A single nucleotide polymorphism genotyping panel for efficient genetic stock identification of the Teno river Atlantic salmon (*Salmo salar*) population complex

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Abstract

A new single nucleotide polymorphism (SNP) panel for genetic stock identification in the Teno River Atlantic salmon fishery was developed, with a view to improving on an existing microsatellite panel. Twenty-two genetically differentiated reporting units were proposed based on population genetic analyses of 1212 individuals collected at 37 locations in the river and genotyped for > 33,000 genome-wide SNPs. A small subset of these SNPs was selected for GSI using an iterative process that considered their diversity and differentiation across reporting units. A genotyping-by-sequencing assay was developed to simultaneously genotype 180 of these GSI SNPs plus a sexing marker. This new SNP panel showed comparative GSI power to the microsatellite panel, with anticipated improvements in terms of cost, speed, and robustness and transferability across laboratories and genotyping platforms. Mixed stock analysis of the 2018 Teno River salmon catch using the new panel inferred all 22 reporting units to contribute to the fishery. Estimated catch proportions positively scaled with an independent estimate of reporting unit productivity: target spawning female biomass. This demonstrates the usefulness and efficiency of the 180 SNP panel for Atlantic salmon GSI in the Teno River system. If conducted on a regular basis, GSI can enable fine-tuning of management strategies to promote sustainable fishing.

Keywords: fisheries, management, Atlantic salmon, genotyping by sequencing, population structure

Introduction

Fisheries often consist of a mix of stocks – that is, they harvest individuals that originate from multiple genetically distinct populations. Key for effective management of such mixed-stock fisheries is accurate knowledge of how different source populations contribute to the catch (Begg *et al.*, 1999; Palsbøll *et al.*, 2007). This enables stock-specific management strategies aimed at safeguarding vulnerable populations while allowing continued harvest of healthier stocks (Crozier *et al.*, 2004). Genetic stock identification (GSI) has been a mainstay of mixed-stock fisheries management for several decades (Anderson *et al.*, 2008; Koljonen & McKinnell, 1996; Winans *et al.*, 2004). The GSI approach involves first generating a genetic baseline of allele frequencies from populations that may contribute to the fishery. Samples from the mixed stock fishery are then genotyped with the same marker panel, and the likelihood of the baseline population allele frequencies producing the genotype in question in the fishery sample are estimated using individual assignment-based approaches or else overall catch proportions are estimated using mixture modelling (e.g. Anderson *et al.*, 2008; Koljonen *et al.*, 2005).

In recent years, single nucleotide polymorphisms (SNPs) have replaced microsatellites as the marker of choice in a range of population and conservation genetic applications, including GSI e.g. (Larson *et al.*, 2014). Compared to microsatellites, which have commonly been typed by fragment size analysis, SNPs have advantages in terms of contemporary ease, speed and cost of genotyping, improved repeatability among laboratories and platforms, and a simpler mutation model allowing more straightforward interpretation of evolutionary and population genetic processes. One key benefit of SNPs compared to microsatellites for GSI is the potential availability of many thousands of easily genotyped loci, from which a sub-set of highly informative markers can be selected for a specific assignment task. This may enable more efficient stock assignment, improved resolution of more challenging population substructures (Larson *et al.*, 2014) and/or the inclusion of SNPs to address different research and management questions (Aykanat *et al.*, 2016; May *et al.*, 2020).

Atlantic salmon, *Salmo salar* L., is an anadromous salmonid fish natively distributed across the north Atlantic coast of Europe and North America. Atlantic salmon are fished and managed across their range, and many wild populations are in long-term decline from a multitude of threats, some of which are poorly understood (Parrish *et al.*, 1998). Management of the species can be politically and logistically complicated, spanning national borders and involving conflicts about rights of use (Crozier *et al.*, 2004). One of the largest and most diverse wild Atlantic salmon populations in the world (Erkinaro *et al.*, 2019) breeds in the large Teno (Norwegian: Tana, Sami: Deatnu) river system on the border of Finland and Norway in northernmost Europe. Previous genetic studies have revealed a highly structured population complex consisting of up to 28 genetically distinct, and temporally stable populations (Vähä *et al.*, 2011, 2017, 2007, 2008), with evidence for fine-scale local adaptation (Mobley *et al.*, 2019; Pritchard *et al.*, 2018). Annual riverine catches have typically varied between 100-200 metric tons, but weak sea survival and returns in recent years suggest a sharp decline in most populations (Anon. 2024). To aid management, a microsatellite baseline (33 loci) was developed (Vähä *et al.*, 2017) that included over 3,300 individuals from 36 locations within the river system. Based on these data, a maximum of 32 reporting units for GSI was proposed, with an estimated assignment accuracy of 36-100% depending on the reporting unit and

analytical approach applied. The current salmon fishery management in the Teno River system is based on these genetic units and stock-specific status assessment of many of them (Anon. 2024).

The aim of this study was to develop a SNP genotyping panel for Teno River salmon GSI, with a view to improving on the microsatellite panel and producing a tool more robust to changes in genotyping technology , with a view to further improving and future-proofing the stock assessment system. We first genotyped original (Vähä *et al.*, 2017) baseline samples, augmented with a set of more recent samples, for over 38,000 genome-wide SNPs. We used this SNP dataset to re-determine suitable reporting units and develop a 180 SNP genotyping-by-sequencing (GT-seq) panel for GSI. We compared the assignment efficacy of the new GT-seq panel with that of 10,000 genome-wide SNPs, and with the previous 33 microsatellite panel. The new SNP panel was then used to perform mixed stock assignment of salmon caught in the Teno River in 2018. As additional validation, GSI results were compared with an independent proxy of expected population size, target female biomass.

