

## Effect of Osmotic Pressure on the Activation and Storage of Channel Catfish Sperm

MARK C. BATES, WILLIAM R. WAYMAN, AND TERRENCE R. TIERSCH<sup>1</sup>

School of Forestry, Wildlife, and Fisheries, Louisiana Agricultural Experiment Station  
Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA

**Abstract.**—Artificial spawning of channel catfish *Ictalurus punctatus* relies on the removal of testis and suspension of sperm in an extender solution for storage and use in fertilization. Little is known about the relationships among osmotic pressure, sperm activation, motility, and storage. Our objectives were to (1) estimate motility of channel catfish sperm diluted in solutions ranging in osmotic pressure from 8 to 295 mosmol/kg, (2) identify the osmotic pressure that induces threshold activation (10% motility) and the highest pressure that induces complete activation, (3) determine the role of ionic dilution in activation by use of ion-deficient solutions, and (4) evaluate the effect of osmotic pressure on the retention of motility during storage. Motility (percentage of actively swimming sperm) was estimated in diluted Hanks' balanced salt solution (HBSS) and sucrose solutions over a range of osmotic pressures. The HBSS osmotic pressures of threshold and complete activation were  $218 \pm 15$  mosmol/kg and  $132 \pm 9$  mosmol/kg, respectively. We found that osmotic pressures above 220 mosmol/kg induced minimal (<10%) activation and pressures below 130 mosmol/kg induced complete activation. Within the zone of incomplete activation (220–130 mosmol/kg) a decrease of 15 mosmol/kg in osmotic pressure increased motility about 10%. We used sucrose solutions to provide an osmotic environment with minimal ionic influence for the testing of activation. Osmotic pressures for threshold ( $214 \pm 1$  mosmol/kg) and complete ( $125 \pm 2$  mosmol/kg) activation were consistent with those obtained with diluted HBSS, suggesting that reduction in osmotic pressure plays a major role in the activation of channel catfish sperm. Sperm stored in 122 mosmol/kg HBSS lost 82% of initial motility after 2.5 h. However, sperm stored in solutions with higher osmolalities retained motility significantly longer ( $P = 0.0017$ ). A minimum reduction of osmotic pressure to below 130 mosmol/kg is essential to induce complete motility of channel catfish sperm for motility estimates and artificial fertilization.

Sperm of channel catfish *Ictalurus punctatus* cannot be collected by stripping the male. The testis must be removed and homogenized in an extender solution for storage. These suspensions can be used to produce specific crosses or hybrids not possible by natural spawning. Because sperm are activated and become motile upon contact with freshwater, they must be stored in a solution, such

as Hanks' balanced salt solution (HBSS), which has sufficient osmotic pressure to maintain sperm viability, or be stored indefinitely by means of cryopreservation (Tiersch et al. 1994).

The activation of channel catfish sperm has never received direct attention. As part of a study on cryopreservation, Guest et al. (1976a) evaluated three activators for channel catfish sperm: distilled water, 0.05% saline solution, and pond water. While each of these activated sperm, the osmotic pressures were not reported, and the potential for the dilution of specific ions to activate sperm was not investigated. The purpose of the present study was to determine the specific relationship among osmotic pressure, sperm activation, and motility (percentage of actively swimming sperm). Our objectives were to (1) estimate motility of sperm diluted in solutions ranging in osmotic pressure from 8 mosmol/kg (deionized water) to 295 mosmol/kg (1.1× full-strength HBSS), (2) identify the osmotic pressure that induces threshold activation (10% motility) and the highest pressure that induces complete activation in diluted HBSS, (3) determine the role of ionic dilution in activation by use of ion-deficient (sucrose) solutions instead of HBSS, and (4) evaluate the effect of osmotic pressure on motility retention of channel catfish sperm during storage.

