

# Spermatozoa motility in the Persian sturgeon, *Acipenser persicus*: effects of pH, dilution rate, ions and osmolality

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## Abstract

Sperm motility is a prerequisite factor determining semen quality and fertilizing capacity. The effects of environmental factors including pH, cations and osmolality as well as the role of dilution rate on sperm motility parameters in *Acipenser persicus* were studied. The best pH and dilution rate for activation of spermatozoa were pH 8.0 and dilution ratio 1:50. Ionic factors can stimulate the initiation of sperm activation. The maximum percentage of motile sperm and total duration of sperm motility were observed in solutions containing 25 mM NaCl, 0.2 mM KCl, 3 mM CaSO<sub>4</sub>, 10 mM MgSO<sub>4</sub> and sucrose with an osmolality of 50 mosmol kg<sup>-1</sup>. The present study provides us with some basic knowledge about sturgeon spermatozoa biosensitivity to ionic and osmolality effects. A sensitivity of *A. persicus* sperm was observed after induction of activation of sperm motility in solution containing cations or sucrose with high osmolality. Concentrations more than 50 mM Na<sup>+</sup>, more than 1 mM K<sup>+</sup>, more than 3 mM Ca<sup>2+</sup> and more than 10 mM Mg<sup>2+</sup> had negative effects on sperm motility. Also, osmolality more than 100 mosmol kg<sup>-1</sup> had an inhibitory effect. It is clear that ions and osmolality stimulate the motility of spermatozoa by changes in the properties of the plasma membrane including its potential and its ionic conductance. The inhibitory role of high osmolality of the swimming medium (more than 100 mosmol kg<sup>-1</sup>) and insufficient osmolality of the seminal plasma to inhibit semen motility suggested that osmolality is not the principal factor preventing sperm motility in seminal fluid but that K<sup>+</sup> is a major inhibitory factor of sperm motility in seminal plasma.

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## Introduction

A major part of the world's sturgeon catch (90–92%) originates from the Caspian Sea (Dettlaff *et al.* 1993). Stocks of sturgeons are decreasing dramatically (Conte *et al.* 1988, Ronyai and Varadi 1995). The world sturgeon catch was nearly 28 000 tonnes in 1982 and less than 2000 tonnes by 1999 (Billard and Lecointre 2001). The Persian sturgeon, *Acipenser persicus*, is an anadromous fish belonging to the genus *Acipenser* and to the family Acipenseridae, which is widely distributed along the Iranian coastal waters of the Caspian Sea (Holci'k 1989, Birstein *et al.* 1997). Since 1971, artificial propagation and rearing of sturgeons has been implemented with a general trend in development of fish culture in the southern part of the Caspian Sea, Iran (Azari Takami 1992). The male sturgeon, like the female, is captured from rivers during the spawning season (Kohneshahri and Azari Takami 1974). The goal of sturgeon cultivation is either the production of fingerlings for restocking

natural waters and populations, or the production of marketable-size fish (Ronyai and Varadi 1995, Chebanov and Billard 2001). Currently, the propagation and cultivation of sturgeon are at the research and development stages in Iran (Alavi *et al.* 2002).

Because of the shortage of sturgeon male broodstock (Kohneshahri and Azari Takami 1974), the availability of goodquality sperm in sufficient amounts at the needed time and the management of semen ultimately determine the success of artificial reproduction in sturgeon farms (Gallis *et al.* 1991, Billard 2000, Linhart *et al.* 2002, Alavi *et al.* 2004). The quality of sperm usually refers to the motility, which is a prerequisite factor determining the semen's fertilizing ability (Billard 1978, Cosson *et al.* 1991, Lahnsteiner *et al.* 1997). Several parameters have been used to evaluate motility. The most commonly used in the past was the total period of sperm motility (Stoss 1983). More recent studies refer to the percentage of motile sperm observed visually (Billard *et al.* 1987,

Cosson *et al.* 1999). More-quantitative approaches to studying sperm motility use the computer-assisted semen analysis (CASA) system (Dreanno *et al.* 1999, Toth *et al.* 1995, Christ *et al.* 1996, Lahnsteiner *et al.* 1996, Cosson *et al.* 1997, Kime *et al.* 2001).

Sturgeon spermatozoa like that of other teleost fishes are immotile in the seminal plasma (Billard 2000, Alavi *et al.* 2002). The inhibition of motility of sperm in semen is mainly due to osmotic pressure in most species (Morisawa and Suzuki 1980, Stoss 1983, Linhart *et al.* 1991, Billard *et al.* 1995a) but  $K^+$  plays a major role in salmonids (Schlenk and Kahmman 1938, Stoss 1983, Billard *et al.* 1995b) and in sturgeons (Gallis *et al.* 1991, Billard 2000, Alavi and Cosson 2004). Several parameters of the swimming medium, such as ion concentration ( $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ), osmotic level, pH and dilution rate, affect the motility duration of fish spermatozoa (Morisawa and Suzuki 1980, Stoss 1983, Linhart *et al.* 1991, Billard *et al.* 1995b, Cosson *et al.* 1999, Tvedt *et al.* 2001, Ciereszko *et al.* 2002, Ingermann *et al.* 2003). Optimum sperm motility was observed in alkaline pH and with a low dilution rate (at 1:50) in *Acipenseridae* (Gallis *et al.* 1991, Alavi *et al.* 2002, Alavi and Cosson 2004). Gallis *et al.* (1991), Cosson and Linhart (1996), Toth *et al.* (1997) and Linhart *et al.* (2002) reported the control of sperm motility by  $K^+$  concentration in sturgeon. According to their results, a  $K^+$  concentration of more than 0.5 mM was an inhibitory factor for the initiation of sperm motility. The biosensitivity of sperm to  $Ca^{2+}$  and  $Na^+$  was reported by Toth *et al.* (1997) and Cosson *et al.* (1999).  $Ca^{2+}$  and  $Na^+$  of more than 10 mM had a negative effect on sperm motility. Sturgeon sperm is motile in the range 0–120 mosmol  $kg^{-1}$  (Gallis *et al.* 1991, Linhart *et al.* 1995), but the osmotic level of seminal plasma is lower than 100 mosmol  $kg^{-1}$  (Gallis *et al.* 1991, Piros *et al.* 2002, Alavi *et al.* 2004). These findings suggest that (1) control of sturgeon sperm motility is due to the ionic content of the medium but that (2) sturgeon sperm shows sensitivity to osmolality and ion concentrations of the medium.

