Chemical composition of seminal and ovarian fluids of chinook salmon (Oncorhynchus tshawytscha) and their effects on sperm motility traits

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A B S T R A C T

The relationships between the compositions of ovarian, seminal fluids and sperm function are not well known in teleostean fish species. The objective of the present study was to determine the concentration of the major inorganic ions (Na+, K+, Ca2+, Mg2+, Cl−), osmolality, and pH of ovarian and seminal fluid of sexually mature chinook salmon (Oncorhynchus tshawytscha), and to determine if the composition of these fluids influences sperm motility traits (swimming speed, duration of forward mobility, swimming path trajectory, and percent motility). Cation concentrations and osmolality were significantly different in the two fluids. The ionic composition of ovarian fluid differed among individual females, and also among samples collected at different times through the spawning season. Carbonate and bicarbonate were the principal buffer ions in ovarian fluid, and its viscosity was considerably greater than that of water and was shear-dependent. The duration of forward motility (longevity) of spermatozoa, swimming speed, percent motility, and path trajectory were measured using milt from 10 males activated in the ovarian fluid from 7 females whose ion concentrations were known. No significant correlations were observed between the composition of the seminal fluid and sperm traits. However, in ovarian fluid, sperm longevity was negatively correlated with variation in [Ca2+] and [Mg2+], while percent motility increased with increasing [Mg2+]. These observations provide a possible chemical basis for cryptic female mate choice whereby female ovarian fluid differentially influences the behaviour of sperm from different males, and thus their fertilization success.

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1. Introduction

The gametes of externally fertilizing fishes, such as salmonids, are released simultaneously into fresh or salt water during spawning. This potentially hostile aquatic environment induces physiological changes that result in the activation of both sperm motility and unfertilized ova (Jamieson, 1991). The initiation of sperm motility in salmonids results from a decrease in external K+ concentration once spermatozoa come into contact with fresh water (Morisawa, 1994). Swelling and lysis of the sperm cells in the hypotonic water then limit the duration of sperm motility (Cossom, 2004). Unfertilized ova are immediately activated once in contact with the fresh water which soon leads to closure of the micropyle due to osmotic swelling eventually preventing fertilization from occurring (Billard and Cossom, 1992). Moreover, the initiation of sperm motility, swimming speed and period of forward motility may also be influenced by the ion concentrations, pH (Stoss, 1983; Wojtczak et al., 2007), viscosity (Brokaw, 1966; Lauga, 2007), and other components of the external media (Yoshida and Nomura, 1972) that spermatozoa encounter on their approach to the egg. As the short period of sperm motility and the closure of the micropyile limit the period during which fertilization is possible to about one minute (Billard, 1986), the properties of the ovarian and seminal fluids that are released with the gametes are expected to play an important role in the fertilization process.

Seminal fluid produced by the efferent duct provides an ionic environment that maintains the viability of spermatozoa after their release from the testis into the sperm duct (Morisawa and Suzuki, 1980; Stoss, 1983; Billard, 1986). In salmonids and most other teleosts (Billard, 1986), spermatozoa are not motile within the testis, and sperm motility is believed to be stimulated primarily by dilution of potassium ions in the seminal fluid. In addition, sperm motility is also affected by the concentration of other cations such as sodium and calcium (Stoss, 1983) in the female’s ovarian fluid. Furthermore, the concentrations of ions in the seminal fluid prior to spawning might influence sperm motility following their release, by altering the intracellular ionic composition, pH, or osmolality (Scott and Baynes, 1991).
The correlation between seminal fluid composition and subsequent sperm motility has been investigated in only a few species: Salmo salar (Hwang and Idler, 1969), Oncorhynchus mykiss (Lahnsteiner et al., 1998), Cyprinus carpio and Alburnus alburnus (Kruger et al., 1984; Lahnsteiner et al., 1996; Alavi et al., 2004), Acipenser persicus (Alavi et al., 2004), and Lota lota (Lahnsteiner et al., 1997).

In the bleak, A. alburnus, Na+ and K+ levels have statistically significant positive and negative relations, respectively, with the percent of motile cells (Lahnsteiner et al., 1996). In some teleost species, the percent of motile cells and duration of sperm motility increased when the K+ ion levels decreased, and Na+ ion levels and osmolality increased in the seminal fluid (Billard and Cosson, 1992; Lahnsteiner et al., 1996).

