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Research Article

Gyrodactylus salaris Malmberg, 1957 (Monogenea, Gyrodactylidae) spreads further – a consequence of rainbow trout farming in Northern Russia

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Abstract

The monogenean freshwater parasite *Gyrodactylus salaris* Malmberg, 1957 is endemic to Atlantic salmon (*Salmo salar*) east of the Baltic Sea, but has spread outside this area via transport and stocking of fish. In Norway and Russia, infections with *G. salaris* have had catastrophic consequences for many salmon populations. The parasite is also common on farmed rainbow trout (*Oncorhynchus mykiss*) where it can persist in low numbers and without clinical signs. The transport and movement of infected rainbow trout is an important factor in the spreading of *G. salaris* in Europe. Due to increasing interest in establishing rainbow trout farms in White Sea drainages in Murmansky Oblast, Russia, and the potential subsequent unintentional spreading of *G. salaris*, parasitological examinations of salmonids were carried out. Farmed rainbow trout (n = 48) and Atlantic salmon (n = 375) from River Tuloma and farmed rainbow trout from Lake Imandra (n = 10), were examined in the period from 2015 to 2019. Additionally, environmental DNA monitoring was conducted for the detection of *G. salaris* in 2018. *Gyrodactylus* specimens were first detected in 2015 on Atlantic salmon from the tributary River Pak. Specimens obtained from Atlantic salmon in River Tuloma and from rainbow trout in River Tuloma and Lake Imandra the following years were confirmed to be *G. salaris* by sequencing of the nuclear ribosomal internal transcribed spacer (ITS rDNA) and mitochondrial cytochrome oxidase 1 (COI). All specimens carried the same COI sequence, which was identical to a strain (GenBank Accession number AF479750) frequently found on farmed rainbow trout. The prevalence varied, but reached 100% in some samples. Maximum intensity observed was 899, but intensities were generally lower than intensities expected to lead to mortalities. There was good correspondence between eDNA monitoring and conventional methods. The results indicate that *G. salaris* has spread to River Tuloma via transport of live rainbow trout.

Key words: OIE, Atlantic salmon, environmental DNA, *Oncorhynchus mykiss*, fish parasite

Introduction

The monogenean *Gyrodactylus salaris* Malmberg, 1957 has been and is responsible for severe epidemics in Atlantic salmon, *Salmo salar* L.,

populations in rivers draining in to the North Atlantic Ocean and the White Sea (Bakke et al. 2007; Paladini et al. 2021). According to the World Organisation for Animal Health (OIE), infection with *G. salaris* is a notifiable disease (http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2014/) and detection of the parasite can result in trading restrictions for their hosts. *Gyrodactylus salaris* is confirmed present in 14 countries (Paladini et al. 2021), and new surveys, as exemplified by the latest from Italy (Paladini et al. 2009), Poland (Rokicka et al. 2007), and Romania (Hansen et al. 2016), will likely extend the number of registered countries for this parasite as well as contribute to increased knowledge on the distribution within each country. Spreading within countries is most likely to occur via stocking of fish in new areas and/or through natural migration of the fish to new rivers via fresh or brackish water (Bakke et al. 2007; Hansen et al. 2016; Paladini et al. 2021). The experience from the Norwegian *G. salaris* epidemics, demonstrates how unintentional spreading can occur, the severity of the consequences of spreading and shows the importance of keeping a high focus on surveillance for this parasite.

Analyses of the mitochondrial cytochrome oxidase I gene (COI) sequences of *G. salaris* from many populations across Europe show the presence of a high number of strains/haplotypes and a high degree of genetic variation between samples (Hansen et al. 2003, 2007; Meinilä et al. 2004). Cytochrome oxidase I sequences have been informative for tracking the invasion history of *G. salaris* in Fennoscandia and Russia (Hansen et al. 2003; Meinilä et al. 2004). However, characterization of parasite individuals according to their haplotype cannot be used to unambiguously distinguish pathogenic from non-pathogenic variants (see Hansen et al. 2007; Olstad et al. 2007; Ramirez et al. 2014). This is especially noteworthy for one mitochondrial variant or strain commonly associated with rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), throughout Europe. Parasites with this haplotype, known from previous studies as haplotype F, III-F, RBT or II-A1 (Hansen et al. 2003; Mieszkowska et al. 2018; Ziętara et al. 2006) have caused epidemics in the Norwegian rivers Lærdalselva and Drammenselva (Johnsen et al. 1999), but are also found as a non-virulent strain on, for example, Arctic char in two lakes in Norway (Olstad et al. 2007). Thus, the current diagnostic method, which is based on the most variable molecular markers available so far, must be complemented by observations from the field and/or by controlled experimental trials to assess the pathogenicity of each strain or new infection.