Until now, little information has been available on the sperm biology of sturgeon, including ultrastructure and morphological functions (Cherr and Clark 1984, 1985, Ciereszko *et al.* 1994, 1996, Billard *et al.* 2000), characterization of motility and fertilization ability (Gallis *et al.* 1991, Linhart *et al.* 1995, Cosson and Linhart 1996, Tsvetkova *et al.* 1996, Toth *et al.* 1997, Cosson *et al.* 2000, Williot *et al.* 2000), respiration and energetics of motility of spermatozoa (Billard *et al.* 1999, Ingermann *et al.* 2002), the correlation between spermatozoan motility parameters and biochemical characteristics of seminal plasma (Gallis *et al.* 1991, Toth *et al.* 1997, Ingermann *et al.* 2002) and cryopreservation and short-term storage (Tsvetkova *et al.* 1996, Jahnichen *et al.* 1999, Billard *et al.* 2000). Unfortunately, data concerning biological aspects of sperm in the Persian sturgeon are rare (Alavi *et al.* 2004).

The objectives of this study were (1) to investigate the effects of environmental factors including pH, cations ( $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ), osmolality of the medium and the dilution rates of semen on motility characteristics of spermatozoa and (2) to determine range of biosensitivity of sperm to ions and osmotic level in *A. persicus*.

## Materials and Methods

### The broodstock

The experiments were carried out at the Dr Beheshti Artificial Sturgeon Propagation and Rearing Center (BASPRC) and the International Sturgeon Research Institute (ISRI), near to the Sangar dam Rasht, Iran, during spawning season, in April 2001 and April 2002. Broodstock of *A. persicus* (119–159 cm total length and 17–20.5 kg weight) were captured from the Sefidroud river, and were then transferred to the broodstock pond of the hatchery at BASPRC. The broodstock were selected and kept together, unexposed to females, in a tank with a water temperature of 14–17 °C and 8.2 mg  $O_2 l^{-1}$ . Male Persian sturgeon were injected intramuscularly with sturgeon pituitary homogenized extract (SPE) at doses of 50–70 mg  $kg^{-1}$  (Kohneshahri and Azari Takami 1974). Milt was collected after spermiation, approximately 24 h after spawning induction, in glass experimental tubes and stored on ice during transportation to the laboratory at the ISRI. Semen of males was stored at 4 °C during motility analysis.

### Motility analysis

Sperm motility was evaluated visually for the percentage of motile spermatozoa after activation and total duration of motility (in seconds). Sperm motility parameters were measured immediately after initiation of sperm activation until 100% of spermatozoa were immotile. To induce the initiation of sperm motility, a 49  $\mu l$  drop of medium placed on a glass slide and then a drop of 1  $\mu l$  fresh sperm was diluted using a microsampler. All experiments were performed in triplicate at room temperature (17–20 °C), using light microscopy under 400 $\times$  magnification. To avoid subjective bias, all measurements were carried out by the same experimenter.

### Effect of pH

Semen of three males was used to determine the pH effect on motility of *A. persicus* spermatozoa. The effect of pH on motility of sperm of Persian sturgeon was assessed using the buffer Tris–HCl (20 mM) adjusted to different values of pH 6.0, 7.0, 8.0 and 9.0 at a dilution rate of 1:50 (1  $\mu l$  semen:49  $\mu l$  diluent). The pH of the diluent was measured with a classical laboratory pH meter (Orion Model 410A pHmeter). The results of this experiment show that pH 8.0 was the optimum for inducing sperm motility and this was retained in subsequent experiments.

### Effect of dilution rate

Semen of three males was used to determine the effect of dilution rate on the motility characteristics of spermatozoa in 2001. The effect of dilution rate on sperm motility was evaluated firstly with fresh water (control) and secondly compared with fresh water containing 20 mM Tris–HCl, pH 8.0, at dilution rates of 1:10, 1:50 and 1:200. The main aim of this experiment was to determine the optimum dilution rate for activating sperm motility. The results show that *A. persicus* sperm motility becomes highly motile at a dilution ratio of 1:50.

### Effects of cations

The semen of four and three males was used to test the effects of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  and to test the effect of  $\text{Mg}^{2+}$ , respectively. Milt samples were suspended with 20 mM Tris–HCl buffer, pH 8.0, containing 0, 25, 50, 100 and 125 mM NaCl, 0, 0.2, 0.5, 1, 2 and 5 mM KCl or 0, 1, 3, 5, 10 and 15 mM  $\text{CaSO}_4$ . To study the effect of  $\text{Mg}^{2+}$ , fresh milt of *A. persicus* was suspended with 20 mM Tris–HCl buffer, pH 8.0, containing 0, 3, 5, 10 and 15 mM  $\text{MgSO}_4$ .

### Effect of osmolality

This experiment also was carried out on three males. Milt was activated with 0–200 mosmol  $\text{kg}^{-1}$  activation solution containing 20 mM Tris–HCl, pH 8.0, to test the effect of osmolality on the motility characteristics of spermatozoa in *A. persicus*. The osmolality of solutions containing sucrose was measured with an osmometer (Melting Point Osmometer no. 961003, Roebling Company, Berlin, Germany) using a freezingpoint depression. Distilled water (0 mosmol  $\text{kg}^{-1}$ ) was used as the control solution.

