



Effects of rotenone on aquatic invertebrate communities in prairie wetlands

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Abstract

We assessed the effects of rotenone on aquatic invertebrate communities by comparing four prairie wetlands treated with rotenone to four control sites. Data collected one week before and three weeks after treatment in the fall of 1998 were paired to assess short-term effects, while data collected in spring 1998 and spring 1999 were paired to assess longer-term effects and recovery rates. Data were collected on 14 taxa of benthic invertebrates collected in Ekman grab samples, and 23 taxa of planktonic-nektonic invertebrates collected in water-column samples. Each data set was analyzed separately with redundancy analysis to assess effects in the two habitats sampled. Significant short-term effects were detected on invertebrates in the water column and abundance of several taxonomic groups declined sharply after treatment. The greatest declines were observed in zooplankton abundance; effects on macroinvertebrates were much less pronounced. Suppression of water-column taxa was short-lived, as significant effects were no longer evident during May 1999. In contrast, no significant short-term effect was evident in the benthic taxa. Our results indicate that fall applications of rotenone may briefly suppress plankton communities, but effects are short-lived. From a fisheries management perspective, fall applications may minimize effects on invertebrate communities and facilitate rapid recovery.

Introduction

Rotenone is a widely applied fish toxicant and has been used since the 1930s (Wiley & Wydoski, 1993). Rotenone kills fish by blocking reoxidation of nicotinamide adenine dinucleotide (NADH) (Horgan et al., 1968), thereby inhibiting respiration at the cellular level. Applications have been made in lakes, rivers, and ponds (Schnick, 1974), and common uses include removal of undesirable fish species (Wiley & Wydoski, 1993) and experiments to assess the ecological role of fish in aquatic ecosystems (Reinertsen et al., 1990). Rotenone is appealing to management agencies and scientists because it detoxifies rapidly

in warm water, is environmentally non-persistent and non-toxic to most mammals and birds, is fairly inexpensive, and readily available (Davies & Shelton, 1983; Wiley & Wydoski, 1993).

Due to the non-specific toxicity of rotenone, potential influences on non-target organisms have been discussed for decades (Zischkale, 1952; Cushing & Olive, 1957). Of particular concern are effects on aquatic invertebrate communities, due to their importance in aquatic food webs and especially their role as a food base for fish introduced following rotenone application. Ideally, a field study assessing the effect of rotenone on invertebrate communities would: (1) assess the influence at the community level as well as

effects on specific taxa, (2) assess the recovery rates of effected taxa, and (3) include replication of control and treated ecosystems to clarify whether observed changes were due to rotenone or unknown variables (Hurlbert, 1984). Numerous studies have assessed the influence of rotenone on invertebrate communities in a variety of habitats (Koksvik & Aagaard, 1984; Rach et al., 1988; Dudgeon, 1990; Reinertsen et al., 1990; Naess, 1991; Beal & Anderson, 1993; Mangum & Madrigal, 1999). To our knowledge, no previous study possessed all three features listed above, making it difficult to fully discern non-target effects of rotenone on invertebrate communities. Considerable uncertainty remains regarding taxon-specific effects (Almquist, 1959; Koksvik & Aagaard, 1984; Mangum & Madrigal, 1999), susceptibility of invertebrates in different habitats (Lindgren, 1960), and recovery rates for specific taxa (Schnick, 1974; Beal & Anderson, 1993).

Here, we assess non-target influences of rotenone application on aquatic invertebrates in prairie wetlands using data collected over two years in four treated and four control sites. This design allowed us to assess short-term responses of invertebrates using community- and taxon-based approaches, and to assess the recovery rates of effected taxa. Our analyses focused on both benthic and planktonic-nektonic taxa.

Methods

Field sampling

The eight wetlands used in our study all had a semi-permanent hydroperiod (following classification of Stewart & Kantrud, 1971) drying approximately once every 10 years. All wetlands were located on US Fish and Wildlife Service lands in west-central Minnesota, U.S.A., and uplands were vegetated mainly by prairie grasses. Four wetlands served as controls (control sites) and four were treated with rotenone (treatment sites). The average surface area and maximum depth of the treatment sites were 5.47 ha (range 4.05–8.1 ha) and 1.34 m (range 1.08–1.66 m), and for the control sites 3.57 ha (range 1.58–5.75 ha) and 1.42 m (range 1.22–1.58 m). A liquid formulation of rotenone (Noxfish, 5% by volume active ingredient) was applied to the treatment sites by aerial application on 8 October 1998, resulting in a 3 mg l^{-1} concentration of rotenone. Our goal was to assess both short- and long-term effects on invertebrates in both the water

column and benthos. To assess short-term effects in both habitats, the study sites were sampled 1 week prior to application and 3 weeks after. For long-term effects and recovery rates of invertebrates, all wetlands were also sampled on six occasions during late spring through summer in both 1998 and 1999.

