An Assessment of the Effect of Rotenone on Selected Non-Target Aquatic Fauna

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Abstract

Rotenone, a naturally occurring ketone, is widely employed for the management of invasive fish species. The use of rotenone poses serious challenges to conservation practitioners due to its impacts on non-target organisms including amphibians and macroinvertebrates. Using laboratory studies, we investigated the effects of different rotenone concentrations (0, 12.5, 25, 37.5, 50, 100 μg L−1) on selected invertebrate groups; Aeshnidae, Belostomatids, Decapods, Ephemeroptera, Pulmonata and zooplankton over a period of 18 hours. Based on field observations and body size, we hypothesized that Ephemeropterans and zooplankton would be more susceptible to rotenone than Decapods, Belostomatids and snails. Experimental results supported this hypothesis and mortality and behaviour effects varied considerably between taxa, ranging from no effect (crab Potamonuates sidneyi) to 100% mortality (Daphnia pulex and Paradiaptomus lamellatus). Planktonic invertebrates were particularly sensitive to rotenone even at very low concentrations. Future research should investigate the recovery time of invertebrate communities after the application of rotenone and conduct field assessments assessing the longer term effects of rotenone exposure on the population dynamics of those less sensitive organisms.

Introduction

Globally, biological invasions are becoming increasingly problematic and are considered potential drivers of biodiversity loss and in some instances are associated with economic threats [1, 2, 3]. Conservation practitioners are increasingly implementing better methods to control the spread of invasive species, particularly in ecologically sensitive areas or areas of high conservation priority [4, 5, 6]. In freshwater ecosystems, biodiversity declines are of particular concern as these environments are considered to be among some of the most threatened systems, with invasions contributing to their deterioration [2, 7]. Non-native fishes contribute considerably in this regard [8]. For the control of non-native fishes, eradication is often considered an option due to the effectiveness of available piscicides [9]. Rotenone, a naturally
occurring ketone, is one such piscicide which has been successfully used for fish biocontrol around the world [6, 9–11].

Rotenone is derived from the roots of plants belonging to the family *Leguminosae* including the jewel vine (*Derris* spp.) and lacepod (*Lonchocarpus* spp.), that grow in Oceania, Central and South America and south-east Asia [12, 13]. Over the last 150 years it has also been used extensively as a commercial insecticide to deter slugs and snails from garden vegetables [12] and for more than 70 years, rotenone has been an important tool both for recreational fisheries management and, more recently, for the restoration of native fish species [6, 9, 10, 14, 15].

The use of rotenone, however, poses a challenge to conservation practitioners because detrimental impacts to non-target freshwater organisms such as amphibians [16] and invertebrates [17] have been reported. Impacts on aquatic invertebrates tend to be highly variable and taxon-specific, making the environmental impacts of proposed rotenone operations difficult to predict [17]. Woodford et al. [18] for example, observed that the immediate impact of rotenone operations on the Rondegat River, South Africa, appeared to have been most severe on the Ephemeropterans, which were among the quickest to respond to rotenone in the water through mass drift and declined significantly in abundance following treatment. While it is recognized that rotenone is likely to affect numerous aquatic taxa, there are few studies that have assessed such impacts [15–18] and as a result the use of rotenone remains contentious as its effect on non-target biota are still largely unknown [6, 9, 11, 17, 19–21].

In headwater streams in South Africa’s Cape Floristic Region, an area of high freshwater fish and invertebrate biodiversity and endemism [22], the primary threat to aquatic biota in the region is considered to be predation by and competition with non-native fish species [6, 23]. While non-native fish eradication using rotenone is currently considered the most appropriate conservation intervention [6, 24], the public as well as the Department of Water Affairs (the South African regulatory authority) have expressed concern on the potential impact of river treatments on non-target organisms (see [6]). These concerns have resulted in delays in the approval of rotenone treatments of several rivers and off-channel impoundments (N.D. Impson, Scientist, CapeNature). To provide guidance for the use of rotenone for future interventions native fish restoration projects [6], the response of insect communities following rotenone treatments are being monitored [18, 25]. A major constraint in applying the results of a field study to predict impacts on other systems, is that treatment concentrations differ between fish species [26] and there are few studies describing the effects of rotenone on invertebrates [10, 15, 27]. Further, laboratory studies typically use exposure durations that are substantially longer than those used in rotenone treatments [27]. This leads to considerable uncertainty regarding taxon-specific effects and susceptibility of invertebrates to rotenone in different habitats [9, 11, 19, 28].

