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NATURAL RESOURCES INSTITUTE FINLAND

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Summary

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The River Vuoksi is the largest Finnish-Russian cross-border river connecting Lake Saimaa in Finland and Lake Ladoga in Russia. The valuable salmonid populations in the river were abundant and healthy still a hundred years ago, enabling also recreational and professional fisheries. The populations, however, started to decline due to human influence such as construction of hydropower plants (HPPs) that obstructed free migration of the fish, dredging of rapids that had been important salmonid breeding and nursery areas, and deterioration of the quality of water by industry. The water quality has during the past decades improved and does not prevent salmonid reproduction anymore. However, the four HPPs in the river still hinder fish migration. In addition, most of the historical salmonid reproduction areas are still non-existent, although the restoration actions carried out have gradually started to improve the situation. However, the short-term river flow regulation and hydropeaking for hydropower production risk salmonid reproduction also in the restored areas. Stocking of hatchery fish has been conducted on the Finnish side, but mainly for the fisheries purposes.

Currently, the Lake Saimaa landlocked salmon *(Salmo salar* m. *sebago*), that is known to have migrated downstream from the lake to breed in the River Vuoksi above Imatrankoski, is critically endangered, and the Lake Ladoga salmon is endangered. Prior to the construction of the HPPs, the Lake Ladoga salmon migrated from the lake to breed, e.g., in rapids as far as right below Imatrankoski. Natural reproduction of brown trout (*Salmo trutta*) is also very scarce in the River Vuoksi, especially on the Finnish side, and not much is known of European grayling (*Thymallus thymallus*) populations existing in the main stream and tributaries.

In this study, we aimed at gaining more understanding of the genetic characteristics of the River Vuoksi salmonids, and possible genetic differentiation of the populations. This information would be important for any conservation and management actions and for sustainable use of the populations in the River Vuoksi and tributaries. We analysed the genetic relatedness and diversity of landlocked salmon, brown trout and European grayling populations from the River Vuoksi and its tributaries. For comparative purposes, a number of populations outside our study area were also analysed.

Only one salmon sample from the River Vuoksi on the Finnish side was obtained and could thus not be included in the analyses. The results of salmon on the Russian side suggest that the population is genetically distinct, and its genetic diversity is relatively high. The genetic diversity of the brown trout on the Finnish side was also rather high, but the sampled populations were in practice similar to the hatchery stock with no genetic differentiation to be found. Stocking has thus had an influence on their population structure. On the Russian side, the studied trout populations in the River Vuoksi tributaries belonged to so called "Lake Ladoga group" but were still genetically distinct and differentiated from each other and the other trout populations in the same group. Grayling in the River Vuoksi watershed formed two groups, one on the Finnish and one on the Russian side. However, the populations within the groups were genetically highly differentiated from each other, which may be a consequence of the small population sizes and genetic drift.

To conclude, there were differences in the level of genetic differentiation and diversity among the River Vuoksi salmonid populations. It seems that on the Russian side, the salmonid populations form more distinct genetic entities. The same holds for grayling on both sides of the border. Stocking on the Finnish side has homogenized the genetic structure of the brown trout populations. In general, however, safeguarding and supporting natural production should be the principle means to preserve the genetic diversity of the salmonid populations on both sides of the border.

The study was part of the "River flows – Life goes" RiverGo project (2019–2022) co-funded by the European Union.

Keywords: Salmonidae, populations, genetic variation, biodiversity, sustainable use

Contents

1. Introduction

The River Vuoksi is the largest Finnish-Russian cross-border river connecting the largest Finnish lake, the Lake Saimaa, and the Europe's largest lake, the Lake Ladoga in Russia. The length of the river is ca. 150 km, with most of the river flowing on the Russian side. The river has historically been a very significant area for catching salmonids, especially landlocked salmon (*Salmo salar* m. *sebago*) and brown trout (*Salmo trutta*). The catch estimates from the turn of the 19th and 20th century (Seppovaara 1984, Titov et al. 2008) clearly imply that these salmonids in the River Vuoksi have been thriving.

Indeed, the river has been a significant breeding area, e.g., for landlocked salmon (Seppovaara 1984, Titov 2004). Saimaa salmon and brown trout migrated downstream from the lake to breed also in the rapids above Imatrankoski (Seppovaara 1984). According to Seppovaara (1984), 90% of the salmonids migrating from the Lake Saimaa to breed in the River Vuoksi were brown trout and 10% salmon. Contrary to this, most of the upstream migrating salmonids from the Lake Ladoga to the river were landlocked salmon (Pravdin 1956, Dirin 2013). The Lake Ladoga salmon migrated from the lake to breed in rapids below the Imatrankoski rapids, but part of the salmon may have bred also in the major tributaries (Jääskeläinen 1923, Seppovaara 1984). Tributaries were, and still are, important breeding areas for migratory brown trout (Titov et al. 2017). Grayling is more local than landlocked salmon and trout, but there have historically been also grayling populations migrating both upwards from the Lake Ladoga and downwards from the Lake Saimaa to breed in the suitable habitats of the River Vuoksi (Jääskeläinen 1917a, b, Seppovaara 1984).

The salmonid populations in the River Vuoksi, and thereby also their importance for recreational and commercial fishery, started to decline from the beginning of 1900's onwards. Industrialisation deteriorated water quality, and hydropower plants (HPPs) were built to produce energy for the industry (Seppovaara 1984). Dredging of rapids for the sake of hydroelectric power production finalised the devastation of almost all the rapid areas important for salmonid breeding in the upper parts of the river by the mid 1950´s (Vehanen et al. 2022). Seppovaara (1984) assessed that there were originally 18 rapids (total length ca. 8.7 km) of which 13 were lost due to hydro power. On the Russian side only, the breeding and nursery area for salmon was estimated to have decreased from 45 ha before water construction to 5–5.5 ha after water construction (Titov et al. 2008). The recent estimation by Vähänäkki and Tapaninen (2022) indicates that the area of rapids has been larger, and that due to hydropower constructions, 189 ha of rapids area were lost in the upper part of the River Vuoksi, 90 ha of this area being on the Russian side.

Currently, the water quality of the river has improved substantially no longer hindering the reproduction of salmonid fishes (Vehanen et al. 2022), but the four HPPs, two on both sides of the current Finnish-Russian border very effectively hinder upward migrating of the salmonids and other species. The lower parts of the main river channel on the Russian side are still "free-flowing" but encompass only few areas suitable for salmonid reproduction. In addition, the short-term river flow regulation and hydropeaking in the River Vuoksi (Vähänäkki 2021) possess a threat especially to the young stages of salmonids (Vehanen *et al.* 2000, Scruton et al. 2008, Casas-Mulet et al. 2015, Puffer et al. 2015, Heggenes et al. 2018, Moreira et al. 2020).

The migratory salmonid stocks have been in decline in the River Vuoksi (Vehanen et al. 2022). Landlocked salmon is currently critically endangered (CR) in the Lake Saimaa region (Hyvärinen et al. 2019, Urho et al. 2019), and it is maintained mainly by hatchery rearing. The Lake Ladoga salmon is endangered (Shurov et al. 2004). The reproduction of the brown trout in the main stream on the Finnish side is weak, and there are only few tributaries that support natural

reproduction (Vehanen et al. 2022). On the Russian side, brown trout reproduces in some areas in the main stream and in the main tributaries (Titov et al. 2017, Vehanen et al. 2022). Grayling exists in the main stream and in many tributaries on both sides of the border, but the status of the populations is poorly known (Vehanen et al. 2022). There have been attempts to improve the conditions for salmonid reproduction by restoration actions in some of the earlier lost breeding grounds on the Finnish side of the river (e.g., Tapaninen 2021). These relatively small-scale restorations have not been as effective as expected due to negative effects from hydropeaking (Vähänäkki 2021). The restorations, however, are of utmost importance for the recovery of the salmonids in the River Vuoksi (Vehanen et al. 2022).

Stocking of hatchery-reared individuals has been the main means of management of the salmonids on the Finnish side of the River Vuoksi watercourse. Stocking is practiced merely for fisheries purposes, to compensate for the lost reproduction to increase the certainty of catching salmonids. Supplementary stocking for supporting the viability and preservation of salmonid species and their populations has recently also been carried out in the Finnish tributaries. The main species that is currently stocked in the River Vuoksi is the brown trout. Stocking of rainbow trout (*Oncorhynchus mykiss*) is practiced only for recreational fisheries purposes (Vehanen et al. 2022). Brown trout and landlocked salmon caught in the River Vuoksi may also originate from stockings of hatchery-born fish to the Lake Saimaa, as is indicated, e.g., by the recovery of tagged individuals from the River Vuoksi (Sundell 1995, Menna et al. 2022). There is also indication that stocked brown trout may be carried downstream through the HPPs to the Russian side of the river (Marttinen 2004, Karels 2020, Menna et al. 2022). On the Russian side of the River Vuoksi, only whitefish (*Coregonus lavaretus* s.l.) releases have been made during recent decades.

Only little is known of the genetics of the salmonids in the River Vuoksi. Previous genetic studies indicate that the Lake Ladoga salmon and the Lake Saimaa salmon are of different origin, the Lake Ladoga salmon of eastern and the Lake Saimaa salmon of western origin (Nilsson et al. 2001, Koljonen 2008). However, they also share some genetic characteristics which hints at a long connection, one option being the River Vuoksi (Lumme et al. 2016). In a phylogenetic analysis, Tonteri et al. (2005) showed that the Lake Saimaa, the Lake Ladoga and the Lake Onega salmon populations are clustered together with the rivers Tornionjoki, Vindelälven and Neva, all being Baltic Sea populations. The non-anadromous salmon populations studied by Tonteri et al. (2005) could be separated into two groups: one including populations from the Baltic Sea Basin (Lake Saimaa, Lake Ladoga and Lake Onega), and the other including the Karelian populations from the White Sea basin. Ozerov et al. (2010) studied the genetic structure of the Lake Onega and the Lake Ladoga salmon populations in rivers flowing into them. They observed a high level of genetic structuring in both lake systems and an apparent low level of migration between the populations. They recommended treating each river as a separate management unit and ensuring sufficient level of natural reproduction in each river would be the best strategy for management (Ozerov et al. 2010). More thorough knowledge of the genetic characteristics of the River Vuoksi salmonids would be important from the perspective of sustainable use of the populations. Mapping the genotypes of salmonid populations and the interpretation of these results into management plans is the foundation for sustainable use of these populations in the River Vuoksi and tributaries.

In this study, we analysed the genetic characteristics of landlocked salmon, brown trout and European grayling populations from the River Vuoksi and its tributaries. These characteristics were compared among the populations in the area (main stream and tributaries) and among the relevant populations outside our study area. The aim was to find out whether there exist genetically distinct populations for conservation efforts and to also reveal genetic distances among populations.

2. Material and methods

2.1. Fish sampling

A tissue sample (a 1 mm^2 clip of a fin from anaesthetised fish) was taken from fish individuals caught by electrofishing. Some of the samples were collected from recreational fishermen. The tissue samples were placed into test tubes and preserved in 95% ethanol. After recovery each electrofished fish was released back to the place of capture.

2.1.1. Atlantic salmon samples

In all, there were 128 new landlocked salmon samples from the River Vuoksi and nearby water-systems, rivers Hiitolanjoki and Vammeljoki and the Lake Ladoga, into which the River Vuoksi and the River Hiitolanjoki drain (Table 1, Fig. 1). One sample from only a single fish from the Finnish side of the River Vuoksi was omitted because it could not be analysed as such.

The River Vuoksi (Fig. 1) drains from the Lake Saimaa into the Lake Ladoga, and from there via the River Neva, across the City of Saint Petersburg (Pietari in Fig. 1), into the Gulf of Finland. Freshwater salmon has occupied both large lakes since the last glaciation. Two sites were sampled from the River Vuoksi in Russia: Kiviniemenkoski rapids and the River Taipaleenjoki. The River Taipaleenjoki is the main branch of the River Vuoksi through which the river drains to the Lake Ladoga (Fig. 1). One small freshwater salmon catch sample (N = 8) was available from the Lake Ladoga itself near the site Vossinoi (Table 1). Freshwater salmon occurs also in the other large Russian lake, the Lake Onega, which drains via the River Svir into the Lake Ladoga (Fig. 2).

The River Hiitolanjoki, a border river draining from Finland into the Lake Ladoga, was also sampled (Hijtola in Fig. 2). Landlocked salmon samples were from Finland (Kangaskoski rapid) and Russia (Kalliokoski, Syrjäkoski, Sahakoski rapids) (Table 1). All three samples from the Finnish section of the River Hiitolanjoki from different years were pooled, as sample sizes did not allow a separate analysis.

For comparison, also an anadromous salmon population was sampled from the Russian River Vammeljoki (Talissalonkoski rapid). The river drains into the Gulf of Finland (Table 1, Fig. 1).

Table 1. The number (N), sampling year and origin of Atlantic salmon samples from the River Vuoksi (FIN/RUS), the River Hiitolanjoki (FIN/RUS), the River Vammeljoki (RUS) and the Lake Ladoga (RUS).

