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EFFECTS OF OSMOLALITY AND POTASSIUM ON MOTILITY OF SPERMATOZOA FROM FRESHWATER CYPRINID FISHES

BY M. MORISAWA

Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164

K. SUZUKI

School of Fisheries Sciences, Kitasato University, Sanriku, Iwate 022-01

H. SHIMIZU

Electrotechnical Laboratory, 1-1-4, Umezono, Sakura-mura, Niihari-gun, Ibaraki 305

S. MORISAWA

Biological Laboratory, St. Marianna University, School of Medicine, 2095, Sugao, Miyamae, Kawasaki 213

AND K. YASUDA

Department of Biology, Japan Women's University, Mejiro-dai Bunkyo 2-8-1, 112 Japan

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SUMMARY

Spermatozoa of freshwater Cyprinidae (goldfish, carp, crucian carp and dace) remained immotile when the semen was diluted in solutions of NaCl, KCl, mannitol or glucose iso-osmolar to the seminal plasma (300 mosmol kg^{-1}). The spermatozoa became motile in media containing these solutes if the osmolality was lower than that of the seminal plasma, suggesting that motility is suppressed by the osmolality of the seminal plasma in the sperm duct and initiated by a decrease of osmolality upon spawning into fresh water. Potassium was a major component of seminal plasma, having a concentration 20–30 times higher than that in the blood plasma in goldfish and carp. Sodium concentration in seminal plasma was lower than that in blood plasma. Potassium increased viability and speed of sperm movement at a concentration below that in the seminal plasma, whereas sodium and the nonelectrolytes were less effective. Potassium released with spermatozoa at spawning may therefore stimulate motility which has already been initiated by the decrease of osmolality.

INTRODUCTION

Since Gray (1928) first reported that the spermatozoa of sea urchin, which are quiescent in the testis, begin to move at spawning because of the increase of free space

Key words: Freshwater fishes, sperm motility, osmolality.

for sperm movement in sea water (dilution effect), many factors have been proposed to initiate sperm motility for marine invertebrates and mammals. Increase of environmental O_2 tension (Nevo, 1965; Rothschild, 1948), decrease of CO_2 tension (Mohri & Yasumasu, 1963; Branham, 1966), exposure to pH of sea water (Rothschild, 1956; Mohri & Horiuchi, 1961), exposure to zinc or copper included in sea water at spawning (Rothschild & Tuft, 1950; Mohri, 1956*a*,*b*) or release from factors inhibitory to sperm motility in the seminal plasma (Hayashi, 1946; Shirai, Ikegami, Kanatani & Mohri, 1982) have been suggested as the responsible factors. These factors have complicated effects on sperm motility and respiration, but it is not clear that any of them are essential to initiate sperm motility.

We have shown previously that spermatozoa of goldfish which are immotile in solutions isotonic to the seminal plasma become motile when they are suspended in hypotonic solutions (Morisawa & Suzuki, 1980).

In the present paper we study the effect of osmolality and of potassium, a major constituent of the seminal plasma, on sperm motility of some freshwater cyprinid fishes.

MATERIALS AND METHODS

Male goldfish, *Carassius auratus*, and carp, *Cyprinus carpio*, weighing about 100 and 250 g, respectively, were obtained from a commercial source or from the Freshwater Laboratory at Hino, Tokyo. The crucian carp, *Carassius carassius*, and dace, *Tribolodon hakonensis* and *Tribolodon taczanowskii*, were captured in the river in Iwate prefecture. The fish were maintained in aquaria with circulating and aerated water at 20 °C for more than a week before use. The semen of goldfish was carefully collected by gently pressing the abdomen of the fishes, 24 or 48 h after intraperitoneal injection of human chorionic gonadtropin (Gonatropin, Teikoku-zoki) at a dose of 50 i.u./10 g body weight. The semen of carp, crucian carp and dace was collected by abdominal pressure without prior hormone treatment. Care was taken to prevent contamination of the semen with water and urine. Semen was kept at room temperature. In the semen, the spermatozoa were immotile. Semen was then taken into a Drummond micropipette and diluted in a buffered medium on a flat glass slide. The slide, without cover, was placed on the stage of a microscope. The duration of sperm motility (the time until all spermatozoa ceased their forward movement) was measured under a light microscope at room temperature. The experiment was repeated with three to seven different samples.

