Effect of fragmentation and lack of precise homing on population structure in European grayling (*Thymallus thymallus)* within a large Norwegian river system

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Kristine Dalen
Oslo, 01.06.16

Abstract

Fragmentation of habitats is one of the major threats to biodiversity. Fragmentations will reduce connectivity and consequently affect the genetic population structure particularly in rivers. In the current study the genetic population structure of grayling *Thymallus thymallus* in a large Norwegian river system with both unobstructed and fragmented sections was investegated. Further, it was assessed whether grayling exhibited homing and thereby were genetically structured within the large unobstructed river section of the river system in question. This was done by using telemetry data of 144 radio tagged grayling, to assign individuals in specific spawning locations. Genotyping data on 12 microsatellite markers was then used to infer, the genetic structure of the individuals from the different spawning locations. To infer the effects of migration barriers on genetic population structure, the population structure in the whole river system was investigated, which contained both a natural and anthropogenic migration barrier. No genetic structuring for grayling in relation to spawning fidelity in the unobstructed river section was found. This may indicate that grayling have no precise homing to particular spawning locations and probably move among different spawning habitats in the river section throughout their lives. Four genetically distinct populations in the whole river system was found, which seemed to be separated by the migration barriers. The migration barriers seemed also to direct the gene flow downstream, which have lead to the highest genetic diversity being found downstream. The anthropogenic migration barrier gave indications of acting as a stronger migration barrier to downstream gene flow compared to the natural migration barrier. Identifying present genetic structure as a consequence of geographic isolation or the use of different spawning habitats is important for understanding and retaining connectivity for fish in rivers.

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Introduction

Fragmentation is a worldwide threat to biodiversity within river systems, destroying connections between habitats and therefore leading to the loss of habitats (Helfman, 2007; Ward, Tockner, & Schiemer, 1999; Zwick, 1992). River systems can be complex and offer many different biotic and abiotic habitats (Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Connectivity in river systems is important for a wide variety of fish species around the world (Ward et al., 1999). In unobstructed rivers fish can move among these habitats to exploit these different resources that may be spatially distributed, and will often use different sections of river systems during seasons and life stages. Fish movements to appropriate habitats can enhance their growth, survival and therefore their fitness (Lucas & Baras, 2001).

Fragmentation of rivers is known to affect connectivity by reducing or completely obstructing the connection between habitats (Collares-Preira, Coelho & Cowx, 2002; Taylor, 1993). Fragmentations could be due to natural causes, like occurrence of a waterfall, or human induced through constructions of weirs or dams. Both natural and human created structures can act as barriers to movements of fish in rivers (Fuller, Doyle & Strayer, 2015), which may result in loss of access to essential habitats (Bunn & Arthington, 2002; Collares-Preira et al., 2002). In turn this may lead to reduced population sizes (Deiner, Garza, Coey & Girman, 2007; Lowe & Allendorf, 2010).

Alteration of connectivity in rivers can affect the genetic population structure of fish in rivers (Poissant, Knight & Ferguson, 2005). Migration barriers often hamper dispersal of fish among habitats, which may reduce gene flow between populations (Helfman, 2007). The gene flow may be directed downstream, while gene flow may be limited or absent in upstream direction (Gouskov, Reyes, Bitterlin, & Vorburger, 2015). Consequently, genetically distinct populations may develop over time (Yamamoto, Morita, Koizumi, & Maekawa, 2004). Fully isolated populations may experience high genetic drift and reduced genetic variability, particularly if they are small (Slatkin, 1985). Combined with a reduction in the number of individuals in populations, this may put populations at risk for the negative effects of inbreeding or local (extinction) (Helfman, 2007).

Salmonids such as salmon and trout are often structured into more or less distinct populations within rivers (Hendry & Stearns, 2004). Besides being geographically structured by natural or human induced fragmentations (Deiner et al., 2007; Wofford, Gresswell, & Banks, 2005), their requirements for spawning habitats with well-defined characteristics may

also lead to individuals being separated in space during spawning (Quinn, 1999). Salmonids also often return to their birthplace for spawning, called homing (Hendry et al., 2004; Stabell, 1984). This implies that population may develop genetic structuring – and even lead to adaptation (Primmer et al., 2006; Hendry et al., 2004; Garant, Dodsen, Bernatchez, 2000). Genetic structuring is often observed within large river systems, where the level of genetic differentiation may increase with geographic distance (Perrier, Guyomard, Bagliniere, & Evanno, 2011; Estoup et al., 1998; Wright, 1943). However, genetic differentiation can also be observed over very short geographic distances (Garant, Dodson, & Bernatchez, 2000; Taylor, 1991) for instance shown for brown trout (*Salmo trutta)* (Carlsson, Olsén, Nilsson, Øverli, & Stabell, 1999; Crozier, & Ferguson, 1986). To summarize, salmonids can be genetically structured both by the process of natal homing and through the effect of physical isolation due to fragmentations (Wofford et al., 2005; Hendry, 2004).

Through the increased demand of renewable energy a large numbers of rivers have become fragmented as a result of hydropower devolvement and dam building. Such constructions heavily affect the connectivity and structure in rivers. Weirs have been shown to act as migration barriers for fish in rivers (Jungwirth, 1998) and to separate fish populations (Wofford et al., 2005). The gene flow between habitats may be hampered or often restricted to only occur in the downstream direction (Gouskov et. al., 2015). The drainage topology naturally also direct the gene flow downstream (Morrissey & Kerckhove, 2009). The allelic richness may then be larger downstream than upstream, resulting in isolated upstream populations with low genetic variability (Gouskov et al., 2015). However, there is still need for more investigation of the effect of dams and weirs on population structure and also compare the effects of anthropogenic and natural migration barriers (Wofford et al., 2005).

Understanding the genetic structure of fish in rivers and how this is affected by fragmentation are important for conservation and management of fish (Helfman, 2007). By knowing the present structure within unobstructed rivers, different genetic populations can be managed separately (Ferguson, 1989) and new hydropower constructions could be avoided in areas with important connectivity (Junge et al., 2014). By identifying the effects of weirs, necessary restorations or actions to establish connectivity can be uncovered.

Therefore in this study I investigate the genetic population structure for a fish species in a large river system in Norway, with both obstructed and unobstructed areas**.** The study system is the river Gudbrandsdalslågen including the Lake Lesjaskogsvatnet, and its main

tributary the river Otta, in the south-eastern Norway. This river system is one of the largest river systems in Norway. I investigated fish movements in a section of this river system that is fragmented by two hydropower stations and a natural waterfall. In between these barriers there is a large unobstructed area. The study species is the European grayling *Thymallus thymallus* (from now on referred to simply as grayling), from the salmonid family. As a potamodromous species, grayling move extensively within freshwater bodies, among preferable feeding, overwintering and spawning habitats (Bass, Haugen, & Vøllestad, 2013; Northcote, 1997; Nykänen, Huusko, & Lahti, 2004). Grayling often make long movements within large river systems (Heggenes, Qvenild, Stamford, & Taylor, 2006), but some individuals move only over shorter distances or are stationary (Northcote, 1997).

Fragmentation by weirs have shown to hamper such movements of grayling and to affect their population structure (Junge, Museth, Hindar, Kraabøl, & Vøllestad, 2014; Meldgaard, Nielsen, & Loeschcke, 2003). The gene flow in such rivers has been observed to be restricted downstream and populations to be genetically separated by the migration barriers (Junge et al., 2014; Meldgaard et al., 2003). However, the effects on genetic population structure is still not fully understood. Another interesting question is also how natural migration barriers could affect the genetic structure of grayling compared to weirs. In unobstructed river systems population structure could be determined by homing to natal birthplace (Hendry et al., 2004). The grayling is iteropareous (i.e. they repeatedly spawn in subsequent years) (Northcote, 1995). In their mark- recapture study, Kristiansen & Døving (1996) showed homing for grayling to adjacent tributaries in a large lake in Norway. Genetic studies have also shown grayling to be genetically structured within lakes and tributaries (Junge et al., 2011; Koskinen, Piironen, & Primmer, 2001) and that they can adapt to local temperature conditions within as little as 10-25 generations (Haugen & Vøllestad, 2000, 2001; Kavanagh, Haugen, Gregersen, Jernvall, & Vøllestad, 2010). Such genetic structuring could indicate that they indeed are homing to natal spawning locations, since one would expect high gene flow and straying among spawning areas if the opposite was true (Hendry, 2004). However, little is known about genetic structure as a consequence of homing within large rivers.

Population structuring of fish in rivers can be studied by many different techniques. Many studies use mechanical fish tags (mark-recapture studies), telemetry or genetic analysis to assess connectivity in rivers (Lowe & Allendorf, 2010). Direct methods such as mechanical fish tags or telemetry give information on the present movements of fish (Slatkin,

1987). Telemetry data can yield very detailed information on movement, since positioning data can be obtained at very small temporally scales (Thorstad, Rikardsen, Ahmet & Økland, 2013). However, the evolutionary outcome of movements and connectivity can perhaps be more precisely revealed using genetic analysis (Lowe & Allendorf, 2010; Slatkin, 1987). Neutral genetic markers such as microsatellites have been used extensively for studying population structure (Vrijenhoek, 1998). A combination of both telemetry and genetics is likely to give the most comprehensive understanding of present and evolutionary connectivity and genetic structure in rivers (Wilson, Hutchings, & Ferguson, 2004).

The aim of the present study was to gain insight into the genetic population structure in a large river system with both unobstructed and fragmented sections. I wanted to assess how fragmentation (both due to natural and anthropogenic migration barrier) affects genetic structure at large spatial scales, and then investigate if homing to local spawning areas within the large unobstructed section of the river system have led to population structuring. Since grayling have shown to move long distances (Heggenes et al., 2006) and to be genetically structured in other systems (Heggenes et al., 2006; Koskinen et al., 2001) I expected to find the same in this system. As fragmentation via barriers to migration have shown to affect population structure elsewhere (Junge et al., 2014; Meldgaard et al., 2003) I assume the natural and anthropogenic barrier in my system to affect the populations similarly. To study this I used a combination of telemetry and microsatellite genotyping.

Materials and methods

Study species

European grayling (Fig. 1) is one of four species in the genus *Thymallus* (Scott & Crossman, 1973) and is distributed throughout Europe and against the Ural mountains in Russia (Northcote, 1995). In Norway grayling are found in rivers and lakes in southern and northern parts of the country (Heggenes et al., 2006; Kristiansen & Dølving, 1996).

Grayling is an spring spawning salmonid (Northcote, 1995). They usually mature at the age of 4-5 years and start their migration in late March and early April when the ice is melted and the temperatures reach 4-7 °C (Northcote, 1995). For spawning, they prefer shallow areas with gravel and relatively high water velocity (Sempeski & Gaudin, 1995). The eggs are deposited in the gravel and hatch after around 276-320 degree-days (Bardonnet & Gaudin, 1991). After hatching, they swim up from the gravel and become fry that starts to drift downstream the river (Bardonnet, Gaudin, & Thorpe, 1993). During spawning the males are the first to arrive at the spawning location to occupy and defend proper spawning territories (Parkinson, Philippart, & Baras, 1999; Poncin, 1996). The grayling have a polygynandrous breeding system, which means that the males often reproduce with several females and females with several males (Haddeland, Junge, Serbezov, & Vøllestad, 2015).

Figure 1. European grayling *Thymallus thymallus,* with an external radio transmitter attached below the dorsal fin. Photo: Jon Museth

Study area

The study area is a section of a large river system in south-eastern Norway, which starts at high elevation at lake Lesjaskogvatnet and flows in a southern direction downstream as the river Gudbrandsdalslågen for 262 km towards Lake Mjøsa. Included in this, is the river Otta, a large tributary which enters this system at Otta City (Fig. 2). The river Otta and the section of river Gudbrandsdalslågen below the Rosten waterfalls was used as a study area in an earlier study by Junge et al. (2014).

