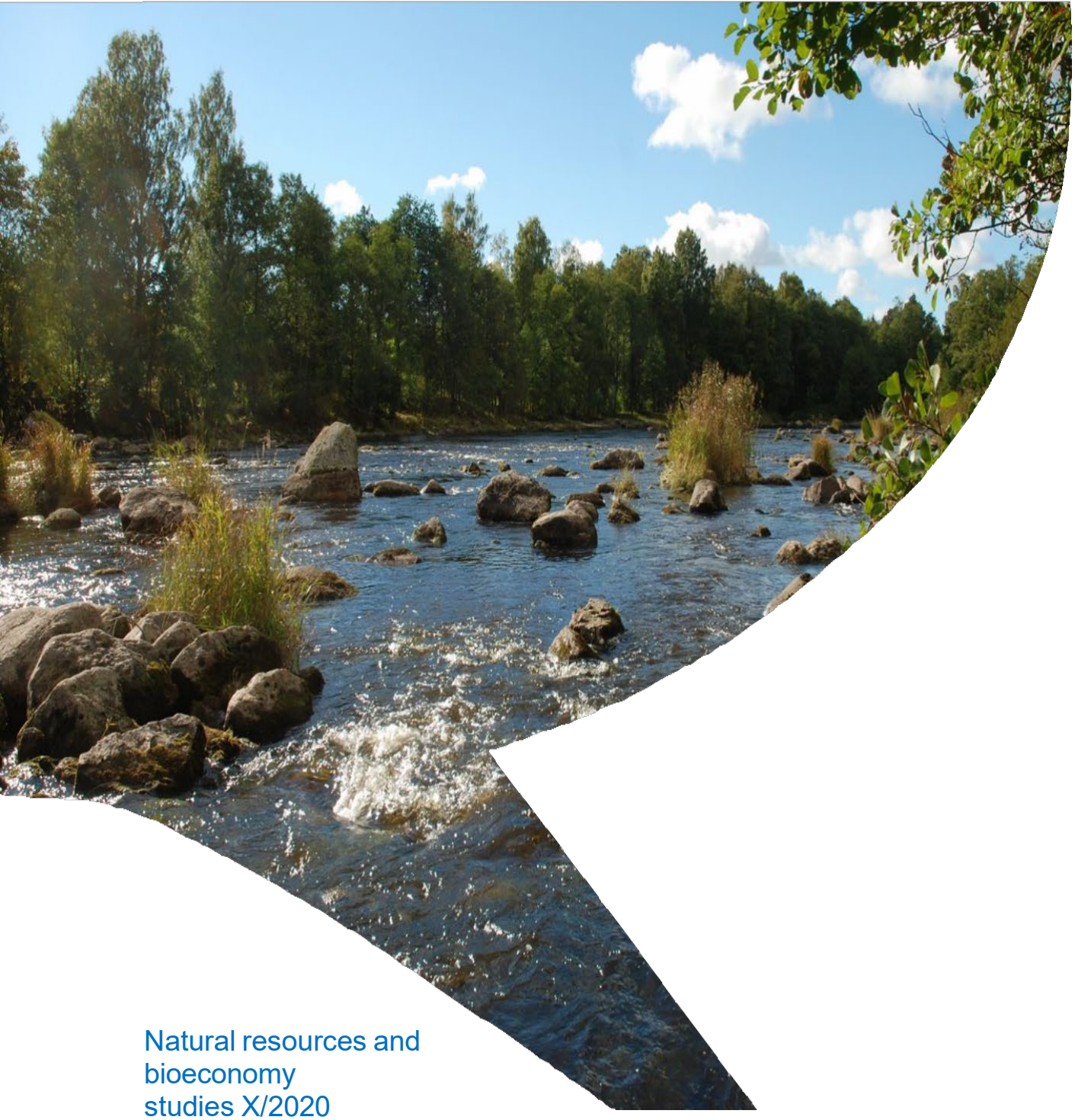

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Marja-Liisa Koljonen
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Abstract

Marja-Liisa Koljonen, Natural Resources Institute Finland (Luke)
Jarmo Koskiniemi, University of Helsinki, Department of Agricultural Sciences

The extent and distribution of genetic diversity, family structure, and genetically effective size of the freshwater form of Atlantic salmon (*Salmo salar m. sebago*) and brown trout (*Salmo trutta*) populations, in the River Gullspångsälven draining into Swedish Lake Vänern, were examined. In addition, the proportion of repeated spawning and potential movements of the spawners between the spawning sites and the level of species hybridization were assessed from offspring genotype data. Sufficient genetic material ensures the viability of by enabling adaptation to a changing environment. The aim of this inventory is to serve as a background for practical planning of future enhancement activities for River Gullspångsälven lake salmon and brown trout populations.

The two species spawn sympatrically in the same rapids along a 5 km long river stretch. Genetic analyses were done mainly using electrofished parr samples, while species hybridization analysis was done in connection with spawning nest counting and egg samplings, where the spawning species of each found salmonid nest could be identified also with genetic markers. In all, 2161 parr or embryo samples were analysed from the years 2014 -2019. From those 1178 were trout, 826 were salmon and 78 (4.8 %) were species hybrids. The salmon data were analysed with 17 DNA-microsatellite loci and the trout data with 16 loci. There are two main rapid sections in the River Gullsångsälven, the Gullspångsforsen and the Åråsforsen rapids. Populations from each section were analysed separately. There was clear genetic differentiation between populations from these rapids in both species.

The mean genetic diversity of the River Gullspångsälven lake salmon was clearly lower than that of most of the compared Atlantic salmon populations. The allelic richness was 63 % of that of the River Torneälven population and less than half (49.7 %) of that of large Russian River Kola population. Genetic diversity has, thus, somewhat decreased from its original level, before the isolation of the population in into the Lake Vänern. The River Gullspångsälven lake salmon populations, however, forms a unique evolutionary branch in the Scandinavian Atlantic salmon phylogenetic tree. Currently, about 45 mature salmon spawn in the River Gullspångsälven annually, and from those only 13 in the Gullspångsforsen rapids. The genetically effective population size (Ne) has annually only been about 20 individuals (8 Gullspf. +12 Åråsf.), which is clearly below the minimum recommended level of Ne = 50. The relatedness of the offspring has also been quite high, above the cousin level (12.5 %). From the salmon spawners, 20 % had been spawning in several years. It is recommended that the rapid populations should be pooled and organise gene flow between rapids to increase the total effective size of the salmon population in the River Gullspångsälven.

The genetic diversity level of the River Gullspångsälven trout population was on average similar to that in the nearby rivers along the same watercourse. The effective population sizes for the brow trout were larger than for the lake salmon. Offspring of 54 spawners were found evenly for both rapid sections (27 + 27). Genetically effective population size (Ne) was 41 (22 for Gullspångsf. and for Årråsf. 19). Respawning of brown trout was much more common, and around 50 % of spawners (54.2 Gullspf., 49.5 % Åråsf) had offspring in several years, which increases the genetically effective population size from that of the annual Ne estimate. The relatedness of the offspring was also somewhat lower (6.9 %, 8.3 %) than in the lake salmon population, although it was still higher than the recommended level of below 3 %.Keywords: Lake salmon, brown rout, genetic diversity, effective population size, relatedness

Keywords: Lake salmon, brown trout, genetic diversity, effective population size, relatedness

Contents

1. Introduction

Landlocked, lake-migratory Atlantic salmon (Salmo salar m. sebago) and brown trout (Salmo trutta) spawn in the same restricted, 5 km long downstream stretch, in the second largest tributary of the Swedish Lake Vänern, the River Gullspångsälven. Both species undergo feeding migrations in Lake Vänern. Lake Vänern salmon spawns also in the River Klarälven. The brown trout population consists of both migrating and resident individuals. Migration from and to the lake is possible as far as the lowest dam. The Gullspång hydroelectric power plant (HPP) is situated close to dam. The status of both salmonid fish populations in the River Gullspångsälven is weak, and extensive enhancement activities and channel constructions have taken place to improve the spawning situation of both species.

The main goal for a conservation strategy of a threatened species is to maintain its genetic diversity in the long term, so that its viability remains, and it has sufficiently genetic material to adapt to the changing environment. For example, global warming will pose a threat to salmonid species as they are generally adapted to cold water, especially during the embryo and juvenile stage. The capacity of populations to maintain their genetic diversity is strongly related to their genetically effective population size, which is usually markedly smaller than the number of spawning individuals, and is estimated to be only about half of population size in a wild, randomly mating fish population. Clear risks of losing genetic diversity arise from declining population sizes, splitting of populations, and even from increasing relatedness and inbreeding in constantly small populations. The risks are especially high for species or ecotypes for which there is no more genetic material available elsewhere.

The goal of this work was to assess the current extent and distribution of genetic diversity, including genetic effective size of the populations, in the Lake Vänern freshwater Atlantic salmon and brown trout populations in the River Gullspångsälven. These species spawn sympatrically in the same rapids and hybridization is also suspected to occur, which is why the identification of potential species hybrids was one of the tasks. Genetic estimation was done mainly with the electrofished parr samples, but species hybridization was done in connection with the spawning nest counting and egg sampling, where the spawning species of each found salmonid nest could be identified also using genetic markers. The tasks of the project included also an analysis of repeated spawning and potential movements of the spawners between the spawning sites on the basis of observed offspring genotypes.

Salmon nests are found in the three rapids of the River Gullspångälven: Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen. Migratory brown trout is spawning in the same rapids and there are some local brown trout in the upper reaches of the river system as well.

This inventory should serve as a background for practical planning of future enhancement activities for River Gullspångsälven lake salmon and brown trout.

This work was funded by European Maritime and Fisheries Fund.

2. Material and methods

2.1. Lake salmon and brown trout sampling

River Gullspångsälven lake salmon and brown trout were sampled over the years 2014-2019. Both electrofishing for parr samplings and egg samplings from spawning nests were used. Samples were taken from three rapid areas Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen (Fig. 1).

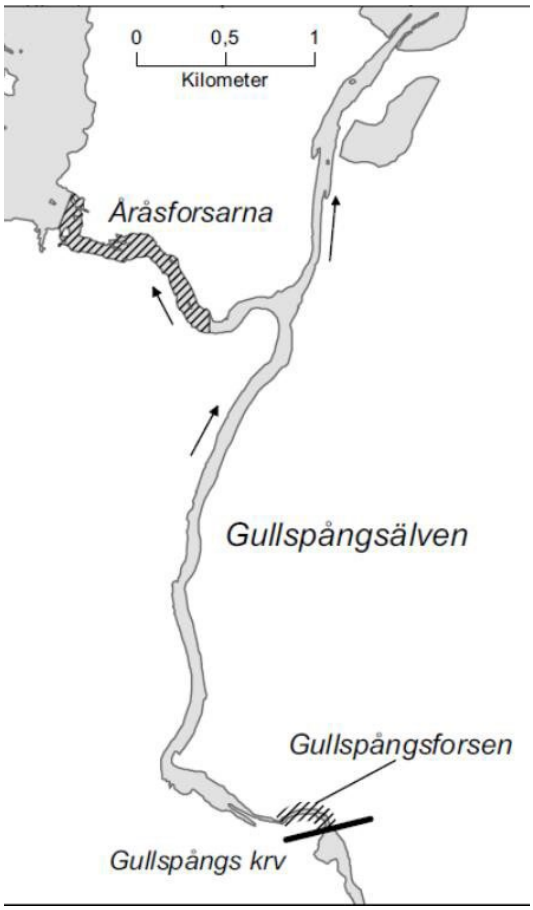

Figure 1. River Gullspångsälven and the sampling rapid areas are shown. Åråsforsarna area includes both Lilla and Stora Åråsforsen rapids.

All, 2161 DNA-samples were analysed from the years 2014 – 2019. From those 1178 were trout samples and 826 were lake salmon samples, while 78 individuals (3.8 %) were species hybrids (Table 1). The DNA quality of all salmon egg samples did not allow for analysing all of the 17 DNA-loci, and these data could not be analysed together with the parr data. These egg DNA-samples were, however, used in the analyses of species identity of the nests and proportion of species hybridisation.

A total of 658 salmon parr samples and 168 salmon egg samples were analysed over the years from the River Gullspångsälven (Table 2). Data from trout egg samplings from the years 2016, 2018, and 2019 could be used for comparison with the parr data, since data for all loci was available for those years. Salmon and trout data were analysed separately and salmon parr data was treated first.

Data for Atlantic salmon stock comparison were from previously published studies (Säisä *et al.*, 2005; Leinonen *et al.*, 2020).

Table 1. Lake-migratory Atlantic salmon and brown trout samples from Lake Vänern drainages. Sampling tissue, sampling year, number (N), speacies, number of hybrids, number of 0 DNA-results and the sample delivery contact person. Borr. = Borrälven, Bratt. = Brattaälven, Treg. = Tregånsbäcken (a tributary of Borrälven), Skag. = Skagersholmsån, Håk. = Håkanbolsbäcken, Rott. = Rottnan, Gammelkr. = Gammelkroppa hatchery stock. Gullspångsälven includes three rapids: Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen.