	Origin	Site	Year	N
	R. Vuoksi, FIN (omitted)	*Railway bridge*	*2014*	*(1)*
1	R. Vuoksi, RUS	Taipaleenjoki	2011, 2013–2016	31
2	R. Vuoksi , RUS	Kiviniemenkoski	2011, 2014–2016	30
3	R. Hiitolanjoki, FIN	Kangaskoski	2003	3
4	R. Hiitolanjoki, FIN	Kangaskoski	2006–2007	2
5	R. Hiitolanjoki, FIN	Kangaskoski	2015–2020	15
6	R. Hiitolanjoki, RUS	Kalliokoski, Syrjäkoski, Saha-koski	2002	21
7	R. Vammeljoki, RUS	Talissalonkoski	2004	17
8	L. Ladoga	Vossinoi	2002	8
	Analysed			**127**
	Total			**128**

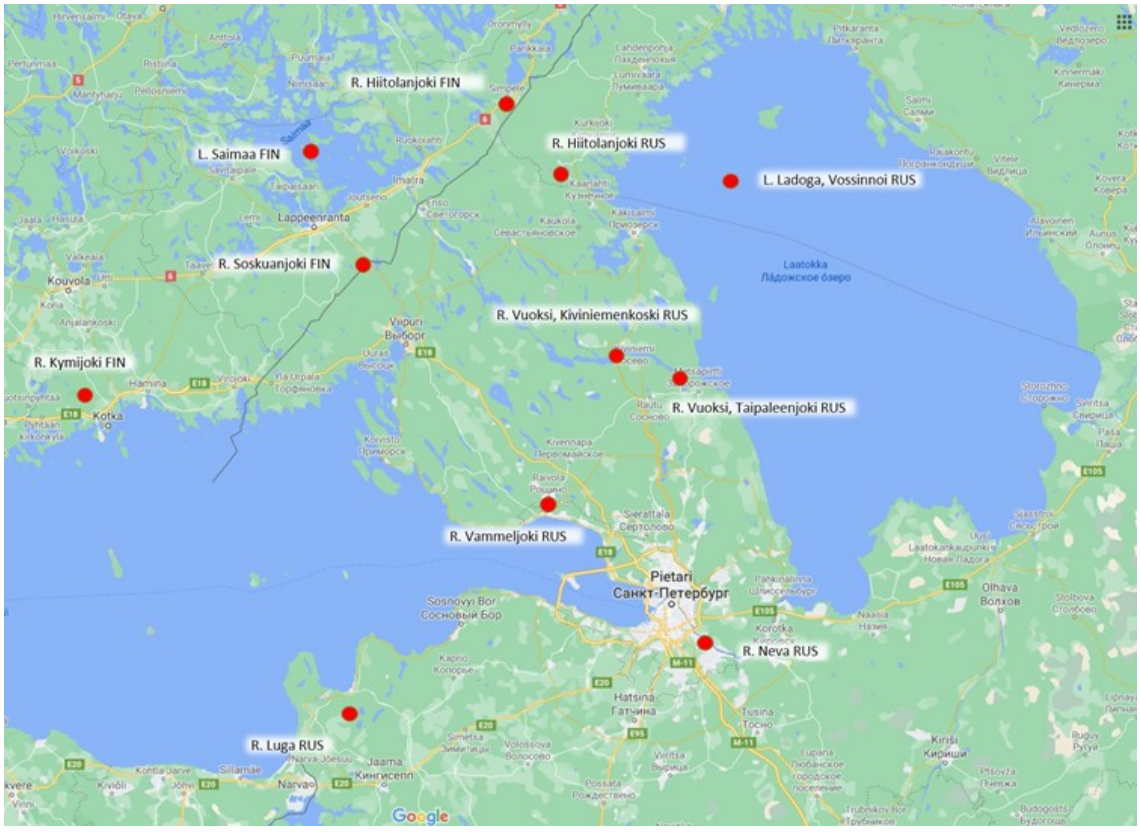

Figure 1. Map of the River Vuoksi and Atlantic salmon sampling sites in South-East Finland and on the Russian side. The River Vuoksi drains into the Russian Lake Ladoga (Ladozkoe Ozero). Soskuanjoki sampling site is on the Finnish side of the border, the River Soskuanjoki draining into the Gulf of Finland. Kiviniemenkoski and Taipaleenjoki sampling sites are in Rus-sia.

In addition, genetic salmon data were available for comparison from previously published papers (Koljonen et al. 1999, Säisä et al. 2005; Table 2, see also Fig. 1). Those included freshwater salmon from the Lake Onega and the Lake Saimaa, and from a Finnish-Russian border river, the River Soskuanjoki. A larger catch sample of the Lake Ladoga freshwater salmon was also included as well as anadromous salmon from the Russian rivers Neva and Luga. Samples were also available from the Finnish River Kymijoki, into which the Russian River Neva salmon has been stocked in the past, and from two northern, also anadromous salmon populations from rivers draining into the Gulf of Bothnia, rivers Simojoki and Tornionjoki. The River Tornionjoki maintains the largest Baltic Sea salmon population.

Table 2. The number (N), sampling year and origin of Atlantic salmon samples used for DNA-analysis comparisons.

	Origin	Drains into	Year	N
1	L. Onega, RUS	Lake Ladoga	1995	19
2	L. Ladoga, RUS	Gulf of Finland	1995	94
3	R. Neva, RUS	Gulf of Finland	1995	50
4	R. Soskuanjoki, FIN	Gulf of Finland	2012–2013	32
5	R. Kymijoki, FIN	Gulf of Finland	2005	100
6	R. Luga, RUS	Gulf of Finland	2003, 2011	147
7	L. Saimaa, FIN	Lake Ladoga	2014–2016	60
8	R. Tornionjoki, FIN	Gulf of Bothnia	2013	70
9	R. Simojoki, FIN	Gulf of Bothnia	2009, 2010	70
	Total			**642**

Figure 2. A map showing the Lake Ladoga and rivers draining into it. The River Hiitolanjoki (Hijtola) is situated in the north-west corner of the lake, the River Vuoksi (Vuoksa) on the western side of the lake.

2.1.2. Brown trout samples

In all, 459 new brown trout samples from 16 sites were obtained for the RiverGo project (Table 3). Four samples were from the Finnish side of the River Vuoksi, four from small tributaries/brooks near the City of Imatra, four from nearby rivers in Finland and three from the Russian rivers (Fig. 3). In addition, there was one catch sample from the Lake Ladoga in 2020. One sample from only a single fish from the Russian side of the River Vuoksi was omitted because it could not be analysed as such.

Table 3. The number (N), sampling year and origin of the brown trout samples used for DNA-microsatellite analysis.

	Origin	Year	N
1	R. Vuoksi, above Tainionkoski rapid, FIN	2018–2020	22
2	R. Vuoksi, Imatrankoski, FIN	2010–2020	62
3	R. Vuoksi, catch 2013, FIN	2013	45
4	R. Vuoksi, catch 2020, FIN	2020	45
5	Voimanpuro 1, tributary draining into R. Vuoksi below Imatra rapid, before 2018, FIN	2010, 2012–2014, 2016–2017	31
6	Voimanpuro 2, tributary draining into R. Vuoksi below Imatra rapid, after 2018, FIN	2019, 2020	21
7	Ylisyöksypuro brook, FIN	2010, 2012, 2014, 2016	30
8	Kaupunkipuro, tributary draining below Imatra rapid, FIN	2016–2019	30
9	Partakoski rapid, FIN	2014–2020	20
10	R. Hiitolanjoki-Lohijoki, FIN	2003, 2014, 2018	30
11	R. Laamalanjoki, FIN	2014, 2020	25
12	R. Unterniskanjoki, FIN	2019, 2020	21
13	R. Semuzhja (Lohijoki), RUS	2012	30
14	R. Losevka, RUS	2014	17
15	R. Volchja (Saijanjoki), RUS	2011	5
16	L. Ladoga, RUS	2002	24
	R. Vuoksi, RUS omitted	*2014*	*(1)*
	Analysed		**458**
	Total		**459**

In addition to the new samples, 13 samples were available from previously analysed data from both Finland and Russia (Table 4, see also Koljonen et al. 2014).

Table 4. The number (N), sampling year, migration type (Anad. = anadromous, Lake-migr. = lake-migrating) and origin of previously analysed comparison samples used for brown trout DNA-microsatellite analysis.

	Origin	Year	N	Type
1	R. Urpalanjoki FIN/RUS	2006, 2010	40	Anad.
2	R. Mustajoki RUS	2006, 2007, 2008	137	Anad.
3	R. Hiitolanjoki FIN/RUS	2002	60	Lake-migr.
4	R. Tsysmu (Puhdaspuro) RUS	2011	23	Lake-migr.
5	R. Notkopuro RUS	2006	51	Anad.
6	R. Inojoki RUS	2006	25	Anad.
7	R. Pikkuvammeljoki RUS	2006	50	Anad.
8	R. Vammeljoki RUS	2006	39	Anad.
9	R. Kuokkalanpuro RUS	2006	23	Anad.
10	R. Luga RUS	2006	64	Anad.
11	Rautalamminreitti FIN, *hatchery*	2006	50	Lake-migr.
12	Kermankoski (Vuoksi *hatchery*) FIN	1999–2006	220	Lake-migr.
13	R. Luutajoki FIN, *hatchery*	2004	40	Resident
	Total		**822**	

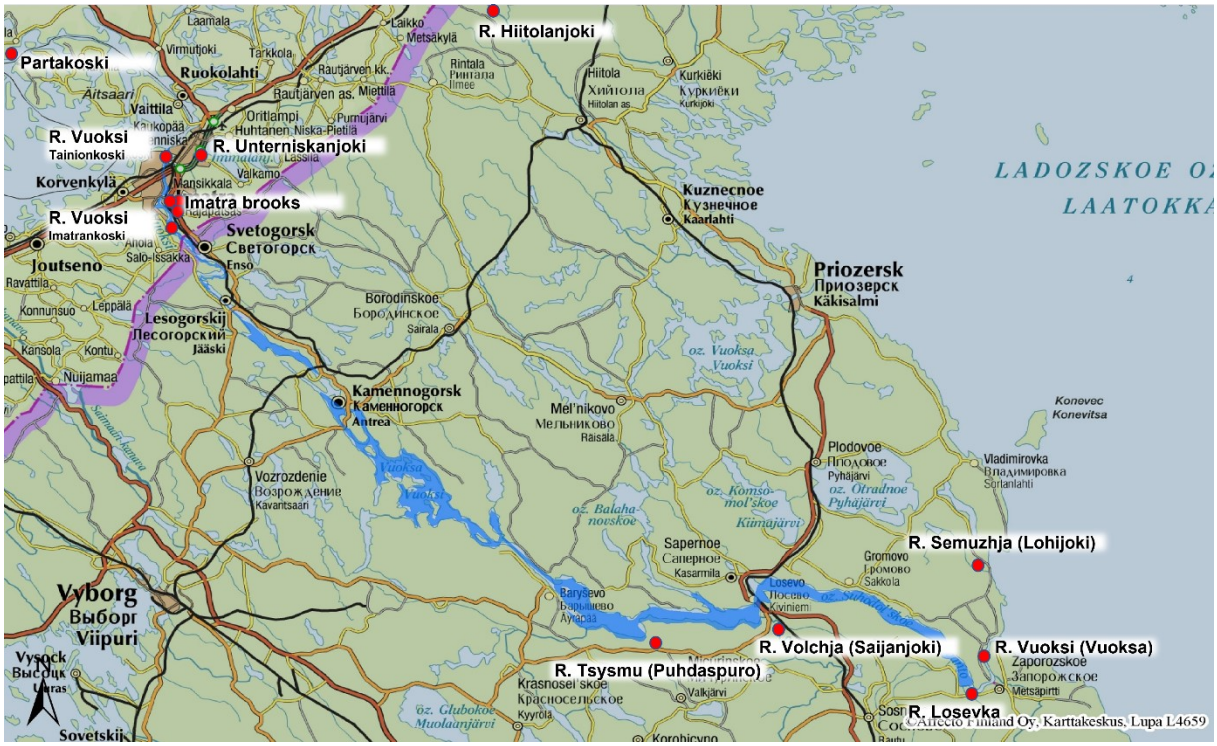

Figure 3. River Vuoksi and the sampling sites of brown trout in the RiverGo project, as well as River Tsysmu (Puhdaspuro), are shown.

2.1.3. Grayling samples

In all, 317 new grayling samples (mostly sampled in the RiverGo project 2019-2020) were obtained from both Finland and Russia. One fish was omitted as it was the only sample from the River Vuoksi above Tainionkoski rapid (Table 5). Most sampling sites were from the Vuoksi river system and many of the samples were small. Of the analysed 316 individual samples, 106 were from the Finnish side of the Vuoksi river system. Of the River Vuoksi samples (incl. tributaries), 53 were from Finland and 67 from Russia. The single Kaupunkipuro sample was pooled in the analysis with the Voimanpuro samples.

In addition to the River Vuoksi river system samples, one small (N = 6) sample from the Russian River Voronka represented a Baltic Sea river sample. From Finland, four comparison samples were analysed from nearby waters: the Southern Lake Saimaa, the River Lieksanjoki and the Lake Puruvesi (hatchery and natural stock samples), all belonging to the Vuoksi watershed. Moreover, a sample from a hatchery stock from Central Finland (from the Päijänne drainage), Rautalamminreitti, was analysed for this project. A previously analysed sample was available from the rare, endangered sea (brackish water)-spawning grayling from the Gulf of Bothnia, the Krunnit islands area.

Table 5. The number (N), sampling year and origin of the grayling samples used for DNA-analysis comparisons.