The medium was buffered at pH 7·7 since this pH was found to be optimal for duration of motility of the sperm of goldfish and carp (data not shown). Composition was NaCl, KCl, mannitol or glucose and 10 mM Hepes-NaOH.

The swimming speed of spermatozoa was measured by using laser light-scattering spectroscopy developed by the Electrotechnical Laboratory (Shimizu & Matsumoto, 1977). Specimens for electron microscopy were fixed with osmium tetroxide, embedded in epoxy resin and observed with a JEOL-100B electron microscope.

The methods for collecting blood and seminal plasma from freshwater fishes and for measuring sodium, potassium, calcium, magnesium and chloride concentrations and osmolality have been described previously (Morisawa, Hirano & Suzuki, 1979).

All chemicals were reagent grade, and water was deionized and glass-distilled.

Table 1. *Inorganic electrolytes and osmolality of blood plasma and seminal plasma of the goldfish* (Carassius auratus) *and the carp* (Cyprinus carpio)

	N	Na^+ (mM)	K^+ (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	Cl^- (mM)	Osmolality (mosmol kg^{-1})
Goldfish							
blood plasma	7	123 ± 2·1	2·4 ± 0·15	3·1 ± 0·06	1·0 ± 0·15	110 ± 3·4	266 ± 6·3
seminal plasma	4	96 ± 4·5	70·2 ± 1·72	2·1 ± 0·10	1·1 ± 0·08	129 ± 5·6	317 ± 10·6
Carp							
blood plasma	5	135 ± 0·7	4·6 ± 0·11	2·9 ± 0·07	1·0 ± 0·02	118 ± 0·8	302 ± 4·5
seminal plasma	5	75 ± 3·2	82·4 ± 3·33	2·0 ± 0·18	0·8 ± 0·04	112 ± 2·6	302 ± 5·4

Values are means ± S.E.

RESULTS

Ion concentrations and osmolality of blood plasma and seminal plasma

As shown in Table 1, the sodium concentration of seminal plasma was lower than that of blood plasma in the goldfish (78%) and carp (56%). In contrast, the potassium concentration of seminal plasma was much higher than that of blood plasma in the goldfish (29 times) and carp (18 times). Calcium, magnesium and chloride concentrations of the seminal plasma were almost the same as that of the blood plasma. The osmolality of seminal plasma was around 300 mosmol kg^{-1} and was slightly higher than that of blood plasma.

Effects of dilution on sperm motility

Spermatozoa of goldfish were immotile in the seminal plasma. They remained motionless when semen was diluted with isotonic NaCl, KCl or mannitol solutions (300 mosmol kg^{-1}) up to and including a dilution ratio of 1:800. When semen was diluted with hypotonic NaCl or KCl (150 mosmol kg^{-1}), sperm became motile at a dilution ratio of 1:25. Motility lasted longer in the KCl than in the NaCl solution. The duration was increased at greater dilutions and reached a maximum with a dilution of 1:100. The maximum duration was maintained at further dilution up to 800 times (Fig. 1).

