Annual Report

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

Veterinærinstituttet
Norwegian Veterinary Institute

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

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## Summary

This report presents results from a surveillance programme that aims to map the potential occurrence of *G. salaris* on fish hosts upstream of the anadromous parts of River Drammenselva and River Lierelva. The aim of the study in 2016 was to assess whether Atlantic salmon juveniles found upstream of the migration barriers in tributaries to the River Lierelva and River Drammenselva were carrying infections with *G. salaris*. As rainbow trout is considered a good host for *G. salaris*, another aim was to evaluate whether rainbow trout was present in the Begna watercourse and if this host carried infections with *G. salaris*.

Altogether 170 hatchery-reared Atlantic salmon juveniles from upstream migration barriers for Atlantic salmon in the tributaries to River Lierelva and River Drammenselva were examined and found negative for *Gyrodactylus salaris.* Environmental DNA samples (water filtrates) were obtained from several localities within the catchment area and some of these samples tested positive for rainbow trout DNA. This indicates that rainbow trout is present in parts of the Drammenselva catchment. However, revisiting the locations where positive samples were obtained, combined with the investigation of additional locations and complementary sampling by electrofishing is required in order to confirm the results of these analyses.

## Introduction

During the period between 1975 to 2015, 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*) (Hytterød et al,. 2017). Furthermore, both pathogenic and non-pathogenic strains of *G. salaris* have been found in lakes on resident Arctic charr (*Salvelinus alpinus*) (Robertsen et al., 2006, Hytterød et al., 2011).

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 (Mo, 1988), 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, as a result, *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 2016 the parasite is confirmed present in only seven rivers (Hytterød et al., 2017). 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 (Anon, 2014), 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 2017. However, before eradication measures can be implemented, the potential occurrence of *G. salaris* in the whole Drammenselva catchment needs to be mapped.

In the River Drammenselva and River Lierelva, several tributaries are stocked with hatchery-reared Atlantic salmon juveniles. To prevent infection with *G. salaris* and thereby increase the survival and migration success of the stocked fish, they are released upstream of the natural migration barriers for anadromous fish. The biological conditions with both *G. salaris* infected salmon and non-infected salmon in the same streams represent a risk of infection for the salmon upstream of the barriers.

Rainbow trout is the only host known to be susceptible to *G. salaris* with a suspected presence in the catchment. This fish species was common in the Begna watercourse in the 1980's (Brabrand 1988), and

while its current presence in the 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 (Thomassen and Norum, 2012) indicating either fish escaping from farms or natural reproduction occurring in the system.

eDNA monitoring is a promising 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 (mucus, epithelial cells) (Thomsen et al., 2012), thus releasing DNA into the water. Using species-specific primers and probes and sensitive PCR-methods, it is possible to detect and identify the presence of DNA from targeted species in water samples. This method is increasingly used by both foreign and domestic agencies (Vrålstad et al., 2016) as a complementary method to traditional monitoring.

## Aims

The first aim of the study in 2016 was to assess whether Atlantic salmon juveniles found upstream of the migration barriers in tributaries to the River Lierelva and River Drammenselva were carrying infections with *G. salaris*. The second aim was to evaluate whether rainbow trout was present in the Begna watercourse and if the rainbow trout carried infections with *G. salaris*. The third aim was to demonstrate the potential use of eDNA monitoring of *G. salaris* and rainbow trout as a complementary method to conventional electrofishing.

## Materials and methods

To assess the possible presence of *G. salaris* on re-stocked Atlantic salmon juveniles upstream the anadromous parts in the Drammenselva and Lierelva watercourse, sampling and examination of salmon was carried out. This was conducted upstream of migrations barriers in all tributaries with known populations of stocked fish. The salmon juveniles were collected by electrofishing, killed and preserved intact in 96 % EtOH. The *Gyrodactylus* examination was conducted at the NVI using a stereo microscope at 10-15 times magnification. Detected *Gyrodactylus* specimen were collected from the fish skin with a micro pipette and stored in 96 % EtOH before species determination was done after the recommendations given by the OiE manual (http://web.oie.int/eng/normes/fmanual/2.3.03_Gyrodactylosis.pdf).

To assess the presence of rainbow trout in the catchment, eDNA samples were collected. At each electrofishing location except in two streams draining to Lake Tyrifjorden, 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 eDNA 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 in Strand et al. (2014). 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 (Manuscript in prep.). Detection of rainbow trout DNA was carried out using the assay described in Wilcox et al. (2015). All real-time PCR analyses were carried out on a Mx3005P qPCR system (Agilent technologies, Santa Clara, CA, United States). To confirm that all parts of the eDNA method and equipment were working in the field, a positive control sample was taken directly from a fish pond at Røn Gård rainbow trout farm. For further confirmation of the analyses, a sub-sample of DNA extracts from the first 6 sample locations were

analyzed for the presence of rainbow trout in a separate laboratory (The National Genomics Center for Wildlife and Fish Conservation in Missoula, MT, USA).
Electrofishing for rainbow trout was conducted in the River Begna, in tributaries to Begna, and in tributaries to the Lake Slidrefjorden, Lake Strondafjorden, Lake Sperillen and Lake Tyrifjorden. Sampling locations and streams were chosen in cooperation with County administrators and residents with knowledge of fish cultivation in the Begna watercourse (see table 2).

