The effect of rotenone on orchard-pond invertebrate communities in the Motueka area, South Island, New Zealand

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ABSTRACT

Rotenone is a naturally derived (organic) fish toxicant used widely in fisheries management. However, because of the non-specific nature of rotenone, non-target animals may also be poisoned. The aim of this study was to determine whether past rotenone poisoning has had detectable effects on pond invertebrate communities and, if so, whether there is any evidence of community recovery. Water-chemistry parameters and invertebrate and plankton communities were investigated in a one-off survey of 18 orchard ponds around Motueka, South Island, New Zealand. Ponds were classified as either rotenone-free ('pest fish present' or 'pest fish absent') or rotenone-addition, where rotenone had been used to eradicate pest fish species '6 months', '1 year' and '3 years' prior to our survey. We found few differences in water chemistry, physical conditions, or invertebrate taxonomic richness between groups of ponds. pH was circum-neutral in all ponds, while conductivity ranged from 112–193 μS_{25}/cm. Zooplankton diversity did not differ between groups: a total of 35 macroinvertebrate taxa were recorded from the 18 ponds, with 12–15 taxa found in each treatment group. However, there were subtle differences in macroinvertebrate and zooplankton community composition. Our results indicated that invertebrate communities in the poisoned study-ponds were able to recover quickly; however, the impact of rotenone on benthic invertebrates is still uncertain, and the results of this study should be interpreted with caution as they were confounded by other variables, such as adjacent land-uses. Ponds in this study were dominated by pollution-tolerant taxa, and were already subjected to a cocktail of chemicals used on the adjacent orchards. Thus, the effect of rotenone may be undetectable in our ponds but more severe in pristine systems.

Keywords: rotenone, piscicides, pond invertebrates, zooplankton, *Gambusia affinis*, mosquitofish

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1. Introduction

Rotenone is obtained from the roots of trees belonging to two genera, *Derris* and *Lonchocarpus* (Meadows 1973; Bettoli & Maceina 1996). It has been used widely as an insecticide (e.g. Derris dust) and piscicide (fish toxicant) (Krumholz 1948; Meadows 1973; Kvenseth & Øiestad 1984; Finlayson et al. 2000), because it is recognised as a relatively benign and naturally derived compound that is a highly effective fish poison. Furthermore, rotenone breaks down very quickly, becoming undetectable in the environment in a matter of days (Dawson et al. 1991; Bettoli & Maceina 1996; Chadderton et al. 2003; Ling 2003). Nevertheless, rotenone was not used extensively as a piscicide in New Zealand until 2000 (but see Rowe & Champion 1994), when the former Pesticides Board granted an experimental-use permit to assist in the eradication of newly discovered populations of introduced pest fish species, specifically mosquitofish (*Gambusia affinis*) from the Nelson–Moteuka region (Chadderton et al. 2003).

Rotenone affects respiration by inhibiting oxygen uptake at the cellular level (Horgan et al. 1968; Singer & Ramsay 1994; Fajt & Grizzle 1998) and is commonly used in fisheries management elsewhere (e.g. Denmark, United States, Australia, England, and Wales) to eradicate coarse fish species (Meadows 1973; Kvenseth & Øiestad 1984). However, due to its non-specific nature, non-target species, such as aquatic invertebrates, are also likely to be affected. Aquatic invertebrates substantially contribute to New Zealand's freshwater biodiversity, play an integral part in freshwater food-webs, and are essential for the maintenance of fish communities. Furthermore, the maintenance of biodiversity is a major objective of the Department of Conservation (DOC). Thus, the long-term effect of rotenone application is of particular concern, and poisoning activities in freshwater environments should be approached with caution.

Research into the effects of rotenone on aquatic invertebrates has been limited primarily to zooplankton and meiofauna (Hamilton 1941; Almquist 1959; Anderson 1970; Meadows 1973; Claffey & Costa 1974; Chandler & Marking 1982; Naess 1991a; Naess et al. 1991; but see Cushing and Olive 1956) and little is known about the responses of macroinvertebrates to this poison (but see Dudgeon 1990; Mangum & Madrigal 1999; Melaas et al. 2001; Lintermans & Raadik 2003). However, there is a great deal of literature on the negative effects of an array of other insecticides and pesticides on freshwater invertebrates (e.g. Wallace et al. 1986; Sibley et al. 2001; Wendt-Rasch et al. 2003). The aim of this study was to determine whether rotenone poisoning has had a detectable effect on pond invertebrate communities and, if so, whether there is any evidence of community recovery up to 3 years after poisoning.

2. Methods

2.1 STUDY SITES

A one-off survey of 18 orchard ponds, primarily around Motueka, was conducted during June 2004. Ponds were selected to represent five rotenone 'treatments': rotenone-free but with pest fish present (i.e. mosquitofish, tench (*Tinca tinca*) and / or rudd (*Scardinius erythrophthalmus*)) (*n* = 4); rotenone-free and without pest fish (*n* = 4); and treated with rotenone 6 months (*n* = 2), 1 year (*n* = 4), and 3 years (*n* = 4) prior to sampling (see Table 1 for pond locations). DOC has carried out regular sampling in each of the ponds analysed in this study since 2001, to ascertain the presence of pest fish species (Dean 2003; S. Elkington, pers. comm.). Therefore, all ponds that had previously been treated with rotenone ('6 months', '1 year' and '3 years') and those that were rotenone-free but without pest fish were assumed to be free of pest fish at the time of sampling. Note that in this study the term 'treatment' is not to be interpreted as the experimental manipulation of ponds; rather it is groups of

TABLE 1. LONGITUDE, LATITUDE, ALTITUDE, SURFACE AREA, MAXIMUM DEPTH AND PERCENT RIPARIAN COVER OF THE 18 ORCHARD PONDS IN FIVE ROTENONE-TREATMENT GROUPS, SAMPLED AROUND MOTUEKA IN JUNE 2004.

