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THE EFFECT OF WATER SALINITY ON THE MOTILITY OF SPERMATOZOA OF THE BROOK TROUT, *SALVELINUS FONTINALIS* (ACTINOPTERYGII: SALMONIFORMES: SALMONIDAE)

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Background. Salmonid spermatozoa are characterised by a very short time of activity in the water, therefore sudden water pollution in the form of increased salinity on the spawning grounds may have a negative effect on the sperm motility parameters, thus affecting the subsequent egg fertilisation and, consequently, the number of hatched individuals of the species. The aim of this study was to determine the effect of water salinity on the motility parameters of spermatozoa of brook trout, Salvelinus fontinalis (Mitchill, 1814).

Material and methods. Sperm motility was monitored with a camera (Basler A312fc) coupled with Nikon Eclipse 50i light microscope from the moment of their activation (contact with water) until the cessation of movement. The following water-salinity treatments were tested: 1.0‰, 3.0‰, 5.0‰, and 10.0‰. The motility parameters: VCL, VSL, VAP, ALH, BCF, LIN, STR, WOB, and MOT, were analysed with Computer Assisted Sperm Analysis (CASA).

Results. The mean values of the studied motility parameters of the brook trout spermatozoa (obtained within 30 s), whose activation took place in the water of 0.35‰ salinity and in water of 1.0‰ and 3.0‰ salinity, did not differ significantly. The highest mean values of motility parameters were recorded for the water of 5.0‰ salinity. The 10.0‰ treatment caused a distinct decrease in the values of all the studied parameters. The percentage of MOT was the highest (37.5%) in the sample activated in the water used for fish rearing (0.35‰). In the remaining samples the MOT was lower, and in the water of 10.0‰ salinity it was only 9.1%. No spermatozoa movement of any kind was recorded in the 35th second of the experiment.

Conclusion. The values of the motility parameters as well as the percentage of motile spermatozoa (MOT) in the semen decrease with increasing salinity of the water used for activation, and with increasing time of exposure.

Keywords: sperm quality, CASA, salinity, brook trout, Salvelinus fontinalis

INTRODUCTION

Different water salinity may have an effect on aquatic organisms, including fishes. It may affect fish gametes (eggs and spermatozoa), developing embryos, as well as juveniles and adults (Swanson 1996, Vetemaa and Saat 1996, Fashina-Bombata and Busari 2003, Bonisławska 2009, Fridman et al. 2012, Khatooni et al. 2012).

Studies on the effect of water salinity on spermatozoa of freshwater fishes have a fairly long history (Ivlev 1940, Lindroth 1946, Dziekońska 1958, Szymelfenig 1979, Landergren and Vallin 1998, Beirão et al. 2015a, 2015b). It has been found that the activity of fish spermatozoa decreases with increasing salinity, and their ability to fertilise eggs decreases accordingly. Spermatozoa of freshwater bream, *Abramis brama* (Linnaeus, 1758) from the Caspian Sea remained motile in the salinity water of 11.0‰, but they were incapable of fertilisation (Ivlev 1940). In the same species from the Vistula Lagoon the spermatozoa were motile at 5.6‰ salinity, but the value of 11.0‰ was too high and no movement was recorded in such samples (Dziekońska 1958). The spermatozoa motility of pike, *Esox lucius* Linnaeus, 1758 at 4.0‰–7.0‰ was the same as in lake water, while at 30.0‰ they were immobile (Lindroth 1946).

Studies on the possibility of reproduction of two salmonid species—the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), and brown trout, *Salmo trutta* Linnaeus, 1758—in the water from the Baltic Sea, showed that water salinity within 4.0‰–7.4‰ prolonged the time of sperm motility, compared to that in freshwater. The spermatozoa of rainbow trout and brown trout moved also in the water of higher salinity, up to 15.0‰ (Szymelfenig 1979).

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Twenty years later, studies by Landergren and Vallin (1998) showed that brown trout spermatozoa displayed their highest motility in freshwater and in the brackish water of 6.7‰ from the coast of Gotland Island (Baltic Sea). Higher salinity (10.0‰ and 14.0‰) caused a decrease in motility, and at the level of 16.0‰ and 18.0‰ the movement ceased. Motility was also observed that at 6.7‰, despite the normal fertilisation, the brown trout embryogenesis was inhibited and no hatching occurred (Landergren and Vallin 1998). The results of Bonisławska (2009) indicated the salinity value of 2.0‰ as the upper limit for normal completion of embryogenesis and hatching. At 3.0‰, despite the 83% of fertilised eggs, there was a mass mortality of embryos before hatching (Bonisławska 2009).

