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Habitat fragmentation has interactive effects on the population genetic diversity and individual behaviour of a freshwater salmonid fish

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Running head: Interactions between salmonid habitat and behaviour

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Abstract

Sufficient genetic diversity can aid populations to persist in dynamic and fragmented environments. Understanding which mechanisms regulate genetic diversity of riverine fish can therefore advance current conservation strategies. The aim of this study was to investigate how habitat fragmentation interacted with population genetic diversity and individual behaviour of freshwater fish in large river systems. We studied a population of the long-distance migratory, iteroparous freshwater salmonid European grayling (*Thymallus thymallus*) in south-eastern Norway. Genotyping (n=527) and radio-tracking (n=54) of adult fish throughout a 169-km river section revealed three major migration barriers limiting gene flow and depleting genetic diversity upstream. Individuals from upstream areas that had dispersed downstream of barriers showed different movement behaviour than local genotypes. No natal philopatry was found in a large unfragmented river section, in contrast to strong fidelity to spawning tributaries known for individuals overwintering in lakes. We conclude that (1) upstream sub-populations in fragmented rivers show less genetic variation, making it less likely for them to adapt to environmental changes; (2) fish with distinct genotypes in the same habitat can differ in their behaviour; (3) spawning site selection (natal philopatry) can differ between fish of the same species living in different habitats. Together this implies that habitat loss and fragmentation may differently affect individual fish of the same species if they live in different types or sections of habitat. Studying behaviour and genetic diversity of fish can unravel their complex ecology and help minimize human impact.

Key-words: barriers, dams and weirs, natal philopatry, radio-telemetry, spawning site fidelity, *Thymallus thymallus*.

1 Introduction

The persistence of many riverine fish species is currently challenged by habitat changes, including fragmentation, destruction, introduction of new species, climatic changes, and eutrophication (Nilsson *et al.*, 2005; Gallardo *et al.*, 2016). Adequate levels of genetic diversity can increase species' resilience to such changes, and increase the chance that at least some individuals in a population survive and reproduce (e.g. Hughes & Stachowicz, 2004). Understanding which mechanisms regulate genetic diversity in fish populations can therefore strongly benefit management and protection of vulnerable species (Piccolo, 2016).

Habitat fragmentation is perhaps the most dominant regulator of genetic diversity in riverine fish populations worldwide (Poff & Schmidt, 2016). Manmade and natural barriers such as dams, weirs and waterfalls often divide larger populations into multiple smaller sub-populations. These smaller sub-populations commonly have reduced genetic diversity, which notably affects upstream sub-populations because of a disproportionate reduction in upstream gene flow (Junker *et al.*, 2012; Gouskov *et al.*, 2015). River fragmentation can therefore increase the extinction risk for small upstream sub-populations (Swatdipong *et al.*, 2010; Junker *et al.*, 2012).

Individual behaviour can also strongly affect genetic diversity in riverine fish. Even in the absence of physical barriers to gene flow, individuals that consistently differ in their (reproductive) behaviour can become genetically differentiated (Waters *et al.*, 2000; Benestan *et al.*, 2015). Many fish species are iteroparous (i.e. have multiple reproductive cycles in their lifetime) and annually return to a particular spawning location, i.e. homing or philopatry (Hendry & Stearns, 2004). Philopatry to natal spawning locations (natal philopatry) can lead to reproductive isolation, which in turn can lead to genetic differentiation among spatially separated clusters. By this mechanism, behaviour can create spatial

patterning in genetic diversity, even in the absence of physical movement barriers (e.g. Waters *et al.*, 2000).

Both habitat fragmentation and individual behaviour affect the genetic diversity of the freshwater salmonid European grayling (*Thymallus thymallus* L.). This makes it a highly suitable species for investigating the combined impact of both processes. The European grayling is a long-distance migratory fish that spawns repeatedly in fast-flowing rivers or tributaries of lakes. There are populations described that live in rivers year-round, spawning in fast-flowing sections and overwintering in slow-flowing sections (Heggenes *et al.*, 2006). Other populations live in lakes, and migrate annually into smaller tributaries to spawn in spring (Barson *et al.*, 2009). Given that individuals rely on multiple habitat types throughout their annual cycle, they generally require high habitat connectivity.