Effects of osmolality and ion concentration on sperm motility

As shown in Fig. 2, when semen was diluted (1:100) with NaCl or mannitol, goldfish sperm were motile for longer periods as the osmolality was increased, up to a maximum of around 6 min at 100 mosmol kg^{-1}. The result is similar to that obtained previously (Morisawa & Suzuki, 1980). When the semen was diluted in a medium containing 100 mosmol kg^{-1} of one solute plus 50, 100, or 150 mosmol kg^{-1} of the other, the duration of sperm motility was decreased with increase of osmolality, and reached zero at 250 mosmol kg^{-1}. The similarity of the duration patterns as a function of the osmolality in NaCl, in mannitol (see Morisawa & Suzuki, 1980), and in a mixture of NaCl and mannitol solutions (Fig. 2) suggests that sodium has a purely osmotic effect on sperm motility. In contrast, when the semen was diluted in a medium containing

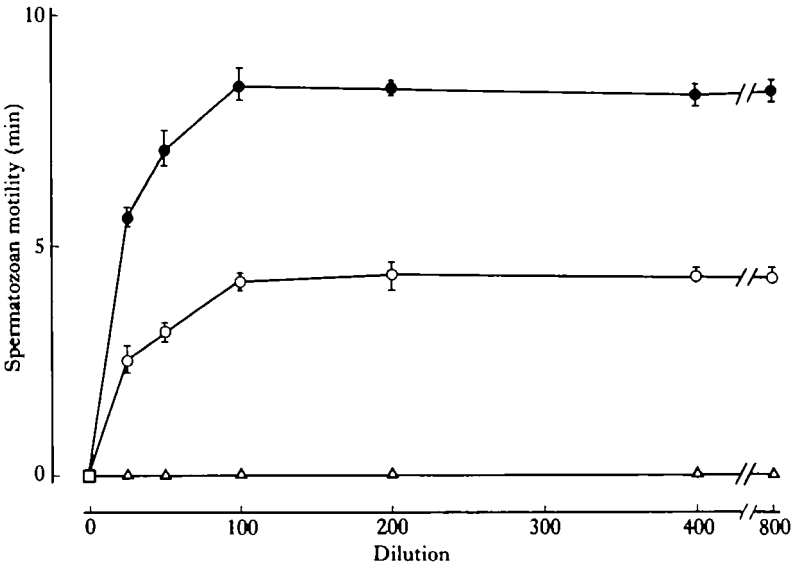

Fig. 1. Effect of dilution on the duration of sperm motility of goldfish. 1 μl semen was added to an appropriate volume of media containing: △, 150 mmol kg^{-1} NaCl, KCl or 300 mmol kg^{-1} mannitol (300 mosmol kg^{-1}); ○, 75 mmol kg^{-1} NaCl (150 mosmol kg^{-1}); ●, 75 mmol kg^{-1} KCl (150 mosmol kg^{-1}). The time taken for all spermatozoa to cease movement was measured. Vertical bars represent the means ±S.E. of three experiments.