### Methods

#### *Experimental animals and collection of sperm.*—

Channel catfish used in this study were of domesticated pond-raised stock. Anterior and posterior testes (Sneed and Clemens 1963) were surgically removed, stored in vials containing HBSS (Tiersch et al. 1994) or sucrose solution (295 mosmol/kg), and transported to the laboratory on ice. For experiments 1–3, the testes were crushed, and sperm were suspended (1 g testis/20 mL of HBSS or sucrose stock solution), stored at 4°C, and studied within 24 h. At this concentration, suspension of sperm in HBSS (295 mosmol/kg) resulted in a reduction of osmotic pressure of the solution by as much as 10 mosmol/kg (mean  $\pm$  SD =  $5 \pm 3$  mosmol/kg). For experiment 4, 1 g of testis was

<sup>1</sup> To whom correspondence should be addressed.

processed in 45 mL of the appropriate storage solution. All experiments were performed outside of the normal spawning season for channel catfish (May–July in southern Louisiana). Experiments 1–3 were performed in August–October, and experiment 4 in January. Motility was 20–90% (mean  $\pm$  SD = 46%  $\pm$  16%), values typically encountered outside of the spawning season (Guest et al. 1976b; Jaspers et al. 1978).

We measured channel catfish blood plasma to enable direct comparison with our results for sperm. Blood was drawn from five channel catfish, transferred immediately to heparinized capillary tubes, and centrifuged at 7,000 revolutions per minute for 15 min before analysis. The osmotic pressure of blood plasma, determined by vapor pressure osmometer (Wescor, Inc., Logan, Utah; model 5500), was 273  $\pm$  7 mosmol/kg (mean  $\pm$  SD). This agrees with the value of 272  $\pm$  3 mosmol/kg reported for blood plasma of channel catfish by Norton and Davis (1976).

*Preparation of solutions.*—We studied sperm activation over a range of values from deionized water to 1.1 $\times$  full-strength HBSS. A stock solution of HBSS was prepared, except that sufficient water was added to yield an osmotic pressure of 295 mosmol/kg (1.1 $\times$  full strength) instead of the full-strength osmolality of 270 mosmol/kg. From this, 10 solutions were prepared by stepwise dilution in increments of 10% ( $\sim$ 30 mosmol/kg). A sucrose stock solution of 295 mosmol/kg was prepared. From this, 10 sucrose solutions were prepared by stepwise dilution in increments of 10% ( $\sim$ 30 mosmol/kg). For Experiment 4, five HBSS storage solutions were prepared at 122, 226, 247, 267, or 285 mosmol/kg. All chemicals were of reagent grade (Sigma Chemical Co., St. Louis, Missouri). Water was deionized (18 M $\Omega$ /cm) with a Barnstead Nanopure ion exchange system (Dubuque, Iowa; model D4741).

*Experiment 1: general curve.*—Motility was scored by placing 1  $\mu$ L of sperm suspension on a slide, activating it by the addition of 19  $\mu$ L of test solution, and viewing under dark-field illumination at 100 $\times$ . Sperm samples from four channel catfish were scored for motility as: 0 = 0 to <10%; 1 = 10 to <25%; 2 = 25 to <50%; 3 = 50 to <75%; and 4 = 75 to 100% for each of the 11 test solutions. Only those sperm engaged in vigorous forward motion were considered motile. Swirling effects were easily distinguished from motility and were minimized by careful addition of the test solution. Osmotic pressure was measured with a vapor pressure osmometer on a 10- $\mu$ L

sample taken directly from the slide. Osmotic pressures and motility scores were averaged for each test solution.

*Experiment 2: threshold and complete activation in HBSS.*—Sperm from 20 channel catfish were used to determine the osmotic pressures of threshold and complete activation in diluted HBSS. The lowest osmotic pressure that induced threshold activation (the initiation of motility in 10% of the cells) and the highest osmotic pressure that induced complete activation (the highest percent motility observed for each sample when activated with deionized water) were recorded for each sample. A two-tailed paired-comparison *t*-test was used to test for a difference between the osmotic pressure of threshold and complete activation; the level for significance was set at  $P < 0.05$ .

*Experiment 3: threshold and complete activation in sucrose solutions.*—To test the effect of osmotic pressure in solutions essentially free of ions, the preceding experiment was repeated for four sperm samples with diluted sucrose solutions instead of HBSS. A two-tailed paired-comparison *t*-test was used to test for a significant difference between the osmotic pressure of threshold and complete activation.

