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Ultrastructural Study of Spermatogenesis and Reproductive Cycle of Male Razor Clam, *Solen grandis* on the West Coast of Korea

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한국 서해산 수컷 대맛조개, *Solen grandis*의 정자형성과정의 미세구조적 연구 및 생식주기

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ABSTRACT : Spermatogenesis and reproductive cycle of the razor clam, *Solen grandis*, were investigated monthly by histological and cytological observations. Samples were collected from natural intertidal population at Oshik-do, Kunsan, Korea, for one year, beginning from January to December, 1993. *Solen grandis* is dioecious. Morphological structures of the spermatozoon of this species are similar to those of other bivalve spermatozoa having a primitive type; i. e., a small head, a cap-shaped acrosome and a short mid-piece with four mitochondria surrounding axial filament. The head of spermatozoon is approximately 2 μm in length and sperm tail is about 20 μm long. The axoneme of tail flagellum consists of nine pairs of peripheral microtubules at the periphery and a pair of central microtubules at the center. Four spherical mitochondria form the paranucleus. Spawning occurs once a year between early June and July, and the main spawning was observed in July when seawater temperature reaches above 20℃. The reproductive cycle of male razor clam can be divided into five successive stages; early active (December to January), late active (January to March), mature (March to early August), partially spawned (June to July), and spent/inactive stage (August to December).

Key words : Spermatogenesis, Germ cell development, Reproductive cycle, *Solen grandis*.

요 약 : 1993년 1월부터 12월까지 한국 서해 군산시 오식도 조간대에서 채집한 대맛조개를 대상으로 정자형성과정 및 생식주기를 조직학적 및 투과형전자현미경으로 조사하였다. 대맛조개 (*Solen grandis*)는 자웅이체이다. 본 종의 완숙 정자의 형태 · 구조는 다른 이매패의 정자들이 갖는 원시형 (primitive type)으로 작은 두부와 한 개의 두모첨체를 가지며, 편모축사를 둘러싸고 있는 4개의 미토콘드리아로 이루어진 짧은 중편을 갖는 것이 관찰되었다. 완숙정자 두부의 길이는 대략 2 μm 정도였고, 정자 미부의 길이는 약 20 μm 정도였다. 정자 미부 편모의 axoneme은 중앙의 2개의 미세소관 (microtubule)과 주변에 위치한 9쌍의 미세소관으로 구성되어 있었다. 본 종의 방정기는 6월과 7월 사이로써 주된 방정시기는 해수수온이 20℃ 이상 상승하는 7월에 일어났다. 생식주기는 초기활성기 (12~1월), 후기활성기 (1~3월), 완숙기 (3~8월초), 부분방정기 (6~7월), 퇴화 및 비활성기 (8~12월)의 연속적인 5단계로 구분할 수 있었다.

INTRODUCTION

The razor clam, *Solen grandis* is distributed along the coasts of Korea, China and Japan. More specifically, this species is found in restricted region in the intertidal zone of the south and west coasts of Korea (Yoo, 1976; Kwon et al., 1993), and it is one of the important commercial food bivalves. However, on account of the recent sharp reduction in the standing stock by reclamation works and reckless overcatching, it has been noted as fisheries resources that should be managed and propagated by a more reasonable fishing method. Therefore, it is important to understand some population characteristics in regard to gametogenesis and the reproductive cycle for propagation and artificial control of important natural resources. Previously, there have been some works on classification (Lee, 1956), and on the reproductive ecological aspect (Yoshida, 1939, 1953), the reproductive cycle (Kawahara and Kato, 1971; Chung et al., 1986), sexual maturation (Chung and Kim, 1989) and life history (Kawahara, 1970) of *Solen* species including the larvae and young shell in Korea and Japan. Though

the reproductive ecological aspects of this species and other *Solen* species have been investigated by some authors, especially, no information is available on ultrasructural study of germ cell differentiation during spermatogenesis of this clam. Therefore, the main purpose of this study is to understand germ cell differentiation during spermatogenesis, the reproductive cycle and the spawning period of this species in order to obtain important information for propagation and natural resource management of this clam based on histological and cytological examinations.

