Japan. J. Ichthyol.
40(2): 273-278, 1993

魚類学雑誌
40(2): 273-278, 1993

Motility and Morphology of Sperm of the Ayu, *Plecoglossus altivelis*, at Different Salinities

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(*Received December 25, 1992; in revised form May 18, 1993; accepted June 30, 1993*)

Teleost spermatozoa are immotile in the testis and often in the seminal plasma. Changes in physical or chemical factors influencing sperm at spawning, such as a decrease in osmolality in cyprinids (Morisawa et al., 1983a), an increase in osmolality in many marine fishes (Morisawa and Suzuki, 1980; Morisawa, 1985; Utsugi, unpublished) and a decrease in concentration of surrounding potassium ions in salmonids (Morisawa et al., 1983b), initiate sperm movement. The effects of ion concentration and osmolarity on sperm motility are not only of biological interest, but are also commercially important for seed production in aquaculture. Spermatozoa lose their potential for motility after movement, and prior to that, become impotent under unfavorable conditions such as extreme hyperosmolarity. Swelling or rupturing of sperm cells due to hyposmolality is a cause of sperm impotency in the carp, *Cyprinus carpio*, and rainbow trout, *Oncorhynchus mykiss* (Morisawa et al., 1983b; Billard, 1983). But sperm also become impotent under hyper- and isotonic conditions. In this study, the effects of sodium and potassium ions on sperm motility in the ayu, *Plecoglossus altivelis*, an amphidromous salmoniform species (Plecoglossidae) were examined, and sperm morphology in different salinities observed with an electron microscope.

Materials and Methods

Semen from five mature males of *Plecoglossus altivelis* obtained from a fish farm in Ozuchi, Iwate Prefecture, Japan, were collected by gently pressing the abdomen. Samples of icecooled semen were taken up on the point of a needle, and diluted and well stirred in about 5 μl of graded sodium and/or potassium chloride solution on a slide glass at concentration intervals of 25 mM, starting from 25 mM. The activity, duration of motility (motility time) and percentage of motile spermatozoa were scored and measured under a light microscope. Sperm activities were scored immediately after dilution and classified as follows: ++, very active (rapid movement); +, active (not very rapid); ±, vibratory (almost still); −, inactive (completely immotile). Sperm motility time was expressed as the duration in seconds from dilution to the cessation of movement in 95% of the sample. All measurements and scores were made on more than ten samples from each individual fish, and averaged. To investigate the influence of changes in chemical factors on the sperm, each semen sample, on which the effects of the NaCl or KCl solutions had been examined, was diluted again with the alternate solution of equal osmolarity and volume to the initial solution, and sperm response measured and scored as above. For electron microscopic observations, semen samples, which were diluted to about 1% and well stirred in NaCl and KCl solutions at several osmolarities, and an undiluted control semen sample were held in test tubes for about 5 minutes at room temperature. Subsequently, they were centrifuged at 800 rpm for 5 minutes, and fixed with 2% glutaraldehyde buffered at pH 7.3 with 0.1 M sodium cacodylate, for 1 h at 4°C. After further centrifugation, they were post-fixed with 1% osmium tetroxide, using the same buffer as above for 1 h at 4°C, dehydrated in an ethanol series and embedded in epoxy resin. Silver or silver-gold sections were made with glass knives on an LKB Ultrotome, stained with uranyl acetate and lead citrate, and observed with a JEOL 100s electron microscope.

Results

Effects of NaCl on sperm motility (Fig. 1).—Most of the ayu sperm (ca. 90%) was very active (++) in NaCl solution at concentrations from 25 to 175 mM, with little difference in motility time overall (ca. 30–50 seconds). Clearly, sodium chloride, as a chemical factor, had little effect on ayu sperm motility. Immotility of the sperm in high NaCl concentrations (more than 200 mM) and a slight shortening of motility time in low concentrations (25–50 mM) were observed, probably caused by hyper- and hyposmolarity.

Sperm samples previously diluted and kept in NaCl solutions for more than 5 minutes were no longer motile upon additional dilution with KCl.