In female salmonids, ovarian fluid comprises 10–30% of the volume of the spawned egg mass (Lahnsteiner et al., 1995), and is formed by filtration from the blood plasma and the secretory activity of ovarian epithelia (Hirano et al., 1978). Several studies have investigated components of the seminal fluid in salmonids (see Alavi and Cosson, 2006) but data on the composition of the ovarian fluid are more limited (see Hirano et al., 1978; Lahnsteiner et al., 1995; Wojtczak et al., 2007). In salmonids, this fluid has electrolyte concentrations similar, although not identical, to blood plasma, and has a rather lower protein concentration. Ovarian fluid also contains various nutrients, metabolites and hormones (Hirano et al., 1978; Lahnsteiner et al., 1995; Ingermann et al., 2002a). In several fish species, this fluid has been found to increase sperm swimming speed and to prolong the period of forward motility of spermatozoa in comparison with activation in fresh water, sea water or a buffered solution (arctic charr Salvelinus alpinus, Turner and Montgomerie, 2002; Atlantic cod Gadus morhua, Litvak and Trippel, 1998; rainbow trout O. mykiss, Dietrich et al., 2008; chinook salmon, O. tshawytscha, P. C. Rosengrave, R. L. Turner and Montgomerie, Y. J. Metcalfe, K. P. McBride, and N. J. Gemmell, unpublished observations). As with seminal fluid, there have been few investigations of the relation between composition of ovarian fluid and sperm motility. A recent study investigated the effects of the pH of ovarian fluid of rainbow trout (Oncorhynchus mykiss) on sperm behaviour and found that the duration of sperm motility and sperm swimming speed increased with an increase in ovarian fluid pH (Wojtczak et al., 2007).

In our recent work we found that sperm swimming speed, path trajectory, and longevity differed significantly among males, and were all differentially affected by the ovarian fluid from different females, suggesting that variation in the composition of female ovarian fluid may be responsible (Rosengrave et al., in press). The aim of the present study was to determine the composition of the ovarian and seminal fluids of chinook salmon (O. tshawytscha) with respect to the concentrations of the major inorganic ions (Na+, K+, Ca++, Mg++, Cl−), osmolality, and pH. Carbonate and bicarbonate concentrations and the viscosity of ovarian fluid were also determined. It is generally believed that, in externally fertilizing fish species, sperm motility is the primary determinant of male fertilization success (Gage et al., 2004; Liljedal et al., in press; Rudolfsen et al., 2008). Therefore, using computer-assisted sperm analysis (CASA), we investigated whether natural variability in the composition of ovarian fluid from individual females, and of the seminal fluid from individual males, influenced sperm motility traits (swimming speed, duration of forward motility, swimming path trajectory, and percent motility).

2. Materials and methods

2.1. Collection of ovarian fluid and milt

Milt samples were collected during the 2004 spawning season (late March to early May) from 15 sexually mature two-year-old male salmon. Fish were netted and dried to avoid activation of sperm by water or urine, and milt was collected by applying gentle bilateral abdominal pressure. Seminal fluid was separated from the milt by centrifugation and transferred to screw–cap tubes. Ovarian fluid was also collected from mature three-year-old female salmon during the 2004 spawning season (n = 20) and on four occasions during the 2005 season (28 April, n = 16; 2 May, n = 4; 5 May, n = 10 and 11 May, n = 14). Fish were killed with a stroke to the head and the eggs were expelled into a dish. The ovarian fluid was then pipetted gently out of the egg batch and into screw–cap tubes with minimal head space to minimize air equilibration. We used ovarian fluid from egg batches that did not contain broken eggs as the breakdown of eggs can lead to changes in ovarian fluid pH (Lahnsteiner, 2000). Seminal and ovarian fluids used for compositional analysis were frozen immediately and stored at −80 °C until processed. In the 2005 season, three aliquots of ovarian fluid were collected from each fish and taken separately through all storage and analytical procedures in order to identify compositional differences among individual fish (coefficients of variation of triplicate measurements were approximately 1–3% for osmolality and all ions except Mg2+ which was about 5%). For sperm motility analysis and some pH measurements (2004 samples), milt and ovarian fluids were immediately refrigerated at 4 °C and transported to the University of Canterbury, where measurements were completed within five h of collection.

