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Research Article

Eradication of *Gyrodactylus salaris* infested Atlantic salmon (*Salmo salar)* in the Rauma River, Norway, using rotenone

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Abstract

The invasive alien ectoparasite *Gyrodactylus salaris* is one of the greatest threats to wild Atlantic salmon (*Salmo salar*) in Norway. Since its introduction in the 1970s the Norwegian Environmental Authorities have applied a piscicide based eradication strategy, using rotenone to eradicate the host species, Atlantic salmon and the parasite. After refining the methods and techniques following several unsuccessful treatments, the program has become a success and a total eradication of *G. salaris* from Norway now seems possible. This paper describes the methods and techniques used in this program during a large eradication operation conducted in the Rauma infection zone in central Norway using different land based peristaltic and boat mounted pumps in combination with continuous drip stations and gardening cans. The eradication was performed in 2013 and 2014 and involved six infected rivers. The largest river, the river Rauma has an anadromous section of 42 kilometers and consists of both rugged fast flowing areas and slow flowing parts characterized by laminar water currents. The piscicide, CFT-Legumine®, containing 3.3% active rotenone was applied at a dose of 1 mg/l using a range of application methods aiming to achieve concentrations of 0.033 mg/l rotenone. To ensure target concentrations were met, rotenone concentrations were monitored using liquid chromatography with UV detection in all treated river in an on-site lab on a daily basis. Target concentration was reached in all treated rivers and while investigations are ongoing, to date they indicate eradication has been effective.

Key words: invasive alien species, CFT-Legumine, fish control, liquid chromatography, piscicide

Introduction

The Atlantic salmon parasite *Gyrodactylus salaris* (Malmberg, 1957) was introduced to Norway during the 1970s on infected Atlantic salmon from Sweden. It is one of the greatest threats to wild Norwegian Atlantic salmon (*Salmo salar* Linnaeus, 1758) (The Norwegian Environment Agency 2014). Average mortality of juvenile Atlantic salmon in studied rivers is estimated to be 86%, with up to 99% mortality in the worst affected populations (Johnsen et al. 1999). Locally adapted Atlantic salmon stocks are typically on the brink of extinction 4–6 years after introduction of the parasite (Johnsen et al. 1999), leading also to severe negative impact for local fishing tourism, recreation and business. Infected rivers pose a serious and continuous risk of spreading the parasite to neighboring river systems.

G. salaris is a monogenean ectoparasite with no intermediate hosts (Olstad 2013). Other than Atlantic salmon, *G. salaris* can survive and reproduce on rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), Arctic char (*Salvelinus alpinus* Linnaeus, 1758), North American brook trout (*Salvelinus fontinalis* Mitchill, 1814), Arctic grayling (*Thymallus thymallus* Linnaeus, 1758), North American lake trout (*Salvelinus*

namaycush Walbaum, 1792) and brown trout (*Salmo trutta* Linnaeus 1758) (in declining order of susceptibility (Bakke et al. 2002, 2007). The only possible hosts present in the infected rivers described are Atlantic salmon and brown trout. The effect of *G. salaris* on Atlantic salmon is characterized by severe infections. Not much is known about the disease Gyrodactylosis and the cause of death in infected individuals, but secondary infections in wounds from attachment and feeding are assumed to be important (Olstad 2013). Often several thousand parasites infect individual fish. Mortality probably results from a combination of *Saprolegnia* spp. infection (Johnsen 1978; Olstad et al. 2006) and penetration of the skin which coupled with feeding activity leads to osmoregulation imbalance (Pettersen et al. 2013).

G. salaris was first identified within the Rauma infection zone in 1980, in the river Henselva. The parasite was subsequently detected in the rivers Rauma (1980), Istra (1982), Skorga (1982), Måna (1985) and Innfjordselva (1991). An attempted eradication was performed in September 1993 (Aspås 1994). This attempt failed, and the parasite was rediscovered in river Rauma in September 1996 (Mo et al. 1997). It is believed that the failure was the result of *G. salaris* infected Atlantic salmon surviving in ground water upwelling areas about 20 km from the river outlet (Johnsen et al. 2008).

The aim of the Norwegian environmental authorities is a total eradication of *G. salaris* in Norway and they have developed a strategy accordingly (The Norwegian Environment Agency 2014). Because of the strong host dependency, eradication of infected fish has been used as a method to remove *G. salaris* from infected rivers. *G. salaris* can only survive for a short time period without a host (Olstad 2013). To date, the only successful method to achieve this has been the application of rotenone based piscicides. CFT-Legumine® containing 3.3% liquid rotenone is the most common formulation used today. Rotenone has been widely used in Norway, especially over the last two decades. Infected rivers are grouped into zones. Infected zones are defined as the geographical area in which infected juvenile fish can move naturally. The parasite has been introduced to a total of 49 rivers in 17 infection zones. Of these 49 infected rivers, 22 have been treated and are parasite free, 18 have been treated and await eradication confirmation and 9 rivers are still untreated and infected (Anon 2015).