POND	EASTINGS (MAP SERIES N26 & 27)	NORTHINGS (MAP SERIES N26 & 27)	ALTITUDE (m a.s.l.)	SURFACE AREA (m^2)	MAX. DEPTH (m)	RIPARIAN COVER (%)
Pest fish present						
Orphange Creek	252887	598705	43	450	2	10
Geoff Rowling's	251139	600439	21	45000	8	5
Strong's	251106	600301	67	4900	6	50
Cave's	250800	600200	43	1200	6	5
Pest fish absent						
Nicholson's (no. 1)	259986	251273	122	875	7	5
Fraser's	251271	600243	62	3500	3	10
Fulford's	251440	600220	35	250	4	50
Syd's Patch	251442	600154	26	150	3.5	15
6 months						
Ian Rowling's	251137	600532	38	420	3	25
Urqhuart's Dam	250863	600533	28	1080	4	85
1 year						
Hansen's	250705	600605	20	2800	8	1
Wood's	251070	600560	63	1000	4.5	10
Devlin's	250621	600808	30	80	2	10
Waiwhero (no. 3)	250333	600122	75	150	2	25
3 years						
Martin's	251276	600387	40	2750	7	1
Chapman's	251603	599960	70	2400	4	10
Goodman's	251144	600920	2	400	1	15
Woodman's	250705	600701	21	2000	6	10

ponds that have been similarly exposed to rotenone over the past 3 years by DOC. Thus, in this study we did not actually add rotenone to pond systems; instead we measured the effect of this piscicide after it had been added. (For rotenone application methodology and concentrations see Chadderton et al. 2003.)

We visually selected ponds with similar-looking riparian cover, macrophyte abundance, and water clarity to control for confounding effects of water quality and physical characteristics. We also avoided any tidally influenced ponds. At each pond, surface area (m^2) and maximum pond depth (m) were measured (as indicators of pond size), percent riparian cover was visually estimated, and altitude (m a.s.l.) was measured with a Garmin etrex GPS (Table 1).

2.2 WATER CHEMISTRY

Basic water-chemistry parameters were assessed for each pond between 1000 h and 1400 h, in June 2004. Specific conductivity (at 25ºC), pH, and water temperature (Oakton CON 10 Series meter), turbidity (HACH 2100P Turbidimeter) and dissolved oxygen content (YSI 550 DO meter) were recorded. Maximum underwater visibility was also determined using a Secchi disc. All measurements were taken 0.5 m from the bottom of the pond, and 2–3 m from the pond edge. Care was taken to avoid stirring up sediments.

Total suspended sediments in the water column were measured from a single grab sample, whereby a 250 mL water sample was collected from the water column approximately 0.5 m from the bottom of each pond and 2–3 m from the pond edge. In the laboratory, water samples were shaken thoroughly and 20 mL was suctioned off, vacuum filtered onto pre-weighed and pre-ashed Whatman glass microfibre filters (GF/C), dried at 50ºC for 24 h, and ashed at 450ºC for 2 h. Dried and ashed filters were weighed (± 0.001 g).

Phytoplankton biomass was estimated by extracting pigments from samples obtained with an 80-µm-mesh net (opening: 20 cm in diameter). Each sample was collected by five sweeps of the top 0.5 m of water column, along a 2-m stretch in each pond. Pigments were extracted in 90% ethanol for 24 h at 4ºC in the dark, and absorbance at 665 and 750 nm was measured using a spectrophotometer. Chlorophyll-*a* concentration was calculated as described in the HACH (1990) manual.

2.3 MACROINVERTEBRATE AND ZOOPLANKTON SAMPLING

To examine whether rotenone affected macroinvertebrate richness and community composition, samples were taken from three microhabitats within each pond. An extensive sweep-net (500-µm-mesh net) sample was taken to examine the substrate and emergent vegetation in the littoral zone ('littoral' sample), whereby the bottom of the pond was disturbed for 45 s. A second sweep-net sample was collected from the water column on and around submerged macrophytes ('macrophyte' sample) . An Ekman grab sampler was used to collect benthic invertebrates from soft sediments at 1.0–1.8 m water depth. All samples were preserved in the field with 90% isopropal-alcohol, and specimens were identified in the laboratory to the lowest practicable taxonomic level, usually species or genus (Winterbourn 1973; Winterbourn et al. 2000).

Zooplankton were sampled as described above for phytoplankton-biomass sampling. Zooplankton samples were preserved in the field with 90% isopropal-alcohol, and specimens were identified in the laboratory to ordinal level at least (Chapman & Lewis 1976).

2.4 STATISTICAL ANALYSES

One-way analyses of variance (ANOVAs) were used to determine whether water chemistry, physical parameters, and macroinvertebrate and zooplankton taxonomic richness differed between groups of ponds. All response variables were log transformed ($x + 1$) where necessary, to meet the assumptions of homoscedasticity and normality (Zar 1999), and analyses were done in Systat version 10. Fisher's Least Significant Difference (LSD) post-hoc tests were carried out where applicable, to determine where pairwise differences lay.

To ascertain whether the two rotenone-treatments or the five pond-treatments had similar community compositions, a non-metric multi-dimensional scaling ordination (NMDS) was performed on relative-abundance macroinvertebrate data, using Primer version 5. As the two factors (rotenone treatment and pond treatment) were hierarchical, a nested analysis of similarities (ANOSIM) was also performed using Primer version 5, where rotenone treatment (either rotenone-free or rotenone-addition) was the main factor being tested, and pond group (pest fish absent, pest fish present, 6 months, 1 year or 3 years) was nested within rotenone treatment. To determine whether particular taxa primarily accounted for any observed assemblage differences between groups, similarity percentages (SIMPER) were calculated where necessary, using Primer version 5.

Mean relative abundances of major taxonomic groups of macroinvertebrates and zooplankton were also compared graphically.

