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Ultrastructural Study of Vitellogenesis during Oogenesis and Sexual Maturation of the Female *Neptunea* (*Barbitonia*) *arthritica cumingii* on the West Coast of Korea

Ee-Yung Chung

School of Marine Life Science, Kunsan National University, Kunsan 573-701, Korea

한국 서해산 암컷 갈색띠매물고둥, *Neptunea* (*Barbitonia*) *arthritica cumingii*의 난자형성과정 중 난황 형성의 미세구조적 연구 및 성 성숙

정 의 영

군산대학교 해양생명과학부

ABSTRACT : Vitellogenesis during oogenesis, reproductive cycle and first sexual maturity of the female *Neptunea* (*Barbitonia*) *arthritica cumingii* was investigated by light and electron microscope observations. In the early vitellogenic oocyte, the Golgi complex and mitochondria were involved in the formation of lipid droplets and yolk granules. In late vitellogenic oocytes, the rough endoplasmic reticulum and multivesicular bodies were involved in the formation of proteid yolk granules in the cytoplasm. A mature yolk granule was composed of three components: main body(central core), superficial layer, and the limiting membrane. The spawning season was between May and August and the main spawning occurred between June and July when the seawater temperature rose to approximately 18~23℃. The female reproductive cycle can be classified into five successive stages: early active stage(September to October), late active stage(November to February), ripe stage(February to June), partially spawned stage(May to August), and recovery stage(June to August). The rate of individuals reaching the first sexual maturity was 53.1% in females of 51.0 to 60.9mm in shell height, and 100% in those over 61.0mm.

Key words : *Neptunea* (*Barbitonia*) *arthritica cumingii*, Oogenesis, Reproductive cycle, First sexual maturity.

요 약 : 갈색띠매물고둥, *Neptunea* (*Barbitonia*) *arthritica cumingii*의 난자 형성과정 중 난황 형성, 생식주기 및 군성숙도를 광학 및 전자현미경적 관찰에 의해서 조사하였다. 초기 난황 형성 단계의 난모세포에서 골지복합체와 미토콘드리아가 지방적 및 난황 과립의 형성에 관여되었다. 후기 난황 형성 단계의 난모세포에서는 조면소포체와 다포체가 세포질 내에서 단백질성 난황 과립 형성에 관여되었다. 성숙 단계 난모세포에서 성숙 난황 과립은 주소체(중앙중심), 표면층, 그리고 이들을 둘러싼 한계막의 3가지로 구성되어 있다. 산란기는 5월과 8월 사이이고, 주 산란은 수온이 대략 18~23℃로 상승하는 6월과 7월 사이에 일어나고 있다. 암컷의 생식주기는 초기 활성기(9~10월), 후기 활성기(9~2월), 완숙기(2~6월), 부분 산란기(5~8월), 회복기(6~8월)의 연속적인 5단계로 구분할 수 있다. 군성숙도는 각고 51.0~60.9mm에서 53.1%이었고 각고 61.0mm 이상에서는 100%를 나타내었다.

INTRODUCTION

Neptunea arthritica cumingii is one of the most important edible gastropods in East Asian countries such as Korea, Japan, China and Russia(Yoo, 1976; Kwon *et al.*, 1993). This species is especially found in silty sand of the subtidal zone of the west coast of Korea. Recently, as the standing stock of this species gradually decreased due to extensive reclamation projects and reckless over-harvesting, it has been designated as one of the important organisms in need of natural resources management.

On *Neptunea* spp. in foreign countries, previously there have been some studies on aspects of reproduction including the reproductive cycle(Takahashi *et al.*, 1972; Takamaru & Fuji, 1981; Fujinaga, 1985; Kawai *et al.*, 1994) and spawning(Miyawaki, 1953; Amio, 1963; Son, 2003), on aspects of ecology including distribution(Ito & Tachizawa, 1981; Ito, 1982; Kwon

