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Assessing Sperm Quality Parameters in Mass-Spawning Norwegian Arctic Charr

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ABSTRACT

Gaps in knowledge exist regarding sperm quality in farmed fish and its variation with age. The literature shows differing results among populations with diverse genetic backgrounds. Data collected from captive mass-spawning Norwegian Arctic charr were analysed to investigate sperm quality parameters and their age-related variations. We conducted a retrospective review of 63 milt analysis records from broodfish aged 2–9 years. Sperm motility and kinetic parameters decreased significantly ($p < 0.05$) with advancing age, whereas no significant changes were observed in sperm concentration. A regression model explaining approximately 30% of the variation (adjusted $R^2 \approx 0.3$) suggested a significant and negative effect of age on sperm swimming velocities ($p < 0.05$). Finally, the analysis of the ddRAD data did not reveal significant genetic associations. Overall, this study provides valuable insights into age-related trends and may serve as a foundation for further investigations into the sperm quality performance of populations with varying reproductive strategies and genetic structures.

1 | Introduction

Arctic charr (*Salvelinus alpinus*) is a species of ecological and economic importance in the northern aquatic systems (Olk 2021). Artificial reproduction of Arctic charr has been practiced in Canada (1980; De March and Baker 1990) and in Nordic countries (Olk 2021) like Sweden (since 1982; Eriksson et al. 2010), Norway (1970s; Jobling et al. 1998), Finland (1983; Pylkkö et al. 1996), and in Iceland since the 1970s (Olk 2021). Hatchery conditions differ significantly from natural habitats, potentially weakening the selective pressures on various phenotypic traits. These changes can impact gamete quality and, ultimately, reproductive success (Gallego et al. 2016).

In Arctic charr, even short-term captive breeding has been shown to affect sperm characteristics. More specifically, within just four generations of Arctic charr captive breeding, Kekäläinen et al. (2013) reported that captive breeding has a negative impact on motility in subsequent generations. Such changes raise concerns about the long-term fertility of cultured stocks.

To counteract these effects and preserve genetic diversity, aquaculture programmes have adopted various reproductive strategies, including controlled single-pair matings, mass spawning with genetic monitoring, cryopreservation of milt or rotational breeding schemes (Gjedrem and Baranski 2009). These methods aim to balance production goals with the maintenance

of reproductive fitness and population viability. For example, in a controlled single-pair mating system commonly used for species like Arctic charr, a high sperm-to-egg ratio is typically applied during in vitro fertilization (Brännäs et al. 2011; Nilsson et al. 2010). This approach may relax natural selection pressures, potentially leading to reduced quality in some semen traits (Kekäläinen et al. 2013). On the other hand, in the mass-spawning strategy, eggs and milt from multiple females and males are combined, which can result in variable sperm competition and differences in sperm quality (motility, velocity and longevity) among males (Haugland et al. 2009; Skjæraasen et al. 2009). Moreover, cryopreservation of milt allows to preserve genetic material and support breeding efforts, but the process can affect post-thaw sperm quality (Judycka et al. 2019; Mansour et al. 2006). Therefore, evaluating sperm quality in broodstock males subjected to different reproductive strategies is essential to understand how hatchery practices influence ejaculate traits and to identify potential risks to fertilization success.

Sperm quality in fish is influenced by a range of extrinsic and intrinsic factors. Environmental conditions, including water temperature (Jeuthe et al. 2015, 2013), photoperiod manipulation (Kurta et al. 2023; Momin and Memiş 2018), nutrition (Yıldız et al. 2021) and hatchery management practices (Kowalski and Cejko 2019; Rurangwa et al. 2004), have been shown to influence sperm motility, velocity and longevity. Additionally, intrinsic factors like male age are also known to affect sperm quality characteristics (Kowalski and Cejko 2019; Kurta et al. 2023). Age-dependent variation in male fertility has been previously demonstrated in the Swedish Arctic charr broodstock where the same males were studied across reproductive seasons (Kurta et al. 2023). More specifically, significant changes in sperm quality, including increased sperm concentration in older males and decreased sperm motility and velocity, were reported (Kurta et al. 2023). These findings highlighted the need for further investigation into how these sperm quality parameters are influenced throughout the reproductive lifespan.

