

# Effects of ions on the motility of fresh and demembrated paddlefish (*Polyodon spathula*) spermatozoa

O. Linhart<sup>1</sup>, J. Cosson<sup>2</sup>, S. D. Mims<sup>3</sup>,  
W. L. Shelton<sup>4</sup> and M. Rodina<sup>1</sup>

<sup>1</sup>Joint Laboratory of Genetics, Physiology and Reproduction of Fish, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, 38925 Vodnany, Czech Republic; <sup>2</sup>Centre National de la Recherche Scientifique, UMR 7009, Université Paris 6, Station Marine, 06230 Villefranche sur Mer, France; <sup>3</sup>Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601, USA; and <sup>4</sup>Zoology Department University of Oklahoma, Norman, OK 73019, USA

This study investigated the effects of different environmental conditions on the motility parameters of paddlefish (*Polyodon spathula*) spermatozoa. Paddlefish spermatozoa demonstrated the following characteristics: (i) all spermatozoa were motile 10 s after activation with a velocity of 130–160  $\mu\text{m s}^{-1}$ ; (ii) after 2 min, velocity decreased to 80–130  $\mu\text{m s}^{-1}$ ; and (iii) motility was maintained for up to 9 min. Concentrations of 0.5–5.0 mmol KCl l<sup>-1</sup> prevented activation of spermatozoa. After transfer into a swimming medium (20 mmol Tris l<sup>-1</sup>, pH 8.2 and 1 mg BSA ml<sup>-1</sup>) containing 0.5 mmol KCl l<sup>-1</sup> (combined with 5 mmol NaCl or MgCl<sub>2</sub> l<sup>-1</sup>), 80–100% of cells were motile with a velocity of

about 120–150  $\mu\text{m s}^{-1}$ . MgCl<sub>2</sub> significantly improved the velocity of spermatozoa at 10, 40, 50 and 60 s after activation and the stable velocity of spermatozoa was about 140  $\mu\text{m s}^{-1}$ . Very low concentrations of CaCl<sub>2</sub> (0.125 mmol l<sup>-1</sup>) combined with 0.5 mmol KCl l<sup>-1</sup> initiated motility in 20% of spermatozoa, whereas all spermatozoa were activated after 2 min with 0.25 mmol CaCl<sub>2</sub> l<sup>-1</sup> in similar medium for the full period of swimming with velocity of about 120  $\mu\text{m s}^{-1}$ . This study demonstrated that potassium (5–15 mmol l<sup>-1</sup>) inhibits demembrated spermatozoa. Thus, initiation of movement in paddlefish spermatozoa is under the reciprocal control of potassium and calcium ion concentrations.

## Introduction

Two species from the family Polyodontidae are extant: the American paddlefish (*Polyodon spathula*) which is confined to North America, and the Chinese paddlefish (*Psephurus gladius*) which is native to mainland China. Both species were commercially important, but numbers have declined markedly over the past 100 years (Carlson and Bonislawsky, 1981; Gengerke, 1986; Liu and Zeng, 1988; Mims *et al.*, 1993a). Artificial propagation and stocking have been successful only for the North American species. Wild stocks have been harvested for flesh and caviar production, and *P. spathula* is a popular sport fish in some areas in the USA (Gengerke, 1986; Liu and Zeng, 1988). *P. spathula* can reach a maximum of 83.5 kg and 216 cm in length (Adams, 1942; Folz and Mezers, 1985) and *P. gladius* grows much larger. *P. spathula* feeds on plankton, especially copepods, some insects, algae and plant fragments, whereas *P. gladius* is piscivorous (Mims *et al.*, 1993a). Adult *P. spathula* filter zooplankton (microscopic food) through their gill rakers while swimming with their mouth open (Mims *et al.*, 1993b;

Kroll *et al.*, 1994). The spawning period of *P. spathula* is in early spring, when adults migrate upstream in the Mississippi River and its major tributaries. Spawning occurs from the middle of March to the end of May at 11–14°C in the Ohio River (Purkett, 1963; Ballard and Needham, 1964) or from April to June at 16°C in the Missouri River (Purkett, 1963; Ballard and Needham, 1964). Needham (1965) and Graham *et al.* (1986) developed artificial reproductive methods for paddlefish in the USA, and Mims and Shelton (1998) have modified this procedure.

Spermatozoa of sturgeons and paddlefish are essentially immotile in the seminal plasma (Linhart *et al.*, 1995; Cosson and Linhart, 1996; Cosson *et al.*, 2000). Spermatozoa are immediately activated when they are transferred into swimming medium, usually fresh water or low salt concentration solutions (Drabkina, 1961), in a similar way to spermatozoa of freshwater teleosts. Sperm motility is greatest immediately after activation. All sperm motility parameters (frequency, velocity and wave amplitude) decrease rapidly during the period after activation and the percentage of motile cells also gradually decreases (Cosson *et al.*, 2000). During the earliest period of motility, spermatozoa of sturgeons and paddlefish move at velocities of 175–250  $\mu\text{m s}^{-1}$

