J. exp. Biol. **107**, 105–113 (1983)
Printed in Great Britain © *The Company of Biologists Limited 1983*

EFFECTS OF POTASSIUM AND OSMOLALITY ON SPERMATOZOAN MOTILITY OF SALMONID 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

AND S. MORISAWA

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

(*Received 15 November 1982–Accepted 28 March 1983*)

SUMMARY

In salmonid fishes, rainbow trout and masu salmon, and the plecoglossid fish, ayu, seminal plasma had an osmolality around 300 mosmol kg^{-1}, isotonic to the blood plasma, and contained a higher concentration of potassium than the blood plasma. Spermatozoa of salmonid fishes were motile when semen was diluted 1:100 with solutions of sodium chloride or mannitol, over the osmotic range of 0–300 mosmol kg^{-1}. They were immotile in sodium chloride solution containing several mM potassium. This indicates that osmolality is not an essential determinant of sperm motility in the Salmonidae, and that sperm motility in these species is suppressed by the seminal potassium in the sperm duct, and initiated by a decrease in potassium concentration surrounding spawned spermatozoa when they are released into fresh water.

INTRODUCTION

We have previously shown that in freshwater and marine fishes, sperm motility is suppressed by the osmolality of the seminal plasma (300 mosmol kg^{-1}) in the sperm duct, and that motility is induced by the decrease or increase in osmolality of the environment when they are released into water at spawning (Morisawa & Suzuki, 1980; Morisawa *et al.* 1983). In rainbow trout, a high concentration of potassium is contained in the seminal plasma and sperm motility is inhibited by potassium chloride (Schlenk & Kahmann, 1938; Benau & Terner, 1980; van der Horst, Dott & Foster, 1980). It has recently been shown that there is a high level of potassium in the seminal plasma of another salmonid fish, chum salmon (Morisawa, Hirano & Suzuki, 1979) and that potassium regulates sperm motility in this species also (Morisawa & Suzuki, 1980).

In the present study, we have examined the effects of osmolality and of potassium and other alkaline metals on sperm motility in three salmonid species.

Key words: Salmonid fishes, sperm motility, potassium.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*), the land-locked masu salmon (*Oncorhynchus masou*), the char (*Salvelinus leucomaenis*) and the ayu (*Plecoglossus altivelis*) were obtained from a commercial source. Chum salmon (*Oncorhynchus keta*) was captured at Otsuchi river, Iwate prefecture. The semen of rainbow trout, masu salmon and ayu was taken directly from the sperm duct by opening the abdomen. Seminal plasma was then obtained by centrifuging the semen at 10 000 rev. min^{-1} for 10 min. Blood was collected from the caudal vessel with a hypodermic syringe, and centrifuged at 10 000 rev. min^{-1} for 15 min to obtain the plasma. The seminal plasma and blood plasma were immediately frozen at −20 °C. Sodium, potassium, calcium and magnesium concentrations were analysed by atomic absorption spectrophotometer (Hitachi 203), and chloride concentration by Buchler digital chloridemeter. Osmolality of blood and seminal plasma were measured, omitting the freezing step, using a melting point Knauer osmometer.

The semen, which was collected by pressing the abdomen of the fishes, was taken up into a 1 μl Drummond micropipette and diluted in 0·1 ml of a buffered medium (1: 100 dilution), then placed on a glass slide without cover at room temperature. The duration of sperm motility and number of moving spermatozoa were observed under the light microscope. Since the duration of sperm motility was very short, it is reasonable to consider that the temperature of the sperm suspension on the glass slide was constant for each measurement. The medium was as used in the preceding study (Morisawa *et al.* 1983) and was buffered with Hepes-NaOH at pH 7·7.

All chemicals were reagent grade, and the water was deionized and glass distilled. Specimens for electron microscopy were fixed with 1 % glutaraldehyde buffered with Hepes-NaOH at pH 7·7 and 1 % osmium tetroxide buffered with phosphate buffer at pH 7·7, and were embedded in epoxy resin. They were examined using a JEOL-100B electron microscope.