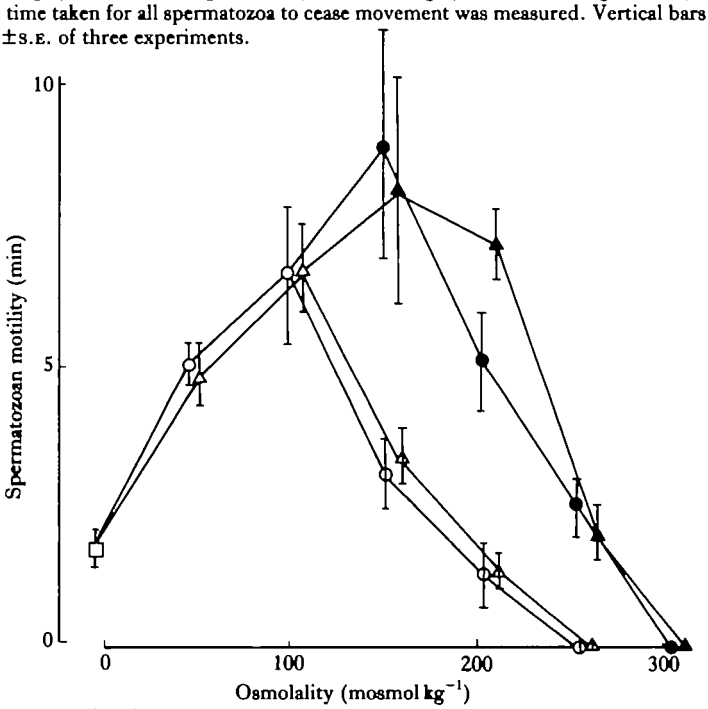

Fig. 2. Effects of Na^+, K^+ and mannitol on the duration of sperm motility of goldfish. 1 μl semen was added to 0·1 ml of the test solutions. ○, NaCl (below 50 mmol kg^{-1}); ⌀, 50 mmol kg^{-1} NaCl + appropriate concentration of mannitol; △, mannitol (below 100 mmol kg^{-1}); ⍋, 100 mmol kg^{-1} mannitol + appropriate concentration of NaCl; ●, 50 mmol kg^{-1} NaCl + appropriate concentration of KCl; ▲, 100 mmol kg^{-1} mannitol + appropriate concentration of KCl. Note that motility is maintained for longer at a wide range of osmolality (100–200 mosmol kg^{-1}) in the presence of K^+. Vertical bars represent the means ±S.E. of three experiments.

100 mosmol kg^{-1} of either solute plus 50, 100, 150 or 200 mosmol kg^{-1} of KCl, sperm motility lasted longest in the range of 100–200 mosmol kg^{-1} (Fig. 2).

Sperm from carp, crucian carp and dace were immotile in 300 mosmol kg^{-1} NaCl, KCl, mannitol or glucose media (Fig. 3), isotonic with the seminal plasma of these