This question is particularly relevant for Arctic charr, as broodfish in commercial aquaculture are frequently used across several consecutive reproductive seasons (Olk et al. 2020). However, identifying the optimal age for male broodstock is complicated by the diverse genetic background of farmed Arctic charr, which makes comparisons between hatcheries or breeding programmes challenging.

In Norway, Arctic charr farming remains a niche but dynamic industry, utilizing both recirculating aquaculture systems (RAS) and net-pens. The most widely farmed strain is of anadromous origin and is characterized by a high degree of genetic heterogeneity. As no family breeding programmes for Arctic charr are currently operational in Norway, pools of eggs from several females are fertilized by a varying number of males. This population has previously been shown to be genetically distinct from other Scandinavian strains of landlocked origin, such as those from Sweden and Finland (Palaiokostas et al. 2024; Pappas et al. 2023). As such, the unique life history and breeding strategy of the Norwegian strain may influence key biological traits, including reproductive performance and sperm quality. Although previous studies, such as Kurta et al. (2023), have explored similar traits in the Swedish population, no data are available on the genetic and phenotypic variation of sperm quality parameters amongst broodfish from the Norwegian commercial strain. Given the distinct genetic background and farming practices in Norway, understanding these variations is essential for improving male fertility.

Advances in genomic technologies, particularly high-throughput DNA sequencing, enable the detection of thousands to millions of single nucleotide polymorphisms (SNPs). These tools have proven invaluable for investigating the genetic basis of male fertility traits across a range of species, like in humans (Kolmykov et al. 2021), livestock (Blaschek et al. 2011; Han and Peñagaricano 2016; Peñ et al. 2012; Schrimpf et al. 2016) and fish (Kurta et al. 2023; Mechaly et al. 2025). Recent studies utilizing double-digest restriction-site associated DNA sequencing (ddRAD-seq) and whole-genome sequencing (WGS) have identified genomic regions associated with key reproductive traits, including sperm motility (Kurta et al. 2022) and individual variability in sperm quality across the spawning season (Kurta et al. 2023). As such, these genomic approaches can provide new insights into the underlying genetic architecture of male reproductive performance.

The current study examined sperm quality parameters such as concentration, motility and swimming velocity and their relationship with age in the most commonly farmed Arctic charr strain in Norway. By utilizing genotype data derived from ddRAD sequencing, we conducted an association study to uncover population-specific genetic markers and phenotypic patterns linked to sperm quality. Our findings aim to support broodstock management strategies that are directly relevant to the Norwegian Arctic charr industry.

2 | Materials and Methods

2.1 | Ethical Statement

This study was carried out with the license (No. A08, 017) from the Norwegian Food Safety Authority (Mattilsynet), which was attributed to the Faculty of Bioscience and Aquaculture, Nord University, to perform experiments on animals.

2.2 | Breeding Population

The studied Arctic charr population originates from a commercial farm located in Sigerfjord Fisk AS in Norway. The broodstock group was established from the Hammerfest strain (approximately 60%–70%), and the remaining part originated from Svalbard (Norway) and Iceland (personal communication with Sigerfjord Fisk AC, January 2021). The Hammerfest strain can grow in full-strength sea water and is classified as anadromous, whereas the majority of Icelandic Arctic charr production occurs in brackish water (Arnesen et al. 1993; Pappas et al. 2023). The reproductive strategy at the hatchery is based on mass spawning, with egg production taking place since 1995. The breeding population consists of 150 males and 700–800 females and is currently on its eighth generation. In this setting, no pedigree records are maintained. Instead, breeding candidates in each generation are selected on the basis of their individual

phenotype (mass selection), with a primary focus on growth. The rearing process occurs in brackish water with varying salinity levels depending on the season, with lower salinity during summer.

