Transactions of the American Fisheries Society, vol. 125, no. 2, 1996, pp. 195-202.
Online ISSN: 1548-8659
Print ISSN: 0002-8487
http://afs.allenpress.com/perlserv/?request=get-archive&ct=1
http://afs.allenpress.com/archive/1548-8659/125/2/pdf/i1548-8659-125-2-195.pdf
DOI: 10.1577/1548-8659(1996)125<0195:COMSIE>2.3.CO;2
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Transactions of the American Fisheries Society 125:195–202, 1996
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Characteristics of Muskellunge Spermatozoa II: Effects of Ions and Osmolality on Sperm Motility

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Abstract.—We investigated the effects of potassium, sodium, glucose, and calcium concentrations, alone or in combinations, on sperm motility in muskellunge *Esox masquinongy*. Sperm motility was evaluated by the duration of sperm movement and the initial percentage of motile sperm. The osmolality of diluents rather than the specific ions or nonelectrolyte played a major role in the regulation of sperm motility in muskellunge. Sperm were fully activated (>80%) when the concentration was lower than 50 mM of KCl and NaCl, or 100 mM glucose (all in 30 mM tris–HCl at pH 8.0). A small percent of spermatozoa could be activated at 150 mM KCl and NaCl, or 300 mM glucose, which were hypertonic to the seminal plasma. The duration of sperm movement was up to 6–7 min at 12°C in a solution of 100 mM glucose or 50 mM NaCl. Spermatozoa had a prolonged duration of movement in potassium solutions, up to 120 min at 12°C in a solution of 100 mM KCl. The prolonged duration of movement might be caused by reactivation of sperm or gradual activation of sperm motility. Calcium had an inhibitory effect on sperm motility in muskellunge, starting at 3 mM $CaCl_2$ with 30 mM tris–HCl at pH 8.0. Semen diluted in calcium-supplemented solutions did not disperse well, and the sperm tended to form clumps. The mechanism involved in muskellunge sperm motility control markedly differs from that in salmonids (inhibitory function of K^+ and activatory role of Ca^{2+}) and cyprinids (no effect of Ca^{2+}).

Artificial fertilization of eggs from muskellunge *Esox masquinongy* has been successfully accomplished in the past (Hasler et al. 1940; Galat 1973) and is currently used in mass propagation of the species (Moore 1991). Information on sperm physiology will aid in perfecting fertilization techniques and reduce the risk of handling muskellunge gametes under hatchery conditions. We reported for the first time some of the physiological and biochemical characteristics of semen and ultrastructure of spermatozoa in muskellunge (Lin et al. 1996). Understanding the regulatory mechanism of sperm motility is essential for implementing chromosome manipulation experiments and may lead to improved fertilization and efficiency of muskellunge propagation.

Sperm of most teleost fishes are quiescent in the semen and initiate their movement after being released into the environment (Billard 1978; Stoss 1983). Sperm motility is one of the most important parameters of sperm quality and is usually expressed in duration of sperm movement and percentage of motile sperm immediately after activation (Redondo-Muller et al. 1991). Changes in osmolality and potassium concentration have been reported as the major factors that initiate sperm motility in cyprinids and salmonids, respectively (Morisawa and Suzuki 1980; Morisawa et al. 1983a, 1983b; Stoss 1983). Calcium also plays an important role in the regulation of sperm motility (Tanimoto and Morisawa 1988; Okuno and Morisawa 1989; Boitano and Omoto 1992). Calcium can prevent abrupt change in the swimming speed and can increase the duration of sperm movement in trout (Christen et al. 1987). However, only a few reports on physiological characteristics of spermatozoa of escoids are available. Billard (1978) found that a physiological solution of about 0.7% salinity, compared with freshwater, increased the motility and fertility of sperm in northern pike *Esox lucius*. Duplinsky (1982) reported the effects of pH values on sperm motility of northern pike and chain pickerel *Esox niger* using buffered tap water. In northern pike, duration of sperm movement at 10–13°C showed a strong trend of increase with increasing pH and reached a maximum duration of 67 s at pH 7.9.

The goal of this study was to investigate the effects of potassium, sodium, glucose, and calcium concentrations, alone or in combination, on sperm motility and thus to understand the regulatory mechanism of sperm motility in muskellunge.

