Genome-wide divergence patterns support fine-scaled genetic structuring associated with migration tendency in brown trout

Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2017-0014.R2
Manuscript Type:	Article
Date Submitted by the Author:	24-Nov-2017
Complete List of Authors:	Lemopoulos, Alexandre; University of Eastern Finland, Department of Environmental and Biological Sciences; University of Turku, Department of Biology Uusi-Heikkilä, Silva; University of Turku, Department of Biology Vasemägi, Anti; University of Turku, Department of Biology; Institute of Veterinary Medicine and Animal Sciences, Department of Aquaculture Huusko, Ari; Natural Resources Institute Finland (Luke), Natural Resources and Bioproduction Kokko, Harri; University of Eastern Finland, Department of Environmental and Biological Sciences Vainikka, Anssi; University of Eastern Finland, Department of Environmental and Biological Sciences
Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	migratory behaviour, salmonids, ddRADSeq, population genomics, Fisheries

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**Genome-wide divergence patterns support fine-scaled genetic structuring
associated with migration tendency in brown trout**

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Abstract: Brown trout *Salmo trutta* exhibit highly diverse life histories varying from resident, slow-growing and early maturing to migratory, fast-growing and late maturing even within single watersheds. We sampled eleven locations within the transboundary Finnish-Russian River Koutajoki watershed to evaluate genomic differences among main stem and headwater sites of which some are isolated by migration barriers. Restriction site associated sequencing (RADSeq) revealed that the most headwater localities supported unique, isolated populations with generally lower heterozygosity compared to the main stem populations. The sampled migratory adults in the three main stems showed signals of admixture despite small but statistically significant genetic divergence while the headwater populations, except for two, showed high level of divergence and a lack of admixture. These results suggest that most of the headwater populations consist of resident brown trout, and that the population genetic structuring is often maintained even in the absence of migration barriers. Our results have clear implications for fisheries management and conservation: each brown trout subpopulation represents an evolutionarily important unit with unique genetic make-up and life history variation.

Key words: migratory behaviour; salmonids; ddRADSeq; fisheries; population genomics

Résumé : Les truites *Salmo trutta* présentent différents types de stratégies de vie, variant entre une forme résidente à croissance lente et maturité retardée ; et une forme migrante à croissance rapide et maturité précoce. Ici, nous échantillonnons onze rivières dans le bassin du Koutajoki Finno-Russe afin d'étudier les différences génomiques entre les populations présentes dans les cours d'eau principaux du système et celles dans leurs affluents, parfois isolées par des barrières de migrations. Le séquençage d'étiquettes associées à des sites de restrictions a révélé que la plupart des affluents contient des populations uniques et isolées avec une hétérozygocité moindre que celles des cours d'eau principaux. Les adultes migrants échantillonnés dans les cours d'eau principaux présentent un mélange génétique entre eux malgré des différences restant significatives. Au contraire, les populations des affluents - sauf deux exceptions - présentent une grande divergence et une absence de mélange génétique. Ces résultats suggèrent que les affluents sont majoritairement constitués de populations résidentes et que les structures génétiques sont maintenues malgré l'absence de barrières de migrations. Notre étude délivre un fort message pour la conservation et la gestion des ressources halieutiques : chaque sous-population représente une unité génétique indépendante avec des stratégies de vie particulières. Ces sous-populations ne peuvent donc pas être soutenue par la réintroduction de poissons exogènes, même provenant d'un affluent proche.

Introduction

Salmonids from genera *Salmo*, *Oncorhynchus* and *Salvelinus* are among the most famous and economically important migratory fishes (Quinn 2005; Harris and Milner 2007). Many species in genus *Salmo* perform migrations from streams to larger rivers, lakes and marine environments for feeding and then return to their natal streams for spawning while many of them can also display an entirely resident life cycle (Groot et al. 1989; Quinn and Myers 2004). Brown trout (*Salmo trutta*) is one of the most diverse species in relation to migration behaviour, as its populations vary from completely resident to partially or fully migratory (Jonsson and Jonsson 2007; Olsson et al. 2006; Wysujack et al. 2009; Dodson et al. 2013; Jones et al. 2015). The migratory brown trout is classified as anadromous, adfluvial or potadromous depending on whether the feeding migration takes place in a sea, a lake or a river, respectively.

Fine-scale genetic structuring is a well-known phenomenon among brown trout populations (e.g. Skaala and Nævdal 1989; Hindar et al. 1991; Hansen and Mensberg 1998; Carlsson and Nilsson 2000; Östergren and Nilsson 2012). Population genetic structure in brown trout has been studied in a variety of countries and continents using different techniques such as allozymes (e.g. García-Marín et al. (1999), microsatellites (e.g. Hansen et al. 2000_{ab}; Pettersson et al. 2001), polymorphic protein-coding loci (Sanz et al. 2000) and mitochondrial DNA (e.g. Pettersson et al. 2001; Lerceteau-Köhler et al. 2013). While often the genetic structuring associates with the geography, i.e. isolation by distance (Griffiths et al. 2009; Vøllestad et al. 2012) or topology related to migration obstacles (Jonsson 1982; Hindar et al. 1991; Sønstebo et al. 2007; Östergren and Nilsson 2012), divergence patterns may be also affected by anthropogenic effects such as stocking (Swatdipong et al. 2010; Hansen et al. 2000_{ab}) or hydroelectric dams (Junge et al. 2014). In addition, several studies have

demonstrated the role of natal homing (Allendorf et al. 1976; Carlsson and Nilsson 2000) and population size (Serbezov et al. 2012) in determining the genetic diversity and population structure in brown trout. Fine-scaled structuring within single rivers also suggests that behavioral mechanisms may be important in restraining gene flow (Carlsson et al. 1999; 2000).

Majority of studies on brown trout have used a small number of assumedly neutral genetic markers, most often microsatellites (e.g., Carlsson et al. 1999; Hansen et al. 2002; Junge et al. 2014). However, to further understand the complex relationships between life history variation and population genetic divergence, it is important to assess genome-wide patterns of divergence and diversity while linking them with landscape topology and potential life-history differences among subpopulations (c.f. Hansen and Mensberg 1998). The emergence of next-generation sequencing techniques has revolutionized the fields of population and conservation genetics (Shendure and Ji 2008; Mardis 2008; Etter et al. 2011; Fumagalli et al. 2013). Studies on fish have been no exception to this trend (e.g., Bruneaux et al. 2013; Keller et al. 2013; Manousaki et al. 2016). By providing large amounts of genetic data, these techniques have unveiled new horizons and possibilities for population geneticists and evolutionary ecologists (Davey and Blaxter 2010). Particularly techniques that reduce the complexity of genomic data, including ways to sequence standard subsamples of the genome, are highly useful for population and conservation genetics (Davey et al. 2011). Restriction site associated DNA (RAD) sequencing (RADSeq) has become one of the most commonly used genome reduction methods (Baird et al. 2008; Hohenlohe et al. 2010). By being able to identify hundreds to thousands of single nucleotide polymorphisms (SNPs) without reference genome data, RADSeq is particularly suited for population genetics studies on non-model organisms (Miller et al. 2007; Baird et al. 2008; Hohenlohe et al. 2010; Davey and Blaxter 2010). Population genetic structure is increasingly being identified using large numbers of

genome wide alleles from SNP panels (Hohenlohe et al. 2010; Davey and Blaxter 2010; Andrews and Luikart 2014; Benestan et al. 2015). As such, RAD sequencing may outperform more traditional methods such as microsatellite analyses while also revealing adaptive differences among populations (Liu et al. 2005; Lao et al. 2006; Helyar et al. 2011).

Several studies have suggested that resident and migratory brown trout are genetically indistinguishable within sympatric populations (Hindar et al. 1991, Carlsson and Nilsson 2000, Charles et al. 2005), and that the genetic differences between watersheds typically exceed those observed within watersheds (Hindar et al. 1991; Hansen and Mensberg 1998). However, while some rivers apparently sustain both migratory and resident trout in sympatry, their proportions may vary along the river continuum. Therefore, the proportion of migratory individuals might mostly depend on environmental conditions such as food availability (Olsson et al. 2006; Jones et al. 2015). On the other hand, migration propensity in salmonids is typically heritable: Doctor et al. (2014) estimated $h^2 = 0.69 - 0.77$ for the smoltification in rainbow trout *Oncorhynchus mykiss* (see also Thériault et al. 2007; Ruzycki et al. 2009). Given the occurrence of resident brown trout populations above waterfalls and manmade migration barriers, migration propensity must be partially either genetically or epigenetically controlled to prevent population sinks due to unidirectional migration (Giger et al. 2006; Hecht et al. 2013; Harris et al. 2015; Kendal et al. 2015). Indeed, genetic differences on causative loci may explain variation in migratory behavior as found in *O. mykiss* (Hale et al. 2013). Still, genome-wide patterns may be influenced by unequal gene flow between resident and migratory brown trout and by genetic drift.

In this study, we investigated if brown trout sampled from 11 localities in different parts of the Finnish-Russian transboundary River Koutajoki watershed would differ from each other genetically, and whether the potential differences would be more pronounced between the three main river watersheds (Rivers Kuusinki, Kitka and Oulanka) than within

151 them using a genome wide SNP dataset. We evaluated if genome-wide data would align with
152 previous electrophoretic study carried out within the same watershed (Huusko et al. 1990),
153 and whether the genetic variation would be best explained by environmental topology or the
154 life-history information on the sampled sites (Huusko et al. 1990, 2017). We hypothesized
155 that headwater brown trout populations typically adopt a resident lifestyle, and are therefore
156 subject to strong isolation, genetic drift and potential locally unique natural selection patterns.
157 As a result, strong genetic divergence patterns would be typically associated with resident life
158 history while migratory populations would be expected to more frequently exchange
159 spawners resulting in lower genetic divergence, higher diversity and more extensive levels of
160 admixture.

