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Do Habitat Restoration Efforts and the Associated Releases of Hatchery-Reared Fish Affect the Genetic Diversity and Integrity of Native Brown Trout Populations?

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ABSTRACT

Habitat restoration may protect brown trout (*Salmo trutta*) populations from loss of genetic diversity by increasing environmental carrying capacity and, consequently, population size. However, the accompanying release of hatchery-reared fish may threaten the genetic integrity of native populations through introgression. We monitored the genetic composition of four native brown trout populations using 16 putatively neutral microsatellite loci in small boreal streams subjected to restoration measures and the release of hatchery-reared brown trout. Sampling was conducted before restoration, during the expected recovery phase, and after recovery. Analysis of population composition over the 19-year study period indicated interbreeding between hatchery and wild fish shortly after the releases, but the most recent samples predominantly represented native, presumably resident genotypes. Restoration treatments had no detectable effects on the genetic diversity of the small study populations. Our results add to the evidence suggesting that stocking with non-native fish is ineffective in enhancing wild populations.

1 | Introduction

Restoration of natural hydrodynamical conditions and salmonid habitats in brooks and rivers, impacted by historical dredging for the transport of timber (Luhta et al. 2012), has become an increasingly popular activity not only by the authorities but by voluntary advocates of aquatic nature and native salmonids such as brown trout *Salmo trutta* and Atlantic salmon *S. salar* (Sarvilinna et al. 2018). Restorations aim at recovering the environmental carrying capacity that was lost due to human activities impacting the hydromorphological quality and associated ecological productivity of the channels (Palmer et al. 2010; Louhi et al. 2011; Marttila et al. 2019). In Finland, restoration efforts have taken place since the late 1970s with the primary aim of increasing brown trout population densities (Vehanen et al. 2010; Louhi et al. 2016). Often, the restoration activities are followed or accompanied by releases of hatchery-reared fish (Luhta et al. 2012), even when original, native, and often resident populations of brown trout with significant conservation value (Araguas et al. 2009) could be present in the restored sites. Whether the stockings have been successful in supporting the recipient populations is often questionable (Marttila et al. 2019; García-Vega et al. 2020), and as such, there is a need to resolve if the stocked fish interbreed with the native fish and how the population genetics parameters of the affected stocks develop during and after restoration and stocking activities (Hansen et al. 2000, 2001, 2006; Gil et al. 2016).

Genetic diversity is essential for the long-term survival of all species (Lande and Shannon 1996; Kardos et al. 2021;

Löytynoja et al. 2023), and an increase in census population size should slow down the loss of genetic variation in small populations (Hoban et al. 2014; Hohenlohe et al. 2021; Bosse and van Loon 2022). Further, heterozygosity can provide a fitness advantage to individuals, and as such, increase population growth rate (Deng and Fu 1998; Stewart et al. 2017). Thus, ecological restoration could improve population's recovery also indirectly through evolutionary mechanisms, that is, through evolutionary rescue due to increased genetic diversity (Williams 2001; O'Brien et al. 2022) or recovery of certain life-history strategies such as migratory behavior (Apgar et al. 2017). While the removal of migration barriers can quickly affect the population composition of salmonids (Wood et al. 2018), the genetic impacts of channel restorations have not been directly studied.

Hatchery fish can differ from wild fish in many significant aspects. While widely used hatchery strains of brown trout can originate from multiple sources and, as such, show relatively high genetic diversity (Berrebi et al. 2021), the genetic diversity of individual hatchery-bred brown trout strains is typically reduced and strongly dependent on the number of fish used to create the broodstock (Aho et al. 2006). Releases of hatchery-reared salmonids have had significant genetic impacts on native populations (McMillan et al. 2023) especially when the released fish have outnumbered the densities of native fish (Hansen and Loeschcke 1994; Hansen 2002). Hatchery-reared fish could decrease overall genetic differentiation and erase local adaptations among populations through genetic homogenization (e.g., Östegren et al. 2021). Hatchery fish can also differ from wild fish in life-history traits such as migration tendency despite being regionally native (Ferguson et al. 2019; Lemopoulos et al. 2019). As such, releases of migratory fish might be used to restore migratory behavior in resident populations that have gained access to historical feeding grounds through removals of migration barriers (Apgar et al. 2017).

Recently, the need for the conservation and restoration of genetic diversity has become more generally acknowledged (Biermann and Havlick 2024), and genetic monitoring of reintroduced and hatchery-supplemented fish populations has become relatively common (e.g., Hansen et al. 2006; Klütsch et al. 2019; Prodöhl et al. 2019; White et al. 2023). Brown trout in particular is a prime model species in conservation genetics studies with significant genetic diversity being present within and among waterbodies, and between life-history types (Skaala and Nævdal 1989; Hansen et al. 2006; Lemopoulos et al. 2018). Between the 1990s and 2000s, the ecological effects of conventional stone restoration (i.e., addition of gravel, cobble and boulders to river channel) and additional wood material restoration were studied using a before-after-control-impact (BACI) design using small, dredged forest streams in North-Eastern Finland (Vehanen et al. 2010; Louhi et al. 2016). Despite of simultaneous supplemental releases of hatchery-bred adfluvial brown trout, the brown trout densities did not acutely increase at the study sites (Vehanen et al. 2010). During the whole 12-year monitoring period, including some years with severe draught and corresponding population declines, densities of one-summer-old brown trout improved at boulder restored sites as did the densities of age-1+ and older brown trout in sites receiving both boulders and large woody debris in comparison to the control sites (Louhi et al. 2016). However, the role of the stocked fish in this increase was not resolved.

In this study, our aim was to examine if small wild resident brown trout populations maintain their genetic integrity better in restored sections of small rivers compared to dredged, unrestored control sites, as based on the experimental design of Vehanen et al. (2010) and Louhi et al. (2016). We also studied if stocked fish contributed to the genetic composition of the receiving wild populations through introgression. We predicted that restorations would locally increase effective population size (N_e) of brown trout compared to non-restored control sites. We further predicted that stockings would increase genetic variation in the receiving populations through natural reproduction and interbreeding with the native fish. Additionally, we anticipated that stocking would not only enhance genetic diversity of local populations but also decrease the genetic divergence among the wild populations due to genetic homogenization. Moreover, we hypothesized that restoration efforts could potentially reduce inbreeding in these populations through increased census population sizes.

2 | Methods

2.1 | Study Area, Study Design, and Sampling of Fish

The Natural Resources Institute Finland (Luke) and the Stream Ecology Research Group of the University of Oulu have conducted a long-term study since 1998 to assess the impact of stream restoration and hatchery-reared brown trout supplementation on native brown trout populations (Vehanen et al. 2010; Louhi et al. 2016). The restoration program involved six boreal Fennoscandian second-order headwater streams representing a larger population of forest streams in the River Oulujoki basin (64° N, 28° E); however, only four historically connected streams within the River Kiehimänjoki basin (Figure 1), also having the best sample coverage, were included in this study. Currently, fish can move freely between the streams Siltapuro and Heikkisenjoki, but all study populations have lost access to their historical migration route to Lake Oulujärvi (928.1 km^2), the principal feeding area for adfluvial brown trout in the region (Figure 1). The streams have mean annual flows, widths, and gradients that vary between 0.29 to 0.70 m^3s^{-1}, 3 to 5.5 m, and 0.7% to 1.2%, respectively. The streams were dredged in the 1950s and 1960s, resulting in the loss of some of their structural complexity. The study sites were selected to cover three swift-flowing sections, ranging from 300 to 1100 m in length, separated by at least 300 m long deep, slow-flowing sections within each of the streams. The length of each section was determined by the natural variability of stream morphology, and a 350 m^2 reach was chosen for fish monitoring within each of the selected sections.

