Sperm morphology, ultrastructure, and motility in pikeperch *Sander lucioperca* (Percidae, Teleostei) associated with various activation media

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ABSTRACT: Spermatozoa morphology, ultrastructure, and spermatozoa motility traits were studied in pikeperch (*Sander lucioperca*) after activation in various media (AM 1 – 45mM NaCl, 5mM KCl, 20mM Tris, pH 8.5; AM 2 – 100mM sucrose, 20mM Tris, pH 8.5; AM 3 – 100mM sucrose, 1mM $CaCl_2$, 20mM Tris, pH 8.5) during a 48-hour storage period. The spermatozoon was acrosomeless and differentiated into a spherical nucleus (head), midpiece, and flagellum. The nucleus length and width measured 1.83 ± 0.03 and 1.63 ± 0.02 mm, respectively. The midpiece was located laterally to the nucleus and possessed proximal and distal centrioles and 2–4 mitochondria. Flagellar length was 33.2 ± 0.90 μm, and a pair of lateral fin-like structures projections was observed. The axoneme consisted of nine peripheral doublet microtubules and a single central pair. After a 24 h storage in all activation media at all sampling times post-activation (15, 45, 90, and 120 s), spermatozoa motility was significantly decreased. Spermatozoa were motile after the 48-hour storage at all sampling times post-activation only in AM 3. After the 48-hour storage, no motile spermatozoa were observed in AM 2 and AM 1 at 90 and 120 s post-activation, respectively. Differences in spermatozoa velocity varied with activation medium during storage. After the 48-hour storage in AM 1 and AM 2, decrease of spermatozoa velocity at 15 s post-activation was observed, while in AM 3, velocity was decreased only after the 48-hour storage. Pikeperch spermatozoa morphology and ultrastructure was found similar to that of most freshwater teleosts, with differences in the arrangement of midpiece, number of mitochondria, and position of centrioles. Viable pikeperch sperm was observed after the 48-hour storage. Motility of spermatozoa was improved by addition of Ca^{2+} to the activation medium, where higher spermatozoa velocity was observed.

Keywords: spermatozoa motility; spermatozoa velocity; calcium; storage time

Pikeperch is a highly valuable fish in commercial aquaculture. Presently, most market size pikeperch are caught from open waters (lakes, rivers, ponds, and lagoons), and relatively few are produced under intensive and/or indoor conditions (Kucharczyk et al., 2007). The total catch of pikeperch was reported about 50 000 t in 1950 and 18 000 t in 2007, global aquaculture production for this species increased from 200 t in 1990 to about 500 t in 2007 (www.fao.org/fishery/statistics/global-capture-production/query/en).

In pikeperch breeding and culture, most studies have focused on improving methods for stimulating ovulation and increasing the quality of the

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eggs and larvae (Zakes and Demska-Zakes, 2005; Ronyai, 2007; Zakes, 2007). Less is known about the biology of sperm and its quality.

In fish farming, good broodstock management is a prerequisite for successful artificial breeding and is important for ensuring consistent production to meet market demand (Billard et al., 1995; Alavi et al., 2008; Mylonas et al., 2010). To date, a large number of studies have focused upon the management of female broodstock in fish farms. However, it is vital to manage also male broodfish, especially in species with a low potential for producing semen and a high risk of sperm contamination by urine during stripping and failure of sperm release in captivity due to stress (Alavi et al., 2008).

Spermatozoa motility in fish, particularly freshwater species such as pikeperch, lasts from several seconds to several minutes (Psenicka et al., 2007; Alavi et al., 2008; Cosson, 2010), and is a key factor in fertilization rate (Billard et al., 1995; Linhart et al., 2008). Due to this short duration of motility and the physiology of the micropyle, which becomes blocked after release of the ova into the aquatic environment (Billard et al., 1995; Alavi et al., 2008; Mylonas et al., 2010), spermatozoa should penetrate the egg within a few seconds of activation (Kudo, 1991). Therefore, use of an activation medium with high potential for triggering activation and identification of factors influencing the viability of sperm after stripping are invaluable for artificial reproduction (Billard et al., 1995; Alavi et al., 2008).

In pikeperch, the quality of spermatozoa differs widely among individuals, and semen production is low (Cejko et al., 2008; Korbuly et al., 2009). The urinary bladder is close to the urogenital pore, which may decrease the initial quality of sperm (Perchec Poupard et al., 1998). Some characteristics of seminal plasma and spermatozoa motility in pikeperch have been reported by Cejko et al. (2008). Significant decrease of spermatozoa viability and motility has been reported after a 3-hour storage of non-diluted sperm or of sperm diluted in various immobilizing media (Korbuly et al., 2009).

