

## EFFECTS OF THREE CONSECUTIVE ROTENONE TREATMENTS ON THE BENTHIC MACROINVERTEBRATE FAUNA OF THE RIVER OGNA, CENTRAL NORWAY

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### ABSTRACT

The effects of piscicides on aquatic invertebrates are often studied after one treatment, even though piscicides may be repeatedly applied within river management. Here we investigate the impacts of repeated piscicide treatment on riverine benthic invertebrates. The River Ogna, Norway, was treated with rotenone three times over a 16-month period. The two first treatments caused temporary density reduction of a few rotenone sensitive benthic invertebrate taxa. Effects of the third treatment were variable with some taxa unaffected while all Plecoptera, were locally extinct. The toxic effect of rotenone increases with water temperature and high water temperature (20 °C) combined with high rotenone concentration was probably the main reason why the benthic community in the third treatment was more negatively affected than during the two previous treatments (4 and 8 °C). Eight months after the treatment benthic densities had not reached pre-treatment levels, but most taxa had recolonized the treated area within a year. Our data suggest that the severe effects of the third treatment were not influenced by the two former ones. This implies that the timing of piscicide treatment has a greater impact on the benthic invertebrate community than the number of treatments. Copyright © 2015 John Wiley & Sons, Ltd.

KEY WORDS: rotenone treatment; benthic invertebrates; EPT; recovery; river

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### INTRODUCTION

The piscicide rotenone has been an important tool for fisheries management and research in the U.S. since the 1930s. Uses include manipulation of fish communities to maintain sport fisheries, quantification of fish populations (sampling), treatment of rearing facilities and eradication of exotic fish (Finlayson *et al.*, 2000). During the two last decades rotenone has been widely used in Norway to eradicate the salmon parasite *Gyrodactylus salaris* Malmberg, which causes high mortality in Atlantic salmon (*Salmo salar* L.) juveniles. The aim of the treatments is to eradicate the parasite by killing all salmon present in a watershed, since the parasite can only survive for a short time period without a host.

Rotenone affects cellular aerobic respiration, blocking mitochondrial electron transport by inhibiting NADH-ubiquinone reductase (Singer and Ramsey, 1994). The toxicity of rotenone to fish is very high (Ling, 2003), although non-target organisms including benthic invertebrates may also be affected during rotenone treatments (Morrison, 1977; Mangum and Madrigal, 1999; Gladsø and Raddum, 2002; Eriksen *et al.*, 2009). A number of studies investigating

the effects of rotenone on invertebrates have been conducted and many report severe impacts (Binns, 1967; Arnekleiv *et al.*, 1997; Hamilton *et al.*, 2009). On the other hand, minor effects have also been registered (e.g. Cook and Moore, 1969; Koksvik and Aagaard, 1984; Dudgeon, 1990). Generally lotic invertebrates seem to be more sensitive to rotenone compared to lentic taxa. Taxa from the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) are particularly sensitive, although some species within these orders are tolerant (Mangum and Madrigal, 1999; Arnekleiv *et al.*, 2001). Some studies report tolerance differences in larval stages, with early stages more sensitive than later stages (Gladsø and Raddum, 2002; Kjærstad and Arnekleiv, 2011). Recovery time of invertebrate densities after rotenone treatments varies, but has been registered as fast as within a year (Binns, 1967; Kjærstad and Arnekleiv, 2004). However, recovery of single taxa may take several years (Arnekleiv *et al.*, 1997; Mangum and Madrigal, 1999). Despite the relative high number of investigations of rotenone effects on invertebrates, the results are somewhat contradictory. This led Vinson *et al.* (2010) to suggest that the true impacts of rotenone on invertebrate assemblages are not well known.

Normally, investigations only report effects of rotenone on invertebrates from a single treatment. In this paper we document the effect of three subsequent treatments over a 16-month period. Other objectives were to assess the effects

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of rotenone on different taxa and the recovery time of the benthic community. We hypothesized that three consecutive rotenone treatments would result in higher short-term toxic effects on the macroinvertebrate community than each of them separately.

## METHODS

### Study area

The River Ognå is situated in Central Norway (Figure 1), draining mainly lowland areas and flowing into Trondheimsfjorden. It has a catchment area of 578 km<sup>2</sup> and a mean annual discharge of 22 m<sup>3</sup> s<sup>-1</sup>.

In 1980 *G. salaris* was found on juvenile salmon in the River Ognå. The river was treated with PW-rotenone in 1993, but for unknown reasons the parasite reoccurred in 1997. This study deals with the rotenone treatments where CFT-Legumin (liquid formulation with a 2.5% by volume active gradient) was applied three times over a two-year period, April and October 2001, as well as in August 2002. The environmental authorities aimed to keep a minimum concentration of 0.5-ppm rotenone solution during the treatments. Rotenone was applied at the upper end of the anadromous river reach at Støafoss, 18 km from the river mouth (Figure 1), as well as other points downstream the river to maintain the desired rotenone concentration. Boats equipped with pumps were used to spray rotenone on gravel banks and river margins parallel to the rotenone application. Drip

barrels were set up in anadromous parts of all tributaries, and backpack sprayers and watering cans were used to treat various types of wetlands adjacent to the rivers. Water temperatures during the treatments in April and October 2001 were approximately 4 °C and 8 °C, respectively. During the treatment in August 2002 the water temperature was approximately 20 °C.

