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# Genomic analysis of anadromous brown trout (*Salmo trutta*) reveals new insights into connectivity patterns and population divergence.

**Espen Danielsen**

## **Supervisors**

Halvor Knutsen and Katinka Bleeker

University of Agder, 2018  
Faculty of Engineering and Science  
Department of Natural Sciences

University of South-Eastern Norway  
Faculty of Technology, Natural Sciences and Maritime Sciences  
Department of Natural Sciences and Environmental Health

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University of Agder  
Faculty of Engineering and Science  
Department of Natural Sciences  
Gimlemoen  
4604 Kristiansand

<http://www.uia.no>

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# Sammendrag

Det finnes i dag mange studier som omhandler brunørret genetikk. Færre av disse studiene har blitt utført på den anadrome brunørreten (sjørret) og har ofte vært begrenset til få genetiske markører av typen allozymer, mtDNA eller mikrosatellitter. I denne studien har vi analysert populasjons struktur og konnektivitet hos sjørret ved bruk moderne genomiske metoder. Vi har analysert single nucleotide polymorphism (SNP) markører på 503 lokus ved bruk av neste generasjons sekvensering. Vi studerte fire bekker langs kysten av Skagerrak og avdekket en signifikant populasjons struktur mellom sjørret fra de ulike bekkene ( $F_{ST} = 0.0161$ ;  $P < 0.01$ ). Sjørreten fra de fire bekkene viste seg å være ytterligere gruppert inn i tre genetisk avgrensede populasjoner som muligens kan regnes som egne forvaltnings enheter. Det var en svak og ikke signifikant trend mellom genetisk differensiering (par-vise multi lokus  $F_{ST}$  estimator) og geografisk avstand (Mantel test:  $r = 0.4823$ ,  $P = 0.1978$ ). Sjørret populasjonene langs denne kyst strekningen viste seg å ha noe varierende genetisk diversitet, men ingen avviket fra Hardy-Weinberg ekvilibrium. En av populasjonene (Mørfjærbekken) hadde noen forskjeller i lengde og k-faktor mot de andre bekkene noe som kanskje antyder et annerledes seleksjons press. Sist så diskuterer vi generelle bevarings og forvaltnings implikasjoner for sjørret på bakgrunn av våre resultater.

# Abstract

A large body of studies on brown trout genetics currently exists. However, fewer of these studies have been conducted on anadromous brown trout (sea trout) and have been limited to only a few genetic markers like allozymes, mtDNA or microsatellites. Here we analyzed population structure and connectivity in sea trout applying a modern genomic approach, analyzing 503 single nucleotide polymorphic variants (SNP's) by next generation sequencing. We studied four streams along the Skagerrak coast and found an overall significant population structure ( $F_{ST} = 0.0161$ ;  $P < 0.01$ ) which further seemed to constitute of three genetically distinct sea trout populations which may constitute separate management units. Pair-wise multi locus  $F_{ST}$  estimates increased with geographical distance, revealed a weak non-significant trend of isolation by distance (Mantel test:  $r = 0.4823$ ,  $P = 0.1978$ ). The sea trout populations along this coastal stretch showed some variation in genetic diversity, but did not deviate from Hardy-Weinberg equilibrium. One population, Mørfjærbekken, displayed differences in some phenotypic traits i.e., length and k-factor, which may possibly be a result of different selection pressure than the other streams. Finally we discuss our findings in relation to conservation and management implications for sea trout.

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# Introduction

The application of new genetic tools has revolutionized our understanding of fisheries biology that has proven most useful for management (for review see: Hauser and Carvalho (2008), (Carvalho *et al.*, 2016)). Genetic markers have over the last decades become important tools when assessing different aspects of population structure in marine fish (Utter, 1991).

Interestingly, the level of genetic diversity and population structure seems to be more defined for those living in freshwater than for marine fish species (McCusker and Bentzen, 2010).

These differences are primarily ascribed to fewer restrictions to gene flow in the marine environment, due to the absence of obvious physical barriers that are more common in freshwater systems. When it comes to anadromous species, they occupy an intermediate position with genetic diversity and population structure less profound than the freshwater species but more than the marine species (Waples, 1998). A combination of higher degree of genetic divergence and smaller population sizes could result in more total genetic diversity lost if an anadromous species go extinct compared to marine species (Ryman *et al.*, 1995).

Analyzing and understanding patterns of genetic diversity, both within and among populations, can be important to gain general ecological/evolutionary insight into how systems work, but results are often also important for managers that may improve their decisions based on such knowledge. Genetic diversity is thought of as the basis for biodiversity which provides the raw material for evolution to act (Fisher, 1999). It has been shown to have important ecological effects on populations like; productivity, growth, stability and recovery from disturbances (Hughes *et al.*, 2008). Additionally, genetic diversity can be directly correlated to the fitness of a population (Szulkin *et al.*, 2010), where endangered species often reveal reduced diversity (Faulks *et al.*, 2017). Migration patterns of anadromous species are influenced both by temperature and water flow (Jonsson and Jonsson, 2009, Jonsson and Jonsson, 2002), hence making genetic patterns susceptible to climate change and global warming (Jonsson and Jonsson, 2009). Additionally migratory species is potentially important in mediating gene flow among populations, thus increasing genetic diversity (Hansen and Mensberg, 1998, Waples, 1998, Westgaard *et al.*, 2016). This highlights the importance of studying genetic diversity in migratory species in order to assess their fitness levels and adaptability to a novel or changing environment. In addition, understanding population structure is crucial to give insight into population dispersal, borders and dynamics like annual recruitment (Ciannelli *et al.*, 2013, Bartolino *et al.*, 2012, Boudreau *et al.*, 2017).

Genetically distinct populations may differ in their resilience to harvest, resulting in a harder impact on the weaker populations (Hutchinson, 2008). Population structure within a species can also stabilize the abundance of the total population, making it less vulnerable to fluctuation in individual subpopulations (i.e., port-folio effect) (Thibaut and Connolly, 2013, Schindler *et al.*, 2010) which further emphasizes the importance of structure when creating management strategies to ensure sustainability and avoid depletion of local populations.

Homing behavior is when fish return to their natal site after migration, even though other sites can be equally probable (Stabell, 1992, Gerking, 1959). This is portrayed as one of the main mechanisms maintaining genetic differentiation in co-existing salmonids (Ferguson *et al.*, 1995). Salmonids in general are renowned for their acute odor discrimination (Halvorsen and Stabell, 1990) giving them the ability to relocate the nursery grounds they originated from with relatively high accuracy (Arnesen and Stabell, 1992). Anadromous species like brown trout (*Salmo trutta*) are often found in sympatric populations, where resident and anadromous populations (sea trout hereafter) coexist (Ferguson, 1989, Hindar *et al.*, 1991). The abundance of sea trout has declined in several parts of its distribution (ICES, 2013). This is suggested to be mainly due to sea lice from salmon rearing facilities (Thorstad *et al.*, 2015) and human engagements on spawning streams (Haraldstad *et al.*, 2014). However, sea trout along the coast of southern Norway, with the absence of salmon rearing facilities, has not experienced severe infestations of sea lice (Nilsen *et al.*, 2018) and appears to be in better condition than elsewhere in Norway (Höjesjö *et al.*, 2017). Many streams along Skagerrak did, however, experience a massive decline in both resident and sea trout numbers in the 1960s to early 1980s (Henriksen *et al.*, 1989, Jensen, 1972). In some spawning streams sea trout even went extinct (Simonsen, 1999). The one major and most plausible reason for this decline was airborne pollutants carrying sulfur and nitrogen leading to acid precipitation in the streams combined with lime poor bedrock offering low buffering capacity (Henriksen *et al.*, 1989). Low pH levels will mobilize aluminum which affects the gill function of fish negatively resulting in mortality when resident sea trout stay in fresh water (Haraldstad *et al.*, 2014). After several successful lime-release projects into the streams during 1983, pH levels were gradually restored back to acceptable levels (Kaste and Håvardstun, 1998) and sea trout has naturally recolonized the extinct streams (Simonsen, 1999). The abundance of sea trout has increased in along Skagerrak over the last 20 years according to long time beach seine series conducted annually by the Flødevigen Institute of Marine Research (Espeland and Knutsen, 2014).

Even though a large body of papers currently exists on genetic diversity and population structure in brown trout there is less studies of their anadromous forms. Also, former studies are largely limited to only a few genetic markers like from allozyme, mtDNA or microsatellites markers (Hansen and Mensberg, 1998, Morán *et al.*, 1995, Carlsson and Nilsson, 2000). Here, we expanded on findings from Knutsen *et al.* (2001) on genetic structure of sea trout in the Skagerrak, by applying a modern genomic approach, analyzing more than 500 single nucleotide polymorphic variants (SNP's) by next generation sequencing. The aim of this approach is to detect more details on genetic structure and compare phenotypic differences among streams possibly displaying different selection signals. within and among sea trout streams along the Norwegian Skagerrak coast. Results will be discussed in relation to potentially important conservation and management implications for this species.

## Materials and methods

### *Species*

Brown trout is a highly polymorphic species, known to exhibit a variety of different phenotypic traits, life- history traits and migration behavior (e.g. see review by (Jonsson and Jonsson, 2009)). It has the ability to adapt to a variety of different conditions and habitat types and three life-history forms are characterized: land-locked trout, resident trout and sea trout (Klemetsen *et al.*, 2003, Pakkasmaa and Piironen, 2001). Land-locked trout is isolated by impassable barriers which restricts gene flow between populations. This results in land-locked trout being genetically diverged from the two other forms and show lower genetic diversity (Hindar *et al.*, 1991). resident trout and sea trout co-exists in spawning streams and could descend from the same parents, but while resident trout remains within their natal stream, sea trout migrates to sea to gain the benefits of increased growth and reproductive output (Klemetsen *et al.*, 2003). Brown trout has a wide geographical distribution with indigenous populations in Europe, North Africa and western Asia (MacCrimmon and Marshall, 1968). Brown trout is important both commercially and for recreational purposes worldwide (Elliott and Elliott, 2010). The combination of their adaptability to new environments and reputation as good food are focal reasons why brown trout has been extensively introduced across the

continents. Currently, the distribution of brown trout is spanning over at least 24 countries outside Europe (Klemetsen *et al.*, 2003).