Email: linhart@vurh.jcu.cz

(Cosson *et al.*, 2000) and then the forward motility gradually reduces to between 50 and 100  $\mu\text{m s}^{-1}$  at 3–6 min after activation, even when considering only the fraction of the population of cells with forward motility. Occasionally, some spermatozoa are motile for up to 9 min (Cosson *et al.*, 2000). The concentration and ratio of ions in seminal fluid is important for maintaining energy. The motility of paddlefish spermatozoa is partly controlled by osmotic pressure (Linhart *et al.*, 1995), as described for freshwater cyprinids (Redondo-Müller *et al.*, 1991) and for marine fish (Morisawa and Suzuki, 1980). When the concentration or ratio of  $\text{Na}^+:\text{K}^+$  or the osmotic pressure change, sperm motility in sturgeon and paddlefish is initiated (Linhart *et al.*, 1995; Cosson and Linhart, 1996; Toth *et al.*, 1997; Cosson *et al.*, 2000). A concentration of  $\geq 0.5 \text{ mmol K}^+ \text{ l}^{-1}$  prevents motility of white sturgeon (*Acipenser fulvescens*) spermatozoa (Toth *et al.*, 1997). The concentration of potassium in seminal fluid of paddlefish prevents sperm motility, but cells can be activated after dilution by about four (Linhart *et al.*, 2002) or eight times (Mims, 1991). Linhart *et al.* (2002) observed a high correlation between osmolality of seminal fluid and concentration of GnRHa, and between velocity of spermatozoa and osmolality of seminal fluid. As a consequence it appears that high osmotic concentration in seminal fluid increases the velocity of paddlefish spermatozoa.

The main aims of the present study were to examine the effects of potassium, calcium and magnesium on the motility of fresh and demembrated spermatozoa of the American paddlefish.

## Materials and Methods

### Induction of spermiation and collection of spermatozoa

The experiments were carried out in March and April at the Aquaculture Research Center, Kentucky State University (Frankfort, KY). Paddlefish of 7.0–12.0 kg were captured below McAlpinne Dam, Louisville, KY.

Five males were selected and held separately in a circular tank (3000 l) with a water flow rate of 12  $\text{l min}^{-1}$ , 9.0  $\text{mg O}_2 \text{ l}^{-1}$ , at controlled water temperature of 17–19°C. Mature male paddlefish can be identified during the spawning period by the presence of tubercles on their head and opercular flaps.

Spermatogenesis was induced hormonally by i.p. injections of the GnRH analogue [des-Gly10 (D-Ala6) GnRH ethylamide] (Sigma, St Louis, MO) at 50  $\mu\text{g kg}^{-1}$  body weight (Linhart *et al.*, 2000). Tygon tubing (5 cm in length) attached to a 10 ml plastic syringe was used to collect spermatozoa. The tubing was inserted into the urogenital pore and the syringe was filled with milt. Spermatozoa were collected and stored in aerobic conditions on wet ice until motility analysis.

### Evaluation of sperm motility parameters

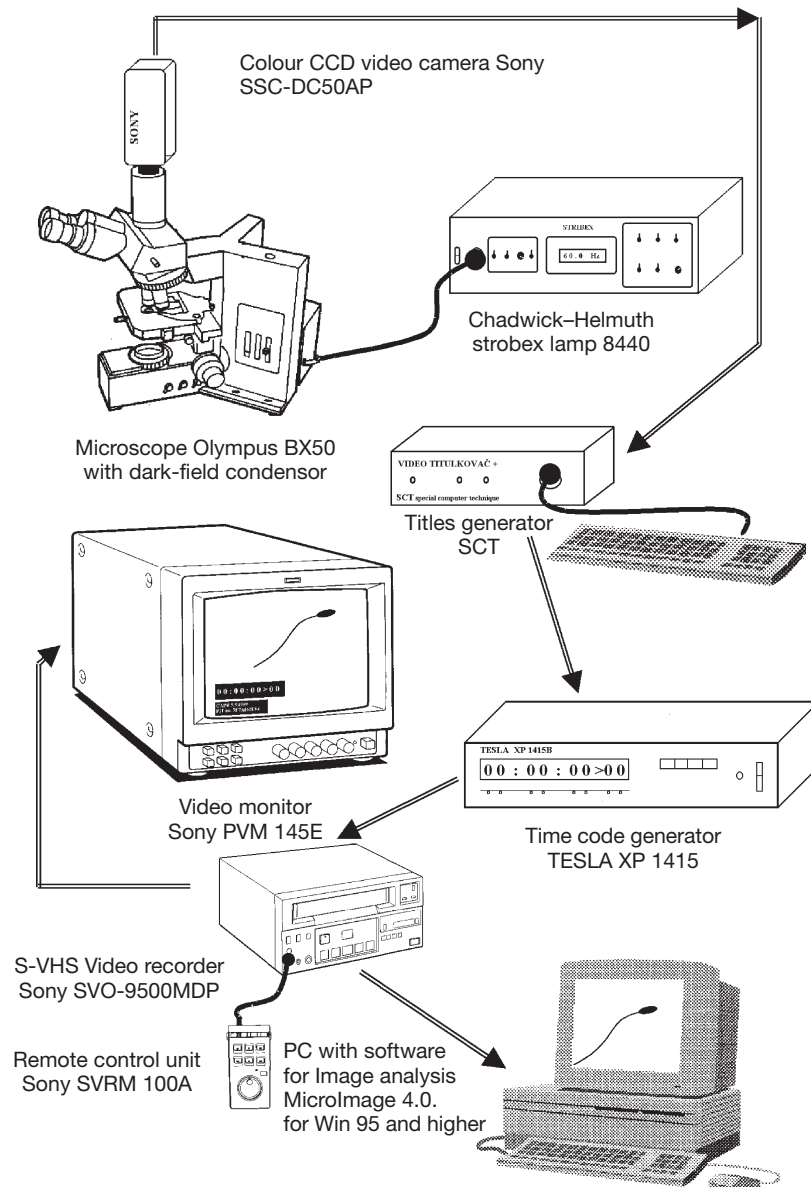
The velocity and percentage of motile spermatozoa were evaluated, and motility parameters were measured under dark field and phase-contrast microscopy (Fig. 1) (Cosson