	Origin	Year	N
	R. Vuoksi, above Tainionkoski rapid, FIN (omitted)	*2020*	*(1)*
1	R. Vuoksi, between HPPs FIN	2011–2019	20
2	R. Vuoksi, below Imatra rapid FIN	2011–2013, 2018–2019	26
3	Voimanpuro, FIN	2019	6
4	Kaupunkipuro, FIN	2020	1
5	R. Suokumaanjoki FIN	2010, 2014–2019	30
6	R. Kupinjoki, FIN	2017–2020	23
7	R. Vuoksi, RUS	2011, 2014, 2016, 2020	8
8	R. Volchja (Saijanjoki), RUS	2011, 2019	33
9	R. Vjun (Viisjoki), RUS	2011, 2019	26
10	R. Voronka, RUS	2005	6
11	Southern Lake Saimaa FIN	2017–2019	30
12	R. Lieksanjoki, FIN	1999	26
13	L. Puruvesi, FIN	2018	13
14	L. Puruvesi, FIN *hatchery*	2020	19
15	Rautalammenreitti, FIN *hatchery*	2020	20
16	Gulf of Bothnia, Krunnit area, FIN	2012, 2013	29
	Analysed		**316**
	Total (RiverGo 256)		**317**

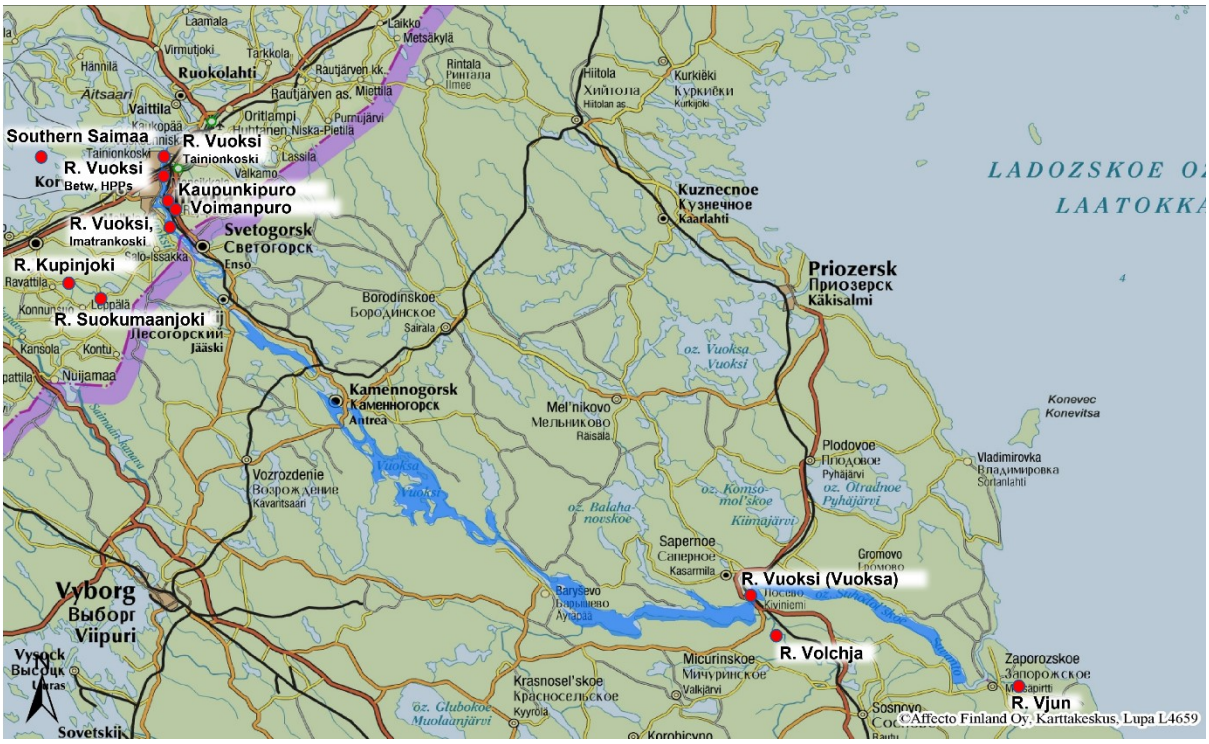

Figure 4. Grayling sampling sites in the RiverGo project from Finnish and Russian side of the River Vuoksi catchment.

2.2. Fish DNA laboratory analysis

The DNA for comparison samples were analysed at the united gene laboratory of the University of Helsinki and Natural Resources Institute Finland (Luke). All DNA samples were analysed according to the previously published methods for brown trout (Koljonen et al. 2014, Koskiniemi 2020a) and Atlantic salmon (Säisä et al. 2005, Vuori et al. 2012, Koskiniemi 2020b, Leinonen et al. 2020). The number of microsatellite loci used was 16 for brown trout, 17 for Atlantic salmon and 22 for grayling. DNA-analysis protocol for grayling in Luke analysis has not been published previously.

For the grayling DNA-analysis, the 22 SSRs were amplified in five PCR reactions according to results from Multiplex Manager v1.2 program (http://multiplexmanager.com). To separate and visualise amplified products, an ABI PRISM® 310 Genetic Analyzer (Thermo Fisher Scientific Ltd, Vantaa, Finland) was used. The forward primer of each primer pair was labelled with a fluorescent dye, FAM™ (5-carboxyfluorescein), NED™, VIC® or PET®. The PCR amplification was performed in a total volume of 10 µl, containing 5 µl Master mix from Qiagen Type-it® Microsatellite PCR Kit (Qiagen, Helsinki, Finland), 10 ng of DNA, and 67-1000 nM each primer. The PCR temperature profile was as suggested in the PCR kit manual except that annealing temperature was 57 °C and number of cycles 32. PCR products were diluted 1/100 for the ABI runs. GeneMapper® software 5 was used for allele size estimation.

Table 6. Microsatellite loci used in Luke for grayling analysis. Locus, multiplex (MP), primer sequences, dye, primer concentration (Pc) and reference are given. References for loci are given in Appendix 1.

Locus	MP	Forward primer seq. (5'-3')	Reverse primer sequence (5'-3')	Dye	Pc(µM)	Ref.
BFRO004	MP1	GCTCCAGTGAGGGTGACCAG	GTTTAGGCCACTGATTGAGCAGAG	6FAM	0.200	R5
Tth_446	MP1	GCCATTCACCCATACTATGC	GTTTCCATTCAGCCACTAGAGC	6FAM	0.400	R1
Tth_403	MP1	CTTGTGCGCGAAGCATACA	GTTTCGACAGAAATTAAGGTTCACA	VIC	0.400	R1
BFRO017	MP1	GCCCCTCTGCTAAACACAC	GTTTCTATTGGGTTGAGGTCTGG	NED	0.200	R7
BFRO005	MP2	CGCATCTGTATGAAAAACCT	GTTTTGGTTTGGTAGGAGTTTCGT	VIC	0.100	R6
BFRO012	MP2	TCTGCACATCCAAAGCCATC	GTTTAATCTCTCTTAATGAATCGT	NED	1.000	R2
Tth_207	MP2	TGATGGCTGGAGGTAATTC	GTTTAATTGCATCTCACGTCAAC	VIC	0.200	R1
Tth_302	MP2	CCGCAGTCATCACCTTATC	GTTTAGTTACAGCCACCGCCTTA	VIC	0.133	R1
Tth_313	MP3	AAACCAGTCCAAGCGAGAG	GTTTCTCCTGTTTATCACATGA	6FAM	0.200	R1
Tth_447	MP3	CTTGATTGCCATTGGATTGT	GTTTCAACATCCTTGTCGCCTCTA	NED	0.200	R1
BFRO007	MP3	AGACCCCCAAAAACTATGCT	GTTTCTTCAGCAGGGGGAGATAAA	6FAM	0.100	R6
Ogo2	MP3	ACATCGCACACCATAAGCAT	GTTTCGACTGTTTCCTCTGTGTTGAG	NED	0.200	R3
BFRO015	MP4	GACTCAGTGAA-GAACTAAAGTACA	GTTTGAAAAGTTATGAAGGTCAACCC	6FAM	0.100	R7
BFRO009	MP4	AAATTGTCCCCGTTGGCAGA	GTTTACATACACCGCAACACCCAG	VIC	0.067	R6
Tth_211	MP4	TGAATTTGTCTGTTTCCGATG	GTTTGACACCTGCTGCTATACCTG	NED	0.200	R1
Tth_305	MP4	CTTTGAATATGATGCGTGAAC	GTTTGAGTATACTGCAGATAGACCA	NED	0.200	R1
BFRO016	MP5	GCACACAATCTCTCTGTGAGTA	GTTTATCAGCCCAAGGTTGTAACA	VIC	0.100	R7
BFRO010	MP5	GGACGGAGCCAGCATCAC	GTTTGCCCCCAGGTTATCATAGCT	NED	0.050	R8
One2	MP5	GGTGCCAAGGTTCAGTTTATGTT	CAGGAATTTACAGGACCCAGGTT	PET	0.670	R4
Tth_433	MP5	AATGATGTCAATTAGCCTAT	GTTTGTTTACAGACTTAGTGAA	VIC	0.400	R1
Tth_214	MP5	TGGTGCCAGTTAGATAGTCA	GTTTGCTTCACATTTATCCTATGATT	6FAM	0.100	R1
BFRO018	MP5	AGAGGGGTCCAGCAACATCA	GTTTGGGGAACCAGTCTAAAGCCT	6FAM	0.067	R7

R1 (Junge et al. 2010), R2 (Koskinen and Primmer, 2001), R3 (Olsen et al. 1998), R4 (Scribner et al. 1996), R5 (Snoj et al. 1999), R6 (Susnik et al. 1999 a), R7 (Sušnik et al. 1999 b), and R8 (Susnik et al. 2000).

2.3. Statistical methods

Diversity measures and pairwise F_{ST} values were calculated with FSTAT version 2.9.3.2. (Feb. 2002) (Goudet 1995, 2001) (http://www2.unil.ch/popgen/softwares/fstat.htm). Analysis of the differences between populations was based on genotype frequencies and was also tested with FSTAT, which includes a Bonferroni correction for multiple tests. Allelic richness was calculated for the smallest population sample sizes.

The genetically effective population sizes (Ne), the number of full-sib families, and the number of male and female spawners in the spawning population samples were calculated with COL-ONY-software (Version 2.0.6.6, June 30th, 2020) (Wang 2004, Wang and Santure 2009), and the mean triadic relatedness within populations was estimated with COANCESTRY-software (Version 1.0.1.9, July 30, 2018) (Wang 2007).

Genetic distances between populations were calculated using Nei's D_A distances (Nei et al. 1983). Phylogenetic trees were constructed using a neighbour-joining (NJ) algorithm (Saitou & Nei 1987, Takezaki 1998) with Populations 1.2.32 software (Copyright (C) 1999, Olivier Langella, CNRS UPR9034 (http://bioinformatics.org/~tryphon/populations/). Bootstrapping with 1 000 replicates was used to test the statistical strength of the branches. The genetic distance tree was drawn with TreeView version 1.6.6. (Page 2000) (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

3. Atlantic salmon

3.1. Genetic differentiation among populations

At first stage, genetic differences were measured among five new salmon samples (Figure 5). The two samples from the River Hiitolanjoki, from Finland and Russia, grouped closely together. Similarly, the two samples from the River Vuoksi main stem, Kiviniemenkoski and Taipaleenjoki on the Russian side, grouped also closely together. The small catch sample obtained from the Lake Ladoga was grouped close to the River Vuoksi. Samples from the anadromous River Vammeljoki salmon were close to the River Hiitolanjoki samples. The bootstrap values were high and over 50% in all cases. Especially the River Hiitolanjoki samples were very similar.

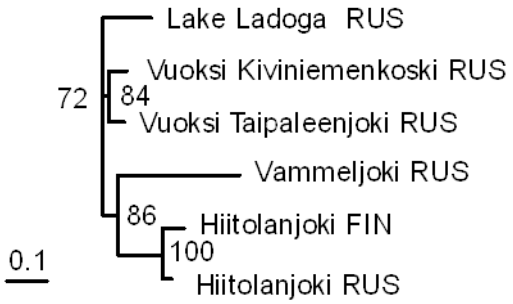

Figure 5. Genetic distances between Atlantic salmon samples analysed in the RiverGo project.

Genetic differentiation among all samples was measured as pairwise F_{ST} -values and it varied with a large range from the minimum of 0.006 between the northern River Tornionjoki and River Simojoki populations to a maximum of 0.331 between the River Simojoki and the Lake Saimaa salmon. In general, both the River Vuoksi landlocked salmon samples and the River Hiitolanjoki samples were very similar (F_{ST} < 0.03), which was logical (Table 7). In addition, the River Vuoksi samples and the small catch sample 2002 from the Lake Ladoga were very similar, as were also both the River Hiitolanjoki samples and the larger catch sample from the Lake Ladoga. In either case, no statistically significant difference could be observed between the river samples and the catch sample. The small catch sample from 2002 (N = 8) is obviously not representative of broader salmon catches in Lake Ladoga. However, the River Vuoksi salmon feeding migrations are to the Lake Ladoga and this sample apparently is a sample of the Vuoksi salmon stock during their feeding migration. The previous catch sample from 1995 was larger (N = 94), and it possibly represents more the average catch in the Lake Ladoga. This sample was more the River Hiitolanjoki type salmon. There are, however, several rivers draining into the Lake Ladoga (Fig. 2), from which we did not have any samples. The salmon sample from the third border river, the River Soskuanjoki, was quite similar (F_{ST} < 0.1) to the 2002 catch sample from the Lake Ladoga.

The most unique was the Lake Saimaa salmon, which differed clearly from several populations. Part of this differentiation is a result from very small Lake Saimaa salmon breeding population. The Gulf of Bothnia anadromous populations differed clearly from the River Vuoksi and the River Hiitolanjoki lake-migrating populations. The anadromous Kymijoki salmon differed also markedly from lake salmon populations (Lake Onega, Lake Ladoga, the River Hiitolanjoki), but also from the geographically more distant anadromous River Vammeljoki salmon (Table 7).

Table 7. Genetic distances between Atlantic salmon samples, measured as pairvise F_{ST} -values. Values higher than 0.2 are highlighted in grey.

Atlantic salmon population	R. Vuoksi Kivin.	R. Vuoksi Taipaleenj. RUS	R. Hiitolanjoki FIN	R. Hiitolanjoki RUS	R. Vammeljoki RUS	L. Ladoga 02 RUS	Onega RUS	L Ladoga 95 RUS	R. Neva RUS	R. Soskuanjoki FIN	R. Kymijoki FIN	R. Luga RUS	L. Saimaa FIN	R. Torniojoki FIN	R. Simojoki FIN
R. Vuoksi Kivin. RUS															
R. Vuoksi Taipaleenj. RUS	0.010														
R. Hiitolanjoki FIN	0.090	0.089													
R. Hiitolanjoki RUS	0.067	0.070	0.021												
R. Vammeljoki RUS	0.170	0.161	0.206	0.181											
L. Ladoga 02 RUS	0.010	0.009	0.099	0.064	0.158										
L. Onega RUS	0.109	0.107	0.117	0.088	0.171	0.070									
L. Ladoga 95 RUS	0.088	0.089	0.021	0.007	0.188	0.092	0.108								
R. Neva RUS	0.123	0.118	0.172	0.151	0.149	0.100	0.142	0.159							
R. Soskuanjoki FIN	0.120	0.119	0.137	0.113	0.118	0.087	0.104	0.127	0.113						
R. Kymijoki FIN	0.194	0.192	0.233	0.217	0.206	0.177	0.211	0.218	0.107	0.170					
R. Luga RUS	0.124	0.120	0.158	0.134	0.035	0.098	0.122	0.142	0.118	0.079	0.176				
L. Saimaa FIN	0.244	0.234	0.296	0.284	0.319	0.245	0.324	0.267	0.290	0.277	0.328	0.250			
R. Tornionjoki FIN	0.211	0.206	0.221	0.208	0.198	0.183	0.175	0.213	0.099	0.096	0.162	0.157	0.325		
R. Simojoki FIN	0.216	0.211	0.220	0.208	0.201	0.191	0.176	0.212	0.108	0.105	0.168	0.158	0.331	0.006	

When genetic distances among Atlantic salmon samples were shown as a genetic tree, four clear clusters were found for which the bootstrap value for the tree branch was over 80% (Fig. 6). Anadromous Russian salmon populations from the rivers Vammeljoki and Luga belong clearly to the same genetic group and they both drain into the Gulf of Finland, southeast and south. The Russian anadromous River Neva salmon and the Finnish River Kymijoki salmon group together as Kymijoki salmon is mostly a derivative of the imported Neva salmon in Finland. Northern anadromous populations from the rivers Tornionjoki and Simojoki group also tightly together. In addition, the two freshwater salmon samples from the River Vuoksi from the Russian side are very similar and they also group near to the more recent small catch sample from the Lake Ladoga. Freshwater salmon samples from the River Hiitolanjoki group also into the same branch, but with somewhat lower bootstrap value, and they group closer to the year 1995 catch sample from the Lake Ladoga.