Sample site and tissue	Year	N	Trout	Salm.	Hyb.	0	0 %	Delivered by
Gullspångsälven parr	2019	234	137	90	7		0,0	F. Nilsson
Gullspångsälven egg	2019/2020	75	61	13	1		0,0	F. Nilsson
Gullspån egg	2018/2019	146	82	55	5	4	2,7	J. Syrjänen, J. Norrgård
Borr. Bratt. Rottnan egg	2018/2019	11	11				0,0	J. Syrjänen, J. Norrgård
Gullspångsälven parr	2018	162	61	71	30		0,0	F. Nilsson
Borr. Bratt. Treg. egg	2017	21	19			2	9,5	J. Syrjänen, J. Norrgård
Gullspångsälven egg	2017	145	72	46	5	22	15,2	J. Syrjänen, J. Norrgård
Gullspångsälven parr	2017	234	87	140	7		0,0	F. Nilsson
Gullsp., Skag., Håk., Rott. egg	2016	102	52	29		21	20,6	J. Syrjänen, J. Norrgård
Borr., Bratt., Granån, Rottnan, Gammelkr. parr	2016	342	340			2	0,6	J. Syrjänen, J. Norrgård
Gullsp., Letälven, Skag., parr	2016	326	125	196	5		0,0	F. Nilsson
Gullspångsälven parr	2015	249	71	161	17		0,0	F. Nilsson
Gullspångsälven egg	2015	61	30	19		12	19,7	J. Syrjänen, J. Norrgård
Gullspångsälven egg	2014	53	30	6	1	16	30,2	J. Syrjänen, J. Norrgård
Total		**2161**	**1178**	**826**	**78**	**79**		

Table 2. Lake salmon DNA samples from 2014-2019 from both egg and parr samples from the River Gullspångsälven. Gullspångsälven includes three rapids: Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen.

Sample site	Tissue	Year	N Salmon	Delivered by
Gullspångsälven	egg	2019	13	F. Nilsson
Gullspångsälven	egg	2018	55	J. Syrjänen, J. Norrgård
Gullspångsälven	egg	2017	46	J. Syrjänen, J. Norrgård
Gullspångsälven	egg	2016	29	J. Syrjänen, J. Norrgård
Gullspångsälven	egg	2015	19	J. Syrjänen, J. Norrgård
Gullspångsälven	egg	2014	6	J. Syrjänen, J. Norrgård
Total			**168**	
Gullspångsälven	parr	2019	90	F. Nilsson
Gullspångsälven	parr	2018	71	F. Nilsson
Gullspångsälven	parr	2017	140	F. Nilsson
Gullspångsälven	parr	2016	196	F. Nilsson
Gullspångsälven	parr	2015	161	F. Nilsson
Total			**658**	

From brown trout, a total of 357 egg samples and 821 parr samples were analysed (Table 3). In addition to samples from the River Gullspångsälven, samples from a few other rivers draining into the Lake Vänern were used for comparison.

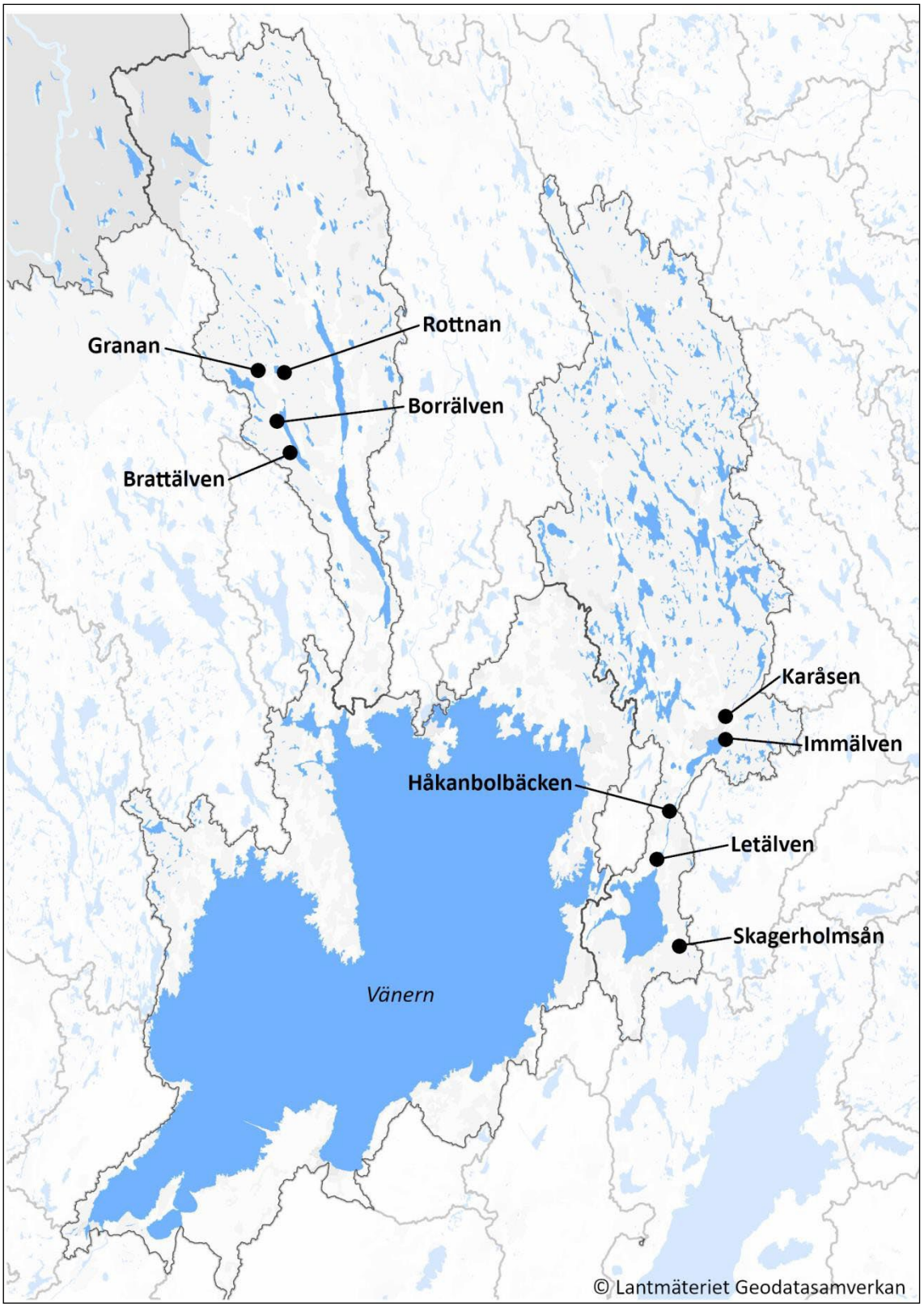

Figure 2. Map of rivers from which brown trout DNA-samples were taken for comparison of the River Gullspångsälven trout.

Table 3. Brown trout egg and parr samples. Borr. = Borrälven, Bratt. =Brattaälven, Treg. = Tregånsbäcken (a tributary of Borrälven), Skag. = Skagersholmsån, Håk. = Håkanbolsbäcken, Rott. = Rottnan, Gammelkr. = Gammelkroppa hatchery stock of Rottnan. Gullspångsälven includes three rapids: Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen.

	Sample site	Tissue	Year	N Trout	Delivered by
1	Gullspångsälven	egg	2019	61	F. Nilsson
2	Gullspångsälven	egg	2018	82	J. Syrjänen, J. Norrgård
3	Borr., Bratt., Rottnan	egg	2018	11	J. Syrjänen, J. Norrgård
4	Borr., Bratt., Treg.	egg	2017	19	J. Syrjänen, J. Norrgård
5	Gullspångsälven	egg	2017	72	J. Syrjänen, J. Norrgård
6	Gullsp., Skag., Håk., Rottnan	egg	2016	52	J. Syrjänen, J. Norrgård
7	Gullspångsälven	egg	2015	30	J. Syrjänen, J. Norrgård
8	Gullspångsälven	egg	2014	30	J. Syrjänen, J. Norrgård
				357	
1	Gullspångsälven	parr	2019	137	F. Nilsson
2	Gullspångsälven	parr	2018	61	F. Nilsson
3	Gullspångsälven	parr	2017	87	F. Nilsson
4	Borr., Bratt., Granån, Rottnan, Gammelkr. parr	parr	2016	340	J. Syrjänen, J. Norrgård
5	Gullsp., Letälven, Skag.	parr	2016	125	F. Nilsson
6	Gullspångsälven	parr	2015	71	F. Nilsson
	Total			**821**	
	All over			**1178**	

2.2. Laboratory analyses

DNA was analysed at the united gene laboratory of the University of Helsinki and Natural Resources Institute Finland (Luke). All parr DNA samples were analysed according to the previously published methods for brown trout (Koljonen *et al.*, 2014) and Atlantic salmon (Säisä *et al.*, 2005; Vuori *et al.*, 2012; Leinonen *et al.*, 2020). The number of microsatellite loci used was 16 for brown trout and 17 for Atlantic salmon. A slightly modified method was used for the egg analyses. DNA was extracted from frozen eggs using Qiagen DNEasy Blood&Tissue Kit with some modifications to kit protocol. The eggs were put in 5 ml Eppendorf tubes with 540 or 720 µl of lysis buffer ATL and 60 or 80 µl of proteinase K (depending on the egg size, 3 or 4 times the volumes in kit protocol). The eggs were punched with pipette tips. The tubes were kept at 56C overnight and mixed 2-3 times. 1230 or 1640 µl of lysis buffer AL-ethanol mix was added (depending on the volumes of ATL and K). The tubes were mixed for a few seconds and centrifuged for 3 minutes at 10 000 rpm. The supernatants were collected and extractions were done from the supernatants following the kit protocol.

Usually one egg from each nest was analysed. The success rate of getting functioning DNA from fertilized eggs in 2014-2017 was about 80 %, when the eggs were collected in the autumn. In 2019, the embryos were collected in spring, in March-May, and the success rates rise to 96 %, as the embryos were then larger. In addition, unfertilized eggs are possible to distinguish from fertilized eggs in the spring, but not in the autumn, which improves the success of spring samplings.

The species (and hybrids) were recognized on the basis of known differences in allele sizes in the two species. The differences in allele sizes are known to be diagnostic or nearly diagnostic in several loci – all salmon and trout differ from each other in these loci (SSosl438, SSa197, SSa289 and SSsp1605). Although not fully diagnostic, the allele sizes in rest of the loci that were analysed were used to give

further support for the species identification. First generation species hybrids can be identified as they have one allele from salmon and one from trout at the diagnostic four loci. Full genotypes could not be identified from all eggs.

The yield and quality of the extracted DNA allowed the identification of the two species and hybrids for all parrs (Table 1.). The two 0 results in one parr sample were contaminations. If eggs were collected in November-December (2014-2017), the identification success varied from 69.8% (Gullspång 2014) to 90.5% (Borr., Bratt., Treg. 2017) of the samples, with the average of 80,9 %. If eggs were collected later in next year of April-May (2019-2020), the identification success varied from 97.3% (Gullspång 2018/2019) to 100% (Gullspång 2019/2020 and Borr., Bratt., Rottnan 2018/2019) of the samples, with the average of 98,3%. The yield and quality of extracted DNA increases significantly from winter to spring samplings, as the embryo is already larger in the spring.

Two species-specific mitochondrial markers for maternal identification of the hybrids were analysed (https://link.springer.com/article/10.1007%2Fs12686-012-9730-6) (Karlsson *et al.*, 2013) in Gullspångsälven parr 2018 and 2019, and Gullspångsälven 2019 egg samples (spawning/2020 sampling).

2.3. Statistical methods

Diversity measures and pairwise Fst 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 fullsib families, and the number of male and female spawners in the spawning population samples were calculated with COLONY-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 and 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. Results from lake salmon genetic analysis

3.1. Genetic diversity of lake salmon parr

3.1.1. Differences between spawning sites and years

Lake salmon parr data from the River Gullpångsälven, from the years 2015-2019, were grouped first into three groups according to the sampling rapids (Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen) and genetic differences among samples from different sampling years and rapids were tested.

Samples from Lilla Åråsforsen and Stora Åråsforsen were in most cases very similar and no statistically significant differences were observed between salmon parr samples of the same year between these close rapids, except in 2019 samples (Table 4). Thus, annual variation between samples from different years was larger than variation between samples from the different rapid sections. This means that there are no temporally stable separate populations in these two rapids and data from those could be pooled. Some spatial stability over years occurred, however, between Gullspångsforsen and the two Åråsforsen samples.

Table 4. Statistical significance of genetic difference between salmon parr samples of three sites in the River Gullspångsälven. Results from comparisons between Lilla and Stora Åråsforsen rapids from the same year are indicated with blue background, other non- significant comparison results are shown with grey background. (Extremely significant = ***, Very significant =**, Significant=*, Not significant = NS).

	Stora Åråsf. 18	Stora Åråsf. 17	Stora Åråsf. 16	Stora Åråsf. 15	Lilla Åråsf. 19	Lilla Åråsf. 18	Lilla Åråsf. 17	Lilla Åråsf. 16	Lilla Åråsf. 15	Gullspångsf. 18	Gullspångsf. 17	Gullspångsf. 16	Gullspångsf. 15
Stora Åråsf. 19	***	***	***	***	NS	NS	***	***	***	***	NS	***	***
Stora Åråsf. 18		***	***	***	***	NS	*	***	***	***	***	***	***
Stora Åråsf. 17			***	***	***	*	NS	***	***	***	**	***	***
Stora Åråsf. 16				***	***	*	***	NS	***	***	***	***	***
Stora Åråsf. 15					***	***	***	***	**	***	***	***	***
Lilla Åråsf. 19						NS	*	***	***	***	*	***	***
Lilla Åråsf. 18							NS	NS	***	NS	NS	***	***
Lilla Åråsf. 17								***	***	***	NS	***	***
Lilla Åråsf. 16									***	***	***	***	***
Lilla Åråsf. 15										***	***	***	***
Gullspångsf. 18											NS	*	***
Gullspångsf. 17												*	***
Gullspångsf. 16													***

This could be seen clearly also in the genetic distance tree, as in all cases salmon parr samples from Lilla Åråsforsen and Stora Åråsforsen from the same year grouped together. Gullspångsforsen rapid samples grouped to a separate group from both Åråsforsen rapids samples (Fig 2). Because of these

results, all the samples from both Åråsforsen rapids were pooled, and the data were divided only into two rapid groups for further analyses.

