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PRIMARY RESEARCH PAPER

Rapid recovery of macroinvertebrates in a South African stream treated with rotenone

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Abstract South Africa's Cape Fold Ecoregion supports a unique freshwater fish assemblage with many endemics. To mitigate impacts of alien invasive fishes on this unique assemblage, nature conservation authority CapeNature used rotenone to remove smallmouth bass (*Micropterus dolomieu*) from the Rondegat River. We investigated whether the rotenone treatments had an adverse impact on the aquatic macroinvertebrate community over the long-term, the first study of its kind in Africa. We monitored macroinvertebrates within treated and untreated (control) sites on multiple sampling events for 2 years before and 2 years after two rotenone treatments. We analysed the difference in invertebrate abundance between treatment and control sites before and after treatment, using generalised linear mixed models with sampling event as a random factor to partition out natural fluctuations in abundances over time. Populations fluctuated widely in control and treatment sites over the study period, and we found no effect that could be clearly attributed to rotenone. We conclude that macroinvertebrates recovered rapidly after treatment, probably through drift from untreated areas upstream, with no long-term adverse effects. We recommend that the presence of uninvaded upstream refuges that may provide demographic rescue be used

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as a key discriminating factor for future conservation purposed rotenone deployments.

Keywords River management · Ecological resilience · Alien fish removal · Non-target effects · Biological monitoring

Introduction

The Cape Fold Ecoregion (CFE, sensu Abell et al., 2008, largely coincident with the Cape Floristic Region, e.g. Weyl et al., 2014) of South Africa is a centre of endemism for freshwater invertebrates, amphibians and fishes (Skelton et al., 1995; Wishart & Day, 2002; Darwall et al., 2009). Non-native fish are recognised as the most significant threat to the long-term survival of indigenous fresh water fish assemblages in the CFE (Tweddle et al., 2009). CapeNature, the conservation authority for the Western Cape Province, is currently using the piscicide rotenone to remove non-native fish from invaded river reaches identified as critical habitat for indigenous fishes (Marr et al., 2012; Weyl et al., 2014). Rotenone is commonly used in North America and Europe to manage undesirable fish populations (Finlayson et al., 2010, 2018). However, the use of rotenone should be weighed against its negative effects on non-target taxa and the broader ecosystem (Vinson et al., 2010). Careful planning before the deployment of rotenone as a conservation tool is essential as non-native fishes support economically valuable recreational fisheries in South Africa (Ellender et al., 2014) that often promote local river stewardship and conservation. Therefore, CapeNature conducted an Environmental Impact Assessment and pilot studies to determine the feasibility and efficiency of using rotenone to manage non-native fish, to monitor the recovery of the native fauna and to assess possible negative consequences (Marr et al., 2012; Impson et al., 2013).

The Rondegat River was the pilot study site, making it the first river in South Africa where a conservation authority used rotenone to remove an invasive fish species (Weyl et al., 2014). Four native fish species are present in the Rondegat River system (Weyl et al., 2014), namely Clanwilliam redfin *Sedercypris calidus* (Barnard 1938), Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist & Thompson 1913), Clanwilliam rock catfish *Austroglanis gilli* (Barnard 1943) and fiery redfin *Pseudobarbus phlegethon* (Barnard 1938). Smallmouth bass *Micropterus dolomieu* Lacepède 1802 had invaded the lower reaches and extirpated native fish species to the extent that the Clanwilliam redfin, fiery redfin and juvenile Clanwilliam yellowfish were restricted to the upper river reaches above the Rooidraai waterfall, which acted as an invasion barrier to smallmouth bass (Woodford et al., 2005; Weyl et al., 2014). Rotenone was applied just below the waterfall in February 2012 and March 2013, following standard operating procedures (Finlayson et al., 2010; Weyl et al., 2014). Smallmouth bass were successfully removed, followed by a rapid recolonization by all four native fish species; reinvasion by bass is prevented by migration barriers below the treated reach (Weyl et al., 2014).

