Carbon dioxide and pH affect sperm motility of white sturgeon (*Acipenser transmontanus*)

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Summary

Maintenance of sperm at pH values less than approximately 7.5 inhibited the onset of motility when sperm were subsequently diluted with water; maintenance at pH values above approximately 8.2 was associated with maximal motility upon dilution with water. Within 5~min of exposure to low pH buffer (pH 6.9), there was a 50% decline in sperm motility upon dilution with water suggesting that exposure to low pH interferes with motility within a time frame that may affect fertilization. In most instances, maintenance of sperm under CO_2 at a pressure of 4–5 kPa almost completely blocked their capacity for motility. Furthermore, exposing semen to increasing partial pressures of CO_2 up to about 1 kPa resulted in a marked decrease in semen pH. These observations are consistent with the findings that the buffering capacity of semen is particularly low at physiological pH, and that this low buffering capacity corresponds to the highest pH sensitivity of the capacity for sperm motility. The low seminal buffering capacity may represent a physiological adaptation in the control of sperm function. It may also represent a vulnerability to environmental hypercapnia or metabolic acidosis.

Key words: *Acipenser transmontanus*, buffering capacity, carbon dioxide, pH, semen, sperm, motility, sturgeon, *Acipenser transmontanus*.

Introduction

The Kootenai River white sturgeon *Acipenser transmontanus* is a landlocked population restricted to a 270 km stretch of the Kootenai River, which flows from western Montana through northern Idaho and into British Columbia. This population has shown an almost complete lack of natural juvenile recruitment since 1974. The total adult population in 1998 was estimated to be about 650 individuals and this fish is currently classified as endangered by the Idaho Department of Fish and Game (http://refuges.fws.gov/fish/KootenaiRiverSturgeon.html). The basis for its lack of juvenile recruitment and declining population numbers is not clear and there is, therefore, a need to better understand the reproductive biology of these animals.

As a species, sturgeon are routinely exposed to hypercapnia in their natural environment (Crocker and Cech, 1998). Environmental hypercapnia is associated with an increase in arterial P_{CO_2} and a decrease in blood pH in the white sturgeon *A. transmontanus* (Crocker and Cech, 1998; Crocker et al., 2000). We have previously noted that maintenance of salmonid sperm at low pH or high CO_2 prevents the onset of motility, and reduces fertility, when sperm are subsequently diluted with water (Bencic et al., 2000a,b, 2001). Further, the maximal pH sensitivity of this capacity for motility corresponds to the pH range over which the buffering capacity of seminal plasma is particularly low (Ingermann et al., 2002). These findings suggest that the functional properties of salmonid sperm may be especially sensitive to hypercapnia and resulting respiratory acidosis, as well as metabolic acidosis, prior to release into the environment. As sturgeon are subjected to environmental hypercapnia, especially in intensive aquaculture settings (Crocker and Cech, 1996, 1998; Farrell et al., 2001), and because fish are occasionally anesthetized with CO_2 (e.g. Fish, 1943; Prince et al., 1995), including during gamete harvesting, our previous findings with salmonids suggest that environmental hypercapnia and respiratory and metabolic acidosis could be associated with reductions in sperm motility and possibly fertility in the sturgeon. In contrast, Gallis et al. (1991) reported that motility of sperm from the Siberian sturgeon *Acipenser baeri* is relatively insensitive to pH changes near physiological semen pH (approximately 8.1). Williot et al. (2000) also found no correlation between sperm motility and pH in *A. baeri*. Finally, Gallis et al. (1991) reported that semen from *A. baeri* shows a strong buffering capacity. As preliminary observations from our laboratory suggested the opposite in the white sturgeon *A. transmontanus*, this study was designed to determine the pH sensitivity of the capacity for motility, the influence of CO_2, and the buffering capacity of semen of the Kootenai River white sturgeon *A. transmontanus*.

Materials and methods

Semen samples of the Kootenai River white sturgeon *Acipenser transmontanus* Richardson were obtained from the Kootenai Tribe (KT) experimental hatchery of Bonners Ferry, ID, USA. Semen samples were collected by syringe and catheter directly from the sperm ducts, placed into plastic bags, gassed with oxygen and stored temporarily on wet ice. Samples were regassed with oxygen just prior to shipment (with wet ice) and arrived at the University of Idaho within 24 h of collection. Several additional *A. transmontanus* samples were obtained from the University of California, Davis, USA (UCD). These were shipped to us under comparable conditions within 24 h of collection and were used exclusively for buffering capacity studies. Experiments were initiated within 24 h of receipt of semen.

