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Comparison of sperm motility subpopulation structure among wild anadromous and farmed male Atlantic salmon (*Salmo salar*) parr using a CASA system

Carina Caldeira[A,B,E], *Almudena García-Molina*[A], *Anthony Valverde*[B,C], *Daznia Bompart*[A], *Megan Hassane*[B], *Patrick Martin*[D] *and Carles Soler*[A,B]

[A]PROISER R+D, Av. Catedrático Agustín Escardino, 9, Building 3 (CUE), Floor 1, 46980 Paterna, Spain.
[B]University of Valencia, Faculty of Biological Sciences, Campus Burjassot, C/ Dr Moliner 50, 46100 Burjassot, Spain.
[C]Technological Institute of Costa Rica, San Carlos Campus, School of Agronomy, 223-21001 Alajuela, Costa Rica.
[D]Conservatoire National du Saumon Sauvage, Larma, 43 300 Chanteuges, France.
[E]Corresponding author. Email: carina.caldeira@proiser.com

Abstract. Atlantic salmon (*Salmo salar*) is an endangered freshwater species that needs help to recover its wild stocks. However, the priority in aquaculture is to obtain successful fertilisation and genetic variability to secure the revival of the species. The aims of the present work were to study sperm subpopulation structure and motility patterns in wild anadromous males and farmed male Atlantic salmon parr. Salmon sperm samples were collected from wild anadromous salmon (WS) and two generations of farmed parr males. Sperm samples were collected from sexually mature males and sperm motility was analysed at different times after activation (5 and 35 s). Differences among the three groups were analysed using statistical techniques based on Cluster analysis the Bayesian method. Atlantic salmon were found to have three sperm subpopulations, and the spermatozoa in ejaculates of mature farmed parr males had a higher velocity and larger size than those of WS males. This could be an adaptation to high sperm competition because salmonid species are naturally adapted to this process. Motility analysis enables us to identify sperm subpopulations, and it may be useful to correlate these sperm subpopulations with fertilisation ability to test whether faster-swimming spermatozoa have a higher probability of success.

Additional keywords: Bayesian method, CASA-Mot system.

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Introduction

Atlantic salmon (*Salmo salar*) is a cultured fish species with a high economic value, which generates significant revenue from both wild catches and fish farming (Hindar *et al.* 2006). However, the total annual catch of wild Atlantic salmon in the North Atlantic has shown a marked decline during recent decades from approximately 12 000 t in 1973 down to 1200 t in 2016 (NASCO 2016). The commercial response to this problem is in the conservation, restoration, enhancement and rational management of wild salmon in the North Atlantic. By focusing on biology, the aims of scientific projects are to increase fish survival and improve their behavioural adaptation to natural conditions. In a conservation program, it is essential to secure the revival of the species by ensuring genetic variability (Rurangwa *et al.* 2004), using wild broodstock from the local habitat. The priority in reproduction for restocking is to ensure that all males contribute towards successful fertilisation. However, it is important to know the differences in sperm motility among males in order to maintain the genetic integrity of the broodstock used (McGinnity *et al.* 1997, 2003; Rurangwa *et al.* 2004). In addition, knowledge of sperm characteristics (velocity, optimal activation time and subpopulation structures) will help improve fertilisation procedures (Bobe and Labbé 2010; Fauvel *et al.* 2010).

In fish species with external fertilisation, the quest for reproductive success among competing males can lead to several adaptations, including behavioural, morphological and physiological, to enhance the competitiveness of their spermatozoa (Beatty *et al.* 1969; Parker 1970; Smith 1984; Järvi 1990; Lahnsteiner *et al.* 1993; Gage *et al.* 1995; De Gaudemar and Beall 1999). The physiological quality of spermatozoa differs between competing males because of differences in investment in gametes and sperm production (Ball and Parker 1996;

Taborsky 1998; Vladić and Järvi 2001). Sperm quality is generally correlated with fertilising ability, which is often used as a determining factor in studies on sperm competition (Stoss 1983; Bencic *et al.* 1999; Levitan 2000). During spawning, the number of spermatozoa released and their swimming speed can affect the probability of fertilisation (Stoltz and Neff 2006; Taborsky 1998). Faster swimming cells may be able to reach the egg first, increasing the probability of successful fertilisation (Gage *et al.* 2004; Stoltz and Neff 2006). Atlantic salmon is an anadromous species that migrates up rivers from the sea in order to breed. In the case of this species, spawning males are characterised by intense sexual competition, which confers strong selective pressure on their reproductive physiology (Vladić and Järvi 2001). The wild salmon population can exhibit a wide natural variation in sperm traits leading to sperm competition (Gage *et al.* 1995; Gage *et al.* 2004). However, mature parr males also participate in the spawning, which means that they need to invest more in sperm production than anadromous males (Parker 1998).