### Sampling

To register benthic invertebrate densities, Surber samples were carried out at two stations, one rotenone treated and one untreated. Six replicates were taken on each station on each sampling occasion. The Surber sampler had an area of 900 cm<sup>-2</sup> and a mesh size of 250 µm. In order to enhance chances of detecting invertebrate taxa, three rotenone treated and three untreated stations were also sampled by a kick net. For each sampling occasion 1–2 kick samples, each of 1-min duration, were taken. The kick net had a square-shaped frame with an opening of 25 × 25 cm and a mesh size of 250 µm. Benthic samples were taken a few days before and a few days after each rotenone treatment, as well as on other occasions (see Table I for details).

### Statistical analyses

To assess the temporal response of the riverine invertebrate fauna to repeated rotenone treatment we used principal response curves. Principal response curves (PRC) are based upon redundancy analysis (RDA), and developed to

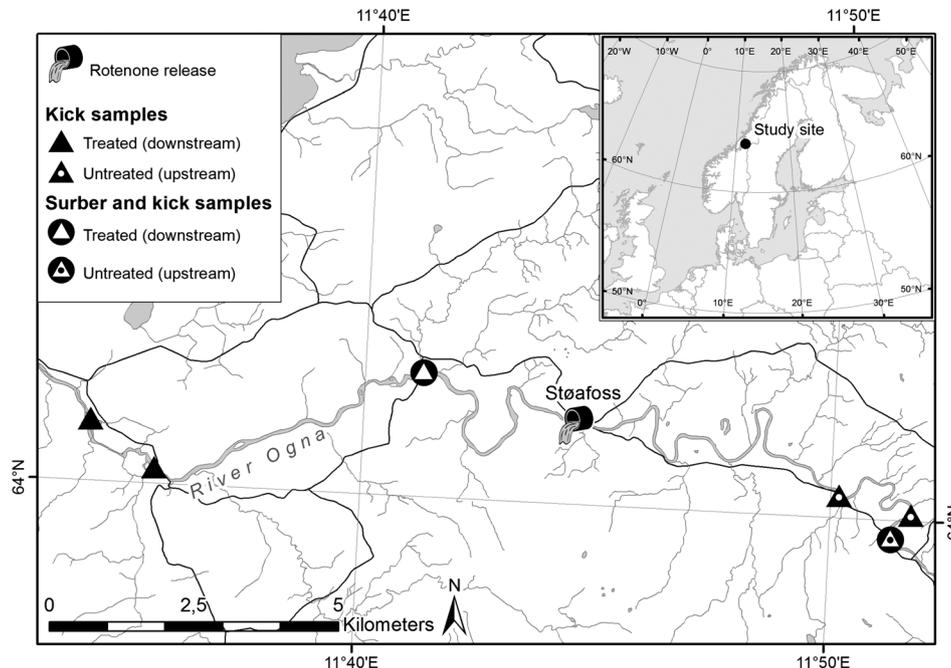


Figure 1. Study area and sampling stations

Table I. Dates of benthic sampling and rotenone treatments and number of days since the first, second and third treatments

Date	Surber sampling	Kick sampling	Rotenone treatment	Days since first treatment	Days since second treatment	Days since third treatment
18.04. 2001	x	x				
21.04. 2001			x			
24.04. 2001	x	x		3		
30.04. 2001	x	x		9		
07.05. 2001	x	x		16		
25.09. 2001	x	x		157		
04.10. 2001			x			
08.10. 2001	x	x		170	4	
15.10. 2001		x		177	8	
31.10. 2001		x		193	24	
07.08. 2002	x	x		473	304	
26.08. 2002			x			
28.08. 2002	x	x		494	325	2
05.09. 2002	x	x		502	333	8
22.10. 2002	x	x		549	280	55
29.04. 2003	x	x		738	569	244
17.06. 2003		x		787	618	293
25.08. 2003	x	x		856	687	362
29.04. 2004		x		1104	935	510

facilitate interpretation of multivariate response to an intervention within a repeated measures design (Van den Brink and Braak, 1999). Principal response curve analysis was carried out using the kick sample data. Counts were  $\log + 1$  transformed prior to analysis.

Invertebrate densities (Surber sampled data) were analysed for the total invertebrate fauna and for each of the EPT orders. Since this data was collected through overdispersed counts, it was analysed using quasi-Poisson family generalized linear modelling. Separate models were run for each sampling date, testing for an effect of treatment on the density of each group. The sample was taken as the unit. The differences in density of individual taxa were estimated using standardized mean differences to allow for comparison of rotenone treatment effect between taxa differing in background abundance. We estimated the standardized mean difference as the difference in mean density (treated – untreated) divided by the pooled standard deviation. Only the most abundant and consistently present taxa were analysed. Data were analysed in the R statistical environment version 3.0.2 (R Core Team, 2013). PRC analysis was carried out using the vegan package (Oksanen *et al.*, 2013)

## RESULTS

The differences in the invertebrate community composition between the treated and untreated stations rapidly increased after each of the three rotenone treatments (Figure 2).

However, a few days after the treatments the difference between the treated and untreated stations was reduced, indicating that the community composition between the sites became again more similar. On day 856, one year after the third and last treatment, the community composition between the two stations was as similar as in the baseline data.

The combined densities of all invertebrates showed only minor differences between the treated and untreated site immediately after the first treatment, but significantly higher densities in the untreated area on the first sampling occasions after the two last treatments (Figure 3). Significant differences in densities between the two sites were also registered 9, 16 and 549 days after the first treatment, but with the greatest densities in the treated area.