2.2. Analytical procedures

The concentrations of sodium, potassium, calcium and magnesium in ovarian fluid were determined by Atomic Absorption Spectroscopy (Model Avanta, GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia) using an air:acetylene flame. For Na and K, 25.0 µL samples were diluted 200-fold and measured at 589.6 nm and 766.5 nm respectively, calibrated with a combined standard (Na 1.2 mmol L−1; K 0.04 mmol L−1). A burner angle of 45° was employed for Na analysis to reduce the sensitivity. Samples and standards contained 1.0 g L−1 Cs (chloride) for ionization suppression. For measurement of Ca and Mg, 100 µL samples were diluted 50-fold and measured at 422.7 nm and 285.2 nm respectively (a deuterium continuum lamp was employed for background correction for Mg). Samples and the combined standard (Ca 0.1 mmol L−1, Mg 0.02 mmol L−1) contained 2.5 g L−1 Na (chloride) as a releasing agent, and 1 g L−1 HNO3 which was necessary for complete recovery of Ca.

The osmolality of undiluted ovarian fluid samples was measured by vapour pressure osmometry (Model 5520, Wescor, Logan, Utah) and chloride was measured using an electrometric chloride titrator (Model CMT10, Radiometer, Copenhagen).

Measurements of the pH of milt and ovarian fluids were made on freshly collected samples in 2004 at the time of the sperm motility analyses. Total CO2 concentration ([CO2]) and pH were also measured at controlled temperature and PCO2 on five ovarian fluid samples collected in 2005. The samples (0.5 mL) were tonomerized for >90 min at 15 °C with humidified 1.0% CO2 in N2 (PCO2 = 1.0 kPa; 7.5 mm Hg) (Cameron Instruments Inc., Dual Equilibrator and Mass Flow Controller) to a stable pH. CO2 and pH measurements were then repeated on the same samples tonomerized with 0.2% CO2 (PCO2 = 0.2 kPa) and with humidified air (PCO2 approximately 0.04 kPa). pH was measured directly in the tonometer using a combination glass electrode and pH meter (Philips PW9145) calibrated at 15 °C (BDS Colorkey buffers). CO2 measurements were made using the method of Cameron (1971).

Samples (10 µL) were injected into a cell containing 10 mmol L−1 HCl thermostatted at 40 °C. The CO2 electrode signal was displayed on a pH meter (Radiometer PHM 84) and calibrated by bracketing ovarian fluid samples between 10 µL standards of 10 mmol L−1 NaHCO3. Concentrations of HCO3− and CO32− in ovarian fluid were calculated from CO2 and pH values using the Henderson–Hasselbalch Equation after subtraction of dissolved [CO2]. For these calculations, the solubility of CO2 at 15 °C was 0.0555 mol L−1 at 15 °C.
(αCO₂=0.0535 mmol L⁻¹ mm Hg⁻¹) was estimated from an empirical formula derived for Rainbow trout plasma (Boutilier et al., 1984). The second dissociation constant of carbonic acid at 15 °C (pK₂=9.46) was obtained using a formula for sea water of equivalent ionic strength (Roy et al., 1993).

Viscosity (mPa s = cps) was measured on 0.5 mL samples of ovarian fluid at 15 °C using a cone plate viscometer (Wells–Brookfield model DV-II, cone spindle CP-40 radius 2.4 cm, 0.8 °C). After temperature equilibration, the viscosity was determined after 1 min at each of four rotation speeds (60, 30, 12, and 6 rpm corresponding respectively to shear rates of 450, 225, 90, and 45 s⁻¹). Calibration was checked using silicone oil viscosity standards and water.

2.3. Sperm motility analysis

The effects of ovarian fluid on sperm motility traits were examined on the samples collected in 2004, for each male (n = 10) activated in the ovarian fluid from each female (n = 7). Ovarian fluid was diluted to 50% using fresh water from the raceway that housed the fish. We used a 50% dilution of ovarian fluid as our activating solution, as it seemed likely that during the natural spawning period, spermatozoa would encounter diluted ovarian fluid as the spermatozoa move from pure freshwater into pure ovarian fluid surrounding the egg, and ovarian fluid at this dilution has close to maximum effect of sperm motility (Rosengrave unpublished data). One µL of milt was thoroughly mixed (for approximately 3 s) with 499 µL of the 50% ovarian fluid, and then pipetted onto a glass slide. A cover slip was then gently placed over the sample for viewing at 400× on a negative phase-contrast microscope (Leica DMR). As sperm swimming speed is dependent upon water temperature (Alavi and Cosson, 2005), a 10× objective was used. As sperm swimming speed was dependent upon water temperature (Alavi and Cosson, 2005), a 10× objective was used. Sperm longevity (DUR, s) for each milt sample was recorded using a stopwatch starting at the contact of the sperm tracks recorded during each trial: mean average path velocity (VAP, µm s⁻¹), mean straight line velocity (VSL, µm s⁻¹), mean curvilinear velocity (VCL, µm s⁻¹), and linearity (LIN; ratio of VSL/VCL, expressed as a percentage). As in other studies, we used VAP as a measure of sperm swimming speed (e.g., Burness et al., 2005; Casselman et al., 2006; Lahnsteiner et al., 1998). We also measured overall sperm motility (MOT) as the percentage of cells in the field of view that were progressively motile at >20 µm s⁻¹ (see Lahnsteiner et al., 1998, for a similar criterion).

