Received: 12 June 2023 | Revised: 3 November 2023 | Accepted: 7 November 2023

DOI: 10.1111/mec.17213

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

New indicators for monitoring genetic diversity applied to alpine brown trout populations using whole genome sequence data

Sara Kurland[1,2] | Atal Saha[1,3] | Naomi Keehnen[1,4] | Maria de la Paz Celorio-Mancera[1] | David Díez-del-Molino[1,5,6] | Nils Ryman[1] | Linda Laikre[1]

[1]Division of Population Genetics, Department of Zoology, Stockholm University, Stockholm, Sweden

[2]Department of Earth Sciences, Natural Resources and Sustainable Development, Uppsala University, Uppsala, Sweden

[3]Centre for Coastal Research, Department of Natural Sciences, University of Agder, Kristiansand, Norway

[4]Department of Ecology, SLU, Uppsala, Sweden

[5]Centre for Palaeogenetics, Stockholm, Sweden

[6]Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Stockholm, Sweden

Correspondence
Sara Kurland and Linda Laikre, Division of Population Genetics, Department of Zoology, Stockholm University, SE-10691 Stockholm, Sweden.
Email: sara.kurland@geo.uu.se and linda.laikre@popgen.su.se

Funding information
Carl Tryggers Stiftelse för Vetenskaplig Forskning; Erik Philip-Sörensen Foundations; Havs- och Vattenmyndigheten; Knut och Alice Wallenbergs Stiftelse, Grant/Award Number: 2014.0278; Svenska Forskningsrådet Formas, Grant/Award Number: 2020-1290; Vetenskapsrådet, Grant/Award Number: 2019-05503

Handling Editor: Andrew P. Kinziger

Abstract

International policy recently adopted commitments to maintain genetic diversity in wild populations to secure their adaptive potential, including metrics to monitor temporal trends in genetic diversity – so-called indicators. A national programme for assessing trends in genetic diversity was recently initiated in Sweden. Relating to this effort, we systematically assess contemporary genome-wide temporal trends (40 years) in wild populations using the newly adopted indicators and whole genome sequencing (WGS). We use pooled and individual WGS data from brown trout (*Salmo trutta*) in eight alpine lakes in protected areas. Observed temporal trends in diversity metrics (nucleotide diversity, Watterson's Θ and heterozygosity) lie within proposed acceptable threshold values for six of the lakes, but with consistently low values in lakes above the tree line and declines observed in these northern-most lakes. Local effective population size is low in all lakes, highlighting the importance of continued protection of interconnected systems to allow genetic connectivity for long-term viability of these populations. Inbreeding (F_{ROH}) spans 10%–30% and is mostly represented by ancient (<1 Mb) runs of homozygosity, with observations of little change in mutational load. We also investigate adaptive dynamics over evolutionarily short time frames (a few generations); identifying putative parallel selection across all lakes within a gene pertaining to skin pigmentation as well as candidates of selection unique to specific lakes and lake systems involved in reproduction and immunity. We demonstrate the utility of WGS for systematic monitoring of natural populations, a priority concern if genetic diversity is to be protected.

KEYWORDS
biodiversity, EBVs, indicators of genetic diversity, microevolution, population genomics, temporal genetic variation

1 | INTRODUCTION

Genetic diversity is the foundation of all overarching levels of biological diversity, from species to entire ecosystems. While the importance of safeguarding biodiversity is recognized in science and policy [most notably by the UN Convention on Biological Diversity (CBD; www.cbd.int)], implementations of these policies lag behind (Bruford et al., 2017; Hoban et al., 2013). This is particularly acute for genetic diversity, for which systematic mapping and monitoring programmes have been lacking (Laikre et al., 2010; Vernesi et al., 2008).

As of late, conditions for safeguarding gene-level diversity are changing for the better, following intense work to develop suggestions for systematic assessment of genetic diversity for global use (Hoban et al., 2020, 2022; Hoban, Bruford, et al., 2021; Hoban, Paz-Vinas, et al., 2021; Hvilsom et al., 2022; Kershaw et al., 2022; Laikre et al., 2020, 2021; O'Brien et al., 2022). The CBD recently presented a 'post-2020' global biodiversity framework, which makes commitments to support genetic diversity, including metrics to monitor temporal trends in genetic diversity (CBD, 2022a, 2022b). Additionally, some countries have initiated programmes for national monitoring of genetic diversity using DNA-based techniques (Switzerland & Sweden; Andersson et al., 2022; Hvilsom et al., 2022; Johannesson & Laikre, 2020; O'Brien et al., 2022; Posledovich et al., 2021a, 2021b).

In Sweden, a science management collaboration involving the Swedish Agency for Marine and Water Management (SwAM) developed a programme for monitoring genetic diversity over contemporary time frames using DNA-based techniques. Temporal trends in genetic diversity are summarized in so-called indicators and three new indicators are included in the programme (Johannesson & Laikre, 2020, 2022): (i) genetic diversity within populations (indicator ΔH), (ii) the effective population size (indicator N_e) and (iii) genetic diversity between populations (indicator ΔF_{ST}). These indicators are based on metrics identified as essential biodiversity variables (EBVs) for genetic diversity (Hoban et al., 2022) and are closely linked to indicators proposed in the CBD context (Hoban et al., 2020). The CBD Headline indicator A.4 – the proportion of populations within species with an effective population size (N_e) >500, is here directly assessed using genome-wide data from which we estimate N_e. Similarly, the presently applied ΔF_{ST} indicator includes quantification of the maintenance of populations similar to the CBD complementary indicator that assesses the proportion of populations within species maintained (CBD, 2022b). However, both of those CBD indicators can also be assessed without DNA data (Hoban et al., 2023; see O'Brien et al., 2022 for a comparison among these and other indicators). The three, presently used, DNA-based indicators developed with SwAM for national use in Sweden were first applied with DNA data by Andersson et al. (2022) using a panel of 96 SNPs. Here, we assess these indicators using whole genome sequencing (WGS) data.

Monitoring conducted with data generated by modern DNA techniques, e.g. WGS, is so far mainly represented by highly threatened and extinct species and extended time frames (Dussex et al., 2021; Palkopoulou et al., 2015; Von Seth et al., 2021). The benefit of WGS data is that it provides vast numbers of loci expected to increase statistical power to detect adaptive change and subtle population divergence, the latter of which is particularly relevant to studies over short time frames, e.g. in monitoring (Allendorf et al., 2010; Garner et al., 2016; Schwartz et al., 2007). Additional genetic monitoring of wild species, using WGS data, and over evolutionarily short (a few generations) time frames is therefore warranted (Elmer, 2016; Jorde et al., 2018). We presently monitor wild populations of brown trout (*Salmo trutta*) over contemporary time using WGS data.

The brown trout is of interest not only due to its cultural and ecological value, but also because a range of anthropogenic stressors threaten the genetic integrity and variability of its wild populations (Ayllon et al., 2006; Bekkevold et al., 2020). The species exhibits a propensity to form genetically distinct populations (Andersson, Jansson, et al., 2017; Bekkevold et al., 2020), making it particularly suitable for monitoring diversity between populations (indicator ΔF_{ST}; Andersson et al., 2022). Furthermore, alpine populations such as those presently studied may be especially affected by climate change, and should be prioritized in monitoring (Urban, 2018).

Specifically, this study monitors genetic diversity using WGS of pooled samples and of individuals from wild populations in temporally stratified samples (from the 1970s and 2010s), covering four decades (roughly six generations). We study brown trout inhabiting alpine mountain lakes in protected areas in Sweden, some of which are part of an ongoing long-term monitoring programme initiated in the 1970s (Andersson, Jansson, et al., 2017; Andersson, Johansson, et al., 2017; Charlier et al., 2011, 2012; Jorde & Ryman, 1996; Kurland et al., 2019, 2022; Palm et al., 2003; Palmé et al., 2013). The study is conducted in association with science management collaborative work with SwAM (Andersson et al., 2022; Johannesson & Laikre, 2020). Our objectives are to (i) study temporal patterns of genome-wide diversity within and between populations of brown trout, (ii) apply the new indicators ΔH, ΔF_{ST} and N_e to quantify and evaluate trends in genetic change and (iii) identify indications of selection over time.

2 | MATERIALS AND METHODS

2.1 | Lakes sampled

We studied temporal genetic variation in brown trout populations from the mid–late 1970s to the 2010s, corresponding to c. six brown trout generations (Palmé et al., 2013), in eight freshwater lakes located in remote mountain areas in the County of Jämtland, central Sweden (Figure 1, Table 1). The lakes are located in the uppermost part of water systems that drain into either of rivers Ångermanälven or Indalsälven that both empty into the Baltic Sea c. 400 km from the sampling sites (Figure 1). The lakes vary in size and degree of isolation (Table 1, Figure 1). We study two metapopulations with creeks potentially enabling genetic exchange among the three lakes: one containing small lakes (below 0.1 km^2) and the

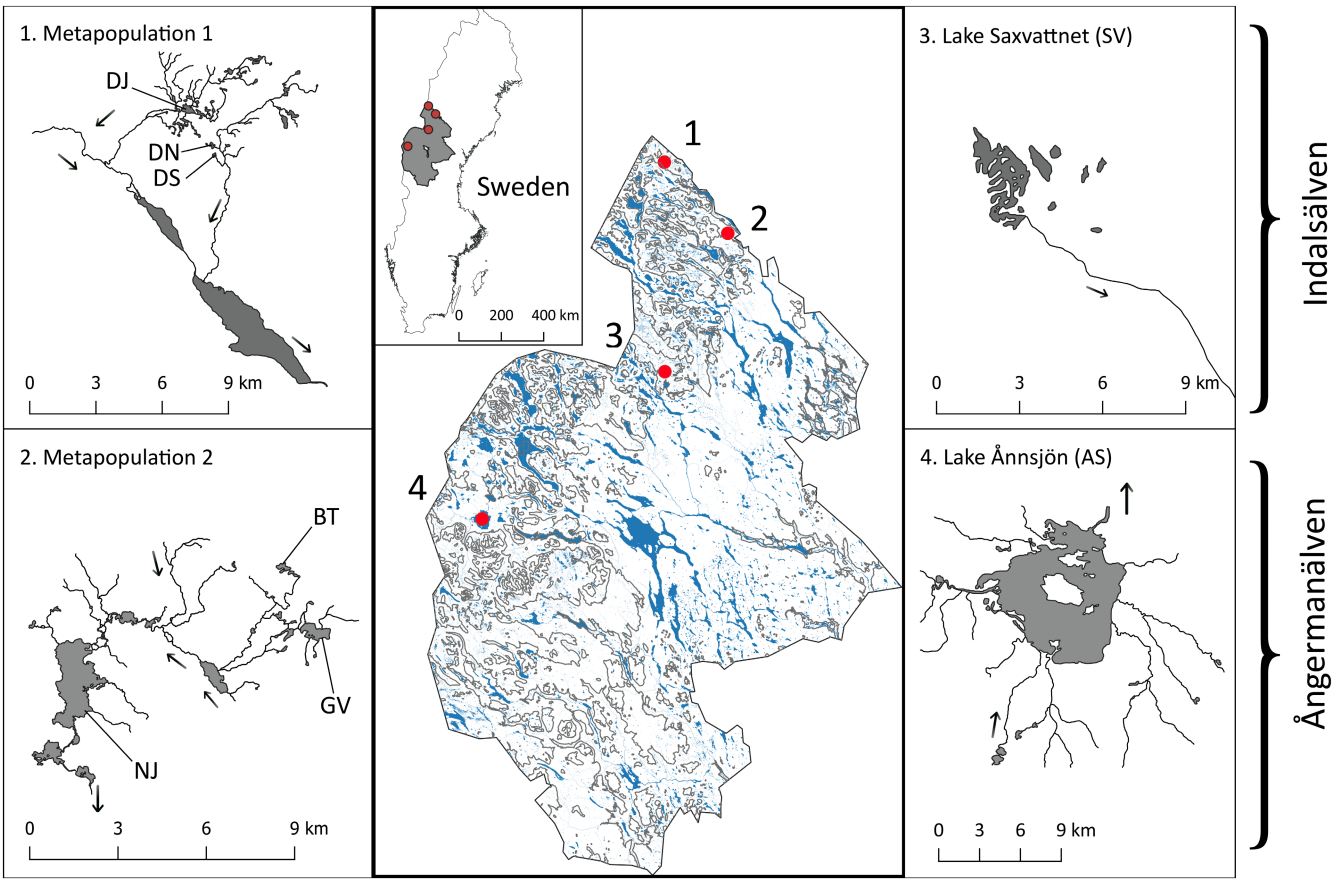

FIGURE 1 Geographic location of the eight lakes monitored for genetic diversity. Red dots in the middle map indicate locations of the two sampled metapopulations (á three lakes each) and two single lakes (cf. Table 1). Numbers within the middle map designate close ups of each lake system. Metapopulation 1 contains lakes Daimanjaure (DJ), Daimanjåppe N (DN) and Daimanjåppe S (DS) and metapopulation 2 lakes Blanktjärnen (BT), Grubbvattnet (GV) and Nils-Jonsa (NJ). Arrows next to the rivers indicate the direction of water flow. The lakes are located in the uppermost part of water systems that drain into either of river Ångermanälven or river Indalsälven, as indicated.

other larger (c. 0.1–3 km^2). Two single lakes are also included: one large (c. 60 km^2 in size) and one small (c. 3 km^2 in size). All three lakes within metapopulation 1 are located above the tree line (>700 m elevation), while the rest of the lakes are found below. All four study systems (metapopulations 1 and 2, single lakes 1 and 2) are located in different protected areas (Table 1) that are also within indigenous Sámi land, remotely located and difficult to access. Fishing in these lakes is prohibited or restricted and stocking or transferals are not allowed.

2.2 | DNA isolation, library preparation and sequencing

DNA from 50 individuals per lake and point in time were combined at equal concentrations for pooled sequencing (Pool-seq). Two individuals per lake and point in time were randomly selected among the $n = 50$ per population pool for individual whole genome sequencing (IWGS). Tissue samples from fish are stored in a frozen tissue bank at the Department of Zoology, Stockholm University. Genomic DNA was extracted from muscle tissue from all individuals using KingFisher Cell and Tissue DNA Kit (Thermo Scientific, MA, USA). DNA samples were sent to the Swedish National Genomics Infrastructure (NGI) at the Science of Life Laboratory (SciLifeLab), Stockholm, Sweden. The NGI conducted the construction of Illumina TruSeq PCR-free libraries with an average insert size of 350 bp followed by the sequencing of paired end reads with 150 bp in length on a NovaSeq6000 instrument.