In late summer 2001, one randomly selected section (out of three) in each stream was restored to its total length using stones (cobbles and boulders), another one using stones and large wood (LW), while the third section was left unmodified as a control (Vehanen et al. 2010). The restoration measures followed typical Finnish stream restoration protocols (see also Turunen

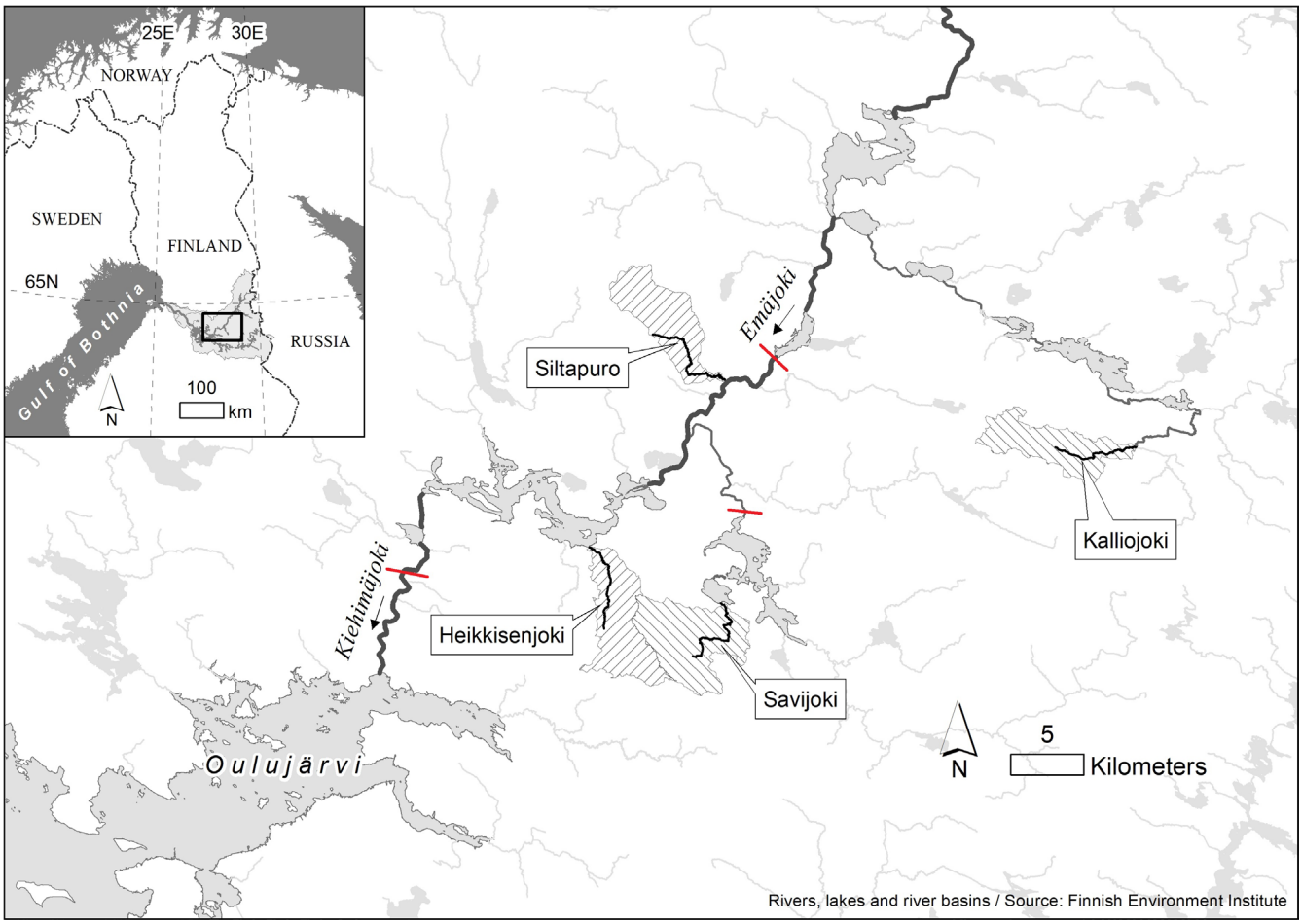

FIGURE 1 | Map of the study area showing the locations of the study streams within the River Kiehimäjoki basin in Finland. Impassable hydropower dams are indicated with red lines.

et al. 2017) with the exception that the use of LW was uncommon in Finland until the 2010s (Syrjänen et al. 2017). The applied measures are known to be highly effective in enhancing physical streambed complexity (Muotka and Syrjänen 2007; Marttila et al. 2016) and restoring natural riparian plant assemblages (Turunen et al. 2017). The restoration design followed a randomized-block design where each treatment was applied to one experimental unit (section) in each block (stream), in a randomized order. Since the expected effect of restoration on population genetics was relatively low, stone-restoration and stone- and wood-restoration were combined as a general restoration treatment in this study.

Between 1998 and 2007, all study sections were supplemented with age-0 hatchery-reared adfluvial brown trout originating from a local hatchery brood stock used to produce fish for releases within the River Oulujoki basin (Lemopoulos et al. 2019) at a density of 0.35 fish m^{-2} every September. Before the release, the adipose fin of the hatchery-reared brown trout was clipped to distinguish the origins of the fish. The density employed in the supplementations was well within the range of densities of age-0 brown trout in Finnish streams (Korsu et al. 2007).

Fish population monitoring began in 1999 in each study section and has since then continued annually (Louhi et al. 2016). Three-pass electrofishing surveys were conducted, and the catch by species was counted and weighted for total mass (0.1 g). Brown trout was the key target species of the study, and each caught brown trout was checked for the condition of the adipose fin to separate stocked and wild fish, measured for total length (1 mm) and mass (0.1 g), and a scale sample was taken and stored in a paper envelope for later determination of age and extraction of DNA. All fish were released back to their capture site in the stream after processing. The average length of the sampled fish was 145.1 ± 39.1 mm (±SD), the average body mass was 38.3 ± 35.1 g and the average age was 1.5 ± 0.7 years.