After pooling the samples from rivers Vuoksi and Hiitolanjoki within rivers and omitting small (N = 8) catch sample from Lake Ladoga 2002, the genetic distance tree remained very similar (Fig. 7). The similarity between the River Hiitolanjoki samples and the Lake Ladoga catch sample was clearer when the River Hiitolanjoki samples were pooled. The River Vuoksi population was located closer to the Lake Ladoga when samples were pooled, and the small catch sample was omitted. The River Vuoksi salmon might have had some genetic influence from the Lake Saimaa salmon. All three salmon populations from rivers draining into the Lake Ladoga were different. The River Soskuanjoki salmon deviated most from the others and was closer to the anadromous populations. All freshwater populations are, however, quite small. All populations in this pooled sample analysis differed statistically highly significantly from each other.

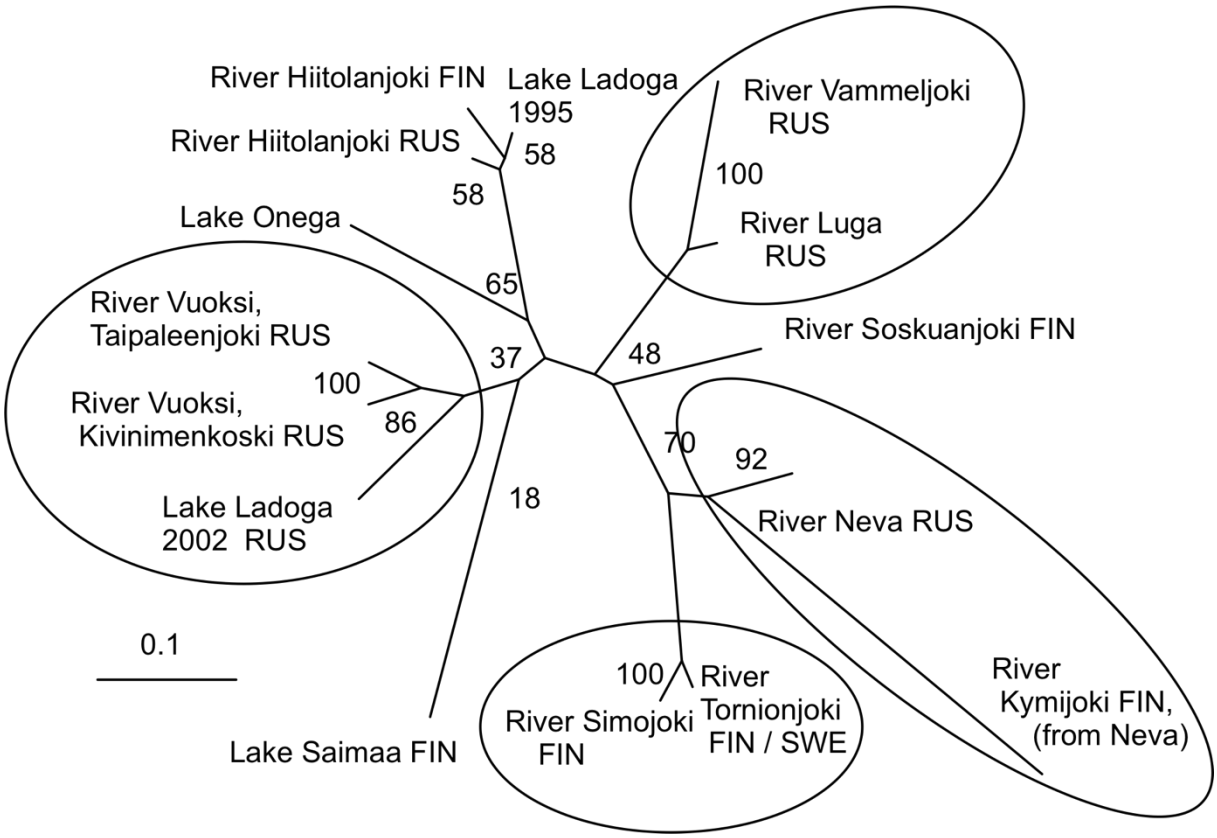

Figure 6. Genetic distances between Atlantic salmon samples from Finnish-Russian border rivers and comparison samples are drawn as an unrooted dendrogram. Sampling year is also indicated for the Lake Ladoga catch samples.

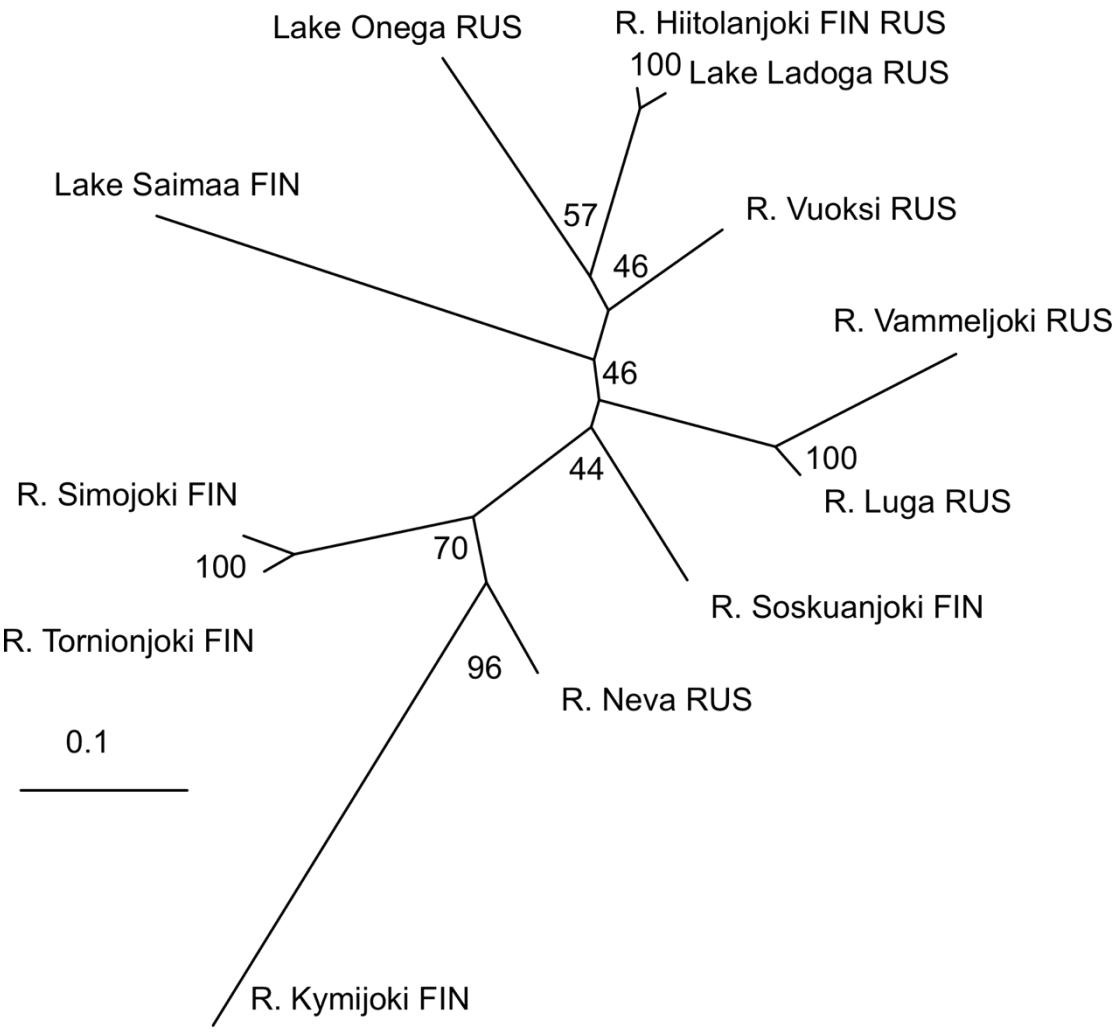

Figure 7. Genetic distances between Atlantic salmon samples from Finnish-Russian border rives and comparison samples are drawn as an unrooted dendrogram.

3.2. Genetic diversity level of the Atlantic salmon populations

The genetic diversity levels of the freshwater salmon populations were compared to those of some other salmon stocks, including both landlocked lake salmon populations as well as sea-migrating anadromous populations (Table 8).

The highest genetic diversity, 77.7%, was observed in the salmon population from the River Soskuanjoki sample and the smallest in the Lake Saimaa population (Table 8). The highest number of alleles was in the River Kymijoki and the River Luga populations, but for those samples, the sample sizes were also the largest. When measured as sample size standardized allele richness on average for 16 individuals, the highest numbers were in the rivers Neva, Soskuanjoki, Kymijoki and Luga.

For freshwater populations, two types of populations occurred. For the River Vuoksi, the River Soskuanjoki and the Lake Onega populations, the diversity levels were quite high, but for the River Hiitolanjoki and the Lake Ladoga, the diversity levels were relatively low, at least on basis of the available samples. Clearly, the lowest diversity measures were for the Lake Saimaa salmon.

Deviations from random mating were rare and were observed only in the Lake Onega and the River Soskuanjoki samples. These may result from unrepresentative samplings.

Table 8. Genetic diversity levels of the studied Atlantic salmon populations. Number of sampled fish (N), percentage of mean diversity over loci (Div %), number of alleles (N All) and mean allelic richness per locus for 16 individuals (All Rich) are shown. In addition, Fis-value measuring random mating deviations and P-values for deviation tests are shown.

Population	N	Div %	N All	All Rich (16)	Fis	P larger	P smaller
L. Onega, RUS	18	72.6	132	7.46	0.154	0.0000	1.0000
L. Ladoga, RUS	94	67.6	113	5.20	-0.005	0.6343	0.3663
L. Saimaa, FIN	60	46.9	74	3.64	-0.017	0.7787	0.2486
R. Hiitolanjoki FIN/RUS	41	67.2	100	5.26	-0.040	0.9690	0.0318
R. Vuoksi, RUS	61	73.7	166	7.33	-0.000	0.5198	0.5146
R. Soskuanjoki, FIN	32	77.7	152	7.61	0.092	0.0000	1.0000
R. Neva, RUS	70	74.4	172	7.73	-0.030	0.9912	0.0114
R. Kymijoki, FIN	100	73.6	187	7.68	0.003	0.4103	0.5916
R. Vammeljoki, RUS	17	67.3	98	5.68	-0.034	0.8565	0.1818
R. Luga, RUS	147	74.7	187	7.59	0.018	0.0398	0.9608
R. Tornionjoki, FIN	70	69.8	178	7.56	0.011	0.2299	0.7944
R. Simojoki, FIN	70	69.0	163	7.12	0.020	0.0897	0.9227
Overall	**780**	**82.7**	**369**	**10.33**			
Mean		69.6	143.5	6.7			
Min	17	46.9	74	3.64			
Max	147	77.7	187	7.73			

3.3. Effective population size and family structure in the salmon population

For the family structure estimation, the salmon samples were pooled for the River Hiitolanjoki and River Vuoksi samples. For sustainable use and maintenance of genetic diversity, minimum sufficient sizes for family number, genetically effective size and relatedness level are given (Table 9). Genetically effective population size should be at least 50 for each individual population; it often means at least 50 individuals and correspondingly over 100 parents. Mating among relatives in the population is harmful for the population and should be avoided. The increased relatedness level indicates already increased mating of relatives, which often happens in small populations. Relatedness varies from 0 to 1 and the relatedness of siblings is 0.5 (50%), half-sibs 0.25 (25%) and between first cousins 0.125 (12.5%). Relatedness less than 0.03 (3%) is regarded as still safe.

Table 9. Family structure of the salmon populations. Number of studied offspring (N), number of observed families (= breeding pairs), number of solved parents (Breeders) for the sexes separately and together (Breed), and maximum number of siblings observed in the families (Max Sib), and number of unrelated clusters (N Clusters) are shown. In addition, the estimate of genetically effective population size of the sampled population (Ne) with its 95% confidence limits (95%), and the mean pairwise relatedness of all individuals in the sample as a percentage (Relatedness %) are shown.

Population	N Offspring	N Family	N Breeders	N Breed	N Max Sib	N Cluster	Ne	Ne 95% CI	Relatedness %
Recommendation		*> 50*	*> 100*	*> 100*			*> 50*		*< 3,0*
Lakes									
L. Onega, RUS	19	**19**	10+12	**22**	1	5	**31**	17–62	**2.6**
L. Ladoga, RUS	94	73	31+33	**64**	4	5	**49**	34–74	5.3
L. Saimaa, FIN	60	57	22+27	**49**	2	4	**48**	32–75	5.8
Lake Ladoga rivers									
R. Hiitolanjoki, FIN/RUS	41	**32**	16+13	**29**	4	**1**	**31**	19–52	4.7
R. Vuoksi, FIN	61	57	22+24	**46**	2	7	81	56–117	2.9
R. Soskuanjoki, FIN	32	**30**	14+18	**34**	2	4	**45**	27–77	3.4
Gulf of Finland rivers									
R. Neva, RUS	70	60	31+35	**66**	4	8	89	63–126	3.2
R. Kymijoki, FIN	100	87	48+49	**97**	6	15	100	75–134	3.2
R. Vammeljoki, RUS	17	**9**	7+5	**12**	5	3	**8**	4–21	5.2
R. Luga, RUS	147	114	62+62	124	6	13	104	80–138	3.4
Gulf of Bothnia Rivers									
R. Tornionjoki, FIN	70	70	37+35	**72**	1	4	101	73–144	**2.7**
R. Simojoki, FIN	70	56	30+29	**59**	7	8	63	44–93	3.3
Mean	65.1	55.3	-	56.2	3.7	6.4	62.5	-	3.8
Min	17	9	7+5	12	1	1	8	-	2.6
Max	147	114	62+62	124	7	15	104	-	5.8

Generally, the effective population sizes of the lake populations were below the threshold level for a viable population (Ne < 50). The Ne was below the threshold also for the River Hiitolanjoki and Soskuanjoki populations. The Ne of the River Vuoksi was above the Ne threshold. The salmon populations of the rivers running into the Gulf of Finland and the Gulf of Bothnia had clearly higher estimated effective population sizes, except for River Vammeljoki, where the sample size was exceptionally low.