162 **Material and Methods**

164 **Study system**

165 River Koutajoki watershed consists of three main stem rivers (Kuusinki, Kitka, and
166 Oulanka), one definitive and one partial natural migration barrier (Jyrävä and Kiutaköngäs
167 waterfalls, respectively) and several small tributaries discharging to the main stem rivers and
168 Lake Kitka that is the main source of water for River Kitka (Fig. 1). All of the main stem
169 rivers discharge first to Lake Panozero via River Oulanka and, from there, via River Olanga
170 to Lake Pyazero which is the main feeding area for the migratory brown trout as known from
171 the tagging studies (Huusko et al. 1999; Saraniemi et al. 2008). The Lake Pyazero eventually
172 discharges to the White Sea via River Koutajoki (River Kovda in Russian), thus geologically
173 separating the studied brown trout populations from the rivers discharging west to the Baltic
174 Sea. However, after the last ice age, Lake Kitka had an outlet to Baltic Sea direction and the
175 brown trout populations above the Jyrävä waterfall may have an evolutionary origin in

ancient Baltic Sea area populations (Heikkinen and Kurimo 1977, Koutaniemi 1999). Large parts of the system are located within two large national parks with certain restrictions for human activity, the Oulanka national park in Finland, and the Paanajärvi national park in Russia. This means that the environment around the rivers is largely in pristine state. The three main stem rivers support extensive recreational angling activity with the estimated economic value of 1-2 million euros for the Kuusamo area (Kuosku et al. 2014).

Kuusinki watershed

The River Kuusinki, originating from the Lake Kiitämö (Fig. 1) is the smallest (45 km in length, mean flow $9.1 \text{ m}^3 \text{ s}^{-1}$) of the studied main rivers having a drainage basin of 830 km^2 . In our study, we consider the main stem (Kuusinkijoki) and its two tributaries below the Myllykoski hydropower plant that forms a significant migration barrier despite the existing artificial fishway (Huusko 1990; Mäki-Petäys et al. 2000). The lakes and rivers upstream from Myllykoski have been intensively stocked with brown trout of various ages and genetic origins questioning their value for population genetic studies. Both Brook Raatepuro and the more upstream River Juumajoki represent relatively long small rivers without any physical barriers for migration (Fig. 1). While the main stem supports migratory brown trout, the presence of small spawners in electrofishing surveys suggests that the tributaries mainly support resident trout (A. Huusko, personal observation).

Kitka watershed - below waterfalls

The 35 km long River Kitka (mean flow $19.7 \text{ m}^3 \text{ s}^{-1}$, drainage basin of 1870 km^2) flows from Lake Kitka. The river is divided into two parts by a definitive migration obstacle, the 9 m high Jyrävä waterfall (Fig. 1). Radiotagging studies performed as a part of data collection in this study confirmed that no fish are able to pass the waterfalls upstream. The

lower Kitka watershed below the Jyrävä waterfall is composed of the main stem and three small tributaries, of which the Brook Pesospuro was sampled in this study. It is a small and steeply sloped brook not accessible by large migratory individuals. The main stem is known from its large spawners and normal downstream smolt migration (Sutela and Huusko 1998; Mäki-Petäys et al. 2000). Brook Pesospuro was known to support wild resident trout (Tammi et al. 1996).

Kitka watershed - above waterfalls

Above the Jyrävä waterfall the upper river Kitka supports a critically endangered, intensively hatchery-supported fish that performs an exceptional upstream smolt migration towards heavily fished Lake Kitka (Keränen 1978). The small brooks River Kirintöjoki and River Lohijoki discharge to Lake Kitka, and support wild brown trout that were taken into pooled hatchery rearing with the intention to increase the number of migratory brown trout in the Lake Kitka. However, whether these small rivers support migratory or resident brown trout was not known. The samples collected for this study represent the pooled hatchery strain.

Oulanka watershed

The River Oulanka is the largest (mean flow $25.5 \text{ m}^3 \text{ s}^{-1}$) and longest (135 km) of the studied rivers (2116 km^2 drainage basin). River Oulanka has a partial migration barrier, Kiutaköngäs waterfalls, but the tributaries are accessible by any migratory trout managing to pass them with or without human help organised since 1965. We focused on the main stem - supporting migratory individuals - and three tributaries: Brook Merenoja, which is found below the waterfalls, and River Maaninkajoki and River Astumajoki which are located above them (Fig. 1). Proportions of migratory individuals in these tributaries were not known.

226

227 **Sampling and DNA extraction**

228 Brown trout were collected by electrofishing from the tributaries in autumn 2015
229 (Table 1). Due to challenging conditions, the Brook Raatepuro samples were collected only
230 from the lower stretches of the brook, while the other tributaries were sampled more
231 representatively along the uppermost 1/3 section of each of the tributaries. Samples from
232 tributaries thus came from individuals with unknown migration tendency. River Kitka and
233 River Kuusinki fish were sampled for scales during a tagging study in which a significant
234 proportion of all ascending fish were trapped during the spawning migration in River Olanga
235 (during summers 2013-2014), and their population of origin could be inferred from the actual
236 spawning site of the radio-tagged individuals. River Oulanka fish were sampled during the
237 human-assisted transfer of ascending trout pass the Kiutaköngas rapids in summer 2015, so
238 that all the sampled River Oulanka fish could also be confirmed migratory and eventually
239 spawning above the Kiutaköngäs rapids. The samples for upper River Kitka and small brooks
240 River Kirintöjoki and Lohijoki sampling sites were taken from hatchery broodstock
241 individuals in anaesthesia in autumn 2015.

242 For each sampling site, fin clips and scales from 16-30 individuals were collected for
243 DNA analysis (Table 1). Total DNA was extracted using the Omega bio-tek E.Z.N.A Tissue
244 DNA kit. We modified the manufacturer's protocol by adding a step in which a solution of
245 24:1 chloroform-isoamyl was used to remove any interfering polysaccharides contained in the
246 tissues immediately after the digestion by the proteinase K. The integrity of isolated DNA
247 was assessed with electrophoresis upon agarose gel (1 %) and by Qubit® dsDNA HS Assay
248 Kit with fluorometric measurement using Qubit 2.0 (Invitrogen, CA, USA).

249

250 **Library preparation and sequencing**

Several modifications have been made since the original RAD sequencing protocol was published by Miller et al. in 2007 (for a review see Andrews et al. 2016): Methods such as 2bRAD (Wang et al. 2012), ddRAD (Peterson et al. 2012) or ezRAD (Toonen et al. 2013) vary mainly in the library preparation process and in genome reduction effectiveness. We used the double digestion RAD (ddRAD) which implies a simplified procedure for library preparation compared to the original RADseq protocol (Baird et al. 2008; Hohenlohe et al. 2010). The usage of two restriction enzymes allows size selection of the sequenced fragments; hence, leading to a more controlled, efficient, robust and cost-efficient sequencing. Stringent size selection enabled efficient genome reduction which resulted in the identification of a low number of contig SNPs with high coverage, resulting into high quality genotype data (for more information about ddRAD see: Peterson et al. 2012; Bruneaux et al. 2013; Pukk et al. 2014; Pukk et al. 2015; Andrews et al. 2016).

To prepare the sequencing library, we followed the five-step protocol used in Pukk et al. (2015). This protocol was derived from the methods described in Bruneaux et al. (2013), and Pukk et al. (2014). We used PstI-HF® (5'CTGCAG 3') and BamHI-HF® (5'GGATCC 3') for the double digestion using restriction enzymes. For each sample, 100 ng of genomic DNA was digested for 20 minutes at 37 °C along with 2.5 µL of each restriction enzyme. The solution was then heat inactivated at 75 °C for 15 minutes. Adaptors were ligated to the cut fragments by adding 1 µL of each adapter (forward and reverse with concentrations of 0.1 pmol) along with 1.5 µL of T4 DNA ligase to the solution obtained in the first step. Forward adaptors contained individual barcodes to identify each sample. The ligation proceeded for 1 hour at 22 °C. It was then heat inactivated for 30 min at 65 °C. Samples were pooled into 4 lanes with 81-82 different barcodes. In order to clean the sequencing libraries, 4 columns (1 per lane) were washed using QIAquick PCR purification kit. The libraries were eluted in 35 µL of elution buffer for a final volume of 165 µL (5x35 µL) per library. For size selection,

with target fragment size of ~300bp, we loaded each library into an E-Gel Size select 2 % Agarose gel (Invitrogen, CA, USA). We extracted fragments from two gels on similar migration times. Upon each e-gel, fragments were extracted at 4 times with correspondence to approximate sizes of 280-320bp, according to a DNA ladder. The products of the two migrations were then pooled together for the amplification of the size-selected products by PCR. For each library, 3 amplifications were done separately with reaction volumes of 69 μ L (15 μ L of size-selected product, 50 μ L of Platinum PCR SuperMix high fidelity and 2 μ L of forward and reverse primers). The products of these reactions were pooled together to form libraries with a total volume of 207 μ L. Finally, libraries were purified using SPRI-beads. They were cleaned-up two times (with 1.1-fold volume of SPRI-beads) in order to remove any fragment smaller than 100 bp. Final library product was eluted in 20 μ L of extremely pure molecular water.

The concentration of the obtained libraries was quantified through fluorometric assessment using Qubit 2.0. Size and quantity of contained fragments were assessed for each library with Agilent 2100 Bioanalyzer. Libraries were sequenced on a Illumina HiSeq 2500 with the rapid run option by the commercial service provider, Turku Centre for Biotechnology (BTK), in Turku, Finland.

RAD analysis and Stacks pipeline

Sequence data were analysed using STACKS, version 1.40 (Catchen et al. 2013). All computations were done using the CSC - IT Center for Science Ltd clusters in Finland. Demultiplexing, cleaning (-c) and quality filtering (-q) was done using the *process_radtags* function. Since barcodes were of different length (5-13bp), we trimmed all reads to 85 bp. *Denovo* pipeline (combination of 3 functions) first assembled putative ortholog tags (*ustacks*).

To assess the best parameters to use in STACKS (Catchen et al. 2013), we used the r80 method as proposed by Paris et al. (2017). We ran iterations of STACKS by varying one parameter at a time and by fixing the other values to default (Table S1). Finally STACKS was run with the optimised parameters. The minimum depth of coverage was -m 3 for the creation of stacks. The maximum nucleotide mismatch we allowed between stacks was -M 2. When a catalogue was created using *cstacks*, we allowed -n 2 mismatches between the sample tags. Individual RAD loci were then matched to the catalogue (*sstacks*). The *Populations* function was used to obtain the population genetics statistics. Extensive filtering was performed in *R* 3.3.1 (The R Foundation) on a data-driven platform, using the *stackr* v0.5.7 (Gosselin and Bernatchez 2016) and *grur* v0.0.3 (Gosselin 2017) packages. The minimum number of population where a locus had to be present to be processed was 9 (-p) and minimum number of individuals per population where a locus had to be present was 60 %. Individuals with more than 30 % of missing data and markers with more than 20 % missing data were blacklisted. Lower threshold for individual heterozygosity was 0.06 and upper threshold was 0.13. To remove homeologous alleles, the maximum heterozygosity for the loci was set to 0.5 (Hohenlohe et al. 2011). The minor allele frequency (maf) was filtered at both local and global scale. In order to be kept, a locus had to present a maf of 0.01 at the local and of 0.005 at the global scale. A locus had to be present in at least one population for this filter. Afterwards, loci that were under Hardy-Weinberg equilibrium as defined by *P*-value threshold 0.05 in at least 6 populations were retained (HW tests made in *R*, using *pegas* (v0.10; Paradis 2010) package).