The European grayling has always been a common species throughout Eurasia (Northcote, 1995), but many local populations are currently endangered due to human modifications of river and lake systems (Koskinen *et al.*, 2001). Among important impacts are habitat loss and reduced connectivity between the remaining habitats (Heggenes *et al.*, 2006; Junge *et al.*, 2014; van Leeuwen *et al.*, 2016). The strongest impact of habitat loss can be expected on fish that repeatedly rely on specific spawning locations as a result of philopatry. European grayling living in lakes repeatedly select the same tributary for spawning (Kristiansen & Døving, 1996), which can lead to genetic differentiation among tributaries differing in ecological conditions (Koskinen *et al.*, 2002; Barson *et al.*, 2009; Junge *et al.*, 2011). This likely makes them especially vulnerable to local habitat loss. It is currently unclear whether or not natal philopatry also occurs in populations inhabiting rivers year-round, and how this affects population vulnerability.

The aims of this study were to (1) expand our knowledge regarding the effects of habitat fragmentation on the genetic diversity in a study population in south-eastern Norway

by combining previous knowledge from two earlier studies with new data, (2) assess whether riverine populations of European grayling show natal philopatry, and (3) explore interactions between habitat fragmentation and the behaviour of individual fish. To achieve our aims, we first reassessed the previously identified sub-populations in our study area (Barson *et al.*, 2009; Junge *et al.*, 2014) by expanding the dataset (from 346 to 527 samples) and improving the methodology. In these two previous studies, we assigned spawning locations to individuals based on their capture locations. We reassess this dataset using known spawning locations. Secondly, we examined the possibility of natal philopatry in the riverine study population. Thirdly, we explored interactions between fish behaviour and habitat fragmentation by analysing behaviour of distinct genotypes in one location. We hypothesized that (1) habitat fragmentation would cause spatial structuring of genetic diversity; (2) natal philopatry would cause spatial structuring of genetic diversity in unfragmented river sections; and (3) distinct genotypes would show similar behaviour in similar habitats, as they originate from the same large population prior to fragmentation. Our approach combined population genetic analyses and radio-tracking of individual fish.

2 Methods

2.1 Study species

European grayling is a spring-spawning, iteroparous, salmonid with a widespread distribution throughout north-western Europe west of the Ural Mountains (Northcote, 1995). Adults migrate over long distances among spawning, feeding, and overwintering locations (Heggenes *et al.*, 2006). In winter, European grayling inhabit slow-flowing parts of rivers or lakes (Nykänen & Huusko, 2002; van Leeuwen *et al.*, 2016). In spring, they migrate to fast-flowing river sections or into tributaries for spawning (Kristiansen & Døving, 1996; Barson

et al., 2009). After hatching, larvae move downstream towards slower flowing nursery areas or into lakes (Nykänen & Huusko, 2003; Van Leeuwen *et al.*, 2017).

2.2 Study area

The study area consisted of Lake Lesjaskogsvatnet, a 169.5 km section of the Gudbrandsdalslågen River and a 15 km section of Otta River in south-eastern Norway (**Fig. 1**). Lake Lesjaskogsvatnet is the most upstream location, and situated 611 m above sea level, with a surface area of 4.52 km^2 and a mean depth of 10 m. Gudbrandsdalslågen River (catchment area: 11567 km^2) drains southwards from Lake Lesjaskogsvatnet, and is joined 82 km downstream by Otta River. The study area included a 15 km stretch of Otta River upstream to the Eidefoss Power Plant (a complete migration barrier for European grayling, Junge *et al.*, 2014). After Gudbrandsdalslågen River is joined by Otta River, the study area continued downstream below Otta City towards the hydropower dam at Harpefoss, and below Harpefoss to Tretten City (**Fig. 1**). The mean annual discharges of Gudbrandsdalslågen River at Rosten Waterfalls and Otta River at Eidefoss Power plant are 33 and 111 $m^3 s^{-1}$, respectively.

Multiple migration barriers can be identified in the study area by combining knowledge from two previous studies (Barson *et al.*, 2009; Junge *et al.*, 2014). Three barriers to upstream gene flow create four sub-populations (**Fig. 1**). The most upstream barrier is a small natural waterfall separating Lake Lesjaskogsvatnet (sub-population A) from Gudbrandsdalslågen River (sub-populations B, C and D). Sub-population B inhabits the section of the river between Lake Lesjaskogsvatnet and “Rosten Waterfalls”: a steep river section with several waterfalls and white rapids alternating with deep pools. The Rosten Waterfalls, Eidefoss Power Station in Otta River and Harpefoss Power Station (hereafter

"Harpefoss") enclose sub-population C. Sub-population D inhabits the river below Harpefoss.

People probably introduced European grayling above the Rosten Waterfalls at an unknown moment before 1880, and made the barrier at the entrance of Lake Lesjaskogsvatnet temporarily passable during the 1880's; allowing colonisation of the lake (Haugen & Vøllestad, 2001). Harpefoss replaced a natural waterfall in the 1960s, which was already considered a natural migration barrier before the hydropower development (Huitfeldt-Kaas, 1918). A fish passage was initially implemented in the dam; but was removed in 1995. Harpefoss is now a complete upstream migration barrier for fish.