Effects of KCl on sperm motility(Fig. 2).—Ayu

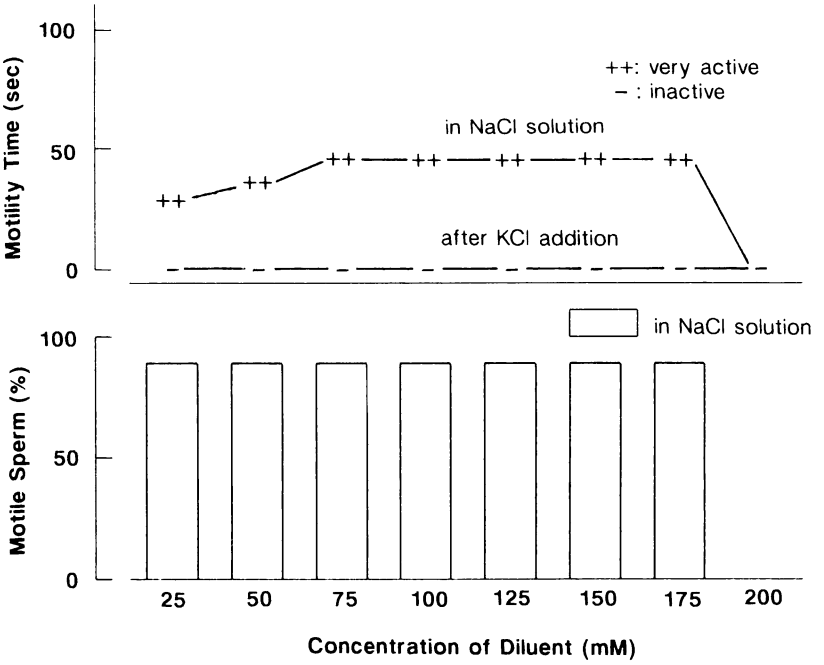

Fig. 1. Effects of dilution with graded NaCl and additional dilution with graded KCl on sperm activity (*top*), sperm motility time (*top*), and percentage of motile sperm (*bottom*) in the ayu, *Plecoglossus altivelis*.

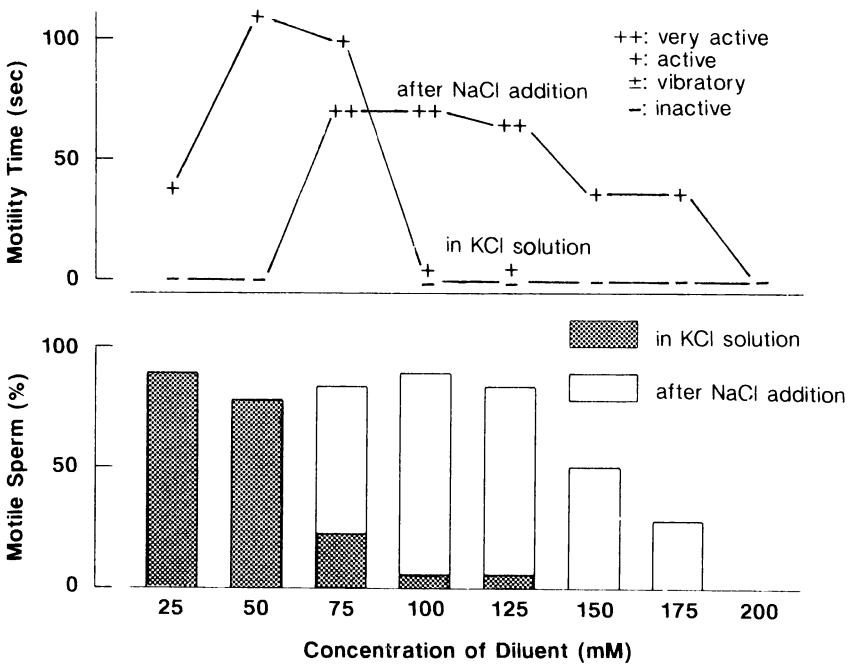

Fig. 2. Effects of dilution with graded KCl and additional dilution with graded NaCl on sperm activity (*top*), sperm motility time (*top*), and percentage of motile sperm (*bottom*) in the ayu, *Plecoglossus altivelis*.