*et al.*, 2000). Spermatozoa were observed for spontaneous movement immediately after collection and before dilution. A drop of 10  $\mu\text{l}$  undiluted spermatozoa was spread on a pre-positioned glass slide and examined at  $\times 200$ . The swimming ability of spermatozoa was measured by the addition of 0.5  $\mu\text{l}$  spermatozoa to 49  $\mu\text{l}$  swimming medium, which was composed of 20  $\text{mmol Tris-HCl l}^{-1}$ , pH 8.2, with 1 mg bovine serum albumin  $\text{ml}^{-1}$  (Sigma A-7511; to prevent cells from adhering to the glass slide) and other component ions, such as  $\text{K}^+$ ,  $\text{Mg}^+$  and  $\text{Ca}^{2+}$ . The swimming medium was placed on a glass slide previously positioned on the microscope stage, and immediately after mixing with spermatozoa, examined at  $\times 200$ . In this part of the study, the final dilution was 1:100. Motile spermatozoa were video-recorded beginning < 5 s after activation for measurement of velocity and percentage of motile spermatozoa. The movements of spermatozoa were observed through a  $\times 20$  lens using dark field microscopy and were recorded at 60  $\text{frames s}^{-1}$  using a CCD video camera (Sony SSC-DC50AP) mounted on a dark field microscope (Nikon Optiphot 2). The focal plane was always positioned near the glass slide surface. Sperm movement was recorded using a tape recorder (Sony VHS, SVO 1520), visualized on a colour video monitor (Sony) and using stroboscopic illumination. The stroboscopic flash (Strobex; Chadwick-Helmut, El Monte, CA) illumination with adjustable frequency was set in automatic register with the video frames (60 Hz). The successive positions of the recorded sperm heads were measured from video frames using a video-recorder (Sony SVHS, SVO-9500 MDP). Velocity and percentage of motile spermatozoa were analysed from five successive frames, each by 'Micro Image Analysis' (Version 4.0 for Windows, special macro of three colours).

For detailed examination of other swimming parameters, such as the flagella beating and wave characteristics, 0.1–0.5  $\mu\text{l}$  spermatozoa (by Eppendorf pipette from 0.1 to 2.5  $\mu\text{l}$ ) was directly mixed on a glass slide with 49  $\mu\text{l}$  distilled water or swimming medium, and a cover slip was added for oil immersion application. Immediately after mixing, sperm motility was recorded under  $\times 400$  or  $\times 1000$ . In this part of the study, the final dilution was between 1:100 and 1:500. Motile spermatozoa were recorded within 6–8 s after activation for measurement of swimming parameters. The movements of the flagellum were recorded with the technique described above, but also using phase-contrast  $\times 40$  or  $\times 100$  oil immersion lenses. The stroboscopic flash illumination was set manually at an adjustable frequency of 150–800 Hz, depending on the time resolution required. During recording, the microscope stage was slowly moved by hand: this allowed the visualization of multiple well-defined successive images of a motile spermatozoon without overlap of flagella images within every video frame (Cosson *et al.*, 1997).

### Demembration and reactivation of spermatozoa

Two microlitres of undiluted spermatozoa were mixed with 50  $\mu\text{l}$  demembration medium, which was composed



**Fig. 1.** Equipment for evaluation of sperm motility in paddlefish.

of 20 mmol NaCl l<sup>-1</sup>, 0.5 mmol EDTA l<sup>-1</sup> (Sigma), 1 mmol dithiothreitol l<sup>-1</sup> (DTT, Sigma D-0632), 20 mmol Tris-HCl l<sup>-1</sup>, pH 8.2 and 0.04% Triton-X100 at 0°C. After 30 s at 0°C, a 2 µl aliquot was pipetted and mixed at room temperature (18–20°C) on the glass slide with 50 µl of reactivation medium (20 mmol NaCl l<sup>-1</sup>, 1 mmol DTT l<sup>-1</sup>, 20 mmol Tris-HCl l<sup>-1</sup>, pH 8.2, 1 mmol MgCl<sub>2</sub> l<sup>-1</sup>, 2 mg BSA ml<sup>-1</sup> and 1 mmol ATP l<sup>-1</sup>, vanadate free from Boehringer). BSA was required to prevent the sperm cells from adhering to glass or particles. The addition of 50–200 µmol cAMP l<sup>-1</sup>, either in demembration or reactivation media, was necessary to initiate motility. The swimming ability of spermatozoa was assessed as described above.