† Correspondence: School of Marine Life Science, Kunsan National University, Miryong-dong, Kunsan, Jeollabuk-do 573-701, Korea. Tel: 82-63-469-4592, Fax: 82-63-465-3917, E-mail: eychung@kunsan.ac.kr

et al., 1993), growth(MacIntosh & Paul, 1977; Fujinaga, 1987; Suzuki *et al.*, 1996) of *N. arthritica*, and feeding(Pearce & Thorson, 1967) of *N. antiqua*. On *N. cumigii*, especially, there has been one study on the spawning season in the East China Sea(Amio, 1963). But, there are still gaps in our knowledge for reproductive biology. So far, little information has been available on ultrastructural study on germ cell differentiation and sexual maturation of *N. arthritica cumingii* in the Korean waters and the Japan Sea(Chung & Kim, 1996). However, there are some information on ultrastructural study of oogenesis in other gastropods(Mccann-Coillier, 1977, 1979; Griffond & Gomot, 1979; Griffond, 1980; Hodgson & Eckelbarger, 2000; Pal & Hodgson, 2002). Therefore, the results of ultrastructural studies on germ cell differentiation of this species and other gastropods will provide important information for the reproductive mechanism. The reproductive cycles of the local populations in marine gastropods vary with environmental factors such as water temperature and food availability(Chung *et al.*, 2002). Understanding the reproductive cycle and the spawning period of *N. arthritica cumingii* will provide necessary information for natural spat collections or the recruitment period and age determination of this population. In addition, data on first sexual maturity and reproductive strategy of this population are very useful information for natural resource management. Therefore, the main aim of the present study is to understand germ cell differentiation during oogenesis, the reproductive cycle and first sexual maturity of this species.

MATERIALS AND METHODS

1. Sampling

Specimens of *Neptunea arthritica cumingii* were collected monthly at the subtidal zone of Maldo, Kunsan, Korea, from January to December, 2002 and 2003(Fig. 1). The snails ranging from 41.0 to 106.8mm in shell height were used for the present study. After the snails were transported alive to the laboratory, shell heights were immediately measured.

2. Germ Cell Differentiation during Oogenesis

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

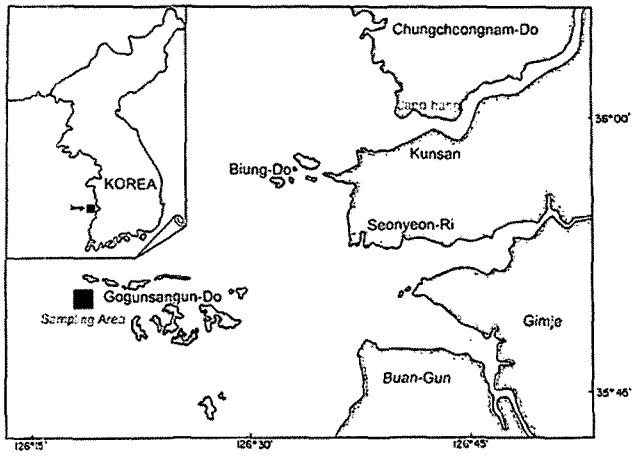

Fig. 1. Map showing the sampling area.

3. Gonadal Development

For light microscopic examination of histological preparations, a total of 456 individuals was used for histological analysis of the gonads from January to December, 2002. Gonad tissues were removed from shells and preserved in Bouin's fixative for 24h and then washed with running tap water for 24h. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5~7μm thickness using a rotary microtome. Sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, Mallory's triple stain and PAS stain, and examined using a light microscope.

4. First Sexual Maturity

The first sexual maturation of a total of 187 female individuals(31.4~90.5mm in shell height) were investigated histologically in order to determine the shell heights of snails reaching

maturation and participating in reproduction from May(ripe stage) to late August(after spawning).

RESULTS

1. Position and Morphology of the Gonads

Neptunea arthritica cumingii is a dioecious species which is composed of well-defined female and male individuals. The ovary is located on the surface of the digestive gland in the spiral posterior region of the shell(Fig. 2). The ovary is composed of numerous oogenic follicles. As the ovary was maturing, it extended to the outer part of the digestive gland. As maturation progresses, the sex of the snailcan be distinguishable easily by color: the ovary being pale yellow and testis yellowish-brown. At this time, if it was slightly scratched with a razor, ripe eggs were readily discharged from the ovary. But after spawning, the ovary degenerated, and it became difficult to distinguish their sexes by external color or dissection.