2.3 Datasets - field sampling and tracking

We reanalysed genotyping and tracking data of an existing dataset (n = 346, hereafter "dataset 1") after expanding it with additional data (n = 181, hereafter "dataset 2"), resulting in a total of 527 analysed fish. For dataset 1, 165 European grayling were trapped as they ascended small tributaries of Lake Lesjaskogsvatnet for spawning. We assigned all these individuals to spawning location 1 (**Fig. 1, Table 1**), as they all spawned in one of the following six tributaries: Sandbekken (n=30), Hyrion Søre (n=30), Sprela (n=15), Skottåe Søre (n=30), Steinbekken (n=30) and Valåe (n=30) entering Lake Lesjaskogsvatnet. The additional 181 fish in dataset 1 were sampled by rod angling between 2008 and 2009 at locations 2 – 12 (**Fig. 1, Table 1**). For dataset 2, 181 adult fish were caught by rod angling just below Harpefoss in 2010 (n=25) and 2013 (n=7), and throughout the area of sub-population C in 2013 (n = 149, **Fig. 1, Table 1**). Thirty-seven individuals of dataset 1 and 38 individuals of dataset 2 were radio-tracked.

Spawning locations were assigned for all individuals in sub-population C to test for possible natal philopatry using two methods. Firstly, some of the fish were caught in

advanced states of maturity during the spawning season in spawning habitats, so we could safely assume they spawned near where we caught them. Secondly, we successfully tracked 54 fish by radio-telemetry (Supporting Information **Table S1**), and used this to assign individuals to spawning locations. The spawning locations for the radio-tagged individuals were assumed to be the most upstream locations visited during the spawning period. This improved our previous analyses, because we previously assumed their capture location was their spawning location, although not all individuals were caught during spawning.

2.4 Genetic data - genotyping and analysis

We assessed genetic diversity and differentiation within and between sampling locations using 12 polymorphic microsatellite markers (**Tables S2 and S3**). DNA was extracted for all new samples for dataset 2 from ~25 mg portions of sampled pelvic fin tissue (stored in 95% ethanol after sampling) using the Qiagen DNeasy Blood and Tissue kit according to manufacturer's standard protocol. After DNA-concentration was quantitatively assessed by a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) to be at least 20 ng μl^{-1}, all samples were genotyped by the company Ecogenics (http://www.ecogenics.ch, labelling details in **Table S2**). Information on the genotyping of the 346 samples in dataset 1 (**Table 1**) is available in earlier publications (Barson *et al.*, 2009; Junge *et al.*, 2011; Junge *et al.*, 2014). Thirty-three samples in dataset 1 were collected and genotyped simultaneously with the samples from Junge *et al.* (2014), but are included in the analyses for the first time.

Dataset 1 and 2 were combined and scored using Genemapper software v4.0 (Applied Biosystems, ABI, USA). Automatic scorings of allele sizes were manually checked and if necessary adjusted to ensure scoring of only true peaks. Twenty samples from dataset 1 were re-genotyped together with dataset 2 from stored DNA, and their identical results ensured safe combining of the two datasets. Genotyping of the samples in dataset 2 by Ecogenics

failed for locus BFRO010, hence, this marker was only included in dataset 1. Because dataset 1 already covers the full geographic extent of the study area (**Table 1**), excluding one of 12 markers in only the individuals in dataset 2 is not expected to have impacts on the results and interpretation.

Before all analyses, data were checked for null alleles based on the methods of Chakraborty *et al.* (1992) and Brookfield (1996). Null alleles frequencies were <10% for all loci. Given that the null alleles were randomly distributed over all loci and had low enough frequencies (Chapuis & Estoup, 2007), we continued the analyses with the full dataset. The total dataset comprised 5% missing data.

Data were analysed in R (R-Development-Core-Team, 2017), using package PopGenReport (Adamack & Gruber, 2014) to calculate all basic population statistics. Measures of population differentiation was calculated using packages hierfstat (Goudet, 2005) and mmod (Winter, 2012). Confidence intervals for G_{ST} -values were calculated by bootstrapping 1000 times, and assumed significant if they did not cross zero. We calculated possible deviations from Hardy-Weinberg equilibrium (HWE) using the method based on linkage disequilibrium restricted to alleles with frequencies >0.02 (Do *et al.*, 2014). We expressed population differentiation as G_{ST} to ensure compatibility with previous studies.

