

## Research Article

## Fast and accurate on-site determination of rotenone in water during fish control treatments using liquid chromatography

Morten Sandvik<sup>1,\*</sup>, Thor Waaler<sup>1,4</sup>, Thomas Rundberget<sup>2</sup>, Pål Adolfsen<sup>3</sup>, Helge Bardal<sup>3</sup> and Roar Sandodden<sup>3</sup>

<sup>1</sup>Norwegian Veterinary Institute, P. O. Box 750 Sentrum, NO-0106 Oslo, Norway

<sup>2</sup>Norwegian Institute for water research, Gaustadalléen 21, NO-0349 Oslo, Norway

<sup>3</sup>Norwegian Veterinary Institute, P. O. Box 5695 Sluppen, NO-7485 Trondheim, Norway

<sup>4</sup>retired

Author e-mails: [morten.sandvik@vetinst.no](mailto:morten.sandvik@vetinst.no) (MS), [thor.waaler@vetinst.no](mailto:thor.waaler@vetinst.no) (TW), [thomas.rundberget@niva.no](mailto:thomas.rundberget@niva.no) (TR), [pal.adolfsen@vetinst.no](mailto:pal.adolfsen@vetinst.no) (PA), [helge.bardal@vetinst.no](mailto:helge.bardal@vetinst.no) (HB), [roar.sandodden@vetinst.no](mailto:roar.sandodden@vetinst.no) (RS)

\*Corresponding author

Received: 17 February 2017 / Accepted: 30 September 2017 / Published online: 17 October 2017

Handling editor: Matthew Barnes

### Abstract

A fast, accurate and simple method using liquid chromatography (LC) with UV detection was used for the on-site determination of the piscicide rotenone in water during fish control treatments. Sample volumes of 10 to 40 µL were loaded onto a Waters XBridge™ C18 2.5 µm 3.0 x100 mm analytical column using a mobile phase of water–acetonitrile (45:55) at a flow-rate of 0.5 mL/min. The method was evaluated using river and estuarine water spiked with rotenone (0.1–330 µg/L) and various preservation methods. The within-assay precision measured as relative standard deviation (RSD, n = 12) was 5.5 to 6.5% and the between assay precision (RSD, n = 4) was 6.5 to 7.5%. The limit of quantification was 1 µg/L, below normal piscicidal treatment rates (5 to 200 µg/L) and regulatory limits (< 2 µg/L) generally considered safe. The analysis time was 6 min/sample allowing for real-time adjustment of rotenone dosages during fish control treatments. The relatively small size (75×60×50 cm) of the LC system made it ideal for transportation and installation in remote treatment areas; it can be operated out of a small trailer in the field with electricity. Our studies indicate that the preservation of water samples with equal quantities of acetonitrile stabilizes rotenone indefinitely (> 170 days) if kept cool (4 °C) and in the dark. Although increased salinity decreased the recovery of rotenone, sample filtration with Spin-X filter membranes negated the effect.

**Key words:** *Gyrodactylus salaris*, high throughput on-site analysis

### Introduction

Rotenone is an isoflavonoid occurring in plants of the Leguminosae family and is used widely as a piscicide (Stenersen 2004). Rotenone's toxic mode of action is as a phosphorylation inhibitor, and fish are highly susceptible because rotenone quickly enters the blood stream via the gills. The efficacy of rotenone as a fish poison is proven and is supported by extensive toxicity data (Johnsen and Jensen 1991; Ling 2002; McClay 2000). The reregistration of rotenone as a piscicide and required standard operating procedures were recently approved by the U.S. Environmental Protection Agency (Finlayson et al. 2010b;