Sperm quality can be assessed using simple methods, such as individual analysis of motility (Billard and Cosson 1992) and morphology (Holstein *et al.* 1988; Munkittrick *et al.* 1992), or sophisticated approaches involving molecular tools (Cabrita *et al.* 2014). Motility is the parameter that is most used to assess sperm quality because it directly reflects the fertilising ability of the spermatozoa. Initially, the percentage of motile spermatozoa and/or the duration of their motility were evaluated using subjective visual estimates. However, computer-aided sperm analysis (CASA-Mot) systems were developed to provide more reliable, repeatable and objective measurements of sperm movement (Rurangwa *et al.* 2004). Even though CASA technology was designed for mammalian species (Rurangwa *et al.* 2004), it is well adapted to fish spermatozoa (Gallego *et al.* 2013; Kime *et al.* 2001), which are characterised by a short period of vigorous motility after activation. In general, fish spermatozoa remain active for less than 2 min in most aquatic species and, in the case of salmonids specifically, the duration of sperm motility is around 20–30 s (Kime *et al.* 2001).

CASA-Mot systems provide a large amount of data based on the kinematic parameters of each spermatozoon. Applying subpopulation analysis to such data allows for the analysis of groups of spermatozoa with similar motility features and to estimate of sperm quality for each male (Gil Anaya *et al.* 2015; Soler *et al.* 2014). A subpopulation characterised by rapid linear movement has been proposed as an indicator of high-quality spermatozoa (Martínez-Pastor *et al.* 2005, 2011; Ferraz *et al.* 2014). Variations in subpopulation distributions have been reported for several species, including boar (Flores *et al.* 2009; Quintero-Moreno *et al.* 2004), bull (Muiño *et al.* 2009; Valverde *et al.* 2016), red deer (Martínez-Pastor *et al.* 2005), stallion (Quintero-Moreno *et al.* 2003), cat (Contri *et al.* 2012; Gutiérrez-Reinoso and García-Herreros 2016), dog (Núñez-Martínez *et al.* 2006), fowl (García-Herreros 2016), rooster (García-Herreros 2016), human (Santolaria *et al.* 2016; Vásquez *et al.* 2016; Yániz *et al.* 2016), gilthead seabream (Beirão *et al.* 2011) and steelhead (Kanuga *et al.* 2012). This statistical methodology has improved knowledge regarding sperm quality, although it is still used primarily as a research tool (Gil Anaya *et al.* 2015).

A new concept of data analysis based on probabilistic modelling with Bayesian networks (BNs) has been proposed to improve the study of associations between variables (Thompson *et al.* 2004; Daly *et al.* 2011). A BN approach represents relationships of independence and dependence among variables and provides a probabilistic distribution between them (Yet *et al.* 2014). The networks established by BN methodology can be applied to almost any type of investigation and help researchers make assumptions with regard to the problem being studied (Jensen and Nielsen 2007).

Frequentist and Bayesian analyses are two different statistical approaches that can be used in biological studies. Frequentist methods consider a fixed parameter of interest and a random parameter of the sampled data. The results, expressed as *P*-values, provide uncertainty about the hypothetical collection of data, helping to reject the original parameter value. Thus, the inability to learn and adapt to new information is a weakness of the frequentist approach. The Bayesian method is completely different in that the data are fixed and the parameter is random or uncertain. In this case, the result identifies all possible parameter values and gives a probability plot for these values. Bayesian estimates are influenced by previously held beliefs and information resulting from a clinical test. The traditional frequentist and Bayesian approaches produce analogous results when there is no useful *a priori* information (Ashby and Smith 2000; Carlin and Louis 2000).

In the present study, fish sperm samples were collected to evaluate motility patterns and the sperm subpopulation structure in wild anadromous male fish and two generations of offspring of farmed mature parr males of Atlantic salmon, using two different statistical approaches. First, an analysis of subpopulations was conducted using motility variables to identify the different sperm subpopulations and clarify their physiology from each group of male fish. Subsequently, the BN method was used to investigate the relative importance of this statistical analysis when determining sperm quality based on motility.

Materials and methods

Broodstock conditions

Wild and cultured male Atlantic salmon were sampled at the Conservatorie National du Saumon Sauvage (Chanteuges, France). The hatchery station is located in the Allier River, which is a tributary of the Loire River. Anadromous fish were captured in the wild and maintained in the hatchery. Wild salmon breeders (WS) were used to produce two different types of offspring (Fig. 1). A cross between wild male and female salmon produced one generation of farmed salmon (FS1), whereas a second generation of salmon offspring (FS2) was produced by crossing wild anadromous male (WS) and farmed salmon female fish (FS1). In the hatchery, photoperiod and temperature were adjusted to simulate the environmental conditions of the area.