Knowledge of spermatozoa morphology and ultrastructure provides information on fish phylogeny and taxonomics and aids in identification of relationships among spermatozoa morphology, reproductive biology, and spermatozoa motility and velocity (Jamieson, 1991, 2009; Psenicka et al., 2006; Lahnsteiner and Patzner, 2008). Spermatozoa morphology and ultrastructure can also be an indicator of male fertility when fish are exposed to endocrine disrupting chemicals or when spermatozoa are manipulated for genome banking such as cryopreservation (Billard et al., 2000; Hatef et al., 2010a; Butts et al., 2010).

The present study was conducted to describe the morphology and ultrastructure of spermatozoa using scanning electron microscopy and to investigate spermatozoa motility and velocity during short-term storage. Ultrastructure of pikeperch spermatozoa has been studied by Lahnsteiner and Mansour (2004). Our results add complementary information to previous studies, particularly by including morphological parameters of spermatozoa and determining motility and velocity within 48 h of sperm collection.

MATERIAL AND METHODS

Broodfish and sperm collection. Sperm was collected from 12 mature pikeperch (body mass 871.58 ± 31.00 g (mean ± standard deviation), total length 466.9 ± 41.0 mm) 48 h after spermiation induction using a single intramuscular injection of hCG at 500 IU/kg (Křišťan et al., 2013). Spermatozoa concentration was assessed by light microscopy, using a Burker haemocytometer (Marienfield, Konigshofen, Germany), following the method of Alavi et al. (2009a).

Sperm morphology. Sperm from four males was fixed in with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.5) and stored at 4°C until transfer to the Electron Microscopy Laboratory, University of South Bohemia in České Budějovice. Samples were post-fixed and washed repeatedly for 2 h in 4% osmium tetroxide at 4°C and dehydrated through an acetone series. Samples for scanning electron microscopy (SEM) were dehydrated in a critical point dryer Pelco CPD 2 (Ted Pella Inc., Redding, USA). Sperm samples were coated with gold under vacuum with a SEM Coating Unit E5100 (Polaron Equipment Ltd., Watford, UK) and examined using a JSM 7401-F (JEOL Ltd., Akishima, Japan) equipped with a three CCD video camera SONY DXC-970MD (Sony, Tokyo, Japan). Samples for transmission electron microscopy (TEM) were embedded in resin (Polybed 812). A series of ultrathin sections were cut using a Leica Ultracut UCT ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany) and double-stained with uranyl acetate and lead citrate. Samples were viewed in a TEM JEOL 1010 (JEOL Ltd.) operated at 80 kV. Micrographs were evaluated using the Olympus Micro Image software

(Version 4.0.1. for MS Windows, 1998) to measure spermatozoa morphological parameters.

Sperm motility assessment. Sperm motility and velocity of non-diluted sperm were analyzed at room temperature during a short-term storage (6°C), at 0, 6, 24, and 48 h after collection. At each storage time, sperm motility was directly activated, without dilution in immobilizing medium, in the following activation media: activation medium 1 (AM 1) was composed of ionic compounds (45mM NaCl, 5mM KCl), while activation medium 2 (AM 2) was a non-ionic compound (100mM sucrose). Activation medium 3 (AM 3) was composed of a non-ionic compound (100mM sucrose) plus 1mM $CaCl_2$. All activation media were buffered by adding 20mM Tris, and pH was adjusted to 8.5. A small drop of sperm was directly diluted in 50 µl of the activation medium at a sperm: AM of about 1 : 2000. Bovine serum albumin (BSA) was added to the activation medium at a final concentration 0.1% w/v to avoid sticking of the sperm cells onto the slide. Immediately after addition of sperm to the drop, sperm motility was recorded 2 min per observation using a three CCD video camera SONY DXC-970MD (Sony) mounted on a dark-field microscope Olympus BX50 (Olympus, Tokyo, Japan) and a DVD-recorder SONY DVO-1000 MD (Sony). The microscope was equipped with a stroboscopic lamp with frequency adjusted to 50 Hz. A computer-assisted image analysis (Olympus Micro Image software) was used to measure percent sperm motility and sperm velocity (µm/s) from five successive video images, which showed positions of sperm heads following Hatef et al. (2010b). Five video frames were captured from a DVD-recorder SONY DVO-1000 MD (Sony) and accumulated in real time post-activation. The output frame showed positions of heads of motile spermatozoa in five spots (red-green-green-green-blue), while the immotile spermatozoa appeared as white. The percentage of motile spermatozoa was calculated by counting red or blue spots versus the number of white spots. To measure velocity, the distance between red and blue spots (five head positions) was measured and divided into the duration of travel. Velocity was calculated only for motile spermatozoa (50–60 spermatozoa per treatment).