Comparison of sperm motility in two species: marine capelin, *Mallotus villosus* (Müller, 1776), and the anadromous rainbow smelt, *Osmerus mordax* (Mitchill, 1814) in water salinities from 0.0‰ to 30.0‰ showed the greatest motility of capelin spermatozoa at 5.0‰, 10.0‰, and 15.0‰ (MOT 75%–80%). In the case of rainbow smelt the greatest MOT values were obtained at 0.0‰, 5.0‰, and 10.0‰ water salinity (MOT 75%–85%). At 15.0‰ and 20.0‰, the MOT of rainbow smelt spermatozoa was 15.0%–20.0%, and at 30.0‰ no movement was observed (Beirão et al. 2013).

The proportion of motile spermatozoa in fish semen determines its quality, which is crucial for fertilisation (Billard 1978, Stoss 1983). Spermatozoa of the majority of fish species are immobile in the seminal plasma and acquire motility only during activation (contact with water). Sperm motility is initiated by the difference in the concentration of potassium ions K^+ between the seminal plasma and the water (salmonids) or by a decrease in osmotic pressure (freshwater teleosts) (Billard 1986, Gatti et al. 1990). The movement of sperm tail requires great amounts of energy, acquired from ATP hydrolysis. The energy (ATP) shortly becomes exhausted, hence the short time of fish sperm motility; the time varies among species (Cosson et al. 1999, Burness et al. 2005). Assessment of sperm motility is necessary, since fertilising capacity depends strictly on motility (Gage et al. 2004). Also important is the fact that sperm motility is influenced by a variety of environmental factors such as temperature, concentration of univalent and bivalent ions in the water (salmonids and acipenserids), osmolality (cyprinids and marine fishes), pH, and magnetic field (Scheuring 1925, Morisawa et al. 1983, Cosson et al. 1999, Inaba et al. 2003, Krasznai et al. 2003, Alavi and Cosson 2005, 2006, Shimoda et al. 2007, Alavi et al. 2008, Ciereszko 2008, Formicki et al. 2013).

Consequently, re-occurring appearance of anthropogenic substances in lakes and rivers may, through the change of water parameters (pH, temperature, or concentration of ions), affect the motility parameters of fish spermatozoa. A very important factor is the increasing salinity of inland waters of Poland, resulting from various kinds of pollution. The main sources of water salinity include mining (79.0%), chemical industry (12.0%), area pollution (7.0%), metallurgic industry (1.5%), and thermal power plant effluents (0.5%) (Szymańska 1990). Pollution of the two largest rivers in Poland—Vistula and Oder, in the form of increased salinity (increased concentration of chlorides and sulphates) is a result of coal-mine draining in Upper Silesia (Janson et al. 2009). In the autumn–winter period the salinity of lakes, rivers, and streams often increases as a result of common usage of salt for winter maintenance of roads. The autumn–winter as well as early spring periods are the times of spawning of economically important and pollution-sensitive salmonids (Salmonidae), including brook trout, *Salvelinus fontinalis* (Mitchill, 1814). This species, like all other representatives of the family, inhabits pure and cold rivers and streams of stony bottom. It was introduced in Europe in the 19th century, and in Poland—in the 1960s (Fopp-Bayat et al. 2010). Individuals found in European rivers may represent intentional stocking or fish-farm escapes.

A thorough literature search shows a lack of studies performed with the use of Computer Assisted Sperm Analysis (CASA) and dealing with the effect of pollution in the form of increased water salinity on the motility parameters of spermatozoa of fishes, including brook trout.

The objective of this study was to determine the effect of increased water salinity (1.0‰–10.0‰) on the motility parameters of brook trout spermatozoa from the moment of their contact with water until the cessation of movement. We hoped to answer the question if and to what extent re-occurring pollution in the form of increased water mineralization affects fertilisation through direct effect on the sperm motility parameters. Our study may help to explain the effect of external factors on the decrease in the number of hatched individuals of various fish species in the rivers of Poland.

MATERIAL AND METHODS

Semen was sampled from brook trout males from a fish farm near the town of Miastko in north-western Poland (54°01′N, 16°59′E) in the second half of October 2013 (water temperature 7.0°C).

