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Sperm Sphere in Unionid Mussels (Bivalvia: Unionidae)

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ABSTRACT—We observed spermatozoa of five freshwater Japanese unionid mussel species under a light microscope. Males discharged spermatozoa as spherical masses. Spermatozoa embedded their heads into a spherical, colorless body with their tails extended around the sphere. We termed this spherical mass of sperm as a "sperm sphere". Just after release the sperm spheres rotated rapidly by synchronous movement of the sperm tails. By the next day, the sperm spheres increased in size and some spermatozoa had detached from the sperm spheres. Spermatozoa embedded in the sperm sphere were active at least forty-eight hours after being discharged. Single spermatozoa in a balanced salt solution were also active until forty-eight hr after extraction from the gonads, while they lost their motility within a few minutes in freshwater. Spermatozoa may maintain their motility for a long time in the form of a sperm sphere and the use of sperm spheres may allow the fertilization of eggs discharged into the gill chambers of females far away from the male.

INTRODUCTION

Unionid mussels are usually dioecious. Sperm are discharged directly into the water and inhaled by females through their inhalant siphons. Fertilization of eggs occurs in the gill chambers of females.

Utterback (1931) observed "hollow globular masses" of sperm being discharged in *Lampsilis ventricosa*, *Lasmigona complanata* and *Utterbackia ohiensis*. Such masses of sperm were also observed in *Margaritifera laevis* (Okada and Ishikawa, 1959). Edger (1965) described in detail the sperm masses being discharged by a male *Anodontoides ferussachianus*: the mass were spherical and made up of hundreds of sperm with their heads embedded in the surface of a spherical colorless body and with their tails extended radially. These sperm masses move slowly through the water in the same manner as *Volvox* moves. They moved actively even at twelve hours after discharge in *Margaritifera laevis* (Okada and Ishikawa, 1959).

Since sperm from freshwater species generally lose their motility within a few minutes after discharge into freshwater (Morisawa, 1985), this suggests that these sperm masses may be a mechanism to transfer sperm to females far away from the males. But little is known about the physiology and how general the use of masses of sperm is in unionid mussels.

In the present study, we examined the role of the sperm masses in unionid mussels by observations on the motility of the spermatozoa in the laboratory.

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MATERIALS AND METHODS

Materials

Five species of unionid mussels were collected from three localities in the Kinki and Chugoku regions in Japan; *Anodonta woodiana* from a creek at Kanmaki, Osaka Prefecture, *Inversidens japanensis* and *Pseudodon omiensis* from a creek at Kinomoto, Shiga Prefecture, *Inversiunio yanagawensis* and *Lanceolaria grayana* from the Gion Creek, Okayama Prefecture. Sex of all individuals collected was determined by observation of gametes under a light microscope, and 10 to 20 males of each species were used in the experiments.

Spawning induction

Males were reared in freshwater kept at 20°C for twenty-four hr, transferred to 4°C freshwater for two hours, and then returned to freshwater at 20°C. Spawning occurred within one hour after this treatment.

Motility of spermatozoa

Spermatozoa released by thermal stimulation were reared in freshwater of 16°C. Sperm collected by dissecting the gonads were diluted by freshwater or balanced salt solution (BSS : 9.6 mM NaCl, 0.7 mM KCl, 1.7 mM Na_2SO_4, 0.3 mM $MgSO_4$, 3.2 mM $CaCl_2$, 7.3 mM $NaHCO_3$, 0.1 mM NaH_2PO_4, 0.2 mM glucose adjusted to pH 7.3) and maintained at 16°C. The balanced salt solution was prepared after Machii (1992). We observed spermatozoa under a light microscope in every twenty-four hours and their motility was examined. Motility was qualitatively quantified into three categories as follows; very active, active and inactive.

RESULTS

Description

Males discharged spermatozoa as spherical masses (Fig. 1A and 1B). Spermatozoa embedded their heads into the spherical colorless body with their tails extended around the