Data analysis. Homogeneity of variance was tested for all data using Levene's test. Repeat measures of Analysis of Variance (ANOVA) were used to understand the effects of storage time and activation medium and their interactions on sperm motility and velocity measured at different times post-activation; alpha was set at 0.05. When significance of storage time was observed, the model was revised to individual ANOVA models for each activation medium to analyze the effects of storage time on spermatozoa motility and velocity using a single-factor ANOVA followed by Tukey's post-hoc test. All data are presented as mean ± standard error of mean (SEM).

RESULTS

Sperm morphology and ultrastructure. The pikeperch spermatozoon is a primitive acrosomeless aquasperm differentiated into a nucleus (head),

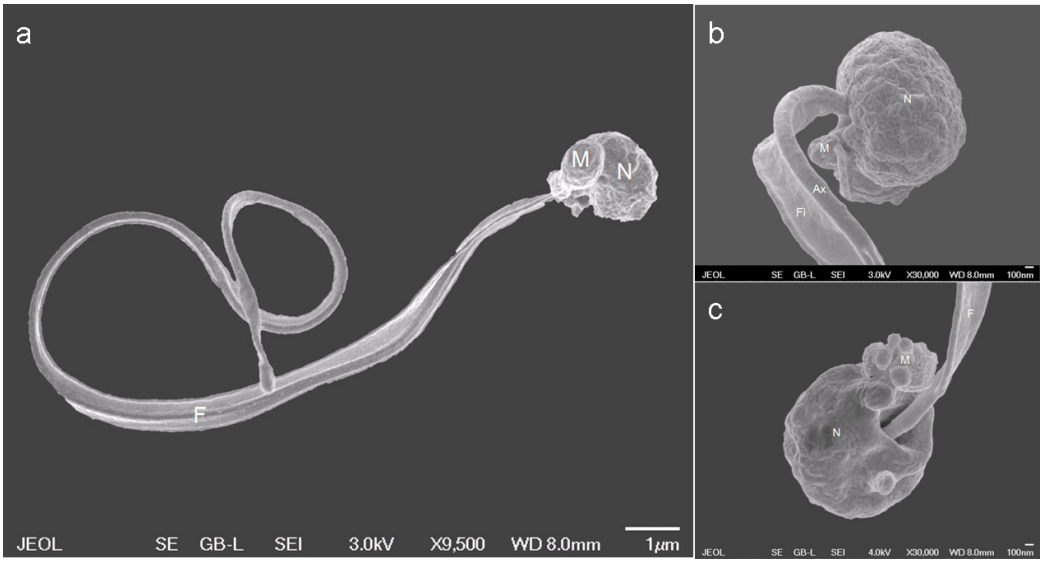

Figure 1. Scanning electron micrograph of pikeperch (*Sander lucioperca*) spermatozoon, **a**–**c** show position of midpiece N = nucleus, M = midpiece, F = flagellum, Ax = axoneme, Fi = fin-like projection