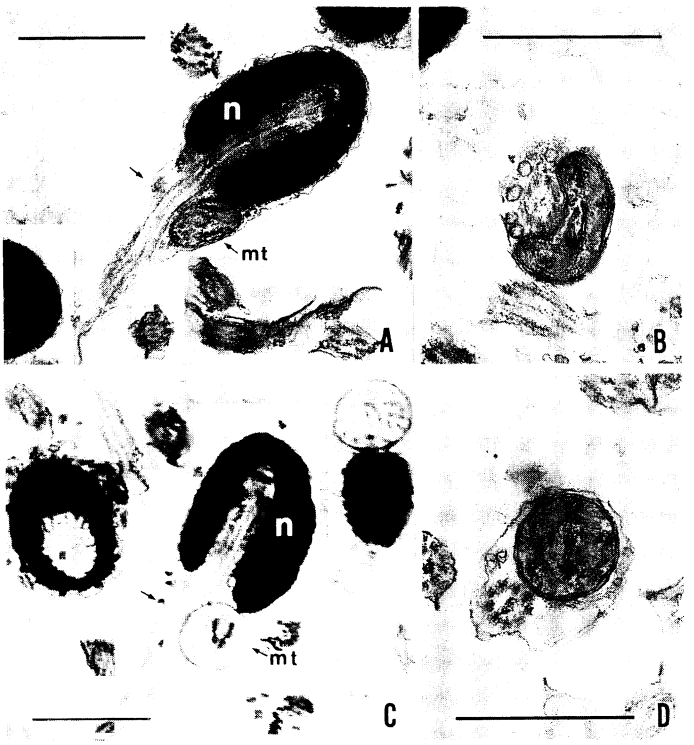

Fig. 3. Electron micrographs of spermatozoa of the ayu, *Plecoglossus altivelis*. A) Longitudinal section of normal (control) spermatozoon; a compressed mitochondrion (*mt*) is attached to the posterior end of the nucleus (*n*). B) Cross section through the mitochondrion at the level marked by arrows in Figure 3A. C) Longitudinal section of a spermatozoon in 150 mM NaCl solution. D) Cross section through the mitochondrion at the level marked by arrows in Figure 3C. Scale = 1 μm.

sperm was motile in low KCl concentrations (25–75 mM) for twice as long (ca. 50–100 seconds) as in NaCl at the same concentrations, the degree of sperm activity being rated +. 80–90% motility occurred in 25–50 mM KCl solutions, compared with 30% motility in 75 mM KCl, although the motility time in the latter was similar to that in 50 mM KCl. In high KCl concentrations (>100 mM), motility was very low, from 100 to 175 mM KCl, the retention of motility potential was demonstrated by additional dilution with NaCl at the same concentrations. As a result, 80–90% of the sperm became very active (++) for about 70 seconds in KCl+NaCl concentrations from 75 to 125 mM. Although motility percentages and times declined in 150 mM KCl (50% motile for ca. 50 seconds) and 175 mM KCl (25% motile for about 50 seconds), sperm became active (+) upon further dilution with NaCl. Sperm samples initially diluted in extremely high concentrations of KCl (more than 200 mM) were no longer motile upon additional dilution with NaCl.

For ayu sperm, the presence of potassium ions in low concentrations (25–50 mM) seemed to prolong sperm motility time and at higher concentrations (100–150 mM), preserve the potential for sperm motility.

Morphology of ayu sperm under different salinity levels.—The fixative used in this study appeared effective, although the osmolarity of the fixative (ca. 400 mOsm/*l*) was somewhat higher than that of the seminal plasma of the species (ca. 300 mOsm/kg—determined with a freezing-point depression osmometer).

Control ayu spermatozoa showed the following morphological features: head with a U-shaped nucleus, a mitochondrion attached to the posterior end of the nucleus (Fig. 3A); mitochondrion crescentic in cross section (Fig. 3B).