Densities within the EPT groups (Ephemeroptera, Plecoptera, Trichoptera) showed that Ephemeroptera had significant differences between the treated and untreated sites on all sampling occasions, except on the first sampling occasion three days before the first treatment, as well as one year after the third treatment (Figure 4a). Apart from the sampling three days prior to and nine days after the first treatment densities were highest at the untreated site. Plecoptera had significant higher densities at the untreated site on day 170 (one day after the second treatment) and on day 738 after the first treatment (8 months after the third treatment) (Figure 4b). However, on day 473 (16 days before the third treatment) densities were significantly higher at the treated site. Due to zero Plecoptera specimens at the treated site on the two first sampling occasions after the third treatment, no calculations of differences between sites were made.

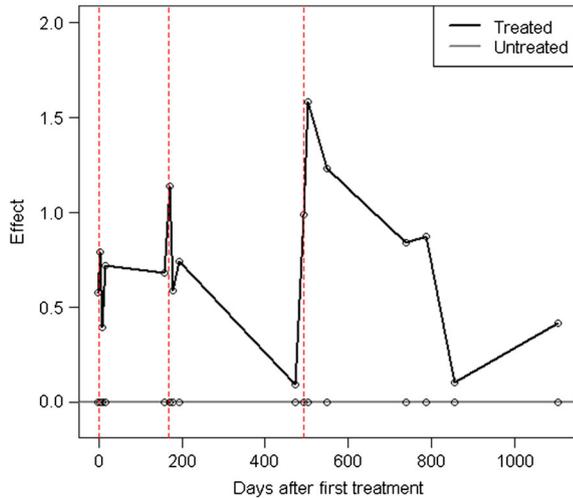


Figure 2. Principle response curve based on kick samples (total material) in the river Oгна (log transformed counts). The dashed vertical lines show the timings of the three treatment applications. The black line shows the effect of the treatment against the untreated samples (grey line at  $y = 0$ ). Points show the dates when samples were taken. This figure is available in colour online at [wileyonlinelibrary.com/journal/rra](http://wileyonlinelibrary.com/journal/rra)

However, at the untreated site densities on the same sampling dates yielded 321 and 45 individuals  $m^{-2}$ , respectively. Significant higher Trichoptera densities were found at the treated site immediately before and after the first treatment,

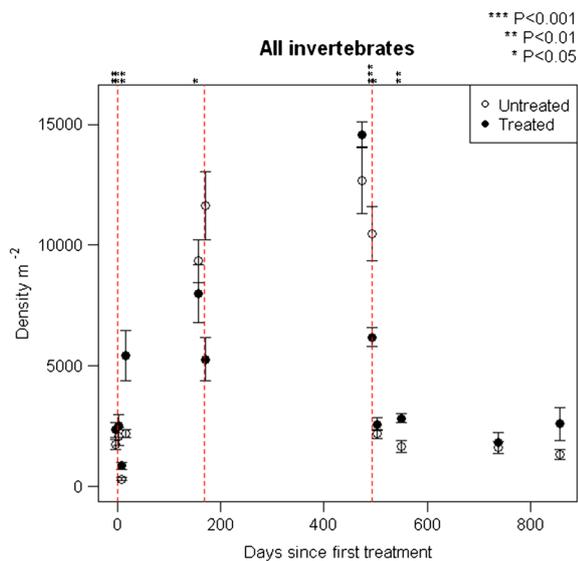


Figure 3. Density (mean  $\pm$  standard error) of total material sampled at stations with and without rotenone treatment. The vertical lines indicate the dates of treatment application. Invertebrates are sampled using Surber. The stars at the top of the figure denote the significance levels from quasi-Poisson GLMs based on the counts rather than densities. This figure is available in colour online at [wileyonlinelibrary.com/journal/rra](http://wileyonlinelibrary.com/journal/rra)

16 days after the first treatment and 157 days after the first treatment (12 days before the second treatment) (Figure 4c). On the three first sampling occasions after the third treatment; on day 494, 502 and 549 after the first treatment, respectively, the densities were significantly higher at the untreated site.

Figure 5 shows the standardized mean differences of selected taxa densities between treated and untreated stations three days after the first, one day after the second and one day after the third rotenone treatment. The density differences between the two stations were smallest in the first treatment and largest in the third treatment. The plots are skewed to the negative side after the third treatment, less after the second treatment and least after the first treatment. This indicates that densities of the treated station has a higher drop than the untreated station, especially after the third treatment, but also after the second treatment, but to a smaller extent after the first treatment.

Out of a total of 28 selected taxa Oligochaeta, Nematoda, Ceratopogonidae and the mayfly *Caenis* sp. densities increased more at the treated than the untreated station after the third treatment (Figure 5). The same situation was true for seven taxa after the first and second treatment. The mayflies *Baetis rhodani*, *Heptagenia dalecarlica* and *Ephemerella aurivillii*, the stoneflies *Diura nanseni* and *Amhinemura* sp., the caddisfly *Rhyacophila nubila* and the beetles *Elmis aenea* and Elmidae (= elmids except *E. aenea*) had decreased densities at the treated station compared to the untreated stations after all three treatments. No taxa had increased densities on the treated station after all three treatments, but Oligochaeta, the stonefly Chloroperlidae (*Siphonoperla burmeisteri* or/and *Xanthoperla apicalis*) and the caddisfly *Agapetus* sp. and Leptoceridae did so after two of the treatments. A detailed taxa list of Surber densities from all sampling occasions is given in Appendix A.