In Russia, *G. salaris* is endemic on wild salmon in the southern parts of Russia (Kudersky et al. 2003; Malmberg 1993) and it was introduced to River Keret, a river draining in to the White Sea, via transport of salmon in 1992 (Ieshko et al. 2008; Kuusela et al. 2007). The species is also present on farmed rainbow trout in Karelia (Ieshko et al. 2016), and was probably introduced to the farm as a result of imported fish from Finland (Evseeva et al. 2009). In recent years the rainbow trout variant of *G. salaris* has been

discovered in rivers belonging to the White Sea basin, on wild landlocked salmon juveniles in several tributaries to Lake Kuito, as well as on migratory salmon juveniles at the mouth of the River Kem (Ieshko *pers. comm.*). Other variants of *G. salaris* have been reported from Lake Kuito (River Pisto), in River Lizhma, the Lake Onega basin and in Lake Ladoga (Barskaya et al. 2009; Ieshko et al. 2016; Ziętara et al. 2006). Although *G. thymalli* Zitnan, 1960, a parasite of grayling, *Thymallus thymallus* (Linnaeus, 1758), has been proposed to be a junior synonym of *G. salaris*, we do not include the presence of strains of *Gyrodactylus* from grayling here.

It is unknown whether the strains present in the rainbow trout farms in Karelia are pathogenic for Atlantic and White Sea populations of Atlantic salmon, but as mentioned above, strains likely originating from rainbow trout have caused severe epidemics in some Norwegian rivers (Hansen et al. 2003).

Within Russia, *G. salaris* has probably been spread between farms in the south-western areas through fish transports. Recently, there has been an increased interest in establishing rainbow trout farms also in watersheds draining in to the White Sea and this could lead to unintentional spread of *G. salaris* to new areas. The annual production of rainbow trout in Russia increased from 9300 tonnes in 2009 to 32500 tonnes in 2019 (Anon 2019), and future growth is planned to take place in areas now currently free of *G. salaris*. In Karelia, fish farms annually import rainbow trout fingerlings for commercial rearing from different neighbouring regions in Russia (e.g. the Leningrad Region, the Republic of North Ossetia-Alania) and from Finland. As *G. salaris* is common on rainbow trout also in many other European countries (Dzika et al. 2009; Hansen et al. 2016; Meinilä et al. 2004; Paladini et al. 2009), and can persist in the farms in low numbers and without clinical signs, movement of infected rainbow trout is considered an important factor in its spread in Europe. Given the potentially catastrophic consequences that infections with *G. salaris* have had in other areas, such movement should thus be avoided or be under strict control. Moreover, the trade in live species susceptible to listed diseases is only permitted between countries, zones or compartments of equivalent health status (or from higher to lower status) (EU L 328/14).

The current knowledge of the monogenean fauna on salmonids in rivers of the Kola Peninsula is very poor. According to Mitenev and Karasev (1995), only *Discocotyle sagittata* (Leuckart, 1842) Diesing, 1850 and *Salmonchus alaskensis* (Price, 1937) have been detected on wild Atlantic salmon and brown trout, *Salmo trutta* L. However, *G. lavareti* Malmberg, 1957 has been found on farmed rainbow trout (Karasev et al. 1997). Surveys of the wild salmonid fish populations and their parasites are carried out annually (Melnik et al. 2019), and in 2015, these surveys discovered *Gyrodactylus* parasites for the first time on young Atlantic salmon from the River Pak, a tributary to River Tuloma (Murmansky oblast). This first discovery initiated