Genotyping and SNP filtering

An average of 278M reads (range 160-340 million) per library were kept after quality filtering (Table S2, Table S3). The average coverage depth per individual after filtering was

53.21x. Based on 10 replicate individuals, genotype error rate at the SNP level was 6 percent. Initially, 31669 putative SNPs were identified. After filtering, 3 972 SNPs and 284 individuals (Table 1) were kept as the final and filtered dataset. Identity-by-missingness analysis was performed on *grur* v0.0.3 (Gosselin 2017) and no clustering bias due to missing data was revealed (Fig. S1).

UPGMA tree and DAPC

Pairwise F_{ST} s were obtained using the *diffCalc* function in the *diveRsity* package (v1.9.89; Keenan et al. 2013) with default parameters. We compiled a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree using the *upgma* (default parameters) function in the *phangorn* v2.0.4 (Schliep 2011) package. The obtained tree was then plotted using Figtree v1.4 (Rambault 2012). Population clustering and structure was analysed using the *adegenet* v2.0.1 (Jombart 2008; Jombart and Ahmed 2011) package. A discriminant principal component analysis (DAPC, Jombart et al. 2010) was performed to form dimensions that capture most of the variation among populations. The number of principal components used for the DAPC was found through cross validation using 1000 permutations from 5 to 100 PC (*xval* function, all other parameters set to default). Cross-validation was ran along with the *tab* function from *adegenet* (*Na.method* = "mean", rest of the parameters to default). 5 runs of cross validation were made in order to get the most consistent possible results. Results indicated that past 14 principle components, the mean successful assignment value reached a plateau where values became stable and were only fluctuating from 83.5 to 84.5. Square rooted errors for assignment followed the same pattern as they reached a plateau at the same value. As we wanted to avoid overfitting of the model using too many components, we chose the lowest value of this plateau - as recommended by Jombart et al.

(2010) - and settled for 14 principal components. Using *adegenet* package, we calculated membership probabilities according to the DAPC to assess distinctiveness among the clusters. DAPC itself was ran with 14 principal components, 5 dimensions and the rest of the parameters were set to default.

Expected heterozygosity

Expected heterozygosity (H_e) was calculated for each inferred population using *adegenet* package (v.2.0.1; Jombart et al. 2010). Based on results from the DAPC, two clusters were then compared for differences in H_e . The first group consisted of four main stems and the second one of the seven tributaries. A second comparison was made without Brooks Raatepuro and Merenoja as they were not genetically distinct enough from the main stem populations according to the DAPC. Both comparisons were done using a Monte Carlo test (999 replications) with a P -value threshold of 0.05.

Parentage inference and effective population size

To infer potential parental and sibship relationships within the samples as well as calculate effective population sizes, we used *COLONY* v2.0.6.2 (Jones and Wang 2010) to analyse each population. Analysis was run using the polygamous mating system prior, the outbreeding model prior and a weak sibship prior on a medium run length. All parents and male/female priors were set to 0. Rest of the parameters were set to default. For comparison, effective population sizes were estimated also based on linkage disequilibrium as in Waples and Do (2008) using the *Nestimator* v2.01 software (Do et al. 2014). *Nestimator* v2.01 was set with all the default values. Effective sizes were compared using a Mann-Whitney test with the *stats* package in R.

Analysis of admixture using *Structure*

We used *Structure*, v2.3.4 (Pritchard et al. 2000; Falush et al. 2007) using the whole dataset. We used the admixture model as an ancestry model. We did not use any previous information about populations (no LOCPRIOR). All the other parameters were set to default values. We ran 20 replicates with a burn in time of 200'000 and 200'000 MCMC iterations according to the recommendations of Porras-Hurtado et al. (2013). As our goal was to investigate the genomic patterns and the population structure associated with different life-histories, we set $K = 11$ as a prior as this reflected our sampling locations and their putative life-history strategies. Results were clustered using *Chumpp* v1.1.2 (Jakobsson and Rosenberg 2007) and plotted using *distruct* v1.1 (Rosenberg 2004).

Results

Population genetic structuring, heterozygosity and effective population sizes

All of the 11 sampling sites showed significant genetic divergence from each other based on F -statistics (Table 2, Fig. 2) and therefore formed separate populations. The average estimated F_{ST} -value across all populations was 0.145. The lowest F_{ST} -value (0.014) was observed between the River Kuusinki and its easily accessible tributary (Brook Raatepuro) while the highest value (0.358) was observed between the two geographically distinct headwaters (Brook Pesospuro and River Maaninkajoki).

Certain river populations showed high F_{ST} -values indicating considerable genetic distances from the others (Table 2, Fig. 2). In particular, the River Juumajoki population differed from the migratory main stem River Kuusinkijoki fish; fish from Brook Pesospuro differed from the migratory fish in the main stem River Kitkajoki, and fish from Rivers Maaninkajoki and Astumajoki differed from the main stem River Oulankajoki migratory

individuals. Migratory hatchery stock originating from the upper River Kitkajoki also showed a clear genetic divergence from the others (Fig. 2).

Migratory main stem populations showed significantly higher genetic diversity than the tributary populations (MCMC permutation test, P -value = 0.01; Fig. 3). However, estimated effective population size N_e (6 – 1300) did not differ between the main stem and tributary populations (Mann-Whitney's test, P = 0.32 for *COLONY* results; P = 0.23 for Nestimator results, Table S4) based on analysis of sibship relationships (range: 15 – 1300 vs. 6 – 137) and linkage disequilibrium (range : 15 – 92 vs. 15 – 66).

Population clustering

Based on the UPGMA-tree (Fig. 2), the majority of the headwater populations formed unique branches. Yet, four populations, the three main stems and the Brook Raatepuro, clustered together (Fig. 2). The main stem River Oulanka was at the base of this cluster while the River Kuusinki and its tributary Raatepuro were forming a branch together. Strikingly, Merenoja and Kirintöjoki-Lohijoki populations were grouped together despite representing geographically distant locations (Fig. 2).

Discriminant principal component analysis (DAPC) suggested clear genetic isolation for four sites on two axes corroborating the UPGMA and F_{ST} -based results (Fig. 4). Hatchery stock originating from the upper River Kitka (above the Jyrävä waterfall) differed from the lower River Kitka on the axes one and three (Fig. S2). The upper River Kitka hatchery stock was separated from all the other sampled sites while the pooled Kirintöjoki-Lohijoki hatchery stock also showed slight signs of differentiation from the other populations on these axes (Fig. S2). The other populations were not clearly differentiated on the five first axes.

Posterior assignments based on principle components from the DAPC showed that five of the studied sites (Juumajoki, Pesospuro, Maaninkajoki, Astumajoki tributaries and Kitkajoki - above falls main stem) exhibit high, over 90 % re-assignment, values (Table S5,

Fig. 5) indicating clear-cut genetic differentiation from the other samples. The Kirintöjoki-Lohijoki hatchery individuals had a relatively high re-assignment value as well with 82 % correct reassignment. On the other hand, migratory Kitkajoki-below individuals showed a medium number of miss-alignment while miss-assigned individuals were highly observed among the migratory Kuusinkijoki, Oulankajoki, and the Raatepuro and Merenoja sites with unknown migration tendency, indicating either natural or stocking-induced admixture.

Admixture

Structure analysis revealed six clear clusters consisting of five tributaries and one main stem group (Fig. 6). The other rivers did not form clear clusters even though some genetic differences among them were present (Fig. 6). In the Kuusinki watershed, the main stem population showed extensive admixture with the River Kitka and River Oulanka populations as well as with its tributary Raatepuro. On the other hand, the River Juumajoki population was well separated and showed a high level of genetic differentiation corroborating the results based on DAPC. In the Kitka watershed, the Brook Pesospuro population showed extreme genetic isolation from the main stem. Above the Jyrävä waterfall, the hatchery populations originating from River Kitka and Rivers Kirintöjoki and Lohijoki were clearly clustered and formed two distinct units. In the Oulanka watershed, only the Merenoja individuals were genetically similar to the main stem while the two others (Maaninkajoki and Astumajoki) were well separated and genetically different from the others.

Discussion

By comparing genetic variation and divergence among brown trout sampled in eleven locations using 3972 SNPs obtained through ddRADSeq, we found that the main stem populations showed significantly higher genetic diversity and admixture than the headwater fish that mainly formed strongly isolated gene pools. Thus, our results suggest that the headwater populations are resident and reproductively isolated from the downstream migratory populations that appear to maintain their greater genetic homogeneity due to feeding migration and occasional exchange of spawners between the rivers. Because the three main stem populations showed smaller genetic differences than the other population pairs (see also Huusko et al. 1990), feeding migration to a lake appeared to induce a small rate of population mixing in the same manner as the sea migration has been found to maintain genetic connectivity among anadromous brown trout populations (Hansen and Mensberg 1998). On the other hand, precise natal homing observed by tagging studies (Huusko et al. 1990) has been enough to create and maintain genetic differentiation among the main stem populations.

Genetic variation with respect to migration strategy

While genetic differences related to the different migration patterns in brown trout have been intensively discussed (e.g. Skaala and Nævdal 1989; Carlsson and Nilsson 2000; Charles et al. 2006; Vøllestad 2017) and demonstrated by transplantation experiments (Jonsson 1982), effects of migration at the genomic level had not yet been elucidated. In this study, we showed how the genetic variation at genome level may reflect the differential migratory strategies among populations within a watershed. Even though we did not have direct movement data on the sampled headwater populations, observations during electrofishing surveys suggested that the fish in these genetically isolated and distinct headwater populations mature at small size and most likely remain resident for their whole

life. Thus, residency might be a mechanism that facilitates local adaptation, and as such, resident fish might be better adapted to their local, typically cold and resource poor environment than the migrants that may be better adapted to the warmer and more resource-rich large river environment: a hypothesis to be tested by a reciprocal transplantation of juveniles.

