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Ovarian Maturation in Female *Ruditapes philippinarum* on the West Coast of Korea

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한국 서해산 바지락, *Ruditapes philippinarum*의 난소 성숙

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ABSTRACT : Germ cell development during oogenesis, ovarian maturation and first sexual maturity in female *Ruditapes philippinarum* were investigated by cytological and histological observations. *R. philippinarum* is dioecious. During vitellogenesis, the Golgi complex, glycogen particles, and mitochondria were involved in the formation of lipid droplets and lipid granules in the cytoplasm of the early vitellogenic oocyte. In the late vitellogenic oocyte, cortical granules, the endoplasmic reticulum, and mitochondria were involved in the formation of proteid yolk granules in the cytoplasm. At this time, exogenous lipid granular substance and glycogen particles in the germinal epithelium passed into the oocyte through the microvilli of the vitelline envelope. The spawning period was once a year between early June and early October, and the main spawning occurred between July and August when seawater temperature was approximately 20℃. The reproductive cycle of this species can be categorized into five successive stages: early active stage(January to March), late active stage(Februaryto May), ripe stage(April to August), partially spawned stage(May to October), and spent/inactive stage (August to February). Percentages of female clams at first sexual maturity of 15.1~20.0mm in shell length were 52.6%(50% of the rate of group maturity was 17.83mm in length), and 100% for the clams >25.1mm.

Key words : *Ruditapes philippinarum*, Oogenesis, Reproductive cycle, First sexual maturity.

요 약 : 암컷 바지락, *Ruditapes philippinarum*의 난 형성과정 중 생식세포 발달과 난소 성숙 및 군 성숙도를 세포 및 조직학적 관찰에 의해 조사하였다. 바지락은 자웅이체이다. 난황 형성 과정 중 골지복합체, 글리코겐 입자들과 미토콘드리아들은 초기 난황 형성 단계의 난모 세포질 내에서 지방적 및 지방 과립 형성에 관여한다. 후기 난황 형성 단계 난모 세포질 내의 피질 과립, 조면 소포체 및 미토콘드리아들은 세포질 내에서 단백질성 난황 과립의 형성에 관여하였다. 이 시기에 생식상피 내의 외인성 지질 과립상 물질들과 글리코겐 입자들이 난황막의 미세 융모를 통해서 난모 세포질 내로 통과해 들어간다. 산란기는 6월 초에서 10월 초 사이로 연중 한 번이었으며, 주 산란은 해수 수온이 대략 20℃인 7월과 8월 사이에 일어났다. 본 종의 생식주기는 초기 활성기(1~3월), 후기 활성기(2~5월), 완숙기(4~8월), 부분 산란기(5~10월), 퇴화 및 비활성기(8~2월)의 연속적인 5단계로 구분되었다. 각장 15.1~20.0 mm인 암컷 조개의 군 성숙도 비율(%)은 52.6%(군 성숙도 50%는 각장 17.83mm)이었고, 각장 25.1mm 이상인 조개는 100%의 군 성숙도를 보였다.

INTRODUCTION

Ruditapes philippinarum(Bivalvia: Veneridae) is widely distributed along the coasts of Korea, China, Japan, the United States, and Spain, etc. It is particularly found in the intertidal and subtidal zones of the south and west coasts of Korea(Yoo, 1976; Kwon *et al.*, 1993; Chung *et al.*, 1994). In Korea, this species is one of the most important marine resources for human consumption. Standing stock of this commercially important clam has been declining for

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decades due to marine reclamation project of tidal areas marine pollution, and reckless over harvesting. Therefore, it is necessary to manage the population of this clam using a proper catching regime and detailed information that will maintain optimal population size in shellfish farm.

So far, many studies have examined various aspects of the reproductive ecology of *R. philippinarum* in Korea, Japan and the other countries(Hur, 1994), population dynamics and secondary production(Ohba, 1959; Choi, 1987; Yoon, 1992), reproduction including maturation(Toba & Miyama, 1995), artificial discharge(Sagara, 1958), the pawning season(Yoshida, 1953; Tanaka, 1954; Ohba, 1959; Holland & Chew, 1974; Ponurovsky & Yakovlev, 1992; Chung *et al.*, 1994), and the reproductive cycle(Toba *et al.*, 1993; Toba & Miyama, 1994; Chung *et al.*, 1994; Tsuji, 1994; Goshima *et al.*, 1996). Neverthless, no information is available for reproductive mechanism of vitellogenesis during oogenesis of this clam. Better understandings of the reproductive cycle and spawning period of this species need to determining population age structure and recruitment period. In addition, data for first sexual maturity and prohibitory measure of the population would be very useful information for aquaculture and the management of natural resources.

The present study provides some information on the reproductive mechanism of vitellogenesis during oogenesis and reproductive ecological data of this clam including some basic information on the ovarian development, first sexual maturity and prohibitory measure for propagation and management.

MATERIALS AND METHODS

1. Sampling

Specimens of *R. philippinarum* were collected monthly at the intertidal zone of Simpo coastal waters of Korea, for two years from January 2000 to December 2001(Fig. 1). Female clams ranging from 8.5mm to 54.8mm in shell length were used for the cytological and histological studies. After the alive clams were transported to the laboratory, shell length and height were measured by a Vernier caliper, and total weight was measured using a top-loading electronic balance(Casbee MW-120).