USEPA 2006). Norway uses rotenone in the management of the ectoparasite *Gyrodactylus salaris* on Atlantic Salmon *Salmo salar* where the parasite can reduce parr density and returning adults by 87% (Johnsen et al. 2008). Although it is difficult to eradicate a pathogenic parasite in the wild, the obligatory dependence of the parasite *G. salaris* on host salmonids and the requirement that the parasite must live on the host in freshwater makes it susceptible to localized eradication through host removal using rotenone. However, the success with these treatments has varied, and one problematic factor is the maintenance of efficacious rotenone concentrations in flowing water during the treatment

interval throughout the target area (Johnsen et al. 2008). A fast and accurate on-site analysis of rotenone in water that would allow for real-time augmentation of the dose rate could greatly increase the efficacy of rotenone treatments. The Norwegian Environmental Agency and Norwegian Food Safety Authority are committed to the eradication of *G. salaris* from infected river systems using rotenone as the only practical method (The Norwegian Environment Agency 2014). A fast and accurate on-site determination of rotenone will not only allow for more efficacious treatments but can also minimize non-target impacts on water quality and biological resources through reducing the need for retreatment and potentially excessive treatment rates.

The determination of rotenone is usually performed using conventional liquid chromatography (LC) with ultraviolet (UV) or mass spectrometric detection (MS) and off-line pre-concentration steps (Cabizza et al. 2004; Draper et al. 1999; Holm et al. 2003). LC-MS with electrospray ionization (ESI) has gained popularity due to the high sensitivity and high mass resolution (Caboni et al. 2008). However MS instrumentation is quite expensive, large and impractical for on-site analysis. In contrast, the development of more compact LC systems has made it suitable for transport and installation on-site. Moreover increased detection sensitivity and improved column technology, eliminates the need for extensive sample concentration prior to determination of rotenone at piscicidal concentrations.

In this study we developed a fast and accurate rotenone analysis that can be used during the treatment to adjust the dosage. Having fast and reliable rotenone concentration data would increase compliance with the regulatory limits and minimize environmental and public health impacts.

## Materials and methods

### *Reagents and materials*

Acetonitrile (MeCN) was of HPLC grade and obtained from Rathburn Chemical (Walkerburn, UK), and water was deionized (MilliQ). Rotenone ( $\geq 95\%$ ) and deguelin ( $\geq 98\%$ ) standards were obtained from Sigma Chemical (St. Louis, MO, USA). The rotenone formulation CFT Legumin 2.5%™ was obtained from Kemira (Espoo, Finland).

Amber Boston round glass bottles (100-mL) were purchased from Fybikon A/S (Kristiansand, Norway). Spin-X® centrifuge filters were purchased from Costar® (0.22- $\mu\text{m}$  pore nylon membrane, polypropylene tube). A micro centrifuge was obtained from VWR (Galaxy miniStar C1413-VWR230). Solid phase extraction

(SPE)-columns were obtained from Thermo Scientific (Hypersep C18, 100 mg/mL). SPE was performed using a Visiprep SPE Vacuum Manifold (Supelco, Bellefonte, USA).

### *Instrumentation and chromatographic conditions*

The UFLC-UV system from Shimadzu (Kyoto, Japan) was equipped with two LC-20AD pumps, a SIL 20A automatic sample injector, and a SPD M20A DAD detector. The separation was performed on a Waters XBridge™ C18 2.5  $\mu\text{m}$  3.0 x100 mm column (Waters, Milford, MA, USA). Separation was achieved using isocratic elution with deionized water- MeCN (45:55) at a constant rate of 0.5 mL/min. The eluent was monitored with the UV/VIS DAD detector at 285–300 nm (wave step 1, slit with 1.2 nm) for rotenone detection. The output wavelength was 290 nm (bandwidth 4 nm, reference wavelength 350 nm).

### *Calibration curves and assay validation.*

Rotenone was quantified using an external four-point calibration curve in the concentration range 1–250  $\mu\text{g/L}$ . The limit of detection (LOD) and the limit of quantification (LOQ) were defined as the minimum concentration generating a signal-to-noise ratios (S/N) equal to 3 and 10, respectively. The precision and accuracy of the method was tested by analysing spiked river water. Within-day assay precision and accuracy were assessed using five replicas of spiked river samples at concentrations of 10, 20, 50 and 100  $\mu\text{g/ml}$  of rotenone. Between-day assay precision and accuracy were assessed using four replicas of spiked river samples during 3 days at concentration of 9.8 ng/ml of rotenone.