Methods

Sexually mature male muskellunge (703–909 mm total length) were captured with trap nets from Clear Fork Reservoir, Ohio, in April 1993 and 1994. Water temperature of the lake at the time of

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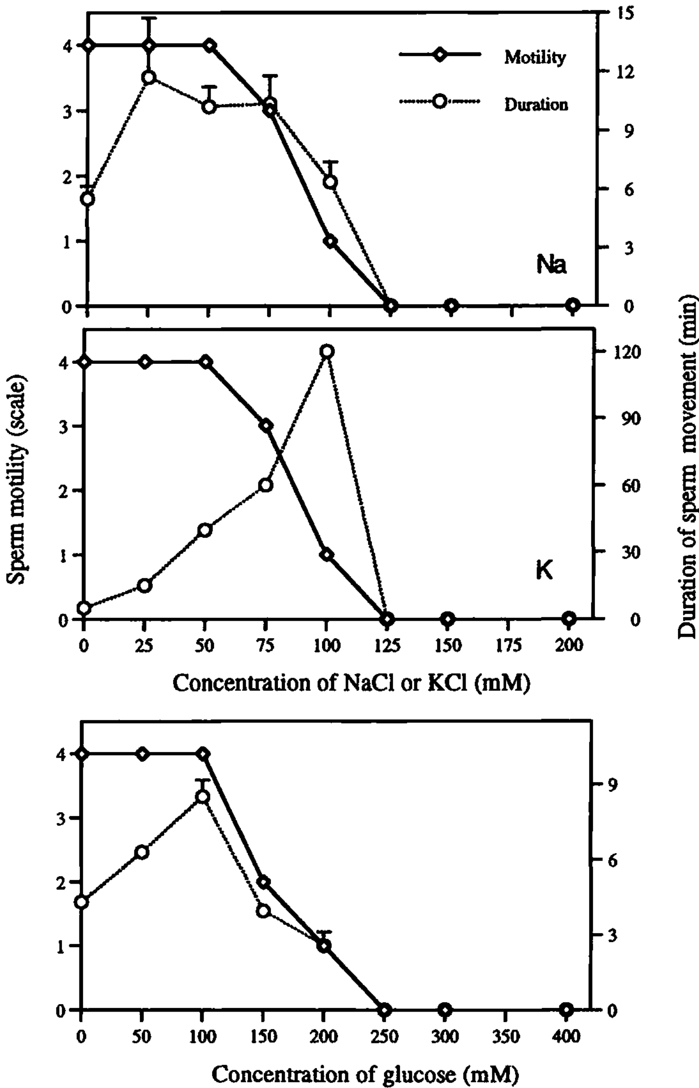

FIGURE 1.—Effects on muskellunge sperm motility of sodium, potassium, and glucose concentrations in the activation solution. Initial percentage of motile sperm (scale, 1993; %, 1994) was estimated at 5–7 s after activation. The arbitrary scale used in 1993 was defined as 4 = 75–100%, 3 = 50–75%, 2 = 25–50%, 1 = 0–25%, 0 = 0%. Duration of sperm movement is the time interval from dilution of semen to the time that all sperm ceased their forward movement. For activation, 1 μL of semen was added to 0.2 mL of diluent containing various NaCl, KCl, or glucose concentrations in 30 mM tris–HCl at pH 8.0. for KCl at 75 and 100 mM, semen was diluted 200× in a plastic tube and sperm motility was estimated at regular intervals. Data points are means; vertical lines represent +SD (*N* = 3–5).

sampling was 10–12°C. The captured fish were aged at 3–6 years old by Ohio Department of Natural Resources personnel. Fish were maintained in a holding pen for several hours or overnight before being stripped for semen. Semen was collected into a vial by hand stripping the unanesthetized fish. Semen was collected from 3 males in 1993 and from 15 males in 1994 for this study. Semen was stored on ice before evaluation, and all measurements were finished within 12 h after gamete