The semen was obtained from 13 males (no anaesthetics were used) at the peak of the spawning season. Their total length (TL) was 25–38 ± 0.5 cm and body mass 280–350 ± 0.5 g.

The semen was sampled from each male separately, using a syringe with silicone catheter; care being taken not to contaminate the sample with faeces, urine, or blood. The semen (not diluted) was transported to the laboratory in test tubes placed in isothermal containers with cooling lining which ensured the adequate, constant temperature of 7.0 ± 0.1°C during the transport. The duration of the transport was 90 min.

Sperm motility. The sperm motility parameters were assessed using Computer Assisted Sperm Analysis (CASA) with computer system for sperm motility analysis (SCA)—Sperm Class Analyzer v. 4.0.0. (Microptic S.L.) software. Sperm motility was monitored with a camera (Basler A312fc) coupled with Nikon Eclipse 50i light microscope (×10 negative phase contrast objective).

The amount of 5 μL of semen and activating liquid mixture (1 : 250) was placed in the Makler chamber (Sefi – Medical Instruments, Israel) and the analysis started after ca. 3 s. The chamber, made with a laser technique, is 10 μm deep, which enables the spermatozoa to move freely but prevents them from making vertical movement and thus disappearing from the field of vision. The motility was recorded at 5-s intervals until the cessation of movement. Each sample was analysed in triplicate.

The following sperm motility parameters CASA System were assessed:

- Curvilinear velocity (VCL) [$\mu m \cdot s^{-1}$];
- Straight-line velocity (VSL) [$\mu m \cdot s^{-1}$];
- Average-pathsperm velocity (VAP) [$\mu m \cdot s^{-1}$];
- Amplitude of lateral head displacement (ALH) [μm];
- Beat cross frequency (BCF) [Hz];
- Linear motion (LIN) [%] ($100VSL \cdot VCL^{-1}$);
- Motion straightness (STR) [%] ($100VSL \cdot VAP^{-1}$);
- Minimum and maximum value of sperm oscillation index (WOB) [%];
- Proportion of motile spermatozoa (MOT) (motility).

Experimental design and hydrochemical analysis

In order to activate the spermatozoa, the semen was mixed with water activators, constituting five experimental treatments differing in salinity: 0.35‰, 1.0‰, 3.0‰, 5.0‰, and 10.0‰. The salinity of 0.35‰ represented water from the fish culture. The aqueous salt solutions used were based on sea salt (hw Marinemix Meersalz Professional, hw Wiegandt, Krefeld, Germany) and deionised water (ultra-pure). Magnetic stirrer (MS HS, Vigo, Spain) was used when preparing the solutions. Analyses of the water from the fish culture and of the salt solutions were performed at the Division of Hydrochemistry and Water Protection of our university, using ion exchange chromatograph Dione ICS 3000 (Table 1).

Statistical analyses. The results were statistically analysed with Statistica v.10 PL software (StatSoft) using univariate variance analysis (ANOVA, $P < 0.01$) and Duncan test ($P < 0.01$) to compare the mean values of the motility parameters.

RESULTS

The values of the studied motility parameters and the proportion of motile spermatozoa in the semen decreased with increasing salinity of the water and with the time of exposure. In all the samples no movement was observed in the 35th second.

Curvilinear velocity (VCL) of brook trout spermatozoa during the first seconds following contact with the culture water and the water of 1.0‰ salinity was the highest (at a similar level), of 92.49 and 87.39 $\mu m \cdot s^{-1}$, respectively. VCL decreased with time elapsing from the moment of activation, and the differences became increasingly pronounced. In the 30th second the VCL of spermatozoa in the culture water was 19.38 $\mu m \cdot s^{-1}$, and in the water of 1.0‰—14.74 $\mu m \cdot s^{-1}$. In case of solutions of 3.0‰ and 10.0‰ the VCL also decreased, and in the water of 5.0‰ in the 5th second from activation the VCL increased to reach its maximum of 96.0 $\mu m \cdot s^{-1}$, while in the 30th second its value was only 16.20 $\mu m \cdot s^{-1}$. The lowest VCL value both at the beginning and 30 seconds after activation was recorded for the spermatozoa in the water of 10.0‰: in the first seconds it was 22.12 $\mu m \cdot s^{-1}$, and in the 30th second only 12.93 $\mu m \cdot s^{-1}$ (Fig. 1a).