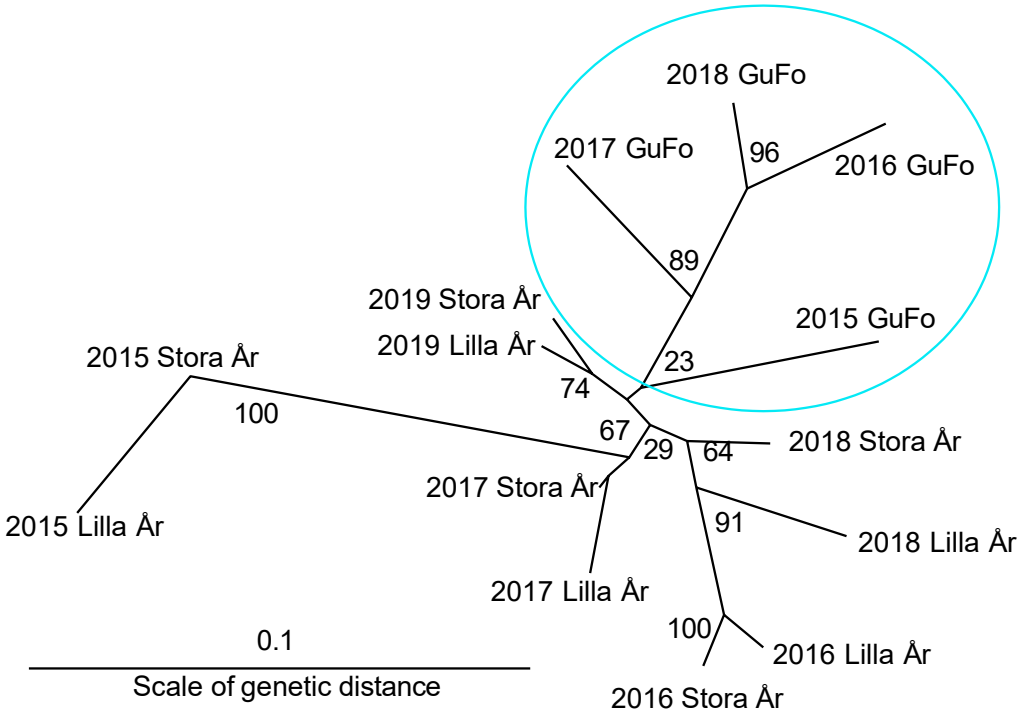

Figure 3. Genetic distances between Atlantic salmon parr samples from the River Gullspångsälven drawn as an unrooted dendrogram. GuFo = Gullspångsforsen, Lilla År = Lilla Åråsforsen, Stora År = Stora Åråsforsen. Sampling year is also shown. The tree branch with the samples from the Gullspångsforsen rapid is circled in light blue. Support for each brancing point is shown as percentages from 1000 bootstrap runs.

Testing of the statistical significance of the genetic differences between parr samples from different years of the two rapid section showed that the spawning populations within rapids are not temporally very stable (Table 5).

Table 5. Statistical genetic difference between salmon parr samples between years of two sites in the Gullspångsälven; Åråsforsen and Gullspångsforsen (Extremely significant = ***, Very significant =**, Significant=*, Not significant = NS).

Site, year	Åråsf 2018	Åråsf 2017	Åråsf 2016	Åråsf 2015	Gullspf 2018	Gullspf 2017	Gullspf 2016	Gullspf 2015
Åråsforsen 2019	***	***	***	***	***	*	***	***
Åråsforsen 2018		***	***	***	***	***	***	***
Åråsforsen 2017			***	***	***	**	***	***
Åråsforsen 2016				***	***	***	***	***
Åråsforsen 2015					***	***	***	***
Gullspångsforsen 2018						NS	*	***
Gullspångsforsen 2017							**	***
Gullspångsforsen 2016								***

Salmon parr samples taken from the Åråsforsen rapids differed statistically very significantly between all years. Gullspångsforsen samples were more similar and no statistically significant differences

could be observed between parr samples from the years 2017 and 2018, and also between 2016 and 2018 the difference was only significant (*)(Table 5). The sample sizes for the Åråsforsen rapids samples were larger than for Gullspångsforsen rapid, which might be reflected in statistical significance being reached more easily. However, the differentiation levels between different years, measured as Fst-values, were not much larger among the Åråsforsen samples than among the Gullspångsforsen samples (Table 6).

Despite the annual variation among samples from different years, the separation into two at least partly separated spawning rapid populations remained, and all samples from each rapid from all years grouped together (Fig. 4). The genetic differences between samples from different years were not always very large, although they were significant, and similarities also occurred between samples from different rapids (Table 6).

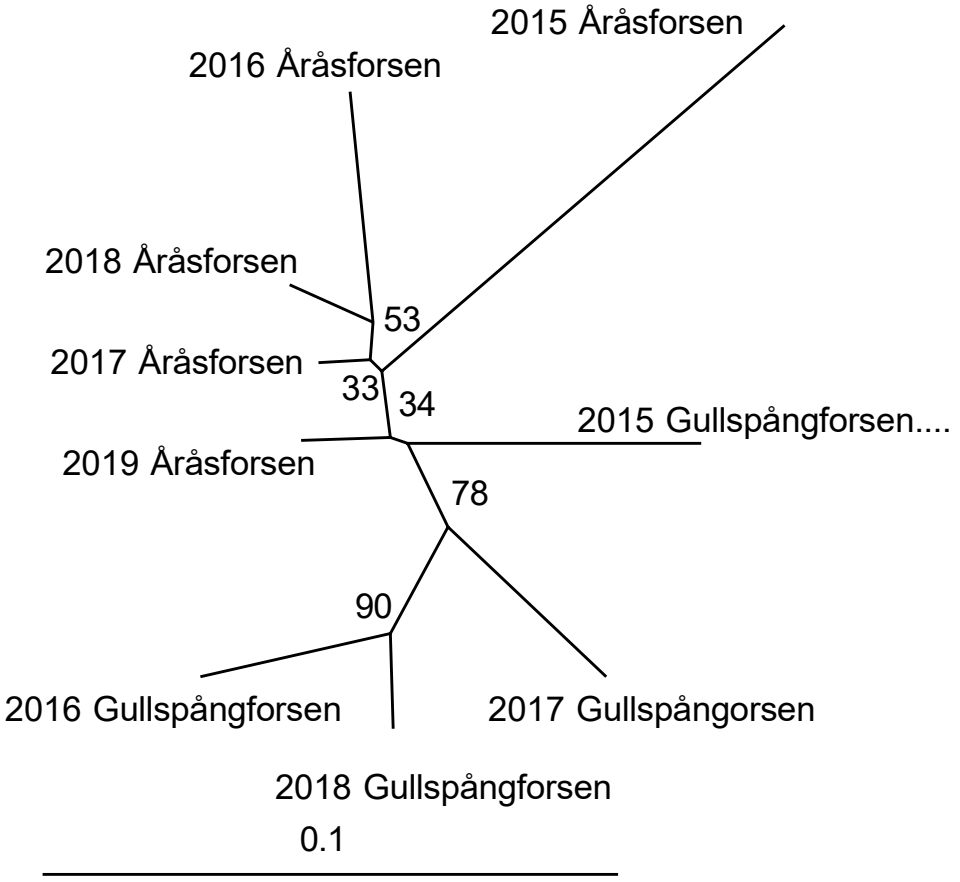

Figure 4. Genetic distances between landlocked Atlantic salmon parr samples from two rapids of the River Gullspångsälven, Åråsfors and Gullspångfors, drawn as an unrooted genetic distance tree. Support for each brancing point is shown as percentages from 1000 bootstrap runs.

Table 6. Genetic distances between Atlantic salmon parr samples from two rapid sections in several years, measured as pairvise Fst –values, which is based on allele frequency variances between samples. It varies from 0 to 1. Small values, below 0.05, are shown on grey background.

Rapid, year	Åråsf_19	Åråsf_18	Åråsf_17	Åråsf_16	Åråsf_15	Gullspf_18	Gullspf_17	Gullspf_16	Gullspf_15
Åråsf_19	------	0.021	0.023	0.055	0.089	0.038	0.025	0.080	0.032
Åråsf_18		------	0.019	0.043	0.112	0.051	0.032	0.090	0.038
Åråsf_17			------	0.058	0.076	0.046	0.025	0.089	0.047
Åråsf_16				------	0.136	0.087	0.077	0.113	0.088
Åråsf_15					------	0.108	0.115	0.163	0.117
Gullspf_18						------	0.022	0.037	0.037
Gullspf_17							------	0.052	0.045
Gullspf_16								------	0.084

3.1.2. Genetic diversity level of the Gullspångsälven salmon

The genetic diversity level of Gullspångsälven salmon was compared to that of some other salmon stocks in the Northern Europe, including both landlocked lake salmon populations as well as sea migratory, anadromous populations in the Baltic Sea and the Barents Sea.

The highest genetic diversity was observed in the Atlantic salmon population from the Russian River Kola draining into the Barents Sea from the Kola Peninsula (Table 7). In this population more than 200 different alleles were observed in the 17 gene loci, while in the Finnish lake salmon the number was below 90. The diversity measures for River Gullspånsgälven salmon were quite low (64.7 %) and clearly below of the mean value (82.6 %), although the diversity was somewhat higher than in the Finnish Saimaa lake salmon (46.9 %).

Diversity levels in lake populations are often low, as the population sizes tend to be smaller than in anadromous populations, which suffered less from isolation following the last glaciation. In the Russian Lake Onega population diversity levels were, however, higher than the mean values, although the sample size was small. The genetic diversity levels of Åråsforsen and Gullspångsforsen were relatively similar (Table 7).

The mean diversity of the River Gullspångsälven lake salmon was about 93 % of that of the largest Baltic Sea River Torneälven and 78 % of that of the large, wild Russian Barents Sea River Kola salmon. The allelic richness was, however, only 63 % of that of the River Torneälven and less than half (49.7 %) of that of River Kola. This indicates that the allelic diversity has somewhat declined from its original level, before the isolation into Lake Vänern. Northern Baltic Sea salmon and Lake Vänern salmon has same origin in ancient Atlantic salmon population and River Kola represent maximum diversity measured in current Northern Europe Atlantic salmon populations.

Table 7. Genetic diversity levels of the Atlantic salmon populations in the northern Europe. Number of samples (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. The lowest values are shown on grey background.

River/Lake	Country	Drainage	N	Div %	N All	All Rich
Åråsforsen	Swe	Lake	523	64.4	114	4.61
Gullspångsforsen	Swe	Lake	135	61.9	103	4.65
Gullspångsälven	**Swe**	**Lake**	**658**	**64.7**	**121**	**4.76**
Torneälven	Fin/Swe	Baltic Sea	70	69.8	178	7.56
Simojoki	Fin	Baltic Sea	70	69.0	163	7.12
Kola	Rus	Barents Sea	59	82.6	219	9.56
Onega	Rus	Lake	18	72.6	132	7.46
Ladoga	Rus	Lake	94	67.6	113	5.20
Neva	Rus	Baltic Sea	70	74.4	172	7.73
Morrumsån	Swe	Baltic Sea	70	74.0	165	7.08
Saimaa R. Pielisjoki.	Fin	Lake	60	46.9	74	3.64
Saimaa R. Ala-Koitajoki.	Fin	Lake	579	47.7	83	3.65
All_W					304	7.19
Min			**18**	**46.9**	**74.0**	**3.64**
Max			**658**	**82.6**	**219.0**	**9.56**
Mean			**200,5**	**66.3**	**136.4**	**6.09**

3.1.3. Genetic distances among North European Atlantic salmon populations

When the genetic distances were calculated for all main drainage areas in the Northern Europe, four to five stock groups or phylogenetic lineages could be formed from all Atlantic salmon population types (Atlantic Barents Sea; Baltic Sea north, east and south; landlocked populations in Finland, Russia and Sweden). In this comparison only a few represantive populations were included from each drainage area.

All the anadromous Baltic Sea Atlantic salmon populations grouped together, although populations from the southern Swedish River Mörrumsån and the eastern Russian River Neva joined with a lower bootstrap value than the northern populations of the Rivers Tornionjoki and Simojoki. These three lines have previously been described as their own phylogenetic lineages within the Baltic Sea (Säisä et al. 2005).

The lake populations of the large Russian lakes, Onega and Ladoga, grouped also together with a high bootstrap value (95 %)(Fig. 5), confirming that they have a common, eastern, phylogenetic history. The most diverse Atlantic salmon population in this study, from the Russian River Kola, draining into the Barents Sea formed a group of its own, without any clear near relatives.

From the Finnish and Swedish lake populations, two samples were analysed and in both cases those two grouped understandably tightly together as they were from the same lake. In addition, the bootstrap value binding the populations of Lake Saimaa and Lake Vänern together was slightly over 50 % (52 %). At least they resampled to each other more than to any other populations, although both must have a long, isolated, and independent evolutionary history from each other, which can be seen in their own long branch in the tree.