2.3 | Samples Collection

Sperm samples were obtained from 63 male broodfish from 4 age groups (2-, 3-, 4- and 9-year-old males). The fish was kept separated by age groups in similar freshwater tanks, and they all received the same commercial diets. Each fish was used only once in the breeding, after which they were sacrificed. The samples were collected simultaneously with the yearly commercial routines during October 2020. In brief, after sedating the fish with benzoate to reduce handling stress, males were stripped by abdominal massage for in vitro fertilization. Sperm samples were directly collected in 15 mL Falcon tubes, where they were kept on a rack on ice at 4°C until further analysis that took place within 1 h from sample collection.

2.4 | Sperm Motility

The sperm was pre-diluted in MIS (motility inactivating solution) solution from Lahnsteiner et al. (1996) prepared with 0.103 M NaCl, 0.049 M KCl, 0.001 M $CaCl_2$, 0.0008 M $MgSO_4$ and 0.02 M Hepes with pH adjusted to 7.4 at the ratio of 1 μL semen to 4 μL MIS. Motility was recorded using CASA (computer-assisted sperm analysis) equipped with the SCA 6.2 motility module from Microptics. A stage temperature controller (Linkam T95-PE, Tadworth, the United Kingdom) set at 8°C was used. The following CASA settings were previously adjusted: head area 2–50 μm, drifting 40 μm/s, fps 100 and number of images 50. Spermatozoa with curvilinear velocity (VCL) < 17 μm/s were considered static. Two-chamber Leja slides with 20-μm depth were used (6 μL each). During recording, 5.5 μL of activation solution and 0.5 μL of sperm sample were used. The usage of an activation solution, instead of tank water, was preferred for reproducibility reasons. The activation solution was the same as recommended by Judycka et al. (2019) and consisted of 1 mM $CaCl_2$, 20 mM Tris, 30 mM glycine and 125 mM NaCl supplemented with 0.5% bovine serum albumin with the pH adjusted to 9. Images were recorded 10 s after motility activation using a digital camera (Basler acA1300-200uc, Ahrensburg, Germany) attached to an optical phase-contrast microscope (Nikon Eclipse Ci, Tokyo, Japan) with ×10 negative phase contrast objective. The following CASA parameters were recorded: total sperm motility (TM) (sum of progressive, medium progressive and non-progressive spermatozoa), progressive sperm motility (PM) (sum of progressive and medium progressive spermatozoa), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s) and straight-line velocity (VSL, μm/s). Two-three readings were taken for each sample, with minimal variation observed between repeats. Individual-level distributions of sperm quality traits illustrating repeatability are shown in Figure S1. Repeated measurements were averaged per sample prior to analysis. The downstream analysis focused primarily on PM, as it explicitly represents the combination of rapid and medium motility compared to TM that also includes non-progressive spermatozoa.

2.5 | Sperm Concentration

The sperm concentration (SC, $\times 10^9$ cells/mL) was recorded manually using a Bürker cell-counting chamber as described in Lahnsteiner et al. (1999). In particular, 10 μL of a 100× prediluted sample with MIS was placed in one of the chambers with a coverslip. The cells in four diagonal squares in the field were counted, covering a total volume of 0.025 mm^3.

2.6 | Genomic DNA Extraction

Genomic DNA was extracted from fin-clip samples using a salt-based precipitation method described in Palaiokostas et al. (2022). Samples were stored in 99% ethanol. Shortly, the tissue was digested at 55°C for 4 h using a lysis solution composed of 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. After digestion, 5 μL RNAse (Thermo Fisher, Vilnius, Lithuania; 2 mg/mL) was added, and the samples were incubated at 37°C for 60 min. To precipitate proteins, 0.7 volumes of 5 M NaCl (Sigma-Aldrich, Darmstadt, Germany) were added, followed by the addition of 0.7 volumes of isopropanol to pellet the genomic DNA via centrifugation (Pico 21, Thermo Fisher, Waltham, MA, USA) at 14,000 *g* for 5 min. After overnight incubation with 75% ethanol, the DNA pellet was dissolved in 30 μL of 5 mM Tris (pH 8.0; Sigma-Aldrich, Darmstadt, Germany). The DNA concentration and quality were evaluated using a NanoDrop 8000 (Thermo Scientific, Waltham, MA, USA), agarose gel electrophoresis and a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The final DNA samples were adjusted to 15 ng/μL in 5 mM Tris (pH 8.0) and stored at 4°C prior to library preparation.

