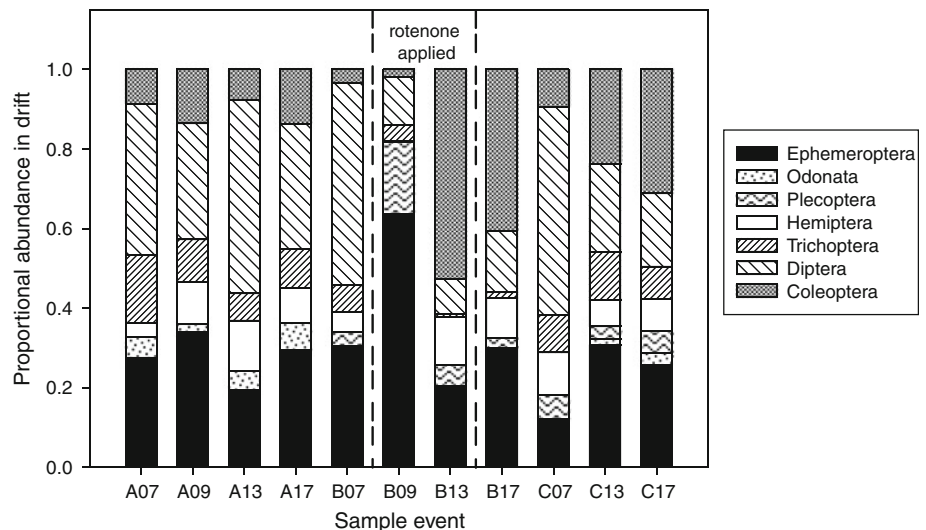


Fig. 5 Proportional densities of key benthic insect orders for all daytime drift samples collected before, during and after rotenone treatment. Sample events are coded according to date of sample (A = 5 days before treatment; B = day of treatment; C = day after treatment), and hour of sampling (24 h scale). The start and end of rotenone application is demarcated by vertical dashed bars



however highlight the difficulty in quantitatively assessing biodiversity losses. Rarefaction analysis showed that the efficacy of SASS in sampling diversity was lower when larger numbers of individuals were captured at a site. While species detection per individual sampled reached an asymptote at all three sites following the rotenone treatment, there was evidence for undetected diversity in the rarefaction trajectories of the pre-treatment samples. This may explain the 14 taxa detected for the first time after the rotenone treatment, when a larger proportion of total diversity appeared to be sampled. Larger overall numbers, together with many more unique records (31 % of total diversity) were recorded in the 2011 survey than in the two 2012 surveys. However, when these incidental records were accounted for by comparing rarefied richness, a significant effect of the rotenone treatment was still detected. This finding suggests that the SASS methodology was sufficient for detecting the piscicide's diversity impacts, even though it likely failed to detect many rare taxa prior to the rotenone treatment. Long-term monitoring and targeted biotope sampling will be needed to determine whether any of these taxa were negatively affected by the rotenone.

Both Ephemeroptera and Trichoptera dropped sharply in abundance in the year between sampling events, and the numbers of insects collected in kick samples followed this trend. While the cause for this annual variability in both densities and diversity is unclear, it does indicate that natural environmental variation had comparable effects on the insect community to the rotenone operation itself. Once again, long-term monitoring of insect community recovery will be needed to determine whether the annual variation seen between 2011 and 2012 was unusual for the stream.

Considering the total richness of the sampled insect community (85 taxa) the short-term loss of 18 taxa (21 % of diversity) appears minor in the context of previous

research. Our results contrast sharply with comparable studies in America, where up to 50 % losses in stream invertebrate diversity have been recorded for up to a year after rotenone treatment (Vinson et al. 2010). Recovery in the treatment zone is expected to be rapid for at least some of this study's missing taxa. Of the 18 species apparently lost to rotenone, nine were recorded upstream of the treatment zone, suggesting re-colonisation via drift is possible for at least some of these taxa. Groups expected to recover rapidly include the mayflies and blackflies. These groups were abundant in pre-treatment drift samples, and all but one missing taxon from each group was found upstream of the treatment zone. Other less mobile taxa, such as the trichopteran larvae, may require re-colonisation by adults to ensure recovery, which in the case of univoltine species could take a year or more to occur.

While these findings suggest a relatively minor long-term impact of rotenone on the conservation status of the Rondegat River fauna, it should be noted that specialised taxonomic expertise is required to identify the species recorded as missing after the rotenone treatment. Such expertise was not available for the Chironomidae, even though they are a numerically dominant component of the insect community, both in the Rondegat River and elsewhere in the CFR (Harrison and Elsworth 1958; Scott 1958). The difficulty of identifying this group to species or even genus means that significant species-level losses may have gone unnoticed in the Rondegat River following rotenone operations.