MATERIALS AND METHODS

1. Sampling

Specimens of *Solen grandis* were collected monthly from natural intertidal population at Oshik-do, Kunsan, Korea, for one year from January to December, 1993 (Fig. 1). A total of 257 male clams were used for histological and cytological studies. After the clams were transported alive to the laboratory, shell lengths and heights were measured by a Vernier caliper, and their total weight was determined using a chemical balance.

2. Histological and cytological analysis

For the analysis of the gonadal phases by light microscopy, histological preparations were made by subjecting the tissues to standard histological procedures and sectioned at 5~7μm using a rotary microtome. Sections were then mounted on glass slides, stained with either Hansen's hematoxylin-0.5% eosin, Mallory's triple stain or PAS stain, and examined.

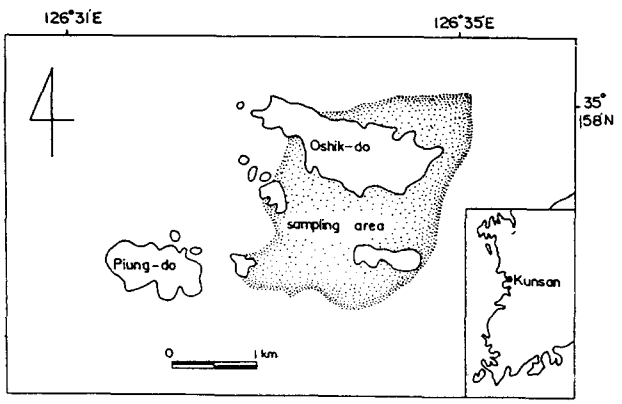

Fig. 1. Location of the sampling area.

For electron microscopical observations, excised pieces of the gonads were cut into small pieces and pre-fixed immediately in 2.5% paraformaldehyde- glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours 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 hour 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

1. Position of the gonad and sex identification

This species is dioecious. The gonads of dioecious *Solen grandis* observed in this study are located between the subregion of the mid-intestinal glands in the visceral cavity and the reticular connective tissues of the foot. The testis comprises several spermatogenic follicles (Fig. 2).

As maturation progresses, external feature of mature testis shows milky white in colour, while the ovary is light brown in colour. At this time, if they are slightly scratched, milky-white sperms and ripe eggs readily flow out. Therefore, their sex of the clams could be distinguishable easily by external features. However, after spawning, gonads degenerate and thus it becomes difficult to distinguish the sex of clams.

2. Electron microscopical observation of spermatogenesis

Based on the testicular development and morphological characteristics of germ cell differentiation, spermatogenesis can be divided into five stages; 1)spermatogonium, 2)primary spermatocyte, 3)secondary spermatocyte, 4)spermatid and 5)spermatozoon stages.

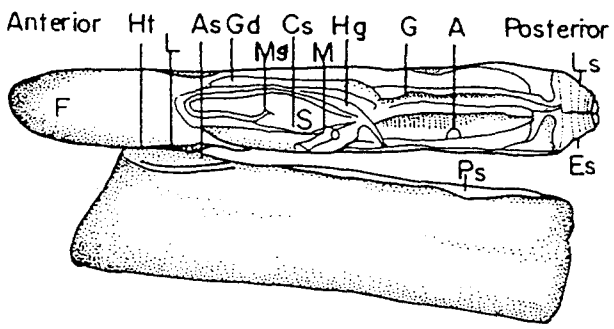

Fig. 2. Anatomy of *Solen grandis*.
A, anus; As, anterior adductor muscular scar; Cs, crystalline style sac; Es, exhalant siphon; F, foot; G, gill; Gd, gonad; Hg, hind gut; Ht, hinge tooth; Is, inhalant siphon; L, ligament; M, mouth; Mg, mid-gut; Ps, post adductor muscular scar; P, palp; S, stomach.