### *Sample treatments*

Water samples during the treatment of the Vefsna Region were collected immediately upstream of downstream rotenone booster stations on the Vefsna River and other streams where rotenone concentrations should be the lowest. Water samples ( $n = 3$ ) were collected at each site at the different sampling times. To avoid cross contamination, new disposable gloves were used for each sample. To provide optimum UV protection for the light sensitive rotenone content, Amber Boston round glass bottles (100-mL) were used. The bottles were rinsed twice with the river water and then filled completely before sealing and transport to the lab that was within the treatment area. Immediately upon arrival, an aliquot of water (1 mL) was transferred from the sample bottle to a HPLC vial containing MeCN (1 mL) and vortexed prior to LC-UV analysis.

**Table 1.** Within-day precision and accuracy for the determination of rotenone in river water.

Concentration of rotenone added	Concentration of rotenone found <sup>a</sup>	Precision (% CV) <sup>b</sup>	Accuracy (%) <sup>c</sup>
10 µg L <sup>-1</sup>	9.6 ± 0.3	3.5	95.6
20 µg L <sup>-1</sup>	19.1 ± 0.7	3.9	95.5
50 µg L <sup>-1</sup>	49.4 ± 0.7	1.5	98.7
100 µg L <sup>-1</sup>	100.5 ± 0.3	0.3	100.5
Average of % CV from different concentrations:		2.3	

<sup>a</sup>Mean ± SD (n = 5)<sup>b</sup>Coefficient of variation (CV) = SD/mean × 100<sup>c</sup>Accuracy = found/added × 100

### Organic and salt interferences

The extraction of estuarine water samples and water samples containing organic matter by visual inspection were evaluated with two different methods:

#### Method 1:

Solid phase extraction (SPE)-columns were conditioned with 1 mL of MeCN, then 1 mL of water. The water sample (1 mL) was loaded onto the column, washed with 1 mL of deionized water, and eluted with 1 mL of MeCN (flow-rate 1–2 mL/min). The samples were then diluted 1:1 with deionized water prior to LC-UV analysis.

#### Method 2:

Water samples (750 µL) were transferred to Spin-X<sup>®</sup> centrifuge filters and centrifuged for 30 sec at 2000 g on a micro centrifuge. For further pre-concentration of water samples, this step was repeated up to 5 times for each membrane. The membranes were then transferred to new tubes before adding 200–750 µL deionized water- MeCN (50:50) and then centrifuged as described above. The eluates were transferred to HPLC vials prior to LC-UV analysis.

### Stability tests

The degradation rate of rotenone under different conditions and storage temperatures was determined by analysing the samples after 2, 7, 15, and 25-days. We conducted a series of stability tests with rotenone (32.4 µg/L) stored in deionized water (pH 6), deionized water- MeCN (50:50) or MeCN only. The test solutions were kept dark at different temperatures (–20 °C, 4 °C and 20 °C). Half-lives of rotenone were determined by fitting the data to the equation:

$$C_t = C_0 e^{-Kt} \quad T_{1/2} = \ln 2/K$$

$C_t$  is the rotenone concentration at time;  $C_0$  is the initial concentration, and  $K$  is the rate constant,  $T_{1/2}$  is the half-life. The data were summarized using the statistical software program Analysis Toolpack for

Excel 2010 (GreyMatter International, Inc., Cambridge, MA, USA).

## Results

### Chromatographic conditions

Different mobile phase compositions of MeCN: water was tested. A concentration of 55% MeCN separated rotenone from both interfering peaks from the water and from deguelin, a rotenoid constituent of CFT Legumin. The retention time of rotenone with isocratic elution at 0.5 mL/min was 5.02 min.

### Precision and accuracy

The standard curves for rotenone was linear within the investigated concentration range (1–1000 µg/L) displaying a calibration curve correlation factor of 0.99. We chose to quantify rotenone using an external four-point calibration curve in the concentration range 1–250 µg/L, covering the piscicidal range of rotenone. The within-day assay precision and accuracy are presented in Table 1. The observed variation of coefficients at all concentrations examined were less than 4%. The accuracy was less than ±5%. The between-day assay precision and accuracy are presented in Table 2. The CV values were within 2% and the accuracy was less than ±3%. The recoveries of spiked water (9.8 µg/L) samples were 99 ± 1% (n = 12). Certified batches of CFT Legumin<sup>™</sup> formulations from the manufacturer Kemira were also analysed, and the recoveries were 99 ± 2% (n = 5).