Samples were collected along five random transects established in each wetland in the summer of 1998, fall of 1998 and summer of 1999. Two sampling stations were established along each transect, one at the interface of emergent vegetation and open water (emergent station) and the other one-half the distance from the emergent station to the center of the wetland (open-water station). We sampled invertebrates in both the water column and benthos along each transect, and each data set (benthos and water column) was analyzed separately. On each sampling date we sampled water-column invertebrates with both activity traps (ATs) (Murkin et al., 1983) and column samples (Swanson, 1978). Ten ATs were deployed for 24 h in each wetland, one at each sampling station, and sample contents were condensed using a $140 \mu\text{m}$ mesh funnel. Column samples were taken concurrently with AT samples at the five open-water stations, and sample contents were condensed with a $68 \mu\text{m}$ funnel. Thus 15 water-column samples were taken in each wetland on each date. On each date, the benthic community was also sampled by taking one Ekman sample at each of the five open-water stations, and sample contents were condensed using a 0.5 mm mesh funnel. Invertebrates in all samples were preserved in 70% ethanol identified to the lowest feasible taxonomic level, and counted. We summed the contents of the 15 water-column samples for each of 24 taxonomic groups in each wetland, resulting in one observation for each taxon on each date. In the same manner, contents of the five Ekman samples in each wetland were summed for each of 14 taxonomic groups, again giving one observation for each taxon on each date. The sum of each taxonomic group in each wetland on each date was then $\ln(n+1)$ transformed to prevent abundant taxa from dominating the results. This was done for both the water column and benthic data sets.

Statistical analysis

We used a matched-pairs design to test for effects of the invertebrate communities in both the water column and benthic data sets, with data from the pre-treatment (Before) period in each wetland paired with data from the post-treatment (After) period. For short-term ef-

fects, data collected in each wetland 1 week before and 3 weeks after treatment were paired and the difference between sampling dates (Before–After) was determined for each taxon in each wetland. This approach resulted in four replicates of both treatment and control sites, with 24 response variables in the water-column data and 14 in the benthic data. If significant short-term effects at the community level were detected, we then assessed long-term effects and recovery rates. To assess long-term effects, data collected on six sampling dates in spring and summer of 1998 (Before) were paired with data collected on the same dates in 1999 (After), with the difference again determined for each date (Before–After). This resulted in six long-term sampling dates, with the dates ranging from early May to late August in 3-week intervals. Each long-term sampling date was then analyzed separately for significant change between 1998 and 1999 in the treated wetlands.

Our goal was to determine whether there was a significant effect at the community level, and to then identify specific taxa most affected by rotenone if a significant community-level effect was detected. Use of MANOVA to test for an overall effect, followed by multiple contrasts on individual taxa, may appear to be a suitable statistical analysis. However, analyses with MANOVA are restricted such that the number of response variables must be less than the error degrees of freedom (Rencher, 1998). In our case, we would be restricted to analysis of only six invertebrate taxa. Thus, we tested for significant effects at the community level using direct-gradient analysis (Ter Braak & Verdonschot, 1995; Van Wijngaarden et al., 1995). Preliminary ordinations with detrended correspondence analysis showed lengths of axes in all data sets to be less than 1.5 standard deviations, and so we chose the linear model of direct-gradient analysis (redundancy analysis, RDA) over the unimodel model (canonical correspondence analysis) (Ter Braak, 1995). RDA has been used in several studies assessing the effects of chemical applications and environmental change on aquatic communities (Ter Braak & Wiertz, 1994; Verdonschot & Ter Braak, 1994; Van Wijngaarden et al., 1995). This technique is similar to MANOVA, but does not restrict the number of response variables (Verdonschot & Ter Braak, 1994). Also, because significance is tested with Monte Carlo permutations, RDA does not require the assumption of multivariate normality (Manly, 1990). RDA integrates ordination and multivariate regression, such that species are analyzed simultaneously and modeled as a function of

axes that are linear combinations of environmental variables (Ter Braak, 1994; Ter Braak & Smilauer, 1998). In our case, we have only one qualitative environmental variable (rotenone), so axis 1 is the only canonical axis. To test for a significant effect of rotenone, the variance in all taxa explained by axis 1 is determined, and the explained variance is then divided by the residual variance to produce a partial F -ratio (Ter Braak & Smilauer, 1998). Significance of the observed F ratio is determined by randomly reassigning wetlands to either treatment group and determining the F ratio of each randomization (Verdonschot & Ter Braak, 1994). Numerous randomizations are performed, and the proportion of randomly generated F -ratios that meet or exceed the observed F -ratio represents the P value.

We used species-centered RDA, and all ordination diagrams are in distance scaling with site scores as linear combinations of environmental variables to fully display effect sizes of the rotenone treatment (Ter Braak & Wiertz, 1994; Ter Braak & Smilauer, 1998). Our use of species-centered RDA and differences of log values between the Before and After sampling dates prevents abundant and/or rare taxa from dominating the results (Ter Braak, 1995). As RDA was performed on the differences of log values between sampling dates (Before–After), the analyses are actually conducted on the change in each species, not on their actual abundance. Thus, species vectors will point in the direction of greatest decrease in abundance over the Before to After time period.

To identify specific taxa effected by rotenone, we estimated the average change in abundance between sampling dates for taxa with greater than 20% of variance fit by the first RDA axis. For this analysis, the average difference of log values between sampling dates (Before–After) and 95% confidence intervals were determined for each taxon in both the treatment and control wetlands. These means and confidence intervals were then back transformed to estimate multiplicative change in abundance of each taxon between the Before and After sampling dates. Confidence intervals that include 1 indicate that no significant change occurred between sampling periods.

Results

RDA on the short-term water column data indicated that the application of rotenone had a significant effect on the invertebrate communities ($P = 0.027$) (Fig.