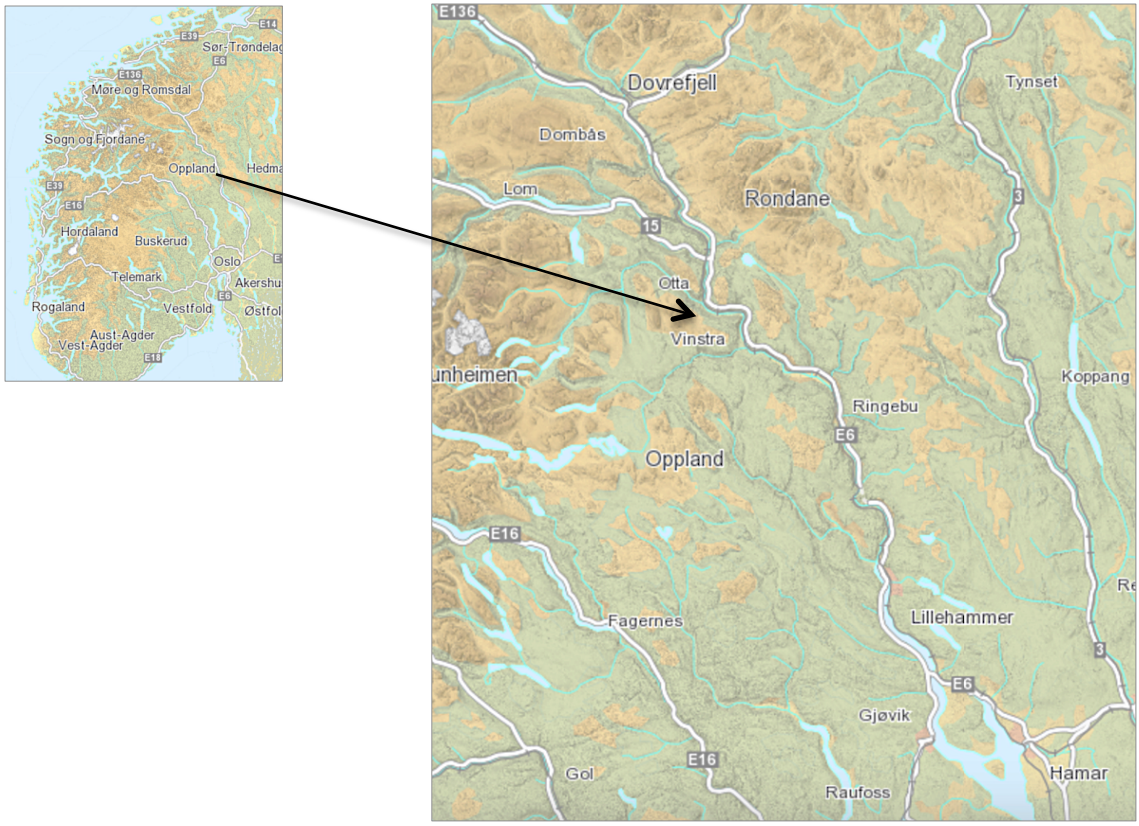


Figure 2. Location of Lake Lesjaskogsvatnet, the River Gudbrandsdalslågen and the River Otta in Norway. Source: kartverket.no

In this study I focus on the section from Lake Lesjaskogvatn and 169 km downstream throughout the mid-section of the river Gudbrandsdalslågen to the settlement at Tretten (Fig. 3). The mid-section of Gudbrandsdalslågen is about 58 km long, and the River Otta tributary, which flows into it, is about 30 km long (Junge et al., 2014). The study system is fragmented by three barriers to upstream migration. The Harpefoss Power Station (61.581°N; 9.840°E) and Rosten waterfalls (61.868°N; 9.411°E) are located in river Gudbransdalslågen and the Eidefoss power station (61.813°N; 9.275°E) in river Otta. The Rosten falls is with high probability a natural migration barrier, whereas the dams at Harpefoss and Eidefoss power station are presently absolute barriers. The waterfalls at Harpefoss and Eidefoss where probably natural migration barriers also before the hydropower constructions (Huitfeldt-Kaas, 1918). Grayling have been observed using overwintering and spawning areas distributed at specific sections throughout the whole system. The food availability is linked to habitat quality and differs strongly with both season and locations (Museth, et al., 2009).

I use telemetry data and genetic samples collected over several years and from different locations in the study area (Fig. 3). The genetic sampling area extends throughout the whole study area, from Lake Lesjaskogvatnet throughout Gudbrandsdalslågen including the river Otta to below Harpefoss power station and Tretten. In this area a total of 610 adult fish have been captured from different locations and an adipose fin-clip have been taken for genetic analysis. Some of the genetic samples have been collected during radio tagging of fish, while others have been collected in separate sampling events. Capturing of grayling for radio-tagging and subsequent tracking have been restricted within the three migration barriers in the mid section of the river Gudbrandsdalslågen and river Otta (total n= 100). However a few individuals (n= 9) were also radio-tagged and followed in the river section above the Rosten Waterfalls.

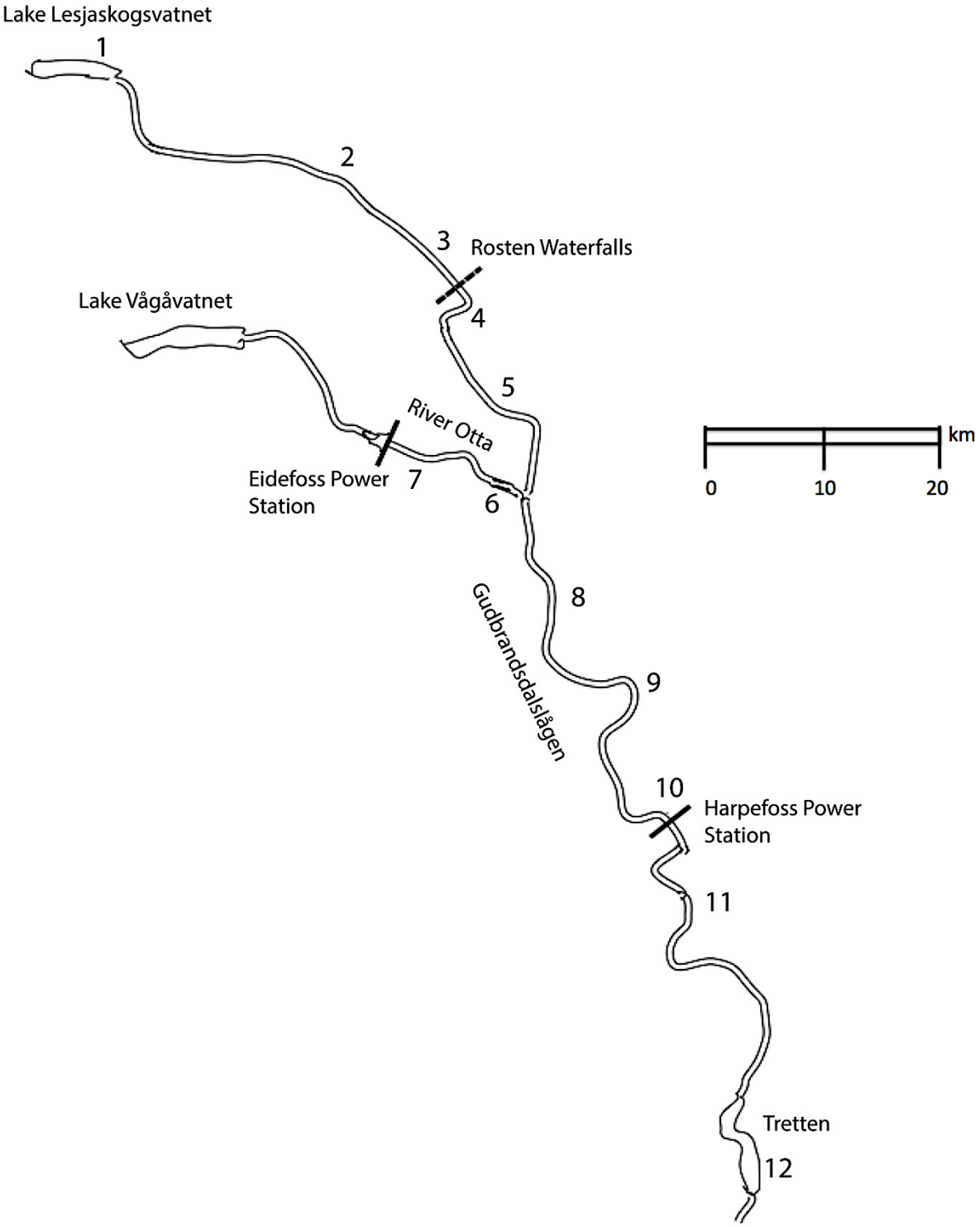


Figure 3. Map of the study system with Lake Lesjaskogsvatnet, the river Gudbrandsdalslågen and the river Otta. The horizontal lines represent three migration barriers (Harpefoss Power Station, Eidefoss Power Station and the Rosten waterfalls). The scale-bar indicates the distances in the river system. The telemetry sampling locations were delimited to the area within the three migration barriers and in the river section above the Rosten waterfalls. The numbers (1-12) illustrate the assigned spawning locations (Table 3).

Radio-Telemetry

A total of 144 grayling were radio-tagged during 2008-2010 and 2013-2014 (Table 1) in the river Gudbrandsdalslågen and the river Otta. All tagging was performed by one person (Jon Museth at NINA, Lillehammer). Some of the data (from 2008 - 2010) used here was also used in an earlier study (Junge et al., 2014). The same methods for sampling and tagging were used throughout.

Table 1. Number of radio-tagged fish from different years, with corresponding estimates of months tracked, fish length and weight as mean and ± Standard Deviations (SD). Gender distribution is also provided.

Radio-tagging information			**Mean (±SD)**			**Gender**	
Tagging year	**Tagging time**	**Number of tagged individuals**	**Tracked (Months)**	**Length (cm)**	**Weight (g)**	**Male**	**Female**
2008	Before spawning	37	8 ± 3	41,1 ± 4	626,5	20	17
2009	Before spawning	41	8 ±3	37,3 ± 2,4	407,3	28	13
2010	Before spawning	26	7.5 ± 4	37 ± 2,6	421	13	13
2013	During spawning	40	-		359,5	-	-
Total all years		**144**	8 ± 3	38,6 ± 3,5	433	62	43

The grayling was captured by rod angling and radio-tagged with either internal or external transmitters from Advanced Telemetry Systems Inc., U.S.A as described in Junge et al. (2014). Weight and fork length were measured and only adult fish were radio-tagged. The weight of the transmitter never exceeded 2% of the fish bodyweight. The internal transmitters were inserted into the abdominal cavity, with the antenna outside the body. These was attached in front of the pelvic fins through a 2-3 cm ventral incision for the transmitter and a 4-5 cm ventral needle incision for the antenna. Two or three stiches of monofilament thread were used to close the wound where the transmitter was inserted. The fish were kept in a V-shaped operation device for better handling during the surgery. A transmitter model F1830 (11 g, 12 ×54 × 12 mm) was used for fish ≥550 g and for fish ≤ 550g model F1580 (3,6 g, 13

× 24 × 7 mm). For the external tagging the fish were placed in a cylindrical tube for better handling, and the transmitter was fastened below the dorsal fin trough the dorsal muscle with two stiches. An attachment device was positioned on the opposite dorsal ventral side to chain the transmitter to the fish. For the external tagging, transmitter model F1970 (4,3 g, 13 × 29 × 7 mm) for fish ≤ 550g was used.

For both the internal and external tagging, the fish were anaesthetized with water administered 2-phenoxyethanol (0,7 ml × L^{-1}) and provided with well-oxygenated water during the procedures. The transmitter attachment procedure lasted for 2-4 min after which the fish were immediately moved to a holding tank for recovery after the tagging procedure. The fish were released to their capture site in the river within 30 minutes of awakening.

Each radio-tagged grayling was followed in the river throughout the year and its position in the river was determined on average once per week. The radio-tracking was done by personnel from NINA, Lillehammer. The fish were tracked either from car, boat or by foot, using a Challenger receiver (model R2100, Advanced Telemetry System USA) and a three-element folding Yagi antenna (model 12762). The exact position of each fish was determined to the nearest map specific 500 meters zone in the rivers. Using a digitalized centreline in the rivers, the positions of each fish was given as distance (m) from upstream the Harpefoss Power Station or the distance from the confluence of river Otta and Gudbrandsdalslågen. The positioning of each fish was given with a 500 meters precision.

Microsatellite genotyping

In total 610 individuals were genotyped and analysed in this study (Table 2). Of these a total of 425 individuals had been genotyped and used in earlier studies (Junge et al., 2011; Junge et al., 2014). The remaining individuals were genotyped in 2015 by the company Ecogenics (http://www.ecogenics.ch) (details about handling of DNA can be found in Appendix 1). All the samples have been genotyped for a set of 12 microsatellite loci (overview of loci can be found in table 1, appendix 1) using markers especially designed for grayling DNA including 214, 309, 313, 414, 415, 433b, 438, 445 (Junge et al., 2010), BFRO010, BFRO011 (Susnik et al., 2000), BFRO013 (Koskinen & Primmer, 2001 – unpublished allele) and BFRO018 (Susnik et al., 1999). The loci were chosen to cover most of the grayling genome.

Table 2. Information on genotyped grayling individuals analysed in this study. Included also an overview of which study that performed the genotyping, the year the samples were genotyped and number of individuals sampled at different years and location in the river system.

Genotyped by	**Year genotyped**	**Year sampled**	**Number of individuals**	**Location**
This study	2015	2013	185	Within barriers
Junge et al. 2014	2013	2008 & 2009	241	Within barriers, Below Harpefoss power station & Above Rosten waterfalls
Junge et al. 2014	2013	2008 & 2009	19	Above Rosten waterfalls
Junge et al. 2011	2011	2008 & 2009	165	Lesjaskogsvatnet
Total			**610**	

Different methods have been used for DNA-extraction and genotyping. For the individuals genotyped in 2013 and 2011 (Table 2) the purification kit E.Z.N.A. Tissue DNA Kit, Omega; or DNeasy® Blood & Tissue Kit, Qiagen (following standard protocol) was used to extract DNA from the grayling adipose fin-clips. The microsatellite loci were amplified with polymerase chain reaction (PCR) for each individual. Five multiplex PCR was used with four, two, three, two and one loci in each multiplex (See Table 1 in Appendix 1). The PCR solution consisted of 2x Qiagen Multiplex PCR Master Mix. The annealing temperatures were between 58 and 60 °C. The annealing process was completed on an ABI3730 Genetic Analyser, ABI.

For the samples genotyped in 2015, the DNA was extracted from the fin-clips using the standard protocol on the purification Qiagen DNeasy Blood and Tissue kit (details about DNA extractions can be found in Appendix 1). Three multiplex PCR was used with three, five and four loci in each multiplex. The PCR solution that was sent for amplification consisted of 10 µl reaction volume containing 2-10 ng of genomic DNA, 5 µl HotStarTaq Master Mix (Qiagen, catalog no. 203445), double distilled water, and appropriate concentrations of forward and reverse primers. The following cycling protocol used was: 35 cycles with 94° C for 30 seconds, 56° C for 90 seconds, and 72° C for 60 seconds. Before the first cycle, a prolonged denaturation step (95 °C for 15 min) was included and the last cycle was followed by a 30 min extension at 72 °C. The genotyping was done on an TC-412 programmable Thermal Controller (Techne). The samples from 2015 were genotyped on a different genetic analyser machine and with a different size standard than the samples from 2013 and 2011. This may induce divergent allele sizes between new and old samples. To address this problem, 20 of the samples genotyped in 2013 were genotyped again in 2015.

