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Ultrastructural Study on Spermatogenesis and Sexual Maturation of the Male Jicon Scallop, *Chlamys farreri* on the West Coast of Korea

Ee-Yung Chung, Ki-Yeol Park[1] and Pal-Won Son[2]

School of Marine Life Science, Kunsan National University, Gunsan 573-701, Korea
[1] Shellfish Research Center, National Fisheries and development Institute, Sangju 668-821, Korea
[2]West Sea Fisheries Research Institute, Inchon 400-420, Korea

ABSTRACT

Gonadosomatic index, reproductive cycle, spermatogenesis and first sexual maturity of *Chlamys farreri* were investigated by cytological and histological observations, from January 1998 to December 1999. The gonadosomatic index (GSI) rapidly increased in April and reached a maximum in May when seawater temperature rapidly increase. Then the GSI gradually decreased from June to August when spawning occur. Accordingly, monthly changes in the GSI in males coincide with the reproductive cycle. The spermatozoon of *Chlamys farreri* is the primitive type found in external fertilization species.

The head of the spermatozoon is approximately 2.75 μm in length including the acrosome measuring about 0. 50 μm in length, and its tail was approximately 20 μm, the axoneme of the tail flagellum consists of nine pairs of microtubules at the periphery and a pair at the center. Five spherical mitochondria around the centriole (the satellite body) appear in the middle piece of the sperm.

The spawning period was from June to August and the main spawning occurs from July to August when seawater temperatures are greater than 20°C.

The reproductive cycle of this species can be categorized into five successive stages: early active stage (January to March), late active stage (March to April), ripe stage (April to August), partially spawned stage (June to August), and spent/inactive stage (August to January). Over 50% of male scallops attained first sexual maturity between 50.0 and 60.0 mm in shell height, and 100% of those over 60.0 mm in shell height achieved maturity. Accordingly, we assume that male individuals begin reproduction at three years of age.

Keywords: Spermatogenesis, Sexual maturation, *Chlamys farreri*.

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Corresponding author: Chung, Ee-Yung
Tel: (82) 63-469-4592 e-mail: eychung@kunsan.ac.kr
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INTRODUCTION

The Jicon scallop, *Chlamys farreri*, is one of the important edible bivalves in East Asian countries including Korea, China and Japan (Kwon *et al.*, 1993). On the west coast of Korea, this species is mainly found in silty sand in the subtidal zone of Heuksando, Jeollanam-do, Korea (Yoo, 1976; Kwon *et al.*, 1993), and it inhabits in depth up to 50-90 m. Due to the recent sharp reduction in the standing stock as a consequence of reckless overharvesting of this scallop, it has been denoted a target organism and fisheries resource that should be managed using a more reasonable fishing regime. For the propagation and management of a living natural resource, it is the important to understand its population characteristics with regard to spermatogenesis and testicular development. Previously, there have been many studies on reproductive aspects including reproductive cycle (Lioa *et al.*, 1983; Yakovlev and Afeichuk, 1995), growth and spawning (Na *et al.*, 1995; Kang and Zhang, 2000), triploid and tetraploid (Yang *et al.*, 1999a), and on ecological aspects including distribution and ecology (Whang and Kim, 1973),

larval growth (Kuang *et al.*, 1997; Yang *et al.*, 1999b), aquaculture experiment (Lim *et al.*, 1995; Sun *et al.*, 1996, 1997) of this species.

Although there have been many studies on reproductive ecology of this species, there are still gaps in our knowledge regarding reproductive biology. In particular, little information is available on reproductive biology such as germ cell differentiation during spermatogenesis and first sexual maturity except for the reproductive cycle of this species. For the study of reproductive mechanism of reproductive biology, above all, it is necessary to study germ cell differentiation during spermatogenesis. Understanding the reproductive cycle and the spawning period of *Chlamys farreri* will be useful information for age determination and the recruitment period of this population. In addition, data for first sexual maturity would be very useful information for the propagation and management of living natural resource of this species.