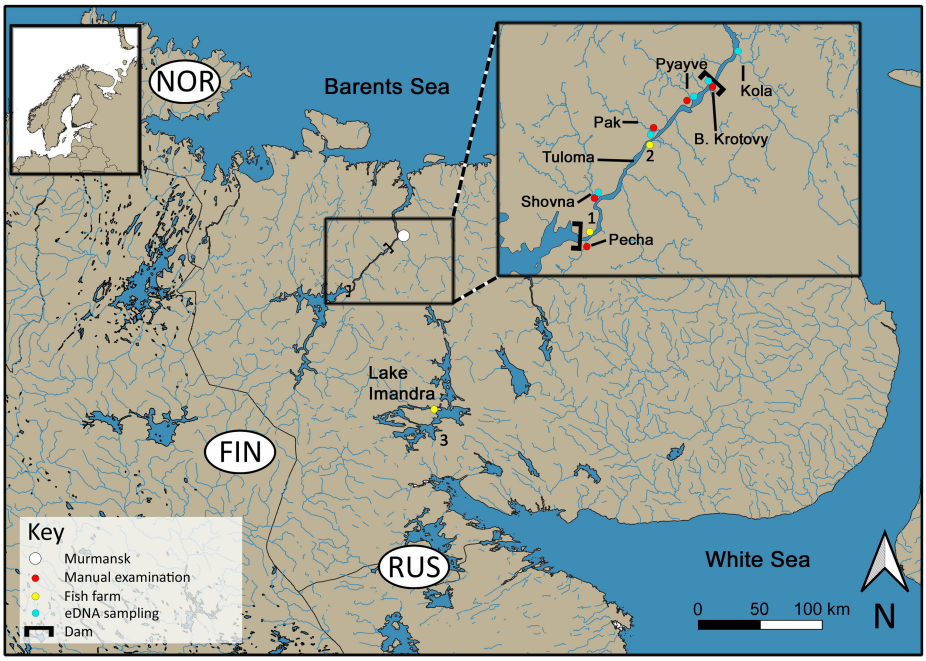

Figure 1. Map of the sampling locations within the River Tuloma and in Lake Imandra in Murmansky Oblast, Russia. Detailed locations within River Tuloma are given in the inset. Numbers are listed beside the different rainbow trout farms (yellow dots) while the names are given for the sampling locations for Atlantic salmon (red dots). The sampling locations for eDNA sampling are indicated with turquoise dots.

the study reported on here, where new sampling and analyses were carried out to detect and diagnose the gyrodactylid specimens from River Tuloma and its tributaries using up-to-date methods.

Materials and methods

Description of the Tuloma river system and sample locations

River Tuloma (see Figure 1) is divided into the upper and lower Tuloma basin by hydropower plants (HPP). The upper basin comprises the Verkhnetulomskoye storage reservoir and its tributaries down to the Verkhne-Tulomskaya HPP, while the lower Tuloma basin, Nizhnetulomskoye storage reservoir and its tributaries, comprises the area from Verkhne-Tulomskaya HPP and down to Nizhne-Tulomskaya HPP. Below the Nizhne-Tulomskaya HPP the river enters the sea through an estuary. The fish fauna of the Tuloma River catchment comprises 12 native and three introduced species (Alekseev et al. 2011); Siberian brook lamprey *Lethenteron kessleri* (Anikin, 1905), (Anikin 1905), Atlantic salmon, brown trout, arctic char, *Salvelinus alpinus* (L.), grayling, whitefish, *Coregonus lavaretus* (L.), vendace, *Coregonus albula* (L.), pike, *Esox lucius* L., ninespine stickleback, *Pungitius pungitius* (L.), minnow, *Phoxinus phoxinus* (L.), perch, *Perca fluviatilis* L., burbot, *Lota lota* (L.), smelt, *Osmerus eperlanus* (L.) (introduced), pink salmon, *Oncorhynchus gorbuscha* (Walbaum) (introduced), and rainbow trout (introduced). Rainbow trout has escaped from fish farm cages in the Nizhnetulomskoye reservoir but natural reproduction of rainbow trout

Table 1. Numbers of fish examined and total length (AB) and weight of the investigated Atlantic salmon juveniles from tributaries of the Lower Tuloma reservoir.

Year	River	No. of fish examined	Fish size, total length (cm) Range (mean)	Fish size, weight (g) Range (mean)
2015	Pak	30	3,6–12,6 (7,31)	0,5–24,7 (6,5)
	Pecha	18	3,6–8,9 (5,9)	0,5–7,9 (2,9)
2016	Pak	47	5,0–12,3 (9,2)	1,5–21,5 (10,1)
	Pecha	25	3,8–11,0 (7,9)	0,5–14,5 (5,7)
2017	Pak	39	2,9–12,1 (6,7)	0,2–18,9 (4,9)
	Pecha	26	4,2–11,4 (5,0)	0,8–18,6 (2,1)
	Shovna	7	5,7–13,0 (10,8)	1,6–22,9 (14,7)
	Pyayve	37	3,7–12,0 (5,3)	0,5–17,2 (2,3)
	Bolshoi Krotovy	14	4,9–15,7 (12,5)	1,1–44,7 (22,6)
2018	Pak	38	3,9–15,7 (7,7)	0,5–38,6 (7,8)
	Shovna	20	4,1–12,6 (9,1)	0,7–20,7 (9,3)
2019	Pak	31	2,9–9,0 (4,3)	0,2–7,5 (1,1)
	Shovna	11	7,5–16,2 (10,3)	5,5–52,8 (14,8)
	Pecha	18	3,9–13,1 (9,0)	0,5–23,0 (9,8)
	Pyayve	14	3,8–11,8 (7,6)	0,6–18,4 (6,3)
	Total number	375		