Relatedness in the salmon populations of the River Vuoksi was on the safe side, below the threshold of 3%. The number of unrelated clusters is relatively high (7), considering the available spawning areas. This hints at an unstable population where migrants or strayers also spawn, causing additional mixing. Excluding the small sample from River Vammeljoki, relatedness among the river populations was highest among the salmon from the River Hiitolanjoki (4.7%). There was also only a single unrelated cluster in the River Hiitolanjoki sample (Table 9), together with the high relatedness indicating at a small population with limited genetic variation. The River Soskuanjoki appears to be in a better state than the River Hiitolanjoki population, although Ne estimate of the River Soskuanjoki population remains below the threshold, and the relatedness is slightly above the recommended 3%.

For salmon lake populations the smallest number of sampled families was for the Lake Onega population (19) (Table 9). Its effective size was, however, clearly larger (Ne = 31). The relatedness among individuals was also below 3%, indicating that the true population size is large, the population is genetically heterogenous and general relatedness within the population is low and the population thus is at good genetic state. The small family number and low number of breeders are a result of small sampling size.

4. Brown trout

4.1. Genetic differentiation among populations

Genetic differentiation among populations was first analysed only for the new RiverGo samples. In the genetic distance tree, Finnish and Russian populations tended to group into different ends of the tree, except for the Finnish Hiitolanjoki-Lohijoki River, which grouped closer to the Russian samples from the Russian rivers Losevka, Volchja (Saijanjoki) and Semuzhja (Lohijoki) (Fig. 8).

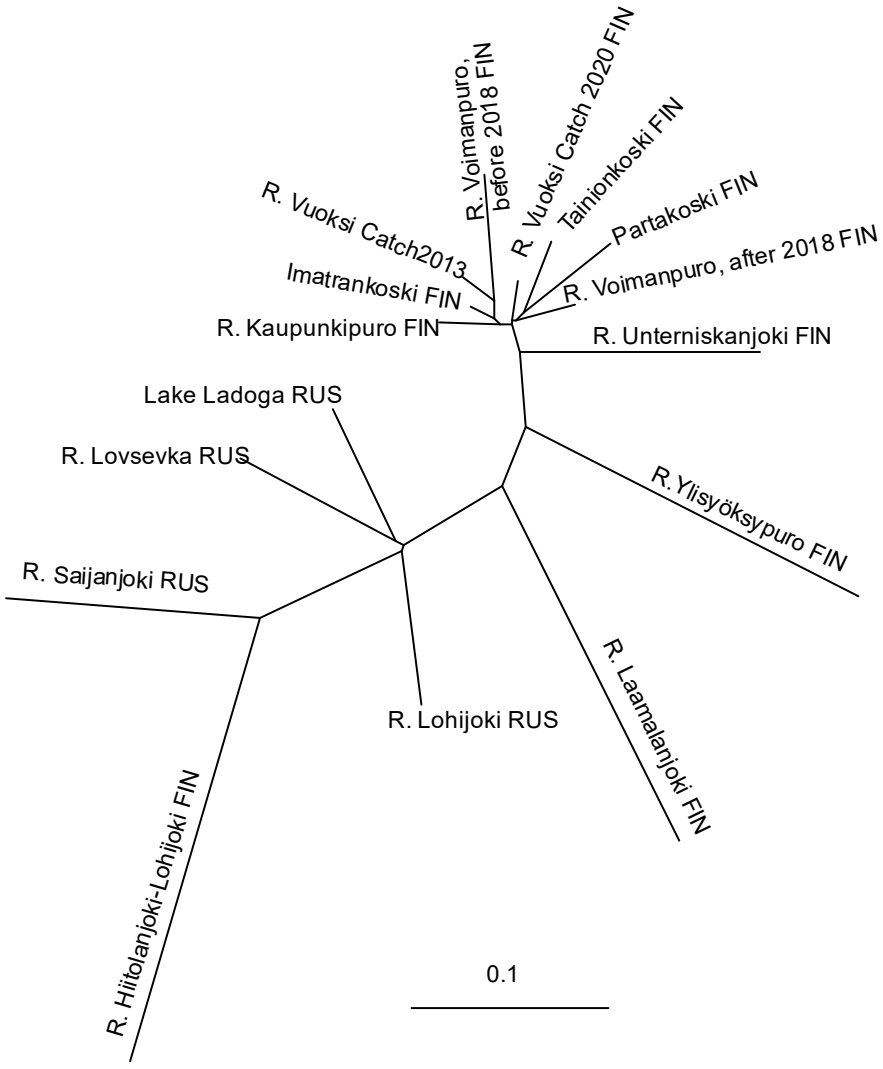

Figure 8. Unrooted genetic distance tree, showing the genetic distances among Finnish and Russian brown trout samples analysed in the RiverGo project.

All samples from the River Vuoksi and Imatra brooks grouped tightly together. The bootstrap values for the locations of the tree branches, shown in the most likely rooted genetic distance tree, were in general low, indicating the uncertainty of the tree structure (Fig. 9). The bootstrap values were over 50% only in few cases and some were only less than ten. This uncertainty is probably resulting from both small sample sizes and high similarity among samples. In those cases, several grouping options remain.

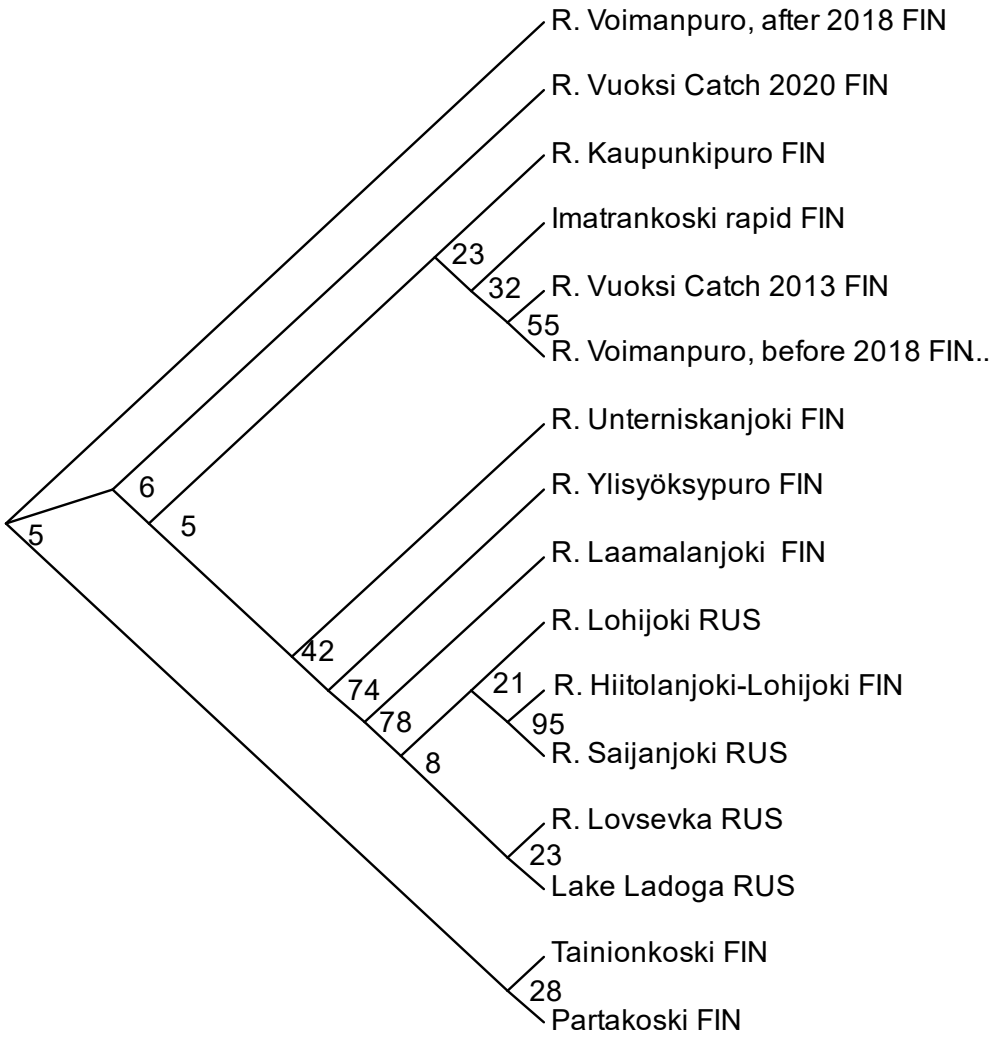

Figure 9. Rooted genetic distance tree showing the genetic distances among Finnish and Russian brown trout samples analysed in the RiverGo project. The numbers indicate the probability (percentage) of the branch location in the tree based on bootstrapping with 1 000 repeats.

When the previously analysed comparison samples (Koljonen et al. 2014) were included the main genetic structure of RiverGo brown trout samples, differentiation between the Finnish and the Russian samples remained the same and the populations formed logically three main groups (Fig. 10 & 11). All the Russian anadromous trout populations from rivers draining into the Gulf of Finland from the Southern Gulf of Finland (the River Luga), from the Bay of Vyborg (the River Mustajoki), and from the Karelian Isthmus, grouped together in one group (Anadromous group, Fig. 10).

The second group of its own was formed by populations from rivers eventually draining into the Lake Ladoga (Lake Ladoga group, Fig. 10). It included three Russian rivers (rivers Semuzhja (Lohijoki), Losevka and Volchja (Saijanjoki)), and the Finnish-Russian border river Hiitolanjoki as well as its tributary, the River Lohijoki in Finland. The long branch for this river indicates larger distance and isolation from the other populations.

The samples from the populations of the River Vuoksi on the Finnish side were mostly in the same third group. Some populations, however, remained outside of those groups: the small Russian River Tsysmu (Puhdaspuro) drains into the River Vuoksi; it did not belong to the anadromous or Lake Ladoga group but remained quite far from the Finnish River Vuoksi populations

also. The Finnish resident Luutajoki hatchery stock grouped close to the River Laamalanjoki population with a very high bootstrap value (99%) (Fig. 11), which might indicate some hatchery fish origin in that sample, causing its deviation from the other populations. Finnish lake-migratory Rautalamminreitti watershed, the hatchery stock, did not group to any of the other sampled populations. The Finnish Vuoksi watershed hatchery stock from the Kermankoski rapid, in contrast, grouped very closely to the Imatrankoski rapid sample and to the Imatrankoski catch sample from 2013.

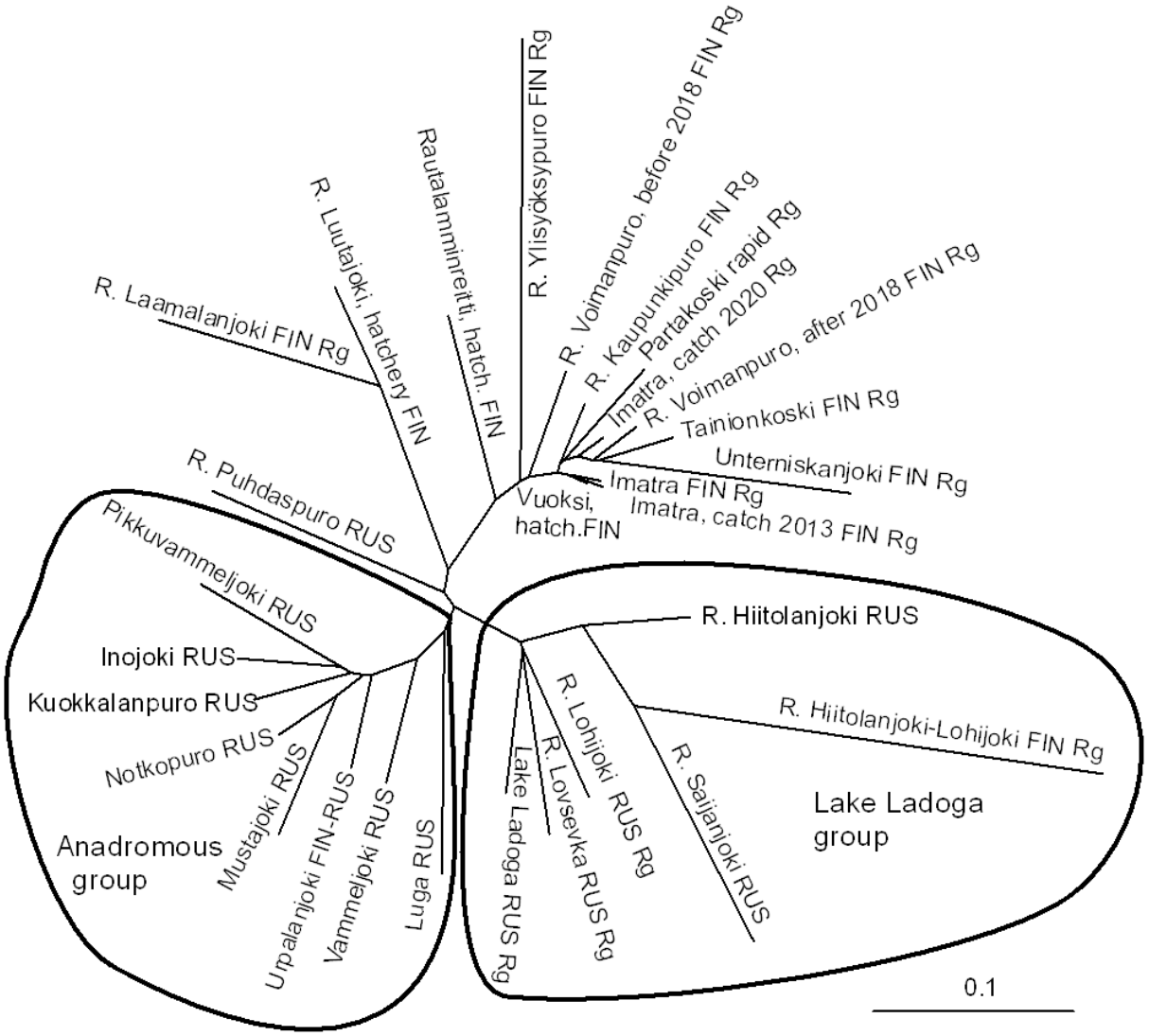

Figure 10. Unrooted genetic distance tree showing relative genetic distances among brown trout populations in the Finnish-Russian border area. RG is indicating RiverGo data.