Materials and Methods

Baseline samples and datasets

Archived DNA samples from juvenile salmon in the (Vähä *et al.*, 2017) baseline collection were used as the basis of the new SNP baseline (Figure 1, Table S1). Samples were quantified using a NanoDrop spectrophotometer (Thermo Scientific) and assessed for degradation using gel electrophoresis. For two of the 32 baseline reporting units in (Vähä *et al.*, 2017) (Bavttájohka and Anarjohka) there were either insufficient samples, the pre-extracted DNA was completely degraded or the DNA did not pass the minimum quality requirements for SNP genotyping. To augment the baseline, DNA was extracted and quantified for juvenile samples collected in 2014 from four localities, Tsarsjoki, Lakšjohka, and the Lakšjohka tributaries Deavkkehanjohka and Gurtejohka. Good quality DNA samples with a minimum concentration of 20ng/ul were sent to the Center for Integrative Genetics (CIGENE), Ås, Norway, for SNP typing on a custom 60,250 (60K) Atlantic salmon SNP genotyping array. All subsequent merging and quality control of SNP datasets was performed using Plink 1.90 (Chang *et al.*, 2015).

To further extend the data set, we added genetic data generated for previous studies of Teno River salmon (Barson *et al*., 2015, Pritchard *et al.*, 2018): adult genotypes from one locality missing from the 60K juvenile baseline (Máskejohka) and additional juvenile genotypes – also part of the original baseline -from ten localities. These samples had been screened using a 220K SNP array, which shares 38,669 named SNPs with the 60K array. Forty-one individuals from Pritchard *et al.* (2018) were typed on both arrays, enabling genotyping repeatability be examined. After merging the 60K and 220K datasets across the 38,669 markers, we removed 364 SNPs with which had > 2/41 mismatching genotypes across arrays and four SNPs with known off-target variants. Subsequently we applied the following filters: SNP minor allele frequency >0.05; SNP missing genotype frequency < 0.03; individual missing genotype frequency < 0.05. Finally, putative full-sibs were identified and removed based on genome-wide patterns of identity-by-descent (--genome) within and across collection locations.

Baseline population substructure

Before analyses of population structure, the baseline SNP dataset was further filtered to prune neighbouring SNPs in high linkage disequilibrium with one another (--indep 50 5 1.5). Population genetic analyses were carried out either in the R statistical environment (R Core Team, 2014), or in the Linux shell.

Overall and pairwise F_{ST}s among samples from different locations were estimated using *StAMPP* (Archer *et al.*, 2017). Significance of pairwise F_{ST} was assessed using 20,000 bootstrap replicates across loci and correcting for multiple testing using the Bonferroni procedure with alpha = 0.05. A neighbour-joining (NJ) tree based on Prevosti's distance (Prevosti *et al.*, 1975), was created and bootstrapped 100 times using *poppr* (Kamvar *et al.*, 2014) and the tree plotted using the package *ape* (Paradis & Schliep, 2019). Prevosti's distance is a model free distance suitable for use with SNP data with missing genotypes.

Admixture (Alexander *et al.*, 2009) was used to identify the most likely number of genetically distinct ancestral populations present in the LD pruned baseline SNP dataset, independent of sampling location. Five replicates of the Admixture analysis were run, each starting from a different random number seed, allowing between 1 and 30 genetically distinct populations in the dataset. The most likely number of populations was determined using the software's cross-validation procedure. Finally, genetic variation within the dataset was also explored using principal component analysis followed by graphical visualization. PCA was performed using the package LEA in R (Frichot & Francois 2015), with number of retained PCs based on Tracy-Widom test results. The combined results from the Admixture and PCA analyses, interpreted with reference to (Vähä *et al.*, 2017) were used to select suitable new reporting units for GSI of the Teno River fishery.

Developing and testing of the GT-seq SNP panel

To identify a sub-set of SNP markers for efficient mixed stock assignment, highly variable SNPs (MAF > 0.45), were first extracted from the full baseline dataset using PLINK 1.9. Following this, genotypes from eight highly differentiated reporting units (Kevojoki, Tsarsjoki, Utsjoki, Lakšjohka, Deavkkehanjohka, Gurtejohka, Yla-Pulmankijoki, Galddasjoki) were removed, as inclusion of these sites greatly skewed the F_{ST} distribution for SNPs, while assignment efficiency should be high for these reporting units regardless of the specific markers used. Closely linked SNPs in this new dataset were LD pruned (indep --50 5 1.5), and the remaining SNPs were ranked by calculating F_{ST} over the remaining reporting units. The 96 highest ranking SNPs were retained as the initial GSI panel. Filtering for highly variable SNPs retains a markers set that is expected to be informative across multiple reporting units, and subsequent selection of SNPs with the highest F_{ST} across units is expected to maximise accuracy for GSI (Ackerman *et al.*, 2011; Storer *et al.*, 2012). This initial SNP panel was tested for its assignment efficacy using the 'leave-one-out' individual re-assignment approach implemented in the R package *rubias* (Moran & Anderson, 2019). Markers were then added to improve discrimination among target subsets of reporting units by selecting additional SNPs with high MAF and high F_{ST} across those units alone. This process was performed iteratively, aiming for a final panel of 220-230 candidate GSI SNPs which was intended to translate into working panel of approximately 200 SNPs. As an additional check to minimize marker redundancy due to linkage, the positions of final candidate SNPs were inspected to exclude any markers within 10,000 bp of one another. Finally, SNPs linked to several genes identified as locally adaptive

164 candidates in the Teno River (Pritchard *et al.*, 2018) and the *sdY* sex marker (Yano *et al.*, 2013)
165 were added to the panel.