2.4. Statistical analysis

Mean values of the ionic concentrations, pH and osmolality were compared between seminal and ovarian fluids using independent t-tests. Correlation analyses were used to measure the association between sperm traits and the ion concentrations, pH and osmolality of the ovarian fluid and seminal fluid. A type II regression analyses equation was used to fit a line to significant correlation analyses as x and y are subject to errors (Dytham, 2003). Differences between ovarian fluid samples collected on different dates during the spawning season with respect to ion concentrations and osmolalities were examined using single factor analysis of variance (ANOVA) on fish mean values. For differences among individual fish, a single factor ANOVA was employed using the triplicate samples for each fish. Statistical significance was set at the p<0.05, mean±standard deviation (SD), is given unless otherwise stated (SEM, the standard error of the mean).

3. Results

The ionic composition and the osmolality of the ovarian and seminal fluids are shown in Table 1. Sodium and chloride ions were the major osmoregulators in both seminal and ovarian fluids but there were highly significant differences between the two fluids (t-tests comparing samples from 2004: seminal fluid samples n = 15; ovarian fluid samples n = 20, apart from Na⁺ n = 13) in the mean concentrations of all four inorganic cations, [K⁺] (t = 26, p = 0.0001), [Ca²⁺] (t = 16, p = 0.001), [Mg²⁺] (t = 4, p = 0.0002), [Na⁺] (t = 8, p = 0.0001), but not of [Cl⁻] (t = 1, p = 0.24). The sum of cations and chloride in ovarian fluid samples indicated an anion deficit of about 64 mmol L⁻¹. This difference was largely explained by bicarbonate and carbonate ions (see below). The mean osmolality of

Table 1
Concentrations of principal inorganic ions (mmol L⁻¹), and osmolality (mmol kg⁻¹), of the seminal and ovarian fluids of Oncorhynchus tshawytscha collected during the 2004 and 2005 spawning seasons

<table>
<thead>
<tr>
<th>Sample and date collected</th>
<th>n</th>
<th>R</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Cl⁻</th>
<th>Osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seminal fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April–May 2004</td>
<td>15</td>
<td>1</td>
<td>110±26</td>
<td>37.3±0.6</td>
<td>0.65±0.22</td>
<td>1.15±0.41</td>
<td>109±17</td>
<td>205±32</td>
</tr>
<tr>
<td><strong>Ovarian fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April–May 2004</td>
<td>20*</td>
<td>1</td>
<td>167±4</td>
<td>3.3±0.3</td>
<td>3.7±0.7</td>
<td>0.67±0.25</td>
<td>114±4</td>
<td>290±11</td>
</tr>
<tr>
<td>28 April 2005</td>
<td>16</td>
<td>3</td>
<td>164±3</td>
<td>3.4±0.4</td>
<td>3.9±0.6</td>
<td>0.76±0.48</td>
<td>113±2</td>
<td>295±4</td>
</tr>
<tr>
<td>2 May 2005</td>
<td>4</td>
<td>3</td>
<td>168±2</td>
<td>3.2±0.1</td>
<td>4.8±0.7</td>
<td>0.72±0.13</td>
<td>110±3</td>
<td>295±2</td>
</tr>
<tr>
<td>5 May 2005</td>
<td>10</td>
<td>3</td>
<td>162±2</td>
<td>3.6±0.4</td>
<td>4.1±0.5</td>
<td>0.77±0.15</td>
<td>110±5</td>
<td>295±3</td>
</tr>
<tr>
<td>31 May 2005</td>
<td>14</td>
<td>3</td>
<td>162±3</td>
<td>3.5±0.4</td>
<td>3.5±0.4</td>
<td>0.73±0.13</td>
<td>109±4</td>
<td>290±4</td>
</tr>
<tr>
<td>All ovarian fluid samples</td>
<td>64*</td>
<td>3</td>
<td>164±4</td>
<td>3.4±0.4</td>
<td>3.8±0.7</td>
<td>0.73±0.29</td>
<td>112±4</td>
<td>292±7</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>156–172</td>
<td>2.7–4.4</td>
<td>2.1–5.5</td>
<td>0.35–5.0</td>
<td>100–119</td>
<td>278–322</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means±standard deviation for n fish sampled on the dates shown. R refers to the number of replicate measurements on separately stored and analyzed sub-samples from each fish.

