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Comparative analysis of genetic diversity in rainbow trout (*Oncorhynchus mykiss*) populations from Denmark and Finland using SNP data☆

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ABSTRACT

Rainbow trout (*Oncorhynchus mykiss*) is a key species in European aquaculture, and the most dominant species in aquaculture production in Denmark, where it accounts for over 70% of total aquaculture production. Despite its economic importance, the precise origins of many European strains remain poorly characterized. This study evaluated the genetic diversity of two Danish farmed subpopulations – 76 conventional (DK-CONV) individuals and 116 organic (DK-ORG) individuals – and compared them with 2952 individuals from the Finnish national breeding program (FI-BP). We analyzed population structure, heterozygosity, inbreeding coefficient, runs of homozygosity (ROH) and inbreeding rates using SNP data. Samples were genotyped on the 57 K Axiom™ Trout Genotyping Array, with 32,162 SNPs remaining after quality control. Principal component analysis (PCA) and Wright's F statistic (F_{ST}) revealed substantial genetic differentiation between the Danish and Finnish populations ($F_{ST} \sim 0.25$) and low differentiation between the Danish subpopulations ($F_{ST} = 0.0254$), indicating historical or more recent gene flow between the two Danish subpopulations. All groups exhibited a slight heterozygosity deficit and positive F_{IS} values, with the DK-CONV subpopulation showing the highest F_{IS}. The ROH analysis showed longer and more frequent homozygous segments in the Danish subpopulations, indicating more recent inbreeding compared to FI-BP. ROH based inbreeding rates (ΔF_{ROH}) were below 1% per generation in DK-ORG and FI-BP. The results of this study demonstrate that sufficient genetic diversity exists within Danish rainbow trout subpopulations to support a sustainable organic breeding program. Enhanced inbreeding management is recommended, such as incorporation of pedigree records or genomic information, to ensure long-term genetic diversity.

1. Introduction

The rainbow trout (*Oncorhynchus mykiss*) is a salmonid species indigenous to North America that has been extensively introduced worldwide. Today, it is among the most widely distributed salmonid species globally (Crawford and Muir, 2008; FAO, 2024; Stanković et al., 2015). Between 1870 and 1970, extensive introduction efforts were undertaken across North and Central America, as well as throughout Europe (Crawford and Muir, 2008; Stanković et al., 2016). In Europe, these introductions were primarily based on the importation of fertilized eggs sourced from a variety of hatcheries, predominantly located in North and South America (Stanković et al., 2016).

Nowadays, rainbow trout is one of the key species for Europe's aquaculture industry (Longo et al., 2024; Paisley et al., 2010), with France, Italy and Denmark as the three main producers (EUMOFA, 2025). In Denmark, rainbow trout is the most dominant species in aquaculture production, accounting for over 70% of the total production of the Danish aquaculture sector (Statistics Danmark, 2025). Farming rainbow trout has a long and well-established tradition in Danish aquaculture, initially cultivated in traditional flow-through ponds and since the 1950s and 1970s also in offshore cages and land-based farms using recirculation technology (Jokumsen and Svendsen, 2010; Paisley et al., 2010).

Despite this long history and economic importance, the precise origins of many European strains remain poorly characterized. Although several studies have investigated the population structure and genetic diversity of *O. mykiss* in Europe (Cadiz et al., 2021; D'Ambrosio et al., 2019; Gross et al., 2007; Longo et al., 2024; Martsikalis et al., 2014), to our knowledge information regarding the population structure and genetic diversity of farmed Danish rainbow trout is lacking.

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Maintaining genetic diversity is crucial to allow populations to adapt to natural and human-induced challenges, resist diseases and adapt to changing environments (Neff et al., 2011). In selective breeding there is a risk of inbreeding and loss of genetic diversity, leading to increased homozygosity and a higher risk of presence of deleterious alleles in the population which can negatively affect long-term genetic progress and reduce the adaptive potential of populations (Falconer, 1996; Neff et al., 2011). For long-term sustainability of a breeding program, it is therefore essential to know the present status of a population with respect to genetic diversity (Nguyen, 2016).

To preserve genetic diversity within a population, it is essential to maximize the effective population size or to minimize the rate of inbreeding. Accurate estimation of inbreeding is therefore critical for effective inbreeding management. Traditionally, inbreeding has been quantified using pedigree information by estimating the expected proportion of loci that are identical by descent (IBD) (Wright, 1922). However, incomplete pedigree information can lead to substantial underestimation of inbreeding (Lutaaya et al., 1999). Moreover, pedigree-based inbreeding estimates may be imprecise, as they represent predictions of the proportion of loci that are IBD and do not account for variation in the realized proportion of loci that are IBD among individuals sharing the same pedigree, arising from Mendelian segregation (Kardos et al., 2015).

