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Spermatogenesis and Reproductive Cycle in Male *Spisula sachalinensis* (Bivalvia: Mactridae) of Korea

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ABSTRACT

Spermatogenesis and the reproductive cycle in male *Spisula sachalinensis* were investigated by cytological and histological observations. The morphology of the spermatozoon has a primitive type and is similar to those of other bivalves in that it contains a short midpiece with four mitochondria surrounding the centrioles. But spermatozoon of this species has not axial rod and satellite body in the midpiece. The morphologies of the sperm nucleus type and the acrosome shape of this species have a globe-shape type and modified cap-like shape, respectively. The spermatozoon is approximately 40-45 μm in length including the sperm nucleus length (about 1.35 μm), acrosome length (about 1.50 μm) and tail flagellum. The axoneme of the sperm tail flagellum consists of nine pairs of microtubules at the periphery and a pair at the center. The axoneme of the sperm tail shows a 9+2 structure. The spawning period of these species lasts from June to July, and the main spawning occurs in July when seawater temperatures are greater than 20° C. The male reproductive cycle of this species can be categorized into five successive stages: early active stage (October to January), late active stage (February to April), ripe stage (April to June), partially spawned stage (June and July), and spent/inactive stage (August to September).

Key words: *Spisula sachalinensis*, Ultrastructure, Spermatogenesis, Germ Cell Differentiation, Reproductive cycle

INTRODUCTION

Spisula sachalinensis is one of the important edible bivalves in East Asia, including Korea, China, and Japan. In Korea, this species is mainly found in sandy bed up to 10 m in water depth in the coastal waters of Jumunjin, Gangwon-Do. For the propagation and management of a living natural resource, it is important to understand its reproductive biology with regard to spermato-

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genesis and reproductive cycle of this species.

Previously, there have been many studies on Mactridae species, especially, *M. veneriformis* on aspects of reproduction, including gametogenesis and sexual maturation (Chung and Ryou, 2000; Kim, 2001), on aspects of ecology, including the structure of tidal flat ecosystem (Choi, 1969), growth (Kim and Ryou, 1991), food organisms (Ryou and Kim, 1995). Regarding *M. chinensis*, several studies have been conducted to investigate aspects of reproductive ecology, including propagation (Sakai, 1976), bioassay study of early development (Lee and Son, 1978), spawning and growth, breeding season (Sakurai *et al.*, 1992), oogenesis and ovarian cycle (Chung and Kim, 2008), and spermatogenesis and sexual maturaion (Kim, 2001; Chung *et al.*, 2007).

However, there have been some studies on *S. sachalinensis* on aspects of reproduction, including the reproductive cycle (Lee *et al.*, 1997) and gametogenesis (Kim, 2001; Lee *et al.*, 2003), on aspects of ecology, including growth (Sasaki, 1981), fecundity (Sasaki, 1981), and life cycle (Sasaki, 1987). Better understanding of the reproductive cycle and the spawning period of this species will provide information needed for the determination of age and recruitment period. In the Mollusca, sperm morphology has been used increasingly in assessing long-standing taxonomic problems (Popham, 1979; Healy, 1988, 1996).

The purpose of the present study is to describe germ cell differentiation during spematogenesis, the reproductive cycle with testicular developmental stages of *S. sachalinensis* using cytological and histological methods.

MATERIALS AND METHODS

Sampling

Male specimens of *S. sachalinensis* were collected by the dredge monthly in the coastal waters of Jumunjin, Korea, from January to December, 2006. A total of 168 clams ranging from 50.0 mm to 72.0 mm in shell length was used for the study. After the clams transported alive to the laboratory, the sizes of the specimens were recorded using a Vernier caliper.

Gonadal development by histological observation

Histological preparations were made for analysis of the gonadal phases by light microscopy, the tissues were subjected to standard histological procedures (dehydrated in alcohol and embedded in paraffin) and sectioned at 5-7 μm using a rotary microtome.

Sections were then mounted on glass slides, stained with either Hansen's hematoxylin-0.5% eosin, and inspected under a light microscope.

Germ cell differentiation by electron microscopic observation

For transmission electron microscopical observations, excised pieces of the gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehydeglutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hr at 4℃. After prefixation, the specimens were washed several times in the buffer solution and then postfixed in a 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) for 1 hr at 4℃. Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and

LKB ultramicrotome at a thickness of about 80-100 nm. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and observed with a JEM 100 CX-II (80-KV) electron microscope.

RESULTS

Position and morphology of the testis

The general morphology of the testis of *S. sachalinensis* is similar to those for the testes in bivalves. The testis is a diffuse organ consisting of branching acini containing differentiating sperm in a variety of stages. Germ cells are distributed in a centripetal pattern from the acinus wall to the lumen (Fig. 1). As gonadal maturation progresses, the external views of the mature testis appear in lemon yellow white, and the ovary is red in color. Therefore, the sexes of the clams can be easily distinguished by external features. At this time, if the testis is slightly scratched with a razor, mature sperm readily flow out after spermatozoa are discharged.