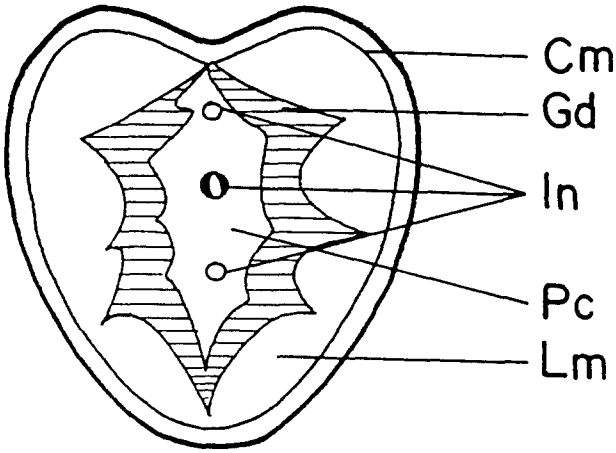

Fig. 3. Schematic cross sectional view of the razor clam, *Solen grandis*.
Cm, circula muscle layer; Gd, gonad; In, intestine; Pc, peritoneal cavity; Lm, longitudinal muscle layer.

1) Spermatogonium stage

Spermatogonia measuring about 7~8 ㎛, each containing a large oval nucleus, are located in the follicular wall of the spermatogenic follicles. The nucleus contains dense, unevenly distributed chromatins. Several mitochondria and rough endoplasmic reticulum are distrbuted throughout the cytoplasm and the undifferentiated auxillary cell locates near the spermatogonia (Fig. 4A).

2) Primary spermatocyte stage

Spermatogonia develop into the primary spermatocytes. The primary spermatocytes, measuring about 5~6 ㎛, have a large nucleus with chromatin and a nucleolus. During the prophase of the first meiotic division, synaptonemal complexes appear in the nucleus of the primary spermatocyte (Fig. 4B).

3) Secondary spermatocyte stage

The primary spermatocytes develop into the secondary spermatocytes by the first maturation division. At this time chromatin becomes more electron-dense in the nucleus (Fig. 4C).

4) Spermatid stage

The second meiotic division of the secondary spermatocytes produces young spermatids measuring about 3~4 ㎛. At this time spermatid nucleus has the typical structure of the nucleus with aggregated heterochromatin. According to the characteristic differentiation of the cell organelles, spermiogenesis can expediently be divided into four phases ; Golgi, cap, acrosome and maturation phases.

The morphology of the spermatid changes gradually during the early Golgi phase in the differentiation of the spermatid. The Golgi apparatus and small acrosomal granules in the spermatid move to a position ahead of the nucleus, while the mitochondria move to a position behind the nucleus of the spermatid (Fig. 4D). Thereafter, during the late Golgi phase, the acrosomal granule and acrosomal vesicle of the spermatid closely aggregates to the tip of the nucleus (Fig. 4E). The small acrosomal granules merge into a larger acrosomal vesicle during the early cap phase in the differentiation of the spermatid, and the large acrosomal vesicle locates around the tip of the nucleus (Fig. 4F). The axial filament is surrounded by four spherical mitochondria which form the paranucleus, and the proximal centriole and distal centriole appear near the mitochondria (paranucleus) (Fig. 4G).

During the late cap phase in the differentiation of the spermatid, the acrosomal granule in the acrosomal vesicle is changed and forml the acrosomal vesicle exhibiting two spines clcsely applied to the tip of the nucleus. Its morphology changes to a cap-shape gradually (Figs. 4H and 4I).