3. Results

3.1 WATER CHEMISTRY AND PHYSICAL PARAMETERS

Comparisons of water chemistry and physical conditions indicated no consistent differences between pond treatments. Pond-water pH was circumneutral in all ponds, and dissolved oxygen content (5.2–11.8 mg/L) was not significantly different between treatments (Table 2). Turbidity was consistently low across all treatments (range 6.6–16.1 NTU), as was the level of suspended sediment in the water column (0.43–0.44 g/L) (Table 2). Ponds treated with rotenone 1 year before sampling had a lower average Secchi depth (0.6 m) than ponds with no pest fish and ponds treated with rotenone 3 years before (both 1.0 m). However, underwater visibility did not differ significantly among the five treatments (Table 2). Specific conductivity ranged from 112 to 193 μS_{25}/cm across groups, while water temperature was between 8.3°C and 11.4°C (Table 2). Although ponds that had been treated with rotenone 1 year prior to sampling had the greatest phytoplankton biomass, this varied substantially between ponds in this group (385 ± 377 μg Chl-*a*/m), and no significant differences were found between the five treatments (Table 2).

TABLE 2. WATER CHEMISTRY AND PHYSICAL PARAMETERS MEASURED IN 18 ORCHARD PONDS IN THE MOTUEKA REGION IN JUNE 2004.

Parameters are shown as means (± 1 SEM) for each of the five groups of ponds; $n = 4$, except '6 months' where $n = 2$. Results of one-way ANOVAs are shown.

PARAMETER	PEST FISH PRESENT	PEST FISH ABSENT	6 MONTHS	1 YEAR	3 YEARS	*F*	*P*
Dissolved oxygen (mg/L)	8.7 ± 0.9	6.7 ± 1.5	11.8 ± 6.2	7.1 ± 1.0	5.2 ± 1.9	0.13	0.30
pH	7.2 ± 0.1	6.9 ± 0.2	7.2 ± 1.2	6.9 ± 0.2	6.9 ± 0.1	1.37	0.97
Specific conductivity (μS_{25}/cm)	118 ± 28	193 ± 11	180 ± 29	112 ± 31	179 ± 38	1.55	0.25
Water temperature (°C)	10.3 ± 0.4	10.8 ± 0.7	11.4 ± 0.2	9.4 ± 0.7	8.3 ± 2.3	0.79	0.55
Secchi depth (m)	0.9 ± 0.2	1.0 ± 0.4	0.7 ± 0.5	0.6 ± 0.1	1.0 ± 0.4	0.48	0.74
Turbidity (NTU)	13.5 ± 8.2	6.6 ± 2.2	11.0 ± 4.5	16.1 ± 3.4	12.0 ± 3.6	0.90	0.49
Phytoplankton (μg Chl-*a*/m)	24 ± 11	69 ± 40	19 ± 18	385 ± 377	13 ± 12	0.65	0.64
Suspended sediments (g/L)	0.44 ± 0	0.43 ± 0	0.44 ± 0	0.43 ± 0	0.43 ± 0.0	1.88	0.18

3.2 MACROINVERTEBRATES

Thirty-eight macroinvertebrate taxa were recorded from the three microhabitats in the 18 ponds sampled (Appendices 1 and 2). Twice as many taxa were found in the macrophyte and littoral sweep-net samples (31 and 34 taxa respectively) than in the bottom-sediment samples (15 taxa). Macroinvertebrate richness ranged from 20 taxa in Geoff Rowling's pond (an unpoisoned pond with pest fish), to only eight taxa in Cave's pond (unpoisoned with pest fish) and nine taxa in Waiwhero pond (poisoned 1 year ago). However, using pooled data from the three microhabitats, taxonomic richness did not differ between treatments (Fig. 1; Table 3). Similarly, it did not differ significantly when microhabitats were analysed separately (Table 3), or when ponds were grouped into rotenone-free and rotenone-addition treatments.

NMDS did not reveal any obvious trends in macroinvertebrate community composition within pond groups or when ponds were separated by the two

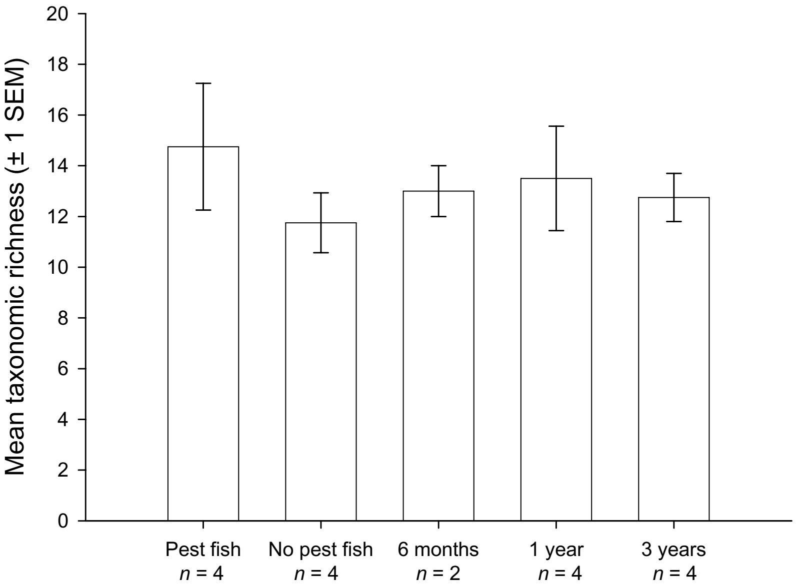


Figure 1. Mean macroinvertebrate taxonomic richness (± 1 SEM) for each group of ponds, based on samples taken from three habitats in June 2004.

TABLE 3. MEAN TAXONOMIC RICHNESS RECORDED IN 18 ORCHARD PONDS IN THE MOTUEKA REGION IN JUNE 2004.

Parameters are shown as means (± 1 SEM) for each of the five groups of ponds; *n* = 4, except '6 months' where *n* = 2. Results of one-way ANOVAs are shown for each treatment, using pooled data as well as data from each of the three microhabitats separately.