Genemapper software version 4.0 (applied Biosystems, ABI) was used to score all the genotypes. The automatic scoring for all allele sizes was manually verified to ensure that only true peaks were scored. Unclear samples dominated by stuttering, split and saturated peaks were deleted from the dataset. For all the samples genotyped in 2015, Locus BFRO010 was excluded from our dataset due to too excessive stuttering and unclear peaks in the samples. The 20 samples genotyped on both machines were used to calibrate and match the allele lengths between the samples genotyped at various time points. The allele lengths mostly differed with between one and three bases. For two allele lengths the difference was between 7 and 8 bases, however the difference was constant for each allele lengths in all the markers,

which enabled appropriate corrections to be made. The difference in allele lengths between the new and old samples were corrected and adjusted in Excel.

Ethics statement

Each step of the sampling, handling and tagging procedure of the fish should be most gentle to avoid effects on their health and natural behaviour (Jepsen, Thorstad, Havn, Lucas, 2015). There was no sign that the sampling or tagging procedure affected the swimming behaviour after the fish were released in our study (Junge et al., 2014). The sampling and handling procedure for the radio tagging was approved by the National Animal Research Authority (Junge et al., 2014). The county and the landowners gave the permissions to capture fish in the rivers. Grayling is not considered as an endangered species in our study area.

Determination of spawning locations

A total of 527 genotyped individuals were assigned to a one of 12 specific geographic spawning location either based on analysis of telemetry data, fish being captured during spawning or on a known spawning location. To determine the specific spawning locations for each fish with telemetry data, their movements from March to the end of June were examined. Grayling is assumed to migrate upstream for spawning during spring (Ovidio, Parkinson, Sonny, & Philippart, 2004; Parkinson et al., 1999). Therefore the most upstream location of the fish in the river during the period end of May to start of June was identified (see example in Fig. 4). The fish typically had a pattern were they migrated upstream some weeks before the assumed spawning, before they stopped and probably spawned. Thereafter the fish either moved downstream again or remained in the spawning area. Individuals with uncertain positioning were excluded from the dataset.

For fish that were clearly in an advanced state of maturity when they were captured, the location at sampling was assigned as the spawning location. For the individuals outside the migration barriers, the sampling locations was assumed to be the spawning location. This because the sampling locations were clearly separated from the spawning locations within the barriers, and these individuals were only used for the large-scale genetic structure. A large part of these samples were also collected during the spawning period.

In the analysis I define 12 spawning locations (Fig. 3) containing at least 14 individuals. Individuals in between were assigned to the closest known geographic spawning locations.

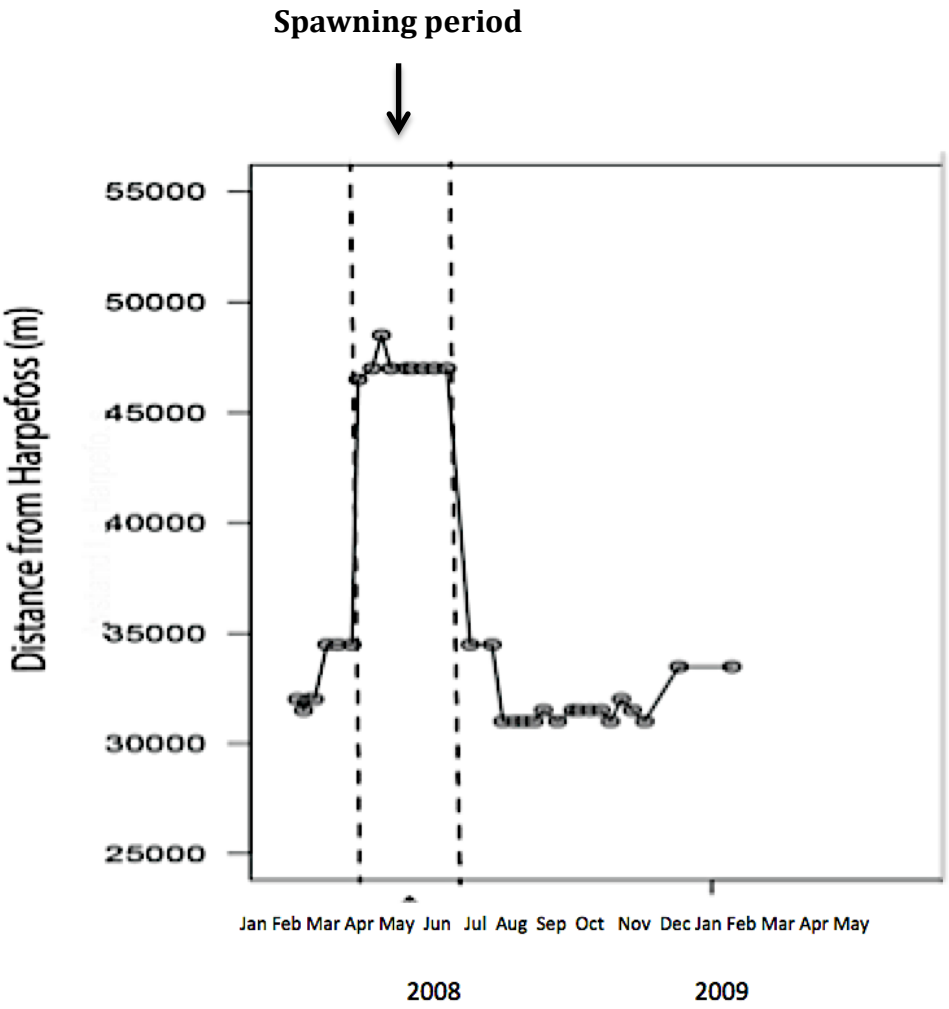


Figure 4. Example demonstrating how an individual could be assigned to a spawning location with telemetry data. The figure illustrates the spawning migration of an individual grayling in river Gudbrandsdalslågen, moving upstream during the end of April before spawning during the end of May, and thereafter returning downstream. The x-axis shows months from year 2008 and 2009. The y-axis is the distance (m) from Harpefoss power station. The dashed vertical lines demonstrate the start and the end of the spawning period.

Table 3. Overview of the 527 individuals assigned to a specific spawning location. The table show the geographic and the specific spawning locations in the river and the number of individuals in each spawning location. The map codes refer to the numbers in figure 3 and the distance between each spawning location is provided in km to Harpefoss Power Station. The distances labelled with a star (*) represent individuals with spawning location in the river Otta. The number of individuals assigned with telemetry, captured during spawning or sampling location for each spawning locations is also provided. For the individuals assigned with telemetry data, brackets around the numbers indicate that the fish were radio tagged during spawning.

Information on spawning locations					Number of individuals assigned by		
Geographic location	Spawning location	Map code	Distance from Harpefoss (km)	Number of individuals	Telemetry	Captured during spawning	Sampling location
Above the Rosten waterfalls	Lesjaskogvatnet	1	124	165		165	
	Dovre	2	63	19			19
	Rosten above	3	58	49	9		40
Within migration barriers	Harrsvaet	4	52	66	36 (21)	30	
	Fevollen/Grenet	5	49	21	19 (3)	1	
	Eidefoss 1	6	42*	15	13	2	
	Eidefoss 2	7	51*	64	24 (16)	40	
	Vegstasjon	8	34,5	48	8	40	
	Kvam	9	15	16		16	
	Harpefoss 1	10	0	32		32	
Below Harpefoss power station	Harpefoss 2	11	0	18			18
	Tretten	12	45,5	14			14
Total		**12**		**527**	**109**	**161**	**271**

Population genetic analysis

GenALEx 6.5 (Peakall & Smouse, 2012) was used to format the genetic data and as a platform to convert data to other analysing programs. Allele richness, linkage equilibrium and number of private alleles per population and locus were estimated in the R-package adegenet version 2.0.0 (Jombart et al., 2008). Pairwise F_{ST} (Weir & Cockerham, 1984) to estimate degree of differentiation, expected heterogozity (He), observed heterogozity (Ho) and deviations from Hardy-Weinberg were also obtained with this package and estimated for the four populations detected by STRUCTURE (Fig. 6).

Population structure

STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) was used to infer population structuring between individuals from the assigned spawning locations in the river. Based on microsatellite data, the software uses a Markov Chain Monte Carlo (MCMC) simulation to find the most likely cluster (K) for each individual (Pritchard et al., 2000). The analysis was used with admixture and with assumed correlated allele frequencies, which means that the software ignores given population of each individual when it assigns an individual to a genetic cluster (François & Durand, 2010). A burn-in of 500 000 with 1000 000 iterations for MCMC was used for all the analyses.

Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/) was used to find the optimal number of clusters (K). This program estimates ΔK and evaluates individual memberships of Q for different values of K, to compare the log-likelihood data given by the number of clusters [$P(X\backslash K)$] (Earl & vonHoldt, 2012).

In total 527 individuals were analysed with STRUCTURE to infer population structuring. The analysis was implemented by including all the individuals from all the spawning locations in the system, from both above and within the migration barriers. Thereafter only individuals within the migration barriers were analysed, by excluding individuals with spawning location and genotypes from below Harpefoss power station and above the Rosten falls.

Isolation by distance

Isolation By Distance (IBD) was used to infer population structuring within the migration barriers in the river system. This method examines if individuals that are close to each other in distance are also closely related genetically (Wright, 1943). A matrix consisting of genetic distance between each individual and a matrix with geographic distance between each individual was compared in GenALEX version 6.5 (Peakall & Smouse, 2012). A Mantel test implemented in R (R Devolvement core team, 2015) was used to correlate the genetic matrix and the geographic distance matrix. 10 000 permutations were set for evaluating the level of significance for the correlation coefficient.

Detection of migrants

Genotyping data on 527 individuals was analysed with STRUCTURE to identify migrants from other populations in the system. Based on the final STRUCTURE results showing four populations (see Results), each individual was assigned to one of the populations (1-4). Individuals spawning below Harpefoss were assigned to population 1, the ones spawning within the migration barriers to population 2, those spawning above the Rosten waterfalls to population 3 and individuals spawning in lake Lesjaskogsvatnet to population 4. The length of the burn-in period was set to 50 000 with 100 000 MCMC repetitions after burn-in, and 10 iterations for each K. The settings were set on "use population information to test for migrants" and there was no admixture. With these settings, the software assumes account that each individual belongs to a given spawning population. The software uses a Bayesian method to estimate the probability (v) of affinity to a population (Pritchard et al., 2000). The software detected if individuals had a 5% probability of being an immigrant, with the default settings set to 0.05.

In addition, Geneclass2 (Paetkau, Slade, Burden, & Estoup, 2004; Piry et al., 2004) was also used to detect migrants, with the following settings: L= L_home/L_max for calculating the likelihood for the individual to belong to the assigned population compared to all the populations analysed (Paetkau et al., 2004) and a Bayesian criterion for likelihood (Rannala & Mountain, 1997). Alpha was set to 0.01, and number of simulated individuals was set to 10 000.

Results:

Spawning locations

A total of 527 genotyped grayling were assigned to a specific spawning location (Fig. 3). The telemetry data showed individual variation in movements within the rivers (Examples of swimming behaviour can be found in Appendix 2). Almost all the fish tracked by telemetry followed a clear pattern: they typically moved upstream to spawn or remained stationary. However, a few fish did also move downstream before the spawning period or showed bizarre movements during the spawning period, which made it challenging to assign them to spawning locations. The fish were excluded when the spawning location was uncertain or if the fish had been tracked over only a short time period. Some grayling was assigned to spawning locations with only a few other individuals. In these instances the fish at that location were assigned to the closest spawning location instead. However, if the distance to the nearest spawning location was more than 2 km, the fish were excluded altogether. In total 35 out of 144 radio tagged individuals were not assigned to a spawning location and excluded from further analyses.

Microsatellite variation

The 12 microsatellites displayed varied degree of polymorphism (Table 4). Expected heterozygosity H_e for the 12 microsatellite loci ranged from 0.87 in locus 438 to 0.313 in locus BFRO11. The highest number of alleles N_a was found in locus 445 and the lowest number was found in locus 214. All the loci were in Hardy Weinberg equilibrium (Bartlett's K-squared: 0.021, df = 1, p-value = 0.8846). In total 106 out of 131 alleles were in linkage equilibrium.

Table 4. Genetic diversity for the 12 loci of European Grayling. The number of alleles per locus (N_a), expected (H_e) and observed (H_o) Heterozygosity is given for each locus. All the loci were in Hardy-Weinberg equilibrium.

Locus	N_a	H_e	H_o
BFRO13	7	0.645	0.587
BFRO18	7	0.698	0.595
BFRO10	6	0.377	0.322
214	3	0.639	0.495
414	10	0.720	0.620
313	11	0.801	0.722
BFRO11	4	0.321	0.290
433b	12	0.826	0.841
415	15	0.787	0.772
309	7	0.313	0.244
455	36	0.924	0.883
438	14	0.879	0.833

Population structure

Fragmented river section

The result from the STRUCTURE analysis showed a clear population structuring (Fig. 5A), when all the geographic spawning populations were included in the analysis. Individuals from Lake Lesjaskogvatnet (green bars) and individuals below the Rosten waterfalls and Harpefoss Power Station (red bars) clustered separately into two different genetic groups (Fig. 5A). The optimal number of population clusters (K) was estimated to be K=2 (Fig. 5C), this was further supported by a significant F_{ST} value of 0.07 between the two clusters. The bar output from the STRUCTURE analysis showing the likelihood for four genetic populations did however indicate that there could be four genetic populations in the river system (Fig. 6A). In this output, the individuals seemed to cluster into four different groups.