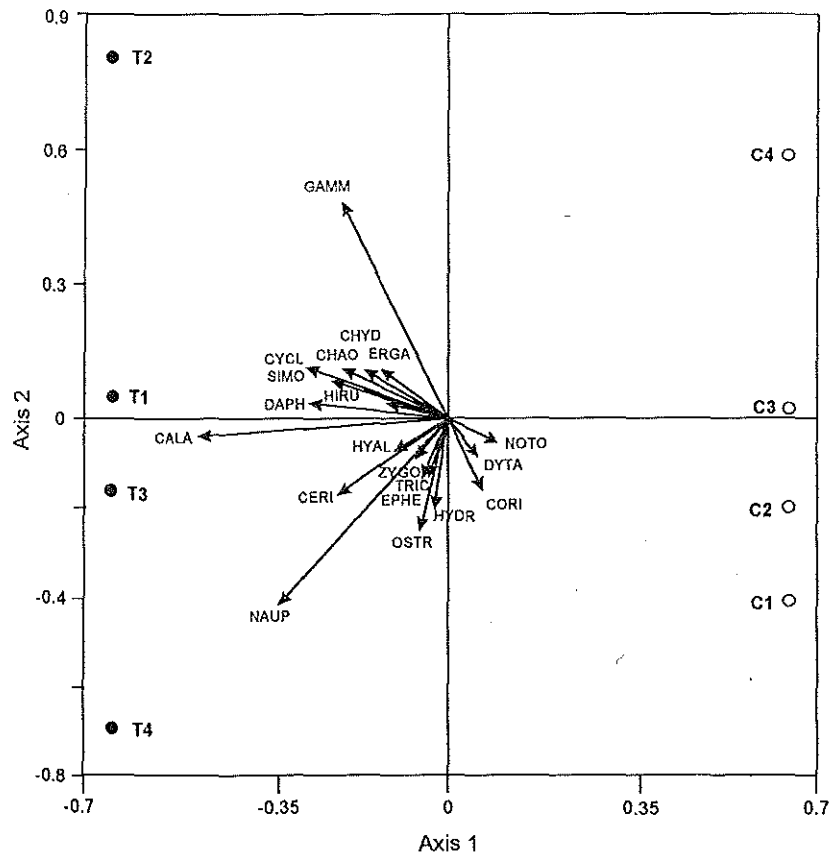


Figure 1. Results of RDA performed on the short-term water column data. The analysis was performed on the changes in abundance of each taxon in each wetland sampled one week before and three weeks after the rotenone application. Because the analysis was performed on the differences (Before-After) of $\ln(n+1)$ values, each taxon vector points toward sites where abundance decreased the most from the Before to After sampling dates. For the site labels, the letter designates the treatment group (T= treatment wetland, C= control wetland) and the number the specific site. Acronyms represent the following invertebrate taxa in this and subsequent figures: CALA= calanoid copepods, CERI= *Ceriodaphnia*, CHAO= *Chaoborus*, CHR= Chironomidae, CHYD= Chydoridae, CORI= Corixidae, CYCL= cyclopoid copepods, DAPH= *Daphnia*, DYTA= adult Dytiscidae, EPHE= Ephemeroptera, ERGA= *Ergasilus*, GAMM= *Gammarus*, HALA= adult Haliplidae, HALL= larval Haliplidae, HIRU= Hirudinoidea, HYAL= *Hyalella*, HYDR= Hydracarina, NAUP= Nauplii, NOTO= Notonectidae, PLAN= Planorbidae, PLEI= Pleidae, SIMO= *Simocephalus*, TRIC= Trichoptera, OSTR= Ostracoda, ZYGO= Zygoptera.

1). Axis 1 (representing differences between treatment groups) explained 42%, and the second axis 21%, of the total variance in change between sampling dates. In our RDA diagrams, species vectors point towards sites in which the decrease in abundance from the Before to After period was greatest, with longer vectors indicating greater differences between sites. The species vectors indicated that the abundances of calanoid copepods, *Daphnia*, cyclopoid copepods, *Ceriodaphnia* and *Simocephalus* were most reduced by rotenone. Nauplii and *Gammarus* also declined following the treatment, but these effects were more variable among the treated sites, as scores of these taxa on axis 2 were further from the origin relative to the species listed above. Axis 2 was largely a gradient of variability in

change in abundances of ostracods, hydracarina, and Corixidae; abundances of these taxa were not affected by the rotenone treatment but were variable among both treated and control sites.

Assessing average change in individual taxa between sampling dates provided results similar to the species vectors in RDA, with the most pronounced effects largely restricted to zooplankton taxa (Table 1). Of the 13 taxa with greater than 20% fit on the first RDA axis, reduced abundance in the treatment sites was evident for calanoid copepods, *Ceriodaphnia*, *Daphnia*, cyclopoid copepods, nauplii, Hirudinoidea, *Chaoborus* and *Simocephalus*. Effect sizes for these taxa ranged from *Simocephalus* being 5 times as abundant before treatment relative to after, to calanoid

Table 1. Multiplicative change observed (95% confidence interval) in the abundance of taxa in the treatment and control wetlands based on water-column data. Multiplicative change represents how many fold greater was the abundance of each taxon in the Before period relative to the After period. Short-term effects represent the change from one week before application of rotenone to 3 weeks after; long-term effects are the change between spring of 1998 (before application) and the same date in 1999 (after application). Confidence intervals that do not include one indicate a significant change between time periods and are indicated with an asterisk. Taxa shown are those with greater than 20% of variation fit by axis 1 in the short-term RDA