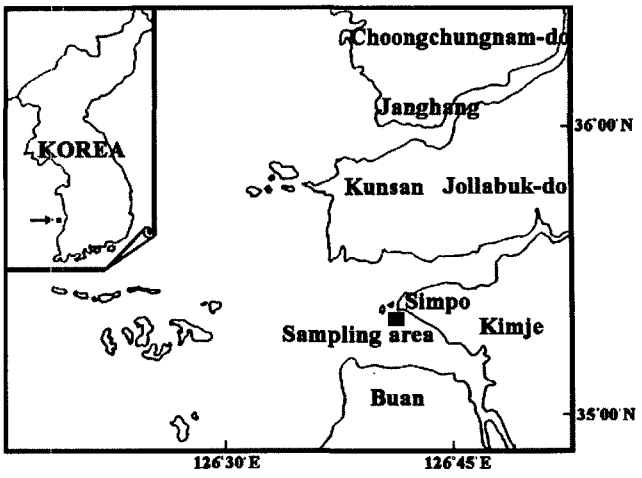

Fig. 1. Map showing the sampling area.

2. Germ Cell Differentiation during Oogenesis by Electron Microscopic Observation

For electron microscopic observation, excised pieces of ovaries were cut into small pieces and fixed immediately in 2.5% paraformaldehyde-prefixation, in 0.1M 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 1% osmium tetroxide solution in 0.2M phosphate buffer solution(pH 7.4) at 4℃ for 1 hour.

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 a LKB Ultramicrotome at a thickness of about 800~1000Å. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrated, and observed with a JEM 100CX-2(80kv) electron microscope.

3. Ovarian Maturation by Histological Observations

Histological preparations of the ovaries were made for analysis of the ovarian developmental phases by light microscopy. A total of 167 female clams over 15.1mm in shell length were used for the histological study. Tissues were removed from shells and preserved in Bouin's fixative for 24 hours and then washed with running tap water

for 24 hours. The tissues were then dehydrated in alcohol, 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, Mallory's triple stain or PAS stain, and were analyzed using a light microscope. Examination of ovary variability in *R. philippinarum* showed no significant differences in reproductive state between 7 random sections taken from different positions in the ovary. Sections were assigned to one of 5 stages: 1) early active stage, 2) late active stage, 3) ripe stage, 4) partially spawned stage, and 5) spent/inactive stage, based on modifications of the staging criteria used by Redfern(1974). Two or more stages often occurred simultaneously within each section, therefore, the staging criteria decisions were based upon the conditions of the majority of the section.

4. Size of First Sexual Maturity and Sexually Matured Length

First sexual maturity: The percentages of first sexual maturity were investigated from the histologically prepared preparations to certify shell lengths of specimens that matured and can be participated in reproduction during the breeding season between April and October, 2001. A total of 210 clams ranging from 8.4 to 54.6mm in shell length were used for first sexual maturity. First sexual maturity was estimated by the proportion of mature individual to the number of females.

Sexually matured length: To calculate sexually mature length, after fitting the rate of group maturity(first sexual maturity) to an exponential equation, the size equivalent to 50% of first sexual maturity was estimated to be the sexually mature length.

RESULTS

1. Position and Structure of the Ovary

R. philippinarum is dioecious. The ovary is located between the subregion of the midintestinal glands in the visceral cavity and the reticular connective tissues of the foot(Fig. 2). The ovary is composed of a number of oogenic follicles.

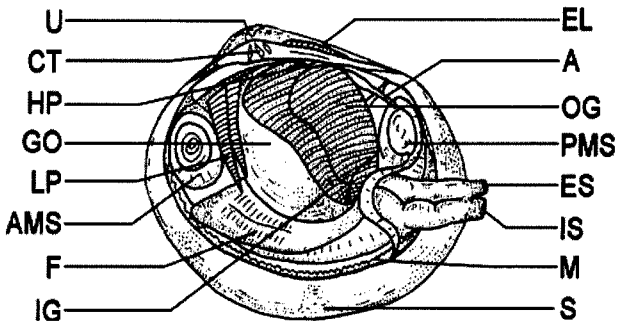

Fig. 2. Anatomy of *Ruditapes philippinarum*.
A, anus; AMS, anterior adductor muscle scar; CT, cardinal tooth; EL, external ligament; ES, exhalent siphon; F, foot; GO, gonad; HP, hepatopancreas; IG, inner gill; IS, incurrent siphon; LP, labial palp; M, mantle; OG, outer gill; PMS, posterior adductor muscle scar; S, shell; U, umbo.

As maturation progresses, the ovary extended to the reticular connective tissue of the foot. At this time, external view of the ovary is light pink in colour. But after spawning, ovaries rapidly degenerated, and then the area of the ovary decreased among the digestive diverticula, muscle tissue.

2. Germ Cell Differentiation during Oogenesis by Electron Microscopic Observation

Oogenesis occurs in the oogenic follicles of the ovary and can be divided into four phases: (1) premeiotic phase, (2) previtellogenic phase, (3) vitellogenic phase, and (4) mature phase.

Premeiotic phase: The stem cells, which constituted the boundaries of the follicle, gave rise to primary oogonia (approximately 9~10 μm.), characterized by a high nuclear-cytoplamic ratio, and the primary oogonia were divided mitotically to produce the secondary oogonia(Fig. 3A). At this phase, granular cells(containing a number of granules and mitochondria) and undifferentiated mesenchymal cells were present around the oogonia. Undifferentiated mesenchymal cells contained long and irregular nucleus with chromatin nucleolus, and have several mitochondria and several vacuoles in the cytoplasm(Fig. 3B).