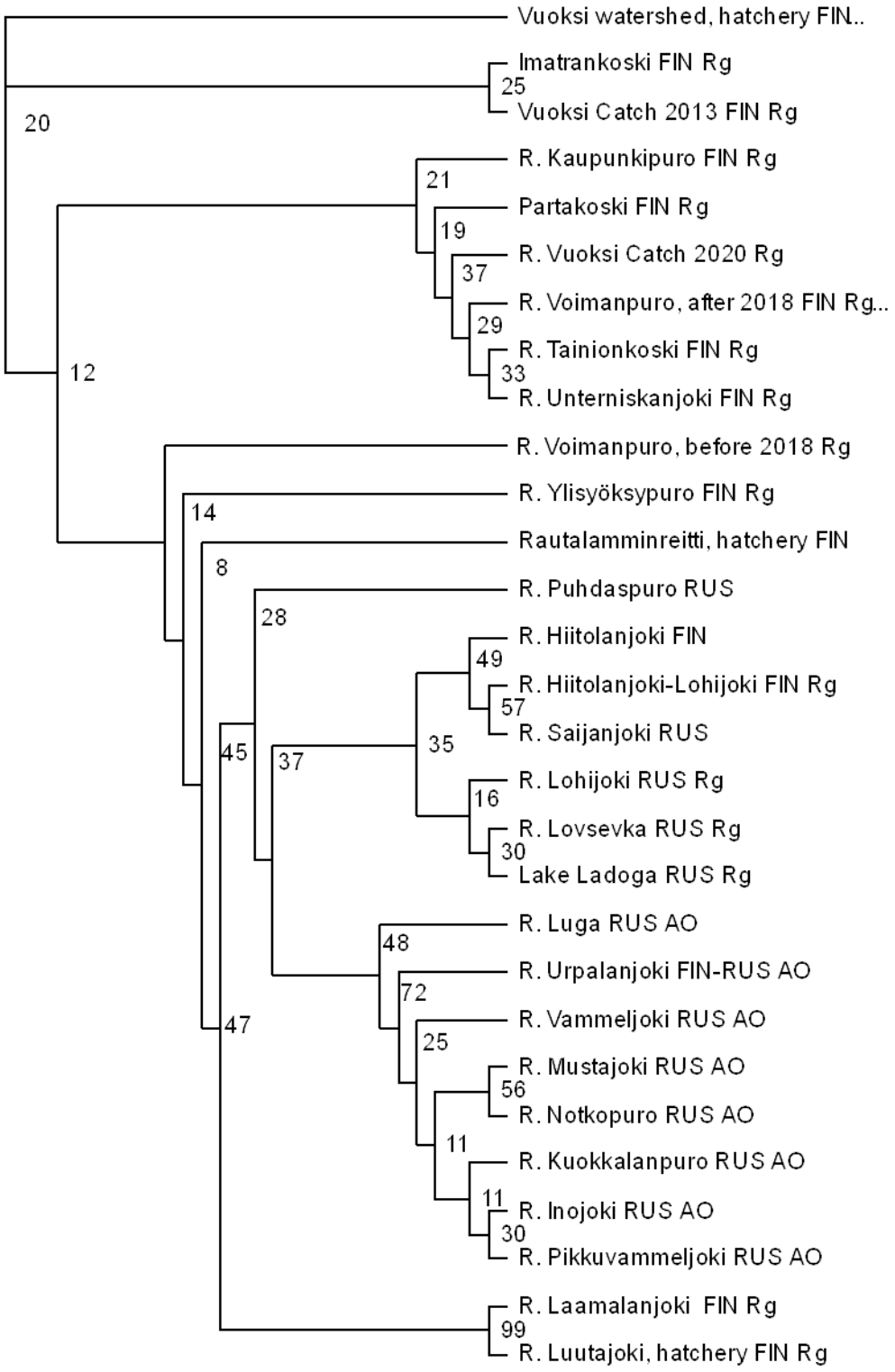

Figure 11. Genetic distances between brown trout samples, presented as a rooted genetic distance tree. AO is indicating anadromous and original population and Rg RiverGo data.

Brown trout populations differed in general systematically statistically significantly (Table 10). The major exception to this were the samples from the River Vuoksi main stream from Finland and the River Vuoksi tributaries Voimanpuro (after 2018) and Kaupunkipuro, as well as the Partakoski rapid, which were generally not different. They were also similar to the Vuoksi hatchery stock. Voimanpuro samples before and after 2018 differed from each other significantly (Table 10). The Voimanpuro sample before 2018 differed from all compared populations. In

contrast, the Voimanpuro sample after 2018 differed from the previous sample and did not differ from the Tainionkoski, Imatrankoski, the later Vuoksi catch sample from 2020, or from the Kaupunkipuro brook sample. This indicates that similarity among populations had increased among studied populations since 2018. However, the level of differentiation in all the above cases was generally low (F_{ST} < 0.08; Table 10). Noticeable also is that the Luutajoki hatchery stock differed from the Laamalanjoki sample, although they grouped closely together in the genetic tree (not shown in Table 10). This means that some differences between them still exist.

The genetic distance estimates between brown trout sample pairs ranged from the non-significant minimum of 0.004 between Voimanpuro after 2018 and Vuoksi catch 2020 to the maximum of 0.433 between rivers Hiitolanjoki-Lohijoki and Ylisyöksypuro (Table 10). In general, the smallest distances occurred among the samples from the Finnish Vuoksi river area. Hiitolanjoki-Lohijoki differed systematically most from all others, which indicates isolation and small population size, and probably genetic drift, which has caused the strong differentiation. This could be seen as a long branch in the genetic tree as well.

As to the hatchery stocks, the Rautalaminreitti watershed and Luutajoki differed from all compared stocks. Especially Vuoksi Imatrankoski and both Vuoksi catches 2013 and 2020 were similar to the Vuoksi Kermankoski hatchery stock (Table 10).

Table 10. Pairwise genetic differentiation among the brown trout populations measured as variance in allele frequency between samples (F_{ST}). The samples from the Russian side are indicated by RUS. F_{ST} values above 0.2 are highlighted in grey. F_{ST} estimates not significant at $p<0.05$ are shown in italics.

Brown trout population	Vuoksi, Tainionkoski	Vuoksi, Imatrankoski	Vuoksi, Catch 2013	Vuoksi, Catch 2020	Voimanp. bef. 2018	Voimanp. aft. 2018	Ylisyöksypuro	Kaupunkipuro	Partakoski rapid	Laamalanjoki	Unterniskanjoki	Semuzhja (Lohijoki) RUS	Losevka RUS	Volchja (Saijanjoki) RUS	Lake Ladoga RUS	Urpalanjoki	Mustajoki	Hiitolanjoki	Hiitolanjoki-Lohijoki	Puhdaspuro RUS	Notkopuro RUS	Inojoki RUS	Pikkuvammeljoki RUS	Vammeljoki RUS	Kuokkalanpuro RUS	Luga RUS	Rautalamminreitti, hatchery	Luutajoki, hatchery
Vuoksi, Imatrankoski	0.02																											
Vuoksi, Catch 2013	0.04	*0.00*																										
Vuoksi, Catch 2020	*0.02*	0.01	0.02																									
Voimanp. before 2018	0.08	0.05	0.04	0.05																								
Voimanp. after 2018	*0.01*	*0.01*	0.03	*0.00*	0.06																							
Ylisyöksypuro	0.25	0.19	0.19	0.21	0.26	0.22																						
Kaupunkipuro	0.02	0.01	0.01	0.01	0.04	*0.01*	0.21																					
Partakoski rapid	0.03	0.02	0.03	0.02	0.07	*0.02*	0.25	0.02																				
Laamalanjoki	0.15	0.15	0.16	0.15	0.22	0.16	0.34	0.15	0.17																			
Unterniskanjoki	0.06	0.07	0.09	0.06	0.12	0.04	0.25	0.06	0.09	0.20																		
Semuzhja (Lohijoki) RUS	0.10	0.07	0.08	0.08	0.14	0.08	0.25	0.08	0.09	0.17	0.13																	
Losevka RUS	0.11	0.09	0.09	0.10	0.14	0.09	0.29	0.09	0.10	0.21	0.15	0.06																
Volchja (Saijanjoki) RUS	*0.17*	0.12	0.12	0.14	0.20	0.14	0.35	0.15	0.17	*0.28*	0.21	0.13	0.09															
Lake Ladoga RUS	0.09	0.05	0.06	0.07	0.13	0.07	0.27	0.06	0.09	0.18	0.15	0.04	0.06	*0.11*														
Urpalanjoki	0.08	0.05	0.06	0.07	0.12	0.07	0.22	0.06	0.09	0.15	0.12	0.05	0.07	0.10	0.04													
Mustajoki	0.13	0.11	0.12	0.12	0.19	0.12	0.26	0.13	0.14	0.19	0.17	0.11	0.13	0.13	0.09	0.06												
Hiitolanjoki	0.09	0.04	0.04	0.06	0.08	0.07	0.21	0.05	0.08	0.17	0.13	0.07	0.08	0.12	0.03	0.07	0.13											
Hiitolanjoki-Lohijoki	0.36	0.28	0.28	0.32	0.37	0.35	0.43	0.33	0.37	0.40	0.41	0.31	0.32	0.32	0.30	0.29	0.32	0.20										
Puhdaspuro RUS	0.16	0.10	0.08	0.12	0.13	0.13	0.29	0.10	0.15	0.22	0.19	0.11	0.13	0.17	0.09	0.08	0.15	0.08	0.32									
Notkopuro RUS	0.11	0.07	0.08	0.09	0.16	0.09	0.23	0.09	0.11	0.16	0.15	0.08	0.10	0.11	0.05	0.04	0.05	0.08	0.28	0.10								
Inojoki RUS	0.10	0.06	0.07	0.08	0.15	0.09	0.24	0.08	0.10	0.18	0.15	0.08	0.11	0.12	0.06	0.04	0.07	0.07	0.30	0.10	0.02							
Pikkuvammeljoki RUS	0.16	0.12	0.12	0.14	0.19	0.14	0.27	0.14	0.16	0.19	0.19	0.11	0.13	0.16	0.10	0.07	0.10	0.12	0.35	0.13	0.06	0.06						
Vammeljoki RUS	0.11	0.08	0.08	0.09	0.16	0.10	0.25	0.10	0.11	0.16	0.15	0.07	0.10	0.11	0.06	0.03	0.05	0.10	0.31	0.11	0.03	0.03	0.06					
Kuokkalanpuro RUS	0.10	0.06	0.08	0.09	0.16	0.09	0.24	0.08	0.10	0.16	0.15	0.07	0.10	0.14	0.06	0.05	0.09	0.07	0.33	0.11	0.02	0.02	0.07	0.04				
Luga RUS	0.12	0.09	0.11	0.11	0.16	0.11	0.29	0.10	0.13	0.17	0.16	0.12	0.11	0.16	0.10	0.09	0.14	0.10	0.32	0.12	0.08	0.08	0.13	0.09	0.07			
Rautalamminreitti, hatchery	0.10	0.07	0.07	0.07	0.06	0.08	0.25	0.07	0.11	0.19	0.13	0.13	0.15	0.22	0.11	0.09	0.17	0.09	0.35	0.12	0.14	0.14	0.15	0.14	0.15	0.15		
Luutajoki, hatchery	0.14	0.12	0.11	0.12	0.18	0.14	0.32	0.12	0.14	0.16	0.23	0.12	0.14	0.18	0.09	0.10	0.15	0.12	0.32	0.14	0.12	0.12	0.16	0.10	0.14	0.17	0.17	
Vuoksi, hatchery	0.03	0.00	*0.00*	0.01	0.04	0.02	0.18	0.01	0.03	0.14	0.07	0.07	0.09	0.12	0.06	0.06	0.12	0.05	0.25	0.10	0.08	0.08	0.13	0.08	0.08	0.10	0.07	0.11

For the temporal samples, some differences could be seen. The later Vuoksi catch sample from 2020 resembled somewhat more the Partakoski sample (D = 0.017) than the older sample from 2013 (D = 0.034). When compared to the sample before 2018, the Voimanpuro sample after 2018 was more similar to Tainionkoski, Imatrankoski, both Vuoksi catch samples, Kaupunkipuro, Partakoski and Unterniska samples. The distances of the later Voimanpuro sample were also slightly smaller on average to the Lake Ladoga, the River Losevka and the River Semuzhja (Lohijoki) with mean distance of 0.083 compared to the mean distance of 0.135 for the earlier sample (Table 10). Voimanpuro trout was slightly more unique before 2018, while after 2018, it resembled more the Vuoksi trout in general

4.2. The amount of genetic diversity in the brown trout populations

The mean diversity of brown trout samples varied from the lowest 38.9% of the Hiitolanjoki-Lohijoki to the highest 73.4% of the anadromous River Urpalanjoki trout (Table 11). The diversity was quite good in the River Vuoksi samples. Some increase in the mean diversity had occurred in the Voimanpuro from 2018 to 2020. Among the hatchery stocks, the Luutajoki hatchery stock had the lowest diversity (58.2%).

The estimates of allelic richness followed the same pattern as the diversity levels. Small allelic richness, below the mean of 5.3 alleles/locus, occurred in the Imatra brook populations (Voimanpuro before 2018 and Ylisyöksypuro) and the other Finnish trout populations, with the lowest mean as a population group (4.9 alleles). Allele richness was higher for the Russian comparison populations from the Lake Ladoga group, with mean of 5.7 alleles/locus. For the anadromous trout population group, there was large variability between populations from the 5.0 alleles of the River Mustajoki trout to the overall maximum of the River Urpalanjoki.

The F_{IS} estimates measuring deviations from the random mating deviated highly significantly in few cases. Samples from the Ylisyöksypuro and Laamalanjoki indicated mixing of populations as F_{IS} was lower than expected. The higher than expected F_{IS} in the River Hiitolanjoki trout population indicated inbreeding.

Table 11. Genetic diversity of brown trout populations. Number of samples (N), percentage of mean diversity over loci (Div %), number of alleles (N All) and mean allelic richness per locus for 14 individuals (All Rich) are shown.