Regardless of the true conservation cost of the rotenone operation, it is important to contrast this with the effect the alien fish may have had on the invertebrates prior to their removal. While no community-wide assessment of alien fish impacts on insect diversity exist in South Africa, a study that compared diversity of Trichoptera in a stream

containing native fish to one containing alien trout, found 20 species in the former stream and only nine in the latter (de Moor 1992). The impact of invasive fish on eliminating the larger, rarer but more visible predatory insects such as Odonata, Hemiptera and Coleoptera, but not the smaller chironomids and baetids, is well known (Weir 1972; Healey 1984).

Most studies on the impact of introduced fish on invertebrate communities have either only investigated taxa at family level (Lowe et al. 2008; Weyl et al. 2010) or have focussed on the specific responses of particular species to introduced fish predators (Englund 1999; Samways 1999; see also Simon and Townsend 2003 for examples). In a notable exception, Englund and Polhemus (2001) assessed the impact of introduced trout on Hawaiian stream insect communities and found little evidence of negative species-level effects, though Englund (1999) earlier found evidence for significant impacts by introduced poeciliid fishes on some odonate species in these streams.

In South Africa, apart from the Trichoptera there is little understanding of the species-level effect of these predators on the community structure, and consequently on the conservation status of these insect assemblages. However, the clear and obvious threat posed by these predators on fish species in the CFR (Tweddle et al. 2009) means that active mitigation of these impacts is a priority for the conservation status of these streams on the whole. The comparatively low number of Cederberg endemics apparently removed by the rotenone (five, all of which occur upstream) matches the number of Cederberg endemic fishes negatively impacted by bass in the same reach of river, which are now expected to re-colonise from upstream following treatment (Marr et al. 2012; Weyl et al. 2013).

Thus the recorded insect diversity losses may be considered acceptable in the greater context of biodiversity conservation within the stream.

Our findings suggest the use of the piscicide rotenone for alien fish removal does not pose an unacceptably high risk to aquatic insect conservation in the CFR at the current scale of operation, given the relatively low number of endemic species negatively affected in this study. The remarkable differences in taxa over all sampling times suggest considerable natural variability in stream community composition. Although this variability may hamper interpretation of rotenone impacts in treated streams, it also suggests a dynamic invertebrate assemblage that may be quite resilient to such disturbances. While setting a species-level baseline for monitoring the impacts of rotenone should be a prerequisite for future planned uses of rotenone for fish conservation, the feared collateral effects of rotenone on this component of the CFR invertebrate fauna should not be used as a reason to block future fish community rehabilitation efforts using the piscicide.

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Appendix

See Table 3.

Table 3 List of taxa collected in stone samples and kick samples in three site visits to the treatment reach of the Rondegat River

