Nofima

Genetic variation in Norwegian Atlantic salmon (*Salmo salar* L.) associated with anthropogenic activity

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Report	ISBN: 978-82-8296-062-5 (printed) ISBN: 978-82-8296-063-2 (pdf)	Report no: 10/2013	Accessibility: **Open**
Title: **Genetic variation in Norwegian Atlantic salmon (Salmo salar L.) associated with anthropogenic activity**		Date: 5 February 2013	
		Number of pages and appendixes: 53	
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By agreement with: Fiskeri- og Havbruksnæringens Forskningsfond (FHF)		Contractors ref.: FHF #900272	
Three keywords: Atlantic salmon, anthropogenic activity, population genomics			

Summary:

Atlantic salmon populations are affected by a number of different anthropogenic activities on local to regional scales. In a recently updated salmon register for Norway maintained by the Directorate for Nature Management (2012), the salmon populations in 54 rivers are categorised as being critically endangered or extinct in the wild. Waterway regulation and acidification are listed as being the most important cause of extinction in 23 and 14 of these rivers, respectively. Given these effects on population viability and productivity, it would be interesting to know whether it is possible to detect effects of waterway regulation and acidification at the genetic level; either through loss of genetic variability or through adaptive responses to altered selective regimes. Therefore, the aim of this study was to assess genetic variation in populations of Atlantic salmon in western Norway that have experienced environmental changes due to river acidification and waterway regulation.

Genomic variation was assessed at 3761 single nucleotide polymorphism (SNP) markers in Atlantic salmon from 25 salmon populations in western and south western Norway. This study identified SNPs which differentiate samples that are affected by acidification and waterway regulation from those that are unaffected, or affected to a lesser degree. It is possible that these SNP markers differentiate the populations due to selection acting on genes closely linked to these loci; however, other mechanisms can also cause such differentiation. Although the rivers in this study were chosen based on their history of acidification and/or regulation, it is likely that these rivers have been affected by other anthropogenic factors. The scope of the present study did not allow for accurate testing of selection of these markers due to the lack of historical samples and low sample sizes, however selection cannot be excluded as the cause of genetic differentiation. In order to determine the fitness consequences in populations of Atlantic salmon affected by these studied anthropogenic activities, it will be necessary to include more samples from a greater distribution to estimate population size changes and changes in genetic variation over a temporal time scale.

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1   Introduction

1.1   Background

Atlantic salmon populations are affected by a number of different anthropogenic activities on local to regional scales. In the recently (2012) updated salmon register for Norway maintained by the Norwegian Directorate for Nature Management, the status and threats for salmon populations in 481 Norwegian rivers have been evaluated, these can be found at: http://www.dirnat.no/content/500042245/Bestandstilstand-for-laks. In this listing, the salmon populations in 54 rivers are now categorised as being critically endangered or extinct in the wild. Waterway regulation and acidification are listed as being the most important cause of extinction in 23 and 14 of these rivers, respectively. Moreover, it is estimated that waterway regulation has a negative effect on productivity of salmon populations in 110 rivers (23 % in total), and that acidification negatively affects 42 rivers (9 % in total). Given these effects on population viability and productivity, it would be interesting to know whether it is possible to detect effects of waterway regulation and acidification at the genetic level, either through loss of genetic variability or through adaptive responses to altered selective regimes. In this report, we used modern molecular genetic techniques to study salmon populations that have been affected by these two factors, all of them situated in an area of south western and western Norway where waterway regulation and acidification have affected salmon populations for more than a century.

The focus of this study does not imply that the populations under study are not affected by other anthropogenic activities. The salmon register of the Directorate for Nature Management lists several other factors negatively affecting Atlantic salmon populations: physical interference, pollution, agricultural effluents, *Gyrodactylus salaris*, over-fishing, sea lice and escaped farmed salmon. The latter two were listed by the Scientific Advisory Committee for Atlantic Salmon Management (Anon, 2011) as the most severe current threats to salmon populations in Norway. Furthermore, the recent study by Glover, *et al.,* (2012) showed evidence of genetic changes likely to be caused by escaped farmed salmon in some wild populations within our study area. Our study design therefore attempts to study genetic effects of waterway regulation and acidification while acknowledging that other anthropogenic activities may also affect the genetic diversity of the study populations.

1.1.1   Acidification

Salmon rivers in Norway have likely been affected by acidification since the late 19th century (Dahl, 1927). As a result of acidification and subsequent increase in aluminium (Al^{3+}) concentration, it is estimated that 14 salmon populations in rivers in southern Norway have become extinct, and many populations in western Norway have been negatively affected (Hesthagen and Hansen, 1991; Hesthagen *et al.*, 2011; Rosseland and Kroglund, 2011). Large-scale liming projects have been conducted to reduce the damage to salmon rivers due to acidification (Hesthagen *et al.*, 2011; Sandøy and Langåker, 2001; Staurnes *et al.*, 1995); Figure 1 shows the location of southern Norwegian salmon populations that were considered extinct, threatened or vulnerable by 2007-2008 as well as the location of rivers being limed).
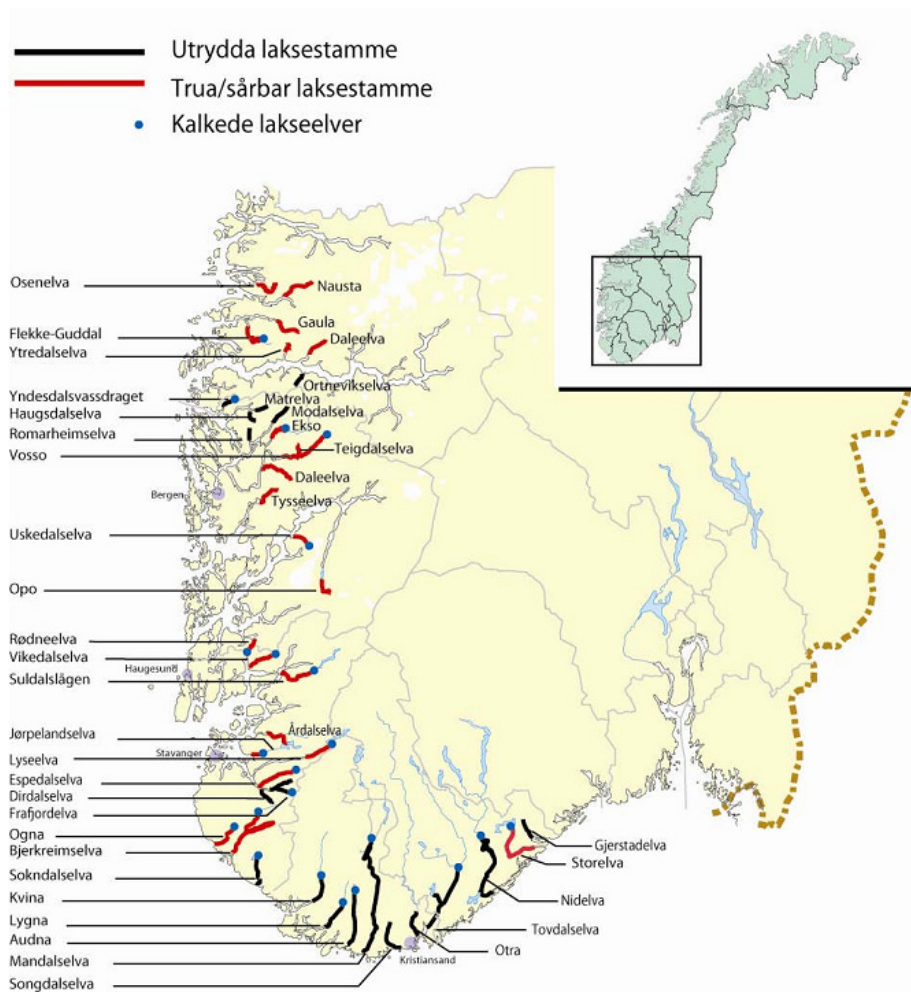
Figure 1   The status of Atlantic salmon populations in rivers in southern and western Norway in 2007-2008. Black lines denote rivers from which Atlantic salmon are extinct. Red lines denote rivers from which Atlantic salmon are threatened or vulnerable. Blue points indicate rivers that are treated by liming. From HANSEN et al., (2008)

Collectively, it has been estimated that the rivers affected by acidification represent a loss of smolt production at 1.5 million smolts (Hesthagen and Hansen, 1991), compared with a total estimated natural smolt production at 6 million (Ståhl and Hindar, 1988). In southern Norway, several rivers have been naturally and artificially recolonised by Atlantic salmon after liming of the rivers and other management actions to reach environmentally benign conditions (Dalziel *et al.*, 1995; Haraldstad and Hesthagen, 2003; Hesthagen *et al.*, 2011). In many cases, the genetic history of the population can be reconstructed by analysing archived scale samples from before local extinction (Hesthagen, 2010). It is also possible to study populations that did not go extinct but existed at very low population levels through the acidification period. Differences in tolerance to acidic water have been documented both among strains of Atlantic salmon (Fraser *et al.*, 2008), and at different life-stages within strains (Rosseland *et al.*, 2001). In addition, it has been established that tolerance to low pH is a heritable trait (Gjedrem and Rosseland, 2012), implying additive genetic variation exists. This finding has potential management use as re-introduction of Atlantic salmon to barren rivers may benefit from choice of acid tolerant individuals.

1.1.2   Waterway regulation

Most hydropower facilities in Norway were constructed in the period between 1950 and 1975 (Heggberget *et al.*, 1999; Johnsen *et al.*, 2010c). Of all Atlantic salmon rivers in Norway, 185 are affected by changes in water flow from hydroelectricity construction. Of these, 110 salmon populations are regarded as being negatively affected, thus: Extinct (23), threatened (9), vulnerable (11), reduced (52), warrant consideration (11), and uncertain (4). The most severe effects are related to dams or other migration barriers on the natural anadromous stretch, or strong reductions in water flow (Johnsen *et al.*, 2010a). More benign effects occur where river regulation occurs above the natural salmon-producing stretch and where mitigatory action (such as environmental flows) has been implemented to secure salmon habitats. Waterway regulation has led to reduced salmon production, suggested to amount to approximately 1 million smolts annually, compared with a natural smolt production of around 6 million smolts (N.O.U., 1999). Changes in water flow usually include changes in how much and when water is flowing, and also changes in water temperature. Such changes may affect the timing of outmigration of the smolts and spawning migration of adult salmon, the timing of spawning and hatching of the eggs, as well as the food availability for the juveniles and juvenile growth rate. In some rivers where most of the water is diverted, strong reductions have been seen in the average body size of spawning salmon. In order to decrease the negative effect of changed water flow conditions, there are restrictions on minimum water flow, and the stability of water flow. Also, restoration and/or enhancement of salmon habitat and construction of fish-ladders have been implemented. Large-scale stock enhancement programs have also been implemented and were, up until the mid-1980s, partly based on releases of non-native stocks (Ståhl and Hindar, 1988). Based on evidence of negative genetic effects of stocking, the release of non-native stocks was banned in Norway by administrative action from 1986 and by legislation from 1992. Recent decades have seen an increased focus on releasing early juvenile stages, rather than smolts, and often in combination with habitat modification (Anon, 2010).

1.2   Detecting genetic changes in populations experiencing environmental threats

In order to manage populations that are exposed to environmental threats, and to assess damage caused by these threats, it can be useful to assess the changes in the population's genetic makeup over time. Such changes may include a loss of genetic diversity following a genetic bottleneck (severe reduction in population size), and a shift in allele frequencies at loci which may be closely linked with a trait that has a differential response to the environmental threat.

If a mutation at a gene locus increases the fitness of an individual in relation to an environmental threat, it is expected that this mutation will become more frequent within the affected population and remain static in non-affected populations. Moreover, if this allele is present across multiple populations, then it may be possible to assess a similar shift in allele frequencies among populations that are similarly affected in contrast to those that are non-affected. Ideally such tests require dense marker sets to increase the probability of identifying markers that are in linkage disequilibrium with the mutation. The identification of genetic markers differentiating populations that are adapted to different environmental conditions typically requires the availability of markers densely spread over the entire

genome. At the commencement of this study the most comprehensive set of characterised DNA markers for Atlantic salmon was a 5.5K Illumina SNP-array (meaning that 5500 single nucleotide polymorphisms (SNPs) could be studied simultaneously in the same individual). This relatively low density SNP-array covers the entire salmon genome with a focus on gene coding regions, and provides the potential to search for SNP markers differentiating salmon from affected and non-affected habitat types.

Genome-wide association studies (GWAS) examine many genetic markers among individuals to assess if any genetic variants are associated with a phenotype or habitat type. These studies involve many individuals that are affected (i.e. by acidification and/or river regulation) and many that are unaffected, and assess allele frequencies among these individuals across the whole genome. If an allele is significantly more frequent in affected individuals than unaffected individuals, this allele is said to be potentially "associated" with the phenotype or habitat type in question, and can thus be used to differentiate individuals and populations based on this habitat type association.

Patterns of genetic diversity among samples can also be used to identify loci potentially acting under selection (Beaumont and Balding, 2004; Excoffier *et al.*, 2009a). This method is based on the population genetics theory that loci influenced by directional selection (e.g. due to local adaptation) will show greater genetic differentiation among samples (higher F_{ST} values), whereas loci affected by balancing selection will show decreased genetic differentiation among samples (lower F_{ST} values). A hierarchical island model of population structuring, in which demes (sub-populations) exchange more migrants within groups than between groups, has been shown to be a more robust method of detecting loci under local adaptation than a simple island model of population differentiation, as this model can account for hierarchical population structuring (Excoffier *et al.*, 2009a).

1.3   The importance of sampling design in studies to detect genetic changes among populations

The sampling design is critical in studies attempting to differentiate between populations of different habitat types. In structured populations, tests of association with habitat type can result in false positives due to genetic structuring among populations and sub-populations (Pritchard and Donnelly, 2001). To overcome this problem, it is recommended to sample populations from a broad distribution with no obvious barriers between affected and non-affected populations that may result in false-positive allele associations.

Due to the structuring of Atlantic salmon populations (Primmer *et al.*, 2006; Schtickzelle and Quinn, 2007; Verspoor, 1997), this study chose a sample design that enabled comparison of affected and control rivers from every geographic region possible, with both affected and non-affected (control) individuals sampled from each region and analyses focusing on differentiating the samples from affected and non-affected habitat types, irrespective of their geographic location.

1.4   Aims

The aim of this study was to assess genetic variation in populations of Atlantic salmon in western Norway that have experienced environmental changes due to river acidification and waterway regulation. Our sub-goals were to:

1. Collect historical information of anthropogenic changes and catch statistics from a large number of salmon rivers in Norway.

2. Assess genomic diversity among samples to identify patterns of genetic diversity and heterozygosity among affected and non-affected rivers

3. Identify loci that show large allele frequency differences among affected and non-affected rivers and that can be used to differentiate samples affected by acidification and/or waterway regulation

4. To further examine samples at the subset of loci found in step 3, to study more-the genomic variation among affected and non-affected samples.

2 Materials and methods

2.1 Anthropogenic changes and catch statistics in western Norwegian rivers.

Historical data was collected for as many rivers in western and southern Norway as possible in order to collate information on anthropogenic changes. These data were progressively updated on a webpage: http://info.vilvitevillaks.no/Shared%20Documents/Changes.aspx – maintained by Cap Mare AS.

Anthropogenic activity collected in rivers in western and southern Norway entailed primarily scientific and anecdotal evidence of river acidification and river regulation; additional information on hatchery re-stocking programs and recorded numbers of escaped aquaculture fish was also collected where available. In addition, historical catch data recorded from recreational fisheries was also collected from as many rivers as possible; however, catch quotas for any particular river can vary on an annual basis and as such direct comparisons among years may not always be a true representative of the actual number of fish.

2.2 Sample collection

Atlantic salmon juveniles and/or adults were collected from 25 rivers in south western and western Norway in one or more years to obtain representative samples. Scales from adult salmon, collected by anglers, and fin tissue from juvenile salmon, collected by electrofishing, were used as sources of DNA.

Three rivers were represented by SNPs analysed previously by KARLSSON *et al.,* (2011): Rivers Figgjo, Suldalslågen, and Lærdalselva. Other rivers were represented by including juvenile or adult salmon from at least two sample years, with one exception (River Vikedalselva which was sampled in 2009 only). The samples, if not at hand at NINA, were obtained with the help of Rådgivende Biologer, Bergen, UNI Research, Bergen, and the Veterinary Institute, Oslo. The sample locations are presented in Figure 2 with numbers of individuals obtained from each presented in Table 1.