2.3 | Read processing and variant calling: Pool-seq data

Quality of raw sequence reads were assessed in FastQC v0.11.5 (Leggett et al., 2013) and MultiQC v1.5 (Ewels et al., 2016). Low-quality bases (phred score <20) and Illumina adapters were trimmed using BBDuk as implemented in BBTools v38.08 (http://sourceforge.net/projects/bbmap/) before mapping to the brown trout assembly (https://www.ncbi.nlm.nih.gov/assembly/GCF_901001165.1/) using BWA v0.7.17 (Li, 2013; Li & Durbin, 2009). The resulting bam files were sorted, merged and filtered for paired reads using SAMtools v1.8 (Li et al., 2009). Quality of bam files

TABLE 1 Eight sampled lakes with information regarding location, protected area, lake size and elevation and sample sizes (n) at two points in time (1970s–1980s and 2010s) for WGS of pools (Pool-seq) and individuals.

Lake system	Protected area	Main river drainage	Lake	Location WGS84 dec		Elevation (MASL)	Lake size (km^2)	Average depth (m)	Sampling year past	n past (pools, individuals)	Sampling year present	n present (pools, individuals)
				North	East							
Metapopulation 1												
	Skäarnja Nature Reserve	Ångermanälven	Daimanjaure	65.00533	14.4733458	792	0.13	3	1976	50, 2	2017	50, 2
			Daimanjåppe N	64.990932	14.497232	800	0.04	NA	1976–77	50, 2	2017	50, 2
			Daimanjåppe S	64.986787	14.499173	800	0.02	NA	1976–77	50, 2	2017	42, 2
Metapopulation 2												
	Hotagen Nature Reserve, N2000: SE0720199	Indalsälven	Blanktjärnen	64.035795	14.643692	670	0.08	3	1980	50, 2	2012	50, 2
			Grubbvattnet	64.015819	14.665005	697	0.34	5	1976	48, 2	2017	50, 2
			Nils-Jonsa	63.999264	14.498902	565	2.98	12	1976	50, 2	2018	50, 2
Single lakes												
	Saxvattnet Nature Reserve	Ångermanälven	Saxvattnet	64.663516	15.187384	527	3.00	12	1977	50, 2	2017	50, 2
	Ånnsjön N2000 SE0720282	Indalsälven	Ånnsjön	63.26016	12.565338	525	57.00	13	1976	50, 2	2017	50, 2

Note: Populations within metapopulation 1 are above the tree line.

was assured using flagstat (SAMtools v1.8) and Qualimap v2.2.1 (García-Alcalde et al., 2012). Variant calling was conducted in SAMtools using minimum mapping and base quality scores of 20 for each SNP and with an active base alignment quality (BAQ) option to avoid false SNPs caused by misalignments. We used the 'identify-genomic-indel-regions.pl' script of PoPoolation2 v1.201 (Kofler, Pandey, et al., 2011) to remove insertions/deletions (indel) and error-prone regions 5 bp upstream and downstream flanking indels from the mpileup file and to convert the mpileup file to synchronized format for downstream analyses. VCF files were created from bam files using BCFools v.1.10 (Li et al., 2009) by calling and genotyping raw site variants in the software's mpileup and call algorithms, using default settings. These variants were compared with those called in the estimation of allele frequencies in PoPoolation2 v1.201 (Kofler, Pandey, et al., 2011), only keeping bi-allelic variants found in both files to account for duplicated genomic regions present in salmonid genomes (Lien et al., 2016). The loci were then controlled for mapping quality, number of high-quality bases, read positional bias, base quality bias and mapping quality versus strand bias, before filtering for mapping quality 100. This final set of SNPs was annotated in SnpEff v.5.0 (Cingolani et al., 2012).

2.4 | Read processing and variant calling: IWGS data

Sequenced reads from two individuals per lake and point in time were aligned against the brown trout reference assembly using BWA mem v0.7.17 (BWA; Li & Durbin, 2009) and sorted using SAMtools v1.8 (Li et al., 2009). PICARD v2.10.3 (broadinstitute.github.io/picard) was used to merge bam files for each individual and to mark PCR duplicates. Quality of bam files was assessed with Qualimap v2.2.1 (García-Alcalde et al., 2012). HaplotypeCaller from the Genome Analysis ToolKit (GATK) v3.8 (McKenna et al., 2010) was used to generate individual genomic variant call format files (gVCFs), and joint genotyping of all brown trout samples was performed with GATK GenotypeGVCFs. We applied a hard filter approach using GATK's VariantFiltration tool to filter out low-quality variants; separately for SNPs (QD <2.0, MQ <40.0, FS >10.0, MQRankSum <−5.0, ReadPosRankSum <−5.0, SOR >5.0) and indels (QD <2.0, FS >10.0, ReadPosRankSum <−5.0, SOR >5.0) in accordance with GATK Best Practices recommendations (gatk.broadinstitute.org; DePristo et al., 2011). VCFtools v0.1 (Danecek et al., 2011) was used to retain bi-allelic SNPs with a minor allele frequency ≥0.01 and a mean depth of coverage 5X–50X, as well as removing variants from un-assigned scaffolds. The joint VCF file was annotated in BCFtools v1.8 (Li et al., 2009).

2.5 | Population genetic diversity

We based most of our measures of population genetic diversity on our Pool-seq data due to the small sample size of IWGS data ($n = 2$ per lake

and point in time). IWGS data were primarily used to estimate metrics not possible for Pool-seq data, e.g. inbreeding (F_{ROH}) and mutational load, and in some instances to complement our Pool-seq data.

2.5.1 | Genetic diversity within populations

Diversity within population pools was estimated in PoPoolation v1.2.2 (Kofler, Orozco-terWengel, et al., 2011) as heterozygosity (H_P), nucleotide diversity (π; Tajima & Tajima, 1983) and Watterson's Θ (Watterson, 1975). Estimates of diversity from Pool-seq data are sensitive to sequencing errors and variation in coverage (Kofler, Pandey, et al., 2011). Inflated coverage over homologous regions is particularly pertinent to partially tetraploid salmonid genomes. Therefore, we took particular precaution by first sub-sampling mpileup files per pool to uniform depths using the 'subsample-pileup.pl' script implemented in PoPoolation v1.2.2 (Kurland et al., 2019; Saha et al., 2022). The 'variance-sliding.pl' script of PoPoolation v1.2.2 was used to estimate π and Θ. To further avoid coverage biased diversity estimates, estimations were performed for non-overlapping 5 kilo base pair (kb) windows across the assembly, with a minor allele count of 2 for a SNP to be called and applying the same depth thresholds as for the sub-sampling. Finally, only windows covered to ≥80% with data within the depth thresholds were used.

Allele frequencies per population pool were calculated using the 'snp-frequency-diff.pl' script of Popoolation2 v1.201 (Kofler, Pandey, et al., 2011), and reformatted to reflect the number of reads corresponding to the most (major) and least (minor) abundant alleles (n_{MAJ} and n_{MIN}) across all populations using a custom script available upon request. Heterozygosity per population pool (H_P) was calculated according to Rubin et al. (2010). Since PoPoolation2 has no option to estimate allele frequencies within windows, allele frequencies and H_P were calculated per variant site.

For measures π, Watterson's Θ and H_P, tests for normality of residuals and homogeneity of variances were conducted with Shapiro–Wilks tests and by visualizing the distribution of quantiles in a QQ plot and were rejected for all three metrics ($p < .05$). Independence of diversity metrics across lakes and time were therefore tested using non-parametric Kruskal–Wallis tests. Pairwise significance tests within lakes over time and between pairs of lakes were performed using non-parametric Wilcoxon signed rank sum tests. All statistical testing was performed in R v4.0.3 (R Core Team, 2020). For genome-wide comparisons of diversity metrics π, Θ and H_P, we use a significance level of $\alpha = 0.05$, and as a comparison, $\alpha = 1 \times 10^{-8}$ which corresponds to $\alpha = 0.05$ corrected for genome size, in this case the c. 300,000 windows underlying our Pool-seq diversity estimates (Pruisscher et al., 2018).

2.5.2 | Genetic diversity between populations

Genetic diversity among population pools was estimated as F_{ST} (Karlsson et al., 2007; Nei, 1973) using the 'fst-sliding.pl' script of PoPoolation2. A minor allele count of 3 and 20–150X depth of coverage was used as threshold for variant calling in order to remove paralogous regions (inflated coverage) and regions represented by a small number of individuals (Kofler, Orozco-terWengel, et al., 2011; their Appendix S1). We used non-overlapping 1 bp and 5 kb windows, respectively, the latter to minimize stochastic errors linked with small window sizes (Kofler, Pandey, et al., 2011) and only including windows with coverage data ≥80%. We report Nei's F_{ST} unless stated otherwise. A dendrogram was constructed to illustrate genetic relationship among all 16 brown trout pools. Allele frequencies per pool obtained from PoPoolation2 were used as input for TreeMix v1.12 (Pickrell & Pritchard, 2012) to generate a maximum likelihood phylogeny. We used blocks of 5000 SNPs (total 5764 blocks = 28,816,918 SNPs) as input for TreeMix; the result was visualized in Mega X (Kumar et al., 2018).

2.6 | Effective population size

Effective population size (N_e) was estimated for each lake with the temporal method using the software TempoFs (Jorde & Ryman, 1995, 1996, 2007). This method measures the variance in allele frequencies between two time points; variance effective size (N_{eV}). We assumed a generation time of 6.8 years, as previously estimated for brown trout in these areas (Palmé et al., 2013). Based on the number of years between sampling occasions for separate lakes (cf. Table 1) this translates into a c. six generation interval over which N_{eV} was estimated. TempoFs was run using a random subset of loci from the Pool-seq data ($n = 150{,}000$) extracted with a minimum of 10 kb distance from one another to avoid linkage.

2.7 | Inbreeding

Inbreeding was estimated per individual using the IWGS data as the summed length of 'runs of homozygosity' (ROH; Gomez-Raya et al., 2015; Kardos et al., 2016; Magi et al., 2014). Homozygosity was determined for 1000 kb (1 Mb) overlapping windows. As suggested for low-coverage data like ours (Ceballos et al., 2018), the minimum length of a ROH was set to 300 kb which must contain at least 50 SNPs and a maximum of three heterozygous genotypes per window were allowed. ROH were estimated for each individual and then categorized into two ROH length classes to reflect different demographic histories: between 300 kb and 1 Mb for deeper co-ancestry and >1 Mb to reflect recent inbreeding. The genomic inbreeding coefficient based on ROH (F_{ROH}) was estimated as the sum of the length of all ROH per individual as a proportion of the total autosomal SNP coverage (c. 2.37 Gb). Total SNP coverage was calculated per individual.

2.8 | Mutational load

We identified mutational load by estimating the number of deleterious mutations in each individual from the IWGS dataset.

Additional filters were implemented in order to avoid biased genotype calls. First, repetitive regions within the brown trout reference assembly were identified with RepeatMasker (Smit et al., 2017) and RepeatModeler (Smit & Hubley, 2015), and all variants within these regions were excluded from the SNP dataset. We only retained loci genotyped in all samples and of these, heterozygote variants were kept if the allelic balance (ratio of alternative/reference supporting reads) was between 0.2 and 0.8 (as in Díez-del-Molino et al., 2020). We performed six separate estimations of mutational load (runs) in order to allow stringent comparison of SNPs. Two runs allow spatial comparisons between lakes, each at a separate point in time: (i) all samples from the 1970s ($n = 16$) and (ii) all samples from 2017 ($n = 16$). We ran four additional runs to allow temporal comparisons within each lake system, including all lakes and time points sampled from (iii) metapopulation 1 ($n = 12$), (iv) metapopulation 2 ($n = 12$), (v) Saxvattnet ($n = 4$) and (vi) Ånnsjön ($n = 4$). Finally, for each run, variants fixed in all individuals were excluded since they are not informative of differences in mutational load among samples. All variant effects were annotated using SNPeff v.4.3 (Cingolani et al., 2012).

2.9 | Indicators

Temporal trends in genetic diversity are summarized in indicators for genetic diversity, as recently suggested for national use in Sweden to systematically assess population genetic health (Johannesson & Laikre, 2020). Here, we follow the guidance for application provided by Andersson et al. (2022). In brief, the first two indicators, ΔH and N_e, reflect temporal changes in within-population genetic diversity, while the third, ΔF_{ST}, concerns change among populations. For the indicator ΔH, we use nucleotide diversity, Watterson's Θ and heterozygosity from Pool-seq data as well as inbreeding based on IWGS data (above) to calculate $\Delta\pi$, $\Delta\Theta$, ΔH_p and ΔF_{ROH}. The effective population size – N_e – indicator was assessed using N_{eV} estimated with TempoFs from Pool-seq data (above). The final indicator, ΔF_{ST}, regards the degree of population retention and temporal change in divergence between populations and is here applied to the two metapopulations based on F_{ST} estimates between lakes within systems from Pool-seq data (above), assessed at each of the two points in time. ΔF_{ST} is obtained by comparing present F_{ST} to past F_{ST}.

Threshold values for indicators are set to evaluate rates of change in accordance with Andersson et al. (2022) and are described in Appendix S3. In brief, three indicator signals are given depending on the occurrence of statistically significant changes of specific magnitudes which are translated to 'traffic lights' signal of green = 'acceptable', yellow = 'warning' or red = 'alarm'. Testing for statistical significance of observed changes in all measures was performed in non-parametric Wilcoxon matched paired tests in R v4.0.3 (R Core Team, 2020).

2.10 | Identifying signatures of selection over time

2.10.1 | Temporal selection inferred from Pool-seq data

In an attempt to detect selection over microevolutionary time, we identified genomic regions exhibiting marked change in allele frequency within lakes over c. six generations in two approaches.

Over time in parallel across all lakes

First, we utilize the vastness of our sample size (eight lakes) in a SNP-based approach to identify shared genes potentially under selection over time within all lakes. Because stochastic processes are unlikely to affect the same genomic regions simultaneously, we identified potentially adaptive SNPs as those showing consistent directional change in allele frequency across all lakes. We tested for parallelism in allele frequency change using PoolFreqDiff (Wiberg et al., 2017). This programme uses a generalized linear model with a quasibinomial error distribution (qGLM) which provides lower false positive rate than the Cochran–Mantel–Haenszel test commonly used in Pool-seq studies of temporal replicates (Garcia-Elfring et al., 2021; Wiberg et al., 2017). We categorized outlier SNPs as those showing significant parallel allele frequency change in all lakes.

To gain insight into potential traits under selection, outlier SNPs were investigated for functional enrichment compared to the rest of the genome in a gene set enrichment analysis (GSEA). This was performed on outlier SNPs within protein coding regions, as identified in the GFF. We looked for enrichment on the molecular function and biological process level using TopGO v.2.36.0 (Alexa & Rahnenführer, 2018). Functional gene ontology (GO) annotation of the brown trout genome was performed using EggNOG v5.0 web interface as described in Saha et al. (2022). Data were further filtered and visualized using Revigo (Supek et al., 2011) and treemaps were drawn in R version 4.0.0 (R Core Team, 2020).