For this reason, we assessed the short-term responses of rotenone exposure on selected aquatic invertebrates, using concentrations and exposure durations typically used in river treatments [29, 30]. To the best of our knowledge, the effect of rotenone concentration on invertebrate taxa has only been assessed to a small extent (see [15]) using field application protocol treatments and exposure times in the laboratory. Our experimental design followed standard piscicide application protocols [12, 15, 29, 30] and this resulted in a unique opportunity to compare the results from short-term exposure experiments to field observations i.e. the Rondegat River. Field studies conducted before and after rotenone treatment in many parts of the world have noted a decline in some members of the macroinvertebrate community [9, 11, 13, 19–21, 25]. Observations in Rondegat River, during rotenone treatments also indicated that other invertebrates, such as *Potamonuates sidneyi*, appeared to be unaffected and were observed feeding on fish that had succumbed to the rotenone during the treatment (Fig 1). Therefore, our aim was to experimentally determine the effects of rotenone exposure on
representative aquatic insect, crustacean and gastropod taxa. We hypothesized that 1.) based on field observations, Ephemeropterans would be more susceptible to rotenone than Aeshnids, Decapods, Belostomatids and snails [18, 25], and 2.) Copepods, Ostracods and Daphniids would be particularly susceptible given their low tolerance for environmental toxicants [31–33].

Material and Methods
Aquatic invertebrates sampling
Collection permits were obtained from Eastern Cape Department of Economic Development and Environmental Affairs (DEDEA permit no. CRO 3/12CR, CRO4/12CR, CRO 12/14CR and CRO 13/14CR) and ethical clearance was obtained from the South African Institute for Aquatic Biodiversity (reference no. 2014/01). Representative species of key selected taxa were collected from natural (i.e. unimpacted) freshwater environments of the Eastern Cape of South Africa. These representatives included the freshwater crab Potamonuates sidneyi (Decapoda, size range 1–1.5 cm), diving beetle Diplonychus capensis (Belostomatidae, size range 0.8–1 cm), mayfly Baetis harrisonii (Ephemeroptera, size range 0.7–0.9 cm), dragon fly Anax imperator (Aeshnidae, size range 2–2.4 cm), freshwater snail Physa acuta (Physidae, size range 0.4–0.7 cm), Cypricercus sp. (Ostracoda, size range 0.2–0.4 cm), Paradiaptomus lamellatus (Copepoda, size range 0.3–0.4 cm) and water flea Daphnia pulex (Cladocera size range 0.1–0.2 cm). Crabs, diving beetles, dragon flies, mayflies and snails were collected using standard SASS kick nets. Ostracods, copepods and water flea were collected by towing a zooplankton net (50 cm Ø, 64 μm mesh) through the water column. All collected invertebrates were sorted in the field and placed in separate labelled buckets for transportation back to the laboratory. Water was
collected from the sampling locations for the invertebrate holding tanks and for conducting the experiments. All animals were acclimatized at 20 ± 0.5°C for a period of 48 hours under constant aeration, prior to experimentation under a 24 h light photoperiod cycle in temperature controlled environmental rooms.

**Experimental design**

The Organisation for Economic Co-operation and Development (OECD) guidelines for acute toxicity studies were followed for the experimental procedures, with four replicates \((n = 4)\) run for each invertebrate group at six rotenone concentrations \([34]\). Within each replicate, 5 individuals were placed in each experimental container. This was done for all experimental taxa except for *P. sidneyi*, which were observed to interact aggressively (fighting, killing and cannibalism) when multiple individuals were placed in single containers during preliminary trials. To avoid confounding effects from deaths not related to rotenone exposures, *P. sidneyi* were individually housed within each container, with 10 replicates employed for each of the experimental rotenone concentrations.

A fresh stock solution of 0.150 mg rotenone L\(^{-1}\) was prepared on the day of each experimental trial from the commercial piscicide CFT Legumine \(^{8}\) (5% active rotenone) which is registered in the United States (EPA Registration number 75338–2). This solution was prepared and diluted using filtered (through a 20 μm mesh sieve) water from the sample location to make up the five treatment concentrations: 12.5, 25, 37.5, 50 and 100 μg L\(^{-1}\). In addition, a no rotenone (0 μg L\(^{-1}\)) treatment was employed using the same filtered water which served as a control. The experimental design included rotenone treatment concentrations (50 and 37.5 μg L\(^{-1}\)) and duration (6-hour) used during the smallmouth bass *Micropterus dolomieu* eradication in the Rondegat River South Africa \([25, 26, 30]\). Additional concentrations (12.5–100 μg L\(^{-1}\)) were included based on recommended concentrations for more susceptible (e.g. rainbow trout *Oncorhynchus mykiss*) and more tolerant fish species such as carp (*Cyprinus carpio*) and bullheads (*Ameiurus spp.*) by Finlayson et al., \([29]\). For each taxon, individuals were placed in a glass container filled with 500 ml of each rotenone concentration, receiving no supplementary aeration during the experiment \([34]\). After introductions of the taxa into the rotenone solution, invertebrates were observed every hour for the first 6 hrs, and again after 18 hrs, at which point the experiment was terminated. Mortality, defined as the cessation or absence of movement after repeated tactile stimulation/prodding \([27]\), was recorded after each observation period. A number of behavioural traits associated with rotenone toxicity, depending on the invertebrate taxa, were also assessed at each time interval, such as loss of equilibrium, location in the jar (surface, middle, bottom, position on glass surface), swimming and cessation of movement (i.e. death).