The number of EPT taxa present in the treated and untreated river section throughout the project period is given in Figure 6. Number of Ephemeroptera, Plecoptera and Trichoptera taxa varied from 11 to 15, 7 to 10 and 6 to 16 in the untreated section, respectively, and from 5 to 16, 0 to 11 and 4 to 12 in the treated section, respectively (Figure 6a). In the treated section all three taxa groups decreased immediately after the third treatment, and the lowest taxa number was registered after the third treatment (Figure 6b). This was, however, not the case in the untreated section.

## DISCUSSION

The River Oгна was treated with rotenone three times in different seasons; spring, summer and fall. Different life stages and different species with different sensitivity will

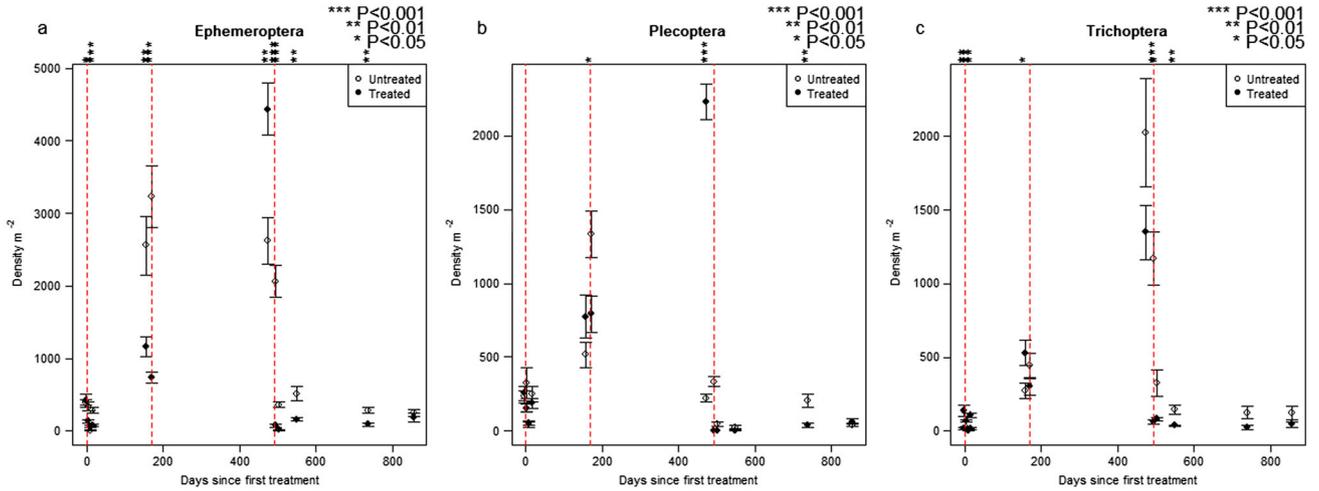


Figure 4. Densities (mean  $\pm$  standard error) of EPT at the treated and untreated station of a) Ephemeroptera, b) Plecoptera and c) Trichoptera. The vertical lines indicate the dates of treatment application. The stars at the top of the figure show the  $P$  values from quasi-Poisson GLMs based on the count data rather than densities. This figure is available in colour online at [wileyonlinelibrary.com/journal/tra](http://wileyonlinelibrary.com/journal/tra)

be present during the different treatments. It is consequently not straightforward to compare rotenone effects between treatments or the effect of three consecutive treatments. Nevertheless many of the same species including many which are rotenone sensitive were present during all three treatments. The first and second treatments were carried out in April and October, respectively, and densities

of a few sensitive taxa decreased, but the species were not locally extinct after the treatments. After the third treatment, however, many taxa remained absent until several months after the treatment. Our data do not suggest that the severe effects of the third treatment were influenced by the two first ones, but rather other factors, including high water temperature during the third treatment. The toxicity of rotenone