### *Study areas and sampling*

Sea trout in this study were caught in four spawning streams along the Norwegian Skagerrak coast in southern Norway (**Figure 1**). This coastline is characterized by numerous skerries, small islands and fjords resulting in a highly heterogeneous environment. The streams, located in Lillesand, Arendal and Tvedestrand, are all recognized as among the most important spawning areas for sea trout in their respective area (Haraldstad *et al.*, 2014). The streams are distributed across an air stretch of approximately 60 km with each site ranging from approximately 1.5 to 70 km apart when measuring along the coastline. The two largest streams in this study, Vallesværelva (**Appendix 2**) and Fjelldalselva, (**Appendix 3**) are located in the municipality of Lillesand, about 1.5 km apart. Vallesværelva and Fjelldalselva have an anadromous stretch of 4680 m and 2335 m, respectively. Mørkfjærbekken (**Appendix 4**) is located in Arendal and has the fourth largest anadromous stretch of 2680 m among the streams in this municipality and the third largest stream of this study. This stream is characterized by more surrounding ponds and small side streams merging into the main stretch compared to the others. This stream is considered to be good for spawning and nursery, although it is influenced by runoffs from agriculture, cultural lands, a fox farm and a car wreck storage area. These influences can affect the environment negatively for fish because supplies of nutrients, erosion particles and organic substances can result in fouling, low oxygen content, smothering of nursery areas (Haraldstad *et al.*, 2014). Østeråbekken (**Appendix 5**) is located inside the Tvedestrand fjord that has been part of a marine protected area (MPA) since 2012. This MPA consists of a no-take zone surrounded by two habitat zones, where the estuary of Østeråbekken is located within the innermost zone. The habitat zones in this area are characterized by only allowing for line and hook fishing, providing additional protection to the sea trout migrating in and out of the stream. This stream has an anadromous stretch of 3500 m and is the second largest stream in the municipality of Tvedestrand. A total of 166 individual sea trout were sampled from the streams, ranging from 22 to 56 individuals (**Table 1**). Sea trout were caught by electrofishing upstream from the estuaries of the streams in October and November 2016. Fish were anaesthetized in clove oil 40 mg/litre (Munday and Wilson, 1997). Fin clips were taken from the caudal fin and stored

in 1.5 ml tubes with 96% ethanol for later molecular analysis. Length and weight was measured for each individual. After sampling, each individual was transferred to a holding tank with oxygen saturation and monitored to provide the best recovery before being released into the stream. Fish were sexed phenotypically by external examination (i.e. development of kype in male fish) or by stripping of eggs and sperm. Individuals that could not be scored at the time of collection were sexed using an Atlantic salmon genetic sex marker.



**Figure 1:** A map of the study area in Southern Norway. Red dots represent the four streams where sea trout were sampled.

### ***Genetic analysis***

DNA extraction, type of kit, ddRAD. All of the 166 sea trout were genotyped for SNP's at 503 loci each. Genomic DNA was extracted from fin clip tissue using the E-Z 96 Tissue DNA kit (Omega Bio-Tek, USA). Individual DNA concentration was quantified using both NanoDrop spectrophotometer (Thermo Fisher Scientific) and Qubit Fluorometer (Invitrogen, Thermo Fisher Scientific). Double digest RAD sequencing (Peterson *et al.*, 2012) and library preparation were outsourced to Beijing Genomics Institute (BGI, Hong Kong, China). Each library contained 24 individuals for which DNA was digested with two restriction enzymes (*SbfI* and *MseI*). Libraries were sequenced with an Illumina HiSeq4000 by including 60 individuals on each sequencing lane, producing a mean of 150 bp paired-end reads per individual (after individuals with less than 1 million reads had been excluded). Sequence reads were aligned to the Atlantic salmon genome and the reference-aligned data were subsequently analyzed using STACKS 2.0 (Catchen *et al.*, 2013). SNPs were called with the following requirements: a minimum depth of 10x for loci to be called and they should be found in at least 90% of the individuals from each population sample. VCFTOOLS (Danecek *et al.*, 2011) was used to create a final dataset containing 503 loci.

### ***Statistical analysis***

All data analyses were conducted in the R software (Huber *et al.*, 2015). The amount of genetic variability was characterized by heterozygosity (observed heterozygosity,  $H_o$ , within samples and expected heterozygosity,  $H_E$ , in the total material for each locus; Nei and Chesser (1983) with the R version of GENEPOP software (Rousset, 2008) and the hierfstat package (Goudet, 2005). Deviations from Hardy-Weinberg genotype proportions were estimated by  $F_{IS}$  (Weir and Cockerham, 1984) and tested using the exact probability test in GENEPOP. In addition, the  $F_{IS}$  values for each population were bootstrapped over a 95% confidence interval with hierfstat. Observed heterozygosity ( $H_o$ ) of each stream were tested for significant differences with two-sided t-tests. Wrights  $F_{ST}$  was used to estimate the level of population structure both overall and among samples (Weir and Cockerham, 1984). An exact G test was applied to test for an overall heterogeneity in the data, i.e. to assess if all alleles were drawn from the same distribution in all population on each locus and the p-values were summarized by Fisher's method. To visualize the spatial genetic structure, we applied two different approaches. First, we correlated genetic and geographical distances. Pairwise calculations of

Weir and Cocherhams  $F_{ST}$  between locations were estimated with hierfstat and tested for isolation by distance with a Mantel test in the ade4 R package (Dray *et al.*, 2007).

Geographical distances were measured in kilometer as the assumed shortest route along the coastline for sea trout to travel between the estuaries. Confidence limits of pair-wise  $F_{ST}$  were calculated by bootstrapping in hierfstat. Secondly, the division in allele frequency differences between the populations were visualized with a principal component analysis with the SNPRelate package (Zheng *et al.*, 2012). All p-values were evaluated at a 0.05 confidence level and controlled for false discovery rates in multiple comparisons with the Benjamini-Hochberg correction method (Thissen *et al.*, 2002). K-factor were calculated by as suggested for salmonid fishes by Barnham and Baxter (2003).

## Results

A total of 166 individuals sampled at four different sites were genotyped at 503 SNP loci. Genetic diversity was measured as the level of observed ( $H_O$ ), expected ( $H_E$ ) and total heterozygosity ( $H_T$ ). The  $H_O$  ranged from 0.1750 to 0.1890 were the lowest estimates belonged to Vallesværelva and Fjelldalselva, which further had significantly lower  $H_O$  as compared to the other streams. Differences of genetic diversity in the total material  $H_T$  (*Appendix I*) varied among loci from 0.0280 (at locus 62472\_60) to 0.5017 (at locus 90497\_114). None of the populations deviated significantly from Hardy-Weinberg equilibrium after correction for false discovery rates at a  $P < 0.05$  level. The four streams  $F_{IS}$  estimates where not significant after summation of the exact probability tests over loci by Fishers method, nor fell outside a bootstrapped 95% confidence interval. There was an overall tendency for heterozygote deficiency. The majority of loci had negative  $F_{IS}$  values (excess of heterozygotes), although the average  $F_{IS}$  (0.0089) from all streams were positive (deficiency in heterozygotes). Except for Mørfjærbekken, all streams displayed an overall deficiency of heterozygotes (positive  $F_{IS}$ ).

**Table 1:** The sample locations and numbers of the individuals. Estimates of genetic diversity are averaged by sample site ( $H_O$  and  $H_E$ ). Deviations from Hardy-Weinberg genotype proportions are measured as average  $F_{IS}$  in each sample site. None of the populations showed significant deviations from Hardy-Weinberg equilibrium after Benjamini-Hochberg false discovery rate correction for multiple tests so  $p$ -values were excluded from the table.

Stream	$n$	$H_O$	$H_E$	Deviation from Hardy-Weinberg equilibrium			
				Average $F_{IS}$	Excess of heterozygotes	Deficiency of heterozygotes	Missing alleles
Vallesværelva	44	0.1753	0.1750	0.0108	307	121	75
Fjelldalselva	44	0.1750	0.1778	0.0280	284	152	67
Mørfjærbekken	22	0.1838	0.1774	-0.0107	251	91	161
Østeråbekken	56	0.1890	0.1886	0.0076	321	145	37
<b>Average</b>		0.1808	0.1850	0.0089	290.75	127.25	85

An overall  $F_{ST}$  value of 0.0161 indicates 1.16% of the genetic variation can be ascribed to genetic difference among the populations (G-test:  $P < 0.001$ , cf. **Appendix 1**). Overall  $F_{ST}$  values in the total sample ranged from -0.0130 to 0.1251, with an average of 0.0161.

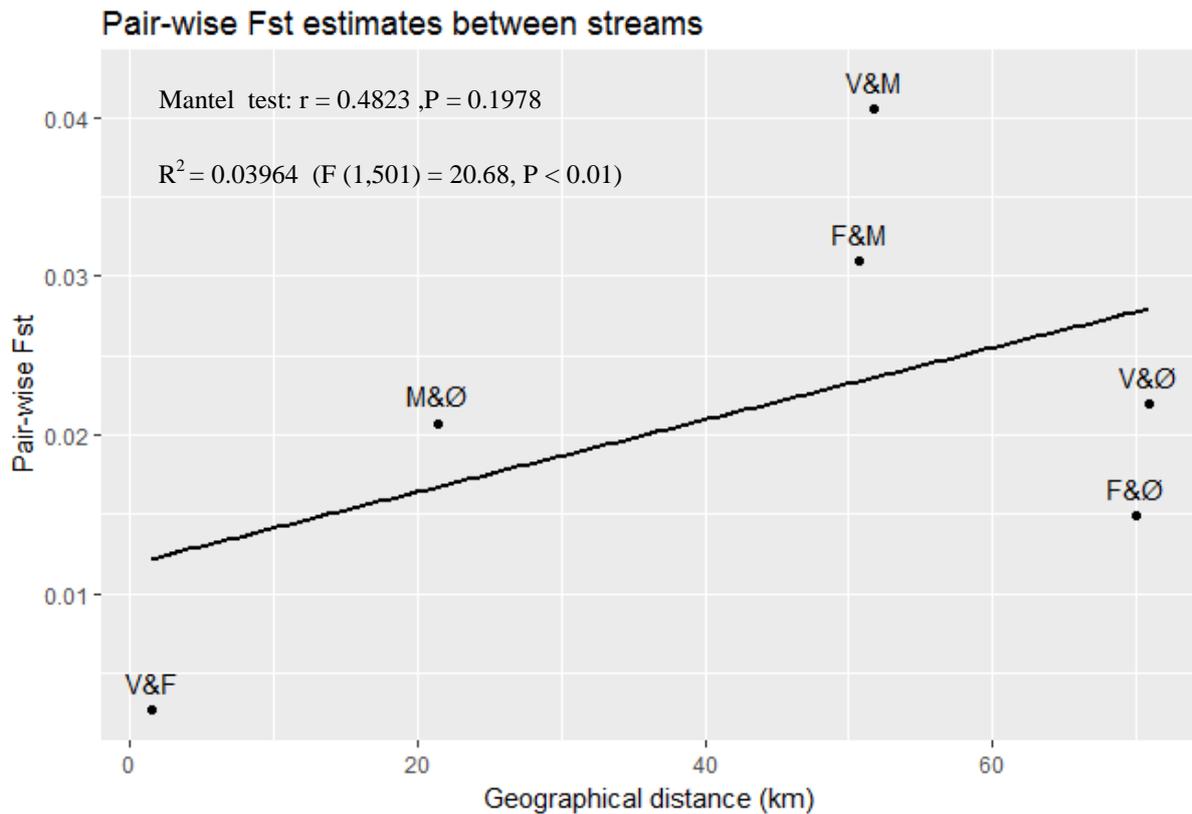
(**Appendix 1**). Out of the 3018 possible loci-population pair combinations in the dataset, the exact G-test for allele frequency heterogeneity across all loci yielded 85  $p$ -values below a 0.05 level after Benjamini-Hochberg correction. Among these 14 loci displayed significant genetic differentiation. All stream pair estimates, except the comparison between the closely located samples from Vallesværelva and Fjelldalselva, were significantly differentiated (**Table. 2**).

**Table. 2:** Pairwise  $F_{ST}$  values calculated of the four populations estimated by the method of Weir and Cockerham (1984).