MICROHABITAT	PEST FISH PRESENT	PEST FISH ABSENT	6 MONTHS	1 YEAR	3 YEARS	*F*	*P*
Pooled	15 ± 2.5	12 ± 1.2	13 ± 1.0	13 ± 0.9	14 ± 2.0	0.25	0.94
Macrophytes	11 ± 1.9	9 ± 1.5	10 ± 0.5	10 ± 1.1	8 ± 2.0	0.49	0.74
Littoral	10 ± 2.1	9 ± 1.5	8 ± 3.0	10 ± 1.2	10 ± 0.8	0.31	0.87
Echman	3 ± 1.7	2 ± 0.6	6 ± 1.5	3 ± 0.9	4 ± 2.3	0.52	0.72

rotenone treatments, rotenone-free and rotenone-addition (Fig. 2). The nested ANOSIM confirmed this. Pond groups, nested within rotenone treatments, were not significantly different from each other ($r = 0.009$, $P = 0.48$; r values < 0.25 indicate that invertebrate communities were virtually indistinguishable between groups). Similarly, ponds grouped according to rotenone treatments were similar in community composition ($r = -0.333$, $P = 0.90$; Fig. 2).

SIMPER did, however, identify some differences in community composition. For example, both Chironominae and Orthocladiinae larvae had higher relative abundances in rotenone-addition ponds than in rotenone-free ponds (Table 4). Conversely, Platyhelminthes and the dytiscid diving beetle, *Antiporus strigosulus*, had higher relative abundances in rotenone-free ponds than in rotenone-addition ponds (Table 4). We also investigated each of the rotenone treatments independently. Rotenone-addition ponds had an average similarity

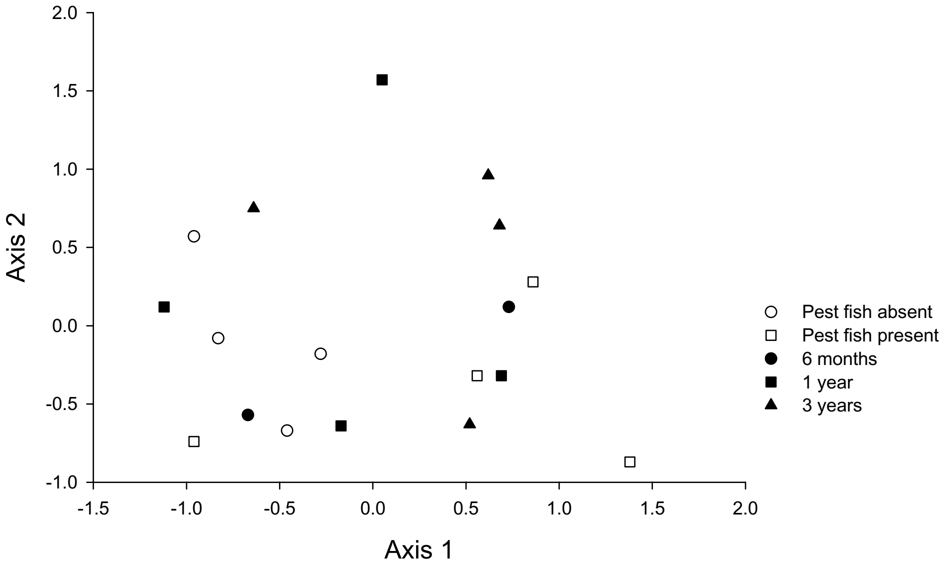


Figure 2. Non-metric multi-dimensional scaling ordination (NMDS) of macroinvertebrate communities using relative-abundance data for each of the 18 ponds divided into the two rotenone-treatments: white symbols indicate the eight rotenone-free ponds, while black symbols are the ten rotenone-addition ponds. NMDS stress value = 0.13. Ponds were sampled in June 2004.

TABLE 4. AVERAGE DISSIMILARITY VALUES, USING RELATIVE-ABUNDANCE DATA (%) IN ROTENONE-ADDITION (ADDITION) AND ROTENONE-FREE (FREE) PONDS.

Average dissimilarity values identify taxa (ranked by importance) that contribute to the observed assemblage differences between the two treatments (rotenone-addition and rotenone-free ponds); the average dissimilarity between ponds = 63.2.

TAXON	AVERAGE ABUNDANCE (%)		AVERAGE DISSIMILARITY	CONTRIBUTION (%)	CUMULATIVE CONTRIBUTION (%)
	ADDITION (n = 10)	FREE (n = 8)			
Oligochaetae	21.50	31.40	13.00	20.57	20.57
Sigara spp.	18.40	28.09	10.92	17.28	37.85
Chironominae	17.31	4.98	8.16	12.91	50.75
Orthocladiinae	8.03	4.56	4.82	7.63	58.38
Platyhelminthes	2.74	6.45	4.03	6.37	64.75
Hirudinea	4.98	3.80	3.61	5.71	70.46
Physella acuta	5.88	3.27	3.43	5.43	75.89
Potamopyrgus antipodarium	3.86	3.06	2.87	4.54	80.43
Gyraulus spp.	4.50	2.19	2.60	4.11	84.55
Xanthocnemis zealandica	4.72	2.19	2.56	4.05	88.60
Antiporus strigosulus	0.61	3.20	1.65	2.61	91.20

value of 33.69, compared with 36.78 for rotenone-free ponds. SIMPER indicated that Orthocladiinae larvae and the snails *Gyraulus* spp. and *Physella* spp. were good discriminators between the two rotenone treatments; however, they contributed to less than 13% of the similarity between rotenone-addition ponds (Table 5). Acarina mites and Hirudinea leeches were good discriminators of rotenone-free ponds (Table 6).

Macroinvertebrate communities were grouped into eight major taxonomic groups and compared graphically; findings were consistent between ANOSIM and SIMPER. For example, waterboatmen (*Sigara* spp.) had fairly consistent relative abundances across the five pond-groups, while beetles (e.g. *Antiporus strigosulus*) and Platyhelminthes were relatively more abundant in unpoisoned ponds than in poisoned ponds. Odonates (particularly the damselflies *Xanthocnemis zealandica* and *Austrolestes colensonis*) were present in all groups of ponds, but had much lower relative abundances 6 months after poisoning than 1 year or 3 years after poisoning (Fig. 3); however, this difference was not tested statistically. Conversely, snails (i.e. *Potamopyrgus antipodarum*, *Gyraulus* spp., *Physella acuta* and *Lymnaea columella*) had higher relative abundances in the two ponds that had been exposed to rotenone as little as 6 months ago (> 23%), but made up a lower proportion of the community over time (< 14% after 3 years) (Fig. 3).