166 Primers for GT-Seq (Campbell *et al.*, 2015) were designed to amplify a 120-155bp DNA sequence
167 surrounding each of the target SNPs using BatchPrimer3 (Rozen & Skaletsky 1998;Untergasser *et*
168 *al.*, 2012), with the following parameters: Tm 58-62°C optimum 60°C; GC% 28-68; all other
169 parameters default. Primers were not assessed *in silico* for dimerization potential.

170 All multiplex PCR was performed in 12ul reactions containing the following: 6ul Qiagen Multiplex
171 PCR mix, 2ul DNA at a concentration of 10-50 ng/ul, volume of multiplexed diluted primers to
172 yield the target concentration of each primer (see below); molecular grade water. Thermocycling
173 conditions were: 98°C for 2 min, 20x [98°C 10s, 62°C 30s, 72°C 15s], 7C for 10 min. PCR
174 products were tagged with individual-specific barcode indices, pooled, and sent to the DNA
175 Sequencing and Genomics Laboratory, University of Helsinki for library preparation and
176 sequencing. Products were single-end sequenced using Illumina150 cycle kits on an Illumina HiSeq
177 or MiSeq. Sequences were demultiplexed by barcode index using Generate FASTQ (Illumina) and
178 3' trimmed on the basis of indexing adaptor sequence using cutadapt 3.2 (Martin, 2011). Trimmed
179 sequences were aligned using bwa mem (BWA 0.7.17, Li & Durbin, 2010) to the *S. salar* reference
180 genome (ICSASG_v2) with an appended *S. salar* sdY sequence (Genbank Accession KT223110.1).
181 Variants at targeted sites were called using samtools mpileup piped to bcftools call (samtools 1.12,
182 Li & Barrett, 2011). The output VCF file was imported into Plink 1.9 with genotypes filtered for a
183 minimum GQ of 15 and a minimum sequencing depth of 8. For genetic sexing, sdY sequencing
184 depth was corrected for overall sequencing depth for each individual. Individuals were then
185 assigned as male (sdY present), female (sdY absent) or unknown on the basis of thresholds chosen
186 by examining the distribution of corrected sdY sequencing depths over all individuals.

187 Two rounds of multiplex PCR optimization were performed for the GT-Seq panel, each using 16
188 samples as the amplification target. For the first optimization round, all primers were combined in
189 equimolar concentrations (0.03 µM) in a single reaction. The output fastq files for the 16 test fish
190 were merged, and a custom bash script was used to count number of sequence reads for each
191 possible forward-reverse primer combination. This information was used to identify primers
192 producing dimer sequence, and primers were split into two multiplex pools that minimized within-
193 pool dimer formation. The subsequent optimization round was used to check pool composition and
194 to adjust primer concentrations (final range 0.03-0.06 µM) with the aim of roughly equalizing
195 sequencing coverage across SNPs. After these two rounds of optimisation the SNP panel was used
196 to genotype 74 baseline individuals to check for genotyping success and consistency of genotype
197 calling between GT-Seq and the 60K SNP array.

198 *Assignment efficacy of SNP panel*

199 The accuracy of GSI across identified reporting units was tested using two methods implemented in
200 *rubias*: the 'leave one out' approach described above, and a '100% mixture' approach. In the latter
201 analysis, baseline allele frequencies are used to simulate the genotypes of fish deriving from
202 different populations and those fish are then assigned back to the full baseline. We simulated 500
203 fish for each population and performed 100 replicates.

204 To compare the performance of the SNP panel to that which could be achieved by a much denser
205 sampling of genetic variation across the genome, we randomly subsampled 10,000 SNPs from the
206 baseline dataset and examined assignment power of this dataset via a leave-one-out analysis
207 implemented in gsi_sim, a Linux command line version of *rubias* suitable for large datasets
208 (https://github.com/eriqande/gsi_sim, (Anderson, 2010; Anderson *et al.*, 2008).

209 To assess the robustness of assignments with smaller numbers of SNPs from the SNP panel, and
210 thus assess the effect of missing genotypes on GSI reliability, random subsets of the 180 SNPs (60,
211 80, 100, 120, 140 and 160 loci) were tested in 'leave-one-out' analyses against the final set of
212 reporting units.

213 *Comparison with the microsatellite baseline*

214 We compared the efficacy of the new SNP GSI panel with that of the previous microsatellite panel.
215 To do this, we used the existing microsatellite baseline, which comprises 3,323 juvenile and adult
216 salmon from 36 sites sampled within the Teno River system and typed at 33 microsatellite loci.
217 Sampling and molecular protocols for this dataset can be found in (Vähä *et al.*, 2011, 2017, 2007,
218 2008). We compared the assignment performance of the SNPs and microsatellites using the *rubias*
219 leave-one-out procedure under two reporting unit scenarios: the 22 units proposed in this paper and
220 the 32 reporting units (two not represented in the SNP baseline) used by Vähä *et al.*, 2011.

221 *Mixed stock assignment and validation*

222 In 2018, scale samples from salmon caught in the Teno River were collected by local fishers,
223 coordinated by Natural Resources Institute Finland (LUKE, Finland) and the Norwegian Institute
224 for Nature Research (NINA, Norway). Fishers also recorded standard data on the fish including
225 length, mass, morphologically determined sex and visually assessed whether they were wild or
226 aquaculture escapee origin (Erkinaro et al. 2010). We extracted DNA from 1-2 scales for each fish
227 (excluding two putative escapees) using a high-salt method, quantified extractions using NanoDrop,
228 and standardized to a concentration of 10-50ng/µl. Samples from which sufficient DNA could be
229 obtained were genotyped for the 180 GSI SNPs and sex marker as described above. Individuals
230 missing genotypes at >60 loci were removed from the data set. Finally, we identified duplicate
231 samples on the basis >of 90% genotype match with another sample using the
232 close_matching_samples function in *rubias* and in each case excluded the duplicate with the fewest
233 missing genotypes.