2.7 | ddRAD Library Preparation and Sequencing

We prepared ddRAD libraries for 63 samples based on the original protocol (Peterson et al. 2012) with modifications described in Palaiokostas et al. (2015). In short, 15 ng of each DNA sample were digested at 37°C for 60 min with the high-fidelity enzyme SbfI (recognizing the CCTGCA = GG motif) and the NlaIII (recognizing the CATG motif) from New England Biolabs, Ipswich, UK. Thereafter, individual-specific P1 and P2 adapters with a unique i5 or i7 bp barcode were ligated to the digested DNA. The ligation reaction was incubated at room temperature for 2 h and quenched by adding 2.5 volumes of PB buffer (Qiagen, Hilden, Germany). Then the samples were combined in a pool and purified with a MinElute PCR Purification kit (Qiagen, Hilden, Germany). Following this, the libraries were size-selected (400–600 bp) via electrophoresis on a 1.1% TAE agarose gel. 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 with QIAquick gel extraction kit (Qiagen, Hilden, Germany), library templates of 40 μL each were obtained. Then, PCR amplification was performed on a T100 thermal cycler (Bio-Rad, Redmond, WA, USA) with the following protocol: 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. The final PCR-amplified library was purified

using an equal volume of AMPure beads (Beckman Coulter, Brea, CA, USA) and eluted (20 μL) with EB buffer (MinElute Gel Purification Kit, Qiagen, Hilden, Germany). The libraries were sent for sequencing in an Illumina NovaSeq 6000 on an SP flow cell (150 base paired-end reads) at the National Genomics Infrastructure Centre in Uppsala, Sweden.

2.8 | 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 BWA-MEM algorithm (Li 2013). Following the Genome Analysis Toolkit (GATK) best practice recommendations, HaplotypeCaller and GenotypeGVCFs from GATK v4.1.4.1 (McKenna et al. 2010) were used to identify SNP variants. First, hard filtering was applied according to GATK recommendations using seven parameters: QualByDepth (QD), FisherStrand (FS), RMSMappingQuality (MQ), MappingQualityRankSumTest (MQRankSum) and ReadPosRankSumTest (ReadPosRankSum). According to the GATK guidelines, SNPs that did not meet the specified criteria: QD > 2.0, QUAL > 30.0, SOR > 5.0, FS < 60.0, MQ > 40.0, MQRankSum>−12.5, ReadPosRankSum>−8.0 were removed using a GATK VariantFiltration tool. Thereafter, the biallelic SNP genotypes were selected by GATK SelectVariants. The retained variants were filtered for minor allele frequency (MAF) above 0.05 and 99% call rate with VCFtools v0.1.16 (Danecek et al. 2011). After the filtering, out of 30,624 SNPs, we retained 1908 SNPs for the downstream analysis.

2.9 | Statistical Analysis

Descriptive statistics for the milt quality characteristics were estimated using the R programming language (R Core Team 2019). The Kruskal–Wallis test was used to compare multiple age groups, whereas Wilcoxon pairwise comparisons were performed to identify statistical differences within groups. A p value less than 0.05 was deemed statistically significant.

In subsequent analysis, all traits were standardized using the following formula:

$$x_{\text{adj}} = \frac{x_i - \mu}{\sigma}$$

Thereafter, a linear regression analysis was conducted to estimate the age effect (predicting variable) on sperm quality characteristics (response variables) using R (lm function) by implementing the following formula:

$$yi = \beta_0 + \beta_1 \times \text{Age}_i + \varepsilon_i,$$

where y_i is the observed value for the individual i, β_0 is the intercept, β_1 is the regression coefficient, and ε_i is the residual error. The residuals (ε_i) were extracted and used as age-adjusted trait values for the subsequent association study.