*a*n = 13 for Na⁺ values.

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all ovarian fluid samples was 292 ± 7 mmol kg⁻¹ (Table 1). The osmolality of seminal fluid was more variable (265 ± 32 mmol kg⁻¹) and was significantly hypo-osmotic to ovarian fluid collected in the same (2004) season (t = −3, p = 0.0026). Differences in seminal fluid osmolality primarily reflected differences in the concentrations of sodium, potassium and chloride which were positively correlated with osmolality ([Na⁺], r = 0.42, p = 0.012; [K⁺], r = 0.82, p = 0.0002; [Cl⁻], r = 0.85, p = 0.0001; [Ca²⁺], r = −0.07, p = 0.81; [Mg²⁺]; r = 0.17, p = 0.56). In the ovarian fluid, there was a statistically significant correlation between [Ca²⁺] and [Mg²⁺] (r = 0.63, p = 0.003).

There were statistically significant differences in ovarian fluid composition between sampling dates during the spawning season with respect to osmolality and all ion concentrations except [K⁺] and [Mg²⁺]. In addition, there were statistically significant differences in osmolality and all ion concentrations among individual fish (Table 2).

The mean pH of fresh seminal plasma and ovarian fluid samples collected in 2004, and measured soon after transport to the laboratory, were identical (8.43 ± 0.13 and 8.43 ± 0.22, respectively; Table 3). For ovarian fluid tonometered at 15 °C and PCO₂ = 10 kPa in N₂, the mean pH was 8.18 ± 0.03 increasing to pH 7.86 ± 0.05 at PCO₂ = 2.0 kPa. After air-equilibration, pH rose further to 8.88 ± 0.08. Substantial pH change occurred within minutes after changing PCO₂, but a new stable value was achieved only after more than an hour in the tonometer, indicating that carbonic anhydrase activity was absent from ovarian fluid.

Mean CO₂ in the tonometered ovarian fluid was 40.8 ± 3.5 mmol L⁻¹ at PCO₂ = 1.0 kPa, decreasing to 38.9 ± 2.6 mmol L⁻¹ at 0.2 kPa, and to 37.4 ± 2.6 mmol L⁻¹ in air (Table 3). These changes were small and not statistically significant. Dissolved CO₂ (including H₂CO₃) comprised <1% of total CO₂ in each case. At the highest PCO₂ (1 kPa), HCO₃⁻ comprised 94% and CO₃²⁻ 5% of the total. These proportions were 82%/17% at 0.2 kPa, and to 79%/21% in air-equilibrated ovarian fluid. The sum of inorganic ion concentrations and CO₂ in this subset of samples was 327 ± 1 mmol L⁻¹. Their mean osmolality was 292 ± (n = 5) mmol kg⁻¹, from which an osmotic coefficient of 0.91 ± 0.01 was calculated. This is close to the theoretical values for salt solutions of this concentration (El Guendouzi et al., 2001) and indicates that all of the major osmolytes were accounted for.

Ovarian fluid exhibited non-Newtonian rheology, its viscosity at 15 °C decreasing from 4.2 ± 1.1 mPa s to 2.7 ± 0.4 mPa s as the shear rate was increased from 45 s⁻¹ to 450 s⁻¹ (Fig. 1). No hysteresis was observed on reversal of the sequence. In the same apparatus, the viscosity of pure water was nearly constant (1.12 to 1.19 mPa s) and agreed with documented values (Weast, 1975).

Correlations between the compositions of seminal fluid (10 males) activated in each of the seven females ovarian fluid prior to activation and subsequent sperm motility traits are shown in Table 4, with the statistically significant correlations shown in Fig. 2. There was a significant negative relation between the [K⁺] of seminal fluid and mean sperm swimming speed (VAP) at 10 s post-activation (Fig. 2a), but this analysis was strongly influenced by one outlier, and with that outlier removed the relation is far from significant (r = 0.081, p = 0.81). Mean sperm longevity (DUR) was significantly negatively correlated with the [Ca²⁺] (slope = −0.19 ± 0.05, SEM, p = 0.01, Fig. 2b), and [Mg²⁺] (slope = −0.50 ± 0.05, SEM, p = 0.005) of ovarian fluids (Fig. 2c). In contrast, there was a significant positive relation between the mean percent of progressively motile cells at 10 s post activation (MOT) and the [Mg²⁺].