2. Germ Cell Differentiation during Oogenesis by Electron Microscopic Observations

Through the ultrastructural observations the germ cell developmental phases during oogenesis could be divided into 4 phases: (1) oogonial phase, (2) previtellogenic phase, (3) vitellogenic phase, and (4) mature phase. Characteristic features in each stage were as follows:

Oogonial phase: Oogonia in the oogonial phase, which propagated on the germinal epithelium(follicular wall), were oval and 15μm in diameter. They commonly were single or formed a cluster on the germinal epithelium. Each oogonium had a large nucleus with chromatin, several mitochondria, and the endoplasmic reticulum, vacuoles in the cytoplasm(Fig. 3A).

Previtellogenic phase: Previtellogenic oocytes were 25~90μm in diameter. With cytoplasmic growth, several small mitochondria, a well-developed endoplasmic reticulum and several vacuoles were concentrated around the nucleus in the cytoplasm of

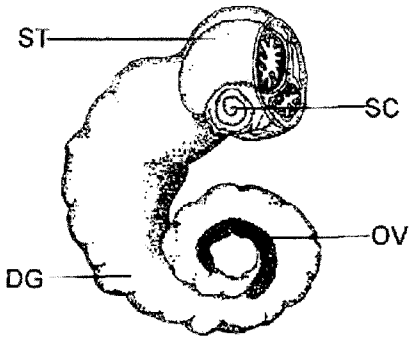

Fig. 2. Anatomy of *Neptunea arthritica cumingii*, removed from its shell. Three sections are spaced equally. DG, digestive gland; OV, ovary; ST, stomach; SC, stomachal caecum.

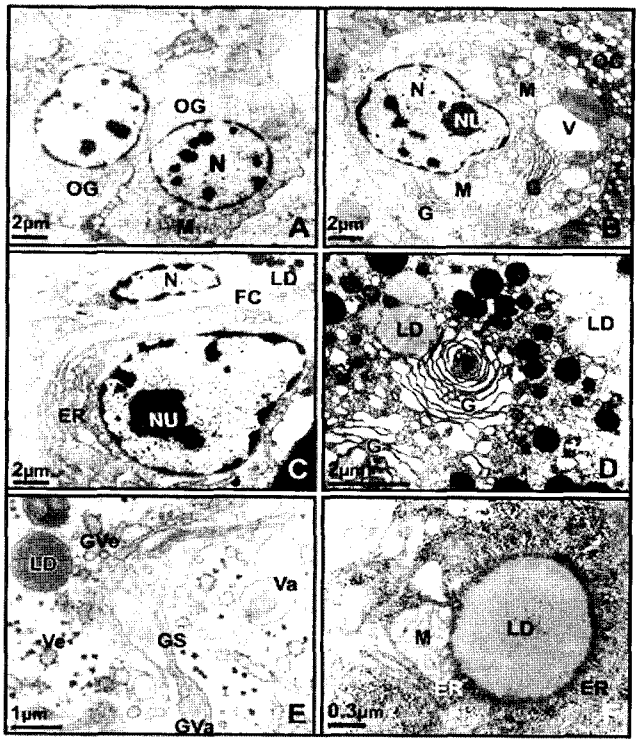

Fig. 3. Electron micrographs of the previtellogenic and early vitellogenic phases during oogenesis of *Neptunea arthritica cumingii*(A~F). A, oogonia in the oogonial phase, with a large nucleus and several mitochondria in the cytoplasm; B, a previtellogenic oocyte, with a large nucleus with a few nucleolus and several mitochondria, the Golgi complex and vacuoles in the cytoplasm; C, an early vitellogenic oocyte attached to a follicle cell, with a large nucleus containing chromatin and a number of vacuoles and well-developed endoplasmic reticulum in the cytoplasm D, the early vitellogenic oocytes, with well-developed Golgi complex, glycogen particles and liped droplets, E, an early vitellogenic oocytes, with lipid droplets formed by secretions in vacuoles and vesicles; F, an early vitellogenic oocyte, with a lipid droplet surrounded by the endoplasmic reticulum and the mitochondria. CR, chromatin; ER, endoplasmic reticulum; G, Golgi complex; GS, Golgi sac; GVa, Golgi vacuole; GVe. Golgi vesicle; LD, lipid droplet; M, mitochondrion; N, nucleus; NU, nucleolus; OC, oocyte; OG, oogonium; ER, Endoplasmic reticulum; Va, vacuole; Ve, vesicle.