Table 2. Infection parameters of *Gyrodactylus salaris* on fins of rainbow trout farms in Murmansky oblast, Russia.

Date	Trout farm	Examined fish (age)	Mean intensity
07.04.2017	Farm 2, Tuloma	5 (1+)	24,2
07.04.2017	Farm 1, Tuloma	20 (0+)	18,5
02.06.2017	Farm 2, Tuloma	3 (1+)	51
02.06.2017	Farm 1, Tuloma	10 (0+)	24,9
08.06.2017	Farm 3, Lake Imandra	10 (0+)	5,1
	Total number	48	

has not been documented. Nizhnetulomskoye reservoir now harbours four trout cage farms. Atlantic salmon is only present in the lower Tuloma basin, and fish counts at Nizhne-Tulomsky HPP fishtrap have recorded an annual average of 6300 breeders or juveniles (min-max – 2875–12784) over the 1971–2018 period. Salmon reproduction is maintained in nine significant streams draining into Nizhnetulomskoye reservoir.

Collection of fish and parasitological examination

From 2015 to 2019 Atlantic salmon juveniles were sampled by electrofishing from a number of the tributaries to River Tuloma with most samples taken from the two tributaries Pak and Pecha (Figure 1, Table 1). In addition samples of rainbow trout were obtained from two farms located in River Tuloma and one sample of rainbow trout was obtained from a farm in Lake Imandra further south (Figure 1, Table 2). After being caught, all fish were killed by a blow to the head, before being preserved whole in 96% ethanol. The total length (± 0.1 cm) and weight (± 0.1 g, measured on a Sartogosm VLT-510P scale, Sartogosm LTD) of the Atlantic salmon was recorded, while only the year class was recorded for the farmed rainbow trout. Whole fish or fins were placed in boxes filled with 96% ethanol and examined for

Table 3. The number of parasites identified by molecular methods from the different localities in Murmansky oblast listed according to date of sample, fish host, number of specimens analysed and the respective GenBank accession numbers.

Locality	Date	Fish host	n *G.* spp. analyzed	n ITS	n COI	GenBank Acc.Nos ITS	GenBank Acc. Nos COI
River Pak	23.08.2016	*S. salar*	7	7	7	MW819680-81, MW819686, MW819691-93, MW819695	MW830301-02, MW830308-09, MW830315, MW830324, MW830326
River Pak	23.10.2017	*S. salar*	5	5	5	MW819679, MW819685, MW819687-89	MW830313-14, MW830306-MW830307, MW830322
River Shovna	26.10.2017	*S. salar*	5	5	5	MW819678, MW819683-84, MW819696-97	MW830305, MW830318-21,
Farm 1, Tuloma	02.06.2017	*O. mykiss*	5	5	5	MW819682, MW819701-02, MW819704-05	MW830303-04, MW830316-17, MW830325
Farm 2, Tuloma	07.04.2017	*O. mykiss*	4	4	4	MW819676-77, MW819690, MW819694	MW830310-12, MW830323
Farm 3, Lake Imandra	08.06.2017	*O. mykiss*	5	5	5	MW819698-99, MW819700, MW819703, MW819706	MW830296-300
		Total number	31	31	31		

the presence of gyrodactylids under a stereomicroscope (see e.g. OIE 2021). Parasites were counted, removed from the fish or fin with a micropipette and placed in individual 1.5 ml or 0.2 ml Eppendorf tubes. The prevalence, abundance and intensity of infection was recorded. Parasitological terms follow Bush et al. (1997).