2.5 Sub-population structure and detection of migrants

The most likely number of sub-populations (K) was assessed using a Markov chain Monte Carlo (MCMC) algorithm in STRUCTURE v2.3.4 (Pritchard *et al.*, 2000). We used an ancestry model of admixture and assumed correlated allele frequencies (Francois & Durand, 2010). The algorithm was run 10 times for each value of K (range: 1-10), with 1000000 iterations after a 500000 iterations burn-in. We used the method of Evanno *et al.* (2005) to find the optimal number of clusters.

After assigning all individual fish to a sub-population, we detected putative migrants using STRUCTURE and GeneClass2 (Piry *et al.*, 2004). First, an assignment test in STRUCTURE used geographical sampling location as prior population information and assumed a user-specified prior probability (v) that an individual was an immigrant (Pritchard *et al.*, 2000). We used the default setting of 0.05, corresponding to individuals having a 5% probability of being an immigrant or having migrant ancestry. Posterior probabilities of immigrant ancestry were calculated one generation back, and models were run with lambda=1.0 and MCMC parameters as previously described. Second, in the Geneclass2 analysis we calculated the likelihood (*L*) that an individual originated from a given population as the ratio between the likelihood of the individual genotype within the population where the individual was sampled (*L_home*) and the highest likelihood value among all available population samples (*L_max*). Alpha was 0.05, and the number of simulated individuals was 10000. Individuals that had both a significant STRUCTURE probability >0.350 and a Geneclass2 likelihood >2.50 were assumed to be true migrants.

To test whether loci assorted independently, linkage disequilibrium was determined over all pairwise combinations of loci for the global dataset and per population using Genepop 4.2 (Rousset, 2008). We estimated effective population sizes (N_e) using the linkage disequilibrium method implemented in NeEstimator V2.01 (Do *et al.*, 2014). We assumed random mating, estimated N_e with the lowest allele frequency of 0.01 (including 72 of 129 alleles) and report confidence intervals as jack-knifed on loci.

2.6 Isolation-by-distance

We tested for patterns of isolation-by-distance among all individuals of sub-population C (**Fig. 1**) by comparing pairwise Nei´s D (Nei, 1972) to geographic distance via water between spawning locations (Rousset, 1997; Rousset, 2000). We compared the two matrices in a

Mantel test with 10000 permutations to evaluate the level of significance for the Pearson correlation coefficient in package ecodist (Goslee & Urban, 2007).

2.7 Radio-telemetry

We equipped 78 adults (28 females, 50 males) with radio-tags during 2008-2009, and could locate 75 (>96%; 28 females, 47 males) individuals multiple times for a mean of 242±120SD days. Data for 54 individuals were sufficient to assign spawning locations (**Table S1**). Weight and fork length were measured of all fish. Transmitter weight never exceeded 2% of fish weight. The study was performed with permission from local county governors and approved by the National Animal Research Authority (permit numbers 2008/26156, 2009/9174). Positions of radio-tagged fish were determined on average once per week for one year. The exact position of each fish was recorded as distance (with a precision of zones of 500 m) in upstream direction from the Harpefoss Power Station (for Gudbrandsdalslågen River) or the distance from the confluence of Otta River and Gudbrandsdalslågen River (for Otta River). Details on the transmitter attachment and tracking are in the footnote of **Table S1** and two previous publications (Junge *et al.*, 2014; van Leeuwen *et al.*, 2016).

3 Results

3.1 Population genetic diversity

The 12 loci displayed different levels of polymorphism, with in total 131 alleles and on average 11 alleles per locus (range: 3–36, **Table S3**). Population differentiation for the global dataset as represented by Nei´s G_{ST} was 0.130 (95%CI: 0.120-0.141), and varied by locus (**Table S3**). The global dataset deviated from HWE, with observed heterozygosity (H_o = 0.60) lower than expected heterozygosity (H_e = 0.66) for 11 of the 12 loci (paired sample t-test: t=-4.93, df=11, p<0.001, locus-specific information in **Table S3**). Tests for linkage equilibrium

revealed low levels and random distributions among loci of interlocus associations. Five of the 66 pairwise comparisons remained significant after sequential Bonferroni correction (Rice, 1989). Within populations, only six of the 264 pairwise comparisons (12 loci with four populations) were significant after sequential Bonferroni correction. We therefore included all loci in the analyses.

3.2 Sub-populations and migrants

Bayesian clustering verified two previously detected distinct clusters (sections A+B and C+D, **Fig. 1**), separated by the Rosten Waterfalls (Junge *et al.*, 2014). Subsequent analyses within each cluster further divided each cluster in two sub-populations, ultimately resulting in the best support for four sub-populations (**Table 2; Fig. 2**) with significant pairwise G_{ST} values in the global dataset (**Table 3**). The Rosten Waterfalls were the strongest barrier.