RESULTS

Ion concentrations and osmolality of blood and seminal plasma

As shown in Table 1, sodium concentration was slightly higher in blood plasma than in seminal plasma in rainbow trout, whereas it was about the same in masu salmon and ayu. Potassium concentration was higher in seminal plasma than in blood plasma, by about ten times in rainbow trout and five times in masu salmon and ayu. Calcium and magnesium were at similar concentrations in seminal plasma and blood plasma in all three species. Chloride had a slightly lower concentration in seminal plasma than in blood plasma for the ayu, but was at similar concentration in the other two species. Osmolality of the seminal plasma (270–300 mosmol kg^{-1}) was isotonic to the blood plasma in the three species.

Effects of NaCl, KCl and mannitol on sperm motility of salmonid fishes

As shown in Fig. 1, maximum duration of sperm motility (spermatozoa always exhibited vigorous and progressive movement) in rainbow trout (20 s, Fig. 1A) and

Table 1. *Inorganic electrolytes and osmolality of blood and seminal plasma of rainbow trout* (Salmo gairdneri), *masu salmon* (Oncorhynchus masou) *and ayu* (Plecoglossus altivelis)

		N	Na^+ (mM)	K^+ (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	Cl^- (mM)	Osmolality (mosmol kg^{-1})
Rainbow trout	Blood plasma	6	146 ± 3·1	3·1 ± 0·74	3·3 ± 0·20	0·9 ± 0·03	115 ± 6·6	292 ± 8·8
	Seminal plasma	6	127 ± 5·4	37·3 ± 4·73	2·6 ± 0·19	1·5 ± 0·14	122 ± 4·9	297 ± 14·5
Masu salmon	Blood plasma	6	133 ± 4·5	2·2 ± 0·21	2·4 ± 0·23	0·7 ± 0·07	128 ± 8·2	296 ± 6·4
	Seminal plasma	6	126 ± 3·5	10·8 ± 1·77	2·1 ± 0·24	1·0 ± 0·10	130 ± 6·0	301 ± 7·5
Ayu	Blood plasma	6	124 ± 0·5	2·9 ± 0·45	3·4 ± 0·10	0·7 ± 0·07	123 ± 4·8	283 ± 6·1
	Seminal plasma	6	130 ± 2·5	11·4 ± 1·14	2·3 ± 0·10	0·6 ± 0·04	107 ± 2·2	271 ± 6·5

Values are means ± S.E.

