Annual Report

Mapping the occurrence of *Gyrodactylus salaris* upstream of the natural anadromous region of the Drammenselva catchment 2017

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Mapping the occurrence of *Gyrodactylus salaris* upstream of the natural anadromous region of the Drammenselva catchment 2017

Contents

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Summary

This report presents results from a surveillance programme that aims to map the potential occurrence of *Gyrodactylus salaris* on fish hosts upstream of the anadromous parts of River Drammenselva and River Lierelva. The aim in 2017 was to assess whether rainbow trout is present in the Begna watercourse and if this host carries *G. salaris*. Further, the presence of *G. salaris* on Arctic char in Lake Randsfjorden was evaluated.

Environmental DNA (eDNA) samples (water filtrates) were obtained from several localities within the catchment area. None of the samples from sites not influenced by waste water from rainbow trout farms tested positive for rainbow trout DNA. This indicates that rainbow trout is not a naturally occurring species in these parts of the Drammenselva catchment. Negative results from electro fishing supports this conclusion.

G. salaris was not detected in eDNA samples from the Begna watercourse. However, the parasite was detected in field samples from River Lierelva, a river with a known presence of *G. salaris,* which was therefore chosen as a positive control. This is the first time the presence of *G. salaris* has been successfully detected from environmental samples using the eDNA methodology.

G. salaris was not detected in any of 500 samples of arctic char obtained from Lake Randsfjorden.

Introduction

During the period between 1975 and 2017, pathogenic strains of *Gyrodactylus salaris* were detected on Atlantic salmon (*Salmo salar*) fingerlings/parr in 50 rivers, in 13 hatcheries/farms with Atlantic salmon parr/smolts and in 26 hatcheries/farms with rainbow trout (*Oncorhynchus mykiss*) (7). Furthermore, both pathogenic and non-pathogenic strains of *G. salaris* have been found in lakes on resident Arctic char (*Salvelinus alpinus*) (5, 11).

In the 1980s *G. salaris* was introduced into several watercourses in the River Drammenselva, Buskerud County. In 1986 and 1987 *G salaris* was detected on rainbow trout in two fish farms in Lake Tyrifjorden (9), a lake draining into the River Drammenselva. These detections led to the examination of rainbow trout in several farms in the watercourses draining into Lake Tyrifjorden, and *G. salaris* was detected in another eight farms. Seven of the farms drained into the Begna watercourse and one drained into Lake Randsfjorden. All fish in *G. salaris* positive farms were eradicated and the farms were thereafter declared free from *G. salaris*. Despite the eradication measures carried out in the farms, *G. salaris* was later found on juvenile Atlantic salmon in the River Drammenselva in 1987, probably due to spread via escaped infected rainbow trout from Lake Tyrifjorden.

The policy of the Norwegian Authorities is to eradicate *G. salaris* from infected watersheds and farms. Action against *G. salaris* has reduced the number of infected rivers in Norway and by the end of 2017 the parasite is confirmed present in only seven rivers (7). Three of these rivers, Drammenselva, Lierelva and Sandeelva, are located in a defined infection region called the Drammenselva Region. According to the action plan against *G. salaris* in Norway (1), the Norwegian Authorities have appointed an expert group to evaluate all potential measures to eradicate *G. salaris* from infected rivers in the Drammenselva Region. This work is scheduled to end in 2018. However, before any measures can be implemented, the potential occurrence of *G. salaris* in the whole Drammenselva catchment needs to be mapped.

Upstream of the migration barriers for anadromous fish in the Drammenselva catchment, rainbow trout and arctic char are the only hosts known to be susceptible to *G. salaris* (2, 5, 10). Rainbow trout was common in the Begna watercourse in the 1980's (3), and while its current presence in the natural water system is unknown, there are several farms that rear rainbow trout in land-based production units alongside the lakes Slidrefjorden and Strondafjorden. The farms are landlocked and thus separated from the lake system. However, rainbow trout was recently detected in Lake Strondafjorden (14) indicating

either fish escaping from farms or natural reproduction occurring in the system. Therefore, it has to be clarified if a permanent rainbow trout population has established itself in the water course, and whether this population is infected by *G. salaris* or not. Natural reproduction is considered a prerequisite for the establishment of a permanent rainbow trout population in the catchment. Rainbow trout DNA was detected at several locations during surveillance in 2016 (6), and positive sites were further investigated in this study.

eDNA monitoring is an efficient new tool that can detect minute amounts of DNA in water samples using a combination of water filtering and molecular detection. All organisms in water shed cells containing DNA into the environment (15). By using species-specific primers and probes and sensitive PCR-methods, it is possible to detect and identify the presence of DNA from specifically targeted species in water samples. This method is increasingly used by both foreign and domestic agencies (16) as a complementary method to traditional monitoring.

The introduced rainbow trout is considered extinct from Lake Randsfjorden (8). However, the lake holds an Arctic char population that experienced a *G. salaris* infection pressure during the introduction of infected rainbow trout in the 1980s. Previous detections of *G. salaris* on Arctic char in landlocked lakes without other known susceptible hosts present (5, 11), highlight the importance of mapping the occurrence of this parasite on arctic char in Lake Randsfjorden.