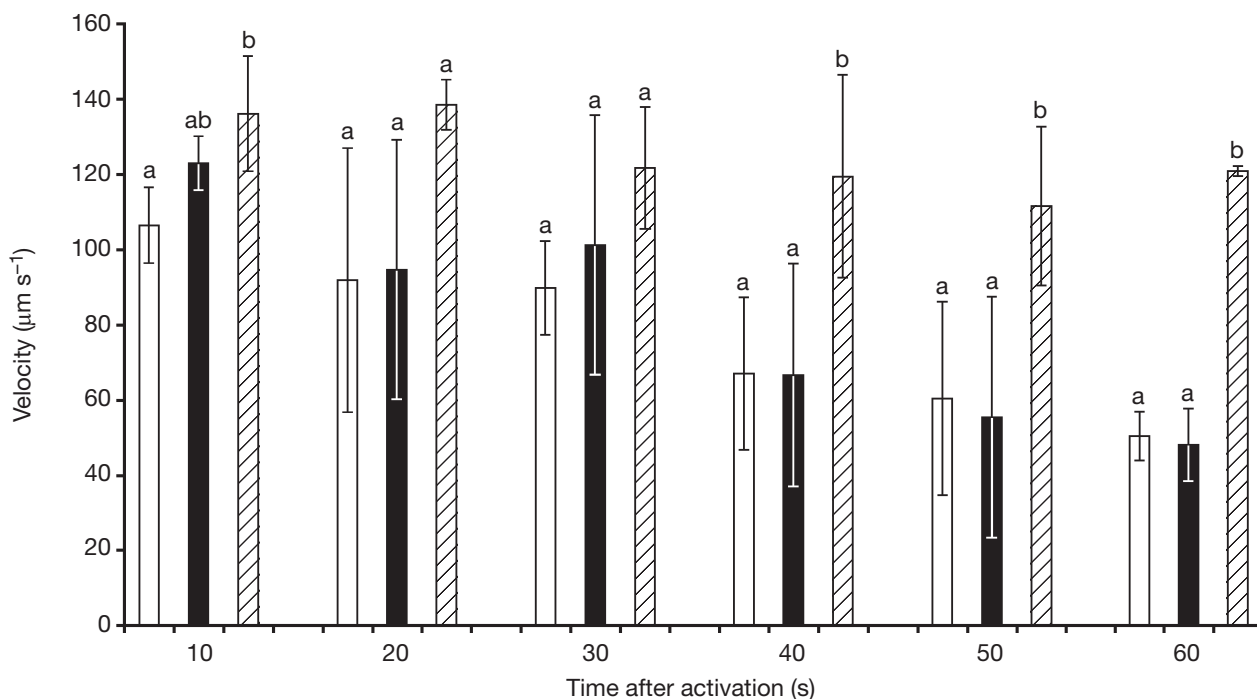
### Statistical analysis

The data acquired from several replications were evaluated and statistical significance was assessed using ANOVA (Statgraphics version 5), followed by multiple comparison tests. Probability values of  $P < 0.05$  were considered significant.

## Results

### Motility of undiluted and diluted spermatozoa in swimming medium

Direct observation of undiluted spermatozoa by dark field microscopy showed that most spermatozoa in the



**Fig. 2.** Velocity of paddlefish (*Polyodon spathula*) spermatozoa activated with 5 mmol CaCl<sub>2</sub> l<sup>-1</sup> (□) and swimming medium (20 mmol Tris-HCl l<sup>-1</sup> at pH 8.2 and BSA), with 5 mmol MgCl<sub>2</sub> l<sup>-1</sup> (■) and swimming medium, or with swimming medium only (control, ▨). Values are mean ± SD. <sup>ab</sup>Columns within the same swimming periods (10, 20, 30, 40, 50 and 60 s after activation) with a common superscript are not significantly different ( $P < 0.05$ ).

seminal plasma of paddlefish were immotile, but that the flagella were straight and shivered slightly. Only a few spermatozoa were motile for < 10 s.

When transferred into swimming medium, paddlefish spermatozoa were immediately activated and displayed the following characteristics: (i) 100% of spermatozoa were motile at 10 s after activation with a velocity of 130–160 µm s<sup>-1</sup>; (ii) after 2 min, the velocity decreased to 80–130 µm s<sup>-1</sup>; and (iii) motility was maintained for up to 9 min.