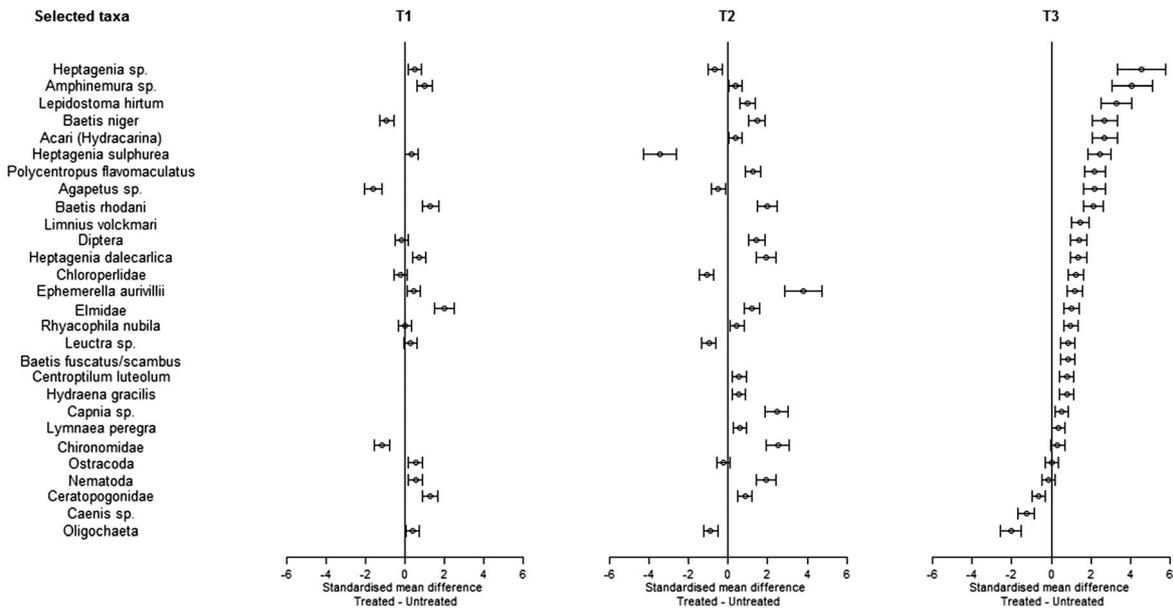


Figure 5. Standardized mean differences (mean difference divided by pooled standard deviation) between taxa densities in treated and untreated stations immediately after the three treatment applications (T1 = 1<sup>st</sup> treatment, T2 = 2<sup>nd</sup> treatment and T3 = 3<sup>rd</sup> treatment). Mean and standard errors presented (estimated across 6 Surber samples per treatment). A positive standardized mean difference indicates that the taxa had a higher density in the treated than untreated samples. Taxa selected are those that are most abundant and consistently present across time periods. Taxa are ordered according to the mean difference after the third treatment application

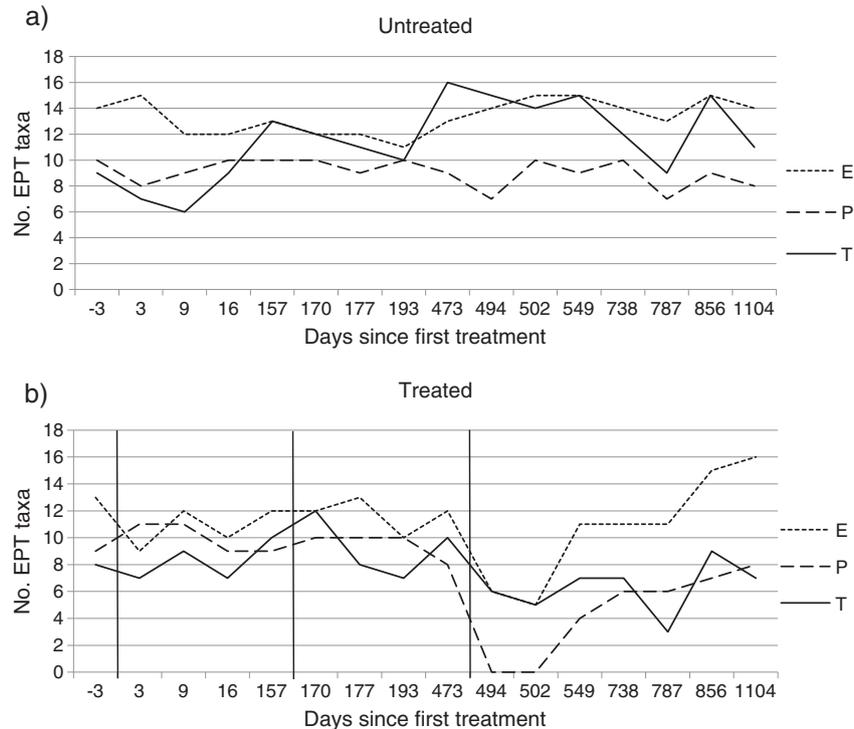


Figure 6. Number of recorded EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in the a) untreated and b) treated area of the River Ognå based on Surber and kick samples. The black vertical lines show the timings of the three treatment applications

increases with temperature (Andreasson, 1963; Meadows, 1973). Water temperature in the first, second and third treatments was 4, 8 and 20 °C, respectively.

The recovery time of lotic invertebrates after rotenone treatment has been shown to vary. Were rotenone treatments include entire watersheds, recovery time is slow and may take several years (Mangum and Madrigal, 1999). However, in many rotenone treatments only a small section of the watershed is treated, and large untreated areas can serve as refuges for dispersal to treated areas. In such cases benthic densities/abundances will often reach pre-treatment levels within a year (Binns, 1967; Gladsø and Raddum, 2000; Arnekleiv *et al.*, 2001; Kjærstad and Arnekleiv, 2004). In the River Ognå several taxa disappeared after the third rotenone treatment, but most of them reoccurred within a year. Only the lowermost 18 km of the river, which constitutes a minor part of the watershed, was treated. Large untreated upstream areas, both of the main river and tributaries, will allow rapid re-colonization through drift. Invertebrate drift is important for downward movement of lotic invertebrates (Maurer and Brusven, 1983; Fenoglio *et al.*, 2002), but also other dispersal mechanisms like ovipositing adults may contribute to recolonization. In addition invertebrates which inhabit the hyporeic zone may have avoided direct contact with rotenone. Invertebrate eggs, which are probably less sensitive to rotenone than later life stages as demonstrated

for fish (Marking and Bills, 1976), may have survived the treatments, but due to the vast untreated upstream areas we believe drift to be the most important factor.