With the availability of high-throughput genomic technologies, inbreeding can now be estimated directly from genomic data, providing measures that reflect the realized rather than the expected proportion of loci that are IBD (Houston et al., 2020; Howard et al., 2017). Genomic-based estimates of inbreeding have been shown to outperform pedigree-based estimates, as kinships between founder animals are often not accounted for in pedigree files (Marras et al., 2015; Purfield et al., 2012), representing an additional advantage of genomic approaches over pedigree-based methods. Moreover, genomic approaches are applicable even in the absence of pedigree information (Nguyen, 2016).

Several methods are available to calculate inbreeding coefficients using genomic data. Estimates based on the genomic relationship matrix (GRM) quantify genome-wide allele sharing among individuals and derive genomic inbreeding coefficients from the diagonal elements of the GRM. Although widely used, GRM-based inbreeding estimates are sensitive to allele frequencies in the base population (Villanueva et al., 2021). An alternative and commonly used method to estimate inbreeding is the use of runs of homozygosity (ROH), which are long stretches of homozygous genome that result from mating between related individuals (Curik et al., 2014). A key advantage of ROH-based inbreeding estimates is their ability to distinguish between distant and recent inbreeding (Purfield et al., 2012). To estimate genetic differentiation between populations, principal component analysis (PCA) and Wright's fixation index (F_{ST}) are effective and commonly used methods (Smaragdov and Kudinov, 2020).

In this study, we evaluated the genetic diversity of two Danish rainbow trout subpopulations and compared them with a rainbow trout population from the Finnish national breeding program. Genetic diversity parameters were computed within and between populations and population structure was assessed. The aims of the study were threefold: (1) to gain insight in the level of genetic diversity present in the three populations, (2) to identify potential differences in genetic diversity between populations managed under different breeding strategies – namely, pedigree-based inbreeding control versus a rotational mating scheme, and (3) to determine whether the Danish organic subpopulation contains sufficient genetic variation to support a sustainable organic breeding program of rainbow trout in Denmark.

2. Materials and methods

2.1.1. Sampling of the Danish population

Fin-clip samples were collected from two farmed rainbow trout (*Oncorhynchus mykiss*) subpopulations representing separate management units within a shared ancestral Danish population at the Ådal Ørred farm in Bredsten, Denmark (November 2024). One subpopulation was reared in a conventional flow-through pond system, while the other was reared in an organically certified flow-through pond system. These subpopulations are hereafter referred to as DK-CONV (conventional) and DK-ORG (organic), respectively. A total of 192 randomly selected fish were sampled from different year classes, comprising 76 individuals from the DK-CONV subpopulation (year classes 2018, 2019, 2021 and 2024) and 116 from the DK-ORG subpopulation (year classes 2017, 2019, 2020, 2021, 2022, 2023 and 2024). Currently, the conventional and organic subpopulations are managed separately; however, they share a common ancestral population, as DK-CONV was separated from DK-ORG in 1991 and an external rainbow trout line was introduced into DK-CONV in 2006 (Ådal Ørred, personal communication). No pedigree records are available for either subpopulation.

2.1.2. DNA extraction and genotyping

The fin-clip samples of DK-CONV and DK-ORG were sent to Eurofins, Galten, Denmark for DNA extraction and genotyping using the 57 K SNP Axiom™ Trout Genotyping Array (Palti et al., 2015) comprising 47,146 SNPs. Quality control was performed using PLINK v1.9 (Chang et al., 2015), and SNPs were excluded based on the following criteria: (i) call rate $< 90\%$, (ii) deviation from Hardy–Weinberg equilibrium ($P < 1 \times 10^{-5}$), and (iii) minor allele frequency (MAF) < 0.05. After quality control 32,162 SNPs and 186 samples (74 and 112 samples from the DK-CONV and DK-ORG subpopulations, respectively) remained in the dataset for downstream analyses.