Therefore, the purpose of the present study is to understand germ cell differentiations during spermatogenesis, the reproductive cycle with testicular developmental stages, first sexual maturity and some basic information for the propagation and management of the male Jicon scallop, using cytological, histological methods and morphometric data.

MATERIALS AND METHODS

1. Sampling

The male Jicon scallop, *Chlamys farreri* was collected monthly from the subtidal zone at Daehuksando, Jeollanam-do, Korea, from January 1998 to December 1999 (Fig. 1). A total of 329 scallops ranging from 31.0 to 100.4 mm in shell height were used for the study. After the scallops transported alive to the laboratory, the sizes of the specimens were recorded using a Vernier caliper, and total wet weights of tissues were weighed using a top-loading electrical balance.

2. Gonadosomatic index (GSI)

A total of individuals were used to calculate the gonadosomatic index (GSI). Monthly changes in the mean GSI were calculated using the following equation:

GSI = Gonad weight (g) × 100 / Meat weight (g)

3. Ultrastructure of germ cells during spermatogenesis

For electron microscope observations, excised pieces of the testis were cut into small pieces and fixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2h at 4℃. After prefixation, the specimens were washed several times with the same buffer (pH 7.4) for 1h at 4℃, and then fixed in 1% osmium tetroxide dissolved in 0.2 M phosphate buffer solution (pH 7.4) for 1h at 4℃. The tissues were dehydrated in a series of increasing concentrations of ethanol, cleared in propylene oxide and embedded in Epon-Araldite mixture. Ultrathin sections of the Epon-embedded specimens were cut with glass knives using a Sorvall MT-2 microtome and a LKB ultramicrotome, at thickness of 800-1000 Å. The tissue sections were mounted on collodion-coated copper grids, double stained with uranyl acetate followed by lead citrate, and observed under a JEM 100 CX-2 (80 kv) electron microscope.

4. Histological analysis

A total of 243 males were used for histological preparation of the testes for light microscopic

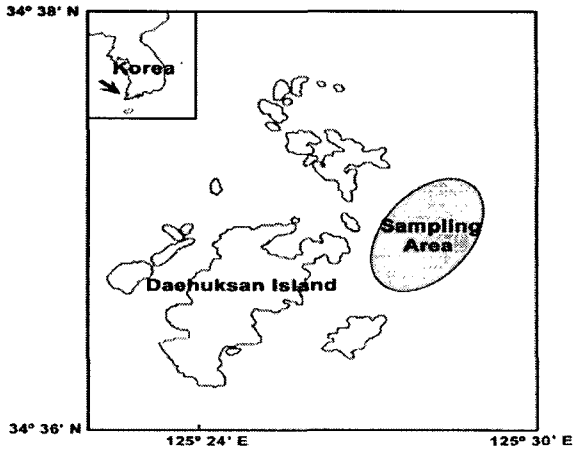


Fig. 1. Map showing the sampling area.

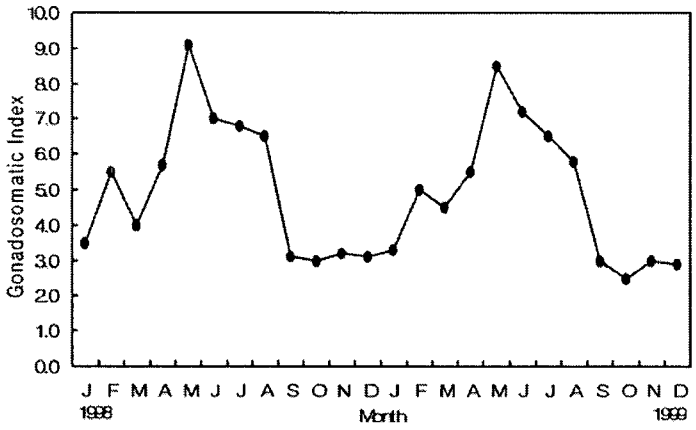


Fig. 2. Monthly changes in the gonadosomatic index in male *Chlamys farreri*.

examination, from January 1998 to December 1999. Testicular tissues were subjected to standard histological procedures (dehydration in alcohol series and embedding in paraffin). The embedded tissues were sectioned at 5-7 μm thickness using a rotary microtome. The sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin and Mallory's triple stain, and examined using a light microscope.