Thereafter, an association study between milt traits and SNPs was conducted using two approaches: linear regression in PLINK v1.9 with the '–linear' flag (Model 1) and restricted maximum likelihood (REML) analysis in GCTA that included a genomic relatedness matrix from SNPs using the '–reml' and '–grm' flags (Model 2). To investigate the potential overlap with previously identified genomic regions (Kurta et al. 2022, 2023), significant SNPs were mapped to annotated genes and compared with previously reported genomic regions.

3 | Results

3.1 | Age-Related Differences in Sperm Quality Parameters

A statistical comparison was performed to evaluate differences in sperm quality among the four age groups, including 2 ($n = 18$), 3 ($n = 10$), 4 ($n = 18$) and 9 ($n = 18$) years. The distribution of measured milt traits across age groups is given in Figures S1 and S2. Sperm concentration had no significant variation across the recorded age groups and ranged from 8.76×10^9 cells/mL (2 years of age) to 7.83×10^9 cells/mL (9 years of age); however, the standard deviation (SD) was higher in older age groups (SD: 3.5–4.3) as compared to the younger males (SD: 1.18–2.56). Average TM, which comprises progressive (PM) and non-progressive (NP) spermatozoa, dropped from 97% to 88% (non-significant, Wilcox test $p > 0.05$), with SD increasing from 7% to 20% across ages. Overall, PM, on average, changed significantly over the age groups. In particular, PM motility dropped from 96% ± 8% to 70% ± 34% (Wilcox test $p < 0.05$) and had a higher SD in the oldest age group (9 years). Spermatozoa velocities (VCL, VAP, VSL) dropped significantly (Wilcox test $p < 0.05$) as age increased: from 247.9 ± 21.9 to 136.3 ± 78.49 μm/s (VCL); from 224.6 ± 25.3 to 116.1 ± 69.8 μm/s (VAP) and from 181.3 ± 31.8 μm/s to 91.2 ± 55.2 μm/s (VSL). The 9-year-old male age group showed the highest SDs (±33.6) in sperm kinematic parameters compared to 2- and 4-year-old males (Figure 1, Table S1).

A linear regression model was employed to assess the effect size of age on sperm quality. Statistically significant results were found for both PM ($\beta = -0.04$, $p < 0.05$) and swimming velocities ($\beta \approx -0.13$, $p < 0.05$). Overall, the fitted model explained approximately 30% of the variation (adjusted $R^2 \approx 0.3$; Table 1).

3.2 | Association Study for Milt Traits

An association analysis was conducted using SNPs to detect genetic variants potentially associated with milt quality traits. No significant or suggestive associations were observed for the studied sperm traits (Figure S3a,b). A minor difference was observed between the results of the two models. More specifically, the GCTA REML analysis (Model 2), which accounted for genetic relatedness using a genomic relationship matrix (GRM), produced slightly lower p values for the PM compared to the linear association analysis in PLINK (Model 1). Additionally, the observed versus expected p value distribution for PM trait was