Rotenone is a naturally occurring substance derived from the roots of tropical plants in the Leguminosae family (USEPA 2007) and has been used for centuries to capture fish for food in Southeast Asia and South America where these plants naturally occur (McClay 2000; Ling 2003). Rotenone is poisonous to organisms to varying degrees, but gill-breathing species are particularly vulnerable. Rotenone is rapidly absorbed across the gill epithelium and blocks oxygen use by cells (Koopman et al. 2005). Rotenone is highly toxic to fish and certain invertebrates (Fukami et al. 1969). Vinson et al. (2010) reviewed the effects of rotenone treatments on invertebrate communities and showed that sensitivity to rotenone varies considerably between taxonomic groups. Studies show that aquatic invertebrates typically experience a dramatic short term reduction in abundance (Arnekleiv et al. 2001). The reestablishment of most taxa is rapid and often complete after a year (Eriksen et al. 2009; Arnekleiv et al. 2015). A comprehensive invertebrate survey before, following and after CFT-Legumine treatments was performed in the river Rauma. Rotenone is non-persistent in the aquatic environment and is degraded by photolysis and hydrolysis (Finlayson et al. 2014).

An essential part of a *G. salaris* eradication program is the reintroduction of the native fish stocks affected by the rotenone treatment. This strategy involves the use of both living genebank and milt bank. The purpose of living genebank is to establish a reservoir of genetic material which can be used for the reestablishment or enhancement of threatened stocks. The living genebank is based on the principal of eggs in – eggs out. Eyed eggs are delivered to local hatcheries or directly to the river for egg planting. Smolts and yearling are also released in smaller numbers the first years after treatment. This ensures a rapid reintroduction of native stock. The most important issue is to create a good founder population in the living genebank. This requires a large number of parentfish, at least 25 of each gender, representing the whole river and including all year classes. Frozen milt from the milt bank is used to increase genetic variation, and frozen milt from the living genebank is used to reduce loss of variation during the production period within the living genebank. A five year program including eyed eggs, one year old yolk sack juveniles and smolt releases are being implemented. The only two other fish species present in the treated rivers are three-spined stickleback (*Gasterosteus aculeatus* Linnaeus, 1758) and European eel (*Anguilla Anguilla* Linnaeus, 1758). Both these species will recolonize the rivers from the Romsdal fjord.

The Norwegian Veterinary Institute, section for Environmental Restoration and Management has been performing fish eradications since 1997. Through experience, international advice (Finlayson et al. 2010) and conclusions made by a Norwegian expert group (Johnsen et al. 2008), a significant number of improvements have been made to the techniques used, the

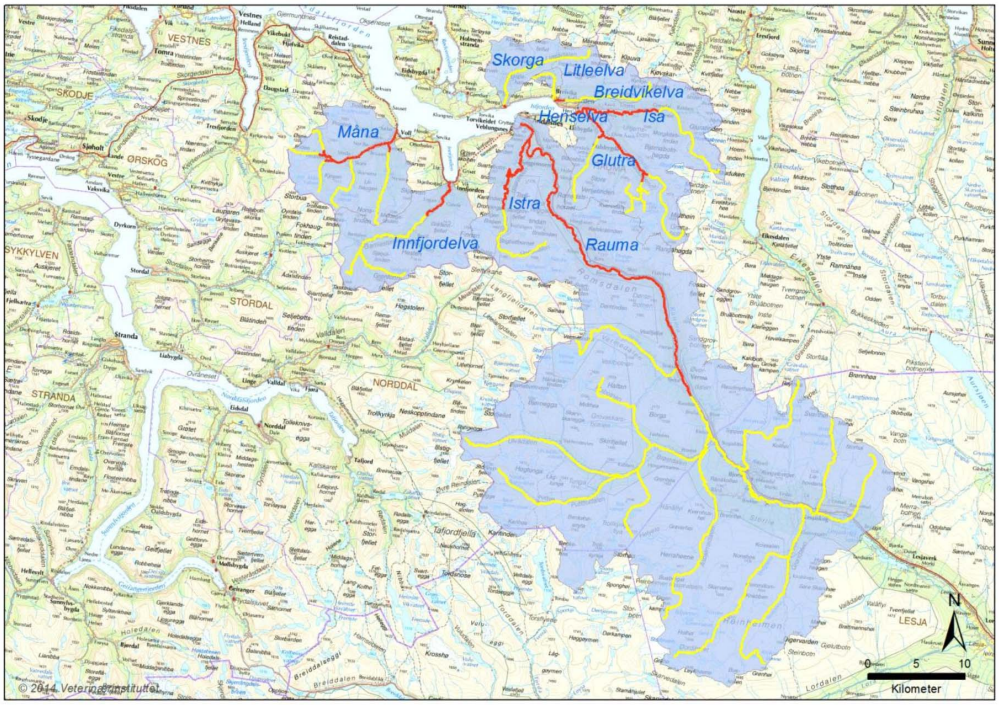

Figure 1. Catchments of the seven largest rivers in the Rauma treatment zone. Anadromous sections of the six infected rivers are marked red.

Table 1. Catchment size, anadromous section and mean annual discharge of six *G. salaris* infected rivers treated.

