

MANAGEMENT BRIEF

Infrared Imagery and Inert Media Used in Treating Upwelling Groundwater with Rotenone

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Abstract

Untreated upwelling groundwater from seeps and springs in and adjacent to surface water bodies has been long suspected of causing failed rotenone treatments by providing a refugia of nontoxic water. A possible solution involves the use of an inert media to carry the liquid rotenone to the source of upwelling groundwater and release rotenone over an extended period of time sufficient to affect the mortality of the target fish. In our initial study to address this problem, we used thermal infrared imagery (FLIR One) on a smartphone to locate groundwater that was subsequently treated with mixtures of the liquid rotenone formulation CFT Legumine (3.3% rotenone) utilizing two commercially available inert carriers: (1) CatSan Hygiene Litter (mixture of quartz sand and calcite) and (2) Vectocarb (fine powder of modified CaCO₃). Trials on the mixtures were conducted in 2015 in upwelling groundwater areas of the Skibotn River drainage, Troms County, Norway, the site of previously failed eradication efforts. Following application, mean concentrations of 75.6 to 131 µg/L rotenone were present at 0.5 h in the pools and the brooks downstream of the upwelling groundwater that decreased and stabilized to 11.5 to 16.8 µg/L rotenone at 3 h. Both carriers have large surface areas (porosity) that transport (through sorption) the rotenone liquid to the source of upwelling groundwater and release (through desorption) concentrations of rotenone over at least 3 h. Both mixtures show promise in treating upwelling groundwater to eradicate fish from those areas and were used successfully in the 2016 retreatment of Skibotn River for the eradication of Atlantic Salmon *Salmo salar* infested with the ectoparasite *Gyrodactylus salaris*.

Most current rotenone treatments are directed towards eradication, the elimination of whole fish populations or fish species from distinct habitats or bodies of water. This

requires maintaining lethal rotenone concentrations in the water over time sufficient to affect the lethality of the target fish (Finlayson et al. 2018). This presents challenges in areas of upwelling groundwater from springs and seeps, particularly in lotic systems. It is not uncommon to find fish in these remote areas away from the major stream channels. The ineffective treatment of groundwater refugia is believed to be one reason that some projects have had incomplete elimination of target species (Leppink 1977; Johnsen et al. 2008). Flowing surface waters are typically treated with drip stations that maintain a continuous concentration of rotenone over time (Finlayson et al. 2018), but it is often not feasible to place drip stations on the many remote small springs and seepage areas.

Upwelling groundwater can confound a rotenone treatment through three potential means: (1) untreated water entering a treated area and lowering the rotenone concentration through dilution, (2) fish inhabiting the stream or lake bottom of an area of upwelling groundwater and avoiding direct rotenone exposure, and (3) fish taking refuge in the hyporheic zone where rotenone is diluted from the mixing of shallow groundwater with the treated surface water. Heggnes et al. (2011) reviewed the literature on the impact of groundwater on critical habitats and the behavior of several salmonid hosts of the ectoparasite *Gyrodactylus salaris*. Salmonids select habitats with low water velocities to reduce the energy needed to maintain position, and in fast moving streams they generally hold position close to the bottom (Nislow et al. 1999;

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Hayes et al. 2000). Salmonids are also known to seek cooler upwelling groundwater habitats for thermoregulation during the critically warm summer months that are typically utilized for rotenone treatments (Ebersole et al. 2001). Conversely, warmwater fish (i.e., centrarchids and ictalurids) may come in contact with upwelling groundwater as they seek refuge in muddy bottoms of lakes during cold winter months (Kerr and Grant 2000). However, there were no studies to substantiate the hypothesis that fish actively take refuge in groundwater in response to environmental stressors like rotenone (Heggenes et al. 2011).