Taxon	Short-term		Long-term	
	Treatment wetlands	Control wetlands	Treatment wetlands	Control wetlands
Calanoid copepods	1708.2 (1074–2717)*	3.6 (2.6–4.9)*	5.7 (3.0–10.6)*	0.3 (0.1–1.1)
<i>Ceriodaphnia</i>	38.0 (14.4–100.0)*	2.7 (0.6–12.6)	1.4 (0.2–10.9)	1.2 (0.1–16.6)
<i>Daphnia</i>	30.4 (9.3–99.6)*	1.2 (0.3–4.5)	1.7 (0.7–4.5)	0.5 (0.1–1.7)
Cyclopoid copepods	32.0 (9.1–113.0)*	1.1 (0.2–4.6)	1.8 (0.6–5.2)	0.6 (0.1–2.9)
Nauplii	92.3 (7.4–1160)*	0.9 (0.03–24.6)	1.6 (0.01–168.7)	0.9 (0.01–57.6)
Hirudinoidea	7.5 (3.8–14.6)*	2.3 (0.7–7.7)	2.4 (0.4–15.1)	2.4 (0.3–18.8)
<i>Chaoborus</i>	5.9 (3.1–11.3)*	0.9 (0.2–4.4)	15.0 (3.8–60.1)*	4.3 (0.9–19.3)
<i>Simocephalus</i>	4.8 (3.0–7.6)*	0.6 (0.08–4.1)	0.6 (0.2–1.9)	13.4 (1.5–121.6)*
<i>Ergasilus</i>	2.6 (0.8–8.2)	0.5 (0.2–1.3)	1.5 (0.1–19.1)	0.5 (0.2–1.2)
<i>Gammarus</i>	5.8 (0.7–44.9)	0.2 (0.01–3.6)	2.6 (0.5–14.9)	0.8 (0.6–1.1)
<i>Hyalella</i>	3.7 (0.6–21.5)	1.2 (0.7–2.0)	3.7 (0.8–16.7)	0.2 (0.01–6.1)
Notonectidae	1.5 (0.6–3.9)	3.4 (1.8–6.3)*	0.9 (0.7–1.3)	1.1 (0.3–3.5)
Chydoridae	5.9 (0.4–95.7)	0.4 (0.02–10.8)	6.0 (0.6–64.8)	7.0 (0.2–267.9)

copepods being 1708 times as abundant before treatment compared to after. In contrast, significantly reduced abundances in the control sites were detected for calanoid copepods and notonectids only, with the change in abundance of calanoid copepods much less than that observed in the impact sites (Table 1).

We subsequently analyzed water-column data paired between spring of 1998 and spring of 1999 to assess long-term effects and recovery rates. RDA on the first sampling date in the long-term water column data indicated no significant effect in the impact sites ($P=0.206$) (Fig. 2). The first axis explained substantially less variance in the changes in abundance of taxa between dates than did the short-term RDA (17% and 42%, respectively), while the second axis in the long-term RDA explained more variation than the same axis in the short-term RDA (30% and 21%, respectively). For the treatment sites, reduced abundances in 1999 relative to 1998 were most pronounced for *Hyalella* and calanoid copepods, while *Simocephalus* and ostracods decreased the most in the control sites. These taxa largely drive axis 1, while axis 2 is a gradient of change in the abundance of nauplii. Abundances of most taxa that exhibited short-term effects were similar between 1998 and 1999; significant long-term effects were detected only for calanoid copepods and *Chaoborus* (Table 1). Excluding these two taxa, long-

Table 2. Multiplicative change observed (95% confidence interval) in the abundance of taxa in the treatment and control wetlands in the benthic data. Multiplicative change represents how many fold greater was the abundance of each taxon in the Before period relative to the After period. The short-term effect represents the change from one week before application of rotenone to 3 weeks after. RDA did not indicate a significant short-term effect at the community level, so long-term effects were not assessed. Confidence intervals that do not include one indicate a significant change between time periods and are indicated with an asterisk. Taxa shown are those with greater than 20% of variation fit by axis 1 in the short-term RDA

Taxon	Short-term	
	Impact wetlands	Control wetlands
<i>Hyalella</i>	13.7 (4.5–41.8)*	1.1 (0.5–2.5)
<i>Chaoborus</i>	3.4 (1.4–8.0)*	1.0 (0.7–1.2)
<i>Gammarus</i>	9.1 (0.7–120.6)	3.3 (0.9–12.3)
Pleidae	1.3 (0.8–2.3)	1.5 (0.6–3.9)
Chironomidae	1.2 (0.9–1.7)	4.6 (0.4–53.7)
Hirudinoidea	1.1 (0.6–2.0)	0.9 (0.7–1.2)
Zygoptera	1.0 (0.6–1.7)	1.7 (0.6–4.6)

term changes observed in the treatment sites were similar to those observed in the control sites, and *Simocephalus* was the only taxon for which a significant long-term change was detected in the control sites (Table 1).