5. First sexual maturity

The percentages of first sexual maturity were investigated with the histological preparations to certify shell heights of specimens that reached maturity and participated in reproduction during the ripe and breeding seasons from May through October, 1998. A total of 94 scallop ranging from 31.4 to 100.4 mm in shell heights were used for the study of first sexual maturity.

RESULTS

1. Position and Morphology of the testis

Chlamys farreri is a dioecious and oviparous species. Morphology of the testis is conical or crescent in shape, and it is separated from the digestive diverticula and the adductor muscle. The testis is located downward of the adductor muscle. With testicular maturation, the testis encircle the adductor muscle in part, and the external color of the testis become milky white or light yellow (the ovary pink). Therefore, their sexes of the scallop can be distinguishable easily by external features. At this time, if the ovary is slightly scratched, ripe sperms readily flow out. After spawning, the testis degenerate, and then the sex becomes difficult to distinguish.

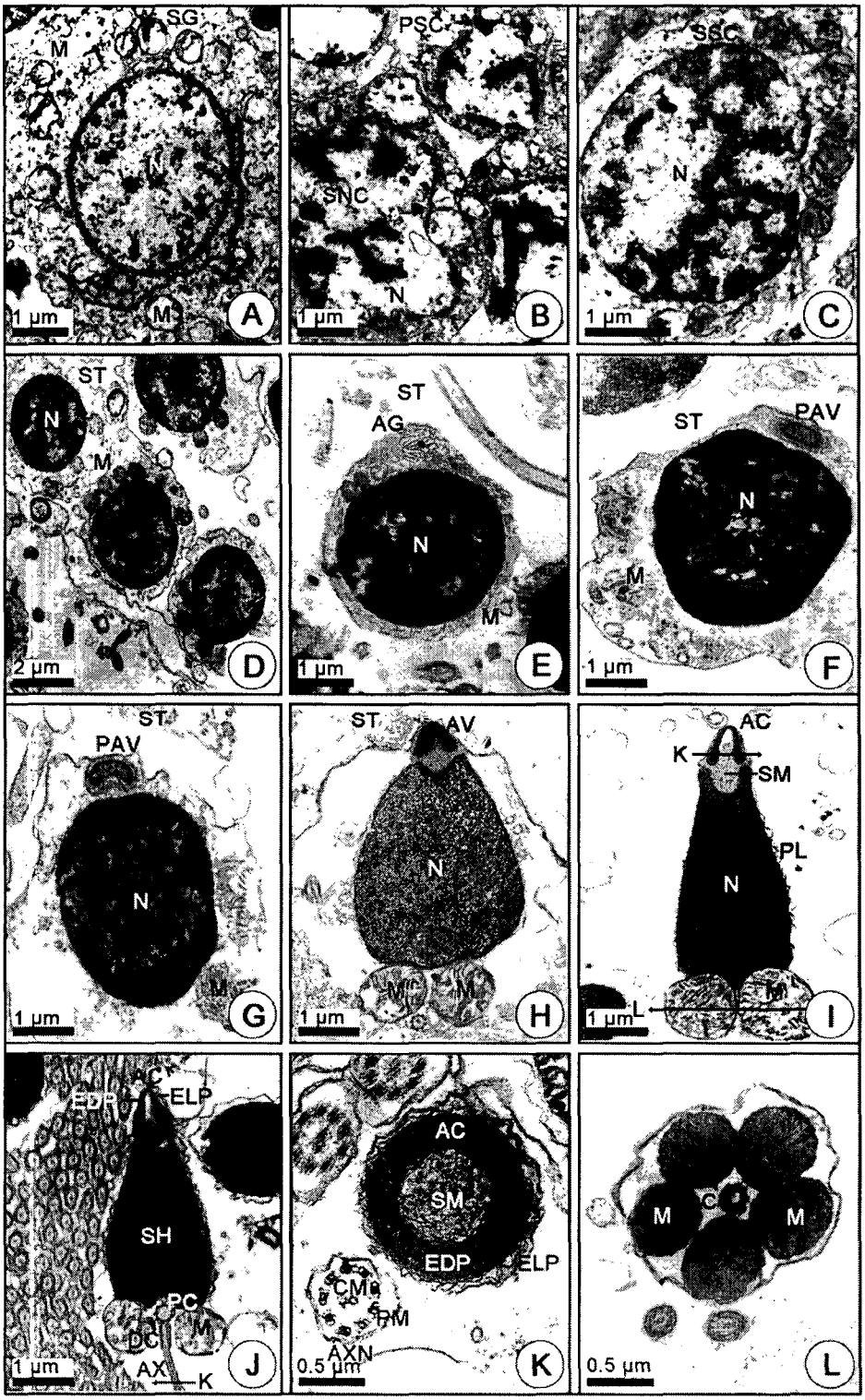
2. Gonadosomatic index (GSI) in males

Monthly GSI changes in males from January 1998 to December 1999 are shown in Fig. 2. In 1998, the GSI rapidly increased from April, and reached a maximum (9.1) in May when seawater temperature rapidly increased. Then the GSI gradually decreased from June to August when relatively high water temperatures was maintained, and spawning occurred continuously. Thereafter, the value temporally reached the minimum (3.0) in October when spawning was completely finished.

The monthly GSI changes in 1999 showed similar patterns to those in 1998.

3. 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) spermatogonial phase, (2) primary spermatocyte, (3) secondary spermatocyte, (4) spermatid, and (5)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spermatozoon phases.

1) Spermatogonial phase