The statistical analysis of the mean VCL of spermatozoa during activation (during 30 seconds) showed significant differences between the mean VCL in the water of 0.35‰, 1.0‰, and 3.0‰ on the one hand and the VCL in the water of 5.0‰ and 10.0‰ on the other (Table 2).

Straight-line velocity (VSL) of the brook trout spermatozoa also decreased with time, but in this case the highest VSL values were reached in the 5th and 10th second of activation at the water of 5.0‰—74.3 and 43.4 $\mu m \cdot s^{-1}$, respectively (Fig. 1b). In the culture water, on the beginning of activation, VSL was lower compared to spermatozoa activated in the water of 1.0‰ and 5.0‰. The highest VSL value in the 30th second of activation was recorded for the spermatozoa activated in the culture water. The lowest VCL values were observed for the spermatozoa in the water of 10.0‰—from 7.24 $\mu m \cdot s^{-1}$ (second 0) to 1.23 $\mu m \cdot s^{-1}$ in the 30th second (Fig.1b). The mean VSL values obtained during 30 seconds differed significantly among spermatozoa activated in the water of 10.0‰ (5.16 $\mu m \cdot s^{-1}$), those activated at 5.0‰ (43.90 $\mu m \cdot s^{-1}$) and

Table 1

Ionic composition and pH of water used in experiments on sperm activity of brook trout, *Salvelinus fontinalis*

Parameter	Water salinity [‰]				
	0.35	1.0	3.0	5.0	10.0
pH	7.7	7.3	8.2	8.4	8.5
Sodium Na^+	0.6016	17.0816	35.1968	78.2918	118.4379
Magnesium Mg^{2+}	0.4168	1.0837	1.9165	5.0002	6.1930
Calcium Ca^{2+}	1.5879	0.3396	0.7867	1.5263	2.2172
Potassium K^+	0.0479	0.2389	0.6799	1.5694	2.0346
Bromine Br^-	0.0065	0.0017	0.0065	0.0177	0.0186
Fluorine F^-	0.0120	0.0043	0.0054	0.0517	0.0221
Chlorine Cl^-	0.5661	15.4666	44.5326	95.9083	145.0728

Concentration of ions expressed in $mmol \cdot dm^{-3}$.

those activated at 0.35‰, 1.0‰, and 3.0‰ (22.68, 25.89, and 22.81 μm·s^{-1}, respectively) (Table 2).

Average-path sperm velocity (VAP). The highest VAP values were observed in second 0 among the spermatozoa activated in the culture water (0.35‰) and at 1.0‰ (83.92 and 81.18 μm·s^{-1}, respectively). In the 5th second of activation in the water of 5.0‰, as in the case of VCL and VSL, the mean VAP was very high, i.e., 89.70 μm·s^{-1} (Fig. 1c). The lowest VAP values were recorded in the sample with spermatozoa activated in the water of 10.0‰—they were within 17.88 μm·s^{-1} (second 0) to 4.29 μm·s^{-1} (30th second) (Fig.1c). The statistical analysis showed no significant differences in the mean values of VAP during 30 seconds among the samples activated at 0.35‰, 1.0‰, 3.0‰, and 5.0‰. Significant differences were demonstrated for the mean VAP of the spermatozoa activated at 10.0‰ (Table 2).

Amplitude of lateral head displacement (ALH). The highest ALH value, amounting to 0.76 μm, was recorded for the spermatozoa activated in the culture water in second 0. With time the ALH decreased in all samples, to drop below 0.40 μm in the 30th second. In the water of the highest salinity (10.0‰), during 30 seconds the ALH was within a narrow range of 0.38–0.42 μm (Fig 1d). Statistically significant differences in the ALH were observed between the spermatozoa activated at 10.0‰ and the remaining samples activated at 0.35‰, 1.0‰, 3.0‰, and 5.0‰ (Table 2).

Beat cross frequency BCF. The values of this parameter for the spermatozoa activated in the water of 0.35‰, 1.0‰, 3.0‰, and 5.0‰ were within the range of 13.0 Hz (20th second at 0.35‰) to 2.8 Hz (20th second at 10.0‰) (Fig 1e). The mean BCF was the highest for the spermatozoa activated at 3.5‰ (11.47 Hz), and the lowest for those activated at 10.0‰ (6.40 Hz) (Table 2).