The sperm traits pH Cl⁻ Na⁺ K⁺ Ca²⁺ Mg²⁺ Osmolality

<table>
<thead>
<tr>
<th>Sperm traits</th>
<th>pH</th>
<th>Cl⁻</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUR (s)</td>
<td>0.08</td>
<td>0.66</td>
<td>0.23</td>
<td>0.03</td>
<td>0.86</td>
<td>0.91</td>
<td>0.48</td>
</tr>
<tr>
<td>VAP (m/s)</td>
<td>0.08</td>
<td>−0.28</td>
<td>0.26</td>
<td>−0.02</td>
<td>0.54</td>
<td>0.29</td>
<td>0.57</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>0.08</td>
<td>0.45</td>
<td>0.50</td>
<td>0.10</td>
<td>0.59</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>MOT (%)</td>
<td>0.19</td>
<td>0.06</td>
<td>0.35</td>
<td>0.54</td>
<td>0.88</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

DUR = longevity, swimming speed (VAP), percent of motile cells (MOT), and linearity of sperm trajectory (LIN) were measured at 10 s post-activation. Seminal fluid and sperm samples for the seminal plasma analyses were obtained from 12 two-year-old male chinook salmon during the 2004 spawning season. For the ovarian fluid analyses, sperm traits from 10 different two-year-old chinook males from the 2004 spawning season were activated in the ovarian fluids from 7 sexually mature three-year-old females. Significant correlations (p ≤ 0.05) are indicated in bold.

*Non-significant after removal of an obvious outlier (Fig. 2a, see text).
of the ovarian fluid (slope=76.20±18 (SEM), p=0.009) in which the sperm was activated (Fig. 2d).

4. Discussion

4.1. Composition of seminal and ovarian fluids

Ion concentrations and osmolality of the seminal fluid of *O. tshawytscha* were similar to those reported for *Salmo gairdneri* (now: *O. mykiss*) (Morisawa et al., 1983), *Salmo salar* (Aas et al., 1991) and *O. mykiss* (Lahnsteiner et al., 1998). The composition of *O. tshawytscha* ovarian fluid was also comparable to that reported for other salmonids (*Salvelinus alpinus, Salmo trutta* and *Hucho hucho*, Lahnsteiner et al., 1995; *O. keta*, Hirano et al., 1978), although there were small differences among these salmonids in the mean values of individual ion concentrations. Lower values for [Na⁺] and [K⁺], and much lower values for [Ca²⁺] reported by Lahnsteiner et al. (1995) probably reflect the use of ion specific electrodes, which sense only the free ions, in that study. Atomic absorption spectroscopy employed here, and by Hirano et al. (1978), measured total metal concentrations, including that bound to proteins and complexed in other ways. The mean concentrations of sodium, calcium, magnesium, chloride and osmolality of the ovarian fluid were generally similar to their concentrations in the blood plasma of this fish in freshwater (Holmes and Donaldson, 1969). In contrast, [K⁺] (3.4±0.4 mmol L⁻¹) was three to four times higher in the ovarian fluid than in the plasma in our fish, as previously observed for freshwater acclimated *O. keta* (Hirano et al., 1978).

The discrepancy between the sum of cations and chloride concentration in ovarian fluid in the present study was largely accounted for by high concentrations of carbonate and bicarbonate. A similar anion deficit was apparent in the ovarian fluid of *O. keta* (Hirano et al., 1978). This indicates that the principal buffer of *O. tshawytscha* ovarian fluid is the CO₂–bicarbonate–carbonate system, as suggested by the relatively low concentrations of other potential buffers such as phosphate and proteins (Ingermann et al., 2002a). These ions were not measured in seminal fluid but a similar discrepancy between the concentrations of cations and chloride suggests that it was also buffered by bicarbonate.