Parasite identification

The current study applied PCR and sequencing of the nuclear ribosomal internal transcribed spacer (ITS rDNA) and the mitochondrial cytochrome oxidase I (COI) as the method for species identification, following the guidelines given in the OIE Manual of diagnostic tests for Aquatic Animals (OIE 2021). Morphological images of the haptoral hard parts were included as supplementary information. Specimens sampled in 2016 and 2017 (Table 3) were prepared for molecular and morphological imaging according to Paladini et al. (2009). In short, the haptors were excised from the bodies and digested with a proteinase K solution and morphological preparations were made (Paladini et al. 2009). DNA was extracted from the remaining cut off bodies of the specimens using the DNEasyKit on a QiaCube automatic extraction machine (Qiagen) following the manufacturer's instructions. The primer pair ITS1A and ITS2 (Matejusová et al. 2001) was used to amplify the ribosomal ITS rDNA fragment, while the primer pair Trp1F and Thr1R (Kuusela et al. 2008) was used to amplify the mitochondrial COI gene. Both PCR reactions were carried out with puRe Taq Ready-to-Go PCR beads (Amersham Biosciences) in a GeneAmp PCR System 9700 (Applied Biosystems) following previously published PCR protocols for ITS rDNA (Matejusová et al. 2001) and COI (Hansen et al. 2003; Kuusela et al. 2008; Meinilä et al. 2004). Samples yielding positive

PCR reactions were sent to Macrogen where they were sequenced with the PCR primers in addition to internal primers (Hansen et al. 2003; Kuusela et al. 2008; Matejusová et al. 2001) to obtain overlapping reads of each nucleotide. Sequences were proofread in Geneious ver. 8.1.9 (Biomatters Limited) and the sequences were then submitted to a GenBank BlastN search to search for identity with known sequences (http://www.ncbi.nlm.nih.gov/) (Zhang et al. 2000).

Preparations of the haptoral hard parts were photographed and measurements of each specimen were made and processed using the software Levenhuk ToupView 3.5 (V. Levenhuk, Inc.). A selection of four measurements describing the total size of the hard parts was taken to compare the size of the hard parts with measurements in previous studies; hamulus total length (HTL), ventral bar total length (VBTL), ventral bar total width (VBTW) and marginal hook sickle length (MHSL) (Shinn et al. 2004).

Environmental DNA sampling and analyses

To supplement conventional surveillance for *G. salaris* using electrofishing and subsequent examination of the fish under a stereomicroscope, we drew upon environmental DNA (eDNA) monitoring. Water filtrates were taken in 2018 in one location in the main river just upstream of the Nizhne-Tulomskaya HPP, and in the tributaries Pak, Shovna and Pyayve (Figure 1). At each sampling location, duplicate water samples of 5 litres were taken according to the sampling protocol established by Strand et al. (2019) and used in Rusch et al. (2018) except for the use of a different pump. To be able to carry out eDNA – sampling as cheaply as possible, we used a field kit where a common battery drill was combined with a water pump (product code 1490-20; Gardena) and attached to garden-hose tubes. As a filter holder, we used filter cups (Nalgene Analytical Test Filter Funnel, 145-0045; Thermo Fisher Scientific) and replaced the original filter provided by the manufacturer with the same type of glass fibre filter (47 mm AP25 Millipore, 2 µm pore size) as is used in Rusch et al. (2018). This same field kit has since also been successfully applied to simultaneously detect native and invasive crayfish and *Aphanomyces astaci* Schikora from environmental DNA samples (Rusch et al. 2020). Using this water filtration system, we were generally able to filter 5 litres of water in less than 5 minutes. The filters were stored on silica gel beads and analysed for the presence of *G. salaris* using duplex ddPCR as outlined in Rusch et al. (2018).

Results

Parasitological analyses and identification

Altogether, 375 salmon parr from the Lower Tuloma reservoir were sampled by electrofishing and examined for the presence of *Gyrodactylus* in the period from 2015–2019 (Table 1). Most samples were taken in the tributaries