2.3 Molecular methods

Genomic DNA was extracted for scale or fin samples using either a salting-out procedure modified from (Miller *et al.*, 1988), or by use of commercial DNA-spin column extraction kits.

Experience has shown us that sample quality is a critical variable affecting the success of genotyping with the 5,500 (5.5K) Illumina SNP-arrays. It is crucial to have a minimum amount of high-molecular weight DNA (un-degraded) in the sample to ensure that whole-genome amplification (WGA) occurs efficiently. For this reason, each individual sample was quality checked (QC'ed) prior to its analysis on the SNP-array by measuring its concentration with picogreen and running a small aliquot on a 1% agarose gel. Visual inspection of the gel will inform us if the sample shows significant degradation and, if it does, will allow us to estimate the concentration of high-molecular weight DNA within the sample. Performing this QC in this way is time consuming, but not only saves money (by avoiding genotyping poor quality samples), but also makes subsequent SNP calling more reliable and streamlined. Due to

practical difficulties associated with sampling in the field, it is not always possible to quickly secure samples and preserve DNA quality, consequently a significant number of samples were found to be of poor quality and deemed unsuitable for analysis. DNA samples were genotyped using one of two Illumina iSelect SNP-arrays; a 15K SNP array or a 5.5K array. The 15K-array represents the first version of CIGENEs Atlantic salmon SNP array and contained approximately 7K "useable" SNP assays (Bourret *et al.*, 2013), a subset of these (5349) were combined with 219 "new" SNPs to produce a second version 5.5K array. Although both array versions were processed using the same Infinium chemistry and the 5349 common SNPs used the same hybridization probe sequences, the genotype calling was performed within-array type. Genotype assignment was performed in two phases, an initial automated calling allowed for many "simple" clusters (e.g. diploid markers) to be assigned genotypes unambiguously, while more complex markers (e.g. multisite variants) were clustered in a second manual phase. Differentiation between simple and complex markers was based on SNP statistic (e.g. imbalance in allele frequency, large numbers of unassigned genotypes) and data from previous large scale genotyping of high-quality pedigree samples.

Only SNP loci in common in both datasets (i.e. arrays) were used for these analyses, this list included a total of 3761 nuclear diploid loci and 8 mitochondrial (mtDNA) haploid loci. Initial diversity and structuring analyses of the mtDNA loci showed little information, and as such these 8 loci were excluded from further analyses.

2.4 Genetic diversity and genetic divergence

Allele frequencies, effective number of alleles, observed and expected heterozygosities for each sample were obtained from GENALEX 6.5b3 (Peakall and Smouse, 2006; Peakall and Smouse, 2012). Deviations from Hardy-Weinberg equilibrium (HWE) expectations were conducted using ARLEQUIN 3.5.1.3 (Excoffier and Lischer, 2010) and its corresponding Unix-command version ARLECORE. Exact tests for deviations from HWE included 1×10^6 Monte Carlo steps and 5×10^4 dememorisation steps and were conducted for each locus separately. Genetic structuring among samples was assessed using pairwise *F*-statistics (F_{ST}) and an analysis of molecular variation (AMOVA) among and within samples using ARLEQUIN. As missing data can be particularly problematic when conducting pairwise distance analyses, the allowed level of missing data was set to 5% in these tests. All tests of genetic structure were performed with 5×10^4 permutations to generate the null-distribution. Pairwise F_{ST} values were calculated to visualise the short-term genetic distances among samples. In the AMOVA tests, groups were defined according to the sampling region (refer to Table 1) with individual genotypes permuted among samples and among groups (F_{ST}), among samples and within groups (F_{SC}), and by permutations of whole samples among groups (F_{CT}).

2.5 Isolation-by-distance

A test of spatial auto-correlation was conducted using a Mantel test in the "ade4" package in the R program (v.2.15.1). Pairwise genetic distances (as F_{ST} values obtained in ARLEQUIN) were tested for correlation with geographic distance in the form of both the natural log of pairwise waterway distances and geographic coordinates. Geographic coordinates used were standard UTM coordinates (Table 1). Waterway distances between samples were

estimated from the mouth of each river using the GIS package QUANTUM GIS V. 1.8.0. Straight-line distances were used along the coastline due to the number of islands of Norway's west coast. Observed R-values were compared with those expected from a null-distribution generated using a Monte Carlo test and 1×10^4 random permutations, with α = 0.05.

2.6 Detection of loci differentiating affected and control samples

2.6.1 Genome-wide association mapping

Genome-wide association mapping using case-control tests was used to identify loci that are non-randomly associated with a particular habitat type. Case-control studies were performed for each habitat type separately using the software PLINK V1.07 (Purcell *et al.*, 2007). Individuals were assigned a 'phenotype' representing their habitat type classification, and allelic association tests were performed using 1×10^9 permutations to obtain the probability of the correlation. Genome-wide association results were plotted using R with the $-\log_{10}$ of permutated *P*-value and the chromosomal positions of each locus (Lien *et al.*, 2011).

2.6.2 F_{ST} and F_{CT}-outlier methods

A hierarchical island model method for detection of outlier loci was also conducted (Excoffier *et al.*, 2009a). Samples were clustered into groups according to their habitat type in two separate analyses for acidified/control and regulated/control samples. Coalescent simulations (2×10^4) were used to generate the null distribution of *F*-statistics, among 10 simulated groups containing 100 demes per group. Results of the joint distribution of F_{CT} and heterozygosity within populations for all loci were plotted alongside one-sided confidence intervals obtained from the simulated data using R.

2.6.3 Genetic diversity and structuring among samples at differential loci

Loci that showed significant differentiation among habitat types using methods outlined in sections 2.6.1 and 2.6.2 were compared and those that were concordant among methods were ranked according to their significance and further analysed at a population level to identify patterns of genetic diversity and divergence among samples. Relative allele frequencies among samples at the top ranked loci were assessed using PLINK. Maximum Likelihood (ML) analyses of individual assignment to populations defined by habitat type were conducted in GENALEX using the "leave-one-out" option when assessing allele frequencies. Bayesian analyses of genetic structure at the top ranked loci were assessed in duplicate for both acidified/control and regulated/control datasets using STRUCTURE v2.3.4 (Pritchard *et al.*, 2000), assuming two populations (*K* = 2). A no-admixture model with no *a priori* information on the sample groupings (i.e. affected or control groups) was used with 5×10^4 repetitions as burn-in followed by a further 5×10^4 repetitions after burn-in. Individual membership (Q-values) to *K* genetically independent clusters at the subset of loci was plotted in STRUCTURE using defined information of sample origin for displaying the plots.

Table 1   Sample attributes and habitat type classifications. Sample ID, identification of samples used in following tables and figures; N, number of fish sampled; Catch data, the mean annual number of salmon caught during 1979-2005 with maximum and minimum numbers in parentheses (PEDER FISKE, NINA, pers. comm.)

Sample	ID	*N*	Region	Year	Catch data	Habitat type classification	Geographic coordinates	
							UTM East	UTM North
Oldenelva	1	38	Nordfjord	2008,2009	63 (135-0)	control	384450	6857950
Gloppenelva (Breimsvassdraget)	2	41	Nordfjord	2008,2009	154 (278-66)	regulation	352325	6851800
Eidselva (Hornindalsvassdraget)	3	11	Nordfjord	2008	426 (831-221)	control	341700	6867150
Nausta	4	31	Sunnfjord	2010,2011	1490 (4983-420)	control	325250	6823575
Jølstra	5	10	Sunnfjord	2011	238 (659-0)	regulation	331200	6818100
Flekkeelva (Guddalsvassdraget)	6	32	Sunnfjord	2009,2011	76 (315-1)	acidification	304900	6801700
Gaula i Sunnfjord	7	33	Sunnfjord	2008,2011	684 (1365-291)	control	322500	6808450
Nærøydalselva	8	36	Sognefjord	2008	149 (265-0)	control	382800	6751200
Aurlandselva	9	38	Sognefjord	2006,2009	30 (133-0)	regulation	401700	6753700
Flåmselva	10	25	Sognefjord	2007,2011	46 (235-0)	control	397900	6749400
Årøyelva	11	16	Sognefjord	2011	64 (135-19)	regulation	401850	6794300
Lærdalselva	12	61	Sognefjord	1977,1978,1997	773 (1654-0)	regulation	417800	6775550
Mørkridselva	13	40	Sognefjord	2006,2008	12 (55-0)	control	425500	6818350
Fortunselva	14	40	Sognefjord	2006,2011	"10"	regulation	425500	6818350
Vosso	15	22	Nordhordland	2011	82 (250-0)	regulation & acidification	333850	6726800
Granvinselva	16	19	Hardangerfjord	2011	34 (84-8)	control	374600	6711950
Kinso	17	25	Hardangerfjord	2011	30 (109-2)	control	374650	6696000
Eio (Eidfjordvassdraget)	18	24	Hardangerfjord	2011	67 (140-9)	regulation	393950	6705500
Vikedalselva	19	40	Rogaland	2009	313 (1262-0)	acidification	324300	6599650
Suldalslågen	20	50	Rogaland	1979,1980	431 (981-120)	regulation & acidification	344250	6597100
Vormo	21	42	Rogaland	2008-2009	200 (638-12)	control	348050	6573500
Figgjo	22	48	Rogaland	1989	2101 (4061-0)	control	300650	6524250
Håelva	23	30	Rogaland	2008,2007	2134 (5369-0)	control	299600	6508500
Ogna	24	28	Rogaland	2007-2008	1617 (4044-0)	acidification	313200	6490900
Bjerkreimselva	25	40	Rogaland	2007,2008,2011	2322 (7914-0)	acidification	324850	6486000

1. Oldenelva
2. Gloppenelva (Breimsvassdraget)
3. Eidselva (Hornindalsvassdraget)
4. Nausta
5. Jølstra
6. Flekkeelva (Guddalsvassdraget)
7. Gaula i Sunnfjord
8. Nærøydalselva
9. Aurlandselva
10. Flåmselva
11. Årøyelva
12. Lærdalselva
13. Mørkridselva
14. Fortunselva
15. Vosso
16. Granvinselva
17. Kinso
18. Eio (Eidfjordvassdraget)
19. Vikedalselva
20. Suldalslågen
21. Vormo
22. Figgjo
23. Håelva
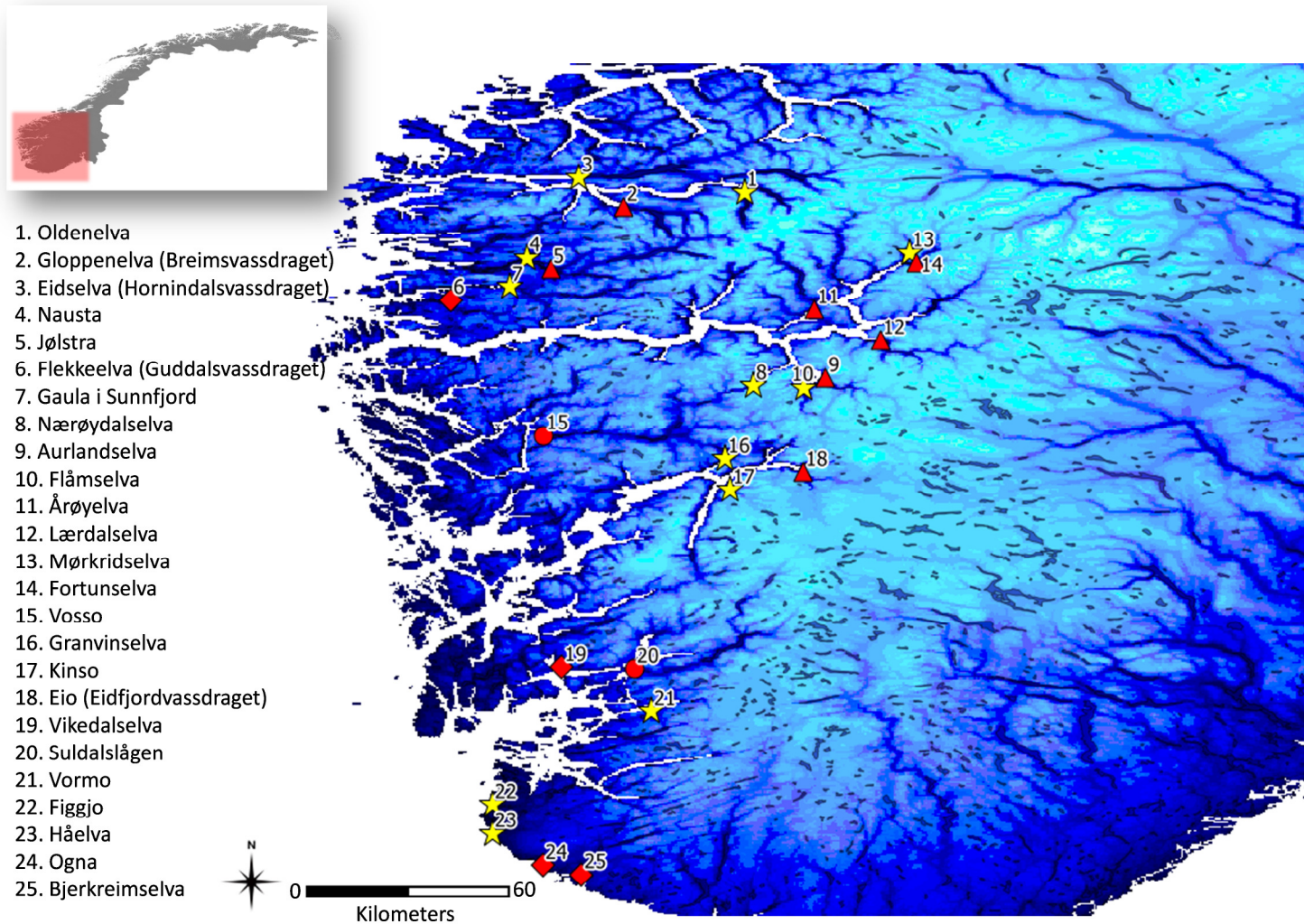24. Ogna
25. Bjerkreimselva

Figure 2   Norway (insert) and study area in western Norway. Sampling locations are indicated by numbers with symbols reflecting habitat type classification; yellow stars denote control rivers (non-affected); red symbols denote locations affected by - river regulation (triangles), acidification (diamonds), and both river regulation and acidification (circles)

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3 Historical river information and habitat-type classification

3.1 Oldenelva

Habitat type classification: Control

Oldenelva is located in Stryn municipality in the county of Sogn & Fjordane and drains into Nordfjord. Oldenelva is known for its large salmon and is popular among anglers. The river drains large glacial areas, and is thus cold from spring until mid-July, but runoff from the Olden Lake (Oldenvatnet) results in the river being relatively warm throughout autumn and early winter. The average water flow is 15.3 m^3/s and is greatest during summer. The water quality of the river is good, and is not affected by acidification. Salmon catches decreased in the 1990s and this is believed to be a consequence of poor survival during the sea-phase. Competition with escaped farmed salmon may also pose a threat to the Oldenelva salmon population (Sægrov and Johnsen, 1998), which was classified as threatened by escaped farmed salmon in a report by Diserud *et al.,* (2012). Oldenelva is protected as one of Norway's 52 national salmon rivers, and is located in one of Norway's 15 national salmon fjords (St.prp.nr.32, 2006).

3.2 Gloppenelva (Breimsvassdraget)

Habitat type classification: River regulation

Gloppenelva drains from Breims Lake (Breimsvatnet) at an altitude of 56 m above sea level (asl), into Gloppenfjord at Sandane. The anadromous salmonid stretch is extended through the use of fish ladders which enable fish to reach above the Eids waterfall (Eidsfossen). Upon the 1995 reparation of the Eidsfossen fish ladder, the third waterfall Trysillfossen serves as a migration barrier. The smolt production area in the Gloppen River system is estimated at 135,000 m^2 in the main river with an additional 15,000 m^2 in tributaries. The total catchment area is 636 km^2 and the average annual water flow is 43 m^3/s. Gloppenelva is a medium-sized river, and because of several large inflows from Jostedals Glacier, it has had relatively stable water flow even during dry summers; nevertheless the water flow peaks in summer due to snow-melting. The river is regulated by a dam about 1 km from the Breimsvatnet. The hydroelectricity stations Eidsfoss kraftverk and Trysilfoss kraftverk use water from the river. There are three waterfalls in Gloppenelva that are utilised for hydroelectricity, fish ladders are present in the lower two waterfalls. Gloppenelva is known for its large trout (*Salmo trutta* L.); the salmon population in Gloppenelva was previously classified as medium-sized, however the size has decreased since the mid-1970s (Sægrov, 1996a). Analysis of scale samples from salmon have indicated that Gloppenelva receives many escaped farmed salmon, with a relative proportion of farmed escapees from 1999-2003 of 24% (Sægrov *et al.*, 2004); this river was classified as 'threatened' by Diserud *et al.,* (2012). Stock-enhancement programs have existed in Gloppenelva since the 1950s however fish surveys have indicated that these programs have had limited success (Sægrov, 1996a).