**Data analysis**

To test for time and treatment effects on mortality rates for the selected invertebrate groups we employed a 2×2 Permutation Analysis of Variance (PERMANOVA; \([35]\)), based on Euclidean dissimilarities as a distance measure, using rotenone concentration and time as factors. Using this analysis, differences in mortality were assessed at the treatment, time, and the treatment × time level (interactions between treatment and time). This analysis was conducted using PRIMER v6 add-on package PERMANOVA+ \([36]\), using 9999 permutations \([37]\), with significant terms investigated using a posteriori pair-wise comparisons with the PERMANOVA \(t\) statistic \([36]\).

The dependence of the distribution of individuals displaying behavioural traits associated with a 6 hour exposure (representing field exposure) to different rotenone concentrations (0, 12.5, 25, 37.5, 50 and 100 μg L\(^{-1}\)) was tested for *A. imperator*, *B. harrisonii*, *Cypricercus sp.*, *D.
capensis and P. acuta using $\chi^2$ contingency tables in Microsoft Excel 2007. For this test, 6 concentrations × 3 traits (B. harrisonii, Diplonychus capensis and Anax imperator) and 6 concentrations × 4 traits (P. acuta, and Cypricercus sp.) were used for the cross-tabulations (see S1 Table).

**Results**

No mortalities were observed in the control treatment for any of the taxa (S1 Table). Paradiapottomus lamellatus and D. pulex were the most rapidly affected species, as all died within the first hour of exposure at each concentration (Fig 2b and 2d). For the remaining groups (Fig 2), exposure time and concentration became more important given that there were significant effects of both variables and interactions between the variables on mortality, with the exception of P. acuta at the Concentration × Time level and D. capensis, where Time and Concentration × Time had no significant effect (Table 1). For P. sidneyi, no mortalities were observed at any of the rotenone concentrations (Fig 2i). There were significant differences ($p < 0.05$) in the rate of mortality in most invertebrate groups across different rotenone concentrations (Table 1). The dragonflies A. imperator, B. harrisonii, Cypricercus sp. and P. acuta mortality rates were found to differ among time and concentrations ($p$-perm < 0.05), while the combined effect of concentration and time was found to have a significant effect on A. imperator, B. harrisonii and Cypricercus sp. mortality rates (Table 1). Using pairwise comparisons, mortality rates was found to differ significantly ($p$-perm < 0.05) between the two time intervals, 6 and 18 hours for selected invertebrates, excluding D. capensis, which had similar mortality rates ($p$-perm = 1; S2 Table). Pairwise comparisons for A. imperator, B. harrisonii, Cypricercus sp., D. capensis and P. acuta at different concentrations and time intervals are highlighted in the S2 Table. Anax imperator mortality rates at 0 μg L$^{-1}$ was found to differ with all concentrations, while other concentrations were not significantly different across concentrations using pairwise comparisons (S2 Table).

Invertebrate behaviour was affected by rotenone concentration and exposure time (Fig 3, S1 Table). A graphical illustration of the proportion of invertebrates exhibiting each behavioural trait (e.g. loss of equilibrium, location in the jar, swimming and cessation of movement) recorded hourly at control (0 μg L$^{-1}$) and field rotenone concentration (37.5 μg L$^{-1}$) are presented in Fig 3.

Chi-square contingency table comparisons at the six hour interval demonstrated that the distribution of individuals exhibiting each behavioural trait was independent of rotenone concentration for A. imperator ($\chi^2 = 5.201$, df = 10, $p = 0.877$), Cypricercus sp. ($\chi^2 = 5.201$, df = 10, $p = 0.877$), D. capensis ($\chi^2 = 2.668$, df = 10, $p = 0.988$) and P. acuta ($\chi^2 = 8.476$, df = 15, $p = 0.903$). The behaviour of B. harrisonii, was however dependent on rotenone concentration ($\chi^2 = 20.84$, df = 10, $p = 0.02$).