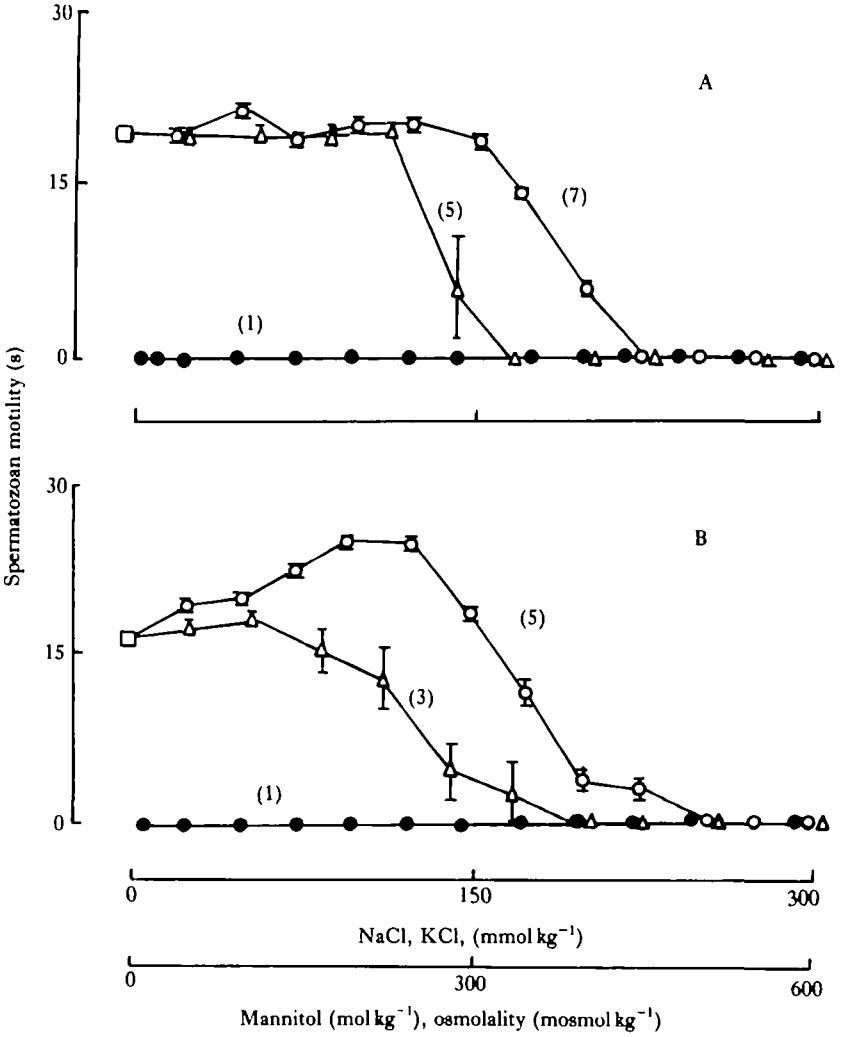


Fig. 1. The effects of sodium, potassium and mannitol on sperm motility in rainbow trout (A) and masu salmon (B). Symbols indicate 10 mM Hepes-NaOH buffer (□), NaCl (○), KCl (●) and mannitol (△). Numbers of experiments are indicated in parentheses, vertical bars represent mean ±S.E.

masu salmon (15–25 s, Fig. 1B) was obtained at a sodium concentration between 0 and 150 mmol kg^{-1} (300 mosmol kg^{-1}). The duration of sperm motility and number of moving spermatozoa decreased with increasing sodium chloride concentrations, and sperm became immotile at 225 mmol kg^{-1} (450 mosmol kg^{-1}) for rainbow trout and at 250 mmol kg^{-1} (500 mosmol kg^{-1}) for masu salmon.

In the nonelectrolyte, mannitol, maximum duration of motility was obtained between 0 and 225 mmol kg^{-1} (225 mosmol kg^{-1}) in rainbow trout, and between 0 and 280 mmol kg^{-1} (280 mosmol kg^{-1}) in masu salmon. The duration and number of moving spermatozoa decreased as mannitol concentration was increased, and reached zero at 340 mmol kg^{-1} (340 mosmol kg^{-1}) in rainbow trout, and 450 mmol kg^{-1} (450 mosmol kg^{-1}) in masu salmon.

In the char (data not shown) maximum duration of sperm motility (around 30 s) was obtained at a sodium chloride concentration between 0 and 150 mmol kg^{-1} (300 mosmol kg^{-1}) and the duration of motility and number of moving spermatozoa decreased with increasing sodium chloride concentration. Sperm became immotile at 275 mmol kg^{-1} (550 mosmol kg^{-1}). In mannitol solutions, spermatozoa were motile at concentrations between 0 and 390 mmol kg^{-1} (390 mosmol kg^{-1}).

Sperm of rainbow trout, masu salmon (Fig. 1) and char (data not shown) were immotile in media containing potassium chloride concentrations between 1 and 300 mmol kg^{-1} (600 mosmol kg^{-1}).

Effects of alkaline metals on sperm motility in rainbow trout

As shown in Fig. 2, the duration of motility of rainbow trout sperm in 100 mmol kg^{-1} sodium chloride was unaffected by the presence of potassium chloride at concentrations between 0·03 and 1·5 mmol kg^{-1}. Sperm motility was completely suppressed at potassium chloride concentrations over 3·0 mmol kg^{-1}. When the medium contained rubidium chloride in addition to 100 mmol kg^{-1} sodium chloride, the duration of motility and number of moving spermatozoa decreased with rubidium concentrations over 0·3 mmol kg^{-1}, and motility ceased at 3·0 mmol kg^{-1}. In contrast, lithium chloride did not inhibit sperm motility in 100 mmol kg^{-1} sodium chloride, at concentrations between 0 and 25 mmol kg^{-1}. Almost all spermatozoa exhibited vigorous and progressive movement. Neither lithium chloride nor sodium chloride alone inhibited sperm motility at concentrations up to 100 mmol kg^{-1} (Fig. 3).