TABLE 5. AVERAGE SIMILARITY PERCENTAGES (SIMPER) IN ROTENONE-ADDITION PONDS, BASED ON RELATIVE-ABUNDANCE DATA.

Average similarity values identify taxa (ranked by importance) that are found consistently in the rotenone-addition ponds (n = 10); the average similarity between ponds = 33.69.

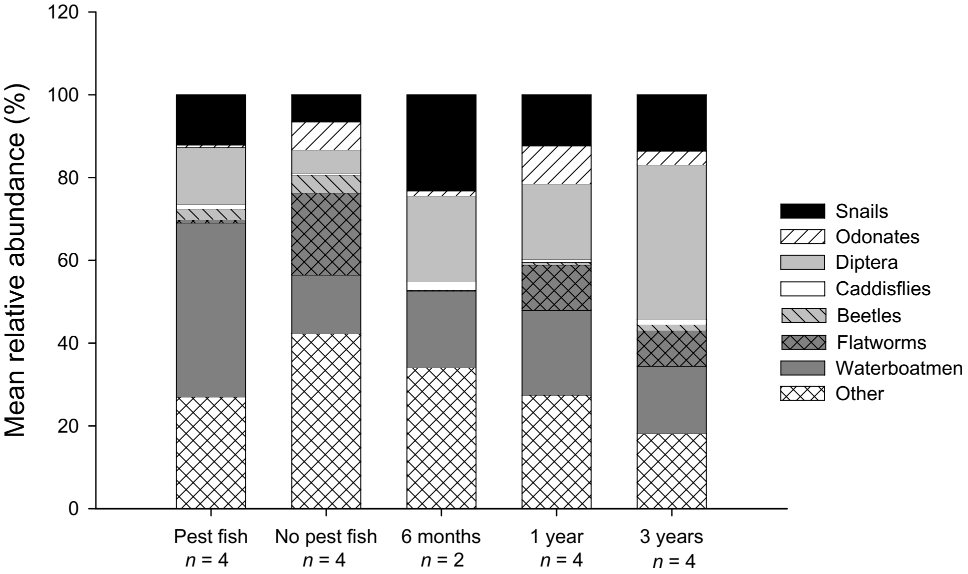
TAXON	AVERAGE ABUNDANCE (%)	AVERAGE SIMILARITY	CONTRIBUTION (%)	CUMULATIVE CONTRIBUTION (%)
Oligochaetae	21.50	10.16	30.15	30.15
Sigara spp.	18.40	9.61	28.54	58.69
Chironominae	17.31	6.28	18.65	77.34
Gyraulus spp.	4.50	1.54	4.57	81.91
Orthocladiinae	8.03	1.49	4.43	86.34
Physella acuta	5.88	1.28	3.81	90.15

TABLE 6. AVERAGE SIMILARITY PERCENTAGES (SIMPER) FOR ROTENONE-FREE PONDS, BASED ON RELATIVE-ABUNDANCE DATA.

Average similarity values identify taxa (ranked by importance) that are found consistently in the rotenone-free ponds (n = 8); the average similarity between ponds = 36.78.

TAXON	AVERAGE ABUNDANCE (%)	AVERAGE SIMILARITY	CONTRIBUTION (%)	CUMULATIVE CONTRIBUTION (%)
Oligochaetae	31.40	16.01	43.54	43.54
Sigara spp.	28.09	13.98	38.02	81.56
Chironominae	4.98	1.14	3.11	84.67
Acarina	2.76	1.11	3.03	87.70
Hirudinea	3.80	0.92	2.49	90.19

Figure 3. Mean relative abundances (%) of eight taxonomic groups in each group of ponds (based on pooled samples from all three microhabitats). Ponds were sampled in June 2004.



3.3 ZOOPLANKTON / MEIOFAUNA

Five zooplankton taxa were recorded in the 18 ponds sampled: two copepods, an ostracod and two cladoceran taxa. Two of the unpoisoned 'pest fish absent' ponds—Syd's Patch and Fraser's—had the greatest zooplankton richness, whereas lowest zooplankton richness was found in Geoff Rowling's, Orphanage Creek (rotenone-free 'pest fish present'), and Urquhart's Dam ('6 months') ponds. Nonetheless, mean zooplankton richness was highly variable across the five treatments, and no significant differences were detected ($F = 1.074$, df = 4, 13, $P = 0.41$; Fig. 4). Similarly, we found no difference in zooplankton richness when ponds were grouped into rotenone-free and rotenone-addition treatments ($F = 0.322$, df = 1, 16, $P = 0.58$).

When investigated visually, zooplankton community composition differed between the two control-groups and the three rotenone-addition pond groups (Fig. 5). Cladocerans and copepods made up approximately equal proportions of the community in the two groups of ponds that had never been treated with rotenone. Conversely, in ponds that had been treated with rotenone 6 months prior to our study, cladocerans were dominant (86%), whereas copepods made up less than 14% of the zooplankton community. Interestingly, 1 year after treatment, cladoceran and copepod abundances were similar to those in untreated ponds. Furthermore, the greatest relative abundances of ostracods (18%) were found in ponds where rotenone had been added 3 years prior to our survey (Fig. 5).

Figure 4. Mean zooplankton taxonomic richness (± 1 SEM) for each group of ponds in June 2004.