234 To estimate the proportional contribution of each of the 22 reporting units to the 2018 Teno River
235 catch, we used *rubias* to perform mixed stock analysis against the SNP baseline. We implemented
236 the maximum likelihood approach with the default options of a uniform prior**,** 20000 sweeps and
237 the first 1000 sweeps discarded as burn-in. We computed 95% credible intervals from the MCMC
238 traces of mixing proportions.

239 To indirectly validate the results of the mixed stock analysis, we used location-specific figures for
240 target spawning female biomass provided in (Falkegård *et al.*, 2014). This is the biomass of females
241 required to produce the target number of eggs for each location, which is estimated by modelling
242 habitat suitability and egg density estimates and taking stock-specific fecundity into account. It can
243 be considered a proxy for the expected numbers of breeding adults returning to different parts of the

Teno River system. In (Falkegård *et al.*, 2014), tributaries were grouped by geography into somewhat different groups than the reporting units that we use here. For the purpose of comparison, the mixed stock analysis proportions were combined to match the (Falkegård *et al.*, 2014) geographic groupings, and female biomass (kg) estimates were transformed into relative proportions of the total for each group (Table S5). A Pearson correlation was performed to compare the estimated female biomass proportions to the proportions from the mixed stock assignment using cor.test in base R. Although not comparing the same thing, as one is based on individuals, and the other on biomass, it provides an indirect assessment of the realism of the GSI proportion estimates.

Results

Genetic baseline

The initial genetic baseline comprised 1,309 samples, collected between 2006 and 2014 from 37 locations in the Teno River (mean per location 35.3, range 11-67) and genotyped at 33,139 post-QC SNPs. Following removal of putative full siblings and LD pruning, a total of 1,212 individuals genotyped at 17,546 loci (Table S1) were used to investigate baseline population structure.

Global F_{ST} demonstrated substantial genetic structuring among sample sites across the Teno River system (F_{ST} =0.055, P=0.0099). Pairwise F_{ST}s ranged from 0.0005 (between Piltamo and Alaköngäs) to 0.1953 (between Deavkkehanjohka and Galddasjoki) and all were statistically significant, with the exception of the comparison between Piltamo and Alaköngäs (Table S2). Nevertheless, the generally very low pairwise F_{ST} among samples from Tana Mainstem localities (Tana bru, Alaköngäs, Piltamo, Sirma, Garnjarga, Kortsham, Yläköngäs, Outakoski) indicated only weak genetic differentiation across most of the Teno River mainstem.

The neighbour-joining tree reflected the pairwise F_{ST} results, showing a strong geographic signal across sampling locations (Figure 2), with strong bootstrap support at many nodes.

Admixture inferred 21 ancestral clusters within the dataset (Figure 3), with a geographic signal in line with the F_{ST} analysis and the neighbour-joining tree and indicative of gene flow among neighbouring locations. The Teno River mainstem and its two large headwater systems (Inarijoki and Kárášjohka) were separately dominated by three large ancestral clusters. The uppermost sample from the mainstem (Outakoski) showed substantial mixing among these clusters. A fourth genetic cluster dominated the lowermost mainstem sample, Tana Bru and the nearby tributary Luovtejohka. Ten smaller tributaries (Karigasjoki, Máskejohka, Galddasjoki, Ylä-Pulmankijoki, Utsjoki, Kevojoki, Váljohka, Kietsimäjoki, Vetsijoki and Geáimmejohka) and two pairs of neighbouring tributaries (Kuoppilasjoki and its tributary Birkejohka and Báišjohka/Nilijoki) were dominated by their own unique ancestral clusters. Akujoki had a mixed ancestry largely from the neighbouring Karigasjoki and Báišjohka/Nilijoki clusters. Two ancestral clusters were inferred for samples from Lakšjohka and its two small tributaries Gurte- and Deavkkehanjohka; examination of the underlying data suggested that this was likely caused by a segregating chromosomal rearrangement not accounted for by LD pruning. The large sample from Tsarsjoki was split into two clusters, which corresponded to year of collection likely reflecting differences in their sampling location in the tributary: these were amalgamated for subsequent analyses.

Twenty three principal component were retained in the PCA. Graphical examination of the position of individuals along these PCs (not shown) supported the conclusions of the Admixture analysis

with three exceptions: the Outakoski sample partly separated from the rest of the Teno River mainstem samples (Alaköngäs, Piltamo, Sirma, Garnjarga, Kortsham, Yläköngäs) along PC5, probably reflecting the genetic mixing in this location, the Iesjohka and Karasjohka samples were separated along PC23, and Akujoki was partly separated from other samples along PC21. Based on the combined Admixture and PCA results, together with consideration of Vähä *et al.*, 2017, 22 reporting units were defined for GSI (Table 2), with Lakš/ Gurte/ Deavkkehanjohka considered a single reporting unit but Outakoski, Akujoki and Kárášjohka treated as separate reporting units.

In some tributaries, a subset of juveniles were assigned very high mainstem ancestry by the Admixture analysis and clustered with mainstem fish in the PCA. Examination of available capture locations showed that these 24 fish were sampled near tributary mouths; they were therefore considered to be migrants from the Teno River mainstem breeding population rather than part of the genetically distinct tributary breeding population and were removed from the final GSI baseline.

SNP panel development

Primers were successfully designed for a final set of 223 candidate GSI SNPs, plus one marker each for candidate adaptively important genes *vgll3*, *six6*, *numa1* and *btg3* and genotypic sex (sdY). Following initial optimization and tests, SNPs were excluded due to complete non-amplification (n=11), >10% mismatching genotypes between GT-Seq genotypes and SNP array genotypes (n=16, mostly missing heterozygotes), and >40% missing genotypes (n= 21). One pair of primers amplified two SNPs that were both included in the GSI panel. Primer sequences for the 180 SNPs in the final GSI panel plus the sdY locus are provided in the Supplementary Materials.