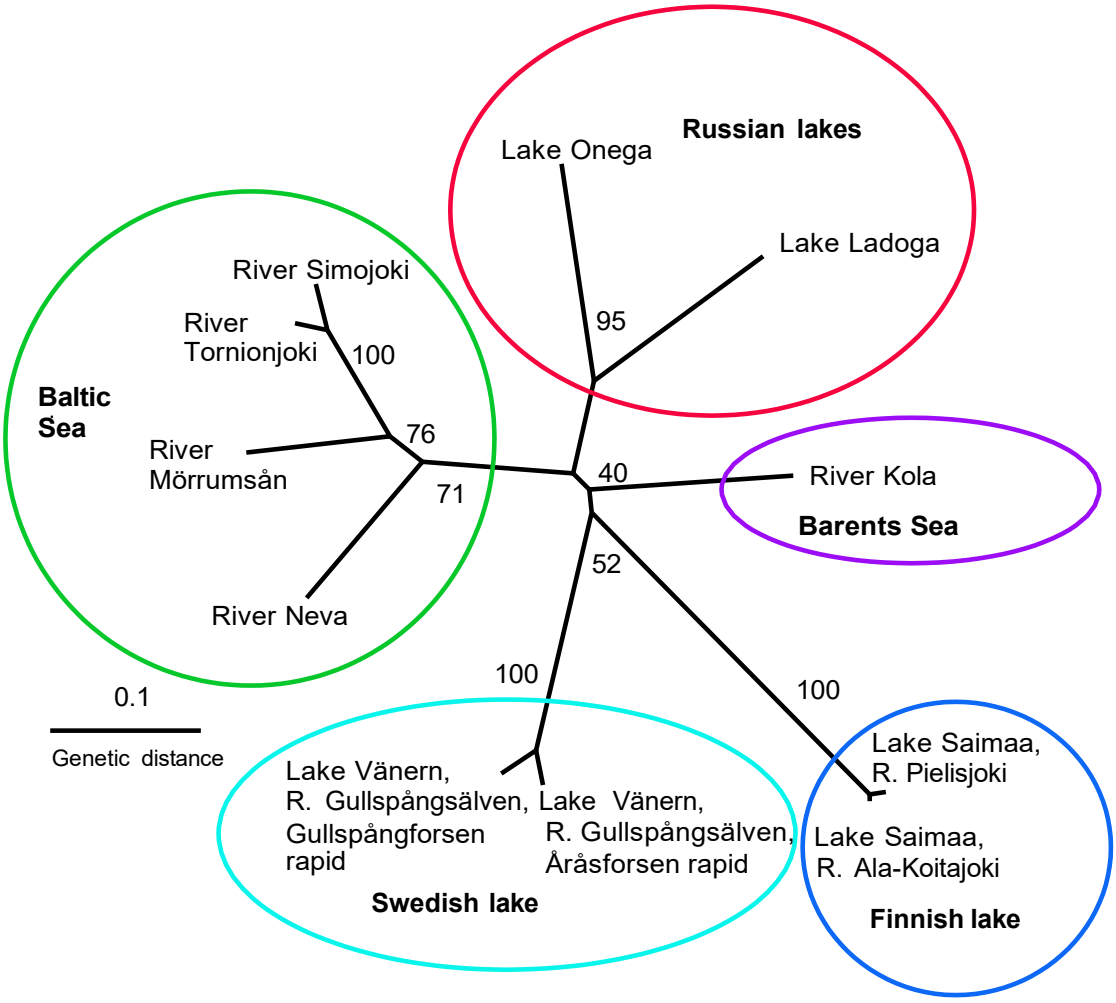

Figure 5. Genetic DA-distances among North European Atlantic salmon populations shown as unrooted phylogenetic tree (NJ-tree). Support for each brancing point is shown as percentages from 1000 bootstrap runs.

3.1.4. Family structure in the Gullspångsälven salmon population

The family structure of the Gullspångsälven salmon was calculated both separately for each sampling year and for both spawning rapids (Gullspångsforsen and Åråsforsen), and also for the whole data to estimate the family structure and the total effective population sizes over the years.

Individual genotype analysis over several years revealed some identical genotypes, which were either contaminations or parr recaptured in different years. The data of recaptured two-year-old parr were removed from the pooled data analysis. Parr length information was used to confirm that the individuals were very likely the same after a year growth period. Similarly, also the second of the identical genotypes in the same year samplings of one-year-old parr were removed (Table 8).

In the Gullspångsforsen rapid, on average 13.3 families were spawning annually (Table 9). Sampled families were smaller in later years, with maximally three siblings in a family, than in earlier years, 2015 and 2016, with 11 and 12 siblings per family. The annual genetically effective population size was less than 10, with an average of 8.0 individuals over the sampling years, with some annual variation. The annual relatedness level was quite high, 12.7 %, which is close to the cousin level (12.5 %). When the data over four years was pooled the total effective population size still remained at the level of only 20 (12-38) individuals, but the relatedness decreased to about half of the annual level (7.2 %).

Table 8. Identical genotypes in the lake salmon parr samples. No difference was found between genotypes of the following numbered offspring samples.

	Sample 1	Length/mm	Sample 2/omitted	Lenght/mm	Reason
1	15_Gulspf.1a_153		15_Gulspf.1a_154		Contamination
2	15_Gulspf.1a_162		15_Gulspf.1a_165		Contamination
3	15_Gulspf.1b_199		15_Gulspf.1b_218		Contamination
4	15_Gulspf.1a_214		15_Gulspf.1a_217		Contamination
5	15_Lilla Åråsf.iC_148		15_Lilla Åråsf.iC_149		Contamination
1	15_Stora Åråsf. Kv_66	84	16_Stora Åråsf. Bi_32	170	Same fish
2	15_Stora Åråsf. el_100	100	16_Stora Åråsf. El_161	195	Same fish
1	16_Stora Åråsf. Bi_4	110	17_Stora Åråsf. B_37	171	Same fish
2	16_Stora Åråsf. Bi_35	102	17_Stora Åråsf. B_39	185	Same fish
3	16_Stora Åråsf. Kv_50	86	17_Stora Åråsf. K_98	171	Same fish
4	16_Stora Åråsf. Kv_60	88	17_Stora Åråsf. K_128	168	Same fish
5	16_Stora Åråsf. Kv_79	155	17_Stora Åråsf.K_135	200	Same fish
6	16_Stora Åråsf. Kv_87	79	17_Stora Åråsf. K_103	173	Same fish
7	16_Stora Åråsf. Kv_89	86	17_Stora Åråsf. K_114	167	Same fish
1	17_Stora Åråsf. B_43	92	18_Stora Åråsf. B_11	149	Same fish
2	17_Stora Åråsf. B_47	88	18_Stora Åråsf. K_50	163	Same fish
3	17_Stora Åråsf. B_52	99	18_Lilla Åråsf._73	164	Same fish
4	17_Stora Åråsf. B_68	93	18_Stora Åråsf. K_49	170	Same fish
5	17_Stora Åråsf. B_70	80	18_Stora Åråsf. K_48	160	Same fish
6	17_Stora Åråsf. B_84	75	18_Stora Åråsf. K_37	150	Same fish
7	17_Stora Åråsf. B_104	75	18_Stora Åråsf. K_43	158	Same fish
8	17_Stora Åråsf. B_107	91	18_Stora Åråsf. K_30	159	Same fish
9	17_Stora Åråsf. B_117	80	18_Stora Åråsf. K_33	153	Same fish
1	18_Stora Åråsf.B_15	87	19_Stora Åråsf._Bk_087	149	Same fish

The salmon population size was over two times larger in the Åråsforsen rapids (31.8) than in the Gullspångsforsen (13.0), with the mean number of families or breeding pairs of 35.4 annually. The number of siblings was also higher in the Åråsforsen rapids population with the mean of the maximum number of siblings 18.2. The number of observed siblings was related to sample size: in 2018 with the small sample size of 54, maximally 5 siblings were observed, although the effective population size was the same 18 as in 2019, when 12 siblings were sampled (Table 9).

The effective size of salmon population in the Åråsforsen rapids did not increase with the same ratio as the number of breeding pairs, as it was annually only on average 12.2 compared to 8.0 in the Gullspångsforsen, although the number of breeding pairs was clearly higher than in the Gullspångsforsen rapid. When the data over five years was pooled, the Ne increased to 30. There was a decreasing tendency in the annual relatedness level from 2015 to 2019, from 24.5 % of half-sib level to 8.9 %, which is less than cousin level, but nevertheless more than the recommended level of about 3 %.

The Ne/Nb ratio was smaller in the Åråsforsen salmon population, so as the difference between the number of breeders and the effective number was larger. It was on average over 0.5 in the Gullsopångsforsen (0.6) and somewhat below that in the Åråsforsen rapids (0.4). In both cases the ratio improved in the later years and true number of breeders were closer to that of the effective number.

Table 9. Family structure of the Gullspångsälven lake salmon populations from the two rapid sections annually, average over the studied years, calculated from the pooled data over the years (2015-2018 for Gullspångsforsen rapid, and 2015-2019 for Åråsforsen rapid), and also for total Gullspångsälven with the pooled data from both rapids and all years (Gullspf + Åråsf.). 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 in the observed families (Max Sib) are shown. In addition, the estimate of genetically effective population size of the sampled population (Ne) with its 95 % confidence limits (95%), the effective size versus the true number of breeders ratio (Ne/Nb) and the mean pairwise relatedness of all individuals in the sample as a percentage (Relatedness %) are shown.

		Breeding pairs	Parents		Family size				
Recommended		**> 50**	**> 100**	**> 100**		**> 50**		**> 0,5**	**< 3,0**
Gullspångsforsen	Ofsp.	Family	Breeders	N Breed	Max Sib	Ne	Ne 95%	Ne/Nb	Relatedness %
2018	17	13	8+6	14	3	11	6-27	0.8	9.8
2017	11	8	4+5	9	3	8	4-24	0.9	13.3
2016	46	11	8+3	11	12	4	2-12	0.4	15.6
2015	56	21	13+5	18	11	9	4-24	0.5	11.9
Mean/year		13,3		13,0	7,25	8,0		0.6	12.7
2015-2018	130	55	19+26	45	11	20	12-38	0.4	7.2
Åråsforsen	Ofsp.	Family	Breeders	Breed	Max Sib	Ne	Ne 95%	Ne/Nb	Relatedness %
2019	89	44	19+19	38	12	18	10-34	0.5	8.9
2018	54	31	18+13	31	5	18	10-34	0.6	10.4
2017	129	49	14+26	40	17	11	6-26	0.3	10.4
2016	150	35	16+17	33	21	9	4-23	0.3	14.0
2015	100	18	9+8	17	36	5	4-20	0.3	25.5
Mean/year		35,4		31,8	18,2	12,2		0.4	13.8
2015-2019	502	147	57+63	120	38	30	19-49	0.3	6.85
Gullsp.+Åråsf.	632	206	81+86	167	38	43	30-67	0.3	6.22

3.2. Proportion of repeat spawning in the lake salmon population

Repeated spawning was analysed from the data including only 1-summer-old salmon parr, as older siblings had caused error in the assumed spawning year. The spawning year of parents of each parr is one years earlier, and the hatching year is the same year. The proportion of repeat spawning could be estimated from the COLONY runs BestConfig tables, in which the most probable parents are assigned to each individual offspring. When all older than 1-year old parr (7) were removed from the total data (130, Table 9) for Gullspångsforsen, the offspring sample size of the remaining Gullspångsforsen parr was 123, meaning that 5.4 % of the individual parr samples were older than 1 year.

From the Gullspångsforsen rapid, a total of 45 salmon spawners were observed for all offspring over the years 2015-2018. In 2019 only one individual offspring was available. From the 45 numbered

spawners, 9 (20.0 %) had offspring among two of the annual 1-year old salmon parr samples (Table 10). In all the cases of repeated spawning, it occurred only in two years, so that in total 9 (= 18 - 9) out of the 45 spawning events were repeated spawning events. This equals 20.0 % out of all spawning events for the Gullspångsforsen lake salmon population.

Overall, in 2015 the population was more independent, while mixing of year classes was more common during 2016-2018. This may reflect the much smaller sample size in the 2017 parr year class. In addition, random similarity of individuals may cause bias in this type of analysis, which is based on an assumed similarity of siblings. This is especially true when the mean relatedness of the individuals is high as is the case. All the individuals, which were so similar that they could be siblings or halfsiblings, were calculated as such. This means that some of the numbers here may be overestimatred.

Table 10. Number of repeat spawning in the Vänern lake salmon population of the River Gullspångsälven Gullspångsforsen rapid, analysed from the salmon parr family structure data.

N	Parent ID	Offspring year	N years
1	Male 3	2015, 2018	2
2	Female 4	2015, 2017	2
3	Male 10	2016, 2017	2
4	Female 12	2015, 2018	2
5	Female 17	2016, 2018	2
6	Male 7	2016, 2018	2
7	Male 9	2016, 2018	2
8	Female 19	2016, 2018	2
9	Female 21	2016, 2018	2
9			**18**

For Åråsforsen rapids, a total of 485 one-year old parr were sampled, when 17 of the 502 parr were 2-year old (3.4 %). For those offspring, 116 (57+59) parents were defined to have been breeding in the rapids over the five sampling years, 2015-2019 (Table 11). From those numbered parents, 24 were spawners, who had offspring in several year classes. This is 20.1 %, which is very near to that in the Gullspångsforsen population (20.0 %). Repeat spawning occurred, in all, 53 (all)-24(first times) = 29 (second or third times), which translates to a respawning percentage of 25 %.

The sex of the parent is only an assumed sex as no true sex data were available. No conclusion of the mating behavior of different sexes can be drawn from this data. Parental genotypes are deduced backwards from the offspring genotypes, and there were no sex markers, so actually only sex 1 and sex 2 were known.

In the Åråsforsen rapids many of the parents had been spawning in both of the rapids in different years (Lilla and Stora Åråsforsen)(Table 11). This was true also for parents spawning in one year and which thus are not listed here, as only spawnings in different years were included here. This confirms the results that these two Åråsforsen rapids function as one spawning ground for salmon, and moving of spawners prevents genetic differentiation between rapids.

Repeat spawning was observed both in successive years and with a gap year. Individuals spawning with a gap year have more likely been females, as their maturation process takes more energy. In conclusion, most of the spawners, about 80 %, reproduced only once. This means also that gene flow over the year classes is not very strong.

In addition to these respawning estimates of the two rapids, there are also individuals, which have changed the spawning rapid between the years, and have spawned in both Åråsforsen and Gullspångsforsen. An analysis of all the data of one-year-old lake salmon parr (608 offspring) revealed that there was very little movement by the spawners between the two rapid sections of the River Gullspångsälven. The data included 168 spawners, and only in a single case the spawner had offspring among both Gullspångsforsen and Åråsforsen parr samples in the same year (Table 12). Overall, only 7 salmon had mated in both spawning areas and in six cases the year was different. There seems to have been a tendency that those repeatedly spawning parents had first spawned in the Gullspångsforsen rapid, but in a later year remained for some reason either in the Stora or Lilla Åråsforsen rapid sections.