3.3 Eidselva (Hornindalsvassdraget)

Habitat type classification: Control

Eidselva flows from Hornindals Lake (Hornindalsvatnet, 52 m asl) and drains into the sea at Nordfjordeid. The river is approximately 12 km long and with an average breadth of approximately 25 m, the total river area approximates 300,000 m^2 (Sægrov, 1996b). The river has a catchment area of 422 km^2 and the average annual water flow was 22.8 m^3/s over the period 1900 - 1986. The river meanders with fine currents and pools and the benthic substrate is of a type well-suited for salmon spawning and fry growth. Eidselva is known for its early migrating, large-sized salmon. The runoff from the large reservoir in Hornindalsvatnet results in relatively warm water during autumn and early winter. Other than Atlantic salmon, the river system also contains trout and eel (*Anguilla anguilla* L.), with Arctic char (*Salvenius alpinus* L.) present in the lake. The salmon and eels migrate from Nordfjord into Eidselva and then further into Hornindalsvatnet. Trout migrate up Storeelva and other smaller tributaries. Water quality tests in 1995 indicated that the Eidselva is not acidic, with a high the buffering capacity and water quality conducive for good survival and growth of salmonids (Sægrov, 1996b). In recent years the river system has been affected by large numbers of escaped farmed salmon, and these potentially pose a threat the Eidselva salmon population, which was classified as vulnerable by Diserud *et al.,* (2012). The whole river system is protected as one of Norway's national salmon rivers (St.prp.nr.32, 2006).

3.4 Nausta

Habitat type classification: Control

Nausta has a catchment area of 277 km^2. There are several large, high-lying lakes in the watershed, but below Vona Lake (Vonavatnet) (466 m asl) there are no larger lakes which dampen the floods from the big valley floors or stabilize the temperature. The average annual discharge is 20.6 m^3/s; with the greatest water flow in May, June and September-October. The river is characterised by very large variations in water flow within short time periods. Nausta is cold water in springtime, with normally low temperatures until late June. The anadromous stretch of the river is 12.4 km, but the lower 1.5 km of this stretch is not considered productive due to the seepage of salt water. The original salmon production stretch was 2.9 km, and in 1975 a salmon ladder in Hove waterfall was opened that increased the salmon stretch by 8 km up to Kalland waterfall and the salmon production area increased by almost a factor of 3. The river area where natural recruitment and smolt production takes place is about 400,000 m^2. In addition, smolts are produced in short reaches of the tributaries Åsedøla and Hyelva, and cultivation by means of release of yolk-sac fry occurs in an approximately 3 km stretch of river above Kalland waterfall. Water quality in Nausta has improved since the 1990s in line with the general reduction of sulphate-rich precipitation and water quality measurements and benthic fauna indicate that the river is no longer acidified. In 1995, the river was included in the national waterway liming program, but was removed from this program in 1998 (Hellen *et al.*, 2000). The Nausta salmon population was classified as warranting consideration in relation to escaped farmed salmon in the report by Diserud *et al.,* (2012). The river is protected as one of Norway's national salmon rivers and is located in a national salmon fjord (St.prp.nr.32, 2006).

3.5 Jølstra

Habitat type classification: River regulation

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The Jølstra River system is located between Nordfjord and Sognefjord and drains into Førdefjord. Jølstra has an average flow throughout the year of 44 m³/s and due to the large reservoir of Jølstra Lake (Jølstravatnet), flow rarely recedes below 5 m³/s. The reservoir also acts to regulate water temperature, with the temperature in the river only occasionally falling below 2 ° C; one such exception was the winter of 2010, with temperatures down to 0 ° C from January to late March. The catchment area is 717 km^2 and the total salmon migration stretch in the river is 6.5 km, with a productive spawning and nursery area of approximately 300,000 m² during average water flow. There are two large river hydroelectricity plants in the Jølstra River system, both are located upstream of the salmon migration stretch. The Bruland falls hydroelectricity plant (Brulandsfossen kraftverk) became operational in 1914, with further development in 1934 and 1989. This hydroelectricity station can cause rapid water level changes in the river and has resulted in stranding of small fish. Approximately 13,000 smolts are annually released from hatchery cultivation (using locally caught salmon) to compensate for smolt loss arising from the hydroelectricity plant. In addition, eyed eggs based on local stock caught in Jølstra and from Norway's genebank for wild salmon are buried in gravel in the river. In the 4.5 km stretch of Jølstra where juvenile fish can be affected by the power station, there is an estimated production potential of 15,000 pre-smolt (8.4 pre-smolt/100 m²), of which 12,000 (80 %) are Atlantic salmon and 3000 (20%) are sea-trout. The Jølstra waterway is not limed and measurements in 1999 showed high pH, even upon flooding (Johnsen *et al.*, 2010b; Sægrov and Urdal, 2011). The Jølstra salmon population was classified as threatened by escaped farmed salmon by Diserud *et al.,* (2012) and is protected as one of Norway's national salmon rivers (St.prp.nr.32, 2006).

3.6   Flekkeelva (Guddalsvassdraget)

Habitat type classification: Acidification

Flekkeelva is the anadromous salmon stretch of the Guddal River system that starts in Guddal and drains into Flekkefjord in the County of Sogn & Fjordane. The anadromous salmonid stretch of the river system is approximately 8.5 km, resulting in an anadromous production area of 125,000 m^2. The catchment area is 66 km ^{2}. The lower regions of the Flekke-Guddal River system are characterised by many large lakes connected by short riverine stretches. The river is affected by agriculture and acidification was documented in the waterway in the 1980s and 1990s. The effects of acidification were most pronounced in the lower regions of the river that include the anadromous salmonid stretch. Liming of the lakes and tributaries has occurred since 1997 (DN, 2000). Flekkeelva contains a population of typically medium and large-size salmon that has increased dramatically since the late 1990s (DN, 2000). The increase in recruitment of salmon is likely due to the improvement in water quality after liming and a reduction in acidification. Flekkeelva has received a relatively small proportion of escaped farmed salmon in recent years (Fiske *et al.*, 2000) and was classified as warranting consideration in relation to farmed escapees in the report by Diserud *et al.,* (2012).

3.7   Gaula

Habitat type classification: Control

The Gaula River system is located predominately in the municipalities of Gaular and Førde in the Sogn & Fjordane County and drains into Dalsfjord. This is one of the largest river systems on the west coast, about 70 km in length and with a catchment area of approximately 630 km². The anadromous salmonid stretch of the waterway is 12.8 km and provides a salmon smolt production area of 1,020,000 m^2, this anadromous stretch has been increased by the presence of four fish ladders at four waterfalls. The salmon population of Gaula is dominated by small and medium-sized salmon, with very few large salmon (Hellen *et al.*, 2000). A study in 1999 indicated low-pH levels in some tributaries of Gaula and liming of three lakes in the Gaula catchment occurred as a result. The lower sections of the Gaula were not affected by acidification to the extent that the upland regions were (Hellen *et al.*, 1997). Water quality in the Gaula has improved in the past decade (Hindar, 2000). The effective population size of Atlantic salmon in Gaula has been estimated in the area of 800-2000, a level considered as sustainable. Scale samples have indicated the presence of farmed salmon in Gaula, at an average relative proportion of 12% over the years 1989-2000 (Gaular-Elveeigarlag); although Glover *et al.,* (2012) found no significant temporal change in microsatellite allele frequencies in the Gaula salmon. The Gaula salmon population was classified as vulnerable in relation to escaped farmed salmon in the report by Diserud *et al.,* (2012). Stock enhancement using juvenile salmon occurred in the river periodically until 1997. The Gaula River system is protected as one of Norway's national salmon rivers and is located in a national salmon fjord (St.prp.nr.32, 2006).

3.8   Nærøydalselva

Habitat type classification: Control

Nærøydalselva is a river situated in the municipality of Voss in Hordaland County and the municipality Aurland in the Sogn & Fjordane County. The catchment area is currently 262 km^2 after 22 km^2 was diverted to the Vikja River system, resulting in 18% of the water being lost from the Nærødalselva. The majority of the salmon migration stretch lies in the municipality of Aurland. The salmon migration stretch is approximately 11 km and extends up to Stalheimskleiva in Hordaland County. The river is relatively gentle with one steep section approximately 2 km from the sea. Nærøydal is a special river with respect to geology, having a light substrate with little fouling; the water in the river is thus extremely clear. This can result in lower potential for hiding and concealment of fish in this river compared with other rivers; thus greater predation pressure is possibly a cause of the limited smolt production in Atlantic salmon. The low winter water flow may conceivably directly impact smolt production, but may also indirectly lead to increased risk of predation (Bremset *et al.*, 2009). The number of spawning Atlantic salmon has been high in recent years (Leif Magnus Sættum, pers. comm.). Nærøydalselva is protected as one of Norway's national salmon rivers, and is located in a national salmon fjord (St.prp.nr.32, 2006).

3.9   Aurlandselva

Habitat type classification: River regulation

Aurlandselva is a river in Aurland municipality in the county of Sogn & Fjordane. The source of the river is located in the mountains northwest of Hallingskarvet, and the river flows through the Aurland Valley and drains into Aurlandsfjord at Aurlandsvangen. The river is 55.4

km long and has a catchment area of 804.22 km². The mean water level at the outlet is 37.6 m³/s. In the period after development of hydroelectricity stations in the Aurland waterway (1983 - 1999), populations of adult salmon and sea trout were reported to have decreased to below approximately 10% and 30% of their pre-regulation (1969 - 1977) population sizes, respectively. In Aurlandselva, the summer water temperatures are reduced due to regulation, and low temperatures in the period after the juvenile salmon emerge from the substrate likely hinders recruitment and production of salmon, and possibly trout, smolts. However in the 1990s, the low number of spawning salmon was deemed the limiting factor in juvenile recruitment, and low sea-survival has likely reinforced the decline in the salmon population (Sægrov *et al.*, 2000). A stock-enhancement program exists in Aurlandselva using locally caught broodstock.

3.10   Flåmselva

Habitat type classification: Control

The Flåm River system is one of the few remaining major waterways in upper Sognefjord that is not strongly influenced by hydroelectric development, but there are nonetheless two small hydroelectricity plants situated in the river (St.prp.nr.53, 2009). The river has a salmon migration stretch of about 4.8 km and is protected as one of Norway's national salmon rives. The annual number of salmon caught normally varies between 60 and 120, but there are occasionally higher or lower catches (Skurdal *et al.*, 2001). Large sections of the Flåm catchment lie at altitudes greater than 900 m asl, and with late snow-melting, the temperature in the river in summer can be low. The low water temperature in the first phase after salmon fry emerge from the gravel is considered to be a contributing factor in fry mortality.

3.11   Årøyelva

Habitat type classification: River regulation

Årøy is a river located between Hafslo Lake (Hafslovatnet) (169-167 m asl) and the bottom of the Sogndalsfjord, in the municipalities of Sogndal and Luster, Sogn & Fjordane County. The main tributary of the Hafslovatnet is Soget from Veitastrond Lake (Veitastrondvatnet) (170.5 to 168 m asl) with sources in Langedals Glacier and Austerdals Glacier, that are both branches of the Jostedals glacier. The catchment area of Årøyelva is 429 km². Prior to river regulation, the Årøy had a relatively short anadromous salmon stretch of 1.1 km; after the establishment of the Årøy hydroelectricity station (Årøy Kraftstasjon), this was further reduced with the uppermost 150 m strongly affected by regulation. Årøy hydroelectricity plant (90 MW, 337 GWh) is situated between Hafslovatnet and the fjord. Årøyelva is known as a river containing large salmon and fish up to 34 kg have been caught in this river, although in recent years the body size of catch has been relatively modest. Drops in water levels due to the hydroelectricity station have been linked to mass-strandings of fish. Escaped farmed salmon have been reported in Årøyelva in higher proportions than in other rivers in the inner Sognefjord; competition with escaped farmed salmon may thus also affect the natural Årøy salmon population (Urdal *et al.*, 2004) which was classified as vulnerable by Diserud *et al.,* (2012). A stock-enhancement program using locally-caught broodstock operates in Årøyelva.

The river is protected as one of Norway's national salmon rivers, and is located in a national salmon fjord (St.prp.nr.32, 2006).

3.12 Lærdalselva

Habitat type classification: River regulation

Lærdalselva lies in Lærdal municipality in the county of Sogn & Fjordane. The river begins at the confluence of Mørkedøla and Smedøla at Æråker, and drains into Sognefjord approximately 44 km downstream. Lærdalselva has a natural salmon and sea trout anadromous stretch up to Sjurhaug waterfalls, 24 km from the fjord, however through the building of four fish ladders this has increased to approximately 40 km (Johnsen *et al.*, 2010b). The catchment area is 1184 km^2 and the anadromous production area in the river is 750,000m^2, making Lærdalselva the largest anadromous waterway in the County of Sogn & Fjordane (Skurdal *et al.*, 2001). Several hydroelectricity plants operate along Lærdalselva. Salmon stock-enhancement occurs in Lærdalselva using locally caught broodstock. The salmon population was classified as good by Diserud *et al.*, (2012) in respect to escaped farmed salmon, and Glover *et al.*, (2012) found no significant temporal change in microsatellite allele frequencies in the Lærdal salmon population. Since the mid-1990s, the Atlantic salmon in Lærdalselva have been affected by the parasite *Gyrodactylus salaris*; periodically since 1997 the river was treated using rotenone in an attempt to eradicate the parasite (Gladsø and Raddum, 2000). Treatment of Lærdalselva to control *G. salaris* shifted to the use of acidic aluminium sulphate in 2005. Lærdalselva is protected as one of Norway's national salmon rivers and is located in a national salmon fjord (St.prp.nr.32, 2006).

3.13 Mørkridselva

Habitat type classification: Control

The Mørkrids River system is located in the municipality of Luster in the County of Sogn & Fjordane. The catchment area is 288 km^2 and flows into Lusterfjord, an inner branch of Sognefjord. Most of the catchment lies at an altitude of greater than 1000 m asl and as such the river is affected by snow-melting, with the heaviest water flow in summer (average summer flow from 1963-1967 29 m^3/s). The anadromous salmonid stretch is approximately 9.5 km long, giving an anadromous production area of 200,000 km^2. The density of salmon in Mørkidselva is low in comparison to some other rivers in Sognefjord (estimated spawning number of 50-70 salmon per year) and it is possible that the cold water temperatures during snow-melting periods are a limiting factor for growth and survival of salmon (Hellen *et al.*, 2000). Mørkridselva is located in a national salmon fjord (St.prp.nr.32, 2006).

3.14 Fortunselva

Habitat type classification: River regulation

The Fortun River system is located in the municipality of Luster, and is formed by the confluence of Nørstedøla and Middøla, before draining into Lustrafjord, an inner branch of Sognefjord. The total catchment area of the river system is 507.7 km^2, while the catchment area utilised by Fortun hydroelectricity plant (Fortun kraftverk) is 379 km^2. In general, it is the

eastern side of the Fortun valley, and large sections of the Sogne Mountains (Sognefjellet) that is regulated. Regulation has resulted in fewer lakes above the power station in comparison with earlier (pre-regulation) times. The Fortun hydroelectricity plant is located at the bottom of Bergselvi and the water from the plant is released immediately upstream from the confluence with Fortunselva. The anadromous salmon stretch of Fortunselva is approximately 16 km and contains a lake, Eidsvatnet, which lies about 500 m upstream of the mouth of the river and has an area of 0.6 km^2. Approximately 8.5 km of the anadromous stretch lies above the Fortun hydroelectricity plant. Thus, regulation has led to a reduction in water flow in the upper part of the anadromous migration stretch, and flow over the seasons has levelled out; consequently, winter water temperatures are higher and summer water temperatures are lower than those recorded prior to hydroelectricity development. (Urdal and Sægrov, 2011). Regulation of Fortunselva has also caused a shift in sediment loads, with gravel spawning areas affected by increased sedimentation and decreased visibility (Johnsen *et al.*, 2010b) (Urdal and Sægrov, 2011). A stock-enhancement program exists in Fortunselva using locally-caught broodstock. Fortunselva is located in one of Norway's national salmon fjords (St.prp.nr.32, 2006).