The morphology of spermatozoa which had ceased movement in 150 mM NaCl differed considerably

from the control specimens, their mitochondria being clearly swollen (Fig. 3C, D), despite the osmotic environment being similar to that of the control spermatozoa in seminal plasma. Mitochondrial migration to the anterior portion of the nucleus, supposedly by flagellar beating, was often observed in 150 mM NaCl solution (Fig. 4A).

Immotile spermatozoa which had retained their motility potential in 100 and 125 mM KCl were morphologically similar to the control spermatozoa. In 150 mM KCl, however, swollen mitochondria were detected in 60% of spermatozoa (counted from ultra-thin sections).

Spermatozoa which had ceased moving in low concentrations (25–50 mM) of both NaCl and KCl solutions, were found to have ruptured (Fig. 4B). Conversely, in a high concentration (300 mM) of both NaCl and KCl in which sperm died without movement, although mitochondria were found to be swollen, the plasma membrane of the sperm cells was undamaged (Fig. 4C).

The conditions of sperm cells and mitochondria

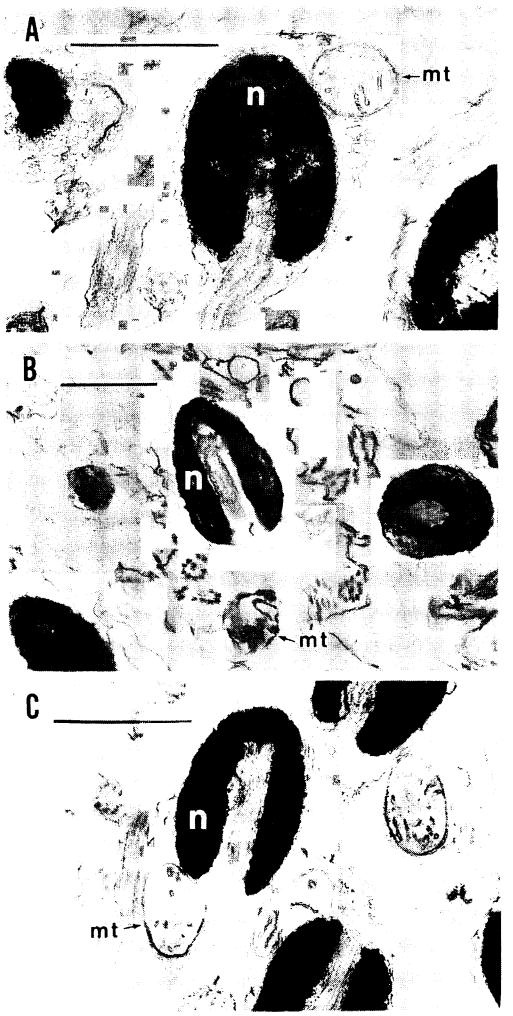

Fig. 4. Electron micrographs of spermatozoa of the ayu, *Plecoglossus altivelis*. A) Longitudinal section of a spermatozoon in 150 mM NaCl solution; mitochondrial migration to the anterior portion of the nucleus was often observed in this solution. B) Longitudinal section of a spermatozoon in 50 mM NaCl solution; sperm cell was ruptured by hyposmosis. C) Longitudinal section of a spermatozoon in 300 mM NaCl; the mitochondrion swelled in spite of the hypertonicity of the solution. Scale = 1 μm.

Table 1. Morphological states of sperm cells and mitochondria of the ayu, *Plecoglossus altivelis*, diluted in different saline solutions for about 5 minutes

Saline conc. (mM)	Osmolarity (mOsm/l)	State of sperm cells	State of mitochondria	Motility potential*
Control	300	normal	compressed	+
50 NaCl	100	injured	swollen	−
50 KCl	100	injured	swollen	−
125 NaCl	250	normal	swollen	−
125 KCl	250	normal	compressed	+
150 NaCl	300	normal	swollen	−
150 KCl	300	normal	compressed or swollen	+
300 NaCl	600	normal	swollen	−
300 KCl	600	normal	swollen	−

* Motility potential of sperm in each medium is given by + or −.

under several saline conditions are shown in Table 1.