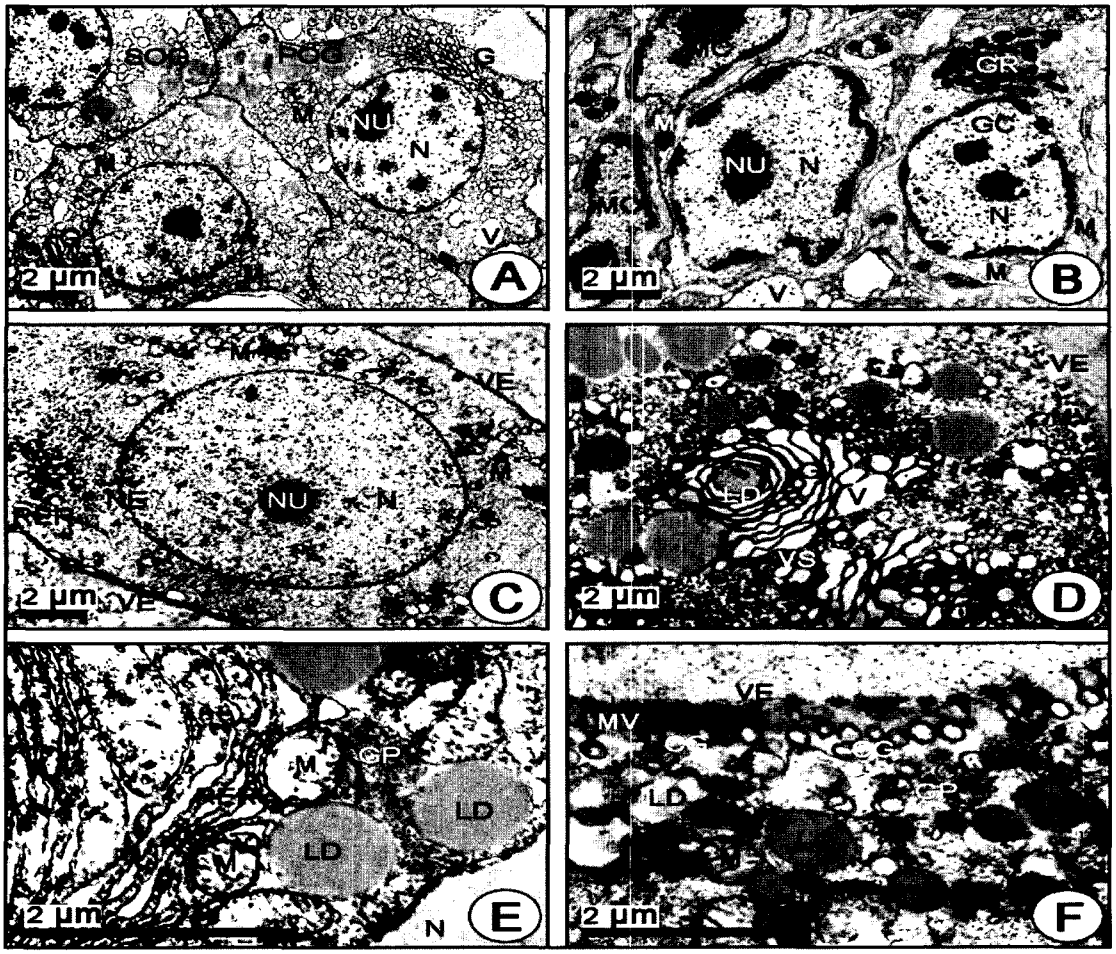

Fig. 3. Electron micrographs of the previtellogenic and early vitellogenic oocytes during oogenesis of *Ruditapes philippinarum* (A~F). A. Oogonia, with a large nucleus, several mitochondria and the Golgi complex in the cytoplasm. B. Undifferentiated mesenchymal cells and the granular cells near the oogonia. C. A previtellogenic oocyte, with a large nucleus, the endoplasmic reticulum and several mitochondria near the vitelline envelope. D. An early vitellogenic oocyte, a number of lipid droplets in the vacuoles and vesicles near the Golgi complex. E. An early vitellogenic oocyte, with lipid droplets which surrounded by the mitochondria, rough endoplasmic reticula and glycogen particles. F. An early vitellogenic oocyte, with the cortical granules, lipid droplets and lipid granules near the vitelline envelope. CG, cortical granule; G, Golgi complex; GC, granular cell; GP, glycogen particle; GR, granule; LD, lipid droplet; LG, lipid granule; M, mitochondrium MC, mesechymal cell; MV, microvilli; N, nucleus; NE, nuclear envelope; NU, nucleolus; POG, primary oogonium; RER, rough endoplasmic reticulum; SOG, secondary oogonium; V, vacuole; VE, vitelline envelope; VS, vesicle.

Previtellogenic phase: As the oogonium enter into the first prophase of meiosis, oogonia developed into the previtellogenic oocytes. At the beginning of cytoplasmic growth, the nucleus and cytoplasm of the previtellogenic oocyte increased in volume at this phase, the nucleus and oocyte diameters were 4~5 μm and 15~25 μm, respectively. A number of mitochondria and endoplasmic reticulum in the cytoplasm were concentrated around the nucleus. But at this phase, the microvilli on the vitelline envelope of the oocyte were not present(Fig. 3C).