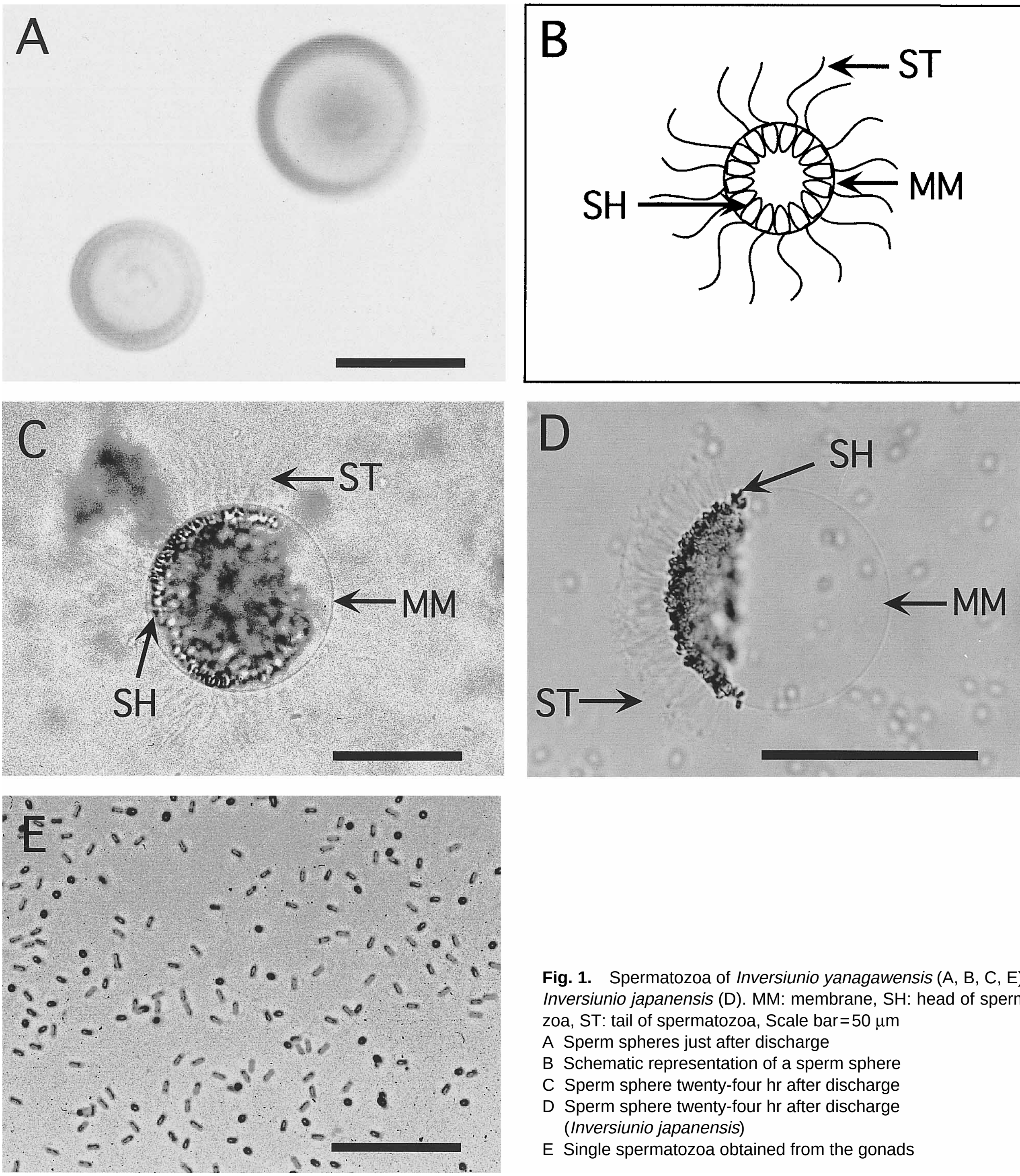

Fig. 1. Spermatozoa of *Inversiunio yanagawensis* (A, B, C, E) and *Inversiunio japanensis* (D). MM: membrane, SH: head of spermatozoa, ST: tail of spermatozoa, Scale bar=50 µm
A Sperm spheres just after discharge
B Schematic representation of a sperm sphere
C Sperm sphere twenty-four hr after discharge
D Sperm sphere twenty-four hr after discharge (*Inversiunio japanensis*)
E Single spermatozoa obtained from the gonads

sphere. We termed this sphere as a “sperm sphere”. Although we tried to fix the sperm spheres by 70% ethyl alcohol, Carnoy’s fixative (ethyl alcohol : acetic acid=4:1) and 10% formalin, we failed to fix them. They disintegrated at once in any fixing fluids, and then only the dispersed spermatozoa were observed, which suggests that the spherical body may be composed of a thin, fragile membrane. At that time, the sperm spheres rotated rapidly and showed active movement through the water. But a part of the surface became bare (Fig. 1C), because some spermatozoa detached from the sphere (Fig. 1D). The sperm spheres gradually increased in size with time (Fig. 2). As a result, the sperm spheres moved forward with the bare surface area at the head.

On the other hand, only single spermatozoa were obtained by dissection from the gonads. The spermatozoa in the balanced salt solution also did not form sperm spheres.