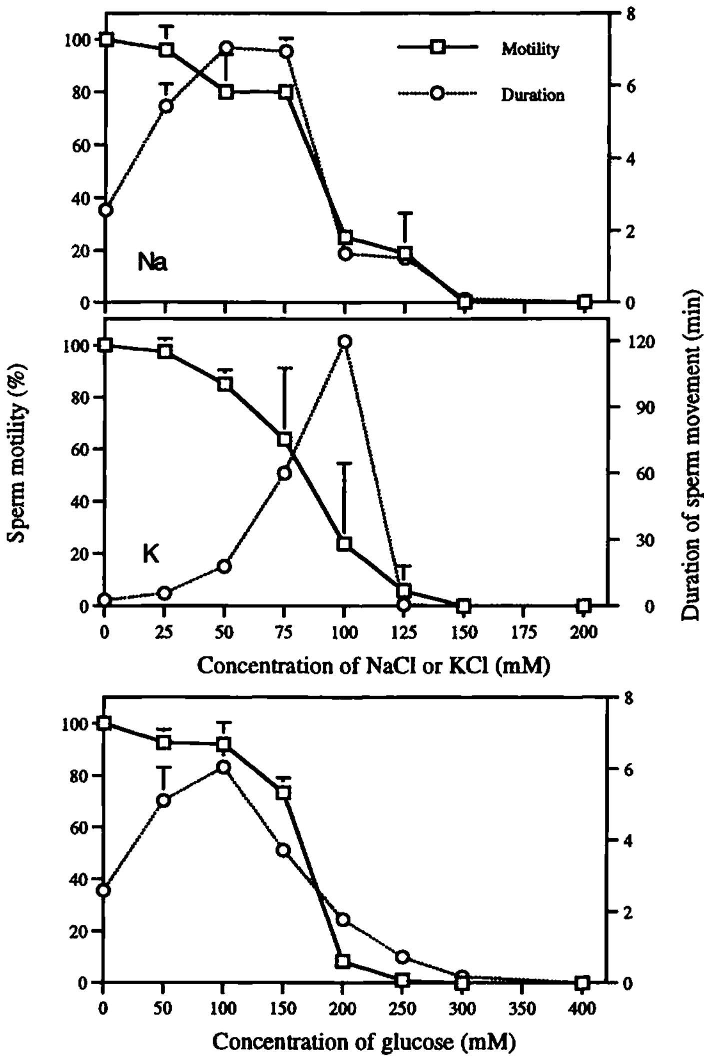

FIGURE 1.—Extended

collection. During this period, there was no difference in sperm motility after semen was activated in 30 mM tris–HCl buffer at pH 8.0 (12°C).

In 1993, semen pooled from 3 males was used with three replicate measurements for each solution. Except as otherwise described, 1 μL of semen was mixed with 0.2 mL of the activation solutions (200× dilution) on a slide and then covered with a cover slip. The activation solutions contained various concentrations of NaCl, KCl, and glucose in 30 mM tris–HCl at pH 8.0 to avoid effects from pH fluctuation. Sperm motility was evaluated by duration of sperm movement and initial percentage of motile sperm. Sperm motility was quantified as quickly as possible with a light microscope at 200× magnification for initial percentage of motile sperm and rated on an arbitrary scale system of 0–4 (Suquet et al. 1992). This procedure required 5 to 7 s from mixing a sperm sample with media to observation of sperm movement. The duration of sperm movement was the time interval from initial mixing to the time that all spermatozoa ceased their forward movement. Because of the long duration of sperm movement in the KCl so-