Sperm collection

Sperm samples were collected from mature WS and farmed parr (FS1 and FS2) males during the reproductive season, from October to December. Six specimens from each type of mature

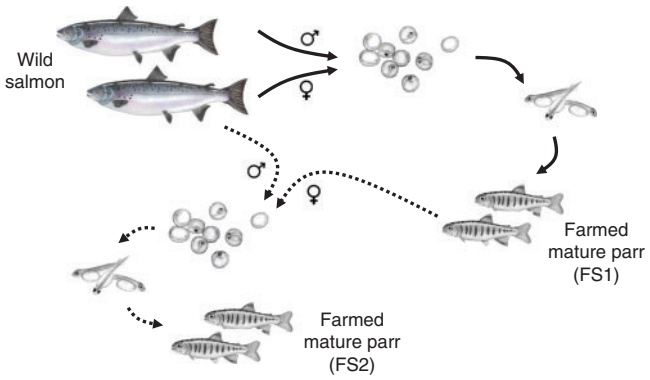

Fig. 1. Diagram of broodstock crossings between anadromous and farmed parr males. FS1 offspring (farmed parr salmon) were produced by a cross between wild anadromous (WS) male and female fish (solid line); FS2 offspring (farmed parr salmon) were produced by crossing WS male and FS1 female fish (dashed line).

male were selected at random ($n = 18$). Sexually mature males were anaesthetised (MS-222; 1 mL L^{-1}) before sperm collection by abdominal massage. Care was taken to avoid contamination of the samples with faeces and urine by cleaning the genital area with fresh water and drying it thoroughly. Samples were stored at 4°C in plastic tubes until analysis.

Sperm motility evaluation

Sperm motility was assessed using a CASA-Mot system (Integrated Semen Analysis System (ISAS) v1; Proiser R&D) coupled with a phase contrast microscope (UOP; Proiser R&D) with a ×10 negative phase contrast objective. Video files were recorded and analysed at a rate of 200 frames s^{-1} (MQ003MG-CM; XIMEA) 5 and 35 s after activation. Sperm motility was activated by mixing a drop of ejaculate with 2 µL distilled water in a commercially produced chamber (Spermtrack; Proiser R&D; depth 10 µL). Each sample was analysed in triplicate. In the present study, particle area (μm^2) and several motility parameters were considered, namely: total motility (MOT; %), defined as the percentage of motile cells within the sample; curvilinear velocity (VCL; µm s^{-1}), defined as the velocity of a sperm head along the real curvilinear trajectory; straight line velocity (VSL; µm s^{-1}), defined as the velocity of a sperm head along the straight line between the first and last detected position; angular path velocity (VAP; µm s^{-1}), defined as the velocity of sperm head along a derived, smoothed trajectory path; straightness (STR; %), defined as the linearity of the angular path distance and calculated as VSL/VAP; and linearity (LIN; %), defined as the ratio of the straight line distance to the real path distance (VSL/VCL). Other parameters that were also determined using the ISAS v1 system included wobble (WOB; %), the amplitude of lateral head movement (ALH; µm) and beat cross frequency (BCF; Hz). Software settings were adjusted for salmon sperm analysis: 2–90 µm for the head area and 6 µm for connectivity.

Statistical analysis

The data obtained from the analysis of all kinematic parameters were first tested for normality and homoscedasticity using the Shapiro–Wilk and Kolmogorov–Smirnov tests respectively. Clustering procedures were performed to identify sperm subpopulations from the complete set of motility data (Vicente Fiel *et al.* 2013). The first step was to perform a principal component analysis (PCA). The number of principal components (PC) that should be used in the next step of the analysis was determined using the Kaiser criterion, namely selecting only those with an eigenvalue (variance extracted for that particular PC) >1. The second step was to perform a two-step cluster procedure with the sperm-derived indices obtained after the PCA. All sperm cells within each generation and time after activation were clustered using a non-hierarchical clustering procedure (*k*-means model and Euclidean distance). This analysis enabled the identification of sperm subpopulations and the detection of outliers.

The effects of clusters between treatments for measuring motility parameters were analysed using the Kruskal–Wallis test, followed by the Mann–Whitney paired non-parametric *U*-test when significant differences were found. The effects of generation, time after activation and individual male fish on the relative distribution frequency of sperm subpopulations was analysed using Chi-squared and Mantel-Haenszel Chi-squared tests. After characterising sperm subpopulations, one-way analysis of variance (ANOVA) was used to explore relationships between the proportions of each sperm subpopulation in the sample. Results are presented as median values with the interquartile range (IQR) or as the mean ± s.d. Statistical significance was set at $P < 0.05$ (two-sided). All data were analysed using InfoStat Software v.2017 (Nacional University of Córdoba, Argentina) for Windows, as described previously (Di Rienzo *et al.* 2017).

Differences in sperm motility indicators were estimated using a model that included the effects of generation and time after activation as permanent effects. All analyses were performed using Bayesian methodology. The posterior median of the difference between generations (D), the highest posterior density region at 95% ($HPD_{95\%}$) and the probability (P) of the difference being positive when $D > 0$ or negative when $D < 0$ were calculated. Bounded uniform priors were used for all effects. Residuals were normally distributed *a priori* with mean of 0 and a variance of σ2e. The priors for the variances were also bounded uniformly. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola 2002) and Monte Carlo sampling errors were computed using the time series procedures described in Geyer (1992). The Rabbit program, developed by the Institute for Animal Science and Technology (Valencia, Spain), was used for all procedures.