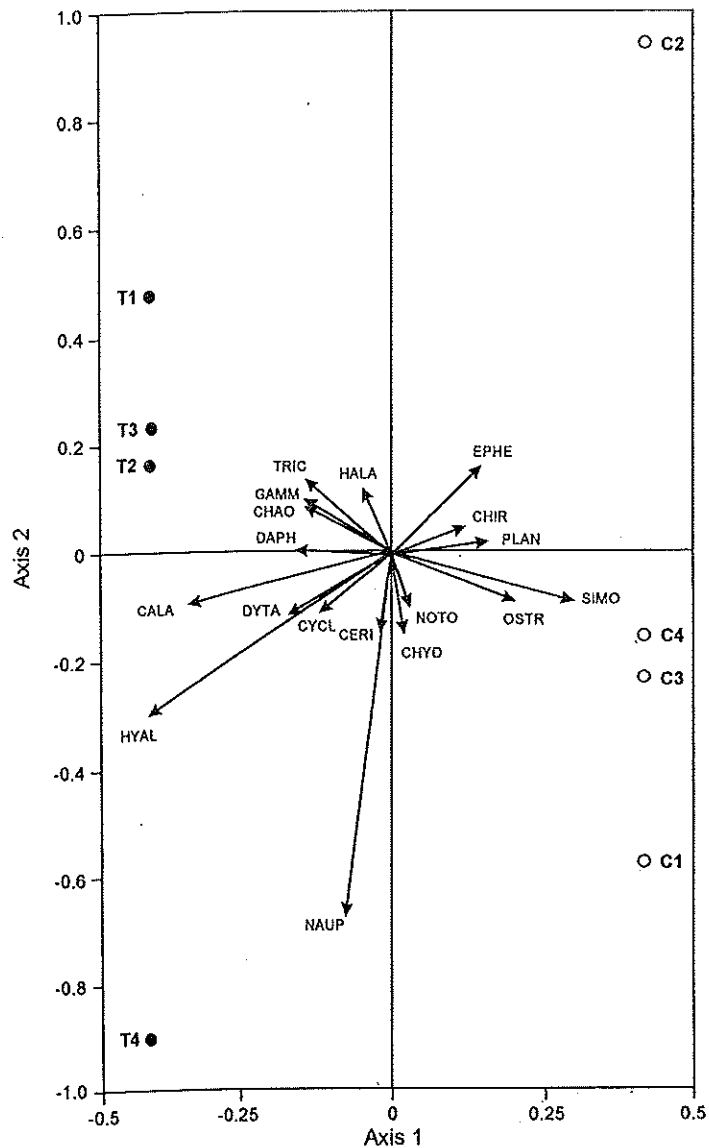


Figure 2. Results of RDA performed on the long-term water column data. The analysis was performed on the changes in abundance of each taxon in each wetland sampled the second week of May in 1998 (Before treatment) and the same date in 1999 (After treatment). Because the analysis was performed on the differences (Before-After) of $\ln(n+1)$ values, each taxon vector points toward sites where abundance decreased the most from the Before to After sampling dates. Site labels and invertebrate acronyms are defined in Figure 1.

We detected no effect of rotenone in the benthic data ($P=0.208$) (Fig. 3). Axis 1 explained only 24% and the second axis 27% of the variance in change between sampling dates. Decreased abundance between dates in the treatment sites was greatest for *Hyaella*, *Chaoborus*, *Gammarus* and Hirudinoidea, while Chironomidae and Zygoptera decreased the most in the control wetlands. Axis 2 was largely driven by differences in the change in abundance of *Gam-*

marus; thus, any effect of rotenone on *Gammarus* appears much more variable between sites than for *Hyaella* and *Chaoborus*. Seven taxa had greater than 20% variation fit by the two RDA axes, and assessment of the change between time periods indicated that abundances of *Hyaella* and *Chaoborus* were significantly reduced in the treatment sites (Table 2). No significant changes were detected in these seven taxa in the control sites. As no significant short-term ef-