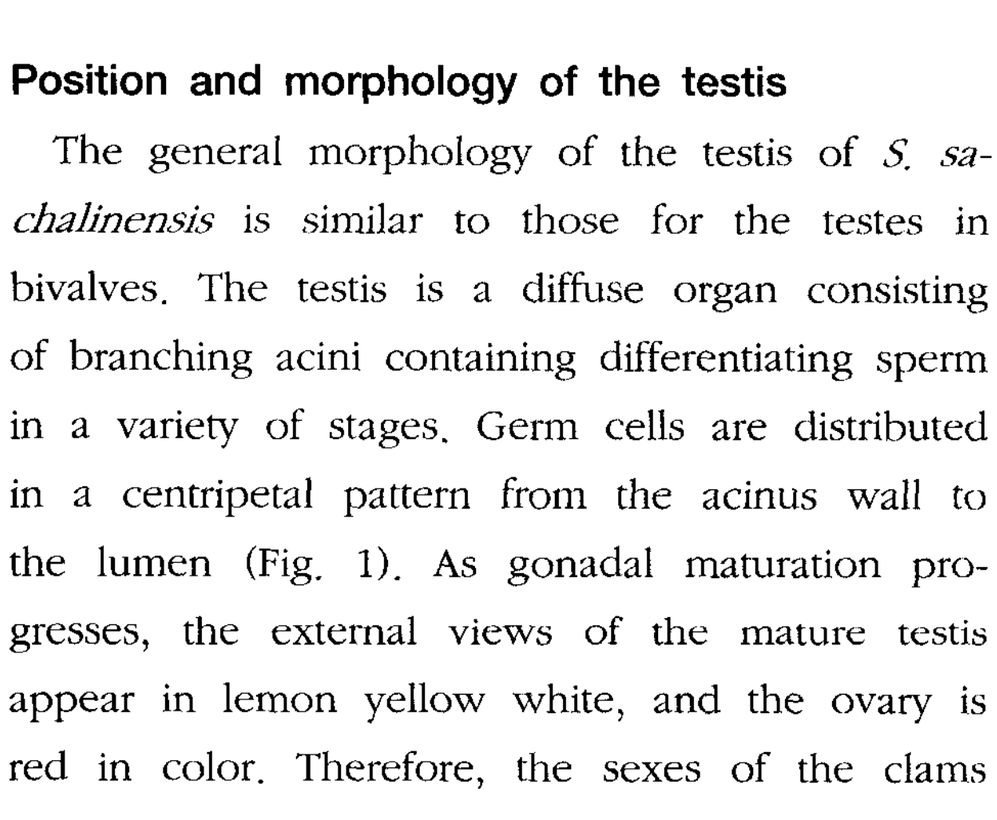

Fig. 1. Photomicrograph of the structure of the testis.Abbreviations: ACN, acinus; AW, acinus wall; SC, spermatocyte; ST, spermatid.

Ultrastructure of germ cells during spermatogenesis

Based on the testicular development and morphological characteristics of germ cells, spermatogenesis can be classified into five phases: (1) spermatogonia, (2) primary spermatocyte, (3) secondary spematocyte, (4) spermatid, and (5) spermatozoon phases.

1) Spermatogonial phase

The primary spermatogonia are located near the accessory cells. They are approximately 7-8 μm in diameter and more or less oval-shaped. Each of the spermatogonia contains a large nucleus with chromatin. The primary spermatogonia divide mitotically to produce the secondary spermatogonia, which are smaller cells with smaller nuclei compared to the primary spermatogonia. At this phase, the mitochondria in the cytoplasm of the accessory cells appear (Fig. 2A).

2) Primary spermatocyte phase

The secondary spermatogonia differentiate into primary spermatocytes. The nucleus of the primary spermatocyte contains slightly denser chromatin than that of the secondary spermatogonium. The synaptonemal complexes in the nucleus appear in the prophase during the first maturation division. Several mitochondria appear in the cytoplasm (Fig. 2B).

3) Secondary spermatocyte phase

The primary spermatocytes develop into the secondary spermatocytes by the first maturation