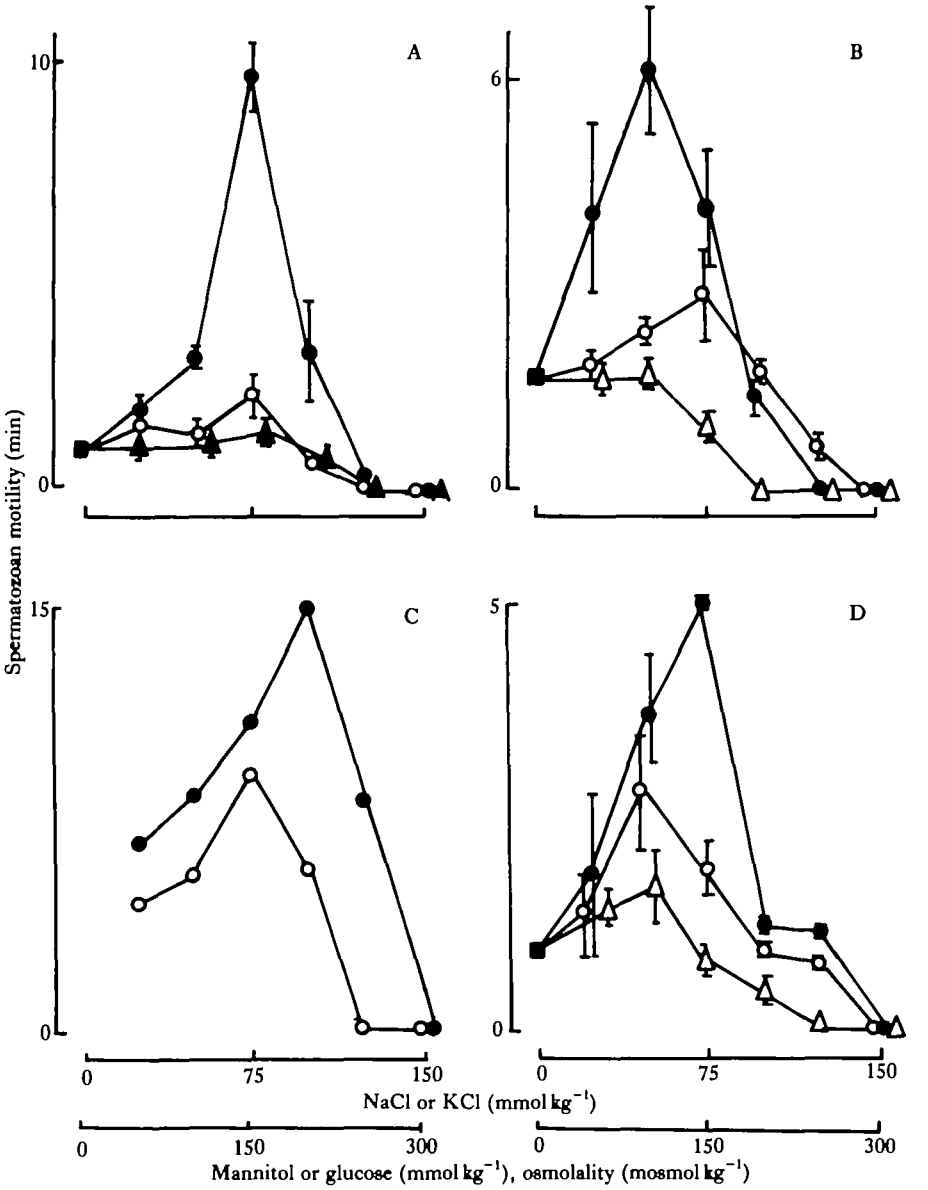

Fig. 3. Effects of Na^+, K^+, mannitol and glucose on the duration of sperm motility of the carp *Cyprinus carpio* (A, $N = 5$); the crucian carp *Carassius carassius* (B, $N = 5$); and two kind of dace *Tribolodon hakonensis* (C, $N = 1$) and *Tribolodon taczanowskii* (D, $N = 4$). 1 µl semen was added to the media containing 0·1 ml NaCl (○), KCl (●), mannitol (▲) or glucose (△). Vertical bars represent the means ±S.E.

species (Table 1). Sperm motility began when osmolality was decreased, and had maximum duration at 100–200 mosmol kg^{-1} (Fig. 3). At 100–200 mosmol kg^{-1}, life-span was longer in the KCl solution than in the other solutions.