### Rotenone stability

The stability of rotenone (32.4 µg/L) stored in deionized water (pH 6), deionized water- MeCN (50:50) or MeCN only are shown in Table 3. Rotenone was most stable in a solution of water-MeCN (1:1). Preservation of water samples with equal quantities of MeCN significantly contributes to the stabilization of rotenone indefinitely (> 170 days) if kept at a temperature of 4 °C in the dark.

**Table 2.** Between-day precision and accuracy for the determination of spiked rotenone ( $9.8 \mu\text{g L}^{-1}$ ) in river water.

Sample	Concentration found ( $\mu\text{g L}^{-1}$ )			mean	SD	Precision (% CV) <sup>b</sup>	Accuracy (%) <sup>c</sup>
	Day1	Day2	Day3				
B1	9.9	9.7	10.0	9.8	0.1	1.4	100.4
B2	9.6	9.5	9.9	9.7	0.2	1.8	98.6
B3	9.6	9.8	9.7	9.7	0.1	0.7	99.0
B4	9.7	9.4	9.5	9.5	0.1	1.3	97.4
mean <sup>a</sup>	9.7	9.6	9.8	9.7	0.1	1.3	98.8
Stdav	0.1	0.1	0.2	0.2			
%CV (within day)	1.3	1.5	2.0	1.6			

<sup>a</sup>Average of % CV for 3 days

<sup>b</sup>Coefficient of variation (CV) =  $\text{SD}/\text{mean} \times 100$

<sup>c</sup>Accuracy =  $\text{found}/\text{added} \times 100$

The test solution ( $32.4 \mu\text{g/L}$ ) was repeatedly analysed for a period of 170 days, and no degradation was observed ( $31.6 \pm 0.7 \mu\text{g/L}$ ,  $n = 68$ ).

### Organic and salt interferences

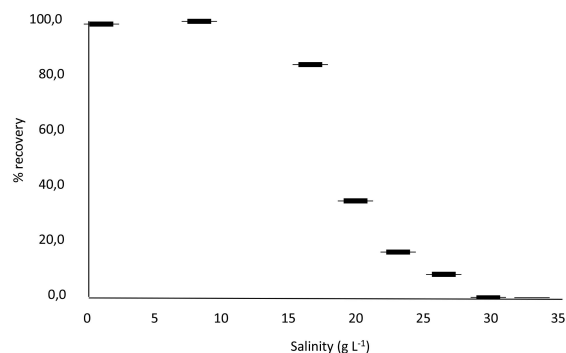
Some samples contained visible particles that were initially removed by filtration with Spin-X<sup>®</sup> filters. However, in all the filtered samples, no rotenone was detected. To check if rotenone was retained on the membrane, river water was spiked with known concentrations of rotenone. When we added water with 50% MeCN or more, and filtered the membrane again, the recoveries of rotenone were approximately 100% (results not shown). We could also add equal amounts of MeCN to the samples before filtering with Spin-X with satisfactory recovery.

Salinity in the lower parts of the Vefsna estuary increases with high tide at the lower 1 km of the river. The possible effect of salinity on rotenone analysis was investigated by spiking rotenone to artificial sea water of varying salinities. The recovery of rotenone decreased drastically with increasing salinity (Figure 1). Initially we desalted saline water using SPE-columns before analysis. The recovery of rotenone ( $30 \mu\text{g/L}$ ) in saline water ( $33 \text{ g/L}$ ) after SPE clean-up was  $99.2 \pm 0.9\%$  (SD,  $N = 4$ ). We also investigated using Spin-X<sup>®</sup> filter membranes to remove rotenone from the sample. Water samples were transferred to Spin-X<sup>®</sup> centrifuge filters and centrifuged before elution with MeCN or deionized water-MeCN (50:50); the recovery in samples eluted with MeCN was lower than water-MeCN (50:50). In samples eluted with deionized water-MeCN (Table 4), the recovery of rotenone ranged from 87 to 106% at rotenone concentrations from 1 to  $330 \mu\text{g/L}$ . We also tested lower rotenone concentrations of 0.1 to  $3.7 \mu\text{g/L}$  with varying sample volumes and different elution volumes. For sample volumes up to  $2250 \mu\text{L}$

