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Evaluating the potential of improving sperm quality traits in farmed Arctic charr (*Salvelinus alpinus*) using selective breeding

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ABSTRACT

Arctic charr (*Salvelinus alpinus*) is a high-value species for the Nordic aquaculture. The highly variable reproductive performance that is commonly observed in commercial farms is hindering the expansion of the Arctic charr industry in Sweden. Traits related to sperm motility (total motility; curvilinear velocity; average path velocity; straight-line velocity) and concentration can play a pivotal role in male fertility. Selective breeding practices could offer solutions and contribute to improving male fertility. The current study aimed to investigate the magnitude of genetic variance for sperm quality traits in a selectively bred population of Arctic charr from Sweden and evaluate the possibility of their improvement through selection. Sperm motility and concentration were recorded using a computer-assisted semen analysis (CASA) system and a NucleoCounter, respectively, in over 400 males from year-class 2017. Double digest restriction-site associated DNA sequencing (ddRAD-seq) was applied in a subset of the recorded animals ($n = 329$), resulting in the detection of over 5000 single nucleotide polymorphisms (SNPs). Moderate heritability estimates were obtained for the recorded semen traits using both pedigree (0.21–0.32; SE 0.09) and genomic (0.23–0.26; SE 0.09) relationship matrices. A genome-wide association study (GWAS) detected a single SNP significantly associated ($P < 1e-05$) with total sperm motility on chromosome LG7 in relatively close proximity (500 Kb) to *PTPN11* a gene previously associated with sperm quality traits in mammals. Moreover, weighted single-step genomic best linear unbiased prediction (WssGBLUP) pinpointed genomic regions explaining more than 3 % of the additive genetic variance for both the motility traits and the sperm concentration. Finally, the efficiency of genomic prediction was tested using a 3-fold cross-validation scheme. Higher prediction accuracy for total motility and velocities (both curvilinear and average path) was obtained using genomic information (0.26–0.29, SE 0.03–0.06) compared to pedigree (0.20–0.28, SE 0.04–0.07), while for sperm concentration a pedigree-based model (0.22 SE 0.03) was more efficient than the genomic model (0.14 SE 0.04). Overall, our results indicate that the recorded sperm quality traits are heritable, and could be improved through selective breeding practices.

1. Introduction

Arctic charr (*Salvelinus alpinus*) is a high-value species for the Nordic aquaculture. Due to its high tolerance to low water temperatures, it has been successfully farmed across the Holarctic region since 1980s (Helgadóttir et al., 2021). Sweden is currently the second largest producer of Arctic charr worldwide with a production volume of approximately 1100 metric tons (SCB, 2021). An ongoing selective breeding program has been operating in Sweden for about 40 years and has been vital through regular seedstock dissemination for the industry (Nilsson et al., 2010). Selection has focused primarily on improving the growth of the animals, reducing the time to reach harvest size (~ 800 g) by one year (Carlberg et al., 2018).

Nevertheless, the highly variable reproductive performance that is

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commonly observed in captivity has hindered the development of the related industry in Sweden (Jeuthe et al., 2019). Furthermore, low fertilization success and prehatch survival have been reported in Arctic charr with values ranging between 30 % and 70 % (Jeuthe et al., 2016; Leblanc et al., 2016; Mansour et al., 2011). In comparison, survival to the eyed stage in Atlantic salmon and rainbow trout often exceeds 90 % (King et al., 2003, 2007; Vehviläinen et al., 2010).

Even though substantial research efforts have been placed so far in studying the role of environmental factors and egg quality parameters in the reproductive success of farmed Arctic charr (Jeuthe et al., 2016), limited research so far has focused on male fertility (Jeuthe et al., 2019). Several semen characteristics have been already reported as quality indicators that can influence fertilization success in fish species, including seminal plasma composition (Kowalski and Cejko, 2019), osmolality (Kowalski and Cejko, 2019), ATP content (Kommisrud et al., 2020), enzyme activity (Jia et al., 2022), DNA fragmentation level (Jeuthe et al., 2021), sperm morphology (Alavi et al., 2008; Mylonas et al., 2017; Tuset et al., 2008), motility and swimming speed (Gallego et al., 2013, 2016; Gallego and Asturiano, 2018) and concentration (Alavi et al., 2008).

Amongst the aforementioned traits, sperm motility and concentration play a pivotal role in determining male fertility (Cabrita et al., 2014; Jeuthe et al., 2019). High correlations ($r = 0.5–0.9$, $P < 0.05$) between motility traits and fertilization or hatching rates have been documented for various fish species (Bozkurt, 2006; Gallego et al., 2016; Gallego and Asturiano, 2018; Jeuthe et al., 2019). Furthermore, through the usage of computer-assisted sperm analysis (CASA) systems several sperm kinematic parameters have been successfully recorded in a wide range of fish species providing key insights regarding sperm quality (Gallego et al., 2018; van der Horst, 2021; van der Horst et al., 2018). Nevertheless, no prior information exists about the underlying genetic factors and the heritability magnitude of sperm motility traits and concentration in fish.

Notably, the potential to improve sperm quality traits in livestock through selective breeding has been demonstrated on several occasions (Butler et al., 2020; Lavara et al., 2012; Marques et al., 2017). Therefore, gaining insights regarding the genetic parameters of sperm quality traits and the magnitude of their inheritance can allow the design and implementation of relevant breeding schemes in Arctic charr.

Moreover, the usage of genomic information either in the form of a pre-customed SNP array (Nugent et al., 2019) or through a species agnostic genotyping by sequencing (GBS) platform (Robledo et al., 2018) can inform about the underlying genetic architecture of various sperm quality traits and expedite genetic gain in farmed Arctic charr. GBS platforms, in particular, like double digest restriction-site associated DNA sequencing (ddRAD-seq) (Peterson et al., 2012), have been applied extensively in various aquaculture species for QTL mapping (Nousias et al., 2022; Taslima et al., 2020), genome-wide association studies (GWAS) (Gong et al., 2021; Jiang et al., 2019; Zhu et al., 2021) and genomic prediction for commercially important traits (Barría et al., 2018).

Our study aimed to evaluate the magnitude of genetic variances for sperm quality traits in selectively bred Arctic charr and assess the possibility of improving them through selective breeding. Sperm quality traits were recorded in Arctic charr males from the same year class. Heritability estimates were obtained for motility-related traits and sperm concentration. SNPs were detected through ddRAD and used as a template for GWAS and a WssGBLUP aiming to detect genomic regions associated with the recorded sperm quality traits. Finally, prediction accuracies for the studied traits were compared between models relying on relationships estimated either through pedigree or SNPs.

2. Materials and methods

2.1. Ethical statement

The current study was performed in accordance with the Swedish legislation described in the Animal Welfare Act 2018:1192 (ethics permit: 5.2.18 – 09859/2019).