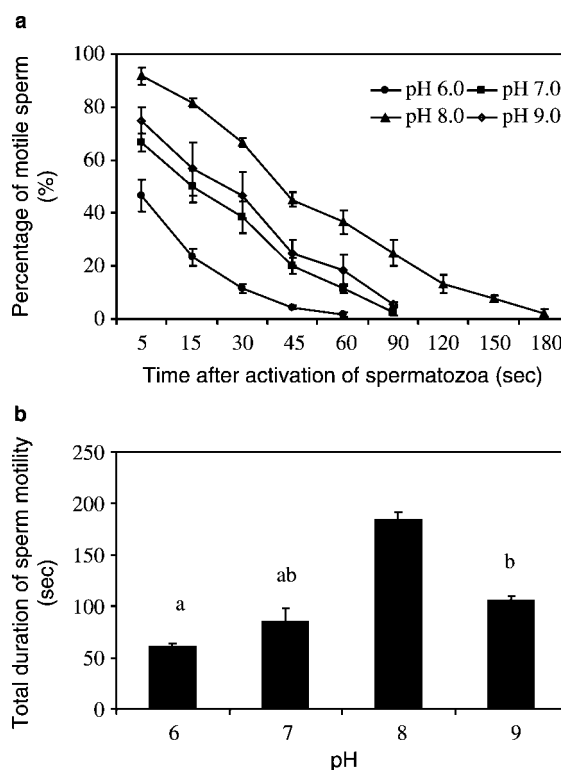
### Statistical analysis

The normal distribution of the data was tested using the Kolmogorov–Smirnov test; data were sufficiently normal. Statistical comparison was made with the independent sample *t* test and the Mann–Whitney U test in the cases of total duration period of sperm motility and percentages of motile spermatozoa, respectively. After testing the equality of variance using Levene's test, statistical comparison of duration of sperm motility was analyzed by independent sample *t* test. Data are presented as means  $\pm$  S.E.M. in the text. All statistical analyses were carried out using SPSS 9.0.

## Results

### Effect of pH

The total duration of sperm motility and the percentage of motile spermatozoa observed after dilution depends on the pH of the dilution medium, distilled water buffered with 20 mM Tris–HCl at pH from 6.0 to 9.0 (Fig. 1). Maximum and minimum percentages and total durations of

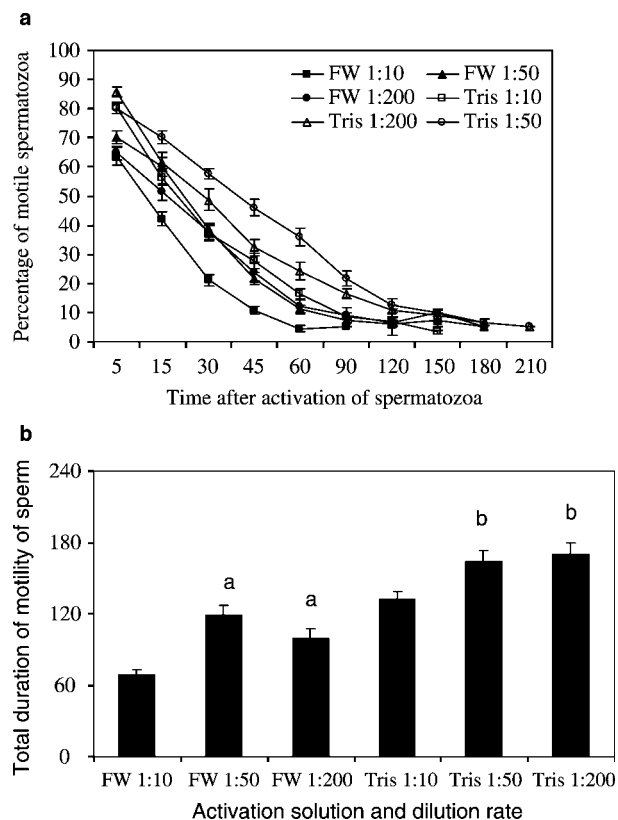


**Figure 1** Percentage of motile spermatozoa (a) and the total duration of sperm motility (b) in *A. persicus* after activation at different pH values. Times are shown in seconds. In b, bars with the same letter above are not significantly different (independent sample *t* test,  $n = 3$ ,  $P > 0.05$ ).

motility occurred at pH 8.0 and pH 6.0, respectively (Fig. 1; Mann–Whitney U,  $P < 0.01$  for percentage of motile sperm; independent sample *t* test,  $P < 0.001$  for total motility duration). There were no significant differences between percentage of motile spermatozoa and duration of activity at pH 7.0 and 9.0 (Fig. 1; Mann–Whitney U,  $P > 0.05$ ).

### Effect of dilution rate

The effects of dilution rate on spermatozoa motility characteristics are shown in Fig. 2. The percentages of motile sperm were 67.63, 70.0 and 64.8% at dilution rates of 1:10, 1:50 and 1:200, respectively, in the fifth second after the initiation of sperm activation in fresh water (Fig. 2a). The observed differences between percentages of motile cells were not statistically significant at this time point (Fig. 2a; Mann–Whitney U,  $P > 0.05$ ). However, the differences were statistically significant between 1:10 and 1:50 (Fig. 2a; Mann–Whitney U,  $P < 0.001$ ), 1:10 and 1:200 ( $P < 0.001$ ) and 1:50 and 1:200 ( $P < 0.05$ ) in 15th second after initiation of sperm motility. The total duration of motility increased after dilution of sperm in fresh water when the dilution rate increased from 1:10 to 1:50 and to 1:200 (Fig. 2b; independent sample *t* test,  $P < 0.01$ ). The percentages of motile spermatozoa increased when the dilution rate increased to



**Figure 2** Effects of dilution rate on the percentage of motile spermatozoa (a) and the total duration of sperm motility (b) in *A. persicus* after activation in fresh water (FW) and buffered fresh water containing 20 mM Tris-HCl, pH 8.0 (Tris). This experiment was carried out in 2001. Times are shown in seconds. In b, bars with the same letter above are not significantly different (independent sample *t* test,  $n = 3$ ,  $P > 0.05$ ).

