



**UiT** The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics, Department of Arctic and Marine Biology

**Recreational fisheries target declining populations of brown trout (*Salmo trutta* L.) in Northern Norway as revealed by a genome wide array of SNP markers**

**Simon Nordblad Schmidt**

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## **Preface and acknowledgements**

This Master's thesis is written as an extended draft of a manuscript intended for publication in a scientific peer-reviewed journal. As an introduction, I here present a few sections on the foundations and the theory behind the project with the aim of familiarising the reader with the subject before presenting the extended manuscript.

The manuscript is scheduled to be presented at Sjøørretkonferansen 2020 (the sea trout conference 2020) in Trondheim this January. The work carried out in this thesis also contributed to a comprehensive report on sea-run brown trout and sea-run Arctic charr in Skjerstadvjorden which covers the marine migration of the species, their habitat use and genetics (see Davidsen et al., 2019), a project lead by Jan G. Davidsen and published electronically by the NTNU University Museum as open access.

I would like to take the opportunity to formally thank my supervisor Kim Præbel and my co-authors Julie Bitz-Thorsen, Shripathi Bhat and Jan G. Davidsen who all have supervised me throughout the process and have been a tremendous resource. Additionally, I extend my gratitude to the Research Genetics Group at UiT whose members have contributed with valuable discussions and feedback over the course of the project.

Thanks to Kim Præbel for funding the study, to the Faculty of Biosciences Fisheries and Economics (BFE) at UiT The Arctic University of Norway for covering fieldwork related travel costs and Jan G. Davidsen for covering living expenses during fieldwork.

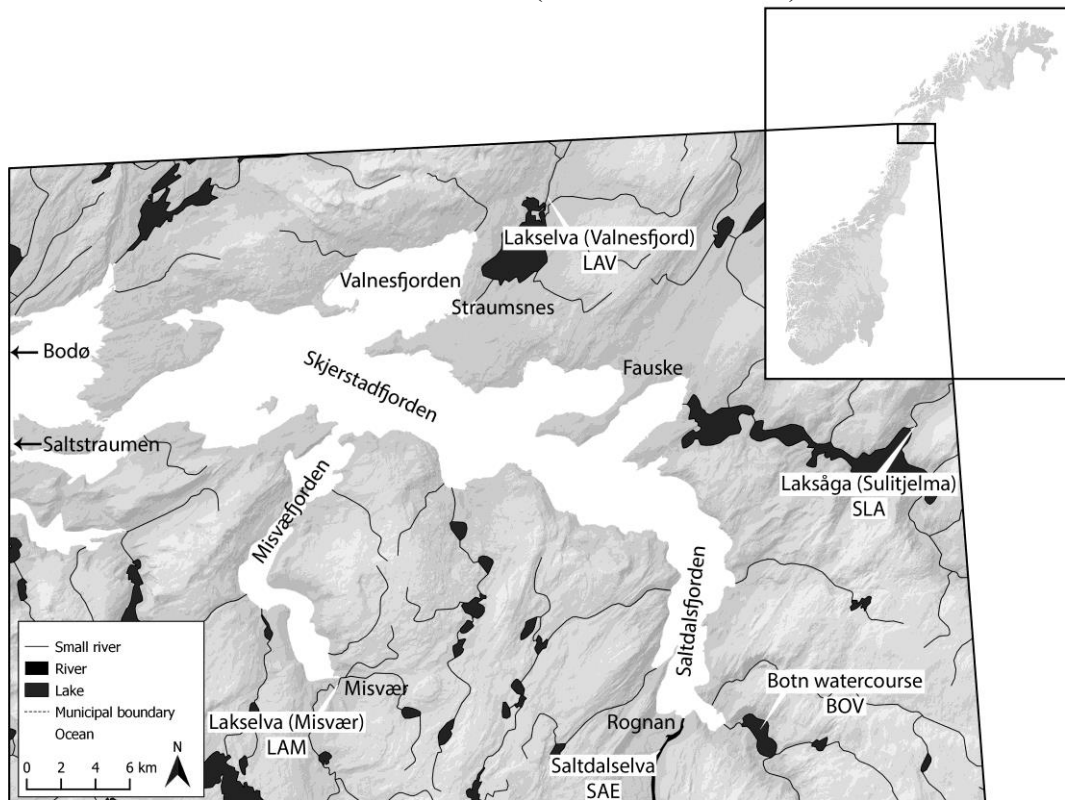
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## General introduction

This study uses single nucleotide polymorphisms (SNPs), a type of DNA marker, to investigate population structure of brown trout (*Salmo trutta* L.) among five watercourses from the Skjerstad fjord system and to establish a genetic baseline. This baseline was then used to determine the contribution of potential source populations in a mixed stock of sea-run brown trout caught by recreational anglers in the fjord system. This general introduction will introduce the reader to the fjord system and its watercourses, the brown trout, its migratory behaviour and philopatry as well as the field of population genetics and how genetic tools are utilised in the context of this thesis.

## Study area

The study area (see **Figure i**) is located within an interconnected fjord system in Nordland county, Northern Norway (67°N 15°E), and comprise of four fjords; Skjerstadfjorden, Saltdalsfjorden, Misfærfjorden and Valnesfjorden, located within the three counties; Bodø, Fauske and Saltdal. The system stretches approximately 50 km from the innermost part of Saltdalsfjorden to the sea through three straits, and reaches depths of more than 500 m in the basin of Skjerstadfjorden (Eliassen et al., 2001). The system is almost entirely landlocked with only three narrow and shallow entrances to the sea at Sundstraumen, Godøystraumen and Saltstraumen, the latter having the strongest tidal current in the world, transporting up to 370 million m<sup>3</sup> water over a six hour tidal interval (Plassen et al., 2015).



**Figure i:** Map of the study area

The system has an inflow of freshwater from several large watercourses; Botn watercourse, river Lakselva (Misvær), Valnesfjord watercourse, Saltdal watercourse and Sulitjelma watercourse which result in seasonal temperature and salinity fluctuations in the upper layers of the water column in the fjord. This qualifies the surface water salinity as a mix of euhaline (>30 PSU) and polyhaline (18-30 PSU) water (Busch et al., 2014). Among recreational anglers, the fjord system and surrounding watercourses are popular destinations for lake-run and sea-run brown trout fisheries. Additionally, the fjord is currently home to six fish farms. The watercourses presented below are all known to harbour brown trout while some have varying numbers of Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.).

The Botn watercourse is located east in the inner part of Saltdalsfjorden just east of Rognan. Here, the lake Botnvatnet is the largest body of water, covering an area of approximately 2.0 km<sup>2</sup>. It receives most of its water from the rivers Ingeborgelva in the north and Knallerdalselva in the south (NVE, 1991b). Lake Botnvatnet was originally a part of Saltdalsfjorden but following the most recent deglaciation period (~10,000 B.P; Andersen et al., 1979) it was dammed up by terminal moraine. Lake Botnvatnet is 118m deep and is known to still have seawater reserves within the bottom water masses (Halvorsen, 2012, NVE, 1991b). Brown trout and Arctic charr mainly occupy this watercourse, while Atlantic salmon rarely occurs. The Botn watercourse was heavily exploited by local fisheries from early 1900s using traps until the 1930s and nets until the 1960s (Davidsen et al., 2019). Since the 1960s little harvesting of brown trout has been carried out in the watercourse.

Saltdal watercourse is one of the largest watercourses (NVE, 1991) and the second largest unregulated watercourse in Nordland (NVE, 2005). Its first part, and the source of the watercourse, is named river Lønselva. This river receives its water from Saltfjellet and several smaller streams before confluence with river Junkerdalselva. From here the river is called Saltdalselva and stretches approximately 36 km north through Rognan to Saltdalsfjorden, reaching a total length of 80 km (Kanstad-Hanssen et al., 2017). Increased surface runoff in spring can cause river Saltdalselva to flood surrounding farmland, roads and settlement. Modifications of the riverbanks have therefore been carried out to prevent flood and erosion and to protect surrounding land over the years. These interventions are thought to have reduced the overall biodiversity of the watercourse (NVE, 2005). The watercourse has historically played an important role in local salmonid fisheries and have several smaller rivers and creeks which are inhabited by anadromous fish, although in general, the low temperatures and nutrient levels found in the watercourse results in relatively low productivity



(Brettum et al., 1980, Kanstad-Hanssen et al., 2017).

The river Lakselva (Misvær) is located in the innermost part of Misvær fjorden by Misvær municipality where it runs approximately 4 km upstream to the lake Skarvatnet. The river is not regulated, however the lower part of the river flows through settlement, where comprehensive modifications has been made to the river and the land adjacent to the watercourse (NVE, 2002). The mouth of the river concludes on a delta, which is partially flooded during high tide. Brown trout and Atlantic salmon can be found in the river and where it meets its tributaries and observations of Arctic charr have been made at the delta. Although river Lakselva receives additional nutrients from nearby agriculture and farmland, it is rich on nutrients by nature and the water quality can generally be characterised as good (NVE, 2002).

The river Laksåga (Sulitjelma) is part of the Sulitjelma watercourse which is a drainage basin with numerous lakes. The basin receives water from a large area which includes two glaciers. Laksåga is a regulated river which flows into Øvrevatnet, a lake downstream of lake Langvatn, and one of the largest lakes of the system (Aanes et al., 1987). Sulitjelma municipality, located near lake Langvatnet, was previously home to Sulitjelma Gruber, a mining company which exploited the copper- and zinc-rich iron ores in the area which resulted in poor water quality of the lake and downstream basins (Iversen et al., 2009). Following the termination of the mining activity in 1991, conditions have improved and today fish have come back to the entire lake. This stands in contrast to lake Øvrevatnet which still is affected by the previous mining activity, showing nearly identical surface concentrations of copper and zinc as those measured back in 1993. Lake Øvrevatnet is affected by the tide, which results in a significant increase in salinity at 15 meters depth and below. A report carried out in 2008 found hypoxia from 25m and anoxia at 40m (Iversen et al., 2009).

River Lakselva (Valnesfjord) is a part of Valnesfjord watercourse, with lake Kosmovatnet (8.3 km<sup>2</sup>) being the largest bed of water in the system. The lake is not regulated and is connected to the fjord by Laukåsstraumen, a one kilometer long strait (Miljødirektoratet, 2007). Lake Kosmovatnet is located one meter above sea level and is affected by inflow of seawater through the strait resulting in brackish water in the lake. River Lakselva flows into the lake on the opposite side of the lake through a waterway which split by a relatively large island, Flatøya. River Lakselva is known to harbour Atlantic salmon, brown trout as well as a small population of Arctic charr. The lake and lower parts of river Lakselva is surrounded mostly by agriculture and forest.

## **Life history of brown trout**

Brown trout is an iteroparous salmonid; one of several salmonid species which long has been recognised for their partial migration. Partial migration is where a population is divided between migration strategies. In brown trout, individuals can choose a resident life, where they remain in their natal streams throughout their life, or they can migrate to connecting waterways, lakes, or to the sea for feeding opportunities (Chapman et al., 2012). Specifically the brown trout is known for its complex plasticity in sympatric migration strategies, where migration strategies within a population varies from residence to anadromy along a continuum, often referred to as the brown trout migration continuum in the literature (Boel et al., 2014, Cucherousset et al., 2005). In addition to varying migration strategies, the duration of migration can vary from a few weeks to permanent residency in the sea only interrupted by occasional spawning events in freshwater (Thorstad et al., 2016). Not surprisingly, much variation has also been observed in the distance travelled during migration, especially during occupation of the marine habitat (Eldøy et al., 2015). The result of such behaviour is a polymorphic population, where the anadromous individuals dwarf their resident (fluvial) counterparts, although indistinguishable during early ontogeny (Jonsson and Jonsson, 1993). The polymorphism is presumably caused by richer feeding opportunities at sea where productivity exceeds that of freshwater in the natural distribution range of the brown trout (Gross et al., 1988, MacCrimmon et al., 1970). Achieving a larger size-at-age probably has no downsides, however, migration and life at sea is energetically costly due to development of a novel osmoregulatory system, as well as adaptations in behaviour, morphology and physiology (Binder et al., 2011, Chapman et al., 2012). Additionally, migrating fish face higher mortality rates and exposure to predators, diseases and infections (Acolas et al., 2008, Goodwin et al., 2016, Solomon, 2006). Especially in areas with fish farms, migrating individuals risk infections with a parasite commonly known as the salmon louse (*Lepeophtheirus salmonis*). This parasite is known to cause skin damage in fish which in turn can cause severe osmoregulatory problems at sea and secondary infections (Grimnes and Jakobsen, 1996). This can directly cause premature return to freshwater (Birkeland and Jakobsen, 1997) and dramatically increase mortality (Hansen et al., 2007, Thorstad and Forseth, 2015).

Following any type of migration, the brown trout also exhibits strong philopatry i.e. homing behaviour, where migrating individuals return to their natal river to spawn, a behaviour shared among salmonids (Stabell, 1984). This behaviour likely causes reproductively isolated and

locally adapted population where specific environmental factors increase the frequency of specific traits through natural selection (Griffiths et al., 2010, Taylor, 1991). The behaviour may also be the main contributing factor to the significant population differentiation consistently observed among rivers (e.g. Mäkinen et al., 2015, Sønstebo et al., 2007, Swatdipong et al., 2013), and on small geographical scales (Carlsson et al., 1999).