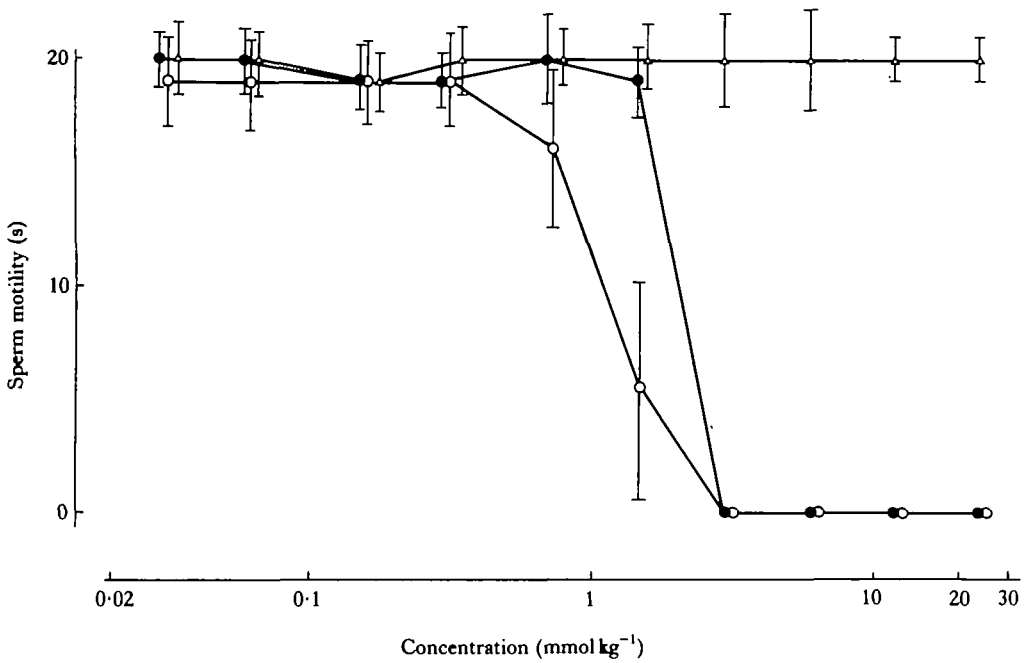


Fig. 2. The effects of potassium, rubidium and lithium on sperm motility in rainbow trout. Symbols indicate KCl (●), RbCl (○) and LiCl (△) in the presence of 100 mmol kg^{-1} NaCl. Vertical bars represent mean ±S.E. in three experiments.