*Experiment 4: storage study.*—To test the effect of osmotic pressure on storage over time, sperm samples from five channel catfish were stored in each of five HBSS solutions of different final osmotic pressures (122, 226, 247, 262, and 285 mosmol/kg). Five replicates were used per treatment level, except for the 247 mosmol/kg treatment, which had 10 replicates. Data were reported as percent motility; otherwise procedures were the same as described above. Percent motility was estimated by activation with distilled water at the start of storage, after 2.5 and 18 h of storage, and every 24 h thereafter until sperm were no longer capable of being activated. The data were arcsine-square-root-transformed and analyzed with a repeated measures split-plot design. A Fisher's least significant difference test was used to determine if significant differences ( $P < 0.05$ ) existed among the five osmolality levels. All statistical analyses were performed with Data Desk software (version 4.2; Data Description, Inc., Ithaca, New York).

## Results

In experiment 1, few (<5%) vibrating (without forward motion) or motile (1–2%) sperm were observed at osmolalities of 240–270 mosmol/kg. Between 220 and 240 mosmol/kg, low levels (<10%) of activation and sluggish swimming were ob-

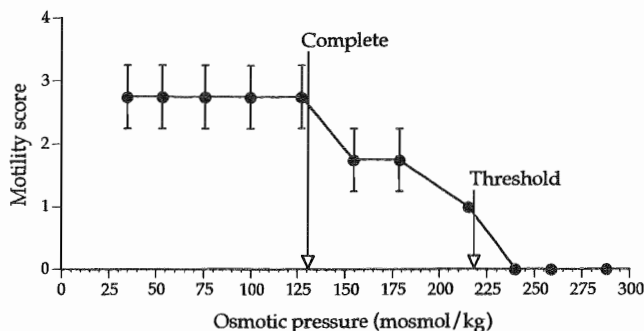


FIGURE 1.—Motility of channel catfish sperm activated in diluted Hanks' balanced salt solution. Each point represents the mean value of samples from four fish (experiment 1). The results of experiment 2 (arrows) are superimposed and represent the mean value of 20 fish. The arrows indicate the osmotic pressure of threshold (10% motility) and complete activation.

served (Figure 1). Vigorous swimming by sperm was observed between 35 and 220 mosmol/kg. A zone of partial activation was observed between 130 and 220 mosmol/kg where a decrease in osmotic pressure of 15 mosmol/kg resulted in an increase in motility of about 10%. Osmotic pressures <130 mosmol/kg induced complete activation in all samples.

The osmotic pressure that induced threshold activation ( $218 \pm 15$  mosmol/kg) in diluted HBSS was significantly higher ( $P < 0.0001$ ;  $N = 20$ ) than the osmotic pressure ( $132 \pm 9$  mosmol/kg) that induced complete activation (experiment 2). These values agreed with the values observed for threshold and complete activation for experiment 1 (Figure 1).

The osmotic pressure that induced threshold activation ( $214 \pm 1$  mosmol/kg) in diluted sucrose solution was significantly higher ( $P < 0.0001$ ;  $N = 4$ ) than the osmotic pressure that induced complete activation ( $125 \pm 2$  mosmol/kg). These values are indistinguishable from those observed for activation in diluted HBSS.

The effect of osmotic pressure of storage buffer on motility over time (experiment 4) was highly significant ( $P = 0.0017$ ). Motility percentages in the lowest (122 mosmol/kg) and highest (285 mosmol/kg) osmotic pressures were significantly different from those in the intermediate pressures (226–262 mosmol/kg; Figure 2).

### Discussion

This study demonstrates the importance of osmotic pressure in the storage and activation of channel catfish sperm. By manipulating osmotic pressure we were able to activate sperm motility over a range of 35–270 mosmol/kg. Partial acti-

vation (sperm vibrating or swimming in place) was triggered by osmotic pressures as high as 270 mosmol/kg. Although motility does not necessarily correlate with fertility, these findings have implications in the selection of storage buffers, and in the dilution of sperm suspensions to assure complete motility for fertilization or assessment of sperm quality.