1:200 in buffered fresh water (80.33% at 1:10, 80.13% at 1:50 and 85.67% at 1:200; Fig. 2a). The observed differences were statistically significant between 1:10 and 1:200 and 1:50 and 1:200 (Fig. 2a; Mann-Whitney U,  $P < 0.05$ ). After dilution of sperm in buffered fresh water, the total duration of motility increased when the dilution rate increased from 1:10 to 1:50 and to 1:200 (Fig. 2b; independent sample *t* test,  $P < 0.01$ ).

Comparison of the percentage of motile sperm (by Mann-Whitney U test) and total duration of motility (by independent sample *t* test) showed that the observed decreases were statistically highly significant between fresh water and buffered fresh water (Fig. 2a and b;  $P < 0.001$ ). These findings suggest that dilution rate 1:50 is optimum for activation of sperm motility. Therefore, dilution rate of 1:50 was used to study the effect of ionic concentration and osmolality on sperm motility.

### Effects of cations

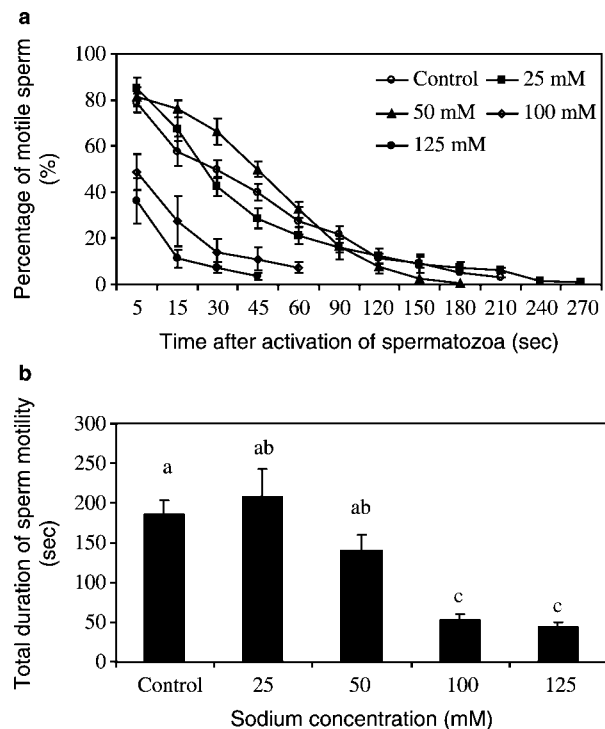
#### Effect of sodium ( $\text{Na}^+$ )

Maximum and minimum percentages of motile spermatozoa and total durations of sperm motility were observed in

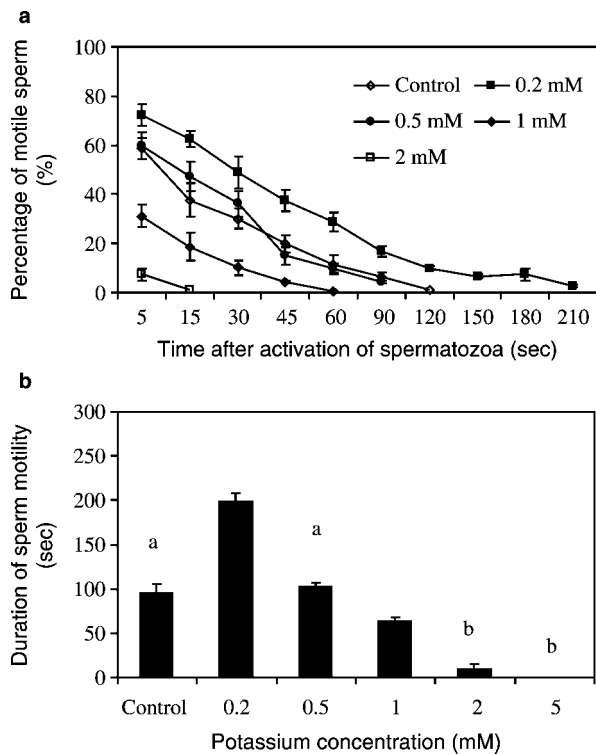
solutions containing 25 and 125 mM NaCl, respectively, 5 s after the initiation of sperm motility ( $85.0 \pm 4.56$  s and  $207.75 \pm 35.0\%$  in 25 mM and  $36.25 \pm 10.11$  s and  $43.75 \pm 5.96\%$  in 125 mM; Fig. 3). The percentage of motile spermatozoa was found significantly different between solutions of 25 and 100 mM or more (Fig. 3a; Mann-Whitney U,  $P < 0.05$ ). However, the percentage of motile spermatozoa decreased rapidly 30 and 45 s after activation in solutions containing 25 and 50 mM NaCl (Fig. 3a). At 3 min post-activation, NaCl in the range 0–20 mM had a positive effect, but over 50 mM it was inhibitory (Fig. 3b). There were no differences (independent sample *t* test,  $P > 0.05$ ) in the duration of sperm motility among samples in the range of 0–50 mM and 100–125 mM NaCl (Fig. 3b).