In addition to the current study, monitoring for long-term impacts of rotenone on the macroinvertebrate assemblages of the Rondegat River, studies also examined the effect of rotenone on aquatic macroinvertebrates in the short-term, while an independent study monitored the recovery of fish populations, to evaluate negative impacts on non-target taxa and general ecosystem health (Woodford et al., 2013; Bellingan et al., 2015; Weyl et al., 2014). Aquatic macroinvertebrates are widely used to detect changes in water quality and are suitable for rapid impact assessments because they are sensitive to environmental perturbations and easy to collect and identify to an adequate taxonomic level (Reynoldson & Metcalfe-Smith, 1992; Resh & Jackson, 1993; Ollis et al., 2006). Certain macroinvertebrate taxa of the orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), are more susceptible to water quality changes than others, and Ephemeroptera are particularly sensitive to rotenone (Mangum & Madrigal, 1999; Whelan, 2002; Finlayson et al., 2009; Vinson et al., 2010; Booth et al., 2015; Dalu et al., 2015). Woodford et al. (2013) observed a reduced richness and density of macroinvertebrate species coupled with mass drift events immediately after rotenone applications in the Rondegat River, especially for Ephemeroptera, whereas Bellingan et al., (2015), using a rapid bioassessment scoring system, observed the medium-term loss of some sensitive taxa from the Ephemeroptera, Plecoptera and Trichoptera orders.

Toxicity data for fish and aquatic macroinvertebrates from Marking & Bills (1976), Chandler & Marking (1982) and Finlayson et al. (2009) suggest that some invertebrates are as sensitive to rotenone as fish are. Thus, using rotenone for fish eradication is likely to produce comparable impacts on invertebrates. Effects persisting for up to 1 year after application have been reported (Hamilton et al., 2009; Vinson et al., 2010). Conversely, the short-term assessments by Woodford et al. (2013) and Bellingan et al. (2015) indicated that macroinvertebrates could rapidly recolonise rotenone-treated Rondegat River reaches from untreated upstream reaches within a year of treatment. This indicates that longer term monitoring of macroinvertebrate populations is necessary to distinguish between natural fluctuations in macroinvertebrate populations and to contextualise short-term impacts of rotenone on the macroinvertebrate assemblage.

We aimed to examine whether a negative effect of rotenone on aquatic macroinvertebrate abundance and diversity would be detected over the longer term. We tested this with macroinvertebrates sampled periodically over the course of 2 years prior to the first rotenone treatment, to 2 years after the second rotenone treatment. Using an adapted Before-and-After Control-and-Impact (BACI) experimental design (cf. Underwood, 1994), we could capture the natural variation in invertebrate abundance and diversity and distinguish effects that could be attributed to rotenone treatment. We hypothesised that a recovery in macroinvertebrate density, or abundance, and diversity, comparable to pre-treatment conditions would be observed within the time limit of the study period.

Methods

Study area, monitoring sites and sampling methods

CapeNature applied rotenone to the 4 km-long stretch of the Rondegat River below the Rooidraai waterfall invasion barrier in February 2012 and March 2013 (Fig. 1; Slabbert et al., 2014; Weyl et al., 2014). To study the impact of rotenone on the macroinvertebrate community of the Rondegat River, we selected three sampling sites in the control reach above the waterfall that had never been invaded, and three sampling sites in the invaded treatment reach below the natural waterfall barrier (Fig. 1). Sampling sites comprised approximately 20 m of river length and were selected to be similar in terms of proportion of suitable habitat available for sampling of macroinvertebrates, to facilitate comparisons of invertebrate assemblages before, during and after rotenone treatment (Table S1; Underwood, 1994; Underwood & Chapman, 2003). Sampling sites in the treatment reach were located at least 200 m downstream of the nearest rotenone application point, to ensure that the rotenone passing through each site was as evenly mixed with the river water as possible.

Sampling events were carried out on multiple occasions between May 2010, approximately 22 months before the first rotenone treatment, and February 2015, approximately 23 months after the second rotenone treatment, to capture natural variation in macroinvertebrate populations that were not related to rotenone. This comprised four sampling events before the first rotenone treatment, four events between the two treatments and five events after the second treatment. All sampling sites were sampled at each sampling event, except for May 2010 and March 2012, when flooding and logistical constraints prevented sampling the control sites (see Woodford et al., 2013 and Bellingan et al., 2015). The treatment and control sites were sampled across 3 days, working in a downstream to upstream order (T1–T3; C1–C3; Fig. 1).

Two methods were used to sample macroinvertebrates. First, four stones were randomly sampled from each of the six sampling sites at each sampling event. Invertebrates on each stone were carefully removed by visual inspection of the stone, and thereafter scrubbing the entire stone surface (Wrona et al., 1986; Woodford et al., 2013; Bellingan et al., 2015). To estimate a stone's surface area, the stone was measured across the three longest orthogonal axes (X, Y and Z) to the nearest millimetre, and the measurements used in the following equation (Graham et al., 1988):

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

Second, we collected kick samples following the SASS5 (South African Scoring System, version 5) method (Dickens & Graham, 2002), an ISO-certified protocol that is commonly used in South Africa to