Table 11. Number of repeat spawning in Vänern lake salmon population of River Gullspångsälven Åråsforsen Rapids, analysed from the salmon parr data. Displayed are the parent numbers, sex assigned by the program, solved offspring years with the rapid section, and mating times in years.

	Sex	**Rapid**	**N Year**
1	Male1	2015 Stora Å., 2015 Lilla Å., 2017 Stora Å.	2
2	Male2	2015 Lilla Å., 2015 Lilla Å., 2017 Lilla Å. Lilla Å., 2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	3
3	Male4	2015 Stora Å., 2017 Stora Å., 2018 Stora Å.	3
4	Male5	2015 Stora Å., 2019 Stora Å.	2
5	Male7	2016 Lilla Å., 2016 Stora Å., 2018 Lilla Å., 2018 Stora Å.	2
6	Male10	2016 Lilla Å., 2016 Stora Å., 2018 Lilla Å., 2018 Stora Å.	2
7	Male11	2016 Lilla Å., 2016 Stora Å., 2018 Stora Å.	2
8	Male15	2016 Lilla Å., 2016 Stora Å., 2018 Stora Å.	2
9	Male16	2016 Stora Å., 2017 Stora Å.	2
10	Male20	2017 Lilla Å., 2017 Stora Å., 2018 Stora Å., 2019 Lilla Å., 2019 Stora Å.	3
11	Male21	2017 Stora Å., 2019 Lilla Å.	2
12	Male26	2017 Lilla Å., 2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
13	Male27	2017 Lilla Å., 2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
14	Male29	2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
15	Male30	2017 Lilla Å., 2017 Stora Å., 2019 Lilla Å.	2
16	Male34	2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
17	Male43	2018 Lilla Å., 2019 Stora Å.	2
18	Fem10	2015 Stora Å., 2018 Stora Å.	2
19	Fem12	2016 Lilla Å., 2016 Stora Å., 2017 Stora Å.	2
20	Fem23	2017 Stora Å., 2019 Stora Å.	2
21	Fem30	2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
22	Fem34	2018 Lilla Å., 2019 Lilla Å.	2
23	Fem7	2015 Stora Å., 2017 Lilla Å., 2018 Stora Å., 2019 Stora Å.	4
24	Fem8	2015 Stora Å., 2019 Stora Å.	2
			53

The moving of spawners between rapid sections was quite rare, as in all 7 out of 168 spawners, 4.2 %, changed their spawning site within the river, which supports the result of clear natural isolation between spawning site populations. Even this 4.2 % is, however, potentially enough to maintain the same alleles in these two populations, although the allele frequencies in the different rapid sections remain somewhat different over the years.

Table 12. Moving of the lake salmon spawners between rapid sections in the River Gullspångsälven. Parr years of offspring and spawning sites are given.

N	Parent ID	Year and rapid
1	Female 6	2015 Gullspångsf., 2016 Åråsf.
2	Male 8	2016 Gullspångsf., 2018 LIlla Åråsf.
3	Male 10	2016 Gullspångsf., 2016 Åråsf., 2018 Gullspångsf.
4	Female 19	2017 Gullspångsf., 2019 LIlla Åråsf., 2019 Stora Åråsf.
5	Female 23	2018 Gullspångsf., 2018 LIlla Åråsf.
6	Male 17	2018 Gullspångsf., 2019 Stora Åråsf.
7	Female 28	2016 Stora Åråsf., 2018 Gullspångsf.

4. Conclusion of lake salmon genetic analysis

The spawning population in the Gullspångsforsen is small. The annual number of breeder varied between 9 to 18, and the annual effective size of the population varied between 4 and 11 (Table 9). Those numbers are clearly below the recommended minimum effective population size of 50, for even the short term conservation and maintenance of genetic diversity of a population. For long term conservation of unique populations the recommended Ne is 500. Some repeated spawning occurred and 9 out of the 45 salmon spawners (20.0 %) had reproduced twice in the Gullspångsforsen (Table 10), thus most about 80 % of spawners reproduced only once. This same was true for Åråsforsen, altought spawning occurred more often during several years. The effective population size estimate for all years was only 20 (12-38), and even this is an overestimate as all year classes do not contribute similarly to forthcoming offspring year classes. The average annual Ne was 8.

In the Åråsforsarna rapids the number of spawners varied annually from 17 to 40 (Table 9). This is corresponding with the range of effective population sizes from 5 to 18 (Table 9). The effective size over the years from the pooled population was 30 (19-49). These numbers are also below the recommended minimum of Ne over 50 (Frankel and Soule, 1981; Meffe, 1986). Even after pooling of both rapids and year classes the total effective size remains below 50, with an estimate of 43 (30-67)(Table 9). The number of individual spawners in the Åråsforsen rapids (120) over the years was over twise of that of Gullspångsforsen (45). Salmon population in the Åråsforsen rapid was stronger than in the Gullspångforsen and hybrization disturbed reproduction there also less (Table 25).

The pairwise relatedness between the salmon parr had increased from the 0-level of wild, randomly mating, nonrelated population. It means that the parents of the studied offspring were already related to some extent, and relatedness tends to increase from generation to generation, if no new genetic material is available. For Gullspångsforsen the pairwise relatedness of the salmon parr varied from about 10 % to 16 % and for Åråsforsarna from 9 % to even 25 %. The relatedness for siblings is 50 %, for halfsibs 25 %, for first cousins 12.5 %, and for second cousins 3.125 %. The recommended value for accepted level of relatedness is below second cousinlevel 3 %. The observed values indicate that the relatedness in the populations is often at the cousin level and sometimes even halfsiblevel.

The current effective population size of the Gullspånsgälven salmon population is unlikely to increase with the current spawning stock, and the population cannot maintain its genetic diversity in long term, as it is vulnerable to stochastic variation, population bottlenecks, constant loss of gene forms, and increasing relatedness. There was some genetic differentiation between the two salmon populations from the Gullspånsgälven, but the difference was, however, quite small, especially when compared to large scale differences among Atlantic salmon populations of other evolutionary lineages. These two small populations represent part of the last remaining genetic material of the lake salmon of the Lake Vänern, in addition, to Klarälven population. Since these populations are anyway from the same river, and some gene flow between them occur, pooling the populations with organized gene flow, rescues probably more diversity than keeping them separately in the current situation.

The recommendation is to do a inventory of all the genetic resources of lake migrating salmon in the Lake Vänern including Klarälven, and also all the current and potential breeding ground options both in the wild and in hatcheries including their production volumes and to combine that information. Then, a common long-term conservation Action Plan for the Lake Vänern salmon as a whole should be created, with a sufficiently large, safe and permanent gene pool. If the population is small, it is often just a random set of individual fishes and not any true result of any evolutionary meaningful adaptation. Splitting the population is often harmful, as it multiplies the risks of losing genes and increasing relatedness, when the remaining populations are too small.

5. Results from genetic analysis of brown trout

5.1. Genetic diversity of brown trout

5.1.1. Differences between spawning sites and years

The brown trout parr data were analysed first for the River Gullspångsälven alone. Data from the Lilla Åråsforsen rapid section was pooled already in the first analysis, because only 27 parr were available from all the sampling years altogether (Table 13). In addition to the samples from Gullspångsforsen and Åråsforsen, 40 samples from local brown trout populations from the upper tributaries of the Gullspångsälven were included for comparison (Håkanbolbäcken, Immälven, Svartälven).

Table 13. Brown trout parr samples from the River Gullspångsälven over the years 2015 – 2019, grouped according to sampling sites.

Years	Site	N
2019	Gullspångsforsen	50
2018	Gullspångsforsen	35
2017	Gullspångsforsen	22
2016	Gullspångsforsen	33
2015	Gullspångsforsen	43
All		**183**
2019	Lilla Åråsforsen	9
2018	Lilla Åråsforsen	3
2017	Lilla Åråsforsen	8
2016	Lilla Åråsforsen	5
2015	Lilla Åråsforsen	2
All		**27**
2019	Stora Åråsforsen	36
2018	Stora Åråsforsen	22
2017	Stora Åråsforsen	55
2016	Stora Åråsforsen	43
2015	Stora Åråsforsen	25
All		**181**
All over		**391**
2019	Håkanbolbäcken	20
2019	Immälven	14
2019	Svartälven	6
		40

When differences among years and rapids were analysed, the results were very similar to those of the lake salmon populations in the same area. Samples from the Gullspångsforsen rapid from all years grouped together, as did all the samples from the Åråsforsen rapids (Figure 5). Temporal variation among spawning populations was smaller than spatial variation between rapids, which indicates spatial genetic differentiation of rapid populations over the years. Both of these populations seem to have their own patterns of homing behaviour. The populations from these two spawning sites were, however, more similar to each other than to the assumedly local brown trout populations of the upper reaches of Gullspångsälven.

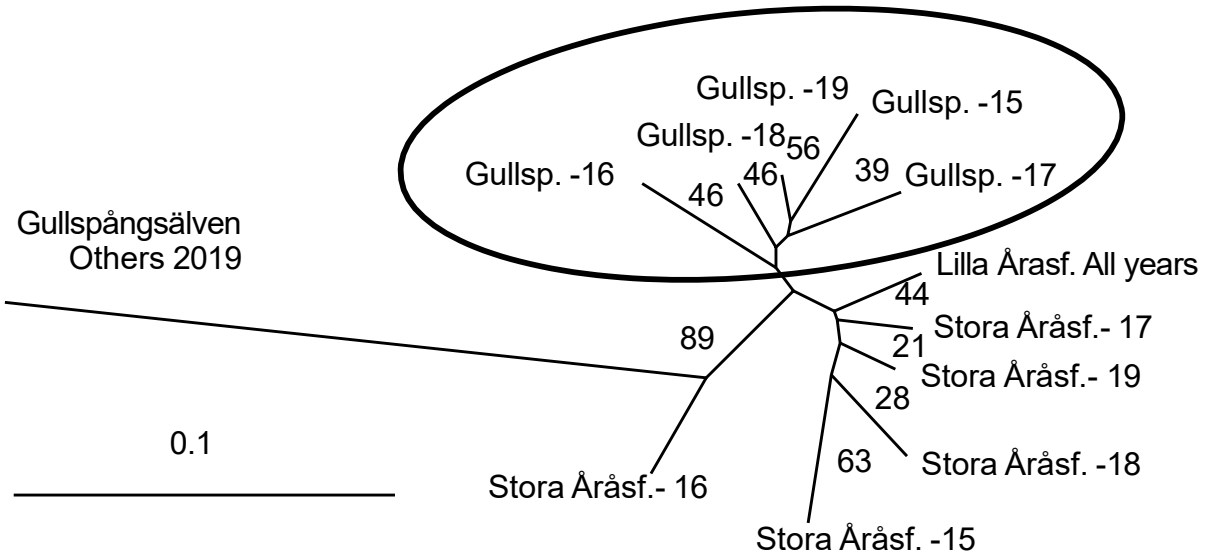

Figure 6. Genetic distances between brown trout parr samples from the River Gullspångsälven. Gullsp. = Gullspångsforsen, Lilla Åråsf. = Lilla Åråsforsen and Stora Årås. = Stora Åråsforsen. Gullspångsälven Others 2019 = Håkanbolbäcken, Immälven, Svartälven. Support for each brancing point is shown as percentages from 1000 bootstrap runs.

Several of the genetic differences between samples from different years of the same rapid section were statistically nonsignificant (NS) (Table 14). Also, the pooled sample of Lilla Åråsforsen did not differ at all from three (2017, 2018, 2019) of the annual Stora Åråsforsen samples, indicating a single brown trout gene pool in those two close spawning rapids..

Table 14. Statistical significance of genetic difference between brown trout parr samples from different sites and years in the River Gullspångsälven. Extremely significant = ***, Very significant =**, Significant=*, Not significant = NS.

Site and year	Stora Åråsf._18	Stora Åråsf._17	Stora Åråsf._16	Stora Åråsf._15	Lil. Åråsf._all	Gullspångsf._19	Gullspångsf._18	Gullspångsf._17	Gullspångsf._16	Gullspångsf._15	Others
Stora Åråsforsen 2019	NS	**	***	*	NS	***	***	***	***	***	***
Stora Åråsforsen 2018		NS	***	NS	NS	***	***	**	***	***	***
Stora Åråsforsen 2017			***	***	NS	***	***	***	***	***	***
Stora Åråsforsen 2016				***	***	***	***	***	***	***	***
Stora Åråsforsen 2015					***	***	***	***	***	***	***
Lilla Åråsforsen all years						***	***	**	***	***	***
Gullspångsf. 2019							NS	NS	***	*	***
Gullspångsf. 2018								NS	**	**	***
Gullspångsf. 2017									***	NS	***
Gullspångsf. 2016										***	***
Gullspångsf. 2015											***

Genetic differences between populations of Gullspångsforsen and Åråsforsen rapids were, however, statistically significant in all cases, and the pooled, probably local, upper river populations differed

also very clearly from all the other samples. As a result, brown trout samples from the Åråsforsen and Gullspångsforsen rapids were treated separately, but samples from the same rapids were pooled over the years in the further analyses.

For the integrated brown trout analysis, comparison of samples were added from other nearby brown trout populations draining into the Lake Vänern and from a hatchery population of Rottnan trout from the Gammelkroppa fish hatchery (Table 15).

Table 15. Brown trout parr samples from other tributaries draining into the Lake Vänern used for comparison to River Gullspångsälven brown trout population analysis.