To investigate the potential for further clustering, all the individuals from above Rosten and all the individuals from below Rosten were analysed in two separate analyses. When excluding the individuals from below the Rosten waterfalls, there was clear structuring with the individuals from Lake Lesjaskogsvatn (red bars) being differentiated from the individuals sampled in the river section between the Rosten waterfalls and Lake Lesjaskogsvatn (green bars) (Fig. 7). The optimal number of population clusters (K) was estimated to be K=2 (Fig. 7). When only analysing the individuals from below the Rosten waterfalls, there was also a clear structuring into two populations, one population containing all individuals from within the three migration barriers (red bars) and one population containing individuals from below Harpefoss Power Station (green bars) (Fig. 8). This was supported by results from Structure Harvester (Fig. 8).

It is therefore likely to assume that there are four genetically differentiated populations in the river system, with a strong separation between clusters by the Rosten waterfalls and a slightly weaker separation forming two further sub-clusters. These four populations are 1) individuals from below Harpefoss power station, 2) within the barriers, 3) above the Rosten Falls and 4) from Lake Lesjaskogsvatnet. Significant genetic differentiation between the four genetic populations detected by STRUCTURE (Fig. 6) was further supported by the pairwise F_{ST} analysis (Fig. 9). The allelic richness increased downstream the river system, with the highest richness located below Harpefoss power station and the lowest located above the Rosten falls (Table 5). The number of private alleles was also highest for the most downstream genetic population (Table 5).

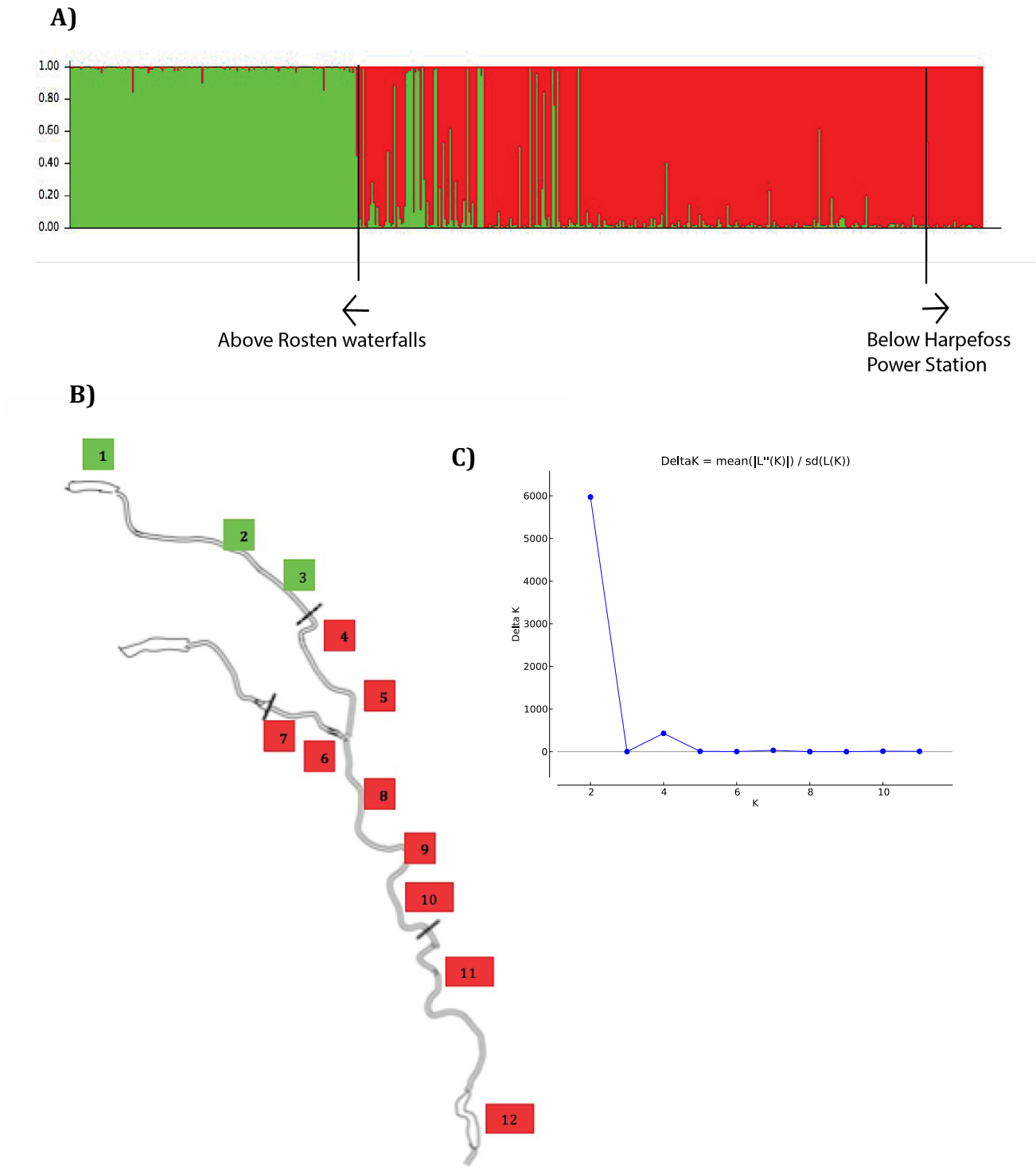
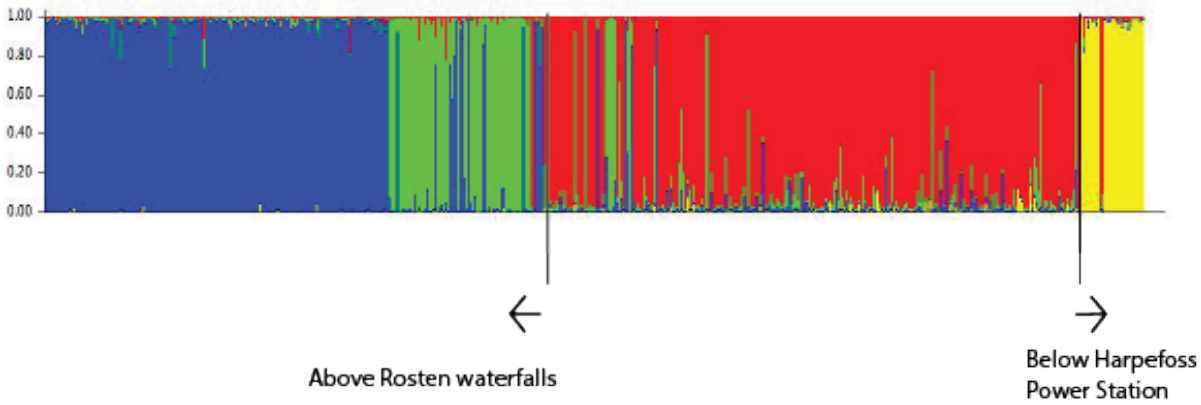


Figure 5. STRUCTURE result for the analysis that included grayling from all the assigned spawning locations in the river system. **A** shows the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, where each line represents an individual with a colour for their putative cluster. The green lines represent the individuals with spawning locations above the Rosten waterfalls and the red lines represent all the individuals with spawning locations below the Rosten waterfalls. The black lines illustrate the migration barriers, with arrows indicating upstream or downstream direction. **B** illustrates a corresponding map to the bar output, showing each spawning location (1-12) in the river system with the colour of their putative cluster. **C** show the Structure Harvester graph with the optimal number of populations clusters (K), estimated to be K=2.

A)



B)

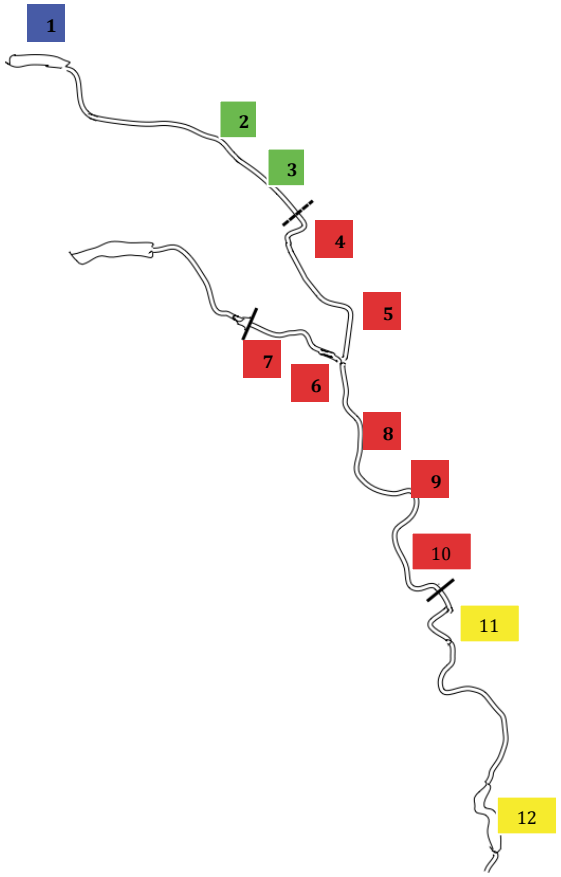


Figure 6. STRUCTURE result for the analysis that included grayling from all the assigned spawning locations in the river system. **A** shows the bar output for the STRUCTURE analysis for K=4. The bar output consists of several lines, where each line represents an individual with a colour for their putative cluster. The blue lines represent the individuals with spawning locations in Lake Lesjaskogsvatnet, green lines above the Rosten waterfalls, red lines within the three migration barriers and yellow lines represent all the individuals with spawning locations below Harpefoss Power Station. The black lines illustrate the migration barriers, with arrows indicating upstream or downstream direction. **B** illustrates a corresponding map to the bar output, showing each spawning location (1-12) in the river system with the colour of their putative cluster.

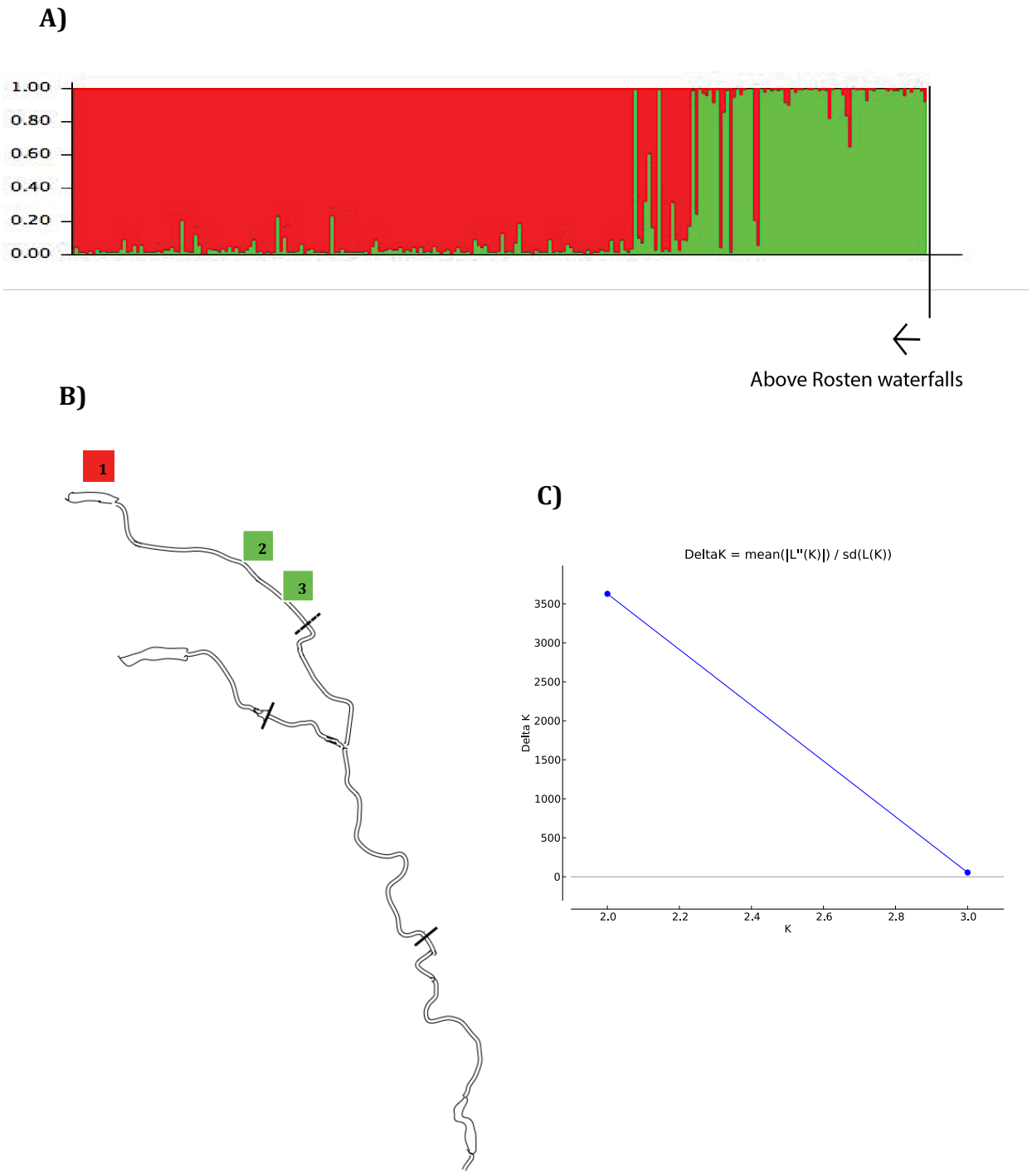


Figure 7. STRUCTURE result for the analysis that included grayling from all the assigned spawning locations above the Rosten waterfalls. **A** shows the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, were each line represents an individual with a colour for their putative cluster. The red lines represent the individuals with spawning locations in Lake Lesjaskogsvatnet and the green lines represent all the individuals with spawning locations in the river section between the Rosten waterfalls and Lake Lesjaskogsvatnet. The black line illustrates the migration barrier, with arrows indicating upstream or downstream direction. **B** illustrates a corresponding map to the bar output, showing each spawning location (1-3) in the river system with the colour of their putative cluster. **C** showss the Structure Harvester graph with the optimal number of populations clusters (K), estimated to be K=2.