	Vallesværelva	Fjelldalselva	Mørfjærbekken
Fjelldalselva	0.0027	-	-
Mørfjærbekken	0.0405*	0.0310*	-
Østeråbekken	0.0219*	0.0149*	0.0207*

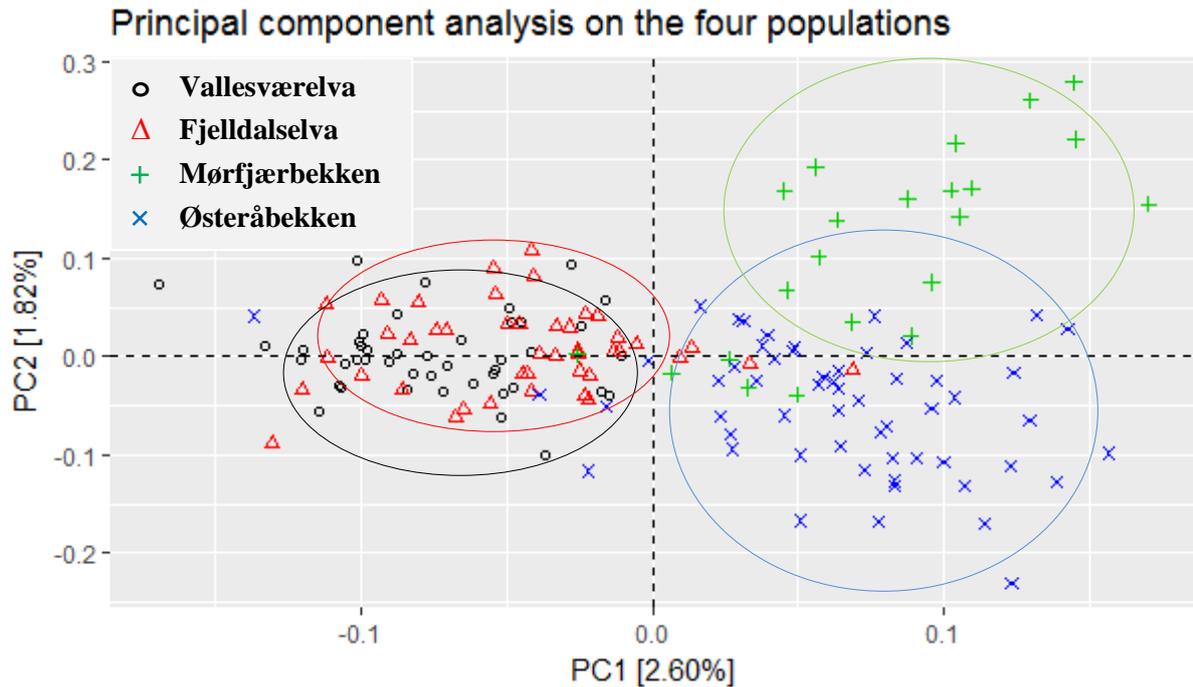
\* denotes highly significant in the *GENEPOP* software.

There was a trend for higher  $F_{ST}$  at increasing distance between populations. The trend was, however, not significant when tested for isolation by distance with a Mantel test (Mantel's  $r = 0.4823$ ,  $P = 0.1978$ ; **Figure 2**). In addition, none of the values fell outside the limits of a 95% bootstrapped interval. Although the patterns of similarity increased in a positive correlation with geographical distance the population pairs did not increase in an entirely step-wise manner. All population pair comparison involving Mørfjærbekken displayed  $F_{ST}$  estimates above the regression line while the other pairs were below.



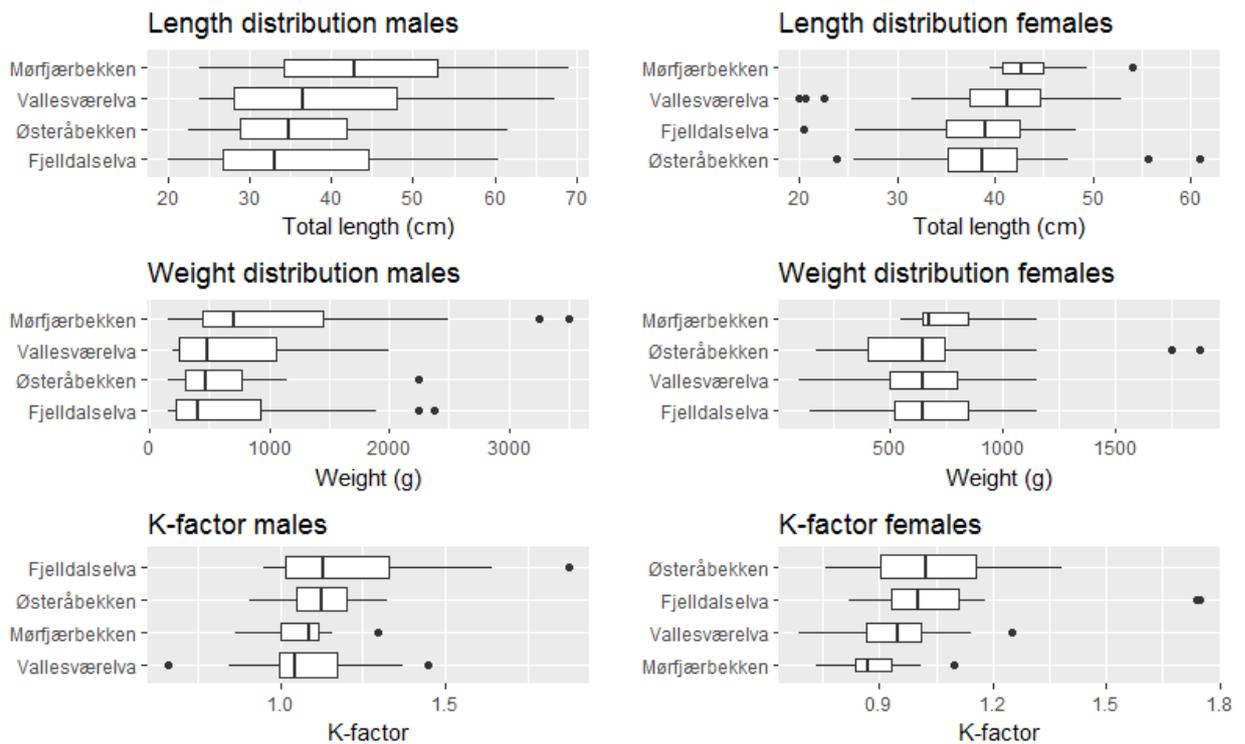
**Figure 2:** Genetic differentiation ( $F_{ST}$ ) between samples regressed to geographic distance (km). The capital letters is the first of each population. A Mantel test demonstrates a non-significant but positive trend for isolation by distance.

The trend that genetic differentiation increases along with geographical distance could be further visualized by a PCA plot (**Figure 3**). There were mainly two prominent clusters in the plot where geographically close populations tend to cluster more together. The further apart they are, the more variation can be observed in relation to both principal components. The first principal component explains 2.60% of the genetic variation in our dataset and reveals patterns where Vallesværelva and Fjelldalselva are clustered in one group at the right side of the plot and Mørfjærbekken and Østeråbekken are clustered on the left side. Vallesværelva and Fjelldalselva were clustered very close and could not be differentiated using the PCA. The second principal component axes explain 1.82% of the genetic variation and makes a distinction between Mørfjærbekken and Østeråbekken.



**Figure 3:** Principal component analysis (PCA) based allele frequency on 385 SNP markers on the four sea trout populations, 118 markers were removed due to linkage disequilibrium. Each population is denoted by a unique color and shape. Circles around each population are drawn subjectively and only meant for visualization purposes.

Significant differences in total length and k-factor were observed among populations (**Figure 4**). When assessing the genders independently all significant length and k-factor differences were ascribed to female sea trout. T-tests comparing the length differences among the streams revealed that the female sea trout in Mørfjærbekken were significantly longer than female sea trout in Fjelldalselva ( $t(28) = 2.198$ ,  $P = 0.0364$ ) and Østeråbekken ( $t(41) = 2.046$ ,  $P = 0.0472$ ). In addition, the female sea trout from Mørfjærbekken had lower k-factor than Fjelldalselva ( $t(28) = 2.077$ ,  $P = 0.0471$ ) and Østeråbekken ( $t(41) = 2.507$ ,  $P = 0.0162$ ).



**Figure 4:** Boxplots of the differences in phenotypic trait among the stream by gender.

## Discussion

We show that sea trout sampled from coastal streams along the Skagerrak are structured into several populations. Pair-wise comparisons of sea trout samples from the four streams in combination with a PCA suggest the presence of three genetically distinct populations in our material. There was a non-significant but positive correlation between genetic divergence and geographical distance (Mantel test:  $r = 0.4823$ ,  $P = 0.1978$ ) among samples. The PCA results support the mantel test as the genetic divergence is stronger with larger distance among sample pairs (**Figure 3**). None of the streams deviated from Hardy-Weinberg equilibrium expectations, but we observed differences in the level of heterozygosity among streams. There were also clear and interesting phenotypic differences among streams. Below we discuss the phenotypic and genetic patterns within and between the sampled streams and the potential management and conservation implications of these results.

The allele frequency heterogeneity test revealed that 85 of 503 loci (ca 17%) analyzed where significantly differentiated (average  $F_{ST} = 0.016$ ). Thus, the hypothesis of no genetic divergence among populations was rejected with high probability. Our results suggested the

presence of three genetically differentiated sea trout populations among the four streams. Mørfjærbekken and Østeråbekken each seem to inhabit their own distinctly diverged population while the two streams Vallesværelva and Fjelldalselva more likely are part of the same population. Sea trout are known to display genetically divergent populations (Knutsen *et al.*, 2001, Bouza *et al.*, 1999, Hansen and Mensberg, 1998). Population differentiation has been documented for sea trout over a wide variety of different geographical scales (Hashemzadeh Segherloo *et al.*, 2012) as well as between different cohorts from the same river (Laikre *et al.*, 2002). The existence of a heterogeneous population structure in sea trout has also been established along the Skagerrak coast earlier. Knutsen *et al.* (2001) used allozymes to assess population structure at an approximately similar scale in the same area and found several differentiated populations. Overall, the level and patterns of genetic divergence herein, seem to conform reasonably to other genetic studies of anadromous brown trout. In particular, estimates of divergence between Mørfjærbekken and Fjelldalselva were conducted both in the study by Knutsen *et al.* (2001) ( $F_{ST} = 0.046$ ) and present study ( $F_{ST} = 0.031$ ). We found similar highly significant estimates in both studies which further indicate that Mørfjærbekken and Fjelldalselva constitute to different populations.

Natal homing and low frequency for straying have been proposed as the two main reasons why resident trout form genetically diverged populations (Arnesen and Stabell, 1992, Stabell, 1992, Stabell, 1984). Hansen and Mensberg (1998) analyzed mitochondrial DNA differentiation in sea trout around the Danish island Bornholm. When pooling samples from within the river systems they found differentiation among populations between rivers. Although no homing behavior studies have been carried out exclusively on sea trout (to my knowledge) the close genetic resemblance between resident trout and sea trout (Hindar *et al.*, 1991) indicates that this behavior could be a mechanism that creates genetically diverged sea trout populations. Vallesværelva and Fjelldalselva are located only approximately 1.5 km apart and both run into Vallesverdfjorden. So although homing behavior could, to some degree, prevent upstream migration in the wrong river, straying still occurs between neighboring streams at a detectable rate (Svårdson and Fagerström, 1982, Ferguson, 1989). Tagging studies have shown that straying usually occurs between streams in close proximity (Svårdson and Fagerström, 1982). Hence, straying between Vallesværelva and Fjelldalselva could occur at high enough rates to create genetic uniformity and prevent detection of differentiation.