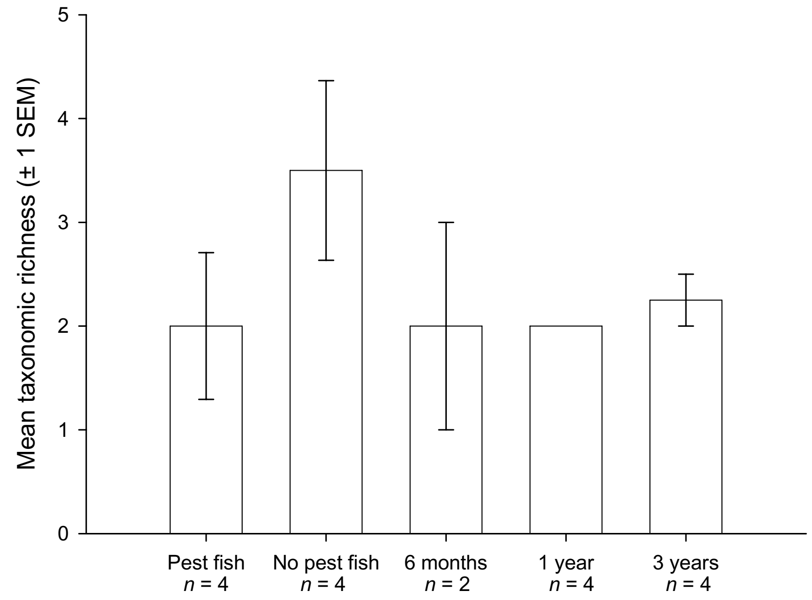
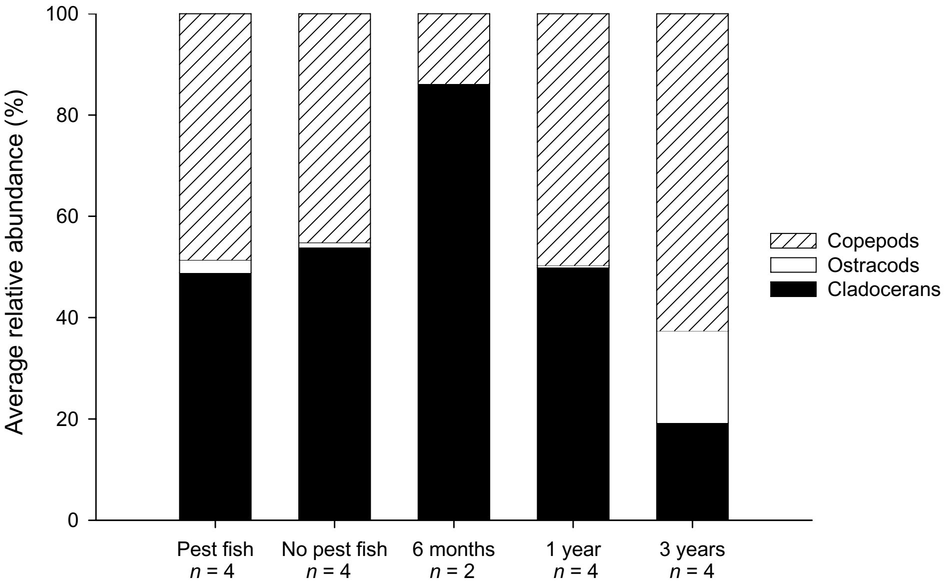


Figure 5. Mean relative abundances (%) of zooplankton collected in sweep-nets at five treatment levels in 18 orchard ponds in the Motueka region, June 2004.



4. Discussion

The principal objective of our study was to determine whether the past use of rotenone has negatively affected invertebrate communities in orchard ponds in the Motueka region and, if so, whether there is any evidence of community recovery. There were subtle differences in community composition between rotenone-free and rotenone-addition ponds, with some invertebrate taxa being more abundant in poisoned ponds, and others being more abundant in non-poisoned ponds. However, the absence of rigorous baseline data on invertebrate communities prior to rotenone addition for each pond means that we can only infer the effects of rotenone, rather than test them directly. Unfortunately, there were other confounding variables that we could not control for in this study, which need to be considered when interpreting these findings. For example, a powdered form of rotenone was used in the ponds that was up to 2.5 years old and consequently was likely to have degraded over time; this would potentially be markedly less toxic than new rotenone-powder stocks. To compensate for the potentially lower toxicity of this piscicide, DOC doubled the application concentrations in some ponds (Chadderton et al. 2003). However, we were unable to distinguish between the concentrations of rotenone used in individual ponds in this study. Furthermore, all of these ponds occur in agricultural landscapes—primarily fruit production—where fungicide, pesticide and fertiliser are commonly applied. There is also anecdotal evidence to suggest that some ponds are given frequent doses of copper. Although this practise might be perceived as necessary to stop macrophyte and algal blooms from blocking irrigation intakes in the orchard ponds, copper can have detrimental effects on aquatic invertebrates (Wiederholm 1984; Hickey & Clements 1998; Hickey 2000). In addition, these orchard ponds have all been artificially created and are used solely for irrigation; hence, they are all typically drawn down to very low levels over summer months, but the level to which the water is drawn down is likely to vary between ponds. As a result of these factors, we would expect a lower diversity of invertebrates in these ponds than in ponds in more pristine landscapes (e.g. Crumpton 1978; Wissinger & McIntosh 2003), and that any taxa present would be strong dispersers and representative of species that are more tolerant of the pollutants and chemicals used in the surrounding environment.

The effects of pollutants on freshwater invertebrates are well documented (e.g. Winterbourn et al. 1971; Winterbourn 1981; Wiederholm 1984; Lenat & Crawford 1994; Beeson et al. 1999): often, pollution-sensitive taxa, such as mayflies (Ephemeroptera) and some caddisflies (Trichoptera), are absent, while more tolerant fauna dominate (see references in Wiederholm 1984). Furthermore, rotenone affects respiration through inhibiting oxygen uptake at the cellular level (Horgan et al. 1968; Singer & Ramsay 1994; Fajt & Grizzle 1998); therefore, it could be expected that some invertebrate taxa might be more susceptible to rotenone than others. For example, taxa that have membranes specific for gas exchange (such as damselflies (Odonata), which have caudal lamellae, or flatworms (Platyhelminthes), in which gases diffuse across the body wall) might be more noticeably impacted by rotenone than those that do not (e.g. waterboatmen (*Sigara* spp.), which regularly swim to the water surface to collect a bubble of air). However, in this study, beetles (predominantly the dytiscid diving beetle, *Antiporus strigosulus*), which also take in air at the water surface, were found less often in the ponds to which rotenone had been added. Interestingly, Orthocladiinae and Chironominae midge larvae made up a larger proportion of the invertebrate community in ponds poisoned with rotenone than in rotenone-free ponds. These taxa are generally considered to be tolerant to pollution (Winterbourn 1981), and their presence in rotenone-addition ponds is more likely to be a reflection of their great dispersal abilities, rather than the effects of rotenone *per se* (Milner et al. 2000; Vieira et al. 2004).