Over time within lake systems

A second approach identified genes putatively under selection within lakes and lake systems by combining measures of divergence and diversity (Kjærner-Semb et al., 2016; Kurland et al., 2022). F_{ST} was used to capture significant allele frequency change within each lake over time. We assume that most loci behave neutrally while overly differentiated loci are shaped by selection and may be located at the tail end of the F_{ST} distribution. This approach avoids tenuous assumptions of demographic history, yet risks including false positives (Akey et al., 2002). Thus, since selection is expected to reduce variation, low levels of nucleotide diversity (π) among later samples were used to corroborate temporal selection inferred from F_{ST}. We employed a window-based approach to avoid increased stochastic error rates associated with small window size (Kofler, Orozco-terWengel, et al., 2011; Kurland et al., 2019). Outlier windows were defined as those exhibiting marked temporal divergence within a

lake (above 95th percentile of F_{ST}) and simultaneously low diversity within that same lake for the second time point (below 20th percentile of π), for each lake. We then quantified the extent of overlap of these outliers between lakes using UpsetR package in R (Conway et al., 2017), for all windows and for windows within coding regions of genes (identified in the GFF). We focused on regions unique to lakes or lake systems.

In summary, we identified candidates of selection from SNPs showing significant allele frequency change in (i) all lakes as identified by PoolFreqDiff and (ii) within lake systems as identified by F_{ST} and π. Functional impact of all outlier SNPs were obtained from the VCF annotated in SnpEff (Cingolani et al., 2012).

2.10.2 | Verifying temporal selection with IWGS data

We used the individual level data to corroborate our findings of temporal selection in Pool-seq data. SNP genotypes within genes putatively under selection identified from Pool-seq data were sought using WGS data of individuals. VCFtools v0.1.16 (Danecek et al., 2011) was used to create a genotype matrix for candidate genes which was then transposed using a custom script (github.com/nimarafati/vcftools). Individuals were clustered according to similarity between genotypes within candidate genes in the R package pheatmap (Kolde & Kolde, 2015).

We also screened the candidates of selection from Pool-seq data for genetic differentiation in haplotype distances (i.e. haplotype differentiation) as implemented in HaploDistScan (Pettersson et al., 2017). The VCF file containing all samples was converted to beagle format using Beagle v5.1 (Browning & Browning, 2007). We scanned within 5 kb windows allowing a minimum of five SNPs per window.

Finally, in an effort to identify genomic patterns of selection surrounding candidate regions of temporal selection identified from Pool-seq data, we scanned the IWGS data for runs of homozygosity (ROH) associated with the candidates using detectRUNS (Biscarini et al., 2019). To do so, ped and map formats were created from the VCF file containing all samples using BCFtools v1.14 (Li, 2011) and VCFtools v0.1.16. We looked for ROH using a sliding window approach in detectRUNS using window size set to 10 bp, minimum length of a ROH 1 kb, with at least 1 SNP per 50 kb and at least 100 SNPs per ROH and otherwise default settings (Biscarini et al., 2019).

3 | RESULTS

3.1 | Data processing

Sequencing and mapping success of Pool-seq and IWGS data are summarized in Appendix S1. Average depth of coverage per pool is 78X (SD = 6.12) and 11X (SD = 1.48) for IWGS data (Tables S1 and S2).

3.2 | Population genetic diversity

Genome-wide diversity per population pool varies among lakes and time points for measures of nucleotide diversity and Watterson's Θ (Kruskal–Wallis test; π; $H = 184{,}847$, $df = 15$, $p < 2.2e{-}16$, Θ; $H = 201{,}070$, $df = 15$, $p < 2.2e{-}16$). Lowest values of π, Θ and H_P are observed in lakes within metapopulation 1 above the tree line (lakes Daimanjaure, Daimanjåppe N, Daimanjåppe S) and highest within metapopulation 2 (lakes Blanktjärnen, Grubbvattnet, Nils-Jonsa; Figure 2, Appendix S2).

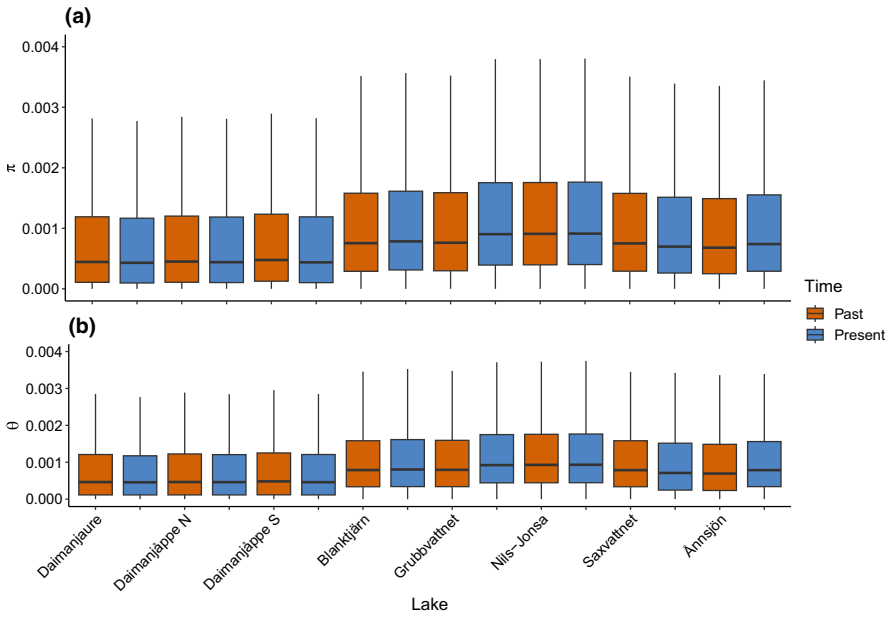

FIGURE 2 Genome-wide diversity per lake and time point (past and present). Box plots of nucleotide diversity and Watterson's theta estimated from Pool-seq data. Changes in diversity over time within lakes are significant (see Table S4 for statistical testing details). Boxes fall within the interquartile range where the median is represented by a horizontal line and whiskers denote minimum and maximum values (past = 1970s–1980s, present = 2010s; Table 1).

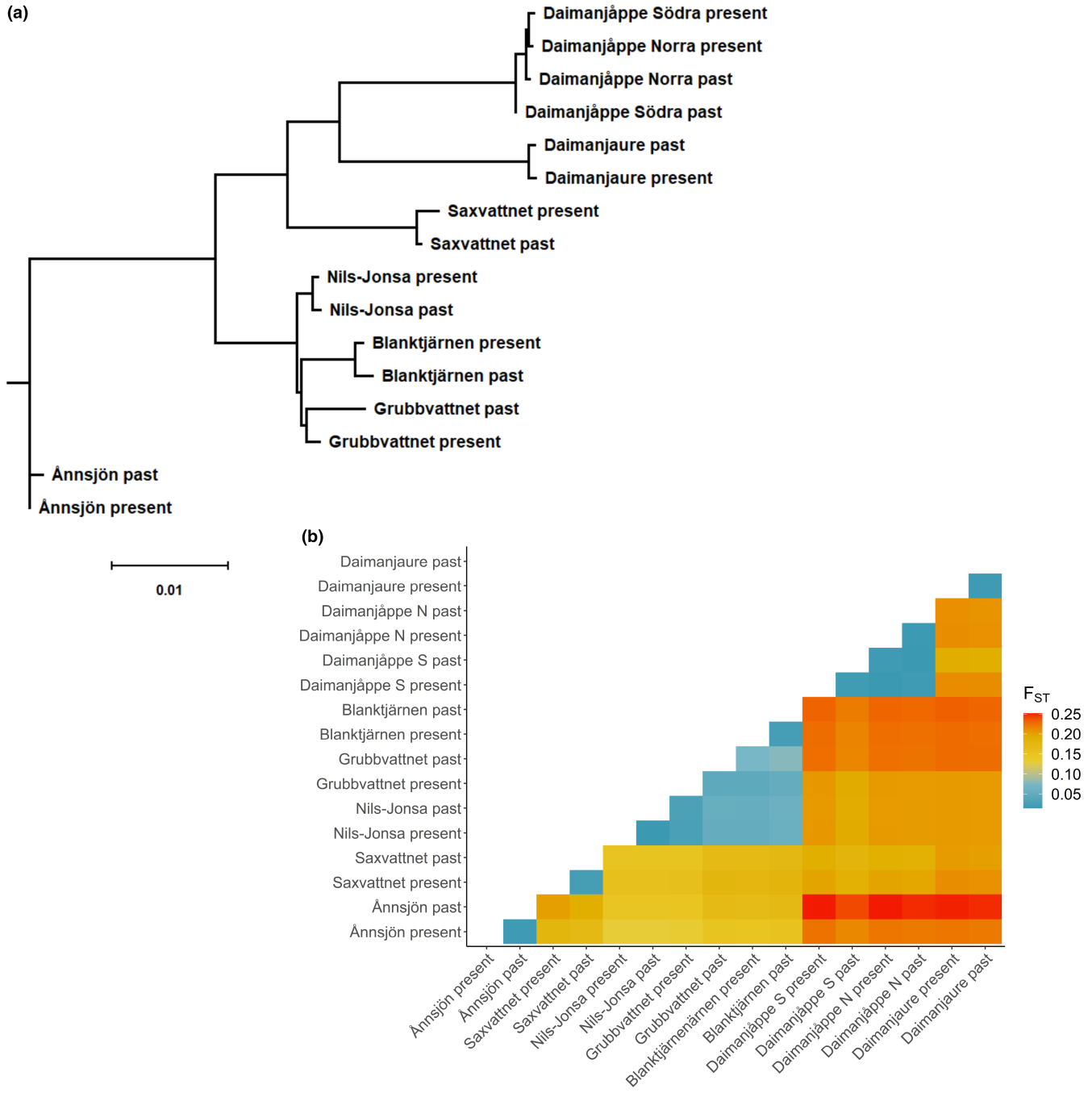

FIGURE 3 Genetic relationships among brown trout populations from eight lakes and two time points (past = 1970s–1980s; present = 2010s (Table 1). (a) Dendrogram constructed using data from Pool-seq. TreeMix analysis was conducted assuming no migration between lakes. The scale indicates the proportion of genetic divergence per unit length of the branch (indicated by the scale length). (b) Heatmap of pairwise F_{ST} between all lakes and time points (Table S5).

Generally, genetic structuring among lakes agrees with geographic proximity (Figure 3). F_{ST} among lakes within the same water system ranges from 0.01 to 0.21, and among lakes of different systems between 0.13 and 0.23 in present-day samples (Table S6). Spatial differentiation between lakes exceeds temporal divergence (F_{ST}; range = 0.01–0.04, mean = 0.02, SD = 0.0094) within lakes, in most cases (Figure 3, Table S6). The exception is divergence between lakes Daimanjåppe N and Daimanjåppe S, which is of the same magnitude as temporal divergence within each lake – suggesting extensive gene flow between the two. Lake Daimanjaure, which belongs to the same metapopulation, clusters separately from Daimanjåppe N and Daimanjåppe S, suggesting limited gene flow to and from this lake (Figure 3). The PCA and dendrogram from IWGS data reflect the same patterns of population differentiation as seen using Pool-seq data (Figure S1, Appendix S3). Estimates of variance effective population size (N_{eV})

from Pool-seq data are low, ranging from 18 (Grubbvattnet) to 75 (Nils-Jonsa; Table 2).

3.3 | Inbreeding and mutational load

Inbreeding (F_{ROH}) exceeds 10% in all lakes and time points (Figure 4, Appendix S3). F_{ROH} is not normally distributed (Shapiro–Wilks test; $W=0.92$, $p=.02$). There is significant difference in F_{ROH} between lakes including both time points (Kruskal–Wallis test; $H=27$, $df=15$, $p=.03$). Highest F_{ROH} of approximately 25% is observed in the most northern lakes Daimanjaure, Daimanjåppe N and Daimanjåppe S in metapopulation 1, found above the tree line (Figure 4, Table S8). Comparably low F_{ROH} (c. 17%) is found in lakes within metapopulation 2.

Generally, a major fraction of ROH include short ROH fragments (<1 Mb). Long ROH fragments (>1 Mb), reflecting recent inbreeding, are retrieved from all samples, but ROH exceeding 2 Mb are only found in three individuals, all from metapopulation 1; one of the individuals from Daimanjåppe N and another from Daimanjåppe S, both sampled in the 1970s, as well as one individual sampled in the 2010s in Daimanjaure (Figure 4, Table S8).

Population levels of highly deleterious mutations (loss of function, LOF) are depicted in Figure 5. We conducted six separate comparisons so as not to lose too much data (Section 2.8) and exact LOF values among Figures 5a–f are therefore not meaningful. We note, however, that among lakes sampled in the past, the highest LOF is observed in Ånnsjön and lowest values in Saxvattnet (Figure 5a). Among present samples, highest values are observed in metapopulation 1; lakes Daimanjaure, Daimanjåppe N and Daimanjåppe S (Figure 5b). With respect to changes within lakes over time, mutational load seems, generally, to have increased in lakes within the two metapopulations, most notably within lake Nils-Jonsa (Figure 5c,d). Only Daimanjaure of metapopulation 1 exhibits lower LOF in the present samples than in the past ones. Reduced LOF over time are observed in lakes Saxvattnet and Ånnsjön (Figure 5e,f).

TABLE 2 Estimates of variance effective population sizes (N_{eV}) by TempoFs (Jorde & Ryman, 2007) using 150,000 SNPs from Pool-seq data.

Lake	N_{eV} (95% CI)
Daimanjaure	53 (52, 54)
Daimanjåppe N	65 (63, 66)
Daimanjåppe S	52 (51, 53)
Blanktjärnen	34 (33, 35)
Grubbvattnet	18 (18, 18)
Nils-Jonsa	75 (74, 77)
Saxvattnet	39 (38, 39)
Ånnsjön	71 (70, 72)

Note: 95% Confidence intervals are given in brackets.