River	Tributary	Catchment, km^2	Anadromous sections, km	Mean annual discharge, m^3/s
Rauma		1203	42	36.3
	Istra	65.5	18	3.1
Måna		109.22	10	5.6
Innfjordselva		104.14	5.5	4.8
Hensvassdraget		176.18		
	Glutra	95.16	11	5.0
	Isa	79.20	12	4.5
Breivikelva		28.89	1.5	1.4
Skorga		43.81	0.4	2.2

training of personnel, the preparatory work, health and safety measures, pre-treatment planning, on-site determination of rotenone concentrations and the implementation of those plans. This paper describes the eradication effort conducted in the Rauma-region and outlines the methods and techniques involved. It aims to contribute to improvements with fish eradication programs at all levels in the future.

Methods

Treatment area

The six infected rivers in the Rauma infection zone all drain into the sea in the Romsdal-fjord in Møre and Romsdal County situated in central Norway (Figure 1). The largest infected river in the region is the Rauma. The Rauma also includes the tributary Istra. The upper sections of the Rauma are steep and fast flowing with several groundwater upwelling areas. The lower sections are slow flowing meandering and characterized by laminar water currents. The characteristics of the infected rivers are summarized in Table 1. The treatment comprised all six infected rivers in the Rauma infection zone.

Treatment plan and design

65 operatives were involved in the 2014 treatment. This consisted of personnel conducting rotenone application, collecting and analyzing water samples, water discharge measurements, dead fish removal and

equipment operation. The treatment in Rauma was performed according to the plan outlined in Table 2.

The eradication was performed over two consecutive years (2013–2014) in the relatively stable hydrological period between August and September, characterized by water discharge close to the annual mean. Typical water temperatures during this period are 10–15 °C. Each treatment was performed over the course of 10 days. Hydrological surveys on various water flow scenarios were performed and water transport times were measured in the infected rivers during characteristically high and low discharge events. Water discharge was monitored during treatment of the infected rivers and the rotenone dose was refreshed every three to four hours transport time downstream towards the outlet. This paper describes the 2014 eradication. Some minor rotenone application adjustments were made between 2013 and 2014, due largely to improved survey techniques and experience gained during the different phases of the initial eradication.

During the rotenone treatment several parallel application stations were set up downstream from the top treatment station at Slettafossen towards the outlet (Figure 2). This enabled treatment of different sections simultaneously in one river. The total treatment effort was coordinated in a manner which ensured that boat and tributary teams never treated areas downstream of the rotenone plume from the main peristaltic dosing stations in the main rivers. The treatment comprised of the anadromous zone of all infected rivers, hence no neutralization of the rotenone was performed.

During the preparations, detailed mapping of all water bodies which could potentially hold infected fish was performed. All areas was visited by foot or boat and mapped by the help of GPS. Treatment maps with numerical codes characterizing specific sites in the treatment zone were produced using ArcMap. Accompanying these maps were detailed instructions describing each number on the map and how the sites should be treated by the treatment teams.

The river Rauma was treated over three days. On the first day, the river was treated from the top treatment station at Slettafossen down to station at Remmem Bridge (Figure 2). All tributaries with outlets in this area were treated accordingly. On the second day, Rauma was treated from the station at Remmem Bridge downstream to the station at Storøya, and on day three the rest of the river including the large tributary Istra was treated. The application timing was planned on discharge readings and water transport times recorded for different discharge regimes and estimated travel times to the next application stations (Table 3).

Table 2. Treatment plan for the Rauma infection zone.

Day	Work conducted
Day -1.	Crew arrival, initial meeting
Day 0.	Crew training, inspection of rivers and handing out equipment
Day 1.	Treatment Rauma upper sections
Day 2.	Treatment Rauma middle sections
Day 3.	Treatment Rauma lower sections and Istra
Day 4.	Treatment Isa upper sections. Inspection of rivers and preparations for day 7
Day 5.	Treatment Isa, Glutra and Hensvassdraget
Day 6.	Treatment Innfjordelva, Skorga, Breivikelva and Litleelva
Day 7.	Treatment Måna

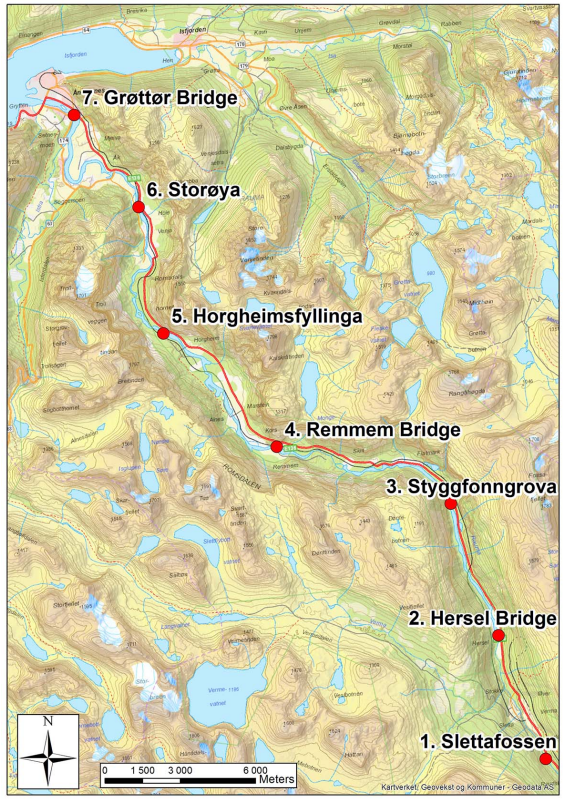

Figure 2. Main CFT-Legumine application stations for during the 2014 treatment in the river Rauma.