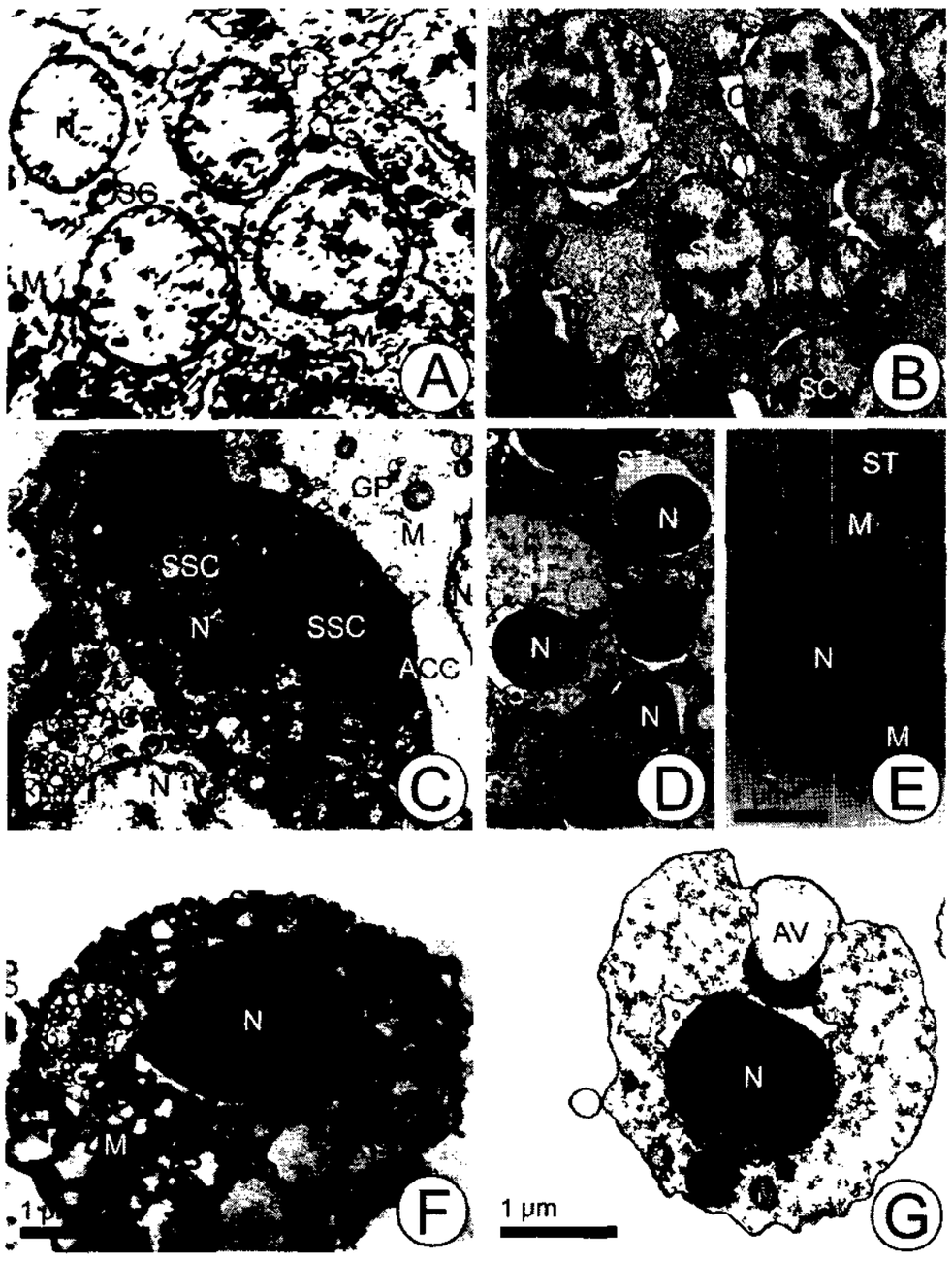

Fig. 2. Transmission electron micrographs of spermatogenesis in male *Spisula sachalinensis*. A, Spermatogonia near the accessory cell, with a nucleolus in the nucleus and the mitochondria in the cytoplasm; B, The primary spermatocytes, with synaptonemal complexes in the nucleus during the prophase of the primary maturation division; C, The secondary spermatocytes and the accessory cells, with glycogen particles, lipid droplets, and the mitochondria in the cytoplasm; D, Spermatids, with high electron dense heterochromatin in the nucleus; E, A spermatid in the early stage of differentiation during spermiogenesis, with the mitochondria and centrosome in the cytoplasm; F, A spermatid in the early stage of differentiation, with acrosomal granule) near the Golgi complex and the mitochondrion beneath the nucleus; G. A spermatid in the middle stage of differentiation during spermiogenesis, with the acrosomal vesicle, the proximal centriole, distal centriole and the mitochondria beneath the nucleus. Abbreviations: ACC, Accessory cell; AG, acrosomal granule; AV, acrosomal vesicle; G, Golgi complex; GP, glycogen particle; LD, lipid droplet; M, mitochondria; N, nucleus; PSC, primary spermatocyte; SC, synaptonemal complex; SG, spermatogonium; SSC, secondary spermatocyte; ST, spermatid.

division. The heterochromatin materials in the nucleus of the secondary spermatocyte are denser and more highly concentrated than those of the primary spermatocytes. At this phase, several mitochondria are present in the cytoplasm of the accessory cell near the secondary spermatocytes (Fig. 2C).

4) Spermatid phase

After the secondary meiotic division, the secondary spermatocyte is transformed into the spermatids with electron-dense heterochromatin materials in the nucleus, and several mitochondria appear in the cytoplasm of the spermatid (Figs. 2D, E). Acrosome formation of the spermatids during spermiogenesis can be simply divided into four phases based on the characteristics of cell organelle differentiation: the Golgi, cap, acrosome, and maturation phases. The morphology of the spermatid changes gradually during the Golgi phase in the differentiation of the spermatid. During this phase, the Golgi complex and small acrosomal granules in the cytoplasm move to a position just in front of the nucleus, while the mitochondria move to a position just behind the nucleus (Fig. 2F). During the cap phase, the morphology of the spermatid nucleus is elongated (Fig. 2G).