The location of upwelling groundwater in dry stream courses is obvious but much more difficult if the source is submerged and the stream is flowing. Submerged areas can be located by measuring concentrations of dye (i.e., Rhodamine WT), by finding areas of open water during winter ice-over, or by manually measuring the water temperature. These techniques are labor intensive and often give imprecise locations. A quicker and more precise method utilizes widely available low-cost infrared collectors that can be attached to smart phones to pinpoint discharges based on the natural contrasts in surface and groundwater temperatures (Fullerton et al. 2015; Hare et al. 2015; USGS 2017; Zhou et al. 2018).

The successful treatment of seeps and springs, similar to streams, requires that the source of the upwelling groundwater be treated with rotenone over a duration necessary to affect lethality on the target fish (Finlayson et al. 2018). As a result, most stream treatments apply rotenone over a minimum of 4 h, including a 1- to 2-h lag between consecutive drip stations, to ensure that lethal levels of rotenone are present throughout the treatment zone for a minimum of 2 h. Miniature drip stations could be deployed in these areas but that would require retrieving the equipment at the end of the treatment. Another approach has been to use a mixture of sand, gelatin, and powdered rotenone that sinks to the source of the groundwater and releases rotenone gradually to maintain toxicity (Spateholts and Lentsch 2001). In trials this mixture performed well, producing 100% mortality of salmonids in areas of upwelling groundwater for a minimum of 12 h after application. It was successfully used in the treatment of >450 spring and seeps in the Strawberry River drainage in Utah (Spateholts and Lentsch 2001).

The influence of upwelling groundwater on the surface discharge from west coast Norwegian rivers is significant. These deeply confined and glacially formed valleys have steep terrain, and runoff through the fluvial and alluvial deposits results in a high water table; groundwater inflow may constitute 40–100% of the total discharge during low flow periods (Heggenes et al. 2011). Norway uses rotenone for the control of the ectoparasite *G. salaris*, which causes fjordwide (adjacent rivers) epidemics that have reduced

Atlantic Salmon *Salmo salar* parr density and returning adults by an average of 86–87% (Johnsen 2006; Sandodden et al. 2018). However, not all rotenone treatments have been successful and some may have failed due to upwelling groundwater (Johnsen et al. 2008), but little research has been done on treating groundwater to achieve eradication of *G. salaris* salmonid hosts in these systems. Powdered rotenone is not available in Norway, but the liquid rotenone formulation CFT Legumine (3.3% rotenone) can be used. In the United States, stream treatments utilize liquid rotenone, and a mixture that uses liquid rotenone would forgo the necessity of having to also purchase powdered rotenone since powdered rotenone use is now severely restricted in streams (USEPA 2007). Here, we report on the field trials to treat upwelling groundwater with two commercially available products, Vectocarb and CatSan Hygiene Litter, which acted as carriers for CFT Legumine.

METHODS

Study area.—The trials were completed in the Skibotn River drainage, Troms County, located in northwestern Norway (Figure 1). The river, which has a catchment area of 784 km², originates from a series of lakes and flows in a northwesterly direction into the Storfjord of the North Sea. The study area was located at the source of cold (4.7°C), upwelling groundwater that came to the surface in two adjacent pools that flowed into two small, parallel brooks. The upwelling of groundwater in these pools was diffuse, and the surface flow from each pool was approximately 0.06 m³/s during the trials. We chose an experimental plot size of 5 m² consistent with the small size of the parallel brook pools. The parallel brooks had additional groundwater upwelling along their courses and were approximately 150 m in length before they merged into a single channel and then entered the Skibotn River. The Skibotn River was treated with rotenone in 1988 and again in 1995 in failed attempts to eradicate *G. salaris* (Johnsen et al. 2008), and the ineffective treatment of upwelling groundwater was strongly believed to be the reason for these failures. This site had been previously investigated by Brabrand et al. (2005), who found Arctic Char *Salvelinus alpinus*, a known host of *G. salaris* (Johnsen et al. 2008), present in these upwelling groundwater areas.