#### Effect of potassium ( $\text{K}^+$ )

The best motility parameters in terms of the percentage of motile cells ( $72.5 \pm 4.33\%$ ) and the duration of sperm motility ( $199.0 \pm 9.11$  s) were observed after dilution of spermatozoa in 0.2 mM KCl (Fig. 4). The sperm motility just after activation was suppressed by concentrations of KCl of 1 mM or more (Fig. 4b). There were no differences in the duration of sperm motility among samples subjected to 2 mM or more (Fig. 4b; independent sample *t* test,  $P > 0.05$ ) and in the percentage of motile spermatozoa



**Figure 3** Effects of  $\text{Na}^+$  on motility characteristics in *A. persicus* spermatozoa: the percentage of motile spermatozoa (a) and the total duration of sperm motility (b). In b, bars with the same letter above are not significantly different (independent sample *t* test,  $n = 4$ ,  $P > 0.05$ ).



**Figure 4** Effects of  $K^+$  (mM) on motility characteristics in *A. persicus* spermatozoa: the percentage of motile spermatozoa (a) and the total duration of sperm motility (b). In b, bars with the same letter above are not significantly different (independent sample *t* test,  $n = 4$ ,  $P > 0.05$ ).

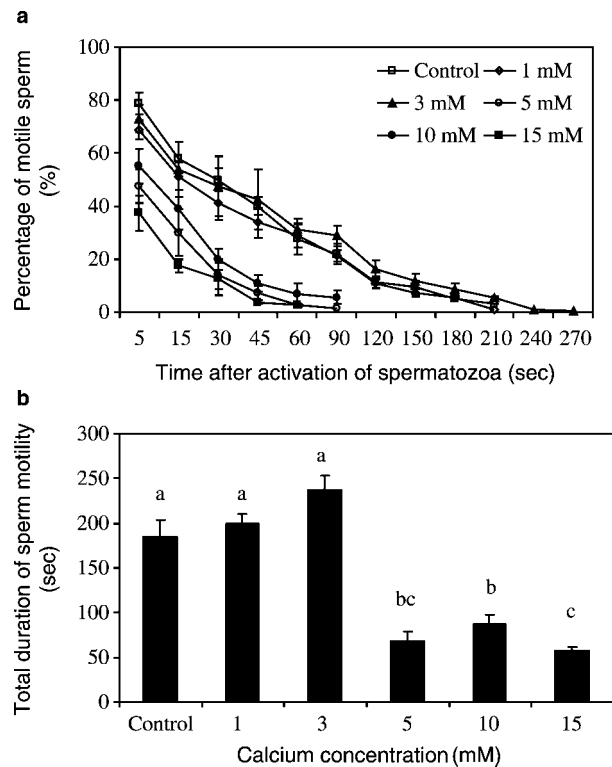
among samples subjected to 1 mM or more (Fig. 4a; Mann–Whitney U,  $P < 0.05$ ).

#### Effect of calcium ( $Ca^{2+}$ )

Different concentrations of  $CaSO_4$  solution combined with 20 mM Tris–HCl, pH 8.0, at a dilution rate of 1:50 were tested (Fig. 5). The maximum percentage of motile spermatozoa and duration of motility of sperm were observed in 3 mM  $CaSO_4$  (Fig. 5). A rapid decrease in the percentage of motile spermatozoa was observed in 5 mM  $CaSO_4$  or more, from 37.5–55.0 after 5 s to 1.25–5.5 after 90 s (Fig. 5a). At 3 min post-activation,  $CaSO_4$  in the range 0–3 mM had a positive effect, but solutions over 5 mM were inhibitory (Fig. 5b). There was no difference in the sperm motility period among samples in the ranges of 0–3 mM  $CaSO_4$  and of 5–15 mM  $CaSO_4$  (Fig. 5b;  $P > 0.05$ , independent sample *t* test for duration of sperm motility).

#### Effect of magnesium ( $Mg^{2+}$ )

The maximum percentage of motile spermatozoa and duration of motility of sperm were observed in a solution containing 10 mM  $MgSO_4$  ( $76.67 \pm 3.33\%$ , Fig. 6a;  $164.33 \pm 15.34$  s, Fig. 6b). However, there were no differences in the sperm motility parameters among samples

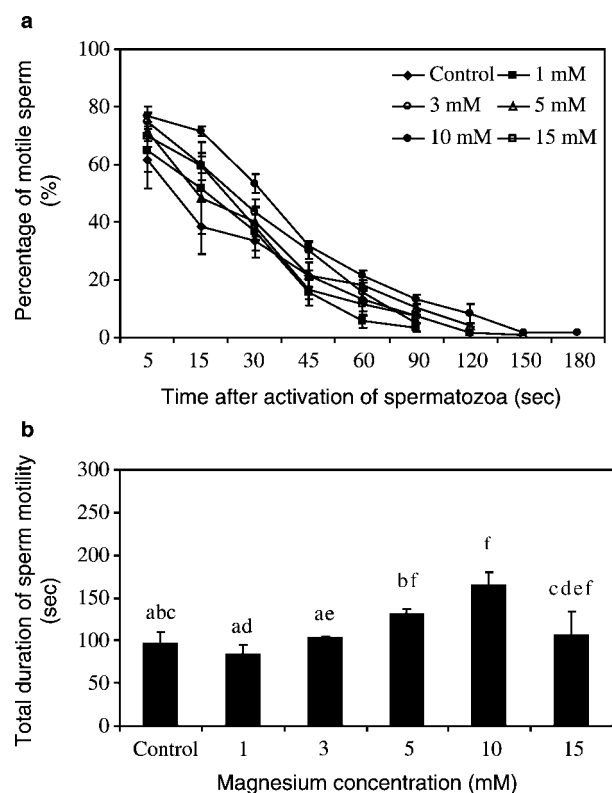


**Figure 5** Effect of  $Ca^{2+}$  on motility characteristics in *A. persicus* spermatozoa: the percentage of motile spermatozoa (a) and the total duration of sperm motility (b). In b, bars with the same letter above are not significantly different (independent sample *t* test,  $n = 4$ ,  $P > 0.05$ ).

subjected to 5 mM  $MgSO_4$  or more (Fig. 6;  $P > 0.05$ , Mann–Whitney U for percentage of motile sperm and independent sample *t* test for duration of motility of sperm). Concentrations over 10 mM had a negative effect on motility parameters in *A. persicus* spermatozoa.