2.2. Sampled animals and rearing conditions

Sampling took place at the facilities of Aquaculture Centre North (ACN, Kälarne, Sweden), where the national Swedish breeding program of Arctic charr is located. A closed breeding nucleus has been kept since the start of the breeding program with no additions of external germplasm (Palaiokostas et al., 2020). Since the core activities of the breeding program require pedigree information, the fish are marked with the PIT (Passive Integrated Transponder) tags.

The studied fish were reared under a natural photoperiod in indoor hexagonal concrete tanks (12 m^3, ~ 1 m depth) supplied with water from the nearby lake Ansjön. The ambient water temperature ranged between 11 ℃ and 13 ℃ during summer, decreasing from mid-September onwards up to 2.6 ℃ in winter. In addition, the fish were fed 2 % of the body weight per day with Vitalis feed (Skretting, Tooele, UT, USA) every 30 min. Feeding occurred from 30 min after lights are turned on in the morning to 30 min before lights are turned off in the evening (natural day length). Portions of feed were calculated and distributed over the day automatically by the feeding system (Arvo-Tec Oy, Huutokoski, Finland) based on data input on the number of fish, starting average body weight, and water temperature.

Sampling took place between October and November 2020 and involved more than 400 males from the 2017 year class ($n = 466$ males for sperm concentration; $n = 441$ males for sperm motility) (Table 1). The sampled animals originated from three different tanks where different rearing conditions were used. More specifically, there was ambient water temperature and regular speed flow (15 cm/s) in tank 1. On the other hand, ambient water temperature and double speed water flow (30 cm/s) was used in tank 2. Finally, in tank 3 a water-cooling system was used (from July to November) reducing the water temperature by 3 ℃ compared to the other tanks, while water flow was of normal speed (15 cm/s). The water exchange rate in all tanks was 200 L/min. During the sampling period, the ambient water temperature ranged from 10.6 ℃ to 4.8 ℃, while in the water-cooling system, it ranged between 7.7 ℃ and 4.3 ℃ (Fig. A1).

Table 1
Number of Arctic charr males (*Salvelinus alpinus*) recorded per tank.

Parameter	Tank 1	Tank 2	Tank 3	Total
Sperm concentration	209	219	38	466
Sperm motility	169	216	56	441

In tanks 1 and 2, the ambient water temperature was used with regular (15 cm/s) and fast (30 cm/s) flow, respectively. In tank 3, animals were held under cooled water (3 ℃ lower than in other tanks) and regular water flow (15 cm/s).

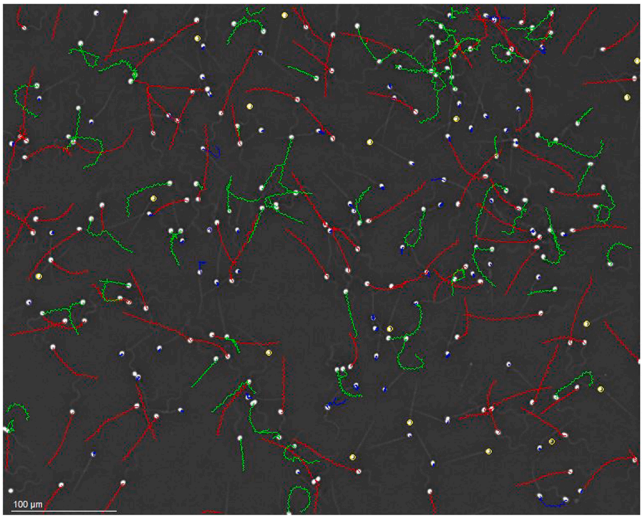

Fig. 1. Automatic analysis and classification of sperm motility and kinematic parameters in Arctic charr (*Salvelinus alpinus*) using a CASA-system with the SCA® Motility imaging software. Spermatozoa with the highest swimming speed are marked with a red trajectory, while the ones with medium and low speed are marked with green and blue trajectories, respectively. Dead spermatozoa are marked with yellow.

Fish were anesthetized using MS-222 (Sigma Aldrich, St. Louis, MO, USA), and semen was collected in disposable sample cups through manual stripping. Additionally, body weight (kg, ± 0.01 kg) and length (cm, ± 0.1 cm) were measured. Finally, the condition factor of the sampled males was calculated using the following equation: $K = BW/BL^3 \times 100$ (Fulton, 1902), where body weight was in grams and total length in centimeters. The average body weight (BW) and total length (TL) of sampled males was 2.97 ± 0.67 kg (range: 1.19–4.56 kg) and 55.1 ± 2.9 cm (range: 43.5–62.5 cm), respectively. The average condition factor was 1.7 (range: 1.2–2.6).

2.3. Sperm collection and analysis

On each sampling occasion, milt was collected over a period of a maximum of one hour from first to the last sire and stored at 4 ℃. Measurements were taken place the same day at the on-site laboratory of ACN. More specifically, sperm motility and kinematic parameters were estimated using a computer-aided sperm analysis (CASA) system equipped with the SCA® Motility imaging software v6.5 (Microptic, Barcelona, Spain) (Fig. 1). In more detail, the CASA system was comprised of a Nikon microscope (Nikon Instruments Inc., NY, USA) with 10 × magnification, Basler Ace acA1300-200uc digital camera (Basler AG, Ahrensburg, Germany), and a cooling stage (Linkam, Tadworth, UK). The installation of the CASA system and the adjustment of the relevant threshold parameters during trait recordings at values suitable for analyzing salmonid sperm including Arctic charr were performed by the manufacturer.

CASA measurements for each sample were taken 2–3 times using standardized 20 µm-depth slides with two counting chambers (CellVision, Heerhugowaard, Netherlands). The slides were pre-cooled at 8 ℃ before loading a sample. Sperm motility and velocity were assessed at 15 s immediately after activation with water (final dilution 1:10). The image capture configurations used for analysis were set at a frame rate of 100 fps with recordings every 5 s (50 frames). The following CASA parameters were recorded: total motility (TM, %), sperm velocity: average path velocity (VAP, µm/s; the average velocity of the smoothed cell path), curvilinear velocity (VCL, µm/s; the average velocity measured over the actual point-to-point track) and straight-line velocity (VSL, µm/s; the average velocity measured in a straight line from the beginning to the end of the track). The minimum velocity for motile sperm was set to VCL ≥ 20 µm/s.

Furthermore, the sperm concentration of each sample (SC × 10^9 cells/mL) was measured using NucleoCounter® SP-100™ (Chemometec, Allerod, Denmark). Before measurement, the semen samples were diluted at 1:1000 with a lysis buffer (20 µL sample and 20 mL buffer; Reagent S-100, Chemometec, Allerod, Denmark). Analyzes were performed using the setting for bull semen, as recommended by the manufacturer.

Thereafter a descriptive analysis was performed for the recorded traits using base R (version 4.0.2) and packages like dplyr and ggplot2 (Team, 2019). Spearman correlation coefficients were estimated using ggcorrplot with *P*-values below the alpha threshold of 0.05 considered statistically significant.