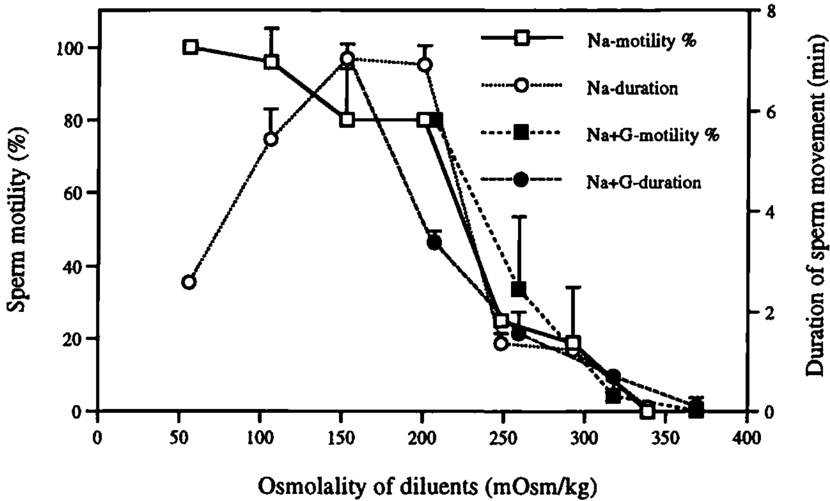

FIGURE 2.—Effects of the combination of sodium and glucose on sperm motility. For activation, 1 μL of semen was diluted 200× with a solution containing equivalent amounts of NaCl or NaCl plus glucose in 30 mM tris–HCl at pH 8.0. The tris–HCl (30 mM) buffer at pH 8.0 had an osmolality of 56 milliosmols/kg. Data points are means; vertical lines represent +SD (*N* = 4).

lutions (75 and 100 mM KCl), semen was activated by 200× dilution in a plastic tube. Then, 0.2 mL sperm suspension was transferred to a slide for estimating the percentage of motile sperm at regular intervals after dilution. All measurements were done in a temperature controlled chamber at 12°C.

In 1994, semen from three to five individual males was used for measurements in each activation solution. Sperm motility was estimated with the same procedures as described above. The percentage of motile sperm was estimated to the nearest 10%. We further investigated the activation solutions containing different concentrations of KCl, NaCl, $CaCl_2$, and glucose, alone or in combinations, all in 30 mM tris–HCl at pH 8.0. Effects of potassium and calcium concentrations on sperm motility was also estimated at a fixed osmolality. Potassium (10 to 50 mM) and calcium (1–30 mM) solutions were adjusted to the equivalent of a 100 mM glucose solution (approximately 160 milliosmols [mosmol]/kg), with glucose solutions at appropriate concentrations. Percentage of motile sperm was estimated at regular intervals after activation. The osmolality of activation solutions was measured with a micro osmometer (μ OSMETTE, Precision Systems, Inc., Natick, Massachusetts) as described previously (Lin et al. 1996).

Parameters of sperm motility were expressed as arithmetical means and standard deviations. One-way analysis of variance (ANOVA) and student *t*-tests were used for comparisons among groups. The time in which 50% of the sperm ceased their forward movement was calculated following the method described by Finney (1971).

Results

Sperm were not activated when the activation solutions had a concentration higher than 125 mM of NaCl and KCl, or 250 mM glucose in 1993 (Figure 1). The pattern of sperm movement was similar in the two consecutive years, with some exceptions. Muskellunge sperm in 1993 seemed to require lower osmolality to arrest sperm movement than did sperm sampled in 1994. We focused on the results of the 1994 experiments because sperm from individual males was used and in vitro interactions among semen samples was avoided. Sperm were not activated when the activation solutions had a concentration of 200 mM of NaCl and KCl, or 400 mM of glucose (Figure 1). Only a small portion of sperm was activated at 150 mM of NaCl and KCl, or 300 mM of glucose. Sperm were fully (>80%) activated when the concentration was lower than 50 mM of NaCl and KCl, or 100 mM glucose. The duration of sperm movement increased to 7 min and 6 min at 50 mM NaCl and 100 mM of glucose, respectively, then gradually decreased as the concentration of solutes decreased. The similarity of both parameters of sperm motility in the activation solutions containing sodium or the equivalent combination of sodium and glucose (30 mM tris–HCl buffer had an