3.15 Vosso

Habitat type classification: River regulation, Acidification

The Vosso River system is located in the municipalities of Voss in the County of Hordaland and and Vik in the County of Sogn & Fjordane. The river drains into the Bolstadfjord. Prior to hydroelectricity development in the river system, the catchment area was 1499 km^2 and this increased after regulation to 1699 km^2 due to diversion from neighbouring river systems, some of which were acidic. The anadromous salmonid stretch of the Vosso River system is approximately 35 km long, of which 18 km is located in lakes. Vosso is a river famous for its large-sized salmon, with catches of approximately 4 tonnes per year recorded; however, the salmon population has been declining since the 1960s, with pronounced reductions since the 1990s. The Evanger hydroelectricity station (Evanger kraftverk) has contributed to acidic aluminium runoff into the Vosso system (Kroglund *et al.*, 1998) and has been continually limed since 1994, as have other small lakes and tributaries (Miljøstatus i Hordaland: http://hordaland.miljostatus.no/msf_widePage.aspx?m=1019). The Vangs Lake (Vangsvatnet) has had periods of poor water quality due in large parts to the evacuation of sewerage and other waste runoff from Vossevangen until the 1970s. This led to changes in the nutrient and organic composition, dissolved oxygen content, turbidity and algae biomass in the lake (Johnsen, 1993). pH in the Vosso River system was reported to have decreased after 1966, with the most significant drop in pH in the tributary Raundalselvi (Kroglund *et al.*, 1998). Improvements in water quality of the Vosso River system were observed by the late 1990s, and attributed to a liming, reduction in sulphur-rich precipitation and salinization in the catchment, and chemical and biological treatment of Vangsvatnet (Kroglund *et al.*, 1998) (Johnsen, 1993). Large numbers of escaped farmed salmon have been found in the Vosso River system and interactions with escapees may also have contributed to the decline in the Vosso salmon population. The Vosso salmon population was classified as threatened by Diserud *et al.,* (2012) in regard to escaped farmed salmon, and Glover *et al.,* (2012) found significant changes in allele frequencies in the population which was attributed to escaped

farm salmon. The Vosso River system is protected as one of Norway's national salmon rivers and is situated in a national salmon fjord (St.prp.nr.32, 2006).

3.16 Granvinselva

Habitat type classification: Control

The Granvin River system flows between Voss and Granvinsfjord in the Hordaland County. The anadromous salmonid stretch of the river is 13 km and Granvin Lake (Granvinsvatnet) makes up 5 km of this stretch. The catchment area is 177 km^2 and its highest point is 1558 m asl. This river system is known predominantly for its sea trout, but Atlantic salmon and Arctic char are also present. Interactions from escaped farmed salmon are considered to be a threat to the Granvin salmonid populations, as is sea lice. Arctic char were not present in the river system until 1967 and the introduction of this species has reduced the nursery areas utilised by sea trout. A stock enhancement program in the Granvin River system existed since the mid-1800s but was closed in 1990 due to the threat of the disease Furunculosis. The water quality of the river system is good and appears to be relatively unaffected by acidification, there is no hydroelectricity regulation in the Granvin River system (Sægrov *et al.*, 1996).

3.17 Kinso

Habitat type classification: Control

The Kinso has a catchment area of 185 km^2 and is the largest river system in the Ullensvang municipality. Large sections of that catchment are at high altitude (> 1000 m asl) in the Hardangervidda region, and the river drains into the Hardangerfjord. The anadromous salmon stretch is approximately 4.5 km. The geology of the region consists mainly of Cambrian Silurian deposits that act as buffers for acidic precipitation, making this river system relatively unaffected by acidification. Nevertheless, pH in the river may drop during flooding, when the buffering capacity is reduced due to the ground saturation. A hydroelectricity plant is positioned upstream of the anadromous salmon stretch and is thus believed to have little or no impact on the anadromous salmon in the river. The river is permanently protected from further hydroelectric development. The density of fish in the river system in relatively low, possibly due to naturally cold water temperatures combined with the steep gradient of the river, or poor survival at sea (Kålås *et al.*, 1996). The Atlantic salmon population in Kinso was classified as critically endangered by Diserud *et al.,* (2012) in regard to escaped farmed salmon.

3.18 Eio (Eidfjordvassdraget)

Habitat type classification: River regulation

The Eio/Bjoreio River system has an anadromous salmon stretch of approximately 13 km and there has previously been many salmon caught in the river. The hydroelectricity development of the Eidfjord River system was completed in 1980 and has led to drastically reduced water flow, with 60% and 20-30% of the original water flow maintained in the Eio and Bjoreio Rivers, respectively. Regulation has in turn altered water temperatures, resulting

in warmer temperatures and decreased flow during winter that has been associated with egg mortality due to desiccation and frost. Reduced temperatures during spring and summer have been associated with higher mortality of fry and lower juvenile growth and higher smolt age. Further, salmon migration patterns appear to have been altered (Berger *et al.*, 2002). Although the hydroelectricity development has doubtless led to a reduction in the fish populations, other causes of the reduced Atlantic salmon and anadromous trout populations may include effects from escaped farmed salmon, sea lice, and fisheries exploitation. The Eio/Bjoreio salmon population was classified as critically endangered in regard to escaped farmed salmon by Diserud *et al.,* (2012).

3.19 Vikedalselva

Habitat type classification: Acidification

The Vikedals River system is situated in the municipality of Vindafjord in the county of Rogaland and drains into Sandeidfjord, a branch of Boknafjord. The catchment area of the river system is approximately 118 km^2 with an average waterflow of 10.3 m^3/s. During the 1980s, the Vikedalelva salmon population was reduced almost to the point of extinction as a result of acidic runoff and an increase in aluminium. Liming of the river was initiated in 1987 and there has been a clear increase in pH since the mid-1990s which is attributed both to the liming and a reduction of sulphur-rich precipitation in the catchment (DN, 2012). The salmon population in Vikedalselva is now re-established, and in 2006 the river was ranked among the seven best salmon rivers in Rogaland County. Vikedalselva is listed as one of Norway's national salmon rivers (St.prp.nr.32, 2006). No hatchery cultivation occurs in this river (County Governor, Rogaland County). The Vikedalselva salmon population was classified as vulnerable in regards to escaped farmed salmon by Diserud *et al.,* (2012).

3.20 Suldalslågen

Habitat type classification: River regulation, Acidification

The Suldalslågen River system is the most water-rich river system on the west coast. The Suldalslågen system, with diversions by Ulla and Førre as well as the upper parts of the Årdal waterway, is highly regulated and has a total of 17 power stations with a combined maximum output of 2621 MW and average annual production of 8924 GWh, which is 7.5% of Norway's total production capacity. Kvilldal power station (Kvildal kraftverk) (1240 MW, 3517 GWh), which also utilises the catchment areas of waterways that flow into the Jøsenfjord, is the country's largest hydroelectricity station. Prior to river regulation, the total catchment area was 1466 km^2, with an annual water flow of 50 m^3/s. The anadromous salmonid stretch of Suldalslågen is approximately 22 km, and the salmon population is currently classified as threatened. In Suldalslågen, below Suldals Lake (Suldalsvatnet), there are no power stations. Regulation of the waterway via the reservoir Blåsjø has contributed to acidification of the waterway, and upper parts of the waterway have been limed since 1985. The liming, in conjunction with improvements in water quality have contributed to a gradual improvement in the river, as illustrated by a trend of increased pH from 1991-2011 (DN, 2012). The Suldal salmon population was classified as vulnerable in regard to escaped farmed salmon by Diserud *et al.,* (2012). Suldalslågen is protected as one of Norway's national salmon rivers and is located in a national salmon fjord (St.prp.nr.32, 2006).

3.21 Vormo

Habitat type classification: Control

The Vormo River system is located in Rogaland County and drains into Jøsenfjord at Tøtlandsvik. The Vormo River system is formed by two rivers – Tøtlandsåna and Kleivalandsåna – with their confluence immediately prior to the mouth at Jøsenfjord. These two rivers have their origin at an altitude of approximately 1100 m asl in the Vormedalsheia conservation area on the south side of Jøsenfjord. The whole system was listed as protected from the fjord to the mountains in 1980, even those regions that do not flow through the Vormedalsheia conservation area. There are no reports of acidification in the Vormo River system (Enge, 2008). The Vormo salmon population was classified as vulnerable in regard to escaped farmed salmon by Diserud *et al.,* (2012).

3.22 Figgjo

Habitat type classification: Control

Figgjo is located at Jæren with its outlet at Honnsvika ca. 10 km southwest of Sandnes. The catchment area is approximately 233 km^2. The anadromous salmonid stretch of the waterway is approximately 23 km up to Ålgård, but the lowest 6 km between the sea and Gruda Lake (Grudavatnet) is possibly of low productivity (Kålås *et al.*, 2003). The upper part of the catchment consists of hilly terrain with many lakes. The highest point in the catchment is Ulvsfjellet to the east at 600 m asl. Nearly 90 % of the catchment is at an altitude of less than 350 m asl. Several small tributaries unite in Edlands Lake (Edlandsvatnet), 104 m asl. From there, the main channel of the river flows approximately 20 km to the northwest and receives only one major tributary, a stream from the north that flows to Grudavatnet. In the Figgjo catchment, there has existed a textile industry, metal industry and intensive agriculture farming in addition to runoff/waste from households in the area. In the Figgjo waterway, which is just one of several rivers in Jæren, agricultural activity has significantly impacted on the ecological state of the river system. Over many years, intensive agricultural activity has diminished natural wetlands and riparian buffer zones along the waterway. This has led to reduced habitat for species relying on both the river, and the riparian zones. Examples of species that have been negatively affected are the freshwater pearl mussel (*Margaritifera margaritifera* L.) and Atlantic salmon. The introduced species Canadian pondweed (*Elodea canadensis*) is also a problem in the river. The Figgjo salmon population was classified as warranting consideration in regard to escaped farmed salmon by Diserud *et al.,* (2012), and Glover *et al.,* (2012) detected significant temporal changes in microsatellite allele frequencies in the Figgjo salmon population. The Figgjo River is protected as one of Norway's national salmon rivers and is situated in a national salmon fjord (St.prp.nr.32, 2006).

3.23 Håelva

Habitat type classification: Control

The Hå River system has its origin in Lake Storamos (244 m asl) and drains into the sea at Hå; the catchment drainage area is 158 km^2, making this one of the Rogaland County's

larger waterways. The length of the anadromous salmonid stretch of the waterway is currently considered to be 29 km up to the Langa Lake (Langavatnet), although this stretch was previously shorter, approximately 16 km, prior to the construction of a fish ladder at Fotlands falls (Fotlandsfossen) (Urdal and Sægrov, 2000). The salmon catch approximates 2000 - 4000 kg salmon pr. year. Håelva has good production conditions for salmon and sea trout as a result of a long growing season, good food availability and favourable substrate and water currents. In good years, Håelva results in catches totalling more than 10 tonnes of salmon, but rarely more than 300 kg of sea trout. Average catches of salmon and sea trout in the five years leading to 2009 (2005-2009) were significantly lower than this; with 3156 kg and 27 kg, respectively. Besides salmon and trout, the Hå River also contains eel, flounder (*Platichthys flesus* L.), river lamprey (*Lampetra fluviatilis* L), sea lamprey (*Petromyzon marinus* L.) and three-spined stickleback (*Gasterosteus aculeatus* L.) (Larsen and Berger, 2010). While the upper and lake-rich portions of the waterway are relatively unaffected by eutrophication, some lower parts are strongly influenced by nutrients and organic matter. For instance, Tverråna, which drains much of the mid- and low-lying regions of the river system, contains phosphorus concentrations of up to 100 µg/L (Larsen and Berger, 2010). The main source of pollution is runoff from agricultural areas. The majority of the waterway is deemed unfit or unsuitable for drinking and bathing water due to agricultural pollution. Fish kills occur from sudden discharges of fertiliser and silage runoff, although such discharges have become less frequent in recent years. The Håelva salmon was classified as vulnerable in regard to escaped farmed salmon by Diserud *et al.,* (2012). The river is protected as one of Norway's national salmon rivers, and is located within a national salmon fjord (St.prp.nr.32, 2006).

3.24   Ogna

Habitat type classification: Acidification

The Ogna River system is situated in the municipalities of Hå and Bjerkreim in the County of Rogaland. The main waterway originates in the uplands of the mountains Laksesvela (536 m asl) and Svartaknuten (498 m asl) west of Vikeså and approximately 23 km from the sea. In the Ogna valley, the river forms three smaller lakes. Average annual rainfall in the region is approximately 2,000 mm, yet due to the relatively smaller lakes with little storage capacity in the catchment, the flow of the river varies with the amount of rainfall. The catchment area is approximately 117 km^2, of which 39 km^2 is diverted to the Helgå river system approximately 3 km from the mouth of the river (Larsen *et al.*, 1992). The anadromous salmon migration stretch in Ogna is approximately 30 km, continuing upstream to Ogna Lake (Ognavatnet) and above Laksesvela. The area is located within the Egersund anorthosite deposits; a field of intrusive igneous rock consisting predominately of anorthosite, and having a chemical composition of varying proportions of $CaAl_2Si_2O_8$ and $NaAlSi_3O_8$. Rock fragments contained in the topsoil are washed away from the high-lying areas and down to the lower reaches of the catchment (Abrahamsen *et al.*, 1972). The vegetation consists mainly of hardy species, peat and heather dominating the upper regions. Agriculture activity increases in the lower regions of the catchment, and in the Ogna valley from Hetland and down to the mouth of the river, the environment is affected by intensive agriculture practices. Acid precipitation has been a threat to the Atlantic salmon population in Ogna and in the 1970s and 1980s it was reported that acidification and poor water quality was affecting the salmon population to the

point that they were classified as threatened (Larsen *et al.*, 1992, and references therein). Agricultural pollution is also considered a major threat to Ogna. Liming has occurred in Ogna regularly since 1991, and there has been an increase in the density of both juvenile and adult salmon following liming, low densities in the years 2007-2009 may be attributable to few adult spawning fish. In addition to acidification, eutrophication is also likely to have affected the Ogna salmonid populations; the Hetland hydroelectricity station (Hetland kraftverk), situated downstream near Hetland, may affect suitable spawning grounds for salmonids between Hetland and Hylland (DN, 2009). Salmon fry from the Håelva population were released in Ogna as a hatchery-supplementation program until approximately 25 years ago. These releases were sporadic and probably functioned much the same as natural straying from neighbouring rivers, the salmon population in Ogna is therefore considered to be genetically "pure" (Driftsplan, 2009, Rogaland County). The Ogna salmon population was classified as vulnerable in regard to escaped farmed salmon by Diserud *et al.,* (2012). The Ogna River is protected as one of Norway's national salmon rivers and is located within a national salmon fjord (St.prp.nr.32, 2006).

3.25   Bjerkreimselva

Habitat type classification: Acidification

The Bjerkreim River system is one of the Rogaland County's largest waterways. It lies mainly in the municipality of Bjerkreim, and is often called Bjerkreimselva (Bjerkreim River). The lower section, from the sea up to Fotland Lake (Fotlandsvatnet), is called Tengselva and is part of the Egersund municipality. Prior to river regulation, the river had a catchment area of 705.8 km^2 and a mean water flow of 54.4 m^3/s. Approximately 20 km^2 of the catchment is diverted into the Figgjo River (Figgjoelva). The anadromous salmon stretch in the Bjerkreim waterway extends upstream to Inner Vinja Lake (Indre Vinjavatn), as well as 7-8 km into Ørsdals Lake (Ørsdalsvatn). The first reports of salmon deaths due to acidification in the Bjerkreim occurred in 1970. pH measurements in the Bjerkreim waterway in the late 1970s and early 1980s showed that the lowest pH occurred during snow melting in spring and with heavy precipitation in autumn. The fish populations in the eastern section of the waterway were found to be non-existent in 1989, while the western sections and lower regions of the main river and its tributaries continued to have naturally sustaining populations. The acidic effects are most pronounced in the north-eastern sections of the river system. Liming of the Bjerkreim waterway has been active since 1996 (DN, 2012). A salmon stock-enhancement program occurs in the Bjerkreim waterway using locally caught broodstock. The Bjerkreimselva salmon population was classified as vulnerable in regard to escaped farmed salmon by Diserud *et al.,* (2012). The Bjerkreim river is protected as one of Norway's national salmon rivers, and is located in a national salmon fjord (St.prp.nr.32, 2006).