2.4. Inbreeding estimation

The INBUPGF90 v1.43 software from the BLUPF90 suite (Misztal et al., 2018) was used to estimate inbreeding coefficients for each animal. A recursive algorithm assuming non-zero inbreeding for unknown parents was used as described by Aguilar and Misztal (2008). The relationship between each individual's inbreeding coefficients and sperm quality traits was assessed through linear regression and from the obtained Spearman correlation coefficient.

2.5. Samples and ddRAD library preparation

Genomic DNA was extracted from fin clips using a salt-based precipitation method as described in Palaiokostas et al. (2022). Briefly, fin tissue was digested at 55 °C for 4 h using a lysis solution containing 200 µL SSTNE (50 mM Tris base, 300 mM NaCl, 0.2 mM each of EGTA and EDTA, 0.15 mM of spermine tetrahydrochloride, and 0.28 mM of spermidine trihydrochloride; pH 9; Sigma-Aldrich, Darmstadt, Germany), 10 % SDS (Bio-Rad, Hercules, USA), and 100 µg proteinase K. Following the digestion, 5 µL RNaseA (Thermo Fisher, Vilnius, Lithuania) (2 mg/mL) was added, and the samples were incubated at 37 °C for 60 min. Proteins were precipitated by adding 0.7 vol of 5 M NaCl (Sigma-Aldrich, Darmstadt, Germany). The genomic DNA was pelleted by the addition of 0.7 vol of isopropanol and centrifugation (Pico 21, Thermo Fisher, Waltham, MA, USA) at 14,000 g for 5 min. Following overnight incubation with 75% ethanol, the DNA pellet was dissolved in 30 µL of 5 mM Tris (pH 8.0; Sigma-Aldrich, Darmstadt, Germany). DNA content and quality were assessed using a NanoDrop 8000 (Thermo Scientific, Waltham, MA, USA) spectrophotometer, agarose gel electrophoresis, and Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Finally, the DNA samples were diluted to 15 ng/µL using 5 mM Tris (pH 8.0) and stored at 4 ℃ before library preparation.

The ddRAD library preparation was performed following a modified version of the original protocol (Peterson et al., 2012) described in detail by Palaiokostas et al. (2015). In short, four ddRAD libraries were prepared for 329 samples. From each individual sample, 15 ng of DNA sample were digested at 37 ℃ for 60 min with the high-fidelity enzyme SbfI recognizing the CCTGCA = GG motif and the *Nla*III recognizing the CATG motif (New England Biolabs, Ipswich, UK). Individual-specific P1 and P2 adapters with a unique 5 or 7 bp barcode were ligated to the digested DNA and incubated at room temperature for 120 min. The ligation reaction was stopped after the addition of 2.5 vol of PB buffer (Qiagen, Hilden, Germany). Thereafter, the samples were combined in a multiplex pool and purified with a MinElute PCR Purification kit (Qiagen, Hilden, Germany).

The libraries were size-selected (400–600 bp) by electrophoresis on a 1.1 % TAE agarose gel followed by gel purification. The gel was run at constant voltages of 45 V for 3 min, 60 V for 3 min, and 90 V for around 70 min. Following gel purification (QIAquick gel extraction kit; Qiagen, Hilden, Germany) library templates of 40 µL each were obtained. Thereafter PCR amplification was performed on a thermal cycler T100

(Bio-Rad, Redmond, WA, USA) using the following cycling conditions: 98 °C for 30 s, 13–14 PCR cycles of 98 °C for 10 s, 65 °C for 30 s, and 72 °C for 30 s, then a final step of 72 °C for 5 min. Each PCR amplified library was purified using an equal volume of AMPure beads (Beckman Coulter, Brea, CA, USA), and elution at 20 μL was followed with EB buffer (MinElute Gel Purification Kit, Qiagen, Hilden, Germany). Finally, the libraries were sequenced in an Illumina NovaSeq 6000 using one SP flow cell (150 base paired-end reads) at the National Genomics Infrastructure center in Uppsala, Sweden.

2.6. SNP identification and genotyping

Sequence reads with low quality (Q < 30) and missing the expected restriction sites were filtered using Stacks software v2.5 (Rochette et al., 2019). The remaining reads were aligned to the *Salvelinus sp*. reference genome assembly [Genbank accession number GCF_002910315.2] using the Bowtie2 program (Langmead and Salzberg, 2012). Following genotyping calling using Stacks, SNPs with a minor allele frequency (MAF) less than 0.05 and a calling rate lower than 70 % were discarded. Finally, SNPs that deviated from the expected Hardy-Weinberg equilibrium (HWE) were discarded using the preGSf90 software from the BLUPF90 suite (Misztal et al., 2018; Wiggans et al., 2009).

2.7. Estimation of genetic parameters

Genetic parameters were estimated with AIREMLF90 (Misztal et al., 2018) using the following model:

$$y = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}, \quad (1)$$

where **y** is the vector of recorded phenotypes (sperm quality traits), **b** is the vector of fixed effects (water temperature during sampling, water flow, and sampling day). **X** is the incidence matrix relating phenotypes with fixed effects, while **Z** is the incidence matrix relating phenotypes with random animal genetic effects. **u** is the vector of random animal genetic effects $\sim N(0, A\sigma_g^2)$, where **A** corresponds to the pedigree-based relationship matrix, which was replaced with the genomic relationship matrix **G** (VanRaden, 2008), in the case of the GBLUP based analysis, and σ_g^2 is the additive genetic variance. **e** is the vector of residuals $\sim N(0, I\sigma_e^2)$, where σ_e^2 is the residual variance and **I** is the identity matrix.

The genomic relationship matrix was created as follows (VanRaden, 2008):

$$\mathbf{G} = \frac{\mathbf{Z}\ \mathbf{Z}'}{\mathbf{2}\ \sum p_i(\mathbf{1}-p_i)}$$

where **Z** is a matrix of centered genotypes and p_i the corresponding MAF for each SNP.

Heritability estimates were obtained for the recorded milt traits using the following equation:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2\ +\sigma_e^2}$$

In the case of sperm concentration, the trait values were standardized using the following formula:

$x_{new} = \frac{x_i-\mu}{\sigma}$

where x_i is the phenotypic record of each animal, μ is the mean value, and σ is the phenotypic standard deviation.

Genetic correlations among the recorded sperm traits were estimated with a bivariate animal model that included the same fixed and random effects as in the model (1). The model had the following format:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}, \quad (2)$$

where $y_{1,2}$ are the vectors of the corresponding phenotypic traits under study; b_i, $u_i \sim N(0, \mathbf{G}_0 \otimes \mathbf{A})$ are the vectors of fixed and random effects, respectively, and $e_i \sim N(0, \mathbf{R}_0 \otimes \mathbf{I})$ the vector of residuals. $\mathbf{G}_0$ and $\mathbf{R}_0$ are the 2 × 2 variance-covariance matrices for random effects and residuals, while ⊗ denotes the Kronecker product. $\mathbf{X_i}$ and $\mathbf{Z_i}$ are the corresponding design matrices for the fixed and random effects.