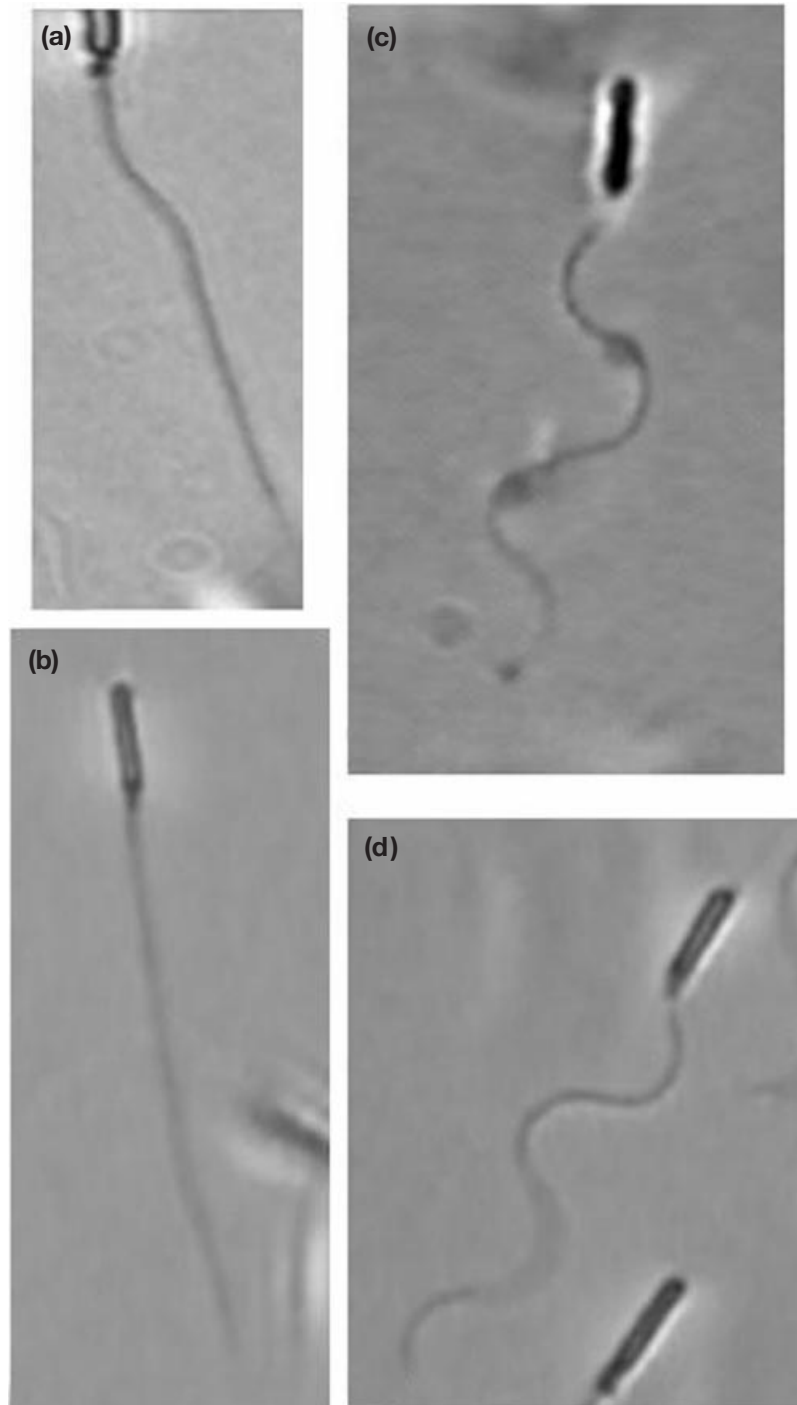
#### *Inhibitory effect of low ionic concentrations*

The motility of paddlefish spermatozoa in low concentrations of KCl, MgCl<sub>2</sub>, NaCl or CaCl<sub>2</sub> solution in swimming medium (20 mmol Tris-HCl l<sup>-1</sup> at pH 8.2 and BSA) was investigated. The spermatozoa were highly motile in swimming medium with low concentrations (0.5–5.0 mmol l<sup>-1</sup>) of NaCl, MgCl<sub>2</sub> or CaCl<sub>2</sub>. Concentrations of 0.5–5.0 mmol KCl l<sup>-1</sup> did not initiate sperm motility. When swimming medium containing 0.5 mmol KCl l<sup>-1</sup> was combined with 5.0 mmol NaCl l<sup>-1</sup> or MgCl<sub>2</sub>, 80–100% of spermatozoa became motile with a velocity of about 120–150 µm s<sup>-1</sup>. MgCl<sub>2</sub> significantly improved velocity of sperm motility at 10, 40, 50 and 60 s after activation (Fig. 2) and stable velocity of spermatozoa was 140 µm s<sup>-1</sup> at 2 min after activation. A low concentration of KCl (1 mmol l<sup>-1</sup>) fully inhibited sperm movement, but motility was re-initiated by the addition of a low concentration of CaCl<sub>2</sub>. A solution of