the previtellogenic oocyte. The number of Golgi complexes, scattered from the perinuclear region to the cortical region of the oocyte, was increased. At this time, many vacuoles formed by the Golgi complex appeared around the endoplasmic reticulum, several mitochondria, and large vesicles were present in the cytoplasm of the previtellogenic oocyte(Fig. 3B).

Vitellogenic phase: In the early vitellogenic oocyte, especially, well-developed endoplasmic reticulum and vacuoles in the cytoplasm were concentrated around the nucleus having nucleoli. At this time, the follicle cell, which lied adjacent to the early vitellogenic oocyte, had an elongated nucleus. In particular, electron-dense granules and several lipid droplets were accumulated in the cytoplasm of the oocyte(Fig. 3C). With the initiation of yolk formation, lipid droplets were accumulated in the vacuoles formed by the Golgi complex in the perinuclear region. Lipid droplets diffused toward the cortical layer, and then glycogen particles appeared around the mitochondria at the cortical region of early vitellogenic oocytes(Fig. 3D). At this time, after electron-dense materials were accumulated in the Golgi complex(Golgi sac, Golgi vacuoles and Golgi vesicles), lipid droplets were formed by secretion of electron-dense materials in the large vacuoles and small vesicles which were formed by the Golgi vacuoles and Golgi vesicles(Fig. 3E). On the other hand, relatively large lipid droplet was surrounded by the endoplasmic reticulum, the mitochondria and glycogen particles in the cytoplasm of the early vitellogenic oocyte(Fig. 3F). In the late vitellogenic oocyte, lots of yolk granules appeared between the rough endoplasmic reticulum and the mitochondria at the cortical layer in the cytoplasm (Fig. 4A). At this time, the multivesicular bodies, which were formed by the modified cristae of the mitochondria, appeared near the nuclear envelope of the nucleus in the late vitellogenic oocyte. Yolk precursors such as glycogen particles, lipid droplets, yolk granules and multivesicular bodies were accumulated in the cytoplasm(Fig. 4B). Eventually, proteid yolk granules were formed by yolk granules and multivesicular bodies(Fig. 4C).

Mature phase: Mature oocytes were about 180~250 × 300~450 μm in diameter. In the mature oocyte, various sizes of proteid yolk granules were intermingled with small lipid yolk granules, and it became a small mature yolk granule(Fig. 4C). Relatively small mature yolk granules were continuously mixed with each other and became large mature yolk granules in the cytoplasm. A fully mature yolk granule is composed of three components: (1) main body, (2) superficial layer, and (3) a limiting membrane(Fig. 4D).