There were two exceptions to the rule: Brook Raatepuro and Brook Merenoja brown trout showed genetic admixture with the main stem populations. This suggests that these brooks are either used for spawning by the main stem migratory individuals or that offspring of migratory parents use these brooks as parr habitats. However, whether these brooks naturally support migratory populations can be questioned (c.f. Hansen et al. 2000_b) as the importance of small brooks for the spawning of large adfluvial trout remains controversial (Huusko et al. 2017). At least the lower stretches of Brook Raatepuro had been stocked with hatchery-reared River Kuusinki fish since 1990's. While the samples used in this study did not include any adipose-fin clipped fish (hatchery-reared fish are fin-clipped), we cannot exclude the possibility that they were offspring of fish with hatchery-origin.

Genetic variation with respect to river characteristics

The studied main stem rivers differ significantly in their size, gradient and other characteristics, and anecdotal evidence suggests that brown trout typically differ in the timing of spawning migration between these rivers with River Oulanka (also the longest) fish ascending first. Thus, it was not a surprise that even the main stem populations showed small but significant level of genetic differentiation. The genetic differentiation between the main stem rivers is allowed by highly precise natal homing (Huusko et al. 1990). However, radiotracking studies in salmonids have typically shown that homing is not 100 % accurate, which can create admixture and genetic connectivity among rivers (Junge et al. 2014). Brown

trout ascending for spawning are known to search for specific spawning habitat types (Saraniemi et al. 2008); hence, constraints in the availability of suitable spawning habitats might also create contact zones and furthermore potential for genetic exchange especially between the main stem and tributary fish. Some brown trout also spawn in the lower stretches of Rivers Oulanka and Olanga creating a potential mixing zone.

Pettersson et al. (2001) suggested that despite a clear genetic difference between headwater resident and downstream partially migrating population, even very low level of downstream gene-flow from resident headwater populations might contribute to the maintenance of resident strategy in partially migrating downstream populations. While we did not observe any direct indications of downstream gene flow, it is puzzling what maintains genetic structuring in headwaters without any migration barriers, as spawning with the closest neighbour is expected to erode genetic structuring (c.f. Moore et al. 2014). Within-river study of Carlsson et al. (1999) suggested that significant, temporally stable genetic differences within a range of only 3 km may be present in brown trout indicating that apparently sympatric populations might in fact be reproductively isolated, potentially due to behavioral reasons that are not yet fully understood (Kraabøl et al. 2013).

Impact of landscape topology on the observed genetic structure

The studied Koutajoki watershed is a dendritic environment as well as most riverine brown trout environments in general. It is common that populations are genetically structured in accordance with the landscape topology. The geological history and exceptional events such as glaciation and land uplifting - which can isolate populations - also play a role in the genetic clustering (e.g Poissant et al. 2005; Guy et al. 2008; Vøllestad 2017). Typically, genetic structuring is evaluated using various differentiation measures, such as pairwise genetic differences (F_{ST}). The values obtained in this study (Table 2) were high compared to

other studies on brown trout. The mean F_{ST} value of 0.145 was higher than the average of 0.078 that Vøllestad (2017) calculated upon 1112 brown trout studies based on different markers such as microsatellites or amplified fragment length polymorphism (AFLP).

The DAPC was used as an explorative analysis here but it revealed similar information as F -statistics. Bayesian analysis in *Structure* revealed further details on the genetic clustering among the studied populations. For example, in the River Kuusinki watershed, Juumajoki population was clearly different from the main stem while the Raatepuro samples were not distinguished from it despite no obvious differences in the size or steepness between these two small rivers. However, parr samples were collected from the headwaters of the River Juumajoki, while the Raatepuro samples were obtained closer to its opening to River Kuusinki. Thus, we cannot definitely conclude whether the fish in Raatepuro are genetically similar with the main stem.

River Kitka populations below and above the Jyrävä Falls have been suggested to originate from different ancestral populations (Heikkinen and Kurimo 1977; Koutaniemi 1999). Pesospuro tributary population likely also originates from the same gene pool as the downstream River Kitka population. Above the Jyrävä falls, the two hatchery populations (River Kitka vs. River Lohijoki/Kirintöjoki) displayed considerable genetic differences between each other suggesting that there has been no natural gene flow between these populations.

The hatchery-maintained above-waterfall River Kitka population performs an exceptional upstream smolt migration towards the Lake Kitka. Populations that descend to downstream rapids for spawning have been known also elsewhere in Finland but are now mostly extinct (Syrjänen et al. 2017). The genetic differentiation between the two River Kitka populations with distinct smolt migration direction was surprisingly low ($F_{ST} = 0.074$) despite a physical barrier preventing bi-directional genetic exchange and the speculated completely

different evolutionary origin of these populations. In comparison, Whiteley et al (2010) found a mean pairwise F_{ST} of 0.28 for above-below waterfall barriers populations pairs of coastal cutthroat trout (*Oncorhynchus clarkii clarkii*). However, the UPGMA-tree (Fig. 2) aligned well with the results of both Gomez-Uchida et al. (2009) and Whiteley et al. (2010), where below waterfall populations were closer to each other than with the upstream populations; but also with previous studies from Huusko et al. (1990) as the upper River Kitka population was found further than the River Kuusinki or Oulanka populations. Altogether, the small F_{ST} between these populations with very different migratory patterns questions the formerly presented hypothesis of their completely different origin (Baltic Sea vs. White Sea).

Below the Jyrävä falls in River Kitka, the Pesospuro population was clearly isolated from the main stem fish. While large migratory trout likely cannot go upstream in the Brook Pesospuro, any smolts could easily migrate downstream and eventually interbreed with River Kitka migrants. The clear genetic divergence ($F_{ST} = 0.214$) suggested that either a very low number of smolts leave this brook or that the population consists to a large extent of resident individuals.

In the Oulankajoki watershed, the two upstream tributary populations in Rivers Maaninkajoki and Astumajoki were isolated from the main stem despite tagging studies having revealed that ascending spawners enter at least the lower stretches of River Maaninkajoki (Saraniemi 2005). However, the more downstream Merenoja population showed admixture signal with the population from the main stem. The divergence of genetic patterns between these tributaries may reflect their connection with the main stem population, as the downstream tributary is more accessible for migratory individuals due to the Kiutaköngas waterfalls in between. The small resident populations may continue contemporary divergence from the main stem population as they are more prone to genetic drift, loss of alleles and inbreeding (Wang et al. 2002; Sato and Harada 2008).

To ensure we sampled migratory individuals in the main stem rivers, we exclusively sampled adults on their way to their spawning grounds. In contrast, the sampling of parr in tributaries may incur a risk of high representation by certain families (Hansen et al. 1997; Ozerov et al. 2015) but it has recently been suggested that siblings should not be purged from population genetic datasets (Waples and Anderson 2017). The sibship analysis confirmed the lack of family bias between tributary and main stem populations as the sampling was spread over relatively long stretches in each river. Interestingly, the number of siblings per population was also lower in our data than in previous studies (Carlsson 2007). Potential exceptions were Rivers Juumajoki, Maaninkajoki and Oulanka, which appeared to contain a considerable proportion of siblings (Table S4), probably indicating the small number of spawners in these rivers.

Implications for conservation

A good understanding of genetic population structure and processes influencing population connectivity is essential for the conservation and evolutionarily enlightened management of natural populations (He et al. 2016). A large number of SNPs, particularly through genome-wide scans, is a particularly informative and important information source for conservation (Morin et al. 2009). Migratory brown trout in main stem rivers showed a higher level of heterozygosity than the brown trout in headwaters. This was in agreement with the general observation that main stem populations display higher level of genetic variation (Vøllestad 2017). However, the mean values for the expected heterozygosity for both migratory ($0.139 \pm 0.021 \sigma$) and resident ($0.099 \pm 0.017 \sigma$) trout were lower than previous studies conducted with different markers as Vøllestad (2017) found values of $0.345 \pm 0.105 \sigma$ and $0.206 \pm 0.105 \sigma$ for the migratory and resident brown trout, respectively. The tentative estimates of effective population sizes did not differ between main stems and

tributaries, which may alarmingly indicate the poor status of the migratory populations that currently consist of at maximum some hundreds of spawners or at maximum some tens of individuals in River Oulanka. Worryingly, also the expected heterozygosity of the hatchery strain originating from the upper River Kitka (above Jyrävä) was at a low level and comparable to the sampled small headwater populations. Typically, a small number of spawners are used for artificial reproduction compared to natural conditions (Franklin 1980). Thus, captive breeding reduces heterozygosity extremely fast (Christie et al. 2012, Rollinson et al. 2014).

Implications for management

Finnish Kuusamo-Salla area and its neighbouring areas in Russia attract thousands of recreational anglers that target the iconic, large (typically 2-8 kg) migratory trout during their spawning migration into the rivers. Recently, also the trolling fishery has increased during the feeding migration on Russian lakes, and the fishing pressure is close to critical for the sustainability (Syrjänen et al. 2017). Based on a recent modelling study (Syrjänen et al. 2017), the number of spawning individuals in Koutajoki watershed before the intensive fishing was likely several thousands. However, the current spawning population is only some tens of individuals in River Oulanka, and a few hundred in Rivers Kitka and Kuusinki. Combined with our results, this information bears a strong message for the management: to maintain the original genetic diversity both within and among rivers the brown trout fisheries must be turned sustainable by reducing the mortality of both spawners and feeding fish. On the other hand, our results suggest that some resident populations could be managed using a considerably lower minimum size limit than the migratory populations, as fishing the resident fish does not interfere with the migratory stocks.

Previously, stockings with hatchery-reared fish have been found to impact the gene pool of resident brown trout more than that of the migratory trout due to the strong selection against hatchery-reared individuals during the feeding migration (Hansen et al. 2000_b). Our results suggest that past stockings, sometimes using non-native fish, have probably not yet eroded the likely original genetic structuring with the potential exception of Brook Raatepuro. However, stockings are not encouraged in the system. Instead, the critically endangered above-waterfall population of River Kitka should be allowed to recover through natural reproduction before the genetic variation is critically lost in the hatchery brood stock.

For the management and conservation of brown trout populations in general, our results, obtained using thousands of markers, confirm that each brown trout sub-population is a unique genetic unit. Thus, declining or even lost populations cannot be readily recovered by stocking fish from any other population, even when located geographically very close. This highlights the importance of using only native fish in any supportive stockings. Our study confirmed the presence of fine-scaled population genetic patterns generally found in brown trout by microsatellite analyses but also identified thousands of SNPs that could bear signatures of selection on ecologically important traits.