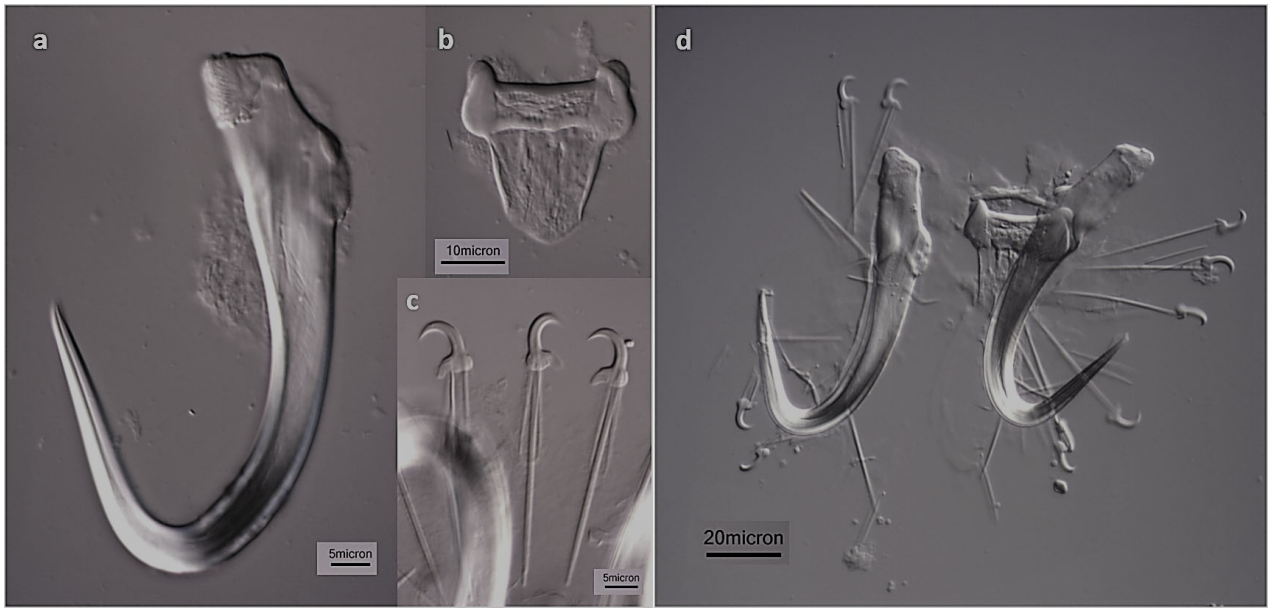

Figure 2. a-d. Light micrographs of the haptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 from farmed *Oncorhynchus mykiss* (a, b, c) and wild *Salmo salar* (d) from River Tuloma, Russia. Note different scale bars. Photomicrographs by Aleksey Parshukov and Sergey Sokolov.

River Pak and River Pecha. In addition, 48 rainbow trout from the fish farms in River Tuloma and 10 from the farm in Lake Imandra were examined (Table 2). Gyrodactylid parasites were first detected on wild salmon juveniles of River Pak in 2015 and these were tentatively identified as *G. salaris* based on examination of morphological features of the haptoral hard parts. Gyrodactylid parasites were detected in River Shovna for the first time in October 2017, the first year this river was examined, while no parasites were detected in the other rivers during the duration of this study. From samples taken from River Pak, River Shovna and the three farms in 2016 and 2017, a total of 31 *Gyrodactylus* specimens were subjected to PCR and sequencing of the internal transcribed spacer and the mitochondrial cytochrome c oxidase subunit I (COI) gene (Table 2).

All specimens analysed in the current study had a morphology corresponding to *G. salaris* (Figure 2) and the four morphometric measurements taken fell within previously recorded measurements for *G. salaris* (data not shown). Sequences of the internal transcribed spacer and the mitochondrial cytochrome *c* oxidase subunit I (COI) gene were obtained for all 31 specimens and identified all specimens, both on salmon and rainbow trout, as *G. salaris*. Not all sequences of COI obtained were of full length (≈ 1600 bp), but the final proof-read sequences varied between 710 and 1544 bp. However, all except nine sequences were more than 1500 bp and full length sequences were obtained from specimens representing all localities. In addition, some sequences, most notably those from Farm 3, contained minor ambiguities that could not be resolved and are thought to be the result of PCR or sequencing errors or heteroplasmy. The same mitochondrial haplotype was present in all individuals and the BlastN-search of the COI sequences (date 15.05.2020) showed that the sequence

Table 4. Infection parameters of *Gyrodactylus salaris* on juvenile Atlantic salmon in the River Pak.

Date	n fish examined	Prevalence, %	Abundance	Intensity, min–max
July, 2015	30	36,7	4,93	1–127
July, 2016	18	11,1	0,22	2–2
August, 2016	18	11,1	0,28	1–4
October, 2016	11	100	46,82	1–140
May, 2017	4	–	–	–
July, 2017	10	50	7,2	1–33
August, 2017	10	50	4,8	1–34
October, 2017	15	100	112,5	9–899
July, 2018	11	–	–	–
September, 2018	15	–	–	–
October, 2018	12	–	–	–
July, 2019	25	–	–	–
October, 2019	6	3 infected	4,8	0–17
Total number	185			

was identical to the GenBank accession number AF479750 representing a haplotype previously known as F, III-F, RBT or II-A1(Hansen et al. 2003; Mieszkowska et al. 2018; Ziętara et al. 2006). All ITS and COI sequences are submitted to GenBank (Table 3).