#### Effect of osmolality

Effects of osmolality on the characteristics of sperm motility in *A. persicus* were shown in Fig. 7. The spermatozoa of the Persian sturgeon were motile in a range of osmolality 0–100 mosmol  $kg^{-1}$ . A rapid decrease in the percentage of motile spermatozoa was observed, from more than 80% to less than 20%, with the increase of diluent osmolality to 200 mosmol  $kg^{-1}$ . The maximum duration of sperm motility was observed in solution containing sucrose at an osmolality of 50 mosmol  $kg^{-1}$  (Fig. 7b). The period of motility increased from  $193.33 \pm 24.21$  s in distilled water (0 mosmol  $kg^{-1}$ ) to  $405.0 \pm 18.93$  s in sucrose solution combined with Tris–HCl activation solution (pH 8.0, 50 mosmol  $kg^{-1}$ ) and then decreased to  $314.0 \pm 62.04$  s in sucrose solution combined with activation solution (100 mosmol  $kg^{-1}$ ). However, there were no differences in the percentage of motile spermatozoa and duration of sperm motility among samples activated in 50 and 100 mosmol  $kg^{-1}$  (Fig. 7;  $P > 0.05$ , Mann–Whitney U for



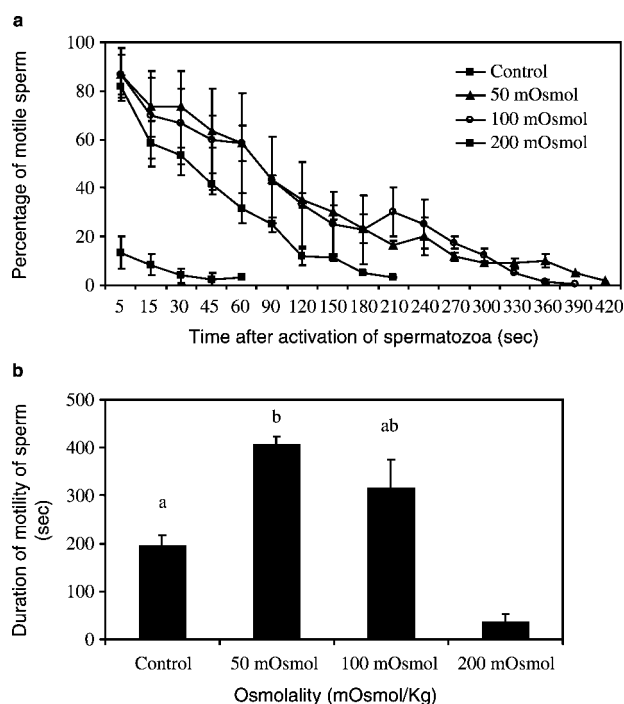
**Figure 6** Effect of  $Mg^{2+}$  (mM) on motility characteristics in *A. persicus* spermatozoa, the percentage of motile spermatozoa (a, %) and the total duration of motility of sperm (b, sec). Values with the same letter above are not significantly different (independent sample *t* test,  $n = 3$ ,  $P > 0.05$ ).

percentage of motile sperm and independent sample *t* test for duration of motility of sperm).

## Discussion

The initiation of motility of fish spermatozoa is induced after delivery of sperm into an aqueous environment (in natural reproduction) or into swimming medium (in artificial reproduction); Stoss 1983, Cosson *et al.* 1999). The new surrounding medium usually has different ionic/osmotic characteristics from the seminal fluid (Cosson *et al.* 1999, Ciereszko *et al.* 2000) where the sperm is stored and the fish spermatozoa have to adapt to a new environment (Cosson *et al.* 1999). In fact, the motility of spermatozoa is related to their sensitivity to osmolality and to ions (Toth *et al.* 1997, Cosson *et al.* 1999). Therefore, determination of optimum parameters for swimming medium is very important to increase the efficiency of artificial reproduction (Alavi and Cosson 2004). The present study on the effects of environmental factors on motility parameters in *A. persicus* spermatozoa provides us with new knowledge about stimulating factors that induce the initiation of sperm motility and the prolong the period of motility.

pH has been reported as one of the major spermactivating factors in fish species (Stoss 1983, Billard *et al.*



**Figure 7** Effect of osmolality ( $\text{mosmol kg}^{-1}$ ) on motility characteristics in *A. persicus* spermatozoa: the percentage of motile spermatozoa (a) and the total duration of sperm motility (b). In b, bars with the same letter above are not significantly different (independent sample *t* test,  $n = 3$ ,  $P > 0.05$ ).