The osmolality of sturgeon seminal plasma is low relative to that of teleosts (Gallis et al., 1991; Williot et al., 2000). Therefore, to determine the effect of pH on sperm motility, 250 μl semen samples were combined with 500 μl of low osmolality, sperm immobilizing buffer (SI buffer; in mmol l^{-1}: NaCl 10; KCl 4; CaCl$_2$ 0.0125; Bis-Tris-Propane 50, titrated to various pH values with HCl). These mixtures were maintained in 2 ml sealed microcentrifuge tubes placed on their sides at 10°C. After 2 h, a portion of each sample was centrifuged for 3 min at 12 000 g (Marathon MicroA microcentrifuge, Fisher Scientific) and the pH of the supernatant determined at 10°C (Accumet 815 MP pH meter, Fisher Scientific). Also, the motility of a small portion of each sample was assessed. The motility of sperm samples was determined using a BH2 Olympic light microscope. Semen was diluted (>1:1000) using dechlorinated water and sperm motility was estimated visually at room temperature (approximately 20°C) and expressed as a percentage of motile sperm relative to the total number of sperm (Terner, 1986; Moccia and Munkittrick, 1987; Munkittrick and Moccia, 1987; Bencic et al., 2000b.) The identities of the treatment groups were unknown by the person evaluating motility.

To examine the relationship between semen P_{CO_2} and pH, 0.7 ml semen samples were maintained at 10°C in 600 ml metallized polyester bags (Kapak Corp., Minneapolis, MN, USA) filled with humidified air either with or without various amounts of injected CO$_2$. Bags were flaccid, not taut. After 4 h, CO$_2$ levels were determined with a Model 920D DualTrak CO$_2$/O$_2$ analyzer from Quantek Instruments (Northboro, MA, USA). Bags were subsequently cut open and the pH of semen samples determined immediately at 10°C. Similarly, to examine the effect of CO$_2$ on sperm motility, 0.7 ml semen samples were maintained for 4 h in either the presence or absence of 4–5 kPa CO$_2$. After 4 h at 10°C, bags were cut open and sperm motility assessed immediately.

The time course of sensitivity to low pH was assessed in the following manner. 250 μl semen was added to 500 μl SI buffer titrated to high or low pH. Within 1 min of mixing, a small portion of suspension was added to water and motility estimated. The remainder of the suspension was kept on wet ice and motility assessed periodically thereafter. At the end of the experiment, the pH of the centrifuged samples was measured at 10°C.

Buffering capacity analyses were conducted on semen and seminal plasma at 10°C. (Seminal plasma was obtained by centrifuging semen at 12 000 g for 3 min.) The buffering capacities of the fluid samples were quantified as described (Ingermann et al., 2002). Basically, 10 ml of BC saline (in mmol l^{-1}: NaCl 110, KCl 40) was added to 1 ml of semen or seminal plasma. The pH of this solution was then monitored at 10°C before and upon addition of small volumes of 0.1 mol l^{-1} HCl in BC saline. Buffering capacity was quantified as μmol HCl 1 ml^{-1} semen or seminal plasma pH unit^{-1} (Slyke; Wolters-Everhardt et al., 1986). Buffering capacities were calculated from linear regressions of plots of pH *versus* HCl added over the ranges 7.5–8.5 and 6.0–7.0.

14 values of water pH taken from the Kootenai River near Bonners Ferry, ID, USA were obtained from Genevieve Hoyle (Kootenai Tribe of Idaho Fisheries Department) and four additional pH values of Kootenai River water were analyzed in our laboratory. The pH measurements were collected periodically from June 2001 to April 2002.

Chemicals were from Sigma Chemical Co. (St Louis, USA). All data were analyzed by one-way analysis of variance (ANOVA) followed by Newman–Keuls' test, with significance set at $P<0.05$. Data are presented as mean ± S.E.M.

Results

Sperm maintained for 2 h with SI buffer titrated to high pH showed appreciable motility when subsequently diluted with water. Sperm maintained with SI titrated to low pH showed zero or reduced motility once diluted with water (Fig. 1A,B). Sperm transitioned between appreciable and no (or little) capacity for motility (upon subsequent addition to water) over a pH change of about 1 pH unit, although the specific pH range varied somewhat among males. Inhibition of the capacity for sperm motility occurred relatively rapidly upon exposure to low pH; within 5 min of exposure to pH 6.85, sperm showed a decrease of 49±11% (*N*=5) in motility once added to water (Fig. 2).