0.125 mmol CaCl<sub>2</sub> l<sup>-1</sup> and 0.5 mmol KCl l<sup>-1</sup> resulted in movement of 20% of the spermatozoa, whereas 100% of the spermatozoa were activated by the addition of 0.25 mmol CaCl<sub>2</sub> l<sup>-1</sup> plus 0.5 mmol KCl l<sup>-1</sup>; spermatozoa swim for 2 min at a velocity of about 120 µm s<sup>-1</sup>. In addition, spermatozoa were activated in higher concentrations of KCl (5 mmol l<sup>-1</sup>) and 3 mmol CaCl<sub>2</sub> l<sup>-1</sup> final concentration, resulting in 50–60% motile sperm cells. All spermatozoa were fully motile after combining 5 mmol KCl l<sup>-1</sup> plus 5 mmol of CaCl<sub>2</sub> l<sup>-1</sup>; velocity was about 120 µm s<sup>-1</sup> at 2 min after activation. Paddlefish spermatozoa inhibited with 0.5 mmol KCl l<sup>-1</sup> could be activated in a solution composed of 40 mmol sucrose l<sup>-1</sup>, 2 mg BSA ml<sup>-1</sup> (to prevent spermatozoa adhering to the glass slide) and at 125 µmol CaCl<sub>2</sub> l<sup>-1</sup>. Low concentrations of Ca<sup>2+</sup> appeared to reverse the inhibitory effects of 0.5 mmol KCl l<sup>-1</sup>. In addition, the EGTA used to complex Ca<sup>2+</sup> could abolish the protective calcium ion effect. In the latter case Ca<sup>2+</sup> combined with the calcium ionophore A23187 completely re-established the inhibitory effect of K<sup>+</sup> probably by increasing of Ca<sup>2+</sup> permeability of the sperm membrane.

#### *Demembration and reactivation of spermatozoa*

In this experiment, the inhibitory effect of K<sup>+</sup> on the axonemes of demembrated spermatozoa (Fig. 3) in the reactivating solution containing ATP was examined. The presence of KCl or K<sup>+</sup> at 5–15 mmol l<sup>-1</sup> was used to inhibit



**Fig. 3.** (a) Shivering, not activated spermatozoa with membrane, (b) resting demembrated spermatozoa, (c) fully-activated spermatozoa with membrane damaged by low osmotic shock (distilled water) and (d) fully activated demembrated spermatozoa of the paddlefish (*Polyodon spathula*).

demembrated flagella. The addition of 20 mmol NaCl l<sup>-1</sup> allowed reactivation of fully demembrated sperm flagella after the addition of 50–200 μmol cAMP l<sup>-1</sup> to the reactivated medium. The effect on motility was greater when the spermatozoa were demembrated and reactivated in

solution demembrated medium and reactivated medium, respectively, both containing 0.5 mmol CaCl<sub>2</sub> l<sup>-1</sup>. The functional sensitivity of sturgeon axonemes to Ca<sup>+</sup> was confirmed. Even when using demembrated medium and reactivated medium without K<sup>+</sup> but with ≤ 100 μmol CaCl<sub>2</sub>

$I^{-1}$  in reactivated medium, the flagella of demembrated ATP-reativated spermatozoa had only twitching motion with a very small wave amplitude. In contrast, waves with large amplitude developed when the reactivated medium contained  $\geq 250 \mu\text{mol CaCl}_2 I^{-1}$ .

## Discussion

Spermatozoa of sturgeons and paddlefish are essentially immotile in the seminal plasma (Cosson and Linhart, 1996), but are fully activated when transferred into swimming medium. Fish spermatozoa are generally immotile in the testes and seminal plasma. Environmental factors, such as ions, pH or osmolality, may lead to the depolarization of the cell membrane and, therefore, stimulate motility of spermatozoa (Cosson *et al.*, 1999). Several generations of paddlefish spermatozoa are present in the testis at the same time. The motility of paddlefish spermatozoa is inhibited by an increase in  $K^+$  concentration even at pH values of 7.0 (Cosson and Linhart, 1996). No cumulative effect of  $Ca^{2+}$  was observed in paddlefish as described for salmonids by Scheuring (1925), Billard (1978), Cosson *et al.* (1986, 1989) and Christen *et al.* (1987). In the case of paddlefish spermatozoa, motility is sensitive to very low concentrations of  $K^+$  ( $0.5 \text{ mmol } I^{-1}$ ), which is lower than that for salmonids (Cosson *et al.*, 1986; Morisawa *et al.*, 1983). The potential for movement in paddlefish spermatozoa was preserved after dilution in a  $5 \text{ mmol KCl } I^{-1}$  plus  $20 \text{ mmol Tris-HCl } I^{-1}$  at pH 8. This solution also regenerated the sperm potential for movement, especially when the pH of fresh spermatozoa was  $< 7$ , which might be due to urine contamination (Cosson and Linhart, 1996). A similar regenerative effect of  $K^+$  was reported by Redondo *et al.* (1991) for carp spermatozoa, but using a medium of high osmotic pressure ( $380 \text{ mOsmol}$ ), in contrast to paddlefish spermatozoa, for which the effect of regeneration was observed at a lower osmotic pressure ( $40 \text{ mOsmol}$ ). The spermatozoa of paddlefish were motile in a range of osmotic pressures from 0 to  $120 \text{ mOsmol kg}^{-1}$ . There was a trend of a rapid reduction from 80 to 0% sperm motility with increases in osmotic pressure from 70 to  $120 \text{ mOsmol kg}^{-1}$  (Linhart *et al.*, 1995). The effects of the KCl,  $CaCl_2$ , glucose and  $Li_2CO_3$  were also tested by Cosson and Linhart (1996). Sperm movement was inhibited in solutions of  $120 \text{ mmol glucose } I^{-1}$  or  $10 \text{ mmol } CaCl_2 I^{-1}$  in combination with  $20 \text{ mmol Tris-HCl } I^{-1}$  at pH 8. The spermatozoa were motile in solutions of  $20 \text{ mmol } Li_2CO_3 I^{-1}$ , which indicates a specific effect for  $K^+$ . No cumulative effect on storage time of spermatozoa was observed between  $1 \text{ mmol KCl } I^{-1}$  and  $100 \text{ mmol glucose } I^{-1}$  (Cosson and Linhart, 1996).