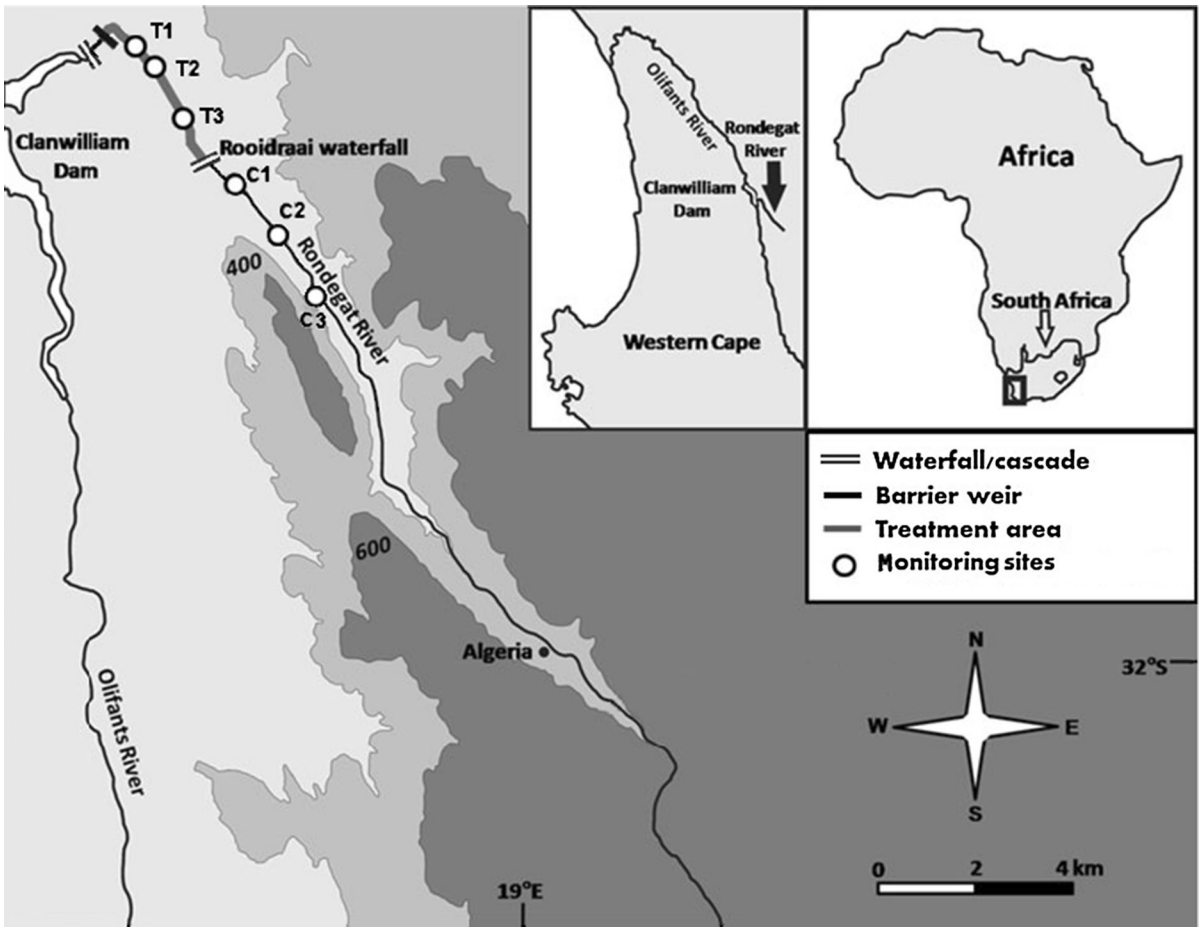

Fig. 1 The Rondegat River, Western Cape Province, South Africa, showing the location of monitoring sites in the control (C1–C3) and treatment (T1–T3) reaches (Modified from Woodford et al., 2013). Darker shades in the main map indicate higher altitudes

rapidly determine macroinvertebrate assemblage response to changes in water quality. Briefly, the SASS5 method consists of sampling three primary instream biotopes: stones-in-current (SIC), marginal vegetation (MV) and gravel/sand/mud (GSM). These were targeted within the 20 m reach of each monitoring site. Kick sampling was carried out for the SIC biotope for 2 min, and for 1 min in the GSM biotope, and marginal vegetation was sampled along 2 m of stream bank. All sampling was performed using a standard SASS5 kick net ($30 \times 30 \times 50$ cm^3 frame; 1 mm mesh size) (Dickens & Graham, 2002; Woodford et al., 2013; Bellingan et al., 2015). All collected macroinvertebrates were preserved on site using 95% ethanol and returned to the laboratory for counting and identification to morphospecies level.