The level of genetic diversity and allele frequencies differed among the four sub-populations (**Table 2**), but each sub-population was in Hardy-Weinberg equilibrium. The global dataset deviated from HWE, indicating a reduction of observed heterozygosity caused by sub-population structure, i.e. the Wahlund effect (Wahlund, 1928). This confirms the presence of geographic barriers to gene flow in combination with genetic drift in the sub-populations. Allelic richness increased in a downstream direction of the river system, with the more private alleles found in sub-population A than D ($X^2 = 16.9$, $df = 1$, $P < 0.001$). Below the Rosten Waterfalls, nine individuals with genotype A and six individuals with genotype B were detected, with their genotypes assigned based on both the STRUCTURE and Geneclass2 analyses (**Table S4**). Two individuals with genotype C were detected downstream Harpefoss. No individuals with genotypes from below barriers were observed above barriers.

3.3 Spawning site fidelity and fish behaviour

We tested for a possible isolation-by-distance (IBD) relationship as a result of natal philopatry (hypothesis 2) for the 245 individuals genotyped as belonging to sub-population C. Within this area enclosed by the three barriers, no pattern of IBD was observed (simple Mantel correlation test: $r = 0.059$ (95%CI: 0.014–0.106), two-tailed p-value=0.33).

We also tested for possible behavioural differences between individual fish of distinct genotypes spawning in the same river section (hypothesis 3). In total we radio-tracked 54 individuals long enough to enable assigning spawning locations to them, and sixteen of these individuals spawned immediately downstream of Rosten Waterfalls. The remaining 38 individuals all had genotype C and showed expected spawning behaviour for European grayling lower in the river system. Among the sixteen individuals spawning at Rosten Waterfalls, three individuals had genotype A, one individual genotype B and 12 individuals genotype C (**Table 4**). This enabled us to compare individual behaviour of distinctive genotypes all spawning in the same location just below Rosten Waterfalls. The four fish that genetically originated from above the Rosten Waterfalls (genotypes A or B) stayed close to the waterfalls throughout the season and moved only short distances between subsequent relocations (1460 m ±1485SD, n=25 movements on four individuals, positioned every 6.4±2.0SD days during May and June, **Fig 3**). However, the 12 individuals with genotype C moved extensively throughout the area enclosed by the three barriers, particularly during the spawning season (3000 m ±6576SD, n=65 recorded movements on 12 individuals, positioned every 7.3±3.3SD days during May and June, **Fig. 3**). Individuals with genotype A or B used a smaller section of the river system throughout the year (mean range=6625 m ±2955SD) than individuals with genotype C (22083 m ±8928SD, Welch´s Two Sample t-test, t=-5.20, df=13.91, p<0.001, **Fig. 3**).

4 Discussion

4.1 Habitat fragmentation and natal philopatry

Combined radio-telemetry and genetic analyses on a European grayling population in a large Nordic river system confirmed our first hypothesis: i.e. that the structure of genetic diversity was affected by disturbed connectivity of the studied system. The strongest or oldest barrier to gene flow was a natural waterfall: Rosten Waterfalls. Rosten Waterfalls consists of a series of cascades and rapids, clearly passable in the downstream direction, but likely completely blocking upstream migration for European grayling. The two other migration barriers in the system also constrained upstream gene flow, but historically there must have been some upstream gene flow to allow colonization of the river and lake after the last ice age. These observations build on our two previous studies in this system (Barson *et al.*, 2009; Junge *et al.*, 2014), and confirm other studies on the effects of river fragmentation on fish populations (Fagan, 2002; Swatdipong *et al.*, 2010; Junker *et al.*, 2012; Gouskov *et al.*, 2015).

No further genetic differentiation occurred in the large unfragmented section of the river system. This refutes our second hypothesis, i.e. that sub-population structuring would occur in the large unfragmented river section due to natal philopatry. This is surprising, because natal philopatry has been documented extensively for European grayling populations living mainly in lakes (Kristiansen & Døving, 1996), including in Lake Lesjaskogsvatnet (sub-population A) upstream in our study system (Barson *et al.*, 2009). European grayling colonized this lake in the 1880's, when an earlier physical migration barrier was removed due to human activity (Haugen & Vøllestad, 2001). This barrier was later re-established, explaining the current genetic differentiation with the sub-populations in the river. The fish live most of their life in the lake, but spawn in a large number of small tributaries that differ in size and environmental conditions, leading to patterns of isolation-by-distance among the individuals with natal philopatry to the different tributaries (Barson *et al.*, 2009; Junge *et al.*,

2011). This strong philopatric behaviour has even facilitated development of life-history differentiation among individuals spawning in the various tributaries (Kavanagh *et al.*, 2010; Thomassen *et al.*, 2011; Papakostas *et al.*, 2014). All this evidence suggests natal philopatry for the individuals overwintering in the lake, in contrast to the absence of isolation-by-distance in the river system.