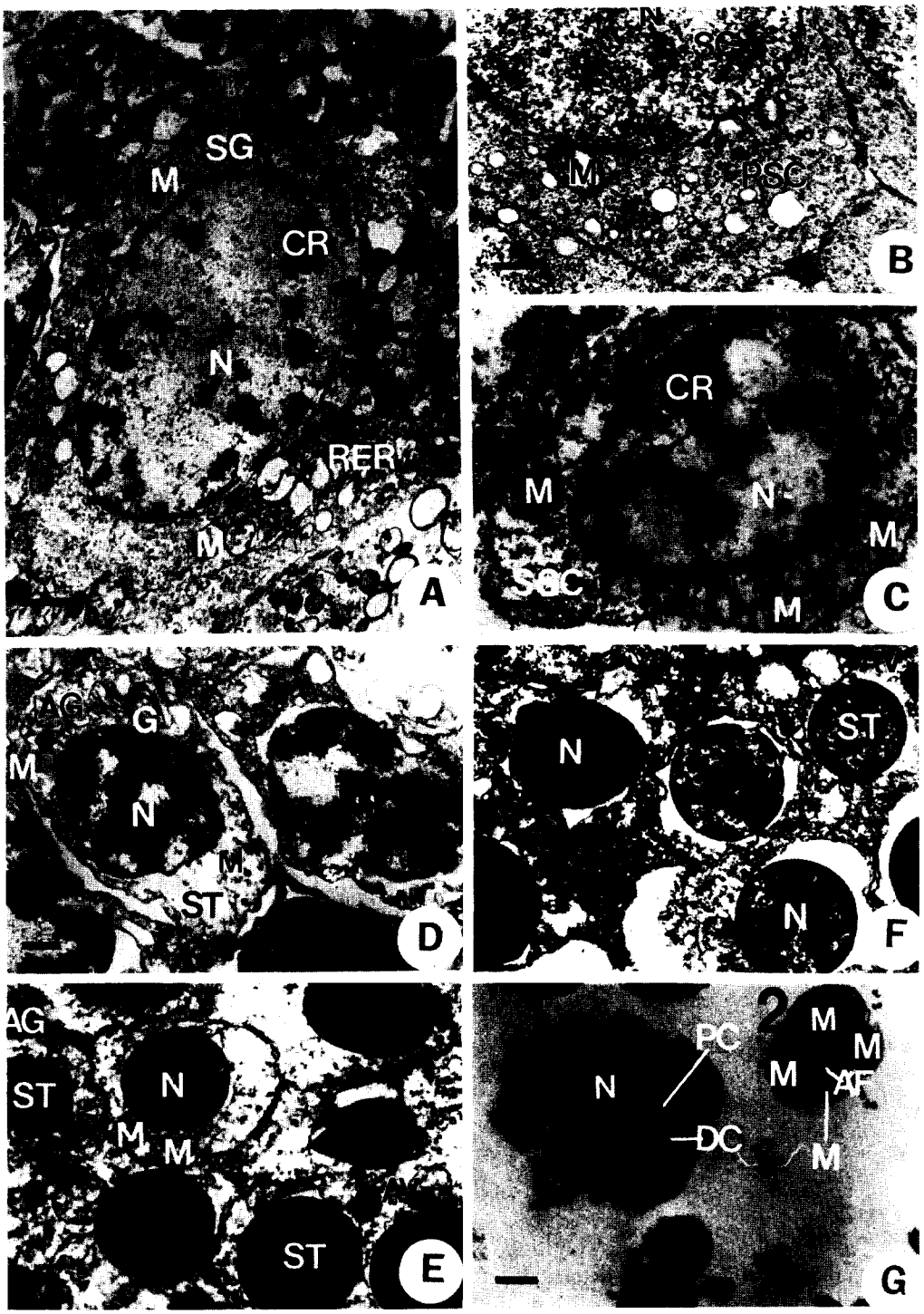

Fig. 4. Electron micrographs of male germ cells of *Solen grandis*.

A, a spermatogonium near the auxillary cell; B, a primary spermatocyte with synaptonemal complexes in the nucleus during the first maturation division; C, a secondary spermatocyte during the second maturation division; D, spermatids during the Golgi phase in the differentiation of the spermatid; E, a spermatid with concentrated heterochromatin; F, spermatids in the early cap phase; G, a spermatid during the spermiogenesis. G-1: a spermatid containing the mitochondria, proximal centriole and distal centriole, G-2: cross sectional view of midpiece of a spermatid containing the axial filament and four mitochondria forming the paranucleus. Abbreviations: AC, auxillary cell; AG, acrosomal granule; AV, acrosomal vesicle; CR, chromatin; DC, distal centriole; G, Golgi apparatus; M, mitochondria; N, nucleus; PSC, primary spermatocyte; PC, proxiaml centriole; RER, rough endoplasmic reticulum; SC, synatonemal complex; ST, spermatid. Scale bars represent 0.5 μm in A-E and G and 1 μm in F.