**Table 3.** Predicted half-lives of rotenone ( $32.4 \mu\text{g L}^{-1}$ ) in Water, water – MeCN (50:50) and MeCN.

Temperature °C	Solvent		
	Water	Water- MeCN (50:50)	MeCN
-20	173	$\infty^*$	$\infty^*$
4	32	$\infty^*$	13
20	18	376	6

\* Half-lives could not be determined.

**Figure 1.** Effect of salinity on the recovery of rotenone ( $30 \mu\text{g L}^{-1}$ ) by dilution with MeCN (water-MeCN 50:50). The results are given as means  $\pm$  SD ( $n = 3$ ).

and elution volumes as low as  $200 \mu\text{L}$ , the recovery was  $102 \pm 4\%$  (SD,  $n = 16$ ) and the limit of quantification was  $0.2 \mu\text{g/L}$ .

### Rotenone analysis

Mean concentrations of rotenone at the different sampling points in Vefsna, Fusta and Drevja are shown in Figure 2 and Figure 3. Water samples were collected in triplicates and the concentrations are given as  $\mu\text{g/L}$  (mean  $\pm$  SD). The horizontal lines represent piscicidal rotenone concentration set to  $12.5 \mu\text{g/L}$  (corresponding to 0.5 ppm legumin – 2.5%

**Table 4.** Effect of desalting seawater (33g L<sup>-1</sup>), and pre-concentration of rotenone using Spin-X<sup>®</sup> centrifuge filters. The eluate was discarded and rotenone eluted with 50 or 100% MeCN. The results are given as means ± SD (n = 3).

Rotenone concentration (µg L <sup>-1</sup> )	sample volume (µL)	elution volume (µL)	concentration factor	% recovery	
				50% MeCN	100% MeCN
330,0	750,0	750,0	1	104 ± 2	90 ± 1
33,0	750,0	750,0	1	91 ± 6	84 ± 2
3,3	750,0	750,0	1	87 ± 6	81 ± 5
3,7	700,0	700,0	1	100*	
3,7	700,0	450,0	1,5	102*	
3,7	700,0	350,0	2,5	104*	
3,7	700,0	200,0	3,5	103*	
3,7	700,0	150,0	4,7	93*	
1,0	750,0	750,0	1	106*	
1,0	2250,0	750,0	3	102*	
1,0	3750,0	750,0	5	113*	
0,4	2250,0	200,0	10,5	105 ± 2	
0,2	2250,0	200,0	10,5	99 ± 3	
0,1	2250,0	200,0	10,5	92 ± 34	

\* n = 1

rotenone). According to Finlayson et al. (2010a), this concentration is assumed to cause complete mortality to salmonids and minimal impact on non-target organisms with an exposure time of 6 to 18 hours. The dosing strategy was designed to maintain concentrations between 12.5 and 35.0 µg/L (0.5 and 1.4 ppm) during the treatment period.

The level of rotenone measured in our study was generally within the range of 12.5–60 µg/L.