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Acknowledgements

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This study (materials, A.L. and A.V.) was funded by the Academy of Finland (decision #286261). S. Uusi-Heikkilä was funded by the AXA Research Fund and A. Vasemägi by the Estonian Ministry of Education and Research (target financed project no. SF1080022s07 and institutional research funding project IUT8-2), and the Academy of Finland. N. Alioravainen and A. Hatanpää are acknowledged for their constructive comments on the manuscript.

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References

[667](#)

[668](#)

Allendorf, F., Ryman N., Stennek, A., and Ståhl, G. 1976. Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations. *Hereditas*, **83**: 73-82.

[669](#)

[670](#)

[671](#)

Andrews, K.R., and Luikart, G. 2014. Recent novel approaches for population genomics data analysis. *Mol. Ecol.* **23**(7): 1661–1667. doi:10.1111/mec.12686.

[672](#)

- [673](#) Andrews, K.R., Good, J.M., Miller, M.R., Luikart, G., and Hohenlohe, P.A. 2016. Harnessing
[674](#) the power of RADseq for ecological and evolutionary genomics. *Nat Rev*
[675](#) *Genet* **17**(2): 81–92. doi:10.1038/nrg.2015.28.
- [676](#) Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U.,
[677](#) Cresko, W.A., and Johnson, E.A. 2008. Rapid SNP Discovery and Genetic
[678](#) Mapping Using Sequenced RAD Markers. *PLoS One* **3**(10): e3376.
[679](#) doi:10.1371/journal.pone.0003376.
- [680](#) Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., and Bernatchez, L.
[681](#) 2015. RAD genotyping reveals fine-scale genetic structuring and provides
[682](#) powerful population assignment in a widely distributed marine species, the
[683](#) American lobster (*Homarus americanus*). *Mol. Ecol.* **24**(13): 3299–3315.
[684](#) doi:10.1111/mec.13245.
- [685](#) Bruneaux, M., Johnston, S.E., Herczeg, G., Merilä, J., Primmer, C.R., and Vasemägi, A.
[686](#) 2013. Molecular evolutionary and population genomic analysis of the nine-
[687](#) spined stickleback using a modified restriction-site-associated DNA tag
[688](#) approach. *Mol. Ecol.* **22**: 565–582. doi:10.1111/j.1365-294X.2012.05749.x.
- [689](#) Carlsson, J., Olsén, K.H., Nilsson, J., Øverli, Ø., and Stabell, O.B. 1999. Microsatellites
[690](#) reveal fine-scale genetic structure in stream-living brown trout. *J. Fish Biol.*
[691](#) **55**: 1290-1303.
- [692](#) Carlsson, J., and Nilsson, J. 2000. Population genetic structure of brown trout. *Hereditas*,
[693](#) **132**: 173–181. doi:10.1111/j.1601-5223.2000.00173.x.
- [694](#) Carlsson, J. 2007. The effect of family structure on the likelihood for kin-biased distribution :
[695](#) an empirical study of brown trout populations. *J. Fish Biol.* **71**: 98–110.
[696](#) doi:10.1111/j.1095-8649.2007.01546.x.

- [697](#) Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., and Cresko, W. A. 2013. Stacks: an
[698](#) analysis tool set for population genomics. *Mol. Ecol.* **22**: 3124–3140.
[699](#) doi:10.1111/mec.12354
- [700](#) Charles, K., Guyomard, R., Hoyheim, B., Ombredane, D., and Baglinière, J. 2005. Lack of
[701](#) genetic differentiation between anadromous and resident sympatric brown
[702](#) trout (*Salmo trutta*) in a Normandy population. *Aquat. Living Resour.* **18**: 65–
[703](#) 69. doi:10.1051/alr.
- [704](#) Charles, K., Roussel, J-M., Lebel, J-M., and Baglinière, J-L. 2006. Genetic differentiation
[705](#) between anadromous and freshwater resident brown trout (*Salmo trutta* L.):
[706](#) insights obtained from stable isotope analysis. *Ecol. of Freshw. Fish*, **15**: 255-
[707](#) 263.
- [708](#) Christie, M.R., Marine, M.L., French, R.A., and Blouin, M.S. 2012. Genetic adaptation to
[709](#) captivity can occur in a single generation. *Proc. Natl. Acad. Sci.* **109**(1).
[710](#) doi:10.1073/pnas.1111073109.
- [711](#) Davey, J.W., and Blaxter, M.L. 2010. RADSeq: next-generation population genetics. *Brief.*
[712](#) *Funct. Genomics* **9**(5–6): 416–423. doi:10.1093/bfgp/elq031.
- [713](#) Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., and Blaxter, M.L.
[714](#) 2011. Genome-wide genetic marker discovery and genotyping using next-
[715](#) generation sequencing. *Nat. Rev. Genet.* **12**(7): 499–510.
[716](#) doi:10.1038/nrg3012.
- [717](#) Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., and Ovenden, J. R. 2014.
[718](#) NeEstimator V2: re-implementation of software for the estimation of
[719](#) contemporary effective population size (N_e) from genetic data. *Mol. Ecol.*
[720](#) *Resour.* **14**: 209-214.
- [721](#) Doctor, K., Berejikian, B., Hard, J.J., and Van Doornik, D. 2014. Growth-mediated

- [722](#) life history traits of steelhead reveal phenotypic divergence and plastic
[723](#) response to temperature. *Trans. Am. Fish. Soc.* **143**: 317–333. doi:10.1080/
[724](#) 00028487.2013.849617.
- [725](#) Dodson, J.J., Aubin-Horth, N., Thériault, V., and Páez, D.J. 2013. The evolutionary ecology
[726](#) of alternative migratory tactics in salmonid fishes. *Biol. Rev.* **88**(3): 602–625.
[727](#) doi:10.1111/brv.12019.
- [728](#) Etter, P.D., Bassham, S., Hohenlohe, P.A., Johnson, E.A., and Cresko, W.A. 2011. Molecular
[729](#) Methods for Evolutionary Genetics. *Methods Mol Biol.* **772**(2): 1–19.
[730](#) doi:10.1007/978-1-61779-228-1.
- [731](#) Falush, D., Stephens, M., and Pritchard, J.K. 2007. Inference of population structure using
[732](#) multilocus genotype data: Dominant markers and null alleles. *Mol. Ecol.*
[733](#) *Notes.* **7**(4): 574–578. doi:10.1111/j.1471-8286.2007.01758.x.
- [734](#) Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135 – 140 in Soule, M.
[735](#) E. and Wilcox, B. A. (eds.), *Conservation Biology: An Evolutionary -*
[736](#) *Ecological Perspective*. Sunderland, Mass.: Sinauer Associates.
- [737](#) Fumagalli, M., Vieira, F.G., Korneliussen, T.S., Linderöth, T., Huerta-Sánchez, E.,
[738](#) Albrechtsen, A., and Nielsen, R. 2013. Quantifying population genetic
[739](#) differentiation from next-generation sequencing data. *Genetics*, **195**(3): 979–
[740](#) 992. doi:10.1534/genetics.113.154740.
- [741](#) García-Marín, J.L., Utter, F., and Pla, C. 1999. Postglacial colonization of brown trout in
[742](#) Europa based on distribution of allozyme variants. *Heredity*, **82**: 46–56.
- [743](#) Giger, T., Excoffier, L., Day, P.J.R., Champigneulle, A., Hansen, M.M., Powell, R., and
[744](#) Largiadèr, C.R. 2006. Life history shapes gene expression in salmonids. *Curr.*
[745](#) *Biol.* **16**(8): 281–282. doi:10.1016/j.cub.2006.03.053.

- [746](#) Gomez-Uchida, D., W, Knight, T.W., and Ruzzante, D.E. 2009. Interaction of landscape and
[747](#) life history attributes on genetic diversity, neutral divergence and gene flow in
[748](#) a pristine community of salmonids. *Mol. Ecol.* **18**: 4854–4869.
[749](#) doi:10.1111/j.1365-294X.2009.04409.x.
- [750](#) Gosselin, T., and Bernatchez, L. 2016. stackr: GBS/RAD Data Exploration, Manipulation
[751](#) and Visualization using R. R package version 0.4.6.
[752](#) <https://github.com/thierrygosselin/stackr>. doi:doi :10.5281/zenodo.154432.
- [753](#) Gosselin, T. 2017. grur: an R package tailored for RADseq data imputations. R package
[754](#) version 0.0.1. doi:10.5281/zenodo.496176.
- [755](#) Griffiths, A.M., Koizumi, I., Bright, D., and Stevens, J.R. 2009. A case of isolation by
[756](#) distance and short-term temporal stability of population structure in brown
[757](#) trout (*Salmo trutta*) within the River Dart, southwest England. *Evol. Appl.*
[758](#) **2**(4): 537–554. doi:10.1111/j.1752-4571.2009.00092.x.
- [759](#) Groot, C., Bailey, R.E., Margolis, L., and Cooke, K. 1989. Migratory patterns of sockeye
[760](#) salmon (*Oncorhynchus nerka*) smolts in the Strait of Georgia, British
[761](#) Columbia, as determined by analysis of parasite assemblages. *Can. J. Zool.* **67**:
[762](#) 1670–8.
- [763](#) Guy, T.J., Gresswell, R.E., and Banks, M.A. 2008. Landscape-scale evaluation of genetic
[764](#) structure among barrier-isolated populations of coastal cutthroat trout,
[765](#) *Oncorhynchus clarkii clarkii*. *Can. J. Fish. Aquat. Sci.* **65**: 1749–1762.
[766](#) doi:10.1139/F08-090.
- [767](#) Hale, M.C., Thrower, F.P., Berntson, E.A., Miller, M.R., and Nichols, K.M. 2013. Evaluating
[768](#) Adaptive Divergence Between Migratory and Nonmigratory Ecotypes of a