4 Results

4.1 Genetic diversity and divergence

There was no obvious trend in departures from HWE with any markers among affected and non-affected samples, with all samples departing from HWE expectations at some loci at the α = 0.05 level (Table 2). Most deviations were in the direction of heterozygote deficiency. There was no obvious geographical trend in samples that departed from HWE expectations at the same SNP locus; however, locus-specific factors cannot be ruled out, as there were several instances of the same samples departing from HWE at identical SNP loci. This may be indicative of linkage among loci, selection acting upon these loci, or simply genotyping errors at these loci (e.g. null-alleles and/or bad assays/clustering). Appendix A, Table 3 contains a table with SNP loci that showed departures from HWE at more than one sample at P < 0.001. There was no obvious trend in overall heterozygosity or diversity (n_e) levels among samples, regions, or habitat types, yet two affected samples (Flekkeelva, and Suldalslågen) exhibited significantly lower heterozygosity than other samples when averaged over all loci (Table 2, Figure 3).

In addition to the lower genetic heterozygosities of the Flekkeelva and Suldalslågen samples, these samples also showed the largest genetic differentiation in all pairwise genetic distance comparisons (Figure 4). Evidence of regional-based genetic structuring was low among samples (Figure 4 and Figure 5) with only 0.67% of the total genetic variance explained by regional groupings. The vast majority of genetic variation was explained by variation within samples (97%). Yet, significant genetic differences were detected both among rivers within regions and among regions.

Table 2   *Genetic statistics. N, number of individuals per sample; n_e, effective number of alleles; He ± se, mean expected heterozygosity over all loci with standard error; N HWE departures, number of loci in Hardy-Weinberg disequilibrium at α =0.05, α =0.01 and α =0.001; % P.L., percent of polymorphic loci (from 3761 total)*

| Sample	N	n_e	mean He	N HWE departures			%P.L.
				P<0.05	*P<0.01*	*P<0.001*	
Oldenelva	38	1.593	0.345 ± 0.00272	96	16	0	96.60 %
Gloppenelva	41	1.596	0.342 ± 0.00266	184	0	0	97.53 %
Eidselva	11	1.568	0.340 ± 0.00328	54	11	0	91.15 %
Nausta	31	1.586	0.341 ± 0.00279	86	16	1	95.40 %
Jølstra	10	1.565	0.335 ± 0.00347	79	24	0	89.63 %
Flekkeelva	32	1.543	0.311 ± 0.00291	104	24	0	91.81 %
Gaula	33	1.585	0.338 ± 0.00279	121	35	14	95.72 %
Nærøydalselva	36	1.583	0.343 ± 0.00280	100	18	1	95.03 %
Aurlandselva	38	1.577	0.340 ± 0.00291	186	42	12	94.87 %
Flåmselva	25	1.580	0.335 ± 0.00292	151	46	10	94.26 %
Årøyelva	16	1.574	0.339 ± 0.00317	130	36	12	92.24 %
Lærdalselva	61	1.576	0.333 ± 0.00268	172	65	15	96.46 %
Mørkridselva	40	1.582	0.340 ± 0.00277	90	10	0	94.79 %
Fortunselva	40	1.593	0.342 ± 0.00272	153	38	6	96.52 %
Vosso	22	1.575	0.337 ± 0.00302	121	34	11	93.62 %
Granvinselva	19	1.582	0.331 ± 0.00300	135	50	15	94.50 %
Kinso	25	1.590	0.346 ± 0.00285	108	24	1	96.09 %
Eio	24	1.583	0.343 ± 0.00295	142	26	2	95.35 %
Vikedalselva	40	1.592	0.344 ± 0.00273	120	18	2	96.28 %
Suldalslågen	50	1.561	0.325 ± 0.00280	118	30	11	93.91 %
Vormo	42	1.594	0.346 ± 0.00272	97	13	2	96.04 %
Figgjo	48	1.580	0.338 ± 0.00275	132	26	6	95.83 %
Håelva	30	1.577	0.340 ± 0.00288	104	19	1	94.42 %
Ogna	28	1.574	0.340 ± 0.00292	109	28	3	94.26 %
Bjerkreimselva	40	1.573	0.330 ± 0.00278	169	49	8	95.05 %
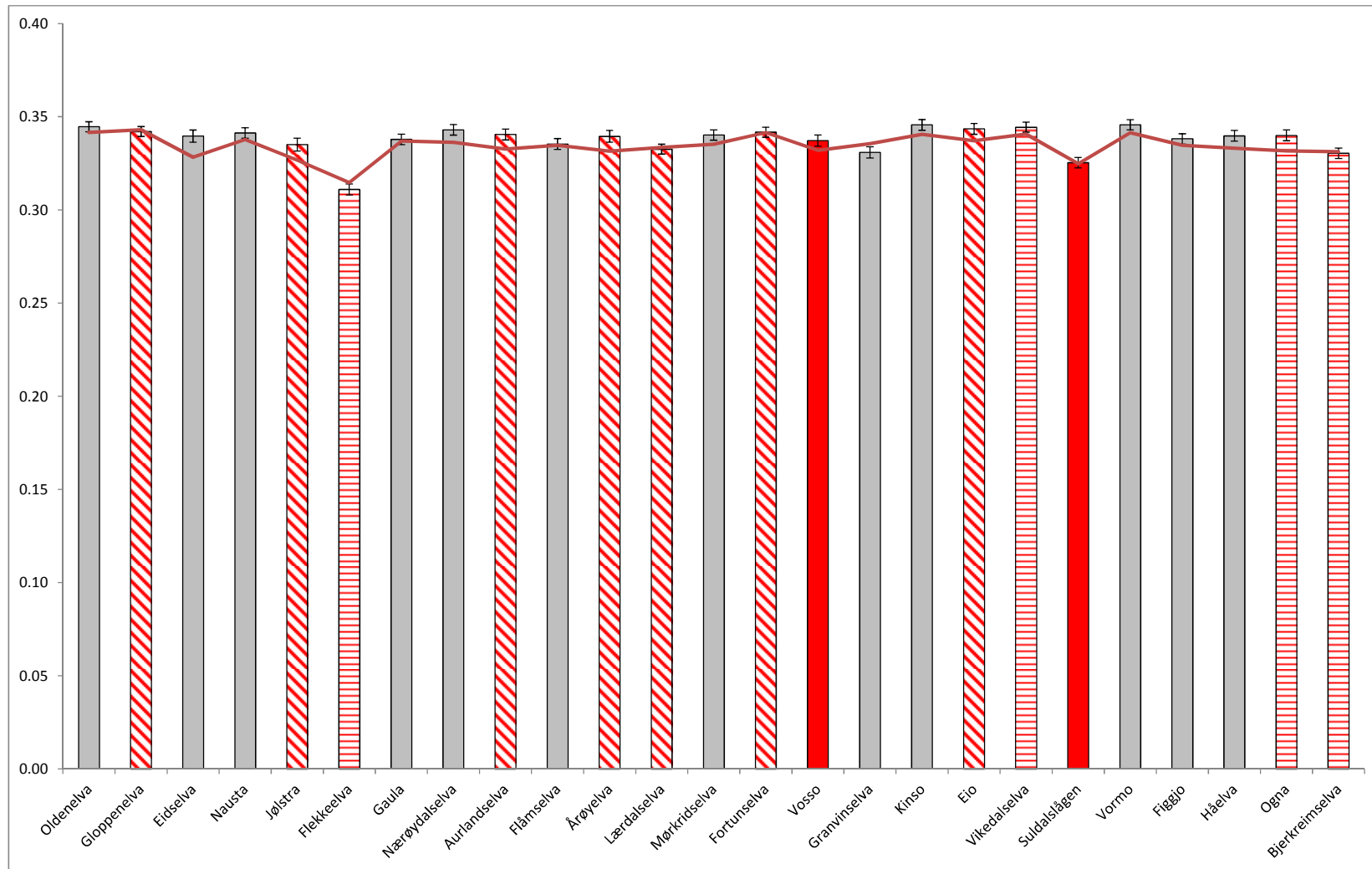
Figure 3   Relative heterozygosity estimates among samples for 3761 diploid loci. Bars represent observed heterozygosity (Ho); line represents expected heterozygosity (He). Samples are coded according to habitat type: grey represents control samples, red represents affected samples – regulated (diagonal stripes), acidified (horizontal stripes), both acidified and regulated (solid red)
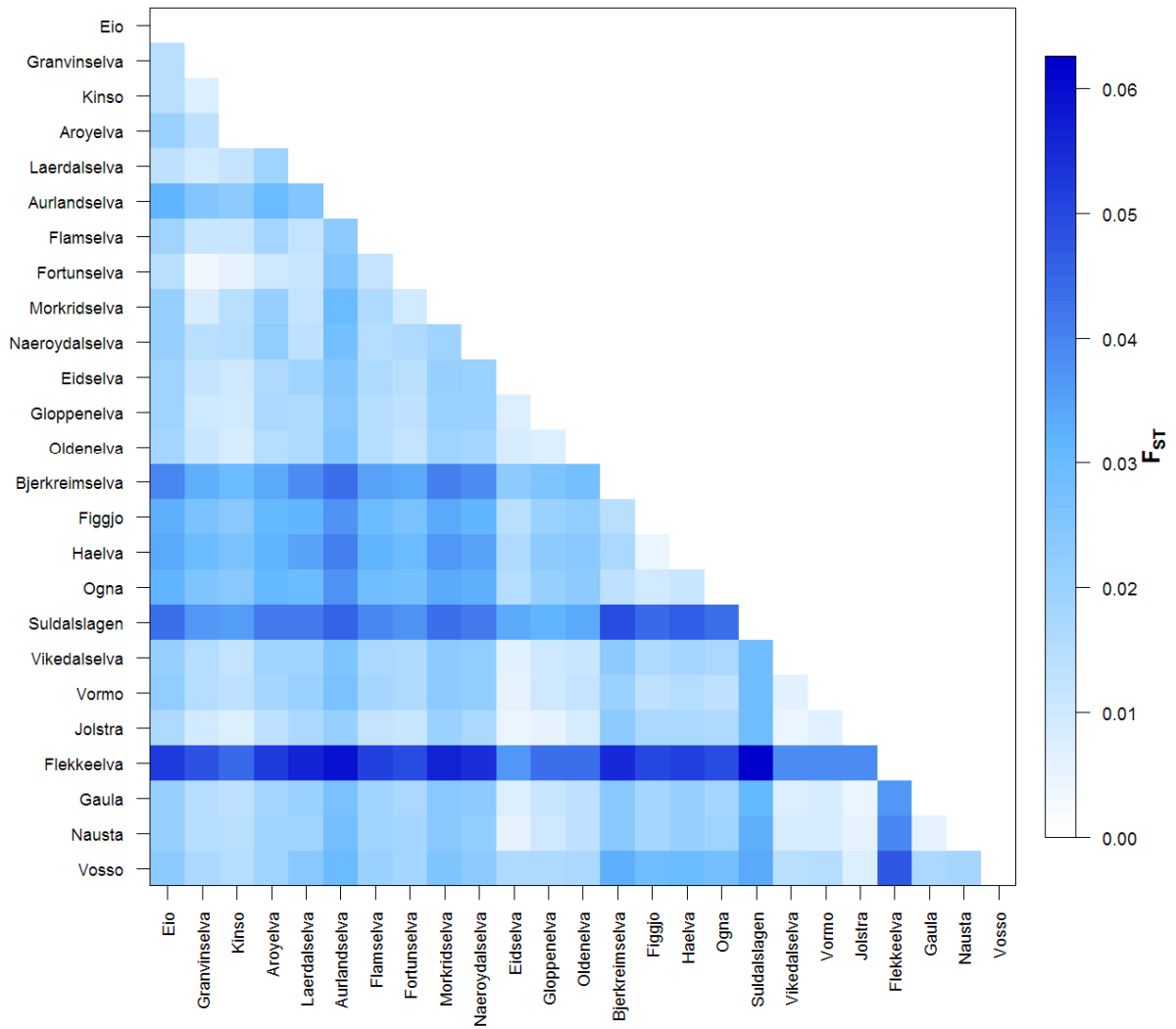
Matrix of pairwise F$_{ST}$

Figure 4 Heat-map matrix of pairwise F$_{ST}$ values among samples obtained from 3638 diploid loci
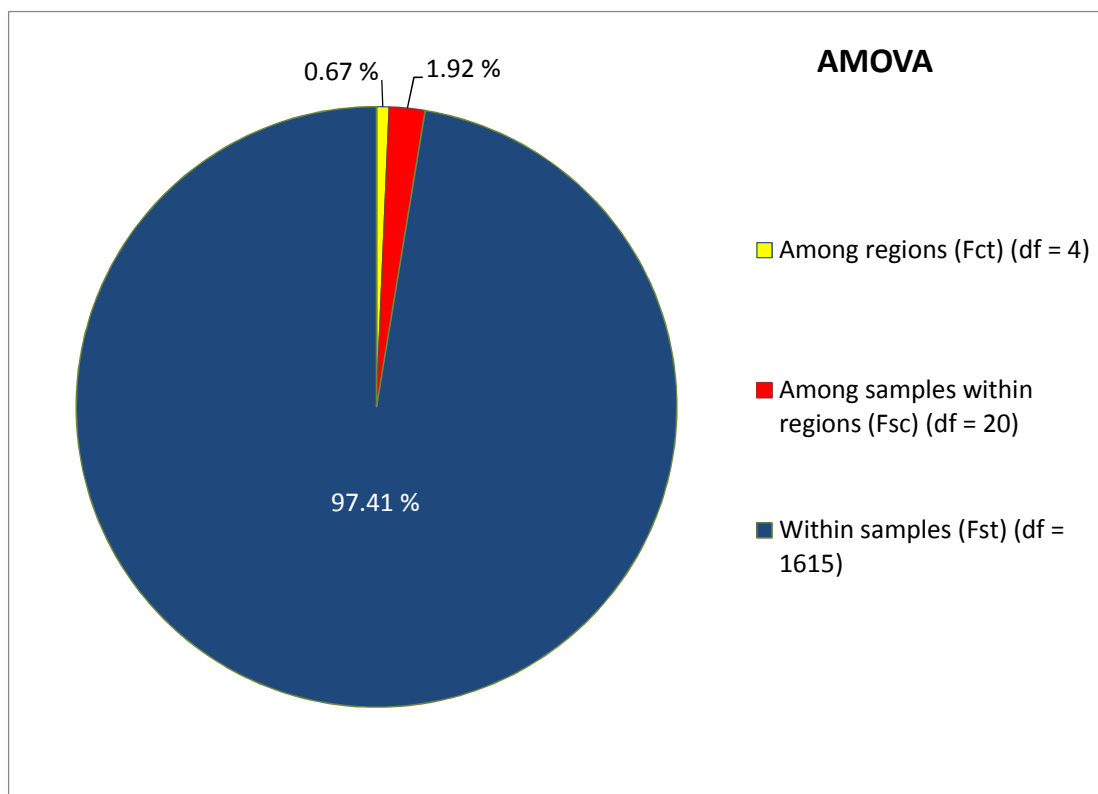
Figure 5   Analysis of molecular variance (AMOVA) for samples at 3638 loci considering various components of genetic variance. Groups are defined according to fjord system/region (refer to Table 1). Sources of variation, degrees of freedom (df) percentage of the variation explained by the specific component (%). The probability against the null-hypothesis (P-value) was P < 0.001 for all tests.

4.2   Isolation by distance

Tests for spatial auto-correlation among samples used both pairwise log natural (Ln) waterway distances and geographic (UTM) coordinates. The null-hypothesis of no spatial correlation among genetic distance and geographic distance could be rejected by the Mantel test for both Ln waterway distance (R = 0.144, *P* < 0.01, Figure 6, A); and the geographic coordinate distances (R = 0.284, *P* < 0.01 Figure 6, B). These results indicate that the gene-flow among Atlantic salmon populations decreases with increasing geographic distances between populations.