Sampling time	Kicks n		Kicks % total taxa		Kicks n		Kicks % total taxa		Stones n		Stones % total taxa		Stones n		Stones % total taxa		Present u/s of treatment
	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year post	1 week post	
Ephemeroptera																	
Baetidae																	
<i>Afroptilum</i> sp. CED120AE	24	3	1.18	0.62	31	0	2.44	0.00	18	2.90	0	0.00	0	0.00	0	0.00	Y
<i>A. sudaffricanum</i>	21	1	1.03	0.21	1	0	0.08	0.00	0	0.00	0	0.00	0	0.00	0	0.00	Y
<i>Baetis harrisoni</i>	93	31	4.56	6.40	29	0	2.28	0.00	12	1.94	0	0.00	0	0.00	0	0.00	Y
<i>Chelecloleon excisum</i>	40	15	1.96	3.10	16	26	1.26	4.54	5	0.81	2	0.60	2	0.30	0	0.00	Y
<i>Cloeon</i> sp.	10	0	0.49	0.00	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Dabulamanzia media</i>	1	0	0.05	0.00	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Demoulinia crassi</i>	2	0	0.10	0.00	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Glossidion</i> sp.	1	0	0.05	0.00	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Peuhlella</i> sp. CED120AF	15	3	0.74	0.62	3	0	0.24	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Pseudopannota maculosa</i>	35	20	1.72	4.13	7	1	0.55	0.17	12	1.94	0	0.00	0	0.00	0	0.00	Y
<i>Pseudopannota</i> sp. CED114E	1	0	0.05	0.00	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Pseudocloleon glaucum</i>	11	0	0.54	0.00	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>P. piscis</i>	169	30	8.29	6.20	3	12	0.24	2.09	0	0.00	0	0.00	0	0.00	0	0.00	Y
<i>P. vinosum</i>	13	9	0.64	1.86	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Securiops macaffertiorum</i>	1	0	0.05	0.00	0	0	0.00	0.00	23	3.71	15	4.52	0	0.00	0	0.00	N
Caenidae																	
<i>Afrocaenis</i> sp. CED 104A	78	47	3.83	9.71	0	90	0.00	15.71	2	0.32	6	1.81	0	0.00	0	0.00	Y
<i>Caenis</i> sp. CED 104B	109	9	5.35	1.86	56	20	4.41	3.49	2	0.32	0	0.00	0	0.00	0	0.00	Y
Heptageniidae																	
<i>Afronurus</i> sp.	11	1	0.54	0.21	1	0	0.08	0.00	4	0.65	0	0.00	0	0.00	0	0.00	Y
Leptophlebiidae																	
<i>Euthraulus elegans</i>	23	13	1.13	2.69	76	13	5.98	2.27	20	3.23	28	8.43	0	0.00	0	0.00	Y
Teloganodidae																	
<i>Lestagella penicillata</i>	0	0	0.00	0.00	1	0	0.08	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Odonata																	
Aeshnidae																	
<i>Aeshna</i> sp.	10	0	0.49	0.00	2	1	0.16	0.17	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Anax</i> sp.	0	0	0.00	0.00	0	1	0.00	0.17	0	0.00	1	0.30	0	0.00	0	0.00	N
Chlorocyphidae																	
<i>Platycypha</i> sp.	1	1	0.05	0.21	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Coenagrionidae																	
<i>Pseudagrion</i> sp.	22	46	1.08	9.50	0	48	0.00	8.38	0	0.00	0	0.00	0	0.00	0	0.00	N
Gomphidae																	
<i>Ictinogomphus</i> sp. CED343B	0	1	0.00	0.21	0	0	0.00	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Paragomphus</i> sp.	54	36	2.65	7.44	5	53	0.39	9.25	0	0.00	0	0.00	0	0.00	0	0.00	Y
Libellulidae																	

Table 3 continued

Sampling time	Kicks n		Kicks % total taxa		Kicks n		Kicks % total taxa		Stones n		Stones % total taxa		Stones n		Stones % total taxa		Present u/s of treatment
	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year post	1 week post	
<i>Sympetrum fonscolombii</i>	1	0.05	1	0.21	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Trithemis</i> sp.	29	1.42	5	1.03	1	0.17	2	0.16	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Zygonyx</i> sp.	0	0.00	1	0.21	1	0.17	1	0.08	0	0.00	0	0.00	0	0.00	0	0.00	N
Protonneuridae																	
<i>Ellatoneura</i> sp.	1	0.05	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Plecoptera																	
Notonemouridae																	
<i>Aphanicerella</i> sp.	0	0.00	0	0.00	0	0.00	3	0.24	0	0.00	0	0.00	0	0.00	0	0.00	N
Hemiptera																	
Veliidae																	
<i>Rhagovelia</i> sp.	76	3.73	4	0.83	27	4.71	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Naucoridae																	
<i>Neomacrocoris</i> sp.	1	0.05	5	1.03	1	0.17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Nepidae																	
<i>Borborophilus afzelii</i>	2	0.10	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Notonectidae																	
<i>Anisops</i> sp.	2	0.10	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Corixidae																	
<i>Micronecta</i> sp.	0	0.00	1	0.21	0	0.00	0	0.00	1	0.16	1	0.30	1	0.30	0	0.00	Y
<i>Enithares</i> sp.	0	0.00	0	0.00	1	0.17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Trichoptera																	
Ecnomidae																	
<i>Ecnomus</i> sp. CED105L	5	0.25	4	0.83	4	0.70	10	0.79	12	1.94	9	2.71	9	2.71	0	0.00	Y
Hydropsychidae																	
<i>Cheumatopsyche afra</i>	4	0.20	3	0.62	2	0.35	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	Y
<i>Cheumatopsyche</i> sp. CED42AA	101	4.96	32	6.61	11	1.92	31	2.44	20	3.23	9	2.71	9	2.71	0	0.00	Y
<i>C. thomasseri</i>	0	0.00	0	0.00	0	0.00	1	0.08	1	0.16	0	0.00	0	0.00	0	0.00	N
<i>Macrostemum capense</i>	8	0.39	0	0.00	5	0.87	0	0.00	1	0.16	2	0.60	2	0.60	0	0.00	N
Hydroptilidae																	
<i>Oxyethira velocipes</i>	6	0.29	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Hydropila cruciata</i>	94	4.61	1	0.21	0	0.00	44	3.46	0	0.00	0	0.00	0	0.00	0	0.00	Y
Leptoceridae																	
<i>Athripsodes harrisoni</i>	12	0.59	17	3.51	32	5.58	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Athripsodes prionii</i>	12	0.59	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Athripsodes</i> sp.	0	0.00	0	0.00	1	0.17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Leptecho</i> sp.	3	0.15	0	0.00	0	0.00	3	0.24	0	0.00	0	0.00	0	0.00	0	0.00	Y
<i>Leptecho helicotheca</i>	1	0.05	0	0.00	1	0.17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N