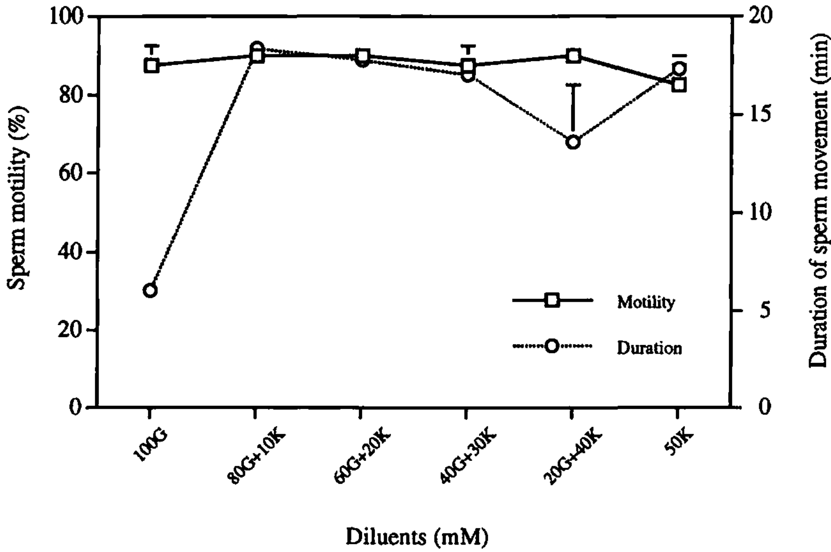

FIGURE 3.—Effects of potassium concentration on muskellunge sperm motility at a fixed osmolality. Osmolality of diluents was adjusted by glucose to the equivalent of 100 mM glucose in 30 mM tris–HCl at pH 8.0 (approximately 160 milliosmols/kg). Data points are means; vertical lines represent +SD ($N = 3$).

osmolality of 56 mosmol/kg) indicated that the effects of sodium on sperm motility were primarily osmotic (Figure 2).

The observed initial percentage of motile sperm in potassium solutions was similar to those in sodium and glucose solutions. However, sperm had a prolonged duration of movement in KCl solutions, up to 120 min at 100 mM KCl (Figure 1). The effect of potassium was also studied at a fixed osmolality equivalent to a 100 mM glucose solution (160 mosmol/kg). Potassium significantly ($P < 0.001$) increased the duration of sperm movement regardless of its concentration from 10 to 50 mM (18 min) compared with 100 mM glucose alone (6 min; Figure 3).

Calcium concentration as low as 3 mM significantly ($P < 0.001$) inhibited sperm motility (Figure 4). Initial percentage of motile sperm de-

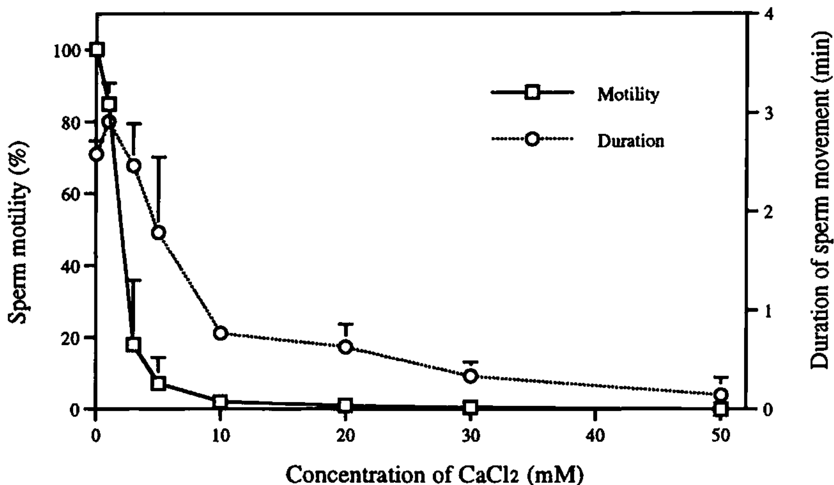

FIGURE 4.—Effects of calcium concentration on sperm motility. Semen was diluted (200×) in activation solutions containing various calcium concentrations in 30 mM tris–HCl at pH 8.0. Data points are means; vertical lines represent +SD ($N = 3$).