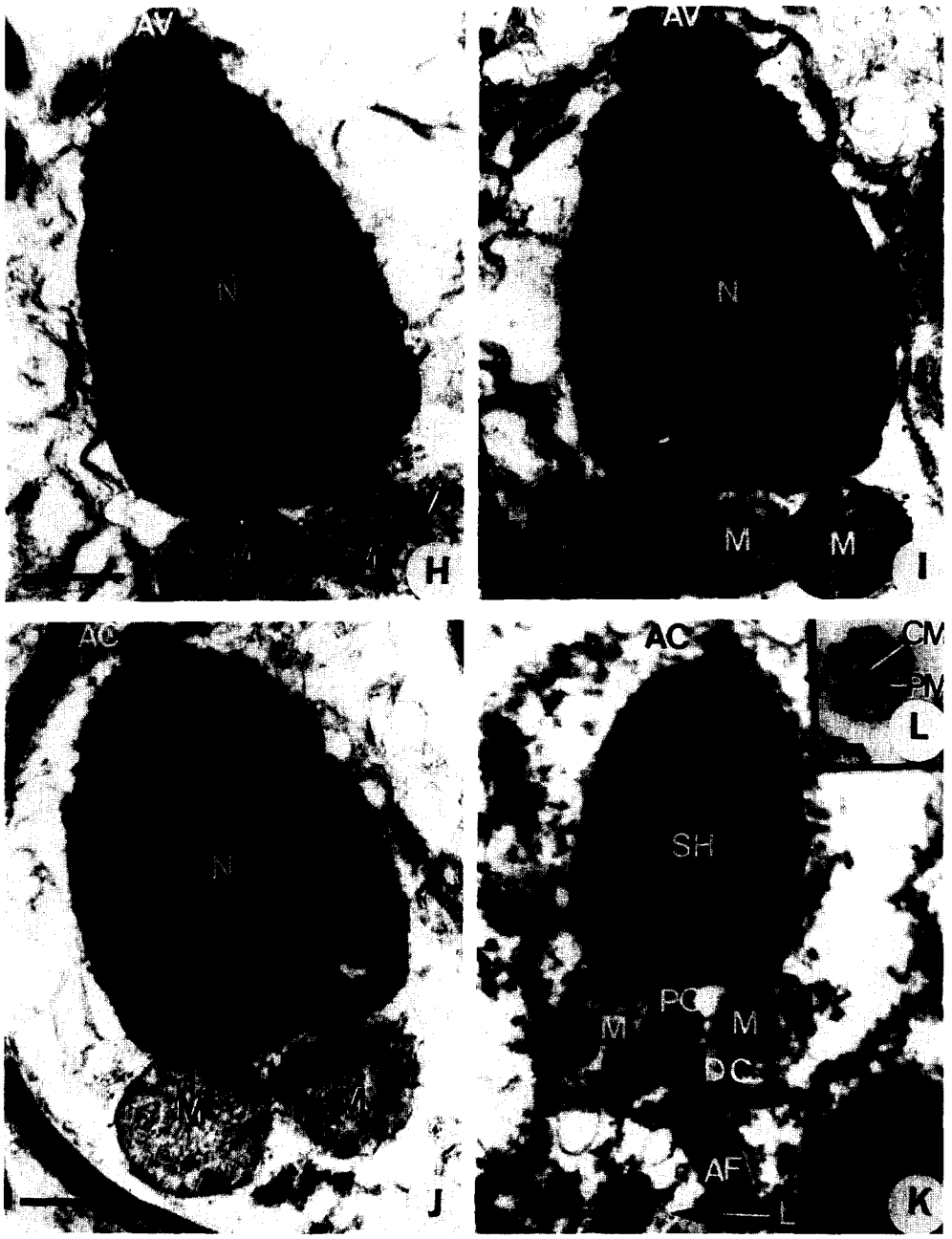

Fig. 4. Continued.