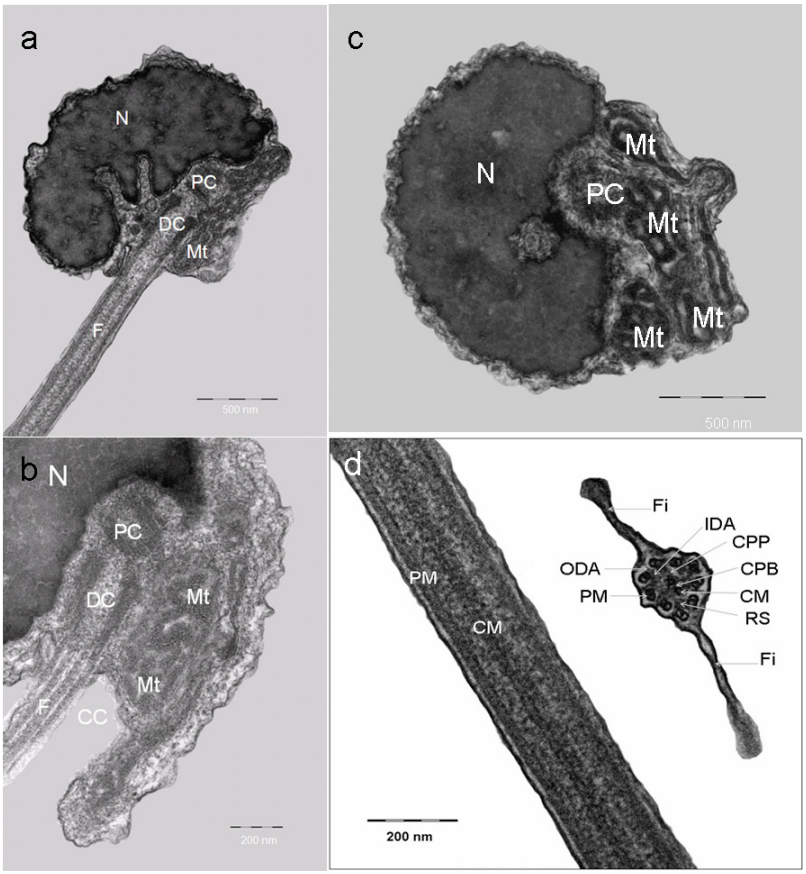

Figure 2. Transmission electron micrograph of longitudinal section (**a, b**), cross section in the midpiece region (**c**), and axonemal ultrastructure of pikeperch (*Sander lucioperca*) spermatozoon (**d**)

N = nucleus, Mt = mitochondria, CC = cytoplasmic channel, DC = distal centriole, PC = proximal centriole, F = flagellum, Fi = fin-like projection, IDA = inner dynein arm, ODA = outer dynein arm, CPB = central pair bridge, CM = central microtubule, PM = peripheral microtubule, RS = radial spoke, CPP = central pair projections

a small midpiece, and a flagellum (Figures 1a–c, 2a–d). The nucleus dimensions were measured at 1.83 ± 0.03 µm length and 1.63 ± 0.02 µm width. The midpiece was located laterally to the nucleus (Figure 1a–c). Longitudinal (Figure 2 a, b) and cross (Figure 2c) sections of midpiece showed the presence of proximal and distal centrioles consisting of nine triplets of microtubules, at right angle to each other. In some cases, the proximal centriole was inclined to the distal centriole at an angle 110° (Figure 2b). The distal centriole served as the basal body of the flagellum. Two to four mitochondria were observed in the midpiece between the plasma membrane and centriole complex (Figure 2a–c). A cytoplasmic channel was clearly visible close the plasma membrane around midpiece and flagellum. A lateral pair of fin-like projections was observed along the flagellum, originating from extension of the plasma membrane around the flagellum. The flagellum, 33.2 ± 0.90 µm in length, was composed of a typical eukaryote microtubule-based axoneme. The axoneme was composed of nine peripheral doublet microtubules and a single central pair (Figure 2d). Outer and inner dynein arms, radial spokes, and other fine structure of the axoneme were observed in pikeperch spermatozoa (Figure 2d).

Sperm concentration, motility, and velocity. Mean ± SE sperm concentration was found to be 14.87 ± 1.36 per ml. Only storage time showed significant effects on spermatozoa motility at different times post-activation (Table 1). Therefore, the model was re-run with the storage time × activation medium interaction effects removed and the effects of storage time and activation medium were interpreted. Results showed significant effects of storage time on sperm motility ($F_{\text{df, 15 s post-activation}} = 29.68_3$; $F_{\text{df, 45 s post-activation}} = 27.06_3$; $F_{\text{df, 90 s post-activation}} = 29.64_3$; $F_{\text{df, 120 s post-activation}} = 13.33_3$; $P < 0.001$). Activation media did not influence sperm motility ($F_{\text{df, 15 s post-activation}} = 0.07_2$; $F_{\text{df, 45 s post-activation}} = 0.06_2$; $F_{\text{df, 90 s post-activation}} = 0.43_2$; $F_{\text{df, 120 s post-activation}} = 0.11_2$; $P > 0.05$). Therefore, the models were revised to calculate the effects of storage time on motility after activation in each activation medium separately for each time post-activation. At all times post-activation (15, 45, 90, and 120 s), motility was significantly affected by storage time in all media (Table 2) and decreased at 24 h storage (Figure 3). Spermatozoa were motile after the 48-hour storage in AM 3 at all times post-activation (Figure 3). After the 48-hour storage, no motile spermatozoa were observed in AM 2 at

Table 1. Summary of statistics (F_{df}) obtained from repeated ANOVA models used to study the effects of storage time, activation medium, and their interaction (storage time × activation medium) on spermatozoa motility and velocity in pikeperch (*Sander lucioperca*) at various times post activation