During the acrosomal phase, three horn-like acrosomal vesicle (composed of two basal rings and one acrosomal filament) makes contact with the nucleus, and the acrosomal vesicle is then formed by way of morphological changes of the acrosomal vesicle. At this phase, of the two centrioles lying in the midpiece of the spermatozoon, the distal centriole assumes a position behind the proximal and distal centrioles, giving rise to the axial filament of the flagellum of the spermatozoon (Fig. 3A). the mass of four mitochondria (paranucleus) is localized in the midpiece of the spermatid (Fig. 3B). But spermatozoon of this species has not axial rod and satellite body in the midpiece.

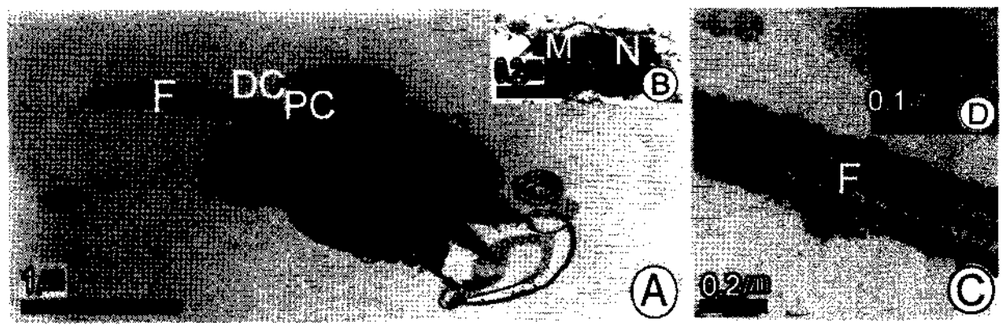

Fig. 3. Electron micrographs of spermatogenesis in male *Spisula sachalinensis*. A, A spermatozoon, with the proximal centriole, and the mitochondria in the midpiece beneath the sperm head and a tail flagellum; B. Cross sectioned midpiece of a spermatozoon, with four mitochondria forming the paranucleus around the centriole; C, Longitudinal sectioned sperm tail flagellum. D, SEM micrographs of the spermatozoa being composed of acrosome, sperm head, and tail flagellum. Abbreviations: AC, acrosome; AV, acrosomal vesicle; BR, basal ring; CM, central microtubule; DC. distal centriole; F, flagellum; M, mitochondria; N, nucleus; PC, proximal centriole; PM, perypheral microtubule; T, tail.

5) Spermatozoon phase

In the maturation phase, the differentiation of spermatozoon is completed and sperm morphology shows the primitive type, as found in species that perform external fertilization. At this time, a cross-sectioned tail flagellum shows that the axoneme of the tail flagellum of the spermatozoon consist of nine pairs of peripheral microtubules at the periphery and one pair of central microtubules at the center. The axoneme of the sperm tail shows a 9+2 structure (Figs. 3C, D).

Reproductive cycle with testicular developmental stage

Based on the morphological features and size of the germ cells and the tissue cells around them, the reproductive cycle with gonadal phases can be classified into five successive stages: (1) early active, (2) late active, (3) ripe, (4) partially spawned, and (5)spent/inactive stages. The stages and the criteria used in defining them are as follows.