A: Mantel test distribution using log natural waterway distances
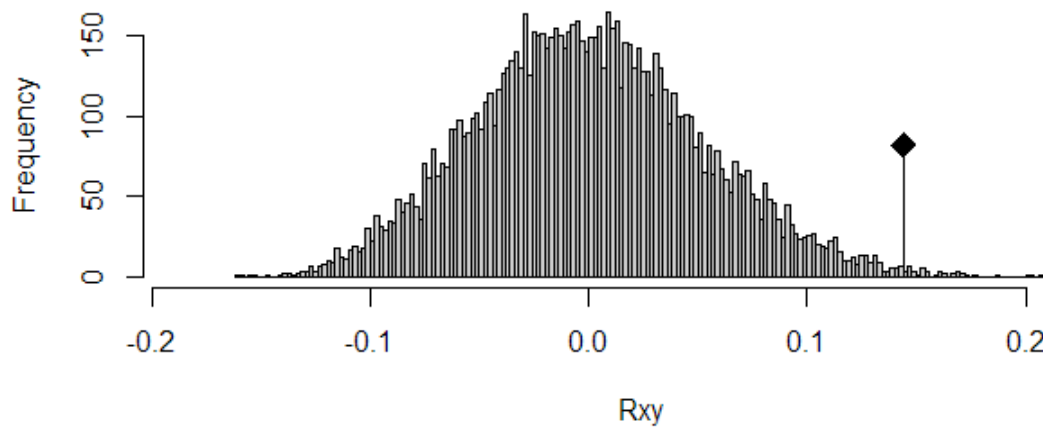
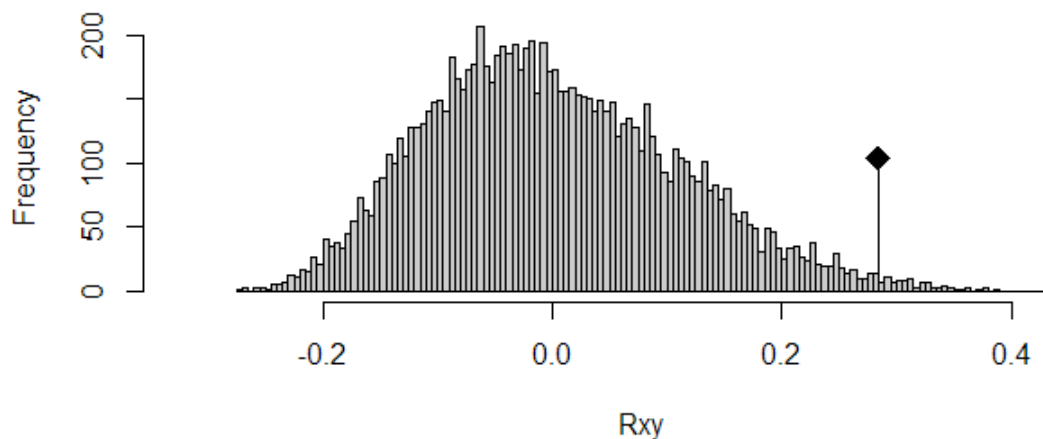**B: Mantel test distribution using geographic co-ordinates**

*Figure 6   Mantel test distribution graphs for testing of isolation by distance using pairwise genetic distance (F_{ST}) among samples and pairwise geographic distance in the form of **A**: Ln waterway distances, and **B**: geographic coordinates. Filled diamonds denote the observed Rxy in relation to the simulated distribution of Rxy*

4.3   Genomic differentiation among habitat types

Genome-wide association mapping tests found several significant associations of loci with affected habitat types (Figure 7 and Figure 8). Association with the acidified habitat type was strongest, yielding more SNPs of highly significant associations. Detection of differentiating loci using the hierarchical analysis of genetic heterozygosity within samples and among groups also detected outlier loci with higher than expected F_{CT} values among groups (Figure 9). SNPs identified using both approaches were largely concordant, although the F_{CT} outlier method detected a greater number of outlier SNPs, possibly indicating false positives.

A subset of SNPs of highest significance using both methods were further analysed to identify the allelic patterns and clustering of individuals. There were no fixed allelic differences found in comparisons among the control and affected groups; however, large frequency differences were observed (Figure 10, Table 4 and Table 5). Principle coordinates analysis (PCoA) of samples at these subset of loci show clear differentiation of habitat types, with coordinates 1 and 2 explaining 56% and 61% of the variation among samples in the control/acidified, and control/regulated datasets, respectively (Figure 11) The PCoA also highlights some indications of regional-structuring, with samples in close proximity clustering more closely together than other samples of the same habitat type. However, within-habitat type clustering was strongest and some samples, e.g. Flekkeelva and Vikedalselva (Figure 11, A), both of an acidified habitat type, clustered closely together despite large geographic distances separating these rivers.

Maximum likelihood analysis of individual assignment to the designated habitat type using a subset of SNP loci significant at α = 0.001 correctly assigned 94% and 90% of individuals in the control and acidified groups, respectively (Figure 12, A) and 84% and 87% of individuals in the control and regulated groups, respectively (Figure 12, B). Bayesian analysis of individual membership to two theoretical clusters using the same subset of loci showed clear structuring of affected individuals into a single cluster, yet control individuals generally showed less clear patterns of structuring (Figure 13).
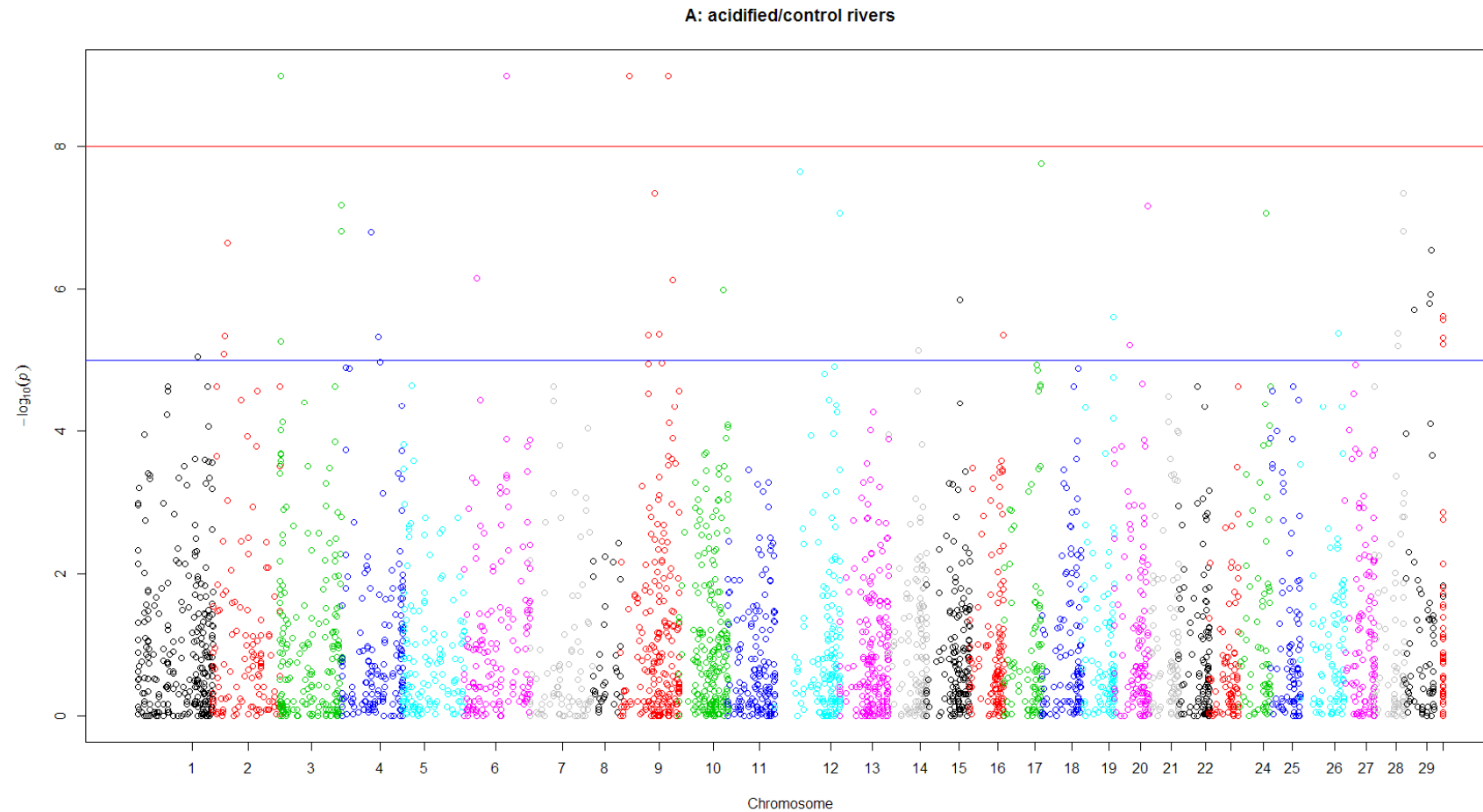
Figure 7   Genome-wide association mapping for detection of loci associated with an acidified habitat type. Genomic coordinates (position on chromosomes) are represented along the X-axis with the negative logarithm of the association P-value for each SNP on the Y-axis. SNP loci without known chromosome positions were designated position 0; the chromosome to the far right (red, un-numbered) was designated for SNP loci that have not yet been mapped to a chromosome
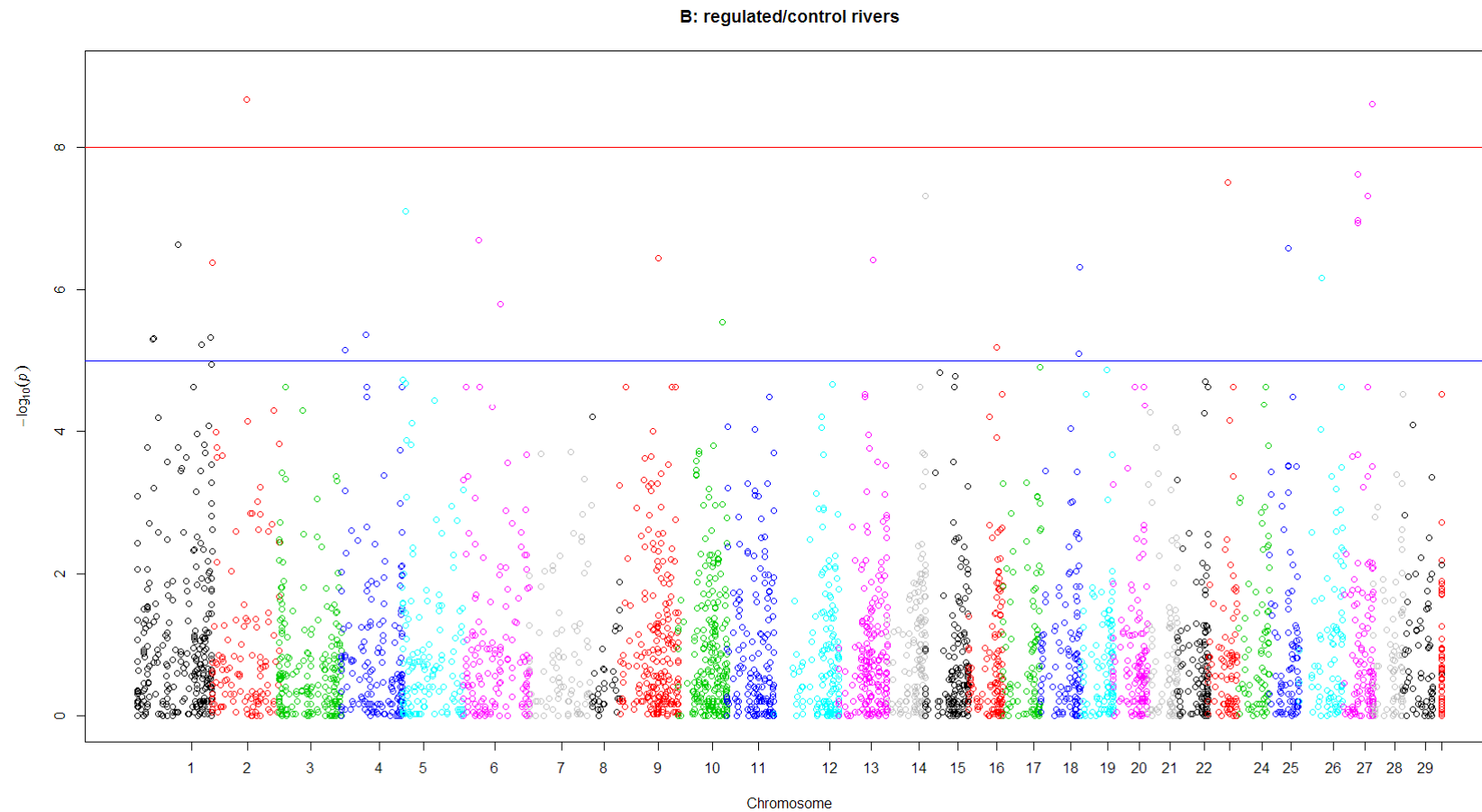
Figure 8   Genome-wide association mapping for detection of loci associated with a regulated habitat type. Genomic coordinates (position on chromosomes) are represented along the X-axis with the negative logarithm of the association P-value for each SNP on the Y-axis. SNP loci without known chromosome positions were designated position 0; the chromosome to the far right (red, un-numbered) was designated for SNP loci that have not yet been mapped to a chromosome

A:

Detection of loci under selection from genome scans based on F_{CT}

B:

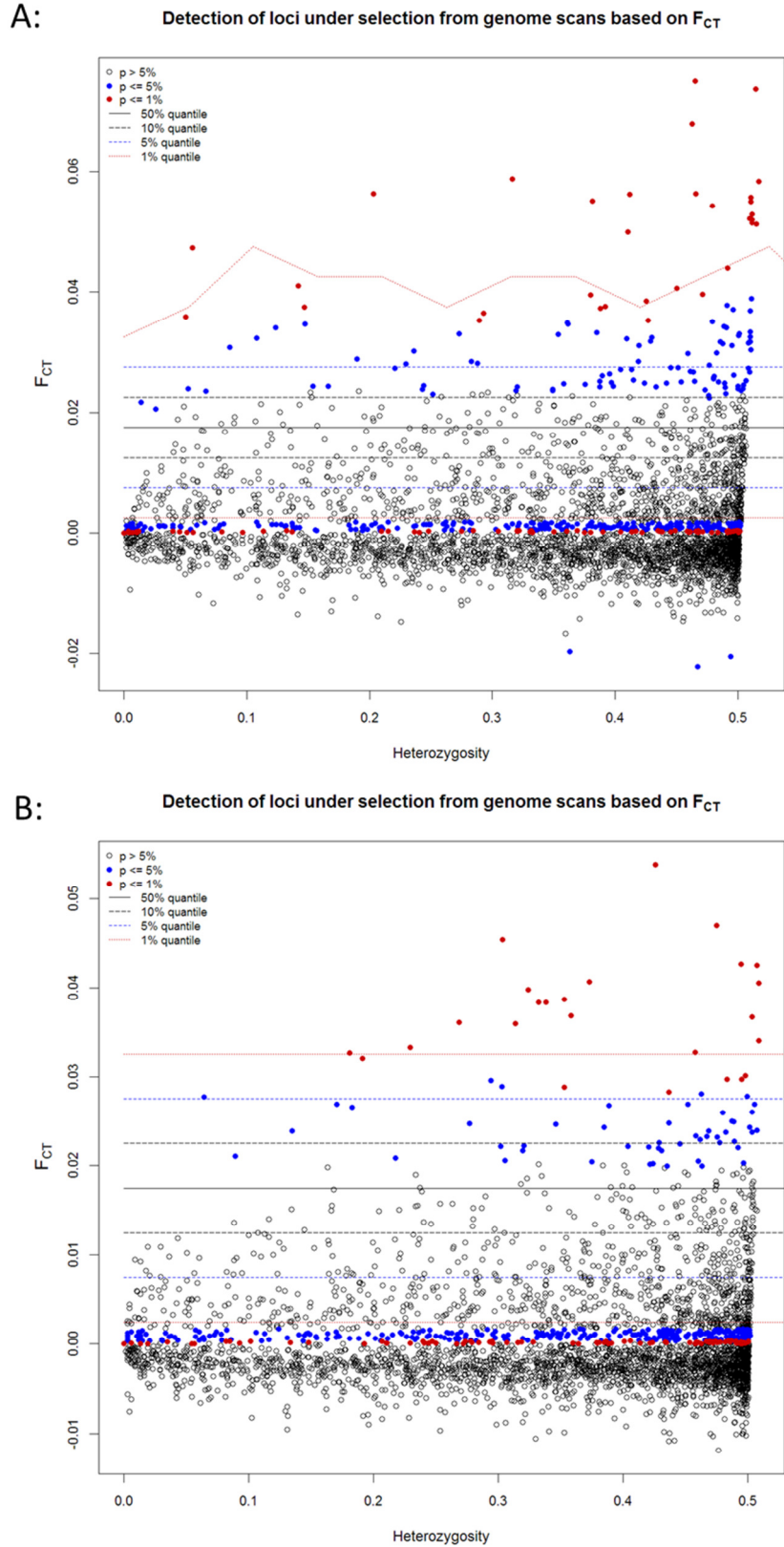Detection of loci under selection from genome scans based on F_{CT}

*Figure 9   Detection of outlier loci using an F_{CT} outlier approach; **A**, Acidified/control groups; **B**, Regulated/control groups. Each SNP locus is represented by a point, with blue and red points denoting those that are significantly different from the simulated distribution at α = 0.05 (blue) and α = 0.001 (red)*

A.