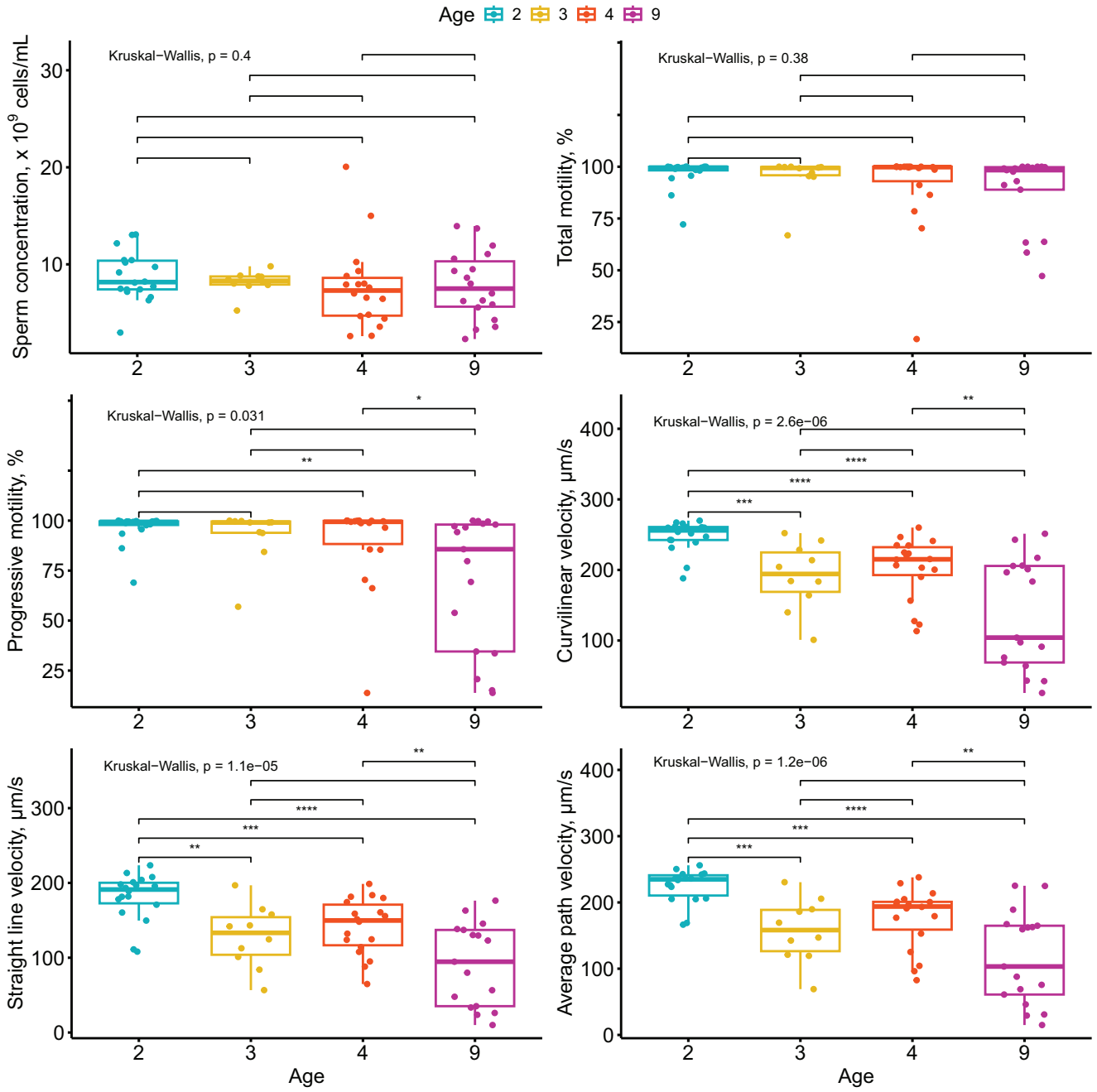

FIGURE 1 | Sperm quality parameters of Arctic charr (*Salvelinus alpinus*) males of the Norwegian population at different ages. Asterisks indicate statistically significant differences (Wilcox test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.00001$).

more inflated in Model 1 (Figure S3a), suggesting potential issues with model performance.

4 | Discussion

In the current study, we assessed milt quality parameters in mass-spawned Arctic charr broodstock from Norway across ages (2, 3, 4 and 9 years). Our findings revealed no significant variation in sperm concentration across age groups. However, sperm motility declined significantly in 9-year-old males compared to those aged 2–4 years. Notably, although PM remained high in 2- and 4-year-old males, sperm swimming velocities exhibited a steady and significant decline in the other groups. Furthermore, linear regression confirmed a significant negative effect of age on spermatozoa motility ($\beta \approx -0.04$, $p < 0.05$) and on velocities ($\beta \approx -0.13$, $p < 0.05$), indicating a progressive decay in sperm function with increasing age. As already mentioned, age-related sperm quality changes were also studied in Arctic charr males from the Swedish breeding programme (Kurta et al. 2023). Unlike the Swedish Arctic charr, where sperm production significantly increased in 5-year-old males compared to 4-year-olds, our findings in the Norwegian broodstock are limited by a gap in age data between 4 and 9 years, making it unclear when reproductive parameters reached their peak. However, in terms of sperm motility, the results of the two studies are consistent,

TABLE 1 | Summary from fitting a univariate linear model using male age as predictor and sperm quality traits as the response variables.