**Discussion**

The toxicity of rotenone to invertebrates varied considerably among taxa ranging from no effect (P. sidneyi) to 100% mortality even at low concentrations (D. pulex and P. lamellatus) (Fig 2). Such information is important for managers and conservation practitioners using piscicides as management tools, particularly in areas of high invertebrate endemicity, because it allows for the evaluation of the risk to non-target biota during rotenone treatments intended to eradicate fishes when alternative methods are either not cost effective, not feasible or ineffective [13, 27, 29]. In addition, the current paper adds potamonautid crab responses to the available knowledge on the impacts of rotenone applications on non-target biota, which have largely focused on insects [16, 17, 25].
Fig 2. Effect of different rotenone concentrations on selected invertebrate fauna at 6 hrs and 18 hrs.

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Typical rotenone applications for river treatments last for 4–8 hrs at concentrations that are at least twice the experimentally-derived minimum effective dose (MED) that result in 100% mortality of the target organism in a 4 h period [29]. The Rondegat River for example, was treated twice, one year apart, using rotenone concentrations of 50 μgL\(^{-1}\) (4 × MED for *M. dolo-mieu*, Jordaan and Weyl [26]) and 37.5 μgL\(^{-1}\) for 6 hrs (3 × MED), respectively [30]. The first 50 μgL\(^{-1}\) rotenone treatment resulted in a substantial invertebrate drift event and a large depletion of gill-respiring EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa but not in plastron respiring groups such as the Corixidae [18]. The second, more conservative 37.5 μgL\(^{-1}\) rotenone treatment also resulted in similar depletion of EPT taxa [25] suggesting mortality at significantly lower concentrations than those used during the treatment. These field observations are consistent with laboratory toxicity trials which have found the Ephemeroptera to be particularly vulnerable to rotenone exposure [15, 17, 38, 39] and the current laboratory study which demonstrated that *B. harrisonii* was more sensitive (100% mortality at >25 μgL\(^{-1}\) rotenone concentration) than other insect taxa (Figs 2 and 3). In addition, this was the only test species of which differences in behaviour were observed at the 6 hr exposure treatment.

Benthic Aeshnid *A. imperator*, gastropod *P. acuta* and decapod *P. sidneyi* demonstrated the lowest mortality rates (Fig 2). Of particular interest was that for the decapod *P. sidneyi*, no deaths were observed at experimental concentrations which were as high as 100 μgL\(^{-1}\). These observations are similar to field [11, 17, 25, 40, 41] and laboratory observations reported in the

Table 1. PERMANOVA test results of the effects of rotenone concentration (0–100 μgL\(^{-1}\)) and time (6 and 18 hrs) on the behavioural traits of selected invertebrates. Significant differences at *p*-perm < 0.05 are indicated in bold. Abbreviations; df = degrees of freedom, MC = Monte Carlo, MS = mean squares, perm = permutation.

| Source                | df  | MS     | Pseudo-F | *p*-perm | *P*(MC) |
|-----------------------|-----|--------|----------|----------|---------|
| **Anax imperator**    |     |        |          |          |         |
| Concentration         | 5   | 0.1720 | 3.1918   | 0.0199   | 0.0179  |
| Time                  | 1   | 2.0833 | 38.6600  | 0.0001   | 0.0001  |
| Concentration×Time    | 5   | 0.1213 | 2.2515   | 0.0302   | 0.0456  |
| Residual              | 36  | 0.0539 |          |          |         |
| **Baetis harrisonii** |     |        |          |          |         |
| Concentration         | 5   | 0.9633 | 42.2930  | 0.0001   | 0.0001  |
| Time                  | 1   | 1.2033 | 52.8290  | 0.0001   | 0.0001  |
| Concentration×Time    | 5   | 0.0853 | 3.7463   | 0.0075   | 0.0078  |
| Residual              | 36  | 0.0228 |          |          |         |
| **Cypricercus sp.**   |     |        |          |          |         |
| Concentration         | 5   | 0.9993 | 58.0260  | 0.0001   | 0.0001  |
| Time                  | 1   | 0.8533 | 49.5480  | 0.0001   | 0.0001  |
| Concentration×Time    | 5   | 0.0873 | 5.0710   | 0.0012   | 0.0016  |
| Residual              | 36  | 0.0172 |          |          |         |
| **Diplonychus capensis** |   |        |          |          |         |
| Concentration         | 5   | 0.0513 | 4.8632   | 0.0020   | 0.0015  |
| Time                  | 1   | 0.0000 | 0.0000   | 1.0000   | 1.0000  |
| Concentration×Time    | 5   | 0.0000 | 0.0230   | 0.7892   |         |
| Residual              | 36  | 0.0106 |          |          |         |
| **Physa acuta**       |     |        |          |          |         |
| Concentration         | 5   | 0.1055 | 8.8326   | 0.0001   | 0.0001  |
| Time                  | 1   | 0.0675 | 5.6512   | 0.0219   | 0.0226  |
| Concentration×Time    | 5   | 0.0055 | 0.4605   | 0.8064   | 0.8028  |
| Residual              | 36  | 0.0119 |          |          |         |

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Fig 3. Behaviour of selected invertebrate taxa over an 18 hour exposure period at rotenone concentrations of 0 and 37.5 μg L⁻¹.