The spermatogonia are located near the auxiliary cells. They are approximately 9 – 10 μm in diameter and more or less oval shaped. Each of them contains a large nucleus with electron dense chromatin. (Fig. 3A).

2) Primary spermatocyte phase

Spermatogonia differentiate into primary spermatocytes. The nucleus of the primary spermatocyte contains slightly more denser chromatin. The synaptonemal complexes in the nucleus appear in the prophase during the first maturation division. Several mitochondria appear in the cytoplasm (Fig. 3B).

3) Secondary spermatocyte phase

The primary spermatocyte develops into the secondary spermatocyte through the first maturation division. The heterochromatin in the nucleus of the secondary spermatocyte showed more concentrated and denser than that of the primary spermatocyte. In this phase, several mitochondria are present in the cytoplasm (Fig. 3C).

4) Spermatid phase

After the secondary meiotic division, the secondary spermatocyte is transformed into the spermatid with electron dense heterochromatin materials in the nucleus, and several mitochondria appear in the cytoplasm (Fig. 3D). Spermiogenesis can expediently be divided into four phases based on the characteristics of cell organelle differentiations: Golgi, cap, acrosome and maturation phases. The morphology of the spermatid changes gradually during the Golgi phase in the early differentiation of the spermatid. At this phase the Golgi complex and small acrosomal granules in the spermatid move to the position of the nucleus, while the mitochondria move to a position just behind the nucleus (Fig. 3E). During the cap phase, morphology of the nucleus is elongated, and the granule in the proacrosomal vesicle at the end of the nucleus is gradually changed and formed a slightly larger acrosomal vesicle. At this phase, especially the mid-piece (which are surrounded by the two or more mitochondria) appears (Fig. 3F-H). Five spherical mitochondria formed the paranucleus around the centrosome (Fig. 3L).