Vitellogenic phase: As the development of previtellogenic oocytes proceed, the oocytes entered into vitellogenesis in the early vitellogenic phase. The early vitellogenic oocytes further enlarged to 30~40 μm in diameter, and the Golgi complex was present in the peirnuclear region of the cytoplasm, and numerous vacuoles and vesicles were scattered from the perinuclear region to the vitelline envelope of the oocyte. Lipid droplets were present in the vacuoles and vesicles which were formed by the Golgi complex in the perinuclear cytoplasm and were dis-

persed toward the cortical layer near the vitelline envelope (Fig. 3D). On the other hand, lipid droplets appeared among the mitochondria, well-developed rough endoplasmic reticula and glycogen particles near the nucleus (Fig. 3E). At this phase the microvilli on the vitelline envelope appeared, and the contours of the microvilli were round or oval in shape. Round cortical granules began to appear among lipid droplets, lipid granules, mitochondria and glycogen particles in the cortical layer near the vitelline envelope(Fig. 3F).

In the late vitellogenic oocyte, lipid droplets and lipid granules which occupy the area around the nuclear envelope, dispersed toward the cortical layer, and accumulation of cortical granules occurred in the cortical layer near the vitelline envelope autosynthetically. A number of proteid yolk granules, which were formed by well-developed rough endoplasmic reticula and cortical granules, appeared in the cortical layer(Fig. 4A). At this time, an amphi