A demembrated flagellum is directly in contact with chemicals and the potential of the motility 'effectors' can be tested directly. Demembration is obtained by application of a mild non-ionic detergent (for example Triton-X100) and reactivation is initiated by addition of ATP- $Mg^{2+}$ , the substrate of flagella dynein-ATPases (Gibbons and Gibbons, 1972). In this respect, the present study demonstrated that

inhibition of fresh or demembrated spermatozoa was effected with low concentration of  $K^+$  ( $0.5 \text{ mmol } I^{-1}$ ) and that this effect was eliminated usually with low  $Ca^{2+}$  concentration or replacement of  $K^+$  by  $Na^+$  in demembrated flagella.

The results from the present study indicate that movement of paddlefish spermatozoa is not significantly influenced by osmotic pressure (Linhart *et al.*, 1995; Cosson and Linhart, 1996), but is under reciprocal control of the concentration of  $K^+$  and  $Ca^{2+}$ . In this respect, paddlefish spermatozoa are more similar to those of salmonids than cyprinids (Billard, 1978; Billard *et al.*, 1995 and Morisawa *et al.*, 1983).

This work was supported by USDA 1890 Capacity Building Grant Program (KYX-01-11469) Kentucky State University, Frankfort, KY. This project was funded by the CNRS-France (J. Cosson), Czech Grand Foundation and Ministry of Education no. J06/98:126100001, Czech Republic (O. Linhart and M. Rodina) and Embassy of France in Prague.

## References

- Adams LA (1942) Age determination and rate of growth in *Polyodon spathula* by means of the growth rings of the otoliths and dentary bones *American Wildlife Nature* **28** 617–630
- Ballard WW and Needham RG (1964) Normal embryonic stages of *Polyodon spathula* (Walbaum) *Journal of Morphology* **114** 465–478
- Billard R (1978) Changes in structure and fertilizing ability of marine and fresh water fish spermatozoa diluted in media of various salinities *Aquaculture* **14** 187–198
- Billard R, Cosson J, Percec G and Linhart O (1995) Biology of sperm and artificial reproduction in carp *Aquaculture* **129** 95–112
- Carlson DM and Bonislavsky PS (1981) The paddlefish (*Polyodon spathula*) fisheries of the midwestern *Fisheries* **6** (2) 17–22, 26–27
- Christen R, Gatti JL and Billard R (1987) Trout sperm motility *European Journal of Biochemistry* **166** 667–671
- Cosson J and Linhart O (1996) Paddlefish (*Polyodon spathula*) spermatozoa: effects of potassium and pH on motility *Folia Zoologica* **45** 361–370
- Cosson MP, Billard R, Carre D, Letellier L, Christen R, Cosson J and Gatti JL (1986) Control of flagellar movement in '9 + 2' spermatozoa: calcium and cyclic AMP dependent chemotactic behavior *Cell Motility and the Cytoskeleton* **6** 237
- Cosson MP, Billard R and Letellier L (1989) Rise of internal  $Ca^{2+}$  accompanies the initiation of trout sperm motility *Cell Motility and the Cytoskeleton* **14** 424–434
- Cosson J, Billard R, Cibert C, Dreanno C, Linhart O and Suquet M (1997) Movements of fish sperm flagella studied by high speed videomicroscopy coupled to computer assisted image analysis *Poliskie Archiwum Hydrobiologii* **44** 103–113
- Cosson J, Billard R, Cibert C, Dreanno C and Suquet M (1999) Ionic factors regulating the motility of fish sperm. In *From Basic Science the Male Gamete to Clinical Application* pp 161–186 Eds C Gagnon. Cache River Press, Vienna, IL
- Cosson J, Linhart O, Mims SD, Shelton WL and Rodina M (2000) Analysis of motility parameters from paddlefish (*Polyodon spathula*) and shovelnose sturgeon (*Scaphirhynchus platyrhynchus*) spermatozoa *Journal of Fish Biology* **56** 1348–1367
- Drabkina BM (1961) Effect of different water salinities on the survival rate of spermatozoa, eggs and larvae of sturgeon *Doklady AN SSSR* **138** 492–495 (in Russian)
- Folz DJ and Mezers LS (1985) Management of the lake sturgeon, *Acipenser fulvescens*, population in the Lake Winnebago system, Wisconsin. In *Natural American Sturgeons Biology Aquaculture and Potential* pp 135–146 Eds FP Binkowski and SI Doroshov. Junk Publishers, Dordrecht