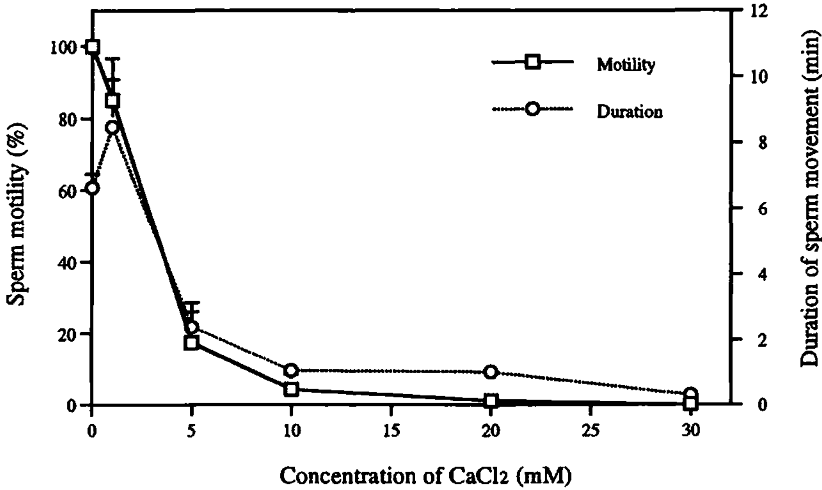

FIGURE 5.—Effects of calcium concentration on muskellunge sperm motility at a fixed osmolality. Osmolality of diluents was adjusted by glucose to the equivalent of 100 mM glucose in 30 mM tris–HCl at pH 8.0 (160 milliosmols/kg). Data points are means; vertical lines represent +SD (N = 3).

creased from 80% to 20% when calcium concentration was increased from 1 to 3 mM. At 10 mM, calcium almost completely inhibited the sperm motility (2% motile sperm). However, a few spermatozoa were still activated at 50 mM calcium. The duration of sperm movement also decreased with increasing calcium concentration. Similar inhibitory patterns were found in the effects of calcium concentrations at fixed osmolality (160 mosmol/kg) adjusted by glucose (Figure 5). Calcium caused clumping of the sperm and decreased the swimming speed.

Percentage of motile sperm decreased over time (Figure 6). Most of the sperm ceased their forward movement within 2 min, but a few spermatozoa swam for a few more minutes in tris buffer (30 mM) and sodium (50 mM) media. The decline in percentage of motile sperm was less sharply pronounced in the potassium solution (50 mM); at 2 min after activation, 20% motile sperm were ob-

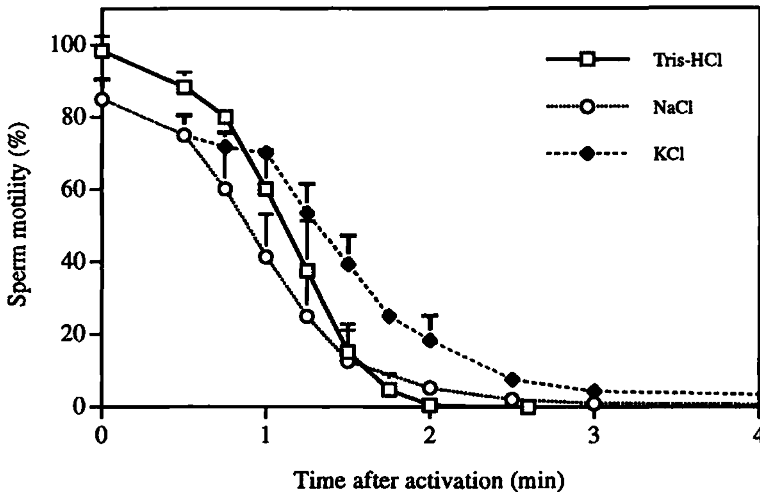

FIGURE 6.—Change in percentage of motile sperm over time after activation. Semen was diluted (200×) in activation solutions of tris–HCl, NaCl, or KCl (50 mM). Percentage of motile sperm was estimated at regular intervals after activation. Tris = 30 mM tris–HCl at pH 8.0, NaCl = 50 mM NaCl in 30 mM tris–HCl at pH 8.0, KCl = 50 mM KCl in 30 mM tris–HCl at pH 8.0. Data points are means; vertical lines represent +SD (N = 4).

served. The average time that 50% sperm ceased their forward movement in sodium, tris, and potassium media was 50, 64, and 74 s, respectively. However, there was no significant difference among these three groups.