Assignment efficacy

The leave-one-out analysis using the 180 SNP panel correctly assigned 86% of individuals to their reporting unit of origin. (Table 1). Thirteen out of the 22 reporting units had assignment accuracies between 90-100%, and only Outakoski, Tana Bru and Akujoki reporting units had assignment success < 70%. Mis-assigned fish were generally assigned to geographically adjacent reporting units (Table 1). Assessment of assignment efficacy using the 100% mixture approach gave very similar results, with slightly better accuracy inferred for all reporting units (93% of simulated individuals correctly assigned). (Table S2).

When testing both with the leave-one-out analysis, the 180 SNP panel performed almost as well as 10,000 genome-wide SNPs to assign samples to reporting unit, indicating that it is well captures genome-wide patterns of population substructure (Table S3). Overall assignment accuracies decreased gradually with each reduction in SNP number for sub-sets of the 180 GT-Seq panel however even with 100 SNPs 80% of fish were assigned back to their correct reporting unit (Table S3). Assignment success for the different size panels generally showed consistent patterns across the same reporting units.

The same two analyses applied to the larger microsatellite baseline split by the new reporting units demonstrated comparable assignment efficacy of the microsatellites (88% correct assignment using the leave-one-out approach, 90% correct assignment for the 100% mixture approach, Table 1 & S2). Similarly to the SNP panel the lowest assignment accuracy was for the Outakoski, Tana Bru and Akujoki reporting units.

Mixed stock analysis

Of 2,116 scale samples received from the 2018 mixed stock fishery, 187 failed genotyping and a further 16 were identified as duplicate samples. This left 1,913 available for mixed stock analysis against the 22 reporting unit SNP baseline (mean proportion of missing genotypes = 0.04, range = 0 – 0.32). Three of the largest reporting groups of the mixed stock analysis (Table 2) were each assigned >10% of the fishery samples, totalling almost 55% of the total catch: Teno River mainstem (25.1%), Inarijoki (15.9%) and Iešjohka (13.6%). Three additional reporting groups were assigned >5% each: Kárášjohka (7.5%); Vetsijoki (7.4%) and Outakoski (5.9%) with the remaining 16 reporting groups each being assigned 3.2% or less of the catch (24.6% of the total catch). The MSA proportions were highly and significantly correlated with the proportions of female biomass target abundance (Figure 3, r= 0.97, p<0.000). About 9% fewer fish were assigned to Tana Bru/Tana Mainstem/Outakoski than expected based on the female biomass target abundance proportions, similarly, 4.5% less fish were assigned to Kárášjohka than expected. Conversely, about 5% more fish were caught from Vetsijoki, and 3% more from Utsjoki/Tsarsjoki/Kevojoki than expected from the target abundance biomass estimates. All other localities had less than 2% difference in proportions between MSA and target abundance female biomass estimates (Table S6).

Discussion

We have designed a new GT-seq panel of 180 SNPs for genetic stock identification within the large Teno River Atlantic salmon fishery. The full panel performs equivalently to the previous microsatellite panel, with advantages in terms of cost and speed of genotyping, transferability among laboratories and flexibility for adding additional markers of interest. For example, in 2024 with an established pipeline and using a desktop short-read sequencer (Illumina MiSeq), generating the 180 SNP genotypes from 1,000 DNA extracts is estimated to require five days of staff time and €3,000 of laboratory consumables. It will also be more robust to anticipated future changes in genotyping technologies – in particular, greatly reduced availability of platforms and reagents currently used for microsatellite typing. Smaller sub-panels of the same SNPs also show good performance indicating that the full panel will be robust to a proportion of missing genotypes, a common outcome in GT-seq due to batch effects causing insufficient read depth over some targeted sites.

Our re-investigation of population structure within the Teno River, for which we re-genotyped archived samples for a large set of genome-wide SNPs, recapitulated the patterns previously found using microsatellites. Examination of genome-wide variation can reveal population genetic substructure that was not previously identified in studies using a smaller number of markers (reviewed in Sunde et al. 2020). However, this was not the case for our study - the existing 33 microsatellites appear to have been sufficient to resolve the population genetic substructure within the Teno River Atlantic salmon population. We caution against a general assumption that examining more variation across the genome will resolve increasingly more ecological- and management-relevant substructure within a target species.

Vähä *et al.* (2008), genotyping archived scale samples, demonstrated that this broad-scale population substructure in Teno River Atlantic salmon had remained stable over a minimum of four generations. Our results also support this, albeit on a smaller temporal scale: samples collected from

the same site 3-6 years apart almost invariably clustered together in the Admixture analysis and PCA. Such temporally stable substructure is expected to arise and be perpetuated by the natal homing of salmon to their birth river. This in turn can enable the establishment of local adapted genotypes, which further reduces gene flow among populations by reducing the fitness of migrants. There is both genetic and demographic evidence for local adaptation within the Teno River salmon stock (Mobley *et al.*, 2019; Pritchard *et al.*, 2018), further emphasizing the importance of population-specific managing the fishery to avoid over-exploitation of certain subpopulations.

Despite this broad-scale stability of population structure, Vähä *et al.* (2008) did document change in allele frequencies across timepoints particularly in smaller tributary populations. We recommend additional juvenile collections to augment the baseline presented here, both to increase the sample size for poorly represented reporting units and to ensure that it accurately represents the contemporary genetic make-up of the Teno River salmon stock. A long-term monitoring programme should include plans to systematically update the baseline at pre-planned intervals and re-test the performance of the 180 SNP panel against the modified baseline.