Our findings are consistent with the somewhat limited research that has focused specifically on macroinvertebrate responses to rotenone use. Several studies have documented the impact of piscicide and insecticide applications on zooplankton communities, with one study indicating that rotenone had a much greater effect on zooplankton than on macroinvertebrates (Melaas et al. 2001). Melaas et al. (2001) suggested that benthic invertebrates can seek refuge from piscicides in organic sediments, whereas nektonic species (organisms in the water column, e.g. zooplankton, damselflies and diving beetles) cannot. This may explain why, in this study, rotenone seemed to have very little impact on some benthic taxa, such as snails (which sometimes reside in organic sediments), whilst appearing to reduce the relative abundances of zooplankton, diving beetles and other nektonic taxa. We also noted differences in zooplankton community composition between the three groups of ponds treated with rotenone; however, these differences were not statistically tested.

Nevertheless, there is a suggestion that the differences in zooplankton composition we observed were not due solely to the direct effects of rotenone exposure. For example, our finding that cladocerans (water fleas) were more abundant than copepods shortly after rotenone exposure is not entirely consistent with previous research, which suggests that cladocerans suffer much higher mortality from rotenone than copepods (Almquist 1959; Meadows 1973; Claffey & Costa 1974; Chandler & Marking 1982; Naess 1991b). It is possible that the removal of mosquitofish had a short-term, positive effect on cladoceran populations, which could have masked the effects of the piscicide application. Hurlbert & Mulla (1981) documented that mosquitofish had a more dramatic impact on cladocerans than on other zooplankton taxa in southern California ponds. Similarly, Cook & Moore (1969) and Wiederholm (1984) present other examples of indirect effects of pesticide applications on aquatic organisms, whereby elimination of a top-predator led to a marked increase in invertebrate prey.

Rotenone has been found to increase water clarity, as a direct result of a reduction in zooplankton and phytoplankton populations (Bradbury 1986, cited in Dawson et al. 1991). Such a trend was not apparent in our study. Furthermore, although zooplankton community composition differed between groups of ponds, zooplankton abundance did not. These findings, combined with the fact that zooplankton communities can recover from disturbances very quickly (Almquist 1959; Anderson 1970; Ling 2003), suggest that the zooplankton communities in our study ponds had already largely recovered from the impact of the rotenone treatment.

5. Conclusions and recommendations

Although there were subtle differences in community composition between rotenone-free and rotenone-addition ponds, these findings need to be interpreted with caution as there were many confounding factors that could not be controlled for. Thus, the impact of rotenone on invertebrate fauna is still uncertain. It is well understood that rotenone breaks down rapidly after exposure to light and water (Willis & Ling 2000; Ling 2003) with a half-life of only 1–3 days (Bettoli & Maceina 1996), and is reduced to very low concentrations in both the water column and sediments in just 10–14 days (Dawson et al. 1991). The application of rotenone to fresh waters can also cause significant declines in zooplankton and certain benthic fauna; however, some invertebrates would normally be expected to recover in a few months (Melaas et al. 2001; Ling 2003), although the recovery rates are largely dependent upon each taxon's recolonisation ability.

To better understand the impact of rotenone on New Zealand's aquatic invertebrate communities, it is essential that future poisoning programmes incorporate rigorous pre- and post-rotenone invertebrate sampling (i.e. commence invertebrate sampling before rotenone application). This is particularly important when these rotenone-addition operations occur in more natural water-bodies, such as near-pristine wetlands and lakes. Furthermore, it is imperative that further studies include sampling immediately after rotenone application and continue for at least 1 year, at regular time-intervals. It is also essential that when rotenone is applied to waterways, concentrations are held constant where possible, to reduce the number of confounding factors that might give contradictory results.

Finally, although there were subtle differences in invertebrate communities between the two treatments (i.e. rotenone-free and rotenone-addition), our study indicated that the addition of rotenone had limited, short-lived impacts on invertebrate communities in the Motueka region. The intensive horticultural and agricultural land-use of the area probably contributed to the low invertebrate species-richness in ponds; therefore, our results are not necessarily indicative of faunal responses expected in other parts of New Zealand.

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Appendix 1

LIST OF ALL INVERTEBRATES FOUND IN EIGHT ROTENONE-FREE MOTUEKA ORCHARD PONDS

+ and 0 indicate the presence and absence of taxa respectively. The following ponds were sampled in June 2004: C = Cave's; GR = Geoff Rowling's; OC = Orphanage Creek; S = Strong's; Fr = Fraser's; Fu = Fulford's; NN1 = Nicholson no. 1; and SP = Syd's Patch.

	PEST FISH PRESENT (*n* = 4)				PEST FISH ABSENT (*n* = 4)			
	C	GR	OC	S	Fr	Fu	NN1	SP
Cnidaria								
Hydra	0	0	0	+	0	0	0	0
Platyhelminthes								
Tricladida	0	+	+	+	+	+	+	+
Mollusca								
Potamopyrgus antipodarum	0	+	+	0	0	0	0	0
Gyraulus spp.	0	+	0	0	0	0	+	0
Physella acuta	+	+	+	+	+	0	0	0
Lymnaea columella	0	+	0	0	+	0	0	0
Musculium novazelandiae	0	0	+	0	0	0	0	0
Annelida								
Oligochaetae	+	+	+	+	+	+	+	+
Hirudinea	0	+	+	+	+	+	+	+
Chelicerata								
Acari	+	+	0	+	+	+	+	+
Crustacea								
Cladocera	0	+	+	+	+	+	+	+
Ostracoda	0	+	+	+	+	+	+	+
Copepoda	0	0	+	0	0	0	+	+
Odonata								
Austrolestes colensonia	+	+	0	+	0	+	0	+
Xanthocnemis zealandica	0	+	+	+	+	+	0	+
Aeshna brevistyla	0	+	0	0	0	0	0	0
Hemicordulia australiae	0	0	0	0	0	0	0	0
Procordulia grayi	0	0	0	0	0	0	0	0
Lepidoptera								
Hygraula nitens	0	+	0	0	0	0	0	0