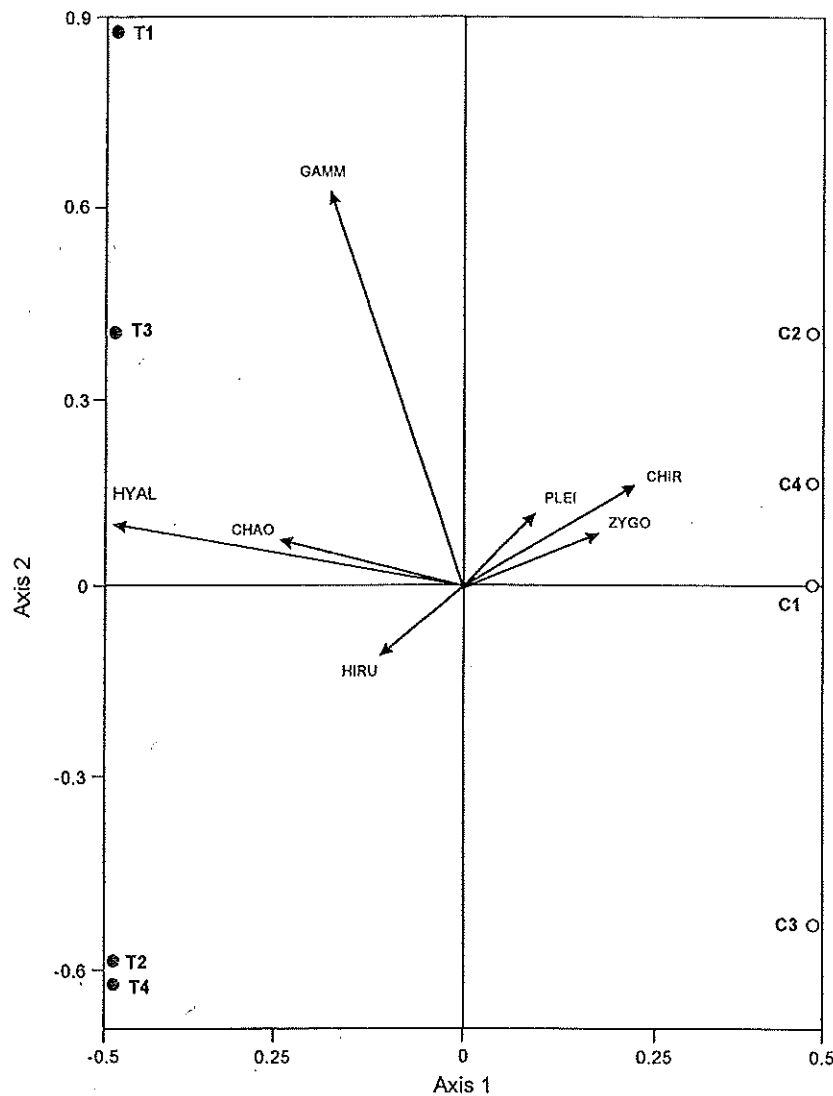


Figure 3. Results of RDA performed on the short-term benthic data. The analysis was performed on the changes in abundance of each taxon in each wetland sampled one week before and three weeks after the rotenone application. Because the analysis was performed on the differences (Before-After) of $\ln(n+1)$ values, each taxon vector points toward sites where abundance decreased the most from the Before to After sampling dates. Site labels and invertebrate acronyms are defined in Figure 1.

fect was detected in the benthic data at the community level, we did not test for long-term effects.

Discussion

Influences of rotenone differed between water column and benthic habitats; significant short-term effects were detected only in the water column. However, even in the water-column, strong effects were restricted largely to zooplankton, and nearly all taxa re-

covered by May the following spring. Overall, our results indicate that fall applications of rotenone appear to have no long-term effects on aquatic invertebrates we considered in prairie wetlands.

Sharp reductions in zooplankton abundances appear to be a common consequence of rotenone application, and have been documented in previous studies (Anderson, 1970; Schnick, 1974; Rach et al., 1988; Beal & Anderson, 1993). However, zooplankton recovery rates vary considerably, with time to full recovery varying from 8 months (Beal & Anderson,

1993) to 3 years (Anderson, 1970). We did not sample between October of 1998 and May of 1999, and so we were unable to assess whether affected taxa recovered prior to May of 1999. However, with the exception of calanoid copepods, our results are consistent with those of Beal & Anderson (1993) in that we observed full recovery in a matter of months instead of years. Variations in zooplankton recovery times are likely due to different species assemblages and their respective life-history strategies, as well as to differences between habitats. Anderson (1970) assessed effects on zooplankton in two mountain lakes in Alberta, whereas the work of Beal & Anderson (1993) and our study were conducted in shallow, productive ecosystems in the Midwestern US. Fall applications of rotenone were assessed in all three studies, and both cladocera and copepods commonly produce resting stages at this time of the year and these stages may be resistant to rotenone (Beal & Anderson, 1993). However, at least one taxon had not reached sexual maturity at the time of treatment in the Alberta study, and so the application may have occurred when a greater proportion of taxa were vulnerable relative to the two Midwestern studies. Colder water temperatures in the Alberta lakes would also lengthen generation times of zooplankton relative to zooplankton in the Midwestern US (Pennak, 1989), leading to lengthened recovery times. Additionally, recovery rates in the Midwestern sites might be further stimulated by higher rates of primary production.

In contrast to results for zooplankton, we did not detect a strong effect on macroinvertebrate abundances, as significant reductions were observed in only *Chaoborus* and Hirudinoidea. These results are similar to those of Koksvik & Aagaard (1984), who reported that *Chironomus* was the only benthic macroinvertebrate reduced in a eutrophic lake following rotenone application. Though most studies report sharp reductions in zooplankton following rotenone treatment, results for macroinvertebrates are much more varied, both between studies (summarized in Lindgren, 1960) and among taxonomic groups (Almquist, 1959; Lindgren, 1960; Meadows, 1973; Koksvik & Aagaard, 1984; Mangum & Madrigal, 1999). Overall, it seems that rotenone effects on macroinvertebrates are much less pronounced and more variable than effects on zooplankton, and our results strongly support this notion. We observed considerable variability in our macroinvertebrate data; a significant short-term effect on the abundance of Hirudinoidea was evident in the water column, but not in the benthic data. Previous stud-