Table 3 continued

Sampling time	Kicks n		Kicks % total taxa		Kicks n		Kicks % total taxa		Stones n		Stones % total taxa		Stones n		Stones % total taxa		Present u/s of treatment
	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year post	1 week post	1 year post	1 week post	
<i>Oecetis modesta</i>	15	0	0.74	0.00	0	0.00	18	1.42	2	0.32	0	0.00	0	0.00	0	0.00	Y
Polycentropodidae																	
<i>Paranyctiophylax</i> sp.	0	2	0.00	0.41	0	0.00	10	0.79	3	0.48	0	0.00	0	0.00	0	0.00	Y
Sericostomatidae																	
<i>Petroplax</i> sp.	0	0	0.00	0.00	0	0.00	2	0.16	0	0.00	0	0.00	0	0.00	0	0.00	N
Coleoptera																	
Dytiscidae																	
<i>Uvarus</i> sp.	0	1	0.00	0.21	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Sharpshyrus</i> sp.	0	0	0.00	0.00	1	0.17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Elmidae																	
<i>Tropidelmis hintoni</i>	0	0	0.00	0.00	0	0.00	0	0.00	1	0.16	0	0.00	0	0.00	0	0.00	N
<i>Elpidelmis capensis</i>	40	0	1.96	0.00	7	1.22	87	6.85	74	11.94	35	10.54	35	10.54	35	10.54	Y
<i>Helminthopsis</i> sp.	0	0	0.00	0.00	0	0.00	0	0.00	1	0.16	1	0.30	1	0.30	1	0.30	N
<i>Microdinodes</i> sp.	2	0	0.10	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Petrololus</i> sp.	5	5	0.24	1.02	0	0.00	86	6.34	48	7.19	35	9.54	35	9.54	35	9.54	Y
Gyrinidae																	
<i>Autonyrus</i> sp.	0	0	0.00	0.00	2	0.35	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Orectogyrus</i> sp.	0	0	0.00	0.00	0	0.00	0	0.00	2	0.32	1	0.30	1	0.30	1	0.30	N
Hydraenidae																	
<i>Mesoceratium</i> sp.	0	2	0.00	0.41	5	0.87	1	0.08	1	0.16	11	3.31	11	3.31	11	3.31	Y
<i>Parhydraena</i> sp.	2	0	0.10	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	Y
<i>Prosthetops</i> sp.	2	0	0.10	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Hydrophilidae																	
<i>Hydrophilid</i> sp.	1	0	0.05	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Ptylodactylidae																	
<i>Ptylodactylid</i> sp.	6	0	0.29	0.00	10	1.75	6	0.47	4	0.65	4	1.20	4	1.20	4	1.20	Y
Scirtidae																	
<i>Scirtid</i> sp.	10	0	0.49	0.00	9	1.57	15	1.18	5	0.81	14	4.22	14	4.22	14	4.22	Y
Diptera																	
Blephariceridae																	
<i>Elporia</i> sp.	1	0	0.05	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
Ceratopogonidae																	
<i>Bezzia</i> sp.	8	0	0.39	0.00	1	0.17	1	0.08	0	0.00	0	0.00	0	0.00	0	0.00	Y
<i>Atrichopogon</i> sp.	0	1	0.00	0.21	1	0.17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	Y
Culicidae																	
<i>Anopheles</i> sp.	24	11	1.18	2.27	7	1.22	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	N
<i>Culex</i> sp.	199	0	9.76	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	Y