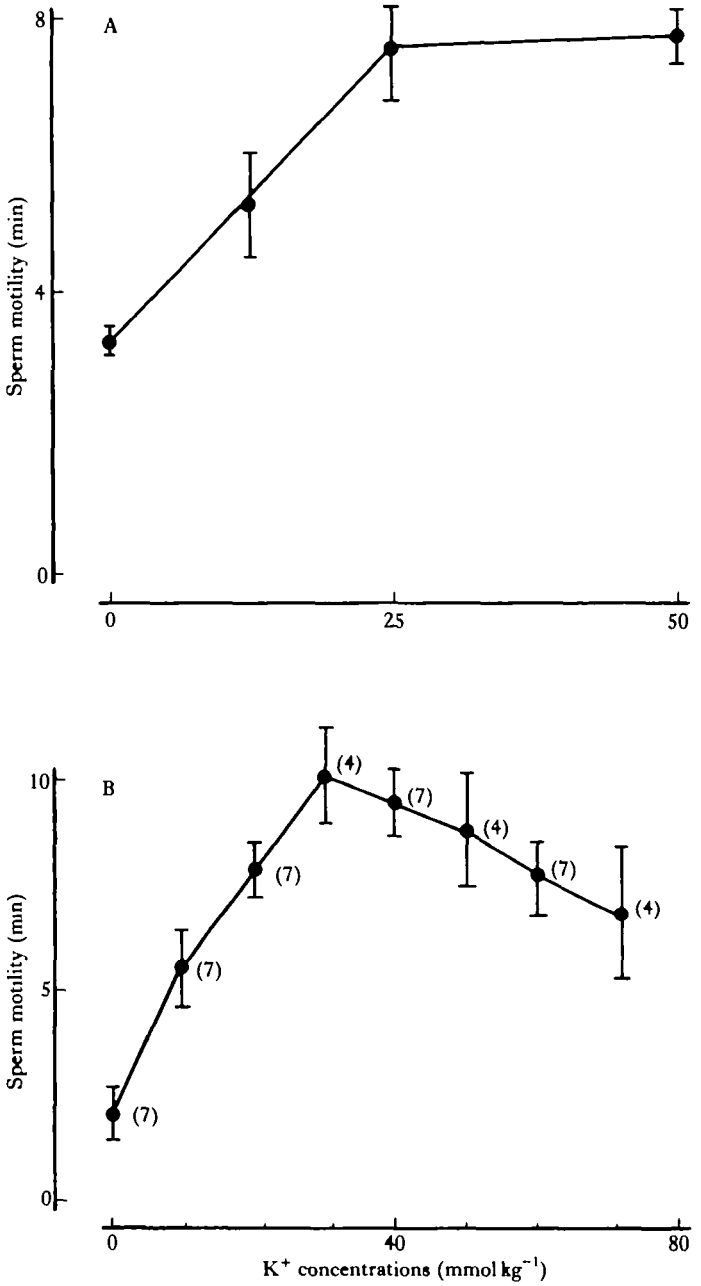

Fig. 4. Effect of K^+ on the duration of sperm motility of goldfish (A) and carp (B). 1 μl semen was added to 0·1 ml media containing various concentrations of KCl. Osmolality was fixed at 150 mosmol kg^{-1} by mannitol. Vertical bars represent the means ±S.E. of three experiments in (A); numbers of experiments is shown in parentheses in (B).

Fig. 6. Electron micrographs of carp spermatozoa at various osmolalities. Semen was suspended for 3 min in the solution constituting the same ion concentrations as the seminal plasma (A), 1/2 (B), 1/5 (C) of the medium, or 10 mM Hepes buffer at pH 7·7 (D). Scale bar = 1 μm.

The concentration of potassium required to maintain maximum duration of sperm motility of goldfish and carp was determined in solutions in which osmolality was fixed at 150 mosmol kg^{-1} by adding the appropriate concentration of mannitol (Fig. 4). Maximum duration of sperm motility was obtained at a concentration of 25–50 mmol kg^{-1} KCl in goldfish (Fig. 4A) and 30–70 mmol kg^{-1} KCl in carp (Fig. 4B). In the case of carp, duration was slightly decreased at higher KCl concentrations. For both fishes, these potassium concentrations at which maximum duration was obtained correspond to about one-third of the concentration in the seminal plasma.

Speed of movement of goldfish spermatozoa reached about 100 $\mu m\ s^{-1}$, 30 s after dilution in 100 mmol kg^{-1} KCl (Fig. 5A). A higher rate has been found for pig (180 $\mu m\ s^{-1}$) and a lower rate for abalon sperm (60 $\mu m\ s^{-1}$) (Shimizu & Matsumoto, 1977). The speed slowed down to 30 $\mu m\ s^{-1}$, 60 s after dilution, and maintained this level until 120 s. The speed was higher in KCl solutions than NaCl solutions at the concentration of 100 mmol kg^{-1} ($P < 0{\cdot}005$) but not significantly different at 50 and 75 mmol kg^{-1} (Fig. 5B).

Morphological changes in carp spermatozoa in media of various osmolalities

The decrease in duration of motility observed at osmolalities less than 150 mosmol kg^{-1} was correlated with sperm structure, as observed in the electron microscope (Fig. 6). When carp semen was diluted and sperm were allowed to swim for 3 min in a medium containing the same concentrations of ions as in the seminal plasma (75 mmol kg^{-1} NaCl, 80 mmol kg^{-1} KCl, 2 mmol kg^{-1} $CaCl_2$, 1 mmol kg^{-1} $MgCl_2$, 10 mM Hepes-NaOH buffer at pH 7·7), the structure of the head, midpiece and tail