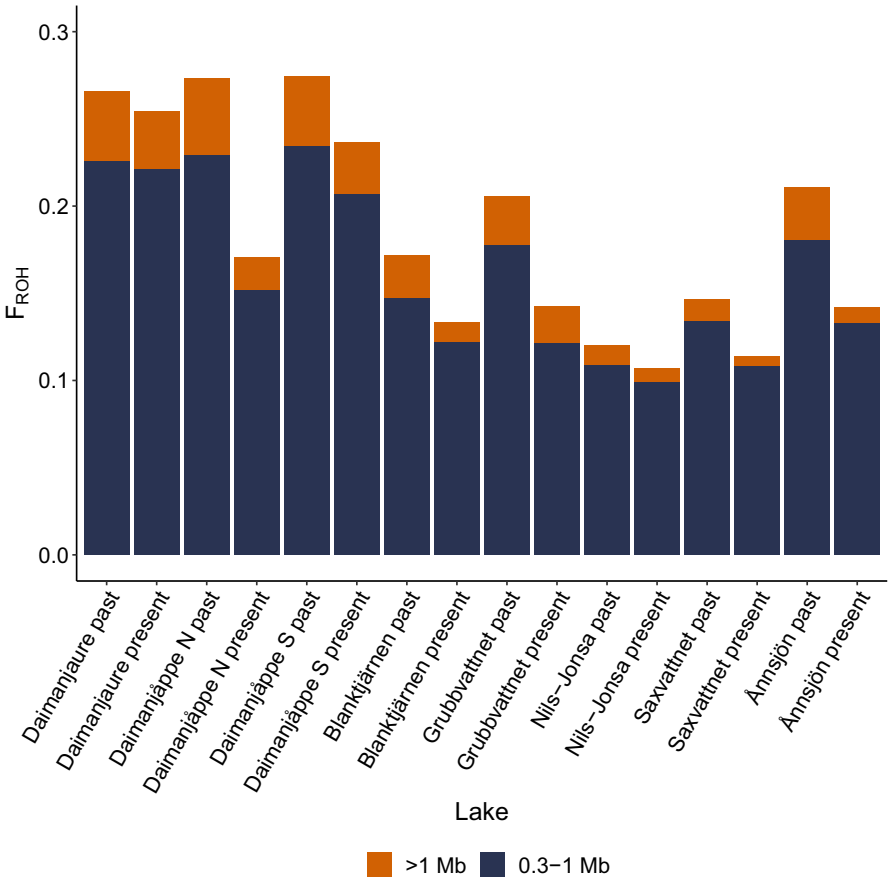

FIGURE 4 Inbreeding estimated as the average proportion of the genome in runs of homozygosity (F_{ROH}) using individual whole genome sequencing data from each of two individuals per lake sampled at two time points. Estimates are shown for short (300 kb–1 Mb; blue) and long (>1 Mb; orange) ROH segments.

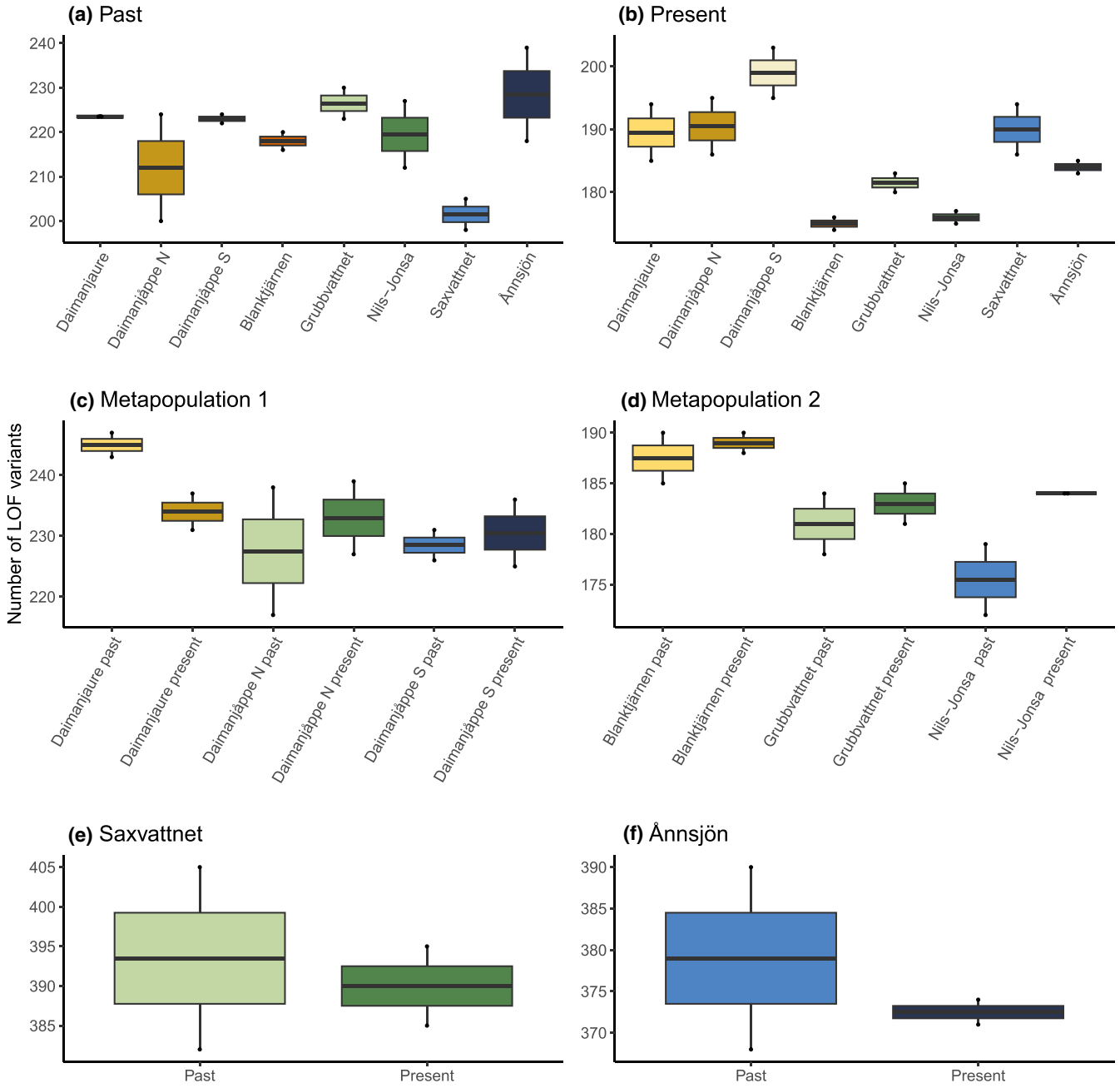

FIGURE 5 Mutational load within lakes. Mutational load is estimated as the number of loss-of-function (LOF) variants within each individual (IGWS data) in six separate runs. Two runs compare spatial trends among (a) past and (b) present samples. Four runs compare temporal trends within each (c, d) metapopulation and (e, f) single lake. Dots correspond to the number of LOF variants per sample, box plots represent first and third quantiles and average values of LOF for samples within the same lake and/or time period in each run. Beware truncated and different *y*-axes.

3.4 | Temporal trends in diversity

The general temporal trends for population genetic diversity are that π, Θ and H_P decrease within the three lakes within metapopulation 1 and single, small, lake Saxvattnet, but increase in lakes belonging to metapopulation 2 and single, large, lake Ånnsjön (Figure 2, Appendix S2).

Generally, we observe little change in divergence between lakes over time (Figure 3b). Within metapopulations, pairwise F_{ST} decreases over time within metapopulation 2 (Appendix S2, Table S5). In metapopulation 1, F_{ST} increases when comparing lake Daimanjaure to either of the other two lakes but remains unchanged between Daimanjåppe N and Daimanjåppe S – again suggesting gene flow between these latter two lakes and limited exchange to Daimanjaure.

Highest temporal differentiation (F_{ST}) is observed within lake Grubbvattnet in metapopulation 2 (F_{ST}=0.045) indicating strikingly higher allele frequency change over time than any other lake

(Table 3, Figure 6). Lake Saxvattnet also shows a relatively high temporal F_{ST} (F_{ST} = 0.027). F_{ST} over time within each of the lakes belonging to metapopulation 1 is low in comparison ($F_{ST} \approx 0.017$). Lowest temporal divergence is observed within lake Nils-Jonsa of metapopulation 2 (F_{ST} = 0.014; Table 3, Figure 6).

F_{ROH} is lower in all present-day samples compared to the historic ones (Figure 4; Wilcoxon signed rank test; W = 186, p = .03). No change within lake over time is, however, statistically significant (Table S8).

TABLE 3 Allele frequency change over time within lakes.

Lake	F_{ST} (95% CI)
Daimanjaure	0.017 (0.0169, 0.0170)
Daimanjåppe N	0.015 (0.0153, 0.0154)
Daimanjåppe S	0.018 (0.0175, 0.0176)
Blanktjärnen	0.021 (0.0211, 0.0212)
Grubbvattnet	0.043 (0.0426, 0.0428)
Nils-Jonsa	0.014 (0.0142, 0.0143)
Saxvattnet	0.022 (0.0223, 0.0224)
Ånnsjön	0.017 (0.0174, 0.0175)

Note: Lake-wise F_{ST} is based on Pool-seq data. 95% Confidence intervals are given in brackets.

3.5 | Indicators

Indicator classifications are depicted in Figure 7 (for details, see Appendix S4, Table S9 for estimates and p-values from statistical tests). The within population genetic diversity indicator, ΔH, shows consistent decrease in all measures included for Pool-seq data in the lakes of metapopulation 1 (lakes Daimanjaure, Daimanjåppe N, Daimanjåppe S) as well as for lake Saxvattnet also located in the northern part of the sampled area (Figure 1). Declines are of a magnitude to result in warning signals in two lakes (Daimanjåppe S, Saxvattnet). In contrast, we find trends of increase in metapopulation 2 (Blanktjärnen, Grubbvattnet, Nils-Jonsa) and in lake Ånnsjön. None of the temporal comparisons of F_{ROH} are significant (Figure 7a, Table S9). The N_e indicator is classified as 'Warning' and 'Alarm' as $N_{eV} < 50$ or $50 < N_{eV} < 500$ in all cases (Table 2, Figure 7a).

The indicator for between-population genetic diversity, ΔF_{ST}, is deemed acceptable for both metapopulations (Figure 7b). Firstly, observed changes in gene flow within metapopulations 1 and 2, respectively, lie within acceptable ranges (Appendix S4). Additionally, both metapopulations are classified as 'Acceptable' with regard to the number of populations that remained over time (i.e. all three).

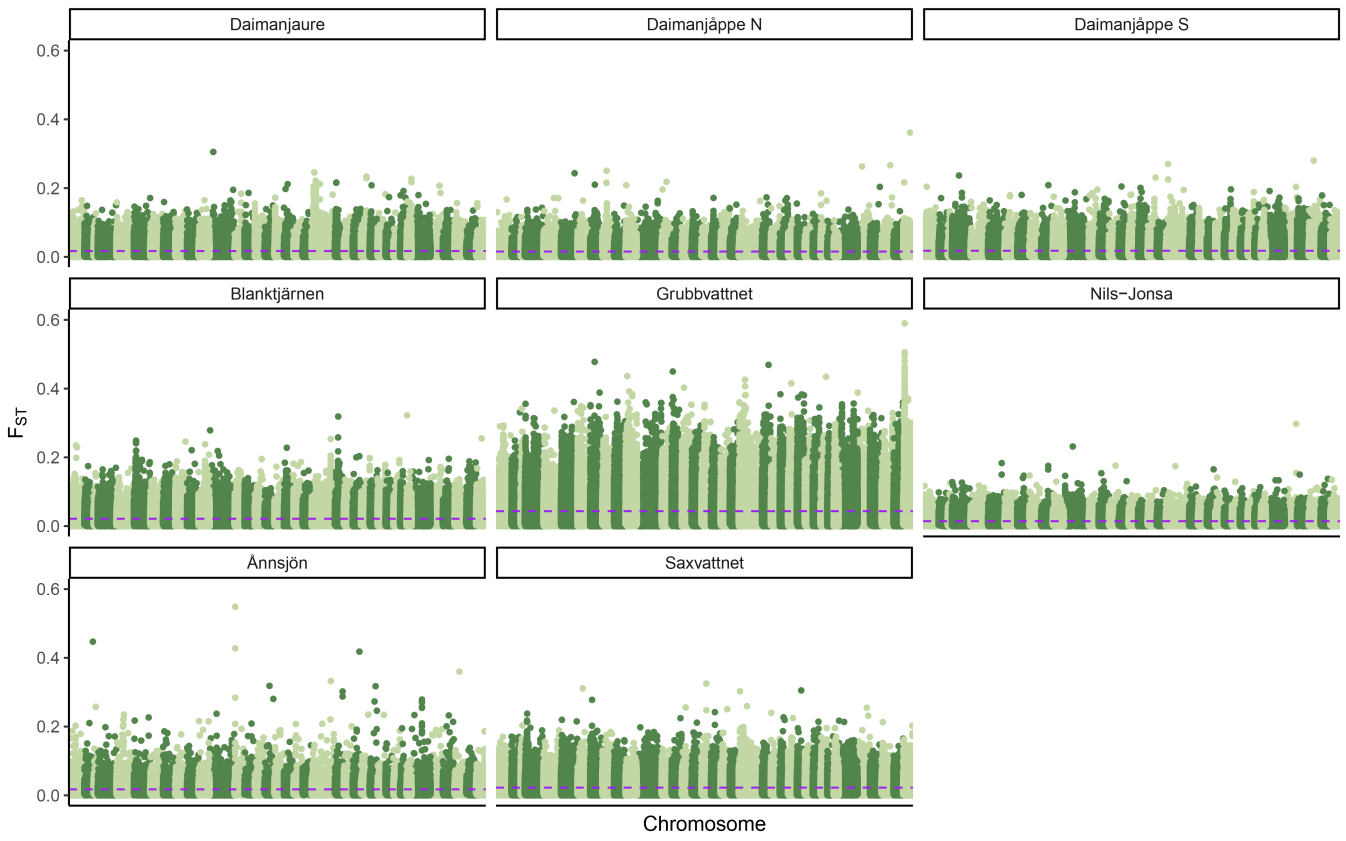

FIGURE 6 Change in allele frequency (F_{ST}) over time within each lake using Pool-seq data. F_{ST} was estimated within 5 kb windows, here represented by a dot each. Chromosomes are presented in order 1–40 and windows mapped to each chromosome in alternating shades of green. The dashed line represents the average temporal F_{ST} per lake.

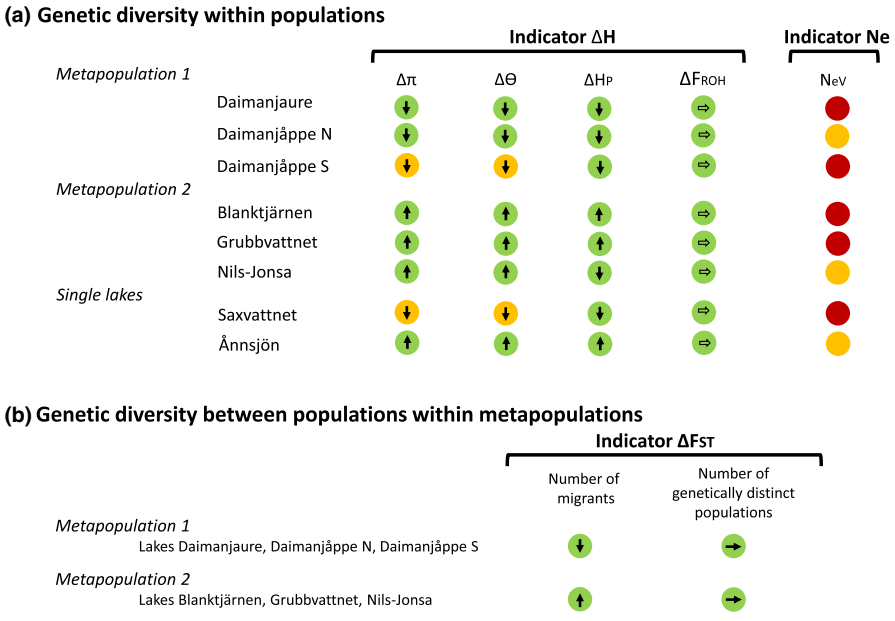

FIGURE 7 Genetic indicator classifications for brown trout populations from eight lakes. Genetic diversity (a) within and (b) between populations. The coloured circles indicate the following classifications; green = 'acceptable', yellow = 'warning' and red = 'alarm'. Arrows inside circles show the direction of change, with horizontal arrows meaning apparent stability (no change); filled arrows indicate that the change is statistically significant (using approaches suggested by Johannesson & Laikre, 2020, and further developed and applied in Andersson et al., 2022). See Appendix S1 and Appendix S2 for details and calculations respectively.