B.
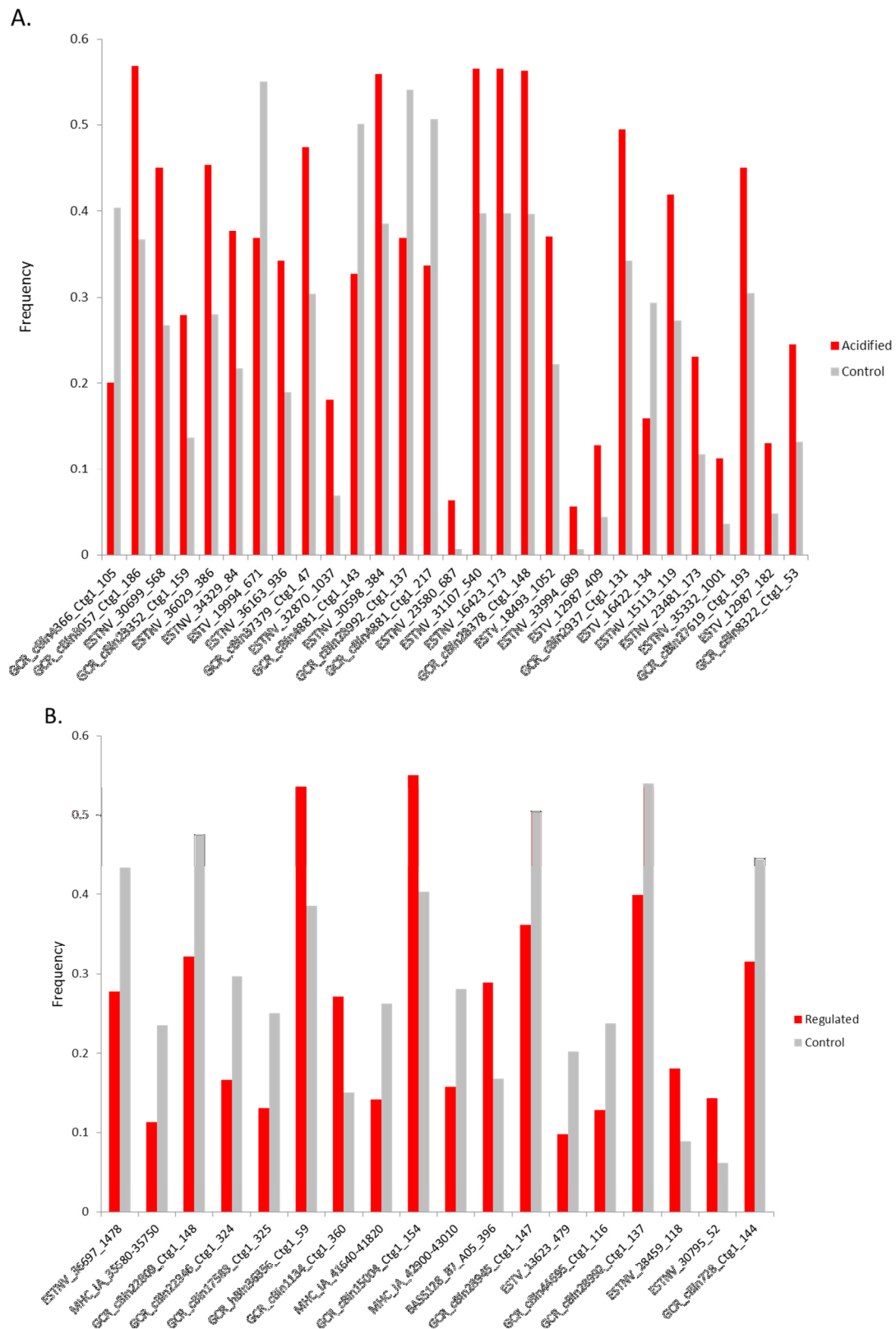
Figure 10   Relative allele frequencies among habitat types at top-ranked loci with association with habitat type significant at α =1x10^{-6}; **A**,: acidified and control habitat types; **B**, regulated and control habitat types. Loci are identified by their corresponding SNP ID. Refer to Appendix A - Table 4 and Table 5 for chromosome and position information for each of these SNPs
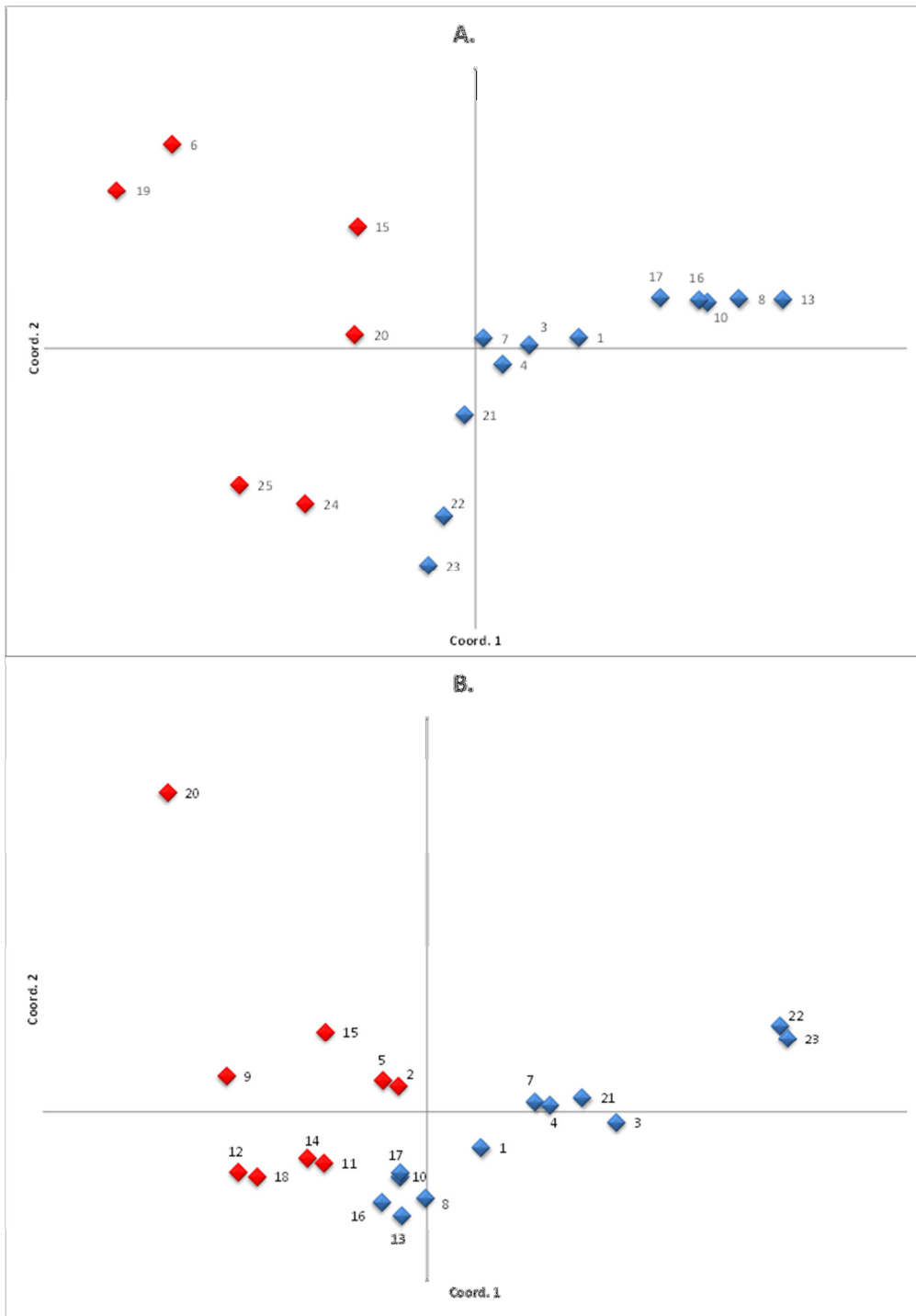
*Figure 11 Principle Coordinates Analysis using genetic distance among samples at a subset of loci found to be significantly associated with habitat type at α = 0.001 using GWAS. **A**, acidified (red) and control (blue) samples; **B**, regulated (red) and control (blue) samples. Refer to Table 1 for corresponding sample codes*
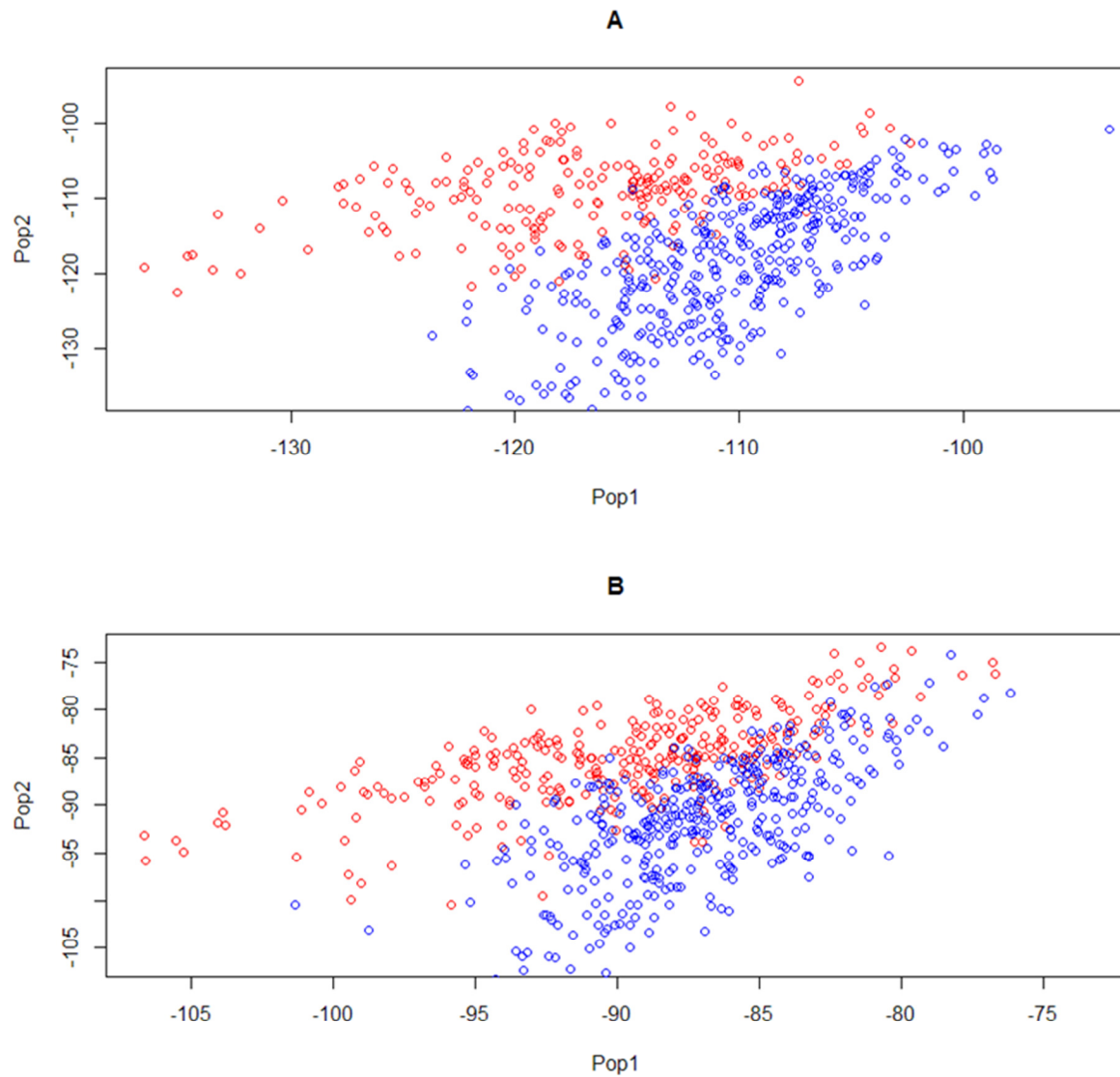
*Figure 12   Maximum Likelihood analysis of assignment of individuals to habitat type groups at loci significantly associated with habitat type at α = 0.001 using GWAS method. **A**, acidified (red) and control (blue) individuals; **B**, regulated (red) and control (blue) individuals*
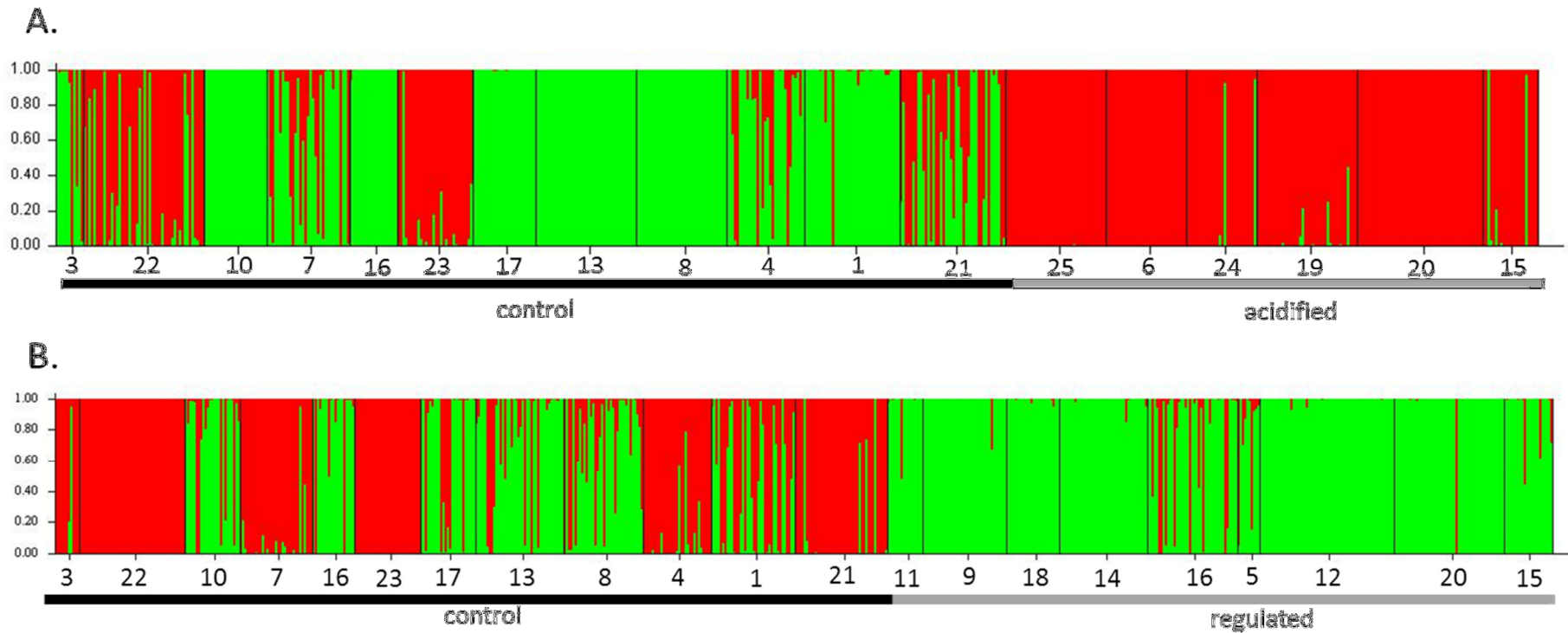
Figure 13   Bayesian plots of individual genotype membership (Q-values) from a subset of loci to K=2 genetically independent clusters
(represented by different colours). *A*: acidified/control habitat type samples; *B*: regulated/control samples. Samples defined a priori
are separated by black vertical lines, refer to Table 1 for corresponding sample codes. Loci used were those that displayed
significant associations to habitat type at α = 0.001 in genome wide association mapping tests (see Figure 7 and Figure 8) and that
were also concordant with outlier loci detected using the hierarchical procedure outlined in 2.6.2 (see results in Figure 9)

5 Discussion

5.1 Genetic diversity and differentiation among samples

Assessment of population diversity among 25 river samples in this study at 3761 SNP loci did not find any obvious trend in levels of genetic diversity associated with habitat type. However, two affected samples, Flekkeelva (acidified) and Suldalslågen (acidified and regulated) both showed significantly lower levels of genetic diversity. In assessing genetic variation shared among and within samples, the majority of the total genetic diversity was found among individuals within populations, consistent with other studies of Atlantic salmon (Dionne *et al.*, 2009; Verspoor, 1997), and diadromous fish in general (Hedgecock, 1994). Reductions in the genetic effective population size could not be estimated due to the small sample sizes. Tests of isolation by distance (Mantel's test) showed a significant negative correlation between pairwise genetic divergence and geographic distance, indicating meta-population structuring among Norwegian rivers as has been shown in other countries for salmonids (Primmer *et al.*, 2006; Schtickzelle and Quinn, 2007; Verspoor, 1997).