The ovarian fluid was distinctly alkaline, a property noted in several other salmonids and in cyprinids (Hirano et al., 1978; Lahnsteiner et al., 1995, 2001; Wojtczak et al., 2007). Lahnsteiner et al. (1995) highlighted the importance of alkalinity of the ovarian fluid under natural spawning conditions, as this would tend to stabilise the micro-environment around the egg, especially in acidic waters. Importantly, pH of the ovarian fluid in...
our fish was dependent on the partial pressure of dissolved carbon dioxide, rising sharply as PCO₂ decreased (Table 2). Therefore, unless efforts are taken to control PCO₂, loss of CO₂ to the atmosphere during sample processing could increase the apparent variability of the pH of salmonid ovarian fluids (e.g. Wojtczak et al., 2007). Aegerter and Jalabert (2004) noted large changes in the pH of O. mykiss ovarian fluid stored in contact with the air and cautioned against the use of pH as an egg quality indicator unless measurement protocols are carefully standardised. The CO₂ dependence of ovarian fluid pH (Table 2) indicates that its mean PCO₂ soon after collection was about 0.7 kPa, which approximates the venous PCO₂ of salmonids (Stevens and Randall, 1967).

Carbonates buffer poorly between pH 7.5 and 8.0, consistent with observations of the buffer capacity of O. tshawytscha ovarian fluid (Ingermann et al., 2002a). However, the [HCO₃⁻]/[CO₂⁻] equilibrium would buffer effectively between pH 8.5 and 9.0 where salmonid sperm motility is maximal (Wojtczak et al., 2007). Diffusion of CO₂ from these fluids into air during artificial fertilization would tend to elevate pH into this range.

The ovarian fluid was more viscous than water, as has been noted for the ovarian fluid of other salmonids (Hirano et al., 1978; Turner and Montogmery, 2002). High viscosity might impede dislodgement of the eggs from the redd by flowing water (McDowell, 2000), and would maintain high ionic concentrations close to the egg surface. Additionally, it would provide low shear, laminar flow conditions adjacent to the eggs, properties which have been shown to be necessary for successful fertilization in another external fertilizer, the red abalone (Haliotis rufescens) (Riffell and Zimmer, 2007). Viscosity was markedly shear rate dependent (Fig. 1) and was highly variable among individuals (range 3.2–6.9 cPs at 45 s⁻¹ for the 16 fish represented by Fig. 1). Lauga (2007) pointed out that the rheological properties of non-Newtonian fluids theoretically could be tuned to allow selection of appropriately motile spermatozoa and proposed such a mechanism within the cervical mucus of the female reproductive tract. Possibly rheological differences among individual fish ovarian fluids likewise contribute to differential fertilization success and perhaps could provide a basis for cryptic female choice.

The ovarian fluid is nearly iso-osmotic to seminal fluid, and thus to the sperm cells (Table 1; Lahnsteiner et al., 1995), and activation in an ovarian fluid solution prolongs sperm motility compared to activation in fresh water (Rosengrave, unpublished data), despite an apparent requirement for higher energy expenditure in the more viscous medium (Hirai et al., 2004). Viscosity was markedly shear rate dependent (Fig. 1) and was highly variable among individuals (range 3.2–6.9 cPs at 45 s⁻¹ for the 16 fish represented by Fig. 1). Lauga (2007) pointed out that the rheological properties of non-Newtonian fluids theoretically could be tuned to allow selection of appropriately motile spermatozoa and proposed such a mechanism within the cervical mucus of the female reproductive tract. Possibly rheological differences among individual fish ovarian fluids likewise contribute to differential fertilization success and perhaps could provide a basis for cryptic female choice.

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The ionic composition of the ovarian fluid varied among individual females with the largest relative differences being observed in [K⁺], [Ca²⁺] and [Mg²⁺] (see ranges in Table 1). Lahnsteiner et al. (1995) also found considerable inraspecific variation in the composition of the ovarian fluid in four salmonid species. Intraspecific variation in ovarian fluid composition could result partly from variation in post-ovulatory maturation within the coelomic cavity, in the physiological status of the female, and in egg quality (Lahnsteiner, 2000; Lahnsteiner et al., 1999). Aegerter and Jalabert (2004) observed progressive changes in the osmolality, pH and protein concentration of ovarian fluid following ovulation in O. mykiss. Correspondingly, we noted significant differences in the ionic composition of O. tshawytscha ovarian fluid collected at different periods during the spawning season. Such seasonal variation could be due to changes in a female’s physiological status over the spawning season, along with changes in egg quality and maturity which will all affect the composition of the ovarian fluid (Lahnsteiner et al., 1999; Lahnsteiner, 2000; Aegerter and Jalabert, 2004). Such variation in the quality of a female’s ovarian fluid could be expected to affect sperm motility, fertilization success and, perhaps, the development and survival of embryos.