Possible explanations for this lack of genetic differentiation in the unfragmented river section firstly include the more homogeneous habitat in river systems than in lake-tributary systems. In Lake Lesjaskogsvatnet for example, tributaries strongly differ in their spring temperatures, increasing the benefits of selecting a particular tributary. Water temperature is likely more similar among the various spawning habitats in the large river system, which could lower the necessity of selecting one particular spawning location. A second possible reason is that adult fish could be repeatedly faithful to a particular spawning location, but if this is not their natal spawning location, no pattern of IBD occurs. Hence, individuals possibly also repeatedly spawn at the same location in rivers, but this does not give rise to genetic differentiation within the river because this is not their natal site. Thirdly, European grayling fry drift downstream extensively after hatching (Van Leeuwen *et al.*, 2017). Those hatching in tributaries generally drift to lakes, while those hatching in large rivers will drift to other river sections. Drift of riverine fry could cause more mixing than drift of fry hatching in tributaries connected to lakes. This could mask possible patterns of genetic diversity, but this idea remains to be further tested.

4.2 Behavioural differences between genotypes

We expected fish of different genotypes to behave similarly in similar habitats (hypothesis 3), because all fish in the different sub-populations originate from the same large sub-population. However, individuals genetically belonging to sub-populations upstream the Rosten

Waterfalls that had descended the waterfalls showed very little movement during the periods of observation. Although we only monitored four migrant individuals, none of them moved beyond seven kilometres downstream of Rosten Waterfalls. In contrast, local individuals from below Rosten Waterfalls (genotype C) showed extensive downstream overwintering migrations. All individuals spawned in a large spawning area just below the waterfalls (previously described in Museth *et al.*, 2011), but downstream wintering migration was only observed for genotype C. This demonstrates how fish of different sub-populations can show different behaviour, even though they once originated from the same source population. Such dependence of individual behaviour on genotype can for instance be compared to behavioural differences between wild and hatchery-type grayling (Horká *et al.*, 2015), but might have important consequences when deliberately relocating fish from lakes to river systems or vice versa. Individual genotypes with distinct behaviours likely require different habitat types.

5 Conclusion

This study confirms that river fragmentation can cause strong population differentiation in European grayling populations, and newly shows that natal philopatry (as known for populations inhabiting lake-tributary systems) is not found in unfragmented river sections. This implies that loss of spawning habitat in lake-tributary systems might differently impact the spawning possibilities of European grayling than loss of spawning habitat in large river systems. If habitat is lost, riverine individuals may be more opportunistic in finding new spawning locations than lake-dwelling individuals that appear to rely on particular tributaries. Within rivers, more downstream sub-populations – thanks to higher genetic diversity – may have greater plasticity and adaptability in their reproduction in response to changes in local conditions.

This illustrates how habitat loss and fragmentation may differently affect individual fish of the same species (1) inhabiting different sections of one habitat (up- or downstream in a river) and (2) inhabiting different habitat types (lakes or rivers). Furthermore, behaviour can differ between genotypes of the same species within one habitat. Behavioural differences between individuals from different sub-populations imply that individuals passing barriers in fragmented rivers may not necessarily adjust easily to their new habitat. How long it takes individuals to adjust their behaviour to new environments, and whether or not their reproductive performance differs from local genotypes, remain interesting avenues for further study. To predict the impact of human-induced habitat changes in a world that is increasingly interested in green energy by hydropower plants, it is essential to study the behaviour and genetic diversity of the fish populations present combined.

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Tables

Table 1: The 527 European grayling captured and genotyped in the study area. The location numbers refer to locations as depicted on the map in **Fig. 1**, and distances from Harpefoss dam (in Gudbrandsdalslågen River if not indicated that in Otta River). The indicated distances are from Harpefoss Power Station and either upstream (positive) or downstream (negative), or upstream in Otta River if specifically indicated. Telemetry indicates the number of individuals for which their spawning locations were assigned based on radio-telemetry tracking.