For each monitoring event at each site, we measured environmental variables including temperature, conductivity and dissolved oxygen with an Aqualytic AL15 hand-held water quality meter (Table S1). The stream's profile perpendicular to the bank was characterised near the upstream limit, middle and downstream limit of each site (i.e. three replicates) by measuring the stream's width and its depth at five points evenly spaced along each width. From these data, the surface area, average width, average depth and volume of each site were estimated (Table S1).

Rotenone dose concentration during treatments was monitored by independent studies for both applications. Target concentrations of 50 µg l^{-1} and 37.5 µg l^{-1} were used for the 2012 and 2013

treatments respectively (Jordaan & Weyl, 2013; Slabbert et al., 2014).

Analyses

For both stones and kick samples, we compared macroinvertebrate abundance (density) and Shannon diversity between control and treatment sites at three treatment phases, i.e. before rotenone application, between rotenone applications and after rotenone application. We considered all taxa together, as well as only those taxa known to be especially sensitive to rotenone and water quality, namely, Ephemeroptera, Plecoptera and Trichoptera (EPT taxa; Vinson et al., 2010; Bellingan et al., 2015; Booth et al., 2015). Additionally, we observed a strong correlation between larval chironomids and total abundance (Pearson's $R = 0.85$) in stone samples; therefore, larval chironomids may have a disproportionate influence on patterns of variation in total abundance and may obscure variation in abundance of other taxa. Therefore, we analysed larval chironomids separately and excluded the taxon from total abundance.

We used generalised linear mixed effects models (GLMM) on the unpooled samples with sampling site and sampling event as random effects to account for natural population fluctuations among sampling sites and over time and to avoid pseudoreplication (R package 'lme4', Bates et al., 2015). Unlike a classical repeated-measures Analysis of Variance (ANOVA, Underwood, 1994), GLMM can handle non-normal and discrete data, as well as the unbalanced design and missing samples that were present in our data without omitting incomplete cases (Nelder & Wedderburn, 1972; Lindstrom & Bates, 1988). Treatment phase, the main effect, was examined as a categorical fixed effect with six levels, i.e. before, between and after rotenone for control, and before, between and after rotenone treatment. In a GLMM, this is almost identical to a control/treatment predictor and a before/between/after predictor with their interaction effect; however, it is easier to apply post hoc tests to one predictor without an interaction effect. We included the environmental variables temperature, dissolved oxygen, average pool depth and average pool surface area (Table S1) as fixed effects to examine the influence of environmental variation. Likelihood Ratio-based χ^2 test statistics and P-values for each predictor were obtained with parametric bootstrapping, by comparing models with and without each relevant predictor. In this way, output equivalent to Type II ANOVA was obtained from the mixed models, where each term is adjusted for all other terms (Langsrud, 2003). We then used Tukey's Honestly Significant Difference (HSD) post hoc test to specifically examine differences between the control and test sites within each rotenone treatment phase (R package 'multcomp', Hothorn et al., 2008). We used the negative binomial distribution for all models with discrete values (i.e. abundances) as a response variable, as it was a consistently better fit to the overdispersed data compared to models based on the Poisson distribution. For example, Bayesian information criterion (BIC; lower is better) values for total macroinvertebrate abundance on the stones samples was 9344.2 for the Poisson model and 2881.1 for the negative binomial model. We used the Gaussian distribution for models with the Shannon diversity or the equivalent species number (Jost, 2006) as a response variable. Stone area reflects sampling effort in the stone samples, therefore stone area was analysed as an offset term.

Results

The 212 kick samples collected produced 144 macroinvertebrate species from 122 genera, 60 families and 15 orders (Table S2), whereas the 288 stone samples produced 83 species of invertebrate from 72 genera, 41 families and 13 orders (Table S3). The two sampling methods shared 74 species, 9 taxa were unique to the stone samples, and 70 were unique to the kick samples, with 153 morphospecies in total. Temperature, dissolved oxygen, depth and surface area were not significant predictors of macroinvertebrate diversity or abundance for stone or kick samples after controlling for sampling event and sampling site, and models with these environmental variables were not presented in Table 1 for the sake of brevity. Residual plots indicated that the models presented in Table 1 were appropriate for the data (Figure S1).