The genetic correlations among traits were calculated as follows:

$$r_{i,j} = \frac{\sigma_{ai,aj}}{\sqrt{\sigma_{ai}^2 \times \sigma_{aj}^2}}$$

where $\sigma_{ai,aj}$ the genetic covariance variance between the two traits, while σ_{ai}^2 and σ_{aj}^2 are the corresponding additive genetic variances.

2.8. Genome-wide association study and WssGBLUP

A genome-wide association study was performed using the "–mlma", "–grm", and "–reml" options implemented in GCTA software v.1.92.3 (Yang et al., 2011) to investigate genomic regions associated with milt quality traits. The following model was applied:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z\alpha} + \mathbf{g} + \mathbf{e}, \quad (3)$$

where **Z** is the incidence matrix linking the recorded animals with their genotypes, **α** is a vector of an allele substitution effect for the candidate SNP, **g** is a vector of the total SNP effects with $\sim N(0, A\sigma_g^2)$, where **A** stands for the pedigree relationship matrix and σ_g^2 is variance explained by all the SNPs. The genome-wide significance threshold was calculated using a Bonferroni correction:

$$bonf = \frac{0.05}{N},$$

where N is the number of SNPs that passed the quality control.

In addition, a weighted single-step genomic best linear unbiased prediction (WssGBLUP) analysis (Wang et al., 2012) was performed using the preGSf90 v1.21 and postGSf90 v1.70 from the BLUPF90 software suite. A genomic relationship matrix was created following VanRaden (VanRaden, 2008) using the equation:

$$\mathbf{G} = \frac{\mathbf{Z}\ \mathbf{DZ}'}{\mathbf{2}\ \sum p_i(\mathbf{1}-p_i)}$$

where **Z** is a matrix of centered genotypes, **D** is a weight matrix for all SNPs, and p_i is the corresponding MAF for each SNP. SNP weights were calculated using the nonlinearA method (VanRaden, 2008).

The percentage of explained additive genetic variance was estimated by non-overlapping windows of 10 adjacent SNPs as follows:

$$\frac{\mathrm{Var}(\alpha_i)}{\sigma_g^2} \times 100\% = \frac{\mathrm{Var}(\sum_{i}^{i=10} z_i\ \ \widehat{a}_1)}{\sigma_g^2} \times 100\%$$

where $\mathrm{var}(\alpha_i)$ is the additive genetic variance of the tested window of adjacent SNPs and σ_g^2 the total additive genetic variance.

2.9. Comparison of prediction accuracies

Estimated breeding values (EBVs) for sperm quality traits were obtained using the BLUPF90 software suite (Misztal et al., 2018) using the model denoted in Eq. (1). The potential of genomic selection for sperm quality traits was assessed by comparing the accuracy of the estimated breeding values using either GBLUP or PBLUP following a three-fold cross-validation scheme. More specifically, the genotyped animals were randomly assigned to three groups. Each subsequent group (n = 147) was treated as the validation set, and the remaining animals (n = 294) were used as the training set. In the validation set, phenotypes of the animals were masked, and their (G)EBVs were estimated using

Table 2
Means, standard deviations (SD), minimum (min) and maximum (max) values for sperm quality parameters of Arctic charr (*Salvelinus alpinus*) males recorded per tank.

Parameter	Tank 1			Tank 2			Tank 3		
	Mean ± SD	min	max	Mean ± SD	min	max	Mean ± SD	min	max
SC, × 10^9/mL	3.1 ± 2.2	0.05	13.5	3.4 ± 1.9	1.52	7.11	2.8 ± 1.6	0.02	7.38
TM, %	76 ± 23	5	99	72 ± 23	9	99	67 ± 22	14	99
VCL, μm/s	77.9 ± 22.0	23.2	125.4	75.8 ± 22.5	11.9	130.7	69.8 ± 20.7	30.4	120.4
VAP, μm/s	53.9 ± 21.9	3.9	98.4	52.1 ± 21.8	2.4	100.8	44.9 ± 20.3	4.2	93.3
VSL, μm/s	39.5 ± 18.6	1.3	74.3	37.8 ± 17.8	11.4	57.1	31.5 ± 15.5	1.0	66.8

In tanks 1 and 2, animals were held with the ambient water temperature and regular (15 cm/s) or fast (30 cm/s) flow speed, respectively; in tank 3 animals were held with cooled water temperature and regular (15 cm/s) flow speed. SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity.

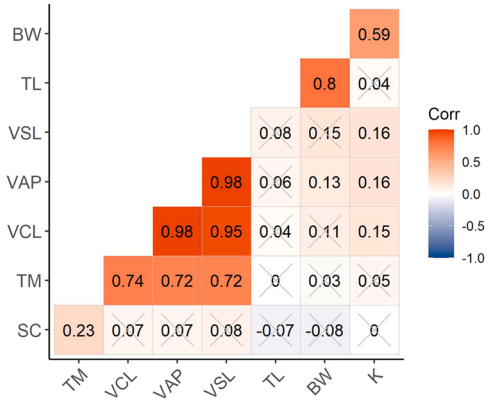

Fig. 2. Correlation matrix between sperm concentration, motility, and growth-related traits in farmed Arctic charr *(Salvelinus alpinus)*. SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity; BW: body weight; TL: total body length; K: condition factor. Crossed values denote non-significant correlation ($P > 0.05$). "Corr" stands for Spearman correlation coefficient.

data from the training set. To minimize random sampling variation, the procedure was repeated five times.

The prediction accuracy (r) of each model was calculated as the correlation between the predicted (G)EBVs in the validation group and the corresponding phenotypes (pre-corrected for the fixed effects). The following formula was used:

$$r = (\text{correlation}((\mathbf{G})\mathbf{EBV}, \mathbf{y}))$$

where ***y*** corresponds to the vector of pre-corrected for the fixed effects phenotype and ***(G)EBV*** the vector of corresponding breeding values.

3. Results

3.1. Descriptive analysis of the recorded sperm traits

The mean sperm concentration ranged from 2.8 ± 1.6 × 10^9/mL to 3.4 ± 1.6 × 10^9/mL across the three tanks; mean total motility ranged from 67 ± 22 % to 76 ± 23 %; mean velocities were between 69.8 ± 20.7 μm/s and 77.9 ± 22.0 μm/s (VCL), 44.9 ± 20.3 μm/s and 53.9 ± 21.9 μm/s (VAP), 31.5 ± 15.5 μm/s and 39.5 ± 18.6 μm/s (VSL) (Table 2).

Values of phenotypic correlations between sperm quality traits are presented in Fig. 2. The correlations were positive between TM and velocities ranging from 0.72 to 0.98 ($P < 0.001$). The SC and velocity parameters had low and non-significant phenotypic correlations (− 0.07 to 0.08, $P > 0.05$). A positive phenotypic correlation (0.23, $P < 0.001$) was obtained between SC and TM (Fig. 2).