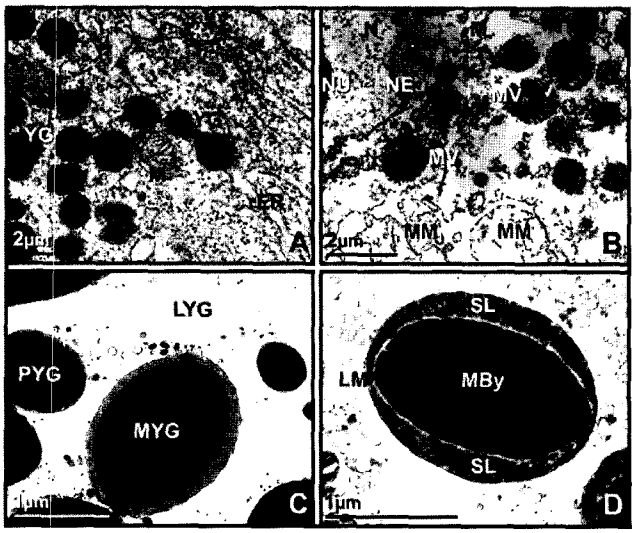

Fig. 4. Electron micrographs of late vitellogenic and mature phases during oogenesis of *Neptunea arthritica cumingii*(A~D). A, a late vitellogenic oocyte, with yolk granules between the rough endoplasmic reticulum and the the mitochondria; B, a late vitellogenic oocyte, with a number of multivesicular bodies formed by modified mitochondria C, a late vitellogenic oocyte, with proteid yolk granules formed by yolk granules and multivesicular bodies; D, mature oocytes, with a mature yolk granule being composed of the main body(central core), superficial layer and a limiting membrane of a yolk granule. LD, lipid droplet; LM, limiting membrane; LYG, lipid yolk granule; M, mitochondrion; MBy, main body; MM, modified mitochondrion; MYG, mature yolk granule; MV, multivesicular body; N, nucleus; NE, nuclear envelope; NU, nucleolus; PYG, proteid yolk granule; rER, rough endoplasmic reticulum; SL, superficial layer.

3. Reproductive Cycle with the Gonad Developmental Stage

Based on the morphological features and sizes of germ cells and the tissue cells around them, the reproductive cycle with gonadal phases could be classified into five stages in females. Especially, the reproductive cycle and monthly changes in water temperatures showed similar patterns in 2002 and 2003(Fig. 5). The criteria in defining of each stage are as follows:

Early active stage: The gonadal volume was small, and the follicles occupied approximately 25% of the gonad. The follicular walls were relatively thick. Oogonia and the previtellogenic oocytes propagated along the oogenic follicular walls and mesenchymal tissues of the ovary. The oogonia and previtello-

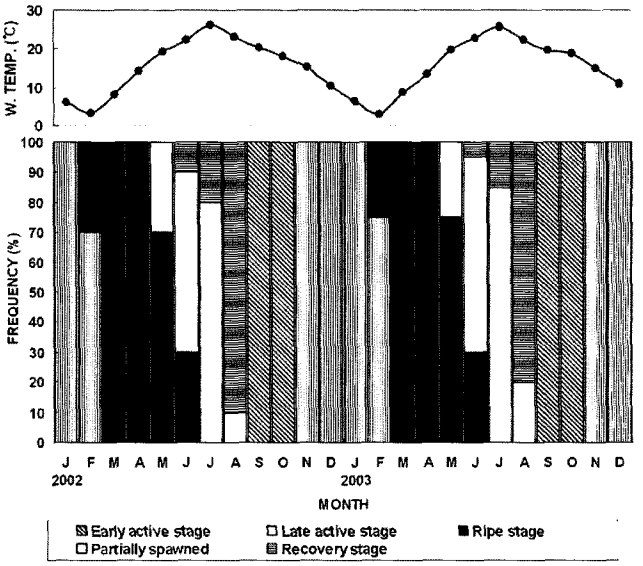

Fig. 5. Frequency of gonadal phases of *Neptunea arthritica cumingii* and the mean water temperatures, for two years, from January 2002 to December 2003.