		Mean	Div.	N	14/Ind All.	All.		P	P
Population	N	N/L	%	All.	Rich/16L	Rich./L	F_{IS}	F_{IS} <	F_{IS} >
River Vuoksi FIN									
R. Vuoksi, Tainionkoski	22	22	68.3	96	86.6	5.4	-0.027	0.8278	0.2101
R. Vuoksi, Imatrankoski	62	62	71.9	133	100.0	6.3	0.023	0.0941	0.9192
R. Vuoksi, catch 2013	45	41	71.2	128	98.8	6.2	-0.021	0.8469	0.1784
R. Vuoksi, catch 2020	45	45	69.8	118	96.8	6.1	-0.023	0.8876	0.1325
Total/mean	**174**		**70.3**	**118.8**	**95.6**	**6.0**			
Imatra brooks FIN									
Voimanpuro 1, before 2018	31	29	63.4	102	82.6	5.2	0.078	0.0043*	0.9970

Voimanpuro 2, after 2018	21	21	71.4	105	96.0	6.0	-0.029	0.8546	0.1817
Ylisyöksypuro	30	30	45.6	53	44.6	2.8	-0.156	1.0000	0.0000***
Kaupunkipuro	30	30	72.7	111	94.3	5.9	0.008	0.3880	0.6559
Total/mean	**112**		**63.3**	**92.8**	**79.4**	**5.0**			
Others FIN									
Partakoski rapid	20	20	66.5	92	85.0	5.3	0.018	0.3139	0.7363
R. Hiitolanjoki-Lohijoki	30	30	38.9	40	38.7	2.4	-0.065	0.9442	0.0715
R. Laamalanjoki	25	25	53.2	46	45.3	2.8	-0.160	1.0000	0.0001***
R. Unterniskanjoki	21	21	63.5	65	63.5	4.0	0.015	0.3590	0.6885
Total/mean	**96**		**63.6**	**92.9**	**79.1**	**4.9**			
Others RUS									
R. Semuzhja (Lohijoki)	30	30	67.0	98	87.0	5.4	0.009	0.3926	0.6526
R. Losevka	17	17	66.3	97	91.4	5.7	-0.009	0.6340	0.4303
Lake Ladoga	24	14	69.1	94	94.0	5.9	0.044	0.1329	0.9013
Total/mean	**71**		**67.5**	**96.3**	**90.8**	**5.7**			
Comparison samples									
R. Urpalanjoki FIN/RUS	40	39.8	73.4	134	106.0	6.6	0.001	0.4856	0.5181
R. Mustajoki FIN/RUS	135	134.3	63.5	114	79.2	5.0	-0.016	0.8929	0.1074
R. Hiitolanjoki FIN/RUS	60	59.7	66.8	128	88.7	5.5	0.097	0.0000***	1.0000
R. Puhdaspuro RUS	23	23	63.6	95	84.7	5.3	0.091	0.0025*	0.9983
R. Notkopuro RUS	51	50.5	66.5	121	95.4	6.0	0.016	0.2043	0.7960
R. Inojoki RUS	25	24.9	68.0	109	97.4	6.1	-0.018	0.7631	0.2404
R. Pikkuvammeljoki RUS	50	49.2	60.3	109	84.6	5.3	0.038	0.0365*	0.9636
R. Vammeljoki RUS	39	38.9	65.8	116	92.9	5.8	0.000	0.4978	0.5073
R. Kuokkalanpuro RUS	23	22.8	66.8	106	94.0	5.9	0.036	0.1207	0.8808
R. Luga RUS	64	58.8	64.8	112	84.1	5.3	0.051	0.0048*	0.9952
Total/mean	**510**		**65.9**	**114.4**	**90.7**	**5.7**			
Hatchery FIN									
Rautalamminr., *hatch*.	50	50	64.4	138	98.6	6.2	0.030	0.0677	0.9445
R. Luutajoki, *hatch*.	40	40	58.2	81	69.2	4.3	0.017	0.2670	0.7663
Kermankoski (Vuoksi, *hatch*.)	220	220	71.0	158	97.5	6.1	0.009	0.1686	0.8421
Total/mean	**350**		**64.5**	**125.7**	**88.4**	**5.5**			
Total/mean overall	**1273**	**1249**	**73.8**	**256**	**120.3**	**7.5**			
Min	17	14	38.9	40	38.7	2.4			
Max	220	220	73.4	158	106.0	6.6			
Mean	45.5	44.6	64.7	103.5	84.9	5.3			

4.3. Effective population size and relatedness level in the brown trout population samples

When analysing family structure in the brown trout samples, some individual fish with identical genotypes were found. Those are very unlikely to occur in nature so those may be sampling mistakes, in which the same individual was sampled twice, or contamination of samples so that same DNA was in two tubes. Identical genotypes were found for Imatrankoski sample individuals 6 and 8, Ylisyöksypuro individuals 1 and 4, and for four pairs of fishes in Vuoksi catch sample 2020: individuals 202 and 205, 207 and 210, 208 and 213, 223 and 233. Here, the identical samples are treated as siblings, and this may cause slightly increased uncertainty in the estimates from those populations.

In general, population sample sizes were quite small, and most were under 50 individuals (Table 12). This affected the effective population size estimates (Ne). Because of the small sample sizes, Ne could not easily reach the minimum level of 50 recommended for a minimum size of a viable population. Given this, it is essential to know how well the sample represents the assumed spawning population and its size. The Ne/N ratio gives some idea of the relatedness state in the population. If it remains over 0.5, relatedness is not very high, and the spawning population may well be larger than the Ne estimate. In some cases, the Ne/N ratio, however, was so small that increased relatedness was evident. Ne/N ratio was below 0.5 for river populations from Ylisyöksypuro, Hiitolanjoki-Lohijoki, Unterniskanjoki, Pikkuvammeljoki, and even from the River Luga.

The number of spawning pairs is also recommended to be at least 50 for each viable population, but it often remained below this in the brown trout samples because of small sample sizes. The number of sampled families varied also quite clearly among sampled population groups. It was small for the Imatra brooks (mean 16.5) and the other Finnish populations (mean 14.3) (Table 12). The maximum number of sampled siblings describes how random the sample is in relation to the spawning population. In a small population, increased sampling only increases the number of siblings in the samples and as there are no unrelated individuals left to sample. In a large unrelated population, no siblings are found in the samples. For the Ylisyöksypuro brook, the number of siblings was as high as 24, representing only six families with effective population size of the whole sample of only three unrelated individuals.

The percentage of mean relatedness in the sample is an overall value, which is recommended to remain below 3% for unrelated populations. The percentage of relatedness for siblings is 50%, for half sibs 25%, and for cousins 12.5%.

Somewhat increased relatedness could be observed in almost all populations. In some Finnish populations the value even exceeded the cousin level: Ylisyöksypuro (17%), Hiitolanjoki-Lohijoki (12.8%), Unterniskanjoki (12.6%). The relatedness value was also high for Laamalanjoki (11.6%) (Table 12). This decreases the genetic value of the populations. The only population for which the mean relatedness remains below 3% was the Russian River Losevka, despite the sample size of only 17 individuals.

For the two temporal sample pairs some differences occurred. The Vuoksi catch (2013 and 2020) samples were quite similar in their relatedness level. For the Voimanpuro sample pair, the older sample was on average more related (8.1%) than the later sample (3.5%) and it included also more siblings (5) than the later sample (1). The effective population sizes were roughly similar.

Table 12. Family structure of the brown trout populations and means for sampling groups. Number of individuals (N), the estimate of genetically effective population size (Ne) with its 95% confidence interval (95% CI), the effective size versus the sample size ratio (Ne/N), number of observed families (= breeding pairs), and maximum number of siblings in the observed families (Max Sib) are shown. In addition, the mean pairwise relatedness of all individuals in the sample as a percentage (Relatedness %) is shown.

Population	N sample	Ne	95% CI	Ne/N	N Family	Max sib.	Related. %
Recommendation		*> 50*		*> 0,5*	*> 50*		*< 3,0*
River Vuoksi FIN							
R. Vuoksi, Tainionkoski	22	17	9–38	0.77	14	4	5.32
R. Vuoksi, Imatrankoski	62	69	48–011	1.11	58	3	3.31
R. Vuoksi, catch 2013	41	37	22–62	0.90	38	2	3.46
R. Vuoksi, catch 2020	45	44	27–68	0.98	37	2	3.94
Total/mean	**170**	**41.8**		**0.94**	**36.8**	**2.8**	**4.01**
Imatra brooks FIN							
Voimanpuro 1, before 2018	29	28	16–50	0.97	16	5	8.1
Voimanpuro 2, after 2018	21	26	16–54	1.24	21	1	3.5
Ylisyöksypuro	30	3	3–∞	0.10	6	24	17.0
Kaupunkipuro	30	24	14–46	0.80	23	2	4.0
Total/mean	**110**	**32.2**		**0.78**	**16.5**	**8**	**8.1**
Others FIN							
Partakoski rapid	20	12	6–30	0.60	15	4	4.7
R. Hiitolanjoki-Lohijoki	30	10	5–24	0.33	20	5	12.8
R. Laamalanjoki	25	13	7–30	0.52	18	3	11.6
R. Unterniskanjoki	21	5	2–20	0.24	4	10	12.6
Total/mean	**96**	**10.0**		**0.42**	**14.3**	**6**	**10.42**
Others RUS							
R. Semuzhja (Lohijoki)	30	30	18–53	1.00	29	2	3.83
R. Losevka	17	15	8–35	0.88	15	2	2.64
L. Ladoga	14	14	7–34	1.00	13	2	4.18
Total/mean	**61**	**19.7**		**0.96**	**19.0**	**2**	**3.55**
Comparison samples							
R. Urpalanjoki FIN/RUS	40	32	20–54	0.80	33	4	4.18
R. Mustajoki FIN/RUS	137	69	50–97	0.50	103	5	5.74
R. Hiitolanjoki FIN/RUS	60	33	20–53	0.55	50	5	4.77
R. Puhdaspuro RUS	23	24	13–49	1.04	21	2	3.39
R. Notkopuro RUS	51	55	36–83	1.08	47	2	3.63

R. Inojoki RUS	25	16	9–35	0.64	17	6	4.42
R. Pikkuvammeljoki RUS	50	19	11–39	0.38	35	8	5.35
R. Vammeljoki RUS	39	39	25–65	1.00	33	2	4.51
R. Kuokkalanpuro RUS	23	24	13–30	1.04	21	2	3.17
R. Luga RUS	64	19	11–39	0.30	37	8	6.61
Total/mean	**512**	**33.0**		**0.73**	**39.7**	**4.4**	**4.58**
Hatchery FIN							
Rautalamminr., hatch.	50	47	31–72	0.94	50	1	3.22
R. Luutajoki, hatch.	40	24	14–44	0.60	36	2	6.01
Kermankoski (Vuoksi hatch.)	220	188	158–235	0.85	199	4	4.05
Total/mean	**310**	**86.3**		**0.80**	**95.0**	**2**	**4.43**
Total overall	**1259**						

5. European grayling

5.1. Genetic differentiation among grayling populations

Except for the phylogenetic analysis of genetic relationships among the grayling populations, the samples from the main streams and the tributaries, River Volchja (Saijanjoki) and River Goryunets, and River Suokumaanjoki and River Kupinjoki, were combined (in later analyses: the River Volchja (Saijanjoki) and the River Suokumaanjoki). The analysis of genetic differentiation among grayling populations revealed four clusters when all sampling sites were kept separate (Fig. 12). Samples from the Russian rivers Volchja (Saijanjoki) and Vjun (Viisjoki) formed a cluster with the samples from the Russian part of the River Vuoksi main stream, while the samples from the Finnish side of the River Vuoksi formed a cluster of their own (Fig. 12). The third clear cluster was formed by the samples from the Lake Saimaa and the wild Lake Puruvesi samples (Fig. 12). The hatchery samples from Lake Puruvesi and the Rautalamminreitti watershed also clustered together, but their position in relation to other samples could not be determined with any confidence. The same applies to the samples from the rivers Lieksanjoki (FIN), Voronka (RUS), and the samples from the Gulf of Bothnia (FIN) as well as to the samples from the Finnish River Suokumaanjoki (Fig. 12). The position of all these samples varied in replicate phylogenetic analyses (bootstrap value <50, i.e., in less than half of the 1 000 replicate phylogenies their position was the same as in the tree displayed in Fig. 12).

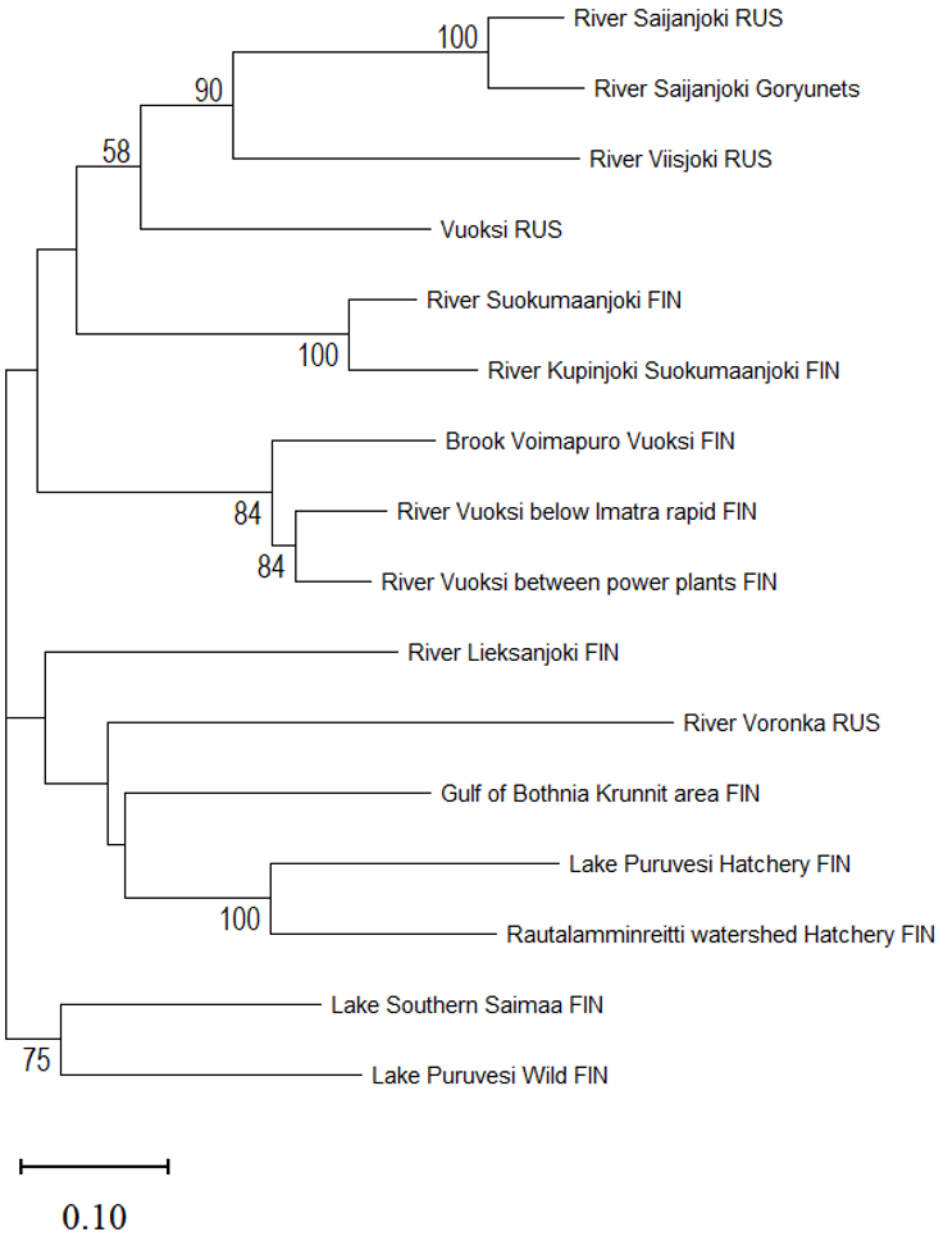

Figure 12. A rooted dendrogram of the genetic distances (DA; Nei et al. 1983) among grayling samples. Only bootstrap values >50 are shown.