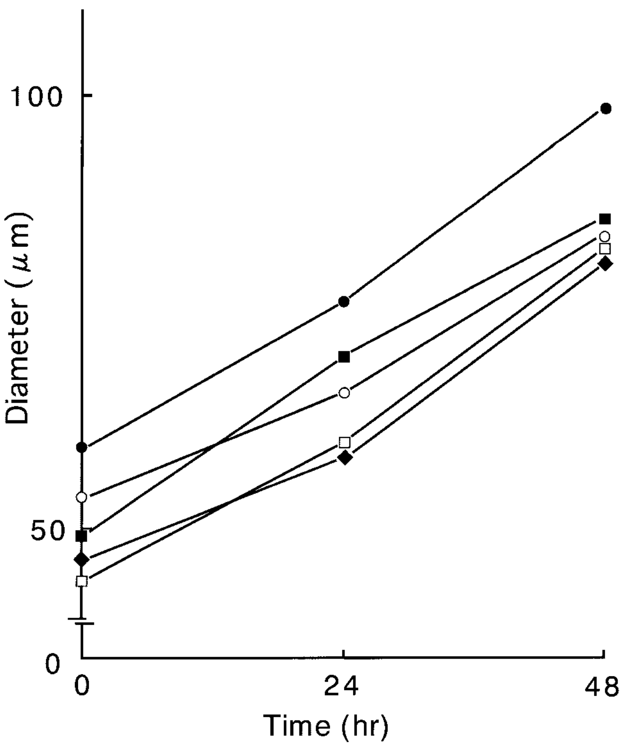

Fig. 2. Increase in diameter of sperm spheres. ●: *Anodonta woodiana*, ◆: *Inversidens japanensis*, □: *Inversiunio yanagawensis*, ○: *Lanceolaria grayana*, ■: *Pseudodon omiensis*.

Mean diameter of sperm sphere just after discharge was 44.4±3.0 μm in Lanceolaria grayana, 47.9±19.1 μm in *Inversiunio yanagawensis*, 49.3±6.8 μm in *Inversidens japanensis*, 53.3±1.8 μm in *Pseudodon omiensis* and 59.6±8.5 μm in *Anodonta woodiana*.

Motility

Spermatozoa in the sperm spheres of five species were very active until forty-eight hours after discharge (Table 1). Spermatozoa detached from the sperm sphere and became inactive within a few minutes (Fig. 1E).

In four species except for *Inversiunio yanagawensis*, no sperm sphere was observed at seventy-two hr. However, in *Inversiunio yanagawensis*, sperm spheres were active seventy-two hr after discharge. Some spheres had no spermatozoa, and others had active spermatozoa but with very weakly lashing tails.

Table 1. Change in the motility of spermatozoa after discharge.

Species	0 hr	24 hr	48 hr	72 hr
Anodonta woodiana	++	++	++	–
Inversidens japanensis	++	++	++	–
Inversiunio yanagawensis	++	++	++	+
Lanceolaria grayana	++	++	++	–
Pseudodon omiensis	++	++	++	–

++: very active, +: active, –: no sperm spheres observed

The motility of spermatozoa extracted from the gonads is shown in Table 2. In balanced salt solution single sperm maintained their motility kept at forty-eight hours while in all species in freshwater, single sperm lost their motility within a few min.

DISCUSSION

Sperm sphere

Sperm sphere observed in the present study are the same as the “hollow globular masses” in *Lampsilis ventricosa*, *Lasmigona complanata* and *Utterbackia ohiensis* described by Utterback (1931) and the “spherical masses of sperm” in *Anodontoides ferussachianus* by Edger (1965). Since all these species belong to the family Unionidae, the use of sperm spheres may be of widespread occurrence in this family. Moreover, sperm spheres were also observed in *Margaritifara laevis* (Okada and Ishikawa, 1959). This species belongs to the family Margaritiferidae, which suggests that sperm sphere may be common in the superfamily Unionacea.

As sperm spheres have not been found in other freshwater bivalves such as the “Seta clam”, *Corbicula sandai* and freshwater mytilid mussel, *Xenostrobus securis* (Ishibashi, unpublished data), they may be unique to Unionacea.

The sperm sphere of unionid mussels is similar to “sperm ball” in the California oyster, *Ostrea lurida* (Coe, 1932), to the “sperm-discs” in the tubucolous polychaete, *Arenicola cristata* (Okada, 1941) and to the “disc of mature sperm” in *Arenicola mariana* (Newell, 1948). But these structures disintegrate immediately when they were released into salt water. On the contrary, sperm spheres moved through the freshwater for a long period. Therefore, sperm spheres appear to play a different role compared with sperm balls and sperm-discs of

Table 2. Change in the motility of spermatozoa in the water and in the balanced salt solution (BSS) after extraction from gonads.

Species	0 hr		24 hr		48 hr	
	Water	BSS	Water	BSS	Water	BSS
Anodonta woodiana	+	+	–	+	–	+
Inversidens japanensis	+	+	–	+	–	+
Inversiunio yanagawensis	+	+	–	+	–	+
Lanceolaria grayana	+	+	–	+	–	+
Pseudodon omiensis	+	+	–	+	–	+

+: active, –: inactive

marine lamellibranchs and polychaetes.

Spermatozoa in the balanced salt solution did not form sperm spheres, which suggest that spheres may form in gonads. We suggested that sperm spheres may form just before spawning in the gonads or gonoducts.

Motility of spermatozoa

In the present study, single spermatozoa dissected from the gonads in freshwater lost their motility within a few minutes, while they were active in the balanced salt solution for forty-eight hr. Further, spermatozoa with their heads embedded in sperm spheres were also active for two days, and sperm spheres increased in size with time. These results suggest that the membrane of the sperm sphere may be semipermeable and the composition of the fluid in the sperm sphere may be similar to the balanced salt solution.

As the concentration of the salt in the balanced salt solution is higher than freshwater, the sperm spheres may protect spermatozoa from the lower osmotic pressure of ambient freshwater and enable them to maintain motility for a long time in order to transport them to females far away from the male.

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