ies have also reported mixed results for susceptibility to rotenone among taxa of Hirudinoidea, with results varying from 0 to 100% mortality (summarized in Lindgren, 1960). Such discrepancies may reflect behavior differences among taxa, with affected leeches more likely to be found in the water column and resistant taxa more restricted to the benthos. Lindgren (1960) suggested that highly organic sediments provide a refuge for benthic invertebrates from rotenone, and our results may be due to differential effects between benthic and nektonic-orientated species of Hirudinoidea. In contrast to Hirudinoidea, significant effects on *Chaoborus* were detected in both the water column and benthic data. Reasons for the reduction in these insects are unclear. Tolerance to rotenone varies widely among macroinvertebrates (Almquist 1959; Mangum & Madrigal, 1999) and fish (Marking & Bills, 1976), and *Chaoborus* may simply be less tolerant than the other insects we studied. Additionally, the meroplanktonic behavior of *Chaoborus* may increase its exposure to rotenone relative to other insects, as sediments and macrophytes are thought to provide a refuge from rotenone (Lindgren, 1960). The longer recovery time for *Chaoborus* relative to most zooplankton taxa was likely due to their reproductive cycle. Most *Chaoborus* species in temperate climates overwinter as larvae and reproduce in late spring (Saether, 1997); thus, it is unlikely that any reproduction occurred between the fall treatment and our May sampling date.

It may be notable that effects on certain invertebrate taxa were highly variable among wetlands particularly for *Gammarus*. Though effects were not significant, sharp reductions were observed in some wetlands while little change was observed in others. This may reflect ecological interactions between rotenone toxicity and chemical or physical features of different ecosystems, and highlights the importance of replication in evaluating rotenone effects. Had we sampled only one treated wetland, we might have concluded that rotenone sharply reduced the abundance of *Gammarus*, while a different wetland may have led us to the conclusion that rotenone had no effect. Instead we reach the more informed conclusion that effects of rotenone on *Gammarus* appears to be highly variable among ecosystems.

We sampled benthic invertebrates less intensively than water-column invertebrates, and this may have reduced our ability to detect significant effects in the benthic habitat. However, we did detect significant short-term effects on two taxa in the benthic data

while estimates of change in other taxa (excluding *Gammarus*) were close to zero with relatively narrow confidence-intervals (see Table 2). This suggests that our sampling intensity was sufficient to detect significant effects on these benthic taxa. Nonetheless, increased sampling intensity might have clarified whether rotenone affected *Gammarus* differently than the related *Hyalella*. Thus, results for our benthic data should be interpreted in light of our sampling intensity.

Our results have considerable relevance to questions regarding non-target effects on aquatic invertebrates in shallow eutrophic waters. Rotenone application is quite common in shallow lakes throughout North America and Europe (e.g. Reinertsen et al., 1990; Hanson & Butler, 1994). The physical, chemical and biological characteristics of shallow lakes are likely quite similar to the prairie wetlands we studied, so results here are probably more applicable to shallow lakes than results obtained in deeper, less productive lakes. Our results have additional utility in that the treated wetlands were fishless prior to rotenone application, thereby eliminating confounding influences of fish predation. Eliminating fish populations results in functionally different food webs, and the composition of the invertebrate communities are likely to change dramatically regardless of chemical effects. This may confound assessment of recovery and community structure during the post-treatment period (Koksvik & Aagaard, 1984). This difficulty is avoided in our study, as treated communities were expected to return to pretreatment compositions and major differences were likely due to the rotenone application.

One potential source of error, especially in the short-term data, was distinguishing animals in samples that were killed by the preservative from those that were killed by rotenone. However, this difficulty exists only with our Ekman samples, as invertebrates collected in the column samples and activity trap samples had to be present in the water column to be captured. We also chose a 3-week delay after treatment to allow decomposition of animals killed by rotenone, and we found that we could readily distinguish between freshly killed animals (those that were alive in our samples) and those killed previously by rotenone.

Our results indicate that fall applications of rotenone in prairie wetlands have significant, short-term effects on some invertebrates. However, sharp reductions in abundance are largely restricted to zooplankton, and nearly all affected taxa recovered by the following spring. From a fisheries management per-

spective, fall applications may be desirable for two reasons. First, fall applications minimize impacts on zooplankton because many taxa exist primarily as resistant resting stages at this time of the year, and these resting stages provide stock for population recovery the following spring. Second, fall applications appear to provide adequate recovery time for zooplankton, such that by spring, there is a considerable forage base for fish stocked subsequent to rotenone treatment.

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References

- Almquist, Z., 1959. Observations of the effects of rotenone emulsives on fish food organisms. *Inst. Freshw. Res. Drottningholm* 40: 146-160.
- Anderson, R. S., 1970. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. *J. Fish Res. Bd Can.* 27: 1335-1356.
- Beal, D. L. & R. V. Anderson, 1993. Response of zooplankton to rotenone in a small pond. *Bull. envir. Contam. Toxicol.* 51: 551-556.
- Cushing, S. F., Jr. & J. R. Olive, 1957. Effects of toxaphene and rotenone upon the macroscopic bottom fauna of two northern Colorado reservoirs. *Trans. Am. Fish. Soc.* 86: 294-301.
- Davies, W. M. & W. L. Shelton, 1983. Sampling with toxicants. In Nielsen, L. A. & D. L. Johnson (eds), *Fisheries Techniques*. American Fisheries Society, Bethesda (M.D.): 199-214.
- Dudgeon, D., 1990. Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing rocks on two Papua New Guinea streams. *Arch. Hydrobiol.* 119: 35-53.
- Hanson, M. A. & M. G. Butler, 1994. Responses of plankton, turbidity, and macrophytes to biomanipulation in a shallow prairie lake. *Can. J. Fish. Aquat. Sci.* 51: 1180-1188.
- Horgan, D. J., T. P. Singer & J. E. Casida, 1968. Studies on the respiratory chain-linked reduced nicotinamide adenine dinucleotide dehydrogenase. XIII. Binding sites of rotenone, piericidin A and amyltal in the respiratory chain. *J. Biol. Chem.* 243: 834-846.
- Hurlbert, S. H., 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54: 187-211.