In sperm of rainbow trout *Oncorhynchus mykiss*, the dilution of extracellular  $K^+$  and the release of intracellular stores of  $Ca^{++}$  are involved in initiation of motility (Boitano and Omoto 1991). Motility of salmonid sperm can be induced at the osmolality of seminal fluid ( $\sim 300$  mosmol/kg) if the concentration of  $K^+$  is below that of seminal fluid (Morisawa et al. 1983a). In contrast, sperm activation in the freshwater catfish *Rhamdia sapo* (Cussac and Maggese 1988) and in several species of freshwater cyprinids (e.g., common carp *Cyprinus carpio*) is triggered by a decrease in osmotic pressure, independent of extracellular  $K^+$  (Morisawa et al. 1983b). In our study of channel catfish, threshold and complete activation occurred in ion-deficient sucrose solutions at osmotic pressures consistent with those for the highly-ionic HBSS. Mechanisms involving the dilution of a specific ion would have predicted activation of sperm at higher osmotic pressures in ion-deficient solutions. While the role of specific ions in the activation process requires further study, it would appear that reduction in osmotic pressure is important in the activation of channel catfish sperm, a condition perhaps common to the superorder Ostariophysii.

Careful dissection and removal of the catfish testis eliminates contamination of sperm with urine and water, which could reduce osmotic pressure and storage time. The immediate addition of

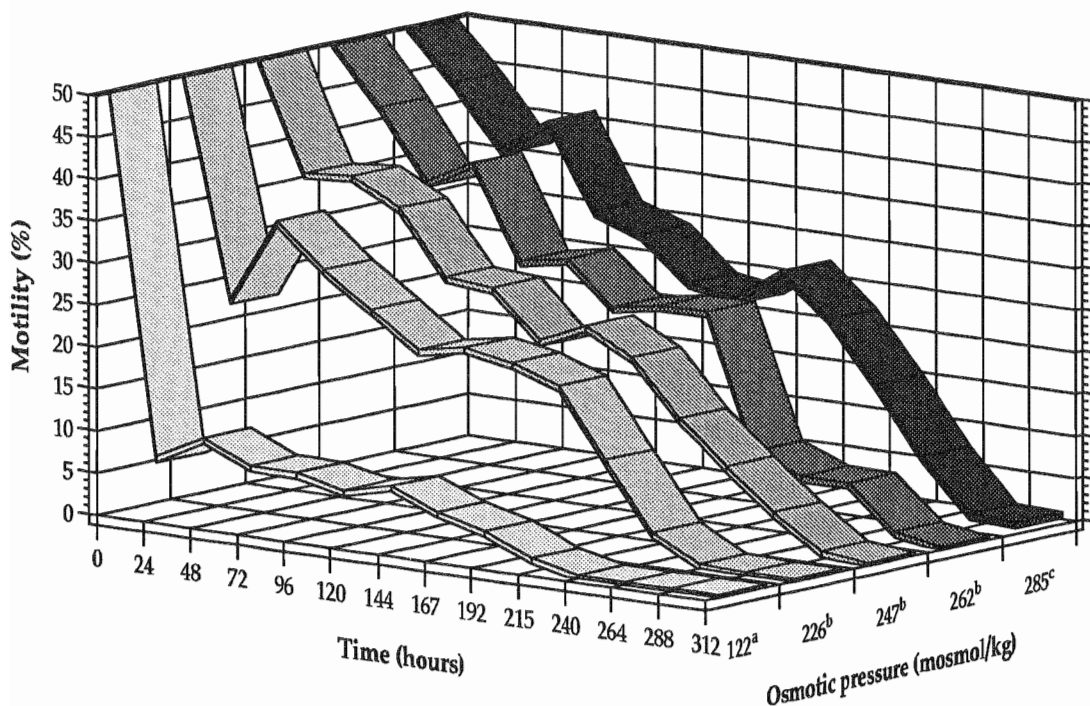


FIGURE 2.—Motility of channel catfish sperm during storage at 4°C in Hanks' balanced salt solutions prepared at five osmolalities (all SDs  $\leq 20\%$ ). Each data point represents the mean value of five fish. Osmolality values followed by the same superscript letter were not significantly different ( $P > 0.05$ ) with respect to storage time as determined by the Fisher's least significant difference means separation test.

an extender to sperm stripped from other species would reduce the likelihood of activation of sperm cells by dilution with urine and water.