The acrosomal vesicle changes into an acrosome during the acrosome phase. The sperm nucleus and acrosome are 2.75 μm and 0.50 μm, respectively. The

Fig. 3. Electron micrographs of spermatogenesis of *Chlamys farreri* (A-L). **A**: Section of spermatogonia, with a large nucleus with chromatin; **B**: the primary spermatocytes during the meiosis, with synaptonemal complex in the nucleus and several mitochondria during the prophase of the primary maturation division; **C**: the secondary spermatocyte, with gradually condensed heterochromatin in the nucleus; **D**: spermatids in the early stage of differentiation during spermiogenesis, with condensed heterochromatin in the nucleus and several mitochondria in the cytoplasm; **E**: spermatids in the Golgi phase during spermiogenesis, with the Golgi complex and acrosomal granule on the nucleus and several mitochondria just behind the nucleus; **F & G**: spermatids in the cap phase during acrosome formation, with the proacrosomal vesicle in the cytoplasm on the nucleus; **H**: a spermatid in the middle stage of differentiation during spermiogenesis, with the acrosomal vesicle on the nucleus and the mitochondria just behind the nucleus; **I**: transformations of the acrosome in the late stage (acrosome phase) of differentiation, with the acrosome and subacrosomal material on the nucleus; **J**: a completed spermatozoa in the maturation phase, with the acrosome (being composed of electron dense part and electron lucent part) and subacrosomal material between the acrosome and the sperm head; **K**: cross sectioned acrosome and tail of the sperm, with subacrosomal material, acrosome (which is composed of electron dense part and electron lucent part) and the axoneme of the sperm tail showing 9+2 structure; **L**: cross sectioned the middle piece of the sperm, with the satellite body and five mitochondria being composed of the paranucleus.
Abbreviations: AC, acrosome; AG, acrosomal granule; AV, acrosomal vesicle; AX, axial filament; AXN, axoneme; C, centriole; CM, central microtubule; DC, distal centriole; EDP, electron dense part; ELP, electron lucent part; M, mitochondrion; N, nucleus; PAV, proacrosomal vesicle; PC, proximal centriole; PL, plasma membrane; PSC, primary spermatocyte; SG, spermatogonium; SH, sperm head; SM, subacrosomal membrane; SNC, synaptonemal complex; SSC, secondary spermatocyte; ST, spermatid.

sperm nuclear type is vase in shape, and the acrosome type shows cone type. The acrosome is composed of two parts with the density of the acrosome: 1) the acrosome with electron dense part in its top or front of the acrosome, 2) subacrosomal materials with low electron dens granules between the nucleus and front part of the acrosome (Fig. 3I).

A gap between the nucleus and acrosome was observed. At this time, of the two centrioles lying in the middle piece of the spermatozoon, the distal centriole take a position behind, and the proximal centriole and the distal centriole give rise to the axial filament of the flagellum of the spermatozoon (Fig. 3J). During the acrosome phase, a cross sectioned tail flagellum shows that the axoneme of the tail flagellum of the spermatozoon consists of nine pairs of peripheral microtubules at the periphery, and one pair of central microtubules at the center (Fig. 3K). After the acrosome formation is completed, a prominent subacrosomal material surrounded by the nucleus is present at a part of cross sectioned sperm head near the acrosome (Fig. 3K). The satellite body (which is composed of the centriole) and five mitochondria appear in the mid-piece (Fig. 3L).

5) Spermatozoon phase

During the maturation phase, the spermatozoon differentiation is completed, The head of a spermatozoon is approximately 2.8 μm in length including the acrosome measuring about 0. 50 μm in length, and its tail is approximately 20 μm (Fig. 3J).

4. 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 (Fig. 4). The stages and the criteria used in defining them are as follows:

1) Early active stage

Spermatogenesis occurs in the acini of the testis.