H and I, a spermatid in the late cap phase; J, a spermatid in the acrosome phase; K, a spermatozoon in the spermatozoon stage; L, cross sectioned tail flagellum of a spermatozoon. Abreviations: AF, axial filament; AV, acrosomal vesicle; DC, distal centriole; M, mitochondria; N, nucleus; PC, proximal centriole; SH, sperm head. Scale bars represent 0.5 μm in H-K and 0.1 μm in L.

The acrosomal vesicle changes to an acrosome during the acrosome phase (Fig. 4J).

5) Spermatozoon stage

The mass of mitochondria which later surrounds the mid region of the spermatozoon initially forms the paranucleus around two centrioles. The proximal centriole and the distal centriole appeared near the axial filament of the flagellum during the acrosome and maturation phases. After spermiogenesis, the head of a ripe spermatozoon is approximately 2

μm in length, and sperm tail is about 20 μm (Fig. 4K). The axoneme of the tail flagellum consists of nine pairs of microtubules at the periphery and one pair at the center (Fig. 4L).

3. Light microscopic observation of testis development

Based on the morphological features and sizes of the germ cells and the somatic cells around them, the reproductive cycle with the gonadal phases of the testis can be classified into five successive stages (Fig. 5). The stages and criteria used to define them are the followings.

1) Early active stage

Spermatogenesis occurs in the spermatogenic follicles of the testis. The spermatogonia and spermatocytes locate in the layer along the follicular wall (Fig. 6A). Compared with the visceral mass, the volume of the testis is small. Individuals in the early active stage appear from December to January when seawater temperature is very low (below 7℃).

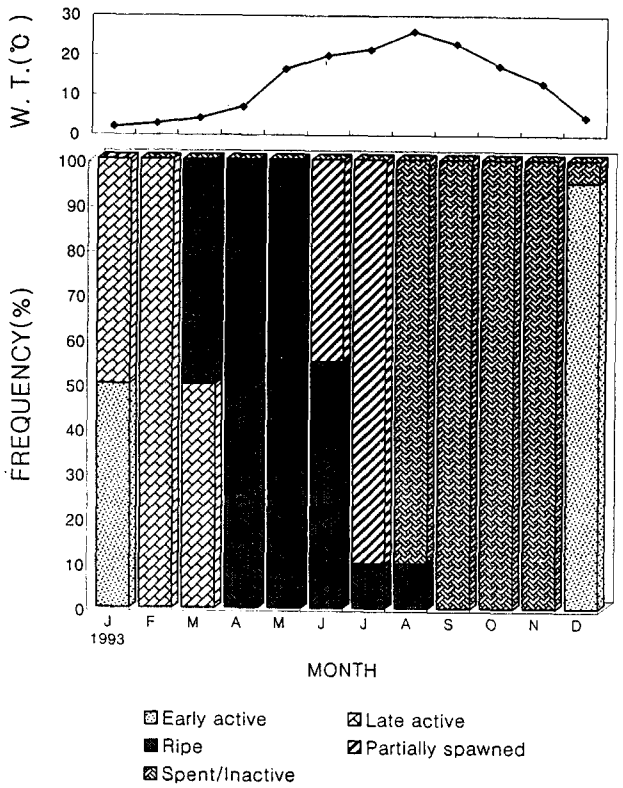

Fig. 5. Frequency of gonadal phases of *Solen grandis* and the monthly seawater temperature (W. T.) from January to December, 1993.

2) Late active stage

Spermatocytes develop into spermatids. The spermatids move toward the center of the lumen, and show the layers. As the testis develops, a dense area of spermatocytes and spermatids occupy approximately one-third to one-half of the lumen in the spermatogenic follicles (Fig. 6B). Individuals in the late active stage are found from January to March when seawater temperature gradually rises.