The sample selection in this study followed the original study design (Figure 2) but was also constrained by the availability of original scale samples. Since brown trout is known to live for at least 10 years and smoltify at ages from 2 to 4 (Syrjänen et al. 2017; Vainikka et al. 2023), we assumed that random selection of samples for genotyping among all available samples and pooling of cohorts would not cause significant biases to the analyses yet estimates of effective population size could show some downward bias (Waples et al. 2014). First, samples of wild brown trout ($N = 120$) from the time before restorations and any potential genetic impacts from stockings, called "background" (Figure 2), were selected to represent the background situation (period I). Stocked fish of Lake Oulujärvi ("OUV") origin (Lemopoulos et al. 2019) were observed in electrofishing between 1999 and 2011, but all genotyped samples represented

FIGURE 2 | A graphical illustration of the experimental design used in this study.

released survivors, that is, fish from no-stocking years 2008 and 2009 ($N = 94$). Sample numbers during the final time of potential interbreeding between released and wild fish (2008–2011) were very small especially in Heikkisenjoki and Siltapuro (average $N = 7.5$ per year). Thus, we used the samples from years 2014 to 2015 ($N = 120$) to represent period II, the "recovery phase" (Figure 2), and the most recent samples from 2016 to 2018 ($N = 240$) to capture the "contemporary phase", period III (Figure 2). Given earliest maturation at age 4 and longevity of 10 years, both the recovery period and contemporary period samples could have contained 1st–4th generation crosses between the native and hatchery fish. As such, these two periods could also be considered as replicates of the situation after restorations and stockings. The samples grouped by period (I-III), treatment (restored vs. control), and stream (Heikkisenjoki (Heik), Kalliojoki (Kall), Savijoki (Savi), Siltapuro (Silt)) and hatchery fish (OUV), formed 26 population units ($N = 5$–64). To overcome the issue of very small sample sizes and to better assess genetic impacts of stocking, the habitat restoration treatments were pooled for alternative analyses, yielding 13 gene pools of interest ($N = 23$–92 individuals in each).

DNA was extracted from the scale samples (total $N = 574$) and analyzed for allelic variation at 16 microsatellite loci using methods developed for brown trout (Koljonen et al. 2014; Koskiniemi et al. 2020; Tanhuanpää et al. 2021). Isolation of DNA and genotyping of the samples was done at the Jokioinen laboratory of the Natural Resources Institute Finland in June–August 2019. Qiagen DNEasy Blood & Tissue kits were used for DNA isolation and Qiagen Type-it Microsatellite PCR kits were used for PCR runs, both following manufacturer's manuals. The products of the PCR runs were separated by capillary electrophoresis using the ABI 3500XL Genetic Analyzer (Applied Biosystems, USA), and the gel images were analyzed with GeneMapper software (Applied Biosystems, USA). A total of 524 genotyped unique samples were obtained for further analysis after omission of 30 redundant duplicate samples of same individuals (fish were not tagged and identifiable otherwise) and 20 samples that showed mixed DNA from several individuals as a result of carry-over contamination during sampling.

2.2 | Within Population Genetic Diversity

The basic population genetic analyses were performed in R 4.6.0 (The R foundation for Statistical computing, 2026) using packages *adegenet* (Chessel et al. 2004; Jombart and Ahmed 2011), *hierfstat* (Goudet 2005), *dplyr*, *pegas* (Paradis 2010), and *poppr* (Kamvar et al. 2014). Both expected (H_e) and observed (H_o) heterozygosities, total and private allele counts, allelic richness, and the inbreeding coefficient (F_{IS}) were calculated for all population units and population units pooled over treatments using the commands *basic.stats* and *ar_global*, and manual tabulation of the allele frequencies. The 95% confidence intervals were estimated by bootstrapping the analysis over individuals ($N = 1000$) and picking the 2.5% and 97.5% percentiles of the bootstrapped distributions. The effective population sizes (N_e) were estimated to approximate the development of breeding population sizes. Here, we used the linkage disequilibrium (LD) estimator as implemented in NeEstimator v.2 (Do et al. 2014) with the lowest accepted allele frequency of 0.020 and non-parametric jackknifed confidence intervals. Wilcoxon signed rank-test (Pratt 1959) was used to test if the recovery and contemporary values differed between the restoration and control treatments over the population units, and whether the F_{ST} values between hatchery and wild populations differed between background and contemporary time periods.

2.3 | Population Structure

A principal component analysis (PCA) was conducted using *ade4* and *adegenet* packages in R to visualize the grouping of the 26 population units using *ggplot2* (Wickham 2016). To investigate genetic differentiation within and among populations, we estimated pairwise F_{ST} values using the Weir and Cockerham (1984) method implemented in the *hierfstat* and *poppr* packages. The confidence intervals for the F_{ST} values were estimated by bootstrapping ($N = 1000$). Bayesian algorithms implemented in Structure (v.2.3.4) software (Pritchard et al. 2000) were applied to examine population genetic structure of fish populations in the four streams over time in relation to the hatchery population. We performed 20 replicate runs with 500,000 burn-in iterations and 1 000,000 Markov Chain Monte Carlo (MCMC) repetitions for each value of K ranging from 1 to 10, using the LOCPRIOR model with estimated lambda. Evanno's delta K (Evanno et al. 2005) was used to determine the statistically most likely number of genetic clusters ($K = 5$ was strongly supported, Figure S1). The replicate run results were inspected visually (Figure S2) and merged using the *alignK* function in the R package *pophelper* (Francis 2017) to account for label switching among runs, and a consensus ancestry matrix (Q-matrix) was generated by averaging across aligned runs using *mergeQ*. The final plot was produced using *ggplot2* (Wickham 2016).

TABLE 1 | Population units pooled over the treatments with their relative sample sizes (N), observed and expected heterozygosities (H_o and H_e, respectively), inbreeding coefficient (F_{IS}), total private allele count (N_p), and mean allele richness (A_R) and effective population size (N_e), and their respective bootstrapped ($N = 1000$) 95% confidence intervals (CI) across the three study periods.

Pop.	Period	Years	Code	N	H_o (95% CI)	H_e (95% CI)	F_{IS} (95% CI)	N_p	A_R	N_e (95% CI)
OUV	Hatchery	2008–2009	OUV	92	0.67 (0.65 to 0.70)	0.69 (0.67 to 0.70)	0.03 (−0.02 to 0.05)	13	6.60	58.4 (40.9 to 89.5)
Heik	Background	1999–2000	Heik I	26	0.64 (0.59 to 0.69)	0.68 (0.63 to 0.70)	0.08 (−0.03 to 0.14)	5	6.36	9.5 (3.2 to 23.9)
Heik	Recovery	2014–2015	Heik II	27	0.67 (0.60 to 0.73)	0.65 (0.61 to 0.67)	−0.03 (−0.14 to 0.06)	3	4.91	10.3 (7.0 to 15.2)
Heik	Contemporary	2016–2018	Heik III	55	0.61 (0.57 to 0.65)	0.63 (0.59 to 0.65)	0.03 (−0.04 to 0.08)	0	4.68	3.4 (2.9 to 3.9)
Kall	Background	1999–2000	Kall I	26	0.66 (0.62 to 0.70)	0.58 (0.54 to 0.61)	−0.11 (−0.23 to −0.05)	4	4.33	11.7 (7.0 to 20.5)
Kall	Recovery	2014–2015	Kall II	26	0.63 (0.57 to 0.69)	0.59 (0.54 to 0.62)	−0.07 (−0.15 to −0.03)	0	4.85	11.3 (7.1 to 18.5)
Kall	Contemporary	2016–2018	Kall III	51	0.55 (0.52 to 0.58)	0.55 (0.52 to 0.56)	0.00 (−0.07 to 0.06)	3	3.97	5.8 (3.7 to 8.1)
Savi	Background	1999–2000	Savi I	29	0.59 (0.53 to 0.64)	0.57 (0.53 to 0.60)	−0.03 (−0.12 to 0.03)	1	4.39	47.3 (19.7 to 73.4)
Savi	Recovery	2014–2015	Savi II	28	0.55 (0.51 to 0.59)	0.57 (0.54 to 0.58)	0.03 (−0.05 to 0.07)	2	4.30	82.4 (24.9 to 73.9)
Savi	Contemporary	2016–2018	Savi III	57	0.61 (0.58 to 0.64)	0.59 (0.57 to 0.61)	−0.03 (−0.08 to 0.00)	1	4.41	37.6 (21.1 to 81.1)
Silt	Background	1999–2000	Silt I	23	0.61 (0.55 to 0.66)	0.66 (0.61 to 0.67)	0.08 (−0.01 to 0.13)	0	5.81	33.6 (16.3 to 158.5)
Silt	Recovery	2014–2015	Silt II	27	0.68 (0.65 to 0.72)	0.65 (0.60 to 0.66)	−0.04 (−0.14 to 0.01)	1	5.54	17.5 (8.2 to 52.2)
Silt	Contemporary	2016–2018	Silt III	57	0.64 (0.61 to 0.67)	0.63 (0.61 to 0.65)	0.00 (−0.06 to 0.04)	0	5.33	53.4 (28.8 to 140.5)