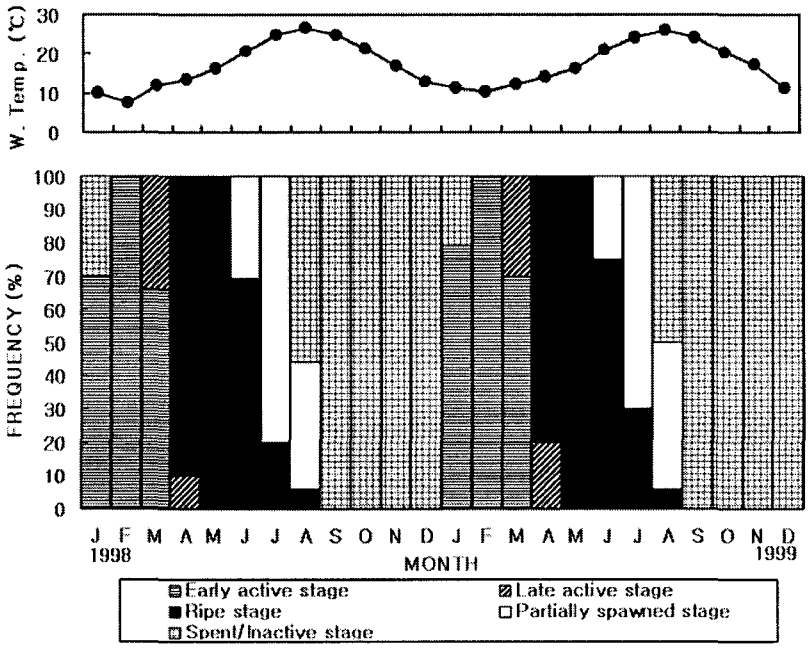

Fig. 4. Frequency of gonadal phases in male *Chlamys farreri* and the mean seawater temperature from January 1998 to December 1999.

The spermatogonia and spermatocytes are 7–8 μm and 5–7 μm in diameter, respectively. The spermatogonia and spermatocytes appear along the germinal epithelium (Fig. 5A). At this time, the volume of the testis is small. The individuals in the early active stage appear from January to March when seawater temperatures are very low.

2) Late active stage

Spermatocytes develop into spermatids. The spermatids moved 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 occupy approximately one–third to one–half of the lumina in the acini (Fig. 5B). Individuals in the late active stage are found between March and April when seawater temperature begins to increase.

3) Ripe stage

A large number of spermatids undergo transformation into differentiated spermatozoa in the