- Koksvik, J. I. & K. Aagaard, 1984. Effects of rotenone on the benthic fauna of a small eutrophic lake. *Verh. int. Ver. Limnol.* 22: 658-665.
- Lindgren, P. E., 1960. About the effect of rotenone upon benthonic animals in lakes. *Inst. Freshw. Res. Drottingholm* 39: 172-184.
- Mangum, F. A. & J. L. Madrigal, 1999. Rotenone effects on aquatic macroinvertebrates of the Strawberry River, Utah: a five-year summary. *J. Freshwat. Ecol.* 14: 125-135.
- Manly, B. F. J., 1990. *Randomization and Monte Carlo Methods in Biology*. Chapman & Hall, London: 399 pp.
- Marking, L. L. & T. D. Bills, 1976. Toxicity of rotenone to fish in standardized laboratory test. U.S. Fish Wildl. Serv., Invest. Fish Control 72, Washington (D.C.): 11 pp.
- Meadows, B. S., 1973. Toxicity of rotenone to some species of coarse fish and invertebrates. *J. Fish Biol.* 5: 155-163.
- Murkin, H. R., P. G. Abbott & J. A. Kadlec, 1983. A comparison on activity traps and sweep nets for sampling nektonic invertebrates in wetlands. *Freshwat. Invertebr. Biol.* 2: 99-106.
- Naess, T., 1991. Marine calanoid resting eggs in Norway: abundance and distribution of two copepod species in the sediment in an enclosed marine basin. *Mar. Biol.* 110: 261-266.
- Pennak, R. W., 1989. *Fresh-water Invertebrates of the United States: Protozoa to Mollusca*, 3. John Wiley & Sons, New York: 628 pp.
- Rach, J. J., T. D. Bills & L. L. Marking, 1988. Acute and chronic toxicity of rotenone to *Daphnia magna*. U.S. Fish Wildl. Serv., Invest. Fish Control 92-94, Washington (D.C.): 5 pp.
- Reinertsen, H., A. Jensen, J. I. Koksvik, A. Langeland & Y. Olsen, 1990. Effects of fish removal on the limnetic ecosystem of a eutrophic lake. *Can. J. Fish. aquat. Sci.* 47: 166-173.
- Rencher, A. C., 1998. *Multivariate Statistical Inference and Applications*. John Wiley & Sons, New York: 559 pp.
- Saether, O. A., 1997. Diptera Chaoboridae, phantom midges. In Nilsson, A. N. (ed.), *Aquatic Insects of North Europe*. Apollo Books, Stenstrup, Denmark: 149-161.
- Schnick, R. A., 1974. A review of the literature on use of rotenone in fisheries. U.S. Fish and Wildl. Serv., Lit. Rev., 74-15. NTIS (National Technical Information Service) PB-235 454/AS, Spring Field (V.A.): 130 pp.
- Stewart, R. E. & H. A. Kantrud, 1971. Classification of natural ponds and lakes in the glaciated prairie region. U.S. Fish Wildl. Serv. Res. Publication Number 92, Washington (D.C.): 57 pp.
- Swanson, G. A., 1978. A plankton sampling device for shallow wetlands. *J. Wildl. Manage.* 42: 670-672.
- Ter Braak, C. J. F., 1994. Canonical community ordination. Part I: Basic theory and linear methods. *Ecoscience* 1: 127-140.
- Ter Braak, C. J. F., 1995. Ordination. In Jongman, R. H. G., C. J. F. Ter Braak & O. F. R. van Tongeren (eds), *Data Analysis in Community and Landscape Ecology*. Cambridge University Press, Cambridge: 91-173.
- Ter Braak, C. J. F. & P. Smilauer, 1998. *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination*, 4. Microcomputer Power, Ithaca New York: 351 pp.
- Ter Braak, C. J. F. & P. F. M. Verdonschot, 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquat. Sci.* 57: 255-289.
- Ter Braak, C. J. F. & J. Wiertz, 1994. On the statistical analysis of vegetation change: a wetland affected by water extraction and soil acidification. *J. Veg. Sci.* 5: 361-372.
- Van Wijngaarden, R. P. A., P. J. Van Den Brink, J. H. Oude Voshaar & P. Leeuwangh, 1995. Ordination techniques for analysing response of biological communities to toxic stress in experimental ecosystems. *Ecotoxicology* 4: 61-77.
- Verdonschot, P. F. M. & C. J. F. Ter Braak, 1994. An experimental manipulation of oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions: multivariate analyses with Monte Carlo permutation tests. *Hydrobiologia* 278: 251-266.
- Wiley, R. W. & R. S. Wydoski, 1993. Management of undesirable fish species. In Kohler, C. C. & W. A. Hubert (eds), *Inland Fisheries Management in North America*. American Fisheries Society, Bethesda (M.D.): 335-354.
- Zischkale, M., 1952. Effects of rotenone and some common herbicides on fish-food organisms. *Field Lab.* 20: 18-24.