The maximum rotenone concentration was 59  $\mu\text{g l}^{-1}$  in the first treatment and 32  $\mu\text{g l}^{-1}$  in the third treatment (no measurements were taken during the 2nd treatment) (Guttvik *et al.*, 2008). However, only occasional measurements were taken, so the true rotenone concentration in terms of ppm-hours cannot be calculated. The total use of CFT-Legumin was much higher in the third treatment with 33001, compared to 11451 in the first and 16751 in the second treatment. Since the water discharge was much lower during the third treatment compared to the two previous ones, the rotenone concentration was probably highest during the third treatment. Additionally low water discharge during the third treatment resulted in extensive dewatered river banks being sprayed with rotenone. Rotenone leaking from the banks probably gave an additional and prolonged effect, compared to the two other treatments where exposed areas of dried banks were smaller because of higher water discharge. On the other hand, due to high water temperatures the breakdown of rotenone would have been faster in the third treatment. It is therefore difficult to predict the effects of different amounts of rotenone in the three treatments on the benthic fauna. However, during the third treatment both high water temperature and a high rotenone

concentration resulted in a more toxic effect on the macro-invertebrates than the two former treatments.

The sensitivity to rotenone is highly variable between invertebrate taxa (e.g. Engstrom-Heg *et al.*, 1978; Chandler and Marking, 1982; Arnekleiv *et al.*, 2001; Eriksen *et al.*, 2009). Although some species within the EPT groups are relatively rotenone tolerant, these orders are often reported to be among the most sensitive invertebrate groups (Binns, 1967; Arnekleiv *et al.*, 1997; Mangum and Madrigal, 1999). All EPT groups had a marked drop in densities immediately after all treatments, except Plecoptera which had a slight increase after the second treatment. The decrease in densities was most pronounced after the third treatment, especially for Ephemeroptera and Plecoptera. Ephemeroptera densities in the treated area dropped from 4200 individuals per  $m^{-2}$  before the third treatment to 80 individuals after the treatment. Plecoptera, which had a density of 2100 individuals per  $m^{-2}$  immediately before the third treatment, was not registered immediately after the treatment. At the reference station the densities were lower, but relatively stable throughout the treatment. For Trichoptera there was also a high density decline, but this was the case at both the treated and untreated stations. The mayfly *Baetis rhodani* had a marked decrease in densities at the treated station immediately after all three treatments, but densities remained stable or increased at the untreated site during the same time period. In fact no specimen of *B. rhodani* was registered in the Surber samples immediately after the third treatment (337 individuals  $m^{-2}$  before treatment). *Baetis* is known to be relatively rotenone sensitive (Engstrom-Heg *et al.*, 1978; Dudgeon, 1990; Eriksen *et al.*, 2009). The difference in tolerance between *B. rhodani* and *B. muticus* has been documented, the former being the most sensitive (Kjærstad and Arnekleiv, 2011). Other mayfly species which seemed to be negatively affected by the treatments included *Ameletus inopinatus*, *Heptagenia dalecarlica* and *H. suphurea*. *Ameletus* has shown to be rotenone sensitive (Gladstø and Raddum, 2000) and Heptageniidae to have an intermediate tolerance (Engstrom-Heg *et al.*, 1978; Kjærstad and Arnekleiv, 2011). Even the relative rotenone tolerant *Ephemerella* (Engstrom-Heg *et al.*, 1978) had a marked density decrease after the third treatment.

Among stoneflies *Isoperla* sp. and *Leuctra* sp. showed decreased densities during the first treatment. According to Kjærstad and Arnekleiv (2011) these taxa have low rotenone tolerance. No Plecoptera taxa appeared to be negatively affected by the second treatment. However, immediately after the third treatment no Plecoptera specimen was recorded. Plecoptera taxa present in Surber samples immediately before the third treatment included *Amphinemura* sp., *Isoperla* sp., Chloroperlidae, *Capnia* sp., *Diura nanseni* and *Leuctra* sp. The two first of these taxa occurred in high

densities at the treated station immediately before the third treatment; 1700 and 287 ind.  $m^{-2}$  respectively.

For Trichoptera, the two taxa *Rhyacophila nubila* and *Agapetus* sp. had reduced densities at the treated station after all three treatments, while the densities at the untreated station had a slight increase during the same time period. *Rhyacophila* is known to be rotenone sensitive (Engstrom-Heg *et al.*, 1978; Arnekleiv *et al.*, 1997; Gladstø and Raddum, 2002), and *Agapetus ochripes* has been cited with an intermediate rotenone tolerance (Kjærstad and Arnekleiv, 2011). During the third treatment all Trichoptera taxa showed decreased densities at the treated station, but all were present immediately after the treatment, but in low densities. The third treatment seemed to have the most severe effect on the benthic community, and most taxa in the treated area showed a decline in densities. Only four taxa, Chironomidae, Ceratopogonidae, the snail *Lymnaea peregra* and the mayfly *Caenis* sp. had higher densities at the treated station immediately after the third treatment, compared to immediately before the treatment. This indicates a high tolerance to rotenone for these taxa. However, within Chironomidae certain genera, especially *Chironomus*, are known to be sensitive to rotenone (Koksvik and Aagaard, 1984; Melaas *et al.*, 2001). Engstrom-Heg *et al.* (1978) reported an intermediate rotenone tolerance of Chironomidae. There are obviously substantial differences in the sensitivity within this species rich group, and this may also be the case for Ceratopogonidae. High rotenone tolerance in *Lymnaea peregra* is in accordance with other investigations involving freshwater snails, e.g. Arnekleiv *et al.* (1997), Chandler and Marking (1982), Holcombe *et al.* (1987) and Kjærstad and Arnekleiv (2011).

Overall benthic densities at the treated and untreated sites were fairly stable between samples taken immediately before and immediately after the first treatment. After the second treatment densities dropped at the treated station and increased at the untreated station. Densities at both stations decreased after the third treatment, but most at the treated station. The reduction in overall benthic densities seemed to be highest immediately after the third treatment and lowest after the first treatment.