Based on our examination of population structure, we chose to use 22 reporting units for the SNP-based GSI, fewer than the maximum of 32 that were used by (Vähä *et al.*, 2017). Specifically, we amalgamated Ylakongas with the Teno River mainstem reporting unit, Luovtejohka with Tana Bru, Báišjohka with Nilijoki, Upper Iešjohka with Lower Iešjohka, and Iskurasjoki, Goššjohka, Vuomajoki and Cáščemjohka with the Inarijoki mainstem. Additionally, due to sample loss our baseline did not include two reporting units, Bavttájohka – a tributary of Kárášjohka and Anarjohka – a headwater tributary of Inarijoki. Nevertheless, investigation of the performance of the 180 SNP panel and the 33 microsatellite panel with the baseline divided into either 22 or 30 reporting units showed very similar results. Both demonstrated better overall assignment accuracy when there were fewer reporting units, while the SNPs actually performed better than the microsatellites for Báišjohka, Niljohka, Lower Iešjohka, Iskurasjoki and Goššjohka. Due to DNA degradation, our SNP baseline omitted two reporting units used by Vähä *et al.*(2017): Kárášjohka tributary Bavttájohka and Inarijoki tributary Anarjohka. Given the known patterns of genetic structure, we expect fish originating from these locations to be misassigned to a Kárášjohka/Iesjohka and an Inarijoki reporting unit, respectively. Bavttájohka contributes 1.8% of the target female biomass for the Teno River, while the biomass of Anarjohka is included in the Inarijoki and not calculated separately (Falkegård et al. 2014); thus, while we recommend addition of these locations into the SNP baseline we do not anticipate that their current absence is substantially biasing our results.

Average accuracy rates for other Atlantic salmon GSI systems range from 87% (33 microsatellites, 182 rivers in the northernmost Norway and Finland and in the northwest of Russia; (Ozerov *et al.*, 2017) and between the upper and lower reaches of a large Baltic salmon river system (Miettinen et al. 2024), to 53.1 % at the level of the individual river and 72.1% at the regional level (31 microsatellite markers, 14 Norwegian rivers (Harvey *et al.*, 2019). Considering the spatial scale of the Teno River system, the resolution using this GSI system is very high. Further increases in accuracy would be possible by merging rivers into broader, regional, reporting units, however, for management purposes tributary-based reporting units are more informative.

We caution that results from the microsatellites and SNP panel are not entirely comparable. In addition to differences in baseline sample sizes, the panel of GSI SNPs were selected and tested

using the same individuals, an ascertainment bias that is expected to somewhat over-estimate their true assignment efficacy. We used this approach instead of dividing the baseline into training and test datasets in order to more accurately capture the true population allele frequency where we had low numbers of samples. Nevertheless, the proportion of the Teno River 2018 salmon catch assigned to the 22 baseline reporting units in the Mixed Stock Analysis closely scaled with the target female spawning biomass of those reporting units, suggesting that the SNP panel is indeed performing well: only three comparisons deviated more than 2% from the expected. Based on the comparison in this study alone, the outtake of fish in 2018 reflected quite accurately the potential size in female biomass of the populations (Falkegård *et al.*, 2014) and the stock assessment status (Anon. 2024) .

The Teno River 180 SNP panel, being easily modified with the addition of new SNPs, also has the potential to form the foundation of a more broadly applicable panel that could replace the current microsatellite panel for Genetic Stock Identification of Atlantic salmon throughout western Europe (Gilbey et al. 2021).

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Contributions

Project conceptualization: CRP, VLP, HJ, JE, MF, PO
Project management/ Sample coordination: - PO, MF, AF, JE, CRP, VLP
Methods development: VLP
Laboratory analysis: VLP, HJ
Bioinformatics: VLP
Data analysis: HJ, VLP
Manuscript drafting: HJ, CRP, VLP
Visualization: HJ, VLP
Manuscript finalization: all authors.

Figure 1 Map of the Teno river system baseline sampling localities and Neighbour-joining tree using Prevosti's distance on 17,546 SNPs. Geographic regions indicated by colour in map and in tree.