Locating upwelling groundwater.—Thermal infrared imagery was used to locate upwelling groundwater in these two parallel brooks by attaching a thermal camera (i.e., FLIR One) to a smartphone and taking both a normal picture and a thermographic picture. The pictures were placed on top of one another using colors to differentiate temperatures. The thermal image shows the relative temperatures over the image area using a color

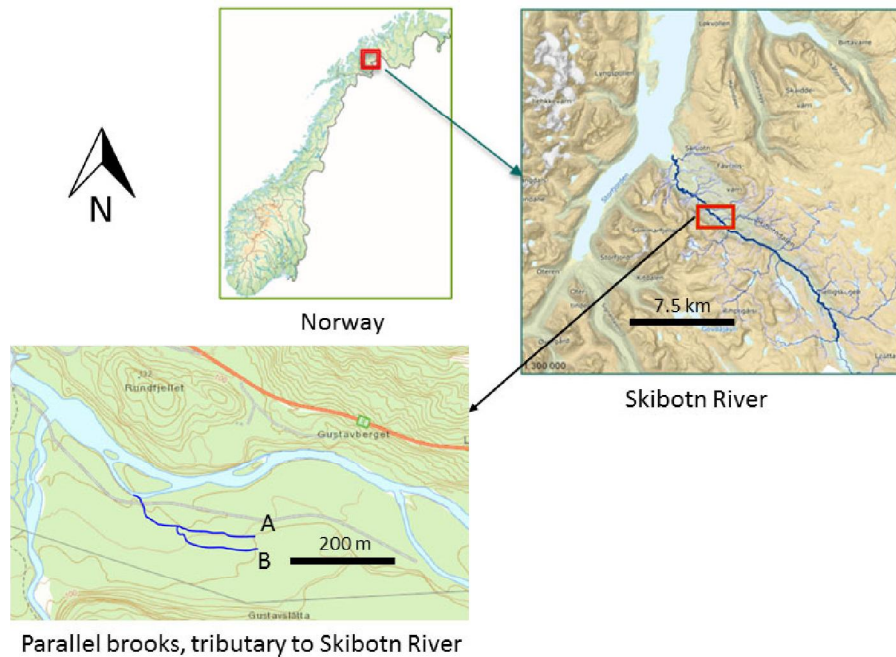


FIGURE 1. Maps of Norway showing the Storfjord, the Skibotn River drainage, and the unnamed parallel Brooks A and B where the trials were located. Brook A was treated with the CatSan mixture, and Brook B was treated with the Vectocarb mixture.

gradient, and the camera can be used to take a temperature measurement at a specific point on the image (Figure 2). This allowed for the easy interpretation of the thermal gradients and the source of the upwelling groundwater. This equipment can detect a temperature difference of 0.1°C , but typically differences of several degrees are needed to conclusively attribute water surface thermal heterogeneity to a groundwater discharge. The picture was then stored on the smartphone and its exact location identified by GPS. The thermal camera FLIR One comes in two versions, one for the iPhone system (e.g., iOS version 8.2 or higher) and one for the Android system (i.e., Samsung Galaxy, version S6 or higher).

Rotenone mixtures.—The mixtures used the CFT Legumine (3.3% rotenone) formulation (product of VESO) as the rotenone source. The CatSan Hygiene Litter mixture was prepared by adding 1 L of the product to 100 ml of CFT Legumine. CatSan Hygiene Litter (product of Mars Petcare) consists of natural quartz sand and chalk. Previous experimentation with this mixture indicated that the volume ratio of 1:10 (CFT Legumine : CatSan) was the ideal consistency (separate granules with no residual liquid rotenone) for broadcast application by hand. The mixture was prepared immediately prior to use as it has a tendency to turn into a sticky cooked-oatmeal consistency, which prevents good broadcast application, if not used within the hour. Approximately 2 L of the mixture was applied by hand,

broadcast to a 5-m^2 area of the pool in unnamed Brook A (Figure 1). The granules sank immediately after application to the bottom of the pool.