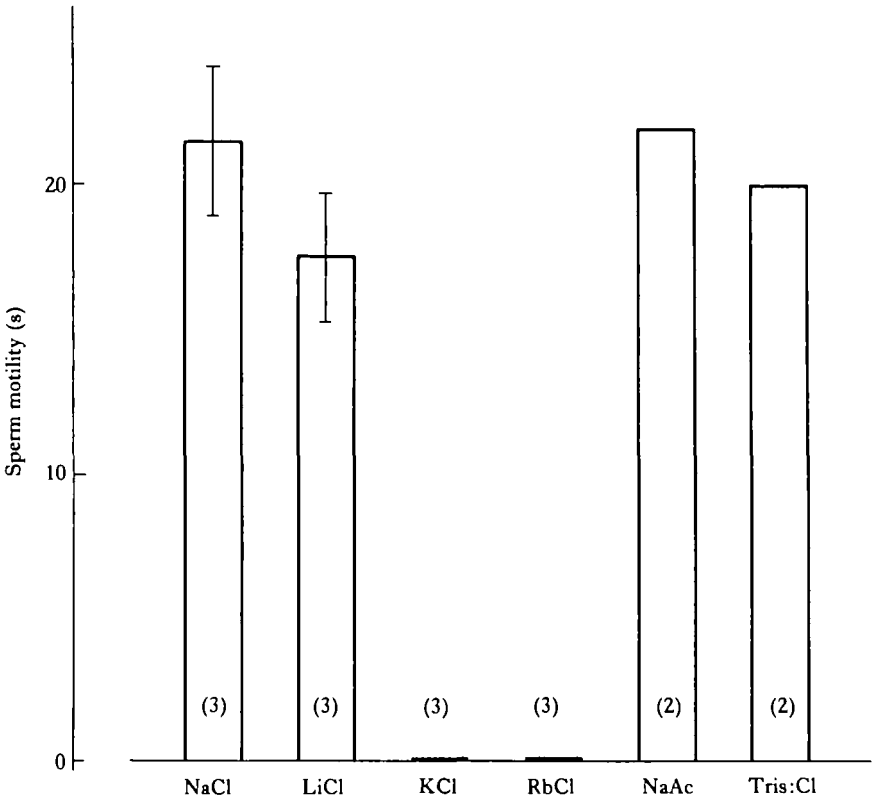


Fig. 3. The effects of alkaline metals, sodium acetate and Tris chloride on sperm motility of rainbow trout. The concentrations of reagents are 100 mmol kg^{-1}. 20 mM Tris-HCl buffer (Tris:Cl) was added in chloride salt solutions and 20 mM Hepes-NaOH buffer was added in sodium acetate solution (NaAc). Numbers of experiments are indicated in parentheses, vertical bars represent means ±S.E.

Potassium and rubidium chloride at concentrations of 100 mmol kg^{-1} completely suppressed sperm motility. Substitution of the sodium chloride by Tris chloride or sodium acetate had little effect on sperm motility.

Effect of sodium upon potassium sensitivity

The concentration of potassium required for inhibition of sperm motility of rainbow trout depended on sodium concentration (Fig. 4). Potassium suppressed sperm motility at concentrations between 6·0 and 6·5 mmol kg^{-1} in the presence of 150 mmol kg^{-1} sodium. At lower sodium concentrations, less potassium was needed to suppress motility. Only 0·25–0·5 mmol kg^{-1} potassium was needed to suppress sperm motility in the absence of sodium chloride.

Effects of calcium and magnesium on sperm motility

Duration of sperm motility in 100 mmol kg^{-1} sodium chloride solution was unaffected by the presence of calcium or magnesium at concentrations between 0 and 16 mmol kg^{-1}, as shown for chum salmon (Fig. 5).