Results

General results

Sperm motility of WS and both FS1 and FS2 parr males was observed over time and the percentage of motile spermatozoa in samples from all fish evaluated was remarkably high (Fig. 2). The percentage of motile spermatozoa 5 s after activation was 82.9 ± 15.5%, 86.5 ± 12.3% and 82.3 ± 14.0% for WS and FS1 and FS2 parr males respectively. The percentage motile

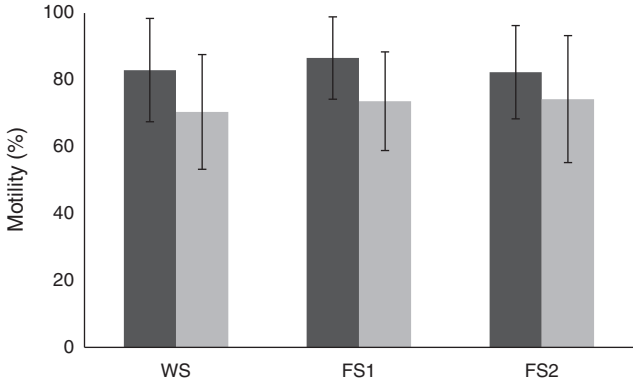

Fig. 2. Percentage of motile spermatozoa in samples from wild anadromous male (WS) and the two groups of farmed parr male offspring (FS1 and FS2) 5 s (dark grey) and 35 s (light grey) after activation. FS1 offspring were produced by crossing male and female WS fish; FS2 offspring were produced by crossing WS males and FS1 females. Data are the mean ± s.d.

spermatozoa in samples 35 s after activation decreased to 70.4 ± 17.2%, 73.6 ± 14.7% and 74.2 ± 19.0% for WS and FS1 and FS2 parr males respectively. Farmed parr males showed lower variability in the percentage of motile cells 5 s after activation.

A preliminary analysis using Pearson's correlation coefficient revealed that the nine CASA-Mot kinematic variables had negative correlations with time, whereas the provenance of the male fish had no substantial effect on these variables (data not shown). In addition, velocity (VCL, VSL and VAP), progressivity (LIN and STR), ALH and BCF showed the strongest correlations with time, with values for all parameters decreasing over time ($P < 0.0001$).

PCA and subpopulations

The PCA was performed to reduce the dimensionality of the present study, which included nine CASA-Mot kinematic variables (VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF and area) for anadromous males and farmed parr males for each time after activation (5 and 35 s). Two PCs with a smaller number of variables were selected and explained 76% of the total variance of all data (Table 1). PC1, designated 'velocity', was positively related to the velocity parameters (VCL, VSL, VAP) and BCF, whereas PC2, designated 'linearity', was positively related to progressivity parameters (LIN, STR) and negatively related to ALH and area.

These two PCs were used to identify three well-defined subpopulations (SP1, SP2 and SP3; Fig. 3), characterised by median values of the nine CASA-Mot parameters (Table 2). SP1 included spermatozoa characterised by low speed (VCL, VSL and VAP) and low linear trajectories (LIN and STR). This subpopulation represented 47.3% of the total sample and was considered as a slow non-linear subpopulation. SP2 accounted for 34.9% of total motile cells and was characterised by high velocity but low linear trajectories; this group was designated as a fast and non-linear subpopulation. Spermatozoa in SP3 had both high velocity and linear trajectories, and this group was

Table 1. Values of the two principal components obtained in the study of sperm motility for wild anadromous (WS) males and two groups of farmed parr male offspring (FS1 and FS2) 5 and 35 s after activation

FS1 offspring were produced by crossing male and female WS fish; FS2 offspring were produced by crossing WS males and FS1 females. PC1, principal component designated 'velocity'; PC2, principal component designated 'linearity'; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; LIN, linearity; STR, straightness; WOB, wobble; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; Area, particle area. The table shows only values of covariance >0.3 for each principal component. Important values are n bold font

	PC1	PC2
VCL (μm s^{-1})	**0.40**	
VSL (μm s^{-1})	**0.42**	
VAP (μm s^{-1})	**0.41**	
LIN (%)	0.32	**0.49**
STR (%)	0.30	**0.48**
WOB (%)		
ALH (μm)	0.31	**−0.44**
BCF (Hz)	**0.41**	
Area (μm^2)		−0.36
Explained variation (%)	57	19

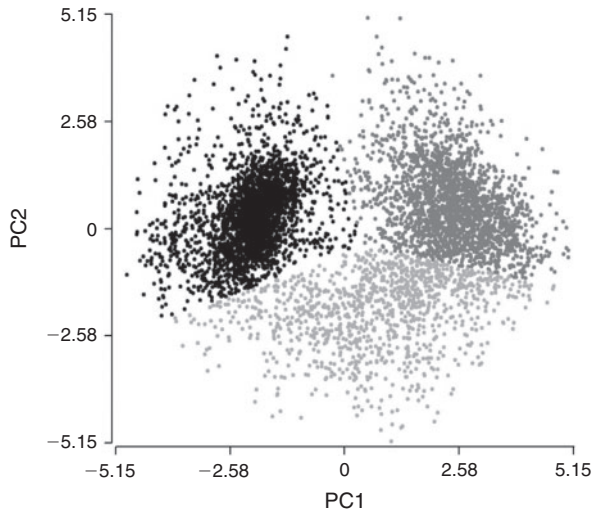

Fig. 3. Distribution of the motile sperm subpopulations after principal component and Kruskal–Wallis analysis. Three sperm subpopulations were identified: SP1 (black), with low speed and low linear trajectories; SP2 (grey), a fast and non-linear subpopulation; and SP3 (dark grey), with high velocity and linear trajectories.

designated as a fast and linear subpopulation and accounted for 17.8% of all cells. Non-parametric comparisons showed the existence of statistically significant differences ($P < 0.0001$) between the three subpopulations for eight of the CASA-Mot variables. ALH was the only variable that did not differ significantly between SP1, SP2 and SP3.