- [769](#) Salmonid Fish, *Oncorhynchus mykiss*. *G3 Genes Genomes Genet.* **3**(8): 1273–
[770](#) 1285. doi:10.1534/g3.113.006817.
- [771](#) Hansen, M.M., Nielsen, E.E., and Mensberg, K-L.D. 1997. The problem of sampling families
[772](#) rather than populations: relatedness among individuals in samples of juvenile
[773](#) brown trout *Salmo trutta* L. *Mol. Ecol.* **6**(5): 469–474. doi:10.1046/j.1365-
[774](#) 294X.1997.t01-1-00202.x.
- [775](#) Hansen, M.M., and Mensberg, K-L.D. 1998. Genetic differentiation and relationship between
[776](#) genetic and geographical distance in Danish sea trout (*Salmo trutta* L.)
[777](#) populations. *Heredity*, **81**: 493-504.
- [778](#) Hansen, M.M., Nielsen, E.E., Ruzzante, D.E., Bouza, C., and Mensberg, K-L.D. 2000_a.
[779](#) Genetic monitoring of supportive breeding in brown trout (*Salmo trutta* L.),
[780](#) using microsatellite DNA markers. *Can. J. Fish. Aquat. Sci.* **57**(10): 2130–
[781](#) 2139. doi:10.1139/cjfas-57-10-2130.
- [782](#) Hansen, M.M., Ruzzante, D.E., Nielsen, E.E., and Mensberg, K-L.D. 2000_b. Microsatellite
[783](#) and mitochondrial DNA polymorphism reveals life-history dependent
[784](#) interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Mol.*
[785](#) *Ecol.* **9**: 583-594.
- [786](#) Hansen, M.M., Ruzzante, D.E., Nielsen, E.E., Bekkevold, D., and Mensberg, K-L.D. 2002.
[787](#) Long-term effective population sizes, temporal stability of genetic
[788](#) composition and potential for local adaptation in anadromous brown trout
[789](#) (*Salmo trutta*) populations. *Mol. Ecol.* **11**: 2523-2535.
- [790](#) Harris, G., and Milner, N. 2007. Frontmatter, in *Sea Trout: Biology, Conservation and*
[791](#) *Management*, Blackwell Publishing Ltd, Oxford, UK.
[792](#) doi: 10.1002/9780470996027.fmatter

- [793](#) Harris, L.N., Bajno, R., Gallagher, C.P., Koizumi, I., Johnson, L.K., Howlan, K.L., Taylor,
[794](#) E.B., and Reist, J.D. 2015. Life-history characteristics and landscape attributes
[795](#) as drivers of genetic variation, gene flow, and fine-scale population structure
[796](#) in northern Dolly Varden (*Salvelinus malma malma*) in Canada. Can. J. Fish.
[797](#) Aquat. Sci. **72**(10): 1477–1493. doi:10.1139/cjfas-2015-0016.
- [798](#) He, Z., Johansson, M.L., and Heath, D.D. 2016. Role of genomics and transcriptomics in
[799](#) selection of reintroduction source populations. Conserv. Biol. **0**(0): 1–9.
[800](#) doi:10.1111/cobi.12674.This.
- [801](#) Hecht, B.C., Campbell, N.R., Holeced, D.E., and Narum, S.R. 2013. Genome-wide
[802](#) association reveals genetic basis for the propensity to migrate in wild
[803](#) populations of rainbow and steelhead trout. Mol. Ecol. **18**(9): 1199–1216.
[804](#) doi:10.1016/j.micinf.2011.07.011.Innate.
- [805](#) Heikkinen, O. and Kurimo, H. 1977. The postglacial history of Kitkajärvi, northeastern
[806](#) Finland, as indicated by trend-surface analysis and radio-carbon dating. Fennia,
[807](#) **153**: 1-32.
- [808](#) Helyar, S.J., Hemmer-Hansen, J., Bekkevold, D., Taylor, M.I., Ogden, R., Limborg, M.T.,
[809](#) Cariani, A., Maes, G.E., Diopere, E., Carvalho, G.R., and Nielsen, E.E. 2011.
[810](#) Application of SNPs for population genetics of nonmodel organisms: New
[811](#) opportunities and challenges. Mol. Ecol. Resour. **11**(SUPPL. 1): 123–136.
[812](#) doi:10.1111/j.1755-0998.2010.02943.x.
- [813](#) Hindar, K., Jonsson, B., Ryman, N., and Ståhl, G. 1991. Genetic relationships among
[814](#) landlocked, resident, and anadromous brown trout, *Salmo trutta* L. Heredity
[815](#) (Edinb). **66**(1): 83–91. doi:10.1038/hdy.1991.11.

- [816](#) Hohenlohe, P. A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E. A., and Cresko, W. A.
[817](#) 2010. Population genomics of parallel adaptation in threespine stickleback
[818](#) using sequenced RAD tags. PLoS Genet. **6**(2): e1000862.
[819](#) doi:10.1371/journal.pgen.1000862.
- [820](#) Hohenlohe, P.A., Amish, S.J., Catchen, J.M., Allendorf, F.W., and Luikart, G. 2011. Next-
[821](#) generation RAD sequencing identifies thousands of SNPs for assessing
[822](#) hybridization between rainbow and westslope cutthroat trout. Mol. Ecol.
[823](#) Resour. **11**(SUPPL. 1): 117–122. doi:10.1111/j.1755-0998.2010.02967.x.
- [824](#) Huusko, A. 1990. Fisheries and fish stocks in the Kuusinkijoki river system, northern Finland,
[825](#) with remarks on the adverse effects of a small hydropower station located on
[826](#) the upper reach of the river. Kalatutkimuksia - Fiskundersökningar Finnish
[827](#) Game and Fisheries Research Institute. Helsinki. Finland. **14**:1–238. (in
[828](#) Finnish)
- [829](#) Huusko, A., van der Meer, O., and Koljonen, M.-L. 1990. Life history patterns and genetic
[830](#) differences in brown trout (*Salmo trutta* L.) in the Koutajoki river system. Pol.
[831](#) Arch. Hydrobiol. **37**: 63-77.
- [832](#) Huusko, A., Jurvelius, J. and Louhimo, J. 1999. Characterizing the pelagic fish assemblage in
[833](#) Lake Paanajärvi: scarce stocks and large individuals. Fennia **177**(1): 36-43.
- [834](#) Huusko, A., Vainikka, A., Syrjänen, J.T., Orell, P., Louhi, P., and Vehanen, T. 2017. Life-
[835](#) history of the adfluvial brown trout (*Salmo trutta* L.) in eastern Fennoscandia.
[836](#) In: Lobon-Cervia, J. and Sanz, N (Eds.), Brown trout: Biology, Ecology and
[837](#) Management. Wiley, in press.
- [838](#) Jakobsson, M., and Rosenberg, N.A. 2007. CLUMPP: A cluster matching and permutation
[839](#) program for dealing with label switching and multimodality in analysis of

- [840](#) population structure. *Bioinformatics*, **23**(14): 1801–1806.
[841](#) doi:10.1093/bioinformatics/btm233.
- [842](#) Jombart, T. 2008. Adegnet: A R package for the multivariate analysis of genetic markers.
[843](#) *Bioinformatics*, **24**(11): 1403–1405. doi:10.1093/bioinformatics/btn129.
- [844](#) Jombart, T., Devillard, S., and Balloux, F. 2010. Discriminant analysis of principal
[845](#) components : a new method for the analysis of genetically structured
[846](#) populations. *BMC Genet.* **11**(95).
[847](#)
- [848](#) Jombart T., and Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide
[849](#) SNP data. *Bioinformatics*. doi: 10.1093/bioinformatics/btr521
- [850](#) Jones, O.R., and Wang, J. 2010. COLONY: A program for parentage and sibship inference
[851](#) from multilocus genotype data. *Mol. Ecol. Resour.* **10**(3): 551–555.
[852](#) doi:10.1111/j.1755-0998.2009.02787.x.
- [853](#) Jones, D.A., Bergman, E., and Greenberg, L. 2015. Food availability in spring affects
[854](#) smolting in brown trout. *Can. J. Fish. Aquat. Sci.* **6**(July): 1–6.
- [855](#) Jonsson, B. 1982. Diadromous and resident trout *Salmo trutta*: is their difference due to
[856](#) genetics? *Oikos*, **38**: 297-300.
- [857](#) Jonsson, B., and Jonsson, N. 2007. Life History of the Anadromous Trout *Salmo trutta*, in
[858](#) Sea Trout: Biology, Conservation and Management (eds G. Harris and N.
[859](#) Milner), Blackwell Publishing Ltd, Oxford, UK.
[860](#) doi: 10.1002/9780470996027.ch14

- [861](#) Junge, C., Museth, J., Hindar, K., Kraabøl, M., and Vøllestad, L.A. 2014. Assessing the
[862](#) consequences of habitat fragmentation for two migratory salmonid fishes.
[863](#) Aquat. Conserv. Mar. Freshw. Ecosyst. **24**(3): 297–311. doi:10.1002/aqc.2391.
- [864](#) Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., and Prod, P.A. 2013. diveRsity: An R
[865](#) package for the estimation and exploration of population genetics parameters
[866](#) and their associated errors. : 782–788. doi:10.1111/2041-210X.12067
- [867](#) Keller, I., Wagner, C.E., Greuter, L., Mwaiko, S., Selz, O.M., Sivasundar, A., Wittwer, S.,
[868](#) and Seehausen, O. 2013. Population genomic signatures of divergent
[869](#) adaptation, gene flow and hybrid speciation in the rapid radiation of Lake
[870](#) Victoria cichlid fishes. Mol. Ecol. **22**: 2848–2863. doi:10.1111/mec.12083.
- [871](#) Kendall, N.W., Mcmillan, J.R., Sloat, M.R., Buehrens, T.W., Quinn, T.P., Pess, G.R.,
[872](#) Kuzishchin, K. V, McClure, M.M., and Zabel, R.W. 2015. Anadromy and
[873](#) residency in steelhead and rainbow trout (*Oncorhynchus mykiss*): a review of
[874](#) the processes and patterns. Can. J. Fish. Aquat. Sci. **342**(November 2014):
[875](#) 319–342. doi:10.1139/cjfas-2014-0192.
- [876](#) Keränen, M. 1978. Migrations and population structure of adfluvial brown trout, *Salmo trutta*
[877](#) L. in the Lake Kitka Basin. Licentiate thesis. 69p. Department of Zoology,
[878](#) University of Oulu, Oulu, Finland (in Finnish).
- [879](#) Koutaniemi, L. 1999. Physical characteristics and palaeogeography of the Oulanka-
[880](#) Paanajärvi region on the Finnish-Karelian border. Fennia, **177**: 3-7.
- [881](#) Kraabøl, M., Johnsen, S.I., Museth, J., Skurdal, J., and Dervo, B. 2013. Telemetristudie av
[882](#) ørret i Hemsil - Kartlegging av leveområder, effekter av fang-ogslipp fiske og
[883](#) kraftverkstekniske inngrep i vassdraget. Research report. (In Norwegian)