1995a). The pH of the activating solution also affects sperm's fertilizing capacity (Billard *et al.* 1995b). Optimum sperm motility has been reported at pH 9.0 in *Oncorhynchus mykiss* (Billard and Cosson 1988) and *Scaphthalmus maximus* (Chauvaud *et al.* 1995) and at pH 7.0 and 8.0 in *Cyprinus carpio* (Cosson *et al.* 1991). The duration of sperm motility in *Petromyzon marinus* decreased with an increase in pH, but the percentage of motile cells did not change over the pH range 6.0–9.0 (Ciereszko *et al.* 2002). Gallis *et al.* (1991) reported that the optimum motility of *Acipenser baeri* spermatozoa was occurred at pH 8.2. This study shows that pH is a prerequisite factor determining sperm motility in *A. persicus*. Our data suggest that the optimal pH for sperm motility induction and prolongation is pH 8.0. According to the results, it is confirmed again that the alkaline conditions of diluents enhance the motility parameters of sperm in *A. persicus*, similar to other sturgeon species such as *Polyodon spathula* (Cosson and Linhart 1996), *A. baeri* (Gallis *et al.* 1991) and *Scaphirhynchus platyrhynchus* (Linhart *et al.* 1995). It has been reported that a change in the external pH value induces a change in the internal pH. Krasznai *et al.* (1995) observed that the intracellular alkalinization (0.15 pH units) is often caused by activation of a  $\text{Na}^+/\text{H}^+$  exchanger. But this phenomenon does not play a key role in triggering axonemal movement as in trout (Gatti *et al.* 1990, Boitano and Omoto 1991). An increase in intracellular pH has been suggested to be a conserved step in the activation of sperm motility (Boitano and

Omoto 1991). Research by Ingermann *et al.* (2002) on pH sensitivity of sperm motility in *Acipenser transmontanus* demonstrated that sperm maintained at high pH (more than 8.2) had appreciable motility when added to water but that the motility was inhibited when the semen was maintained at low pH (less than 7.5). They reported that the low buffering capacity of seminal plasma corresponded with the high sensitivity of sperm motility to pH. They also suggested that the low buffering capacity of seminal fluid allowed the epithelial cells of the reproductive tract to exert control on sperm motility by regulating semen pH via bicarbonate secretion. These findings show the major role of spermatid-duct epithelium in the sperms' acquisition of motility on exposure to water. Little information is available about the role of the spermatid duct in the sensitivity of sperm to pH.

Sperm dilution is a major factor in the induction of sperm motility (Stoss 1983, Billard and Cosson 1992) and the maintenance of fertilizing ability of diluted fish sperm including freshwater fish (Ginzburg 1968, Billard 1983) and marine fishes (Suquet *et al.* 1992, 2000). Since the duration of motility is short, and since the quality of sperm movement varies during the phase of motility, dilution rate becomes a key issue, because the volume of diluent added determines the dynamics of sperm activation (Billard and Cosson 1992, Billard *et al.* 1995a). A relatively high dilution (1:1000 or 1:2000) is necessary to initiate simultaneous motility of all of the spermatozoa (Billard *et al.* 1995b). After high dilution a homogenous sperm suspension is obtained which is suitable for observation of synchronous motility and for studies of the biochemical changes that occur during and after activation. In Salmonidae the period of active movement of spermatozoa decreases when the dilution rate decreases (Ginzburg 1968). Compared with teleosts, there are several studies show that *Acipenseridae* sperm becomes motile at low dilution rates (Gallis *et al.* 1991, Linhart *et al.* 1995, Toth *et al.* 1997, Alavi *et al.* 2002). Gallis *et al.* (1991) reported that the duration of sperm motility and intensity of spermatozoa in *A. baeri* increased when the dilution rate increased from 1:6 to 1:100. This study shows again that the period of sperm motility in *A. persicus* depends on the dilution ratio and the best sperm activity, in terms of both period of motility and percentage of motile cells, was observed at a dilution ratio of 1:100. These results can be explained by the low concentrations of inorganic cations and/or the low spermatocrit of the semen in the sturgeons compared with the teleosts (Gallis *et al.* 1991).

In addition to pH, other environmental factors such as ions and osmolality pressure stimulate the motility of spermatozoa by changes in the properties of the plasma membrane including its potential and its ionic conductance (Morisawa 1985, Cosson *et al.* 1999, Linhart *et al.* 1999). The percentage of motile spermatozoa was inhibited by 50% when more than 1 mM  $K^+$  was added the Tris-HCl buffer, pH 8.0. This observation confirms and extends those of Gallis *et al.* (1991), Toth *et al.* (1997) and Linhart

*et al.* (2002) who reported complete inhibition in the presence of 0.1 mM  $K^+$  in *A. baeri*, 50% inhibition of motility following the addition of 0.5 mM  $K^+$  to Tris-glycine buffer in *A. fulvescens* and prevention of the activation of spermatozoa motility in *P. spathula* at concentrations of 0.5–5.0 mM. Occasionally these results suggest that motility of sturgeon sperm is sensitive to very low concentrations of  $K^+$ , which is lower than that for salmonid sperm (Cosson *et al.* 1999) and which is in contrast to carp (Perchec *et al.* 1993). It is also confirmed that  $K^+$  can control sperm activation in sturgeon sperm at very low concentrations, in the range of 0.01–0.3 mM in *A. baeri* (Gallis *et al.* 1991), *P. spathula* (Cosson and Linhart 1996), *A. fulvescens* (Toth *et al.* 1997) and *A. persicus* (Fig. 4, this study).

In the case of the effect of  $Na^+$ , the results show (1) sperm biosensitivity to  $Na^+$  when the concentration reaches 50 mM or more and (2) optimum duration of motility and percentage of motile spermatozoa at 25 mM  $Na^+$  (Fig. 3). Toth *et al.* (1997) reported inhibition of activation of sperm motility in *A. fulvescens* at  $Na^+$  concentrations of 40 mM or more. In the case of *A. baeri*, sodium ions at concentrations in the range of that in seminal plasma (20 mM) had no effect on sperm motility (Gallis *et al.* 1991). Toth *et al.* (1997) observed a lower percentage of motile spermatozoa just at the time of initiation of motility (58%) and the maximum duration of motility (more than 1700 s) in swimming medium containing 10 mM  $Na^+$ , pH 9.0, at a dilution rate of 1:500. In addition, the optimum percentage of motile cells just after initiation of activation was observed in swimming medium containing 0 and 25 mM  $Na^+$  (87.0 and 78.5%, respectively) in *A. fulvescens* (Toth *et al.* 1997). They also reported that sperm motility was unchanged after 5 min in activation solution containing 10 mM  $Na^+$ . In fact, this study confirms that swimming medium containing  $Na^+$  stimulates and controls spermatozoa motility in sturgeon but it seems that it is dependent on the seminal plasma composition and  $Na^+$  content, which confirms a species-specific character of sperm biosensitivity in sturgeon to  $Na^+$  and other ions and osmolality (Alavi *et al.* 2004).