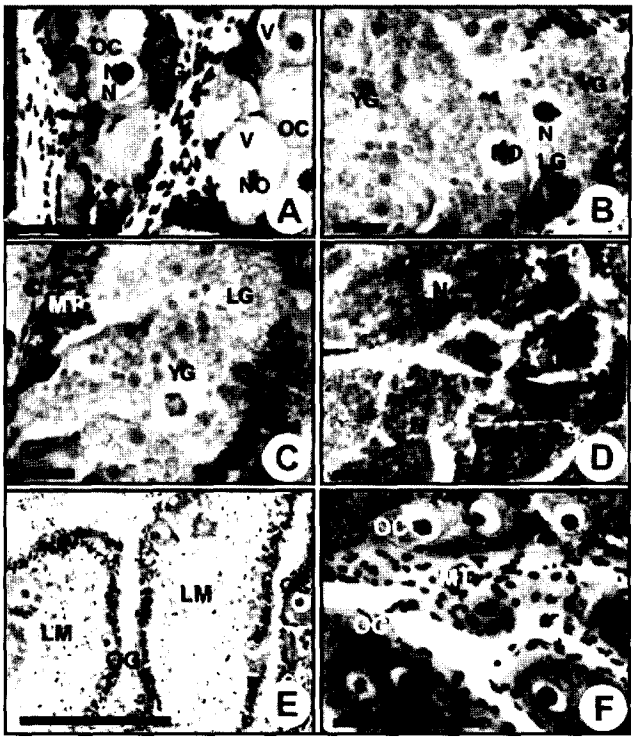

Fig. 6. Photomicrographs of the gonadal phases of female *Neptunea arthritica cumingii*. A, Transverse section of oogenic follicles in the early active stage; B, C, section of follicles in the late active stage; D, section of ripe oocytes in the ripe stage; E, section of follicles in the partially spawned stage; F, section of the follicles in the recovery stage. Scale bars = 50μm. LG, lipid granule; LM, lumen; MT, mesenchymal tissue; N, nucleus; NO, nucleolus; OC, oocyte; OG, oogonium; V, vacuole; YG, yolk granule.

genic oocytes are about 15~25μm in size, respectively. At this time, early vitellogenic oocytes of 25~50μm in diameter formed an egg-stalk attached to the walls(Fig. 6A). The individuals in the early active stage were found from September to October when seawater temperatures were gradually decreasing.

Late active stage: This stage is characterized by the presence of developing early vitellogenic oocytes. Follicular walls(germinal epithelium) were thin. A number of early vitellogenic oocytes of 100~140μm in diameter were attached to the follicular walls through each egg-stalk. With the initiation of yolk formation, there were numerous yolk granules in the cytoplasm of late vitellogenic oocytes of 150~200 × 250~300μm in diameter. Some fully mature oocytes were free in the lumen of the follicle(Figs. 6B, C). The individuals in the late active stage appeared from November to February.

Ripe stage: In females, the majority of oocytes grew to 160~180μm in diameter, occupied over 70% of the gonad, and follicular walls became very thin. Mature oocytes growing up to 180~250 × 300~450μm in diameter became tetragonal or polygonal in shape and contained a number of mature yolk granules (Fig. 6D). Mature or ripe ovaries were found in February through June when seawater temperatures gradually increased.

Partially spawned stage: Since about 50~70% of the oocytes in the follicles were discharged, the lumen of the follicles became considerably empty. Spawned ovaries were characterized by the presence of a few undischarged vitellogenic oocytes as well as previtellogenic oocytes in the follicles(Fig. 6E). The individuals in this stage appeared from May to August, and the main spawning occurred between June and July when the seawater temperature rose to approximately 16~23℃.

Recovery stage: After spawning, the undischarged vitellogenic oocytes in the lumen of the follicle undergo cytolysis, each follicle was contracted, and then degeneration or resorption of undischarged vitellogenic or mature oocytes occurred. Thereafter, the rearrangement of newly formed connective tissues, a few oogonia and previtellogenic oocytes appeared on the newly formed follicular walls(Fig. 6F). The individuals in the recovery stage appeared from June to August.

4. First Sexual Maturity

Before and after spawning, a total of 187 female individuals

Table 1. Shell height and first sexual maturity of the female *Neptunea (Barbitonia) arthritica cumingii* from May to August, 2002

Shell height (mm)	Gonadal developmental stage*						
	EA	LA	RI	PS	RE	Total	Mature(%)
31.4~40.9	34					34	0.0
41.0~50.9	26	2	2			30	13.3
51.0~60.9	15	2	10	5		32	53.1
61.0~70.9		3	21	6		30	100.0
71.0~80.9		2	22	9		33	100.0
81.0~90.5			16	12		28	100.0
Total						187	100.0

*Abbreviations: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage; RE, recovery stage.

(31.4~40.9mm in shell height) was histologically examined to certify whether they reached maturity and participated in reproduction. The rate of shells of different sizes that reached first sexual maturity is summarized in Table 1. The breeding season of this species was from May to August. In the case of some individuals with gonad developmental stage in the late active stage in May through August, it is supposed that they can be reached maturity except for individuals in the early active stage during the breeding season. First sexual maturity was 0% in female snails of 31.4~40.9mm in shell height if they were at the early active stage during the breeding season.