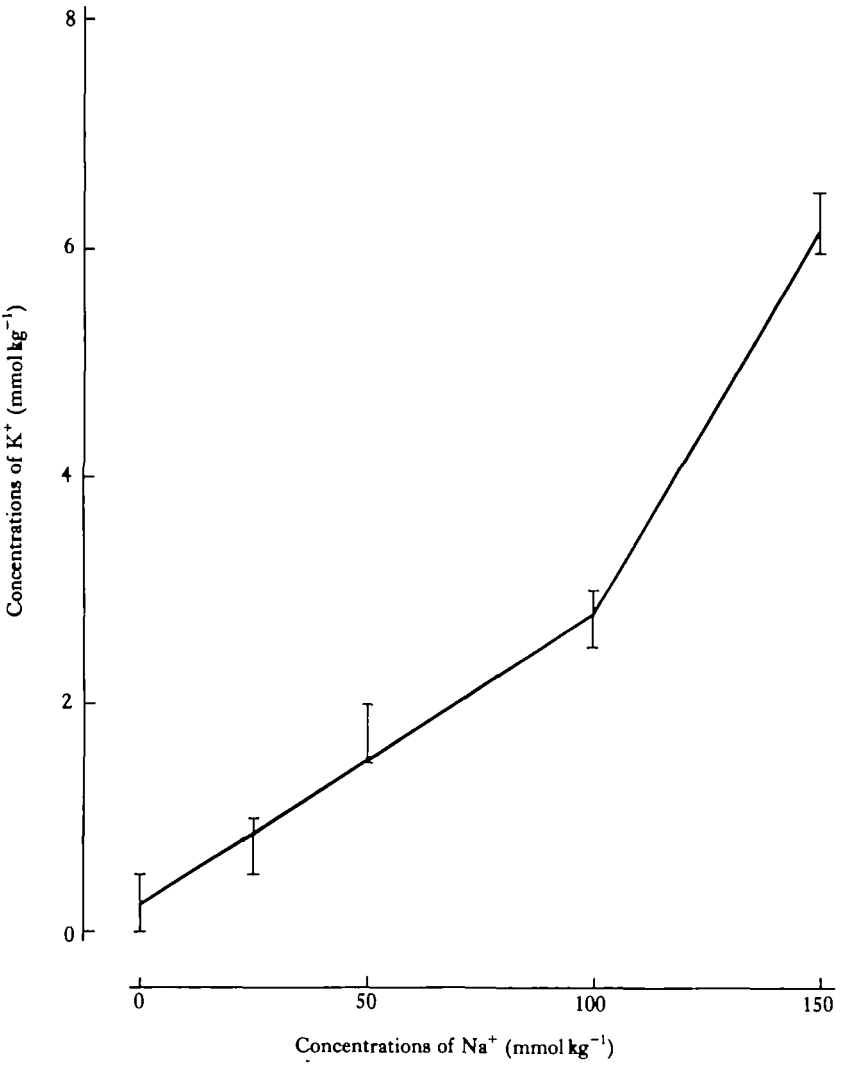


Fig. 4. Potassium concentrations required to inhibit sperm motility of rainbow trout in the presence of various concentration of sodium. At each sodium concentration, potassium concentration was varied in 0·5 mmol kg^{-1} steps. Upper ends of vertical bars represent minimum concentrations to inhibit sperm motility; lower ends of vertical bars represent maximum concentrations to allow sperm motility.

Morphological changes in rainbow trout sperm in different osmolalities

Rainbow trout spermatozoa in the semen had a bullet-shaped head, a midpiece which contained several mitochondria, and a tail. The plasma membrane tightly covered the head, midpiece and tail (Fig. 6A). When semen was diluted and the spermatozoa were allowed to swim in 100 mmol kg^{-1} sodium chloride solution for 90 s, the plasma membrane of the tail became swollen and the space between the plasma membrane and axoneme became considerably wider (Fig. 6B). When semen was diluted in the buffer solution, the head maintained the original shape although the