Application rates

The application rates of CFT-Legumine® 3.3% (http://www.veso.no) was based upon a combination of empirical data and operational experience from similar lotic treatments in Norway. A bioassay with Atlantic salmon fry using the 3.3% CFT-Legumine® formulation with 24 h exposure, produced an LC_{50} and LC_{90} of 2.99 µg/l and 3.25 µg/l respectively at temperatures ranging from 5,8 to 7.8 °C during the test (Sevatdal 2013, unpublished). The aim was to achieve 100%

Table 3. CFT-Legumine dosage regime for all main application stations in the river Rauma during the 2014 treatment.

Day	Appl. station	Discharge m^3	Time	CFT-Legumine l/h^{-1}	CFT-Legumine mg/l	Rotenone µg/l	Transport-time, min.
1	1	18	08:00-09:00	120	2	66	120
			09:00-16:00	65	1	33	
	2	18	08:00-09:00	130	2	66	300
			09:00-11:00	65	1	33	
	3	18	08:00-09:00	130	2	66	390
			09:00-15:00	65	1	33	
			15:00-16:00	35	0.5	12.5	
2	4	17	07:30-08:30	130	2	66	240
			08:30-15:30	60	1	33	
	5	17	07:30-08:30	130	2	66	300
			08:30-12:30	60	1	33	
			12:30-15:30	30	0.5	12.5	
3	6	32	08:00-09:00	230	2	66	330
			09:00-16:00	115	1	33	
	7	107_a	10:00-13:00	200	0.5	12.5	

[a]Water discharge was measured and represents a sum of the actual water discharge of the river and the effect of tide.

fish mortality during a theoretical exposure time of 4–6 hours. To account for degradation, biological variability, inaccuracies of water discharge measurements and uneven mixing of water and CFT-Legumine under natural conditions, we applied a dosage of 1 mg/l CFT-Legumine® (33 µg/l of rotenone) for 8 hours. The applied dose is high according to the LC_{50} cited, but using lower concentrations has previously led to unsuccessful treatment in similar rivers (Johnsen et al. 2008). The high dose also reflects a relative short application period. In order to mitigate a diluted front of the rotenone plume we applied 2 mg/l CFT-Legumine® (66 µg/l of rotenone) during the first hour at the top stations only. A further reduction in dosage was applied to avoid rotenone accumulation downstream following dosage from several stations. (Table 3).

Application equipment

CFT-Legumine® was applied using different equipment suited for different purposes and challenges. Some of the infected rivers in the Rauma infection zone are characterized by rugged, stony and fast flowing white water. The equipment used was adapted to the challenges met in individual rivers. At the main application stations we used peristaltic pumps of different sizes to accommodate the discharge at each site. For creeks and tributaries we used peristaltic pumps. To ensure a quick mix of water and CFT-Legumine®, we aimed to locate application stations at sites characterized by turbulence, rapids, upstream turns or waterfalls. At sites where this was not possible, we set up stations with multiple emission points across the whole section of river. The hoses were fixed and attached to a bridge, or on a wire stretched across the river. Drip stations were used in seeps. They supplied a linear and constant dosage of 20 liters of diluted (according to discharge) CFT-Legumine® over four hours. In the smallest of seeps, small pockets of standing water and small upstream creeks, common watering cans used in gardens were used to dispense the diluted (1000 ppm) CFT-Legumine®. Boat mounted pumps were used for treating river- and gravel banks. For treating rapid flowing waters a white water raft mounted pump system was used and was comprised of a professional rafting guide, one team leader conducting the pumping and a two man crew. For the smaller rivers we used smaller rafts with crews of two to three (Figure 3). All river banks and gravel banks were flushed with diluted (100 ppm) CFT-Legumine® by the use of the boat mounted pumps.

Rotenone concentration analysis

Rotenone concentration analysis was performed to confirm that target dose levels had been achieved in the treated rivers and in areas with upwelling ground water. An on-site lab was set up by the treatment headquarters and water samples were withdrawn on a daily basis according to a predetermined plan. This allowed daily rotenone concentration analyzes and discussion of the results with the treatment planning group. Fast, simple and accurate liquid chromatography with UV detection was used for on-site determination of rotenone in water.