Parameter	Time post activation (s)	Storage time	Activation medium	Storage time × activation medium
Spermatozoa motility	15	26.51_3^{***}	0.75_2	0.50_6
	45	22.12_3^{***}	0.19_2	0.57_6
	90	23.05_3^{***}	0.30_2	0.50_6
	120	10.44_3^{***}	0.05_2	0.42_6
Spermatozoa velocity	15	18.57_3^{***}	8.42_2^{***}	1.37_6
	45	6.72_3^{***}	10.48_2^{**}	0.62_6
	90	7.10_3^{***}	4.47_2	0.38_6
	120	3.91_3^{*}	0.97_2	1.19_6

$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

90 s post-activation and in AM 1 at 120 s post-activation (Figure 3c–d).

Significant effects of both storage time and activation medium were observed on spermatozoa velocity (Table 1). As no significant effect of storage time × activation medium interactions was observed (Table 1), this effect was removed from the model, and the effects of storage time and activation medium were interpreted. Results showed significant effects of storage time on spermatozoa velocity at all evaluated times post-activation ($F_{df,\,15\,s\,post\text{-}activation} = 11.39_3, P < 0.001; F_{df,\,45\,s\,post\text{-}activation} =$

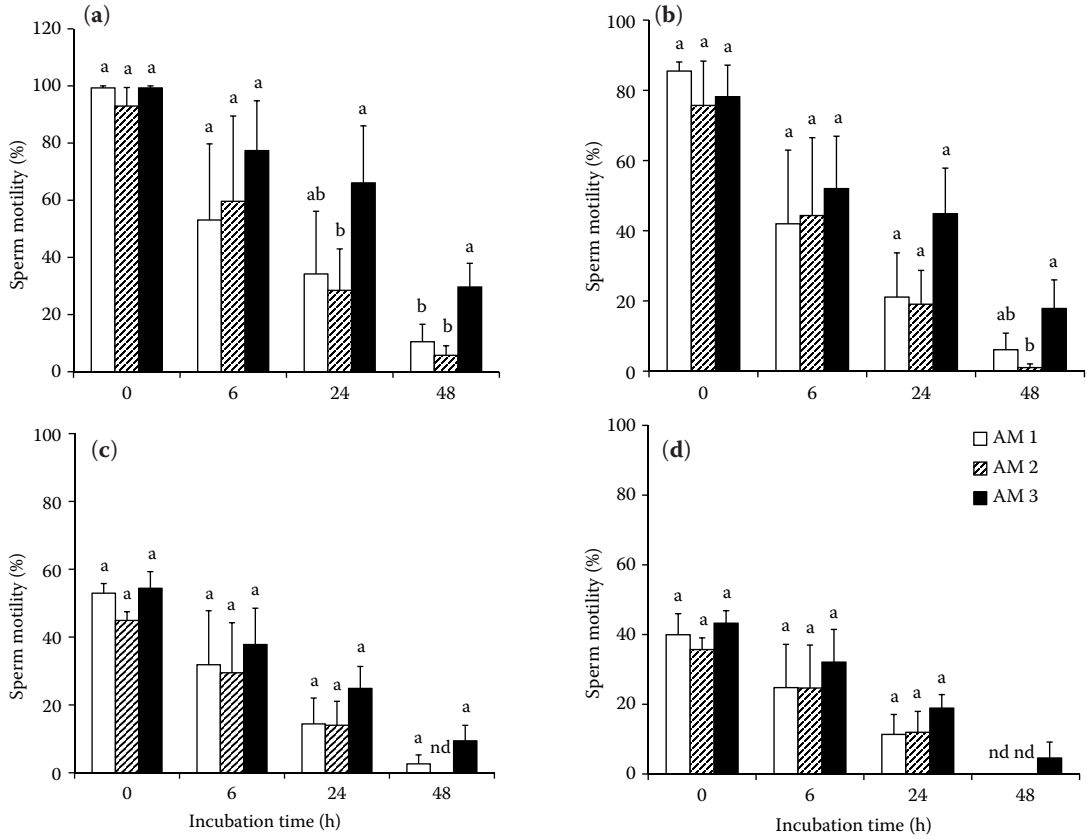

Figure 3. Spermatozoa motility in pikeperch (*Sander lucioperca*) under 4 storage periods measured at 15 (**a**), 45 (**b**), 90 (**c**), and 120 (**d**) s post activation in different activation media (AM 1: 45mM NaCl, 5mM KCl, 20mM Tris, pH 8.5; AM 2: 100mM sucrose, 20mM Tris, pH 8.5; AM 3: 100mM sucrose, 1mM $CaCl_2$, 20mM Tris, pH 8.5). For each activation medium, values with similar superscripts are not significantly affected by incubation time ($P > 0.05$)