Table 3 continued

Sampling time	Kicks n		Kicks % total taxa		Kicks n		Kicks % total taxa		Stones n		Stones % total taxa		Stones n		Stones % total taxa		Present u/s of treatment
	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year pre	1 week pre	1 year post	1 week post	
Tabanidae																	
<i>Tabanid</i> sp.	0	1	0.00	0.21	0	0	0.00	0.00	0	0	0.00	0.00	0	0	0.00	0.00	N
Tipulidae																	
<i>Antocha</i> sp.	5	4	0.25	0.83	2	2	0.35	0.83	46	30	3.62	4.84	12	12	3.61	3.61	Y
<i>Limmophila</i> sp.	2	0	0.10	0.00	0	0	0.00	0.00	0	0	0.00	0.00	0	0	0.00	0.00	Y
Simuliidae																	
<i>Simulium impukane</i>	17	1	0.83	0.21	0	0	0.00	0.21	1	2	0.08	0.32	0	0	0.00	0.00	Y
<i>S. medusaeforme</i>	139	45	6.82	9.30	10	10	1.75	9.30	5	4	0.39	0.65	0	0	0.00	0.00	Y
<i>S. unicornutum</i>	10	0	0.49	0.00	0	0	0.00	0.00	5	1	0.39	0.16	0	0	0.00	0.00	N
<i>Simulium (Pomeroyellum)</i> sp.	3	0	0.15	0.00	0	0	0.00	0.00	0	0	0.00	0.00	0	0	0.00	0.00	N
<i>S. ruficorne</i>	0	0	0.00	0.00	0	0	0.00	0.00	0	0	0.00	0.00	1	1	0.30	0.30	N
<i>S. bequaerti</i>	0	0	0.00	0.00	0	0	0.00	0.00	1	0	0.08	0.00	0	0	0.00	0.00	Y
Malacostraca																	
<i>Potamonautes</i> sp.	9	6	0.44	1.24	6	6	1.05	1.24	1	1	0.08	0.16	3	3	0.90	0.90	N
Gastropoda																	
Ancylidae																	
<i>Ferrissia</i> sp.	1	0	0.05	0.00	0	0	0.00	0.00	0	0	0.00	0.00	0	0	0.00	0.00	N
<i>Burnupia</i> sp.	0	0	0.00	0.00	1	1	0.17	0.00	0	0	0.00	0.00	0	0	0.00	0.00	N
Unidentified taxa																	
Ephemeroptera																	
Baetidae																	
Small baetids	0	3	0.00	0.01	0	0	0.00	0.01	329	179	0.26	0.29	9	9	2.71	2.71	
Caenidae	0	1	0.00	0.21	0	0	0.00	0.21	24	0	1.89	0.00	0	0	0.00	0.00	
Small caenids	0	1	0.00	0.21	0	0	0.00	0.21	24	0	1.89	0.00	0	0	0.00	0.00	
Plecoptera																	
Notonemouridae																	
Small notonemourids	2	0	0.10	0.00	1	1	0.17	0.00	3	8	0.24	1.29	19	19	5.72	5.72	
Trichoptera																	
Hydropsychidae																	
Small hydropsychids	0	0	0.00	0.00	1	1	0.17	0.00	0	17	0.00	2.74	23	23	6.93	6.93	
Leptoceridae																	
Small leptocerids	0	8	0.00	1.65	3	3	0.52	1.65	6	3	0.47	0.48	13	13	3.92	3.92	
Coleoptera																	
Dytiscidae																	
Dytiscid larvae	5	2	0.25	0.41	2	2	0.35	0.41	0	0	0.00	0.00	1	1	0.30	0.30	
Diptera																	
Chironomidae																	

Table 3 continued

Sampling time	Kicks n		Kicks % total taxa		Kicks n		Kicks % total taxa		Stones n		Stones % total taxa		Stones n		Stones % total taxa		Present u/s of treatment	
	1 year pre	1 year pre	1 week pre	1 week pre	1 week post	1 week post	1 year pre	1 year pre	1 year pre	1 year pre	1 week pre	1 week pre	1 week post	1 week post	1 week post	1 week post		
Chironomid larvae	421	20.66	48	9.92	150	26.18	173	13.61	61	9.84	63	18.98						
Simuliidae																		
Small simuliids	0	0.00	0	0.00	0	0.00	10	0.79	10	1.61	2	0.60						
Oligochaeta																		
Various oligochaetes	3	0.15	2	0.41	0	0.00	66	5.19	3	0.48	2	0.60						
Acari																		
Various hydracarinae	5	0.25	0	0.00	1	0.17	27	2.12	11	1.77	10	3.01						
Total individuals	2,040		484		573		1,271		620		332							

Bolded taxa comprise more than 1 % of all individuals captured across all sites in a particular visit, for either stone or kick samples. "Stones n" and "Kicks n" represent raw numbers per taxon collected from respective samples. Final column indicates whether an identified taxon was recorded in reaches upstream of the treatment zone

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