In present study we detected a weak but non-significant trend of isolation by distance (IBD). These results contrasts to several other studies conducted on sea trout (Hansen and Mensberg, 1998, Bouza *et al.*, 1999, Morán *et al.*, 1995). However, many of these studies used genetic methods that may not be directly compared to ours or population systems that are more complex or at a different geographic scale (see below). Hansen and Mensberg (1998) documented a significant correspondence between genetic and geographical distance in sea trout populations located in large river systems in northern Denmark and Bornholm. However, their results were only significant when the large distance between the river systems were considered, meaning the within river system IBD patterns were of limited importance for the genetic structure of their sea trout samples. They further argued that the relatively small distance between rivers within systems, as opposed to between river systems, caused the different IBD patterns. This study operated on a much larger scale than our study. The distance between the large river systems in this study spanned from approximately 120 km to 500 km whereas in our study the maximal distance among the stream pairs were approximately 70 km. Thus, one of the most reasonable explanations to why this study detected IBD, is that they were conducted over a larger geographical scale and with large sample sizes. similar correlations between genetic and geographical distance have also been found in the southern most parts of sea trout distribution. A study by Morán *et al.* (1995) assessed genetic differentiation through allozyme electrophoresis to detect IBD in sea trout inhabiting large river systems in Spain. This study operated at a much smaller scale than Hansen and Mensberg (1998), but reached similar conclusions regarding less gene flow between rivers than within river systems. The river systems in the study by Moran were located at a maximum distance of approximately 43 km apart, meaning it is more comparable to our study than to Hansen and Mensberg (1998). When looking closer at the regression line of pair-wise  $F_{ST}$  estimates in present study and the study by Morán *et al.* (1995) we see that our values are slightly lower. The values ranged from  $F_{ST} = 0.02$  to  $F_{ST} = 0.09$  at a geographical distance of 43 km in (Morán *et al.*, 1995) study, while our values at the same distance ranged from  $F_{ST} = 0.01$  to  $F_{ST} = 0.02$ . This conforms to the differences in correlation of genetic and geographical distance between our studies.

The aforementioned studies are different from ours since they were conducted in large river systems instead of relatively small streams located adjacently along linear stretch. The samples within each river in these studies were pooled and compared to other river systems with more individuals in each river compared to our study. The streams in our study were

located adjacently without the numerous tributaries seen in the other studies. Hence, geographical scale and numbers of streams could both be major factors explaining our lack of IBD. However, large complex river systems and larger geographical scale are not necessarily equivalent to detecting IBD. In a mtDNA analysis on sea trout samples from 13 stream on the Swedish island Gotland did not reveal significant IBD (Laikre *et al.*, 2002). It is worth mentioning though, that mtDNA markers lack power, compared to microsatellites and SNPs which makes this study not entirely comparable to our SNP analysis. We can also compare present study to Knutsen *et al.* (2001) which were conducted in the same area at almost the exact same geographical scale. Knutsen *et al.* (2001) found IBD among the long standing streams (those surviving the acid period in the 1980s), while not when including the newly recolonized ones after liming and return of trout. Those streams were recolonized from a mix of several streams and distorted the overall pattern. Thus, this indicates that the sea trout population in this region indeed could display a true IBD. One major difference from this study and ours is the limited numbers of streams analyzed in present study, reducing statistical power in detecting a pattern. Also, with few streams, comparing those at the extreme ends with moderately large differentiation could be mistaken for a cline (Carlsson and Nilsson, 2000), Petrou *et al.* (2014). Another potential reason for not detecting correspondence between genetic and geographical distance could be natural or anthropogenic disturbances. Human induced selection pressure through alterations of the environment and introgression from non-local individuals could change the structure of populations and have been argued as alternative explanations to altered IBD patterns in salmonids (Nielsen *et al.*, 1999, Petrou *et al.*, 2014).

Genetic diversity, as evaluated by expected and observed heterozygosity, was quite similar within the streams. Further, none of the streams deviated from Hardy-Weinberg genotype proportions. Thus, indicating that all the streams in our study consist of randomly mating populations. This is consistent with previous analysis of Fjelldalselva and Mørfjærbekken, using allozyme variation (Knutsen *et al.*, 2001). We detected a slight deficiency of heterozygotes in Fjelldalselva ( $F_{IS} = 0.028$ ), while Knutsen *et al.* (2001) detected excess ( $F_{IS} = -0.037$ ). The excess of heterozygotes (though not significant) combined with phenotypic differences in Mørfjærbekken could be a weak indication of the presence of more than one population within this stream. This stream stands out from the other streams as it is surrounded by several small upstream ponds with side streams branching into the main anadromous stretch. Stream systems are known to potentially house several distinct

populations of brown trout (Hansen and Mensberg, 1996, Carlsson *et al.*, 1999, Lehtonen *et al.*, 2009). Additionally, sub structuring of brown trout have been detected in Langgangsvassdraget, a stream nearby Mørfjærbekken (Knutsen *et al.*, 2001). It has previously been argued that genetically differentiated sea trout with homing to an area further upstream could become part of a sample taken further downstream (Carlsson and Nilsson, 2000), meaning a sample could show signals of being more outbred. This could be a potential reason for why we observed more genetic diversity in Mørfjærbekken compared to the other streams in our study. The two steams, Vallesværelva and Fjelldalselva displayed very similar estimates of genetic diversity which further conforms that these streams probably are inhabited by the same population. However, this population in Lillesand did have less genetic diversity compared to the other two populations, Mørfjærbekken and Østeråbekken. One possible explanation to this is the massive decline in trout abundance the Lillesand area experienced in the 1960s to early 1980s (Jensen, 1972, Henriksen *et al.*, 1989) were many streams went extinct, or nearly so (Simonsen, 1999). Knutsen *et al.* (2001) argued that the original brown trout population in Fjelldalselva, and other nearby streams in this region had either been nearly extinct and underwent a bottleneck event or has been recolonized after total extinction by sea trout of mixed origins. Additionally, it has been argued that low estimates of genetic diversity in sea trout from Karup river are caused by pollution (Hansen and Mensberg, 1998), supporting that the lower genetic diversity observed in the Lillesand population may have been caused by the mass reduction of sea trout in the 1960s to 1980s.

We observed differences in phenotypic traits like total length and k-factor among the streams. All significant differences were ascribed to female trout in Mørfjærbekken. Comparisons revealed that female trout from Mørfjærbekken were significantly longer and conversely had lower k-factor than Fjelldalselva and Østeråbekken. Brown trout are known to exhibit differences in phenotypic traits among genetically differentiated populations (Pakkasmaa and Piironen, 2001). The phenotypic differences in Mørfjærbekken further indicate that this population has limited contact/gene flow with the other populations and that these differences arguably could be caused by selection pressure.

In conclusion, the present study has assessed genetic diversity and population structure among four spawning streams along the Skagerrak coast with novel genetic methods with many markers. The study supports the existence of several genetically different subpopulations among sea trout streams in Skagerrak. Since our samples seem to constitute three genetically distinct populations, we do expect them to respond independently to

exploitation and need to be considered in conservation strategies. For instance, by treating each genetically distinct population as isolated units requiring independent management (Carlsson *et al.*, 1999). This knowledge is useful for management on how to recognize population boundaries and avoid depletion of weaker stocks. Furthermore, knowledge on differentiation within species is an important tool when assessing mixed stock catchment to address which population is being harvested (Koljonen *et al.*, 2014). We did not detect significant patterns of IBD, but the streams followed a stepping stone model where migrations mainly occur between neighboring streams (Kimura and Weiss, 1964). This could be valuable for further assessment on connectivity of sea trout populations and how recolonization operates in this species. The low connectivity observed among the populations in present study could present a challenge if any of these populations were depleted. Rebuilding an extinct population could be a slow process potentially resulting in lower genetic diversity in the recolonized population.

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## Appendix

*Appendix 1: A table showing the loci with their corresponding observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), total heterozygosity ( $H_T$ ),  $F_{ST}$  estimates per locus and  $F_{IS}$  estimates per locus.*

<b>Locus</b>	<b><math>H_O</math></b>	<b><math>H_E</math></b>	<b><math>H_T</math></b>	<b><math>F_{ST}</math></b>	<b><math>F_{IS}</math></b>
352186_29	0.1523	0.1519	0.1419	0.0030	-0.0019
352186_31	0.1523	0.1519	0.1419	0.0030	-0.0019
352593_36	0.4067	0.4002	0.4004	-0.0028	-0.0106
353717_146	0.0530	0.0516	0.0452	-0.0063	-0.0192
234461_134	0.0455	0.0444	0.0513	0.0048	-0.0236
234461_18	0.2133	0.1906	0.1778	-0.0041	-0.1128
235740_142	0.0533	0.0519	0.0476	0.0059	-0.0285
236450_25	0.1830	0.1975	0.1968	0.0496	0.0415
237129_74	0.0588	0.0571	0.0657	-0.0022	-0.0254
237341_32	0.3179	0.3263	0.3335	-0.0074	0.0344
100189_89	0.1908	0.1935	0.2196	0.0249	-0.0008
100189_71	0.3600	0.3783	0.3815	0.0294	0.0307
100216_91	0.2680	0.2949	0.2836	-0.0041	0.0972
100216_115	0.2680	0.2949	0.2836	-0.0041	0.0972
92330_19	0.1776	0.1619	0.1575	-0.0074	-0.0883
92330_100	0.1503	0.1390	0.1365	-0.0063	-0.0731
94104_131	0.1373	0.1390	0.1283	0.0154	0.0047
95698_25	0.4013	0.4792	0.4758	-0.0044	0.1684
96150_46	0.0596	0.0578	0.0566	0.0399	-0.0586
97610_83	0.1613	0.1696	0.1564	0.0003	0.0520
98058_30	0.0968	0.0921	0.0982	-0.0009	-0.0469
98058_70	0.1364	0.1271	0.1313	0.0007	-0.0705
98058_123	0.1529	0.1519	0.1362	0.0393	-0.0334
98058_139	0.3226	0.3042	0.3257	0.0151	-0.0691
46168_9	0.4026	0.4339	0.4489	0.0371	0.0492
46168_98	0.3896	0.4291	0.4457	0.0343	0.0716
46168_100	0.4026	0.4339	0.4489	0.0371	0.0492
46168_121	0.0519	0.0506	0.0479	0.0066	-0.0284
46249_125	0.0375	0.0488	0.0722	0.0352	0.2134