Inhibition of the capacity for motility could also be achieved by prior maintenance of semen with CO$_2$ (Table 1). With the exception of semen from 2 of 7 males, sperm maintained under 4–5 kPa CO$_2$ for 4 h at 10°C showed little, if any, motility upon addition to water (Table 1). Sperm from the single male that showed the lowest CO$_2$ sensitivity in the capacity for motility did show marked pH sensitivity, but over a lower pH range than sperm from other males (Fig. 1; male, filled circle).

The pH of semen maintained for 4 h at 10°C without added CO$_2$ was 8.93±0.06 (*N*=6, each for two determinations per male; Fig. 3). Addition of CO$_2$ to semen under these conditions resulted in acidification, with the greatest change in semen pH occurring upon CO$_2$ addition at relatively low P_{CO_2} values (Fig. 3A); semen pH appeared less sensitive to changes in P_{CO_2} values above approx. 1 kPa. Nonetheless, pH plotted against the logP_{CO_2} demonstrated a linear relationship over a range of
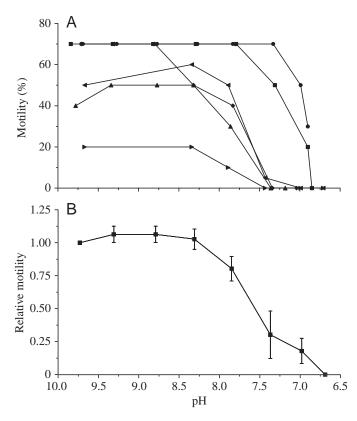
Fig. 1. Effect of 2 h maintenance at various pH values on the ability of sperm to become motile upon addition to water. (A) Percentage motility of sperm from 6 males as a function of pH. (B) Same data set with motility expressed relative to that associated with the highest pH.

0.01 to 5.3 kPa CO_2 that corresponded to a semen pH of approximately 9.0–7.3 (Fig. 3B).

Addition of HCl to semen and seminal plasma resulted in multiphasic titration curves that demonstrated a lower buffering capacity at high pH than at low pH (Fig. 4A). This can be seen more clearly in Fig. 4B, which shows the buffering

Table 1. *Effect of CO_2 on sperm motility*

Male	Percentage motility	
	<0.02 kPa CO_2	4–5 kPa CO_2
1	50	0
2	70	0
3	50	0
4	70	60
5	70	5
6	70	0
7	70	30
Mean±S.E.M.	64±4	14±9

Semen samples were maintained for 4 h at 10°C in the presence and absence of added CO_2. Sperm motility was subsequently assessed upon dilution of semen with water.

Semen from male 4 also demonstrated pH sensitivity over the lowest pH range (shown in Fig. 1, filled circles).
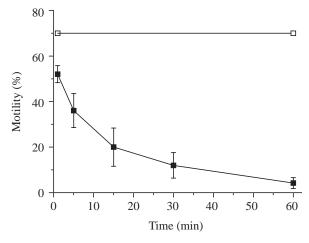

Fig. 2. Motility *versus* time. Sperm maintained in low pH (6.91±0.02; filled squares) or high pH (9.72±0.03; open squares) SI buffer prior to water activation. *N*=5 for each data set. Earliest motility measurements were within 1 min of combining semen and SI buffers. If the *t*=0 data point is taken as 70±0% (the earliest motility value in high pH SI), all low pH motility values are significantly different from *t*=0. (Motility of sperm in high pH SI at 60 min was 70±0%, *N*=5.)

capacity relative to the pH of the solution. Quantification of buffering capacities as reciprocals of linear regressions of titration curves indicated that buffering capacities of semen and seminal plasma over the pH range 7.5–8.5 are about 20% of those over the pH range 6.0–7.0 (Fig. 4C). (Statistically indistinguishable results were obtained from four UCD semen samples; data not shown.) The ratio of buffering capacity of semen to that of seminal plasma from the same male was 1.11±0.02 (*N*=6) and 1.04±0.02 (*N*=4) over the pH range 7.5–8.5 for KT and UCD samples, respectively. The ratio of buffering capacity of semen to that of seminal plasma was 0.97±0.02 (*N*=6) and 0.99±0.02 (*N*=4) over the pH range 6.0–7.0 for KT and UCD samples, respectively.

Water samples taken from the Kootenai River over a calendar year had pH values of 8.4±0.1 (*N*=18).