Linear motion (LIN). The highest LIN values were recorded in the 15th second for the spermatozoa activated in the culture water (0.35‰) 62.3%. In that sample the LIN was observed to increase until the 15th second, and then it decreased. However, the LIN value in the 30th second was 49.99% and was higher than immediately after activation (35.10%). The LIN decreased with time in the case of the salinity water samples (Fig 1f.). The mean LIN (Fig. 1f) differed statistically significantly between the sample activated at 10.0‰ and the remaining samples activated in the water of lower salinity. The values of LIN in the samples of 0.35‰–5.0‰ were within 38.75‰–42.60‰, and at 10.0‰ the value was 24.07% (Table 2).

Motion straightness (STR). Like in the case of LIN, the values were the highest in the 15th second, for the spermatozoa activated in the culture water (0.35‰)—73.22%. In that sample the STR increased till the 15th second of activation, and then decreased. The lowest STR was recorded for the spermatozoa activated at 10.0‰ (Fig 1g). Like in the case of LIN, the mean STR differed statistically significantly between the sample activated at 10.0‰ and the remaining samples—salinities: 0.35‰, 1.0‰, 3.0‰, and 5.0‰ (Table 2).

Minimum and maximum value of sperm oscillation index (WOB). The parameter was the highest in the sample activated in the culture water (0.35‰)—in the 5th second it was 86.87%. In the remaining samples the WOB was lower and decreased with time to 38.26% in the 30th second in the water of 10.0‰ (Fig. 1h). The mean WOB values were the highest in the sample of 0.35‰, and the lowest at 10.0‰. In the samples of 1.0‰, 3.0‰, and 5.0‰ they were similar (no statistically significant differences) (Table 2).

Percentage of motile spermatozoa (MOT) was the highest (37.5%) in the sample activated in the culture water (0.35‰). In the remaining samples (1.0‰, 3.0‰, 5.0‰, and 10.0‰) the MOT was within 19.2% (1.0‰)—9.1% (10.0‰) (Table 3). The MOT decreased with time, and in the 10.0‰ sample no motile spermatozoa were observed in the 35th second.

Table 2

Mean values of motility parameters of spermatozoa of brook trout, *Salvelinus fontinalis*, activated in water of different salinity

Parameter	Water salinity [‰]				
	0.35	1.0	3.0	5.0	10.0
VCL	60.68^{b}	58.34^{b}	56.28^{b}	69.21^{c}	19.09^{a}
VSL	22.68^{b}	25.89^{b}	22.80^{b}	43.90^{c}	5.16^{a}
VAP	54.99^{b}	52.00^{b}	50.42^{b}	62.48^{c}	12.32^{a}
ALH	0.57^{b}	0.55^{b}	0.53^{b}	0.54^{b}	0.40^{a}
BCF	11.30^{c}	8.92^{b}	9.37^{bc}	10.32^{bc}	6.38^{a}
LIN	41.93^{b}	38.46^{b}	38.74^{b}	39.02^{b}	24.62^{a}
STR	48.65^{b}	46.10^{b}	48.87^{b}	46.68^{b}	38.41^{a}
WOB	83.06^{c}	74.23^{b}	74.51^{b}	68.97^{b}	54.64^{a}

The mean values of all measurement performed during 30 second; ANOVA $P < 0.01$; mean values marked with identical superscripts are not significantly different at $P < 0.01$, Duncan's multiple range test; VCL = curvilinear velocity [μm · s^{-1}], VSL = straight-line velocity [μm · s^{-1}], VAP = average-pathsperm velocity [μm · s^{-1}], ALH = amplitude of lateral head displacement [μm], BCF = beat cross frequency [Hz], LIN = linear motion [%](100VSL · VCL^{-1}), STR = motion straightness [%](100VSL · VCL^{-1}), WOB = minimum and maximum value of sperm oscillation index [%].