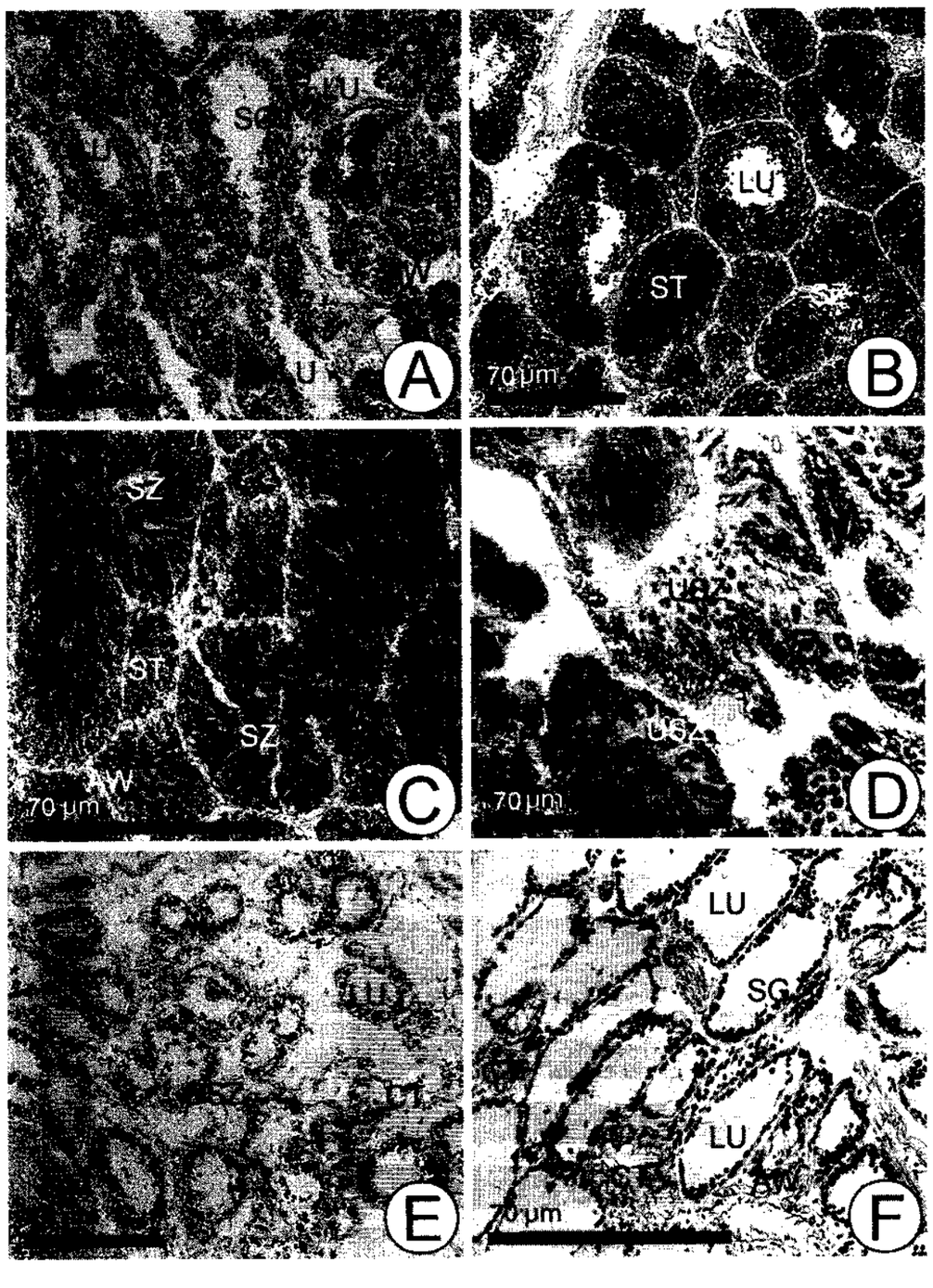

Fig. 4. Photomicrographs of gonadal phases in male *Spisula sachalinensis*.

A. Section of the acini in the early active stage, with spermatogonia and spermatocytes) in the lumen near the acinus wall; B. Section of the acini in the late active stage, with spermatocytes, spermatids (ST), and a few spermatozoa in the lumen; C. Section of the acini in the ripe stage, with spermatids and numerous spermatozoa; D, section of the acini in the partially spawned stage, with partially spawned lumen of the acini. E. Section of the acini in the spent stage, with degenerated spermatozoa in the lumen of acini near the connective tissues; F. Section of the acini in the inactive stage, with the acini containing a few spermatogonia in vacant lumen near the connective tissues. Abbreviations: AW, acinus wall; CT, connective tissue; DSZ, degenerated spermatozoon; LU, lumen; SC, spermatocyte; SG, spermatogonium; ST, spermatid; SZ, spermatozoon; USZ, undischarged spermatozoon.

1) Early active stage

Spermatogenesis occurs in the acini of the testis. The spermatogonia and spermatocytes are about 7-8 μm and 5-7 μm in diameter, respectively.

Compared with the visceral mass, the volume of the testis is small (Fig. 4A). Individuals in the early active stage appear from October to January, when seawater temperatures are very low.

2) Late active stage

Spermatocytes develop into spermatids. The spermatids move toward the center of the lumen, measuring 3-4 μm in diameter, and show layers. As the testis develops, a number of spermatocytes, spermatids, and small number of spermatozoa occupied the lumina in the acini (Fig. 4B). Individuals in the late active stage are found from February to April, the time of the year in which seawater temperatures begin to increase.

3) Ripe stage

A large number of spermatids undergo transformation into differentiated spermatozoa in the center of the lumen. The ripe testis is characterized by the formation of a number of spermatozoa in the center of the lumen (Fig. 4C). Ripe testes are found from April to June, when seawater temperatures are relatively high.

4) Partially spawned stage

A large number of spermatozoa in the acini are discharged into the surrounding water, and the lumen is emptied. However, a number of spermatozoa, as well as spermatids and spermatocytes, still remain in the lumen (Fig. 4D). The spawning period occurs once a year from June and July, and the main spawning occurs in July when seawater temperatures are greater than 15.7° C (Fig. 5).

5) Spent/inactive stage

A small number of undischarged spermatozoa and residual spermatids are degenerated at this stage (Fig. 4E). Thereafter, a few newly formed spermatogonia on the germinal epithelium and connective tissues are rearranged

between the acini in this stage (Fig. 4F). Individuals in this stage appear from August through September, when seawater temperatures rapidly decrease.