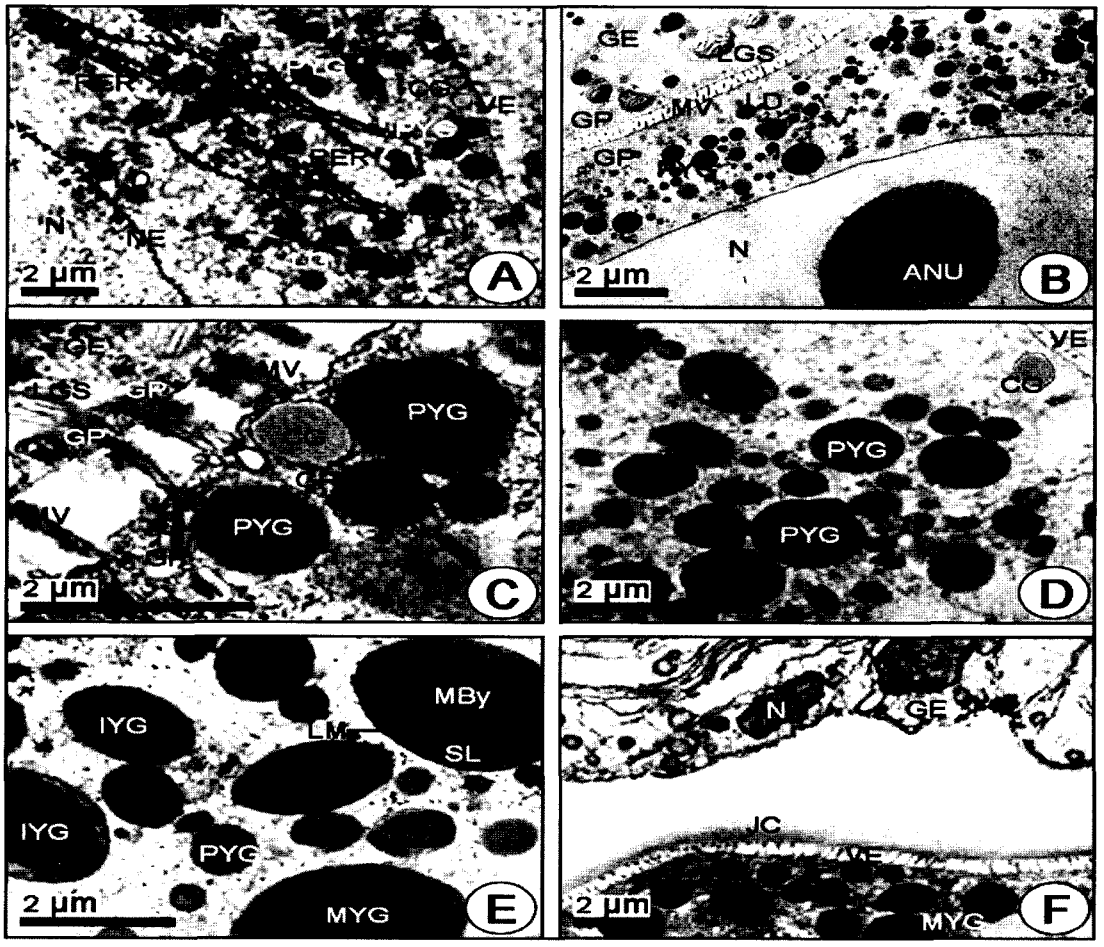

Fig. 4. Electron micrographs of late vetellogenic and mature oocytes during oogenesis of *Ruditapes philippinarum*(A~F). A. the late vitellogenic oocyte, with a number of proteid yolk granules among the lipid droplets, well- developed endoplasmic reticula, and several cortical granules near the vitelline envelope. B. A late vitellogenic oocyte attached to the germinal epithelium, with an amphinucleolus in the nucleus. C. A late vitellogenic oocyte, with a number of lipid granular substances and glycogen particles in the germinal epithelium passing into the ooplasm in the oocyte through the microvilli of the vitelline envelope. D. A late vitellogenic oocyte, with a number of proteid yolk granule in the perinuclear cytoplasm. E. A mature oocyte, with several immature and mature yolk granules. F. A mature oocyte, which was separated from the germinal epithelium, with a number of mature yolk granule and the vitelline envelope surrounded with the jelly coat. ANU, amphinucleolus; CG, corticalgranule; GE, germinal epithelium; GP, glycogen particle; IYG, immature yolk granule; JC, jelly coat; LD, lipid droplet; LG, lipid granule; LGS, lipid granular substance; LM, limiting membrane; MBY, main body; MV, microvilli; MYG, mature yolk granule; N, nucleus; NE, nuclear envelope; PYG, proteid yolk granule; RER, rough endoplasmic reticulum; SL, superficial layer; VE, vitelline envelope.

nucleolus appeared in the nucleus of the late vitellogenic oocyte, and especially, exogenous electron dense lipid granular substances and lots of glycogen particles in the germinal epithelium were passed into the ooplasm of the oocyte through the microvilli of the vitelline envelope (Figs. 4B, C). However, proteid yolk granules, which were formed by the cortical granules, endoplasmic reticula, and glycogen particles(exogenous substance) in the cytoplasm, are dispersed from the cortical layer near the vitelline envelope to the perinuclear cytoplasm(Fig. 4D). And proteid yolk granules containing several different components were intermingled and became immature yolk granules in the oocyte(Fig. 4E).

Mature phase: In the mature phase, small immature yolk granules were fused to each other and became larger mature yolk granule. A mature oocyte was composed of three parts: 1) main body, 2) superficial layer, and 3) limiting membrane(Fig. 4E). At this phase, the tip of the microvilli, some of which bifurcate, protrude and extend just beyond the outer border of the vitelline envelope. The thick vitelline envelope of the mature oocyte was about 0.60 μm thick and covered with thick jelly coat. And then mature oocyte was separated from germinal epithelium(Fig. 4F).

3. Ovarian Dvelopmental Stages and Reproductive Cycle

Based on morphological characteristics, differentiation of the germ cells, and surrounding tissues during oogenesis, ovarian developmental stages can be divided into 5 successive stages: 1) early active stage, 2) late active stage, 3) ripe stage, 4) partially spawned stage, and 5) spent/inactive stage. Ovarian developmental stages of this species showed a periodicity through the year. Frequency of ovarian developmental stages of female clams was shown in Fig. 5.

4. Early Active Stage

This stage was characterized by the expansion of the follicle and the appearance of oogonia, previtellogenic oocytes, undifferentiated mesenchymal tissues, and eosinophilic granular cells along the follicular wall. No free oocytes were present in the lumen. At this time, the mean

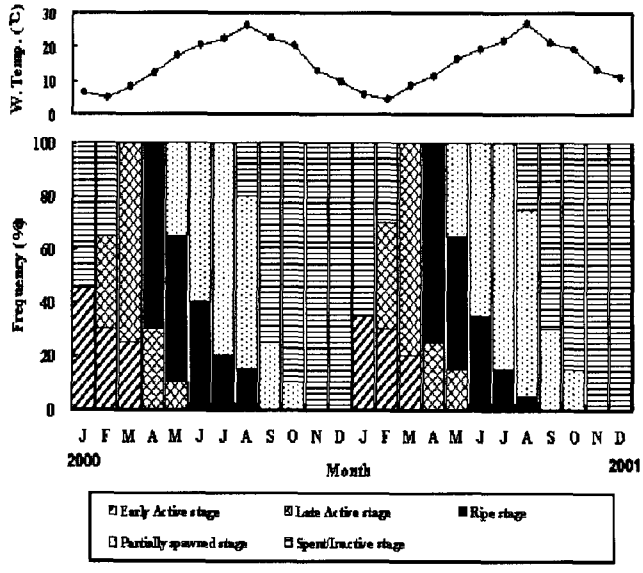

Fig. 5. Frequency of the ovarian developmental phases of female *Ruditapes philippinarum* and the mean seawater temperature, for two years from January 2000 through December 2001.

oogonium and previtellogenic oocyte were 10~11 μm and <25 μm in diameter, respectively(Fig. 6A). Female indi-viduals in the early active stage appeared from January to March in 2000 and 2001.

5. Late Active Stage

The undifferentiated mesenchymal tissues and eosinophilic granular cells in the follicle were gradually decreased, the early and late vitellogenic oocytes and a few free mature oocytes were present in the lumen of the follicle. More than half of the oocytes attached to the follicular wall, vitellogenic oocyte diameters were 40~50 μm (Fig. 6B). The individuals in the late active stage were observed from February to May in 2000 and 2001.

6. Ripe Stage

The ripe ovary exhibited distended follicles with mature and fully ripe oocytes. Undifferentiated mesenchymal cells and eosinophilic granular cells disappeared, and follicular wall was thin. Half or more than half of mature oocytes were free in the lumen of the follicle and the mean ripe oocyte diameter was 60~65 μm in diameter(Figs. 6C and D). The individuals in the ripe stage appeared from April to August in 2000 and 2001.