The percentage of spermatozoa in each subpopulation varied over time in the WS, FS1 and FS2 groups (Fig. 4). The proportional size of SP1 increased significantly 35 s after

Table 2. Descriptive statistics for the nine CASA-Mot variables for each sperm subpopulation in samples from wild anadromous (WS) and farmed parr (FS1 and FS2) males 5 and 35 s after activation (n = 6447 total spermatozoa)

Unless indicated otherwise, data are given as the median (interquartile range). Within rows, values with different superscript letters differ significantly ($P < 0.05$). FS1 offspring were produced by crossing male and female WS fish; FS2 offspring were produced by crossing WS males and FS1 females. SP1, slow non-linear subpopulation; SP2, fast and non-linear subpopulation; SP3, fast and linear subpopulation; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; LIN, linearity; STR, straightness; WOB, wobble; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; Area, particle area

	SP1	SP2	SP3
No. **spermatozoa** (%)	3050 (47.3)	2250 (34.9)	1147 (17.8)
VCL (μm s^{-1})	110.10^{a} (96.40–122.40)	250.30^{b} (218.30–284.20)	242.94^{b} (218.30–270.60)
VSL (μm s^{-1})	36.60^{a} (28.30–44.60)	72.60^{c} (31.40–107.00)	159.70^{b} (139.90–178.50)
VAP (μm s^{-1})	73.10^{a} (63.20–81.20)	183.30^{c} (162.60–200.70)	183.81^{b} (168.40–198.50)
LIN (%)	33.80^{a} (27.00–40.80)	28.60^{c} (14.30–41.40)	65.70^{b} (59.90–71.30)
STR (%)	51.80^{a} (41.20–64.10)	41.60^{c} (19.60–58.30)	88.70^{b} (82.90–92.60)
WOB (%)	66.90^{a} (61.10–71.30)	71.30^{c} (66.40–76.60)	75.10^{b} (71.10–79.50)
ALH (μm)	1.10 (1.00–1.10)	1.50 (1.40–1.71)	1.40 (1.20–1.50)
BCF (Hz)	21.90^{a} (15.90–27.80)	89.34^{c} (71.60–103.20)	90.90^{b} (76.92–103.50)
Area (μm^2)	14.20^{a} (12.50–16.50)	16.30^{c} (14.60–18.40)	14.50^{b} (12.90–16.40)

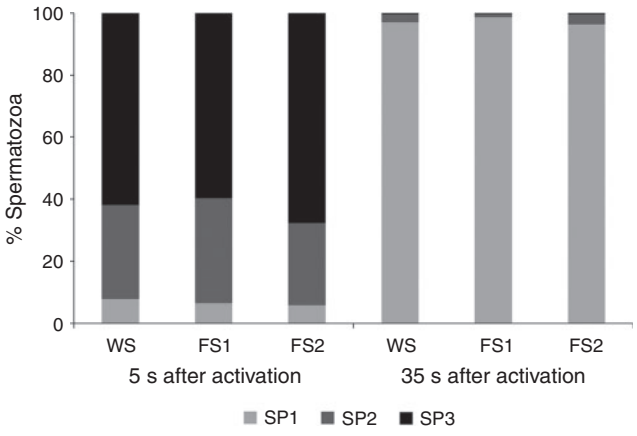

Fig. 4. Percentage of each sperm subpopulation in samples from wild anadromous (WS) and two groups of farmed parr (FS1 and FS2) males 5 and 35 s after activation). FS1 offspring were produced by crossing male and female WS fish; FS2 offspring were produced by crossing WS males and FS1 females. Three sperm subpopulations were identified: SP1 (grey), with low speed and low linear trajectories; SP2 (dark grey), a fast and non-linear subpopulation; and SP3 (black), with high velocity and linear trajectories.

activation, whereas the opposite was observed for SP2 and SP3. At the beginning of the analysis, most of the spermatozoa from all analysed males were classified as either SP2 or SP3 (i.e. 30.4% and 61.8% respectively for WS males; 33.8% and 59.7% respectively for FS1 males; and 26.4% and 67.7% respectively for FS2 males). In the second period (35 s after activation), the proportion of these subpopulations declined substantially to values less than 4% and most spermatozoa were then classified as SP1 (i.e. 97.0%, 98.6% and 96.4% in the WS, FS1 and FS2 groups respectively). There were significant differences in the percentage of spermatozoa in each subpopulation between the two periods after activation in each of the WS, FS1 and FS2 groups ($P < 0.0001$).