Table 2. Summary of statistics (F_{df}) obtained from ANOVA models used to study the effects of storage time on spermatozoa motility and velocity in pikeperch (*Sander lucioperca*) at various times post activation in different activation media

Parameter	Time post-activation (s)	Activation medium		
		AM 1	AM 2	AM 3
Spermatozoa motility	15	4.62_3^*	4.49_3^*	4.40_3^*
	45	7.58_3^{**}	5.63_3^*	4.56_3^*
	90	5.87_3^*	5.49_3^*	7.26_3^{**}
	120	5.33_3^*	4.81_3^*	8.14_3^{**}
Spermatozoa velocity	15	4.01_3^*	2.49_3^*	52.42_3^{***}
	45	3.56_3^*	2.24_3^*	2.53_3^*
	90	12.20_3^{**}	0.07_3	4.25_3^{**}
	120	1.00_3	0.02_3	5.53_3^{**}

$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

5.13_3, $P < 0.01$; $F_{df,\ 90\ s\ post\text{-}activation} = 7.49_3$, $P < 0.001$; $F_{df,\ 120\ s\ post\text{-}activation} = 3.96_3$, $P < 0.05$). Activation media influenced spermatozoa velocity at 45 s ($F_{df} = 7.13_2$, $P < 0.01$) and 90 s ($F_{df} = 3.39_2$, $P < 0.05$) post-activation, but not at 15 s ($F_{df} = 2.21_2$, $P > 0.05$) and 120 s ($F_{df} = 0.56_2$, $P > 0.05$) post-activation. Therefore, for each time post-activation, the models were revised to separately analyze the effects of storage time on velocity after activation in each medium. At 15 and 45 s post-