### **A brief introduction to population genetics**

The field of population genetics studies genetic variation within and among populations through the distribution and differences in allele frequencies (Maia and de Araújo Campos, 2019). Population genetics crosses over many scientific disciplines and can thus be applied to many of the existing branches within biology and is commonly utilised in the industry through breeding programmes or cultivation of plants (Crow and Kimura, 1970, Hartl et al., 1997). Genetic variation arises in the scope of evolution where genetic material of a population changes over time, thus the variation represents the outcome of evolutionary processes. The observed variation in genes can be independent, as under the Hardy-Weinberg equilibrium (HWE) and linkage equilibrium, or dependent, where the frequencies of alleles at two or more loci are associated (linkage disequilibrium, LD; Delves and Roitt, 1998). Additionally, frequencies and distributions of alleles can be altered by forces such as natural selection, random genetic drift, mutations, non-random mating and gene flow. The Hardy-Weinberg principle states that without the presence of these forces, allele frequencies will remain constant throughout future generations, at HWE, however, this assumes diploid organisms with sexual reproduction and non-overlapping generations and requires that the considered genes have two alleles and that their frequencies are identical for males and females (Hartl et al., 1997). The HWE can be expressed through the frequencies of two alleles of a gene by the following equation:

$$p^2 + 2pq + q^2 = 1$$

Here,  $p$  represents the dominant allele and  $q$  the recessive, where  $p^2$  and  $q^2$  represents the frequencies of the dominant and recessive homozygote genotypes, respectively, and  $2pq$  represents the heterozygote genotypes.

Departure from the HWE can for instance be caused by introduction of new alleles through mutations, through gene flow as a result of interbreeding or by population sub-structuring. Similarly, changes in gene frequencies could be accounted natural selection or non-random mating where alleles increase or reduce individual fitness or even by random genetic drift which increases or lowers frequencies by chance, a force which typically only takes place in

small populations (Ellstrand and Elam, 1993). HWE rarely occurs in nature since it describes an idealised condition, and any genetic divergence can therefore be measured as the changes in allele frequencies through departure from HWE.

### **Methods used to investigate population structure**

In fisheries, various methods and characteristics have been used to differentiate between populations such as number of fin rays or gill rakers, body shape or dimensions of body parts (Begg and Waldman, 1999), scale patterns (Fryer and Kelsey, 2002), otolith shape (Campana and Casselman, 1993), or parasite composition (MacKenzie and Abaunza, 1998), to mention some. Modern day techniques, however, mainly rely on DNA markers such as microsatellites (Beacham et al., 2006, Sønstebo et al., 2007, Swatdipong et al., 2013) or single nucleotide polymorphisms (SNPs; Kalinowski, 2004, Smith et al., 2005) to identify patterns of genetic variation within or between populations. A SNP is the substitution of a single nucleotide at a specific position in the genome, for instance a substitution of an adenine with a cytosine. In this case, the cytosine variant might spread throughout one population over time while another population maintains the adenine and the populations could in theory be separated based on the observed variation at that SNP. In this study an array of SNPs are used to address the research questions.

The variation in SNPs can assist us in identifying underlying population structure much more accurately than before (Anderson et al., 2008, Beacham et al., 2006). Because of the large number of individuals often genotyped (i.e. the process of identifying genetic variation in the genetic make-up) at thousands or even sometimes at millions of SNPs, analysis and comparison of data can often be difficult to handle. Today, however, a variety of genetic computational tools are readily available to researchers which can analyse such data. Among the most reputed is the model-based software, STRUCTURE (Pritchard et al., 2000), but also non-model approaches such as the principle component analysis (PCA; Jombart, 2008) or discriminant analysis of principal components (DAPC; Jombart and Collins, 2015).

STRUCTURE is typically the first step in an examination of population structure and works by analysing the distribution of genetic variation between a putative number of groups. The software then places each sample into one of these groups based on the variations in their genetic patterns determined by a Bayesian algorithm (Porras-Hurtado et al., 2013). The PCA summarises the global genetic variability of the dataset while maintaining the genetic variance observed. The DAPC, on the other hand, aims to emphasise variation between groups while minimising variation within the groups. While STRUCTURE is a very powerful tool, it is

equally computationally demanding. PCA and DAPC analyses, on the other hand, can visualise data in minutes and are therefore also great initial approaches.

Genetic tools, however, can only identify population structure if there is any population structure present. Fortunately, as mentioned previously, this is generally the case in brown trout. The high level of differentiation between the potential populations can increase the power of the analyses and enable good resolution, even in cases where genotyping quality might be subpar due to poor DNA integrity, or if limited sample sizes are available.

For the assignment of individuals with unknown origin and identification of the contribution of potential source populations to a mixed stock (i.e. a stock containing individuals with mixed origins), a mixed stock analysis (MSA) is regularly utilised, especially in salmonids (Bradbury et al., 2016, Crozier et al., 2004, Shaklee et al., 1999). The present study implements the use of STRUCTURE, *geneplot* and *rubias*. To carry out a MSA, a representative genetic baseline (i.e. a group of reference populations whose genetic backgrounds can be separated from other reference populations) is required. This is important, since a mixed stock analysis do not infer population structure but assigns samples based on the provided allele frequency estimates for each population. STRUCTURE uses these estimates to compute a likelihood value for each sample as described above but allows for detection of admixed individuals (i.e. individuals whose genotypes arise from more than one of the populations) (Porras-Hurtado et al., 2013, Pritchard et al., 2000). *geneplot* similarly assigns individuals but does so by providing a measure of fit for each sample to each group using approaches similar to that of the GeneClass2 software. In contrary to GeneClass2, it provides visualisation of the results by scatterplots (McMillan and Fewster, 2017). In *rubias* the *infer\_mixture* module calculates a score for all samples which can be used to indicate whether a mixed stock fits well within the baseline. The scores calculated by *rubias*, however, are not designed to enable direct assignment of samples; they just indicate whether scores fit the expected values of the model or not (Anderson et al., 2008).

### **Authors contributions**

KP conceived the study; SNS, KP, SB, and JGD designed the study; SNS and JGD (and co.) performed sampling of juvenile salmonids; JGD collected samples from recreational anglers; SNS, JBT, SB and KP performed the lab work; SNS performed the scoring of species and individual sex determination; SNS and SB carried out the computational genomic analyses; SNS, SB, and KP performed the statistical analyses and interpreted the results; SNS wrote the draft and all authors critically contributed to the final manuscript.

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## References

- Aanes, K. J., Iversen, E. R., Johannesen, M. & Mjelde, M. 1987. Overvåkning av Sulitjelmavassdraget 1985. *NIVA-rapport*.
- Acolas, M. L., Roussel, J. M. & Baglinière, J. L. 2008. Linking migratory patterns and diet to reproductive traits in female brown trout (*Salmo trutta* L.) by means of stable isotope analysis on ova. *Ecology of Freshwater Fish*, 17, 382-393.
- Andersen, B., Bøen, F., Rasmussen, A. & Vallevik, P. 1979. The deglaciation between Skjerstadvjord and Svartisen, north Norway. *Boreas*, 8, 199-201.
- Anderson, E. C., Waples, R. S. & Kalinowski, S. T. 2008. An improved method for predicting the accuracy of genetic stock identification. *Canadian Journal of Fisheries and Aquatic Sciences*, 65, 1475-1486.
- Beacham, T. D., Candy, J. R., Jonsen, K. L., Supernault, J., Wetklo, M., Deng, L., Miller, K. M., Withler, R. E. & Varnavskaya, N. 2006. Estimation of stock composition and individual identification of Chinook salmon across the Pacific Rim by use of microsatellite variation. *Transactions of the American Fisheries Society*, 135, 861-888.
- Begg, G. A. & Waldman, J. R. 1999. An holistic approach to fish stock identification. *Fisheries research*, 43, 35-44.
- Binder, T., Cooke, S. & Hinch, S. 2011. The biology of fish migration. *Encyclopedia of Fish Physiology: From Genome to Environment*. Elsevier Inc, 1921.
- Birkeland, K. & Jakobsen, P. J. 1997. Salmon lice, *Lepeophtheirus salmonis*, infestation as a causal agent of premature return to rivers and estuaries by sea trout, *Salmo trutta*, juveniles. *Environmental biology of fishes*, 49, 129-137.
- Boel, M., Aarestrup, K., Baktoft, H., Larsen, T., Søndergaard Madsen, S., Malte, H., Skov, C., Svendsen, J. C. & Koed, A. 2014. The physiological basis of the migration continuum in brown trout (*Salmo trutta*). *Physiological and Biochemical Zoology*, 87, 334-345.
- Bradbury, I. R., Hamilton, L. C., Chaput, G., Robertson, M. J., Goraguer, H., Walsh, A., Morris, V., Reddin, D., Dempson, J. B. & Sheehan, T. F. 2016. Genetic mixed stock analysis of an interceptory Atlantic salmon fishery in the Northwest Atlantic. *Fisheries Research*, 174, 234-244.
- Brettum, P., Erlandsen, A. H., Faafeng, B., Løvik, J. E. & Aanes, K. J. 1980. Vurdering av planlagte vassdragsreguleringer i Saltdalsvassdraget. *NIVA-rapport*.
- Busch, K. E. T., Iversen, K. R., Nashoug, B. F., Kiele, M. R., Gitmark, J., Remen, V., Velvin, R. & Krogstad, M. 2014. Miljøundersøkelse av Skjerstadvjorden. *SALT rapport*.
- Campana, S. E. & Casselman, J. M. 1993. Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 50, 1062-1083.
- Carlsson, J., Olsen, K., Nilsson, J., Øverli, Ø. & Stabell, O. 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. *Journal of Fish Biology*, 55, 1290-1303.
- Chapman, B., Hulthén, K., Brodersen, J., Nilsson, P. A., Skov, C., Hansson, L. A. & Brönmark, C. 2012. Partial migration in fishes: causes and consequences. *Journal of fish biology*, 81, 456-478.
- Crow, J. F. & Kimura, M. 1970. An introduction to population genetics theory.
- Crozier, W., Schön, P., Chaput, G., Potter, E., Maoileidigh, N. & MacLean, J. 2004. Managing Atlantic salmon (*Salmo salar* L.) in the mixed stock environment: challenges and considerations. *ICES Journal of Marine Science*, 61, 1344-1358.
- Cucherousset, J., Ombredane, D., Charles, K., Marchand, F. & Baglinière, J.-L. 2005. A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 1600-1610.

- Davidson, J. G., Eldøy, S. H., Meyer, I., Halvorsen, A. E., Sjørusen, A. D., Rønning, L., Schmidt, S. N., Præbel, K., Daverdin, M., Bårdsen, M. T., Whoriskey, F. & Thorstad, E. B. 2019. Sjørørret og sjørøye i Skjerstadvfjorden - Marine vandringer, områdebruk og genetikk. *NTNU Vitenskapsmuseet naturhistorisk rapport*.
- Delves, P.-I. & Roitt, I. 1998. *Encyclopedia of immunology*, Academic Press.
- Eldøy, S. H., Davidson, J. G., Thorstad, E. B., Whoriskey, F., Aarestrup, K., Næsje, T. F., Rønning, L., Sjørusen, A. D., Rikardsen, A. H. & Arnekleiv, J. V. 2015. Marine migration and habitat use of anadromous brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*, 72, 1366-1378.
- Eliassen, I. K., Heggelund, Y. & Haakstad, M. 2001. A numerical study of the circulation in Saltfjorden, Saltstraumen and Skjerstadvfjorden. *Continental Shelf Research*, 21, 1669-1689.
- Ellstrand, N. C. & Elam, D. R. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual review of Ecology and Systematics*, 24, 217-242.
- Fryer, J. K. & Kelsey, D. A. 2002. Identification of Columbia Basin sockeye salmon stocks using scale pattern analyses in 2001.
- Goodwin, J. C., Andrew King, R., Iwan Jones, J., Ibbotson, A. & Stevens, J. R. 2016. A small number of anadromous females drive reproduction in a brown trout (*Salmo trutta*) population in an English chalk stream. *Freshwater biology*, 61, 1075-1089.
- Griffiths, A. M., Machado-Schiaffino, G., Dillane, E., Coughlan, J., Horreo, J. L., Bowkett, A. E., Minting, P., Toms, S., Roche, W. & Gargan, P. 2010. Genetic stock identification of Atlantic salmon (*Salmo salar*) populations in the southern part of the European range. *BMC genetics*, 11, 31.
- Grimnes, A. & Jakobsen, P. 1996. The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *Journal of Fish Biology*, 48, 1179-1194.
- Gross, M. R., Coleman, R. M. & McDowall, R. M. 1988. Aquatic productivity and the evolution of diadromous fish migration. *Science*, 239, 1291-1293.
- Halvorsen, M. 2012. Sjørøyevassdragene i Nord-Norge; 100 av 400 mulige. En zoogeografisk analyse av de aktuelle vassdragene. *DN-utredning 1-2012*. Direktoratet for naturforvaltning.
- Hansen, M. M., SKAALA, Ø., Jensen, L. F., Bekkevold, D. & MENSBERG, K. L. D. 2007. Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Fjord, Norway. *Molecular Ecology*, 16, 1413-1425.
- Hartl, D. L., Clark, A. G. & Clark, A. G. 1997. *Principles of population genetics*, Sinauer associates Sunderland, MA.
- Iversen, E. R., Kristensen, T. & Aanes, K. J. 2009. Oppfølging av forurensningssituasjonen i Sulitjelma gruvefelt, Fauske kommune. Undersøkelser i 2008. *NIVA-rapport*.
- Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403-1405.
- Jombart, T. & Collins, C. 2015. A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0. 0. *London: Imperial College London, MRC Centre for Outbreak Analysis and Modelling*.
- Jonsson, B. & Jonsson, N. 1993. Partial migration: niche shift versus sexual maturation in fishes. *Reviews in Fish Biology and Fisheries*, 3, 348-365.
- Kalinowski, S. T. 2004. Genetic polymorphism and mixed-stock fisheries analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 1075-1082.