2.2. Genotypes from the Finnish national breeding program

To compare the genetic diversity of the Danish rainbow trout subpopulations with another population, we used publicly available genotype data from the Finnish national rainbow trout breeding program, (described in Fraslin et al. (2023)). The dataset was accessed at https://figshare.com/articles/dataset/Genotypes_and_phenotypes_of_rainbow_trout/21814602/2 (accessed: 11/March/2025). The Finnish breeding program was established in the early 1990s and fish are bred for growth, maturity age, external appearance, deformities, fillet color, cataract, visceral percentage and survival. Optimum contribution selection (OCS) is used to control inbreeding rate in this population (Fraslin et al., 2022). The dataset used for this study contains genotypes of 2952 individuals, including both offspring and parents from 105 families, all genotyped using the 57 K SNP Axiom™ Trout Genotyping Array (Palti et al., 2015). For the purpose of this study, this population is referred to as FI-BP. To ensure consistency across datasets, genotypes from the FI-BP population were pruned to match the 32,162 SNPs retained for the DK-CONV and DK-ORG subpopulations.

2.3. Population structure and genetic diversity

Population structure and genetic diversity were assessed using multiple complementary methods. Principal component analysis (PCA) was performed using PLINK v1.9 (Chang et al., 2015) and results were

visualized using R version 4.4.2 (R Core Team, 2024). Additionally, admixture analysis was performed using ADMIXTURE version 1.3.0 (Alexander et al., 2009). As the cross-validation error decreased linearly with increasing K (see Fig. S1), admixture analysis results were interpreted alongside PCA results, with emphasis on biologically meaningful patterns rather than the absolute minimum cross-validation error. Genetic diversity was assessed by calculating several summary statistics like observed and expected heterozygosity and Wright's F_{IS}. The F_{IS} is based on the expected versus observed heterozygosity and provides a measure of heterozygote deficiency, where $F_{IS} = 0$ indicates no heterozygote deficiency or excess, $F_{IS} > 0$ indicates heterozygote deficiency and inbreeding and $F_{IS} < 0$ indicates excess of heterozygotes and inbreeding avoidance (Nei, 1977). To assess the significance of deviations between observed and expected heterozygosity within populations and subpopulations, a paired *t*-test was performed with a significance threshold of $p < 0.01$. Differences in F_{IS} values among populations and subpopulations were tested using the non-parametric Kruskal-Wallis test (McKight and Najab, 2010), also applying a significance level of $p < 0.01$.

Furthermore, Wright's F statistic (F_{ST}) was calculated using PLINK v1.9 (Chang et al., 2015), with default settings. Prior to calculation of F_{ST}, genotypes from the Danish subpopulations and the Finnish population were filtered independently based on call rate (threshold: 90%) and deviation from Hardy–Weinberg equilibrium (HWE; threshold: $p < 1 \times 10^{-5}$), without applying a filter on MAF. According to Wright's (1984) guidelines, F_{ST} values can be interpreted as follows: 0–0.05 indicates little genetic differentiation, 0.05–0.15 moderate genetic differentiation, 0.15–0.25 large genetic differentiation and above 0.25 very large genetic differentiation. To account for unequal sample sizes across groups, weighted F_{ST} values were used. To validate the robustness of the observed population structure and genetic differentiation with respect to substantial differences in sample size between the Danish subpopulations and the Finnish population, an additional analysis was performed. Individuals from the FI-BP population were randomly subsampled to match the sample size of the corresponding Danish subpopulation ($n = 74$ for DK-CONV and $n = 112$ for DK-ORG), followed by recalculation of F_{ST}. This subsampling procedure was repeated 10 times, and the resulting F_{ST} estimates were used to assess whether the observed population structure and genetic differentiation were independent of sample size effects.

2.4. Runs of homozygosity

Runs of homozygosity (ROH) and the ROH-based inbreeding coefficient (F_{ROH}) were calculated using PLINK v1.9 (Chang et al., 2015) and the *detectRUNS* package (version 0.9.6, https://cran.r-project.org/web/packages/detectRUNS/detectRUNS.pdf, accessed: 08/May/2025) in R version 4.4.2 (R Core Team, 2024). For ROH calculation the default settings in PLINK v1.9 (Chang et al., 2015) were used, with a window length of 50 SNPs, the maximum gap at 1000 kb and the minimum ROH length at 1000 kb. The inbreeding coefficient based on ROH was calculated with the *detectRUNS* package in R version 4.4.2 (R Core Team, 2024) using Eq. (1) as proposed by McQuillan et al. (2008).

$$F_{ROH} = \sum L_{ROH} \Big/ L_{genome} \tag{1}$$

where $\sum L_{ROH}$ is the sum of the length of all ROH detected in an individual, and L_{genome} is the total length of the genome that was used, comprising 1,900,017 kb in this study. A non-parametric Kruskal-Wallis test was used to test for differences in F_{ROH} values among populations, applying a significance level of $p < 0.01$, using R version 4.4.2 (R Core Team, 2024).