Reagents

Acetonitrile (MeCN) was of HPLC grade and obtained from Rathburn Chemical (Walkerburn, UK), and water

Figure 3. Large (yellow) and small (blue) white water rafts equipped with fixed pumps for spraying water diluted CFT-Legumine. Photograph by Dag H. Karlsen.

was deionized (MilliQ). Rotenone (≥ 95%) and deguelin (≥ 98%) standards were obtained from Sigma Chemical (St. Louis, MO, USA). The rotenone formulation CFT Legumin 2.5%™ was obtained from Kemira (Espoo, Finland).

Instrumentation and chromatographic conditions

The UFLC-UV system from Shimatzu (Kyoto, Japan) was equipped with two LC-20AD pumps, a SIL 20A automatic sample injector, and a SPD 20A DAD detector. The separation was performed on a Waters XBridge™ C18 3.5 µm 2.1 x100 mm column or a Waters XBridge™ C18 2.5 µm 3.0 x100 mm (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.

Rotenone quantification

Rotenone was quantified using an external four-point calibration curve in the concentration range 1–250 µg/L (Sandvik 2018). 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 limit of quantification was 1 µg/l in water samples diluted with acetonitrile (water-acetonitrile, 50:50). A pre-concentration step using Spin-X® centrifuge filters allowed quantification levels of 200 ng/l. Analysis time was 6 min per water sample and all samples were analyzed within a few hours of collection, making it possible to adjust the nominal rotenone concentration to desired levels during treatment. When treating the upper tributaries of the river Glutra this showed necessary.

Health and safety

A health and safety plan for the treatment was developed. Risk assessments were produced for all major tasks. All personnel received safety instructions and equipment training before the treatment. Every treatment team handed in daily reports describing the treatment conducted according to a quality assurance plan. All reports were checked on a daily basis to make sure all treatment areas were covered. All clothes and equipment used were disinfected with a 2% solution of the disinfectant Virkon-S® on a daily basis during the treatment. All personnel and equipment had to pass a disinfection sluice in order to ensure no spread of *G. salaris* infection. Dead fish collected were stored in leak proof containers at a dead fish laboratory set up by the treatment headquarters. To kill any possible surviving *G salaris* on the dead fish, 35‰ salts based on fish weight was added to the fish containers and disposed of according to Norwegian regulations for potential infected waste.

Dead fish removal and evaluation of results

Dead fish removal was conducted in order to document possible juvenile salmon surviving the first treatment in 2013 and to identify possible areas in need of extra eradication effort. The search was mainly concentrated in areas considered difficult to treat. These areas are characterized with upwelling

groundwater, areas with large stones and boulders difficult to reach and large gravel banks in and adjacent to rivers. In these areas water is filtered through sand and gravel with possible long retention times. Earlier treatments have shown that fish in these areas stay alive longer than in more central parts of the river. To further evaluate the effect of the treatment leading to a possible declaration of successful removal of the parasite, a five year electrofishing program following the treatment will be performed (Hytterød et al. 2015; Hytterød et al. 2016). Atlantic salmon juveniles are sampled along the whole anadromous part of the river. The program recommends sampling of at least 10 salmon juveniles near the river outlet to the sea, and further 10 salmon at every second kilometer, all the way up to the migration barrier in the main river as well as in the tributaries. Thus, the total number of sampled fish is dependent of the length of the anadromous part of the river system. Fingerlings and parr ranging in size from 7–12 cm are caught by means of electrofishing. The fish are killed and then preserved whole in 96% ethanol. All the samples are sent to the Norwegian Veterinary Institute where the whole fish surface including body, head and fins are examined under a stereo microscope at 10–15 times magnification. When *Gyrodactylus* specimens are detected, species identification is performed by morphology and molecular methods. The Norwegian Veterinary Institute is the OIE reference laboratory for the disease and the methods used for species identification follows those in the Gyrodactylosis (*G. salaris*) chapter in the Manual of diagnostic tests for aquatic animals from the World Organization for Animal Health (OIE).

Results

In total 8198 liters of CFT-Legumine® was applied during the 2014 treatment. 5302.4 liters were applied in Rauma and the tributary Istra. 4395 liters were applied from the main application stations; whereas 568.5 liters were applied from the boat mounted pumps and 338.9 liters were applied in the tributaries using peristaltic pumps continuous drip stations and watering cans.

The peristaltic pumps and continuous drip stations were reliable and precise. The peristaltic pumps were tested every morning and the flow was controlled by the applicators every hour. The drip stations were controlled four hours after start to make sure they were empty. The white water rafts fitted with pumps proved suitable for saturating large areas with diluted CFT-Legumine®. Rotenone concentrations in all six infected rivers were monitored. Water samples were taken immediately upstream of all main application stations and at the outlet into the Romsdal fjord. Concentrations at all stations exceeded the target application value of 33 µg/l rotenone. The results from the River Rauma are presented in Figure 4. All water samples collected from areas considered difficult to treat were also above the target dose of 33 µg/l rotenone.