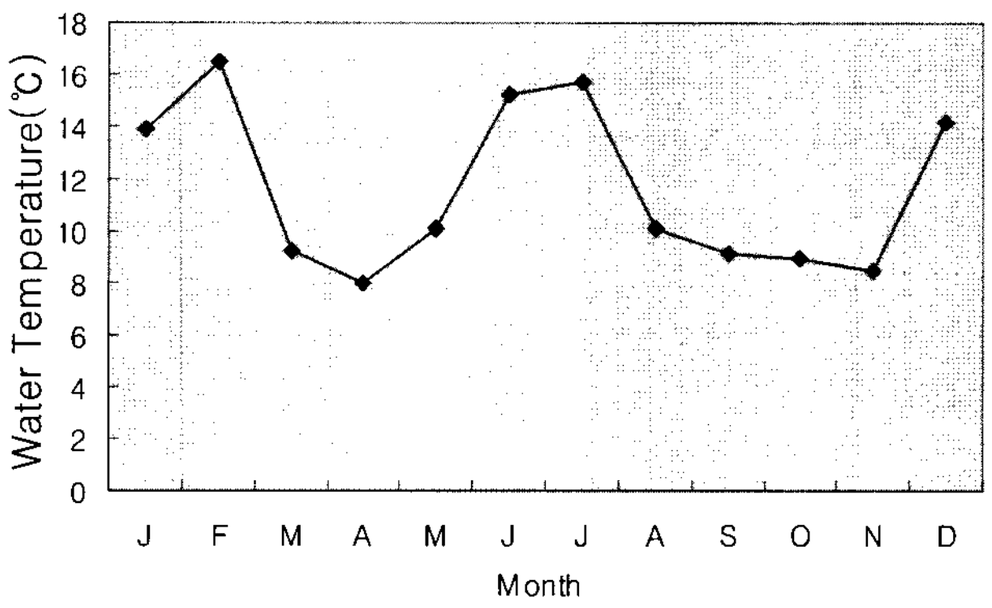

Fig. 5. Monthly changes in the mean seawater temperature at the sampling area from January to December, 2006.

DISCUSSION

Most of the bivalves have a primitive type of spermatozoa with a small head and cap shaped acrosome, and a short mid-piece with four to five mitochondria surrounded the centrioles (Longo and Dornfield, 1967; Chung, 2006; Chung *et al.*, 2007).

Early investigations of bivalve sperm ultrastructure demonstrated the taxonomic value of comparative studies (Franzen, 1983; Eckelbarger and Davis, 1996), and such studies are now widely used in taxonomic analyses. Healy (1989) showed that different subclasses of bivalves each have unique acrosomal morphologies. Regarding molluscan sperm morphology, Franzen (1970) div-

ided molluscan sperm morphology into two types: 1) the primitive type found in species that perform external fertilization, and 2) the modified type found in internal fertilization species.

Also, Verdonk *et al.* (1983) divided sperm morphology into four types: 1) primitive, 2) modified, 3) biflagellate, and 4) aflagellate types. In addition to the primitive type and partially modified type of molluscan sperm, the biflagellate type is seen in the triploid *Corbicula fluminea* and *C. leana* in natural populations (Komaru and Konishi, 1996; Komaru *et al.*, 1997). The aflagellate type is also found in a few crustaceans (Kim, 2001). In this study, *S. sachalinensis* undergoes external fertilization and possesses the primitive spermatozoon type, unlike the modified type found in most gastropods that perform internal fertilization. The acrosome morphology of the sperm head differs markedly among the species (Popham, 1979).

The acrosome shape can be classified into four types: cone, cap, elongate modified cone, and modified cap types. Moreover, the sperm nucleus type varies along molluscan species. Regarding the morphology of the sperm nucleus, Kim (2001) reported that sperm nuclei are cylindrical-shaped in *Septifer virgatus*, some *Mactra* spp., and *Pernidia venulosa*, globe-shaped in *Tersus keenae*, oval-shaped in the Ostreidae, *Pinctata fucata martensii*, and *Atrina pinnata* japonica, jar-shaped in *Solen grandis*, and arrow-shaped in *Corbicula japonica*.

In the present study, the morphology of the sperm nucleus type and acrosomal shape of *S. sachalinensis* are of the globe-shaped and a modified cap-like shape, respectively. Compared with the formation of the acrosomal vesicles in species of other families, one of the morphological or phylogenetical characteristics of Mactridae species is the presence of acrosomal vesicle being composed of two basal rings and axial filament during spermatogenesis. Therefore, we assume that the presence of a special acrosomal vesicle during spermatogenesis could be used as a key characteristic for identification of species of the family Mactidae. Some authors (Chung and Ryou, 2000; Chung *et al.*, 2006) described that the number of mitochondria in the midpiece of the spermatozoon is four in families Ostreidae, Veneridae, Solenidae, Mactridae, and Corbiculidae, while this number is five in the Arcidae, Mytilidae, Pinnidae, and Veneridae. The number of mitochondria in the midpiece of the spermatozoon of *Patinopecten yessoensis* are four, except in *Argopectin irradians*. *Argopectin irradians* has five mitochondria in the midpiece of the spermatozoon. In the present study, we found that there are four mitochondria in the midpiece of the sperm in *S. sachalinensis*. Sometimes, within one species, we assume that the number of mitochondria in the midpiece of the sperm show slight differences in number.