For both sampling methods, macroinvertebrate abundance varied widely among sampling events for control and treatment sites, and sampling event is a significant predictor in all models (ANOVA output, Table 1). For stone samples, after controlling for the influence of sampling site and event, no significant differences were found between control and treatment

Table 1 Generalised linear mixed models (GLMM) were used to examine the relationship between macroinvertebrate abundance and Shannon diversity, and treatment phase (before, between and after rotenone for control and treatment sites) with sampling event and site as random effects, for both the stones and the kick sampling methods

Sampling method	Response variable	Sampling event		Sampling site		Treatment phase		BIC	Null BIC
		χ^2	P	χ^2	P	χ^2	P		
Stones	Abundance	28.037	**< 0.001**	8.743	**< 0.001**	6.031	0.410	2881.1	2858.8
Stones	Larval chironomids	72.889	**< 0.001**	1.166	0.055	32.253	**< 0.001**	2637.8	2641.7
Stones	EPT abundance	13.089	**< 0.001**	7.089	**< 0.001**	14.893	**0.027**	2584.6	2571.1
Kick	Abundance	36.620	**< 0.001**	4.348	**0.003**	7.383	0.276	2407.4	2388
Kick	EPT abundance	43.161	**< 0.001**	6.185	**0.002**	10.755	0.097	2664.6	2648.5
Kick	Shannon diversity	22.415	**< 0.001**	0.032	0.342	−0.99	0.085	331.88	315.31
Kick	EPT Shannon diversity	15.838	**< 0.001**	1.331	0.099	−2.436	0.110	303.76	286.06

The GLMM models were used to produce a Type II ANOVA with bootstrapped likelihood ratio Chi-squared (χ^2) values and P-values. Numerator degrees of freedom were 5 for all models, stone samples were based on 288 observations and kick samples on 212 observations. Bayesian Information Criterion (BIC) values for the full model and the null model that excluded treatment phase is supplied, to further evaluate the effect of treatment phase while taking into account random site and time effects. Probability values in bold indicate significant differences between the response variable and sampling event, sampling site and treatment phase, respectively, for the two sampling methods employed

reach for macroinvertebrate abundance excluding larval chironomids (Table 1, Fig. 2). Larval chironomid abundance was significantly lower along the treatment reach compared to the control reach throughout the sampling period, and this difference was greater after treatment (Fig. 2). Treatment phase was a significant but weak predictor of EPT taxon abundance from stone samples (Table 1); we found no significant difference when the treatment and control reaches were compared within each particular treatment phase (Fig. 2). For kick samples, after controlling for the influence of sampling site and event, no significant differences were found between control and treatment reach for total macroinvertebrate abundance or for the abundance of EPT taxa (Table 1, Fig. 3).

As kick sampling produced many more invertebrate morphospecies than stone sampling, we decided that kick samples were better for examining biodiversity; therefore, we present the results of the Shannon diversity analysis based on the kick samples only. Although Shannon diversity varied widely over time and sampling event was a significant predictor, we found no significant effect of sampling site, and no significant difference between control and treatment reach before, between or after rotenone treatment (Table 1, Fig. 4). The analysis of equivalent species numbers derived from the Shannon index (Jost, 2006) gave the same result and are not reported further here.

Discussion

The Rondegat River pilot project was considered a conservation success, given that it substantially increased the habitat and distribution of Clanwilliam redfin and fiery redfin minnows, and the suitable juvenile habitat for Clanwilliam yellowfish (Weyl et al., 2014), despite some short-term negative effects for sensitive macroinvertebrate taxa (Woodford et al., 2013; Bellingan et al., 2015). Concerns about aquatic macroinvertebrates' responses to rotenone treatment are valid because macroinvertebrates are considered to be good indicators of water quality and ecosystem health (Dickens & Graham, 2002). In particular, CFE rivers have high macroinvertebrate endemicity that is threatened by human activities and introduced fish and plants (de Moor & Day, 2013).

There is a paucity of studies on the long-term effects of rotenone on aquatic macroinvertebrates, and those studies that do exist reported negative effects up to a year after application (Vinson et al., 2010). Whelan (2002) noted that short-term impacts on macroinvertebrates, attributable to rotenone treatment, occurred 1 year after treatment, but the observed impacts were indiscernible by the 2nd year after treatment. In the current study, sustained monitoring captured natural variation in macroinvertebrate abundance and diversity over time in both the control and the treatment sites, with no decreases clearly