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literature [27, 42, 43, 44]. In acute toxicity tests, Chandler and Marking [42] for example, demonstrated that the tolerance of Dragonfly naid Macromia sp., gastropods including Physa pomilia and the freshwater prawn Palaemonetes kadiakensis far exceeded the tolerances of the water flea D. pulex and Ostracod Cypridopsis sp. Similarly, our data suggest that potamonautid crab responses are consistent with observations on other decapods such as crayfish. For example, Vinson et al. [17] noted that benthic organisms were less sensitive to rotenone when compared to pelagic organisms. Melas et al. [11] also highlighted that during piscicide applications, benthic organisms can seek refuge in organic sediments. However, as the current experiment did not include sediment, the use of refugia does not adequately explain low impacts on benthic taxa (e.g. A. imperator, P. acuta and P. sidneyi). Similarly, Recsetar and Bonar [44] observed 0% mortality in crayfish at recommended rotenone dosages and Wujtewicz et al. [43] demonstrated that rotenone concentrations required to kill crayfish Procambrus acutus (4 mg L\(^{-1}\)) were > 20× higher than those resulting in 100% mortality in white perch Morone americana (0.15 mg L\(^{-1}\)). The absence of any mortality in crabs (observed in this study) and crayfish [43, 44] can likely be attributed to the open circulatory system in Decapods [10, 44]. Öberg [10] highlighted that rotenone cause’s death at a cellular level and not at the water–blood interface. Hence, one will expect death by tissue anoxia to take longer and require higher rotenone concentrations for decapods [45]. While additional range testing would be necessary to determine lethal concentrations of rotenone to P. sidneyi, our data suggest that river treatments typically using concentrations <100 \(\mu\)gL\(^{-1}\) are unlikely to impact on Potamonautes spp. populations. This observation is supported by field observations of Potamonautes sp. consuming dead fish in the Rondegat River during rotenone treatments (Fig 1).

Our findings on high mortalities for zooplankton (e.g. D. pulex and P. lamellatus) at relatively low rotenone concentrations (25 \(\mu\)gL\(^{-1}\)) are consistent with field research that has focused specifically on zooplankton responses to rotenone use [11, 13, 46, 47, 48]. Recent literature e.g. [9, 11, 44, 49–55] have reported short-term extirpation of zooplankton after rotenone application, followed by a relatively rapid recovery of zooplankton communities. Examples include two lakes in Jasper National Park, Canada [47], Upper Karori Reservoir, New Zealand [46], Fern [52] and Diamond [55] Lakes in the USA, and Lakes Salmo and Alm in Sweden [53]. The high mortalities observed for the zooplankton species during this investigation are therefore, not unexpected (Fig 2). As much of the crustacean zooplankton produce resting or dormant eggs that reside and persist in the sediment through periods of unfavourable environmental conditions [49], the presence of an “egg reservoir” may have assisted in the lack of long term effects of rotenone on zooplankton in previous field studies [11, 13, 46]. In addition, other post-treatment factors such as lack of fish predation pressure and shifting invertebrate community dynamics likely aided in zooplankton recovery in these studies [11, 13, 46]. While this suggests that zooplankton communities may be largely impervious to effects of rotenone treatment, this resilience would likely be dependent on the presence of healthy resting egg propagules within a system. In lotic systems i.e. rivers, re-colonisation from upstream is likely also to be rapid. Impacts on zooplankton communities are however mostly a concern for treatments of natural lentic waterbodies that have endemic zooplankton communities [49, 50]. In southern Africa, zooplankton communities in such environments are poorly studied and there is a need for further studies on aspects of the reproductive biology of certain zooplankton groups.