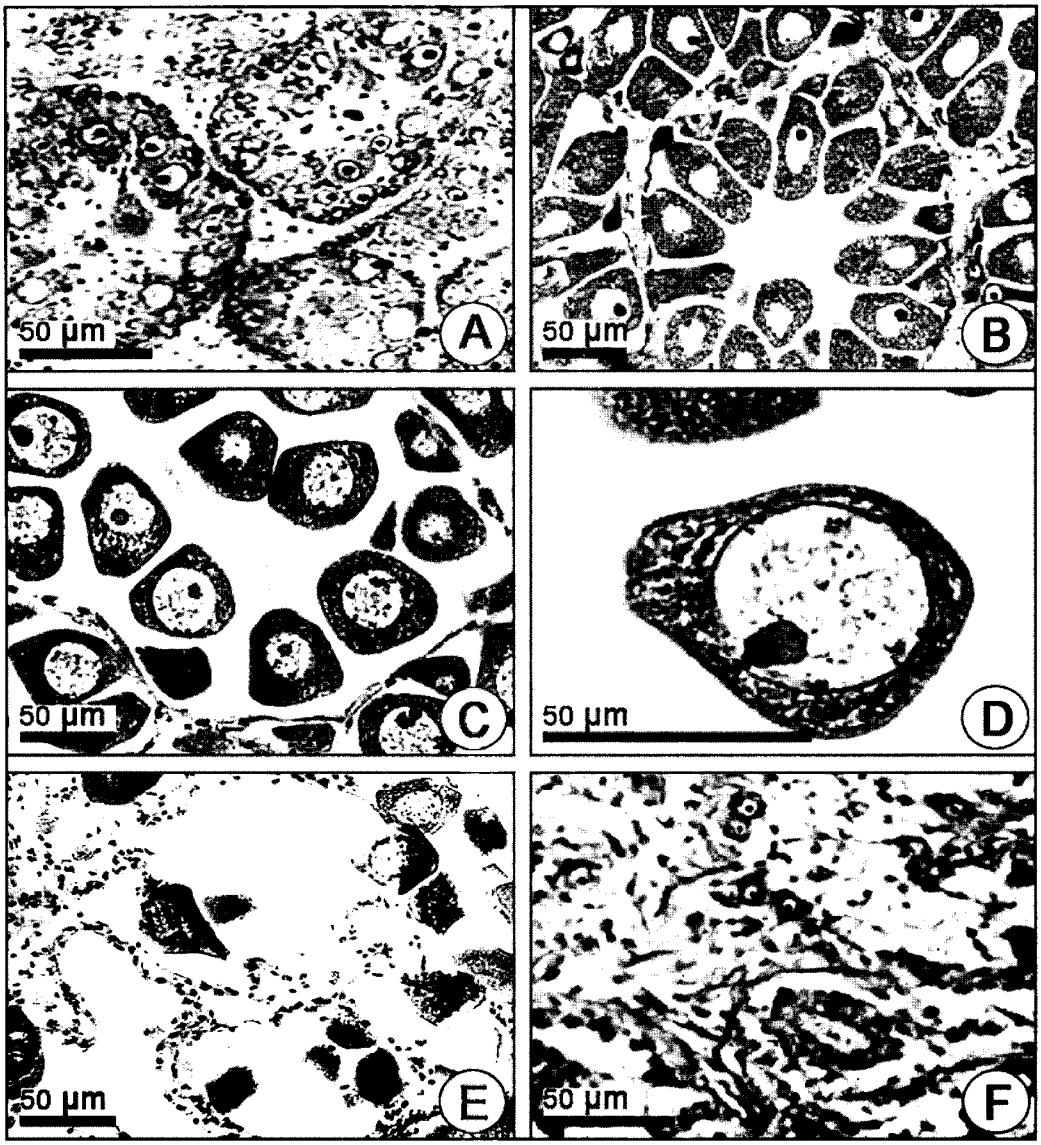

Fig. 6. Photomicrographs of the ovarian developmental phases of *Ruditapes philippinarum*(A~F). A. Transverse section of the oogenic follicles in the early active stage. B. Section of the follicles in the late active stage. C. Section of the follicles in the ripe stage. D. Section of a fully mature oocyte. E. Section of the follicles in the partially spawned stage. F. Section of the follicles in the spent and inactive stage. Scale bars=50 μm.

7. Partially Spawned Stage

The number of free mature oocytes in the follicle decreased because of discharging fully matured oocytes, and empty and ruptured follicles appeared. Some oocytes underwent cytolysis(Fig. 6E).

Spawning in females occurred from late May to October, and peak spawning occurred between July and August in 2000 and 2001.

8. Spent/Inactive Stage

After spawning, follicles were shrunk and disorganized, and there was no sign of gonadal activity. Half or more than half of the follicles were empty. Follicles became contracted, and undischarged oocytes in the lumen underwent cytolysis. Thereafter, newly formed oogonia appeared among the connective tissues(Fig. 6F). The individuals in the spent/inactive stage appeared from August to February in 2000 and 2001.

9. First Sexual Maturity and Sexually Matured Length

First sexual maturity: During the breeding season, a

total of 210 female individuals (8.4~54.6mm in shell length) were histologically examined to check whether they reached maturity and participated in reproduction. The rate of shells of different size that reached first sexual maturity is summarized in Table 1.

The breeding season of *R. philippinarum* was found to be from late May to October. In the case of some individuals with ovarian developmental stage in the late active stage in May through October, it is supposed that they can reach maturity except for individuals in the early active stage during the breeding season. First sexual maturity was 0% in female clams of 8.4 to 10.0mm in shell length if they were at the early active stage during the breeding season. The percentages of first sexual maturity of female clams of 10.1~15.0mm in shell length were 14.3%: most of the individuals were still in the early active stage. Percentage of first maturity in 15.1 to 20.0mm in shell length were over 50%, all of which were at the late active, ripe or partially spawned stage. Sexual maturity was 100% for clams.