Overall benthic densities before the third treatment were relatively high with 14000 individuals per  $m^{-2}$  at the treated site and 12000 individuals per  $m^{-2}$  at the untreated site. The high density is probably partly due to the very low water discharge, only 1–2  $m^3 s^{-1}$ , which was lower than on the other sampling occasions (4–58  $m^3 s^{-1}$ ). As water discharge decreases the submerged area will decrease, resulting in higher benthic densities. The highest water discharge was registered nine days after the first treatment, and densities both at the treated and untreated stations were by far the lowest registered, and much lower than on the previous sampling occasion, three days after the first treatment.

The overall densities of the benthic fauna in the treated river section in April 2003 ( $1700 \text{ ind. m}^{-2}$ ), eight months after the third treatment, did not reach pre-treatment densities ( $2200 \text{ ind. m}^{-2}$ ). This was also the case in the untreated section. However, the water discharge was much higher with  $28 \text{ m}^3 \text{ s}^{-1}$  in April 2003, compared to  $8 \text{ m}^3 \text{ s}^{-1}$  before the first treatment in April 2001. If the water discharge had been equal on both sampling occasions, densities in the treated section may have been more close to, or perhaps exceeding, the pre-treatment densities. However, seasonal changes in the abundance of various taxa may also be considered as an explanation for differences in densities between sampling occasions.

EPT taxa richness was relatively stable for the whole period in the untreated section, and also in the treated section before the third treatment. However, after the third treatment taxa numbers of all three groups decreased in the treated section. Plecoptera had the highest drop from eight taxa prior to the treatment to zero on the first sampling occasion, two days after the treatment. On the second sampling occasion, eight days after the third treatment, we again did not find any Plecoptera specimens in the treated river section. On the third sampling occasion, 55 days after the third treatment, four Plecoptera taxa were found in the treated area (Surber and kick samples combined). One year after the treatment seven taxa were present, which is close to the number immediately before the third treatment. For Ephemeroptera and Trichoptera taxa numbers reached pre-treatment levels on the third sampling occasion, 55 days after the third treatment. Six EPT taxa in the treated area and two in the untreated area detected before treatment were absent after the third treatment. Moreover, two EPT taxa in the treated area and three in the untreated area were only registered after the third treatment. However, densities of these taxa were low, and their absence may as well be due to chance than other factors, including treatment effects. Missing taxa after rotenone treatments have been reported earlier (Mangum and Madrigal, 1999; Kjærstad and Arnekleiv, 2004; Hamilton *et al.*, 2009).

In conclusion the two first treatments had only minor effects on the benthic invertebrate fauna, but the third treatment caused severe effects. Much higher water temperature combined with high rotenone concentration during the third treatments compared to the two former ones is the most likely explanation for the observed differences. Despite the severe effects during the third treatment, with parts of the benthic fauna being locally extinct, most taxa registered before the treatment were present in the treated river section within a year of the final treatment. Since only a minor part of this watercourse was treated large untreated upstream areas allowed rapid re-colonization through drift, with the exception of those species restricted to the lower reaches. The three consecutive treatments do not appear to have

had a greater influence on the invertebrate fauna community than one single treatment.