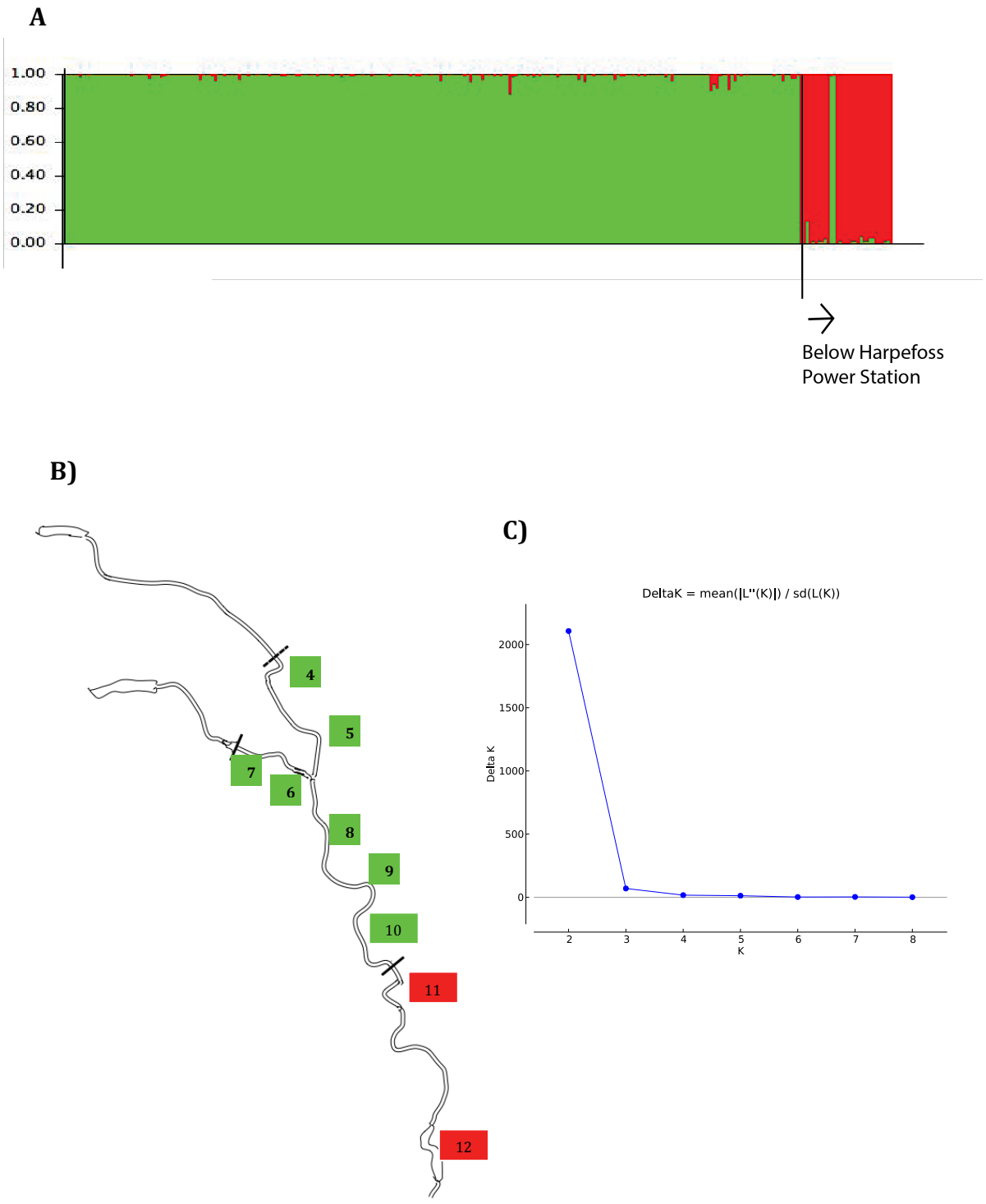


Figure 8. STRUCTURE result for the analysis that included grayling from all the assigned spawning locations below the Rosten waterfalls. **A** shows the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, were each line represents an individual with a colour for their putative cluster. The green lines represent the individuals with spawning locations within the three migration barriers and the red lines represent all the individuals with spawning locations below Harpefoss Power Station. The black lines illustrate the migration barriers, with arrows indicating upstream or downstream direction. **B** illustrates a corresponding map to the bar output, showing each spawning location (4-12) in the river system with the colour of their putative cluster. **C** shows the Structure Harvester graph with the optimal number of populations clusters (K), estimated to be K=2.

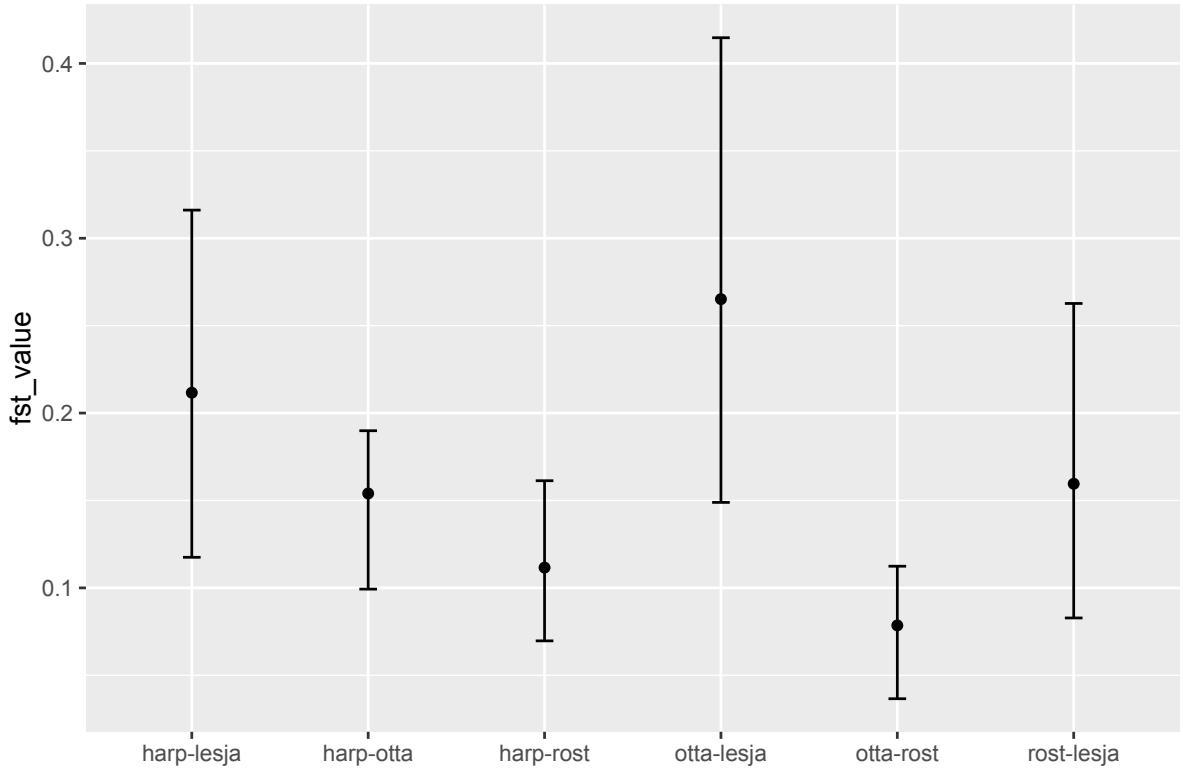


Figure 9. Plot form pairwise F_{ST} analysis, comparing F_{ST} values with confidence intervals between the four populations detected by STRUCTURE (Fig. 6B). The x-axis shows comparisons of each populations against the other. Explanation of the population abbreviations: "harp" = below Harpefoss Power Station, "Lesja" = Lake Lesjaskogsvatnet, "rost" = above the Rosten waterfalls and "otta" = the population within the three migration barriers. The y-axis shows an increasing F_{ST} value, indicating increasing genetic differentiation. All the F_{ST} values were significant.

Table 5. Overview of genetic the diversity of the four populations detected by STRUCTURE (Fig. 6B). The table shows number of individuals (*N*), Mean Allelic richness (*Ar*), Private Alleles (*Pa*), Number of alleles (*Na*) for each population.

Population	***N***	***Ar***	***Pa***	***Na***
1	185	4.13	5	69
2	67	3.66	-	60
3	245	5.11	15	93
4	30	6.11	29	94

Large river stretch without migration barriers

To investigate in more detail if the individuals with spawning locations in the large unobstructed river stretches above Harpefoss Power Station and below Eidefoss Power Station and the Rosten waterfalls indeed belonged to one genetic population, a new STRUCTURE analysis based on this subset of the data was performed. The results from the STRUCTURE analysis showed no population structuring within this river section (Fig. 10A) and Structure Harvester gave a low likelihood for the presence of more than one population in this river section (Fig. 10C). To investigate this further, several STRUCTURE analyses excluding different sections of the rivers within the three barriers were run. None of the STRUCTURE analyses showed population structure within the three migration barriers (results from these STRUCTURE analyses can be found in Appendix 3). The Isolation By Distance (IBD) analysis (Fig. 11) further supported this result, finding no correlation between genetic and geographic distance. The graph indicated no significant correlation between genetic differentiation and geographic distances (r^2= 0.0031, P=0.177).

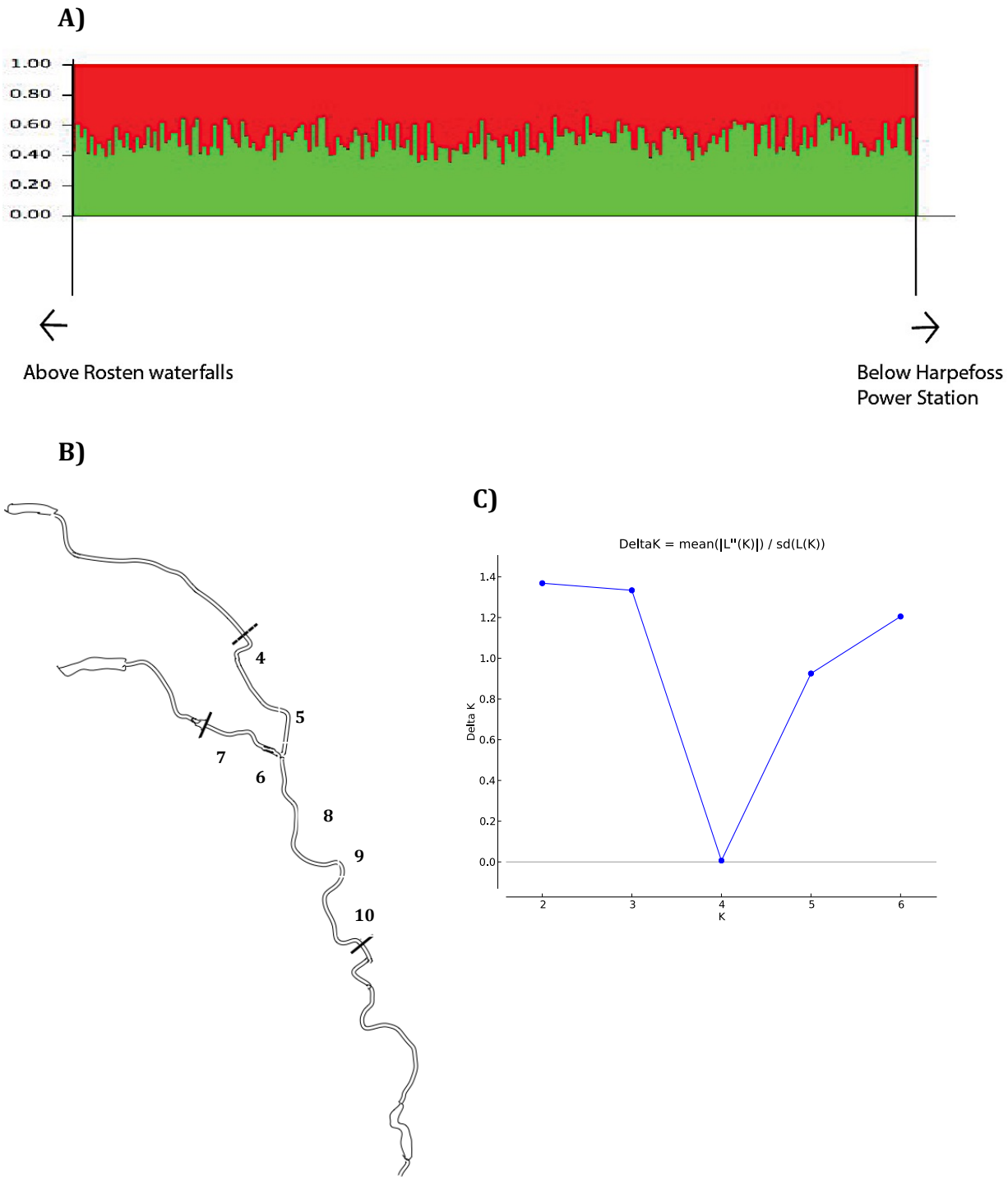


Figure 10. STRUCTURE result for the analysis that included grayling from all the assigned spawning locations within the three migration barriers. **A** show the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, were each line represents an individual with a colour of their putative cluster. In this output each line contains two colours indicating that individuals belong to both the putative clusters. The black lines illustrate the migration barriers, with arrows indicating upstream or downstream direction. **B** illustrates a corresponding map to the bar output, showing each spawning location (4-10). Fig. **C** shows the Structure Harvester graph with the optimal number of populations clusters (K), estimated to be K=2.