The primary action of rotenone is to block important biochemical pathways of cell metabolism, via the re-oxidation of nicotinamide adenine dinucleotide (NADH), and thereby inhibiting respiration at the cellular level [10, 56]. Fish are particularly susceptible to rotenone due to the efficiency of entry of the toxin through their gills [10], but other taxa such as gill-respiring aquatic organisms [16] and aquatic invertebrates that absorb rotenone through their tracheal gills and cuticles have also been shown to be susceptible to exposure to it, as highlighted in this
and other studies e.g. [9, 13, 19, 27, 57]. In the current study, taxa that have membranes specific for gas exchange (e.g. *B. harrisonii*, *Cypricercus* sp., *D. pulex* and *P. lamellatus*), which have gill-like lamellae were noticeably more impacted by rotenone than those that had different breathing structures i.e. the plastron breathers (e.g. *A. imperator* and *D. capensis*).

To fully understand and minimize rotenone effects on non-target taxa, more laboratory studies should be carried to determine survivorship of several macroinvertebrate and zooplankton taxa at different stages of their development and assess how they are impacted by rotenone. In addition, assessments of whether animals would recover after exposure times would be useful and were not conducted in the present study. Latent toxicity effects may have resulted in higher mortalities in certain groups than those observed at the end of the experiment. This is a relevant consideration as in field exposures, rotenone is neutralised after a given period of time [18, 25]. The latent effects of invertebrates exposed to rotenone after neutralisation is, therefore, a consideration that is yet to be assessed for invertebrates of the region. Since most aquatic insects have terrestrial life forms, re-colonisation levels in aquatic systems treated with rotenone are likely high. While no analytical confirmation of rotenone exposure levels were assessed in the laboratory, the procedure employed was reflective of field trials where theoretical levels are determined based on point source introductions of rotenone stock solution at a known concentration [18, 30]. As such, controlled field studies such as those by Melaas et al. [11], Blakely et al. [13] and Beal and Anderson [46] assessing *in situ* effects of rotenone on macroinvertebrate and zooplankton communities should be conducted to give us an understanding of which particular taxa may be vulnerable in the long term.

**Supporting Information**

S1 Table. Mean proportion (± standard error) Behaviour traits of selected invertebrates after exposure to different rotenone concentrations (0–100 μgL⁻¹) and time intervals (1–18 hours).

(XLSX)

S2 Table. Pairwise comparisons for mortality rates at different concentrations (0–100 μgL⁻¹) at 6 and 18 hours for selected invertebrate groups. Significant differences at $p_{-perm} < 0.05$ are indicated in bold. Abbreviation: MC = Monte Carlo, perm = permutation, t = test statistic.

(DOCX)

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**Author Contributions**

Conceived and designed the experiments: TD RJW MJ PWF OLFW. Performed the experiments: TD RJW. Analyzed the data: TD RJW MJ PWF OLFW. Contributed reagents/materials/analysis tools: TD RJW MJ PWF OLFW. Wrote the paper: TD RJW MJ PWF OLFW.
References