The Vectocarb 30-OM mixture was prepared by adding 1 L (~300 g) of the product to 1 L of CFT Legumine; the resulting slurry had a consistency of cream. Vectocarb 30-OM (product of Omya International AG) is a fine powder of hydroxyapatite-modified CaCO_3 . Previous experimentation with this mixture indicated that the volume ratio of 1:1 (CFT Legumine : Vectocarb) was the ideal consistency (creamy slurry with no residual liquid rotenone) for pumping. The mixture was prepared off site and stored in a plastic bucket. Approximately 2 L of the mixed slurry was applied with site water at a 1:10 ratio using a semiclosed application system (Finlayson et al. 2018) to a 5-m^2 area of the pool in unnamed Brook B (Figure 1). The slurry followed the current of water settling to the bottom of the pool.

Rotenone dosing.—The primary objective of this initial study was to determine whether the two media could be used to carry liquid rotenone to the source of upwelling groundwater and release rotenone over an extended period of time sufficient enough to affect mortality of salmonids. We adjusted the dosing so that rotenone concentrations in the water would be in the range of 10 to $100\ \mu\text{g/L}$, the lethal range for salmonids (Marking and Bills 1976; Finlayson et al. 2009) and easily verified by chemical analysis (see below). For comparative purposes, we applied the same amount of both mixtures (2 L) to the 5-m^2 test plots.

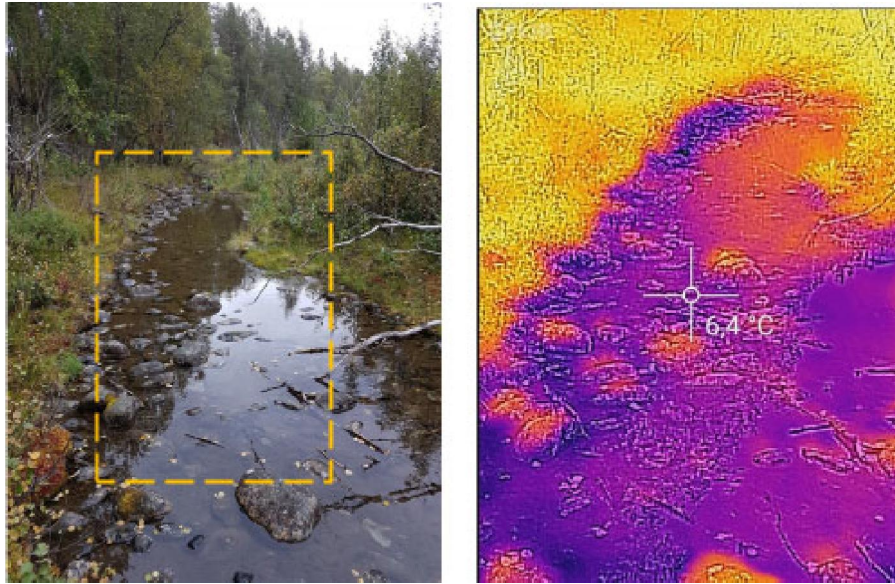


FIGURE 2. A normal image (left panel) and its thermographic replicate (right panel) of a groundwater seep in an unnamed tributary to the Skibotn River using a smartphone and the FLIR One thermal imaging system. The darker blue color (lower left of right image) indicates the location of cooler, upwelling groundwater. The system can also provide the water temperature at a specific point (white symbol and number; 6.4°C).

These conditions resulted in approximately 33 g (6.6 g/m²) and 6.6 g (1.3 g/m²) of rotenone applied to the test plots for the Vectocarb and CatSan mixtures, respectively. Over the 3-h test period, with the brooks discharging at approximately 0.06 m³/s (60 L/s), we would expect average rotenone concentrations of 51 µg/L from the Vectocarb mixture and 10 µg/L for the CatSan mixture if all the material dissipated within the 3-h period.