- Kanstad-Hanssen, Ø., Lamberg, A., Bentsen, V., Bjørnbet, S. & Gjertsen, V. 2017. Gytefiskregistrering i Saltdalselva i 2017 - Resultater fra drivtelling av laks, sjøørret og sjørøye. Skandinavisk naturovervåking, Prosjekt Utmark, Nordland bondelag.
- MacCrimmon, H. R., Marshall, T. L. & Gots, B. L. 1970. World distribution of brown trout, *Salmo trutta*: further observations. *Journal of the Fisheries Board of Canada*, 27, 811-818.
- MacKenzie, K. & Abaunza, P. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. *Fisheries Research*, 38, 45-56.
- Maia, R. T. & de Araújo Campos, M. 2019. Introductory Chapter: Population Genetics-The Evolution Process as a Genetic Function. *Integrated View of Population Genetics*. IntechOpen.
- Mäkinen, H., Niva, T., Koljonen, M.-L., Swatdipong, A. & Primmer, C. R. 2015. temporal variation in lake-run brown trout (*Salmo trutta*) mixed-stock fishery catches in a large Fennoscandian lake.
- McMillan, L. & Fewster, R. 2017. Visualizations for genetic assignment analyses using the saddlepoint approximation method. *Biometrics*, 73, 1029-1041.
- Miljødirektoratet 2007. Lakselva/Valnesfjordelvas utløp i Valnesfjorden/Kåsmovatnet.
- NVE 1991. 163/1 Saltdalsvassdraget NOU 1991 12B.
- NVE 1991b. 163/2 Botnelva NOU 1991 12B.
- NVE 2002. 162/2 Lakselva, Misvær (Vassdragsnr. 162.7Z).
- NVE 2005. Helhetlig tiltaksplan for Saltdalselva. *Faktaark - NVE Fakta*.
- Plassen, L., Van Son, T. C., Lepland, A. & Longva, O. 2015. Kartlegging av Saltstraumen marine verneområde. *NGU-rapport*.
- Porrás-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á. & Lareu, M. 2013. An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in genetics*, 4, 98.
- Pritchard, J. K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.
- Shaklee, J. B., Beacham, T. D., Seeb, L. & White, B. A. 1999. Managing fisheries using genetic data: case studies from four species of Pacific salmon. *Fisheries Research*, 43, 45-78.
- Smith, C. T., Templin, W. D., Seeb, J. E. & Seeb, L. W. 2005. Single nucleotide polymorphisms provide rapid and accurate estimates of the proportions of US and Canadian Chinook salmon caught in Yukon River fisheries. *North American Journal of Fisheries Management*, 25, 944-953.
- Solomon, D. 2006. Migration as a life-history strategy for the sea trout. *Sea trout: biology, conservation and management*. Blackwell, Oxford, 224-233.
- Sønstebø, J. H., Borgstrøm, R. & Heun, M. 2007. Genetic structure of brown trout (*Salmo trutta* L.) from the Hardangervidda mountain plateau (Norway) analyzed by microsatellite DNA: a basis for conservation guidelines. *Conservation Genetics*, 8, 33-44.
- Stabell, O. B. 1984. Homing and olfaction in salmonids: a critical review with special reference to the Atlantic salmon. *Biological Reviews*, 59, 333-388.
- Swatdipong, A., Vasemägi, A., Niva, T., Koljonen, M. L. & Primmer, C. 2013. Genetic mixed-stock analysis of lake-run brown trout *Salmo trutta* fishery catches in the Inari Basin, northern Finland: implications for conservation and management. *Journal of fish biology*, 83, 598-617.
- Taylor, E. B. 1991. A review of local adaptation in Salmonidac, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, 98, 185-207.

- Thorstad, E. B. & Forseth, T. 2015. Råd om beskatning av laks og sjøørret for perioden 2016 til 2018.
- Thorstad, E. B., Todd, C. D., Uglem, I., Bjørn, P. A., Gargan, P. G., Vollset, K. W., Halttunen, E., Kålås, S., Berg, M. & Finstad, B. 2016. Marine life of the sea trout. *Marine Biology*, 163, 47.

# Recreational fisheries target declining populations of brown trout (*Salmo trutta* L.) in Northern Norway as revealed by a genome wide array of SNP markers

Simon N. Schmidt<sup>1,2\*</sup>, Shripathi Bhat<sup>1</sup>, Julie Bitz-Thorsen<sup>1</sup>, Jan G. Davidsen<sup>3</sup> & Kim Præbel<sup>1</sup>

<sup>1</sup>The Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, Tromsø, Norway, <sup>2</sup>Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, Norway, <sup>3</sup>NTNU University Museum, Norwegian University of Science and Technology, Trondheim, Norway

\*Corresponding author: Simon N. Schmidt, e-mail: [simon.n.schmidt@gmail.com](mailto:simon.n.schmidt@gmail.com)

## Abstract

Marine recreational and commercial exploitation of salmonids often target a mixed stock consisting of genetically distinct and independent populations with varying abundances, life histories, standing genetic variation, and conservation status. In this study we use SNP markers to investigate the genetic population structure of juvenile brown trout (*Salmo trutta* L.) from five distinct watercourses in order to identify the contribution of different source populations in a mixed stock fishery on sea-run brown trout. We identify significant genetic population structure between the watercourses, even between those with little geographical separation. While the Saltdal watercourse in recent years has seen a decline in brown trout catchments, we discover that it is the main contributor to the mixed stock; supplying 63% of sea-run brown trout longer than 50 cm and 76% longer than 70 cm. Sea-run brown trout shorter than 50 cm mainly exhibited genetic patterns dissimilar to those of our reference populations. For a holistic view of the sea-run brown trout fisheries and to obtain a better understanding of the dynamics of the fjord system, further research is therefore warranted.

**Keywords:** Genetic population structure, mixed stock analysis, recreational fisheries, *Salmo trutta*, Skjerstadfjorden

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## Introduction

Marine recreational and commercial exploitation of salmonids often target a mixed stock consisting of genetically distinct and independent populations with varying abundances, standing genetic variation, and conservation status (ESUs; Bekkevold et al., 2011, Shaklee et al., 1999, Swatdipong et al., 2013). Given the complexity of mixed stocks, outcomes of conservation plans and stock rebuilding programmes become unpredictable without the ability to divide mortality between stocks, which in turn complicates management (Griffiths et al., 2010). Identification of the relative contribution of different source populations in a mixed stock fishery is therefore essential for an effective and sustainable management of fisheries (Begg et al., 1999, Cadrin et al., 2013).

While early applications of stock identification have utilised a range of approaches (as reviewed in Begg and Waldman, 1999), modern day techniques mainly rely on DNA markers such as microsatellites (e.g. Beacham et al., 2006, Sørstebø et al., 2007, Swatdipong et al., 2013) or single nucleotide polymorphisms (SNPs; e.g. Kalinowski, 2004, Smith et al., 2005) both which have provided much greater resolution than earlier methods (Anderson et al., 2008, Beacham et al., 2006). Mixed stock fisheries have been extensively studied in anadromous salmonids, especially the Pacific and Atlantic salmonid species (Bradbury et al., 2016, Crozier et al., 2004, Shaklee et al., 1999); sea-run brown trout (*Salmo trutta* L.) on the other hand has, to the best of our knowledge, yet to be studied. Salmonids have long been recognised for their partial migration, where part of a population remain resident in their natal streams while others migrate to sea (Chapman et al., 2012). Compared to other salmonids, the brown trout is known for its complex variation in sympatric migration strategies, where migration strategies within a population varies from residency to anadromy along a continuum (Boel et al., 2014, Cucherousset et al., 2005). This behaviour naturally complicates appropriate management, as one population can be extensively distributed and exploited throughout its range, being it by anglers in rivers, through netting along the coast or in estuaries, or catches in a common fjord system (Griffiths et al., 2010). Previous studies on the brown trout have further found significant genetic population structure between watercourses even where these are geographically adjacent (Carlsson et al., 1999, Hansen et al., 2002). This calls for approaches to further increase the understanding of population dynamics within systems and the need for accurate stock identification and identification of management units. These goals can be achieved through genetic approaches such as those introduced in present study.

The concept of mixed stock analysis (MSA) is based on identification of the possible contributing populations to the mixed stock (i.e. the possible origins of the mixed stock individuals, the baseline) through surveying of a system. Individuals from the mixed stock can then be assigned a reference population based on the population in which the individuals genotype has the highest probability of occurring (individual assignment (IA); Cornuet et al., 1999) or through mixture modelling where genotypes are related to expected genotype frequencies in the baseline (Koljonen et al., 2005) The accuracy of a MSA has been shown to depend on several factors such as the quality of the baseline and the sample size of each population, genotyping quality, the temporal stability of the populations, genetic differentiation between the populations and the number of alleles (Anderson et al., 2008, Beacham et al., 2006)

Brown trout is an abundant species distributed widely throughout Europe (MacCrimmon et al., 1970). However, recent declines in brown trout populations has led to an increased interest in the species (ICES, 2013) for conservational concerns. Unreported catches and lack of information on mixed stocks complicates quantification of harvesting pressures on populations and thus sustainable management (Höjesjö et al., 2017), especially for brown trout (Fiske and Aas, 2001).

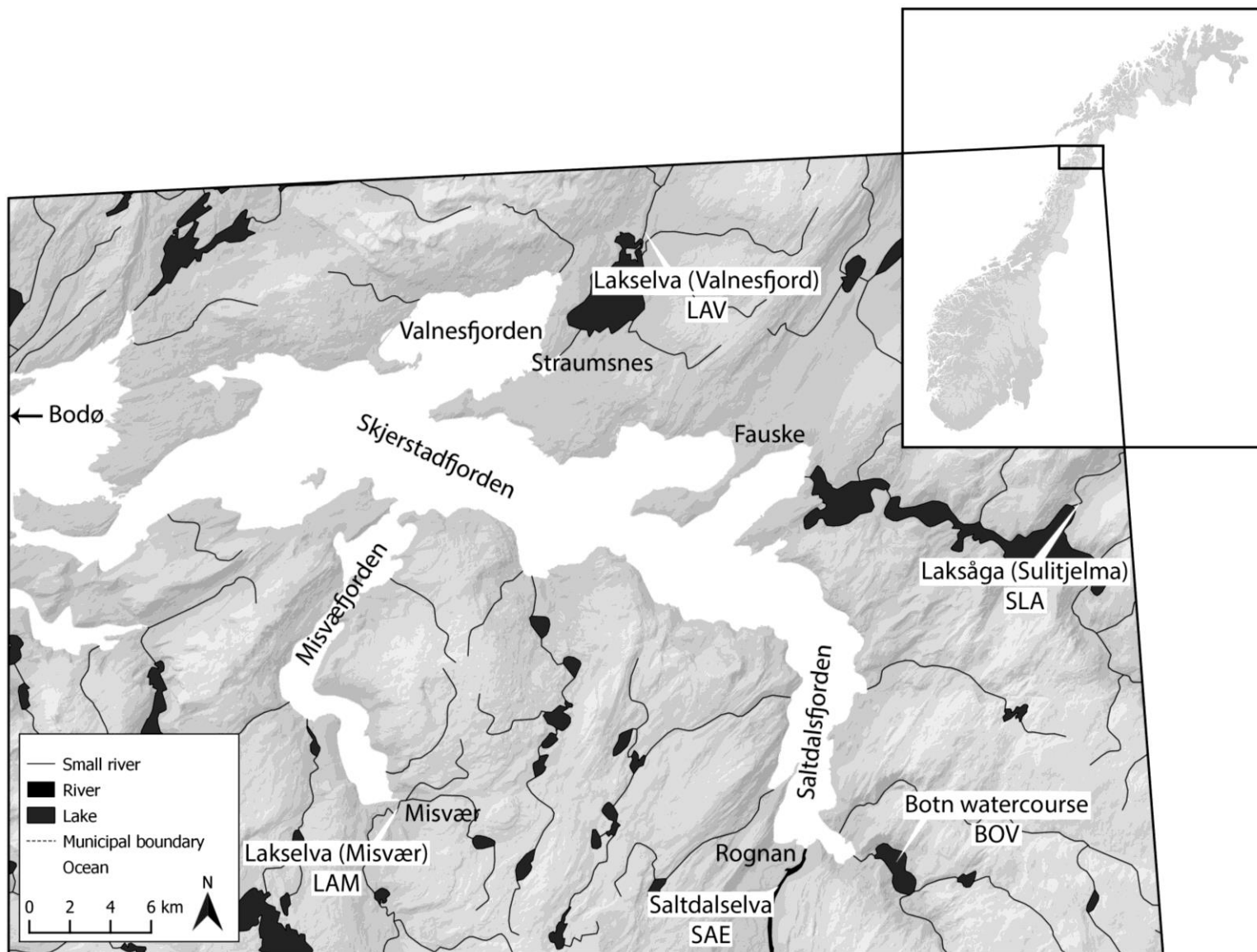
In this study, we investigate population structure among five watercourses known to harbour brown trout in the almost entirely landlocked Skjerstad fjord system in Northern Norway and hypothesise that significant population structure can be observed between them. Further, we carry out a MSA on the large-scale recreational fishery of sea-run brown trout in the fjord system, reputed for its big game sea-run brown trout. We hypothesise that individuals from Saltdal watercourse will be strongly represented in the mixed stock, as it historically has been an important local resource (Kanstad-Hanssen et al., 2017).

## Materials and methods

### Study area

The study was performed within an interconnected fjord system in Nordland county, Northern Norway (67°N 15°E), which comprise of four fjords; Skjerstadvfjorden, Saltdalsfjorden, Misvær fjorden and Valnesfjorden. The system stretches approximately 50 km from the innermost part of Saltdalsfjorden to the open sea through three narrow straits and has an inflow of freshwater from several large watercourses. Sampling took place at seven locations within five watercourses: Botn watercourse, river Lakselva (Misvær), river Lakselva (Valnesfjord), river Laksåga (Sulitjelma) and Saltdal watercourse (see **Figure 1**).