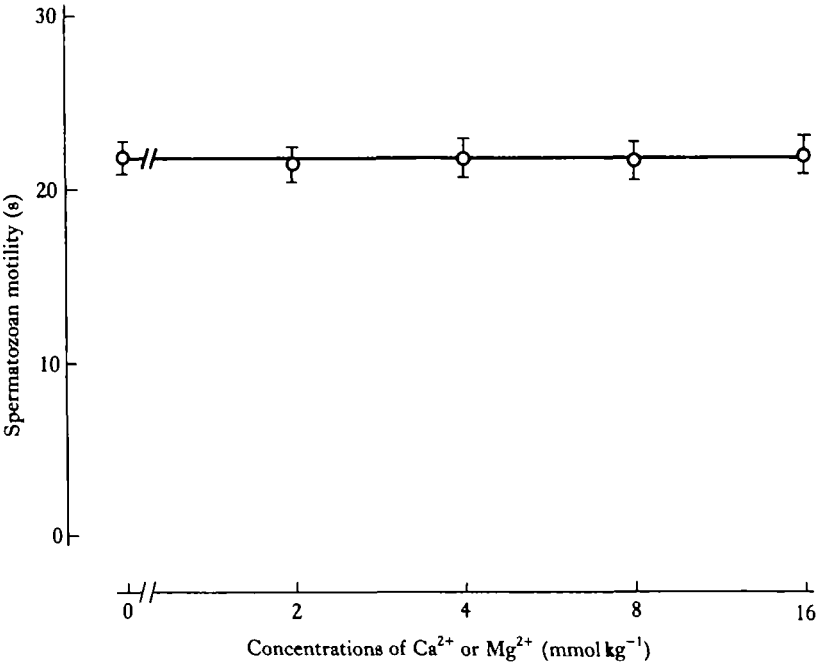


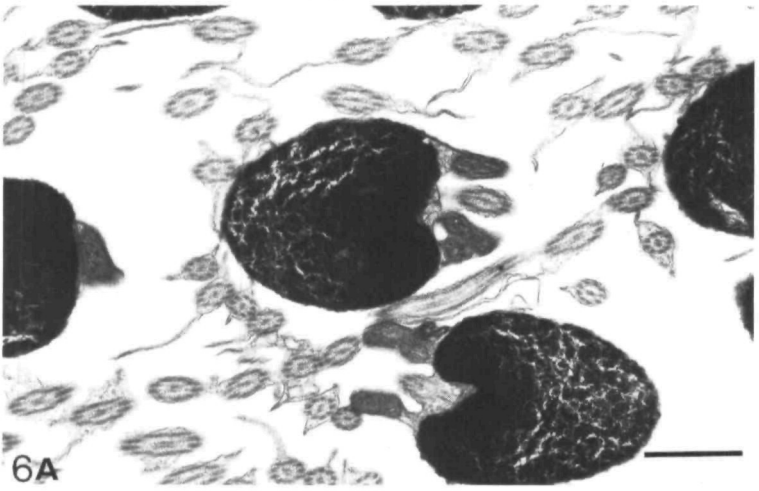
Fig. 5. The effects of calcium and magnesium on sperm motility of chum salmon, *Oncorhynchus keta*. Calcium and magnesium at the concentration indicated was added simultaneously. Vertical bars represent means ±S.E. in five experiments.

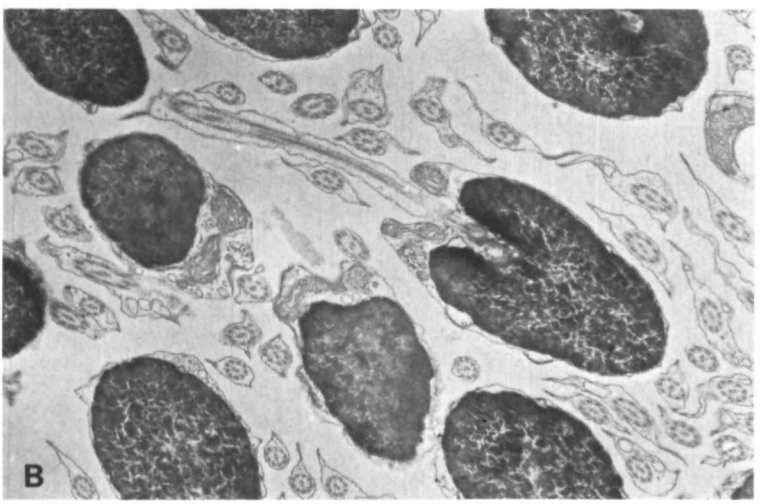
electron-sparse region increased. The plasma membrane became swollen, membranes fused with each other, and the envelope enclosed several axonemes (Fig. 6C). These morphological alterations are identical to those occurring in fresh water (Billard, 1978).

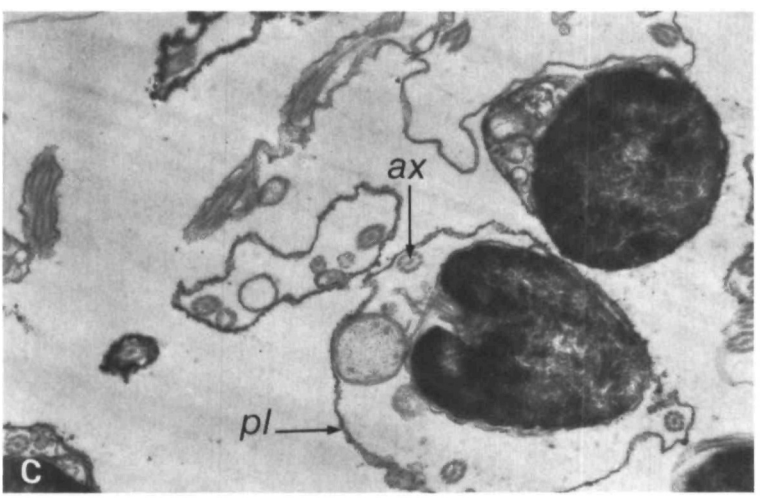
DISCUSSION

Spermatozoa of the salmonid fishes, rainbow trout, masu salmon and char, were motile in sodium chloride and mannitol solutions hypertonic to the seminal plasma (Fig. 1), in agreement with previous observation on chum salmon (Morisawa & Suzuki, 1980). Spermatozoa of rainbow trout, for example, were able to move at sodium chloride concentrations up to at least 225 mmol kg^{-1} (450 mosmol kg^{-1}) or at mannitol concentrations up to 340 mmol kg^{-1} (340 mosmol kg^{-1}). This suggests that the osmolality of the seminal plasma is not the factor inhibiting sperm motility in the sperm duct of salmonid fishes, and that sodium chloride may slightly stimulate motility. This contrasts with the situation in freshwater cyprinid fishes, where sperm motility is inhibited at the osmolality of the seminal plasma, and where sodium chloride and mannitol have similar effects on motility (Morisawa & Suzuki, 1980; Morisawa *et al.* 1983).