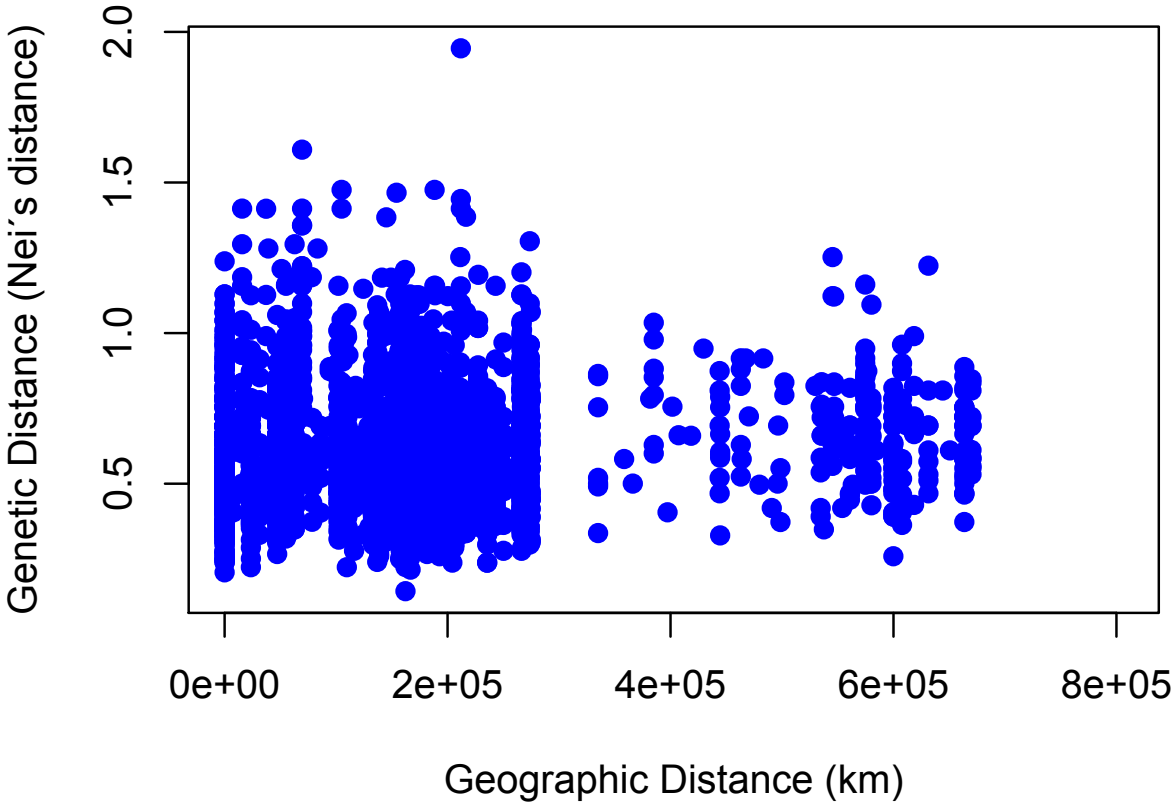


Figure 11. Showing pairwise genetic distances (Nei`s distance) plotted against pairwise geographic distances (km) between each grayling individual with spawning location within the three migration barriers. The x-axis show the geographic distance (km) from Harpefoss Power Station and the y-axis show the genetic distance (Nei´s distance).

Detection of migrants

The results from the STRUCTURE analysis showing four populations in the whole river system (Fig. 6A) indicated that some individuals might move between populations. The STRUCTURE bar output indicated that some individuals with spawning location below the Rosten waterfalls had a genotype from Lake Lesjaskogsvatnet (blue bars) or the river section above the Rosten waterfalls (green bars). Further, a few individuals with spawning locations below Harpefoss Power Station seemed to have a genotype originating from within the three migration barriers.

To investigate this further, the four populations were analysed again using STRUCTURE with settings for detecting migrants between the specified populations. The results showed that two individuals with spawning location below Harpefoss Power Station had greater than 90 % probability of having a genotype from within the three migration barriers (Fig 13). An additional 12 individuals with spawning location within the three

migration barriers were detected with probabilities greater than 70 % for being immigrants. Of these, 6 individuals were detected with a genotype from lake Lesjaskogvatnet and 6 individuals with a genotype from Rosten above (Fig. 13). Geneclass2 supported the results from the STRUCTURE analysis and identified the same individuals as migrants. In addition, three more individuals with spawning locations from within the three migration barriers were detected with a genotype from the river section above Rosten. (Details about the detected migrants and the probabilities for their inferred populations can be found in Appendix 4).

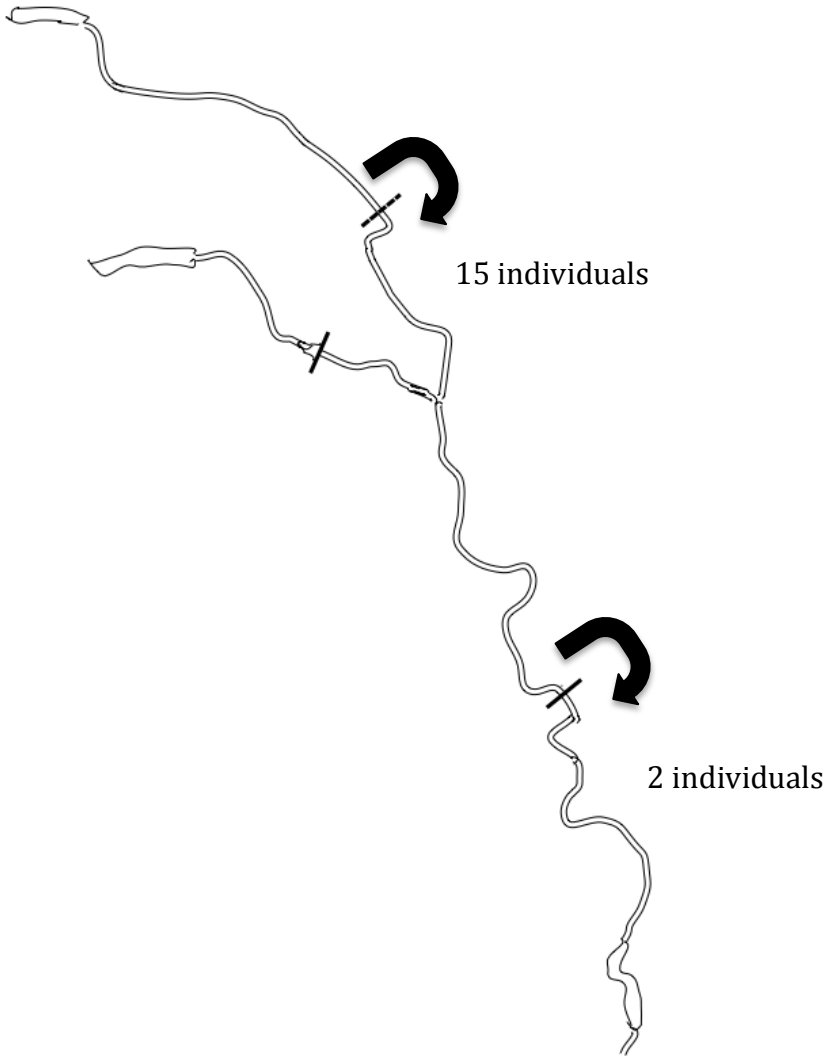


Figure 12. Map illustrating total numbers of individuals detected with either STRUCTURE or Geneclass2. Illustrating individuals with a different genotype detected downstream the Rosten waterfalls or Harpefoss Power Station. 15 individuals with spawning location within the three migration barriers were detected with a genotype from above the Rosten waterfalls. Two individuals with spawning location below Harpefoss Power Station were detected with a genotype from within the three migration barriers.

All the individuals detected with a genotype from Lesjaskogsvatnet or the river section above the Rosten waterfalls had their spawning location in the upper part of river Gudbrandsdalslågen below the Rosten waterfalls (Fig.12). Telemetry data were available for five of these individuals, which indicated that they also preferred to reside in this area during the rest of the year (Fig. 13B). For the individuals with a genotype from within the three migration barriers that also spawned in the upper part of Gudbrandsdalslågen, the telemetry data showed more long-distance movements during the year. It seems like these individuals move upstream for spawning and return downstream after their spawning period (Fig. 13A). The sample size is too small for a formal analysis.

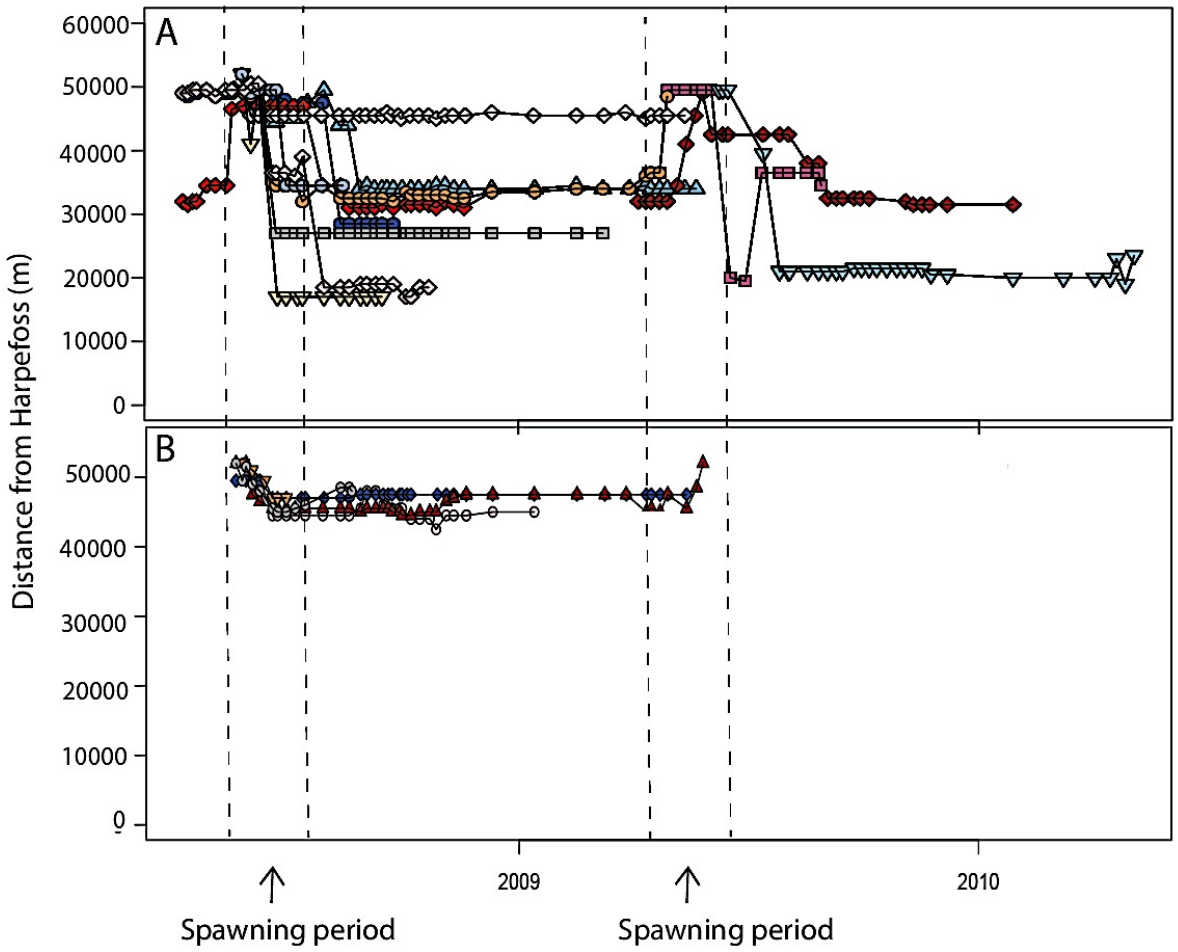


Figure 13. Seasonal movements of grayling with spawning location below the Rosten waterfalls but with different genotypes. A illustrate individuals with a genotype from within the three migration barriers. B shows individuals with a genotype from above the Rosten waterfalls. The y-axis is the distance from Harpefoss Power Station (m) and the x-axis show the years the grayling were tracked. The dashed vertical lines demonstrates the start and the end of each spawning period.

Discussion

This study has shown, using a combination of population genetics and telemetry methods, that the grayling in Gudbrandsdalslågen are structured into four distinct populations. The populations seem to be structured by both natural and anthropogenic migration barriers that hamper upstream migration and direct the gene flow downstream. There were no signs of population structure within the large stretch of the river where there is no migration barriers. This may indicate that the grayling do not home to particular spawning locations, but instead utilize a variety of spawning locations. Large individual variation in movement patterns was found, which together with the lack of population structuring in this part of the river indicates the importance of retaining connectivity here and in large rivers in general.

Population structure as a consequence of fragmentation

Four genetically differentiated populations of grayling were found within the whole river system, which consisted of a lake, two anthropogenic migration barriers and a natural waterfall. This corresponds well with an earlier study in the same system by Junge et al. (2014). The STRUCTURE analyses were first conducted at a broader scale including all the individuals assigned to a spawning location within the river system. In this way an overview of all the genotypes in the system could be obtained. The results indicated two populations, one population above and one population below the Rosten waterfalls. However, the STRUCTURE software is often only able to detect populations that are very genetically different, and may not detect populations within that are more genetically similar (Evanno, Regnaut, & Goudet, 2005). When the two populations were analysed separately, four genetically differentiated populations were found in the whole river system. Lesjaskogsvatnet and the river section above the waterfalls clustered as two separated populations, while individuals within the three migration barriers and below Harpefoss Power Station also clustered as two populations.