3 | Results

3.1 | Within Population Genetic Diversity

The estimated values of expected and observed heterozygosity were generally moderate to relatively high and well balanced (Tables 1 and 2). The average observed heterozygosity was 0.63 for control and 0.59 for restoration treatment (Wilcoxon signed rank test, $Z = 1.41$, $N = 8$, $p = 0.079$) during recovery and contemporary periods. The average expected heterozygosity was 0.60 for control and 0.60 for restoration treatment (Wilcoxon signed rank test, $Z = 0.140$, $N = 8$, $p = 0.444$) during recovery and contemporary periods. The corresponding F_{IS} values, suggesting a lack of any significant inbreeding or introgression from hatchery fish, were −0.05 and −0.03 ($Z = 0.774$, $N = 8$, $p = 0.219$), A_r values were 3.27 and 3.23 ($Z = 0.210$, $N = 8$, $p = 0.417$) and N_e estimates were 15.11 and 23.46 ($Z = 1.268$, $N = 7$, $p = 0.102$). The estimated effective population sizes were very small and smaller than in the hatchery strain (Tables 1 and 2). The restored sections seemed to support more hatchery fish than the control sections (Table 2). The striking background values for control site fish in Kalliojoki (Table 2) could result from a low sample size combined with a strong immigrant effect. None of the populations showed a statistically significant positive response in effective population size to restoration treatment (Table 2). In the end of the study, Siltapuro and Savijoki supported larger effective populations than the other two streams (Table 1).

3.2 | Population Structure

The sampled wild individuals clustered into four population groups, while the used hatchery population grouped in the middle with significant overlap with Heikkisenjoki and Siltapuro populations (Figure 3). The only visually observable effect was the migration of the Heikkisenjoki population further away from the center over time (Figure 3). Accordingly, F_{ST} estimates indicated that all the study populations were distinct from each other and the hatchery strain (Table 3; Table S1). Aligning with the clustering (Figure 3), the Heikkisenjoki population showed statistically significant F_{ST} estimates across time periods,

TABLE 2 | Comparison of control (C) and restoration (R) treatment (Tre.) site samples by the population (Pop.) and time period (Per: background B, recovery R and contemporary C).

Pop.	Per.	Tre.	Acr.	*N*	H_o (95% CI)	H_e (95% CI)	F_{Is} (95% CI)	N_p	A_R	N_e (95% CI)
OUV		C	ORC	28	0.69 (0.64 to 0.73)	0.69 (0.65 to 0.70)	−0.00 (−0.07 to 0.04)	3	4.16	36.1 (17.8 to 149.4)
OUV		R	ORR	64	0.66 (0.63 to 0.69)	0.69 (0.66 to 0.69)	0.04 (−0.02 to 0.08)	4	4.09	61.5 (44.3 to 92.4)
Heik	B	C	HBC	14	0.65 (0.57 to 0.72)	0.65 (0.56 to 0.67)	0.03 (−0.11 to 0.09)	1	3.73	5.1 (1.6 to 40.5)
Heik	B	R	HBR	12	0.62 (0.55 to 0.70)	0.68 (0.57 to 0.71)	0.10 (−0.10 to 0.19)	4	3.90	4.1 (2.1 to 12.7)
Heik	R	C	HRC	22	0.71 (0.65 to 0.77)	0.64 (0.59 to 0.66)	−0.11 (−0.22 to −0.05)	3	3.29	8.5 (6 to 11.8)
Heik	R	R	HRR	5	0.49 (0.37 to 0.56)	0.68 (0.42 to 0.68)	0.26 (−0.08 to 0.27)	0	3.94	inf. (14.9 to inf.)
Heik	C	C	HCC	21	0.70 (0.63 to 0.76)	0.62 (0.56 to 0.64)	−0.13 (−0.24 to −0.07)	0	3.11	6.4 (3.2 to 10.2)
Heik	C	R	HCR	34	0.56 (0.51 to 0.62)	0.53 (0.48 to 0.55)	−0.04 (−0.14 to 0.03)	0	2.82	3.3 (2.5 to 5.7)
Kall	B	C	KBC	8	0.70 (0.63 to 0.77)	0.54 (0.47 to 0.56)	−0.28 (−0.45 to −0.24)	0	2.80	inf. (28.7 to inf.)
Kall	B	R	KBR	18	0.65 (0.59 to 0.69)	0.58 (0.52 to 0.62)	−0.09 (−0.23 to −0.03)	1	3.27	5.1 (2.2 to 13.7)
Kall	R	Cl	KRC	8	0.56 (0.47 to 0.66)	0.55 (0.43 to 0.57)	−0.03 (−0.24 to 0.01)	0	2.95	15.1 (2.7 to inf.)
Kall	R	R	KRR	18	0.66 (0.58 to 0.74)	0.61 (0.54 to 0.63)	−0.10 (−0.21 to −0.04)	0	3.42	10.9 (6.5 to 19.6)
Kall	C	C	KCC	18	0.55 (0.50 to 0.59)	0.51 (0.44 to 0.54)	−0.08 (−0.22 to 0.01)	0	2.76	2.6 (1.6 to 6.4)
Kall	C	R	KCR	33	0.55 (0.51 to 0.59)	0.55 (0.52 to 0.57)	0.01 (−0.08 to 0.08)	3	2.96	5.9 (3.5 to 9.5)
Savi	B	C	VBC	10	0.58 (0.51 to 0.64)	0.52 (0.45 to 0.54)	−0.10 (−0.24 to −0.06)	0	2.90	12.4 (1.9 to inf.)
Savi	B	R	VBR	19	0.59 (0.52 to 0.66)	0.59 (0.53 to 0.62)	−0.01 (−0.14 to 0.06)	1	3.24	7 (1.8 to 60)
Savi	R	C	VRC	10	0.56 (0.49 to 0.63)	0.56 (0.47 to 0.58)	0.01 (−0.15 to 0.05)	0	3.08	12.8 (4.5 to 112.3)
Savi	R	R	VRR	18	0.55 (0.49 to 0.60)	0.57 (0.53 to 0.58)	0.03 (−0.08 to 0.09)	2	3.17	37.5 (17.2 to 393.8)
Savi	C	C	VCC	20	0.63 (0.57 to 0.69)	0.58 (0.53 to 0.59)	−0.10 (−0.22 to −0.05)	0	3.12	24.7 (14.5 to 54.3)
Savi	C	R	VCR	37	0.60 (0.57 to 0.63)	0.60 (0.57 to 0.61)	−0.01 (−0.07 to 0.03)	1	3.25	25.2 (12.4 to 69.2)
Silt	B	C	SBC	8	0.70 (0.60 to 0.77)	0.68 (0.57 to 0.69)	−0.03 (−0.25 to 0.01)	0	3.86	inf. (14.4 to inf.)
Silt	B	R	SBR	15	0.56 (0.50 to 0.62)	0.63 (0.56 to 0.65)	0.13 (0.01 to 0.18)	0	3.69	39.2 (10.8 to inf.)