2.5. Inbreeding rate

The inbreeding rate based on F_{ROH} in different year classes was calculated with Eq. (2).

$$\Delta F = 1 - \left(\frac{1 - F_{ROH_{t0+n}}}{1 - F_{ROH_{t0}}} \right)^{\frac{L}{n}} \tag{2}$$

where F_{ROH} is the inbreeding coefficient based on ROH, t_0 is the birth year of the parental generation, n is the number of years after t_0, and L is the generation interval in years. For calculation of the inbreeding rate discrete generations and a generation interval of 3 years were assumed.

3. Results

3.1. Genetic differentiation among populations and subpopulations

The PCA revealed a clear genetic differentiation between the Finnish and Danish populations (Fig. 1A). The two Danish subpopulations clustered closely together and were distinct from the Finnish population. The first and second principal components explained 64.23% and 3.87% of the variance, respectively. Consistent with this, admixture analysis (Fig. 2) supported two ancestral populations as the primary level of structure, with higher K values capturing no clear differentiation across the Danish subpopulations. A separate PCA comparing the two Danish subpopulations (Fig. 1B) indicated partial genetic similarity, although some degree of differentiation was visible. In this analysis, the first two principal components explained 27.5% and 10.8% of the variance, respectively. Within DK-CONV, three genetic clusters were identified: year class 2021 formed a distinct cluster, year class 2024 formed a second cluster, and year classes 2018 and 2019 clustered together with the second cluster identified within DK-ORG. Within DK-ORG, two clusters were observed: one comprising year classes 2019, 2020, 2021 and 2023, and another comprising year classes 2017, 2018, 2019, 2020, 2021, 2022 and 2024. A detailed visualization of these clustering patterns is provided in Appendix A (Fig. S2).

These findings are in line with pairwise F_{ST} estimates, which indicated a large genetic differentiation ($F_{ST} = 0.2534$–0.2540) between the Finnish and Danish populations, and relatively low genetic differentiation between the two Danish subpopulations ($F_{ST} = 0.0254$) (Table 1). F_{ST} estimates from the validation analysis were consistent with those obtained using the full FI-BP dataset, with a mean F_{ST} of 0.2521 (0.2510–0.2536) between FI-BP and DK-CONV, and a mean F_{ST} of 0.2520 (0.2507–0.2529) between FI-BP and DK-ORG.

3.2. Genetic diversity within populations and subpopulations

Observed and expected heterozygosity were very similar across the three groups, with overall average values of 0.34 and 0.41, respectively (Table 2). All groups showed a positive value for the F_{IS} coefficient, where the FI-BP population had the lowest value ($F_{IS} = 0.126$) and the DK-CONV subpopulation the highest value ($F_{IS} = 0.186$) (Table 2).

3.3. Analysis of ROH

A total of 15,920 ROH segments were identified across 3139 samples of rainbow trout. ROH statistics as well as inbreeding coefficients based on ROH are presented in Table 3. Among the three groups analyzed, the FI-BP population showed the lowest average number of ROH (4.78), while the two Danish subpopulations showed higher and similar averages (9.53 and 10.03, respectively). The average size of ROH per individual ranged from 8238 kb in the FI-BP population to 13,004 kb in the DK-ORG subpopulation. The average inbreeding coefficient was very low for the FI-BP population ($F_{ROH} = 0.022$) and was 0.062 and 0.066 for DK-CONV and DK-ORG, respectively.

As illustrated in Fig. 3A, there was a strong positive correlation ($r = 0.87$) between the number of ROH segments and their total length across individuals. The length of ROH segments differed clearly between groups (Fig. 3B). In the Danish subpopulations, a substantial proportion