Fig. 6. Electron micrographs of rainbow trout spermatozoa. (A) Spermatozoa in the semen, (B) spermatozoa in hypotonic (100 mmol kg^{-1}) NaCl solution, (C) spermatozoa in buffer solution: note that the plasma membrane (*pl*) became swollen although the chromatin in the head and axoneme (*ax*) in the tail maintained the original shape. Scale bar = 1 μm.


6A


B


ax
pl
C

Potassium chloride suppressed sperm motility as found previously for rainbow trout (Schlenk & Kahmann, 1938; Benau & Terner, 1980; van der Horst *et al.* 1980) and chum salmon (Morisawa & Suzuki, 1980). Even in the presence of 100 mmol kg^{-1} sodium chloride, 3 mmol kg^{-1} potassium chloride could inhibit motility. This effect could not be due to chloride, since up to 225 mmol kg^{-1} sodium chloride, and removal of sodium or chloride, have no effect. Thus potassium, which is a major constituent of the seminal plasma, is the factor inhibiting sperm motility in the sperm duct, and sodium as well as chloride can maintain sperm motility.

In freshwater cyprinid fishes, potassium accelerates sperm motility (Morisawa *et al.* 1983). Another difference between cyprinid and salmonid spermatozoa is the resistance to hypotonicity even though both groups of teleosts release spermatozoa into fresh water at spawning. As shown in the previous paper (Morisawa *et al.* 1983), in hypotonic solutions, sperm motility is reduced and sperm structure is disrupted in cyprinid fish; spermatozoa of carp become completely amorphous in fresh water. In rainbow trout, sperm head chromatin and flagellar axoneme (9+2 structure) retain the original shape even in fresh water (Fig. 6; see also Billard, 1978), and sperm lifespan is almost the same in fresh water and in isotonic solution. Salmonid fishes are thought to belong to a primitive group of teleosts. They migrate in the ocean for several years and transfer to fresh water to reproduce. Freshwater cyprinid fishes live all their life in fresh water. Thus it seems probable that the difference of potassium effect on sperm motility and of resistance to hypotonicity between Cyprinidae and Salmonidae is related to the course of adaptation and evolution of teleosts.

Rubidium also inhibited motility of rainbow trout sperm. In contrast, lithium and sodium had no inhibiting effect. Indeed, an increase in sodium concentration reduced the sensitivity to potassium (Fig. 4). Detailed experiments using the potassium-sodium system to regulate sperm motility may provide further insight into the events which occur at the plasma membrane during the initiation of sperm motility of salmonid fishes.

The authors wish to thank Jean Gilder, Stazione Zoologica di Napoli, for her help in preparing the manuscript and Yabe Hatchery, Yamanashi Prefecture and Tokyo for supplying the materials. This work was supported in part by grant in aid from Ministry of Education Japan to M. M. We would like to thank M. Di Genova for drawing.

REFERENCES

Benau, D. & Terner, C. (1980). Initiation, prolongation and reactivation of the motility of salmonid spermatozoa. *Gamet Res.* **3**, 247–257.

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.

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, M., Suzuki, K., Shimizu, H., Morisawa, S. & Yasuda, K. (1983). Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. *J. exp. Biol.* **107**, 95–103.

Schlenk, W., Jr. & Kahmann, H. (1938). Die chemische Zusammensetzung des Spermatliquors und physiologische Bedeutung. *Biochem. Z.* **295**, 283–301.

van der Horst, G., Dott, H. M. & Foster, G. C. (1980). Studies on the motility and cryopreservation of rainbow trout (*Salmo gairdneri*) spermatozoa. *S. Afr. J. Zool.* **15**, 275–279.