Botn watercourse (BOV) is located in the inner part of Saltdalsfjorden just east of Rognan municipality. It receives most of its water from the rivers Ingeborgelva in the north and Knallerdalselva in the south (NVE, 1991b). The river Lakselva (in Misvær; LAM) is located in the innermost part of Misvær fjorden and runs through Misvær municipality, stretching 4 km upstream to the lake Skarvatnet. The river Lakselva (in Valnesfjord; LAV) can be found in Valnesfjord watercourse by the lake Kosmovatnet which is connected to the fjord system by Laukåsstraumen strait. The river Laksåga (in Sulitjelma; SLA) flows into the northern part of Øvrevatnet which is one of the largest lakes in the system (Aanes et al., 1987). Saltdal watercourse (SAV) is Nordlands fourth largest watercourse (NVE, 2005) and has its source in Lønselva river (LOE), which receives water from Saltfjellet and several smaller streams before confluence with Junkerdalselva river (JUE). From here the river is called Saltdalselva (SAE) and stretches approximately 36 km north through Rognan to Saltdalsfjorden, reaching a total length of 80 km (Kanstad-Hanssen et al., 2017). All watercourses are known to harbour brown trout while some have varying numbers of Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.).



**Figure 1:** Map of the study area. The area comprise of four fjords; Skjerstadfjorden, Saltdalsfjorden, Misvær fjorden and Valnesfjorden and is located just east of Bodø in Nordland, Northern Norway.



## **Sample collection**

To determine patterns of genetic population structure among the rivers and establish a genetic baseline, tissue from juvenile salmonids ( $n=317$ ) in age groups 0 to 2/3+ were collected using electrofishing equipment. Samples were preserved in 96% ethanol and stored at  $-20^{\circ}\text{C}$  until extraction. Individuals were collected from river Saltdalselva (SAE,  $n=60$ ), Botn watercourse (BOV;  $n=59$ ), river Lakselva (Misvær, LAM;  $n=45$ ), river Laksåga (Sulitjelma, SLA;  $n=42$ ), river Junkerdalselva (JUE;  $n=42$ ), river Lønselva (LOE;  $n=35$ ), and river Lakselva (Valnesfjord, LAV;  $n=34$ ).

Local recreational anglers contributed to the study by collecting scale samples from sea-run brown trout ( $n=102$ ) caught in the fjord system. For each fish they collected 5-15 scales and measured the length and weight of the each individual. As length and weight is strongly correlated in brown trout (Arslan et al., 2004), and since weight is often rounded off, we focus on given length measurements. As these fish were caught in the fjord system, the population of origin was unknown, and this catchment of fish will thus be referred to as the mixed stock hereafter.

## **DNA extraction, quality assessment and quantification**

Genomic DNA was extracted from adipose fin tissue and from fish scales using the DNeasy 96 blood and tissue kit (QIAGEN), following the manufacturers protocol with some modifications i.e. heating of the AE buffer to  $60^{\circ}\text{C}$  and increasing centrifugation speed to  $20.000 \times g$  during elution to increase DNA yield.

As our downstream SNP genotyping assay required high quality DNA, we determined the DNA integrity of all samples through gel electrophoresis. DNA quantity was estimated in PicoGreen dsDNA concentration assays (ThermoFisher). Ten per cent of the samples were also selected by random and checked on a Qubit 4 fluorometer (average of 3 readings) using the dsDNA BR (broad range) Assay Kit (ThermoFisher).

## **Species and sex determination**

All locations sampled in this study were known also to harbour Atlantic salmon.

Morphologically differentiating between Atlantic salmon and brown trout at their juvenile stage can be very difficult, especially if hybridisation between the species occur (Pendas et al., 1995). To ensure that only brown trout were included in the genotyping an assay was developed to differentiate brown trout from Atlantic salmon and identify any hybridisation between the species. The assay consisted of two markers for species identification, 5S in the ribosomal DNA (dye: VIC; Pendas et al., 1995), *Salmo\_Mito951* in the mitochondrial DNA

(dye: NED; Karlsson et al., 2013) and one marker for identification of males (sdY, dye: 6FAM; Yano et al., 2012). The amplification of 5S and Salmo\_Mito951 also served as positive PCR controls.

Firstly, DNA was diluted to 0.05-0.025 ng DNA/ $\mu$ l. Polymerase chain reaction (PCR) was then performed in 3 $\mu$ l reactions at the following thermal cycling conditions: 15 minutes at 95°C (denaturation), 3 minutes at 60°C (primer annealing), 1 min at 72°C (extension), 27 cycles of 30s at 94°C, 3 minutes at 60°C and 1 minute at 72°C and a final elongation step for 30 minutes at 60°C. PCR products were separated on an Applied Biosystems Genetic Analyzer 3130xl using GeneScan LIZ 500 (Applied Biosystems) size standard. To correctly interpret results, adult individuals of brown trout and Atlantic salmon with known genotypes were used as references. Fragment size for the male marker was 223.5 base pairs (bp), the mitochondrial species marker was 230/238 bp (Salmon/trout) and the ribosomal species marker was 257/278 bp (Salmon/trout). All samples were visually scored using these allele sizes in Genemapper 4.0.

### **Genotyping**

Samples identified as brown trout through the species and sex determination assay were analysed at a 5509 SNP array using the Illumina Infinium assay (Illumina, San Diego, CA, USA; Drywa et al., 2013). The genotyping was performed at the Centre of Integrative Genetics (CIGENE; Ås, Norway). This SNP array consists of SNPs obtained through whole genome sequencing of brown trout individuals representing wild populations and domestic families (Andersson et al., 2017). The SNP subset was selected based on inter-SNP physical distance (60%), their similarity to Atlantic salmon cDNA sequences (20%) and homology to greater genetic Atlantic salmon scaffolds (20%; Andersson et al., 2017).

### **Evaluation of genotyping quality**

Thirteen replicates were initially checked to confirm the absence of cross contamination by manual comparison of single genotypes. To improve the quality of the final SNP dataset, we followed the filtering steps used in Andersson et al. (2017). Briefly, we removed multisite-variant loci (MSV-3s), samples with <96% call rate, loci present in <95% of samples and loci exhibiting alternate alleles with allele frequencies of < 0.01 (MAF, **Table 1**). The SNP genotype matrix was converted into Genepop format using the *snp2gen* function in the R package *diveRsity* (Keenan et al., 2013), a comprehensive package useful in estimating various population genetic parameters and converting file formats. We also used PGDSpider (v. 2.1.1.5; Lischer and Excoffier, 2011) to convert genepop files into other formats required

in downstream analyses.

**Table 1:** Number of SNPs retained following each filtering step and individuals used in various parts of the analysis.

	SNP	Individuals ( <i>n</i> )
CIGENE brown trout assay	5509	294
Filtering steps		
<96% call rate, bad samples, relevant SNP	4069	279
Removal of MSV-3	3694	
<95% call frequency	3667	
MAF 0.01	3509	
Removal of replicates		266
Loci under selection	1532	
Reference individuals	1532	179
Selection of reference individuals	1532	119
Mixed stock	1532	87

### Test for locus under selection and estimation of basic population genetic parameters

BayeScan (v. 2.1) uses a Bayesian method to determine the selection status of loci by calculating posterior probability for each locus being under selection based on other alternative models with and without selection components (Foll and Gaggiotti, 2008). We ran BayeScan with default chain parameters. Prior odds (PO) were set to 1000 and a false discovery rate (FDR) q-value threshold of 0.05 was used. In order to explore the resolution power of the markers and to discriminate the populations for assignment, we initially tested three datasets consisting of all, only neutral and only markers presumably influenced by divergent selection. We tested each putatively neutral loci for deviation from HWE in genepop (Keenan et al., 2013) in R. Obtained p-values was further corrected for multiple testing using the Bonferroni correction test (Holm, 1979). We used the *divBasics* function from the *diveRsity* package (Keenan et al., 2013) to estimate basic genetic population parameters such as observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{is}$ ) as a measure of deviation from Hardy-Weinberg equilibrium (HWE).

### Establishment of reference populations

To establish the genetic baseline required for assignment of individuals in the mixed stock we used three different population genetic methods; two non-model based methods, PCA and DAPC, as well as a model based STRUCTURE analysis (Pritchard et al., 2000), using the three datasets. The PCA and DAPC analyses were performed using the *adegenet* package

(Jombart, 2008). While the PCA aims to compress the data while maintaining variability, the DAPC tries to emphasise the differences between groups while minimising variation within each cluster. DAPC analysis was performed through two approaches. Firstly, using the *find.clusters* module, and secondly, using location as a prior. The *find.clusters* identifies the number of putative groups present in the dataset based on Bayesian information criterion (BIC) scores. Further, we used the *xvalDapc* module to identify the optimum number of principle components (60) to be used for the DAPC.

STRAUTO V1.0 (Chhatre and Emerson, 2017) which enables parallel computing, was used to execute STRUCTURE V2.3.4 by assuming admixture model (NOADMIX = 0), correlated allele frequency (FREQSCORR = 1) and use of sampling location as prior (LOCPRIOR = 1). The program was run for variable  $K$  from 1 to 10 i.e. presumed true number of  $K$ ;  $7 + 3$ , (Evanno et al., 2005) in 5 replicates, each with a burn-in period of 200000 and 500000 iterations. The STRUCTURE results were summarised and visualised using structure harvester (Earl, 2012) and CLUMPAK (Cluster Markov Packager Across  $K$ , Kopelman et al., 2015). In order to increase the power of assignment analyses, individuals with  $q$ -values (i.e. membership coefficients) higher than 0.8 were used to establish the final reference populations (Vähä and Primmer, 2006).

To confirm the genetic integrity of the selected individuals, a self-assignment test was implemented using STRUCTURE with STRAUTO (Chhatre and Emerson, 2017) and the *self\_assign* module from the R package *rubias* (Moran and Anderson, 2018). *rubias* performs genetic stock identification (GIS) using Bayesian inferences. The *self\_assign* module assigns individuals back to the reference populations using the leave-one-out method which leaves one sample out for each consecutive training set, resulting in the entire dataset being tested as explained more detailed in Anderson et al. (2008).

### **Assignment of individuals of recreational migratory brown trout fisheries**

To determine the population origin of individuals caught by recreational anglers, individuals from mixed stock which had good genotyping quality ( $n=87$ ; see **Table 1**) were analysed using STRUCTURE, *rubias* and the *geneplot* package (McMillan and Fewster, 2017). STRUCTURE was run in both assignment mode with population identifiers (POPFLAG = 1) for  $K = 5$  and in standard mode without assumptions for  $K = 10$  each for 5 iterations per  $K$ . The *infer\_mixture* function in *rubias* was used with 25,000 MCMC iterations and a burn-in period of 10,000. The package calculates z-scores for all individuals derived from the log-likelihood values and the expected standard deviation thus taking missing data into account. If

individuals in the mixed stock look like those in the reference populations a normal distribution of z-scores is expected. To visualise this results values can be plotted alongside simulated random variables to compare distributions. It should be noted, however, that the z-score cannot directly assign individuals, it merely identifies whether individuals fit the model or not (Anderson et al., 2008). The *geneplot* package is based on the GeneClass2 model and uses the saddlepoint approximation and the Rannala and Mountain (1997) model but enables visualisation of the results as 2D scatterplots.

### **Effective population size**

NeEstimator (v. 2.1 March 2018; Do et al., 2014) was used to estimate the contemporary effective population size. We utilised the bias-corrected linkage disequilibrium (LD) method for single samples as previously implemented in the LDNe software (Waples and Do, 2008). The software utilises the amount of LD generated within a population with random mating to estimate the effective population size and corrects for any bias by using various sample sizes (Waples and Do, 2008).