Eckelbarger *et al.* (1990) described that bivalve testes contain accessory (somatic) cells that may play some role in sperm maintenance and nutrition. In an utrastructural study of spermatogenesis in three species of galeommatoidid bivalves they described, two types of accessory cells: the first, "a pleomorphic follicle cell was confined glycogen and lipid deposits, the second was a phagocytic cell that was scattered throught the acinus in close association with developing sperm" . So far, the ultrastructural descriptions of testicular accessory cells are so limited. Sousa *et al.* (1989) reported that accessory cells were observed to be connected to adjacent germ cells via desmosomes in the testes of *Scrobicularia plana*. And these observations show that the interaction

between germ cells and accessory cells. They described very active, phagocytic support cells from the testis of *S. plana*, which are autolysed at the end of spermatogenesis. Gaulejac *et al.* (1995) reported that in *Pinna nobilis* the auxiliary cells with pseudopodia-like projections between germ cells appeared to serve a resorptive function near the end of spermatogenesis. One common feature of these accessory cells is that they appear to have a phagocytic or resorptive function. In the present study, we have easily observed accessory cells near germ cells during spermatogenesis in the acini. However, no junctions were observed between germ cells and accessory cells in *S. sachalinensis*. In particular, glycogen particles and lipid droplets were easily observed in the accessory cells during spermatogenesis as in *M. chinensis* (Chung *et al.*, 2007), therefore, it is assumed that the accessory cells may play some role in nutrition to developing germ cells. But we could not confirmed phagocytic or resorptive functions during the period of germ cell degeneration. Henceforth, we should study the functions of accessory cells during spermatogenesis and germ cell degeneration in detail.

As in most other marine bivalves (Chung *et al.*, 1991; Chung *et al.*, 2006), spermatogonia and spermatocytes appear in the early active stage, and a number of spermatids in spermiogenesis and a small number of spermatozoa appear in the late active stage. Numerous fully-matured spermatozoa appear in the ripe stage, and they are released in the partially spawned stage. After they are discharged, small numbers of residual spermatozoa become degenerated and are resorbed. Thereafter, newly formed spermatogonia on the acini (germinal epithelium) occur in the spent/inactive stage.

Most marine invertebrate have unique breeding patterns. According to Boolootian *et al.* (1962), breeding patterns of mollusks can be classified into three large categories based on their spawning behavior or seasonality: 1) year-round breeders, 2) winter breeders, and 3) summer breeders. According to histological observations of its gonad, the spawning season of *S. sachalinensis* are between June and July in Jumunjin, Korea and between mid April and May in Sendai Bay, Japan (Sasaki, 1981, 1987). Therefore, this species belongs to the summer breeder category.

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REFERENCES

Boolootian, R.A., Farmanfarmain, A. and Giese, A.C. (1962) On the reproductive cycle and breeding habits of two western species of *Haliotis*. *Biological Bulletin*, **122**: 183-192.

Choi, K.C. (1969) Studies on the structure of tidal flat ecosystem for increasing commercial clam yield. *Korean Journal of Limnology*, **2**: 1-21.

Chung, E.Y., Lee, T.Y. and An, C.M. (1991) Sexual maturation of the venus clam, *Cyclina sinensis*, on the west coast of Korea. *Journal of Medical and Applied Malacology*, **3**:

125-136.

Chung, E.Y. and Ryou, D.K. (2000) Gametogenesis and sexual maturation of the surf clam *Mactra veneriformis* on the west coast of Korea. *Malacologia*, 42: 149-163.

Chung, E.Y., Kim, H.J., Kim, J.B. and Lee, C.H. (2006) Changes in biochemical components of several tissue in *Solen grandis*, in relation to gonad developmentalphases. *Korean Journal of Malacology*, 22: 27-38.

Chung, E.Y. (2006) Ultrastructure of germ cells during spermatogenesis and the reproductive cycle in male *Meretrix petechialis* on the west coast of Korea. *Korean Journal of Malacology*, 22: 115-124.

Chung, E.Y., Kim, E.J. and Park G.M. (2007) Spermatogenesis and sexual maturation in male *Mactra chinensis* (Bivalvia: Mactridae) of Korea. *Integrative Biosciences*, 11: 227-234.

Chung, E.Y. and Kim E.J. (2008) Ultrastructure of germ cells and follicle cells during oogenesis and oocyte degeneration, and the ovarian cycle in female *Mactra chinensis* Philippi (Bivalvia: Mactridae) in western Korea. *The Yellow Sea*, 9: 18-26

Eckelbarger, K.J., Bieler, R. and Mikkelsen, P.M. (1990) Ultrastructure of sperm development and mature sperm morphology in three species of commensal bivalves (Mollusca: Galeommatoidea). *Journal of Morphology*, 205: 63-75.

