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Sexual Maturation and Artificial Spawning of the Hard Clam, *Meretrix lusoria* (Bivalvia: Veneridae) on the West Coast of Korea

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ABSTRACT

Reproductive cycle with the gonadal phases, first sexual maturity, artificial spawning amount by the size and spawning interval of the hard clam, *Meretrix lusoria* were investigated by histological observations and morphometric data by artificial spawning induction.

Meretrix lusoria is dioecious and oviparous. The reproductive cycle of this species can be classified into five successive stages: early active stage (January to March), late active stage (February to May), ripe stage (April to August), partially spawned stage (June to September), and spent/inactive stage (September to February). The spawning period was from June to September, and the main spawning occurred between July and August when the seawater temperature exceeds over 20℃. Percentage of first sexual maturity of female and male clams ranging from 40.0 to 45.0 mm in shell length was over 50%, and all clams over 50.0 mm in shell length sexually matured. Female and male clams ranging from 40.0 to 45.0 mm in shell length are considered to be two years old. Therefore, we assume that the hard clams of both sexes begin reproduction from two years of age. The mean number of the spawned eggs increased with the increase of size (shell length) classes. In case of artificial spawning induction, the number of spawned eggs from the clams of a sized class was gradually decreased with the increase of the number of the spawning frequencies (the first, second, and third spawnings). In the experiments of artificial spawning induction during the spawning season, the interval of each spawning was estimated to be 15-18 days (average 17 days).

Keywords: *Meretrix lusoria*, Reproductive cycle, First sexual maturity, Artificial spawning, Spawning.

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INTRODUCTION

The hard clam, *Meretrix lusoria* (Pelecypoda: Veneridae) is distributed along the coasts of Korea, China and Japan. Especially, it is found in the intertidal and subtidal zones of the south and west coasts of Korea (Kwon *et al.*, 1993). In Korea, the hard clam is one of the most important marine resources for human consumption. Due to reclamation projects of tidal flats and reckless overharvesting of this clam, its standing stock has been reduced during the past decade. Therefore, it is necessary to manage the population of this clam with a proper harvesting regime. Previously there have been many studies of *Meretrix lusoria*: on reproductive aspects, including artificial fertilization and development (Choi and Song, 1974), early embryonic development and growth (Choi, 1975; Hur, 1994), reproductive cycle (Lee, 1997), on ecological aspects, including production (Chun *et al.*, 1981). There are still gaps in our knowledge regarding reproductive biology of this clam. No information on spawning amount by the individual size and spawning interval are available. Understanding the reproductive cycle and the spawning period of this species will provide necessary information for age

determination and the recruitment period of a population. In addition, first sexual maturity, artificial spawning, and spawning intervals of a population are very useful information for aquaculture, natural resource management and reproductive potential in the conservating this species.

The purpose of the present study is to understand reproductive cycle, first sexual maturity, the number of spawned eggs by size class, spawning interval, and some basic aquaculture information for propagation and management in a shellfish farm.

MATERIALS AND METHODS

1. Sampling

Specimens of *Meretrix lusoria* were collected monthly from the intertidal and subtidal zones in Simpo coastal waters of Korea (Fig. 1), for one year, from January through December, 2002. After the clams were transported alive to the laboratory, shell length and height were measured by a Vernier caliper, and total weight was measured using a top-loading electric balance. Unpublished data for seawater temperatures measured at 10:00 a.m. by Kunsan Regional Maritime Affairs and Fisheries office were used for the present study.

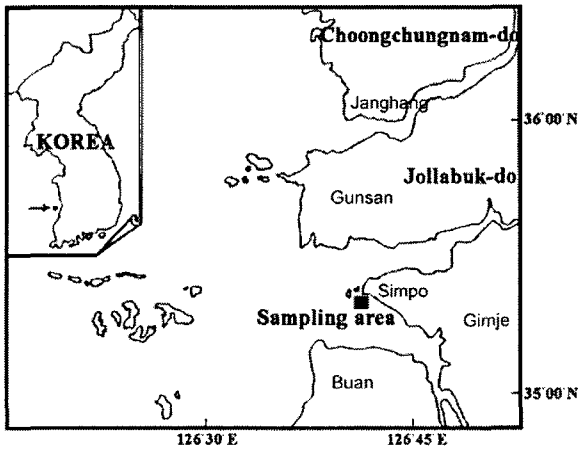

Fig. 1. Map showing the sampling area.