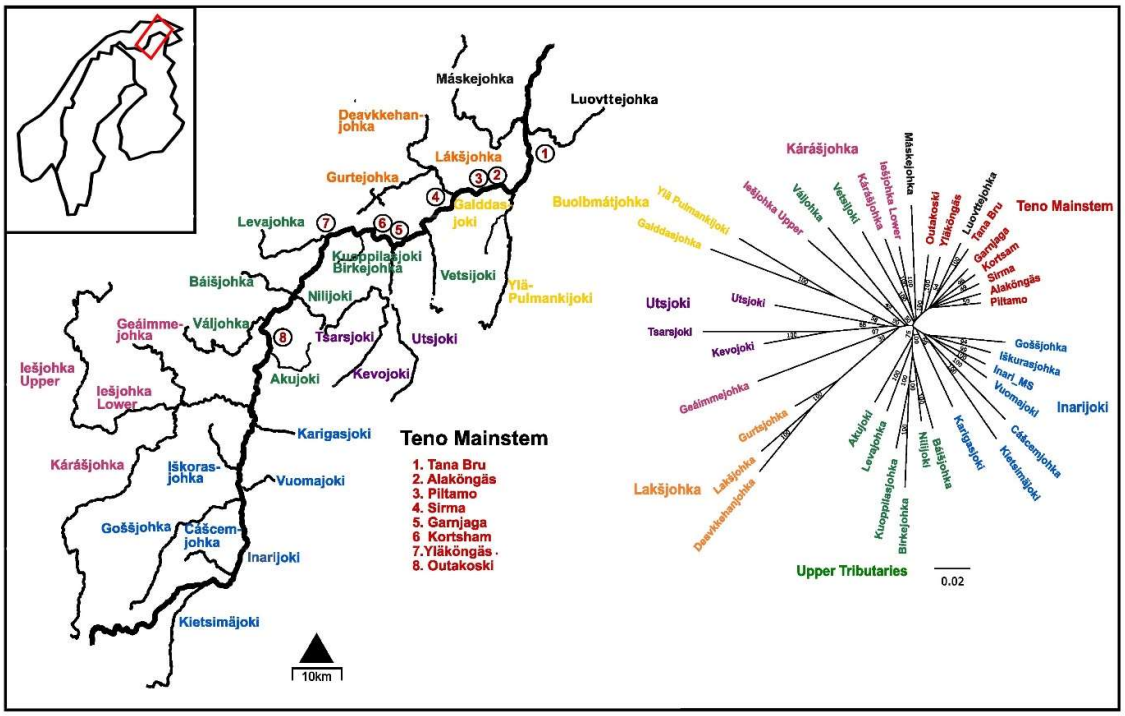


Figure 2: Results of Admixture analysis of Teno River Atlantic salmon individuals. Each column represents an individual fish, ordered by sampling location, indicated on the y-axis. Colours indicate inferred proportion of ancestry (Q) from each of 21 possible ancestral clusters.

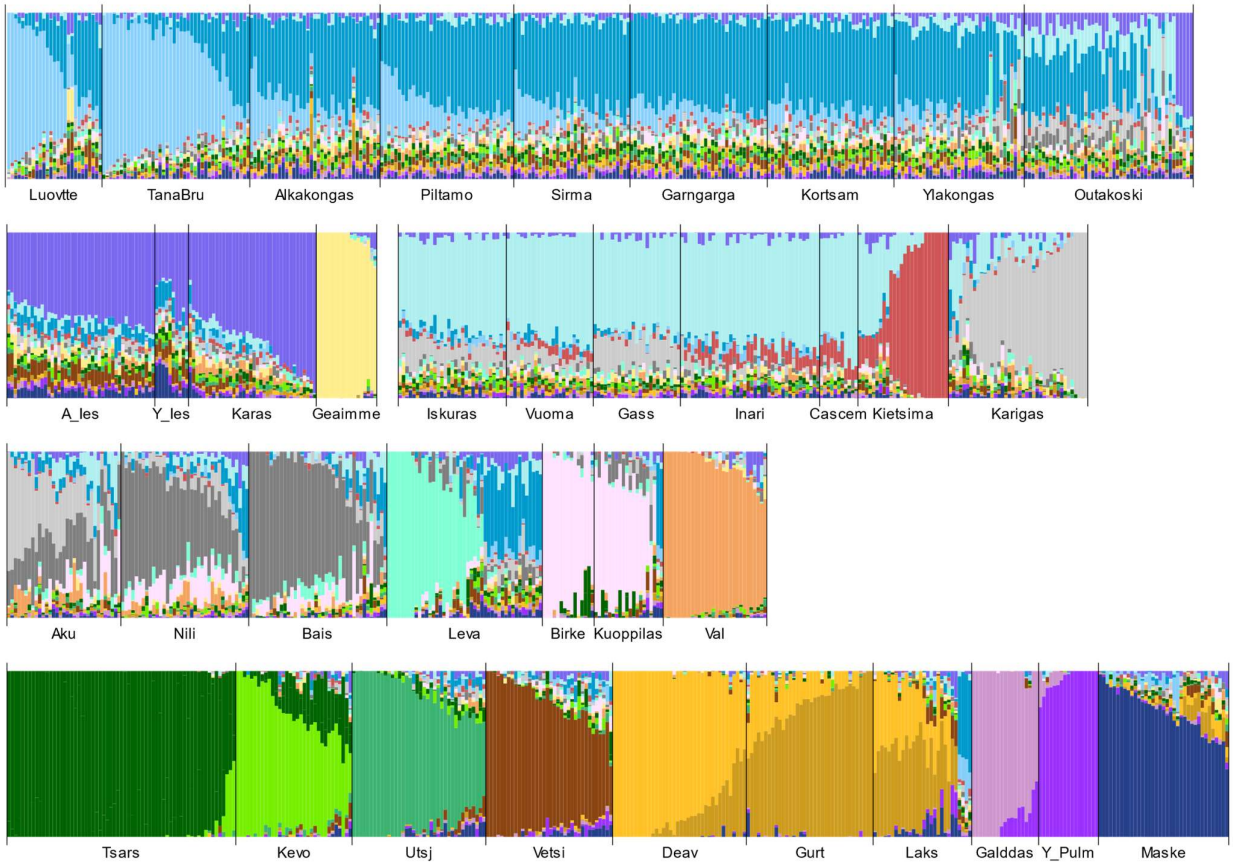
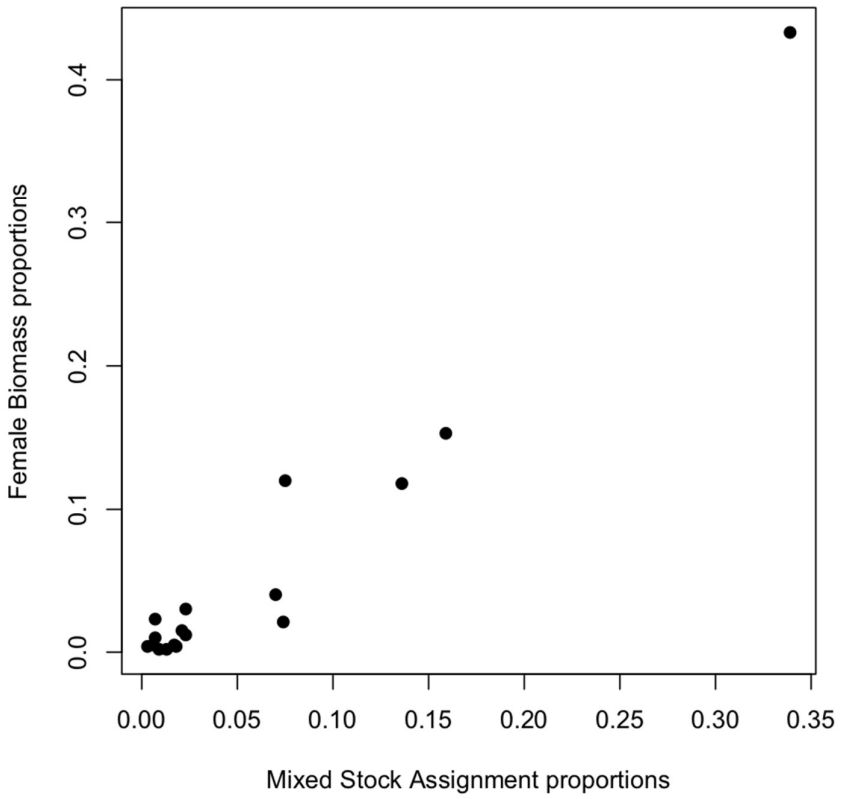