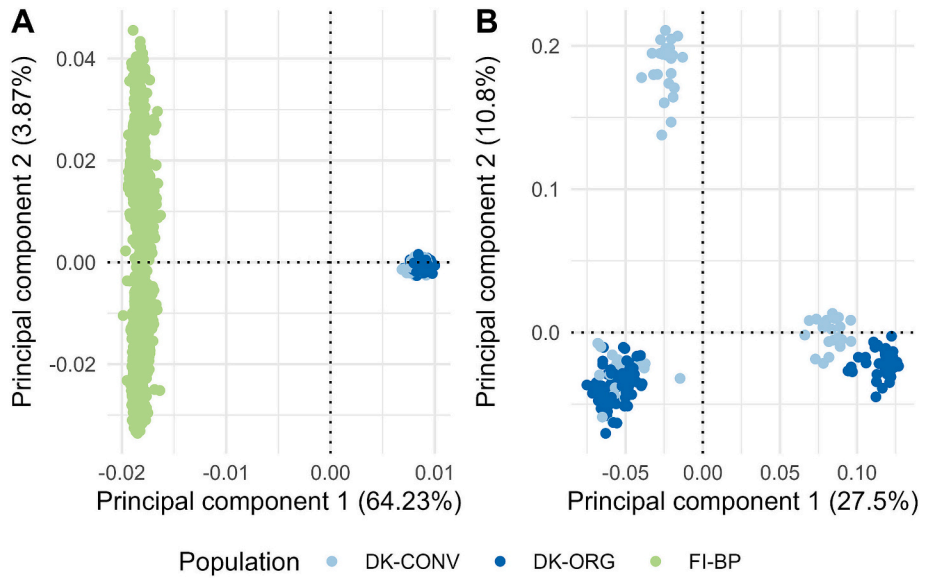

Fig. 1. (A) Principal component analysis (PCA) of the Finnish population (FI-BP) and the two Danish subpopulations (DK-CONV and DK-ORG). (B) Principal component analysis (PCA) of two Danish subpopulations (DK-CONV and DK-ORG).

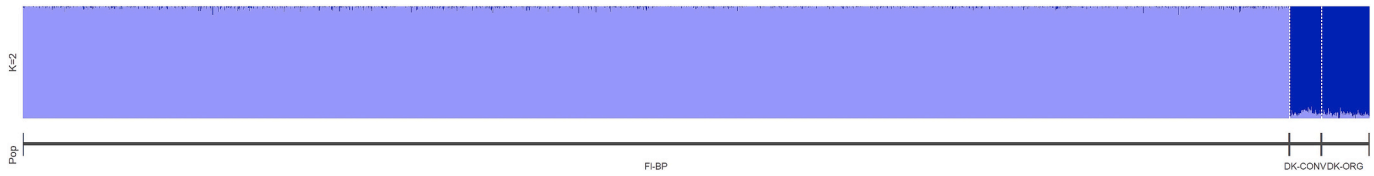

Fig. 2. Admixture analysis with individuals from the Finnish population (FI-BP) and the two Danish subpopulations (DK-CONV and DK-ORG) for K = 2.

Table 1
Wright's F statistic (F_{ST}), weighted for unequal sample sizes, among the Danish subpopulations and the Finnish population.

	DK-ORG	FI-BP
DK-CONV	0.0254	0.2534
DK-ORG		0.2540

of ROH segments exceeded 16 Mb in length – 44% in DK-CONV and 56% in DK-ORG – whereas in the FI-BP population, 39% of ROH segments fell within the 4–8 Mb category. Population-specific patterns were also visible in the chromosomal distribution of ROH segments (Fig. 3C). In both Danish subpopulations the largest proportion of ROH segments was observed on chromosome 20 (8.36% in DK-CONV and 7.69% in DK-ORG). In contrast, chromosome 5 accounted for the highest proportion of ROH in the FI-BP population (7.41%).

3.4. Inbreeding rate

Inbreeding rates were estimated for all three groups and are summarized in Table 4. The DK-CONV subpopulation had an average inbreeding rate of 0.016 per generation. The DK-ORG subpopulation showed a lower average inbreeding rate of −0.007, with values ranging from −0.028 to 0.006, indicating a reduction in inbreeding in some year classes. The average inbreeding rate in the FI-BP population was 0.001. The inbreeding rates in both DK-ORG and FI-BP remained below the generally recommended threshold of a 1% increase in inbreeding per generation (Meuwissen and Woolliams, 1994).

Table 2
Genetic diversity measures (observed heterozygosity (H), expected heterozygosity and Wright's F_{IS}) within the Danish subpopulations and the Finnish population with the standard error in parentheses.

Population	H observed	H expected	F_{IS}
DK-CONV	0.33 (<0.01)*	0.41 (<0.01)	0.186 (0.01)**
DK-ORG	0.34 (<0.01)*	0.41 (<0.01)	0.179 (0.01)**
FI-BP	0.36 (<0.01)*	0.41 (<0.01)	0.126 (<0.01)

* Significant p-value (<0.01) for paired t-test for observed heterozygosity to expected heterozygosity.
** Significant *p*-value (<0.01) for Kruskal-Wallis test for F_{IS} value in comparison to F_{IS} value for FI-BP.