- Gengerke TW** (1986) Distribution and abundance of paddlefish in the United States. In *The Paddlefish: Status, Management and Propagation* pp 22–35 Eds JG Dillar, LK Graham and TR Russell. North Central Division, American Fisheries Society Special Publication 7, Bethesda, MD
- Gibbons BH and Gibbons IR** (1972) Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton-X100 *Journal of Cell Biology* **54** 75–97
- Graham LK, EJ Hamilton, TR Russell and CE Hicks** (1986) The culture of paddlefish – a review of methods. In *The Paddlefish: Status, Management and Propagation* pp 78–94 Eds JG Dillard, LK Graham and TR Russell. North Central Division, American Fisheries Society Special Publication 7, Bethesda, MD
- Kroll KJ, Vaneennaam JP, Doroshov SI and Linares J** (1994) Growth and survival of paddlefish fry raised in the laboratory on natural and artificial diets *Progressive Fish-Culturist* **56** 169–174
- Linhart O, Mims SD and Shelton WL** (1995) Motility of spermatozoa from shovelnose sturgeon (*Scaphirhynchus platyrhynchus*, Rafinesque, 1820) and paddlefish (*Polyodon spathula*, Walbaum, 1792) *Journal of Fish Biology* **47** 902–909
- Linhart O, Mims SD, Gomelsky B, Hiott AE, Shelton WL, Cosson J, Rodina M and Gela D** (2000) Spermiation of paddlefish (*Polyodon spathula*, Acipenseriformes) stimulated with injection of LHRH analogue and carp pituitary powder *Aquatic Living Resources* **13** 455–460
- Linhart O, Mims SD, Gomelsky B, Hiott AE, Shelton WL, Cosson J, Rodina M, Gela D and Bastl J** Ionic and osmolality level of paddlefish (*Polyodon spathula* Acipenseriformes) seminal fluid with sperm motility parameters *Aquaculture International* (in press)
- Liu C and Zeng Y** (1988) Notes on the Chinese paddlefish *Psephurus gladius* (Martens) *Copeia* 484–487
- Mims SD** (1991) Evaluation of activator solutions, motility duration and short-term storage of paddlefish spermatozoa *Journal of World Aquaculture Society* **22** 224–229
- Mims SD and WL Shelton** (1998) Monosex culture of paddlefish and shovelnose sturgeon. In *Symposium on Harvest, Trade and Conservation of North American Paddlefish and Sturgeon* pp 67–76 Eds DF Williamson, GW Benz and CM Hoover. TRAFFIC North America/World Wildlife Fund, Washington DC
- Mims SD, Georgi TA and Chen-Han L** (1993a) The Chinese paddlefish, *Psephurus gladius*: biology, life history and potential for cultivation *Journal of World Aquaculture Society* **24** 45–48
- Mims SD, Clark JA, Williams JC and Rouse DB** (1993b) Comparisons of two by-products and a prepared diet as organic fertilizers on growth and survival of larval paddlefish *Polyodon spathula* in earthen ponds *Journal of Applied Aquaculture* **2** 171–187
- Morisawa M and Suzuki K** (1980) Osmolality and potassium ion: their roles in initiation of sperm motility in teleosts *Science* **210** 1145–1147
- Morisawa M, Suzuki K and Morisawa S** (1983) Effects of potassium and osmolality on spermatozoa motility of salmonid fishes *Journal of Experimental Biology* **107** 105–113
- Needham RG** (1965) Spawning of paddlefish induced by means of pituitary material *Progressive Fish-Culturist* **27** 13–19
- Purkett CA, Jr** (1963) Artificial propagation of paddlefish *Progressive Fish-Culturist* **25** 31–33
- Redondo-Müller C, Cosson MP, Cosson J and Billard R** (1991) *In vitro* maturation of the potential for movement of carp spermatozoa *Molecular Reproduction and Development* **29** 259–270
- Scheuring L** (1925) Biologische und physiologische Untersuchungen an Forellensperma *Archiv Hydrobiologie Supplement* **4** 181–318
- Toth GP, Ciereszko A, Christ SA and Dobrowski K** (1997) Objective analysis of sperm motility in the lake sturgeon (*Acipenser fulvescens*): activation and inhibition conditions *Aquaculture* **154** 337–348

---

Received 4 March 2002.

First decision 18 April 2002.

Revised manuscript received 23 July 2002.

Accepted 1 August 2002.