1. Kolar CS, Lodge DM. Progress in invasion biology: predicting invaders. Trends Ecol Evol. 2001; 16: 199–204. PMID:11245943
2. Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C et al. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev. 2006; 81: 163–182. PMID:16336747
3. Pimentel D, Lach L, Zuniga R, Morrison D. Environmental and economic costs of nonindigenous species in the United States. BioSci. 2000; 50: 53–65.
4. Britton JR, Davies GD, Brazier M, Chare S. Case studies on eradicating the Asiatic cyprinid Pseudorasbora parva from fishing lakes in England to prevent their riverine dispersal. Aquat. Conserv. Mar Freshwat Ecosys. 2008; 18: 867–876.
5. Vander Zanden MJ, Olden JD. A management framework for preventing the secondary spread of aquatic invasive species. Can J Fish Aquat Sci. 2008; 65: 1512–1522.
6. Weyl OLF, Finlayson B, Impson ND, Woodford DJ, Steinkjer J. Threatened endemic fishes in South Africa’s Cape Floristic Region: a new beginning for the Rondegat River. Fisheries 2014; 39: 270–279.
7. Cohen AN. Success factors in the establishment of human-dispersed organisms. In: Bullock JM, Kenward RE, Hails RS. (eds.) Dispersal Ecology. Blackwell, London, 2002.
8. Moyle PB, Marchetti MP. Predicting invasion success: freshwater fishes in California as a model. Biosci. 2006; 56: 515–524.
9. Mangum FA, Madrigal JL. Rotenone effect on aquatic macroinvertebrates of the Strawberry River, Utah: a five-year summary. J Freshwat Ecol. 1999; 14: 515–524.
10. Öberg KE. The reversibility of the respiratory inhibition in gills and the ultrastructural changes in chloride cells from the rotenone-poisoned marine teleost, Gadus callarias. Exp. Cell Res. 1967; 45: 590–602. PMID: 6022569
11. Melaas CL, Zimmer KD, Butler MG, Hanson MA. Effects of rotenone on aquatic invertebrate communities in prairie wetlands. Hydrobiologia. 2001; 459: 177–186.
12. Finlayson BJ, Siepmann S, Trumbo J. Chemical residues in surface and ground waters following rotenone application to California lakes and streams. American Fisheries Society, Bethesda, Maryland. 2001. Available: www.fisheriessociety.org/rotenone/rewards/04finlayson.pdf. Accessed 20 May 2015.
13. Blakely TJ, Chaderton WL, Harding JS. The effect of rotenone on orchard-pond invertebrate communities in the Motueka area, South Island, New Zealand. Research and Development Series 220 Science and Technical Publishing, New Zealand Department of Conservation, Wellington, 2005.
14. Lintermans M. Recolonization by the mountain galaxias Galaxias olidus of a montane stream after the eradication of rainbow trout Oncorhynchus mykiss. Mar Freshwat Res. 2000; 51: 799–804.
15. Finlayson BJ, Somer WL, Vinson MR. Rotenone toxicity to rainbow trout and several mountain stream insects. N Am J Fish Manage. 2010; 30: 102–111.
16. Billman HG, Kruse CG, St-Hilaire S, Koel TM, Arnold JL, Peterson CR. Effects of rotenone on Columbia spotted frogs Rana luteiventris during field applications in lentic habitats of southernmost Montana. N Am J Fish Manage. 2012; 32: 781–789.
17. Vinson MR, Dinger EC, Vinson DK. Piscicides and invertebrates: after 70 years, does anyone really know? Fisheries 2010; 35: 61–71.
18. Woodford DJ, Barber-James HM, Bellingan TA, Day JA, de Moor FC, Gouws J et al. Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage: a lesser of two evils? J Insect Conserv. 2013; 17: 959–973.
19. Kjærstad G, Arnekleiv JV. Effects of rotenone treatment on lotic invertebrates. Internat Rev Hydrobiol. 2011; 96: 58–71.
20. Skorupski Jr JA. Effects of CFT Legumine™ rotenone on macroinvertebrates in four drainages of Montana and New Mexico. MSc thesis, University of North Texas. 2011.
21. Pham L. Rotenone use for native fish conservation: Macroinvertebrate community recovery and the reintroduction of a native galaxiid (Galaxias fasciatus) following piscicide treatment in two streams. MSc Thesis. University of Otago, Dunedin, New Zealand. 2012.
22. Linder HP, Johnson SD, Kuhlman M, Matthee CA, Nyffeler R, Swartz ER. Biotic diversity in the South- ern African winter-rainfall region. Cur Opin Environ Sustain. 2010; 2: 109–116.
23. Ellender BR, Woodford DJ, Weyl OLF. The invisibility of small headwater streams by an emerging invader, Clarias gariepinus. Biol Invasions. 2014; 17: 57–61.
24. Marr SM, Impson ND, Tweedle D. An assessment of a proposal to eradicate non-native fish from priority rivers in the Cape Floristic Region, South Africa. Afr J Aquat Sci. 2012; 37: 131–142.
25. Bellingan TA, Woodford DJ, Gouws J, Villet MH, Weyl OLF. Rapid bioassessment of the effects of repeated rotenone treatments on invertebrate assemblages in the Rondegat River, South Africa. Afr J Aquat Sci. 2015; 40: 89–94.

26. Jordaan MS, Weyl OLF. Determining the minimum effective dose of rotenone for eradication of alien smallmouth bass Micropterus dolomieu from a South African river. Afr J Aquat Sci. 2013; 38: 91–95.

27. Booth AJ, Moss S, Weyl OLF. Effect of rotenone on gill-respiring and plastron-respiring insects. Afr J Aquat Sci. 2015; 40: 95–100.

28. Huribert SH. Pseudoreplication and the design of ecological field experiments. Ecol Monogr. 1984; 54: 187–211.

29. Finlayson B, Schnick R, Skaar D, Anderson J, Demong L, Duffield D et al. Planning and standard operating procedures for the use of rotenone in fish management—rotenone SOP manual. American Fisheries Society Publication, Bethesda, Maryland. 2010.

30. Slabbert E, Jordaan MS, Weyl OLF. Analysis of active rotenone concentration during treatment of the Rondegat River, Cape Floristic Region, South Africa. Afr J Aquat Sci. 2014; 39: 467–472.

31. Cooman K, Debels P, Gajardo M, Urrutia R, Barra R. Use of Daphnia spp. for the ecotoxicological assessment of water quality in an agricultural watershed in South-Central Chile. Arch Environ Contamin Toxicol. 2005; 48: 191–200.