Less information is available about the effects of bivalent cations on sperm motility in sturgeon sperm. It is clear that the inhibition of motility by  $K^+$  concentration can be overcome by an increase in the external  $Ca^{2+}$  concentration (Billard *et al.* 1999). The data in the literature indicated that (1) external  $Ca^{2+}$  ions are a prerequisite for the initiation of motility of sperm in salmonids (Christen *et al.* 1987, Billard *et al.* 1995b), carp (Krasznai *et al.* 2000) and sturgeons (Linhart *et al.* 2002), (2) the concentration of intracellular  $Ca^{2+}$  increased upon initiation of motility in salmonids (Christen *et al.* 1987, Billard *et al.* 1995b) and in carp (Krasznai *et al.* 2000), (3) the increase of intracellular free  $Ca^{2+}$  was produced by a flux of external  $Ca^{2+}$  into the cell rather than by a mobilization of internal  $Ca^{2+}$  stores (Krasznai *et al.* 2000), and (4) the  $Ca^{2+}$  similar to  $Na^+$  can reduce inhibitory effects of  $K^+$  in activation of spermatozoa in teleosts (Stoss 1983, Cosson

*et al.* 1999, Linhart *et al.* 1999) and sturgeon (Billard *et al.* 1999, Alavi *et al.* 2002, Linhart *et al.* 2002). In the case of sturgeons,  $\text{Ca}^{2+}$  at 100  $\mu\text{M}$  could reverse the  $\text{K}^{+}$  inhibitory effect but, as in salmonid sperm, EGTA could abolish the  $\text{Ca}^{2+}$  effect (Cosson *et al.* 1999). The sturgeon spermatozoa are sensitive to  $\text{Ca}^{2+}$ , which was confirmed by the use of demembrated spermatozoa (Cosson *et al.* 1999). The results of this study show the high sensitivity of *A. persicus* spermatozoa to the concentrations of  $\text{Ca}^{2+}$  in the swimming medium. Although these data confirm a key role of  $\text{Ca}^{2+}$  in the activation of sperm motility in fish, including sturgeon, there are many questions that must be answered about the mechanisms and function of intercellular and extracellular calcium signaling and the interactions between cAMP, calcium and protein phosphorylation in sperm motility. There is less information about the effects of  $\text{Mg}^{2+}$  ions on sperm motility in teleosts and sturgeon. Linhart *et al.* (2002) reported that the velocity of spermatozoa and the percentage of motile sperm could be improved in *P. spathula*. Studies on the intracellular mechanisms of sperm motility in teleosts confirm a key role of  $\text{Mg}^{2+}$  in the initiation of activation of sperm motility, especially in demembrated sperm (Cosson *et al.* 1999). However, this is the first report about the negative effects of  $\text{Mg}^{2+}$  on motility characteristics of sturgeon spermatozoa when the concentrations of  $\text{Mg}^{2+}$  are increased to 15 mM.

High osmotic pressure (400 mosmol  $\text{kg}^{-1}$ ) inhibits sperm motility of salmonids and the osmotic pressure of the seminal plasma (approximately 300 mosmol  $\text{kg}^{-1}$ ) is not sufficiently high to account for the inhibition of motility in semen (Billard and Cosson 1992). Motility of carp sperm is fully initiated in media of osmotic pressure below 150–200 mosmol  $\text{kg}^{-1}$  (Plouidy and Billard 1982), and the osmolality of seminal plasma is higher than necessary for activation of sperm; for example, the osmolality of carp seminal plasma is 286 mosmol  $\text{kg}^{-1}$  (Plouidy and Billard 1982). In the case of sturgeon, spermatozoa from Siberian sturgeon (Gallis *et al.* 1991), shovelnose sturgeon (Linhart *et al.* 1995) and paddlefish (Linhart *et al.* 1995) were motile in a range of osmotic pressures; 0–100 mosmol  $\text{kg}^{-1}$ , and 0–120 mosmol  $\text{kg}^{-1}$  and more than 100 mosmol  $\text{kg}^{-1}$ , respectively. But, the average values of osmotic pressure of seminal plasma were reported to be lower than the osmotic pressure needed for induction of sperm motility in sturgeon ( $38 \pm 3$  mosmol  $\text{kg}^{-1}$  in *A. baeri* (Gallis *et al.* 1991) and more than 80 mosmol  $\text{kg}^{-1}$  in *A. persicus* (Alavi *et al.* 2004)). Therefore, it is suggested that osmolality is not the principal factor preventing sperm motility in seminal fluid. This study also reported sperm biosensitivity to osmotic pressure in sturgeon but there are no data on its effects on motility patterns, sperm morphology or physiological functions during motility.

In conclusion, the mechanisms of initiation of motility in sturgeon spermatozoa are not completely elucidated, especially the events occurring in the intracellular

environment. In addition,  $\text{K}^{+}$  is major inhibitory factor of sperm motility in sturgeon. Ionic factors can stimulate the initiation of activation of sperm, but the biological sensitivity of sperm to ionic concentrations in the swimming medium must be of concern during determination of diluent composition in fish farms.

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