2. Histological analysis and histological staging

A total of 452 clams over 40.0 mm in shell length were used for the histological study. Histological preparations were made for analysis of the gonadal phases by light microscopy. Tissues were removed from shells and preserved in Bouin's fixative for 24 h and then washed with running tap water for 24 h. 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 and PAS stain, and were analyzed using a light microscope. Examination of gonad variability showed no significant differences in reproductive state between 7 random sections taken from different positions in the gonad. Sections were assigned to one of 5 stages: 1) early active, 2) late active, 3) ripe, 4) partially spawned, 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.

3. First sexual maturity

The percentage of first sexual maturity was investigated from the histologically prepared preparations to certify shell lengths of specimens that reached maturity and participated in reproduction from April through October, 2002. A total of 440 clams (221 females and 219 males) ranging from 26.3 to 95.4 mm in shell length were used for the study of first sexual maturity.

4. Induction of artificial spawning

1) Preparations before the spawning experiment

The hard clams of 40.0-79.4 mm in shell length, which were collected from Simpo coastal waters, were used for artificial spawning experiment. Adult clams were sorted into 8 size classes with a 5.0 mm interval. The first size class (1) represent individuals that are 1 year of age, while the last class (8) being composed of 7 years old.

For acclimation of adult hard clams in the

laboratory conditions for 3 days without food before the beginning of the experiment, clams were placed in rearing mesh containers (40 cm x 40 cm x 10 cm) with a 10 cm-deep layer of sand substrate: after sand substrates were collected from the shellfish bed in Simpo, they were sieved to remove any coarser particles (particle size > 1.0 mm in diameter) and were put into rearing containers after washing with tap water and drying.

Several beakers (200 ml) were placed in the water-bath equipped with automatic water temperature control system, and several aeration apparatus were installed.

Sufficient amount of cultured microalgae-supplemented seawater (*Tetraselmis tetrathele, Isochrysis galbana, Nitzschia* sp., *Chaetoceros gracilis, Chlorella ellipsoidea,* and *Nannochloris oculata*) were supplied as food (approximately 4-6 x 10^8 cells · G^{-1} · day^{-1} were ingested) before artificial spawning experiment. Cell density of phytoplankton were measured using a particle counter (TA. Coulter Electronics Ltd.) with 100 μm orifice aperture tube.

Salinity, the velocity of running seawater and initial seawater temperature in the FRP rearing aquarium during artificial spawning experiment were 31.5 psu, 0.5 l/ min., and 25 ± 0.5℃, respectively. Seawater in the FRP aquarium was replaced daily during the experiment.

2) First spawning experiment by artificial induction

A total of 300 clams ranging from 40.0 to 79.4 mm in shell length (over size of 50% of first sexual maturity) were reared for 3 days in two FRP rearing aquaria (1.0 m x 1.5 m x 0.5 m) for 3 days without food supply before the beginning of the experiment. A design for the first spawning experiment on June 1-2, 2003 was shown in Table 3. Sequences of several stimuli for spawning induction and the method for counting of the number of spawned eggs per individual are as follows:

A. Exposure stimulus to air and feeding

For the first artificial spawning induction, the sizes (shell length, cm) and total weights (g) of the adult clams were measured in advance during the period of exposure stimulus to the air for two hours. Then, each individual was transferred into a beaker (200 ml), sufficient amount of cultured microalgae-supplemented seawater were supplied for them as food (6 species of phytoplankton) for 5 h.

B. Thermal shock (water temperature stimulus)

After exposure stimulus and food supply, water temperatures were continuously raised up to 29℃ for 40 minutes from the initial level of 25℃ (by the modified methods of Hur (1994) and Toba and Miyama (1994)).

C. Biological stimulus by the sperm fluid

After receiving thermal shock, female clams were exposed to the sperm fluids released from male individuals for simultaneous artificial spawning.

D. Counting of the number of spawned eggs per individual

One ml of the total spawned eggs per individual by the shell size was transferred into a cell counter, and the number of spawned eggs were repeatedly counted from 5 fields using a particle counter (TA. Coulter Electronics Ltd.) and a light project (Nikon V12).

3) Second spawning experiment by artificial induction

To estimate the number of the second spawned eggs and spawning interval, a total of 216 clams (102 females and 114 males) which were already first spawned on June 1-2, 2003 were used for the second spawning on June 15-21, 2003 (at intervals of 14-19 days by the modified method of Toba and Miyama, 1994) as shown in Table 3. Environmental conditions in the FRP aquarium for the second induction of spawning were maintained as in the first spawning induction. The first spawned individuals were exposed to the air and provided sufficient cultured microalgae-supplemented seawater (the same amount of microalgae ingested for the first spawning experiment). After sufficient feeding, they received thermal shock from the initial water temperature of 25℃ up to 29℃, and female individuals were then exposed to the sperm fluid released from male ones at the intervals of 14-18 days (by the modified method of Toba and Miyama, 1994). And then the total number of the second spawned eggs/per individual by the shell size class were counted using the same methods for the first spawning experiment.