46249_44	0.1582	0.1457	0.1593	-0.0031	-0.0803
46337_54	0.4211	0.4720	0.4666	-0.0006	0.1115
46613_22	0.2115	0.2377	0.2393	0.1362	0.0102
48359_119	0.0769	0.0740	0.0651	0.0075	-0.0426
48359_60	0.2949	0.2945	0.3049	-0.0016	0.0033
49111_123	0.5260	0.4888	0.4946	0.0074	-0.0786
49175_86	0.0728	0.0702	0.0766	0.0024	-0.0363
50726_96	0.0526	0.0636	0.0617	0.0601	0.1375
50776_53	0.0397	0.0389	0.0441	0.0047	-0.0205
50776_54	0.0397	0.0389	0.0441	0.0047	-0.0205
50776_146	0.4733	0.4696	0.4676	-0.0025	-0.0029
283269_40	0.0987	0.0938	0.1020	0.0689	-0.1051
271302_88	0.0467	0.0582	0.0649	-0.0112	0.2078
272202_142	0.4740	0.4610	0.4460	0.0536	-0.0674
272202_116	0.4610	0.4770	0.4661	0.0767	-0.0217
272953_128	0.2549	0.2864	0.2617	0.0189	0.1008
272953_57	0.2549	0.2864	0.2617	0.0189	0.1008
275471_47	0.1429	0.1437	0.1393	-0.0090	0.0156
275471_120	0.4800	0.4743	0.4807	-0.0056	-0.0046
78533_109	0.3533	0.3682	0.3824	0.0278	0.0239
78533_83	0.2733	0.3240	0.3120	-0.0042	0.1622
78533_44	0.3867	0.4252	0.4245	-0.0130	0.1025
89251_148	0.0392	0.0509	0.0487	0.0345	0.2128
91562_110	0.2258	0.2389	0.2409	-0.0046	0.0613
91562_93	0.0510	0.0617	0.0834	0.0225	0.1630
79456_122	0.1200	0.1128	0.1091	0.0069	-0.0659
80613_45	0.0533	0.0519	0.0592	-0.0083	-0.0179
80737_149	0.2200	0.2162	0.2074	-0.0086	-0.0077
80737_44	0.1987	0.1998	0.1913	-0.0095	0.0159
81770_118	0.3400	0.3160	0.3332	0.0122	-0.0824
86278_78	0.0461	0.0450	0.0451	-0.0110	-0.0122
86278_103	0.0461	0.0450	0.0451	-0.0110	-0.0122
86710_26	0.3922	0.3599	0.3646	0.0023	-0.0883
87316_74	0.4359	0.4356	0.4534	0.0479	-0.0343

87316_117	0.0449	0.0439	0.0465	-0.0061	-0.0152
87316_120	0.1895	0.1821	0.1662	0.0105	-0.0457
89077_24	0.0980	0.0932	0.0853	0.0025	-0.0502
89077_71	0.1307	0.1335	0.1402	-0.0093	0.0304
89310_19	0.1133	0.1186	0.1274	-0.0093	0.0545
89310_18	0.5000	0.4920	0.4909	0.0337	-0.0388
89497_8	0.1667	0.1638	0.1611	0.0002	-0.0143
90177_107	0.0844	0.0809	0.0720	-0.0010	-0.0401
90497_58	0.4065	0.4993	0.5017	0.0594	0.1513
90497_95	0.0844	0.1043	0.1541	0.2331	0.0131
90497_114	0.4065	0.4993	0.5017	0.0594	0.1513
91085_141	0.0795	0.0884	0.1113	0.0324	0.0825
91143_99	0.2200	0.2550	0.2648	-0.0114	0.1476
91143_30	0.2252	0.2582	0.2680	-0.0120	0.1389
165947_16	0.0654	0.0632	0.0761	0.0236	-0.0487
165947_29	0.0654	0.0632	0.0761	0.0236	-0.0487
165947_98	0.0461	0.0450	0.0402	-0.0056	-0.0161
161195_95	0.0968	0.1037	0.0989	0.0060	0.0656
162193_144	0.3510	0.3597	0.3588	-0.0010	0.0281
162193_88	0.1667	0.1528	0.1541	-0.0100	-0.0797
162193_3	0.0464	0.0453	0.0588	0.0221	-0.0373
162887_25	0.1355	0.2003	0.2021	-0.0086	0.3307
162887_114	0.0764	0.1081	0.1127	0.0081	0.2915
164138_109	0.0733	0.0706	0.0754	-0.0069	-0.0295
167598_102	0.1267	0.1416	0.1515	0.0046	0.1056
167598_96	0.1267	0.1416	0.1515	0.0046	0.1056
167598_92	0.1267	0.1416	0.1515	0.0046	0.1056
167598_76	0.0533	0.0519	0.0484	0.0216	-0.0407
168761_124	0.0533	0.0519	0.0495	0.0206	-0.0399
168881_101	0.1533	0.1416	0.1432	0.0019	-0.0812
169689_29	0.0909	0.0868	0.0802	0.0338	-0.0712
172246_34	0.0523	0.0632	0.0566	0.0310	0.1569
172846_125	0.3026	0.3399	0.3342	0.1011	0.0399
172846_130	0.2961	0.3208	0.3197	0.0703	0.0294

329636_122	0.1299	0.1214	0.1278	-0.0052	-0.0622
322620_134	0.2484	0.3072	0.3073	-0.0154	0.2037
322665_107	0.4200	0.4589	0.4742	0.0168	0.0767
322676_17	0.0596	0.0578	0.0579	-0.0128	-0.0179
326633_63	0.0701	0.0676	0.0551	0.0080	-0.0392
328988_141	0.1000	0.1069	0.1132	0.0087	0.0619
329288_17	0.2867	0.3160	0.3251	0.0095	0.0898
245601_106	0.4000	0.3848	0.3563	0.0367	-0.0650
246643_122	0.2152	0.2019	0.2120	0.0562	-0.1089
246643_147	0.2152	0.2019	0.2120	0.0562	-0.1089
246932_24	0.0400	0.0392	0.0464	0.0064	-0.0219
239129_81	0.0458	0.0447	0.0405	0.0049	-0.0238
240015_140	0.0458	0.0693	0.0634	-0.0017	0.3436
11481_16	0.0325	0.0319	0.0505	0.0375	-0.0420
15606_147	0.4172	0.4157	0.4174	0.0003	-0.0006
15606_96	0.1333	0.1244	0.1190	0.0249	-0.0882
15737_109	0.4481	0.4452	0.4441	-0.0122	0.0056
183335_51	0.0658	0.0636	0.0638	0.0074	-0.0364
183374_98	0.1883	0.1810	0.1723	-0.0016	-0.0357
183381_40	0.3816	0.3473	0.3455	0.0061	-0.1004
186315_71	0.0728	0.0824	0.0919	0.0111	0.1118
186502_76	0.2649	0.2582	0.2638	0.0330	-0.0480
187446_115	0.1733	0.1906	0.1998	-0.0089	0.0996
189336_61	0.0855	0.0819	0.0954	0.0033	-0.0439
192757_131	0.1176	0.1222	0.1166	0.0039	0.0376
192757_21	0.0458	0.0447	0.0435	0.0120	-0.0292
193930_102	0.0588	0.0693	0.0888	0.0298	0.1355
175757_73	0.0467	0.0456	0.0470	-0.0131	-0.0109
69781_14	0.3046	0.3103	0.3087	0.0601	-0.0242
69781_38	0.3377	0.3666	0.3585	0.0523	0.0448
69781_134	0.4267	0.4399	0.4357	0.0070	0.0285
67442_138	0.3806	0.4300	0.4380	0.0399	0.0912
67442_84	0.3057	0.3603	0.3520	0.0789	0.1015
70845_93	0.0513	0.0500	0.0609	0.0339	-0.0492

70974_45	0.2839	0.2706	0.2723	0.0421	-0.0799
70974_117	0.0654	0.0632	0.0695	0.0093	-0.0376
71031_128	0.3444	0.2937	0.2884	0.0132	-0.1809
72534_9	0.1097	0.1374	0.1247	0.0141	0.1964
72690_13	0.1457	0.1463	0.1382	0.0074	0.0021
73322_136	0.1613	0.1590	0.1405	0.0298	-0.0337
75315_73	0.0993	0.1179	0.1018	0.0325	0.1401
78210_57	0.0395	0.0387	0.0355	-0.0008	-0.0162
32516_37	0.0993	0.0944	0.1089	0.0245	-0.0684
33878_121	0.1267	0.1302	0.1160	0.0103	0.0231
37120_114	0.0400	0.0392	0.0386	0.0049	-0.0207
28866_109	0.2208	0.2355	0.2607	0.0246	0.0487
29700_131	0.0667	0.0890	0.0788	0.0100	0.2483
30517_114	0.3733	0.3911	0.3875	-0.0084	0.0546
30517_53	0.0519	0.0506	0.0467	0.0176	-0.0369
30553_129	0.0662	0.0763	0.0743	-0.0113	0.1426
30553_116	0.2781	0.2582	0.2300	0.0466	-0.1124
30553_60	0.0662	0.0763	0.0743	-0.0113	0.1426
30553_13	0.0662	0.0763	0.0743	-0.0113	0.1426
30833_51	0.2600	0.2822	0.2653	0.0338	0.0586
30833_28	0.3200	0.3279	0.3174	0.0027	0.0255
30833_13	0.1111	0.1049	0.0995	-0.0029	-0.0533
30955_117	0.1133	0.1302	0.1277	-0.0151	0.1422
31186_134	0.1060	0.1351	0.1505	0.0112	0.2123
336415_97	0.0641	0.0620	0.0548	0.0316	-0.0544
337686_100	0.1921	0.2150	0.2063	-0.0125	0.1181
337686_40	0.0728	0.0944	0.1049	-0.0093	0.2366
330963_141	0.0195	0.0444	0.0339	0.0437	0.5491
331862_13	0.1935	0.2151	0.2056	0.0160	0.0927
332621_102	0.1688	0.1758	0.1650	0.0836	-0.0211
332700_77	0.1060	0.1121	0.1328	0.0595	0.0148
334634_112	0.1711	0.1672	0.1594	0.0334	-0.0451
334634_6	0.0784	0.0754	0.0671	0.0090	-0.0444
336105_13	0.1111	0.1049	0.0962	0.0359	-0.0844

344450_149	0.3026	0.3324	0.3158	-0.0017	0.0939
344450_129	0.3026	0.3324	0.3158	-0.0017	0.0939
344450_83	0.0654	0.0632	0.0611	-0.0117	-0.0218
345193_67	0.0588	0.0571	0.0521	0.0114	-0.0357
345525_110	0.0855	0.0819	0.0723	0.0216	-0.0581
348723_59	0.1126	0.1407	0.1196	0.0870	0.1474
52627_39	0.2194	0.2248	0.2257	0.0254	0.0087
52627_65	0.0455	0.0567	0.0447	0.0193	0.1904
53019_149	0.2000	0.1902	0.1870	0.0011	-0.0490
53019_30	0.1795	0.1738	0.1725	0.0021	-0.0310
54245_44	0.0592	0.0575	0.0623	0.0299	-0.0503
54330_8	0.0733	0.0706	0.0726	-0.0048	-0.0311
54330_122	0.0733	0.0706	0.0726	-0.0048	-0.0311
54330_126	0.0733	0.0706	0.0726	-0.0048	-0.0311
307106_134	0.3400	0.3613	0.3379	0.0208	0.0477
310289_18	0.4474	0.4057	0.4367	0.0549	-0.1458
310289_94	0.0633	0.0847	0.0770	0.0483	0.2279
313222_36	0.1589	0.1682	0.1665	-0.0140	0.0681
313222_57	0.1133	0.1302	0.1223	0.0000	0.1329
304962_147	0.0974	0.1158	0.0979	0.0480	0.1309
307818_65	0.2157	0.2224	0.2209	0.0723	-0.0217
307818_52	0.2208	0.2260	0.2244	0.0708	-0.0281
307818_41	0.2208	0.2260	0.2244	0.0708	-0.0281
308305_39	0.1800	0.1853	0.1983	0.0134	0.0225
310326_11	0.1169	0.1327	0.1288	0.0385	0.0962
310326_78	0.0645	0.0624	0.0636	0.0141	-0.0409
311282_138	0.0467	0.0456	0.0450	-0.0044	-0.0173
311282_143	0.0467	0.0456	0.0450	-0.0044	-0.0173
311870_22	0.1267	0.1186	0.1204	0.0100	-0.0721
311870_35	0.0461	0.0450	0.0371	-0.0025	-0.0184
1365_34	0.1250	0.1286	0.1211	0.0010	0.0307
1676_22	0.0596	0.0824	0.1034	0.0503	0.2519
3436_139	0.0392	0.0384	0.0415	-0.0096	-0.0097
3436_72	0.0392	0.0384	0.0415	-0.0096	-0.0097