Table 1. Shell length of first sexual maturity in female *Ruditapes phillippinarum* from April to October (before and after the spawning period)

Shell length (mm)	Number of individuals by Gonadal stage*					Female	
	EA	LA	RI	PS	SP/IA	Total	Mature(%)
8.5 ~ 10.0	22					22	0
10.1 ~ 15.0	18	2	1			21	14.3
15.1 ~ 20.0	7	5	4	3		19	52.6
20.1 ~ 25.0	5	6	7	2		20	75.0
25.1 ~ 30.0		7	8	6		21	100.0
30.1 ~ 35.0		2	13	5	2	22	100.0
35.1 ~ 40.0			13	7	4	24	100.0
40.1 ~ 45.0			11	8	4	23	100.0
45.1 ~ 50.0	2		7	11	2	20	100.0
50.1 ~ 54.8			10	5	3	18	100.0
Total						210	

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

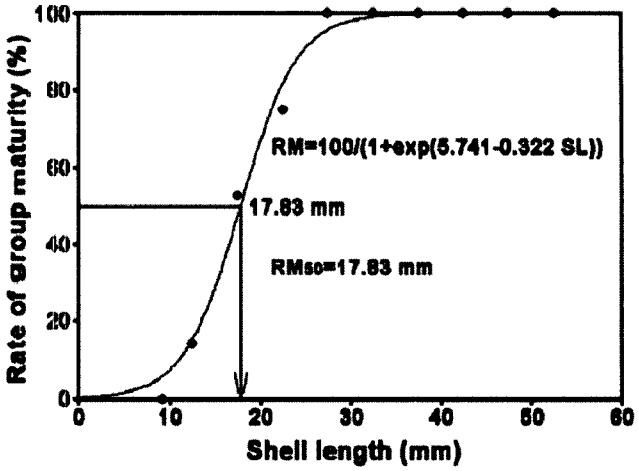

Fig. 7. Relationship between the rate of group maturity(%) and shell length(mm) of *Ruditapes philippinarum*.

Shell length of sexually mature clams: Shell lengths of sexually mature clams(50% of the rate of group maturity) in females that fitted to an exponential equation was 17.83mm in shell length(Fig. 7).

DISCUSSION

1. Germ Cell Differentiation and Vitellogenesis during Oogenesis

It has been well-known that in the early vitellogenic oocytes of bivalves, the Golgi apparatus present in the perinuclear region is involved in lipid droplet formation, as in *Mytilus edulis*(Reverberi, 1971), *Mactra chinensis*(Chung, 1997) and *Mactra veneriformis*(Chung & Ryou, 2000). In the present study, a similar result was observed that the Golgi complex present in theregion are involved in lipid droplet formation(referred as autosynthetic). Beside this finding, our electron microscope observation of early vitellogenic oocytes suggests that the mitochondria, endoplasmic reticula, and glycogen particles in the perinuclear cytoplasm are also involved in the formation of lipid droplets in the cytoplasm autosynthetically.

Light microscope observations with the PAS stain showed a strong positive reaction at the site of the egg-stalk of the late active oocyte connected to the germinal epithelium(follicular wall). At this time, according to the results by electron microscope observation, electron dense lipid granular substances and glycogen particles in the

germinal epithelium are passed into the ooplasm of the oocyte through the microvilli of the vitelline envelope (referred as heterosynthetic). Therefore, we assume that some vitellogenic substances in the late vitellogenic oocyte are originated from exogenous substances in the germinal epithelium during vitellogenesis, as in *Mactra veneriformis* (Chung & Ryou 2000) and *Mactra chinensis*(Chung 1997).

Bottke *et al.*(1982) and Medina *et al.*(1986) reported that gastropod species, *Planorbarius corneus*, *Lymnaea stagnalis*, *Hypselodoris tricolor*, and *Gordiva banyulensis* synthesize yolk by a combination of autosynthetic and heterosynthetic processes. Judging from our electron microscopical observations, it is assumed that *R. philippinarum* synthesizes yolk by a combination of autosynthetic and heterosynthetic processes.

Associated with the formation of proteid yolk granules during vitellogenesis, some authors reported that the endoplasmic reticula and multivesicular bodies, as seen in the opisthobranchs *Hypselodoris tricolor* and *Godiva banyulensis*(Medina *et al.*, 1986), and another snails, *Physa acuta* (Terakado, 1974) and *Rapana venosa*(Chung *et al.*, 2002) are involved in the formation of proteid yolk granules in some gastropods(Taylor & Anderson 1969). In the present study, we could not find multivesicular bodies which is formed by the modified mitochondria in the late vitellogenic oocyte, as seen in *Rapana venosa*(Chung *et al.*, 2002).

However, in the late vitellogenic oocyte of this species, especially, the proteid yolk granules appeared near the endoplasmic reticula, mitochondria and cortical granules in the cortical layer. Therefore, it is assumed that the endoplasmic reticulum, mitochondria, and cortical granules are involved in the formation of proteid yolk granules during vitellogenesis.