The water transport time from treatment station Slettafossen to Hersel Bridge on day 1 is short and the rotenone concentration increased sharply before stabilizing at lethal levels between 33 and 38 µg/l rotenone. Due to dilution of the front of the rotenone plume, the rotenone concentration increased more slowly at Styggfonngrova and Remmem Bridge on day 1 before stabilizing between 40 and 37 µg/l rotenone respectively. The concentration at Horgheimsfyllinga was 30 µg/l rotenone when the application started from Remmem Bridge and measurement commenced on day 2. This was probably due to a combination of starting to sample after the rotenone plume had reached from the above station at Remmem Bridge and residues from day 1. The curve stabilized between 40–50 µg/l rotenone. The rotenone concentrations at Storøya on day 3, when the application started from Horgheimsfyllinga were 25 µg/l rotenone and also stabilized between 40–50 µg/l rotenone. This was also probably due to a cumulative effect from stations Remmem Bridge and Horgheimsfyllinga and residues from day 1. The rotenone concentration at Grøttør Bridge, situated in the tidal zone of river Rauma dropped in the middle of the day due to dilution from the influx of tidal water on day three. The rotenone concentration increased towards the end of the measured period on the above station Storøya and would continue to rise after the end of the measured period both due to the cumulative effect from Storøya and the falling tide.

Rotenone flushed through the Rauma River to the sea resembling the water transport speed downstream. The transport time between stations Slettafossen and Hersel Bridge was 1 hour and 50 minutes at the discharge measured on the first day of the 2013 treatment. Dosage at Slettafossen was started at 07:00 and water samples were collected every hour between 09:00 and 19:00. The dosage target during the first hour from 07:00 and 08:00 was 66 µg/l and 33 µg/l between 08:00 and 14:00 the following hours. Rotenone was flushed out of the river upstream of Hersel Bridge by 19:00 (Figure 5).

Areas considered difficult to treat in all pre infected rivers were visited during the second year of treatment to search for possible surviving salmon from the 2013 treatment and subsequently any salmon older than one year. Only Atlantic salmon older

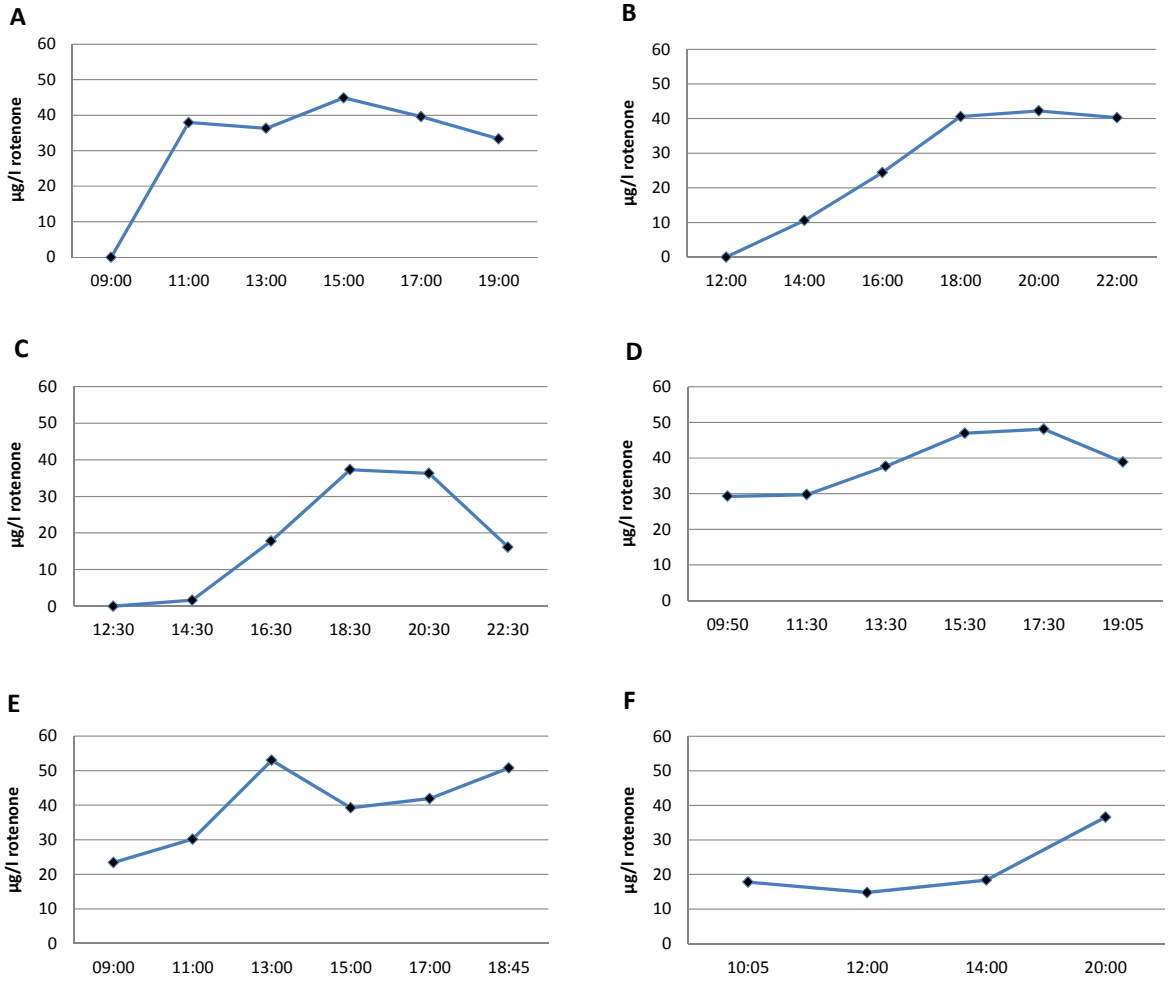

Figure 4. Rotenone concentrations measured during three treatment days in river Rauma during the 2014 treatment: **A** – at Hersel bridge, **B** – at Styggfonngrova, C – at Remmem bridge; D – Horgheimsfyllinga; E – Storøya; F - Grøttør bridge .