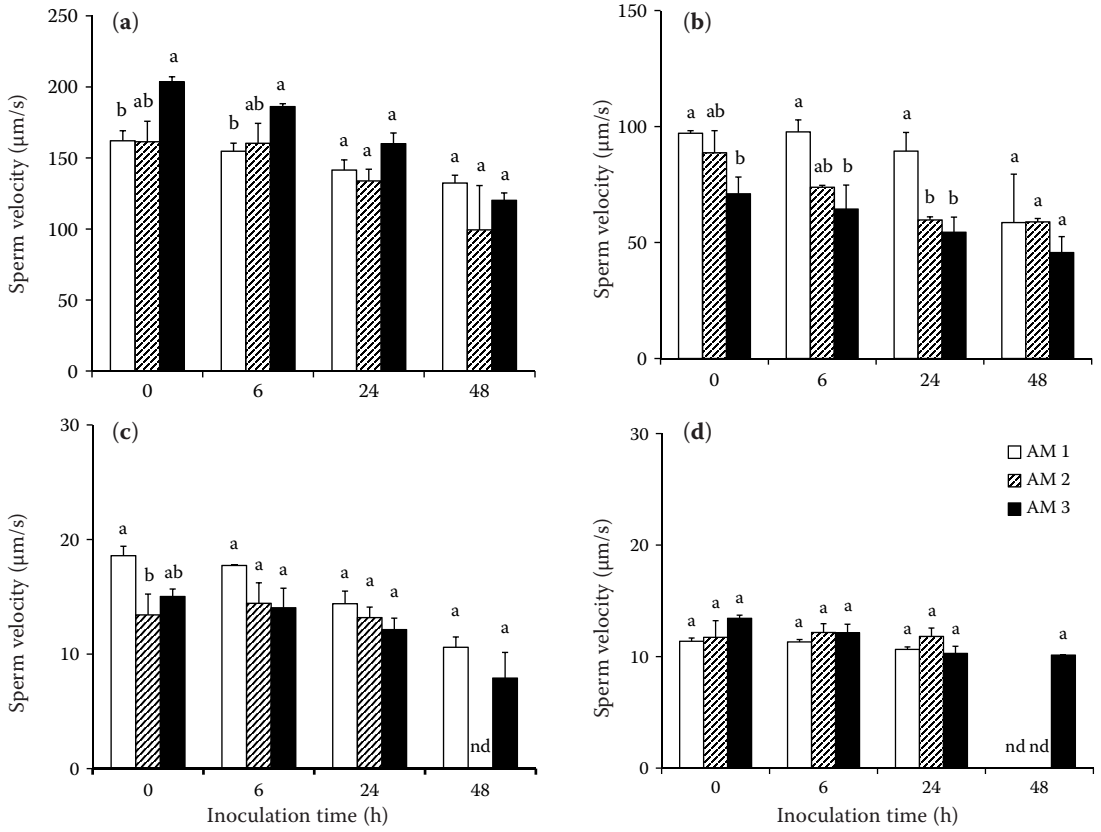

Figure 4. Spermatozoa velocity in pikeperch (*Sander lucioperca*) under 4 storage periods measured at 15 (**a**), 45 (**b**), 90 (**c**) and 120 (**d**) s post activation in different activation media (AM 1: 45mM NaCl, 5mM KCl, 20mM Tris, pH 8.5; AM 2: 100mM sucrose, 20mM Tris, pH 8.5; AM 3: 100mM sucrose, 1mM $CaCl_2$, 20mM Tris, pH 8.5). For each activation medium, values with similar superscripts are not significantly affected by incubation time ($P > 0.05$)

activation, spermatozoa velocity was significantly affected by storage time in all activation media (Table 2). After the 48-hour storage, activation in AM 1 resulted in decreased spermatozoa velocity at 15, 45, and 90 s post-activation, but no difference was observed among storage times at 120 s post-activation (Figure 4). After the 48- and 24-hour storage in AM 2, spermatozoa velocity at 15 and 45 s post-activation, respectively, was significantly decreased, while no velocity was observed at 90 and 120 s post activation (Figure 4). Spermatozoa velocity showed significant decrease at 15 s post-activation after the 24-hour storage (Figure 4).

DISCUSSION

In the present study, spermatozoa morphology, ultrastructure, and motility traits were studied in pikeperch after activation in different media during storage period. The motility of spermatozoa was evaluated within 2 min of activation during the storage period. Storage time showed significant effects on motility, and both storage time and activation medium showed significant effects on sperm velocity.

To date, among 159 species in 9 genera of Percidae (Lahnsteiner and Patzner, 2008), spermatozoa morphology and ultrastructure have been studied only in Eurasian perch *Perca fluviatilis* (Lahnsteiner et al., 1995; Hatef et al., 2010b) and pikeperch (Lahnsteiner and Mansour, 2004). Nucleus shape seems to be similar in Eurasian perch and pikeperch, both exhibiting a spherical nucleus (Lahnsteiner et al., 1995; Lahnsteiner and Mansour, 2004; Hatef et al., 2010b). Previous studies have shown a larger spermatozoa nucleus in Eurasian perch (1.9 × 1.8 μm) (Lahnsteiner et al., 1995) compared to that of pikeperch (1.6 × 1.3 μm) (Lahnsteiner and Mansour, 2004), and the present study shows similar dimensions (1.8 × 1.6 μm). The minimal difference observed between the present study and that of Lahnsteiner and Mansour (2004) might be related to inter-individual differences as reported previously (Alavi et al., 2008; Psenicka et al., 2008; Hatef et al., 2011). Midpiece in pikeperch was found to be located lateral to the nucleus, in agreement with Lahnsteiner and Mansour (2004), while in Eurasian perch it is located at the base of the nucleus where the flagellum originates (Lahnsteiner et al., 1995; Hatef et al., 2011). The present study and that of Lahnsteiner and Mansour (2004) showed similar ultrastructure of centrioles consisting of nine triplets of microtubules, but the organization of centrioles seems to differ between Eurasian perch and pikeperch. The proximal centriole was inclined to the distal centriole under the angle of 90 or 110° in pikeperch (Lahnsteiner and Mansour, 2004; the present study), but the angle was 90° in Eurasian perch (Lahnsteiner et al., 1995; Hatef et al., 2010b). One or 2 mitochondria were previously reported in Eurasian perch (Lahnsteiner et al., 1995; Hatef et al., 2011), but 2–4 mitochondria were observed in the present study. In both species, cytoplasmic channel was observed between the flagellum and midpiece. The present study presents the first record of flagellar length at 33.2 μm, similar to that of Eurasian perch at 30–35 μm (Lahnsteiner et al., 1995). In both Eurasian perch and pikeperch, the axoneme consists of 9 peripheral doublet microtubules and a central pair, similar to other eukaryotes (Inaba, 2008). A lateral fin-like projection was observed in pikeperch, but it was not reported in Eurasian perch (Lahnsteiner et al., 1995; Hatef et al., 2010b). This structure was previously reported in sturgeon (Psenicka et al., 2008; Hatef et al., 2011) and pike, *Esox lucius* (Alavi et al., 2009b).