(Continues)

TABLE 2 | (Continued)

Pop.	Per.	Tre.	Acr.	N	H_o (95% CI)	H_e (95% CI)	F_{Is} (95% CI)	N_p	A_R	N_e (95% CI)
Silt	R	C	SRC	8	0.70 (0.66 to 0.76)	0.68 (0.57 to 0.69)	−0.01 (−0.23 to 0.03)	0	3.92	9.8 (2.9 to 113.4)
Silt	R	R	SRR	19	0.67 (0.63 to 0.72)	0.61 (0.56 to 0.62)	−0.12 (−0.21 to −0.09)	1	3.39	28.6 (14.4 to 110.5)
Silt	C	C	SCC	12	0.66 (0.61 to 0.71)	0.67 (0.58 to 0.69)	0.03 (−0.12 to 0.06)	0	3.95	34.4 (14.2 to inf.)
Silt	C	R	SCR	45	0.63 (0.60 to 0.67)	0.62 (0.60 to 0.63)	−0.02 (−0.09 to 0.01)	0	3.59	52.8 (31 to 118.2)

Note: This table shows the sample sizes (N), observed and expected heterozygosity (H_o and H_e, respectively), inbreeding coefficient (F_{IS}), total private allele count (N_p), and mean allele richness (A_R) and effective population size (N_e), and their respective 95% confidence intervals (CI).

suggesting some unexplained temporal changes in population structure. Similarly, a statistically significant change occurred in Kalliojoki stream between the background and the recovery periods (Table 3), while the other populations remained temporally stable (Table 3). The wild populations showed no homogenization with the hatchery strain as the average F_{ST} values between the hatchery and wild population units increased marginally from the background value 0.095 ± 0.025 (±SD) to 0.114 ± 0.014 in the most recent period (Wilcoxon signed rank test, $Z = 1.657$, $N = 8$, $p = 0.049$).

Fish from control and restored sites in Heikkisenjoki showed significant genetic divergence during all time periods (Table 4; Table S2) indicating strong within-stream structuring. The generally more stable Siltapuro and Savijoki populations showed some statistically significant but biologically irrelevant differentiation values across time periods and restoration treatments (Table 4; Table S2).

Structure analysis (Figure 4) produced five genetic clusters, four of which corresponded to the wild populations and the fifth to the OUV strain used in stockings. The OUV strain formed a clear background for the analysis of potential admixture over time (Figure 4). There were signs of interbreeding between hatchery and wild fish in all streams, and the Heikkisenjoki population had likely hatchery fish already before the experimental stockings (Figure 4). None of the contemporary samples were assigned to have full hatchery origin, but particularly Kalliojoki and Siltapuro had a few individuals that were likely second or third generation crosses with the hatchery fish (Figure 4). Some cross-assignments among Heikkisenjoki, Siltapuro, and hatchery fish likely reflect their close genetic relatedness instead of true physical movements or hybridization (Figures 3 and 4).

4 | Discussion

Our study of small stocking-supplemented resident brown trout populations in experimentally restored forest streams revealed signs of some introgression soon after the stockings but suggested that the introduced fish did not significantly impact the genetic structure of the native populations over multiple generations. Since the ecological effects of the restoration measures on the brown trout populations were rather mild (Vehanen et al. 2010; Louhi et al. 2016), it was not a surprise that the restorations did not have significant impacts on the genetic diversity of the very small local populations. These results are generally in line with former results on the genetic impacts of brown trout stockings (Hansen and Loeschcke 1994; Caudron et al. 2011, Linløkken et al. 2021; McMillan et al. 2023) but also point to unaccounted life-history differences between the native and stocked fish at the time. Brown trout migration decisions were long assumed to be environmentally driven (e.g., Olsson et al. 2006), but more recent research has demonstrated a strong intrinsic and genetic contribution (Ferguson et al. 2019; Vainikka et al. 2023; Mottola et al. 2026) suggesting that in this study, the introduced, genetically migratory fish likely simply left the systems without never returning and spawning.

When considering the genetic divergence among populations, both geographically and over time, our results generally align with the commonly observed pattern of significantly diverged resident brown trout populations even in geographically very close rivers (Bouza et al. 1999; Swatdipong et al. 2010; Lemopoulos et al. 2018, 2019). The measures of heterozygosity, allelic richness, and effective population sizes were at par with three other streams in the same River Oulujoki system (Lemopoulos et al. 2019) as well as with the brown trout populations inhabiting rivers and streams discharging to Lake Inarijärvi (Swatdipong et al. 2010). The Heikkisenjoki and Siltapuro streams, being tributaries of the main stem River Kiehimänjoki, have had a historical connection to the Lake Oulujärvi before the construction of the Leppikoski hydropower dam closing the River Kiehimänjoki in 1963 (Figure 1). Reflecting the habitat connectivity and potential common ancestry of the contemporary populations, these two populations showed close relatedness to the hatchery population particularly in the PCA-figure, yet the axes explained relatively little of the total variance. All the study populations showed statistically significant divergence both in F_{ST} statistics and STRUCTURE analysis. The observed divergence among populations was stable over time in the two streams with the largest population size, but the extremely small populations of Heikkisenjoki and Kalliojoki streams showed divergence both over time and between the treatment sections, which likely reflected strong genetic drift and demographic stochasticity. Generally, the results imply that these two populations are likely not able to persist over time unless recovering quickly in population size (Serbezov et al. 2012; Prunier et al. 2023). The two other populations barely met the