	Sampling site		**N**
1	Borrälven, below and above migration hindrance, Tregångsbäcken	2016	84
2	Brattaälven, Ängsälven, Näveråsen, Lövfallsbäcken, below migration hindrance	2016	111
3	Granån, below Gransjön, above Bredsjön, above Lilla Gransjön, above Stora Gransjön	2016	79
4	Letälven, Aatorp	2016	21
5	Rottnan, roadside upstream	2016	36
6	Skagersholmsån, Kyrkvägen	2016	22
7	Gammelkroppa, hatchery stock	2016	30
	Total		**353**

5.1.2. Genetic diversity level in the brown trout populations

The mean diversity level varied between the lowest (47.2 %) of the brown trout from the Gammelkroppa hatchery stock and the highest (68.7 %) of the Gullspångsforsen and Skagersholmsån (Table 16). The diversity levels of all brown trout populations in the River Gullspångsälven (66.6 %, 68.7 %, 65.1 %) were above the overall mean level (61.2 %). The number of observed different alleles was highest in the Åråsforsen rapid population, but the sample size there was also large. The highest allelic richness per studied gene locus (6.2) was observed in the Borraälven brown trout population and the lowest, only nearly half of that, in the Gammelkroppa (3.2) hatchery reared population.

Table 16. Genetic diversity levels of brown trout populations in selected watersheds draining into Lake Vänern. Number of samples (N), percentage of mean diversity over loci (Div %), number of alleles (N All) and mean allelic richness per locus for 16 individuals (All Rich) is shown.

Population	**N**	**Div %**	**N All.**	**All. Rich.**	**Fis**
Åråsforsen	208	65.1	142	5.95	0.01
Gullspångsforsen	183	68.7	136	6.26	-0.01
Gullspångsälven upper reaches	40	66.6	108	5.88	0.16
Borraälven	84	65.5	126	6.20	0.04
Brattaälven	111	61.8	118	5.80	0.08
Granån	79	62.5	91	4.87	0.24
Letälven	21	55.0	67	4.19	-0.29
Rottnan	36	50.8	67	3.86	0.02
Skagersholmsån	22	68.7	95	5.89	0.01
Gammelkroppa, hatchery	30	47.2	52	3.20	0.01
All_W	**814**		**205**	**7.63**	
Mean		**61.2**	**100.2**	**5.21**	
Max		**68.7**	**142.0**	**6.26**	
Min		**47.2**	**52.0**	**3.20**	

5.1.3. Genetic differentiation among brown trout populations

All pairwise genetic differences among the analysed brown trout populations were statistically highly significant (P< 0.001), except that between Gammelkroppa hatchery population and Rottnan population, which was only significant (P < 0.05) (Table 17). Statistcal significance between population allele frequnecies occur relatively easily, even with small Fst-values with large sample sizes, so that genetic similarity results are more informative from the population differentiation point of view.

Table 17. Statistical significance of genetic difference between brown trout parr samples of different populations around the Lake Vänern. Extremely significant = ***, Very significant =**, Significant=*, Not significant = NS.

	Gullspångsforsen	Gullsp. Upper reaches	Borrälven	Brattaälven	Granån	Letälven	Rottnan	Skagersholmsån	Gammelkroppa
Åråsforsen	***	***	***	***	***	***	***	***	***
Gullspångsforsen		***	***	***	***	***	***	***	***
Gullsp. Upper reaches			***	***	***	***	***	***	***
Borrälven				***	***	***	***	***	***
Brattaälven					***	***	***	***	* **
Granån						***	***	***	***
Letälven							***	***	***
Rottnan								***	*
Skagersholmsån									***

When genetic differentiation between population pairs was measured with Fst-index, the smallest distance (Fst = 0.02) was found between the two rapids of Gullspångsälven; Gullspångsforsen and Åråsforsen (Table 18). The other populations in the upper reaches of the Gullspångsälven differed clearly more (Fst = 0.115 and 0.143) from those. Genetic differentiation was also low between the Gammelkroppa hatchery reared population and the Rottnan population, which are supposed to have the same origin. Other distances less than 0.1 were found between the population pairs: Åråsforsen - Letälven (Fst = 0.087), Borrälven - Rottnan (Fst = 0.079), Skagerholmsån - Åråsforsen (Fst = 0.098) and Skagerholmsån - Gullspångsforsen (0.077).

On average, the Gammelkroppa hatchery population was differentiated furthest from all the other populations. Its genetic differentiation from the Letälven brown trout population especially high (Fst = 0.322).

Table 18. Pairwise Fst-values among the studied brown trout populations in the Lake Vänern watershed. Values below 0.1 are highlighted with light blue colour.

Population	Gullsoångforsen	Gullspångsälv-others	Borrälven	Brattälven	Granån	Latälve	Rottnan	Skagerholmsån	Gammlekroppa
Åråsforsen	0.020	0.143	0.124	0.172	0.201	0.087	0.228	0.098	0.250
Gullspångsforsen	----	0.115	0.107	0.150	0.167	0.112	0.200	0.077	0.216
Gullspälv._others		----	0.122	0.129	0.134	0.216	0.222	0.142	0.242
Borrälven			----	0.091	0.101	0.180	0.079	0.149	0.103
Brattälven				----	0.167	0.245	0.198	0.181	0.210
Granån					----	0.253	0.112	0.198	0.125
Letälven						----	0.302	0.166	0.322
Rottnan							----	0.257	0.022
Skagersholmsån								----	0.273

The genetic distance tree, drawn on the basis of pairwise genetic distances (DA)(Nei et al., 1983) among the populations reflects the patterns of differentiation seen already in the FST values (Fig. 7). The Letälven population groups together with Åråsforsen population and they both are probably migratory populations, as is the Gullspångsforsen population, which groups together on the same branch. The Letälven population is most probably offspring from hatched Gullspångsälven trout released in Lake Skagern.

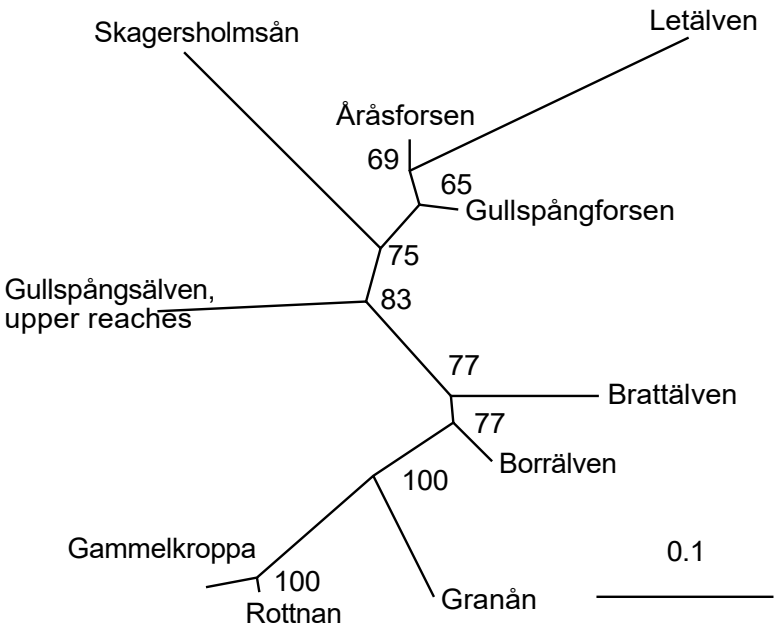

Figure 7. A neighbour-joining tree (NJ) with the genetic distances (D_A) among the brown trout populations from the drainages flowing into the Lake Vänern. Support for each brancing point is shown as percentages from 1000 bootstrap runs.

At the opposite end from the River Gullspångsälven trout in the genetic distance tree, is the Gammelkroppa hatchery population (originating from Rottnan), which resembles the Rottnan, Granån and Borrälven and Brattaälven populations. All bootsrap values were over 50 % and in general high.

The same genetic structure, in principle, remained when the egg samples collected from brown trout spawning nests, in 2016, 2018, and 2019, were added into the genetic distance analysis (Fig. 8). Both parr and egg samples from Gullspångsforsen and Åråsforsen grouped into their own groups and the resident brown trout populations formed a separate group from those. However, the populations from the upper reaches of the Gullspångsälven grouped closer to the populations from the other rivers from the Lake Vänern drainage than Gullspångsälven. Samples from adjacent years grouped mainly close to each other. Letälven grouped close to Åråsforsen here also.

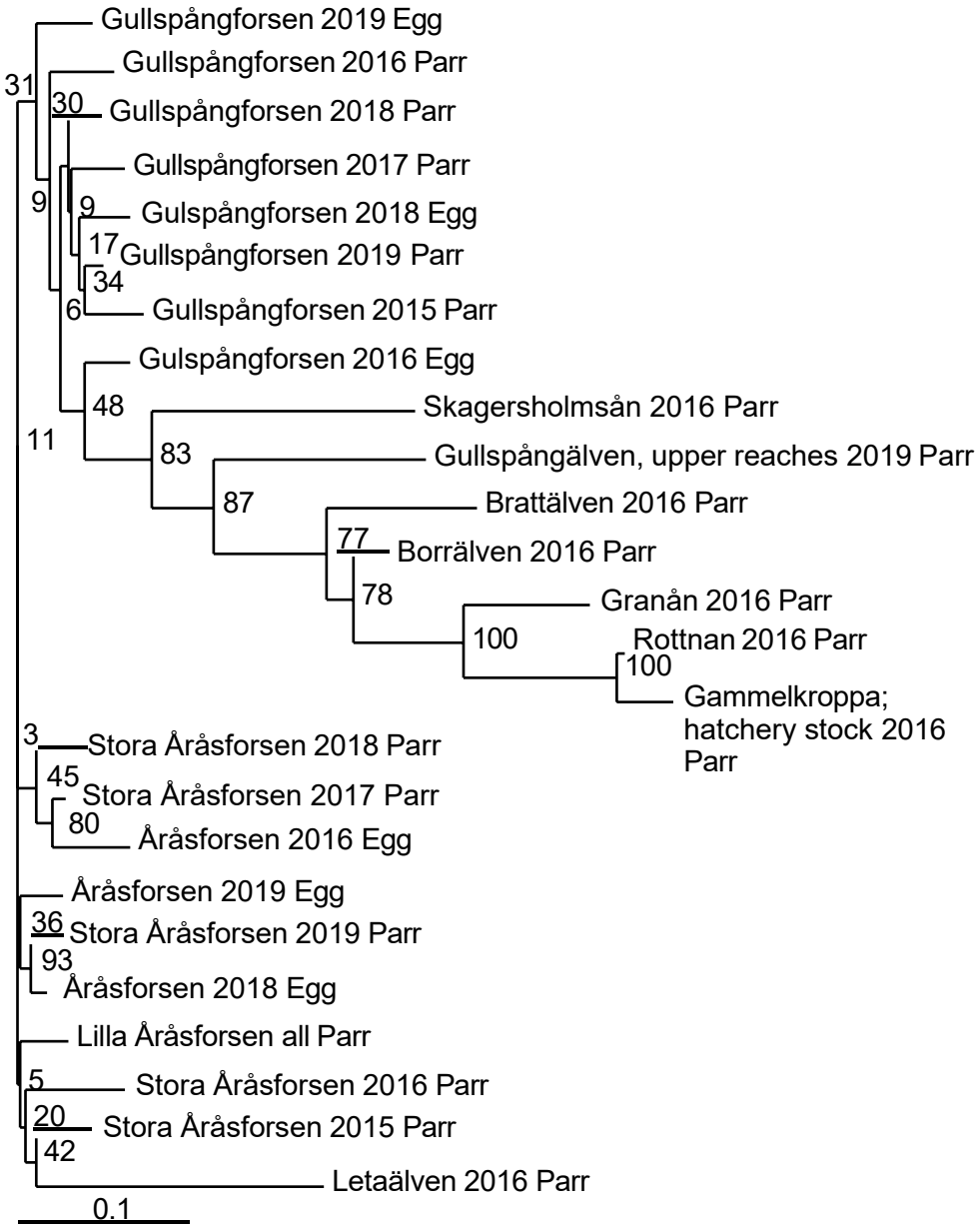

Figure 8. Genetic distances (D_A) among brown trout populations from the River Gullspångsälven and from other selected rivers of the Lake Vänern drainage.

5.1.4. Family structure of the brown trout populations

The individual genotype data of the brown trout parr samples included six recaptured two-year-old individuals, and the second samples of those individuals were omitted before analysing the data (Table 19). Suspected contaminations were also omitted, which resulted in slightly smaller sample sizes in these analyses.

Table 19. Identical genotypes in the dataeither contaminations in the samplings or the same individual brown trout parr samples recaptured in different years – which were omitted from the family structure analysis.

	Sample 1	Lenght/mm	Sample 2, removed	Lenght/mm
1	2015 Gullspångsf. 1a_150	-	2015 Gullspångsf. 1a_151	contamination
2	2015 Gullspångsf. 1a_157	-	2015 Gullspångsf. 1a_158	contamination
3	2015 Gullspångsf. 1a_196	-	2015 Gullspångsf. 1a_207	contamination
1	2018 Stora Årårsf. B_22	89	2019 Stora Årårsf. _Bk_076	192
2	2017 Stora Årårsf. Bi_14	115	2018 Stora Årårsf. B_7	185
3	2017 Stora Årårsf. Bi_55	90	2018 Stora Årårsf. B_21	163
4	2017 Lilla Årårsf. Bi_195	92	2018 Lilla Årårsf. B_80	154
5	2016 Stora Årårsf. Kv_123	260	2017 Stora Årårsf. Kv_61	290
6	2016 Lilla Årårsf. Kv_61	93	2017 Stora Årårsf. Kv_98	212

The annual number of brown trout families, or breeding pairs, spawning in the Gullspångsforsen was less than 30 (26.2), and the number of the breeders was about the same (27.2) (Table 20). The genetically effective population size (Ne) was only about half (22.0) of the minimum recommendation of over 50. Relatedness among the offspring was 5.2 %, which also exceeded the recommended level (3%), but it was not as high as among the Gullspångsforsen salmon, whose relatedness was 12.7 % on average.