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## APPENDIX A

Days since first treatment	Untreated												
	-3	3	9	16	157	170	473	494	502	549	738	856	
Nematoda		29±29		75±28	246±68	116±22	287±37	385±191	20±7	12±6			4±2
Oligochaeta	160±31	208±36	62±8	178±27	834±109	967±135	510±77	290±49	371±55	203±30	337±48	235±57	
Acari (Hydracarina)	2±2			2±2	78±12	107±41	367±56	894±145	7±4	5±2	2±2	7±2	
Ostracoda		7±7		2±2	53±11	118±23	25±10	2±2					
Ameletus inopinatus	25±12	20±8		32±16	219±32	251±39	271±96	118±40	25±8	14±8	12±7	5±2	
Centropilum luteolum					23±19	46±44	1302±425	69±45	29±24			4±4	
Baetis niger	2±2			2±2	102±28	125±45	141±41	201±40	53±22	45±34	4±2	7±2	
Baetis rhodani	150±28	150±45		77±9	105±47	121±32	5±4	96±24	5±2	93±30	71±10	27±13	
Heptagenia sp.	36±6	21±10		14±6	301±93	264±101	273±66	691±80					
Heptagenia dalearica	21±9	7±4		14±4	32±10	36±9	39±8	59±23	45±13	59±13	9±5	59±18	
Heptagenia sulphurea	9±6	4±2	2±2		9±4	2±2	78±5	116±25	110±31	169±29	43±15	80±15	
Ephemera aurivillii	115±5	11±4	4±2	11±3	32±8	125±16	45±11	30±13	25±13	5±4	11±3	18±8	
Ephemera mucronata	46±8	45±12	4±2	64±7	1589±366	2102±373	337±101	533±157	18±6	80±32	103±26		
Caenis sp.													
Diura nanseni	2±2	5±5	9±7	20±3	11±6	27±7	18±4	9±5	12±5		9±4	36±13	
Isoperla sp.	11±4	32±13	4±2	16±2	23±17	34±16	14±5	71±13	11±5	9±6	20±9		
Chloroperlidae	5±2	2±2			11±4	4±2	18±4	53±23					
Siphonoperla burmeisteri	4±2	5±4							11±6	5±4	27±8	2±2	
Taeniopteryx nebulosa					7±5	5±4	11±4	16±5		2±2			
Amphinemura sp.	116±16	166±43	18±9	139±44	226±60	821±166	114±25	166±22		5±2	132±40		
Capnia sp.					200±49	369±70	27±8	2±2		4±4		2±2	
Capnia pygmaea	4±2			2±2									
Leuctra sp.	80±20	86±54		61±7	9±3	4±2	2±2	4±2	5±4		7±7		
Elmidae	23±5	107±28		16±9	586±130	360±123	992±151	962±181	349±64	228±34	61±42	333±66	
Elmis aenea		7±4		5±4	176±36	148±45	351±53	269±47	78±20	30±8	27±19	46±8	
Limnius volckmari					5±4		11±4	11±4	4±2	5±2	4±2	4±2	
Oulimnius tuberculatus					2±2	2±2	2±2	2±2	2±2	2±2		4±2	
Rhyacophila nubila	2±2	11±7		2±2	9±5	16±9	2±2	36±16		5±4	5±4	5±4	
Agapetus sp.	5±4	9±3		5±4	102±33	130±38	447±184	463±111	123±47	66±20	69±28	39±14	
Hydroptila sp.					87±32	144±25	1144±180	280±87	98±29	16±9	12±10	5±4	
Oxyethira sp.				2±2	11±6	30±8	73±23	48±29	20±9	5±2		2±2	
Polycentropus flavomaculatus	2±2			2±2	20±5	23±10	52±12	53±13	14±5	9±4			
Hydropsyche contubernalis					4±2	12±7	4±2	25±5	2±2	9±6	12±6	9±7	
Hydropsyche nevae					7±4	4±2	23±15	45±14			5±2	5±5	
Lepidostoma hirtum					2±2	7±2	41±11	46±7	7±5	7±4	2±2	5±2	
Leptoceridae					11±7		41±15	21±7	5±4	7±5		9±4	
Diptera		16±8	2±2	14±5	98±28	126±27	50±9	77±29	39±13	21±5	11±7	34±10	
Chironomidae	786±129	894±193	157±38	1272±123	3560±472	4296±668	4659±742	3497±641	349±31	362±73	451±86	139±20	
Simuliidae	9±7	14±6	4±2	4±2	2±2	4±2	5±2			5±5		2±2	
Ceratopogonidae	37±10	50±15		12±4	102±18	93±34	128±30	205±38	69±18	41±15	25±9	25±5	
Lymnaea peregra					12±5	21±11	105±49	36±13	110±33	21±6	37±12	39±10	

## EFFECTS OF ROTENONE ON BENTHIC MACROINVERTEBRATES

Treated											
-3	3	9	16	157	170	473	494	502	549	738	856
2±2		4±4		135±57	29±11	700±161	444±77	23±6	2±2		4±2
353±94	151±71	162±33	862±204	2020±428	1848±506	1881±54	1249±245	1163±157	625±71	387±179	1481±455
5±2			4±2	173±33	75±16	494±132	134±40	5±2	2±2		11±5
	11±7	12±6	16±16	410±169	167±110	5±2	2±2				
			4±4	9±6	4±2	52±34					
	9±5				2±2	2±2	±2		2±2	4±2	
237±31	30±12		7±4	306±49	2±2	337±61		2±2	2±2		25±17
37±17	11±6	4±2	29±8	483±56	401±46	290±322	4±2		123±21	84±23	30±10
2±2	2±2			29±12	4±2	27±5					43±9
7±5	2±2	2±2	5±2	87±16	53±8	151±23					75±30
	5±5	4±2		2±2	4±4						
87±45	57±10	34±6	29±7	132±36	210±32	353±67	18±7		7±4		
11±11						11±9	52±22				
		2±2	2±2	5±2	2±2	18±7					14±5
21±6	4±4	2±2	9±5	4±2	2±2	287±36				5±2	2±2
9±4	4±4		4±2	21±13	18±7	78±17					
		2±2									
				2±2	2±2						2±2
93±37	80±17	29±10	87±22	673±132	677±111	1698±145				25±11	
				34±9	43±11	50±13					
2±2	2±2	2±2	2±2								
105±27	55±9	9±6	59±10		12±4	4±4				4±2	2±2
4±4			36±20	278±63	84±11	693±55	538±123	273±57	41±12	41±11	189±39
18±12				4±2	9±4	9±9	20±8	25±11	11±4	5±4	9±4
				4±2		12±6		11±7	4±2		7±4
18±8	11±6	2±2	5±2	20±8	7±5	89±13	5±4	2±2	12±5	5±4	14±7
66±33	34±8	5±4	78±18	401±84	192±56	1049±167	5±4	2±2	7±2	12±12	14±8
				25±8	16±10	23±4	11±5	43±9	4±2	2±2	2±2
									2±2		
	2±2										5±2
32±22	7±4	2±2	9±3	34±11	41±12	50±12	18±7	16±5	9±3	4±4	5±2
2±2					2±2	12±4	4±2				
2±2	4±2		2±2	2±2	2±2	30±8	20±12	16±6	2±2	2±2	
18±8	20±6	7±5	41±15	121±30	43±17	109±20		5±4	9±3	23±10	5±4
983±215	1853±399	535±98	3822±841	2083±343	1005±207	2152±180	3091±172	780±73	1714±217	645±102	387±96
43±25	20±9		32±9	12±7	11±3	9±3			34±8	346±142	4±2
16±5	12±5		39±17	39±17	36±12	185±17	303	±71	46±11	45±15	53±20
11±7	2±2			27±11	9±3	18±7	25	±7	55±26	45±21	25±10