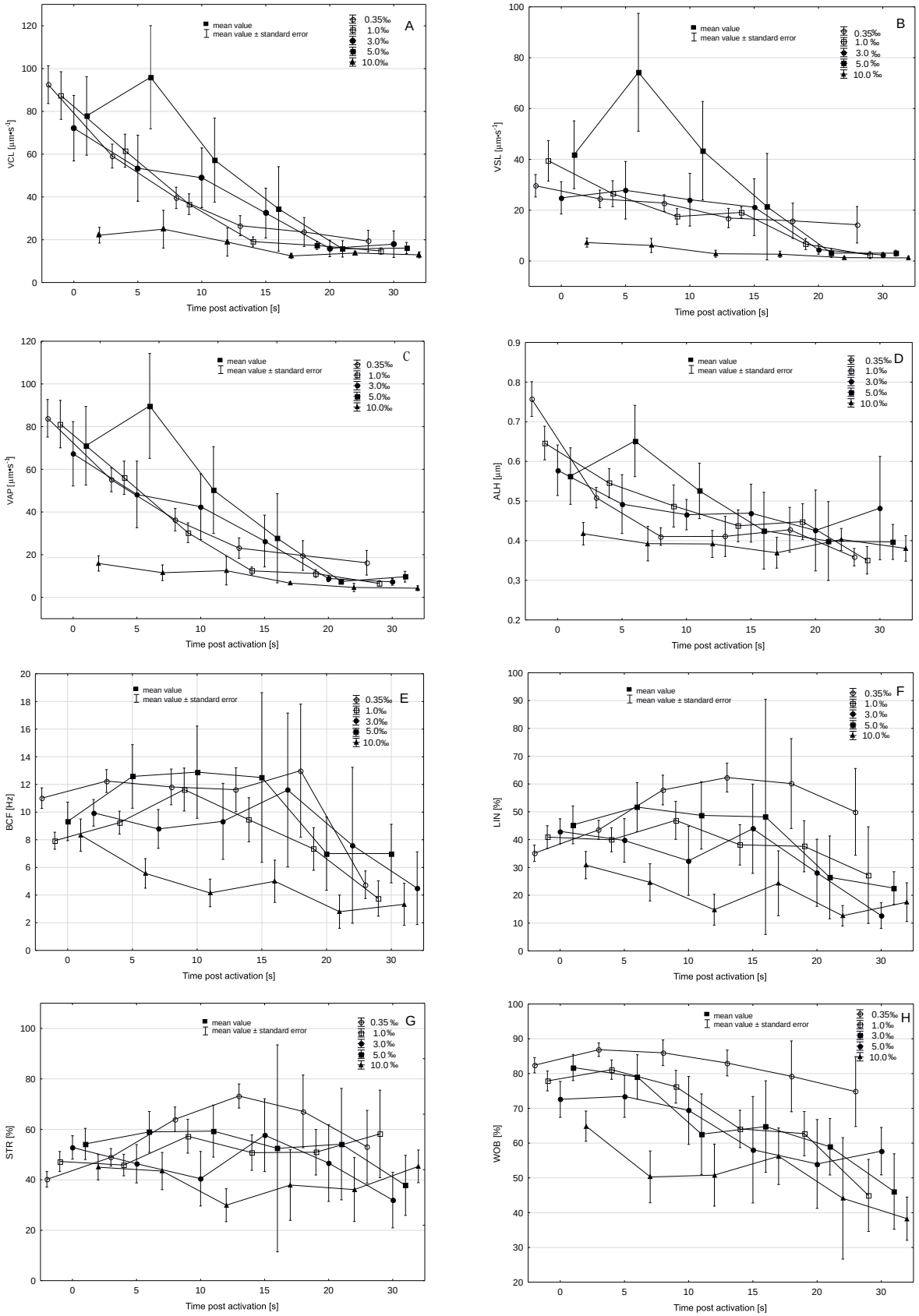

Fig. 1. Motility parameters of spermatozoa of brook trout, *Salvelinus fontinalis*, in different water-salinity treatments (0.35‰, 1.0‰, 3.0‰, 5.0‰, and 10.0‰) analysed with CASA; **A**, curvilinear velocity (VCL); **B**, straight-line velocity (VSL); **C**, average-path sperm velocity (VAP); **D**, amplitude of lateral head displacement (ALH); **E**, beat cross frequency (BCF); **F**, linear motion (LIN); **G**, motion straightness (STR); **H**, minimum and maximum value of sperm oscillation index (WOB)

Table 3

Percentage of motile spermatozoa (MOT) of brook trout, *Salvelinus fontinalis*, in different water-salinity treatments

Time [s]	Water salinity [‰]				
	0.35	1.0	3.0	5.0	10.0
0	37.5^{c}	19.2^{b}	11.1^{a}	11.5^{a}	9.1^{a}
5	17.8^{b}	12.5^{b}	8.9^{b}	12.1^{b}	5.2^{a}
10	10.4^{b}	8.3^{b}	5.6^{a}	5.5^{a}	3.4^{a}
15	9.3^{b}	6.3^{a}	5.0^{a}	5.8^{a}	3.9^{a}
20	7.8^{b}	7.6^{b}	7.1^{ab}	4.6^{a}	3.6^{a}
30	4.2^{b}	3.6^{ab}	4.2^{b}	4.7^{b}	2.0^{a}

Mean values marked with identical superscripts are not significantly different at $P < 0.01$; Duncan's multiple range test.