Correlation coefficients ranging from − 0.08 to 0.15 were estimated between body weight or length and the recorded sperm traits. These values were generally not significant ($P > 0.05$), with the exception of VAP and body weight which had a significant positive correlation (0.13, $P < 0.05$). Furthermore, the condition factor and velocities (VCL, VAP, VSL) had a positive phenotypic correlation (~ 0.16, $P < 0.05$).

3.2. Correlation between inbreeding and sperm quality traits

The mean inbreeding coefficient of the sampled animals was 0.07, with the range amongst individuals between 0.03 and 0.18. A Spearman correlation coefficient close to zero (range: − 0.06 to 0.02) was obtained between the individual inbreeding coefficients and the sperm quality traits. Furthermore, the regression coefficient of the recorded sperm traits on the inbreeding was not statistically significant.

3.3. Distribution of detected SNPs

In total, 5418 SNPs were identified. Out of those SNPs, 1140 did not have a physical map location and were assigned to the "Pseudo" chromosome. The rest of the SNPs were mapped on the 39 chromosomes of the *Salvelinus sp*. genome (Fig. 3). During the quality control step, 5191 SNPs passed the QC filters and were used for downstream analysis. In addition, three animals with a genotyping call rate below 70 % were discarded. As such, 326 males were retained for further analysis.

3.4. Genetic parameter estimates for sperm quality traits

Moderate heritability estimates were obtained with both pedigree (PBLUP) and genomic-based models (GBLUP), with values ranging from 0.21 (SE 0.09) to 0.32 (SE 0.10) and from 0.23 (SE 0.09) to 0.26 (SE 0.09) respectively (Table 3, Table 4). Sperm concentration had the highest heritability among the PBLUP derived estimates. While in the case of GBLUP, the highest heritability was obtained for the VCL parameter. On average, the heritabilities estimated using the genomic data were 9–24 % higher than the estimates obtained with the pedigree-based model. However, the opposite was observed in the case of SC, where the PBLUP estimate was 28 % higher. Furthermore, the accompanying standard errors were comparable across the two models (0.09–0.10).

Genetic correlations amongst the sperm quality traits varied widely (− 0.13 to 0.99) for both models (Table 3, Table 4). High and positive genetic correlations were observed between TM and velocities using pedigree- (0.80 – 0.87; SE 0.47–0.60) or genomic-based (0.91–0.93; SE 0.49–0.23) approaches. The lowest genetic correlation was between SC and velocities (− 0.13 to − 0.09; SE 0.39 – 0.53). Finally, a positive genetic correlation was between TM and SC (~ 0.20 SE 0.38).

3.5. Genome-wide association study and WssGBLUP for sperm quality traits

GWAS was performed for sperm quality traits recorded on 326 animals using 5191 SNPs identified through ddRAD. The genome-wide association analysis identified a single SNP significantly associated

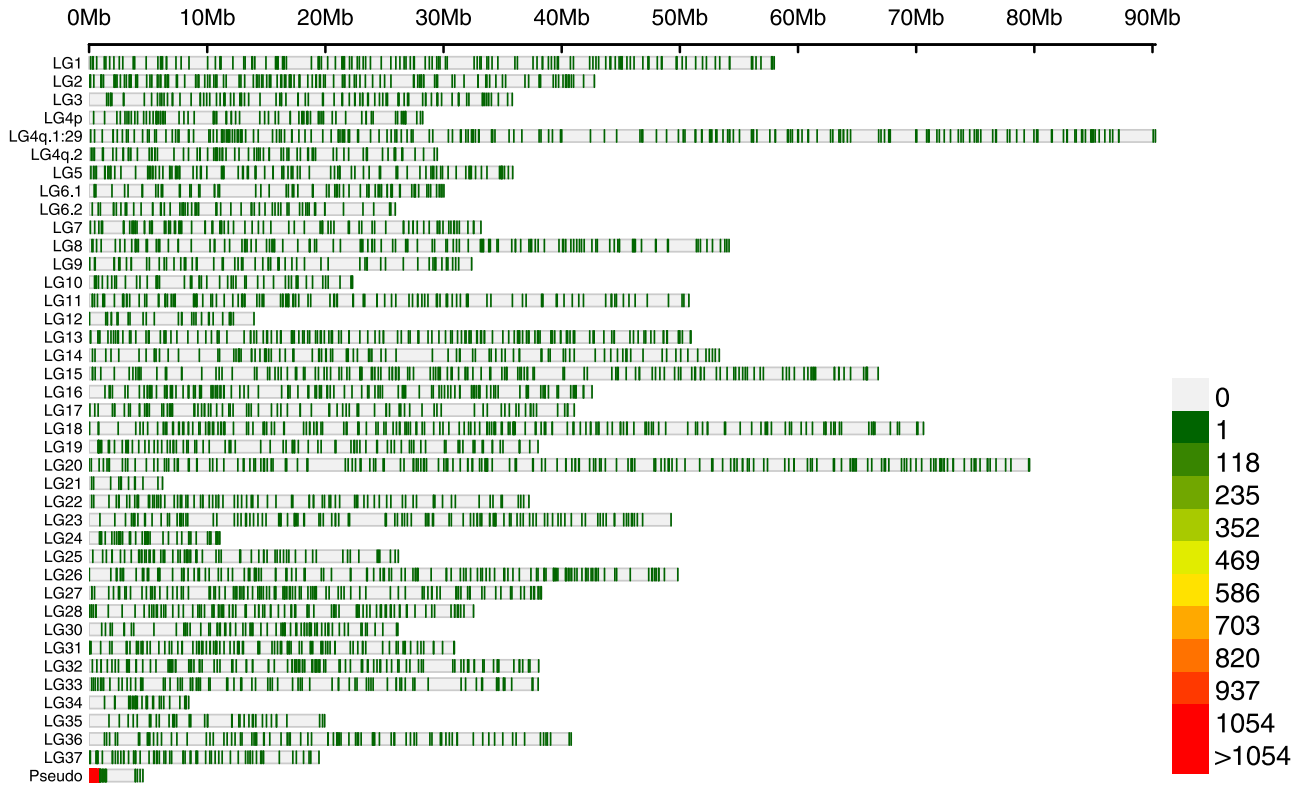

Fig. 3. SNP density within 1 Mb window size across each chromosome in farmed Arctic charr *(Salvelinus alpinus)*.

Table 3
Heritability (diagonal), genetic correlations (below diagonal), and corresponding standard errors (superscripts) for sperm traits in farmed Arctic charr *(Salvelinus alpinus)* using the pedigree relationship matrix.