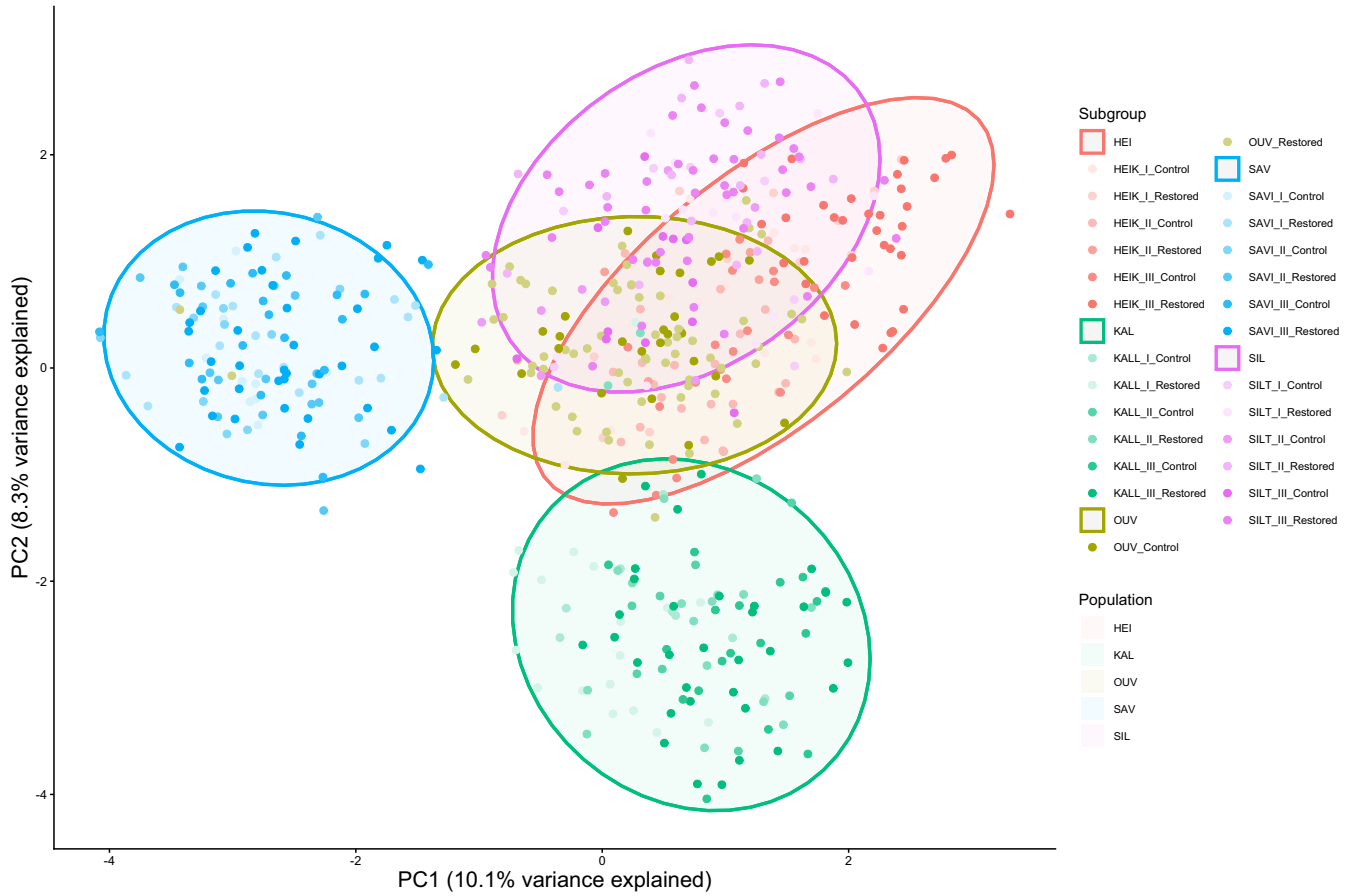

FIGURE 3 | Genetic variation of individuals in 26 population units, as plotted on the two principal component analysis axes using data from 16 microsatellite markers. Each point on the biplot corresponds to an individual, with proximity between points reflecting genetic similarity.

TABLE 3 | Pairwise genetic differentiation (F_{ST} values) among the four color-coded wild brown trout populations (Heikkisenjoki, Kalliojoki, Savijoki, and Siltapuro), across three time periods in comparison to the hatchery strain (OUV) sampled in the streams 2008–2009.

	Silt III	Silt II	Silt I	Savi III	Savi II	Savi I	Kall III	Kall II	Kall I	Heik III	Heik II	Heik I
OUV	**0.10**	**0.09**	**0.08**	**0.12**	**0.13**	**0.11**	**0.13**	**0.11**	**0.12**	**0.11**	**0.09**	**0.07**
Heik I	**0.11**	**0.11**	**0.09**	**0.17**	**0.18**	**0.16**	**0.16**	**0.15**	**0.16**	**0.07**	**0.09**	
Heik II	**0.16**	**0.14**	**0.13**	**0.19**	**0.19**	**0.18**	**0.18**	**0.17**	**0.17**	**0.08**		
Heik III	**0.16**	**0.15**	**0.12**	**0.22**	**0.23**	**0.22**	**0.20**	**0.19**	**0.20**			
Kall I	**0.19**	**0.19**	**0.17**	**0.18**	**0.19**	**0.19**	**0.07**	**0.06**				
Kall II	**0.16**	**0.16**	**0.15**	**0.20**	**0.21**	**0.20**	0.01					
Kall III	**0.19**	**0.19**	**0.18**	**0.23**	**0.24**	**0.23**						
Savi I	**0.18**	**0.17**	**0.17**	0.01	0.00							
Savi II	**0.19**	**0.18**	**0.18**	0.00								
Savi III	**0.17**	**0.16**	**0.16**									
Silt I	0.01	0.01										
Silt II	0.01											

Note: The time periods are represented by I (1999–2000), II (2014–2015), and III (2016–2018). Statistically significant F_{ST} values with confidence intervals entirely above zero are bolded. Color shading is used to help interpreting the values: The greener the cell, the higher the F_{ST} value.

TABLE 4 | Pairwise genetic differentiation (F_{ST} values) among the 26 population units.