For River Pak, from where most samples were taken, the prevalence and abundance of parasites of juvenile salmon were the highest in the autumn season (Table 4). The distribution of parasites was aggregated: the fish population was, also in autumn, dominated by individuals with a relatively low intensity of parasites, and only a few highly infected individuals (max number of parasites was 899, Table 4). *Gyrodactylus* spp. were recorded from 2015 to 2017, but in 2018 they were not detected, neither in summer nor in autumn samples. However, the parasites were again detected in 2019. The infection parameters on rainbow trout in these farms obtained for the spring and summer seasons of 2017 are presented in Table 2.

Environmental DNA detection

From the four locations sampled, eDNA of *G. salaris* was unambiguously detected in two. We detected eDNA of *G. salaris* in both samples taken in River Tuloma and in one of two samples taken in River Shovna, but failed to detect eDNA of *G. salaris* in River Pyayve and River Pak. In all samples, we detected eDNA of *Salmo salar*, indicating that Atlantic salmon was or had recently been present in the rivers. The eDNA results are summarized in Table 5.

Discussion

This study reports on the first detection of *Gyrodactylus salaris* on both wild Atlantic salmon and farmed rainbow trout in a new area of Russia and the results show that it is highly likely that *G. salaris* has been recently introduced to this area by movement of live rainbow trout between fish farms. The presence of identical haplotypes (parasite strains) on both farmed rainbow trout and wild salmon within the same river and the fact

Table 5. Results from eDNA analyses for the presence of *Gyrodactylus salaris* and *Salmo salar* for each sampling site. List of sampling sites including amount of water filtered, number of samples per site (each sample constitutes one filter), the number of eDNA copies per litre (ddPCR) from all filters taken at each point, respectively. eDNA copies per litre are abbreviated as eDNA/l. Non-detection is indicated with a zero.

Sample	River	Date	Volume (L)	eDNA/L *G. salaris*	eDNA/L *S. salar*
1A	Tuloma	27.09.2018	5	52	39
1B	Tuloma	27.09.2018	5	47	196
2A	Pyayve	27.09.2018	5	0	2356
2B	Pyayve	27.09.2018	5	0	862
3A	Pak	27.09.2018	5	0	15920
3B	Pak	27.09.2018	5	0	8284
4A	Shovna	27.09.2018	5	0	4462
4B	Shovna	27.09.2018	5	382	4809

that the strain in question is a strain that is associated with rainbow trout farms elsewhere (Hansen et al. 2003; Paladini et al. 2009; Ziętara et al. 2010), supports this conclusion. In addition, previous yearly studies of the parasite fauna of the wild salmonids in River Tuloma have never detected *G. salaris* (Melnik et al. 2019). However, in 1996, monogeneans were recorded from rainbow trout grown in one farm in the Nizhnetulomskoye storage reservoir, but on the basis of morphology the specimens were identified as *G. lavareti*, a species infecting whitefish *Coregonus lavaretus* (Karasev et al. 1997). The findings in the present study thus once again show that new investigations in new areas and countries will result in new discoveries of *G. salaris* (Hansen et al. 2016; Paladini et al. 2009). Such introductions have previously resulted in disastrous consequences, especially in Norway (see Bakke et al. 2007; Johnsen and Jensen 1986; Johnsen et al. 1999), but also in the River Keret in Russia (Ieshko et al. 2008). The finding of *G. salaris* in the River Tuloma highlights the importance of rainbow trout as a vector, as has been shown and noted by other authors (Hansen et al. 2016; Paladini et al. 2009, 2021; Ziętara et al. 2010). This is yet another argument for caution and more control of movement of live fish between aquaculture facilities (Peeler et al. 2006) and between areas of different infection status. Since the introduction to River Tuloma there has been fear of *G. salaris* spreading to new rivers and areas and recent observations indicate that *G. salaris* has now also infected the nearby River Kola although the species identification is not confirmed (E. Ieshko *pers. comm.*). This spreading is not surprising as River Kola enters the sea through the same estuary as River Tuloma, but nevertheless shows that the infection has potential to spread further.