Trait	SC $^{(SE)}$	TM $^{(SE)}$	VCL $^{(SE)}$	VAP $^{(SE)}$	VSL $^{(SE)}$
SC$^{(SE)}$	**0.32$^{(0.10)}$**	–	–	–	–
TM$^{(SE)}$	0.20$^{(0.38)}$	**0.21$^{(0.09)}$**	–	–	–
VCL$^{(SE)}$	– 0.09$^{(0.45)}$	0.87$^{(0.60)}$	**0.21$^{(0.09)}$**	–	–
VAP$^{(SE)}$	– 0.13$^{(0.39)}$	0.80$^{(0.47)}$	0.98$^{(0.24)}$	**0.22$^{(0.09)}$**	–
VSL$^{(SE)}$	– 0.12$^{(0.39)}$	0.82$^{(0.42)}$	0.96$^{(0.26)}$	0.99$^{(0.24)}$	**0.22$^{(0.09)}$**

SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity.

Table 4
Heritability (diagonal), genetic correlations (below diagonal), and corresponding standard errors (superscripts) for sperm traits in farmed Arctic charr *(Salvelinus alpinus)* using the genomic relationship matrix.

Trait	SC $^{(SE)}$	TM $^{(SE)}$	VCL $^{(SE)}$	VAP $^{(SE)}$	VSL $^{(SE)}$
SC$^{(SE)}$	**0.23$^{(0.09)}$**	–	–	–	–
TM$^{(SE)}$	0.22$^{(0.52)}$	**0.24$^{(0.09)}$**	–	–	–
VCL$^{(SE)}$	– 0.09$^{(0.53)}$	0.93$^{(0.23)}$	**0.26$^{(0.09)}$**	–	–
VAP$^{(SE)}$	– 0.11$^{(0.63)}$	0.91$^{(0.49)}$	0.99$^{(0.16)}$	**0.25$^{(0.09)}$**	–
VSL$^{(SE)}$	– 0.11$^{(0.57)}$	0.92$^{(0.46)}$	0.99$^{(0.18)}$	0.99$^{(0.20)}$	**0.24$^{(0.09)}$**

SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity.

with total motility (TM) on chromosome LG7 (position 10,471,337 bp) (Fig. 4), with the corresponding quantile-quantile (QQ) plots showing no indications of inflation for the observed *P* values (Fig. B.1).

Notably, within a 500 kb flanking window from the significant SNP of chromosome LG7 resides the gene *PTPN11* which has been previously associated with sperm quality traits in mammals. Furthermore, using WssGBLUP genomic regions that explained more than 1 % of additive genetic variances were suggested across 21 chromosomes. More specifically, the highest proportions of explained additive genetic variance (above 3 %) were found on chromosomes LG7 and LG13 for the TM and VCL parameters, respectively. Moreover, chromosomes LG9 and LG37 explained more than 1 % of the additive genetic variance for all the recorded sperm quality traits simultaneously (Fig. 5).

3.6. Prediction accuracies with pedigree and genomic relationships

The prediction accuracies for the recorded sperm traits ranged between 0.20 and 0.28 (SE 0.03–0.07) and 0.14–0.29 (SE 0.03–0.08) using pedigree and genomic BLUP, respectively. A 7–30 % improvement was achieved for TM and velocities (VAP and VCL) when models used genomic information. On the other hand, the pedigree model showed 36% better prediction accuracy for sperm concentration. Finally, in the case of VSL, similar accuracies from both models (Fig. 6).

4. Discussion

Traits related to fertility have been regularly targeted in livestock breeding programs (Burren et al., 2019; Khattab et al., 2021; Morton et al., 2017; Olsen et al., 2021; Rostellato et al., 2021; Sarakul et al., 2018). To the best of our knowledge, no aquaculture breeding program has placed so far particular emphasis on directly including traits related to fertility in the selection process. Notably, farmed fish have been recently highlighted as the category of farmed animals with the most evident drop in reproductive success compared to their wild counterparts (Farquharson et al., 2018). Despite the fact that a wide range of factors (e.g., related with egg quality) play a role in fertility, determining whether sperm quality traits can be improved through selection is expected to be beneficial for the Arctic charr industry.