Bayesian analysis

Probabilistic modelling with the Bayesian method describes each treatment separately and provides the probability of each possible combination within treatments (i.e. WS, FS1 and FS2; and 5 and 35 s) for the nine CASA-Mot descriptors. Descriptive analysis of all variables showed variations according to the provenance of the male fish and the time period studied (Table 3). WS males were characterised by smaller particle areas (median 14.6 μm), as well as the lowest velocity (172.1, 78.9 and 118.5 μm s^{-1} for VCL, VSL and VAP respectively) and linearity (41.9% for LIN). Farmed parr males showed higher sperm speeds, although spermatozoa from FS2 offspring were the fastest. In the FS2 group, the median values of the velocity variables VCL, VSL and VAP were 176.9, 87.0 and 125.9 μm s^{-1} respectively. In addition, the FS2 group had a high particle area (median 14.9 μm^2). With regard to the time after activation (5 or 35 s), the velocity and progressivity parameters, as well as particle area declined over time. For example, 5 s after activation VCL and LIN were 240.5 μm s^{-1} and 52.8% respectively, declining to 108.5 μm s^{-1} and 32.9% respectively 35 s after activation. The area of motile cells also decreased from 15.0 μm^2 at 5 s to 14.5 μm^2 at 35 s.

Investigating relationships between WS and FS1 and FS2 male revealed that the farmed fish had higher values for sperm velocity variables (VCL, VSL and VAP) compared with anadromous males (Table 4). Compared with WS males, VCL and VAP were higher in the FS1 males (median 2.4 μm s^{-1} and 7.3 μm s^{-1} respectively) and VCL, VSL and VAP were higher in FS2 males (median 4.8, 8.1 and 7.4 μm s^{-1} respectively). Among the farmed mature parr male offspring, those in the FS2 group had the highest VCL (>2.40 μm s^{-1}) and VSL (>8.31 μm s^{-1}). In contrast, linearity (LIN and STR) was lower in FS1 males (i.e. decreases of 0.8% for LIN and 4.2% for STR) but higher in FS2 males (i.e. an increase of 2.7% for LIN and 1.27% for STR) compared with WS males. The area was greater for both FS1 and FS2 males compared with WS males, but was highest for the FS2 group, being 0.24 μm^2 greater than in WS

Table 3. Bayesian analysis of the nine sperm motility variables for wild anadromous (WS) and farmed parr (FS1 and FS2) males and 5 and 35 s after activation

FS1 offspring were produced by crossing male and female WS fish; FS2 offspring were produced by crossing WS males and FS1 females. VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; LIN, linearity; STR, straightness; WOB, wobble; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; Area, particle area; *D*, median of the marginal posterior distribution of each generation and time periods after activation; $HPD_{95\%}$, highest posterior density region at 95%; *k*, limit of the interval ($(-\infty, k]$ when $D < 0$ and $[k, +\infty)$ when $D > 0$).

	VCL ($\mu m\ s^{-1}$)	VSL ($\mu m\ s^{-1}$)	VAP ($\mu m\ s^{-1}$)	LIN (%)	STR (%)	WOB (%)	BCF (Hz)	ALH (μm)	Area (μm)
WS									
D	172.06	78.92	118.47	42.24	62.00	67.38	51.65	1.29	14.63
$HPD_{95\%}$	170.17, 173.93	77.00, 80.81	117.12, 119.89	41.44, 43.01	61.00, 62.97	66.97, 67.78	50.78, 52.51	1.28, 1.30	14.47, 14.79
***k* (80)**	171.26	78.06	117.84	41.90	61.57	67.20	51.27	1.28	14.56
FS1									
D	174.47	78.66	125.78	41.44	57.85	70.81	53.19	1.21	14.79
$HPD_{95\%}$	172.70, 176.31	76.66, 80.40	124.41, 127.11	40.69, 42.20	56.81, 58.77	70.40, 71.20	52.28, 53.96	1.20, 1.22	14.63, 14.94
***k* (80)**	173.66	77.84	125.18	41.11	57.44	70.64	52.81	1.21	14.72
FS2									
D	176.88	86.97	125.86	44.93	63.27	70.30	54.88	1.23	14.87
$HPD_{95\%}$	175.15, 178.48	85.32, 88.70	124.54, 127.07	44.25, 45.67	62.41, 64.22	69.92, 70.66	54.12, 55.71	1.22, 1.24	14.71, 15.02
***k* (80)**	176.15	86.21	125.32	44.63	62.88	70.14	54.54	1.23	14.80
5 s after activation									
D	240.45	127.63	175.87	52.82	71.62	73.25	85.03	1.41	14.98
$HPD_{95\%}$	239.00, 241.70	126.20, 129.04	174.89, 176.87	52.24, 53.39	70.83, 72.34	72.94, 73.55	84.37, 85.67	1.41, 1.42	14.86, 15.11
***k* (80)**	239.86	127.04	175.44	52.57	71.30	73.12	84.75	1.41	14.93
35 s after activation									
D	108.51	35.37	70.85	32.92	50.48	65.74	21.44	1.08	14.54
$HPD_{95\%}$	106.88, 110.10	33.66, 37.00	69.70, 72.06	32.27, 33.62	49.60, 51.33	65.39, 66.12	20.74, 22.21	1.07, 1.09	14.39, 14.67
***k* (80)**	107.78	34.67	70.33	32.63	50.09	65.58	21.12	1.07	14.48