Rotenone mixture monitoring and analysis.—Following the application of the mixtures to the pools, water samples for rotenone analysis were collected for the next 3 h, 3 cm above the bottom substrate using a hand-operated siphon pump in the pools and at several locations along the brooks using standard techniques (Sandvik et al. 2018). To provide optimum UV protection for the light-sensitive rotenone, water samples were placed in amber glass bottles. The bottles were rinsed twice with the stream water and then filled completely before sealing and transporting to the laboratory. Immediately upon arrival, an aliquot of water (1 mL) was transferred from the sample bottle to a high-performance liquid chromatography vial containing acetonitrile (1 mL; high-performance liquid chromatography grade) and vortexed prior to liquid chromatography–UV analysis. Rotenone was analyzed by liquid chromatography with UV detection; typical analytical performance was 1 µg/L rotenone quantification, within-assay precision (relative standard deviation) of 5.5 to 6.5%, and 99 ± 2% recovery of rotenone from certified

batches of CFT Legumine from the manufacturer (Sandvik et al. 2018).

RESULTS

Both mixtures released rotenone for at least 3 h following application (Tables 1, 2), with the highest (up to 214 µg/L rotenone) and most variable (19.8 to 214 µg/L rotenone) concentrations occurring in the initial samples collected at 0.5 h. As expected based on the difference in rotenone dosing between the two mixtures, the CatSan mixture produced lower rotenone concentrations (Table 1) compared with the Vectocarb mixture (Table 2). The mean concentrations of rotenone during the 3-h trials varied from 9.7 to 75.6 µg/L for the CatSan trial (Table 1) and from 16.8 to 131 µg/L for the Vectocarb trial (Table 2).

In both trials, the highest concentrations generally subsided in subsequent samples suggesting a large release of rotenone soon after application that later stabilized. It appears that the Vectocarb mixture had a tendency to move with the water current downstream, whereas the CatSan mixture stayed in place. Similar rotenone concentrations were found in the CatSan trial pool and brook downstream, whereas higher concentrations from the Vectocarb trial occurred in the brook downstream of the application pool, particularly the samples collected at 0.5 h.

TABLE 1. Resulting rotenone concentrations ($\mu\text{g/L}$) in the treated pool and brook from the CatSan mixture at various times after application. The mixture was applied to the plot at a rate of $1.3 \text{ g rotenone/m}^2$, and the brook discharge was $0.06 \text{ m}^3/\text{s}$.

Time after application (h)	Sample location				Mean \pm SD
	Pool	Brook 10 m downstream	Brook 20 m downstream		
0.5	59.0	105.0	62.7		75.6 ± 25.6
0.75	19.8	24.1	19.1		21.0 ± 2.7
2.0	8.7	9.2	11.2		9.7 ± 1.3
3.0	7.9	12.5	14.2		11.5 ± 3.2

TABLE 2. Resulting rotenone concentrations ($\mu\text{g/L}$) in the pool and brook from the Vectocarb mixture at various times after application. The mixture was applied to the plot at a rate of $6.6 \text{ g rotenone/m}^2$, and the brook discharge was $0.06 \text{ m}^3/\text{s}$.

Time after application (h)	Sample location				Mean \pm SD
	Pool	Brook 10 m downstream	Brook 20 m downstream	Brook 30 m downstream	
0.5	19.8	127.0	162.0	214.0	131.0 ± 82.1
0.75	10.8	61.4	72.9	74.6	54.9 ± 30.0
2.0	15.5	27.3	30.4	31.0	26.1 ± 7.2
3.0	7.6	20.8	19.5	19.1	16.8 ± 6.1