Except for lake Lesjakogsvatnet, the populations seemed to be separated by migration barriers. Lakes are different habitats than rivers, and as differences in spawning habitats have been shown to affect the genetic population structure (McGlauflin et al., 2011), this may explain why lake Lesjaskogsvatnet clustered as a population. The anthropogenic Power Station at Harpefoss and the natural waterfall at Rosten appeared to separate the three other populations within the rivers. Previously Meldegaard et al. (2003) observed similar genetic

structuring of grayling affected by weirs in a Danish river system and such structuring has also found for other salmoinds (Kitanishi, Yamamoto, Edo, & Higashi, 2012; Stelkens, Jaffuel, Escher, & Wedekind, 2012; Wofford et al., 2005). Furthermore, natural waterfalls are also known to act as migration barriers and may lead to a population structuring (Campos, Posada, & Morán, 2006; Deiner et al., 2007).

Significant levels of genetic differentiation (F_{ST}) was also found between the four populations, and thus supports the results from the STRUCTURE analysis. The genetically difference between the populations did also correspond to F_{ST} levels found in an earlier study of grayling from the same study system (Junge et al., 2014 see supplementary data, table 4). Although, the analysed populations from these two studies were not exactly the same, comparison could be made since the populations were conducted in the same areas within the river system. The genetically difference were also high compared to genetic differentiated populations found in an earlier study of grayling recently introduced to a lake (Junge et al., 2011). Individuals from Lesjaskogsvatnet and within the barriers were the most genetic different and the comparison of Lesjaskogvatnet and below Harpefoss Power station were the second most genetic different. The lowest F_{ST} value was found between the population within the migration barriers and the population in the river section above the Rosten waterfalls. This may indicate that more gene flow has occurred across the natural migration barrier at Rosten compared to the barrier at Harpefoss. The migration barrier at Harpefoss is a huge dam and a clear barrier to upstream migration for fish, but was also assumed to be a strong migration barrier before the constructions of the dam (Huitfeildt-Kaas, 1918). Historically this may suggest that the migration barrier at Harpefoss could have been a stronger migration barrier to gene flow than the natural migration barrier at Rosten. Although the natural migration barrier at Rosten prevents upstream movement for fish today, it may still be important for the contribution of gene flow downstream.

The allelic richness was observed to be directed downstream, with the highest richness being located below the Harpefoss Power Station. The analyses of the allelic richness did not take into account the sample sizes between the populations. A larger sample size can lead to the automatic detection of a larger numbers of alleles, since more individuals can contribute with alleles (Leberg, 2002). Therefore analysis of allelic richness should preferably be standardized after the smallest sample size (Leberg, 2002). This was not done here. However, the most downstream population below Harpefoss Power Station had a very low sample size compared to the uppermost population at Lesjaskogsvatnet, although the highest richness was still found below Harpefoss Power Station, indicating that it was not

critical to standardize in this case. This pattern has been found in studies of other species, were they uncovered the highest genetic diversity downstream in drainage systems (Yamamoto et al., 2004). This is to be expected, seeing as the drainage topology, will naturally direct the gene flow downstream in most cases (Morrissey & Kerckhove, 2009). Gouskouv et al., (2015) recently also observed that weirs could also affect the direction of gene flow of European chub (*Squalius cephalus*). Scenarios such as those may promote up stream isolation of individuals, leading to a decreased genetic diversity upstream (Gouskov et al., 2015; Neville, Dunham & Peacock, 2005).

Historically, the grayling invading Gudbrandsdalslågen following the last glaciation may have been stopped several times on their way up the river, which may contribute to the explanation of the observed present genetic structure. Harpefoss Power Station was built upon a natural waterfall, which also was assumed to act as a natural migration barrier for fish before the constructions were made (Huitfeldt-Kaas, 1918). The first grayling that came upstream Gudbrandsdalslågen were therefore probably not able to move upstream the waterfall barrier and were forced to stop, but were later moved upstream by human interference (Huitfeldt-Kaas, 1918). Upstream the waterfall the fish would have been able to spread, but meet another barrier against upstream movement in the Rosten waterfalls. Also here fish were moved upstream by humans, allowing them to further disperse towards lake Lesjaskosvatnet (Huitfeldt-Kaas, 1918). However, a third natural migration barrier were also present at the outlet of lake Lesjaskogsvatnet, and fish were not able to move into the lake before a channel was created in late 1880s (Haugen & Vøllestad, 2001). The continuous delay of movement upstream may have resulted in several cases of founders effect, were a few individuals from the larger population downstream Harpefoss have been moved upstream and established new populations with reduced genetic variability from that of their initially population (Sirkkomaa, 1983; Nei, Maruyama, Chakraborty, 1975). This may therefore perhaps also explain the observed reduced genetic variability (Shaw, Carvalho, Magurrant & Seghers, 1994; Nei et al., 1975).

Absence of population structure within a large unobstructed river section

No population structuring was found for the individuals with spawning location within the large unobstructed area in the river system. This is somewhat surprising, since salmonids have been found to exhibit genetic structuring in other studies (Carlsson & Nilsson, 2000; Garant et al., 2000) which is also observed in earlier studies for grayling (Junge et al., 2011). The individuals were assigned to specific spawning locations by telemetry data or after being

captured during spawning. The telemetry data revealed individual variation in swimming behaviour throughout the season. Some individuals were stationary, while other displayed a variety of movement in the river system. A typical pattern was upstream migration for spawning, followed by movement to other habitats short time after spawning was completed. Similar movement patterns have previously also been identified in other studies of grayling (Ovidio et al., 2004; Parkinson et al., 1999). Long movements after spawning to feeding habitats has been observed to be beneficial for their growth and probably therefore also for their overall fitness (Van Leeuwen, Museth, Qvenild & Vøllestad, submitted).

Previously mark-recapture studies have shown grayling to exhibit homing in lakes to adjacent tributaries (Kristiansen & Dølving, 1996). However, direct methods may not explain the population genetic structure (Lowe & Allendorf, 2010). Grayling may probably exhibit homing within the present study system, but the STRUCTURE results suggest that it did not lead to a population structuring. The isolation by distance analysis also confirmed the STRUCTURE analysis and showed no correlation between geographic and genetic distance. When the level of genetic differentiation does not vary with geographic distance individuals are thought to be closely related due to gene flow (Wright, 1943). High gene flow may indicate that the individuals probably move among more spawning locations instead of spawning only at their birthplace, and that they also are reproductively successful on their new spawning locations (Hendry et al., 2004).

However, population structuring in grayling has mostly been found in lakes and adjacent tributaries (Barson, Haugen, Vøllestad, & Primmer, 2009; Junge et al., 2011). Koskinen et al., (2001) observed a genetic structuring of grayling within an unobstructed lake system, and claimed that homing in addition to reduced fitness of hybrids or limited dispersal behaviour probably could be a possible explanation. There is still little available documentation of genetic structuring of grayling within large unobstructed rivers. Heggenes et al., (2006) observed a genetic structuring of grayling over long distances within three large rivers, but found that the populations in closer proximity of each other within the rivers appeared to be more genetically similar. Salmoinids have been observed to adapt to environmental differences in tributaries (Taylor, 1991). Different environment may lead to local adaptations (Hendry et al., 2004) which has also been observed for grayling in different tributaries (Kavanagh et al., 2010). Reilly et al., (2015) recently observed genetic population structuring and patterns of isolation by distance for the artic grayling (*Thymallus arcticus*) within a large unobstructed river system. They suggested that the structuring could be due to sampling sites with different environmental conditions, which could have lead to genetic

diversity. However, when spawning populations are in close proximity to each other with similar environments it could be difficult for the individuals to distinguish between spawning sites (Hendry, 2004), which could perhaps explain the lack of precise homing of grayling in the present river system. Although, Strong population structuring has been found for brown trout with spawning populations in close proximity in rivers (Carlsson et al., 1999). Brown trout are usually stationary as young and only move short distances from their birthplace (Elliot, 1994). Although, in contrast young grayling drift downstream the rivers short time after they swim up as fry from the gravel (Bardonnet et al., 1993). To achieve experience at natal sites as young may be important for precise homing (Dittman et al., 2010; Dittman & Quinn, 1993). Shorter time period on the birthplace may affect the success of homing to particular locations in the river (Hendry et al., 2004), which could perhaps also be another possible explanation for the lack of precise homing in the present study.

The grayling in this river system probably move among different spawning habitats and the telemetry data showed individual variation during the rest of the season. Such movements of grayling within large rivers is previously also observed (Heggenes et al., 2006). Eventually fragmentation of unobstructed rivers may have severe effects on the ability of fish to move to essential habitats, and maintaining connectivity in rivers may therefore be important for their viability (Bunn & Arthington, 2002). Today, permission for construction of a tunnel from the existing Power station at Eidefoss with an outlet in the lower part of the river Otta is provided. Additionally also development of a Power Station above the Rosten waterfalls is under constructions and is planned to be finished in 2018 (www.eidsivaenergi.no).

Detection of migrants

A total of 14 individuals were identified as migrants between the above Rosten to the below Rosten population. Two individuals with a genotype from within the barriers were also detected downstream Harpefoss Power Station. Most of the gene flow came downstream the natural waterfall, which corresponds to the earlier study by Junge et al. (2014) from the same river system. Fish ways may enable movements upstream dams and within rivers (Clay, 1995). The efficiency of the fish ways have shown to be important (Noonan, Grant, & Jackson, 2012), but also that they are operationally throughout spawning migrations (Van Leeuwen, Museth, Sandlund, Qvenild, & Vøllestad, 2016). Although many studies mainly focus on the upstream movements in rivers, however allowing for downstream movement and gene flow may also be equally important (Lucas & Baras, 2001). Damage or death by the turbines or spill ways, is a problem for fish moving downstream dams (Østergren & Rivinoja,

2008). Several studies have tried to understand and facilitating downstream passage (Calles, Karlsson, Hebrand & Comoglio, 2010: Arnekleiv, Kraabøl, & Museth, 2007).

All the 14 individuals that were detected with a genotype from above the Rosten waterfalls had been assigned to a spawning location in close distance to the waterfalls in the upper part of the river Gudbrandsdalslågen. Five of these individuals had also been radio-tagged, which made it possible to investigate their swimming behaviour during a season. All these fish remained more or less stationary during the season, in close distance to the Rosten waterfalls. However, the individuals with same spawning locations but with a genotype from within the barrier displayed a lot more long distance movement during a season. These individuals seemed to move upstream for spawning and downstream shortly after spawning. This again confirms the isolation by distance results that indicated a lot of movement by the individuals within the barriers. This may question that the grayling with different genotypes have different swimming behaviour, or that they simply residue below the waterfalls because they want to move back upstream. Although all the 14 individuals had their spawning location in the upper part of the river Gudbrandsdalslågen, the seasonal behaviour could only be detected for five of the individuals, which is a low sample size for a formal analysis. The nine individuals without telemetry information may also have remained stationary during the season. Further, investigations of the swimming behaviour of individuals that are migrants to systems with different genotypes would be interesting.

Conclusion

This study shows four populations of grayling within a large river system, separated by migration barriers that hamper upstream migration and direct the gene flow downstream. Within the large stretch of the river where there is no migration barriers, the grayling were not genetically structured, indicating that the grayling not home to particularly spawning locations. However, large variation in individual movements were observed. The study therefore highlights the importance of retaining connectivity within large rivers, allowing individuals to move among essential habitats that may enhance their fitness. Insight into how the genetic population structuring within rivers are shaped either by geographical barriers or the use of different spawning habitats in rivers, may give knowledge about the present connectivity.

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Appendix 1 - Microsatellite genotyping

Extraction of DNA from adipose-fin clips:

For the present study the DNA was extracted from the fin-clips using the purification Qiagen DNeasy Blood and Tissue kit. Protocol: purification of total DNA from animal tissues (Spin-column protocol). Before beginning the protocol, the working bench and necessary equipment were cleaned with 70% Ethanol. Lab coat and gloves were used during the entire purification protocol, to avoid contamination from DNA other than the DNA from the grayling fin - clip.

The storing tubes with grayling fin-clips were taken out of the fridge and kept on ice to preserve the samples. Each fin-clip was then taken out of the storing tube (with 95% ethanol) using a tweezers. Approximately 25 mg of the fin-clip was cut off and placed in a 1.5 ml micro-centrifuge tube. (The rest of the fin-clip was put back into the storing tube and kept on ice.). The scissors and tweezers were cleaned with 95% ethanol between each individual sample, to avoid mixing the DNA between the different individuals. Next 180 µl Buffer ATL and 20 µl were added to the micro-centrifuge tubes and mixed by vortexing for 10s. The samples was then placed in a thermomixer for incubation at 56 ° C, until the tissue were completely lysed after minimum 15 hours and maximum 20 hours.

After incubation, the tubes were taken out and mixed by vortexing for 15 s. Subsequently 200 µl Buffer AL was added to each sample and mixed by vortexing 10 s, before adding 200µl ethanol (%) and mixing by vortexing. The mixture was then pipetted into the DNeasy Mini spin column placed in a 2 ml collection tube. The tubes were placed in a micro-centrifuge and centrifuged at 8000 rpm for 1 min. The flow- through and the collection tube were discarded and the spin column was placed in a new 2 ml collection tube. 500 µl Buffer AW1 was added and the mixture was centrifuged again for 1 min at 8000 rpm. The flow–through and collection tube were discarded and the spin column was placed in a new collection tube. 500 µl Buffer Aw2 was then added and the mixture was centrifuged for 3 min at 14000 rpm, the collection tube and flow- through were discarded. The spin column was then placed in a 1.5 ml LoBindtube and 100 µl Buffer AE was pipetted directly onto the DNeasy membrane to dilute the DNA from the membrane. The samples incubated for 1 min at room temperature, and were then centrifuged for 1 min at 8000 rpm. This time the flow through with the DNA was saved in loBind tubes labeled “Elution 1”. The spin columns were

then placed in new loBind tubes labeled as "Elution 2" and the last step was completed a second time to elute more of the DNA from the DNeasy membrane.