Aims

The first aim of the study in 2017 was to evaluate the presence of a rainbow trout population in the Begna watercourse, and if the rainbow trout carried infections with *G. salaris*. The second aim was to assess the potential occurrence of a *G. salaris* infection on Arctic char in Lake Randsfjorden. The current study is a part of a larger project, aiming to map the occurrence of a *G. salaris* infection in the whole watercourse upstream anadromous parts of River Drammenselva. This work is considered essential to the further planning of measures against *G. salaris* in the region.

Materials and methods

To assess the presence of rainbow trout and *G. salaris* in the catchment, electrofishing was applied in potential spawning and breeding areas of this species, and water samples for eDNA analysis were collected at the same sites. Duplicate water samples were taken from 5 cm above the riverbed. Five liters of water for each sample replicate were filtered through a 2 µm glass fibre filter (Millipore, Balerica, MA, United States) using a peristaltic pump (Masterflex E/S portable sampler, tygon tubes, Masterflex, Gelsenkirchen, Germany). After sampling at one location, the entire equipment was disinfected with a 10 % chlorine-solution. This was done to break down any residual DNA and prevent contamination. Subsequently, the tubes were rinsed with Na-Thiosulphate to neutralize the chlorine-solution. Each filter was placed in a separate clean 15 ml Falcon tube and stored on ice directly after filtration. Upon arrival at the laboratory the samples were stored at -20 ºC until further analysis.

The extraction of DNA from the filters was carried out according to a CTAB protocol described previously (13). Each filter yielded two DNA subsamples (A & B). Both an environmental control and a blank extraction control were included during DNA-extraction and qPCR analysis as a control in the event of carry-over contamination. Detection of DNA from *G. salaris* was carried out using a newly developed species-specific real-time PCR assay that targets the internal transcribed spacer region (12) Detection of rainbow trout DNA was carried out using the assay described previously (17).

All real-time PCR analyses were carried out on a Mx3005P qPCR system (Agilent technologies, Santa Clara, CA, United States). To confirm the functionality of the eDNA method in the field, a positive control sample was taken from River Lierelva, where there is a known presence of *G. salaris*.

Electrofishing for rainbow trout was conducted in River Begna, in tributaries to River Begna, and in tributaries to Lake Slidrefjorden and Lake Strondafjorden. Sampling locations were chosen in cooperation with County administrators and residents with knowledge of fish cultivation in the Begna watercourse, and by taking positive detections of rainbow trout DNA during surveillance in 2016 (6) into account (Table 2).

To exclude the presence of *G. salaris* with an acceptable degree of certainty in lakes containing Arctic char, with a previously known presence of *G. salaris*, a surveillance involving the screening of a large number of fish is required. This is due to the likely low prevalence and intensity *of G. salaris* infections in Arctic char (11). Thus, this study was designed with a sample size of approximately 500 Arctic char, distributed around different locations in Lake Randsfjorden.

Arctic char was caught using gillnets (mesh size 35 to 39 mm) at 1,5 - 40 meters depth in autumn 2017 by local fishermen who were commissioned to carry out the fieldwork. Live fish were removed from the nets and killed by a blow to the head. All fins from 500 fish were clipped and preserved in 96% ethanol.

The *Gyrodactylus* examination was conducted at the Norwegian Veterinary Institute by use of a stereo microscope at 10 - 15 times magnification. Single *Gyrodactylus* specimens was collected from the fish skin with a micro pipette, and stored in 96% ethanol until species determination was performed by molecular methods. A maximum of five *Gyrodactylus* specimens were sampled from each fish/fin for species determination. To maximize the likelihood of detecting *G. salaris* among the identified *Gyrodactylus* specimens, at least one parasite was determined to species level in every *Gyrodactylus* positive fish.

Results

Altogether, 500 Arctic char from 14 locations in Lake Randsfjorden were collected and the fins were examined (Table 1). A total of 300 *Gyrodactylus* individuals were determined to species level. *G. salaris* was not detected within this selection of *Gyrodactylus* specimens.

Table 1. Locations in Lake Randsfjorden where Arctic char was caught.

Location	**GPS (WGS 84)**	*G. salaris* **detected**
Randsfjorden site 1	60.17234, 10.21949	0
Randsfjorden site 2	60.17026, 10.22208	0
Randsfjorden site 3	60.16636, 10.22257	0
Randsfjorden site 4	60.16641, 10.22927	0
Randsfjorden site 5	60.16485, 10.22622	0
Randsfjorden site 6	60.16226, 10.22988	0
Randsfjorden site 7	60.16064, 10.23201	0
Randsfjorden site 8	60.15881, 10.23216	0
Randsfjorden site 9	60.15800, 10.23536	0
Randsfjorden site 10	60.17331, 10.23353	0
Randsfjorden site 11	60.17245, 10.23445	0
Randsfjorden site 12	60.15935, 10.23552	0
Randsfjorden site 13	60.47742, 10.11188	0
Randsfjorden site 14	60.17425, 10.23465	0
Total		0

Molecular analysis of the water samples yielded no positive results for *G. salaris* in any of the water samples from the Begna watercourse or any of its tributaries. eDNA of *G. salaris* was detected in all four samples taken from River Lierelva, the positive control site. Four samples from the Begna watercourse contained rainbow trout eDNA (Table 2), all of which were taken downstream of rainbow trout farms. In this study, no rainbow trout was detected by electrofishing (Table 2).