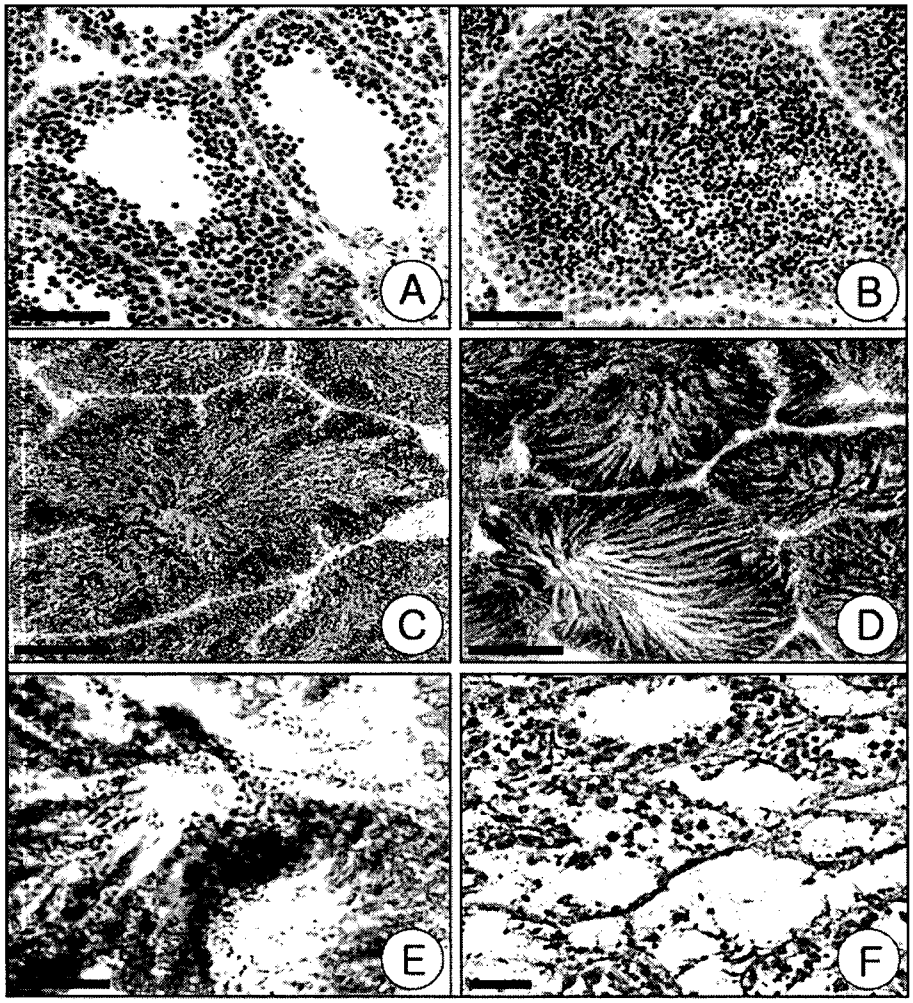

Fig. 5. Gonadal phases in male *Chlamys farreri* as seen by light microscopy. **A**: transverse section of the acini in the early active stage; **B**: section of the acini in the late active stage; **C** & **D**: sections of the acini in the ripe stage; **E**: section of the acini in the partially stage; **F**: section of the acini in the spent and inactive stage.

center of the lumen. The ripe testis is characterized by the formation of a number of spermatozoa in the center of the lumen (Fig. 5C, D). Mature and ripe testes are found from April to August when seawater temperature is relatively high.

4) Partially spawned stage

A large number of spermatozoa in the acini are discharged into the surrounding water, and the lumen becomes empty. However a number of spermatozoa, as well as spermatids and spermatocytes, still remain in the lumen (Fig. 5E). The spawning period occurs from June to August, and the main spawning occurs from July to August when seawater temperature is greater than 20°C.

5) Spent/inactive stage

A few remaining spermatozoa and spermatids are degenerated. Thereafter, the rearrangement of a few newly formed connective tissues occurs in the acini in this stage (Fig. 5F). The individuals in this stage appeared from August to January when seawater temperature decreased gradually.

5. First Sexual Maturity

First sexual maturities of a total of 94 male individuals of *Chlamys farreri,* ranging from 31.0 to 100.4 mm in shell height, were investigated histologically in order to certify shell heights of scallop participating in reproduction from April to August. As shown in Table 1, from the results of histological observations, it was found that although the specimens were collected during the breeding season, gonadal development of smaller individuals, ranging from 31.0 to 40.9 mm in shell height, were in the early active stage, as spermatogonia and spermatocytes were present in the acini of the testis. Judging from histological observations, the group in this size cannot be considered to have reached maturity until late August when spawning was finished. The percentage of first sexual maturity of male scallop ranging from 40.0 to 50.0 mm in shell height is 38.5%. But, gonadal development of those were in the early active, late active or ripe stages during the breeding season. Percentages of first sexual maturity of individuals in 50.0 to 60.0 mm in shell height group that are composed of individuals being in the late active, ripe and partially spawned stages, was 64.3%, and 100% in clams over 60.0 mm in shell height group that were in the late active, ripe and partially spawned, and spent/inactive stages. Accordingly, it is assumed that most individuals can reach to maturity before late August if they are larger than 60.0 mm in shell height.

DISCUSSION

Spermatogenesis in *Chlamys farreri* is very similar

Table 1. Number of male *Chlamys farreri* at each gonadal stage in their first sexual maturing period from May through October, 1999.

Shell length (mm)	No. clam	Number of individuals by gonadal stage*					Percentage of matured clam
		EA	LA	RI	PS	SP/IA	
31.4-40.0	16	16					0
40.0-50.0	13	8	2	3			38.5
50.0-60.0	14	5	2	5	2	1	64.3
60.0-70.0	15		3	6	5	1	100.0
70.0-80.0	17		3	9	4	1	100.0
80.0-90.0	14		2	7	3	2	100.0
90.0-100.4	5			3	1	1	100.0
sum	94	29	12	33	15	6	

*Gonadal stage: EA, early active stage; LA, late active stage; RI, ripe stage; PS partially spawned stage; SP/IA, spent/inactive stage.

to that of other bivalves that undergo external fertilization (Chung and Ryou, 2000). However, fine structural differences in molluscan sperm structures, which are associated with the evolution of th species, are sometimes used as criteria for classification (Popham, 1979).