Discussion

Salmonid spermatozoa are able to move in 0–200 mM NaCl and/or 0–400 mM sugar solutions, but are never motile in solutions containing several mM KCl and/or in hypertonic solutions of more than 400 mOsm/kg independent of its components (Morisawa et al., 1983b). Considering fishes generally, only in salmonids are potassium ions known to suppress the onset of sperm movement. On the contrary, the initiation of sperm motility in cyprinids and many marine fishes depend only upon changes in osmolality of the surrounding medium (Morisawa and Suzuki, 1980; Utsugi, unpublished). In fact, the presence of potassium ions is somewhat favorable for sperm motility in cyprinids (Morisawa et al., 1983a). In the ayu, sperm motility patterns resembled those of salmonids, an anadromous group (motility time <1 minute, immotile in >200 mM NaCl, motility suppressed by potassium), except in the degree of suppression of sperm motility by potassium ions (>100 mM in *Plecoglossus altivelis* vs. several mM in salmonids). However, promotion of motility by potassium, as seen in cyprinids, was observed.

The physiological nature of ayu sperm, such as low sensitivity to potassium, permits successful spawning downstream and at river mouths, where potassium (from sea water) exists in high concentrations, and suggests a close relationship of the species with other anadromous groups such as the Osmeridae, which include sea-spawning species (e.g. *Hypomesus pretiosus*). It is considered that other similarities in sperm motility patterns between the ayu and salmonids reflect their phyletic closeness.

Promotive effects of potassium on sperm motility are likely to be common in fishes, because the sperm motilities of many sea-spawning species are low in potassium-free media (Utsugi, unpublished). Furthermore, incapacitated sperm of marine-captured chum salmon, *Oncorhynchus keta*, were capacitated and their motility accelerated by dipping sperm into isotonic KCl for a few minutes prior to dilution for the purpose of sperm activation (Utsugi, unpublished). Accordingly, it may be said that the suppressive effects of potassium on the initiation of sperm motility is specific in salmoniform fishes among teleosts.

In the present study, all of the spermatozoa which had lost their motility potential had swollen mitochondria (Table 1). Even in 150 mM KCl, 60% of spermatozoa, which were probably directly related to the immotile faction recorded during experimentation, possessed swollen mitochondria. Therefore, swelling of the mitochondria is considered as indicative of the death of the spermatozoa. It is obvious that such swelling was not caused by osmotic factors, because the phenomenon was detected in a wide range of saline concentrations, from isotonic to hypertonic, leading to the conclusion that impotency of spermatozoa in *Plecoglossus altivelis* under any conditions is caused primarily by disfunction of the mitochondria. Osmotic injuries of sperm cells such as swelling and rupturing in hypotonic conditions, rather than being direct factors causing sperm impotency, should be considered as secondary events.

Acknowledgments

I am grateful to Professor Yasuhiko Taki, Tokyo University of Fisheries, for his critical reading of the manuscript. I wish to dedicate this work to the late Dr. Motoyoshi Yokote, who was my technical adviser.

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種々の塩分条件下におけるアユ精子の運動性と形態

打木研三

アユの精子の運動性に対する K^+, Na^+, およびそれぞれのイオ

ンの浸透濃度の影響を調べた．その結果，アユの精子は塩分組成とは無関係に 400 mOsm/*l* 以上の浸透濃度下では全く運動せず，潜在的運動能力をも失った．精子の運動を許容する濃度範囲において，Na^+ は精子の活性や運動時間に影響を与えなかったが，一方 K^+ は低濃度では僅かに活性を低下させるかわりに運動時間を延長させ，中濃度では強く運動を抑制するかわりに潜在的運動能力をむしろ高め，高濃度では強く運動を抑制するとともに潜在能力をも低下せしめるという，濃度の違いに応じた様々な影響を与えた．またこれと平行して，透過型電子顕微鏡で諸条件下における精子の形態観察を行なったところ，潜在的運動能力を喪失したすべての精子にミトコンドリアの著しい膨潤が観察されたことから，精子の死はミトコンドリアの機能不全によるものと判断された．

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