Discussion

Because of the precarious status of the Kootenai River white sturgeon, study into all aspects of the reproductive biology of this fish is particularly warranted. As motility correlates well with fertility in sperm from at least several teleosts (Billard, 1978; Moccia and Munkittrick, 1987; Ciereszko and Dabrowski, 1994; Lahnsteiner et al., 1998; Bencic et al., 2000b; Rurangwa et al., 2001), impairment of sperm motility could have deleterious effects on fertility in the white sturgeon. Therefore, we have examined the sensitivity of sturgeon sperm motility to pH and CO_2, variables known to have a negative impact on salmonid sperm motility (Bencic et al., 2000b; Ingermann et al., 2002).

Sturgeon sperm maintained for several hours at low pH demonstrated little, if any, motility when subsequently added
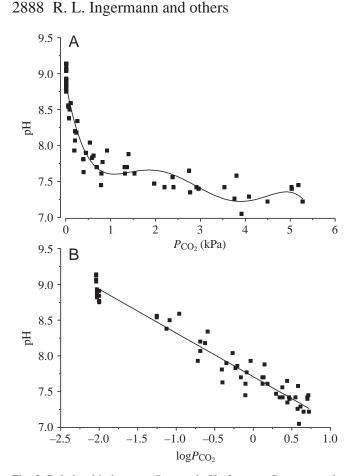
Fig. 3. Relationship between P_{CO_2} and pH of semen. Semen samples were equilibrated for 4 h at 10°C prior to pH analysis. 5–12 data points were collected from each of 6 males. (A) Curve represents the fourth order polynomial equation generated with all data. (B) Linear regression of pH as a function of the log of the P_{CO_2}. The line is given by the equation: pH=7.708–0.612 (logP_{CO_2}); r^2=0.94 with 95% confidence limits for the slope being –0.653 to –0.571.
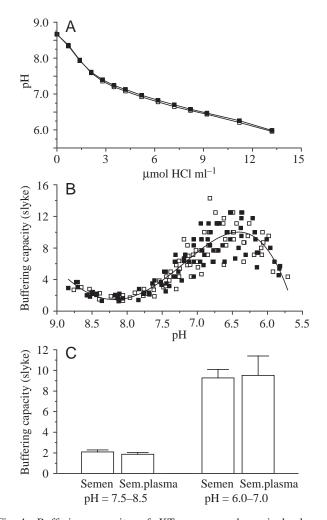

Fig. 4. Buffering capacity of KT semen and seminal plasma. (A) Representative titration of semen (filled squares) and seminal plasma (open squares) with HCl at 10°C. (B) Buffering capacity of semen (filled squares) and seminal plasma (open squares) as a function of pH. Each value shown was calculated as the added HCl (in μmol HCl ml^{-1} semen or seminal fluid) divided by the resulting change in pH plotted against the median pH of each addition. Data represent 12–23 measurements from each of 6 males; curve represents the fourth order polynomial equation generated using all data points. (C) Buffering capacity of semen and seminal (Sem.) plasma determined by linear regression analysis of titration curves over the pH range 7.5–8.5 and 6.0–7.0; N=6. Buffering capacities of semen and seminal plasma were not different over either pH range. (Comparable data were obtained for UCD samples, N=4; data not shown.)

to water. In contrast, sperm maintained at high pH demonstrated appreciable motility when added to water. Although the specific range of pH that controlled this capacity for motility varied among males it was, nonetheless, relatively narrow, approximately 1 pH unit. This is consistent with observations in salmonids (Ingermann et al., 2002) but appears to contrast with the results of studies of sperm collected from *A. baeri* by Williot et al. (2000). They found no correlation between semen pH and sperm motility. However, the pH values of all of their semen samples exceeded 7.5 and thus correspond approximately to the range of pH values in the current study (>7.8) that had little effect on sperm motility.

Crocker and Cech (1998) found that maintenance of *A. transmontanus* under 3.3–4.7 kPa CO$_2$ resulted in a decrease in arterial blood pH of approximately 0.7 pH units. Gallis et al. (1991) reported that the normal pH of *A. baeri* seminal plasma is approximately 8.1. Therefore, if the pH of the semen of *A. transmontanus* is comparable to that of *A. baeri*, the current data suggest that exposure of the animal to acute hypercapnia will have significant deleterious effects on the functional properties

of *A. transmontanus* sperm. Whether chronic hypercapnia is associated with such putative effects is not clear because fish appear to regulate pH upward under these exposures by adjusting bicarbonate levels (Lloyd and White, 1967; Cameron and Randall, 1972; Crocker and Cech, 1998).