## Results

Altogether, 170 salmon from five tributaries in the River Lierelva and River Drammenselva were collected and examined in 2016 (Table 1). A total of five *Gyrodactylus* specimens were found on salmon in Hoenselva, a tributary to Drammenselva and they were all determined to be *Gyrodactylus derjavinoides*. *G. salaris* was not detected (Table 1).
None of the 26 water samples tested positive for *G. salaris*. Four samples (including the positive control from Røn Gård fish farm) tested positive for rainbow trout (Table 2). The positive results were verified by an independent laboratory. In the study, no rainbow trout was detected by electrofishing (Table 2).

**Table 1**. Number of fish examined for *G. salaris* in tributaries to the River Lierelva and the River Drammenselva.

| River | Tributary | Species | No. of fish examined | Detections |
|---|---|---|---|---|
| Lierelva | Nykjua | Atlantic salmon | 39 | 0 |
| Lierelva | Tverrelva | Atlantic salmon | 36 | 0 |
| Drammenselva | Hoenselva | Atlantic salmon | 43 | 0 |
| Drammenselva | Sagelva | Atlantic salmon | 41 | 0 |
| Drammenselva | Kolbrekkbekken | Atlantic salmon | 0 | - |
| Drammenselva | Bingselva | Atlantic salmon | 11 | 0 |
| **Total** | | | 170 | 0 |

**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* |
| Storåna oppstr. Ryfoss | 61.15232, 8.72498 | 0 | Yes | No |
| Røn Gård fish farm (pos. contr.) | 61.0381, 9.04688 | - | Yes | No |
| Neselvi | 60.99255, 9.22073 | 0 | No | No |
| Sundheimselva v/Sundheim | 60.99817, 9.09423 | 0 | No | No |
| Vasetvatnet outlet | 60.9957, 8.98599 | - | No | No |
| Leirelvi v/Leira Camping | 60.96783, 9.28887 | - | Yes | No |
| Reina | 60.82764, 9.51901 | 0 | No | No |
| Urula | 60.55034, 9.95092 | - | No | No |
| Begna v/Nes bru | 60.56252, 9.9931 | - | Yes | No |
| Sperillen v/Kongsstrømmen | 60.36915, 10.07998 | - | No | No |
| Hørtebekken (Tyrifjorden) | 59.90871, 10.29361 | 0 | No | No |
| Sandsbekken (Tyrifjorden) | 59.90741, 10.29504 | 0 | - | - |
| Dragbekken (Tyrifjorden) | 59.93282, 10.3031 | 0 | No | No |
| Nordelva (Tyrifjorden) | 59.93028, 10.33553 | 0 | No | No |
| Stream south of Nordelva (Tyrifjorden) | 59.92929, 10.3349 | 0 | - | - |
| **Total** | | 0 | 4 | 0 |

## Discussion

Atlantic salmon was caught in all the examined tributaries to the River Drammenselva, except in the small stream Kolbrekkbekken (Table 1). As part of the restocking programme in the River Drammenselva, 10 000 salmon juveniles, age 0+, were stocked in this tributary in 2015. However, no salmon juveniles were caught during three hours of electrofishing effort in august 2016.
*G. salaris* was not detected in any of the samples from upstream of the migration barriers in the tributaries.

Rainbow trout was not detected by electrofishing in any of the examined locations in the River Begna watercourse, or in the examined tributaries to Lake Slidrefjorden, Lake Strondafjorden, Lake Sperillen and Lake Tyrifjorden.

To demonstrate the viability of the eDNA assay for rainbow trout under field conditions we analysed a water sample from a fish tank at Røn Gård fish farm. The analysis gave a strong positive signal (low CT-value) in this water sample which was similar to signals obtained from DNA extracts from muscle tissue samples.

The positive sample at Storåna oppstr. Ryfoss indicates presence of rainbow trout at or upstream of this sampling site. These findings are corroborated by anecdotal evidence of sports-fishing for rainbow trout in the area in the 1990s (personal communications). No rainbow-trout farms are located upstream of this sampling location, thus excluding the possibility of a false positive through contamination with eDNA from farmed rainbow trout.

The positive sample obtained from the River Begna, at the location at Nes Bru is one of two sampling sites from an area situated downstream of fish farms. Sampling downstream from any 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 (Deiner & Aldermatt, 2014). The location at Nes Bru is situated more than 85 km downstream from the nearest fish farms, and the stretch includes lakes with unknown retention time. We therefore consider the probability of "contamination" from the fish farms upstream as highly unlikely. Re-testing along a longitudinal transect through the watercourse should lead to clarification and possibly help pinpointing the location of the DNA source.

Natural reproduction was observed in the early 1990s in Hørtebekken, Dragbekken and the stream south of Nordelva, the streams that run into Lake Tyrifjorden (Morten Eken pers. med). We did not catch rainbow trout with electrofishing equipment and all eDNA samples from these locations were negative. This strongly indicates that rainbow trout is not present in the respective waterbodies and that natural reproduction of rainbow trout in Lake Tyrifjorden is no longer occurring.