Table 4. Features of the estimated marginal posterior distributions of differences between different groups of males (wild anadromous (WS) and farmed parr (FS1 and FS2) males) and the two points after activation (5 and 35 s) for kinematic sperm variables

FS1 offspring were produced by crossing male and female WS fish; FS2 offspring were produced by crossing WS males and FS1 females. T1, 5 s after activation; T2, 35 s after activation; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; LIN, linearity; STR, straightness; WOB, wobble; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; Area, particle area; *D*, median of the marginal posterior distribution of the differences between generations and time periods after activation; $HPD_{95\%}$, highest posterior density region at 95%; *P*, probability of the difference being less than zero when $D < 0$ and greater than zero when $D > 0$; *k*, limit of the interval ($(-\infty, k]$ when $D < 0$ and $[k, +\infty)$ when $D > 0$).

	VCL ($\mu m\ s^{-1}$)	VSL ($\mu m\ s^{-1}$)	VAP ($\mu m\ s^{-1}$)	LIN (%)	STR (%)	WOB (%)	BCF (Hz)	ALH (μm)	Area (μm^2)
WS–FS1									
D	−2.37	0.23	−7.32	0.81	4.16	−3.43	−1.54	0.07	−0.16
$HPD_{95\%}$	−4.87, 0.39	−2.36, 2.88	−9.17, −5.27	−0.29, 1.87	2.69, 5.46	−4.01, −2.85	−2.79, −0.38	0.06, 0.09	−0.39, 0.06
k (80)	−1.28	0.57	−6.53	0.34	3.54	−3.18	−1.04	0.07	−0.06
P	0.96	−0.87	1.00	0.93	1.00	1.00	0.99	1.00	0.92
WS–FS2									
D	−4.79	−8.07	−7.40	−2.70	−1.27	−2.93	−3.23	0.06	−0.24
$HPD_{95\%}$	−7.36, −2.36	−10.65, −5.58	−9.29, −5.57	−3.73, −1.63	−2.55, 0.09	−3.45, −2.35	−4.46, −2.07	0.04, 0.07	−0.48, −0.03
k (80)	−3.70	−6.97	−6.61	−2.24	−0.68	−2.68	−2.73	0.05	−0.15
P	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	0.98
FS1–FS2									
D	−2.40	−8.31	−0.06	−3.49	−5.42	0.51	−1.70	−0.02	−0.08
$HPD_{95\%}$	−4.84, 0.04	−10.76, −5.78	−1.85, 1.75	−4.43, −2.41	−6.70, −4.12	−0.02, 1.05	−2.80, −0.52	−0.03, −0.00	−0.30, 0.13
k (80)	−1.33	−7.25	0.69	−3.05	−4.85	0.27	−1.21	−0.01	0.01
P	0.97	1.00	0.53	1.00	1.00	0.97	1.00	0.99	0.77
T1–T2									
D	131.95	92.27	105.02	19.91	21.14	7.52	63.59	0.33	0.45
$HPD_{95\%}$	129.75, 134.05	89.97, 94.38	103.47, 106.56	19.03, 20.81	19.97, 22.24	7.00, 7.97	62.55, 64.53	0.32, 0.34	0.26, 0.62
k (80)	131.03	91.30	104.35	19.52	20.67	7.30	63.16	0.33	0.36
P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

males. Values for all nine CASA-Mot variables decreased over time. The velocity (VCL, VSL and VAP) and linearity (LIN and STR) parameters were higher 5 s after activation, as was sperm area, which was 0.45 μm^2 higher for the same time period.

Discussion

Male Atlantic salmon are characterised by intense sexual competition, with a strong selective pressure on male reproductive features, which could lead to multiple paternity (Weir *et al.* 2010). It is known that parr males invest more in the physiological competitiveness of spermatozoa during spawning (Vladić and Järvi 2001; Gage *et al.* 2004). In a natural population, females may mate with many males, including small mature parr and large anadromous males (Weir *et al.* 2010). In the present study, analysis of the percentage of motile spermatozoa showed that small mature males, such as the farmed parr males, produced competitive spermatozoa with a similar proportion of motile cells. This may be explained by the greater concentrations of ATP in farmed parr male ejaculates (Vladić and Järvi 2001), indicating greater sperm vigour.