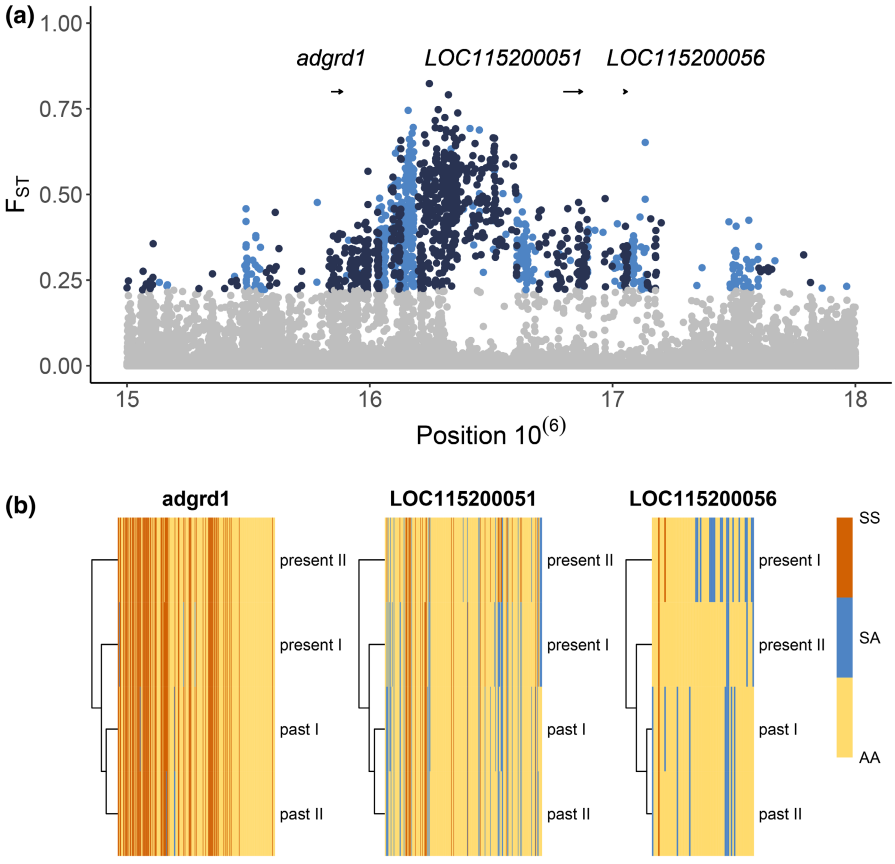

FIGURE 8 Putative selective sweep over the six generations studied in lake Grubbvattnet on chromosome 9. (a) Pairwise F_{ST} estimated between the two time points from Pool-seq data using SNP-based F_{ST} calculations. The dark and light blue dots indicate SNPs with F_{ST} values above the top 99.5th distribution of F_{ST}, of which light blue ones lie within 5 kb of coding regions. Arrows indicate three genes overlapping non-synonymous SNPs. (b) Genotypes within each of the three genes for the four individuals sampled from this lake – two individuals from the past (1976; Table 1) and two individuals from the present (2017). Roman numerals denote individuals sampled at each time point. The *x*-axis denotes genome coordinates within each gene. Cell colours denote SNP genotypes: homozygotes for the reference (red; SS) and alternative allele (yellow; AA), heterozygotes (blue; SA). Two samples are included from each lake and point in time.

3.6 | Signatures of selection over time

3.6.1 | Temporal selection in parallel across all lakes

First, to detect common changes acting across all lakes over time, candidates of selection are identified from SNPs exhibiting significant temporal change in allele frequency in all lakes; 106,983 such SNPs are identified. Average temporal change within lakes for these SNPs is $F_{ST} = 0.006$ (SD = 0.009), none of the SNPs have become fixed in any population, and the by far highest temporal F_{ST} based on these SNPs is found within lake Grubbvattnet with temporal $F_{ST} \approx 0.04$ (just as for the genome-wide F_{ST} results).

The gene functions present among these 106,983 SNPs reveal several superclusters that show an enrichment of higher F_{ST} in genes involved with several key processes (GSEA analysis), e.g. presynaptic activity, tyrosine phosphatase signalling pathways and SAM domain

binding (Figure S2). Further, a total of 2318 SNPs (c. 2%) are predicted to be within coding regions and these are mostly intergenic or intronic (Figure S3a). The functional category exhibiting highest F_{ST} is the one in which variants mutate a stop codon and encompasses four disruptive SNPs (Figure S3a). Three gene models overlap these SNPs (Table S10). In one of the genes (*vps18*), the disruptive (stop gained) mutation is located c. 800 bp from the start of the gene and is flanked by two non-synonymous SNPs that also exhibit parallel allele frequency change in time across all lakes. *Vps18* regulates skin pigmentation in other brown trout (Djurdjevič et al., 2019; Sivka et al., 2013). Here, genotypes within individually sequenced samples were obtained for this gene and the dendrogram based on these does not mirror the dendrogram from the whole genome (Figure S3b, cf. Figure 3a). Instead, populations are scrambled with respect to geography and lake system. However, no apparent clustering of time is either observed.

Of the SNPs exhibiting parallel change in time across all lakes, we also identify genes overlapping those encoding missense mutations (Figure S3a). Fifty-seven missense SNPs are identified, overlapped by 37 genes. Three genes contain a minimum of three SNPs (Table S10), of which two are described in Atlantic salmon (*Salmo salar*): *loc115173703* which is predicted to encode *protocadherin gamma-C5-like* possibly linked to immunity (Dettleff et al., 2017) and *loc115192652* which encodes *plexin-b2-like*, previously reported as possibly under selection in domesticated Canadian populations of Atlantic salmon (López et al., 2019).

3.6.2 | Temporal selection within lakes

We combined measures of F_{ST} over time within lakes and temporal changes in π within lakes to identify signatures of temporal selection acting within lakes. Windows (17,644, 5 kb in size) are identified as candidates of temporal selection within any lake, of which most (12,567) fall within 5 kb of coding regions. A majority of these windows are unique to a single lake, most of them to lakes Ånnsjön or Nils-Jonsa (Figure S4). We focus on selection acting within metapopulations; finding 54 uniquely shared windows between Daimanjåppe N and Daimanjåppe S in metapopulation 1 within protein coding regions, of which 26 contain non-synonymous SNPs, overlapped by six genes. The protein predictions for these are as far as we know not described in fish (Table S11).

Candidates of selection unique to lakes belonging to metapopulation 2 are identified, namely five windows in coding regions uniquely shared between lakes Blanktjärnen, Grubbvattnet and Nils-Jonsa (Table S12). These windows are overlapped by seven genes, of which three are described in fish: *tsn* associated with sperm quality in rainbow trout (*Oncorhynchus mykiss*; Nynca et al., 2014), *loc115151923* which is near a SNP involved in immune response in brown trout (Ahmad et al., 2018) and meat tenderness in rainbow trout (Ali et al., 2019), and *loc115195360* which is implied in immune response in stickleback (*Gasterosteus aculeatus*; Fuess et al., 2021).

For the gene candidates of selection within either metapopulation, dendrograms based on genotypes from IWGS data show no discernible temporal or spatial pattern (not shown).

3.6.3 | Suggested selective sweep in lake Grubbvattnet

A region of strong divergence over time is observed on chromosome 9 in lake Grubbvattnet (Figure 8). This region exhibits higher density of SNPs with marked temporal differentiation (above 99.5th percentile of F_{ST} within this lake) in coding regions compared to the genome-wide average, suggesting a selective sweep (Figure 8a). A total of 20 missense mutations are found here, overlapped by seven genes (Table S13). Two of these are described in teleost fish: *loc115200043* which is an immune effector described in Atlantic salmon (*Salmo salar*; Robinson et al., 2021) and *loc115199627* which has functions related to seminal fluid composition in rainbow trout and common carp (*Cyprinus carpio*; Shaliutina-Kolešová et al., 2016). An additional gene, *adgrd1*, may have a sex-specific expression; it regulates oviductal fluid in mammals (Bianchi et al., 2021) and is linked to sex-specific response to hypoxia in medaka (*Oryzias melastigma*; Lai et al., 2020).

Genotypes within individually sequenced fish from lake Grubbvattnet were sought for these seven genes. Genotypes within three genes, including *adgrd1*, exhibit temporal differentiation among time points (Figure 8b). In four other genes, no differentiation is discerned (not shown).

We are unable to find regions of outstanding genetic differentiation based on genotype frequencies (haplotype differentiation) with IWGS data with HaploDistScan and detectRuns for any region or chromosome surrounding candidates of selection, nor when scanning the full genome (not shown).

4 | DISCUSSION

Here, wild brown trout populations in eight mountain lakes are genetically characterized in temporally separated samples spanning over four decades (corresponding to c. six trout generations), in a novel attempt to monitor genetic variation using genome-wide data. Present results show temporal trends in diversity (nucleotide diversity, Watterson's Θ and heterozygosity) with an overall decrease in the northern most metapopulation (located above the tree line) and the northernmost single lake, while the larger metapopulation (located below the tree line) and the largest single lake show increase in genetic diversity. The populations do not seem to be hampered by inbreeding effects, as inbreeding (F_{ROH}; spanning 10%–30%) is mostly represented by ancient (<1 Mb) runs of homozygosity and we observe little change in mutational load. However, lower levels of genetic diversity and higher levels of inbreeding are observed in the above tree line metapopulation and continued monitoring is therefore important.

We apply indicators for genetic diversity recently adopted for national use in Sweden. Proposed threshold values for these indicators generally show acceptable maintenance of genetic diversity within (indicator ΔH) and between (ΔF_{ST}) populations, but with warnings exhibited for ΔH in two lakes. Local effective population sizes (indicator N_e) are too small, with all lakes below the acceptable level 500 required for our present indicator as well as the CBD Headline Indicator A.4 of the new Kunming-Montreal Global Biodiversity Monitoring Framework (CBD, 2022b), which also uses $N_e > 500$ as a critical threshold for assuring populations of adequate size to maintain adaptive potential (CBD, 2022a). The low N_e observed here suggest that the studied populations may be genetically vulnerable, highlighting the need for continued protection of large, interconnected systems for their long-term viability.

Neutral and putatively adaptive loci suitable for the ongoing elaboration of monitoring programmes of genetic diversity within this species are identified. We identify putative parallel selection across all lakes within a gene pertaining to skin pigmentation as well as candidates of selection unique to specific lakes and lake systems involved in reproduction and immunity. We identify one lake in particular (Grubbvattnet), where selection seems to be particularly proponent.

Overall, this study demonstrates the benefit of WGS data for monitoring wild populations over contemporary time frames.

4.1 | Population genetic diversity

Genome-wide diversity per population pool ranges between $\pi = 0.001–0.002$, $\Theta = 0.0009–0.0010$ and $H_P = 0.06–0.09$ in the studied populations (Figure 2, Appendix S2). This is comparable to previous genomic observations from brown trout populations inhabiting the same geographic area (Kurland et al., 2019, 2022; Saha et al., 2022), yet lower than observed in French populations ($\pi = 0.004–0.005$; Leitwein et al., 2016).

The smallest and northern most populations within the three lakes belonging to metapopulation 1, found above the tree line, exhibit the lowest diversity (π, Θ and H_P) and the highest inbreeding (F_{ROH}) of all populations. Diversity decreases over time within these populations while between population divergence (F_{ST}) increases, suggesting that gene flow has been reduced which is expected to result in reduced diversity within populations due to restricted population size. Reduced variation may also be expected at range margins, e.g. if limited gene flow to marginal population results in small, isolated populations prone to drift (e.g. Kawecki, 2008). These findings indicate that these populations are particularly sensitive and warrant particular focus in continued monitoring efforts. Conversely, recent literature has highlighted populations which flourish despite restricted genetic diversity, e.g. when the number of founders is limited (Kinziger et al., 2021; Kurland et al., 2022), and demonstrating local adaptations (Willoughby et al., 2018), suggesting that low levels of genetic diversity are not inevitably negative.

4.2 | Effective population size (N_e)

Evidence of significant change in allele frequencies within lakes is found over the c. six generations studied. Temporal, genome-wide F_{ST} within lakes averages 0.02, ranging from 0.01 in Nils-Jonsa to 0.04 in Grubbvattnet (Table 3, Figure 6). These allele frequency shifts are reflected in exceedingly small measures of N_{eV}; most lakes exhibit $N_{eV} < 50$. Further, N_{eV} is not correlated to diversity (π; $r^2 = .01$, $t = 0.22$, $p = .84$). At the same time, several lakes retain genetic diversity over time or even show increased diversity (e.g. lakes of metapopulation 2 and Lake Ånnsjön). However, the N_{eV} estimates we obtain are expected to reflect local effective sizes in isolation (Ryman et al., 2019). Metapopulation structure governs the rate at which genetic diversity is maintained in a population, suggesting that if connectivity is disrupted this will result in rapid genetic diversity loss (Kurland et al., 2023; Laikre et al., 2016). All of the present lakes are connected to nearby ones, even the single lakes are connected via creeks to other water bodies. In cases such as these, where gene flow does occur, local N_{eV} underestimates the actual rate of inbreeding (Ryman et al., 2019).

4.3 | Inbreeding and mutational load

Inbreeding (F_{ROH}) exceeds 10% in all lakes over time, but little evidence of recent inbreeding is found, since a majority of the runs of homozygosity (ROH) are below 1 Mb and longest ROH (>2 Mb) are identified in only a few individuals (Figure 4, Table S8). In comparison, D'Ambrosio et al. (2019) find stretches exceeding 10 Mb in domestic lines of rainbow trout (*O. mykiss*). Our comparable lack of recent inbreeding again suggests gene flow between the studied populations.

The 1970s samples show no obvious spatial trend in genetic load, but for present-day samples we observe a tendency for above tree line lakes to have higher LOF (Figure 5a,b). However, differences are small and little to no temporal change in mutational load is observed in most lakes (Figure 5c–f), which may be due to small samples sizes (Díez-del-Molino et al., 2020). Further sampling of genomes from present populations is needed to fully link their genome variability with population viability.