32. Shuhaimi-Othman M, Yakub N, Ramle N-A, Abas A. Toxicity of metals to a freshwater Ostracod: Stenocypris major. J Toxicol. 2011; 2011: 136104 doi:10.1155/2011/136104 PMID: 21559091

33. Ward DJ, Perez-Landa V, Spadaro DA, Simpson SL, Jolley DF. An assessment of three harpacticoid copepod species for use in ecotoxicological testing. Arch Environ Contamin Toxicol. 2011; 61: 414–425.

34. OECD (Organisation for Economic Cooperation and Development). OECD Environmental health and safety publications series for testing and assessments. Test no. 50. January, 2005. OECD Environment Directorate, Paris. 2003.

35. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001; 26: 32–46.

36. Anderson MJ, Gorley RN, Clarke KR. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK. 2008.

37. Anderson MJ, ter Braak CJF. Permutation tests for multi-factorial analysis of variance. J Stat Comput Simul. 2003; 73: 85–113.

38. Amnkleiv JV, Dolmen D, Rønning L. Effects of rotenone treatment on mayfly drift and standing stocks in two Norwegian rivers. In: Dominguez E. (ed.), Trends in Research in Ephemeroptera and Plecoptera. Kluwer Academic/Plenum Publishers. 2001; pp. 77–88.

39. Lintermans M, Raadik TA. Local eradication of trout from streams using rotenone: the Australian experience. Managing invasive freshwater fish in New Zealand. Proceedings of a workshop hosted by Department of Conservation. 2001; pp. 10–12.

40. Winterbourn MJ. The use of aquatic invertebrates in studies of stream water quality. Water Air Soil Poll. 1981; 22: 5–16.

41. Naess T. Tolerance of marine calanoid resting eggs: effects of freezing, desiccation and rotenone exposure—a field and laboratory study. Mar Biol. 1991; 111: 455–459.

42. Chandler JH Jr, Marking LL. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. Prog Fish-Cult. 1982; 44: 78–80.

43. Wujtewicz D, Petrosky BR, Petrosky DL. Acute toxicity of 5% non-synergized emulsifiable rotenone to white river crayfish Procambarus acutus acutus and white perch Morone americana. J World Aquacult Soc. 1997; 28: 249–259.

44. Recsetar MS, Bonar SA. Effectiveness of two commercial rotenone formulations in the eradication of virile crayfish Orconectes virilise. N Am J Fish Manage. 2015; 35: 616–620.

45. Ling, N. Rotenone: a review of its toxicity and use for fisheries management. New Zealand Department of Conservation, Science for Conversation 211, Wellington. 2003.

46. Beal DL, Anderson RV. Response of zooplankton to rotenone in a small pond. Bull Envir Contam Toxicol. 1993; 51: 551–556.

47. Anderson S. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. J Fish Res Board Can. 1970; 27: 1335–1356.

48. Duggan IC, Wood SA, West DW. Brown trout (Salmo trutta) removal by rotenone alters zooplankton and phytoplankton community composition in a shallow mesotrophic reservoir. New Zeal J Mar Fresh. 2015;
49. Hairston NG, VanBrunt RA, Kears M, Engstrom DR. Age and survivorship of diapausing eggs in a sediment egg bank. Ecol. 1995; 76: 1706–1711.

50. Marcus NH, Lutz R, Burnett W, Cable P. Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. Limnol Oceanogr. 1994; 39: 154–158.

51. Morrison BRS. The effects of rotenone on the invertebrate fauna of three Hill Streams in Scotland. Fish. Mgmt. 1977; 8: 128–139.

52. Kiser RW, Donaldson JR, Olson PR. The effect of rotenone on zooplankton populations in freshwater lakes. Trans Am Fish Soc. 1963; 92: 17–24.

53. Almquist E. Observations on the effect of rotenone emulsives on fish food organisms. Institute of Freshwater Research Drottingholm. 1959; 40: 146–160.

54. Rach JJ, Bills TD, Marking LL. Acute and chronic toxicity of rotenone to Daphnia magna. U.S. Fish and Wildlife Service Investigation Fish Control 92–94, Washington, D.C. 1988.

55. Eilers J, Truemper H. Diamond Lake Recovery—Again. LakeLine, Summer 2010; pp. 23–26.

56. Horgan DJ, Singer TP, Casida JE. Studies on the respiratory chain-linked reduced nicotinamide adenine dinucleotide dehydrogenase. XIII. Binding sites of rotenone, piericidin A and amytal in the respiratory chain. J Biol Chem. 1968; 243: 834–846. PMID: 4295606

57. Hamilton BT, Moore SE, Williams TB, Darby N, Vinson MR. Comparative effects of rotenone and antifungal on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. N Am. J Fish Manage. 2009; 29: 1620–1635.