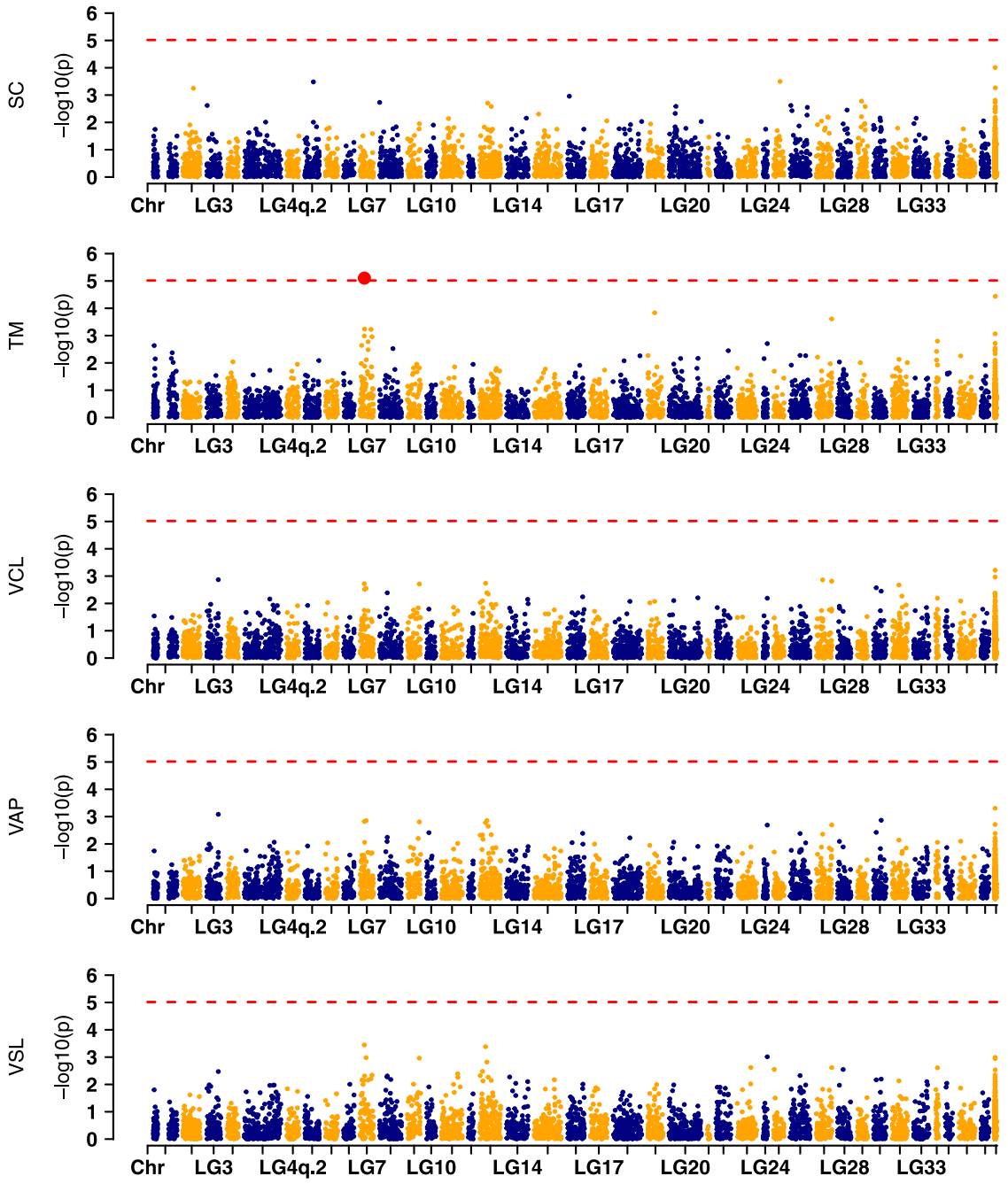

Fig. 4. Genome-wide association analysis for sperm quality-related traits in farmed Arctic charr (*Salvelinus alpinus*). SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity. The x-axis shows the SNP markers in each chromosome, and the y-axis shows the negative logarithm of *P*-values. The red horizontal line denotes the genome-wide significance threshold (Bonferroni correction).

4.1. Impact of breeding for increased growth in sperm quality traits

As previously mentioned, the Swedish Arctic charr breeding program has been selecting for increased growth rate aiming to reduce the required rearing time until harvest. Even though no universal relationship exists between growth-related traits and sperm quality, prior studies in livestock reported negative associations between these traits (Chang et al., 2017; Lavara et al., 2012). Furthermore, a significantly negative association between growth-related traits and sperm concentration ($r \sim -0.61$, $P < 0.05$) was previously reported in rainbow trout (*Oncorhynchus mykiss*), while a correlation close to zero was noted between growth parameters (weight and length) and sperm motility (Bozkurt, 2006).

Generally, no linear association was found in our study between the recorded sperm quality traits and body weight or length. However, a low positive correlation was obtained between velocities and body weight or condition factor ($r = 0.13$–0.16). Meanwhile, a low but negative correlation ($r \sim -0.10$) between curvilinear velocity and condition factor was previously reported in Atlantic cod (*Gadus morhua*) (Tuset et al., 2008). Nevertheless, the varying rearing conditions coupled with the moderate sample size of our study could have acted as confounding factors masking the true relationships among the aforementioned traits.

4.2. Effect of inbreeding accumulation on sperm quality traits

Inbreeding can negatively impact traits related to fertility in farmed

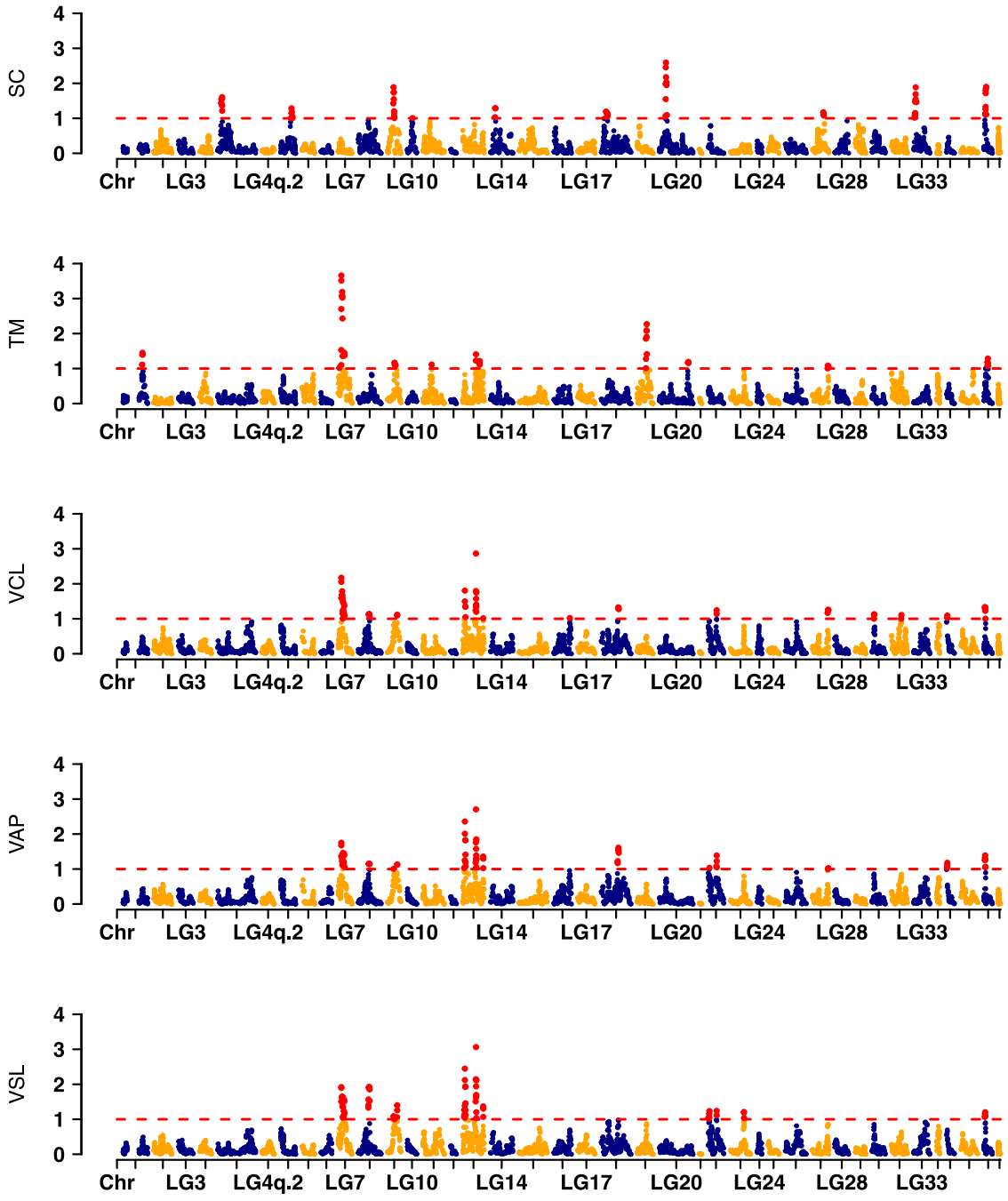

Fig. 5. Manhattan plot for WssGBLUP with the explained additive genetic variance calculated using non-overlapping windows of 10 adjacent SNPs in farmed Arctic charr (*Salvelinus alpinus*). SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity. The x-axis shows the SNP markers in each chromosome, and the y-axis shows the percentage of explained additive genetic variance. The red horizontal line denotes 1% of the explained additive genetic variance.

animals (Leroy, 2014). Particularly in the case of farmed fish, due to their high fecundity, inbreeding can accumulate rapidly if relationships amongst sires and dams are not accounted for during artificial stripping, for instance. Even though, in the case of the Swedish Arctic charr, the breeding schemes did not allow crossings between close relatives (e.g., between either full- or half-sibs), as is the case with any closed breeding nucleus, inbreeding will unavoidably increase over generations (Falconer and Mackay, 1996). Currently, the mean inbreeding coefficient of the studied Arctic charr population is approximately 7 % (Palaiokostas et al., 2020). However, males with an inbreeding coefficient of up to 18 % were used in our study.