Parameter	Estimate	Standard error	*t* value	*p* value	Adjusted R^2	*F*-statistic	Residual standard error	CI lower	CI upper
SC	−0.14	0.15	−0.92	0.36	0.003	0.84	3.24	−0.43	0.16
PM	−0.04	0.01	−3.84	0.00	0.18	14.71	0.22	−0.06	−0.02
VCL	−14.26	2.41	−5.92	0.00	0.35	34.99	52.97	−19.08	−9.44
VAP	−13.39	2.35	−5.69	0.00	0.34	32.38	51.72	−18.10	−8.69
VSL	−11.19	1.99	−5.63	0.00	0.33	31.69	43.72	−15.18	−7.22

Abbreviations: PM, progressive motility; SC, sperm concentration; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity.

with younger males exhibiting higher motility and swimming velocity.

The effect of age on milt quality was extensively studied in other salmonids, like rainbow trout *Oncorhynchus mykiss* (Risopatrón et al. 2018; Shamspour and Khara 2016). Unlike our results for Arctic charr, a prior study on rainbow trout brood males demonstrated a significant drop in sperm concentration from 13.5 in 3-year-olds to 5.7 × 10^9 cells/mL in 5-year-old males (Shamspour and Khara 2016). Similar to our study, sperm motility in 4-year-old trout broodstock had lower motility (80%) than 2- and 3-year-old males (92%–97%) (Gallego and Asturiano 2018; Risopatrón et al. 2018).

Direct comparisons of milt quality between studies on Arctic charr are challenging due to differences in milt assessment methods and handling protocols. In the present study, sperm concentration was assessed manually, whereas an automated system was used in the Swedish population. Despite the difference in methods, we believe that this had minimal impact on sperm concentration values. However, for sperm motility and velocity traits, different activation solutions and CASA systems have been employed across the aforementioned studies on Norwegian, Swedish and Finnish Arctic charr populations. These methodological differences are known to significantly influence measures of sperm quality characteristics and must be considered when interpreting the results.

Nevertheless, literature data from these hatchery-reared populations provide a valuable reference, offering insight into the general scale of variation in milt quality among Arctic charr broodstocks. For instance, sperm concentration in the mass-spawned Norwegian charr (7.6–8.7 × 10^9 cells/mL) is considerably higher than those previously reported in the Swedish Arctic charr strain of 2.01–4.68 × 10^9 cells/mL for 4- and 5-year-old males (Kurta et al. 2023). Similarly, progressive sperm motility was substantially higher in the Norwegian population (91%–97%) compared to the Swedish broodstock males (52%–84%). Records of the spermatozoa velocity on average were also higher in the Norwegian (VCL ≈ 191.3–247.9 μm/s) than in the Swedish strain (VCL ≈ 99–150 μm/s) when comparing relatively similar age groups (3–5 years old). A similarly lower range of VCL values (VCL ≈ 110–135 μm/s) was observed in Arctic charr from a Finnish hatchery (Janhunen et al. 2009). Observed differences in sperm quality characteristics between populations can, at least in part, be attributed to the different genetic backgrounds, as many traits related to sperm function, such as motility and concentration, have a heritable genetic basis (h^2 ≈ 0.25; Kurta et al. 2022). The Norwegian, Swedish and Finnish populations are genetically distinct (Palaiokostas et al. 2024; Pappas et al. 2023), reflecting adaptations to different environments, mating systems and demographic histories, including variation in origin, inbreeding levels and selection pressures. In addition, reproductive management practices differ: Paired fertilization is used in Swedish (Brännäs et al. 2011) and Finnish (Janhunen et al. 2009) broodstock, whereas mass spawning is practiced in the studied Norwegian population. These contrasting fertilization strategies may impose distinct selective pressures on male fertility traits (Haugland et al. 2009; Kekäläinen et al. 2013), potentially contributing to the observed variation in sperm quality.