Although subjective (Turner 1986), motility is the most used indicator of sperm quality. Accurate assessment of sperm quality in channel catfish by estimation of motility is dependent on testing at osmotic pressures below 130 mosmol/kg (i.e., a minimum 60% reduction in osmotic pressure of full-strength HBSS). Sufficient dilution is also essential to ensure optimal fertilization rates, although it remains to be determined if channel catfish eggs are activated in the same fashion as channel catfish sperm.

Guest et al. (1976a) reported that the percent motility of channel catfish sperm yielded reasonable estimates of sperm viability. In our study, sperm retained motility significantly longer when stored at 285 mosmol/kg than when stored at  $\leq 262$  mosmol/kg. This suggests that storage of sperm in HBSS at osmotic pressures above 270 mosmol/kg (the osmolality of blood plasma) would provide protection from activation by osmotic mechanisms. Therefore, we recommend that channel catfish sperm be stored in HBSS prepared at 295 mos-

mol/kg. This provides a margin of safety given our experience that the addition of sperm and testicular fluids can result in a 10 mosmol/kg reduction in the osmolality of the storage solution. Because commercially prepared HBSS can vary among lots by as much as 35 mosmol/kg (Gibco BRL, Inc., and Sigma Chemical Co., personal communications) storage solutions should be tested directly under the microscope to ensure that they do not activate sperm. When an osmometer is not available, use of a  $1.1 \times$  full-strength HBSS solution is advised.

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### References

- Boitano, S., and C. K. Omoto. 1991. Membrane hyperpolarization activates trout sperm without an increase in intracellular pH. *Journal of Cell Science* 98:343–349.
- Cussac, V. E., and M. C. Maggese. 1988. Effects of salt solutions on the fertilizing ability and activation of gametes in the catfish, *Rhamdia sapo*, (Pisces, Pimelodidae). *Revista Brasileira de Biologia* 48:203–211.
- Guest, W. C., J. W. Avault, Jr., and J. D. Roussel. 1976a. Preservation of channel catfish sperm. *Transactions of the American Fisheries Society* 105:469–474.
- Guest, W. C., J. W. Avault, and J. D. Roussel. 1976b. A spermatological study of channel catfish, *Ictalurus punctatus*. *Transactions of the American Fisheries Society* 105:463–468.
- Jaspers, E. J., J. W. Avault, Jr., and J. D. Roussel. 1978. Testicular and spermatozoal characteristics of channel catfish, *Ictalurus punctatus*, outside the spawning season. *Transactions of the American Fisheries Society* 107:309–315.
- Morisawa, M., K. Suzuki, and S. Morisawa. 1983a. Effects of potassium and osmolality on spermatozoan motility of salmonid fishes. *Journal of Experimental Biology* 107:105–113.
- Morisawa, M., K. Suzuki, H. Shimizu, S. Morisawa, and K. Yasuda. 1983b. Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. *Journal of Experimental Biology* 107:95–103.
- Norton, V. M., and K. B. Davis. 1976. Effect of abrupt change in the salinity of the environment on plasma electrolytes, urine volume, and electrolyte excretion in channel catfish, *Ictalurus punctatus*. *Comparative Biochemistry and Physiology* 56A:425–431.
- Sneed, K. E., and H. P. Clemens. 1963. The morphology of the testes and accessory reproductive glands of the catfishes (Ictaluridae). *Copeia* 1963:606–611.
- Terner, C. 1986. Evaluation of salmonid sperm motility for cryopreservation. *Progressive Fish-Culturist* 48:230–232.
- Tiersch, T. R., C. A. Goudie, and G. J. Carmichael. 1994. Cryopreservation of channel catfish sperm: storage in cryoprotectants, fertilization trials, and production of channel catfish with cryopreserved sperm. *Transactions of the American Fisheries Society* 123:580–586.

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