The percentage of first sexual maturity of the female snail of 41.0 to 50.9mm in shell height was 13.3%. The percentages of first sexual maturity of the female individuals of 51.0 to 60.9cm in shell height were over 50%, all of which were at the late active, ripe or partially spawned stages. First sexual maturity was 100% for snails over 61.0mm in height.

DISCUSSION

1. Vitellogenesis during Oogenesis

As vitellogenesis commences the nucleus of the oocytes increased in size. Early vitellogenesis is characterized by proliferation of endoplasmic reticulum and mitochondria, both of which are closely associated with lipid droplets. According to our electron microscope observations of early vitellogenic oocytes of *N. arthritica cumingii*, the Golgi apparatus is thought to be involved in a number of vacuoles and small vesicles in the perinuclear region in the cytoplasm, with carbohydrate(glycogen particles) filling the vacuoles. Lipid droplets and lipid yolk granules are then added to the vacuoles and vesicles formed by the Golgi complex(referred as autosynthetic by Taylor & Anderson, 1969), as in *Ilyanassa obsoleta*(Taylor & Anderson, 1969), *Biomphalaria glabrata*(de Jong-Brink *et al.*, 1976), *Mytilus edulis* (Reverberi, 1971), *Rapana venosa*(Chung *et al.*, 2002), *Siphononaria capensis*(Pal & Hodgson, 2002), *Pattella Barbara*, *P. argenvillei*, *P. granularis*, *P. oculus*, *P. miniata* and *Helcion pectunculus*(Hodgson & Eckelbarger, 2000). Therefore, this study suggests that the Golgi complex and various sizes of vacuoles are involved in the formation of lipid droplets in the early vitellogenic oocytes. From our observations of oogenesis, it is assumed that the mitochondria and the endoplasmic reticulum near lipid droplets are involved in the formation of lipid droplets in the early vitellogenic oocyte. However, we did not find pinocytotic tubules which are thought to be involved in yolk production as seen in the vitellogenic oocytes of *Agriolimax reticulatus* (Hill & Bowen, 1976; Domen, 1983). In the late vitellogenic oocyte we also did not observe microvilli on the vitelline envelope which is thought to be involved in helping in absorption, transportation and secretion of egg envelopes(Nørrevang, 1968) as seen in *Mactra chinensis*(Chung, 1997), *M. veneriformis*(Chung & Ryou, 2000), and *Siphonaria serriata*(Pal & Hodgson, 2002).

Formation of cortical granules is a prominent feature of late vitellogenic oocytes in most bivalves such as *Mactra chinensis* (Chung, 1997) and *M. veneriformis*(Chung *et al.*, 2000). Regarding formation of cortical granules during oogenesis, Hodgson & Eckelbarger(2000) described that Golgi complexes appeared predominately in the cortical region of the ooplasm and secrete electron dense, cortical granule-like organelles in the vitellogenic oocytes of *Patella barbara*. And they stated that Golgi complexes synthesize cortical granules. In the present study, however, such structures were not observed in the vitellogenic oocytes as in *Iliana obsoleta*(Taylor & Anderson, 1969) and *Rapana venosa*(Chung *et al.*, 2002). Compared with *Patella barbara*, that is prominent characteristics during oogenesis and a remarkable difference of *N. arthritica cumingii*. In the present study, proteid yolk granules, which appeared near the rough endoplasmic reticulum and modified mitochondrial structure(mul-

tivesicular bodies) as seen in *Hypselodoris tricolor* and *Godiva banyulensis*(Medina *et al.*, 1986), were observed at the cortical region of the cytoplasm. Accordingly, it is assumed that the endoplasmic reticulum and multivesicular bodies are involved in the formation of proteid yolk granules(Taylor & Anderson, 1969) as yolk precursor. In the present study, although the follicle cell, which lied adjacent vitellogenic oocyte, contains electron-dense granules and lipid droplet, we could not observe clear evidence of secretion into the vitellogenic oocyte. Therefore, it is assumed that *N. arthritica cumingii* synthesize yolk autosynthetically as in the majority of gastropods except for some gastropod species (*Planorbarius corneus*, *Lymnaea stagnalis*, *Hypselodoris tricolor*, *Godiva banyulensis*, *Siphonaria capensis* and *S. serrata*) which synthesize yolk autosynthetically and heterosynthetically(Bottke *et al.*, 1982; Medina *et al.*, 1986 Pal & Hodgson, 2002).