3436_27	0.0392	0.0384	0.0415	-0.0096	-0.0097
3436_24	0.0392	0.0384	0.0415	-0.0096	-0.0097
5382_107	0.5267	0.4982	0.4998	0.0006	-0.0542
5382_35	0.2105	0.2088	0.1993	0.0138	-0.0153
5699_87	0.0844	0.0927	0.0874	0.0398	0.0647
5720_130	0.2053	0.2347	0.2360	0.0088	0.1227
5720_118	0.2185	0.2442	0.2491	0.0086	0.1027
8714_51	0.2895	0.3399	0.3387	-0.0138	0.1601
8714_50	0.2895	0.3399	0.3387	-0.0138	0.1601
9145_95	0.0600	0.0582	0.0609	0.0310	-0.0516
9145_4	0.0933	0.1010	0.1036	0.0047	0.0758
9325_15	0.1133	0.1302	0.1583	0.0470	0.1014
9913_5	0.2848	0.2894	0.2827	0.0229	0.0024
220499_31	0.3974	0.3631	0.3788	0.0137	-0.1021
220628_131	0.2384	0.2582	0.2686	-0.0110	0.0874
221390_63	0.3533	0.3682	0.3870	0.0903	-0.0259
221390_111	0.3600	0.3783	0.4010	0.1111	-0.0353
221438_120	0.3831	0.3876	0.3736	0.0393	-0.0147
223808_66	0.0733	0.0829	0.0691	-0.0069	0.1232
224037_9	0.1447	0.1779	0.1854	-0.0025	0.1910
224037_45	0.2400	0.2311	0.2315	-0.0129	-0.0256
224037_97	0.2384	0.2395	0.2396	-0.0108	0.0154
224037_125	0.2384	0.2395	0.2396	-0.0108	0.0154
224037_138	0.2384	0.2395	0.2396	-0.0108	0.0154
37835_10	0.0993	0.0944	0.0850	0.0122	-0.0584
38080_53	0.0909	0.0868	0.0926	-0.0060	-0.0398
38476_116	0.0861	0.0824	0.0829	0.0056	-0.0460
38571_117	0.1467	0.1359	0.1372	-0.0122	-0.0664
38571_112	0.2053	0.2347	0.2284	0.0426	0.1001
42719_112	0.5526	0.4986	0.5010	0.0039	-0.1083
313713_87	0.0526	0.0512	0.0454	0.0039	-0.0266
313793_67	0.5686	0.4753	0.4860	0.0350	-0.2250
313965_110	0.0800	0.0768	0.0822	-0.0080	-0.0323
315111_17	0.0774	0.0744	0.0798	-0.0027	-0.0350

316197_93	0.0933	0.0890	0.0972	-0.0080	-0.0395
317211_19	0.5267	0.4466	0.4400	-0.0044	-0.1721
317370_138	0.2933	0.3036	0.3333	0.0441	0.0046
319443_147	0.0987	0.1056	0.1178	0.0106	0.0613
319443_24	0.1523	0.1519	0.1398	0.0181	-0.0133
32241_81	0.2089	0.2164	0.2111	0.0128	0.0287
33516_53	0.1634	0.1716	0.2045	0.0436	0.0193
33516_24	0.1842	0.1884	0.1727	0.0267	0.0058
35098_62	0.0533	0.0519	0.0445	0.0014	-0.0251
35936_29	0.1046	0.1222	0.1222	-0.0050	0.1503
35936_24	0.0523	0.0509	0.0371	0.0566	-0.0685
239001_96	0.0800	0.0768	0.1033	0.0851	-0.1089
225526_63	0.1987	0.2200	0.1999	0.0031	0.0982
225526_12	0.2800	0.3356	0.3214	0.0223	0.1553
229133_117	0.0867	0.0950	0.1053	0.0017	0.0899
229935_41	0.3421	0.4272	0.4246	0.0017	0.2014
230310_14	0.1523	0.1736	0.1882	0.0235	0.1105
232941_126	0.0392	0.0384	0.0377	0.0042	-0.0198
232941_118	0.1948	0.1964	0.1842	-0.0046	0.0148
232941_77	0.0724	0.0697	0.0777	0.0143	-0.0452
233013_19	0.2450	0.2249	0.2056	0.0206	-0.1029
233013_105	0.2450	0.2249	0.2056	0.0206	-0.1029
233265_147	0.1503	0.1500	0.1385	0.0435	-0.0317
233792_136	0.2895	0.2748	0.2975	0.0260	-0.0705
55406_32	0.0714	0.0689	0.0767	-0.0053	-0.0298
57344_91	0.3400	0.3682	0.3602	0.0529	0.0423
57344_142	0.1111	0.1049	0.0947	0.0153	-0.0676
57391_140	0.0455	0.0444	0.0400	0.0048	-0.0236
57391_49	0.0897	0.1087	0.1275	0.0079	0.1729
57580_37	0.4248	0.4508	0.4561	-0.0086	0.0667
57580_87	0.0764	0.0967	0.1191	0.0454	0.1856
57593_80	0.0833	0.0915	0.0984	-0.0018	0.0940
57593_28	0.5097	0.4859	0.4817	0.0058	-0.0501
58084_143	0.1733	0.1583	0.1512	0.0018	-0.0930

58446_49	0.5600	0.4978	0.5015	0.0292	-0.1465
60138_130	0.0461	0.0450	0.0589	0.0191	-0.0348
60528_34	0.3026	0.3006	0.2655	0.0809	-0.0681
60685_96	0.0784	0.0754	0.0718	0.0374	-0.0670
62472_60	0.0263	0.0387	0.0280	0.0341	0.3055
65215_69	0.0705	0.0915	0.0911	-0.0083	0.2373
118514_104	0.1355	0.1263	0.1453	0.0206	-0.0860
119650_132	0.2649	0.2674	0.2663	0.0098	0.0054
120350_145	0.2566	0.2614	0.2499	0.0369	-0.0058
120638_86	0.2800	0.2952	0.3130	0.0294	0.0337
120638_124	0.1126	0.1294	0.1182	-0.0059	0.1370
121031_11	0.1176	0.1222	0.1443	0.0474	0.0053
121089_115	0.0331	0.0326	0.0311	0.0089	-0.0202
121128_13	0.1000	0.0950	0.1047	-0.0048	-0.0457
121160_34	0.5232	0.4912	0.4955	0.0444	-0.0979
122059_77	0.0461	0.0450	0.0453	-0.0107	-0.0124
122689_108	0.3000	0.3078	0.2913	0.0167	0.0166
124638_109	0.0592	0.0575	0.0629	-0.0088	-0.0206
106618_11	0.0844	0.0809	0.0690	0.0846	-0.1113
110657_101	0.1200	0.1244	0.1224	-0.0068	0.0437
286514_120	0.4867	0.4226	0.4127	0.0262	-0.1709
288527_10	0.2933	0.2952	0.3058	-0.0093	0.0164
288606_93	0.2053	0.2049	0.1852	0.0609	-0.0461
289208_6	0.4510	0.4070	0.4052	-0.0096	-0.0972
291952_57	0.2517	0.2937	0.3017	0.0295	0.1273
140823_25	0.5200	0.4928	0.4926	0.0065	-0.0569
140823_26	0.2237	0.2188	0.2205	-0.0107	-0.0113
140980_149	0.1558	0.1862	0.2274	0.1060	0.0941
142074_28	0.4400	0.3911	0.4071	0.0050	-0.1258
142122_104	0.1118	0.1056	0.1145	-0.0024	-0.0541
142613_26	0.4600	0.4728	0.4730	-0.0053	0.0341
143985_56	0.3684	0.3815	0.3768	-0.0043	0.0406
144861_132	0.0600	0.0582	0.0544	0.0137	-0.0380
126461_4	0.0987	0.0938	0.0910	-0.0030	-0.0463

126461_138	0.0592	0.0575	0.0685	0.0415	-0.0597
126461_145	0.0397	0.0389	0.0369	0.0133	-0.0270
126774_144	0.0467	0.0456	0.0413	0.0264	-0.0408
126874_14	0.0458	0.0447	0.0397	-0.0057	-0.0159
127056_11	0.0833	0.0799	0.0694	-0.0039	-0.0373
127114_53	0.1118	0.1056	0.1098	-0.0076	-0.0501
303418_70	0.0915	0.0873	0.0804	0.0064	-0.0496
303418_72	0.0915	0.0873	0.0804	0.0064	-0.0496
303418_73	0.0915	0.0873	0.0804	0.0064	-0.0496
304665_147	0.3667	0.3942	0.4314	0.1065	-0.0082
304878_128	0.0789	0.0879	0.0982	-0.0016	0.1058
294988_19	0.0645	0.0624	0.0486	0.0341	-0.0567
299397_131	0.0464	0.0453	0.0406	-0.0052	-0.0166
299397_129	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_107	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_103	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_94	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_82	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_60	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_38	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_37	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_36	0.0458	0.0447	0.0400	-0.0053	-0.0162
299397_5	0.0458	0.0447	0.0400	-0.0053	-0.0162
300502_39	0.2903	0.2749	0.2494	0.0179	-0.0669
300502_34	0.2903	0.2749	0.2494	0.0179	-0.0669
300502_26	0.3442	0.3097	0.2797	0.0333	-0.1358
300502_12	0.0641	0.0620	0.0566	0.0055	-0.0341
302001_117	0.0581	0.0564	0.0856	0.0705	-0.0832
302001_141	0.3684	0.3683	0.3867	0.0080	-0.0028
175575_21	0.1569	0.1555	0.1607	-0.0116	0.0028
176895_147	0.0933	0.1010	0.0883	-0.0012	0.0799
178516_60	0.0592	0.0575	0.0456	0.0283	-0.0492
180914_75	0.0867	0.0829	0.0803	-0.0005	-0.0416
181989_18	0.3444	0.3831	0.3925	0.0020	0.1032