Sperm motility analysis using a CASA-Mot system provides information about a large number of kinematic variables for each individual spermatozoon. This objective analysis generates a great deal of data that should be examined statistically using techniques that allow for spermatozoa to be classified into subcategories with certain motility characteristics. The cluster approach conducted for several species confirms that ejaculate samples are heterogeneous in that they contain spermatozoa with different motility patterns (Abaigar *et al.* 1999; Quintero-Moreno *et al.* 2003, 2004; Chantler *et al.* 2004; Miró *et al.* 2005; Núñez-Martínez *et al.* 2006; Martínez-Pastor *et al.* 2008; Muiño *et al.* 2008; Beirão *et al.* 2009; Dorado *et al.* 2010, 2011; Contri *et al.* 2012; Kanuga *et al.* 2012). In the present study, we identified three different sperm subpopulations in samples from wild anadromous Atlantic salmon and two groups of farmed parr male offspring. However, the proportions of the different subpopulations varied with the time after activation. The distribution of motile spermatozoa in each subpopulation was used to evaluate differences between the provenance of the male and/or time. These results demonstrated that there was some variability within the groups of males, which could be reflect a connection between variability in sperm velocity and male genetic quality (McGinnity *et al.* 1997, 2003; Fleming *et al.* 2000; Fitzpatrick *et al.* 2007; Beirão *et al.* 2009, 2011; Kanuga *et al.* 2012). Fertilisation success is correlated with sperm velocity and progressivity (Gage *et al.* 2004; Casselman *et al.* 2006; Tuset *et al.* 2008; Kanuga *et al.* 2012). The presence of a fast and linear sperm subpopulation may be advantageous for fecundity and the selection of high-quality spermatozoa (Kime *et al.* 2001; Rurangwa *et al.* 2004; Martínez-Pastor *et al.* 2005, 2011; Beirão *et al.* 2009; Ferraz *et al.* 2014). High proportions of fast spermatozoa (SP2 and SP3 in the present study) at the beginning of activation (5 s after activation) may be considered biologically important because of the limited time spermatozoa have to locate and enter the egg's micropyle (Kime *et al.* 2001; Rurangwa *et al.* 2004). In the case of salmonids, the eggs are large with a diameter of approximately 5 mm and, during the first 30 s, motile spermatozoa swim a distance of 3–4.9 mm around the egg (Perchec *et al.* 1993). However, fast spermatozoa with non-linear progressivity could play an important role in the successful fertilisation of salmonid eggs. Trout spermatozoa exhibit circular trajectories, which maximise the chances of sperm–egg contact during the short period of sperm motility (Cosson *et al.* 1989). In this regard, cluster analysis to identify sperm subpopulations based on velocity and progressivity could be proposed as an indicator of high-quality breeders.

In the present study, a new statistical approach was used for the sperm motility analysis. The aim of the Bayesian analysis was to study the relationship between variables based on the probability of distribution. This distribution gives the probability that the variables are integrated into a range of specific values for that variable. However, it not only considers individual probabilities, but also probabilities for all variables jointly.

The results from the present study indicated that there is a relationship between the velocity and progressivity variables (VCL, VSL, VAP, LIN and STR) and particle area, the provenance of the male fish and time after activation. These results were consistent with the cluster analysis described above. An important advantage of the Bayesian analysis is the ability to make inferences. In the present study, wild anadromous males had small spermatozoa with low velocity and progressivity, whereas mature farmed parr males had larger spermatozoa with higher speeds. However, the FS2 group of offspring that came from a cross between wild males and farmed female FS1 fish had the fastest spermatozoa with higher progressivity. Genotype–environment interactions and growth conditions in the fish farm are important for sexual maturation in salmonids (Garant *et al.* 2003; Vladić *et al.* 2010). The combination of these factors determines the precocious maturation of Atlantic salmon parr males. For successful participation in spawning, precocious parr males invest more in their gonads with regard to sperm motility and quality (Vladić and Järvi 2001; Vladić *et al.* 2010).

As expected, sperm velocity declined to less than half over time, which is in accordance with the depletion of sperm ATP stores (Vladić and Järvi 2001). Despite spermatozoa remaining active 35 s after activation, motile cells did not show vigorous movement, thereby decreasing fertilising ability. A long duration of sperm motility could not be considered an advantage because, for salmonids, the eggs are fertilised within 30 s after activation (Ginsburg 1968; Kime *et al.* 2001). However, under natural conditions, the egg and micropyle of salmonids are surrounded by ovarian fluid and the pH of this fluid could enhance the swimming speed, trajectory and duration of sperm motility (Wojtczak *et al.* 2007). Therefore, the spermatozoa swimming behaviour is a determinant trait in Atlantic salmon reproduction.

Conclusions

In the present study, a Bayesian approach was used to identify the relationship between all sperm variables after identification of three sperm subpopulations based on motility parameters. These analyses confirmed some expectations. The provenance of male fish and time proved to be determinant variables in the characterisation of Atlantic salmon sperm physiology.

Different statistical methods for analysing sperm motility can be used to increase knowledge regarding fish sperm characteristics and sperm speed in a species, such as the structure of sperm subpopulations and sperm competition. However, it is important to obtain fertility data to understand the relationship between sperm velocity and sperm fertilisation ability. These approaches could help with sperm selection processes and fertility potential, and consequently improve artificial reproduction for fish species.

Conflicts of interest

The authors declare no conflicts of interest.

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