4.4 | Selection over time

We find allele frequencies for SNPs exhibiting levels of temporal differentiation above what is expected for neutrality, including parallel shifts across all eight lakes and those unique to specific lakes. This finding is striking, given the narrow time frame and limited genetic diversity, including small effective population sizes, suggesting drift may be proponent within the studied populations.

4.4.1 | Parallel allele frequency shifts across all lakes

The observed significant allele frequency change acting in parallel across all eight lakes, possibly suggests directional selection having occurred over the present sampling period. Of the identified SNPs, those within coding regions are enriched for GO terms associated to presynaptic activity (Figure S2). Genes regulating immunity as well as skin pigmentation are also implied (Table S10). One gene, *vps18*, is a candidate for labyrinthine skin pattern in the sister taxon marble trout (*Sebastiscus marmoratus*; Sivka et al., 2013), the expression of which also results in different pigmentation intensity in marble and brown trout by (Djurdjevič et al., 2019). Skin coloration and pattern are among the most diverse phenotypical characteristics of salmonid fishes, playing important roles in the survival of animals and suggested to be among the driving forces of speciation (Braasch et al., 2007). If the observed parallel allele frequency shifts are explained by selection, identified SNPs may exhibit local adaptive importance to these lakes. However, for individual genomes, we find no temporal clustering of individual samples in the past as compared to present-day individuals within this gene as expected given the contention of parallel change over time. Neither is temporal differentiation supported in cluster analyses of individuals from separate lakes (not shown). This may in part be due to the small sample size of individual genomes per lake and time point. Further validation of the putatively adaptive role of this gene, e.g. through environmental association studies, is needed to validate our hypothesis of directional selection.

4.4.2 | Selection within lakes and lake systems

Lake Grubbvattnet stands out in measures of temporal change; exhibiting lowest N_e ($N_{eV}=18$, Table 2) and highest temporal F_{ST} (c. 0.05, Table 3), suggesting marked change in allele frequencies. We also observe a region of marked temporal divergence within Grubbvattnet, suggesting a putatively selective sweep. Two of the seven genes identified encode reproductive traits and one immunity (Figure 8a, Table S13). This lake belongs to metapopulation 2, and for the genes that are potentially under selection in this lake system, reproductive and immune traits are also predicted (Table S12). It is therefore possible that we have detected local selective pressures unique to this lake system. Further validation of potential fitness effects of the present candidates for local adaptation is required to test our hypothesis. However, variation surrounding genes regulating immunity and reproduction are associated with fine-scale local adaptation in salmonids (Pritchard et al., 2018), and may fluctuate over short time frames (Fraser et al., 2011; Kjærner-Semb et al., 2016). Additionally, reproductive success has even been linked to genotypes in immune genes in salmonids (Fraser et al., 2011; Gessner et al., 2017).

Our definitions of candidates of selection are highly conservative, which lends strength to the contention of selection. However, separating selection from drift in natural settings and over microevolutionary time frames is challenging (e.g. reviewed by Hoban et al., 2016). Inference of selection from measures of divergence between populations is susceptible to false signals of selection as divergence patterns are also shaped by population ancestry and genome characteristics, e.g. linkage disequilibrium (LD). For instance, local inflations in differentiation may be due to reduced diversity in regions shaped by recombination (e.g. surrounding centromeres) or increased background selection (e.g. in regions with high gene density; Jacobs et al., 2020). Although recombination is prevalent in other brown trout populations (Leitwein et al., 2016), it is most likely not a prominent disturbance in the current study, given so few generations. In addition, our scans for selection utilize samples from the same lake taken at different points in time which may involve comparing descendant fish genomes to those from preceding generations. However, we err on the side of caution and continuously described our candidates of selection as candidates.

4.5 | Comparing Pool-seq to IWGS

Generally, we find agreement between Pool-seq and IWGS data for genome-wide patterns of diversity and divergence when comparing between lakes. However, temporal trends captured by Pool-seq data are not reflected in the individuals, neither genome wide nor for regions surrounding our candidates of selection. The discrepancy between pooled and individual samples may be due to the fact that the two individuals sampled per time point and lake for IWGS data are inadequate to reflect subtle shifts in allele frequency. For Pool-seq data, c. 50 individuals were sampled which should give accurate allele frequency estimates (Schlötterer et al., 2014). An increasing body of research demonstrates that even small changes in allele frequency can be detected using Pool-seq (Lima & Willett, 2018). Additional explanations for incongruence between Pool-seq and IWGS data for putatively adaptive regions include no real underlying selection (i.e. SNPs are false positives) and that selective differences are highly polygenic.

4.6 | Indicators

Our results suggest that continued protection of large, interconnected systems is crucial for the long-term viability of presently studied populations. This contention is based on findings using newly formulated indicators for genetic monitoring in Sweden (Johannesson & Laikre, 2020). Most prominently, effective population sizes of the studied populations are well below suggested thresholds ($N_{eV}<50$ and $50<N_{eV}>500$), and all lakes are marked with 'warning' or 'alarm'. While these estimates reflect N_{eV} under isolation and therefore over-estimate the actual rate of inbreeding with migration (Ryman et al., 2019), they suggest a vulnerability to genetic erosion if the lakes should become isolated.

The presently studied lakes were also monitored by Andersson et al. (2022) using a 96 SNP panel. We generally find our indicator

assessments to agree with theirs, though some discrepancies exist. In some cases, direct comparisons are not possible, as Andersson et al. (2022) consider genetic clusters within lakes, while we focus exclusively on population pools sampled from lakes (the SNP array generates genotype data, while Pool-seq regards allele frequencies for all populations pooled within a lake). However, we generally observe more cases exhibiting significant temporal changes in diversity when using Pool-seq data than the SNP array (Figure 7 cf. Andersson et al., 2022; Figure 6). This is not completely unexpected, given the added statistical power provided by increased number of neutral loci in WGS (Allendorf et al., 2010; Garner et al., 2016; Schwartz et al., 2007).

In particular, differences occur between the N_{eV} estimates in our study and those in Andersson et al. (2022). Our N_{eV} estimates are based on 150,000 random, putatively unlinked SNPs from Pool-seq data and are consistently lower than those based on the 96 SNP array (cf. Andersson et al., 2022). There are several likely reasons for these deviations. First, Andersson et al. (2022) assess N_e of genetic clusters identified from the 96 SNP data within lakes, while we assess N_e after pooling all individuals from each lake. Because the clusters do not occur in equal frequency in the samples from the two separate time points, we expect allele frequency shifts in our present data that are not due to genetic drift but due to sampling effects. Further, we suggest an additional sample variance to be present in the Pool-seq data due to sample sizes not being exactly 50 genomes, only approximately 50. Such sample variance might also inflate estimation of allele frequency differences over time, thus reducing N_{eV} (cf. Figure S5). However, further analyses are needed to fully understand these potential effects and we plan to return to this topic in forthcoming work.

No indicator for adaptive genetic diversity has of yet been suggested, but present findings suggest that selective changes can be detected even over a few generations (see also Enbody et al., 2023). In fact, we identify temporal, putatively selective change in multiple genes likely involved in local adaptation and speciation in salmonid fishes (Djurdjevič et al., 2019; Pritchard et al., 2018; Sivka et al., 2013). Thus, WGS data, including from Pool-seq, can contribute valuable information not possible to detect with traditional markers (such as microsatellites or limited numbers of SNPs) for contemporary monitoring purposes. WGS data also contributes information on rates of inbreeding and genetic load (Díez-del-Molino et al., 2018); costlier individual WGS is then needed, however, and sample sizes in the present case (only $n = 2$ per sampling lake and locality) are clearly too small. We intend to continue investigating the benefit of WGS for conservation genetics monitoring and management, including comparisons of indicator values from different approaches which is highly warranted.

AUTHOR CONTRIBUTIONS

L.L., N.R. and S.K. designed the study. N.R. and L.L. provided the material. S.K., M.C. and N.K. carried out the population genomic and bioinformatic analyses using Pool-seq data. A.S., D.D.M. and S.K. performed analyses on IWGS data. S.K. wrote the first draft of the manuscript. All authors contributed to writing. L.L. funded the study.

ACKNOWLEDGEMENTS

We thank the following persons for fieldwork to collect present-day samples: Anastasia Andersson, Jens Andersson (County Administrative Board of Jämtland), Kristoffer Andersson, Rolf Gustavsson, Tina Hedlund (Aquanord AB), Kurt Morin, Randi Olofsson-Lund, Håkan Lund (Almdalen Fjällgård), Jan Oscarsson (Sport fishing club D30), Gunnar Ståhl, Karin Tahvanainen. We also thank Anastasia for valuable input when comparing our indicator results to previous work. We acknowledge long-term support from the National Genomics Infrastructure (NGI) in Stockholm funded by Science for Life Laboratory (SciLife), the Knut and Alice Wallenberg Foundation and the Swedish Research Council. Computations and data storage were enabled by resources provided by the Swedish National Infrastructure for Computing (SNIC) at Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) partially funded by the Swedish Research Council through grant agreement no. 2018-05973. This research was supported by the Swedish Research Council Formas (2020-01290; L.L.), the Swedish Research Council (2019-05503; L.L., N.R.), the Carl Trygger (grant 10:192; L.L.), the Erik Philip-Sörensen Foundations (L.L.) and the Swedish Agency for Marine and Water Management (L.L.), the SciLifeLab Bioinformatics Long-Term Support (L.L.) funded by the Knut and Alice Wallenberg foundation (grant no. 2014.0278). In particular, we thank Verena Kutchera and Diana Ekman from the Long-Term Support (WABI) team for supervision. S.K. expresses gratitude to support from SciLife's Swedish Bioinformatics Advisory Program and her advisor Nima Rafati.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Illumina raw sequences from this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under project accession number PRJEB61943. Processed data are available at Dryad (https://doi.org/10.5061/dryad.x69p8czqt). Scripts are available at https://github.com/sarkur/Pool-seq.

BENEFIT SHARING STATEMENT

A research collaboration was developed between scientists from various institutions and all collaborators are included as co-authors. The research is closely linked to biodiversity management in Sweden and the indicators applied here were elaborated within ongoing science–management–policy implementation work in Sweden focused on monitoring genetic diversity of key species for management. These collaborations are not only primarily with the Swedish Agency for Marine and Water Management and the Swedish Environmental Protection Agency, but also with local County Administrative Boards (in the present case the Jämtland County Administrative Board). Sampling was conducted in collaboration with indigenous

Sámi villages and local sport fishing clubs. Results are shared with these communities as well as other stakeholders, nationally and internationally, and the broader scientific community (see above). Furthermore, the research addresses a priority concern, the case of how to systematically monitor wild populations for sustainable management.

ORCID

Sara Kurland https://orcid.org/0000-0002-5370-1236
Atal Saha https://orcid.org/0000-0003-1334-928X
David Díez-del-Molino https://orcid.org/0000-0002-9701-5940
Nils Ryman https://orcid.org/0000-0003-3342-8479
Linda Laikre https://orcid.org/0000-0001-9286-3361

REFERENCES

Ahmad, F., Debes, P. V., Palomar, G., & Vasemägi, A. (2018). Association mapping reveals candidate loci for resistance and anaemic response to an emerging temperature-driven parasitic disease in a wild salmonid fish. *Molecular Ecology*, *27*(6), 1385–1401. https://doi.org/10.1111/mec.14509

Akey, J. M., Zhang, G., Zhang, K., Jin, L., & Shriver, M. D. (2002). Interrogating a high-density SNP map for signatures of natural selection. *Genome Research*, *12*(12), 1805–1814. https://doi.org/10.1101/GR.631202

Alexa, A., & Rahnenführer, J. (2018). Gene set enrichment analysis with topGO. *Bioconductor Improv*, *27*, 1–26.

Ali, A., Al-Tobasei, R., Lourenco, D., Leeds, T., Kenney, B., & Salem, M. (2019). Genome-wide association study identifies genomic loci affecting filet firmness and protein content in rainbow trout. *Frontiers in Genetics*, *10*, 386. https://doi.org/10.3389/fgene.2019.00386

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, *11*(10), 697–709. https://doi.org/10.1038/nrg2844

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*(5), 1213–1227. https://doi.org/10.1007/s10592-017-0972-4

Andersson, A., Johansson, F., Sundbom, M., Ryman, N., & Laikre, L. (2017). Lack of trophic polymorphism despite substantial genetic differentiation in sympatric brown trout (*Salmo trutta*) populations. *Ecology of Freshwater Fish*, *26*(4), 643–652. https://doi.org/10.1111/eff.12308

Andersson, A., Karlsson, S., Ryman, N., & Laikre, L. (2022). Mapping and monitoring genetic diversity of an alpine freshwater top predator by applying newly proposed indicators. *Molecular Ecology*, *31*, 6422–6439. https://doi.org/10.1111/mec.16710

Ayllon, F., Davaine, P., Beall, E., & Garcia-Vazquez, E. (2006). Dispersal and rapid evolution in brown trout colonizing virgin Subantarctic ecosystems. *Journal of Evolutionary Biology*, *19*(4), 1352–1358. https://doi.org/10.1111/j.1420-9101.2005.01075.x

Bekkevold, D., Höjesjö, J., Nielsen, E. E., Aldvén, D., Als, T. D., Sodeland, M., Kent, M. P., Lien, S., & Hansen, M. M. (2020). Northern European *Salmo trutta* (L.) populations are genetically divergent across geographical regions and environmental gradients. *Evolutionary Applications*, *13*, 400–416. https://doi.org/10.1111/eva.12877

Bianchi, E., Sun, Y., Almansa-Ordonez, A., Woods, M., Goulding, D., Martinez-Martin, N., & Wright, G. J. (2021). Control of oviductal fluid flow by the G-protein coupled receptor Adgrd1 is essential for murine embryo transit. *Nature Communications*, *12*(1), 1–12. https://doi.org/10.1038/s41467-021-21512-w

Biscarini, F., Cozzi, P., Gaspa, G., & Marras, G. (2019). detectRUNS: An R package to detect runs of homozygosity and heterozygosity in diploid genomes. *The R Project. Retrieved June*, *8*, *2019*.

Braasch, I., Schartl, M., & Volff, J.-N. (2007). Evolution of pigment synthesis pathways by gene and genome duplication in fish. *BMC Evolutionary Biology*, *7*(1), 1–18. https://doi.org/10.1186/1471-2148-7-74

Browning, S. R., & Browning, B. L. (2007). Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. *The American Journal of Human Genetics*, *81*(5), 1084–1097. https://doi.org/10.1086/521987

Bruford, M. W., Davies, N., Dulloo, M. E., Faith, D. P., & Walters, M. (2017). Monitoring changes in genetic diversity. In M. Walters & R. J. Scholes (Eds.), *The GEO handbook on biodiversity observation networks* (pp. 107–128). Springer International Publishing.

CBD. (2022a). Kunming-Montreal Global Biodiversity Framework. Decision adopted by the Conference of the Parties to the Convention on Biological Diversity. Fifteenth meeting – Part II, Montreal, Canada, 7–19 December 2022. CBD/COP/DEC/15/4.