257585_42	0.1419	0.1643	0.1566	0.0337	0.1176
257585_41	0.1419	0.1643	0.1566	0.0337	0.1176
258102_119	0.1513	0.1509	0.1507	-0.0087	0.0072
247550_110	0.2171	0.2237	0.2319	0.0050	0.0290
247629_137	0.0733	0.0829	0.0646	0.0340	0.0961
247873_88	0.1933	0.1853	0.2034	0.0076	-0.0458
249479_101	0.0850	0.0814	0.0952	0.0048	-0.0448
250571_113	0.1104	0.1271	0.1150	-0.0060	0.1382
250571_110	0.2434	0.2614	0.2884	0.0934	0.0025
251207_80	0.0658	0.0758	0.0840	0.0022	0.1343
251726_14	0.0592	0.0575	0.0586	0.0173	-0.0404
337787_124	0.0927	0.1121	0.1605	0.1044	0.1063
342454_102	0.1765	0.1716	0.1611	0.0151	-0.0367
343631_5	0.0728	0.0702	0.0740	0.0365	-0.0633
343730_102	0.1533	0.1416	0.1526	-0.0041	-0.0765
338234_85	0.2733	0.2550	0.2799	0.0415	-0.1025
338234_19	0.2733	0.2550	0.2799	0.0415	-0.1025
338364_106	0.1046	0.0991	0.0860	0.0092	-0.0589
338364_20	0.4800	0.4911	0.4843	0.0223	0.0101
338448_7	0.4667	0.4956	0.4920	0.0086	0.0559
338448_64	0.1200	0.1244	0.1222	0.0371	0.0121
339066_109	0.1118	0.1286	0.1119	0.0236	0.1183
340906_105	0.4533	0.4743	0.4867	0.0635	0.0002
340906_94	0.3247	0.2892	0.2795	-0.0051	-0.1154
341223_117	0.1364	0.1271	0.1265	-0.0046	-0.0663
341755_118	0.3247	0.2892	0.3062	0.0239	-0.1397
17972_92	0.0592	0.0575	0.0509	-0.0061	-0.0227
18436_102	0.4733	0.4920	0.4972	0.0293	0.0202
18651_149	0.1842	0.1987	0.2261	0.0309	0.0546
18679_52	0.1258	0.1294	0.1453	0.0410	0.0007
18679_107	0.1258	0.1294	0.1453	0.0410	0.0007
19420_60	0.0458	0.0447	0.0404	0.0001	-0.0202
19420_30	0.1250	0.1286	0.1191	-0.0079	0.0370
19420_14	0.0458	0.0447	0.0404	0.0001	-0.0202

22562_36	0.1908	0.2038	0.1995	0.0501	0.0312
25358_99	0.5232	0.4495	0.4462	0.0069	-0.1667
25358_14	0.0850	0.0814	0.0772	-0.0089	-0.0343
105695_53	0.1987	0.1895	0.1823	-0.0087	-0.0388
105695_147	0.0867	0.0829	0.0707	-0.0020	-0.0405
110598_98	0.0331	0.0578	0.0651	-0.0140	0.4359
110598_14	0.0331	0.0578	0.0651	-0.0140	0.4359
112289_16	0.1133	0.1069	0.1221	0.0026	-0.0588
112289_38	0.1133	0.1069	0.1221	0.0026	-0.0588
112289_90	0.0719	0.0693	0.0904	0.0210	-0.0501
112289_137	0.1133	0.1069	0.1221	0.0026	-0.0588
112588_65	0.1623	0.1810	0.1767	-0.0065	0.1107
112588_132	0.1218	0.1144	0.1000	0.0025	-0.0636
112816_55	0.3533	0.3880	0.3772	0.0040	0.0899
112816_36	0.0596	0.0702	0.0750	0.0255	0.1379
112816_24	0.2267	0.2408	0.2444	-0.0007	0.0625
112816_23	0.2318	0.2442	0.2478	-0.0030	0.0563
113095_91	0.3618	0.4195	0.4136	-0.0042	0.1433
113762_5	0.2941	0.3032	0.2942	0.0090	0.0267
113808_4	0.0600	0.0582	0.0900	0.0692	-0.0833
114298_140	0.1046	0.1222	0.1140	0.0075	0.1426
114564_123	0.0467	0.0456	0.0612	0.0159	-0.0326
116402_43	0.1623	0.1600	0.1661	0.0201	-0.0268
116621_5	0.0458	0.0571	0.0501	0.0057	0.1984
116757_130	0.5267	0.4982	0.5001	0.0197	-0.0694
116830_34	0.3750	0.3970	0.4051	0.0740	0.0038
116830_84	0.3709	0.3956	0.4048	0.0725	0.0126
194667_134	0.1579	0.1564	0.1617	-0.0057	-0.0020
196335_97	0.1457	0.1351	0.1368	-0.0070	-0.0697
200985_101	0.4503	0.4473	0.4569	0.0133	-0.0132
200985_15	0.4503	0.4473	0.4569	0.0133	-0.0132
208411_46	0.0701	0.0910	0.1025	0.0266	0.2175
194161_125	0.0795	0.0763	0.0674	0.0281	-0.0598
194927_139	0.1867	0.1800	0.1635	0.0758	-0.0962

194927_137	0.1133	0.1069	0.0975	0.0065	-0.0618
194927_72	0.1267	0.1528	0.1424	0.0018	0.1730
196126_23	0.0867	0.0829	0.0719	0.0626	-0.0929
197774_30	0.0705	0.0680	0.0795	0.0117	-0.0423
197774_32	0.0577	0.0560	0.0442	0.0451	-0.0622
198615_16	0.0784	0.0873	0.0723	0.0341	0.0818
199742_144	0.0596	0.0578	0.0621	-0.0110	-0.0192
200504_142	0.1053	0.0997	0.0966	0.0545	-0.0967
201281_73	0.5098	0.4938	0.4886	0.0250	-0.0484
201281_149	0.4379	0.4692	0.4580	0.0735	0.0156
103299_105	0.2053	0.2049	0.1904	-0.0048	0.0050
103466_39	0.2600	0.2456	0.2575	0.0025	-0.0573
103786_78	0.0458	0.0571	0.0777	0.0382	0.1788
103786_37	0.0728	0.0702	0.0701	-0.0109	-0.0264
103903_121	0.0519	0.0628	0.0669	-0.0136	0.1844
100747_30	0.0467	0.0456	0.0452	0.0127	-0.0301
100982_23	0.0861	0.0824	0.0735	-0.0009	-0.0409
100982_88	0.0461	0.0450	0.0505	0.0030	-0.0225
277349_78	0.0199	0.0453	0.0369	-0.0017	0.5641
278164_27	0.0719	0.0693	0.0610	-0.0030	-0.0318
278540_45	0.4065	0.4947	0.4947	0.0036	0.1793
279262_28	0.2252	0.2298	0.2215	0.0324	-0.0004
280217_89	0.0867	0.0829	0.0684	0.0176	-0.0556
280636_95	0.0400	0.0519	0.0497	0.0222	0.2200
128216_32	0.1987	0.2200	0.2020	0.0164	0.0892
128837_45	0.2252	0.2489	0.2482	0.0399	0.0712
129969_146	0.2067	0.2061	0.1925	0.0025	-0.0012
130527_31	0.4305	0.4613	0.4509	0.0162	0.0589
130527_47	0.2252	0.1998	0.1785	0.0195	-0.1400
131069_54	0.1111	0.1049	0.1034	-0.0123	-0.0461
132564_30	0.0662	0.0640	0.0586	0.0053	-0.0349
132707_112	0.3333	0.3432	0.3600	0.0115	0.0239
132707_28	0.3400	0.3469	0.3632	0.0094	0.0165
135398_9	0.0464	0.0453	0.0552	0.0071	-0.0257

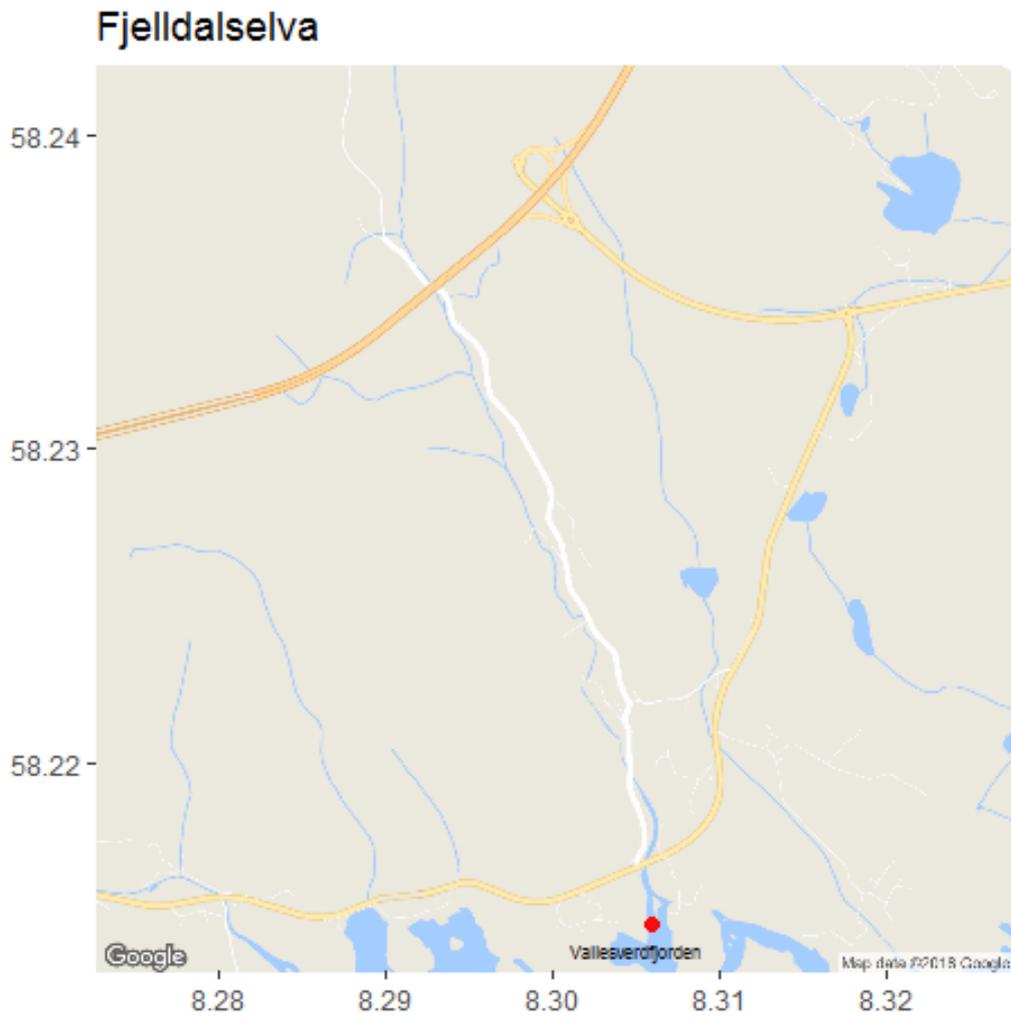
136240_104	0.3974	0.4384	0.4503	0.0227	0.0817
136656_45	0.1067	0.1128	0.1059	-0.0102	0.0646
136656_44	0.1067	0.1128	0.1059	-0.0102	0.0646
156953_67	0.2792	0.2934	0.2946	0.0274	0.0318
148027_112	0.3510	0.3453	0.3694	0.0162	-0.0254
148027_95	0.0933	0.0890	0.0992	-0.0020	-0.0441
148529_75	0.0662	0.0763	0.0581	0.0467	0.1047
149300_63	0.2645	0.2749	0.2618	-0.0055	0.0448
149550_102	0.0658	0.0636	0.0723	0.0259	-0.0507
152197_145	0.0795	0.0884	0.0785	0.0002	0.1043
152197_41	0.2133	0.2503	0.2426	-0.0113	0.1579
152197_15	0.2133	0.2503	0.2426	-0.0113	0.1579
152459_36	0.4333	0.4626	0.4692	0.0178	0.0543
152459_37	0.4333	0.4626	0.4692	0.0178	0.0543
154184_40	0.2222	0.2273	0.2073	0.0083	0.0195
155643_71	0.2645	0.2482	0.2610	-0.0074	-0.0570
156588_104	0.0662	0.0763	0.0691	0.0086	0.1300
156855_121	0.1250	0.1399	0.1690	0.0441	0.0794
156855_46	0.1250	0.1399	0.1341	-0.0087	0.1152
157112_129	0.3660	0.4306	0.4116	0.0193	0.1410
157813_30	0.0510	0.0497	0.0509	-0.0108	-0.0150
157813_138	0.1090	0.1144	0.1248	0.0093	0.0440
158022_8	0.0728	0.0702	0.0632	0.0053	-0.0385
158028_21	0.3377	0.3292	0.3140	-0.0020	-0.0209
158028_22	0.3377	0.3292	0.3140	-0.0020	-0.0209
158666_113	0.0719	0.0693	0.0789	0.0036	-0.0368
158705_6	0.4575	0.4098	0.4172	-0.0017	-0.1119
158918_59	0.0577	0.0560	0.0520	0.0085	-0.0329
203500_70	0.0774	0.0744	0.0866	0.0555	-0.0819
203500_13	0.1935	0.1748	0.1935	0.0733	-0.1682
204703_84	0.1600	0.1583	0.1526	0.0128	-0.0168
205047_27	0.3444	0.3631	0.3689	0.0439	0.0235
205924_56	0.5629	0.4495	0.4509	-0.0026	-0.2469
205924_107	0.4733	0.4509	0.4631	0.0312	-0.0709