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Appendix 1—continued

	PEST FISH PRESENT (n = 4)				PEST FISH ABSENT (n = 4)			
	C	GR	OC	S	Fr	Fu	NN1	SP
Trichoptera								
Oxyethira albiceps	0	0	+	0	0	0	0	0
Paroxyethira sp.	0	0	0	0	0	0	+	0
Paroxyethira hendersoni	0	+	0	0	0	0	0	0
Ocetis unicolor	0	+	0	0	0	0	0	0
Triplectides cephalotes	0	0	0	+	0	0	0	0
Hemiptera								
Sigara spp.	+	+	+	+	+	+	+	+
Coleoptera								
Antiporus strigosulus	+	+	0	+	+	0	+	0
Lancetes lanceolatus	0	0	0	0	0	0	+	0
Scirtidae	0	0	0	0	0	0	0	0
Enochrus tritus	+	0	0	0	0	0	+	0
Limnoxenus zelandicus	+	0	0	0	0	0	0	0
Diptera								
Zelandotipula sp.	0	0	0	0	0	0	0	0
Limonia sp.	0	0	0	0	0	0	0	0
Paralimnophila skusei	0	0	0	0	0	0	0	0
Tanypodinae	0	0	+	0	0	0	0	0
Chironominae	0	+	0	+	0	+	+	0
Orthocladiinae	0	+	+	+	0	0	+	0
Corynoneura scutellata	0	0	+	0	0	0	0	0
Neolimnia sp.	0	0	0	+	0	0	0	0

Appendix 2

LIST OF ALL INVERTEBRATES FOUND IN TEN ROTENONE-ADDITION MOTUEKA ORCHARD PONDS

Rotenone was added to the ponds 6 months, 1 year or 3 years prior to the study. + and 0 indicate the presence and absence of taxa respectively. The following ponds were sampled in June 2004: IR = Ian Rowling's; UD = Urqhuart's Dam; H = Hansen's; W = Wood's; D = Devlin's; WN3 = Waiwhero (no. 3); M = Martin's; C = Chapman's; G = Goodman's; and Wm = Woodman's.

	6 MONTHS ($n = 2$)		1 YEAR ($n = 4$)				3 YEARS ($n = 4$)			
	IR	UD	H	W	D	WN3	M	C	G	Wm
Cnidaria										
Hydra	0	+	0	0	0	0	0	0	0	0
Platyhelminthes										
Tricladida	0	0	0	+	0	+	+	0	0	0
Mollusca										
Potamopyrgus antipodarum	0	0	0	+	0	0	0	+	0	+
Gyraulus spp.	+	+	+	+	0	+	+	0	+	+
Physella acuta	+	+	+	+	0	0	+	0	0	+
Lymnaea columella	+	+	0	+	0	0	0	0	+	0
Musculium novazelandiae	0	0	+	0	0	0	+	0	0	0
Annelida										
Oligochaetae	+	+	+	+	+	+	+	+	+	+
Hirudinea	+	0	+	0	0	+	+	0	+	+
Chelicerata										
Acari	+	+	+	+	0	+	0	+	+	+
Crustacea										
Cladocera	+	+	+	0	+	+	+	0	0	0
Ostracoda	0	+	+	+	+	+	+	+	+	0
Copepoda	0	+	0	0	0	0	+	0	+	0
Odonata										
Austrolestes colensonia	0	0	+	0	+	0	0	0	0	+
Xanthocnemis zealandica	+	+	+	+	+	+	0	+	0	+
Aeshna brevistyla	0	0	0	0	+	0	0	0	0	0
Hemicordulia australiae	0	0	0	0	0	0	0	0	0	0
Procordulia grayi	+	0	0	0	0	0	0	0	0	0

Continued on next page

Appendix 2—continued

	6 MONTHS (*n* = 2)		1 YEAR (*n* = 4)				3 YEARS (*n* = 4)			
	IR	UD	H	W	D	WN3	M	C	G	Wm
Lepidoptera										
Hygraula nitens	0	0	+	+	0	0	0	0	0	+
Trichoptera										
Oxyethira albiceps	0	0	0	0	+	0	0	0	0	0
Paroxyethira sp.	0	0	0	0	0	0	0	0	0	0
Paroxyethira hendersoni	+	0	0	+	0	0	0	0	0	0
Ocetis unicolor	0	0	0	0	0	0	0	0	0	0
Triplectides cephalotes	0	+	+	0	0	0	+	+	+	0
Hemiptera										
Sigara spp.	+	+	+	+	+	+	+	+	+	+
Coleoptera										
Antiporus strigosulus	0	0	0	+	0	+	0	0	+	+
Lancetes lanceolatus	0	0	0	+	0	0	0	0	0	0
Scirtidae	0	0	0	0	0	0	0	+	0	0
Enochrus tritus	0	0	0	0	0	0	0	0	+	+
Limnoxenus zelandicus	0	0	0	0	0	0	0	0	0	0
Diptera										
Zelandotipula sp.	0	0	0	+	0	0	0	0	0	0
Limonia sp.	0	0	0	0	0	0	0	0	0	+
Paralimnophila skusei	0	0	+	0	0	0	0	0	0	0
Tanypodinae	0	0	+	0	0	0	0	+	0	0
Chironominae	+	+	+	+	0	+	+	+	+	+
Orthocladiinae	0	+	+	+	+	0	+	+	+	0
Corynoneura scutellata	0	0	0	0	0	0	0	0	0	0
Neolimnia sp.	0	0	0	0	0	0	0	0	+	0