Seminal fluid [K⁺] was ten times that of ovarian fluid, but the [Na⁺] of seminal fluid was only two thirds that of ovarian fluid, in agreement with previous research in salmonids (Scott and Baynes, 1980). High [K⁺]/[Na⁺] ratio in the seminal fluid is expected as this inhibits sperm motility in the reproductive tract (Billard, 1986; Billard et al., 1995, Morisawa et al., 1983). The [Ca²⁺] of seminal fluid was less than one fifth of that in ovarian fluid, possibly reflecting calcium bound to protein at higher concentration in the latter (Ingermann et al., 2002a). Seminal fluid osmolality was more variable than that of ovarian fluid and, on average, was hypo-osmotic to ovarian fluid and blood plasma. Correlations of seminal fluid osmolality with [Na⁺], [K⁺] and [Cl⁻] but not with [Ca²⁺] or [Mg²⁺] could indicate contamination with diluted urine from the adjacent urinary bladder (Holmes and Stainer, 1966; Wood et al., 1999), although care was taken to avoid this. However, similarly variable osmolality and ion concentrations were observed in O. mykiss seminal fluid when urine contamination was rigorously excluded (Lahnsteiner et al., 1998). Ion reabsorption in the sperm ducts is therefore a possible explanation of the hypotonicity. As fertilization success is markedly dependent on seminal fluid electrolytes and pH (Lahnsteiner et al., 1998; Bencic et al., 2000; Ingermann et al., 2002a,b), the possible significance of modifications to the seminal fluid composition prior to spawning also deserves further study.

4.2. Relationship with sperm traits

There were significant negative correlations between the mean duration of forward motility (DUR) of sperm and the [Ca²⁺] and [Mg²⁺] of ovarian fluid from seven females (Fig. 2b and c). Conversely, the mean percent of progressively motile sperm (MOT) at 10 s post-activation increased as [Mg²⁺] in the ovarian fluid increased (Fig. 2d). Ovarian fluid [Ca²⁺] and [Mg²⁺] were significantly correlated with each other, perhaps reflecting variation in protein concentration. Although positive correlation coefficients for [Ca²⁺] with swimming speed (VAP) and (MOT) (Table 4) were not significant, the sperm from all males swam slower and exhibited the lowest percent motility in the ovarian fluid of one female that had the lowest [Ca²⁺] at 2.10 mmol L⁻¹. Wojtczak et al. (2007) found that the percentage of motile sperm, as well as the swimming speed and duration of motility of the spermatozoa from a single male rainbow trout were positively correlated with the pH of the ovarian fluid from 31 different females. We did not find these relations to be significant, possibly due to our smaller sample size (n=7 females), narrower pH range observed in ovarian fluid. In addition, the relation between sperm VAP and ovarian fluid pH was negative (Table 4).

After correction for an outlier (Fig. 2a), there were no statistically significant correlations between sperm motility traits and any component of seminal fluid (Table 4). Nonetheless, correlations between VAP and both [Cl⁻] and [Na⁺] were both negative and reasonably large, and the small sample size would have resulted in low power to detect a real relation. Kusa (1950) found that a high potassium concentration in the seminal fluid of chum salmon (O. keta) inhibited sperm motility resulting in a decrease in fertilization success, but the correlation with [K⁺] in our study was small and far from significant (Table 4, Fig. 2a). The effect of spermatozoa sensitivity to [K⁺] in the seminal fluid may also vary through the reproductive season (see Alavi and Cosson, 2006). In contrast, Alavi et al. (2004) observed no statistically significant correlations between seminal fluid composition and sperm motility traits for Acienser persicus. There appears to be considerable inter- and intra-specific variability in the ionic composition of seminal fluid in fish (see Alavi and Cosson, 2006). This may imply that different ions and ion concentrations are involved in regulating and initiating sperm motility for different fish species (Billard and Cosson, 1992; Scott and Baynes, 1980).

Recent studies have shown that the ovarian fluids from chinook salmon, rainbow trout and arctic char of individual females differentially enhance the sperm mobility characteristics of different males (e.g., swimming speed; Urbach et al., 2005, Dietrich et al., 2008, Rosengrave et al., in press). Thus, differences in ovarian fluid composition provide a mechanism of cryptic female choice, whereby fertilization is biased towards certain males (Thorhill 1983; Eberhard 1996). The present study lends support to this hypothesis. Intraspecific variations in the ionic...
composition of seminal and ovarian fluids, and differences in ovarian fluid through the spawning season, are large enough to differentially enhance sperm motility and longevity. However, whether such selection operates during natural mating still remains to be demonstrated.

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