Furthermore, reduced genetic diversity has been suggested as a potential factor negatively affecting reproductive traits (de los Ríos-Pérez et al. 2017; Gallardo et al. 2004; Tsheten et al. 2023; Zhang et al. 2022), particularly in populations subjected to selective breeding for production-related traits (D'Ambrosio et al. 2019; Rauw et al. 1998). This is particularly relevant when comparing Arctic charr broodfish from the Norwegian and Swedish populations. The Norwegian broodstock has undergone mass selection based on phenotypic traits (Pappas et al. 2023), whereas the Swedish broodstock has been selectively bred using pedigree-based methods (Nilsson et al. 2010). These distinct breeding strategies may influence genetic diversity and, consequently, milt quality characteristics in these populations. Our previous study (Pappas et al. 2023), on the basis of WGS data, estimated genomic inbreeding levels for the same Norwegian population used in the present study at 6% and 9% in the Swedish population (Pappas et al. 2023). These values indicate that slightly higher inbreeding in the Swedish breeding population could contribute to lower milt quality. Unfortunately, the available data in this study were insufficient to assess the impact of inbreeding levels on sperm quality metrics. Our previous estimates in the Swedish selectively bred strain found no significant association between individual inbreeding levels (3%–18%) and recorded sperm quality traits, with correlation coefficients (r^2) ranging from −0.06 to 0.02 (Kurta et al. 2022). Similarly, Johnson et al. (2015) reported no inbreeding depression on sperm quality in hatchery-reared lake trout (*Salvelinus namaycush*) with inbreeding levels of about 25%. In addition, relaxed selective pressures and minimal or entirely absent male competition under hatchery conditions may reduce the evolutionary effect to maintain high sperm quality, potentially contributing to the decline in traits such as sperm velocity (Kekäläinen et al. 2013).

Finally, in this study, we also attempted to identify population-specific genetic markers and genomic regions associated with the recorded sperm quality traits and to investigate potential overlaps with those previously reported in the Swedish broodstock (Kurta et al. 2022, 2023). However, factors like insufficient sample size and the relatively low number of SNPs ($n = 1908$) led to the lack of significant or suggestive associations.

5 | Conclusions

Overall, sperm motility in mass-spawning Arctic charr from Norwegian broodfish declined significantly with age, whereas sperm concentration remained relatively stable across age groups. This suggests that although sperm production does not diminish, the functional quality of sperm, particularly traits critical for fertilization such as motility and velocity, deteriorates in older males, potentially reducing their reproductive success. Regression analyses further supported these patterns, which confirmed a significant negative effect of age on sperm motility and velocity but not on sperm concentration. The general overview of sperm quality parameters in different Arctic charr broodstocks suggests that reproductive strategies and the population genetic background may partially explain the observed variations. However, environmental factors such as feeding regimes, photoperiod manipulation and other hatchery management practices could also have contributed. Future research combining controlled environmental data with genetic analyses would be valuable for understanding these effects and optimizing broodstock management across aquaculture settings.

Author Contributions

Khrystyna Kurta: Data analysis, Data Visualization, Writing - original draft, Writing - review & editing. **José Beirão**: Writing - review & editing. **Benjamin Thomason**: Data collection. **Christos Palaiokostas**: Funding acquisition, Writing - review & editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1 Individual-level distributions of sperm quality traits measured two to three times in Norwegian Arctic charr (*Salvelinus alpinus*) males.

Figure S2 Distributions of sperm quality parameters measured in Norwegian Arctic charr (*Salvelinus alpinus*) males across 2, 3, 4 and 9 age groups.

Figure S3 Manhattan plot showing the −log10(*p* values) (*y*-axis) for each single nucleotide polymorphism (SNP) identified through ddRAD sequencing in relation to sperm quality parameters in Norwegian Arctic charr (*Salvelinus alpinus*). Model 1 presents result from the PLINK linear association analysis, whereas Model 2 displays results from the GCTA REML analysis. The horizontal red and blue lines indicate the Bonferroni-corrected significance thresholds corresponding to significance levels of $\alpha = 0.05$ and $\alpha = 0.3$, respectively.

Table S1 Descriptive statistics for Norwegian Arctic charr (Salvelinus alpinus) sperm quality parameters samples across four age groups.