## Discussion

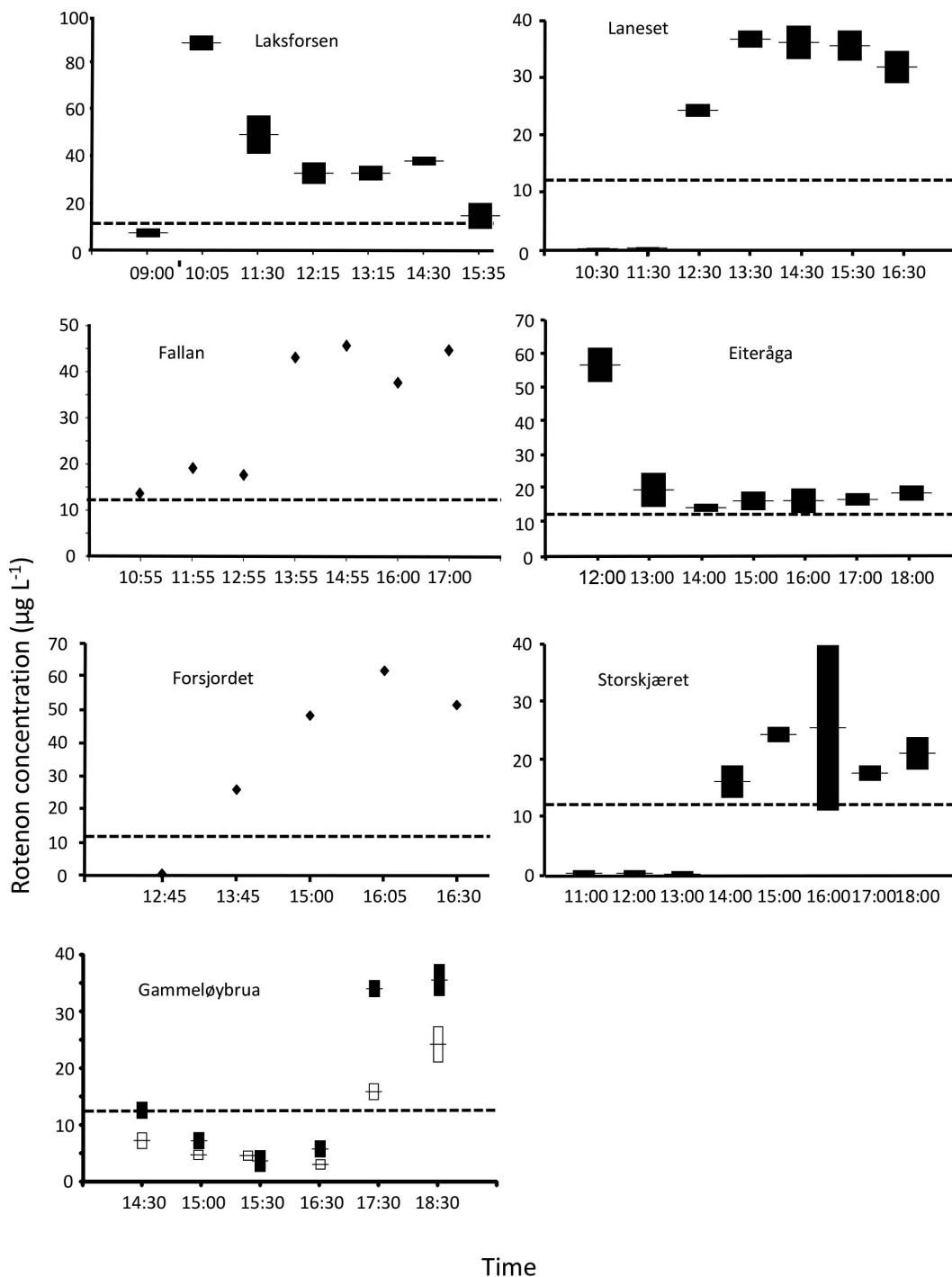
The results from our study demonstrated a clear potential for including on-site LC-UV methodology monitoring real-time rotenone concentrations in fish eradication projects.

Transportation and installation of the LC system in the treatment area combined with high throughput analysis allowed monitoring of the efficacy of the dosing. The rotenone treatments of the streams were 6–10 hours in duration with 1 to 2 hours travel time between consecutive rotenone booster stations. Thus, it will take 1 to 2 hours for the rotenone in the treatment area to come to equilibrium as the rotenone moves downstream to the next station. Samples taken after that time will show how close to theoretical the rotenone levels are with 4 to 6 hours left in the treatment interval for augmenting the dose. With the lab on site we could provide an answer in less than 1 hour. This would likely increase the efficacy of the treatment and improving the success rate for *G. salaris* control. A fast and accurate analysis of rotenone will result in more