According to the conducted analysis, no negative association was found between inbreeding and any of the recorded sperm quality traits. Even though it would not be prudent to use strict thresholds upon which inbreeding accumulation may have a negative effect on sperm quality traits, no inbreeding depression was observed on sperm motility and velocity in a hatchery-reared lake trout (*Salvelinus namaycush*) population with an average inbreeding of 25 % (Johnson et al., 2015). On the other hand, a significantly negative impact of inbreeding on male reproductive success was reported in Nile tilapia (*Oreochromis niloticus*) (Fessehaye et al., 2009). Therefore, although no negative associations were found between inbreeding and sperm quality traits, continuous monitoring of the former is required.

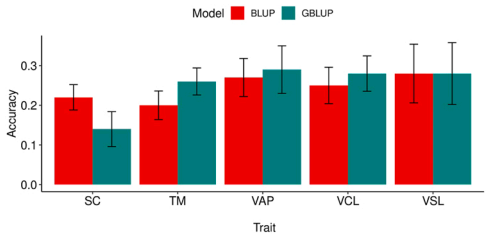

Fig. 6. Accuracy comparison between pedigree-based (BLUP) and genomic (GBLUP) prediction models for estimating breeding values in farmed Arctic charr (*Salvelinus alpinus*) with threefold cross-validation and five replicates. SC: sperm concentration; TM: total motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity.

4.3. Genetic parameters of sperm quality traits

The recorded sperm quality parameters exhibited moderate heritability, suggesting that through selective breeding sperm quality traits can be improved in Arctic charr. Since no prior heritability estimates of sperm quality traits exist for any farmed fish, no comparisons were possible. Nevertheless, our heritability estimates were comparable with ones from livestock species. More specifically, the heritability estimate for sperm concentration using the GBLUP model (0.22) was in close proximity with previous studies in livestock (0.18–0.22; Olsen et al., 2021). In terms of total motility and velocities, the heritability estimates of our study (0.21–0.24) were also within the previously reported range of 0.02–0.43 for bulls (Druet et al., 2009; Olsen et al., 2021; Yin et al., 2019) and rabbits (Lavara et al., 2012).

On the other hand, the genetic correlations estimated among the studied sperm traits were inaccurate as they were accompanied by high standard errors. Since a more complex animal breeding model is required for estimating genetic correlations, a higher sample size was most likely needed (Bijma and Bastiaansen, 2014). Therefore further investigating whether the sperm quality traits can be improved simultaneously using a larger sample size should be prioritized before breeding schemes are implemented.

4.4. Genome-wide association study on sperm quality traits

The present work is the first study aiming to detect genomic regions associated with sperm quality traits in farmed fish. Even though no major QTLs were detected through GWAS, a SNP marker located on LG7 (at 10,471,337 bp) reached the genome-wide significance threshold ($P < 1e\text{-}05$) for total motility (TM). Interestingly, within a range of 500 Kbp from the putative QTL are located genes with functions related to sperm biology and directly affecting male fertility (motility, sperm development, and maturation, fertilization ability) in mammalian species. More specifically, the group of the aforementioned genes includes PTPN11 which has been shown to be associated with sperm motility in boar, dog, and stallion spermatozoa (González-Fernández et al., 2009).

Following GWAS, we conducted a WssGBLUP analysis aiming to increase the statistical power for detecting QTL through the most efficient usage of all available data. In particular, since only a subset of the animals ($n = 466$) with phenotypic information was genotyped ($n = 326$), a WssGBLUP analysis offered concrete advantages as both pedigree and genomic relationships are taken into account during QTL scanning (Zhang et al., 2016). Obtained results suggested that the genomic region where the significant SNP was identified (LG7) explained above 3% of the additive genetic variance, while additional genomic regions associated with the sperm quality traits were suggested.

However, it should be stressed that considering the relatively low genotyping density of our study (~ 5000 SNPs), additional genomic regions affecting sperm traits may have remained undetected. As such, a higher SNP density and a larger number of genotyped animals would be required for further elucidating the underlying genetic architecture of the recorded sperm quality traits.

4.5. Prediction accuracy for sperm quality traits

A pedigree-based (PBLUP) and genomic model (GBLUP) were compared to determine the most effective method for breeding value estimation for sperm quality traits. In cases where the trait(s) of interest are controlled by major QTL(s), using genomic information is clearly the preferred option (Barría et al., 2021; Griot et al., 2021), as genotyping costs are outbalanced by the genetic gain increase. A plethora of recent studies suggested that the inclusion of genomic information can increase prediction accuracy on a wide range of commercially important traits in farmed fish (Cáceres et al., 2021; Faggion et al., 2022; Vallejo et al., 2021; Vela-Avitúa et al., 2022).

Nevertheless, especially when the trait(s) of interest have a polygenic architecture, as appears to be with the recorded sperm quality traits of our study, assessing the value of including genomic information in the selection process is of paramount importance. In terms of motility and velocity related traits, GBLUP was the preferred option with prediction accuracies 7–30 % higher compared to ones obtained through PBLUP. On the other hand, the pedigree model showed 36 better prediction accuracy for sperm concentration, indicating that genomic information, in that case, did not capture as efficiently the additive genetic variance as PBLUP.

Even though there are benefits in increasing the genotyping density, the related costs tend to increase as well. Recent reports in the literature where low genotyping density (e.g., in the magnitude of a couple of thousand SNPs) in many cases is sufficient for accurate predictions are of particular relevance to aquaculture breeding (Kriaridou et al., 2020; Tsairidou et al., 2020). On the other hand, as genomic-based models are more complex than pedigree PBLUP, a sufficiently large sample size is necessary. Therefore a follow-up study with a larger sample size, preferably spanning across year classes, would be needed for deriving more concrete conclusions about the efficiency of genomic-based models in accurately predicting sperm quality traits of breeding candidates.

5. Conclusions

Our study assessed the possibility of improving sperm quality traits in Arctic charr using selective breeding practices. The estimated genetic parameters suggested that the recorded sperm quality traits were moderately heritable and, therefore improvements through selective breeding can be achieved. No negative associations were found between inbreeding and any of the recorded sperm quality traits. Finally, a genomic region associated with sperm motility was detected in relatively close proximity (500 kbp) with genes previously linked with male fertility in mammals.

CRediT authorship contribution statement

Khrystyna Kurta: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Henrik Jeuthe:** Conceptualization, Data curation, Funding acquisition, Writing – review & editing. **Dirk Jan de Koning:** Funding acquisition, Project administration, Writing – review & editing. **Christos Palaiokostas**: Conceptualization, Data curation, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices A and B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2022.101234.

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