CBD. (2022b). Monitoring framework for the Kunming-Montreal Global Biodiversity Framework. Decision adopted by the Conference of the Parties to the Convention on Biological Diversity. Fifteenth meeting – Part II, Montreal, Canada, 7–19 December 2022. CBD/COP/DEC/15/5.

Ceballos, F. C., Joshi, P. K., Clark, D. W., Ramsay, M., & Wilson, J. F. (2018). Runs of homozygosity: Windows into population history and trait architecture. *Nature Reviews Genetics*, *19*(4), 220–234. https://doi.org/10.1038/nrg.2017.109

Charlier, J., Laikre, L., & Ryman, N. (2012). Genetic monitoring reveals temporal stability over 30 years in a small, lake-resident brown trout population. *Heredity*, *109*(4), 246–253. https://doi.org/10.1038/hdy.2012.36

Charlier, J., Palmé, A., Laikre, L., Andersson, J., & Ryman, N. (2011). Census (NC) and genetically effective (N_e) population size in a lake-resident population of brown trout *Salmo trutta*. *Journal of Fish Biology*, *79*(7), 2074–2082. https://doi.org/10.1111/j.1095-8649.2011.03124.x

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly*, *6*(2), 80–92. https://doi.org/10.4161/fly.19695

Conway, J. R., Lex, A., & Gehlenborg, N. (2017). UpSetR: An R package for the visualization of intersecting sets and their properties. *Bioinformatics*, *33*, 2938–2940. https://doi.org/10.1093/bioinformatics/btx364

D'Ambrosio, J., Phocas, F., Haffray, P., Bestin, A., Brard-Fudulea, S., Poncet, C., Quillet, E., Dechamp, N., Fraslin, C., Dupont-Nivet, M., & Charles, M. (2019). Genome-wide estimates of genetic diversity, inbreeding and effective size of experimental and commercial rainbow trout lines undergoing selective breeding. *Genetics Selection Evolution*, *51*(1), 1–15. https://doi.org/10.1186/s12711-019-0468-4

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., McVean, G., Durbin, R., & Sherry, S. T. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., del Angel, G., Rivas, M. A., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., Daly, M. J., & Hanna, M. (2011). A

framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, *43*(5), 491–498. https://doi.org/10.1038/ng.806

Dettleff, P., Moen, T., Santi, N., & Martinez, V. (2017). Transcriptomic analysis of spleen infected with infectious salmon anaemia virus reveals distinct pattern of viral replication on resistant and susceptible Atlantic salmon (*Salmo salar*). *Fish & Shellfish Immunology*, *61*, 187–193. https://doi.org/10.1016/j.fsi.2017.01.005

Díez-del-Molino, D., Sánchez-Barreiro, F., Barnes, I., Gilbert, M. T. P., & Dalén, L. (2018). Quantifying temporal genomic erosion in endangered species. *Trends in Ecology & Evolution*, *33*(3), 176–185. https://doi.org/10.1016/j.tree.2017.12.002

Díez-del-Molino, D., von Seth, J., Gyllenstrand, N., Widemo, F., Liljebäck, N., Svensson, M., Sjögren-Gulve, P., & Dalén, L. (2020). Population genomics reveals lack of greater white-fronted introgression into the Swedish lesser white-fronted goose. *Scientific Reports*, *10*(1), 18347. https://doi.org/10.1038/s41598-020-75315-y

Djurdjevič, I., Furmanek, T., Miyazawa, S., & Bajec, S. S. (2019). Comparative transcriptome analysis of trout skin pigment cells. *BMC Genomics*, *20*(1), 1–15. https://doi.org/10.1186/s12864-019-5714-1

Dussex, N., van der Valk, T., Morales, H. E., Wheat, C. W., Díez-del-Molino, D., von Seth, J., Foster, Y., Kutschera, V. E., Guschanski, K., Rhie, A., Phillippy, A. M., Korlach, J., Howe, K., Chow, W., Pelan, S., Mendes Damas, J. D., Lewin, H. A., Hastie, A. R., Formenti, G., … Dalén, L. (2021). Population genomics of the critically endangered kākāpō. *Cell Genomics*, *1*(1), 100002. https://doi.org/10.1016/J.XGEN.2021.100002

Elmer, K. R. (2016). Genomic tools for new insights to variation, adaptation, and evolution in the salmonid fishes: A perspective for charr. *Hydrobiologia*, *783*(1), 191–208. https://doi.org/10.1007/s10750-015-2614-5

Enbody, E. D., Sendell-Price, A. T., Sprehn, C. G., Rubin, C. J., Visscher, P. M., Grant, B. R., Grant, P. R., & Andersson, L. (2023). Community-wide genome sequencing reveals 30 years of Darwin's finch evolution. *Science*, *381*(6665), eadf6218.

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Fraser, D. J., Weir, L. K., Bernatchez, L., Hansen, M. M., & Taylor, E. B. (2011). Extent and scale of local adaptation in salmonid fishes: Review and meta-analysis. *Heredity*, *106*(3), 404–420. https://doi.org/10.1038/hdy.2010.167

Fuess, L. E., Weber, J. N., den Haan, S., Steinel, N. C., Shim, K. C., & Bolnick, D. I. (2021). Between-population differences in constitutive and infection-induced gene expression in threespine stickleback. *Molecular Ecology*, *30*(24), 6791–6805. https://doi.org/10.1111/mec.16197

García-Alcalde, F., Okonechnikov, K., Carbonell, J., Cruz, L. M., Götz, S., Tarazona, S., Dopazo, J., Meyer, T. F., & Conesa, A. (2012). Qualimap: Evaluating next-generation sequencing alignment data. *Bioinformatics*, *28*(20), 2678–2679. https://doi.org/10.1093/bioinformatics/bts503

Garcia-Elfring, A., Paccard, A., Thurman, T. J., Wasserman, B. A., Palkovacs, E. P., Hendry, A. P., & Barrett, R. D. H. (2021). Using seasonal genomic changes to understand historical adaptation to new environments: Parallel selection on stickleback in highly-variable estuaries. *Molecular Ecology*, *30*(9), 2054–2064. https://doi.org/10.1111/mec.15879

Garner, B. A., Hand, B. K., Amish, S. J., Bernatchez, L., Foster, J. T., Miller, K. M., Morin, P. A., Narum, S. R., O'Brien, S. J., Roffler, G., Templin, W. D., Sunnucks, P., Strait, J., Warheit, K. I., Seamons, T. R., Wenburg, J., Olsen, J., & Luikart, G. (2016). Genomics in conservation: Case studies and bridging the gap between data and application. *Trends in Ecology and Evolution*, *31*(2), 81–82. https://doi.org/10.1016/j.tree.2015.10.009

Gessner, C., Nakagawa, S., Zavodna, M., & Gemmell, N. J. (2017). Sexual selection for genetic compatibility: The role of the major histocompatibility complex on cryptic female choice in Chinook salmon (*Oncorhynchus tshawytscha*). *Heredity*, *118*(5), 442–452. https://doi.org/10.1038/hdy.2016.116

Gomez-Raya, L., Rodríguez, C., Barragán, C., & Silió, L. (2015). Genomic inbreeding coefficients based on the distribution of the length of runs of homozygosity in a closed line of Iberian pigs. *Genetics Selection Evolution*, *47*(1), 1–15. https://doi.org/10.1186/s12711-015-0153-1

Hoban, S., Archer, F. I., Bertola, L. D., Bragg, J. G., Breed, M. F., Bruford, M. W., Coleman, M. A., Ekblom, R., Funk, W. C., Grueber, C. E., Hand, B. K., Jaffé, R., Jensen, E., Johnson, J. S., Kershaw, F., Liggins, L., MacDonald, A. J., Mergeay, J., Miller, J. M., … Hunter, M. E. (2022). Global genetic diversity status and trends: Towards a suite of Essential Biodiversity Variables (EBVs) for genetic composition. *Biological Reviews*, *97*(4), 1511–1538. https://doi.org/10.1111/brv.12852

Hoban, S., Bruford, M., D'Urban Jackson, J., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P. A., Paz-Vinas, I., Sjögren-Gulve, P., Segelbacher, G., Vernesi, C., Aitken, S., Bertola, L. D., Bloomer, P., Breed, M., Rodríguez-Correa, H., Funk, W. C., Grueber, C. E., Hunter, M. E., Jaffe, R., … Laikre, L. (2020). Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. *Biological Conservation*, *248*, 108654. https://doi.org/10.1016/j.biocon.2020.108654

Hoban, S., Bruford, M. W., Funk, W. C., Galbusera, P., Griffith, M. P., Grueber, C. E., Heuertz, M., Hunter, M. E., Hvilsom, C., Kalamujic Stroil, B., Kershaw, F., Khoury, C. K., Laikre, L., Lopes-Fernandes, M., MacDonald, A. J., Mergeay, J., Meek, M., Mittan, C., Mukassabi, T. A., … Vernesi, C. (2021). Global commitments to conserving and monitoring genetic diversity are now necessary and feasible. *BioScience*, *71*(9), 964–976. https://doi.org/10.1093/biosci/biab054

Hoban, S., da Silva, J. M., Mastretta-Yanes, A., Grueber, C. E., Heuertz, M., Hunter, M. E., Mergeay, J., Paz-Vinas, I., Fukaya, K., Ishihima, F., Jordan, R., Köppä, V., Latorre-Cárdenas, M. C., MacDonald, A. J., Rincon-Parra, V., Sjögren-Gulve, P., Tani, N., Thurfjell, H., & Laikre, L. (2023). Monitoring status and trends in genetic diversity for the Convention on Biological Diversity: An ongoing assessment of genetic indicators in nine countries. *Conservation Letters*, *16*, e12953. https://doi.org/10.1111/conl.12953

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., Poss, M. L., Reed, L. K., Storfer, A., & Whitlock, M. C. (2016). Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions. *The American Naturalist*, *188*(4), 379–397. https://doi.org/10.1086/688018

Hoban, S., Paz-Vinas, I., Aitken, S., Bertola, L. D., Breed, M. F., Bruford, M. W., Funk, W. C., Grueber, C. E., Heuertz, M., Hohenlohe, P., Hunter, M. E., Jaff'e, R., Lopes Fernandes, M., Mergeay, J., Moharrek, F., O'Brien, D., Segelbacher, G., Vernesi, C., Waits, L., & Laikre, L. (2021). Effective population size remains a suitable, pragmatic indicator of genetic diversity for all species, including forest trees. *Biological Conservation*, *253*, 108906. https://doi.org/10.1016/j.biocon.2020.108906

Hoban, S. M., Hauffe, H. C., Pérez-Espona, S., Arntzen, J. W., Bertorelle, G., Bryja, J., Frith, K., Gaggiotti, O. E., Galbusera, P., Godoy, J. A., Hoelzel, A. R., Nichols, R. A., Primmer, C. R., Russo, I.-R., Segelbacher, G., Siegismund, H. R., Sihvonen, M., Vernesi, C., Vilà, C., & Bruford, M. W. (2013). Bringing genetic diversity to the forefront of conservation policy and management. *Conservation Genetics Resources*, *5*, 593–598. https://doi.org/10.1007/s12686-013-9859-y

Hvilsom, C., Segelbacher, G., Ekblom, R., Fischer, M. C., Laikre, L., Leus, K., O'Brien, D., Shaw, R., & Sork, V. (2022). *Selecting species and populations for monitoring of genetic diversity*. IUCN.

Jacobs, A., Carruthers, M., Yurchenko, A., Gordeeva, N. V., Alekseyev, S. S., Hooker, O., Leong, J. S., Minkley, D. R., Rondeau, E. B., Koop, B. F., Adams, C. E., & Elmer, K. R. (2020). Parallelism in eco-morphology and gene expression despite variable evolutionary and genomic backgrounds in a Holarctic fish. *PLoS Genetics*, *16*, e1008658. https://doi.org/10.1371/journal.pgen.1008658

Johannesson, K., & Laikre, L. (2020). Monitoring of genetic diversity in environmental monitoring (in Swedish). Report to the Swedish Agency for Marine and Water Management (dnr. HaV 3642-2018, 3643-2018).

Johannesson, K., & Laikre, L. (2022). Monitoring of genetic diversity in environmental monitoring (in Swedish). Report to the Swedish Agency for Marine and Water Management (dnr. HaV 02213-2020, 02212-2020).

Jorde, P., & Ryman, N. (1996). Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics Society of America*, *143*(3), 1369–1381. https://doi.org/10.1093/genetics/143.3.1369

Jorde, P. E., Andersson, A., Ryman, N., & Laikre, L. (2018). Are we underestimating the occurrence of sympatric populations? *Molecular Ecology*, *27*(20), 4011–4025. https://doi.org/10.1111/mec.14846

Jorde, P. E., & Ryman, N. (1995). Temporal allele frequency change and estimation of effective size in population with overlapping generations. *Genetics*, *139*(2), 1077–1090. https://doi.org/10.1093/genetics/139.2.1077

Jorde, P. E., & Ryman, N. (2007). Unbiased estimator for genetic drift and effective population size. *Genetics*, *177*(2), 927–935. https://doi.org/10.1534/genetics.107.075481

Kardos, M., Taylor, H. R., Ellegren, H., Luikart, G., & Allendorf, F. W. (2016). Genomics advances the study of inbreeding depression in the wild. *Evolutionary Applications*, *9*(10), 1205–1218. https://doi.org/10.1111/eva.12414

Karlsson, E. K., Baranowska, I., Wade, C. M., Salmon Hillbertz, N. H. C., Zody, M. C., Anderson, N., Biagi, T. M., Patterson, N., Pielberg, G. R., Kulbokas, E. J., 3rd, Comstock, K. E., Keller, E. T., Mesirov, J. P., von Euler, H., Kämpe, O., Hedhammar, A., Lander, E. S., Andersson, G., Andersson, L., & Kulbokas, E. J. (2007). Efficient mapping of mendelian traits in dogs through genome-wide association. *Nature Genetics*, *39*(11), 1321–1328. https://doi.org/10.1038/ng.2007.10

Kawecki, T. J. (2008). Adaptation to marginal habitats. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 321–342. https://doi.org/10.1146/annurev.ecolsys.38.091206.095622

Kershaw, F., Bruford, M. W., Funk, W. C., Grueber, C. E., Hoban, S., Hunter, M. E., Laikre, L., MacDonald, A. J., Meek, M. H., Mittan, C., O'Brien, D., Ogden, R., Shaw, R. E., Vernesi, C., & Segelbacher, G. (2022). The Coalition for Conservation Genetics: Working across organizations to build capacity and achieve change in policy and practice. *Conservation Science and Practice*, *2022*, e12635. https://doi.org/10.1111/csp2.12635

Kinziger, A. P., White, J. L., Nakamoto, R. J., & Harvey, B. C. (2021). Recent, small beginnings: Genetic analysis suggests *Catostomus rimiculus* (Klamath smallscale sucker) in the Smith River, California, are introduced. *Journal of Fish Biology*, *98*(5), 1321–1328. https://doi.org/10.1111/jfb.14664

Kjærner-Semb, E., Ayllon, F., Furmanek, T., Wennevik, V., Dahle, G., Niemelä, E., Ozerov, M., Vähä, J. P., Glover, K. A., Rubin, C. J., Wargelius, A., & Edvardsen, R. B. (2016). Atlantic salmon populations reveal adaptive divergence of immune related genes – a duplicated genome under selection. *BMC Genomics*, *17*(1), 610. https://doi.org/10.1186/s12864-016-2867-z

Kofler, R., Orozco-terWengel, P., de Maio, N., Pandey, R. V., Nolte, V., Futschik, A., Kosiol, C., & Schlötterer, C. (2011). Popoolation: A toolbox for population genetic analysis of next generation sequencing data from pooled individuals. *PLoS One*, *6*(1), e15925. https://doi.org/10.1371/journal.pone.0015925

Kofler, R., Pandey, R. V., & Schlötterer, C. (2011). PoPoolation2: Identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics*, *27*(24), 3435–3436. https://doi.org/10.1093/bioinformatics/btr589

Kolde, R., & Kolde, M. R. (2015). Package 'pheatmap'. *R Package*, *1*(7), 790.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547.