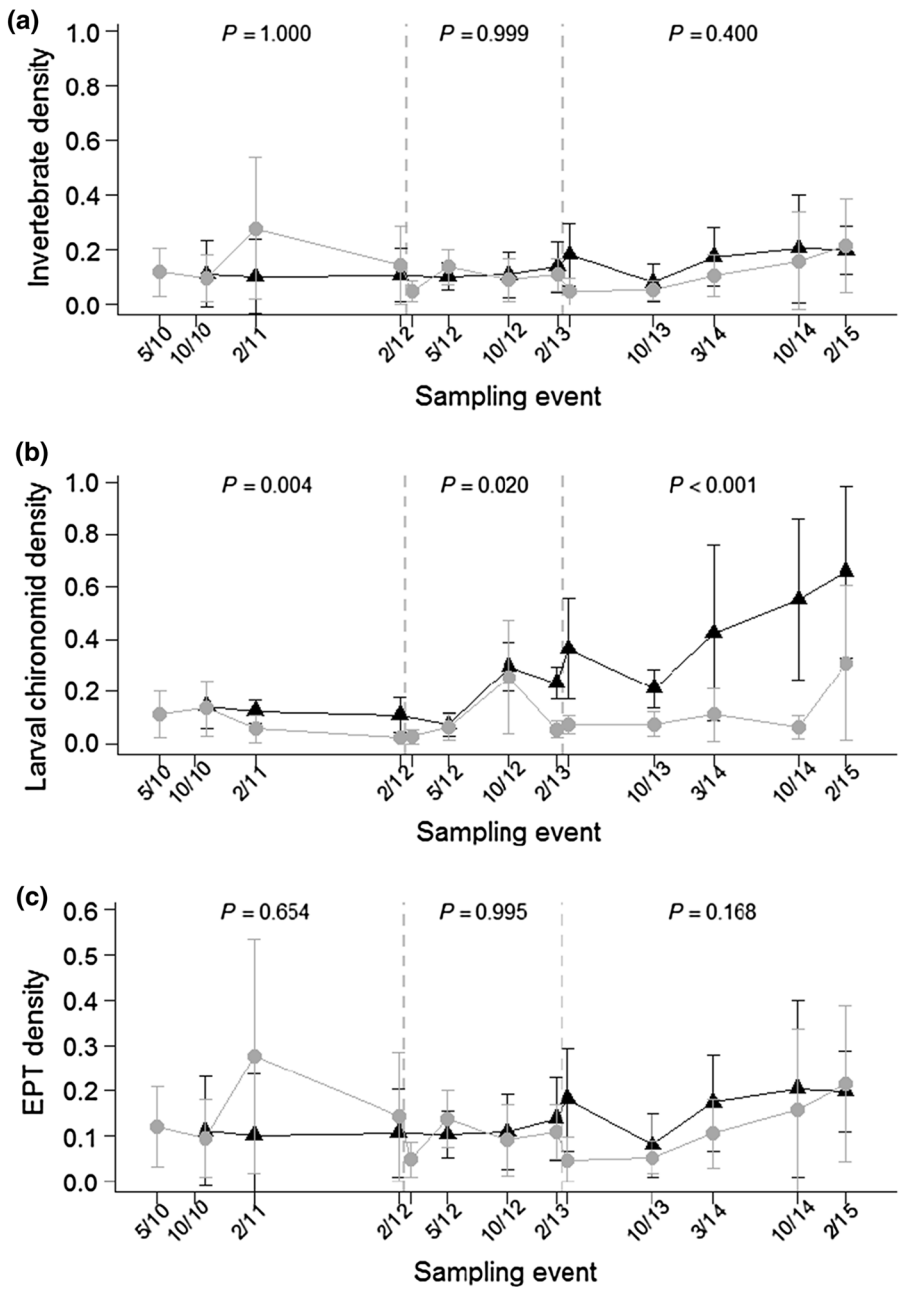

Fig. 2 Variation in invertebrate abundance per unit area of stone surface (species density) collected through the stone sampling method across the 5-year sampling period. Mean and standard deviation is given for each sampling event (month/year) from the control reach (black) and treatment reach (grey), for **a** invertebrate density without larval chironomids, **b** larval chironomid abundance only and **c** abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT). The dashed lines represent rotenone application events. *P*-values represent Tukey HSD pairwise post hoc tests from the mixed models reported in Table 1, comparing treatment and control sites within each sampling phase

attributable to rotenone. In particular, EPT taxa that were demonstrably sensitive to rotenone on an individual basis (Booth et al., 2015; Dalu et al., 2015) were extremely resilient from a population basis, returning to pre-treatment levels along the treatment zone within a single year. Larval chironomid abundance increased beyond the pre-treatment baseline in the control reach after rotenone treatment without a matching increase in the treatment reach (October 2014; Fig. 2). High abundances of chironomid larvae may be indicators of lower oxygen concentrations and increased organic input (cf. Dickens & Graham, 2002); such conditions along the control reach may be associated with effects of an alien riparian vegetation removal intervention (Impson et al., 2013; Fig. 2a, b) that may promote insolated, warmer water and runoff bearing sediment