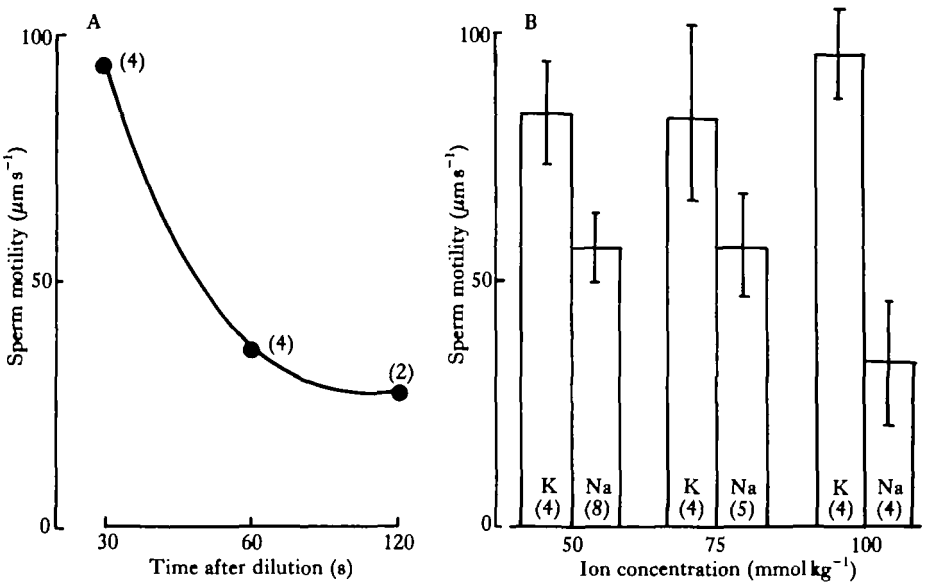

Fig. 5. Swimming speed of goldfish spermatozoa in NaCl and KCl solutions. 20 μl semen was added to 1 ml of the solution. (A) Time course in 100 mmol kg^{-1} KCl. Data represent the average of two or four experiments. (B) Speed in various concentrations of NaCl or KCl. Vertical bars represent the means ±S.E., number of experiments is indicated in parentheses.

remained almost intact (Fig. 6A; also see Morisawa, 1979). If this medium was diluted by one-half, a dilution at which the sperm motility is almost at a maximum (Figs 2, 3), each sperm part appeared to retain its original shape even after swimming (Fig. 6B). However, as ion concentrations and osmolality were lowered to one-fifth of the initial medium, the head and midpiece appeared swollen and the plasma membrane ruptured (Fig. 6C). In the buffer solution, spermatozoa became completely amorphous (Fig. 6D). These observations indicate that the decrease in duration of sperm motility at osmolalities below 100 or 150 mosmol kg^{-1} (see Figs 2, 3) is probably the result of disruption of sperm structure by osmotic shock.

DISCUSSION

Spermatozoa of goldfish, carp, crucian carp and dace were immotile when the semen was mixed with either an electrolyte (NaCl or KCl) or nonelectrolyte (mannitol or glucose) solution isotonic to the seminal plasma (300 mosmol kg^{-1}). The sperm became motile only when the semen was diluted in hypotonic solutions. These observations confirm earlier observations in goldfish (Morisawa & Suzuki, 1980). It was further found that goldfish spermatozoa remain immotile when the semen was diluted with isotonic media at ratios up to 1:800. It therefore seems unlikely that dilution is the factor that initiates sperm motility at spawning. It has previously been shown that changes in gas tension or pH at spawning cannot initiate sperm motility (Morisawa & Suzuki, 1980). This suggests that sperm of freshwater Cyprinidae become motile at spawning because of a reduction in the osmolality of their environment. Progressive reduction of the osmolality of the environment of spermatozoa, spawned into fresh water, may cause sperm disruption (Fig. 6). The male of freshwater cyprinid fish approaches near the female before spawning and releases spermatozoa immediately after oviposition (Stacy & Liley, 1974), and thus spermatozoa probably can reach eggs within a short period before sperm disruption.

It was also found that potassium maintained motility of sperm from goldfish, carp, crucian carp and dace (Figs 2, 3, 4), and increased the speed of swimming of goldfish sperm (Fig. 5). The high concentration of potassium in the seminal plasma (Table 1, see also Grant, Pang & Griffith, 1969; Clemens & Grant, 1965) may thus help maintain sperm motility as the semen is diluted.