4. Discussion

4.1. Population and subpopulation differentiation

Results from the PCA and the admixture analysis revealed substantial genetic divergence between the FI-BP and the two Danish subpopulations, consistent with the observed F_{ST} values (0.2534–0.2540). As the Finnish and Danish populations represent distinct farmed populations without recent shared ancestry, a high level of genetic differentiation is expected. In contrast, the DK-CONV and DK-ORG subpopulations showed low genetic differentiation, as observed in both PCA and F_{ST} analyses (F_{ST} = 0.0254). Given their shared origin, this low level of differentiation is expected; however, a slightly higher degree of genetic differentiation was anticipated due to the separation of these subpopulations several generations back. The observed genetic similarity may reflect historical or more recent gene flow between the two subpopulations, as they share a common ancestry and occasional crosses

Table 3
Runs of homozygosity (ROH) summary statistics (average number of ROH, average size of ROH in kb) and ROH-based inbreeding coefficient (F_{ROH}) within the Danish subpopulations and the Finnish population. The standard error is provided in parentheses.

Population	Average number of ROH	Average size of ROH (in kb)	Average F_{ROH}
DK-CONV	10.03 (0.41)	11,729 (335)	0.062 (0.003)*
DK-ORG	9.53 (0.30)	13,004 (379)	0.066 (0.002)*
FI-BP	4.78 (0.04)	8238 (56)	0.022 (<0.001)

* Significant p-value (<0.01) for Kruskal-Wallis test for F_{ROH} value in comparison to F_{ROH} value for FI-BP.

between subpopulations have occurred in recent years, up until 2023 (Ådal Ørred, personal communication). In the absence of pedigree records, the timing and extent of such gene flow cannot be determined.

Similar findings have been reported in other studies. For example, Longo et al. (2024) reported a F_{ST} value of 0.015 between two commercial rainbow trout populations in Sweden, suggesting that those two populations might share a common origin. Similarly, D'Ambrosio et al. (2019) reported F_{ST} values ranging from 0.08 to 0.150 among four commercial lines of rainbow trout in France, which share a common ancestry but had been maintained as closed populations for at least five generations at the time of sampling. In Liu et al. (2017), eight commercial rainbow trout breeding populations (four strains with separate odd- and even-year classes) showed moderate genetic differentiation, with F_{ST} values ranging from 0.056 to 0.195. Odd- and even- year class of the same strain showed moderate genetic differentiation (F_{ST} = 0.056–0.102), whereas comparisons between different strains showed moderate to large genetic differentiation (F_{ST} = 0.087–0.195). Together, these results indicate that observed patterns of genetic differentiation are closely linked to population formation and breeding management, with high F_{ST} values reflecting long-term separation without shared ancestry, and low F_{ST} values reflecting a shared origin and ongoing or recent gene flow.

4.2. Genetic diversity within populations and subpopulations

All groups exhibited a slight heterozygosity deficit, with a mean H_O of 0.34 and a mean H_E of 0.41. Similar patterns were observed in other farmed rainbow trout populations by D'Ambrosio et al. (2019) and Longo et al. (2024). F_{IS} values were positive across all groups, indicating a general excess of homozygosity. Positive F_{IS} values are expected in aquaculture breeding programs, which typically consist of a closed nucleus breeding structure. In such systems, sires and dams are selected from a group of breeding candidates within the nucleus to produce the next generation, thereby increasing F_{IS} over time (Gjedrem, 2005).

Table 4
Inbreeding rate (ΔF_{ROH}) per generation in the Danish subpopulations and the Finnish population.

Population	Parent generation	Offspring generation	ΔF_{ROH}
DK-CONV	2018	2021	0.023
	2021	2024	0.009
DK-ORG	2017	2020	0.006
	2019	2022	−0.028
	2020	2023	0.006
	2021	2024	−0.012
FI-BP	2016	2019	0.001