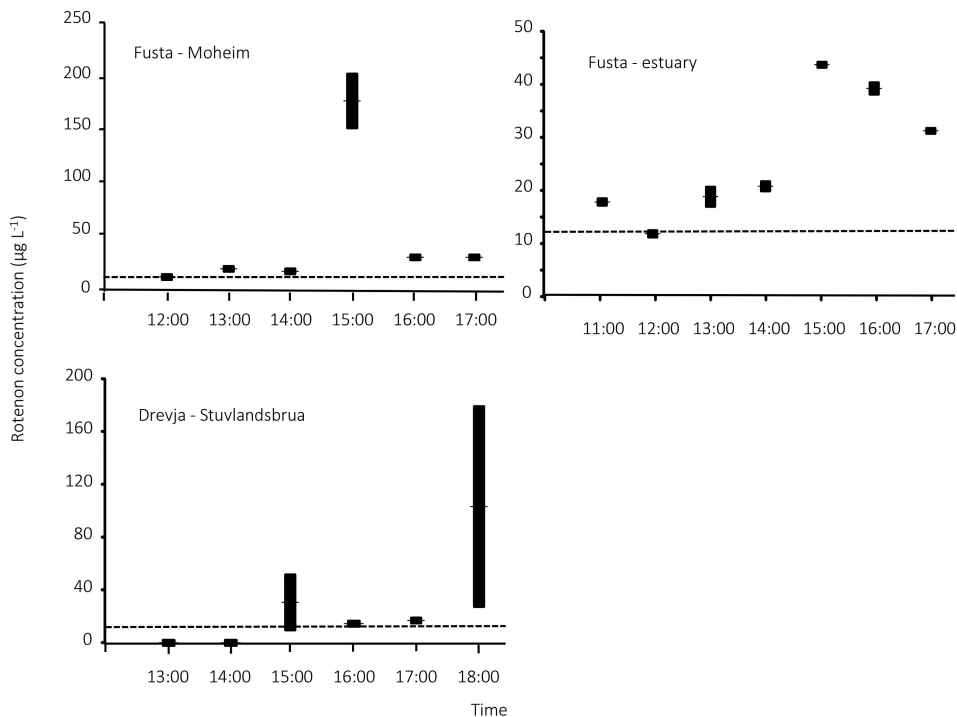
efficacious treatments that are not overtreatments resulting in greater environmental impacts, or undertreatments that require retreatment and thus, additional environmental impacts. The lack of real-time rotenone monitoring data is likely one cause for previously failed treatments, resulting in not maintaining efficacious rotenone levels throughout the target area for the entire treatment duration. The effect of the water sampling regime with continuous sampling is further discussed in a following *G. salaris* eradication operation conducted in the Rauma infection zone in central Norway (Sandodden et al. 2018).

Although increased salinity decreased the recovery of rotenone, sample filtration with Spin-X filter membranes negated the effect. Pre-concentration with Spin-X<sup>®</sup> had several advantages compared to SPE clean-up. In addition to the satisfactory recovery, it was less time consuming, easy to operate and more cost effective.

Rotenone has low to moderate mobility in soil and sediment, has a relatively low potential for bio-concentrating in aquatic organisms, and is unstable in the environment, with hydrolysis and photolysis half-lives measured in days and hours, respectively (Finlayson et al. 2010b; USEPA 2006). Our findings suggest that preservation of water samples with equal quantities of MeCN should be considered when samples can't be analysed within the first 24 hours. Preservation of water samples with equal quantities of MeCN significantly contributes to the stabilization of rotenone indefinitely (> 170 days) if kept at a temperature of 4 °C in the dark.



**Figure 2.** Mean values of rotenone concentrations ( $\mu\text{g L}^{-1}$ ) during the treatment of river Vefsna. ■ (right bank). □ (left bank) = Mean  $\pm$  SD ( $n = 3$ ). ♦ Represents single measurements. The horizontal dotted line represents  $12.5 \mu\text{g L}^{-1}$  rotenone, corresponding to 0.5 ppm legumin (2.5% rotenone).