The number of spawning families in the Åråsforsen brown trout population was on average somewhat higher (30.2) than in the Gullspångsforsen (26.2), but the number of breeders was about the same (27.2 in Gullspångsforsen and 26.8 in Åråsforsen). The effective population size was not higher (mean 19.4) than in the Gullspångsforsen (mean 22.0)(Table 20). The mean relatedness in the Åråsforsen population was even slightly higher (8.3 %) than in the Gullspångsforsen (6.9 %), meaning that the same number of spawners produced more offspring in the Åråsforsen.

In case all brown trout spawners had mixed evenly over the five years the estimated number of spawners had increased over 100 spawners for both rapids (Gullspf. 116; Åråsf. 109), and their effective sizes had increased the minimum requirement of Ne = 50, recommended to prevent inbreeding depression (Gullspf. Ne = 79 (59-109); Åråsf. Ne = 68 (48-96)) (Table 20). If all the brown trout spawners would have mixed evenly over the five years and across the two rapids, the total effective number of spawners would have been 137 (109-175), which is more than double the recommended level of 50 spawners.

Overlapping of year classes increases the total effective sizes of total spawning population from that of the annual level somewhat, but complete overlapping of five years did not occur, altought overlapping of year classes was much more common in brown trout than in lake salmon population. The estimate of whole spawning population effective size is thus probably somewhat over the annual level for each rapid, but less than the pooled five year estimate, over 20 but less than 70.

Table 20. Family structure of the Gullspångsälven brown trout populations from the two rapid sections each sampling year with the means calculated from pooled data over all the years (2015-2018 for Gullspångsforsen rapid, and 2015-2019 for Åråsforsen rapid), and the mean estimate for the pooled data from both Gullspånsgälven rapids from all the sampling years (Gullspf + Åråsf.). Shown are the total Number of studied offspring in each river (N), the number of observed families (= breeding pairs; N Family), the number of solved parents (N Breeders) for each sex separately and together (N Breed), and the maximum number of siblings in the observed families (Max Sib). In addition, the estimate of genetically effective size of the sampled population (Ne) with its 95 % confidence limits (95%), the effective size versus the true number of breeders ratio (Ne/Nb), and the mean pairwise relatedness of all the individuals in the sample as a percentage (Relatedness %) are shown.

Brown trout			Parents		Family size				
Recommends		**> 50**	**> 100**	**> 100**		**> 50**		**> 0.5**	**< 3.0**
	N Offsp.	N Family	N Breeders	N Breed	Max Sib	Ne	Ne CI 95%	Ne/Nb	Related. %
Gullspf. 2019	50	34	19+19	38	6	32	20-54	0,8	5,7
Gullspf. 2018	35	29	14+16	30	3	27	16-48	0,9	6,2
Gullspf. 2017	22	22	11+10	21	1	20	11-39	1,0	5,7
Gullspf. 2016	33	20	10+11	21	8	14	7-30	0,7	8,7
Gullspf. 2015	40	26	11+13	26	9	17	9-34	0,7	8,0
Mean/ year	36	26,2		27,2	5,4	22,0		0,8	6,9
Gullspångf- 15-19	**180**	**138**	**56+60**	**116**	**9**	**79**	**59-109**	**0,7**	**4,9**
Åråsf. 2019	44	36	15+18	33	7	24	15-43	0,7	7,1
Åråsf. 2018	22	22	11+11	22	1	21	11-42	1,0	6,2
Åråsf. 2017	62	40	14+20	34	4	24	14-42	0,7	7,8
Åråsf. 2016	47	36	13+16	29	4	18	10-35	0,6	8,7
Åråsf. 2015	27	17	6+10	16	7	10	5-23	0,6	11,9
Mean/ year	40,4	30,2		26,8	4,6	19,4		0,7	8,3
Åråsf. 15-19	**202**	**154**	**53+56**	**109**	**7**	**68**	**48-96**	**0,6**	**5,3**
Gullspf.+Åråsf. 2015-2019	**382**	**285**	**109+116**	**225**	**9**	**137**	**109-175**	**0,6**	**4,8**
Gullspälv_Local	40	18	11+10	21	7	16	9-34	0,8	9,2

5.1.5. Proportion of repeat spawning in the Gullspångsälven brown trout population

For repeat spawning analysis, all the 2-year-old parr samples were removed from the data as they cause bias in the analysis. Consequently, 175 (-5 2-year-old) offspring were available for Gullspångsforsen and 184 (-18 2-year-old) for Åråsforsen. Some 2-year old parr were already removed in connection to omitting identical genotypes (-6). In the whole Gullspångsälven data set, the percentage of 2-year old brown trout smolt samples was 2.3 % (29/391).

Overall, 107 brown trout spawners (50 males and 57 females; sex designated by the program; see methods) were identified in Gullspångsforsen during the years 2015-2019. More than half of the trout spawners, 58 (54.2 %), had offspring in several year classes of one-year-old parr. In all, 72 out of 107 spawning events involved previously spawned parents. Thus, over half (67.3 %) of all the spawning events were repeated spawnings (Table 21).

Table 21. List spawners, deduced from the offspring data, which had had offspring in several years in the Gullspångsforsen brown trout population.

	Parent ID	Year	N year
1	Female 12	2015, 2018, 2019	3
2	Female 14	2016, 2019	2
3	Female 15	2015, 2019	2
4	Female 16	2017, 2019	2
5	Female 17	2016, 2019	2
6	Female 18	2015, 2018, 2019	3
7	Female 19	2018, 2019	2
8	Female 20	2016, 2019	2
9	Female 21	2017, 2018, 2019	3
10	Female 22	2017, 2019	2
11	Female 23	2016, 2019	2
12	Female 25	2016, 2018	2
13	Female 27	2017, 2018	2
14	Female 27	2017, 2018	2
15	Female 28	2015, 2016, 2018	3
16	Female 30	2015, 2018	2
17	Female 32	2016, 2018	2
18	Female 34	2015, 2018	2
19	Female 37	2015, 2017, 2018	3
20	Female 38	2015, 2017, 2018	3
21	Female 40	2015, 2018	2
22	Female 44	2016, 2017	2
23	Female 47	2015, 2016	2
24	Female 48	2015, 2016	2
25	Female 5	2017, 2019	2
26	Female 51	2015, 2016	2
27	Female 52	2015, 2016	2
28	Female 6	2017, 2019	2
29	Female 7	2016, 2019	2
30	Female 8	2015, 2017, 2019	3
31	Female 9	2016, 2019	2
32	Male 1	2016, 2017, 2018, 2019	4
33	Male 13	2018, 2019	2
34	Male 14	2017, 2019	2
35	Male 15	2017, 2019	2
36	Male 16	2017, 2019	2
37	Male 17	2018, 2019	2
38	Male 18	2015, 2019	2
39	Male 19	2018, 2019	2
40	Male 22	2018, 2019	2
41	Male 24	2015, 2016, 2019	3
42	Male 25	2015, 2017, 2019	3
43	Male 27	2017, 2018	2
44	Male 28	2016, 2018	2
45	Male 29	2015, 2018	2
46	Male 3	2016, 2019	2
47	Male 30	2017, 2018	2
48	Male 32	2015, 2018	2
49	Male 33	2017, 2018	2
50	Male 34	2017, 2018	2
51	Male 35	2017, 2018	2
52	Male 36	2015, 2018	2

53	Male 38	2015, 2018	2
54	Male 39	2016, 2018	2
55	Male 41	2015, 2017	2
56	Male 45	2015, 2016	2
57	Male 5	2018, 2019	2
58	Male 7	2017, 2018, 2019	3
			128

For Åråsforsen rapid brown trout population, the situation with respawning was very similar to that of the Gullspångsforsen rapid. From the 97 spawners, 51 were classified as males and 46 as females. From those 97 spawners, 48 individuals (49.5 %) had offspring in more than one year class. From the total of 97 spawning events, 65 (63 % of all matings) involved fish that had spawned already once previously (Table 22).

Half-sib offspring from different years were usually only from two different year classes, but in some cases from three and even four year classes. Respawning was much more common in the brown trout population than in the lake salmon population. This may be a result from the possibly more common precociousness of males in brown trout population. In some cases, offspring was found among samples spanning so many years that precocious spawning is likely to explain the results. There may also be non-migratory individuals, which may partly explain the high respawning numbers.

The sexes of the parents assigned by the software are arbitrary, so they cannot be used for explaining spawning behaviour of the sexes. In addition, all the individuals with relatedness values corresponding to that of halfsibs were interpreted as being true half-sibs, so that these estimates probably overestimate respawning to some extent. However, respawning is likely to be more common in the brown trout populations than in salmon populations, based already on previous information on the life cycle of these two species. In the Baltic Sea, salmon respawning is quite rare and the recovery of salmon from spawning is weaker than that of brown trout, possibly because the longer fasting and more stressful spawning migration. Baltic salmon stops feeding when they start spawning migration from the Baltic Main Basin.

Moving of spawners between the Gullspångsforsen and Åråsforsen rapids was much more common among the mature brown trout than among the lake salmon. As many as 36 out of 226 spawners (15.9 %) had spawned in both rapids. This number is so high that, if it describes the true situation, no temporally stable genetic differentiation should occur among the brown trout populations of these two rapids (Table 23).

Table 22. List of the offspring of the deduced spawners, who had had offspring in more than one year in the Åråsforsen brown trout population.

	Parent ID	Year/rapid	N year
1	Female 02	2015 Lilla Å., 2016 Lilla Å., 2017 Stora Å., 2018 Stora Å.	4
2	Female 03	2015 Lilla Å., 2017 Stora Å., 2018 Stora Å.	3
3	Female 05	2015 Lilla Å., 2019 Stora Å.	2
4	Female 06	2015 Lilla Å., 2018 Stora Å., 2019 Stora Å.	3
5	Female 08	2015 Lilla Å., 2017 Stora Å.	2
6	Female 09	2015 Lilla Å., 2017 Stora Å.	2
7	Female 11	2015 Lilla Å., 2019 Stora Å.	2
8	Female 13	2016 Stora Å., 2019 Stora Å.	2
9	Female 17	2016 Stora Å., 2019 Stora Å.	2
10	Female 19	2016 Stora Å., 2017 Stora Å., 2018 Stora Å.	3
11	Female 22	2017 Stora Å., 2018 Lilla Å., 2018 Stora Å.	2
12	Female 24	2017 Stora Å., 2018 Stora Å., 2019 Stora Å.	3
13	Female 26	2017 Stora Å., 2019 Stora Å.	2
14	Female 27	2017 Stora Å., 2019 Stora Å., 2019 Lilla Å.	2
15	Female 29	2017 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
16	Female 30	2017 Stora Å., 2017 Lilla Å., 2019 Stora Å.	2
17	Female 34	2018 Stora Å., 2019 Stora Å.	2
18	Female 36	2018 Stora Å., 2019 Lilla Å.	2
19	Female 37	2018 Stora Å., 2019 Lilla Å., 2019 Stora Å.	2
20	Female 38	2018 Stora Å., 2019 Lilla Å.	2
21	Male 03	2015 Lilla Å., 2017 Stora Å., 2018 Stora Å., 2019 Stora Å.	4
22	Male 04	2015 Lilla Å., 2016 Stora Å., 2017 Stora Å.	3
23	Male 05	2015 Lilla Å., 2017 Stora Å., 2019 Stora Å.	3
24	Male 07	2015 Lilla Å., 2018 Stora Å., 2019 Stora Å.	3
25	Male 08	2015 Lilla Å., 2016 Stora Å.	2
26	Male 09	2015 Lilla Å., 2018 Stora Å., 2019 Stora Å.	3
27	Male 10	2015 Lilla Å., 2018 Lilla Å., 2018 Stora Å., 2019 Stora Å.	3
28	Male 12	2015 Lilla Å., 2016 Stora Å., 2018 Stora Å.	3
29	Male 13	2015 Lilla Å., 2016 Stora Å., 2019 Stora Å.	3
30	Male 14	2016 Stora Å., 2017 Stora Å.	2
31	Male 15	2016 Stora Å., 2017 Stora Å.	2
32	Male 16	2016 Stora Å., 2019 Stora Å.	2
33	Male 17	2016 Stora Å., 2017 Stora Å.	2
34	Male 20	2016 Stora Å., 2017 Stora Å., 2019 Stora Å.	3
35	Male 21	2016 Stora Å., 2019 Stora Å.	2
36	Male 22	2016 Stora Å., 2018 Stora Å.	2
37	Male 23	2016 Stora Å., 2019 Stora Å.	2
38	Male 25	2016 Stora Å., 2016 Lilla Å., 2018 Lilla Å.	2
39	Male 26	2016 Lilla Å., 2019 Lilla Å.	2
40	Male 29	2017 Stora Å., 2019 Stora Å.	2
41	Male 31	2017 Stora Å., 2019 Stora Å.	2
42	Male 32	2017 Stora Å., 2019 Stora Å.	2
43	Male 34	2017 Stora Å., 2019 Stora Å.	2
44	Male 36	2017 Stora Å., 2018 Stora Å., 2019 Stora Å.	3
45	Male 38	2017 Stora Å., 2019 Stora Å.	2
46	Male 42	2018 Stora Å., 2019 Lilla Å.	2
47	Male 43	2018 Stora Å., 2019 Stora Å.	2
48	Male 45	2018 Stora Å., 2019 Stora Å.	2
			113

Table 23. Brown trout spawners in the Gullspångsälven that had spawned in both the Gullspångsforsen and Åråsforsen rapids.