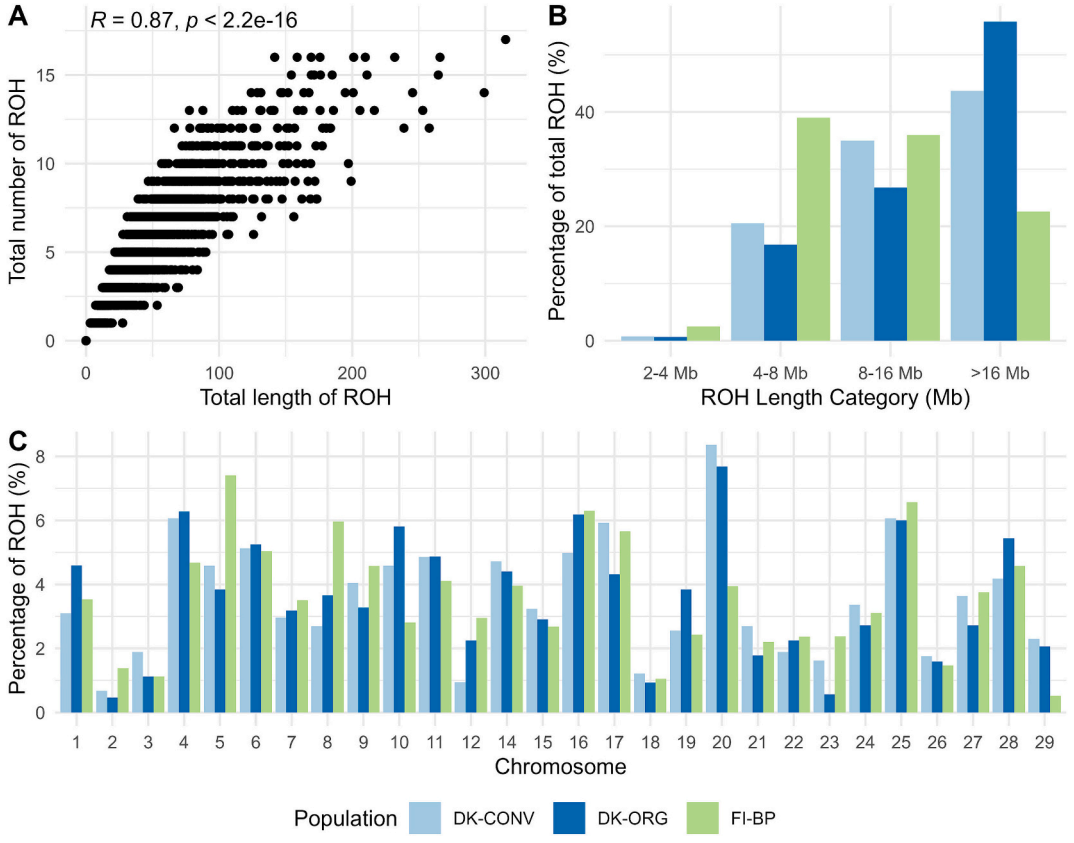

Fig. 3. (A) Correlation between total number of ROH segments and the total length of ROH per individual. (B) Proportion of ROH segments divided into several length categories (2–4 Mb, 4–8 Mb, 8–16 Mb and > 16 Mb) for the three populations. (C) Chromosomal distribution of ROH segments for the three populations in percentages.

The highest F_{IS} value was observed in the DK-CONV subpopulation (F_{IS} = 0.186), suggesting stronger selection pressure and a smaller effective population size relative to the other Danish subpopulation and the Finnish population. Due to the lack of pedigree records for the Danish subpopulations, it was not possible to estimate the effective population size directly. The FI-BP population exhibited the lowest F_{IS} value (0.126), which may reflect the effectiveness of OCS in managing inbreeding. In contrast, Danish subpopulations lack genomic- or pedigree-based inbreeding management but use a rotational mating scheme. As demonstrated by Windig et al. (2019), rotational mating schemes can effectively control inbreeding rates; however, realized inbreeding levels may still exceed theoretical levels due to selection and unequal contributions of animals to the next generation. An alternative approach to inbreeding management is the use of genomic information to estimate inbreeding rates within a population and relatedness among individuals. This information can then be used to restrict inbreeding at both the population and individual levels through methods such as OCS, mate allocation and mate selection (Howard et al., 2017; Nguyen, 2016; Yoshida et al., 2017).

Lower F_{IS} values have been reported in other studies, including an average of 0.075 in Swedish rainbow trout (Longo et al., 2024) and 0.034 in French brown trout (Bohling et al., 2016). The Swedish rainbow trout samples originated from a breeding program established in 2011, which crossed one Swedish strain of Norwegian origin with another Swedish strain; two generations of selection had been performed at the time of sampling. In contrast, the Finnish and Danish populations have a much longer breeding history, with populations established in the early 1990s and 1972, respectively (Ådal Ørred, personal communication; Fraslin et al., 2022). This may explain the lower F_{IS} value reported by Longo et al. (2024) compared to values observed in our study. The low F_{IS} in French brown trout was expected, as domestic stocks have been supplemented with wild fish and some stocks have been directly derived from local rivers (Bohling et al., 2016). Gross et al. (2007) reported negative F_{IS} values (−0.01 to −0.15) in Estonian rainbow trout populations that originated from farmed Danish strains, using microsatellites. These fish were sampled in Estonian fish farms in the year of their introduction, and their breeding history was not reported. It is possible that no selection had been applied in the source populations and that only random mating occurred, which could explain the lower F_{IS} values. Together, these varying F_{IS} levels across populations highlight that breeding strategy and population history influence inbreeding levels and genetic diversity in aquaculture populations.