- [884](#) Kuosku, K., Kauppila, P., and Karjalainen, T.P. 2014. Regional economic impacts of fishing
[885](#) tourism on the rivers Kitkajoki, Kuusinkijoki and Oulankajoki. Riista- ja
[886](#) kalatalous – Tutkimuksia ja selvityksiä 3/2014. 35 p. (In Finnish with English
[887](#) abstract).
- [888](#) Lao, O., van Duijn, K., Kersbergen, P., de Knijff, P., and Kayser, M. 2006. Proportioning
[889](#) whole-genome single-nucleotide-polymorphism diversity for the identification
[890](#) of geographic population structure and genetic ancestry. *Am. J. Hum. Genet.*
[891](#) **78**(4): 680–690. doi:10.1086/501531.
- [892](#) Lerceteau-Köhler, E., Schlieven, U., Kopun, T., and Weiss, S. 2013. Genetic variation in
[893](#) brown trout *Salmo trutta* across the Danube, Rhine, and Elbe headwaters: a
[894](#) failure of the phylogeographic paradigm? *BMC Evol. Biol.* **13**: 176.
[895](#) doi:10.1186/1471-2148-13-176.
- [896](#) Liu, N., Chen, L., Wang, S., Oh, C., and Zhao, H. 2005. Comparison of single-nucleotide
[897](#) polymorphisms and microsatellites in inference of population structure. *BMC*
[898](#) *Genet.* **6**(1): 26. doi:10.1186/1471-2156-6-S1-S26.
- [899](#) Mäki-Petäys, A., Huusko, A., and Mustonen, S. 2000. Management and monitoring of brown
[900](#) trout, grayling and landlocked salmon stocks in the river Oulankajoki and river
[901](#) Pistojoiki Basins in Kuusamo, northern Finland. Kala- ja riistaraportteja
[902](#) Finnish Game and Fisheries Research Institute. Helsinki. Finland. **178**: 1-18.
[903](#) (in Finnish).
- [904](#) Manousaki, T., Tsakogiannis, A., Taggart, J.B., Palaiokostas, C., Tsaparis, D., Lagnel, J.,
[905](#) Chatziplis, D., Magoulas, A., Papandroulakis, N., Mylonas, C.C., and
[906](#) Tsigenopoulos, C.S. 2016. Exploring a Non-model Teleost Genome Through
[907](#) RAD Sequencing - Linkage Mapping in Common Pandora, *Pagellus*

- [908](#) *erythrinus* and Comparative Genomic Analysis. G3 Genes Genomes Genet.
[909](#) **6**(March): g3.115.023432-. doi:10.1534/g3.115.023432.
- [910](#) Mardis, E.R. 2008. The impact of next-generation sequencing technology on genetics. Trends
[911](#) Genet. **24**(3): 133–141. doi:10.1016/j.tig.2007.12.007.
- [912](#) Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A., and Johnson, E.A. 2007. Rapid and
[913](#) cost-effective polymorphism identification and genotyping using restriction
[914](#) site associated DNA (RAD) markers. Genome Res.: 240–248.
[915](#) doi:10.1101/gr.5681207
- [916](#) Moore, J.S., Loewen, T.N., Harris, L.N., and Tallman, R.F. 2014. Genetic analysis of
[917](#) sympatric migratory ecotypes of arctic charr *Salvelinus alpinus*: Alternative
[918](#) mating tactics or reproductively isolated strategies? J. Fish Biol. **84**(1): 145–
[919](#) 162. doi:10.1111/jfb.12262.
- [920](#) Morin, P.A., Martien, K.K., and Taylor, B.L. 2009. Assessing statistical power of SNPs for
[921](#) population structure and conservation studies. Mol. Ecol. Resour. **9**(1): 66–73.
[922](#) doi:10.1111/j.1755-0998.2008.02392.x.
- [923](#) Olsson, I.C., Greenberg, L.A., Bergman, E., and Wysujack, K. 2006. Environmentally
[924](#) induced migration: the importance of food. Ecol. Lett. **9**: 645–651. doi:
[925](#) 10.1111/j.1461-0248.2006.00909.x. PMID:16706909.
- [926](#) Östergren, J., and Nilsson, J. 2012. Importance of life-history and landscape characteristics
[927](#) for genetic structure and genetic diversity of brown trout (*Salmo trutta* L.).
[928](#) Ecol. Freshw. Fish, **21**: 119-133.
- [929](#) Ozerov, M., Jürgenstein, T., Aykanat, T., and Vasemägi, A. 2015. Use of sibling relationship
[930](#) reconstruction to complement traditional monitoring in fisheries management
[931](#) and conservation of brown trout. Conserv. Biol. **29**(4): 1164–1175.
[932](#) doi:10.1111/cobi.12480.

- [933](#) Paradis, E. 2010. pegas : an R package for population genetics with an integrated – modular
[934](#) approach. **26**(3): 419–420. doi:10.1093/bioinformatics/btp696.
- [935](#) Paris, J.R., Stevens, J.R., and Catchen, J.M. 2017. Lost in parameter space: A road map for
[936](#) stacks. *Methods Ecol. Evol.*, doi: 10.1111/2041-210X.12775.
- [937](#) Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., and Hoekstra, H.E. 2012. Double digest
[938](#) RADseq: An inexpensive method for de novo SNP discovery and genotyping
[939](#) in model and non-model species. *PLoS One* **7**(5).
[940](#) doi:10.1371/journal.pone.0037135.
- [941](#) Pettersson, J.C.E., Hansen, M.M., and Bohlin, T. 2001. Does dispersal from landlocked trout
[942](#) explain the coexistence of resident and migratory trout females in a small
[943](#) stream? *J. of Fish Biol.* **58**: 487-495.
- [944](#) Poissant, J., Knight, T.W., and Ferguson, M.M. 2005. Nonequilibrium conditions following
[945](#) landscape rearrangement : the relative contribution of past and current
[946](#) hydrological landscapes on the genetic structure of a stream-dwelling fish.
[947](#) *Mol. Ecol.* **14**: 1321–1331. doi:10.1111/j.1365-294X.2005.02500.x.
- [948](#) Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., and Lareu, M. V. 2013.
[949](#) An overview of STRUCTURE: Applications, parameter settings, and
[950](#) supporting software. *Front. Genet.* **4**(MAY). doi:10.3389/fgene.2013.00098.
- [951](#) Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using
[952](#) multilocus genotype data. *Genetics* **155**(2): 945–959. doi:10.1111/j.1471-
[953](#) 8286.2007.01758.x.
- [954](#) Pukk, L., Kisand, V., Ahmad, F., and Vasemägi, A. 2014. Double-restriction-site-associated
[955](#) DNA (ddRAD) approach for fast microsatellite marker development in

- [956](#) Eurasian perch (*Perca fluviatilis* L.). *Conserv. Genet. Resour.* **c**: 183–184.
- [957](#) doi:10.1111/eva.12060.
- [958](#) Pukk, L., Ahmad, F., Hasan, S., Kisand, V., Gross, R., and Vasemägi, A. 2015. Less is more :
- [959](#) extreme genome complexity reduction with ddRAD using Ion Torrent
- [960](#) semiconductor technology. *Mol. Ecol. Resour.* **15**: 1145–1152.
- [961](#) doi:10.1111/1755-0998.12392.
- [962](#) Quinn, T.P. 2005. The behavior and ecology of Pacific salmon and trout. University of
- [963](#) Washington Press, Seattle, Wash.
- [964](#) Quinn, T.P., and Myers, K.W. 2004. Anadromy and the marine migrations of Pacific salmon
- [965](#) and trout: Rounsefell revisited. *Rev. Fish Biol. Fish.* **14**(4): 421–442.
- [966](#) doi:10.1007/s11160-005-0802-5.
- [967](#) R Core Team. 2016. R: A language and environment for statistical computing. R Foundation
- [968](#) for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>
- [969](#) Rambault, A. 2012. <http://tree.bio.ed.ac.uk/software/figtree/>
- [970](#) Rollinson, N., Keith, D.M., Houde, A.L.E.E.S., Debes, P. V, McBride, M.C., and Hutchings,
- [971](#) J.A. 2014. Risk Assessment of Inbreeding and Outbreeding Depression in a
- [972](#) Captive-Breeding Program. *Conserv. Biol.* **28**(2): 529–540.
- [973](#) doi:10.1111/cobi.12188.
- [974](#) Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population
- [975](#) structure. *Mol. Ecol. Notes* **4**: 137–138. doi:10.1046/j.1471-
- [976](#) 8286.2003.00566.x.
- [977](#) Ruzycki, J.R., Clarke, L.M., Flesher, M.W., Carmichael, R.W., and Eddy, D.L. 2009.
- [978](#) Performance of progeny from steelhead and rainbow trout crosses. Oregon
- [979](#) Department of Fish and Wildlife, Fish Research and Development, NE Region.

- [980](#) Sanz, N., García-Marín, J.-L., and Pla, C. 2000. Divergence of brown trout (*Salmo trutta*)
[981](#) within glacial refugia. Can. J. Fish. Aquat. Sci. **57**: 2201–2210.
- [982](#) Saraniemi, M. 2005. Migrations and population characteristics and structure of adfluvial
[983](#) brown trout in the River Oulankajoki. Naturpolis Kuusamo koulutus- ja
[984](#) kehittämispalvelut, Tutkimuksia **1/2005**: 1-71. (in Finnish)
- [985](#) Saraniemi, M., Huusko, A., and Tahkola, H. 2008. Spawning migration and habitat use of
[986](#) adfluvial brown trout, *Salmo trutta*, in a strongly seasonal boreal river. Boreal
[987](#) Environ. Res. **13**(2): 121–132.
- [988](#) Sato, T., and Harada, Y. 2008. Loss of genetic variation and effective population size of
[989](#) Kirikuchi charr : implications for the management of small , isolated salmonid
[990](#) populations. Anim. Conserv. **11**: 153–159. doi:10.1111/j.1469-
[991](#) 1795.2008.00165.x.
- [992](#) Schliep K.P. 2011. phangorn: phylogenetic analysis in R. Bioinformatics, **27**(4): 592-593
- [993](#) Serbezov, D., Jorde, P.E., Bernatchez, L., Olsen, E.M., and Vøllestad, A.L. 2012. Short-term
[994](#) genetic changes: Evaluating effective population size estimates in a
[995](#) comprehensively described brown trout (*Salmo trutta*) population. Genetics,
[996](#) **191**(2): 579–592. doi:10.1534/genetics.111.136580.
- [997](#) Shendure, J., and Ji, H. 2008. Next-generation DNA sequencing. Nat. Biotechnol. **26**(10):
[998](#) 1135–1145. doi:10.1038/nbt1486.
- [999](#) Skaala, Ø., and Nævdal, G. 1989. Genetic differentiation between freshwater resident and
[1000](#) anadromous brown trout, *Salmo trutta*, within watercourses. J. Fish Biol. **34**:
[1001](#) 597-605.
- [1002](#) Sønstebø, J.H., Borgstrøm, R., and Heun, M. 2007. Genetic structure of brown trout (*Salmo*
[1003](#) *trutta* L.) from the Hardangervidda mountain plateau (Norway) analyzed by