205987_114	0.0467	0.0456	0.0550	0.0115	-0.0291
241889_44	0.3121	0.3357	0.3629	0.0660	0.0258
242243_74	0.2353	0.2273	0.2447	0.0078	-0.0380
243563_48	0.2333	0.2456	0.2499	0.0014	0.0522
244196_80	0.0592	0.0575	0.0502	-0.0033	-0.0247
246051_35	0.2403	0.2212	0.1996	0.0189	-0.0983
214443_77	0.4737	0.4913	0.4967	0.0185	0.0259
223929_11	0.0795	0.0884	0.0808	0.0035	0.1022
208919_23	0.0784	0.0754	0.0660	0.0154	-0.0494
208919_55	0.3444	0.3700	0.3721	0.0332	0.0492
209181_77	0.0701	0.0676	0.0618	0.0094	-0.0403
209181_113	0.0701	0.0676	0.0618	0.0094	-0.0403
212758_36	0.0596	0.0578	0.0800	0.0585	-0.0740
212950_58	0.4248	0.4623	0.4706	-0.0073	0.0892
213595_23	0.3782	0.3585	0.3637	-0.0068	-0.0467
214990_130	0.1410	0.1528	0.1622	0.0035	0.0777
215318_125	0.0968	0.0921	0.1030	0.0072	-0.0532
215356_93	0.2597	0.2631	0.2864	0.0108	0.0083
267941_6	0.2680	0.2600	0.2529	0.0044	-0.0307
265335_3	0.1316	0.1229	0.1242	0.0082	-0.0737
265518_43	0.0724	0.0697	0.0684	0.0315	-0.0589
266696_132	0.1447	0.1454	0.1417	-0.0012	0.0089
267634_28	0.2774	0.2662	0.2489	0.0508	-0.0800
358024_74	0.0588	0.0571	0.0506	-0.0015	-0.0259
358478_51	0.0600	0.0829	0.1219	0.0907	0.2269
360284_97	0.0596	0.0578	0.0497	-0.0054	-0.0234
360284_81	0.0795	0.0763	0.0782	0.0241	-0.0569
361107_68	0.2000	0.1800	0.1785	0.0087	-0.1149
361808_37	0.0467	0.0456	0.0441	0.0005	-0.0210
362451_102	0.0526	0.0512	0.0537	0.0309	-0.0477
364831_84	0.0933	0.1128	0.1023	0.0721	0.1287
368209_122	0.0667	0.0644	0.0643	0.0233	-0.0491
<b>Average</b>	<b>0.1809</b>	<b>0.1850</b>	<b>0.1850</b>	<b>0.0161</b>	<b>0.0141</b>

## Vallesværelva

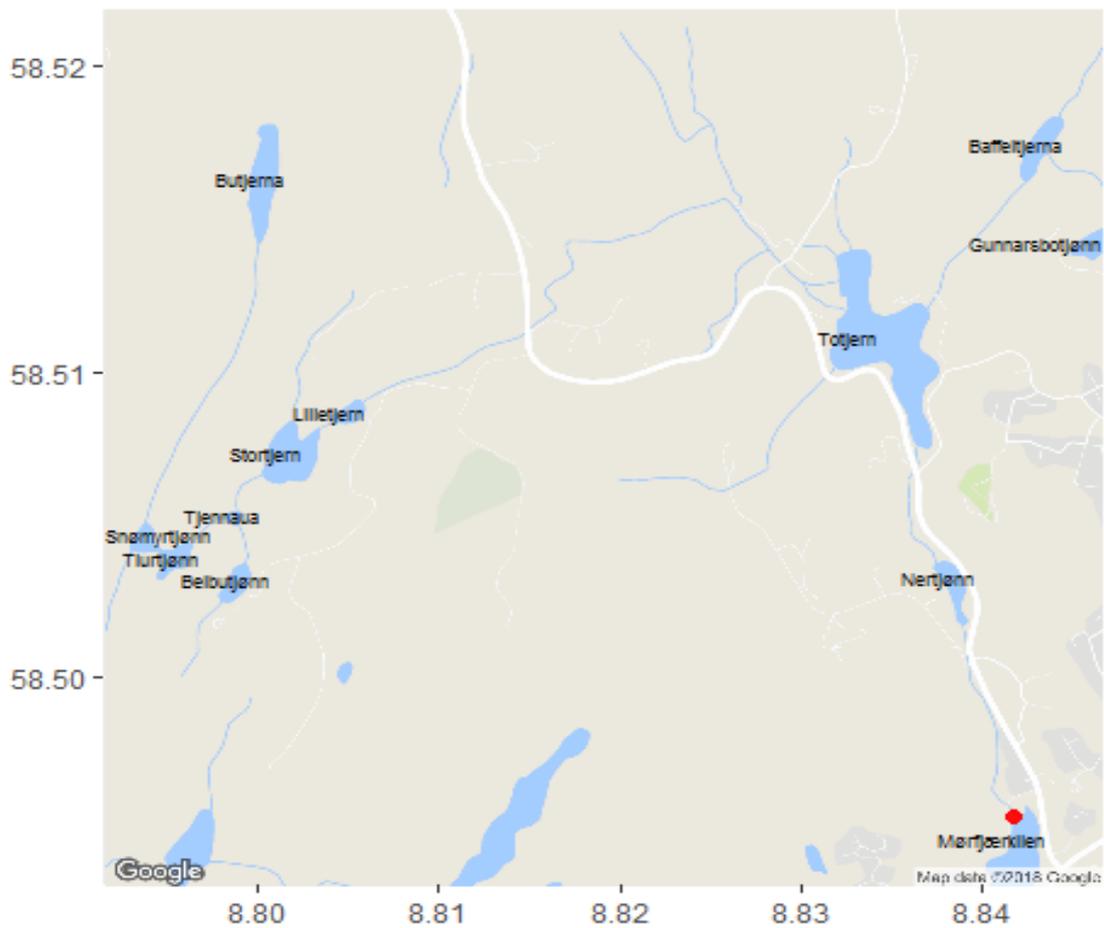


*Appendix 2: Map of Vallesværelva located in Lillesand. The red dot represents the entrance of the stream in Vallesverd fjorden. The red stop sign is a migration barrier.*



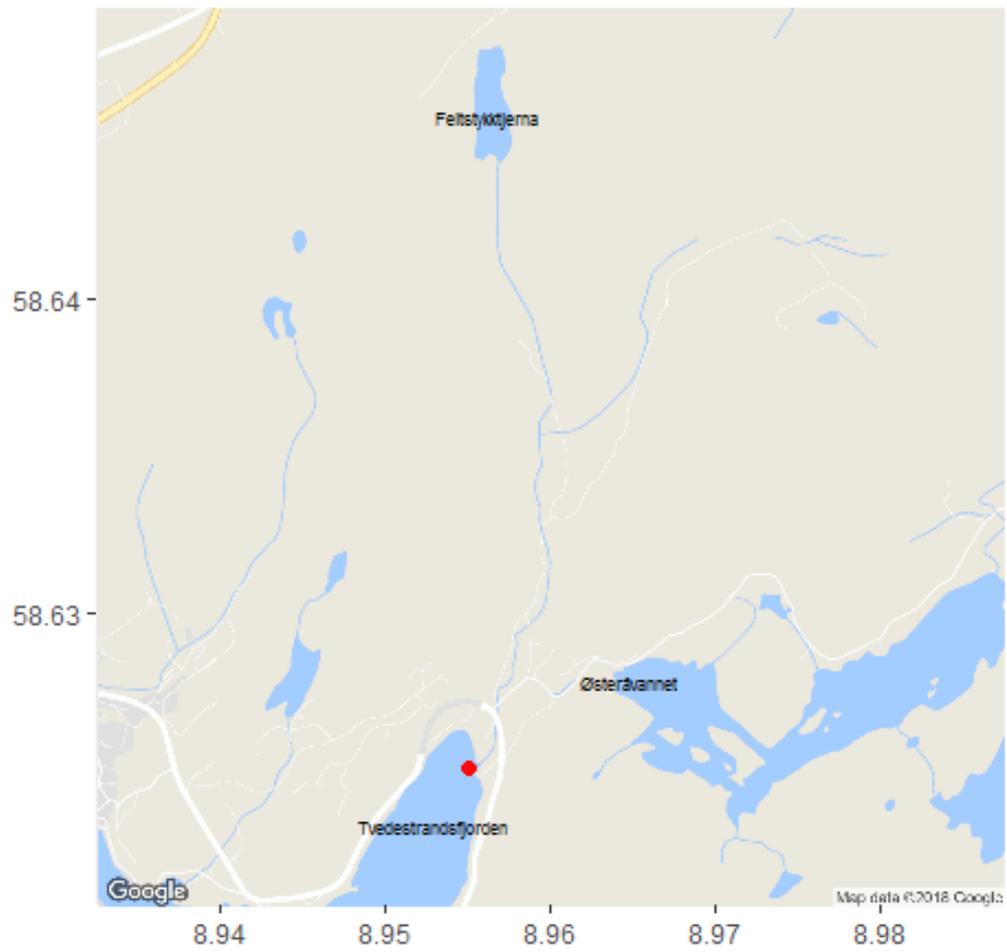
*Appendix 3: Map of Fjelldalselva located in Lillesand. The red dot represents the entrance of the stream in Vallesverdfjorden.*

## Mørjærbekken



*Appendix 4: Map of Mørjærbekken located in Arendal. The red dot represents the entrance of the stream in Mørjærkilen. This stream is characterized by more ponds upstream compared to the other streams.*

## Østeråbekken



*Appendix 5: Map of Østeråbekken located in Tvedestrand. The red dot represents the entrance of the stream in Tvedestrandsfjorden.*