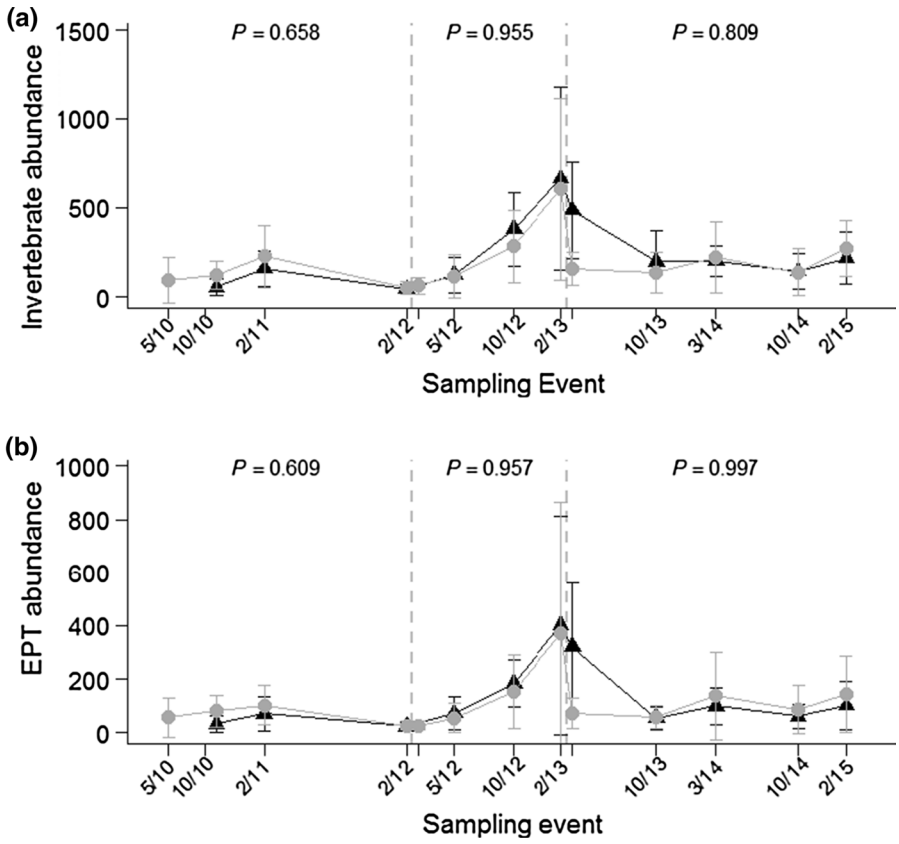

Fig. 3 Variation in invertebrate abundance (density) collected through the kick sampling method across the five-year sampling period. Mean and standard deviation are given for each sampling event (month/year) from the control reach (black) and treatment (grey), for **a** invertebrate abundance, and **b** abundance of EPT taxa. The dashed lines represent rotenone application events. *P*-values represent Tukey HSD pairwise post hoc tests from the mixed models reported in Table 1, comparing treatment and control sites within each sampling phase

and leaf litter, providing favourable instream substratum for Chironomidae and other taxa that take advantage of instream disturbance (see Samways et al., 2011). There is some evidence in Fig. 2b that the boom in chironomid density may be progressing longitudinally downstream. The ability of the samples to reflect such ecological changes suggests a validation of the method as a tool for monitoring.

All of these observations are reasonable because the treatment reach could be recolonised by immature aquatic macroinvertebrates from upstream in the same manner as the native fish have (Weyl et al., 2014), or by their aerially-dispersing, winged adult stage. The treatment and control sites are therefore not biologically or statistically independent as a classical BACI experimental design requires (Underwood, 1996). To generalise this case study would require including sites in several rivers, but this is not possible here because the study was dependent on the CapeNature rotenone pilot project in the Rondegat River. However, it is important to be able to test empirically if macroinvertebrate communities can recover through colonisation from upstream as fishes have been demonstrated to do (Weyl et al., 2014).