These cyprinid fishes thus provide a valuable model for the study of the initiation of sperm motility.

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REFERENCES

BRANHAM, J. M. (1966). Motility and ageing of *Arbacia* sperm. *Biol. Bull. mar. biol. Lab., Woods Hole* **131**, 251–260.

CLEMENS, H. P. & GRANT, F. B. (1965). The seminal thinning response of carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) after injections of pituitary extracts. *Copeia* **2**, 174–177.

GRANT, F. B., PANG, P. K. T. & GRIFFITH, R. W. (1969). The twenty-four-hour seminal hydration response in goldfish (*Carassius auratus*). I. Sodium, potassium, calcium, magnesium, chloride, and osmolality of serum and seminal fluid. *Comp. Biochem. Physiol.* **30**, 273–280.

GRAY, J. (1928). The effect of dilution on the activity of spermatozoa. *J. exp. Biol.* **5**, 337–344.

HAYASHI, T. (1946). Dilution medium and survival of the spermatozoa of *Arbacia puncturata*. II. Effect of the medium on respiration. *Biol. Bull. mar. biol. Lab., Woods Hole* **90**, 177–187.

MOHRI, H. (1956*a*). Studies on the respiration of sea-urchin spermatozoa. I. The effect of 2,4-dinitrophenol and sodium azide. *J. exp. Biol.* **33**, 73–81.

MOHRI, H. (1956*b*). Studies on the respiration of sea-urchin spermatozoa. II. The cytochrome oxidase activity in relation to the dilution effect. *J. exp. Biol.* **33**, 330–337.

MOHRI, H. & HORIUCHI, K. (1961). Studies of the respiration of sea-urchin spermatozoa. III. Respiration quotient. *J. exp. Biol.* **38**, 249–257.

MOHRI, H. & YASUMASU, I. (1963). Studies of the respiration of sea-urchin spermatozoa. V. The effect of pCO_2. *J. exp. Biol.* **40**, 573–586.

MORISAWA, M., HIRANO, T. & SUZUKI, K. (1979). Changes in blood and seminal plasma composition of the mature salmon (*Oncorhynchus keta*) during adaptation to fresh water. *Comp. Biochem. Physiol.* **64**A, 325–329.

MORISAWA, M. & SUZUKI, K. (1980). Osmolality and potassium ion: Their roles in initiation of sperm motility in teleosts. *Science, N.Y.* **210**, 1145–1147.

MORISAWA, S. (1979). The fine structure of the spermatozoa of the carp, *Cyprinus carpio*. *Bull. St. Marianna Univ. School Med.* **8**, 23–28.

NEVO, A. C. (1965). Dependence of sperm motility and respiration on oxygen concentration. *J. Reprod. Fert.* **9**, 103–107.

ROTHSCHILD, LORD. (1948). The physiology of sea-urchin spermatozoa, lack of movement in semen. *J. exp. Biol.* **25**, 344–352.

ROTHSCHILD, LORD. (1956). The respiratory dilution effect in sea urchin spermatozoa. *Vie et Milieu* **7**, 405–412.

ROTHSCHILD, LORD & TUFT, P. H. (1950). The physiology of sea-urchin spermatozoa. The dilution effect in relation to copper and zinc. *J. exp. Biol.* **27**, 59–72.

SHIMIZU, H. & MATSUMOTO, G. (1977). Light scattering study on motile spermatozoa. *IEE Trans. biomed. Engineer.* BME-**24**, 153–157.

SHIRAI, H., IKEGAMI, S., KANATANI, H. & MOHRI, H. (1982). Regulation of sperm motility in starfish. I. Initiation of movement. *Devl. Growth Differ.* **24**, 419–428.

STACY, N. E. & LILEY, N. R. (1974). Regulation of spawning behaviour in the female goldfish. *Nature, Lond.* **247**, 71–72.