Discussion

Spermatozoa of muskellunge were immotile in a solution containing either an electrolyte (NaCl or KCl) or nonelectrolyte (glucose) at a high concentration. A small number of sperm became motile when the diluents had the osmolality of 340 mosmol/kg (150 mM NaCl and KCl, or 300 mM glucose), higher than that of the seminal plasma (290 mosmol/kg, Lin et al. 1996). Sperm motility initiated in isotonic to hypertonic media in northern pike, channel catfish *Ictalurus punctatus*, and salmonids has been reported (reviewed by Stoss 1983). Strussmann et al. (1994) reported that sperm of the freshwater fish pejerrey *Odontesthes bonariensis* became motile when diluted with nonelectrolyte solutions up to 388 mosmol/kg and electrolyte solutions up to 551 mosmol/kg, while the osmolality of seminal plasma was 331 mosmol/kg. The results from 1993 indicated that activation solutions containing 125 mM NaCl and KCl, or 300 mM glucose inhibited sperm motility (Figure 1). Unfortunately, at that time we did not have the equipment to measure the osmolality of the diluents in order to make direct comparisons. The similar patterns of both parameters of sperm motility was found in sodium and glucose solutions, alone or in combinations, in muskellunge, as was the case in freshwater cyprinids (Morisawa et al. 1983b). This similarity suggests that the motility of muskellunge sperm is regulated by the changes of the osmolality in the environment. The duration of sperm movement in muskellunge was up to 6–7 min in a solution of glucose (100 mM) or NaCl (50 mM). During the 1993 experiments, longer duration of sperm movement was observed, perhaps because of variation between seasons and duration of sperm movement of pooled sperm represented the individual with longest motility. The variability between individual males is the major reason we chose to use individual males during the 1994 season. Results for the duration of sperm movement in muskellunge are comparable to those observed by Billard (1978) in northern pike (3–5 min in 0.7% physiological solution) and by Morisawa et al. (1983b) in cyprinids (6 min in 100 mosmol/kg of NaCl). However, Duplinsky (1982) reported a movement duration of 67 s for northern pike sperm in tap water buffered to pH 7.9. However, different observation methods and conditions of sperm activation, including temperature and diluent, limit direct comparison of results.

Morisawa et al. (1983b) reported that potassium maintained motility and increased the swimming speed of cyprinid fish spermatozoa. We found that muskellunge sperm also have a prolonged duration of movement in potassium solutions. The duration of sperm movement increased from 6 min in 100 mM glucose solution to about 18 min in 10 to 50 mM KCl, adjusted by glucose to the equivalent of 100 mM glucose in osmolality (Figure 3). Some motile sperm appeared up to 2 h after activation in 100 mM KCl. Such a long duration of movement is rare in freshwater fish. This extended motility might be caused by reactivation of sperm (Christen et al. 1987) or gradual activation of "subpopulations" of sperm cells during observations. We noticed that sperm could be reactivated with 30 mM tris–HCl buffer 10 min after being activated with a 25 mM KCl solution (our unpublished data). Thus, muskellunge sperm potentially provides a valuable model for the investigation of sperm reactivation and prolonged movement generated by potassium influx. Sperm of rainbow trout *Ohcorhynchus mykiss* is activated by a decrease in external potassium concentration below 3 mM (Morisawa and Suzuki 1980). This contrast represents a major difference between cyprinid sperm and muskellunge sperm.

External calcium has been shown to antagonize the potassium inhibition of motility in rainbow trout (Baynes et al. 1981; Tanimoto and Morisawa 1988). Calcium at 10 mM maintained the swimming speed and increased the duration of movement in rainbow trout sperm (Perchec et al. 1993). Conflicting interpretations have resulted from intensive investigation of the role of calcium in rainbow trout sperm activation. By using Ca^{2+}-channel blockers, a flux of external Ca^{2+} into the cell rather than a mobilization of internal Ca^{2+} stores was demonstrated to be required to activate sperm motility in rainbow trout (Tanimoto and Morisawa 1988; Cosson et al. 1989). The finding that rainbow trout sperm can be activated in the absence of extracellular Ca^{2+} suggests that no influx of extracellular Ca^{2+} is required for activation (Boitano and Omoto 1992). Okuno and Morisawa (1989) have also shown an inhibitory effect of relatively low calcium concentration on reactivation of demembranated rainbow trout sperm. We found that 3 mM calcium had an inhibitory effect on sperm motility in muskellunge. Semen diluted in calcium solutions did not disperse well and

sperm tended to form clumps. A few sperm activated in a solution of high calcium concentration showed slow forward movement that ceased within 30 s. There is no report, to our knowledge, on the mechanism of calcium fluxes in sperm where motility is regulated by osmolality.