Ultimately, this research confirms the previous findings of Woodford et al. (2013) and Bellingan et al. (2015) that macroinvertebrate communities in the CFE are resilient in the long-term to rotenone operations using rotenone concentrations designed to ensure extirpation of smallmouth bass populations (Jordaan & Weyl, 2013). If higher concentrations of active piscicide were to be applied, such as in rivers invaded by African sharptooth catfish (Jordaan et al., 2017), *Clarias gariepinus* (Burchell 1822), the macroinvertebrate community may prove to be less resilient, particularly if key sensitive taxa do not also occur in upstream refugia. Slabbert et al. (2014) found that macroinvertebrate fauna of the Rondegat River were exposed to rotenone for a minimum of 6 h at a minimum dose concentration of 12.5 µg l^{-1} and a maximum dose concentration of 39.2 µg l^{-1}. This dose concentration exposure would be expected to elicit a variable response across differing macroinvertebrate groups (see Woodford et al., 2013). Following

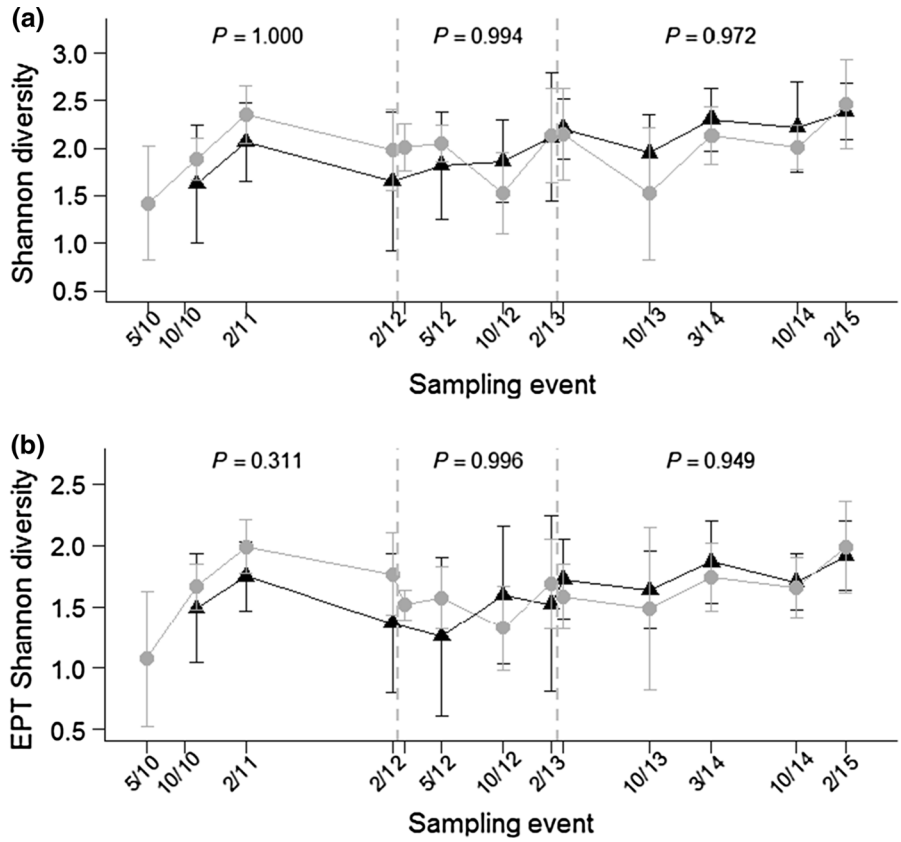

Fig. 4 Variation in Shannon diversity of invertebrates collected through the kick sampling method across the 5-year sampling period. Mean and standard deviation are given for each sampling event (month/year) from the control reach (black) and treatment (grey), for **a** invertebrate diversity, and **b** diversity of EPT taxa. The dashed lines represent rotenone application events. *P*-values represent Tukey HSD pairwise post hoc tests from the mixed models reported in Table 1, comparing treatment and control sites within each sampling phase

experimental sensitivity tests on groups carried out by Dalu et al. (2015), 50% mortality would be expected from the minnow mayfly *Baetis harrisonii* Barnard, 1932 (Baetidae: Ephemeroptera) at the highest concentration observed by Slabbert et al. (2014), for a 6 h exposure duration. For the same (highest) exposure period and concentration, less than 20% mortality would be expected for *Anax imperator* Leach, 1851 (Aeshnidae: Odonata) and 0% mortality for *Potamnautes sydneyi* (Rathbun, 1904) (Potamonautidae: Decapoda), with the three macroinvertebrate species mentioned above each occurring in the Rondegat River (Fig. 2, Dalu et al., 2015).

Therefore, in agreement with what is recommended by Finlayson et al. (2009), for stream treatments in the CFE we recommend that the lowest effective dose concentration of rotenone be used. We also recommend that the existence of uninvaded upstream refuge reaches that can provide demographic rescue to treated reaches should be made a key discriminating factor when deciding whether or not to deploy rotenone for conservation purposes.

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