- [1004](#) microsatellite DNA: A basis for conservation guidelines. *Conserv. Genet.* **8**(1):
[1005](#) 33–44. doi:10.1007/s10592-006-9145-6.
- [1006](#) Sutela, T., and Huusko, A. 1998. Catch, costs and management opinions of anglers in three
[1007](#) rivers in NE Finland. In: Hickley, P. and Tompkins, H. (eds.) *Recreational*
[1008](#) *Fisheries. Social, economic and management aspects*, Fishing News Book.
[1009](#) Blackwell Science Ltd. Oxford: 129-141.
- [1010](#) Swatdipong, A., Vasemägi, A., Niva, T., Koljonen, M.-L., and Primmer, C.R. 2010. High
[1011](#) level of population genetic structuring in lake-run brown trout, *Salmo trutta*, of
[1012](#) the Inari Basin, northern Finland. *J. Fish Biol.* **77**(9): 2048–2071.
[1013](#) doi:10.1111/j.1095-8649.2010.02784.x.
- [1014](#) Syrjänen, J. T., Vainikka, A., Louhi, P., Huusko, A., Orell, P., and Vehanen, T. 2017. History,
[1015](#) conservation and management of adfluvial brown trout stocks in Finland. In:
[1016](#) Lobón-Cerviá, J. and Sanz, N.(Eds.): *Brown trout. Biology, Ecology and*
[1017](#) *Management*. Wiley, in press.
- [1018](#) Tammi, J., Niinioja, R., and Rask, M. 1996. Finnish Long-Term Socio-Ecological Research
[1019](#) network FinLTSER: fish and monitoring in 1992-1994. Kala- ja
[1020](#) riistaraportteja Finnish Game and Fisheries Research Institute. Helsinki.
[1021](#) Finland. **59**: 1-24. (in Finnish)
- [1022](#) Thériault, V., Garant, D., Bernatchez, L., and Dodson, J.J. 2007. Heritability of life-history
[1023](#) tactics and genetic correlation with body size in a natural population of brook
[1024](#) charr (*Salvelinus fontinalis*). *J. Evol. Biol.* **20**(6): 2266–2277.
[1025](#) doi:10.1111/j.1420-9101.2007.01417.x.

- [1026](#) Toonen, R.J., Puritz, J.B., Forsman, Z.H., Whitney, J.L., Fernandez-Silva, I., Andrews, K.R.,
[1027](#) and Bird, C.E. 2013. ezRAD: a simplified method for genomic genotyping in
[1028](#) non-model organisms. *PeerJ* **1**: e203. doi:10.7717/peerj.203.
- [1029](#) Vøllestad, L.A., Serbezov, D., Bass, A., Bernatchez, L., Olsen, E.M., Taugbøl, A., and Grant,
[1030](#) J. 2012. Small-scale dispersal and population structure in stream-living brown
[1031](#) trout (*Salmo trutta*) inferred by mark-recapture, pedigree reconstruction, and
[1032](#) population genetics. *Can. J. Fish. Aquat. Sci.* **69**(9): 1513–1524.
[1033](#) doi:10.1139/f2012-073.
- [1034](#) Vøllestad, L.A. 2017. Understanding brown trout population genetic structure: a Northern-
[1035](#) European perspective. In: Lobón-Cerviá, J. and Sanz, N.(Eds.): *Brown trout.*
[1036](#) *Biology, Ecology and Management.* Wiley, in press.
- [1037](#) Wang, S., Hard, J.J., and Utter, F. 2002. Genetic variation and fitness in salmonids. *Conserv.*
[1038](#) *Genet.* (3): 321–333.
- [1039](#) Wang, S., Meyer, E., McKay, J.K., and Matz, M. V. 2012. 2b-RAD: a simple and flexible
[1040](#) method for genome-wide genotyping. *Nat. Methods* **9**(8): 808–810.
[1041](#) doi:10.1038/nmeth.2023.
- [1042](#) Waples, R.S., and Do, C. 2008. LDNE: A program for estimating effective population size
[1043](#) from data on linkage disequilibrium. *Mol. Ecol. Resour.* **8**(4): 753–756.
[1044](#) doi:10.1111/j.1755-0998.2007.02061.x.
- [1045](#) Waples, R.S., and Anderson, E.C. 2017. Purging putative siblings from population genetic
[1046](#) datasets: A cautionary view. *Mol. Ecol.* **26**(5): 1211–1224.
[1047](#) doi:10.1111/mec.14022.

- [1048](#) Whiteley, A.R., Hastings, K., Martin, J.C., and Allendorf, F.W. 2010. Genetic variation and
[1049](#) effective population size in isolated populations of coastal cutthroat trout.
[1050](#) Conserv. Genet. **11**: 1929–1943. doi:10.1007/s10592-010-0083-y.
- [1051](#) Wysujack, K., Greenberg, L.A., Bergman, E., and Olsson, I.C. 2009. The role of the
[1052](#) environment in partial migration: Food availability affects the adoption of a
[1053](#) migratory tactic in brown trout *Salmo trutta*. Ecol. Freshw. Fish **18**: 52–59.
[1054](#) doi:10.1111/j.1600-0633.2008.00322.x.

Draft

[1055](#) **Table 1.** List of the samples collected from the 11 rivers and the acronyms used for them.

Sample location	River type	Number of sample before /after filtering	Average length (TL) [mm]	Sampling time	Stage	Accronym
Kuusinkijoki,	Main stem	30 / 26	699.13 ± 73.42	Summer 2014	adult	KUU
Raatepuro	Tributary	30 / 25	123.07 ± 37.58	Sep. 2015	parr	Raa
Juumajoki	Tributary	30 / 29	70.83 ± 12.25	Sep.2015	parr	Juu
Kitkajoki	Main stem	30 / 26	767.73 ± 77.32	Summer 2014	adult	KIT
Pesospuro	Tributary	30 / 30	146.20 ± 24.64	Nov. 2015	parr	Pes
Oulankajoki	Main stem	30 / 27	604.33 ± 56.88	Nov.2015	adult	OUL
Maaninkajoki	Tributary	30 / 29	86.42 ± 33.21	Oct. 2015	parr	Maa
Merenoja	Tributary	20 / 19	174.55 ± 34.17	October 2015	parr	Mer
Astumajoki	Tributary	16 / 16	106.00 ± 59.01	Nov. 2015	parr	Ast
Kitkajoki (hatchery line)	Main stem	30 / 29	337.73 ± 53.01	Nov. 2015	parr	Khy
Kirintöjoki -Lohijoki (hatchery line)	Tributary	30 / 28	552.13 ± 23.07	Nov. 2015	parr	Kil

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Table 2. F_{ST} -values for the pairwise differences among the studied brown trout populations as calculated based on 3972 total SNPs. SNPs used for pairwise comparison are shown on the upper right part and F_{ST} on the down left part.

	Kuusinkijoki	Raatepuuro	Juumajoki	Kitkajoki - below	Pesospuro	Oulankajoki	Maaninkajoki	Merenoja	Astumajoki	Kitkajoki - above	Kirintöjoki - Lohijoki
Kuusinkijoki		2946	2934	3179	2880	3555	2820	3009	2890	3181	2978
Raatepuuro	0.014		2729	3062	2675	3511	2595	2854	2683	3093	2816
Juumajoki	0.127	0.134		2978	2420	3415	2279	2708	2384	2855	2636
Kitkajoki - below	0.016	0.046	0.149		2931	3512	2861	3065	2918	3062	3022
Pesospuro	0.201	0.221	0.296	0.214		3402	2172	2611	2517	2871	2514
Oulankajoki	0.057	0.072	0.127	0.060	0.202			3445	3333	3477	3425
Maaninkajoki	0.186	0.202	0.294	0.102	0.358	0.173		2443	1973	2759	2293
Merenoja	0.048	0.067	0.173	0.044	0.239	0.064	0.134		2517	3031	2624
Astumajoki	0.148	0.161	0.254	0.156	0.327	0.125	0.182	0.099		2796	2388
Kitkajoki - above	0.087	0.105	0.164	0.074	0.245	0.063	0.225	0.104	0.183		2990
Kirintöjoki - Lohijoki	0.098	0.114	0.208	0.102	0.275	0.093	0.113	0.029	0.076	0.139	

Figure captions

1069

1070 **Figure 1.** Map of the sampled 11 rivers and river sections in River Koutajoki watershed in
1071 Finland. Jyrävä Falls form a complete natural migration barrier, and all the downstream
1072 migratory populations use River Olanga as migration route to and from Lake Pyaozero in
1073 Russia. Map drawn by tmi Olli van der Meer.

1074

1075 **Figure 2.** Unrooted unweighted pair group method with arithmetic mean (UPGMA) tree of
1076 the studied 11 brown trout populations (c.f. Fig. 1). The tree was constructed using pairwise
1077 genetic differentiation values (F_{ST}) (see also Table 2).

1078

1079 **Figure 3.** Expected heterozygosity for the 11 studied populations of brown trout as based on
1080 3972 SNPs. Grey dots represent main stem populations and the black dots represent
1081 populations from small tributaries.

1082

1083 **Figure 4.** Discriminant principal component analysis of 3972 SNPs. Four tributary
1084 populations stand out from the others. (KUU = Kuusinkijoki; Raa = Raatepuro; KIT =
1085 Kitkajoki below falls; Pes= Pesospuro; OUL = Oulankajoki; Mer = Merenoja; Maa =
1086 Maaninkajoki; Ast = Astumajoki; Khy = Kitkajoki hatchery; Kil = Kirintöjoki- Lohijoki)

1087

1088 **Figure 5.** Graphical visualization of individual group reassignment of the 284 sampled brown
1089 trout in the eleven original populations. A white (0) to red (1) color gradient represents the
1090 proportion of correct assignment for every individual. Blue crosses represent the predicted
1091 origin of the sample.

1092

[1093](#) **Figure 6.** Structure plot of the studied eleven brown trout populations. Every column
[1094](#) represents one individual assigned to its presumed population. A total of 284 individuals
[1095](#) were analyzed.
[1096](#)

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