DISCUSSION

The results of the presently reported study indicate that the water of increased salinity, i.e., 1.0‰, 3.0‰, 5.0‰, and 10.0‰, used for activation affected the values of the studied motility parameters of brook trout spermatozoa. Our observations are compatible with the results of studies on rainbow trout and brown trout, indicating an increase in sperm motility in the water of increased mineralization (Szymelfenig 1979, Landergren and Vallin 1998). A higher salinity, of 14.0‰–15.0‰, could probably completely immobilise brook trout spermatozoa, as was the case of spermatozoa of other species of the same family (Salmonidae), i.e., brown trout (Landergren and Vallin 1998). The spermatozoa of that species displayed their greatest motility in freshwater and the water of 6.7‰ (the highest values of linear and oscillating motion). In the water of 14.0‰, brown trout spermatozoa showed only oscillating movements, and at 16.0‰ and 18.0‰ no movement was observed (Landergren and Vallin 1998). The mean VCL of spermatozoa of the rainbow smelt—a fish of the family Osmeridae which is closely related to Salmonidae—at 15.0‰ and 20.0‰ was 50.0–150 $\mu m \cdot s^{-1}$, but compared to the values observed at 0.0‰, 5.0‰, and 10.0‰ it was by ca. 50 percentage points smaller (Beirão et al. 2013).

The proportion of motile spermatozoa (MOT) immediately after activation at 10.0‰ was more than four times lower (9.1%) than in the culture water (37.5%) (Table 3). The studies by Dietrich et al. (2010) on the European whitefish spermatozoa showed that cations Ca^{2+}, K^{+}, and Na^{+} decreased the percentage of motile spermatozoa, and the decrease was proportional to the cation concentration. Ions Ca^{2+} and K^{+} at the concentration of 2.0 mmol · dm^{-3} decreased MOT by ca. 10 percentage points in the case of calcium and ca. 35 percentage points in the case of potassium. The concentration of Na^{+} of 120 mmol · dm^{-3} decreased MOT by ca. 45 percentage points (Dietrich et al. 2010). In our studies the water of 10.0‰ had a similar content of cations: 2.22 mmol · dm^{-3} (for Ca^{2+}), K^{+} 2.03 mmol · dm^{-3} (for K^{+}), and 118.44 mmol · dm^{-3} (for Na^{+}) (Table 1).

The viability and motility of fish spermatozoa depend on the adequate content of univalent (K^{+}, Na^{+}) and bivalent (Ca^{+2}, Mg^{+2}) ions in the water, on the osmolality, and on pH (Morisawa et al. 1983, Cosson et al. 2000, Cosson 2004, Alavi and Cosson 2005). The prevalent ions in the fish seminal plasma are Na^{+}, K^{+}, Cl^{-}, as well as Ca^{+2} and Mg^{+2}. The ionic composition, however, may change during the spawning period; besides, the concentration of such ions as Na^{+}, K^{+}, and Cl^{-} is higher in salmonids and cyprinids, and smaller in acipenserids (Alavi and Cosson 2006). Scheuring (1925) have already demonstrated that one of the factors initiating sperm movement in a salmonid (rainbow trout) was an adequate (smaller than in the seminal plasma) concentration of potassium ions (K^{+}) in the water. Later studies showed that a solution of an elevated concentration of that ion amounting to 20–40 mmol K^{+}, completely inhibited the motility of rainbow trout spermatozoa (Morisawa et al. 1983, Billard et al. 1986). However, diluting the semen with an isotonic solution of NaCl immediately restored motility (Billard et al. 1986, Gatti et al. 1990). Earlier studies demonstrated also that ions such as Na^{+}, Ca^{+2}, and Mg^{+2} at increased concentrations were antagonistic to K^{+} ions (Scheuring 1925, Baynes et al. 1981) but were indispensable to initiate sperm motility (Christen et al. 1987, Cosson et al. 1989, Billard et al. 1995, Krasznai et al. 2000, Bondarenko et al. 2014)