5.2 Genetic diversity and differentiation among affected and non-affected habitat types

This study identified SNPs that accurately differentiated samples collected from six rivers affected by acidification, and nine rivers affected by regulation, from samples collected from twelve rivers that are non-affected (or effected to a lesser degree). The scope of the study did not allow for in-depth exploration of the cause(s) of this genetic differentiation; yet it is possible that these loci differentiate the samples due to differing selection pressures in the different habitat types, and thus genes linked to these loci may be under selection. However, other mechanisms can cause such differentiation, and as such, many samples from many rivers are required to obtain greater certainty about selection acting on these loci as a result of these anthropogenic activities. It has been shown that genetic structuring and range expansions can mimic a selective sweep and lead to false positives in adaptive genetics studies (Excoffier *et al.*, 2009b). This study attempted to control for genetic structure and range expansion by including many samples from affected rivers in multiple regions and also having samples from non-affected rivers in close proximity to the affected rivers; yet in Norway, southern-most rivers are most affected by acidification and as such four of the six acidified samples are in relatively close proximity to each other, thus genetic structure may have affected these results and this may explain the clustering of the control sample Håelva (ID. 23) with these acidified rivers in the Bayesian clustering analysis (Figure 13). In contrast, it is important to note that two acidified samples (Flekkeelva and Vikedalselva) also cluster together, regardless of the great geographic distances separating these rivers. In the Bayesian analysis (Figure 13), there is a clear trend of membership to a single group for individuals from affected rivers (acidic – Figure A, and regulated – Figure B); yet in the control samples, membership is somewhat mixed. Such a result would be expected if these loci are under directional selection in affected rivers; control rivers experiencing no selection at these loci would be expected to show more heterogeneity, whereas a shift in allele frequencies towards a beneficial (or against a deleterious allele) would be expected where there is directional selection in the affected rivers.

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It is also possible that directional selection may be occurring in some of the rivers used as control samples in this study, and this may explain patterns observed in Figure 13. In this Figure, Figgjo (ID. 22), Håelva (ID. 23), Nausta (ID. 4) and Gaula (ID. 7) all show membership to the acidified-like group (red). These rivers, although included as control rivers in this study, have been heavily affected by pollution and eutrophication (Figgjo and Håelva), or have previously been affected by acidification (Nausta and Gaula). Thus, it cannot be ruled out that the salmon populations in these rivers are, or have previously been under selection due to habitat changes, and that this is reflected in the subset of SNPs identified in this study. Likewise, Figure 13B shows several samples with regulated-like membership (green). It is possible that hydroelectricity stations that were not believed to affect the salmon in some of these control rivers (e.g. Flåmselva, ID. 10 and Kinso, ID. 17) do in fact have an effect. These rivers also are characterised by cold water temperatures which have been suggested to affect salmon fry survival. It may therefore be possible that these control populations (eg. Flåmselva, Kinso, Mørkridselva, Nærøydalselva) are under selection due to habitat changes, and that this is reflected in the subset of SNPs identified in this study.

Gene flow among rivers via natural straying may also result in changes in allele frequencies within a population where straying occurs from an affected to a non-affected river. Given the isolation by distance results (section 4.2), it is likely that migrants are shared among the proximate rivers Håelva, Figgjo, Ogna and Bjerkreimselva, which encompass both control and affected samples. Such straying between rivers has also been shown with tagging studies (Hindar, 1992). Migrants would be expected to contribute their genes to the recipient population if they had as-good or greater fitness than the local inhabitants in the recipient river; thus resulting in a shift in allele frequencies over generations. Further study is needed of these populations to understand the shift in allele frequencies at these SNP loci over time.

Although the rivers in this study were chosen based on their relative history of acidification and/or regulation, it is nonetheless possible, and in some cases likely, that many of these rivers are or have been affected by other anthropogenic factors. In a population dynamic analysis of return of one-sea winter salmon (grilse) to Norwegian rivers, OTERO *et al.,* (2011) showed that the general trend of decreasing catches of returning grilse over the period of 1979-2007 was more pronounced in populations where fish farms were located along the out-migration route of the smolts. In the same analysis, a significant effect on catches of grilse was also observed in rivers that were affected by hydroelectricity development.

It has also possible that interactions with escaped farmed salmon have impacted populations in south and western Norway, as many of these rivers are known to receive large numbers of escaped farmed salmon (Diserud *et al.*, 2012) and temporal genetic changes have been observed in some rivers, likely as a result of this (Glover *et al.*, 2012).

5.3   Future studies

This study identified outlier SNP markers which differentiated Atlantic salmon sampled from six rivers affected by acidification and nine rivers affected by regulation, from 12 non-affected rivers. These markers can therefore be considered as candidate SNPs possibly under selection. However, due to the lack of historical samples and small sample sizes, the present dataset was not sufficient to allow for accurate testing of selection at these markers, or for

identification of the environmental agent(s) responsible for the possible signatures of selection observed. Nevertheless, these candidate SNPs may be used together with "neutral" SNPs in further studies, in order to assess temporal changes in populations subjected to the same anthropogenic influences. The identification of the candidate SNPs in this study will allow future studies to use different SNP genotyping techniques that are less sensitive to DNA degradation, thereby enabling genotyping of historical samples. A temporal study using historical samples collected from rivers before or soon after they experienced anthropogenic changes, in addition to contemporary samples will enable the identification of changes at these candidate SNPs as a response to the changing environmental conditions, a useful indicator of selection acting on these loci. In order to determine the fitness consequences in populations of Atlantic salmon affected by these anthropogenic activities, it will be necessary to include more samples from a greater range to estimate population size changes and linkage disequilibrium in and among samples.

The application of a higher-density SNP-array will provide greater information regarding genetic changes due to anthropogenic effects, as it will enable the detection of markers closely linked to loci under selection. A higher-density SNP-array (approximately 200K SNPs) will be available in early 2013 (S. Lien, pers. comm), a dramatic increase from the 5.5K SNP array used in the present study. A common garden experiment with native fish reared in both low-pH (acidic) and regular-pH environments will provide a way to assess the effects of selection to acidification over a life-cycle. Furthermore, such a study will provide a means by which to assess the differential SNP loci identified in this study, and determine whether there is a true association with tolerance to acidification.

6   Appendix A

Table 3   *SNP loci that showed departures from HWE in more than one sample at α =*
0.001

SNP-ID	Samples with common HWE departures ($P < 0.001$)
ESTNV_35442_128	Lærdalselva, Figgjo, Suldalslågen
GCR_cBin13736_Ctg1_42	Lærdalselva, Figgjo, Suldalslågen
GCR_cBin17484_Ctg1_144	Lærdalselva, Figgjo, Suldalslågen
GCR_cBin25358_Ctg1_191	Lærdalselva, Figgjo,
GCR_cBin47268_Ctg1_70	Lærdalselva, Suldalslågen,
ESTNV_26758_619	Bjerkreimselva, Gaula,
ESTNV_36029_386	Bjerkreimselva, Årøyelva, Gaula, Vosso, Granvinselva,
ESTNV_16657_595	Årøyelva, Gaula,
ESTNV_35178_1774	Årøyelva, Granvinselva,
ESTNV_35810_833	Årøyelva, Gaula,
ESTNV_37714_614	Årøyelva, Vosso, Nærøydalselva, Flåmselva, Fortunselva, Granvinselva
GCR_cBin22840_Ctg1_249	Årøyelva, Vosso,
GCR_cBin30634_Ctg1_159	Årøyelva, Fortunselva, Granvinselva
GCR_cBin46344_Ctg1_63	Årøyelva, Gaula, Granvinselva
GCR_cBin47864_Ctg1_28	Årøyelva, Fortunselva,
GCR_cBin6716_Ctg1_223	Årøyelva, Flåmselva,
ESTNV_27898_719	Gaula, Kinso,
GCR_cBin18076_Ctg1_99	Gaula, Vosso,
GCR_cBin30162_Ctg1_245	Gaula, Fortunselva,
GCR_cBin37930_Ctg1_88	Gaula, Vosso,
GCR_hBin27560_Ctg1_46	Gaula, Vosso, Granvinselva
ESTNV_36692_352	Vosso, Granvinselva,
GCR_cBin33212_Ctg1_226	Vosso, Fortunselva, Granvinselva
GCR_cBin14344_Ctg1_130	Flåmselva, Granvinselva,
ESTV_13197_590	Fortunselva, Granvinselva,

Table 4 *Information for top ranked SNPs identified as significantly differentiating between acidified and control habitat types based on both genome-wide association case-control tests and hierarchical structure analyses. Rank of the SNPs is given according to non-permuted P-values in Plink (where α = 1x10⁻⁶); Chr, chromosome SNP is mapped to; SNP-ID, identification of the SNP; f, frequency of the minor allele over all affected (A) and control (C) individuals*

Rank	Chr.	SNP-ID	f (A)	f (C)
1	9	GCR_cBin4366_Ctg1_105	0.201	0.404
2	6	GCR_cBin8057_Ctg1_186	0.569	0.367
3	3	ESTNV_30699_568	0.45	0.267
4	17	GCR_cBin23352_Ctg1_159	0.279	0.137
5	6	ESTNV_36029_386	0.453	0.28
6	24	ESTNV_34329_84	0.377	0.217
7	9	ESTV_19994_671	0.368	0.55
8	4	ESTNV_36163_936	0.342	0.189
9	3	GCR_cBin37379_Ctg1_47	0.474	0.304
10	9	ESTNV_32870_1037	0.18	0.069
11	28	GCR_cBin4881_Ctg1_143	0.327	0.501
12	12	ESTNV_30598_384	0.559	0.386
13	10	GCR_cBin28992_Ctg1_137	0.368	0.541
14	28	GCR_cBin4881_Ctg1_217	0.337	0.507
15	29	ESTNV_23580_687	0.064	0.008
16	2	ESTNV_31107_540	0.566	0.398
17	20	ESTNV_16423_173	0.566	0.398
18	12	GCR_cBin28378_Ctg1_148	0.564	0.397
19	3	ESTV_18493_1052	0.37	0.222
20	?	ESTNV_33994_689	0.057	0.007
21	?	ESTV_12987_409	0.128	0.045
22	15	GCR_cBin2937_Ctg1_131	0.495	0.342
23	9	ESTV_16422_134	0.159	0.294
24	29	ESTNV_15113_119	0.419	0.273
25	28	ESTNV_23481_173	0.231	0.118
26	29	ESTNV_35332_1001	0.113	0.037
27	2	GCR_cBin27619_Ctg1_193	0.45	0.305
28	?	ESTV_12987_182	0.13	0.049
29	27	GCR_cBin8322_Ctg1_53	0.245	0.132

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Table 5   *Information for top ranked SNPs identified as significantly differentiating between control and regulated habitat types based on both genome-wide association case-control tests and hierarchical F_{CT} outlier analyses. Rank of the SNPs is given according to non-permuted P-values in Plink (where α = $1x10^{-6}$); Chr, chromosome SNP is mapped to; SNP-ID, identification of the SNP; f, frequency of the minor allele over all affected (A) and control (C) individuals*

Rank	Chr.	SNP-ID	f (A)	f (U)
1	27	GCR_cBin21412_Ctg1_181	0.38	0.223
2	2	ESTNV_36697_1478	0.278	0.433
3	27	MHC_IA_35580-35750	0.113	0.236
4	23	GCR_cBin22809_Ctg1_148	0.322	0.476
5	27	GCR_cBin22346_Ctg1_324	0.167	0.297
6	25	GCR_cBin17583_Ctg1_325	0.13	0.251
7	6	GCR_hBin36356_Ctg1_59	0.537	0.386
8	2	GCR_cBin1134_Ctg1_360	0.272	0.151
9	27	MHC_IA_41640-41820	0.142	0.263
10	5	GCR_cBin15004_Ctg1_154	0.551	0.403
11	27	MHC_IA_42900-43010	0.158	0.281
12	9	BASS128_B7_A05_396	0.289	0.168
13	1	GCR_cBin28945_Ctg1_147	0.362	0.505
14	14	ESTV_13623_479	0.098	0.202
15	13	GCR_cBin44695_Ctg1_116	0.128	0.239
16	10	GCR_cBin28992_Ctg1_137	0.399	0.541
17	18	ESTNV_28459_118	0.181	0.089
18	26	ESTNV_30795_52	0.144	0.062
19	19	GCR_cBin728_Ctg1_144	0.315	0.446

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8 Glossary of terms and abbreviations

Term or abbreviation	Definition
Allele	One form of a gene that is present at a single locus. Diploid organisms contain two copies of each gene in nuclear DNA and thus have two possible alleles at each locus
AMOVA (analysis of molecular variance)	A statistical model for assessing the genetic variation within and among a group of individuals
Anthropogenic	Caused by human activity
Diploid	Containing two complete sets of chromosomes, one from each parent
Directional selection	The preferential reproduction or survival of different genotypes under different environmental conditions
Genetic diversity	The number of alleles at a given locus, or averaged over all loci, in a group of individuals
Genotype	The genetic constitution of an individual at a single, or multiple loci
GWAS (genome-wide association study)	An analysis of allelic association for genes throughout a genome
Habitat type	A classification of the environment or habitat occupied by an individual or group of individuals. Used in this report for classifying rivers by anthropogenic activity; habitat types are defined in Table 1
Heterozygosity	The proportion of individuals that are heterozygous (have two different alleles) at a single locus
Heterozygous	Having different alleles at a given locus
Homozygous	Having identical alleles at a given locus
HWE (Hardy-Weinberg equilibrium)	A theory that states that in an ideal population, allele and genotype frequencies will remain in constant proportion (i.e. in equilibrium) over time unless the population is affected by factors including: non-random mating, mutation, selection, genetic drift, gene flow and meiotic drive. In real populations, one or more of these factors is always in effect
IBD (Isolation by distance)	The tendency of individuals to mate with others from the same, or nearby populations, rather than distant populations. The occurrence or success of mating decreases with increasing geographic distances
LD (Linkage disequilibrium)	The non-random association of alleles at closely linked gene loci that deviates from their individual frequencies predicted by the Hardy-Weinberg

	equilibrium
Locus (plural: loci)	The position of a gene, or marker at a chromosome
m asl	metres above sea level
mtDNA (mitochondrial DNA)	DNA in the mitochondria of the cell, haploid in most species (inherited only from the mother)
nuDNA (nuclear DNA)	DNA in the nucleus of the cell, diploid in most animal species
Sample	A group of individuals sampled from a location that are thus assumed to be a representative sample of a single population
Selection	See: Directional selection
SNP (single nucleotide polymorphism)	DNA sequence variation that occurs when a single nucleotide (A,C,T or G) varies among individuals (or between paired chromosomes in an individual)
Waterway	A river, stream, creek, lake, or a natural channel through which water regularly flows, whether or not the flow is continuous