DISCUSSION

Both the Vectocarb and CatSan media were successful in carrying liquid rotenone to the source of upwelling groundwater and releasing rotenone into the water for over 3 h. Further, it appears that these treatments likely would have produced conditions sufficient to kill salmonids within the 3-h exposure, with mean concentrations varying from 9.7 to $75.6 \mu\text{g/L}$ for the CatSan trial and from 16.8 to $131 \mu\text{g/L}$ for the Vectocarb trial. The short-term toxicity of rotenone to Rainbow Trout *Oncorhynchus mykiss* varies from $8.8 \mu\text{g/L}$ rotenone (3-h LC50 [concentration lethal to 50% of test organisms in 3 h] value from Noxfish; Marking and Bills 1976) to $7.4 \mu\text{g/L}$ rotenone (4-h LC50 value from CFT Legumine; Finlayson et al. 2009). However, rotenone can be lethal to salmonids within 0.5 h of exposure. The CatSan and Vectocarb mixtures were used successfully in the 2016 treatment of the Skibotn River to eradicate *G. salaris* (Adolfson et al. 2017).

It is difficult to compare the performance of the Vectocarb and CatSan mixtures with the powdered-rotenone-sand-gelatin mixture described by Spateholts and Lentsch (2001) because of differences in treatment strategies and groundwater conditions. Spateholts and Lentsch (2001) applied the mixture to distinct exposed springs rather than to submerged diffuse upwelling groundwater as was used in this study. Their treatment rates (based on a discharge

over a 24-h period) of 100 , 250 , and $350 \mu\text{g/L}$ rotenone were all releasing $>100 \mu\text{g/L}$ rotenone at 1 h following application, which subsided to 70 to $84 \mu\text{g/L}$ rotenone at 4 h. The release rates from our mixtures were less, but we applied the mixtures to cover the bottom of diffuse upwelling groundwater areas, and we did not treat the entire pool but only a 5-m^2 area of each pool. Both studies did show decreasing rotenone concentrations over time, which likely could be increased in concentration and duration by increasing the dose.

Both carriers used in this study have high surface areas (porosity), which allowed for the adsorption of the rotenone on the surface of the particles that then sank when applied to the water and released rotenone back into the water over an extended period of time. The Vectocarb 30-OM was developed specifically for applications in aquatic pest control and has high porosity (surface area of $27 \text{ m}^2/\text{g}$) and sedimentation behavior in aquatic systems (Omya International AG 2016). Similar information is not available for CatSan, but the silica gel that is commonly used in cat litter products has high porosity (surface area of $\sim 800 \text{ m}^2/\text{g}$). The Vectocarb appeared to move greater distances with the current than did CatSan, likely a function of the size of the individual particles. The median particle size for Vectocarb is 0.0024 mm (Omya International AG 2016), whereas the size of CatSan is approximately 5 mm , suggesting that the former is more likely to

move with water current. Although not tested here, we suspect that cat litter products that utilize clay may not perform well since clay appears to bind to rotenone and would likely interfere with rotenone desorption into the water (Dawson 1986).

Both mixtures show promise in treating upwelling groundwater to eradicate target fish from these areas. Additional experimentation is needed to determine the impact of temperature on the rotenone release rates of the mixtures, the limits of the current affecting the placement of the mixtures, and the effectiveness of infrared technology in locating upwelling groundwater under varying environmental conditions. Although we used the mixtures at an application rate of 0.4 L/m², lower rates should be tested for releasing lower rotenone concentrations for shorter durations and higher rates should be tested for releasing higher rotenone concentrations for longer durations. Both mixtures used the CFT Legumine formulation but other rotenone formulations (e.g., Prenfish Fish Toxicant and Chemfish Regular) are expected to function similarly with the products tested.

In streams with multiple upwelling groundwater areas, the mixtures could be applied over all the upwelling areas so long as the maximum label rate of 200 µg/L rotenone is not exceeded. However, the treatment of the hyporheic zone can also affect nontarget aquatic invertebrates that inhabit those areas (Vinson et al. 2010). In addition to using the lowest effective dose of rotenone needed, another method of limiting invertebrate impacts is to leave headwater reaches of drainages that are above fish barriers and have never inhabited fish as untreated refuges for invertebrates and a source for recolonization of downstream treated reaches (Whelan 2002; Finlayson et al. 2009).

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