In general, differences in allele frequencies among the grayling samples were relatively high (mean F_{ST} among all populations = 0.32). Especially the samples from Russia differed noticeably from the Finnish samples as well as among themselves (Table 13). The grayling population from the Russian River Voronka deviated systematically most from all the other analysed populations with F_{ST} -values over 0.5, although the statistical significance of the difference could not be determined due to the low sample size (N = 6). It is also noticeable that the wild and hatchery samples from the Lake Puruvesi differed clearly and statistically significantly from each other (F_{ST} = 0.2).

The smallest genetic distances were between the samples from the Finnish side of the River Vuoksi (F_{ST} < 0.01), and between the samples from the River Volchja (Saijanjoki)) and its Goryunets tributary (F_{ST} = 0.06; Table 13). The hatchery populations of the Lake Puruvesi and

Rautalamminreitti watershed population were also relatively similar (F_{ST} = 0.08). No genetic difference was found between the Finnish River Vuoksi samples below the Imatra rapid and between the power plants, nor were pairwise differences found between the Finnish River Vuoksi samples and the Voimanpuro brook samples (Table 13). The samples from the River Vuoksi between the power plants and the Voimanpuro samples also did not differ from the Russian River Vuoksi samples (Table 13).

Table 13. Differences in allele frequencies among the grayling samples (F_{ST}). F_{ST} values not statistically significant (after Bonferroni correction for multiple tests) are shown in italics (*statistical significance could not be determined for the River Voronka due to the low sample size).

	R. Vuoksi, below Imatra rapids, FIN	R. Vuoksi, between power plants, FIN	Voimanpuro tributary, FIN	R. Suokumaanjoki, FIN	R- Kupinjoki, FIN	R- Vuoksi, RUS	R- Volchja (Saijanjoki), RUS	R. Vjun (Viisjoki), RUS	R- Voronka*, RUS	L. South-Saimaa, FIN	R. Lieksanjoki, FIN	L. Puruvesi, FIN	L. Puruvesi, FIN *hatchery*	Rautalamminreitti, FIN *hatchery*	Gulf of Bothnia, Krunnit area, FIN
R. Vuoksi, between power plants, FIN	*0.02*														
Voimanpuro, FIN	*0.02*	*0.01*													
R. Suokumaanjoki, FIN	0.26	0.22	0.21												
R. Kupinjoki, FIN	0.33	0.31	0.28	0.03											
R. Vuoksi, RUS	0.19	*0.19*	*0.15*	0.14	0.19										
R. Volchja (Saijanjoki,) RUS	0.48	0.47	0.47	0.38	0.42	0.30									
R. Vjun (Viisjoki), RUS	0.47	0.45	0.45	0.36	0.41	0.28	0.38								
R. Voronka*, RUS	0.56	0.53	0.55	0.45	0.50	0.44	0.63	0.51							
L. South-Saimaa, FIN	0.23	0.23	0.17	0.25	0.29	0.14	0.36	0.37	0.44						
R. Lieksanjoki, FIN	0.35	0.33	0.29	0.28	0.34	0.28	0.48	0.43	0.50	0.22					
L. Puruvesi, FIN	0.27	0.26	0.22	0.21	0.27	0.16	0.43	0.40	0.46	0.10	0.20				
L. Puruvesi, FIN *hatchery*	0.40	0.34	0.33	0.29	0.35	0.28	0.47	0.36	0.38	0.29	0.32	0.29			
Rautalamminreitti, FIN *hatchery*	0.34	0.29	0.25	0.25	0.30	0.21	0.40	0.34	0.39	0.20	0.25	0.20	0.08		
Gulf of Bothnia, Krunnit area, FIN	0.32	0.28	0.26	0.27	0.32	0.22	0.40	0.34	0.34	0.19	0.20	0.22	0.19	0.14	
Goryunets tributary, RUS	0.45	0.43	0.43	0.37	0.41	*0.26*	*0.06*	0.39	0.66	0.32	0.46	0.40	0.45	0.38	0.36

5.2. Genetic diversity within the grayling populations

The level of genetic diversity in the grayling samples was generally low. The mean diversity was below 50% (48.9%) and the allelic richness very low (mean = 2.7; Table 14). This can be partly explained by the small sample sizes, but the diversity levels were relatively low also for the larger samples. In the river Vuoksi samples from both Finland and Russia, there were signs of inbreeding (F_{IS} > 0; Table 14). The rate of inbreeding exceeded the expected level also in the River Suokumaanjoki sample (Table 14).

Table 14. Genetic diversity of grayling populations. Mean number of samples (N/L), percentage of mean diversity over loci (Div %), number of alleles (N All) and mean allelic richness per locus for 6 individuals (All Rich) are shown. In addition, F_{IS} and the significance of deviations are shown (adjusted for multiple tests).

Population	N/L	Div %	N All	All. Rich	F_{IS}	F_{IS} < Exp.	F_{IS} > Exp.
R. Vuoksi, FIN	52.5	54.1	90	2.5	0.173*	1	0
R. Suokumaanjoki, FIN	52.3	50.3	79	2.82	0.110*	1	0
R. Vuoksi, RUS	8.0	50.9	78	3.03	0.199*	1	0
R. Volchja (Saijanjoki), RUS	33.0	34.3	50	1.89	0.141	0.826	0
R. Vjun (Viisjoki), RUS	26	42.0	65	2.37	0.031	0.539	0.204
R. Voronka, RUS	5.8	28.0	37	1.58	-0.018	0.687	0.618
L. South-Saimaa, FIN	30.0	63.2	110	3.29	0.010	0.781	0.354
R. Lieksanjoki, FIN	26.0	45.5	76	2.51	0.028	0.123	0.224
L. Puruvesi, FIN	12.7	50.3	85	2.92	-0.052	0.007	0.892
L. Puruvesi, FIN *hatchery*	18.8	47.4	81	3.20	-0.093	0.748	0.996
Rautalamminreitti, FIN *hatchery*	20.0	55.6	98	2.84	-0.019	0.753	0.296
Gulf of Bothnia, Krunnit area, FIN	28.7	62.5	114	3.22	0.018	1	0.248
Mean	28	48.9	80.3	2.69	0		
Total	**196.8**	**65.2**	**198**	**4.93**	**0.052**		
Min	5.8	28.0	37	1.58	-0.052		
Max	52.5	63.2	114	3.29	0.199		

5.3. Effective population size and relatedness in the grayling populations

Relatedness in all the grayling populations was high, although the relatedness estimates should be treated with caution due to the low informativeness of the loci for relatedness (Table 15). However, the effective population sizes (Ne) and the number of breeders are also low for all the sampled populations – none reach the minimum rule-of-thumb recommendation for a population viable in short term of Ne > 50 (Table 15). Some of the apparent lack of genetic diversity can be explained by the low sample sizes, and bias in sampling, especially in the populations, where the samples included full sibling families with more than two members (Table 15).

Table 15. Family structure of the grayling populations and means for sampling groups. Number of individuals (N sample), the estimate of genetically effective population size (Ne) with its 95% confidence interval (95% CI), number of observed full-sib families (= breeding pairs; N family), the maximum number of siblings in the observed families (Max Sib), and the mean pairwise relatedness (Related. r_{wang}) of all individuals in each sample.

Population	N sample	Ne	95% CI	N family	Max Sib.	Related.* r_{wang}
Recommendation		*> 50*		*> 50*		*< 0.03*
R. Vuoksi FIN	53	26	16–46	29	6	0.30
R. Suokumaanjoki FIN	53	43	4–43	44	2	0.17
R. Vuoksi, RUS	8	11	14–46	5	3	0.48
R. Volchja (Saijanjoki), RUS	33	20	4–∞	20	4	
R. Vjun (Viisjoki), RUS	26	25	24–68	22	2	0.23
R. Voronka, RUS	6	10	15–52	4	3	0.39
L. South-Saimaa FIN	30	38	8–45	24	2	0.26
R. Lieksanjoki FIN	26	28	14–54	23	2	0.38
L. Puruvesi, FIN	13	17	11–43	8	4	0.24
L. Puruvesi, FIN *hatchery*	19	26	14–45	16	2	0.24
Rautalamminreitti, FIN *hatchery*	20	21	16–46	13	4	0.30
Gulf of Bothnia, Krunnit area, FIN	29	24	29–71	23	5	0.28

*The analysis of the informativeness of the genetic data for the inference of relatedness was especially low for the grayling data set (power for distinguishing full siblings from unrelated pairs, mean for all loci = 0.19).

6. Conclusions

The salmon on the Russian side of the River Vuoksi appear to form a genetically relatively variable and viable population which is differentiated from the other studied salmon populations in the rivers draining to the Lake Ladoga. The result is in accordance with Ozerov et al.'s (2010) finding of a high level of genetic structuring in the Lake Ladoga and the Lake Onega lake systems. Although fishing of salmon is prohibited on the Russian side of the River Vuoksi watershed, the Vuoksi salmon population, however, is threatened by poaching in the river (Menna et al. 2022). Therefore, management actions to prevent poaching are needed.

The genetic diversity of brown trout samples from the River Vuoksi area (main stream and the Imatra brooks) was at a relatively good level and the populations were not differentiated from each other, nor from the Finnish Vuoksi watershed hatchery stock. The genetic distances between the populations were small. This indicates that stocking of hatchery fish has had strong influence on the genetic structure of the River Vuoksi trout populations. This is expectable after the relatively long period of brown trout stocking to the River Vuoksi and the Lake Saimaa, and on the other hand, the poor state of the natural trout populations especially due to water construction and the poor quality of the water in the past (Menna et al. 2022). The Voimanpuro population sample taken before the repair of the Fortum power plant dam implies that the population differed from the other populations nearby. However, the original population was destroyed by the repair (Menna et al. 2022) and seems to have been replaced by a more hatchery-like population. The small Ylisyöksypuro differs from the other trout populations in the River Vuoksi area, but a drawback, as comes to its genetic value, is the high relatedness within the population. However, care should still be taken to ensure its preservation and natural reproduction. On the Russian side, the Lake Ladoga group consisting of brown trout from the River Vuoksi tributaries, the Hiitolanjoki-Lohijoki and the Lake Ladoga was distinct but still the populations showed clear genetic differentiation and should thus be treated as their own entities. The same applies to the River Tsysmu (Puhdaspuro) trout that was genetically differentiated from all other trout populations on the Russian side.

Grayling in the River Vuoksi watershed showed differentiation from the other analysed grayling populations. The River Vuoksi watershed group was further divided into two genetically differentiated clusters, one consisting of the sampled rivers on the Finnish side and the other on the Russian side. Interestingly, the between population genetic differences within the clusters, measured by allele frequencies, were still high. The high genetic differentiation among the sampled grayling populations may be explained by genetic isolation of small populations, where the number of close relatives is high, and the resulting inbreeding is followed by genetic drift. The differentiation should, however, be taken into account in the management of the populations. The similarity (although not always statistically significant) in allele frequencies among the samples from the River Vuoksi main stream on the Russian side and the main stream as well as the tributaries on the Finnish side is also notable and may reflect gene flow among the populations.

In conclusion, the studied salmonid species in the River Vuoksi river system differed in their genetic characteristics depending on the species and the geographic area. On the Russian side, the salmon populations appeared to be genetically diverse and differentiated from each other. This was the case also with European grayling on both sides of the border. In contrast, brown trout populations on the Finnish side were genetically more similar and closely resembled the hatchery stock. On the Russian side, the brown trout were more differentiated from each other than on the Finnish side and formed more distinct genetic entities. Among the grayling populations, genetic diversity was low and the effective population sizes small. Despite the

differences in genetic diversity and structure among the different salmonids of the Vuoksi watershed, they are all close to or below a critical level for populations viable in short term. As such, all possible measures to preserve and enhance genetic diversity in all the salmonid stocks are warranted. In general, safeguarding and supporting natural production should be the principle means to preserve the genetic diversity of the salmonid populations on both sides of the border. A sufficiently large, self-sustaining spawning population should be the target for any conservation and management measure as well as when exploiting the populations. It must be noted though that these results are based on neutral genetic variation. Conservation and management decisions should be done also considering variation in adaptive traits, which in the end dictates how well populations adapt to new and changing environments.

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Appendices

Appendix 1.

R1: Junge, C., Primmer, C.R., Vøllestad, L.A. & Leder, E.H. 2010. Isolation and characterization of 19 new microsatellites for European grayling, *Thymallus thymallus* (Linnaeus, 1758), and their cross-amplification in four other salmonid species. Conservation Genetic Resources 2: 219–223.

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