Table 2. Detection of rainbow trout by electrofishing, and detection of rainbow trout and *G. salaris* DNA from eDNA samples.

Location	GPS (WGS 84)	Electro fishing	eDNA detections	
		No. of rainbow trout caught	Rainbow trout	*G. salaris*
Ala Camping	61.14732, 8.71219	0	No	No
Tørpegårdsvegen/bru	61.15224, 8.72507	0	No	No
Leira/Garlivegen	60.97429, 9.29363	0	Yes	No
Leira Camping	60.96801, 9.28844	0	Yes	No
Faselfoss bru	60.96712, 9.28894	0	Yes	No
Bagn	60.81989, 9.56127	0	Yes	No
Nes/bru	60.56281, 9.99294	0	No	No
Lierelva	59.85807, 10.22133	0	-	Yes
Total		0	4	1

Discussion

G. salaris was not detected in any of the eDNA samples from upstream of the migration barriers in the Begna watercourse and its tributaries.

No rainbow trout was detected during electrofishing, neither in any of the locations in the Begna watercourse, nor in the tributaries to Lake Slidrefjorden and Lake Strondafjorden.

To demonstrate the viability of the eDNA assay for *G. salaris* under field conditions we analysed water samples from River Lierelva, a river with a known presence of *G. salaris*. All four samples yielded positive results demonstrating the efficacy of this method in the field. The presence of *G. salaris* was confirmed by examination of Atlantic salmon juveniles collected during electrofishing in River Lierelva, upstream of the eDNA water sampling sites. All collected fish were infected with the parasite (mean intensity 83 parasites). These water samples constitute the first positive detection of *G. salaris* presence from environmental samples using the eDNA methodology. Contamination in the laboratory was controlled for by using extraction-blank and environmental-blank samples in the analyses.

Four of seven samples in the Begna watercourse tested positive for rainbow trout eDNA. According to information from local authorities received after the fieldwork, and which was unknown to the authors at the time of sampling, two locations (3 and 4) were situated downstream of a rainbow trout farm, roughly 400 m and 1200 m respectively. The detection of rainbow trout eDNA in River Leira can therefore be attributed to waste water originating from this farm. This presumption is further corroborated by the fact that no rainbow trout were caught despite extensive electrofishing in the stream. The other two positive samples, obtained from the locations Faslefoss Bru and Bagn, were also taken downstream of an area where fish farms are located. Sampling downstream from a fish farm can theoretically lead to a false positive result, but the chance for such contamination decreases with distance from the farm. Previous research suggests that eDNA signals can be transported up to roughly 10 km from the source (4). However, this study refers to DNA from natural populations of invertebrates rather than the huge amount of biomass concentrated in fish farms. It is therefore likely that the positive samples at Faslefoss Bru and Bagn are caused by diluted DNA from fish farms.

The sampling sites located upstream of Lake Slidrefjorden were both negative for rainbow trout DNA, contrary to the samples taken the previous year (6). Natural reproduction of rainbow trout in this river section would likely result in a permanent presence of rainbow trout DNA in the water, thus the negative samples indicate the absence of rainbow trout spawning upstream of the sampling sites. The absence of rainbow trout detections during electrofishing in 2016 and 2017 further substantiates this assumption.

A high number of *Gyrodactylus* specimens were observed on the fins of Arctic char from Lake Randsfjorden. Due to budget limitations, only 300 of the collected *Gyrodactylus* specimens were determined to species level. Analysing more than the selected 300 Gyrodactylids would confirm the absence of *G. salaris* to an even higher degree of certainty. We recommend to carry out this analysis prior to the termination of the project, in 2018.

In this study no presence of *G. salaris* was detected, neither in the Begna watercourse through eDNA-monitoring and electrofishing, nor in Lake Randsfjorden according to the examination of fins from 500 Arctic char and species determination of 300 *Gyrodactylus* specimens.

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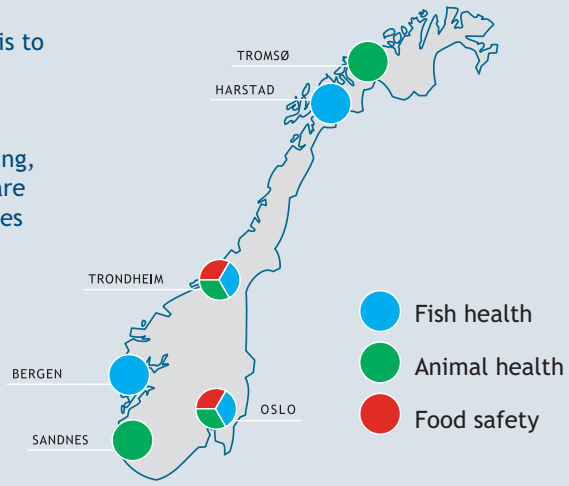

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