Initial spermatozoa motility in pikeperch observed in the present study is similar to that of Eurasian perch, but velocity seems to be higher (160 vs. 115–130 μm/s) (Lahnsteiner et al., 1995; Alavi et al., 2007). The difference in velocity between Eurasian perch and pikeperch spermatozoa might be related to the presence of a lateral fin-like projection, which enhances flagellar movement during sperm activation, or to initial ATP content (Cosson, 2010).

Motility in pikeperch spermatozoa observed in the present study was higher than that reported by Cejko et al. (2008) and Jarmolowicz et al (2010). They reported sperm motility of 15–31%, while in the present study, initial motility ranged 92–100%. This difference may be related to initial quality of sperm rather than the hypo-osmolality required for sperm activation in freshwater teleosts (Alavi and Cosson, 2006; Morisawa, 2008). Perchec et al. (1995) and Poupard et al. (1998) showed significant effects of urine on both spermatozoa motility and velocity via modulation of ATP content of spermatozoa, which is required for sperm activation after triggering by a hypo-osmotic signal.

Storage period significantly affected both motile cells and velocity in pikeperch spermatozoa. Initial sperm motile cells and velocity, analyzed at 15 s post-

activation, was 92–99% and 161–204 µm/s at 0 h storage and decreased to 6–30% and 9–132 µm/s at a 48-hour storage. At 120 s post-activation, spermatozoa motility decreased from 36–43% (0 h storage) to 0–5% (48-hour storage of sperm), but sperm velocity remained unchanged (11–13 µm/s at 0 h and 10 µm/s after 48 h). Decrease in spermatozoa motility and velocity has been frequently documented in fish species including common carp, *Cyprinus carpio* (Saad et al., 1988), halibut, *Hippoglossus stenolepis* (Billard et al., 1993), turbot, *Psetta maxima* (Chereguini et al., 1997), tench, *Tinca tinca* (Rodina et al., 2004), and Eurasian perch (Hatef et al., 2011). These decreases have been attributed to damage of spermatozoa ultrastructure and decrease of ATP content (Billard et al., 1995; Hatef et al., 2011). Rodina et al. (2004) observed lower fertilizing ability of stored sperm of tench and suggested use of an immobilizing medium with osmolality similar or slightly higher than that of seminal plasma for sperm storage. In pikeperch, Korbuly et al. (2009) studied incubation of sperm using various immobilizing media and observed better spermatozoa motility after 3 h incubation compared to a control when the sperm was previously diluted in Ringer's solution or PBS. In the present study, the sperm was not pre-diluted in immobilizing medium, as the aim was to quantify viability of sperm during storage. The results showed that pikeperch spermatozoa usually live for 24 h after sperm collection, and very low sperm motility could be achieved at a 48-hour storage after activation in medium containing 1mM Ca^{2+}. This suggests participation of Ca^{2+} in spermatozoa activation, as has been reported in freshwater teleosts (Alavi and Cosson, 2006; Morisawa, 2008).

The present study showed spermatozoa activation in pikeperch both after dilution in ionic medium (AM 1 and AM 3) and in non-ionic medium (AM 2). This suggests a similar mechanism of sperm activation in Eurasian perch and pikeperch (Alavi et al., 2007). However, activation media in the present study influenced velocity, but not motility. Spermatozoa motility evaluated at 15 s post-activation did not differ with respect to activation media at 0 h storage, but, after the 48-hour storage, the highest motility was observed with activation in AM 3 (30%) compared to that in AM 1 (10%) and AM 2 (6%). At 120 s post-activation, spermatozoa motility did not differ with activation media at 0 h storage, but, after a 48-hour storage, motile spermatozoa were observed only with activation in AM 3. At 15 s post-activation, the highest spermatozoa velocity was observed after activation in AM 3 (204 µm/s), compared to AM 1 (162 µm/s) and AM 2 (161 µm/s) at 0 h storage, and did not differ at the 48-hour storage (132, 99, and 120 µm/s in AM 1, AM 2, and AM 3, respectively). The observed higher velocity with AM 3 might be related to the presence of Ca^{2+}. It is known that Ca^{2+} enhances axonemal beating. Similar effects of Ca^{2+} have been reported in velocity of Eurasian perch spermatozoa (Alavi et al., 2007).

The present study showed similar morphology and ultrastructure of Eurasian perch and pikeperch, with differences observed in the arrangement of midpiece and centrioles and the presence of fins along the flagellum. The present study suggests using an activation medium containing Ca^{2+} for activation of short-term stored sperm.

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