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## Results

### Basic pop parameters and establishment of dataset and test for locus under selection

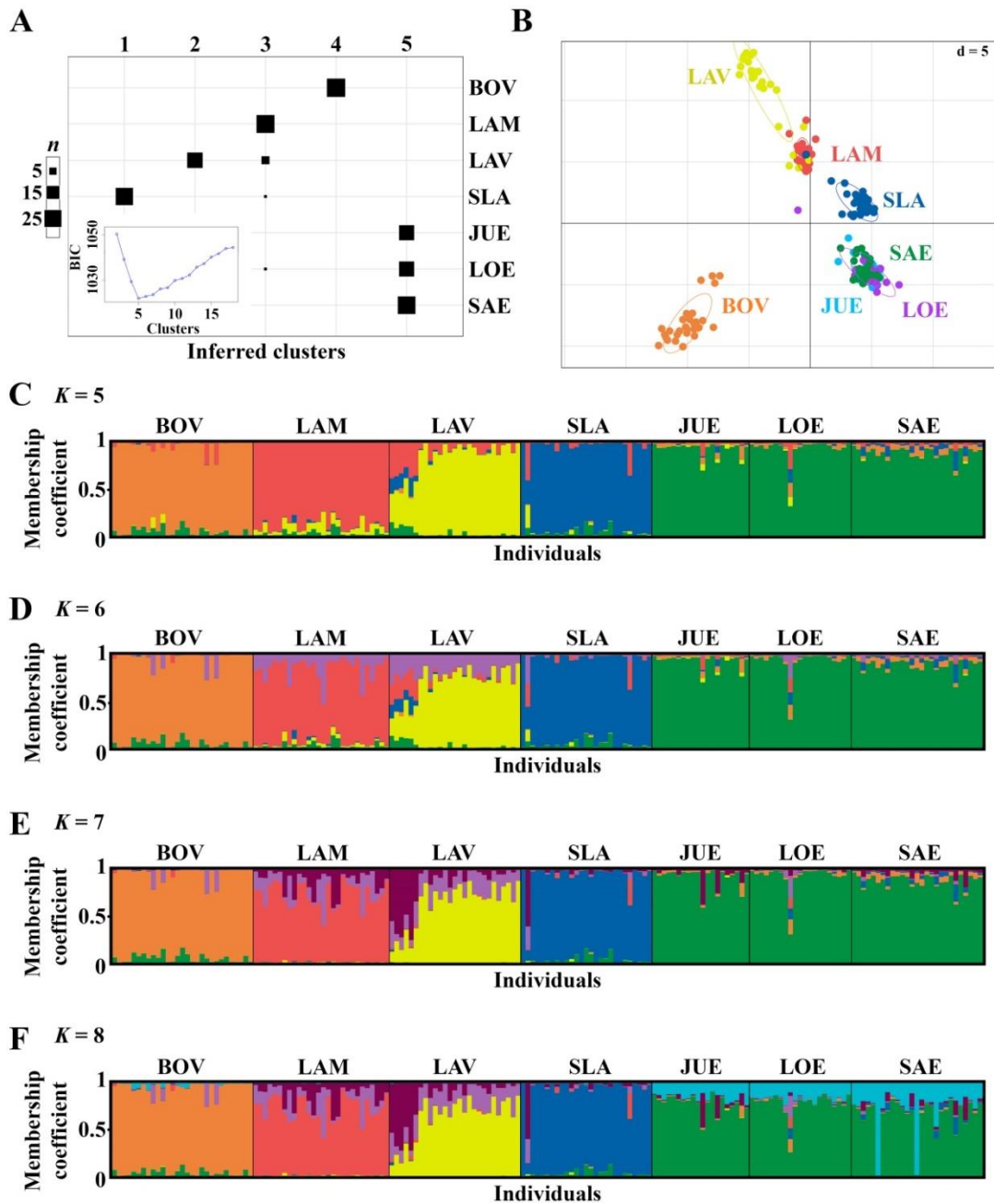
Of the 5509 loci, we received 4096 SNPs from CIGENE as these were deemed relevant for our study. Of the 294 individuals, 15 were removed due to low call rates (<96%) or errors during genotyping. Additionally, 375 MSV-3s, 27 SNPs with less than 95% call frequency and 158 SNPs with MAF of < 0.01 were excluded. Of thirteen replicates, one locus deviated in one replicate (0.002%). After the quality filtering (summary in **Table 1**), 3509 SNPs were retained, of which BayeScan identified 1532 SNPs (43.7%) alleged neutral, 8 SNPs (0.2%) putatively under diversifying selection and 1969 SNPs (56.1%) putatively under balancing selection (**supplementary, Figure S1**). While diversifying markers have potential usefulness and resolution power in discrete populations (Ackerman et al., 2011), the putative divergent SNPs identified in present study suggested low resolution when resolving population structure (**supplementary, Figure S2**). Consequently, we settled on the smaller set containing exclusively putative neutral markers (1532 SNPs) for downstream analyses, a dataset which also can be used in the estimation of effective population sizes. Our tests for deviation from HWE revealed no loci consistently under HWE across the identified populations. Estimations of basic population parameters revealed no significant deviation from HWE in our reference populations. The mixed stock deviated from HW proportions ( $F_{is}$  of 0.088), however this was to be expected as it was assumed to contain individuals from a multitude of populations. Genetic variation (expected and observed heterozygosity, **Table 2**) was approximately similar between watercourses, with the exception of Botn watercourse, and similar to what has been observed in other studies (Drywa et al., 2013, Saint-Pe et al., 2019).

### Population structure and establishment of reference populations

The *find.clusters* module in *adegenet* identified five main populations across the seven sampling locations, clustering the rivers Junkerdalselva, Lønselva and Saltdalselva together and Botn watercourse, river Lakselva (Misvær), river Lakselva (Valnesfjord) and river Laksåga (Sulitjelma) as four separate clusters (**Figure 2 A**). Similar clustering was observed in the PCA plot. Here, individuals from the rivers Junkerdalselva, Lønselva and Saltdalselva clustered together while being separate from the remaining clusters (**Figure 2 B**).

STRUCTURE supported this population clustering as the least negative  $\ln P(K)$  (-195988.64±6.52250) were observed for  $K=5$ , grouping the rivers Junkerdalselva, Lønselva and Saltdalselva together again (**Figure 2 C**). Consequently, individuals from these three rivers were grouped as Saltdal watercourse (SAV) in downstream analyses. While distinct

patterns were identified for each population, some individuals from river Lakselva (Valnesfjord) (**Figure 2 C-F**) revealed signatures of admixture. This, however, could also be stayers from foreign populations which were not identified by our assignment analysis.



**Figure 2:** Analysis of population structure of reference individuals ( $n=179$ ) from seven locations displayed as a (A) contingency table of the *find.clusters* inferred clusters vs. the pre-defined populations (inset shows graph of BIC values vs. number of clusters) (B) PCA where each colour represents one sampling location and (C-F) STRUCTURE in assignment mode with priors for  $K=5$  to  $K=8$ , here each vertical bar denotes one individual and colour represents groupings. Sampling locations are Botn watercourse (BOV), river Lakselva (Misvær, LAM), river Lakselva (Valnesfjord, LAV), river Laksåga (Sulitjelma, SLA), river Junkerdalselva (JUE), river Lønselva (LOE) and river



Saltdalselva (SAE).

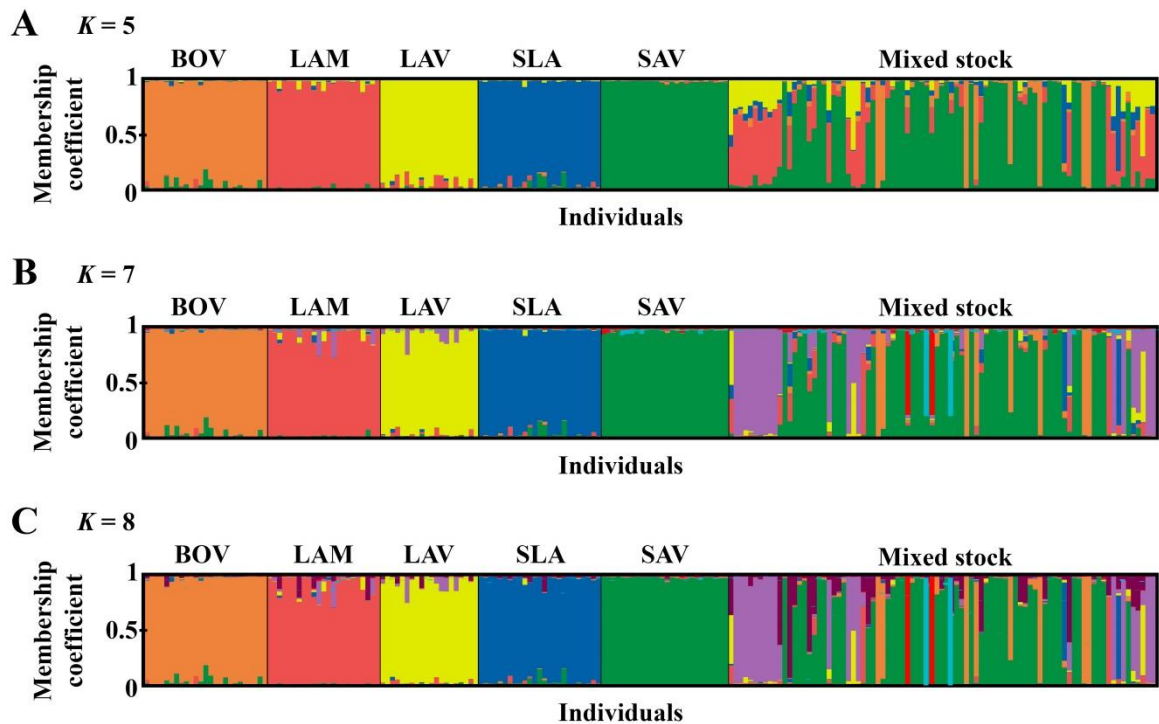
All reference individuals were assigned back to their respective populations with a 100% success rate using the *self\_assign* module in *rubias* (results not shown). STRUCTURE also assigned all selected individuals back to their respective origin (**Supplementary, Figure 3S**) indicating distinct genetic patterns for each population.

### **Genetic mixture analysis of recreational migratory brown trout fisheries**

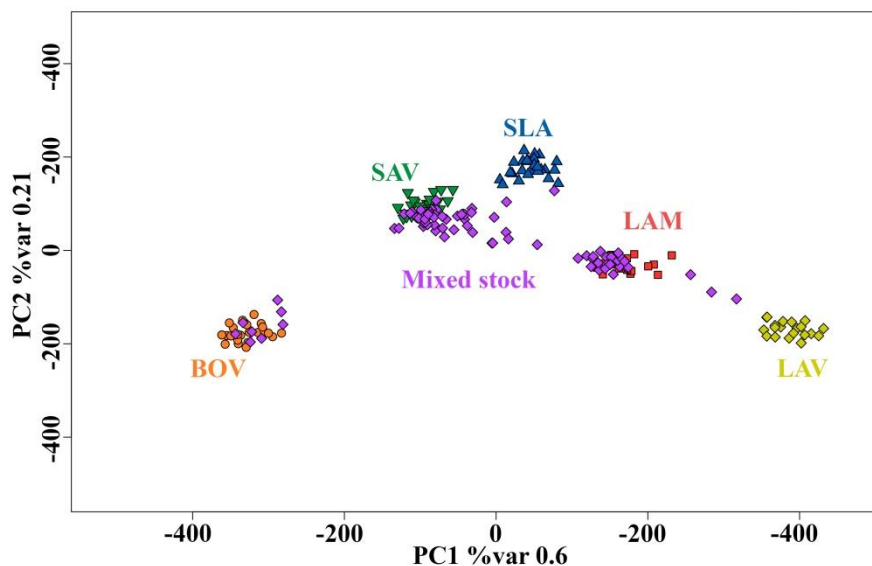
The mixed stock analysis of brown trout from recreational fisheries revealed that the individuals originated from several populations. The STRUCTURE analysis, in assignment mode, found that 45% of the mixed stock had higher than 80% membership probability with Saltdal watercourse, 8% with Botn watercourse and 2% with river Lakselva (Misvær), while the remaining 45% had  $q$ -values of  $<0.8$  (**Figure 3 A**). STRUCTURE analysis, in the standard mode, however, indicated that the remaining individuals might represent populations not included in the baseline. This was also suggested by the geneplot (**Figure 4**) and the z-score analysis in *rubias* (**Supplementary, Figure S4**). The geneplot analysis indicated differentiation between the reference populations while the mixed stock showed partly differentiation from the baseline. As this plot was explained by the two most descriptive principal components, some variation could not be included in the visualisation. However, it was clear that not all individuals from the mixed stock identified with the reference populations (purple colour, **Figure 4**). The STRUCTURE analysis, in standard mode, identified the most likely number of clusters to be  $K=7$  or  $K=8$  (**Figure 3 B-C**, for  $\text{Ln}(P)K$  see **supplementary, Figure S5**)

### **Weight and length distribution of the mixed stock**

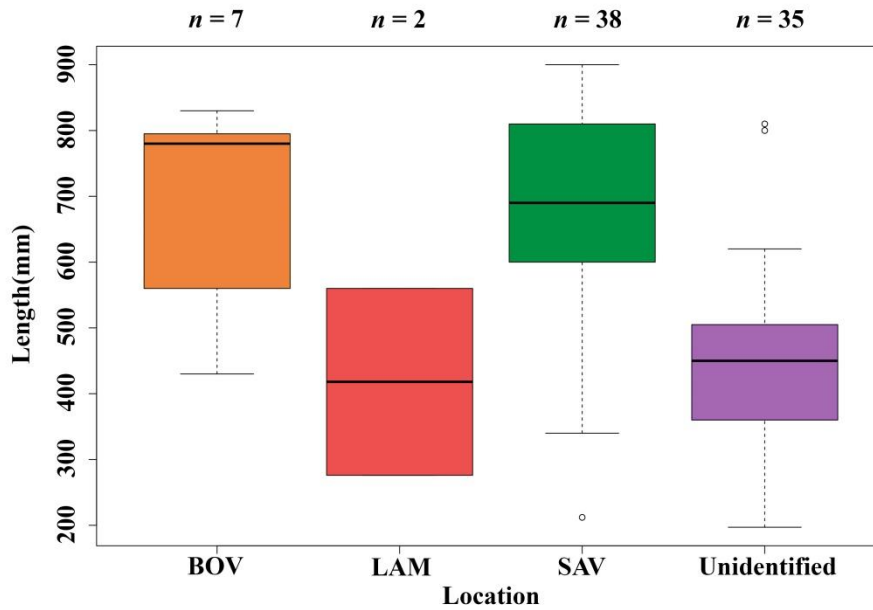
Data on length of the mixed stock revealed that sea-run brown trout from Saltdal watercourse amounted to 63% of the catchment of individuals longer than 50 cm and 76% of individuals longer than 70 cm (**Supplementary, Figure S6**) while individuals shorter than 50 cm mainly came from from unassigned populations (77%). Botn watercourse similarly contributed primarily with individuals longer than 50 cm. Lakselva river (Misvær) only contributed with two individuals while the river Lakselva (Valnesfjord) and the river Laksåga (Sulitjelma) did not contribute to the mixed stock.



**Figure 3:** Analysis of individuals from the mixed stock ( $n=87$ ) alongside the reference individuals ( $n=119$ ) using (A) STRUCTURE in assignment mode, (B-C) STRUCTURE in standard mode for  $K=7$  and  $K=8$  as these were identified as the most likely number of populations by the  $\text{LnP}(K)$ , here each vertical bar denotes one individual and colour represents groups. Reference populations are Botn watercourse (BOV), river Lakselva (Misvær, LAM), river Lakselva (Valnesfjord, LAV), river Laksåga (Sulitjelma, SLA) and Saltdal watercourse (SAV).



**Figure 4:** Genepool analysis of the mixed stock along the reference populations Botn watercourse (BOV), river Lakselva (Misvær, LAM), river Lakselva (Valnesfjord, LAV), river Laksåga (Sulitjelma, SLA) and Saltdal watercourse (SAV). Here axes represent the two principle components which explain 60% and 21% of the variation in the dataset and colours represent plotted groups.