Location	Distance	Study	Dataset	Year	Number of individuals	Telemetry
1	124	(Barson *et al.*, 2009; Junge *et al.*, 2011)	1	2008/09	165	
2	63	This study	2	2008/09	19	
3	58	(Junge *et al.*, 2014)	1	2008	49	
4	52	This study (Junge *et al.*, 2014)	1 and 2	2013 2008/09	49 17	8
5	49	This study (Junge *et al.*, 2014)	1 and 2	2013 2008/09	8 13	2
6	42 (in Otta)	(Junge *et al.*, 2014)	1	2008/09	15	15
7	51 (in Otta)	This study (Junge *et al.*, 2014)	1 and 2	2013 2008/09	37 27	8
8	35	This study (Junge *et al.*, 2014)	1 and 2	2013 2008	40 8	20
9	15	This study (Junge *et al.*, 2014)	1 and 2	2013 2008	15 1	1
10	1	This study This study	1 and 2	2010 2013	25 7	
11	-1	(Junge *et al.*, 2014)	1	2008/09	18	
12	- 45	This study	2	2008/09	14	

Table 2: Genetic diversity statistics and sample sizes for individuals assigned to each of the detected genetic clusters, arranged from upstream to downstream. Number of fish (*N*), allelic richness standardized by rarefaction for the minimum sample size of 30 individuals (A_R), the number of alleles (Na), mean expected (H_e) and observed heterozygosity (H_o) with standard deviation, paired t-tests for deviations from HWE (*t*- and *p*-values for 11 degrees of freedom over 12 loci), the number of private alleles (*Pa*) and estimated effective population size (N_e) with confidence intervals (inf = infinity) are indicated.

Population	***N***	A_R	***Na***	H_e ± SD	H_o ± SD	***HWE***		***Pa***	N_e ***(95%CI)***
						t	***p***		
All	527		131	0.661 ± 0.214	0.600 ± 0.224	-4.93	<0.001	-	-
A	185	4.13	69	0.627 ± 0.175	0.644 ± 0.187	1.57	0.15	5	400 (236-1022)
B	67	3.66	60	0.480 ± 0.250	0.479 ± 0.268	-0.08	0.94	0	41 (29-62)
C	245	5.11	93	0.613 ± 0.260	0.605 ± 0.275	-0.54	0.60	15	598 (352-1570)
D	30	6.11	94	0.693 ± 0.242	0.582 ± 0.290	-1.65	0.13	29	140 (35-inf)

Table 3: Nei´s pairwise G_{ST} values between the four identified sub-populations in the lower triangle, with associated confidence intervals in the upper triangle.

	A	**B**	**C**	**D**
A		0.093-0.195	0.068-0.167	0.117-0.319
B	0.140		0.036-0.112	0.146-0.412
C	0.110	0.070		0.080-0.256
D	0.290	0.270	0.154	

Table 4: Radio-telemetry details for the 16 individual European grayling that spawned in the section of Gudbrandsdalslågen River just below Rosten Waterfalls (location 4 in **Fig. 1**), but originated from sub-populations A, B or C.