In our studies on brook trout spermatozoa the concentration of ions in the water used for activation was diversified (Table 1). The culture water (0.35‰) contained 0.048 mmol · dm^{-3} of K^{+}, while the water of 10.0‰ contained over 42 times more of these ions (2.035 mmol · dm^{-3}). Also the concentration of the remaining ions increased with water salinity, and in case of Na^{+} and Cl^{-} their concentration in the water of 10.0‰, compared to the water of 0.35‰, was by 200–250 percentage points higher (Table 1). The concentrations of ions in the seminal plasma of other salmonids, reported by other researchers, were as follows: 20.0–66.0 mmol · dm^{-3} (for K^{+}), 103.0–140.0 mmol · dm^{-3} (for Na^{+}), 0.3–2.6 mmol · dm^{-3} (for Ca^{+2}), 130.0–135.0 mmol · dm^{-3} (for Cl^{-}), 0.8–3.6 mmol · dm^{-3} (for Mg^{+2}) (Billard et al. 1995, Lahnsteiner et al. 1998, Glogowski et al. 2000). It is therefore possible that the increased concentration of K^{+}, Na^{+}, and Ca^{+2} ions in the water of 10.0‰, though smaller than in the salmonid seminal plasma, does not have to be the reason for the decrease in the values of the studied sperm motility parameters. In case of Mg^{+2} (6.193 mmol · dm^{-3}) and Cl^{-} (145.073 mmol · dm^{-3}) ions in the water of 10.0‰ the concentration is higher than that in the salmonid seminal plasma which may affect the motility parameters which were very low in that sample. It should be noted, however, that ion concentrations in the

seminal plasma varies among salmonid species. The content of K^+ in rainbow trout plasma was within 25.3–30.4 mmol·dm^{-3} (Glogowski et al. 2000) and in chum salmon it was 66.1 mmol · dm^{-3} (Morisawa and Suzuki 1980). The content of Ca^{+2} in rainbow trout plasma was within 1.1–2.2 mmol · dm^{-3} (Glogowski et al. 2000), in chum salmon it is 2.2 mmol · dm^{-3} (Morisawa and Suzuki 1980, Lahnsteiner et al. 1998, Glogowski et al. 2000).

No information is available on the content of the studied ions in brook trout seminal plasma. However, based on the results of studies on other salmonid species it can be suspected that a too high content of such ions in the activation water of 10.0‰ may be a factor inhibiting the movement of spermatozoa of that species.

Another factor which may affect the motility of brook trout spermatozoa is pH. Ciereszko et al. (2010) observed the highest values of sperm motility (%) in brook trout at pH of 8.0–9.0. The solutions used in our experiments had only slightly different pH which increased with increasing salinity (Table 1) and was within 7.68–8.52. Considering the results of Ciereszko et al. (2010) it was the range in which sperm motility should be as high as 85%–90%. In our studies the MOT for freshwater was more than twice lower, reaching 37.5%. Such low values of the sperm motility parameters may be associated with the use of culture water for activation of control sample (0.35‰), and salinity water, as well as the duration of transport of the material to the laboratory (1.5 h), and the semen sampling method—without catheter (Glogowski et al. 2000). This is confirmed by the studies in which NaCl and sucrose solution of varied osmolality (0, 100, and 300 mOsm · kg^{-1}) was used for activation of control sample of brook trout spermatozoa; the sperm motility was high and exceeded 70.0%. When activating liquids were used, the MOT increased to more than 90.0% (Bondarenko et al. 2014).

It can be concluded from our results that the water of 1.0‰ and 3.0‰ salinity, used for activation of brook trout spermatozoa, caused a decrease in the values of the studied motility parameters, while in the water of 5.0‰ salinity the values increased and were higher than in control sample (0.35‰). Activation of spermatozoa in the water of the highest salinity (10.0‰) caused a distinct decrease in the values of all studied parameters which may have resulted from too high ion concentration in the water.

Salmonid spermatozoa are characterised by a very short time of activity in the water, hence sudden water pollution in the form of increased salinity on the spawning grounds may have a negative effect on the sperm motility parameters, thus affecting the subsequent egg fertilisation and, consequently the number of the hatched individuals of the species.

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