**Figure 5:** Boxplot of length (mm) distribution of individuals from the mixed stock. Individuals have been assigned their putative populations (Botn watercourse BOV, river Lakselva (Misvær) LAM and Saltdal watercourse, SAV) based on minimum 80% membership probabilities obtained from STRUCTURE while individuals which did not identify with the baseline have been labelled as unidentified. The black lines indicate the median, boxes represent 95% distribution, T-bars represent the range of the dataset, and dots indicate outliers. Number of individuals for each boxplot can be seen above.

### Effective population size estimates

Single sample estimates of effective population sizes varied among the populations (**Table 2**), revealing the highest effective population size (256.6, CI 229.8-290) in Saltdal watercourse (SAV) and the lowest (48.7, CI 47.2-50.4) in Botn watercourse (BOV).

**Table 2:** Basic population parameters and estimations of the effective population size. Population parameters were estimated using the *diveRsity* package and includes number of genotyped samples ( $N_{\text{geno}}$ ) and samples in the reference population ( $N_{\text{ref}}$ ) in addition to observed- ( $H_o$ ) and expected heterozygosity  $H_e$  and  $F_{is}$  as a measure of deviation from Hardy-Weinberg equilibrium. Effective population size ( $N_e$ ) was estimated with NeEstimator v2.1. Confidence intervals (C.I.) are also given for  $F_{is}$  and  $N_e$ . Groups are Botn watercourses (BOV), river Lakselva (Misvær, LAM), river Lakselva (Valnesfjord, LAV) river Laksåga (Sulitjelma, SLA), Saltdal watercourse (SAV), river Junkerdalselva (JUE), river Lønselva (LOE) and river Saltdalselva (SAE).

Groups	Sampling year	$N_{\text{geno}}$	$N_{\text{ref}}$	Individual age/size(mm)	Heterozygosity		$F_{is}$		Effective population size ( $N_e$ )	
					$H_o$	$H_e$	Estimate	95% C.I.	Estimate	95% C.I.
BOV	2017	32	25	0 to 2/3+ / -	0.25	0.24	-0.016	-0.043, 0.007	48.7	47.2, 50.4
LAM	2017	31	23	0 to 2/3+ / -	0.32	0.32	-0.016	-0.039, 0.001	153.5	142.2, 166.5
LAV	2017	31	20	0 to 2/3+ / -	0.29	0.29	-0.023	-0.049, -0.004	70.7	67.5, 74.3
SLA	2017	30	25	0 to 2/3+ / -	0.32	0.31	-0.036	-0.058, -0.016	167.3	154.4, 182.3
SAV	2017/18	-	26	0 to 2/3+ / -	0.31	0.31	0.006	-0.029, 0.013	256.6	229.8, 290.0
JUE	2018	21	-	0 to 2/3+ / -	-	-	-	-	-	-
LOE	2018	22	-	0 to 2/3+ / -	-	-	-	-	-	-
SAE	2017	32	-	0 to 2/3+ / -	-	-	-	-	-	-
Mixed stock	2017/18	95	87	- / 197 to 900	0.32	0.35	0.088	0.070, 0.104	-	-

## Discussion

Our results reveal genetic differentiation among all the watercourses sampled in the current study. The mixed stock analysis revealed that the Saltdal watercourse was the major contributor (63%) of individuals longer than 50 cm and 76% of individuals longer than 70 cm to the recreational fisheries, and that up to three additional unidentified populations contributed to the mixed stock.

The first objective of present study was to identify genetic differences between populations of brown trout using juvenile (pre-migratory) individuals from five major watercourses and to establish a representative genetic baseline for each identified population, as this is one of the key assumptions in genetic mixed stock assignment studies (Beacham et al., 2006). Previous studies on brown trout have shown significant genetic differentiation even on small geographical scales (Carlsson et al., 1999) and high levels of population structure among rivers (e.g. Mäkinen et al., 2015, Sønstebo et al., 2007, Swatdipong et al., 2013). Similarly, we observed significant genetic differences between juvenile brown trout samples belonging to the different watercourses in the Skjerstad fjord system, even when geographical adjacent, which supports our first hypothesis. However, a handful of juveniles from the river Lakselva (Valnesfjord) were suggested to have a mixed origin. Self-assignment of reference individuals to their respective populations further supported the observation of differentiation among samples from the different watercourses. As also observed in other studies (Charles et al., 2005, Hindar et al., 1991), we did not identify any significant genetic population structure within the watercourses, suggesting gene flow among spawning grounds within watercourses.

The second objective was to identify the relative composition of recreational sea-run brown trout fisheries within the fjord system. Our MSA indicated that Saltdal watercourse was the major contributor (45%) of sea-run brown trout to the Skjerstad fjord system, with the Botn watercourse and the river Lakselva (Misvær) contributing with 8% and 2%, respectively. The remaining individuals in the MSA (45%) were from populations with low (<80%) membership coefficients to populations in our baseline. This is supported by Saltdal watercourse being estimated to have the largest effective population size ( $N_e$ ) in our baseline and the watercourse being one of the largest in Nordland (NVE, 1991). These findings support our second hypothesis, as Saltdal watercourse was expected to be strongly represented in the mixed stock since historical catch records show yearly catches of several tons dating back almost 150 years (Kanstad-Hanssen et al., 2017). Despite the high  $N_e$  for the Laksåga (Sulitjelma) population, no sea-run brown trout were identified as originating from this

population. This could indicate that the population predominantly consists of resident or lake-migrating trout or that migrating individuals occupy areas of the fjord which are not easily accessible from shore. Generally, our  $N_e$  estimates are comparable to those found in other studies on brown trout (Hansen et al., 2007, Østergaard et al., 2003). Interestingly, Lakselva (Misvær) contributed negligible to the mixed stock. This could, however, be related to the sampling bias inherent in samples, as recreational anglers naturally prefer to fish in areas with abundant and large specimen. Data from recreational anglers further showed that none of the investigated sea-run brown trout were caught in Misvær fjorden where Lakselva (Misvær) potentially could be an important contributor to the mixed stock. Despite most sea-run brown trout being taken in Valnesfjorden, Lakselva (Valnesfjord) was not among the contributing populations. This could indicate that brown trout from this river forage in the lake Kosmovatnet, instead of migrating further into the fjord. The remaining sea-run brown trout which did not identify with populations in our baseline were found to originate from two to three putative unsampled populations, suggesting under-surveying of reference populations in the study area. The catch records suggested that individuals contributing to one of the unidentified putative populations (18% of the mixed stock) all were caught at the same location at Straumen/Straumsnes, a strait connecting Kosmovatnet and Valnesfjorden (see **Figure 1**), illustrating the importance of proper spatial sampling in order to identify all contributing populations.

Modern fisheries management practices are moving towards sustainable utilisation of fisheries resources (e.g. Beacham et al., 2019). Accurate identification of stocks makes up a critical component in effective management of fisheries (Begg et al., 1999, Cadrin et al., 2013). The method involves exhaustive genetic surveying of reference populations to establish a representative baseline which can be used in stock composition analyses in the region. As suggested by the results of this study, brown trout populations from each watercourse represent distinct populations. To effectively preserve these populations and the biological diversity of the system, these populations should therefore be managed as separate units, even if they do not qualify as evolutionary significant units by others standards (ESUs; Almodóvar et al., 2006). However, since the mixed stock suggests an incomplete baseline, the presence of unidentified populations should also be considered in management plans.

Sea-run brown trout from the Saltdal watercourse accounted for 63% of the individuals longer than 50 cm and 76% of those longer than 70 cm caught for this project (**Figure 4**) thus being of utmost interest for management and especially recreational anglers. In contrast, the

unidentified populations contributed with individuals of varying sizes, although especially individuals shorter than 50 cm (76%), indicating a relationship between population and fish size. This result also shows that recreational anglers, who naturally target the largest fish possible, are more likely to target the Saltdal watercourse brown trout population. The Saltdal watercourse displayed the largest  $N_e$ , which usually would indicate good resilience towards exploitation. However, recent years have shown a decline in brown trout caught in this watercourse (cf. statistisk sentralbyrå, ssb.no), an unfortunate trend which corresponds to observations in several other European countries (ICES, 2013). Similar to Saltdal watercourse, Botn watercourse also contributed to the mixed stock (8%), especially with individuals longer than 50 cm (12%) and 70 cm (16%). However, a low  $N_e$  (48.7) suggested that this population is under the risk of harmful effects from inbreeding depression following the “50/500 rule” as proposed by Franklin (1980), a rule which since its proposal has been a guiding principle in conservation biology (Jamieson and Allendorf, 2012). The rule states that a minimum population size (i.e. short-term  $N_e$ ) of 50 individuals is required to avoid inbreeding depression and 500 to reduce effects of genetic drift. Kuparinen and Hutchings (2019), however, argued that numerous cases of natural populations exist which are ecologically and genetically stable despite lower  $N_e$  than recommended by the 50/500 rule. The authors further suggest that numerous factors contribute towards forming and maintaining genetic diversity in populations with low  $N_e$ , but that the rule may be useful in cases with limited access to demographic and genetic data. Generally, however, larger  $N_e$  is always better and increases a population's chance of survival (Franklin et al., 2014). It has been shown that mainly individuals longer than 60 cm spawn in the Botn watercourse (Davidsen et al., 2019); this naturally limits the amount of spawners, especially since fish at those lengths are being targeted by recreational fisheries in the fjord. The Botn watercourse was heavily exploited by local fisheries from early 1900s using traps until the 1930s and nets until the 1960s (Davidsen et al., 2019), periods which could have created bottlenecks for the population leading to the limited genetic variation observed today. While other populations which contribute with less or smaller sea-run brown trout to the catchment might not be of great interest for managers or recreational fisheries, these populations should not be neglected. Overexploitation of these populations could harm the complexity of the biological system and reduce biodiversity and long-term stability of the fjord system (Hilborn et al., 2003, Schindler et al., 2010).

Population genomic approaches such as those used in this study have been shown to promote

the understanding of population structure and identify conservation units for management purposes (Laikre et al., 1999). Our study revealed that recreational fishing for big game sea-run brown trout in the Skjerstad fjord system mainly target the Saltdal watercourse. However, for a holistic view of exploitation of sea-run brown trout in the system, we recommend that future studies expand the genetic baseline through thorough spatial sampling and utilise temporal sampling methods to identify supposed variations in the mixed stock composition between years.



## References

- Aanes, K. J., Iversen, E. R., Johannesen, M. & Mjelde, M. 1987. Overvåkning av Sulitjelmavassdraget 1985. *NIVA-rapport*.
- Ackerman, M. W., Habicht, C. & Seeb, L. W. 2011. Single-nucleotide polymorphisms (SNPs) under diversifying selection provide increased accuracy and precision in mixed-stock analyses of sockeye salmon from the Copper River, Alaska. *Transactions of the American Fisheries Society*, 140, 865-881.
- Almodóvar, A., Nicola, G., Elvira, B. & García-Marín, J. 2006. Introgression variability among Iberian brown trout Evolutionary Significant Units: the influence of local management and environmental features. *Freshwater Biology*, 51, 1175-1187.
- Anderson, E. C., Waples, R. S. & Kalinowski, S. T. 2008. An improved method for predicting the accuracy of genetic stock identification. *Canadian Journal of Fisheries and Aquatic Sciences*, 65, 1475-1486.
- Andersson, A., Jansson, E., Wennerström, L., Chiriboga, F., Arnyasi, M., Kent, M. P., Ryman, N. & Laikre, L. 2017. Complex genetic diversity patterns of cryptic, sympatric brown trout (*Salmo trutta*) populations in tiny mountain lakes. *Conservation Genetics*, 18, 1213-1227.
- Arslan, M., Yıldırım, A. & Bektaş, S. 2004. Length-weight relationship of brown trout, *Salmo trutta* L., inhabiting Kan stream, Coruh Basin, north-eastern Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 4, 45-47.
- Beacham, T. D., Candy, J. R., Jonsen, K. L., Supernault, J., Wetklo, M., Deng, L., Miller, K. M., Withler, R. E. & Varnavskaya, N. 2006. Estimation of stock composition and individual identification of Chinook salmon across the Pacific Rim by use of microsatellite variation. *Transactions of the American Fisheries Society*, 135, 861-888.
- Beacham, T. D., Wallace, C., Jonsen, K., McIntosh, B., Candy, J. R., Willis, D., Lynch, C., Moore, J. S., Bernatchez, L. & Withler, R. E. 2019. Comparison of coded-wire tagging with parentage-based tagging and genetic stock identification in a large-scale coho salmon fisheries application in British Columbia, Canada. *Evolutionary applications*, 12, 230-254.
- Begg, G. A., Friedland, K. D. & Pearce, J. B. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research*, 43, 1-8.
- Begg, G. A. & Waldman, J. R. 1999. An holistic approach to fish stock identification. *Fisheries research*, 43, 35-44.
- Bekkevold, D., Clausen, L. A., Mariani, S., André, C., Hatfield, E. M., Torstensen, E., Ryman, N., Carvalho, G. R. & Ruzzante, D. E. 2011. Genetic mixed-stock analysis of Atlantic herring populations in a mixed feeding area. *Marine Ecology Progress Series*, 442, 187-199.
- Boel, M., Aarestrup, K., Baktoft, H., Larsen, T., Søndergaard Madsen, S., Malte, H., Skov, C., Svendsen, J. C. & Koed, A. 2014. The physiological basis of the migration continuum in brown trout (*Salmo trutta*). *Physiological and Biochemical Zoology*, 87, 334-345.
- Bradbury, I. R., Hamilton, L. C., Chaput, G., Robertson, M. J., Goraguer, H., Walsh, A., Morris, V., Reddin, D., Dempson, J. B. & Sheehan, T. F. 2016. Genetic mixed stock analysis of an interceptory Atlantic salmon fishery in the Northwest Atlantic. *Fisheries Research*, 174, 234-244.
- Cadrin, S. X., Kerr, L. A. & Mariani, S. 2013. *Stock identification methods: applications in fishery science*, Academic Press.