Franzen (1970) divided molluscan sperm morphology into two types: 1) the primitive type found in external fertilization species and 2) the modified type found in internal fertilization species. 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, a biflagellate type is seen in the triploid *Corbicula fluminea* and *C. leana* in natural populations (Komaru and Konishi, 1996; Komaru *et al.*, 1997: Choi, 2004). An aflagellate type is also found in a few crustacean (Kim, 2001). *Chlamys farreri* undergoes external fertilization and possesses the primitive type of spermatozoon, unlike the modified type found in most internal fertilization gastropods.

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 vary with molluscan species. In the present study, the morphologies of the sperm nucleus type and acrosome shape of *Chlamys farreri* and other Pectinidae scallops except for *Argopecten irradians* are vase type and cone type, respectively (Kim, 2001). Kim (2001) reported that the sperm nuclei are cylindrical in *Septifer virgatus* and some *Mactra* spp. and *Pernidia venulosa*; global in *Spisula sachalinesis* and *Tersus keenae*; ovoid in the Ostreidae, *Pinctata fucata martensii*, and *Atrina pinnata japonica*; vase shaped in *Solen grandis* and arrow shaped in *Corbicula japonica*.

Kim (2001) described that the number of the mitochondria in the middle piece of the spermatozoon was four in the families Ostreidae, Veneridae, Mactridae, Solenidae and Corbiculidae, while five in the Arcidae, Mytilidae, Pinnidae and Veneridae. Especially, the Pectinidae such as *Patinopecten yessoensis, Chlamys farreri, Chlamys swifti,* and *Argopecten irradians* have the satellite body in the middle piece of the spermatozoa. The number of the mitochondria in the middle piece of the spermatozoon of *Patinopecten yessoensis, Chlamys farreri,* and *Chlamys swifti* was four. However, *Argopecten irradians* has five mitochondria in the middle piece of the sperm.

In the present study, the number of the mitochondria in the mid-piece of the sperm of *Chlamys farreri* was five. Although it is the same species, we assume that the number of the mitochondria shows slight differences in number.

As in most other marine bivalves (Chung *et al.*, 1991; Chung, 1997 Chung and Ryou, 2000), occurrences of spermatogonia and spermatocytes appear in the early active stage, and A number of spermatids and small number of spermatozoa during spermiogenesis occur in the late active stage. Numerous fully mature spermatozoa appeared in the ripe stage, and they are released in the partially spawned stage. After spawning, small numbers of undischarged spermatozoa are degenerated and resorbed. Thereafter, newly formed spermatogonia on the germinal epithelium occur in the spent/inactive stage.

Marine invertebrate have unique breeding patterns. Boolootian *et al.* (1962) categorized the breeding pattern of female Chlamys farreri into three large groups based on their spawning behavior or seasonality: 1) year-round breeders, 2) winter breeders, and 3) summer breeders. According to histological and cytological observations of its gonad, the spawning season of *Chlamys farreri* is from April to August. Therefore, this species belongs to summer breeders because the spawning seasons in *Chlamys farreri* in China (Lioa *et al.*, 1983) and Japan (Yakovlev and Afeichuk, 1995) are approximately from May to July.

Percentages of first sexual maturity of individuals of 50.0 to 60.0 mm in shell height that composed of individuals being in the early active, late active, ripe and partially spawned stages were over 50%, and 100% in those over 60.0 mm in shell height that

composed of individuals being in the late active, ripe and partially spawned, spent and inactive stages. Accordingly, it is assumed that most individuals can reach to maturity before late August if they were larger than 60.0 mm in shell height.

According to the growth curves for the mean shell length fitted to von Bertalanffy's equation by Park (2002), individuals ranging from 50.0 to 60.0 mm in shell height are considered to be three years old. And we assume that male sex begin reproduction at three years of age. This result suggests that catching the Jicon scallop < 50.0 mm in shell height can potentially cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate natural resources management. However, further detailed studies for age determination on this species should be carried out to understand the population dynamics for natural sources.

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