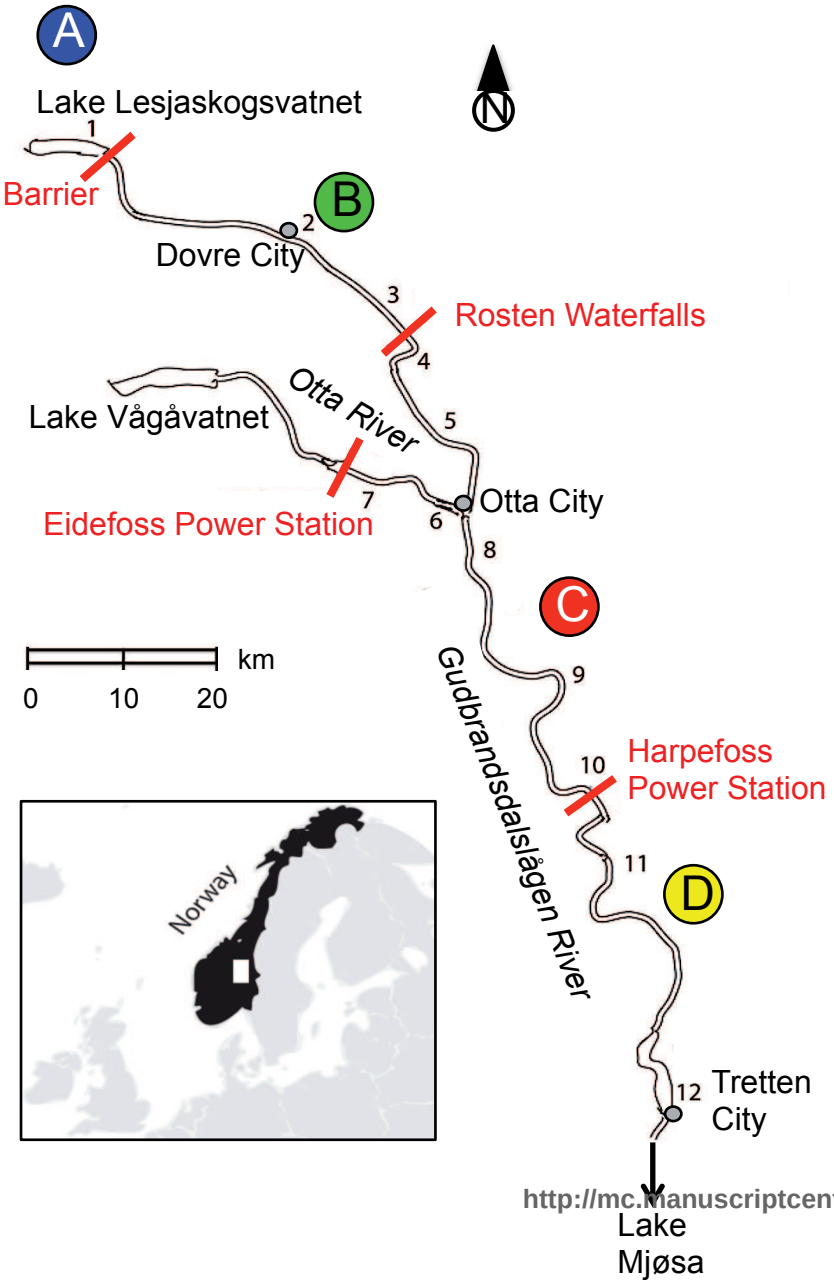
Individual	Genotype	Sex	Spawning location distance from Harpefoss (km)	Start date	End date	# positionings
1	C	F	49.5	21-May-2008	22-May-2009	35
2	C	F	49	10-April-2008	24-Sep-2008	24
3	C	F	48	10-April-2008	14-Jan-2009	34
4	C	F	49	6-Apr-2009	28-01-2010	29
5	C	M	49.5	14-Apr-2009	29-Aug-2009	17
6	C	M	49.5	9-Jun-2009	5-Apr-2010	30
7	C	M	49	21-May-2008	29-Apr-2009	34
8	C	M	49.5	21-May-2008	13-May-2009	38
9	C	M	52	27-May-2008	15-Sep-2008	14
10	C	M	52	27-May-2008	9-Mar-2009	27
11	C	M	52	27-May-2008	15-Aug-2008	11
12	C	M	50.5	10-Apr-2008	22-Oct-2008	28
13	A	F	52	27-May-2008	13-Jan-2009	29
14	A	F	52	21-May-2008	14-May-2009	37
15	A	F	52	21-May-2008	27-May-2009	40
16	B	M	52	21-May-2008	21-Aug-2008	13

Figure legends

Figure 1: Map of the study system with the four sub-populations indicated in blue (A), green (B), red (C) and yellow (D). Red bars crossing the rivers indicate the four migration barriers separating the sub-populations. The numbers refer to the 12 sampling locations indicated in **Table 1**.

Figure 2: STRUCTURE results for inference of the number of genetic clusters in the study system based on the extended dataset, confirming that three barriers form four genetic clusters in the study system (Barson *et al.*, 2009; Junge *et al.*, 2014). The proportional membership (*Q*) to one of the four sub-populations (A, B, C or D) is indicated for each individual fish by one horizontal bar. Individuals are ordered by their geographical sampling location from upstream (top) to downstream (bottom) in the study system.

Figure 3: Movement behaviour for the 16 European grayling spawning just below Rosten Waterfalls and tracked by radio-telemetry. The horizontal axis depicts the time of the monitoring period between 2008 and 2010, and the vertical axis the position of individual fish as distance from Harpefoss dam by water. The horizontal black bar indicates the migration barrier formed by Rosten Waterfalls. Each individual has a different colour-shape combination. Individuals 1-12 clustered to genotype C, 13-15 to genotype A and 16 to genotype B (details in **Table 4**).


A
Lake Lesjaskogsvatnet
1
Barrier
B
2
Dovre City
3
Rosten Waterfalls
4
5
Lake Vågåvatnet
Otta River
7
Eidefoss Power Station
6
Otta City
8
C
km
0
10
20
9
Gudbrandsdalslågen River
10
Harpefoss
Power Station
11
D
Norway
12
Tretten
City
Lake
Mjøsa