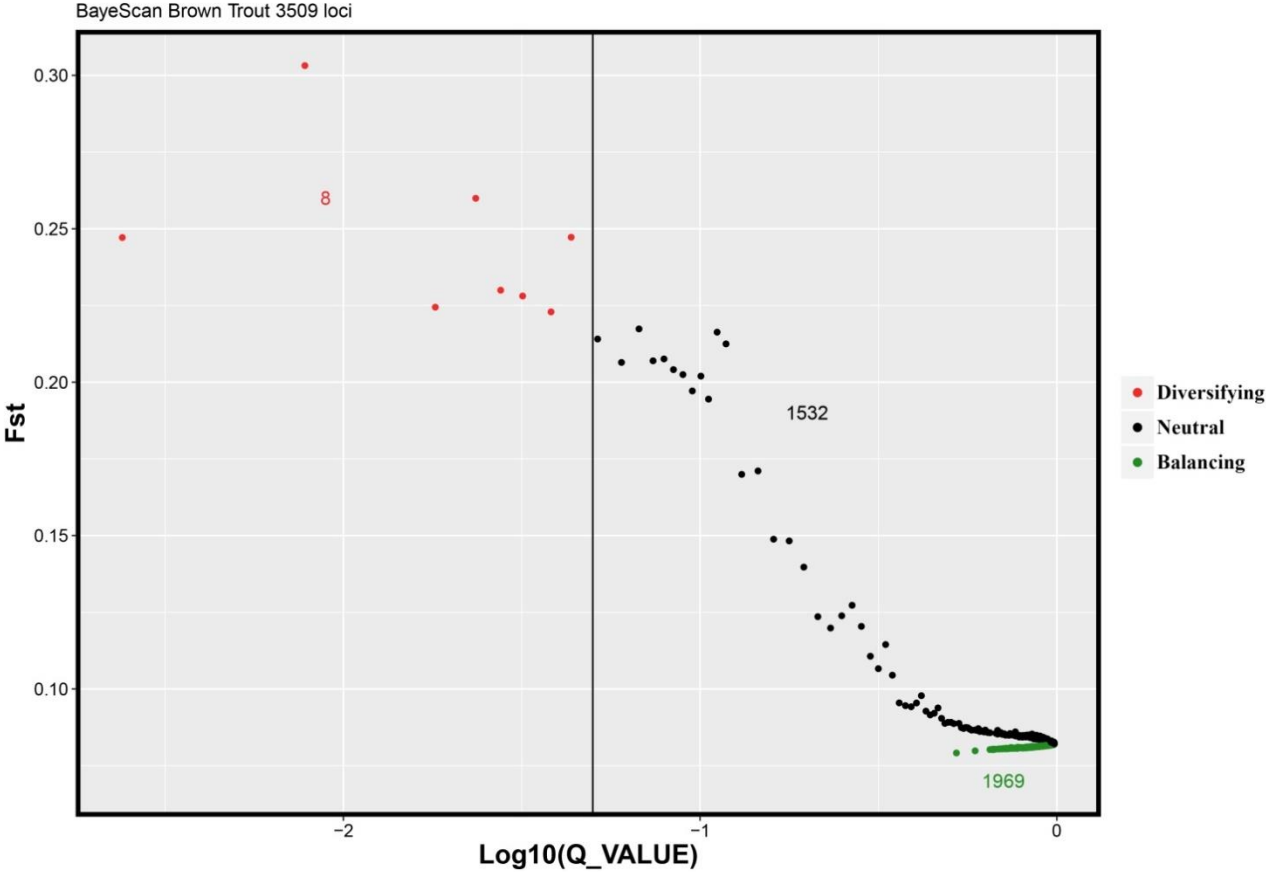
- Carlsson, J., Olsen, K., Nilsson, J., Øverli, Ø. & Stabell, O. 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. *Journal of Fish Biology*, 55, 1290-1303.
- Chapman, B., Hulthén, K., Brodersen, J., Nilsson, P. A., Skov, C., Hansson, L. A. & Brönmark, C. 2012. Partial migration in fishes: causes and consequences. *Journal of fish biology*, 81, 456-478.
- Charles, K., Guyomard, R., Hoyheim, B., Ombredane, D. & Baglinière, J.-L. 2005. Lack of genetic differentiation between anadromous and resident sympatric brown trout (*Salmo trutta*) in a Normandy population. *Aquatic Living Resources*, 18, 65-69.
- Chhatre, V. E. & Emerson, K. J. 2017. StrAuto: automation and parallelization of STRUCTURE analysis. *BMC bioinformatics*, 18, 192.
- Cornuet, J.-M., Piry, S., Luikart, G., Estoup, A. & Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, 153, 1989-2000.
- Crozier, W., Schön, P., Chaput, G., Potter, E., Maoileidigh, N. & MacLean, J. 2004. Managing Atlantic salmon (*Salmo salar* L.) in the mixed stock environment: challenges and considerations. *ICES Journal of Marine Science*, 61, 1344-1358.
- Cucherousset, J., Ombredane, D., Charles, K., Marchand, F. & Baglinière, J.-L. 2005. A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 1600-1610.
- Davidson, J. G., Eldøy, S. H., Meyer, I., Halvorsen, A. E., Sjursen, A. D., Rønning, L., Schmidt, S. N., Præbel, K., Daverdin, M., Bårdsen, M. T., Whoriskey, F. & Thorstad, E. B. 2019. Sjørøret og sjørøye i Skjerstadfjorden - Marine vandringer, områdebruk og genetikk. *NTNU Vitenskapsmuseet naturhistorisk rapport*
- Do, C., Waples, R. S., Peel, D., Macbeth, G., Tillett, B. J. & Ovenden, J. R. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular ecology resources*, 14, 209-214.
- Drywa, A., Poćwierz-Kotus, A., Wąs, A., Dobosz, S., Kent, M. P., Lien, S., Bernaś, R. & Wenne, R. 2013. Genotyping of two populations of Southern Baltic Sea trout *Salmo trutta* m. *trutta* using an Atlantic salmon derived SNP-array. *Marine genomics*, 9, 25-32.
- Earl, D. A. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources*, 4, 359-361.
- Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, 14, 2611-2620.
- Fiske, P. & Aas, Ø. 2001. *Laksefiskeboka: om sammenhenger mellom beskatning, fiske og verdiskaping ved elvefiske etter laks, sjøaure og sjørøye*, Norsk Institutt for Naturforskning.
- Foll, M. & Gaggiotti, O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, 180, 977-993.
- Franklin, I. R. 1980. Evolutionary change in small populations.
- Franklin, I. R., Allendorf, F. W. & Jamieson, I. G. 2014. The 50/500 rule is still valid—reply to Frankham et al. *Biol. Conserv*, 176, 284-285.
- Griffiths, A. M., Machado-Schiaffino, G., Dillane, E., Coughlan, J., Horreo, J. L., Bowkett, A. E., Minting, P., Toms, S., Roche, W. & Gargan, P. 2010. Genetic stock identification

- of Atlantic salmon (*Salmo salar*) populations in the southern part of the European range. *BMC genetics*, 11, 31.
- Hansen, M. M., Ruzzante, D. E., Nielsen, E. E., Bekkevold, D. & Mensberg, K. L. D. 2002. Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology*, 11, 2523-2535.
- Hansen, M. M., SKAALA, Ø., Jensen, L. F., Bekkevold, D. & MENSBERG, K. L. D. 2007. Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Fjord, Norway. *Molecular Ecology*, 16, 1413-1425.
- Hilborn, R., Quinn, T. P., Schindler, D. E. & Rogers, D. E. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences*, 100, 6564-6568.
- Hindar, K., Jonsson, B., Ryman, N. & Ståhl, G. 1991. Genetic relationships among landlocked, resident, and anadromous brown trout, *Salmo trutta* L. *Heredity*, 66, 83.
- Höjesjö, J., Aldvén, D., Davidsen, J. G., Pedersen, S. & Degerman, E. Perspectives on sea trout stocks in Sweden, Denmark & Norway: monitoring, threats and management. 2nd International Sea Trout Symposium, 2017. Troubador Publishing, 443-459.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian journal of statistics*, 65-70.
- ICES 2013. Report of the Workshop on Sea Trout (*Salmo trutta*) (WKTRUTTA). 242p.
- Jamieson, I. G. & Allendorf, F. W. 2012. How does the 50/500 rule apply to MVPs? *Trends in ecology & evolution*, 27, 578-584.
- Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403-1405.
- Kalinowski, S. T. 2004. Genetic polymorphism and mixed-stock fisheries analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 1075-1082.
- Kanstad-Hanssen, Ø., Lamberg, A., Bentsen, V., Bjørnbet, S. & Gjertsen, V. 2017. Gytdefiskregistrering i Saltdalselva i 2017 - Resultater fra drivtelling av laks, sjøørret og sjørøye. Skandinavisk naturovervåking, Prosjekt Utmark, Nordland bondelag.
- Karlsson, S., Hagen, M., Eriksen, L., Hindar, K., Jensen, A. J., de Leaniz, C. G., Cotter, D., Guðbergsson, G., Kahilainen, K. & Guðjónsson, S. 2013. A genetic marker for the maternal identification of Atlantic salmon × brown trout hybrids. *Conservation Genetics Resources*, 5, 47-49.
- Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W. & Prodöhl, P. A. 2013. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, 4, 782-788.
- Koljonen, M.-L., Pella, J. J. & Masuda, M. 2005. Classical individual assignments versus mixture modeling to estimate stock proportions in Atlantic salmon (*Salmo salar*) catches from DNA microsatellite data. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 2143-2158.
- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A. & Mayrose, I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular ecology resources*, 15, 1179-1191.
- Kuparinen, A. & Hutchings, J. A. 2019. When phenotypes fail to illuminate underlying genetic processes in fish and fisheries science. *ICES Journal of Marine Science*.
- Laikre, L., Antunes, A., Apostolidis, A., Berrebi, P., Duguid, A., Ferguson, A., Garcia-Marin, J., Guyomard, R., Hansen, M. & Hindar, K. 1999. *Conservation genetic management of brown trout (Salmo trutta) in Europe*, Danmarks Fiskeriundersøgelser, Afd. for Ferskvandsfiskeri.

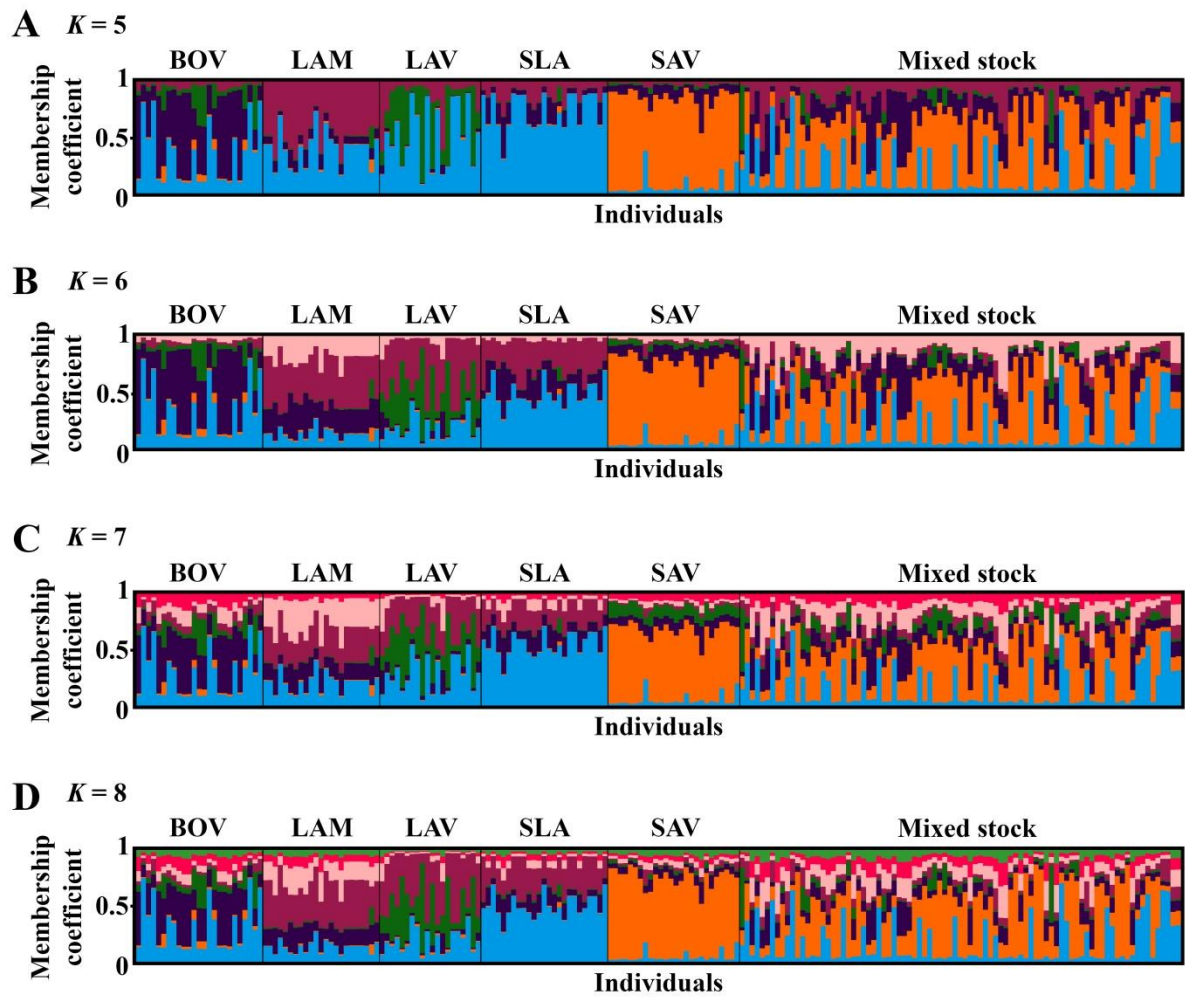
- Lischer, H. E. & Excoffier, L. 2011. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, 28, 298-299.
- MacCrimmon, H. R., Marshall, T. L. & Gots, B. L. 1970. World distribution of brown trout, *Salmo trutta*: further observations. *Journal of the Fisheries Board of Canada*, 27, 811-818.
- Mäkinen, H., Niva, T., Koljonen, M.-L., Swatdipong, A. & Primmer, C. R. 2015. temporal variation in lake-run brown trout (*Salmo trutta*) mixed-stock fishery catches in a large Fennoscandian lake.
- McMillan, L. & Fewster, R. 2017. Visualizations for genetic assignment analyses using the saddlepoint approximation method. *Biometrics*, 73, 1029-1041.
- Moran, B. M. & Anderson, E. C. 2018. Bayesian inference from the conditional genetic stock identification model. *Canadian Journal of Fisheries and Aquatic Sciences*, 76, 551-560.
- NVE 1991. 163/1 Saltdalsvassdraget NOU 1991 12B.
- NVE 1991b. 163/2 Botnelva NOU 1991 12B.
- NVE 2005. Helhetlig tiltaksplan for Saltdalselva. *Faktaark - NVE Fakta*.
- Østergaard, S., Hansen, M. M., Loeschcke, V. & Nielsen, E. E. 2003. Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. *Molecular Ecology*, 12, 3123-3135.
- Pendas, A., Moran, P., Martinez, J. & GARCIA-VAZQUEZ, E. 1995. Applications of 5S rDNA in Atlantic salmon, brown trout, and in Atlantic salmon brown trout hybrid identification. *Molecular Ecology*, 4, 275-276.
- Pritchard, J. K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.
- Rannala, B. & Mountain, J. L. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences*, 94, 9197-9201.
- Saint-Pe, K., Leitwein, M., Tissot, L., Poulet, N., Guinand, B., Berrebi, P., Marselli, G., Lascaux, J.-M., Gagnaire, P.-A. & Blanchet, S. 2019. Development of a large SNPs resource and a low-density SNP array for brown trout (*Salmo trutta*) population genetics. *BMC genomics*, 20, 582.
- Schindler, D. E., Hilborn, R., Chasco, B., Boatright, C. P., Quinn, T. P., Rogers, L. A. & Webster, M. S. 2010. Population diversity and the portfolio effect in an exploited species. *Nature*, 465, 609.
- Shaklee, J. B., Beacham, T. D., Seeb, L. & White, B. A. 1999. Managing fisheries using genetic data: case studies from four species of Pacific salmon. *Fisheries Research*, 43, 45-78.
- Smith, C. T., Templin, W. D., Seeb, J. E. & Seeb, L. W. 2005. Single nucleotide polymorphisms provide rapid and accurate estimates of the proportions of US and Canadian Chinook salmon caught in Yukon River fisheries. *North American Journal of Fisheries Management*, 25, 944-953.
- Sønstebø, J. H., Borgstrøm, R. & Heun, M. 2007. Genetic structure of brown trout (*Salmo trutta* L.) from the Hardangervidda mountain plateau (Norway) analyzed by microsatellite DNA: a basis for conservation guidelines. *Conservation Genetics*, 8, 33-44.
- Swatdipong, A., Vasemägi, A., Niva, T., Koljonen, M. L. & Primmer, C. 2013. Genetic mixed-stock analysis of lake-run brown trout *Salmo trutta* fishery catches in the Inari Basin, northern Finland: implications for conservation and management. *Journal of fish biology*, 83, 598-617.