	SCR	SCC	SRR	SRC	SBR	SBC	VCR	VCC	VRR	VRC	VBR	VBC	KCR	KCC	KRR	KRC	KBR	KBC	HCR	HCC	HRR	HRC	HBR	HBC	ORR
ORC	**0.10**	**0.07**	**0.12**	**0.05**	**0.10**	**0.06**	**0.12**	**0.14**	**0.14**	**0.14**	**0.10**	**0.14**	**0.14**	**0.15**	**0.10**	**0.14**	**0.13**	**0.14**	**0.17**	**0.13**	**0.05**	**0.10**	**0.08**	**0.08**	0.00
ORR	**0.11**	**0.07**	**0.12**	**0.04**	**0.10**	**0.06**	**0.12**	**0.12**	**0.13**	**0.13**	**0.09**	**0.13**	**0.13**	**0.14**	**0.10**	**0.14**	**0.12**	**0.13**	**0.17**	**0.13**	**0.04**	**0.10**	**0.08**	**0.08**	
HBC	**0.13**	**0.10**	**0.15**	**0.07**	**0.12**	**0.08**	**0.18**	**0.17**	**0.18**	**0.20**	**0.16**	**0.19**	**0.17**	**0.18**	**0.14**	**0.17**	**0.18**	**0.16**	**0.15**	**0.13**	**0.06**	**0.11**	**0.04**		
HBR	**0.13**	**0.09**	**0.16**	**0.09**	**0.10**	**0.07**	**0.19**	**0.19**	**0.19**	**0.21**	**0.17**	**0.20**	**0.19**	**0.21**	**0.15**	**0.20**	**0.18**	**0.16**	**0.11**	**0.11**	**0.07**	**0.12**			
HRC	**0.19**	**0.14**	**0.19**	**0.14**	**0.15**	**0.15**	**0.20**	**0.21**	**0.20**	**0.21**	**0.19**	**0.20**	**0.18**	**0.20**	**0.17**	**0.21**	**0.17**	**0.19**	**0.20**	**0.03**	**0.03**				
HRR	**0.13**	**0.09**	**0.14**	**0.07**	**0.10**	**0.08**	**0.15**	**0.17**	**0.16**	**0.17**	**0.14**	**0.18**	**0.18**	**0.20**	**0.15**	**0.20**	**0.17**	**0.20**	**0.13**	**0.10**					
HCC	**0.21**	**0.16**	**0.22**	**0.15**	**0.18**	**0.16**	**0.22**	**0.23**	**0.22**	**0.23**	**0.21**	**0.24**	**0.20**	**0.23**	**0.19**	**0.24**	**0.20**	**0.21**	**0.19**						
HCR	**0.21**	**0.19**	**0.23**	**0.18**	**0.19**	**0.16**	**0.29**	**0.30**	**0.30**	**0.32**	**0.28**	**0.32**	**0.27**	**0.29**	**0.24**	**0.30**	**0.29**	**0.28**							
KBC	**0.21**	**0.13**	**0.24**	**0.16**	**0.19**	**0.17**	**0.20**	**0.22**	**0.21**	**0.23**	**0.21**	**0.24**	**0.08**	**0.11**	**0.07**	**0.13**	0.03								
KBR	**0.21**	**0.14**	**0.23**	**0.15**	**0.18**	**0.18**	**0.17**	**0.20**	**0.18**	**0.19**	**0.18**	**0.21**	**0.07**	**0.09**	**0.05**	**0.08**									
KRC	**0.18**	**0.14**	**0.21**	**0.14**	**0.18**	**0.18**	**0.22**	**0.25**	**0.24**	**0.26**	**0.22**	**0.29**	**0.04**	0.01	0.01										
KRR	**0.16**	**0.11**	**0.18**	**0.12**	**0.15**	**0.13**	**0.18**	**0.21**	**0.19**	**0.21**	**0.17**	**0.23**	0.02	0.01											
KCC	**0.21**	**0.17**	**0.24**	**0.18**	**0.21**	**0.19**	**0.24**	**0.27**	**0.26**	**0.28**	**0.24**	**0.30**	**0.04**												
KCR	**0.21**	**0.15**	**0.23**	**0.15**	**0.19**	**0.18**	**0.22**	**0.24**	**0.23**	**0.25**	**0.22**	**0.27**													
VBC	**0.23**	**0.19**	**0.26**	**0.16**	**0.24**	**0.21**	**0.04**	**0.03**	0.01	0.00	0.02														
VBR	**0.17**	**0.12**	**0.19**	**0.10**	**0.17**	**0.14**	0.00	0.01	0.00	0.00															
VRC	**0.21**	**0.15**	**0.23**	**0.13**	**0.20**	**0.18**	0.01	0.01	0.01																
VRR	**0.19**	**0.14**	**0.21**	**0.12**	**0.20**	**0.16**	0.01	0.00																	
VCC	**0.19**	**0.15**	**0.21**	**0.11**	**0.20**	**0.16**	0.02																		
VCR	**0.18**	**0.12**	**0.19**	**0.12**	**0.18**	**0.14**																			
SBC	0.02	0.01	**0.04**	0.03	0.02																				
SBR	**0.02**	0.00	0.02	**0.05**																					
SRC	**0.05**	0.01	**0.06**																						
SRR	**0.02**	**0.03**																							
SCC	0.01																								

Note: The three-letter acronyms reflect (1) Stream, (2) Time period and (3) Treatment: The color-coded streams are Heikkisenjoki (H), Kalliojoki (K), Savijoki (V), Siltapuro (S) and Oulujärvi hatchery (O), the time periods are represented by B (as background 1999–2000), R (as recovery 2014–2015) and C (as contemporary 2016–2018) and the treatments are Control (C) and Restoration (R). Statistically significant F_{ST} values with confidence intervals entirely above zero are bolded. The respective 95% confidence intervals are presented in Table S2. Color shading is as in Table 3.

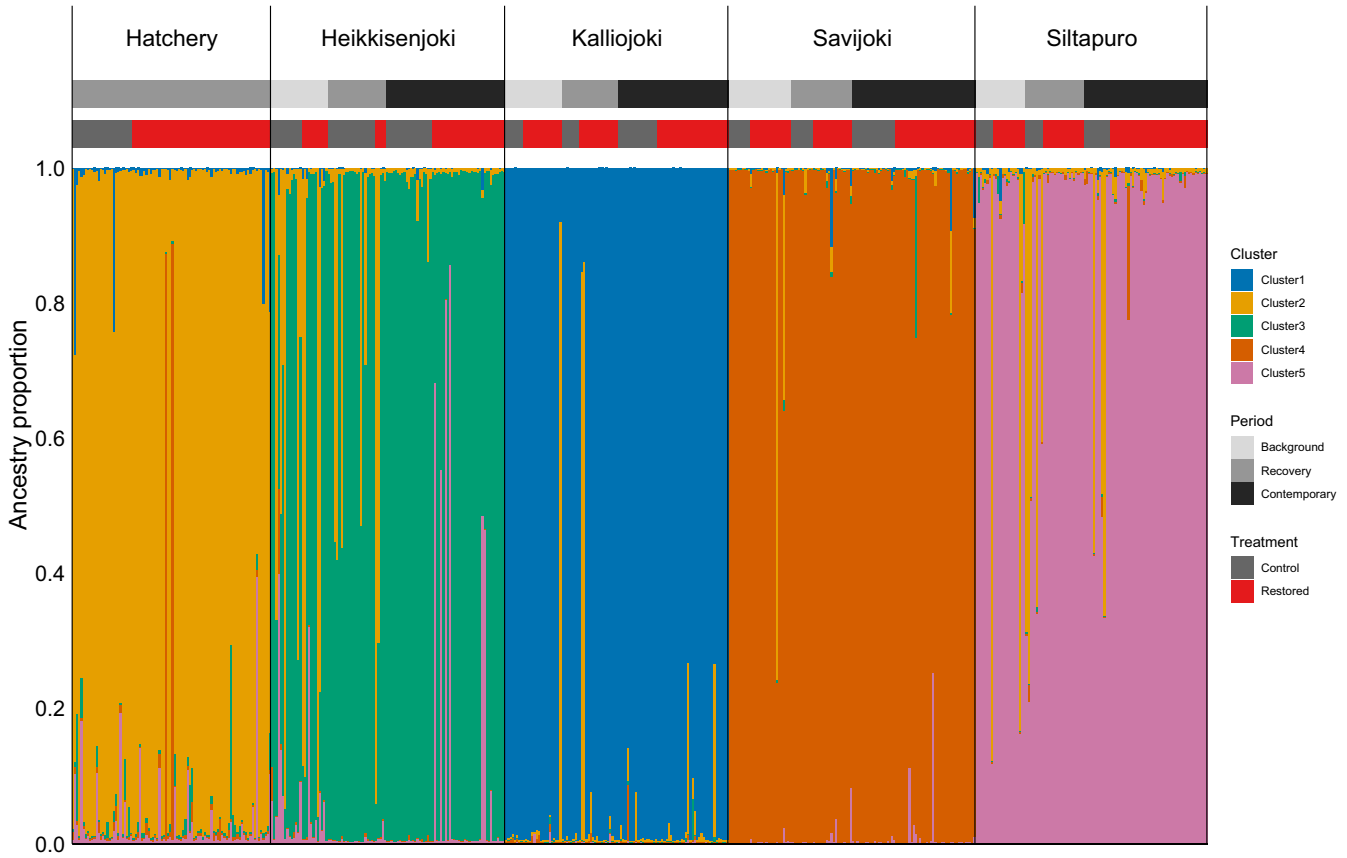

FIGURE 4 | STRUCTURE plot (16 microsatellites; $K = 5$) of electrofished brown trout individuals by the stream of origin (including stocked hatchery fish), time period and treatment.

minimum effective population size of 50, which is not enough to prevent inbreeding depression over five generations (Frankham et al. 2014) but provided some stability over this study.