Eckelbarger, K.J. and Davis, C.V. (1996) Ultrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. II. Testis and spermatogenesis. *Marine and Biology*, 127: 89-96.

Franzén, Å. (1970) Phylogenetic aspects of the morphology spermatozoa and spermiogenesis in Baccetti B (ed): "*Comparative spermatology.*" New York: Academic Press, p. 29-46.

Franzén, Å. (1983) Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa. *Gamete Research*, 7: 199-214.

Gaulejac de, J., Jenry, M. and Vicente, N. (1995) An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus, 1758). II. Spermatogenesis. *Journal of Molluscan Study*, 61: 393-403.

Healy, J.M. (1988) Sperm morphology and its systematic importance in the Gastropoda. In: Ponder W. F. (ed) Prosobranch phylogeny. *Malacological Review*, 4: 251-266.

Healy, J.M. (1989) Spermiogenesis and spermatozoa in the relict bivalve genus *Neotrigonia*: relevance to trigonioid relationships, particularly Unionoidea. *Marine and Biology*, 103: 75-85.

Healy, J.M. (1996) Molluscan sperm ultrastructure: correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia. In: Taylor J (ed) *Origin and evolutionary radiation of the Mollusca*. Oxford University Press, London, pp. 99-113.

Kim, J.H. (2001) Spermatogenesis and comparative ultrastructure of spermatozoa in several species of Korean economic bivalves (13 families, 34 species). Ph. D. thesis, Pukyung National University 161pp.

Kim, Y.H. and Ryou, D.K. (1991) Study on the growth of Mactra veneriformis Reeve in the coast of Kunsan. *Bulletin of Gunsan National Fisheries Junior College*, 25: 41-47.

Komaru, A. and Konishi, K. (1996) Ultrastructure of biflagellate spermatozoa in the freshwater clam, *Corbicula leana* (Prime). *Invertebrate Reproduction and Development* 29: 193-197.

Komaru, A., Konishi, K., Nakayama, I., Kobayashi, T., Sakai, H. and Kawamaru, K. (1997) Hermaphroditic freshwater clams in the genus *Corbicula* produce nonreductional spermatozoa with somatic DNA content. *Biological Bulletin*, 193: 320-323.

Lee, J.Y., Chang, Y.J. and Lee, C.S. (1997) Reproductive cycle of surf clam, *Spisula sachalinensis*. *Journal of Korean Fisheries Society*, 30: 132-138.

Lee, J.Y., Chang, Y.J. and Chang, Y.J. (2003) Ultrastructure of germ cell during the gametogenesis in surf clam, *Spisula sachalinensis*. *Journal of Korean Fisheries Society*, 36: 157-162.

Lee Y.L. and Son, Y.W. (1978) Bioassay study of early development of *Mactra chinensis* Philippi for the water quality evaluation of the coastal waters of Busan. *Bulletin of Korean Fisheries Society*, 11: 115-121.

Longo, F.J. and Dornfield , E.J. (1967) The fine structure of spermatid differentiation in the mussel, *Mytilus edulis*. *Journal of Ultrastructural Research*, 20: 462-480.

Popham, J.D. (1979) Comparative spermatozoon morphology and bivalve phylogeny. *Malacological Review*, 12: 1-20.

Ryou, D.K. and Kim, Y.H. (1995) Studies on the food organisms of Bivalve, *Mactra veneriformis* in shore of Kunsan. *Journal of Aquaculture*, 8: 99-115.

Sakai, T. (1976) Notes on the propagation of round clam *Mactra chinensis* Philippi at the west coast in Awaji-island. *Bulletin of the Hyogo Prefectural Fisheries Experimental Station*, 16: 1-8.

Sakurai, I., Kurata, M. and Miyamoto, T. (1992) Breeding season of the sunray surf clam, Mactra chinensis in Tomakomai, Southwest Hokkaido, *Nippon Suisan Gakkaishi*, 58: 1279-1283.

Sasaki, K. (1981) Fecundity of the Sakahalin surf clam, Spisula sachalinensis (Schrenck) in Sendai Bay. *Tohoku Journal of Agricultural Research*, 33: 76-82.

Sasaki, K. (1981) Growth of the Sakahalin surf clam, *Spisula sachalinensis* (Schrenck) in Sendai Bay. *Tohoku Journal of Agricultural Research*, 33: 168-180.

Sasaki, K. (1987) Life cycle in adult stage of the Sakahalin surf clam, in Sendai Bay. *Nippon Suisan Gakkaishi*, 53: 1959-1963.

Sousa, M., Corral, L. and Azevedo, C. (1989) Ultrastructural and cytochemical study of spermatogenesis in *Scrobicularia plana* (Mollusca, Bivalvia). *Gamete Research*, 24: 393-401.

Verdonk, N.H., Van Den Biggelaar, J.A.M. and Tompa, A. S. (1983) *The Mollusca. Vol. 3. Development.* Academic press. New York, 48 pp.