	Parent ID	Year/rapid/site
1	Female 04	2015 LilÅrBi, 2019 Gullspf.
2	Female 17	2015 Gullspf., 2019 Gullspf., 2019 StorÅBk
3	Female 21	2017 StorÅbi, 2017 LilÅrBi, 2019 Gullspf.
4	Female 22	2015 LilÅrC1, 2015 Gullspf., 2019 Gullspf.
5	Female 24	2015 Gullspf., 2017 StorÅNe, 2019 Gullspf.
6	Female 25	2016 Gullspf., 2016 LilÅrBi, 2016 StorÅkv, 2019 Gullspf.
7	Female 37	2017 StorÅbi, 2018 Gullspf.
8	Female 42	2018 Gullspf., 2019 StorÅne
9	Female 50	2015 LilÅrbi, 2015 Gullspf., 2016 Gullspf., 2017 Gullspf., 2019 StorÅNe
10	Female 52	2016 Gullspf., 2017 Gullspf., 2019 StorÅNe
11	Female 53	2017 LilÅrSN, 2017 Gullspf.
12	Female 54	2016 Gullspf., 2019 LilÅrHi
13	Female 55	2016 Gullspf., 2019 LilÅrC1
14	Female 57	2015 Gullspf., 2016 Gullspf., 2016 LilÅrbi
15	Female 61	2015 LilÅrhi, 2016 Gullspf.
16	Female 65	2015 Gullspf.2018 StorÅB
17	Femalle63	2015 Gullspf., 2016 StorÅLA, 2016 StorÅKv, 2019 StorÅne
18	Male 07	2017 StorÅBi, 2017 StorÅKv, 2017 StorÅne, 2019 Gullspf.
19	Male 10	2017 StorÅbi, 2019 Gullspf.
20	Male 13	2015 Gullspf., 2018 Gullspf., 2019 Gullspf., 2018 StorÅne
21	Male 17	2015 Gullspf., 2019 Gullspf., 2019 StorÅne, 2019 StorÅBk
22	Male 23	2019 Gullspf., 2019 LilÅr
23	Male 24	2016 LilÅrBi, 2016 StorÅKv, 2018 Gullspf., 2019 Gullspf.
24	Male 26	2015 LilÅrhi, 2015 Gullspf., 2018 Gullspf.
25	Male 28	2015 Gullspf., 2015 LilÅc1, 2018 Gullspf., 2018 StorÅB
26	Male 32	2015 LilÅrbi, 2015 Gullspf., 2018 Gullspf.
27	Male 36	2017 StorÅBI, 2017 StorÅkv, 2018 Gullspf.
28	Male 37	2015 Gullspf., 2018 Gullspf., 2019 StorÅBk
29	Male 38	2016 LilÅBi, 2016 Gullspf., 2018 Gullspf.
30	Male 43	2015 Gullspf., 2016 Gullspf., 2016 StorÅLA, 2018 Gullspf.
31	Male 51	2017 Gullspf., 2018 StorÅNE, 2019 StorÅNE, 2019 StorÅBk
32	Male 63	2016 Gullspf., 2019 StorÅBk
33	Male 64	2015 LilÅrBi, 2016 Gullspf.
34	Male 65	2015 Gullspf., 2017 StorÅKv, 2017 StorÅLA
35	Male 67	2015 Gullspf., 2019 StorÅB
36	Male 69	2015 Gullspf., 2017 LilÅbi, 2017 StorÅne

6. Conclusion of brown trout analysis

The genetic diversity level of Gullspångsälven brown trout population was not low, but rather above the level of the other compared populations, some of which, however, were non-migratory, local populations. The only population which had a clearly decreased diversity level was the Gammelkroppa hatchery population (Rottnan trout). Genetic differentiation among the brown trout populations varied considerably, which is understandable as some of the populations were isolated.

The annual genetically effective population sizes were markedly higher for brown trout than for lake salmon. Mean annual effective population size for the Gullspångsforsen salmon was 8.0 individuals, when it was 22.0 individuals for trout. For Åråsforsen the numbers were 12.2 for salmon and 19.4 for trout. Repeated spawning from all spawning events was also clearly more common among brown trout (67 % – 63 %) spawners than salmon spawners (20 % – 25 %), which increases the total effective size of the populations and ensures more efficent transfer of genes, by keeping the number of mixing spawners higher and increasing overlapping of year classes.

Based on the half-sib data the moving of spawners between Gullspångsforsen and Åråsforsen was more common for brown trout than salmon spawners. For the salmon population it seemed to be very rare, about 4 % of spawners had changed the spawning rapid, when for brown trout 16 % of spawners had been spawning in both rapids. According to this, homing of lake salmon is much more precise than that of brown trout. It is possible that part of the brown trout population is local, or remains in the river over the winter and moves there more along the river, or precocious males are more common for brown trout, which markedly increases the number of potential spawning years. The range of spawning years was also wider for brown trout trout.

These spawning behaviour factors increase the total effective population sizes from those of the annual estimates for brown trout more than for lake salmon, so it probably increases the probability of long term survival of the population.

Species hybridization is waste of reproduction potential and is usually an indication of disturbance in the spawning environment or circumstances in general. Increased hybridization between salmon and trout has been observed for example in the Baltic Sea area (in the River Dalälven), when the M74-syndrom incidence is high among salmon spawners, and the condition of the spawners is weak (Jansson *et al.*, 1991). In the Gullspångsälven, one explanation may be that the competition of the spawning grounds between species in the rebuilt environment is disturbing spawning behavior or the two species don’t have equal oportunities to reach the upper river spawning grounds.

The first requirement for an enhancement project is that there is sufficient spawning ground space and parr habitat for effective reproduction. Migration obstacles should be moved as spawners should be able to reach the potential spawning grounds. Spawning ground area limits the population size, as reproduction can occur only to the extent spawning habitat is available.

7. Hybridization between salmon and trout

In the Gullspångsälven, out of 1621 parrs and eggs that could be reliably genotyped, 78 (4.8 %) were genetically identified to be hybrids (Table 24). It is quite common to find hybrids in spawning grounds, where salmon and trout co-exist. The number of hybrid parr has been remarkable in some cases in both Europe (Garcia de Leaniz and Verspoor, 1989; Jansson *et al.*, 1991; Hurrell and Price, 1993; Matthews *et al.*, 2000), and in North America (Verspoor, 1988; McGowan and Davidson, 2011) where brown trout is an invasive species.

A detailed look at the three sites (Gullspångsforsen, Lilla Åråsforsen and Stora Åråsforsen) shows that hybridization occurs mainly at Gullspångsforsen, where 66 of the hybrids were found. Nearly all of the Gullspångsforsen hybrids (60) were identified in the parr samples. Half of these (30) were among the 2018 parrs, and the percentage of hybrids in the 2018 parrs was 36.6 %. There were 15 hybrids (12.7 %) among the 2015 parrs. The percentage of hybrids was also quite high (over 10 %) among the 2019 and 2017 parrs.

No clear trend (increase or decrease) in the number of hybrids over the years was seen. The year 2018 (18.5 %) in the Gullspångsforsen should be seen as an exceptional case, as the percentage of hybrids was in general quite low.

No hybrids were found among the 2015 and 2016 egg samples, and the percentages of hybrids were quite moderate in all egg samples. The highest percentages were in the Gullspångsforsen, but percentages were clearly lower in the egg samples than in the parr samples (Table 24).

The female parents of the hybrids were identified in three sets of samples (parrs from 2019 and 2018, and eggs from 2019, 2019 spawned/2020 sampled). Two of the analysed hybrids had salmon as a mother and all the others (36) a trout as a mother. Thus salmon males succeeded to spawn with trout females more often than vice versa.

Table 24. Atlantic salmon and brown trout hybrids in the River Gullspångsälven. N is the total number of fish sampled in each site, N trout = the number of sampled trout, N salmon = the number of sampled salmon, the number of hybrids (N Hyb.) and their percentage (Hyb. %). The numbers of trout and salmon mothers of the hybrids are also shown for the three samples where the species of the mothers in the hybrid matings were analysed.

Sample site	Year	N all	N trout	N salmon	N Hyb.	Hyb. %	Trout mother	Salmon mother
Gullspångsälven parr	**2019**	**194**	**97**	**90**	**7**	**3,6**	**7**	
Gullspångsforsen		57	50	1	6	**10,5**	6	
Lilla Åråsforsen		38	9	28	1	**2,6**	1	
Stora Åråsforsen		99	38	61				
Gullspångsälven egg	**2019/2020**	**75**	**61**	**13**	**1**	**1,3**		**1**
Gullspångsforsen		26	24	1	1	**3,8**		1
Lilla Åråsforsen		27	21	6				
Stora Åråsforsen		22	16	6				
Gullspångsälven egg	**2018/2019**	**142**	**82**	**55**	**5**	**3,5**		
Gullspångsforsen		28	26		2	**7,1**		
Lilla Åråsforsen		72	33	36	3	**4,2**		
Stora Åråsforsen		42	23	19				
Gullspångsälven parr	**2018**	**162**	**61**	**71**	**30**	**18,5**	**29**	**1**
Gullspångsforsen		82	35	17	30	**36,6**	29	1

Lilla Åråsforsen		13	3	10				
Stora Åråsforsen		67	23	44				
Gullspångsälven egg	**2017**	**123**	**72**	**46**	**5**	**4,1**		
Gullspångsforsen		42	25	14	3	**7,1**		
Lilla Åråsforsen		47	31	15	1	**2,1**		
Stora Åråsforsen		34	16	17	1	**2,9**		
Gullspångsälven parr	**2017**	**234**	**87**	**140**	**7**	**3,0**		
Gullspångsforsen		37	22	11	4	**10,8**		
Lilla Åråsforsen		32	8	23	1	**3,1**		
Stora Åråsforsen		165	57	106	2	**1,2**		
Gullspångsälven egg	**2016**	**74**	**45**	**29**				
Gullspångsforsen		28	23	5				
Lilla Åråsforsen		26	14	12				
Stora Åråsforsen		20	8	12				
Gullspångsälven parr	**2016**	**283**	**82**	**196**	**5**	**1,8**		
Gullspångsforsen		85	34	46	5	**5,9**		
Lilla Åråsforsen		35	5	30				
Stora Åråsforsen		163	43	120				
Gullspångsälven parr	**2015**	**248**	**70**	**161**	**17**	**6,9**		
Gullspångsforsen		118	43	60	15	**12,7**		
Lilla Åråsforsen		40	2	38				
Stora Åråsforsen		90	25	63	2	**2,2**		
Gullspångsälven egg	**2015**	**49**	**30**	**19**				
Gullspångsforsen		21	17	4				
Lilla Åråsforsen		19	12	7				
Stora Åråsforsen		9	1	8				
Gullspångsälven egg	**2014**	**37**	30	6	1	**2,7**		
Gullspångsforsen		15	13	2				
Lilla Åråsforsen		14	10	3	1	**7,1**		
Stora Åråsforsen		8	7	1				
Gullspångsälven parr, all	**2015-2019**	**1121**	**397**	**658**	**66**	**5,9**		
Gullspångsforsen		379	184	135	60	**15,8**		
Lilla Åråsforsen		158	27	129	2	**1,3**		
Stora Åråsforsen		584	186	394	4	**0,7**		
Gullspångsälven egg, all	**2014-2020**	**500**	**320**	**168**	**12**	**2,4**		
Gullspångsforsen		160	128	26	6	**3,8**		
Lilla Åråsforsen		205	121	79	5	**2,4**		
Stora Åråsforsen		135	71	63	1	**0,7**		
Gullspångsälven egg, TOTAL	**2014-2020**	**1621**	**717**	**826**	**78**	**4,8**		
Gullspångsforsen		539	312	161	66	**12,2**		
Lilla Åråsforsen		363	148	208	7	**1,9**		
Stora Åråsforsen		719	257	457	5	**0,7**		

The sampled parrs were visually identified as salmon, trout or hybrid in the field before the samples were delivered to the laboratory in Helsinki for genotyping. In each year there were a few differences between visual and genetic identifications but in the 2018 parr sample the visual and genetic identifications differed significantly (Table 25). A few differences were seen in Lilla Åråsforsen and Stora Åråsforsen, but in Gullspångsforsen, there were many differences. The differences clearly demonstrate the difficulty in identifying hybrid parrs visually, as the visual characteristics of hybrids can be either mainly trout-like or salmon-like.

Table 25. Comparison of visual and genetic species identification of Atlantic salmon and brown trout parr in the 2018 samples, at each sampling site. Field-visual identification and Genetic-DNA columns show the number of observed salmon, trout, and hybrid offspring.

Parr samples	***N***	**Field - visual**			**Genetic - DNA**			**OBS**
		trout	**salmon**	**hybrid**	**trout**	**salmon**	**hybrid**	
Gullspångsforsen, stn 1a	*37*	28		9	9	10	18	**!!!**
Gullspångsforsen, stn 1b	*2*			2		2		**!!!**
Gullspångsforsen, stn 2	*43*	33	2	8	26	5	12	**!!!**
Lilla Åråsforsen, Biotopkanalen	*1*	1			1			
Lilla Åråsforsen, inom C1	*3*	1	1	1	1	2		
Lilla Åråsforsen, mellan oarna H o I	*3*	1	1	1	1	2		
Lilla Åråsforsen, sydsidan nedre	*6*		6			6		
Stora Åråsforsen, Biotopkanalen	*24*	13	11		12	12		
Stora Åråsforsen, Kvarnen	*28*	5	23		5	23		
Stora Åråsforsen, Laxstationen	*3*		3			3		
Stora Åråsforsen, nedan elledning	*12*	5	7		6	6		
Total	***162***	**87**	**54**	**21**	**61**	**71**	**30**	

In one hybrid parr from 2017, alleles from both species could be seen only in 2 out of the 4 diagnostic loci. In the two other loci, only salmon alleles could be identified. Usually F1- species hybrids have one allele from trout and one allele from salmon in all loci, but sometimes the PCR-amplification of DNA results only one allele (either trout or salmon) in a particular locus, this being much stronger than the other. This can be a result of the tetraploidy background of both species.

This hybrid in question can also be a rare backcross between a salmon and a hybrid fish, as the pattern of the alleles is what can be expected from a back-cross individual. It is also possible, that for some reason, the PCR amplification of trout alleles in some loci was so weak, that they could not be identified. Backgrossing could not be verified.

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Natural Resources Institute Finland
Latokartanonkaari 9
FI-00790 Helsinki, Finland
tel. +358 29 532 6000