The observed intensities of infections in River Tuloma and tributaries were quite low (Table 3) and much lower than commonly observed on Atlantic strains of Atlantic salmon in, for example, Norway. Different strains of Atlantic salmon have been observed to demonstrate different resistance towards infections with *G. salaris* (Bakke et al. 1990) but this has never been tested experimentally for the salmon population of River Tuloma. The origin of the Atlantic salmon population in the rivers draining into the Barents Sea and White Sea is not clear (Nilsson et al. 2001). However,

based on the epidemic nature of the infections with *G. salaris* observed in River Keret in the White Sea (Ieshko et al. 2008), it could be suspected that the River Tuloma population was highly susceptible to infections with *G. salaris*. The observed intensities of infections in River Tuloma and tributaries are, however, so far low and an explanation for this could be that the salmon strain in River Tuloma is resistant towards the infections or that the parasite strain in question is less virulent. The mitochondrial haplotype detected on Atlantic salmon and rainbow trout in the present study was identical to a strain of *G. salaris* (GenBank accession number AF479750) common on rainbow trout (Meinilä et al. 2004; Mieszkowska et al. 2018). Parasites with this haplotype or variants of this (see Hansen et al. 2003; Mieszkowska et al. 2018; Ziętara et al. 2006) have caused epidemics in the Norwegian rivers Lærdalselva and Drammenselva, but are also found as a non-virulent strain on e.g. Arctic char in two lakes in Norway (Olstad et al. 2007). As different strains and variants of *G. salaris* vary in their pathogenicity towards Atlantic salmon (Hansen et al. 2007; Karlsson et al. 2020) the biological characteristics of the strain present in Tuloma are unknown. Controlled experimental work is therefore needed to establish whether the strain present in River Tuloma is pathogenic towards Atlantic salmon. Other factors, such as water chemistry (Soleng et al. 1999), can, however, also explain the low intensities observed.

eDNA monitoring

A comparison of eDNA results with those of conventional monitoring shows a good correspondence between both methods. While we failed to detect eDNA of *G. salaris* in River Pak in 2018, no parasites were found during manual observation the same year which suggests very low abundance or absence of the parasite at the time of sampling. Non-detection of *G. salaris* in some samples may result from absence of infection or low parasite intensity, dilution of eDNA concentration (Rusch et al. 2020) through increased precipitation (flooding) and from non-optimal DNA extraction procedure. A recent report where different extraction protocols were applied to the same type of filters used in the present study, found that DNA yield can be increased with a different protocol (Fossøy et al. 2020). The observation of *G. salaris* on juvenile Atlantic salmon in River Shovna in 2018 corroborates the positive detection of *G. salaris* eDNA. With further development, the eDNA-method should be a valuable tool for control of rainbow trout transports and for monitoring the epizootic status of trout farms, particularly because transport containers facilitate a concentration rather than a dilution of eDNA.

Conclusions

This study demonstrates again that rainbow trout is an important vector for the spreading of *G. salaris* and that careful examination of the fish for the presence of this parasite must be carried out before transport and

stocking of rainbow trout. With further development, the eDNA methodology could be applied for such examination. As genetic markers that can differ between pathogenic and non-pathogenic strains of *G. salaris* are lacking, controlled experimental work is needed to establish the pathogenicity of the strain present in River Tuloma. This would be important information when evaluating potential risks of further spreading.

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Authors' contribution

VM, IS, AP, SS and EI all participated in sampling and examination of fishes in the field. AP and SS isolated parasites and carried out the morphological analyses and prepared the parasite samples for further molecular work. JCR, EI and HH carried out the eDNA sampling. HH performed the molecular diagnostics and genetic characterisation and HH, EI and NM were involved in the interpretation of the molecular data. HH and JCR analysed the eDNA samples. HH wrote the first draft of the manuscript and all authors participated in the writing of the manuscript. All authors read and approved the final version of the manuscript.

Ethics and permits

No approval from Institutional Animal Care and Use Committee (IACUC) or ethics committee was necessary. No experiments that involved fish were performed. All fish were killed following the strict codes of practice in force in Europe. Licenses for the collection of fish specifying which rivers, how many and what species of fish can be fished was issued by Barentsevo-Belomorskoe territorial department of Federal Agency of fisheries and permit number(s)): 51 2019 03 0108, 51 2018 03 0124, 51 2017 03 0054, and 51 2016 03 0125.

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