Quantification of DNA:

The Qubit 2.0 Flurometer was used to quantify DNA in our samples. The number of 0.5 ml tubes needed was set up including 2 standards. The Qubit working solution consisted of dsDNA BR regent 1:200 in Qubit dsDNA BR buffer. The final volume in each tube was 200 µl. Each standard tube consisted of 190 µl with Qubit working solution and 10 µl of either standard 1 or 2. Each sample tube consisted of 198 µl Quibit working solution and 2 µl DNA from the samples. The mixtures were vortexed carefully for 5 s. and incubated for 2 min at room temperature. Afterwards the samples tubes were inserted to the Qubit 2.0 flurometer to quantify DNA.

Both elution 1 and elution 2 was analyzed with the Qubit 2.0 Fluorometer to see which solution that gave the highest concentrations of DNA. Elution 2 did also function as a backup in case of damage or loss of elution 1. To prevent fragmentation of the DNA by unnecessary freeze-thaw cycles, 20 µl of elution 1 was stored in the fridge. The residue of 80 µl of elution 1 and 100 µl of elution 2 was stored in the freezer at – 22 °C to protect the DNA from degradation.

Handling of DNA:

The samples from the freezer were slowly thawed on ice and carefully vortexed before 10µl of Elution 1 was transferred to labeled loBind tubes. The samples was then placed together with a cooler inside a Styrofoam box to keep them as cool as possible. The Styrofoam box was carefully wrapped together in a box and a letter that stated that the package did not contain any hazardous material was attached. The package was shipped by DHL (http://www.dhl.no/no.html) to Switzerland for microsatellite genotyping by the company Ecogenics (http://www.ecogenics.ch).

Table 1. Overview of microsatellite loci with genotyping information. Includes the developer of the markers, the allele range, multiplex number (M), primer concentration (μM), PCR temperature (°C) and the Fluorescent dye (dye). The table is based on the table from the study of Junge et al. (2014) (supplementary data Table 2), including new genotyping information for the present study.

			Ecogenics				**Junge**			
Locus	**Devolper**	**Allele range**	**M**	**μM**	**°C**	**dye**	**M**	**μM**	**dye**	**°C**
BFRO13	GenBank: AF151370	235-247	3	0.3	56	Atto550	1	0.09	FAM	58
BFRO18	Sušnik et al. 1999	177-195	2	0.3	56	FAM	1	0.04	VIC	58
BFRO10	Sušnik et al. 2000	96-126	2	0.3	56	Atto565	1	0.08	VIC	58
214	Junge et al. 2010	292-313	4	0.3	56	Atto550	1	0.14	PET	58
414	Junge et al. 2010	393-421	1	0.15	56	FAM	2	0.20	FAM	60
313	Junge et al. 2010	180-204	3	0.3	56	FAM	2	0.18	NED	60
BFRO11	Sušnik et al. 2000	80-102	1	0.3	56	Atto550	3	0.30	NED	59
433b	Junge et al. 2010	283-319	3	0.6	56	Atto550	3	0.18	NED	59
415	Junge et al. 2010	189-221	1	0.3	56	FAM	3	0.33	PET	59
309	Junge et al. 2010	447-451	3	0.3	56	FAM	4	0.57	FAM	59
445	Junge et al. 2010	366-468	4	0.3	56	FAM	4	0.13	NED	59
438	Junge et al. 2010	249-301	2	0.3	56	Atto532	single	0.34	VIC	60

Appendix 2 - Swimming Behaviour

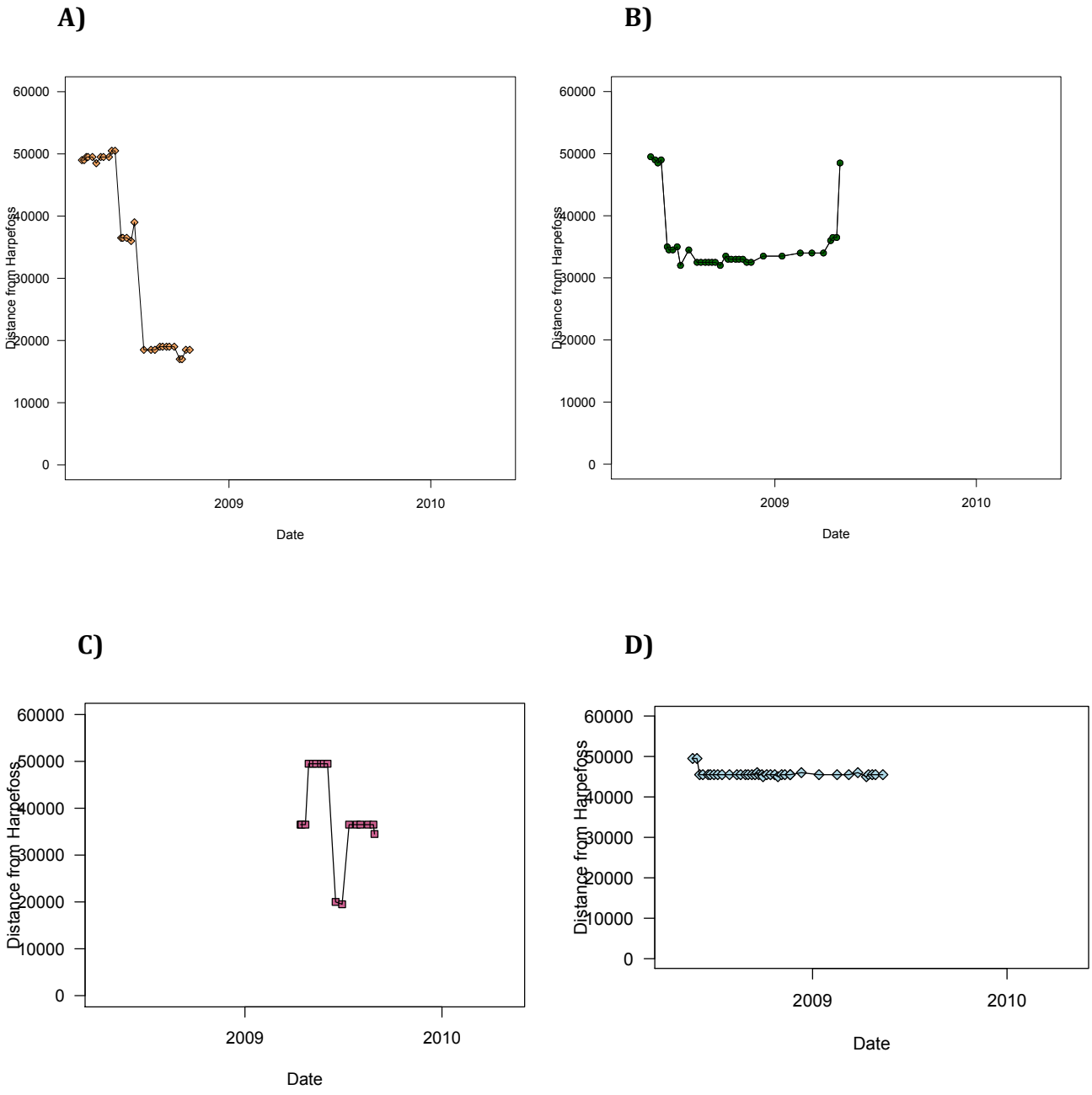


Figure 1. Plot **A-D** illustrating typical swimming behaviour observed for individual radio tagged grayling in Gudbrandsdalslågen, throughout a season. Each point in the graph represents a position in the river. The x-axis shows the year the fish was followed and the y-axis shows the distance from Harpefoss Power Station.

Appendix 3 - STRUCTURE analysis

A)

B)

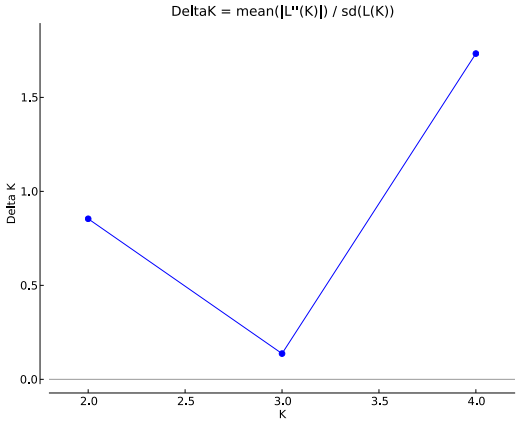


Figure 1. STRUCTURE result for the analysis that included grayling from the river Gudbrandsdalslågen and the river Otta: population 4-5 and 8-10. **A** show the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, were each line represents an individual with a colour of their putative cluster. In this output each line contains two colours indicating that individuals belong to both the putative clusters. **B** shows the Structure Harvester graph with the optimal number of populations clusters (K), estimated low probabilities of K=2. Indicating that there was only one population.

A)

B)

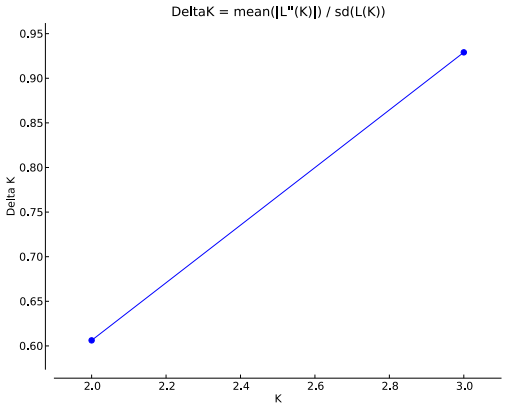
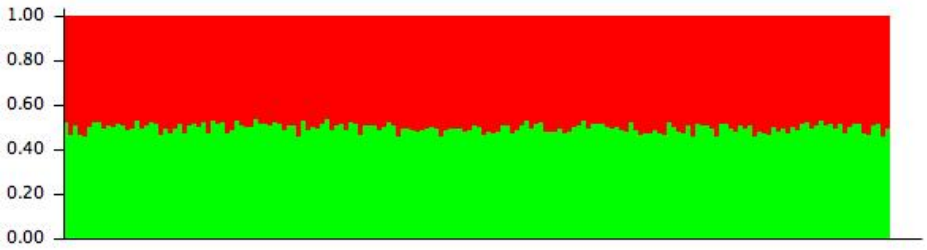


Figure 2. Up STRUCTURE result for the analysis that included grayling from upper part of the river Gudbrandsdalslågen below the Rosten waterfalls and the river Otta: population 4-5 and 8-10. **A** show the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, were each line represents an individual with a colour of their putative cluster. In this output each line contains two colours indicating that individuals belong to both the putative clusters. **B** shows the Structure Harvester graph with the optimal number of populations clusters (K), estimated low probabilities of K=2. Indicating that there was only one population.

A)



B)

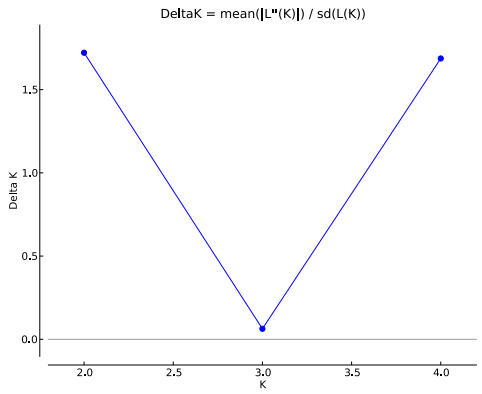


Figure 3. STRUCTURE result for the analysis that included grayling from lower part of the river Gudbrandsdalslågen below and the river Otta: population 4-5 and 8-10. **A** show the bar output for the STRUCTURE analysis for K=2. The bar output consists of several lines, were each line represents an individual with a colour of their putative cluster. In this output each line contains two colours indicating that individuals belong to both the putative clusters. **B** shows the Structure Harvester graph with the optimal number of populations clusters (K), estimated low probabilities of K=2. Indicating that there was only one population.

Appendix 4 - Detection of migrants

Table 1. Individuals detected as migrants with STRUCTURE or Geneclass2. Showing their spawning locations and the probability (%) of belonging to detected genotype location.

		STRUCTURE		**Geneclass2**	
Sample name	**Spawning location**	**Genotype location**	**Probability (%)**	**Genotype location**	**Probability (%)**
HAF00011	Below Harpefoss	Within Barriers	97.0	Within Barriers	99.9
HAF00012	Below Harpefoss	Within Barriers	98.9	Within Barriers	99.9
2.361	Within Barriers	Lesjaskogsvatnet	80.0	Lesjaskogsvatnet	99.9
39	Within Barriers	Lesjaskogsvatnet	75.1	Lesjaskogsvatnet	99.7
21	Within Barriers	Lesjaskogsvatnet	98.7	Lesjaskogsvatnet	99.5
RostBel_36	Within Barriers	Lesjaskogsvatnet	90.2	Lesjaskogsvatnet	100
RostBel_33	Within Barriers	Lesjaskogsvatnet	95.2	Lesjaskogsvatnet	99.9
RostBel_32	Within Barriers	Lesjaskogsvatnet	84.9	Lesjaskogsvatnet	99.8
2.251	Within Barriers	Above Rosten	88.9	Above Rosten	99.9
9	Within Barriers	Above Rosten	86.3	Above Rosten	99.9
20	Within Barriers	Above Rosten	72.2	Above Rosten	99.7
12	Within Barriers	Above Rosten	49.6	Above Rosten	99.5
13	Within Barriers	Above Rosten	60.6	Above Rosten	99.4
11	Within Barriers	Above Rosten	94.3	Above Rosten	99.1
RostBel_28	Within Barriers	Above Rosten	80.0	Above Rosten	76.0
RostBel_31	Within Barriers	Above Rosten	43.1	Above Rosten	99.3
18	Within Barriers	Above Rosten	95.7	Above Rosten	99.8