- Vähä, J. & Primmer, C. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular ecology*, 15, 63.
- Waples, R. S. & Do, C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular ecology resources*, 8, 753-756.
- Yano, A., Guyomard, R., Nicol, B., Jouanno, E., Quillet, E., Klopp, C., Cabau, C., Bouchez, O., Fostier, A. & Guiguen, Y. 2012. An immune-related gene evolved into the master sex-determining gene in rainbow trout, *Oncorhynchus mykiss*. *Current Biology*, 22, 1423-1428.

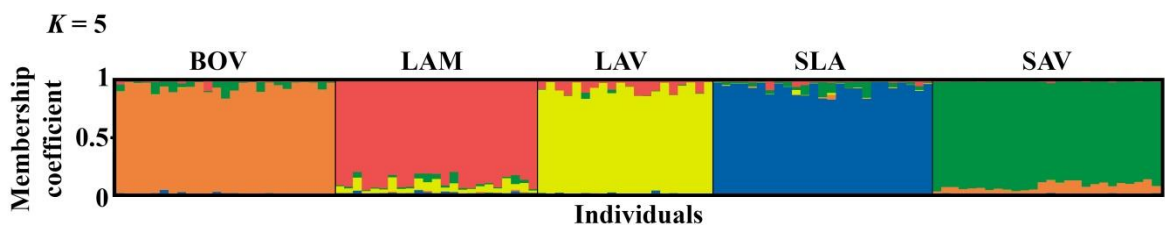
# Supplementary



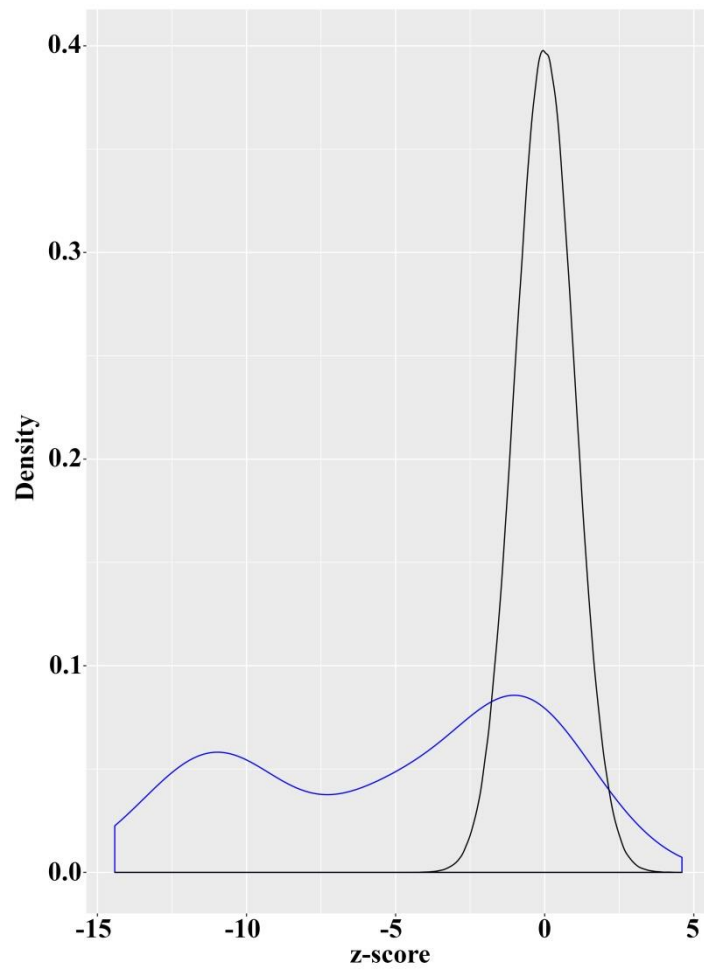
**Figure S1:** BayeScan identification of loci under diversifying (red), neutral (black) and balancing (green) selection.



**Figure S2:** STRUCTURE of 8 divergent SNPs to investigate assignment of the mixed stock for (A)  $K=5$ , (B)  $K=6$ , (C)  $K=7$  and (D)  $K=8$ . Reference populations are Botn watercourse (BOV), river Lakselva (Misvær, LAM), river Lakselva (Valnesfjord, LAV), river Laksåga (Sulitjelma, SLA) and Saltdal watercourse (SAV).

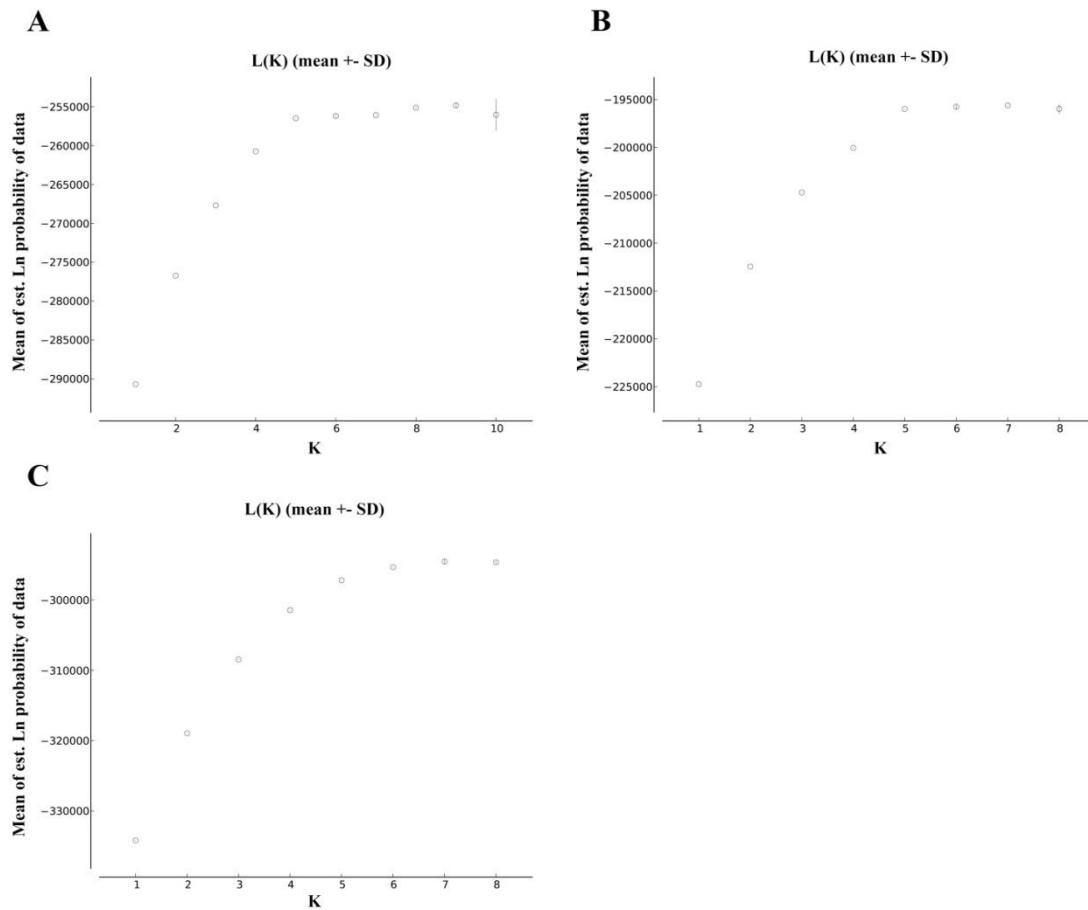


**Figure S3:** Self-assignment of reference individuals for  $K=5$  using STRUCTURE for Botn watercourse (BOV), river Lakselva (Misvær, LAM), river Lakselva (Valnesfjord, LAV), river Laksåga (Sulitjelma, SLA) and Saltdal watercourse (SAV).

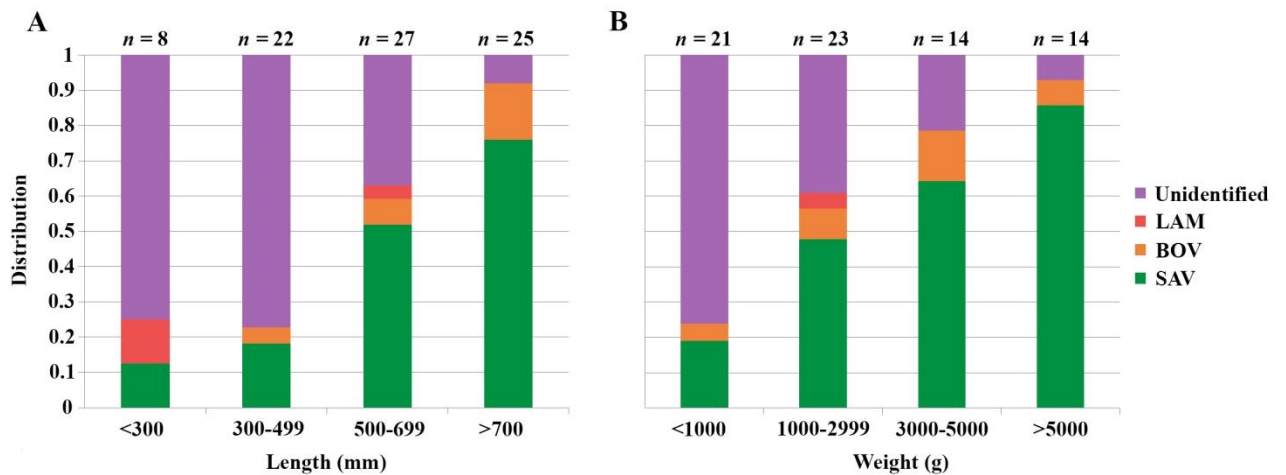


**Figure S4:** Assignment of the mixed stock (blue) and simulated random variables (black) against the baseline using the *infer\_mixture* module in *rubias*.





**Figure S5:** Mean estimated log-normal distribution for number of populations for (A) 7 sampling locations, (B) established reference populations and (C) for reference populations and the mixed stock together.



**Figure S6:** Distribution of (A) length (mm) and (B) weight (g) of the mixed stock. Individuals from the mixed stock were identified with a population if they had  $q$ -values above 0.8. Putative populations assigned to were Botn watercourse (BOV), river Lakselva (Misvær, LAM) and Saltdal watercourse (SAV). Number of individuals per column is displayed above.