Acknowledgments

We thank John D. Harder of the Department of Zoology and Jennifer Tomsen of the School of Natural Resources, The Ohio State University, for their critical reading of this manuscript. Thanks are due to Andrzej Ciereszko for his valuable discussions. We appreciate helpful cooperation in sampling gametes and providing fish information from Richard Day, Frank Kapler, and Bruce Bartens of the Ohio Department of Natural Resources. This work was funded by Piketon Research and Extension Center seed grant program and the Federal Aid in Sport Fish Restoration (project F-69-P, Fish Management in Ohio), adminstered jointly by the United States Fish and Wildlife Service and the Ohio Division of Wildlife. Salaries were partly provided by the state and federal funds appropriated to the Ohio Agriculture Research and Development Center (OARDC). This is OARDC manuscript 48/95.

References

Baynes, S. M., A. P. Scott, and A. P. Dawson. 1981. Rainbow trout, *Salmo gairdnierii* Richardson, spermatozoa: effects of cations and pH on motility. Journal of Fish Biology 19:259–267.

Billard, R. 1978. Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in media of various salinities. Aquaculture 14:187–198.

Boitano, S., and C. K. Omoto. 1992. Trout sperm swimming patterns and roles of intracellular Ca^{++}. Cell Motility and the Cytoskeleton 21:74–82.

Christen, R., J.-L. Gatti, and R. Billard. 1987. Trout sperm motility: the transient movement of trout sperm is related to changes in the concentration of ATP following the activation of the flagellar movement. European Journal of Biochemistry 166:667–671.

Cosson, M. P., R. Billard, and L. Letellier. 1989. Rise of internal Ca^{2+} accompanies the initiation of trout sperm motility. Cell Motility and the Cytoskeleton 14:424–434.

Duplinsky, P. D. 1982. Sperm motility of northern pike and chain pickerel at various pH values. Transactions of the American Fisheries Society 111:768–771.

Finney, D. J. 1971. Probit analysis, 3rd edition. Cambridge University Press.

Galat, D. L. 1973. Normal embryonic development of the muskellunge (*Esox masquinongy*). Transactions of the American Fisheries Society 102:384–391.

Hasler, A. D., R. K. Meyer, and H. M. Field. 1940. The use of hormones for the conservation of muskellunge, *Esox masquinongy imaculatus* Garrard. Copeia 940:43–46.

Lin, F., L. Liu, and K. Dabrowski. 1996. Characteristics of muskellunge spermatozoa I: Ultrastructure of spermatozoa and biochemistry composition of semen. Transactions of the American Fisheries Society 125:

Moore, A. A. 1991. Refrigerated storage and cryopreservation of walleye and muskellunge semen. Iowa Department of Natural Resources, Technical Bulletin 4, Des Moines.

Morisawa, M., and K. Suzuki. 1980. Osmolality and potassium ion: their roles in initiation of sperm motility in teleost. Science 210:1145–1147.

Morisawa, M., K. Suzuki, and S. Morisawa. 1983a. Effects of potassium and osmolality on spermatozoa 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.

Okuno, M., and M. Morisawa. 1989. Effects of calcium on motility of rainbow trout sperm flagella. Cell Motility and the Cytoskeleton 14:194–200.

Perchec, P. G., J. Cosson, F. Andre, and R. Billard. 1993. La motilite des spermatozoides de truite (*Oncorhynchus mykiss*) et de carpe (*Cyprinus carpio*). Journal of Applied Ichthyology 9(3–4):129–149.

Redondo-Muller, C., M.-P. Cosson, J. Cosson, and R. Billard. 1991. In vitro maturation of the potential for movement of carp spermatozoa. Molecular Reproduction and Development 29:259–270.

Stoss, J. 1983. Fish gamete preservation and spermatozoa physiology. Pages 305–350 *in* W. S. Hoar, D. J. Randall, and E. M. Donaldson, editors. Fish Physiology: reproduction, volume 9, part B. Academic Press, New York.

Strussmann, C. A., P. Renard, H. Ling, and F. Takashima. 1994. Motility of pejerrey *Odontesthes bonariensis* spermatozoa. Fisheries Science 60(1):9–13.

Suquet, M., H. M. Omnes, Y. Normant, and C. Fauvel. 1992. Assessment of sperm concentration and motility in turbot (*Scophthalmus maximus*). Aquaculture 101:177–185.

Tanimoto, S., and M. Morisawa. 1988. Roles for potassium and calcium channels in the initiation of sperm motility in rainbow trout. Development, Growth and Differentiation 30:117–124.

Received March 20, 1995
Accepted September 7, 1995