**Figure 3.** Mean values of rotenone concentrations ( $\mu\text{g L}^{-1}$ ) during the treatment of rivers Fusta and Drevja. ■ = Mean  $\pm$  SD ( $n = 3$ ). The horizontal dotted line represents  $12.5 \mu\text{g L}^{-1}$  rotenone, corresponding to 0.5 ppm legumin (2.5% rotenone).

## Acknowledgements

The authors would like to acknowledge Brian Finlayson (California Department of Fish and Game (retired)) for providing comments and direction on previous drafts. This work was supported/granted by the Norwegian Environment Agency.

## References

- Cabizza M, Angioni A, Melis M, Cabras M, Tuberoso CV, Cabras P (2004) Rotenone and rotenoids in cube resins, formulations, and residues on olives. *Journal of Agricultural and Food Chemistry* 52: 288–293, <https://doi.org/10.1021/jf034987a>
- Caboni P, Sarais G, Vargiu S, Luca M, Garau V, Ibba A, Cabras P (2008) LC–MS–MS Determination of Rotenone, Deguelin, and Rotenolone in Human Serum. *Chromatographia* 68: 739–745, <https://doi.org/10.1365/s10337-008-0830-0>
- Draper WM, Dhoot JS, Perera SK (1999) Determination of rotenoids and piperonyl butoxide in water, sediments and piscicide formulations. *Journal of Environmental Monitoring* 1: 519–524, <https://doi.org/10.1039/a904398e>
- Finlayson B, Somer WL, Vinson MR (2010a) Rotenone Toxicity to Rainbow Trout and Several Mountain Stream Insects. *North American Journal of Fisheries Management* 30: 102–111, <https://doi.org/10.1577/M09-078.1>
- Finlayson B, Schnick R, Skaar D, Anderson L, Demong J, Duffield D, Horton W, Steinkjer J (2010b) Planning and standard operating procedures for the use of rotenone in fish management – rotenone SOP manual. American Fisheries Society, Bethesda, Maryland, 143 pp
- Holm A, Molander P, Lundanes E, Greibrokk T (2003) Determination of rotenone in river water utilizing packed capillary column switching liquid chromatography with UV and time-of-flight mass spectrometric detection. *Journal of Chromatography A* 983: 43–50, [https://doi.org/10.1016/S0021-9673\(02\)01693-X](https://doi.org/10.1016/S0021-9673(02)01693-X)
- Johnsen BO, Brabrand Å, Jansen P, Teien H, Bremset G (2008) Evaluering av bekjempelsesmetoder for *Gyrodactylus salaris* – rapport fra ekspertgruppe. Utredning for DN 2008-7 (in Norwegian)
- Johnsen BO, Jensen AJ (1991) The *Gyrodactylus* story in Norway. *Aquaculture* 98: 289–302, [https://doi.org/10.1016/0044-8486\(91\)90393-L](https://doi.org/10.1016/0044-8486(91)90393-L)
- Ling N (2002) Rotenone – a review of its toxicity and use for fisheries management. *Science for Conservation* 211: 40
- McClay W (2000) Rotenone Use in North America (1988–1997). *Fisheries* 25: 15–21, [https://doi.org/10.1577/1548-8446\(2000\)025<0015:RUINA>2.0.CO;2](https://doi.org/10.1577/1548-8446(2000)025<0015:RUINA>2.0.CO;2)
- Sandodden S, Brazier M, Sandvik M, Moen A, Wist AN, Adolfsen P (2018) Eradication of *Gyrodactylus salaris* infested Atlantic salmon (*Salmo salar*) in the Rauma River, Norway, using rotenone. *Management of Biological Invasions* 9: 67–77, <https://doi.org/10.3391/mbi.2018.9.1.07>
- Stenersen J (2004) Chemical Pesticides Mode of Action and Toxicology, CRC Press, Boca Raton, chapter 3, pp 35–65, <https://doi.org/10.1201/9780203646830>
- The Norwegian Environment Agency (2014) Action plan against the salmon parasite *Gyrodactylus salaris* for the period 2014–2016, The Norwegian Environment agency, report M-288
- USEPA (2006) Environmental Fate and Ecological Risk Assessment for the Reregistration of Rotenone. Phase IV (final), 197 pp