Inhibition of the capacity for motility by exposure to low pH occurred relatively rapidly. Within 1 min and 5 min, motility had decreased by about 26% and 50%, respectively (Fig. 2). Although it seems likely that the mechanism underlying this

inhibition is associated with intracellular pH change as in salmonids (Bencic et al., 2000b), the rapidity of response leaves open the possibility that low pH affects sperm motility by an extracellular mechanism. *A. transmontanus* is a broadcast spawner with gametes released into fast water (http://refuges.fws.gov/fish/KootenaiRiverSturgeon.html). Because sturgeon sperm remain motile for several minutes (Gallis et al., 1991; Linhart et al., 1995; Toth et al., 1997), it is possible that the shedding of sperm into acidic waters could be associated with reduced sperm motility and fertility. Indeed, Gallis et al. (1991) reported that although diluting the sperm of *A. baeri* 1:100 in a Tris-buffered activating solution at pH 7.2 had little effect on motility, diluting sperm at pH 6.2 reduced the initial intensity to about 40% of the motility observed in pH 9.2 buffer. However, pH measurements of water samples from the Kootenai River yielded values above 8.0 suggesting that pH sensitivity of sperm motility after semen release from the male is not likely to be a concern with the Kootenai River *A. transmontanus*.

Sperm maintained under high CO_2 levels (4–5 kPa) generally showed poor, if any, motility upon addition to water relative to sperm maintained at low CO_2 levels (<0.02 kPa; Table 1). Furthermore, the pH of semen maintained without added CO_2 was 8.9 and addition of modest levels of CO_2, up to about 1 kPa, resulted in a marked decrease in semen pH (Fig. 3A). Thus, the inhibition of the capacity for motility by CO_2 is likely caused, at least in large part, by acidification of semen and probably an accompanying decrease in intracellular pH.

That modest levels of CO_2 have a marked effect on semen pH suggests that semen possesses low buffering capacity. Indeed, within the approximate normal physiological range of pH≥7.5, the buffering capacity of semen was low, and lower than that associated with pH values less than 7.5 (Fig. 4A,B). Furthermore, the lowest buffering capacity noted in the present study (Fig. 4B) corresponds to the seminal plasma pH of 8.1 reported by Gallis et al. (1991) for *A. baeri*.

Gallis et al. (1991) reported that the buffering capacity for *A. baeri* semen was high. From figure 3 in Gallis et al. (1991), it is clear that over the pH range of approximately 2.5–10.8 there are several pH regions where buffering capacity is relatively high; however, over the more physiologically relevant pH range of 7.5–9.0, their results are consistent with our results and demonstrate a low seminal buffering capacity.

Semen pH was highly sensitive to modest additions of CO_2 up to approx. 1 kPa but appeared less sensitive to additions above that amount (Fig. 3A). However, Fig. 3B indicates that over the pH range of approximately 7.3–9.0, pH changed in a consistent and predictable manner in response to changes in P_{CO_2}. These results suggest that the low and relatively stable buffering capacity of semen over this pH range was responsible for the direct relationship between pH and logP_{CO_2}. The present findings suggest a nonlinear response would be noted if P_{CO_2} values were raised such that the pH was sufficiently shifted into the range where seminal buffer capacity was greater (Fig. 4B).

To determine whether sperm contribute to the buffering capacity of the semen, the ratio of buffering capacity of semen to that of seminal plasma per male was calculated. These data suggest that within the pH range 7.5–8.5, sperm contribute perhaps 5–10% of the buffering capacity of semen whereas they appear to make no contribution over the pH range of 6.0–7.0. The relatively minor contribution of sperm to the semen at the higher pH values seems unlikely to be physiologically significant and may simply be a consequence of the presence of sperm proteins.

That the buffering capacity of sturgeon semen is low within a normal physiological range is comparable to results obtained with salmonids (Ingermann et al., 2002). Morisawa and Morisawa (1988) noted that sperm taken directly from the testes of salmonids are immotile when exposed to water while those taken from the distal portion of the sperm ducts do become motile when exposed to water. The primary differences between semen in the testes and in the reproductive ducts are higher pH values and higher bicarbonate levels in semen in the reproductive ducts (Morisawa and Morisawa, 1986, 1988). We have suggested that a low semen buffering capacity represents a physiological adaptation allowing the epithelial cells of the reproductive tract to exert control over the capacity for sperm motility by regulating semen pH *via* bicarbonate secretion (Ingermann et al., 2002). A comparable situation may exist in the sturgeon as well: the low semen buffer capacity may permit control of the potential for sperm motility *via* pH regulation mediated by acid/base secretions into the semen. However, the low buffering capacity coupled with the high pH sensitivity of sperm motility within the physiological pH range may also make the sturgeon sperm vulnerable to acute environmental hypercapnia and respiratory and metabolic acidosis.

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