4.3. Analysis of ROH

The average length of ROH was above 8 Mb across all three groups, indicating recent inbreeding. Long ROH segments are typically indicative of recent common ancestry, whereas shorter ROH reflect more distant ancestry (Curik et al., 2014). Given that rainbow trout has undergone domestication relatively recently compared to traditional livestock species, evidence of recent inbreeding is not unexpected.

In the Danish subpopulations, more than 40% of ROH segments exceeded 16 Mb in length, with DK-ORG showing a higher proportion of long ROH segments than DK-CONV. The high proportion of long ROH segments suggests recent inbreeding resulting from mating among relatives in both Danish subpopulations, with a more pronounced effect in DK-ORG. Although the estimated inbreeding rate for DK-ORG remains below the commonly recommended threshold of 1% per generation, continued mating among close relatives may pose a risk to the long-term genetic diversity and effective population size of the population (Howard et al., 2017).

It should be noted that with the SNP array density (57 K) used in this study, the detection of shorter ROH may have been limited. Previous research has shown that arrays with 50 K SNPs are insufficient for accurately identifying ROH between 0.5 and 1 Mb but are adequate for detecting ROH longer than 5 Mb (Purfield et al., 2012).

Few studies have investigated inbreeding estimates based on ROH in rainbow trout. One such study by D'Ambrosio et al. (2019) reported F_{ROH} values ranging from 0.094 to 0.186 in French rainbow trout. In comparison, F_{ROH} values in the present study were generally lower across all groups. The Danish subpopulations showed higher F_{ROH} values than the FI-BP population, which is consistent with the results of the F_{IS} values discussed earlier and might be expected when considering the differences in breeding management practices.

4.4. Inbreeding rate

Inbreeding rates of the DK-ORG subpopulation and the FI-BP population were below the recommended threshold of 1% per generation, indicating that these groups currently have sufficient genetic diversity to support a sustainable breeding program. In contrast, the average inbreeding rate in the DK-CONV subpopulation exceeded 1%. However, a notable decline in the inbreeding rate per generation was observed, which may be attributed to gene flow between the two Danish subpopulations (Ådal Ørred, personal communication). Similarly, the negative inbreeding rate observed in the DK-ORG subpopulation is likely due to the introduction of broodstock from the DK-CONV subpopulation during family production.

The inbreeding rate observed in the FI-BP population in this study is considerably lower than the pedigree based inbreeding rate reported by Kause et al. (2005), which ranged from 0.52% to 0.70%. However, their estimates were derived from earlier generations than those examined in the current study. Additionally, Kause et al. (2005) calculated a pedigree based inbreeding rate (ΔF_{PED}), whereas the present study calculated a F_{ROH} based inbreeding rate. As F_{PED} and F_{ROH} measure inbreeding using different approaches, differences between the inbreeding rate estimates are expected, with F_{PED} potentially underestimating inbreeding due to limited pedigree depth, incomplete pedigree information, or underestimation of the proportion of loci that are IBD among individuals (Kardos et al., 2015; Zhang et al., 2015).

5. Conclusion

The results of this study demonstrate that sufficient genetic diversity exists within the Danish rainbow trout subpopulations to support the development of a breeding program for an organic line. The DK-ORG subpopulation exhibits slightly higher genetic diversity than the DK-CONV subpopulation; however, gene flow between the two has contributed to their low genetic differentiation. Among the groups studied, the FI-BP population displays the highest level of genetic diversity, which may reflect more advanced inbreeding management practices relative to those employed in the Danish subpopulations. For a future breeding program, the incorporation of pedigree records or genomic information is recommended to enhance inbreeding management and to preserve genetic diversity across generations.

CRediT authorship contribution statement

Janneke Willemijn Verweij: Writing – original draft, Investigation, Formal analysis. **Hanne Marie Nielsen:** Writing – review & editing, Funding acquisition. **Albert Johannes Buitenhuis:** Writing – review & editing, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.743706.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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