All but two sampling points (Nes Bru and Sperillen) were located in tributaries upstream of any rainbow trout farms in the Begna river system or in tributaries to the lakes Slidrefjorden, Strondafjorden and Sperillen. This excludes the possibility of "false positive" samples due to contamination originating from rainbow trout farms. Likewise, contamination from the equipment can be ruled out as it was disinfected with DNA-degrading chlorine solution between each sampling point. The presence of negative field samples analysed during this study also supports this. Contamination in the laboratory was controlled for by using extraction-blank and environmental-blank samples in the analyses.

The possibility of an anthropogenically induced "false positive" through discarding of food left-overs (e.g. rakfisk) after a picnic, for example, cannot be excluded for any positively tested location. However, re-testing these and additional locations further upstream is considered important to confirm the results and to further locate potential localities where rainbow trout might be present.

# References


1. Anon (2014). Handlingsplan mot lakseparasitten *Gyrodactylus salaris* for perioden 2014-2016. Miljødirektoratet 2014. 114 s.
2. Brabrand, Å. (1988). Fiskeribiologiske undersøkelser i Slidrefjorden. Oppland Fylke: Vurdering av tilslag på settefisk. Laboratorium for ferskvannsøkologi og innlandsfiske (LFI), Zoologisk Museum, Universitetet i Oslo. 44 s.
3. Deiner, K., Altermatt, F. (2014). Transport Distance of Invertebrate Environmental DNA in a Natural River. Plos One, 9, e88786
4. Hytterød, S., Hansen, H., Johansen, K. & Larsen, S. (2017). The surveillance programme for *Gyrodactylus salaris* in Atlantic salmon and rainbow trout in Norway 2016. ISSN 1894-5678. Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2016. Oslo: Norwegian Veterinary Institute; 2017. 4 s.
5. Hytterød, S., Adolfsen, P., Aune, S. & Hansen, H. (2011). *Gyrodactylus salaris* funnet på røye (*Salvelinus alpinus*) i Fustvatnet (Nordland); patogen for laks (*Salmo salar*)? Veterinærinstituttets rapportserie 11, 2011. 14 s.
6. Mo, T.A. (1988). *Gyrodactylus*-undersøkelsene ved Zoologisk museum, Universitetet i Oslo. Virksomheten i 1987 og program for virksomheten i 1988. Rapport nr. 4.
7. Robertsen, G., Plaisance, L. & Bakke, T.A. (2006). *Gyrodactylus salaris* og røye, ørret, regnbueørret og bekkerøye: Utbredelse og potensiell overføring til laks. Naturhistorisk museum Seksjon for zoologi (Zoologisk museum), Universitetet i Oslo. 25 s.
8. Strand, D.A., Jussila, J., Johnsen, S.I., Viljamaa-Dirks, S., Edsman, L., Wiik-Nielsen, J., Viljugrein, H., Engdahl, F. & Vralstad, T. (2014). Detection of crayfish plague spores in large freshwater systems. *J Appl Ecol* 51, 544-553.
9. Thomassen, G. & Norum, I. (2012). Fiskeundersøkelse i Strondafjorden. Fylkesmannen i Oppland, miljøvernavdelingen. Rapp. nr. 17/12, 18 s.
10. Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., Orlando, L. & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology, 21, 11, 2565-257
11. Vrålstad, T., Strand, D.A., Rusch, J.C., Toverud, Ø., Johnsen, S.I,, Tarpai, A., Møller, P.R. & Gjevre, A.G. (2017). The surveillance program for *Aphanomyces astaci* in Norway 2016. ISSN 1894-5678. Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2016. Oslo: Norwegian Veterinary Institute; 2017. 26s.
12. Wilcox, T.M., Carim, K.J., Mckelvey, K.S., Young, M.K. & Schwartz, M.K. (2015). The Dual Challenges of Generality and Specificity When Developing Environmental DNA Markers for Species and Subspecies of *Oncorhynchus*. PLoS ONE, 10, e0142008.

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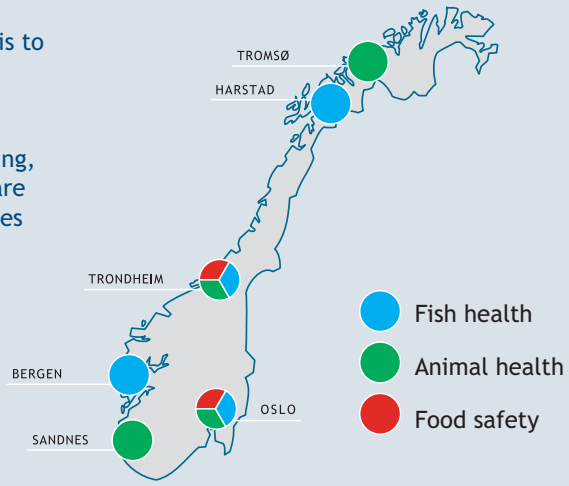


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