Generally, the genetic impacts of salmonid stockings are highly variable (e.g., Annett et al. 2012; Bernaś et al. 2014; Valiquette et al. 2014; Östegren et al. 2021) suggesting that the success of stocking varies depending on the fish strains used in the stocking, type of aquaculture used to produce the stocked fish, and potentially also the system in question (Almodóvar et al. 2006; Araki and Schmid 2010). When stocking ceases, natural selection might favor wild-type genotypes, if still present, and purge imported genetic variation also from introgressed part of the population (Hansen et al. 2006, 2009). However, hatchery strains of brown trout can be genetically diverse compared to their effective population sizes due to the mixing of several founder populations (Aho et al. 2006; Berrebi et al. 2021). As such, introducing hatchery fish to small, inbred natural populations could also increase their genetic diversity with positive effects on their population growth rates through genetic rescue (Robinson et al. 2017; Kovach et al. 2021; Pregler et al. 2023). Very good population recovery results have also been obtained by using translocations of native brown trout (Caudron et al. 2012). The STRUCTURE analysis suggested that some hatchery fish existed in Heikkisenjoki and Siltapuro streams already before the stockings starting in 1999, while predicted hybrids were prevalent also during the recovery period. These results are likely explained by the available migration routes (and past gene flow) since both streams are open for any dispersing fish via the main channel River Kiehimänjoki while the two other streams are locked behind impermissible dams. On the contrary, given that the hatchery fish used in the stockings were genetically predominantly migratory (Vainikka et al. 2023), these results suggest that some migratory fish can still stay in the stocking sites, in the closest reachable main channel river or in some of the small lakes in the system until maturation and interbreed with the wild resident fish. Due to the lack of telemetry data, we cannot directly conclude whether the poor success of stocked fish was due to their different life-history strategy or generally poor fitness in the new environment (O'Sullivan et al. 2020).

Stream restoration should enhance or better maintain the genetic diversity of fish populations by providing more and better habitats, by increasing the environmental carrying capacity and census population size (Smith 2009). However, the impact of habitat restoration on genetic metrics was statistically insignificant in this study, consistent with its relatively weak ecological effects (Louhi et al. 2016). The F_{IS} values varied across populations but were generally very close to zero, suggesting that no major inbreeding or immigration of novel genotypes occurred. Overall, these values reflected small population sizes and an extremely small number of spawning individuals in these isolated streams but could also be biased by our sampling that pooled individuals from several years and exposed the analyses to potential temporal Wahlund effects (Ruzzante et al. 2002). If the hatchery fish induced a Wahlund effect through introgression, the F_{IS} values should have increased towards the end of the study, but this was not observed, and instead, the number of private alleles tended

to decrease during the study period. Similarly, the genetic differentiation between the hatchery strain and the wild populations increased over time, suggesting a lack of any genetic homogenization. To further address the effects of inbreeding, genomic measures such as runs of homozygosity could be used (Kurland et al. 2024).

From a managerial point of view, increasing and ensuring population connectivity would be a way to conserve the genetic diversity of small salmonid populations (e.g., Davis et al. 2018; Kurland et al. 2024). When restoration efforts lead to the removal of migration barriers, expected population genetic consequences can be more evident (Davis et al. 2018; Kurland et al. 2024). However, Neville et al. (2016) did not find any major changes in the population genetic metrics of cutthroat trout after removal of impassable culverts. Moccetti et al. (2024) reported a case where the construction of a fishway led to further differentiation of brown trout subpopulations due to strong philopatric behavior of migratory individuals. These examples demonstrate that improved connectivity does not necessarily always lead of increased admixture and genetic diversity in strongly philopatric species like brown trout. In the STRUCTURE analysis, the fish from Heikkisenjoki and Siltapuro did not show any major signs of admixture despite the proximity of the streams and absence of any migration barriers between them. However, some recent Heikkisenjoki samples from restored sections were assigned to have a genetic contribution from Siltapuro fish suggesting potential for gene flow between these populations.

The potential for genetic rescue and outbreeding or inbreeding depression associated with hatchery releases should be evaluated on a case-by-case basis (Rollinson et al. 2014; Waller 2015). Outbreeding can at best enhance the genetic resilience of populations by introducing novel genetic variation that may offer adaptive advantages in changing environments (White et al. 2023). However, at worst, interbreeding of hatchery and wild fish can result in the loss of local adaptations and unique population-level genetic variation, and generally decreased fitness (Krueger and Menzel 1979; Hansen and Loeschcke 1994; McMillan et al. 2023). Moreover, as an ecological impact, hatchery fish can compete with wild fish for resources (Bagley et al. 1994) and increase predation pressure on all brown trout (Hansen and Loeschcke 1994) through apparent competition. Hybridization with genetically dissimilar fish might also reduce the effective population size by increasing variation in reproductive success of individuals (Hansen et al. 2009). Based on our study, stocking of hatchery brown trout to Heikkisenjoki and Siltapuro would probably not be harmful from a population genetic perspective but appears inefficient.

5 | Conclusion

Our study showed that the studied brown trout populations in small forest streams are extremely small and isolated, and are in continuing danger of losing more genetic diversity and dying out. Physical habitat restoration did not improve the situation for reasons not addressed here, but they include water quantity and quality issues (Louhi et al. 2016). Due to the lack of connectivity to any major lakes or other rivers, these populations are not expected to support brown trout fishery in Lake Oulujärvi or the intermittent watercourses. For the management of fishable adfluvial brown trout populations, the small forest streams appear problematic, as they often support native resident populations, and stockings with migratory individuals can produce poor results as demonstrated here. Whether the situation would change along restoration of connectivity to lake system is unclear and would necessarily require that the migratory parr would largely outcompete the native resident individuals with conservation value on their own. In the current situation with no return routes available for lake-migrating fish, the stocking of migratory brown trout parr to small streams appears unsuccessful and wasteful from the perspective of the stream populations.

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Ethics Statement

Electrofishing was conducted under a permission from the local ELY-center and local fishing right holders. No animal experimentation was performed in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The microsatellite dataset is openly available at https://doi.org/10.23729/fd-8b71500d-0b80-3dd3-b2fd-abe8ee6861f9.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Evaluation of different *K* values for STRUCTURE analysis. **Figure S2:** Raw results of 20 replicate STRUCTURE runs (the order of individual bars does not match the final result figure). **Table S1:** 95%-confidence intervals for the pairwise F_{ST}-values, presented in Table 3 of the main text, among different rivers and time periods. **Table S2:** 95%-confidence interval for the pairwise F_{ST}-values, presented in Table 4 of the main text, among different treatments, rivers and time periods.