2. Gonad Development and Maturation

Many studies(Sastry 1963, 1966, 1968, 1970; Sastry & Blake 1971; Sastry 1979; Simpson 1982; Chung *et al.* 1991) have reported that gonadal development and maturation of bivalves is affected by environmental conditions, with exogeneous factors(water temperature, food organism, and day length) interaction with endogenous factors(neuronal and hormonal) inside the organism.

In the present study, *R. philippinarum* from Simpo coastal waters on the west coast of Korea initiated gonadal development during the late winter-early spring seasons when water temperatures were relatively low, and while chlorophyll-a level was high(Kim, 1999).

The gonadal phases were in the immature stage during the winter season because of lower temperatures and insufficient food organisms.

Sastry(1966, 1968) contended that gonad growth and gametogenesis in *Argopecten irradians* took place under temperature conditions at which nutrient mobilization for the gonad occured and described that temperature acted as a triggering stimulus for initiation of the oocyte growth phase. Therefore, we suggest that temperature and food availability required for active growth of oocytes at the beginning of oogenesis and for attaining maturity ultimately limit the annual period of gonad growth and gametogenesis in nature.

Gonadal development is an energy demanding process, as the mobilization of nutrients to the gonad is essential for gamete development. Although it is still unclear, gonadal development appears to depend on ingested food or stored reserves, or some combination of the two(Sastry, 1979; Baber, 1984). According to the results of Ministry of Maritime Affairs and Fisheries, Republic of Korea(2001), in Komso Bay, food level(phytoplankton) was high in mid spring(April) and early summer(June). In the present study, thus, gonad growth and gametogenesis during mid spring (April) coincided with high food level. The highest food level that occurred in early summer will be necessary for oocyte maturity and spawning in *R. philippinarum*(Fig. 8).

3. Breeding Pattern

Investigations of natural reproductive cycle or spawning cycle are central not only to studies of population dynamics(i.e., age determination and the recruitment period) but also to our understanding of biogeography and speciation. The reproductive cycle comprises the entire sequence of events from activation of the gonad through gametogenesis to spawning and the subsequent recession of the gonad(Chung, 1997). In nature there are considerable variations in the reproductive cycle of *R. philippinarum*. Intra-

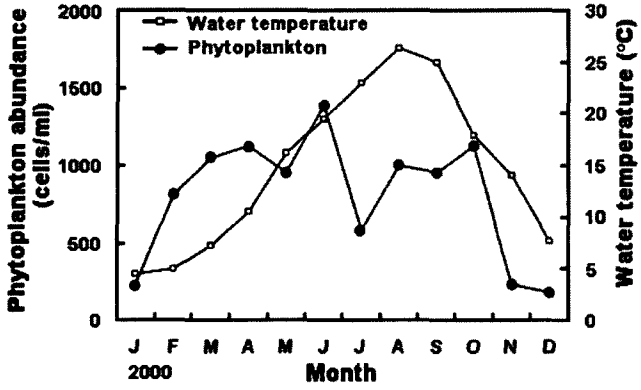

Fig. 8. Monthly changes in phytoplankton abundance and water temperature of Gomso Bay in 2000(Ministry of Marine Affairs and Fisheries, Republic of Korea, 2001).

specific variations in the timing of spawning periods and the amount of produced gametogenic material vary with years and latitudinal gradient due to variations in environmental conditions influencing the reproductive process (Chung *et al.*, 1997).

Rand(1973) stated that breeding strategy varied with latitudinal gradient: i.e., northern climates were characterized by a single synchronous spawning every year, temperate climates by two spawning seasons and tropical ones by year-round spawning.

In case of different populations, there are some differences in the reproductive cycles of *R. philippinarum* in the other areas of the world; there is one spawning period in British Columbia, Canada(Quayle & Bourne, 1972), Hood Canal, Washington, USA(Holland & Chew, 1974), northern Japan(Yoshida, 1953), and Vostok Bay, northwestern part of the Sea of Japan (Ponurovsky & Yakovlev, 1992); while two in southern Japan(Tanaka, 1954; Ohba, 1959).

In the present study, this species has one spawning period as in the northern districts of Tokyo Bay, Japan. Therefore, it is assumed that the number of spawning frequencies in the same species varied with temperature-latitude.

4. First Sexual Maturity

Sexual maturity in this study was assessed as a function of age and shell length, as age or shell length can be used as a convenient indicator.

According to our results, percentages of first sexual maturity for females and males of 15.1~20.0mm in shell length were 56.3% (50% of the rate of group maturity was 17.83mm in shell length) and 100% in those > 25.1mm.

Ko(1957) reported that the size at first sexual maturity ranged from 10.0~15.0mm in shell length in Sasebo Bay, Japan, while Goshima *et al.*(1996) reported that shell lengths at first maturity were 25mm(2 years) and 27mm(2 or 3 years), respectively for males and females in Saroma Lagoon, Hokkaido, northern Japan. Therefore, it is assumed that the size of first sexual maturity of the local population of this species varied with their habitat latitudes.

According to the growth curves for the mean shell length fitted to von Bertalanffy's equation by Chung *et al.* (1994), ages and shell lengths are as follows:

Age (years)	Mean shell length(mm)
1	18.39
2	28.29
3	36.23
4	42.60

Therefore, individuals of 15.0~20.0mm(17.83mm) in shell length are considered to be one year old. We assume that female population begin reproduction at one year of age. For natural resources management of this species, the present study suggests that catching female clam <17.83 mm in shell length or <1 year old cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate natural resources management.

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