3) Ripe stage

A few spermatids begin to undergo transformation into differentiated spermatozoa in the center of the lumen. The ripe testis is characterized by the formation of a number of spermatozoa (Figs. 6C and 6D). Mature and ripe gonads are found in individuals from March to August when seawater temperature is relatively high (13-26.4℃).

4) Partially spawned stage

During spawning, a large number of spermatozoa in the spermatogenic follicles are discharged into the surrounding water, and the lumen becomes empty. However, a significant number of spermatozoa, as well as spermatids and spermatocytes, still remain in the lumen (Fig. 6E). Spawning occurs once a year from early June to July, but mostly occurs in July when seawater temperature is higher than 20℃.

5) Spent / Inactive stage

The remaining spermatozoa and spermatids undergo degeneration (Fig. 6F) at this stage. Thereafter, rearrangement consisting of newly formed connective tissues occurs in the spermatogenic follicles at this stage. Individuals at this stage are found in from August to December when seawater temperature lowers gradually.

DISCUSSION

1. Spermatogenesis

Most bivalves have a primitive type of spermatozoa with a small head and cap-shaped acrosome, a short mid-piece

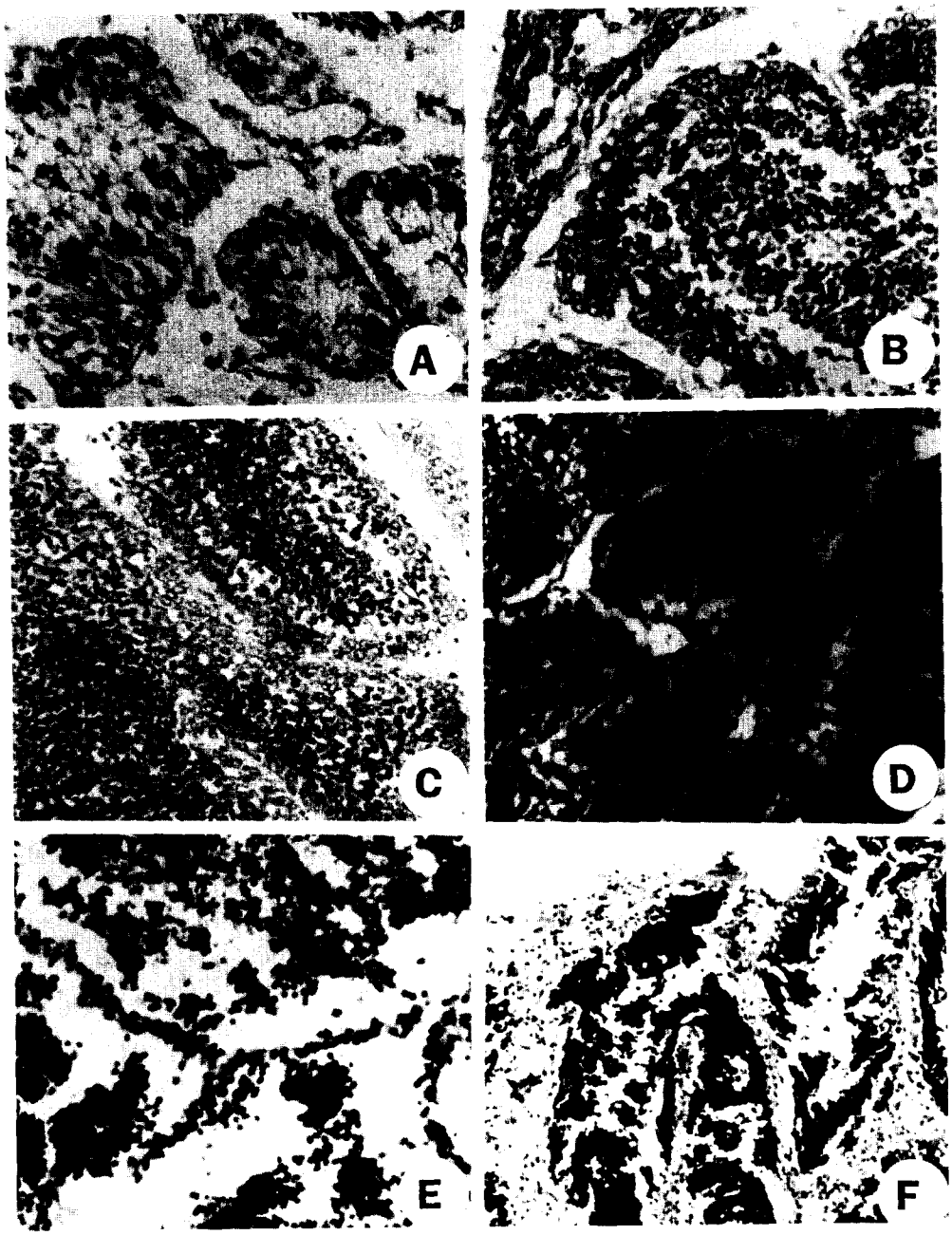

Fig. 6. Gonadal phases of male *Solen grandis* observed under the light microscope.
A, Spermatogenic follicles in the early active stage; B, follicle of the testis in the late stage; C and D, sections of the follicles in the ripe stage; E, follicles of the testis in the partially spawned stage; F, follicles of the testis in the spent /inactive stage. All magnifications, ×300.

with four to five mitochondria surrounding the axial filament (Longo and Dornfield, 1967; Chung et al., 1991; Chung, 1997). In the present study, it was observed that the morphological structures of the spermatozoa of *Solen grandis* found in the west coast of Korea were similar to those of other bivlave spermatozoa having a cap-shaped acrosome, a short mid-piece with four mitochondria surrounding the axial filament. In the mid-piece of the spermatozoon, it is already known that the number of mitochondria varied with species (Franzen, 1983). Number of mitochondria present in sperms of *Mytilus galloprovincialis* is five to six (Hodgson and Bernard, 1986) and that of