than one year and not any other age class or species was collected. In total 43 juvenile fish were collected. 40 juveniles were identified as Atlantic salmon and 3 as brown trout. All fish were younger than one year. As part of the Atlantic salmon post treatment electrofishing monitoring program, only Rauma was sampled in 2015. 125 salmon juveniles were electrofished from 10 different stations on 15.10.2015. *G. salaris* was not found (Hytterød et al. 2015). More comprehensive sampling in all infected rivers was conducted in 2016 after juveniles from the 2015 spawn had reached catchable size after swim-up from the river bed. A total of 656 salmon juveniles were electrofished in the 6 treated rivers. *G. salaris* was not found (Hytterød et al. 2016).

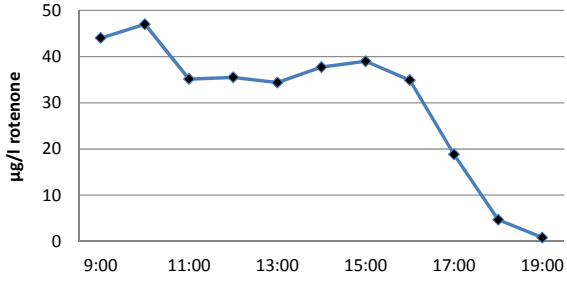

Figure 5. Rotenone concentrations measured at Hersel bridge during day one of the 2013 treatment in River Rauma.

Discussion

The rotenone use presented illustrates the emphasis made on treating river banks and gravel banks from boat mounted pumps. These areas are possible refuges for juvenile fish for a variety of reasons. Fish may swim in isolated pools of water between big rocks or are not possible to see from the river bank by the applicators. In addition, they may also dwell in areas with upwelling ground water and in pools of water filtered through large gravel banks. These factors pooled together represent challenges that must be met by spraying large areas with large volumes of diluted CFT-Legumine. Since not all possible water holding infected fish is fed by the river, the, main application stations alone will not be sufficient to reach all fish. Substantial effort was put into the qualitative dead fish removal during the second year of treatment. We were unable to find fish in these areas that had survived the 2013 treatment. The possible search area is large and the apparent absence of survivors should not be considered a confirmation of a successful eradication. The number of possible survivors would be low and difficult to find. On the other hand, not detecting surviving Atlantic salmon after both treatments after thorough monitoring is very promising.

The results presented show that the equipment and treatment regime used resulted in rotenone concentrations either close to, or above the target dose at the sites investigated. The peristaltic pumps were easy to use, precise and highly adjustable. They also allowed low pressure in all hoses, reducing the risk of spillage in the event of hose breakage. Application stations with multiple emission points allowed even dosage along the entire cross section of the river in areas with laminar currents. The different boat rigs used were suitable and reliable. The pumps allowed dosage of both rugged areas comprised of large stones and groundwater upwelling zones, as well as saturating large sand and gravel banks in and along the river. They also proved suitable for transporting both personnel and rotenone downstream in white water areas. We did not use backpack sprayers due to the CFT-Legumine haze it creates. It is in this form it is most hazardous to applicators. When you use watering cans as a replacement in small seeps and pockets of standing water you can apply without using gas-masks, and that is an advantage when you work in very rugged terrain.

The measurement series that showed deviation from aimed application and above target concentration were mainly due to the overlap between parallel application stations. The results showed a cumulative or "piggy back" downstream effect at several stations. This resulted from an overestimation of degradation of rotenone which in later treatments should be taken into greater consideration. The reasons for low degradation were probably due to low relative low temperatures low solar degradation and low breakdown potential in the Rauma water. This might also facilitate the option of longer travel times between application stations. It should be stated that the breakdown is strongly dependent on water temperature, turbidity and pH (USEPA 2007). The water in Rauma is characterized by cold, crystal clear water, with low turbidity and nutrient content, pH ranging between 6.2 and 7, all of which facilitates low rotenone degradation rates.