2. Gonadal Development and Maturation

We observed that gametogenesis of *N. arthritica cumingii* initiates at a temperature of about 3.0℃, with maximum gonadal maturation occurring in April 2002 and 2003 when water temperatures rose(Fig. 6) and phytoplankton was very abundant. Periods of high food abundance and gonad development were nearly coincident. In Korean coastal waters, growth and production of *Meretrix lusoria* and *Ruditapes philippinarum* are very high in the spring early summer seasons(Kim *et al.*, 1977; Chung *et al.*, 1994; Lee, 1995) due to the abundant phytoplankton that occurs with increasing water temperatures. Especially, *Ruditapes philippinarum*, *Meretrix lusoria* and other clams are commonly used as food organisms of *N. arthritica cumingii*. At this time, abundant food can be supplied to *N. arthritica cumingii* during the period of gonadal development and maturation. Therefore, it is suggested that gonadal development and maturation of *N. arthritica cumingii* is closely related to water temperature and food availability.

3. Breeding Pattern

As shown in Table 2, our histological observations show that spawning of *N. arthritica cumingii* on the west coast of Korea occurs from late May to August 2002 and 2003 when sea water temperatures were high. The spawning season of *N. cumingi* collected by the trawl net in the East China Sea occurs between July and August(Amio, 1963). And *N. arthritica* in Japan has been reported to spawn once a year from May and June in Usu Bay, Japan(Fujinaga, 1985).

Therefore, it is assumed that the spawning period of *N. arthritica cumingii* on the west coast of Korea occurred somewhat earlier than that in the East China Sea. On the whole, *N. arthritica cumingii* in Korea is a summer breeder, based on the criteria outlined by Boolootian *et al.*(1962) for marine mollusks. In general, it is assumed that spawning of *N. arthritica cumingii* and *N. arthritica* in Korea and Japan occurs between May and August. However, spawning of *N. constricta* and *Siphonalia assidariaeformis*(Buccinidae) occurs during December as a winter breeder(Table 2). Therefore, the slight discrepancy in the spawning period between these studies might be related to geographic differences in water temperature and food availability(Chung *et al.*, 2002).

4. First Sexual Maturity with the Gonad Developmental Stage

From the results of histological observations, we found that although the specimens were collected during the breeding season, the gonadal development of smaller individuals ranging from 31.4 to 40.9mm in shell height were in the early active stage as small number of oogonia and the previtellogenic oocytes

Table 2. Comparisons of the spawning season of Buccinidae in each locality

Species	Spawning season	Locality	Author
Neptunea arthritica cumingii	May~August	Kunsan, Korea	Present study
N. cumingii	July~August	East China Sea, China	Amio, 1963
N. arthritica	May~June	Usu Bay, Hokkaido, Japan	Fujinaga, 1985
N. arthritica	May~August	Saroma, Hokkaido, Japan	Kawai *et al.*, 1994
N. constricta	December	East Sea, Korea	Son, 2003
Siphonaria cassidariaeformis	December	East China Sea, China	Habe, 1960

were present in the follicle of the ovary. Judging from histological observations, it is supposed that the size of the oocyte could not have reached maturity until late August when spawning was ended. It was observed that snails of 51.0~60.9mm high were in the late active, ripe and partially spawned stages, and more than 50% reached first sexual maturity. However, all snails in the late active, ripe, or partially spawned stages reached it, if they were larger 61.0mm. This means that larger individuals can be reached maturity earlier than smaller individuals. In the aspect of natural resources management, the present study suggests that because catching the snails<51.0mm can potentially cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate natural resources management. Henceforth, age determination by size of the individuals should be investigated in detail for natural resources management of this species.

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