Crassostrea virginica (Daniels et al., 1971) and *Mactra chinensis* (Chung, 1997) is four, respectively, while sperm of *Mytilus perma* has five mitochondria or very rarely four (Bourcart et al., 1965). Therefore, the difference in the number of mitochondrium among clams doesn't necessarily mean that either one is abnormal or not.

2. Gonad development and maturation

General development and maturation of gonads in bivalves are closely related to environment factors; water temperature (Sastry, 1966, 1968) and food organism (Griffiths, 1977; Chung et al., 1991). In the present study, it was observed that gonad development and maturation of *Solen grandis* occurred in from late spring to summer when the seawater temperature was high enough and the number of planktons available for their food was also abundant. But gonadal development was in the inactive stage when the seawater temperature was low and there was insufficient food organisms. These observations coincide well with others' reports mentioned above.

3. Breeding and spawning

In general, most marine molluscs have their own special characteristics of breeding habits, and they can be divided into three large categories; 1) year-round breeders, 2) winter breeders, and 3) summer breeders (Boolootian et al., 1962). In this study, spawning of this species was found to occur in from early June to July. Therefore, this species belongs to the category of summer breeders.

Although spawning seasons of *Solen species* in most locations in Korea are May, June or July. However, their main spawning season is not the same depending on the seasonal changes (Chung et al., 1986; Chung and Kim, 1989). Some local variations of the spawning period might be related to the geographical differences having different water temperatures (Chang and Lee, 1982), food availability (Chung and Kim, 1989) and some other environmental factors (Chung et al., 1991; Chung, 1997).

4. Fate of germ cell

After spawning, resorption of undischarged spermatozoa is commonly found in the spermatogenic follicles of the testis of most bivalves. Hopeless gametes remaining in the follicles of gonads are believed to be resorbed by way of the gamete atresia for reproductive energy resorption (Xie and Burnell, 1994). Therefore, it is suggested that the Korean clam has a similar mechanism to resorb and utilize the highly nutritive reserves for allocation of the reproductive energy for developing oocytes or to use for other physiological and metabolic purposes (Dorange and LePennec, 1989).

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