Kurland, S., Rafati, N., Ryman, N., & Laikre, L. (2022). Genomic dynamics of brown trout populations released to a novel environment. *Ecology and Evolution*, *12*(7), e9050.

Kurland, S., Ryman, N., Hössjer, O., & Laikre, L. (2023). Effects of subpopulation extinction on effective size (N_e) of metapopulations. *Conservation Genetics*, *24*, 417–433. https://doi.org/10.1007/s10592-023-01510-9

Kurland, S., Wheat, C. W., de la Paz Celorio Mancera, M., Kutschera, V. E., Hill, J., Andersson, A., Rubin, C. J., Andersson, L., Ryman, N., & Laikre, L. (2019). Exploring a Pool-seq-only approach for gaining population genomic insights in nonmodel species. *Ecology and Evolution*, *9*(19), 11448–11463. https://doi.org/10.1002/ece3.5646

Lai, K. P., Tam, N., Wang, S. Y., Lin, X., Chan, T. F., Au, D. W. T., Wu, R. S. S., & Kong, R. Y. C. (2020). Hypoxia causes sex-specific hepatic toxicity at the transcriptome level in marine medaka (*Oryzias melastigma*). *Aquatic Toxicology*, *224*, 105520. https://doi.org/10.1016/j.aquatox.2020.105520

Laikre, L., Allendorf, F. W., Aroner, L. C., Baker, C. S., Gregovich, D. P., Hansen, M. M., Jackson, J. A., Kendall, K. C., McKelvey, K., Neel, M. C., Olivieri, I., Ryman, N., Schwartz, M. K., Short Bull, R., Stetz, J. B., Tallmon, D. A., Taylor, B. L., Vojta, C. D., Waller, D. M., & Waples, R. S. (2010). Neglect of genetic diversity in implementation of the Convention on Biological Diversity. *Conservation Biology*, *24*(1), 86–88.

Laikre, L., Hoban, S., Bruford, M. W., Segelbacher, G., Allendorf, F. W., Gajardo, G., González Rodríguez, A., Hedrick, P. W., Heuertz, M., Hohenlohe, P. A., Jaffé, R., Johannesson, K., Liggins, L., MacDonald, A. J., Orozco-terWengel, P., Reusch, T. B. H., Rodríguez-Correa, H., Russo, I.-R. M., Ryman, N., & Vernesi, C. (2020). Post-2020 goals overlook genetic diversity. *Science*, *367*, 1083–1085. https://doi.org/10.1126/science.abb2748

Laikre, L., Hohenlohe, P. A., Allendorf, F. W., Bertola, L. D., Breed, M. F., Bruford, M. W., Funk, C. W., Gajardo, G., González-Rodríguez, A., Grueber, C. E., Hedrick, P. W., Heuertz, M., Hunter, M., Johannesson, K., Liggins, L., MacDonald, A. J., Mergeay, J., Moharrek, F., O'Brien, D., … Hoban, S. (2021). Author's Reply to Letter to the Editor: Continued improvement to genetic diversity indicator for CBD. *Conservation Genetics*, *22*, 531–532. https://doi.org/10.1007/s10592-021-01359-w

Laikre, L., Olsson, F., Jansson, E., Hössjer, O., & Ryman, N. (2016). Metapopulation effective size and conservation genetic goals for the Fennoscandian wolf (*Canis lupus*) population. *Heredity (Edinb)*, *117*, 279–289. https://doi.org/10.1038/hdy.2016.44

Leggett, R. M., Ramirez-Gonzalez, R. H., Clavijo, B. J., Waite, D., & Davey, R. P. (2013). Sequencing quality assessment tools to enable data-driven informatics for high throughput genomics. *Frontiers in Genetics*, *4*, 288. https://doi.org/10.3389/fgene.2013.00288

Leitwein, M., Gagnaire, P. A., Desmarais, E., Guendouz, S., Rohmer, M., Berrebi, P., & Guinand, B. (2016). Genome-wide nucleotide diversity of hatchery-reared Atlantic and Mediterranean strains of brown trout *Salmo trutta* compared to wild Mediterranean populations. *Journal of Fish Biology*, *89*(6), 2717–2734. https://doi.org/10.1111/jfb.13131

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, *27*(21), 2987–2993.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *ArXiv Preprint ArXiv:1303.3997*.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., Hvidsten, T. R., Leong, J. S., Minkley, D. R., Zimin, A., Grammes, F., Grove, H., Gjuvsland, A., Walenz, B., Hermansen, R. A., von Schalburg, K., Rondeau, E. B., di Genova, A., Samy, J. K. A., … Davidson, W. S. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature*, *533*, 200–205. https://doi.org/10.1038/nature17164

López, M. E., Benestan, L., Moore, J., Perrier, C., Gilbey, J., Di Genova, A., Maass, A., Diaz, D., Lhorente, J. P., Neira, R., Bernatchez, L., Yáñez, J. M., & Correa, K. (2019). Comparing genomic signatures of domestication in two Atlantic salmon (*Salmo salar L.*) populations with different geographical origins. *Evolutionary Applications*, *12*(1), 137–156. https://doi.org/10.1111/eva.12689

Magi, A., Tattini, L., Palombo, F., Benelli, M., Gialluisi, A., Giusti, B., Abbate, R., Seri, M., Gensini, G. F., Romeo, G., & Romeo, G. (2014). H^3 M^2: Detection of runs of homozygosity from whole-exome sequencing data. *Bioinformatics*, *30*(20), 2852–2859. https://doi.org/10.1093/bioinformatics/btu401

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., DePristo, M. A., & Daly, M. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science of the United States of America*, *70*(70), 3321–3323.

Nynca, J., Arnold, G. J., Fröhlich, T., Otte, K., Flenkenthaler, F., & Ciereszko, A. (2014). Proteomic identification of rainbow trout seminal plasma proteins. *Proteomics*, *14*(1), 133–140. https://doi.org/10.1002/pmic.201300267

O'Brien, D., Laikre, L., Hoban, S., Bruford, M. W., Ekblom, R., Fischer, M. C., Hall, J., Hvilsom, C., Hollingsworth, P. M., Kershaw, F., Mittan, C. S., Mukassabi, T. A., Ogden, R., Segelbacher, G., Shaw, R. E., Vernesi, C., & MacDonald, A. J. (2022). Bringing together approaches to reporting on within species genetic diversity. *Journal of Applied Ecology*, *59*(9), 2227–2233. https://doi.org/10.1111/1365-2664.14225

Palkopoulou, E., Mallick, S., Skoglund, P., Enk, J., Rohland, N., Li, H., Omrak, A., Vartanyan, S., Poinar, H., Reich, D., Dalén, L., & Götherström, A. (2015). Complete genomes reveal signatures of demographic and genetic declines in the woolly mammoth. *Current Biology*, *25*(10), 1395–1400. https://doi.org/10.1016/j.cub.2015.04.007

Palm, S., Laikre, L., Jorde, P., & Ryman, N. (2003). Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta*, *L.*). *Conservation Genetics*, *4*(3), 249–264. https://doi.org/10.1023/A:1024064913094

Palmé, A., Laikre, L., & Ryman, N. (2013). Monitoring reveals two genetically distinct brown trout populations remaining in stable sympatry over 20 years in tiny mountain lakes. *Conservation Genetics*, *14*(4), 795–808. https://doi.org/10.1007/s10592-013-0475-x

Pettersson, M. E., Kierczak, M., Almén, M. S., Lamichhaney, S., & Andersson, L. (2017). A model-free approach for detecting genomic regions of deep divergence using the distribution of haplotype distances. *BioRxiv*, 144394. https://doi.org/10.1101/144394

Pickrell, J., & Pritchard, J. (2012). Inference of population splits and mixtures from genome-wide allele frequency data. *Nature Precedings*, *1*, 2560–2575. https://doi.org/10.1038/npre.2012.6956.1

Posledovich, D., Ekblom, R., & Laikre, L. (2021a). Mapping and monitoring genetic diversity in Sweden: Suggestions for pollinating species. Report to the Swedish Environmental Protection Agency, report nr. 6958.

Posledovich, D., Ekblom, R., & Laikre, L. (2021b). Mapping and monitoring genetic diversity in Sweden: A proposal for species, methods, and costs. Report to the Swedish Environmental Protection Agency, report nr. 6959.

Pritchard, V. L., Mäkinen, H., Vähä, J., Erkinaro, J., Orell, P., & Primmer, C. R. (2018). Genomic signatures of fine-scale local selection in Atlantic salmon suggest involvement of sexual maturation, energy homeostasis and immune defence-related genes. *Molecular Ecology*, *27*(11), 2560–2575. https://doi.org/10.1111/mec.14705

Pruisscher, P., Nylin, S., Gotthard, K., & Wheat, C. W. (2018). Genetic variation underlying local adaptation of diapause induction along a cline in a butterfly. *Molecular Ecology*, *27*, 3613–3626. https://doi.org/10.1111/mec.14829

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Robinson, N., Karlsen, C., Ytteborg, E., Krasnov, A., Gerwins, J., Johnsen, H., & Kolarevic, J. (2021). Skin and bone development in Atlantic salmon (*Salmo salar*) influenced by hatchery environment. *Aquaculture*, *544*, 737155. https://doi.org/10.1016/j.aquaculture.2021.737155

Rubin, C.-J., Zody, M. C., Eriksson, J., Meadows, J. R. S., Sherwood, E., Webster, M. T., Jiang, L., Ingman, M., Sharpe, T., Ka, S., Hallböök, F., Besnier, F., Carlborg, Ö., Bed'hom, B., Tixier-Boichard, M., Jensen, P., Siegel, P., Lindblad-Toh, K., & Andersson, L. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*, *464*, 587–591.

Ryman, N., Laikre, L., & Hössjer, O. (2019). Do estimates of contemporary effective population size tell us what we want to know? *Molecular Ecology*, *28*, 1904–1918. https://doi.org/10.1111/mec.15027

Saha, A., Andersson, A., Kurland, S., Keehnen, N. L. P., Kutschera, V. E., Hössjer, O., Ekman, D., Karlsson, S., Kardos, M., Allendorf, F. W., Ryman, N., Laikre, L., & Ståhl, G. (2022). Whole-genome resequencing confirms reproductive isolation between sympatric demes of brown trout (*Salmo trutta*) detected with allozymes. *Molecular Ecology*, *31*(2), 498–511. https://doi.org/10.1111/mec.16252

Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals—mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics*, *15*(11), 749–763. https://doi.org/10.1038/nrg3803

Schwartz, M. K., Luikart, G., & Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution*, *22*(1), 25–33. https://doi.org/10.1016/j.tree.2006.08.009

Shaliutina-Kolešová, A., Kotas, P., Štěrba, J., Rodina, M., Dzyuba, B., Cosson, J., & Linhart, O. (2016). Protein profile of seminal plasma and functionality of spermatozoa during the reproductive season in the common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). *Molecular Reproduction and Development*, *83*(11), 968–982. https://doi.org/10.1002/mrd.22737

Sivka, U., Snoj, A., Palandačić, A., & Bajec, S. S. (2013). Identification of candidate genes involved in marble colour pattern formation in genus Salmo. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *8*(3), 244–249. https://doi.org/10.1016/j.cbd.2013.06.003

Smit, A., & Hubley, R. (2015). RepeatModeler Open-1.0.

Smit, A. F. A., Hubley, R., & Green, P. (2017). 1996–2010. RepeatMasker Open-3.0.

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One*, *6*(7), e21800. https://doi.org/10.1371/journal.pone.0021800

Tajima, F., & Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, *105*(2), 437–460. https://doi.org/10.1093/genetics/105.2.437

Urban, M. C. (2018). Escalator to extinction. *Proceedings of the National Academy of Science of the United States of America*, *115*(47), 11871–11873. https://doi.org/10.1073/pnas.1817416115

Vernesi, C., Bruford, M. W., Bertorelle, G., Pecchioli, E., Rizzoli, A., & Hauffe, H. C. (2008). Where's the conservation in conservation genetics? *Conservation Biology*, *22*(3), 802–804.

Von Seth, J., Dussex, N., Díez-del-Molino, D., Van Der Valk, T., Kutschera, V. E., Kierczak, M., Steiner, C. C., Liu, S., Gilbert, M. T. P., Prost, S., Guschanski, K., Nathan, S. K. S. S., Brace, S., Chan, Y. L., Wheat, C. W., Skoglund, P., Ryder, O. A., Goossens, B., Götherström, A., … Sinding, M.-H. S. (2021). Genomic insights into the conservation status of the world's last remaining Sumatran rhinoceros populations. *Nature Communications*, *12*(1), 1–11. https://doi.org/10.1038/s41467-021-22386-8

Watterson, G. A. (1975). On the number of segregating sites in genetical models without recombination. *Theoretical Popululation Biology*, *7*(2), 256–276. https://doi.org/10.1016/0040-5809(75)90020-9

Wiberg, R. A. W., Gaggiotti, O. E., Morrissey, M. B., & Ritchie, M. G. (2017). Identifying consistent allele frequency differences in studies of stratified populations. *Methods in Ecology and Evolution*, *8*(12), 1899–1909. https://doi.org/10.1111/2041-210X.12810

Willoughby, J. R., Harder, A. M., Tennessen, J. A., Scribner, K. T., & Christie, M. R. (2018). Rapid genetic adaptation to a novel environment despite a genome-wide reduction in genetic diversity. *Molecular Ecology*, *27*(20), 4041–4051. https://doi.org/10.1111/jfb.14664

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kurland, S., Saha, A., Keehnen, N., de la Paz Celorio-Mancera, M., Díez-del-Molino, D., Ryman, N., & Laikre, L. (2024). New indicators for monitoring genetic diversity applied to alpine brown trout populations using whole genome sequence data. *Molecular Ecology*, *33*, e17213. https://doi.org/10.1111/mec.17213