Overall, the results showed that it is possible to dispense CFT-Legumine® very evenly and close to the planned concentrations with the equipment used for the main application stations. The results also showed a high dilution at the front of the CFT-Legumine® plume. In general the concentrations take several hours to peak and to reach plateau concentration. An exception is in fast flowing water as the example from figure 5 illustrates. This leads to the conclusion that a double dose the first hour of treatment should still be applied or increased to two hours in future eradications. Application duration must be set in context with the distance between main application stations in order to ensure desired concentrations for the sufficient amount of time. The effects of a relative short dosage regime with a diluted front can be seen when looking at the concentrations measured above the application station at Styggfonngrova. The stretch between Hersel Bridge and Styggfonngrova is characterized by large water volumes of slow flowing water, leading to the dilution of the front and a stretched front-plume of CFT-Legumine®. A short application period contributes to the depletion in front of the CFT Legumine® plume and the extension of the CFT-Legumine® pulse might not reach plateau concentrations at all. This may facilitate an application strategy that account for these factors and applying a safety margin of setting a 20–30% higher target dose compared with treating stagnant water bodies. The less severe environmental consequences of shorter exposure times when treating running water in comparison with stagnant water should also be taken into consideration and justify a higher rotenone dose. The rotenone concentration at Grøttør Bridge on day 3 close to the river outlet dropped in the middle of the day due to the influx of tidal water and did not reach target concentration until late afternoon when the tide had turned. The discharge at Grøttør brigde doubles during maximum tide due to the tidal effect.

The application at upstream stations Horgheimsfyllinga and Storøya continued until 15:30 and 16:00 respectively, so it is apparent that the concentrations at Grøttør Bridge close to the river outlet were still increasing at 20:00. This is further substantiated by the increasing concentration at Størøya 18:45. We stopped the rotenone concentration measurement too early to be able to document this. The rotenone treatment in river Rauma continued for two days, so the outlet and station Grøttør bridge also received rotenone during a long period.

An effective water sampling regime with continuous sample analysis allows deviations from the planned concentrations to be corrected on a daily basis or the following day. The concentrations obtained are more reliable and accurate than the traditional method of using caged sentinel fish above treatment stations. An ongoing and systematic review of the results from the analysis laboratory as they were available allows control of all critical areas inside the treatment area in time for necessary measures to be made. A clear priority on analysis order is needed. This is a prerequisite to get the answers in time for the relevant measures to be implemented when the results suggest that rotenone dosage had not been satisfactory. A detailed plan must also allow sufficient time to analyze extra and unexpected samples. An unexpected change in water discharge or low rotenone concentrations measured must be taken into account on a daily basis and allow adjustments of the application to be made inside of the treatment period. Adjustment to the application was necessary in the upper tributaries of river Glutra. Rotenone concentration measurement received after during application showed concentrations below target level. The application rate was increased to increase the rotenone concentration. Concentrations increased above target level during late afternoon. The upper tributaries of river Glutra are characterized by large gravel banks and the water is filtered through large areas of gravel and sand. This probably resulted in depleted concentrations which made it necessary to increase the dosage.

The treatments showed a temporary reduction in some species and taxa, while other was not affected. One year after the treatments only one stonefly (*Isoperla* sp.) showed a significant reduction in numbers (Kjærstad and Arnekleiv 2016). Consecutive treatments do not appear to have greater influence on the invertebrate fauna community than one single treatment (Kjærstad et al. 2015). Water temperature and rotenone concentration during treatment is hypothesized to be more important (Kjærstad et al. 2015). The Norwegian Environmental Authorities have not set a regulatory limit for rotenone, but this is well below the regulatory limit set by the U.S. Environmental Protection Agency (USEPA 2007). USEPA uses rotenone concentrations as criteria for requiring deactivation of discharged treated water (> 2 µg/l rotenone), allowing the resumption of public contact (< 90 µg/l rotenone), and allowing potable water consumption (< 40 µg/l rotenone). The results from our study demonstrated a clear potential for including rotenone monitoring with on-site LC-UV methodology in fish eradication projects (Sandvik 2018), to be used during the actual treatment to adjust the dosage for efficacy and to check for regulatory compliance.

The techniques applied, the rotenone concentrations used and treating two consecutive years represents a major shift in the Norwegian battle against *G. salaris*. Treatments have been 100% successful after making this shift. The new method and techniques was implemented before a large treatment in the Rana zone in 2003–2004 which led to eradication confirmation (6 infected rivers) (Moen et al. 2005). We have also had an eradication confirmation in the Steinkjer zone (4 infected rivers) (Moen et al. 2011) and this fall we probably also will get it in the Vefsna zone, since this is the fifth and final year of monitoring (10 rivers) (Stensli and Bardal 2014). Some of our techniques are based on the American SOP manual (Finlayson et al. 2010), but many are further developed and new equipment is used. We believe that our new treatment regime is robust and despite that eradication confirmation in the Rauma-region cannot take place before 2019. The method and techniques used has not been previously published in peer-reviewed journals.

The Norwegian Food Safety Authority has decided that five years of *G. salaris* monitoring is necessary to evaluate the effect of the eradication. After five years, juvenile Atlantic salmon has been present long enough in all parts of the river to facilitate the spread of eventual surviving *G. salaris* or *G. salaris* infected Atlantic salmon in such a magnitude that it should have been detected by the monitoring program. The combination of not being able to find juvenile salmon during the 2014 eradication which survived the 2013 eradication and no *G. salaris* found in the monitoring program 2015 and 2016 are very promising results.

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