Figure 3. The proportion of female biomass per river/tributary (estimated spawning target converted to required female biomass from Falkegård et al. (2014)) plotted against the estimated catch proportion in the mixed fishery for the same reporting units.



481 **Table 1**. Teno River Atlantic salmon GSI correct assignment rates and main unit of mis-assignment using the 180 SNP panel, and comparison of
482 correct assignment achieved using microsatellites.

	SNPs			Microsatellites	
Reporting unit	**Baseline sample size**	**% correct assignment**	**Largest misassignment to:**	**Baseline sample size**	**% correct assignment**
Teno Mainstem					
TanaBru/Luovtejohka	69	61%	Teno Mainstem (37%)	127	66%
Middle Mainstem*	220	83%	Tana Bru (12%)	446	83%
Outakoski	48	52%	Teno MS (18%); Inari HW (15%)	91	54%
Lower Tributaries					
Máskejohka	37	86%	Teno Mainstem (8%)	121	97%
Vetsijoki	36	92%	Iešjohka (5%)	212	96%
Ylä-Pulmankijoki	17	100%	-	279	99%
Galddasjoki	19	100%	-	85	99%
Utsjoki	38	97%	Vetsijoki (3%)	129	99%
Tsarsjoki	65	97%	Kevojoki (3%)	196	97%
Kevojoki	33	100%	-	165	99%
Lakš/Deavkke/Gurtsjohka	98	97%	Karigas; Máske; Vetsi (1% each)	71	100%
Upper Tributaries					
Levajohka	31	74%	Outakoski (6%); Báiš/Nilijoki (6%)	51	69%
Báišjohka/Nilijoki	73	85%	Akujoki (6%)	106	77%
Akujoki	33	70%	Báišjohka/Nilijoki (12%)	53	40%
Karigasjoki	40	83%	Akujoki (10%)	75	79%
Kuoppilasjoki/Birkejohka	33	91%	Báišjohka/Nilijoki (10%)	112	91%
Váljohka	30	93%	Inari HW (7%)	270	94%
Kárášjohka/Iešjohka lower					
Iešjohka	54	93%	Kárášjohka (5%)	201	89%
Kárášjohka	40	83%	Iešjohka (3%); Vetsijoki (3%)	270	94%
Geáimmejohka	18	94%	Akujoki (5%)	52	92%
Inari Headwaters					

Inari Headwaters**	132	91%	Karigasjoki (4%)	177	85%
Kietsimäjoki	26	73%	Inari HW (15%)	61	74%

*Teno River Mainstem reporting group: Alaköngäs, Piltamo, Sirma, Garnjaga, Kortsham and Yläköngäs

**Inari Headwaters reporting group: Inari Mainstem, Cášcemjohka,Vuomajoki, Goššjohka, Iškurasjohka

Table 2. Mixed stock analysis of the Teno River mainstem Atlantic salmon fishery catch in 2018.

Reporting Unit	Estimated mixture proportion	Lower 95% CI	Upper 95% CI
Tana Mainstem			
TanaBru/Luovtejohka	0.029	0.0214	0.0378
Teno Mainstem*	0.251	0.2335	0.2689
Outakoski	0.059	0.0480	0.0700
Lower Tributaries			
Máskejohka	0.023	0.0171	0.0297
Vetsijoki	0.074	0.0641	0.0851
Váljohka	0.021	0.0155	0.0265
Ylä-Pulmankijoki	0.007	0.0044	0.0109
Galddasjoki	0.001	0.0003	0.0030
Utsjoki	0.019	0.0144	0.0250
Tsarsjoki	0.019	0.0140	0.0243
Kevojoki	0.032	0.0253	0.0386
Lakš/Deavkkehan/Gurtsjohka	0.007	0.0044	0.0107
Geáimmejohka	0.013	0.0086	0.0171
Upper Tributaries			
Levajohka	0.018	0.0133	0.0237
Báišjohka/Nilijoki	0.023	0.0171	0.0285
Akujoki	0.009	0.0048	0.0129
Karigasjoki	0.017	0.0120	0.0232
Kuoppilasjoki/Birkejohka	0.006	0.0031	0.0090
Iešjohka Headwaters			
Iešjohka Upper/Lower	0.136	0.1227	0.1507
Kárášjohka	0.075	0.0639	0.0854
Inari Headwaters			
Inari Headwaters**	0.159	0.1445	0.1733
Kietsimäjoki	0.003	0.0009	0.0050

*Teno Mainstem reporting group: Alaköngäs, Piltamo, Sirma, Garnjaga, Kortsham and Yläköngäs
**Inarijoki reporting group: Inarijoki Mainstem, Cášcemjohka,Vuomajoki, Goššjohka, Iškurasjohka

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