4) Third spawning experiment by artificial induction

To estimate the number of the third spawned eggs and spawning intervals, a total of 115 clams (52 females and 63 males which were the second spawned on 30 June to July 8, 2003) were used for the third spawning experiment (Table 3). Environmental conditions in the FRP aquarium for the third induction of spawning were maintained as in the second induction. The total number of the third spawned eggs per individual by shell size class were counted using the same counting method as in the first and second spawning experiments. To confirm the spawning interval of this population, the required days for the third spawning of the second spawned individuals were checked by the same experimental conditions used for the second spawning experiments in the laboratory.

RESULTS

1. Reproductive cycle with the gonad developmental stage

Frequency of gonadal phases of the *Meretrix lusoria* was shown in Fig. 2. Based on morphological characteristics of germ cells and surrounding tissues, gonadal phases of this species can be divided into 5 successive stages. Gonad developmental stages showed a periodicity.

1) Early active stage

In females, the early active stage was characterized by the expansion of the follicle and the appearance of well-defined oogonia and early developing oocytes along the follicle wall. The mean oogonium and oocyte diameters were 10-11 μm and approximately

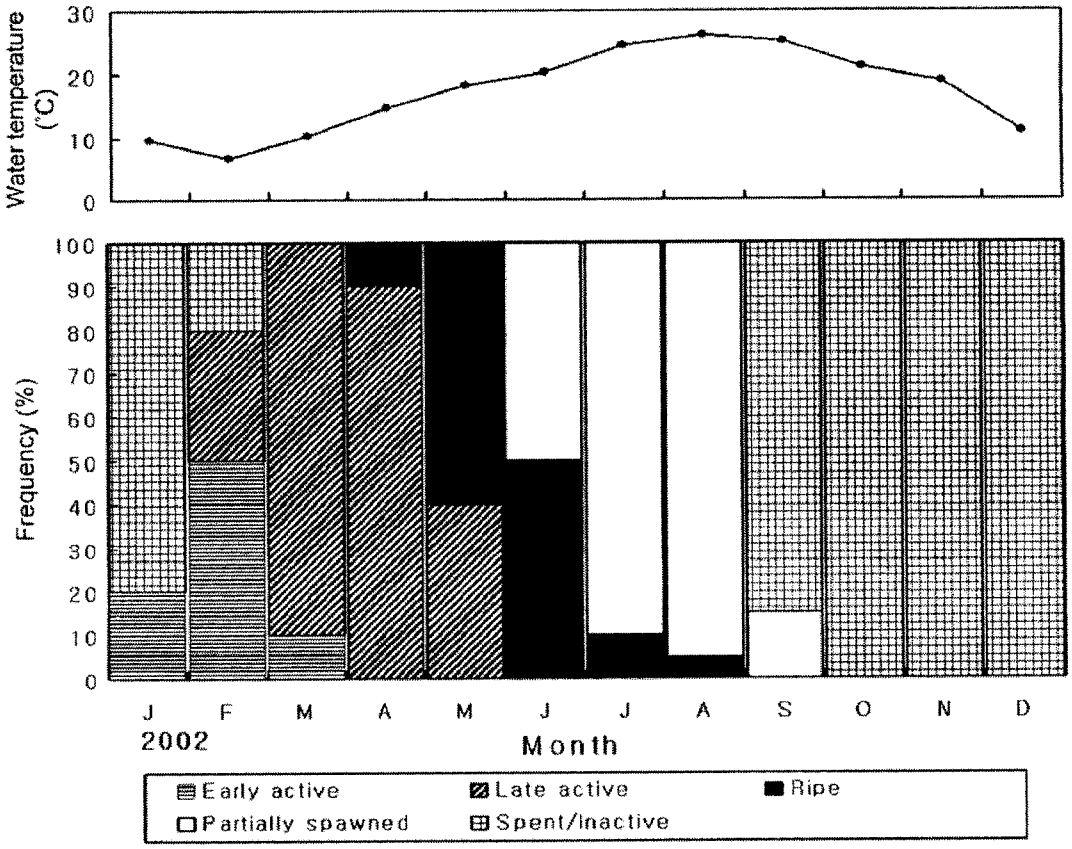

Fig. 2. Frequency of the gonadal phases of *Meretrix lusoria* and the mean seawater temperature from January to December, 2002.

20 μm, respectively. No free oocytes were present in the lumen (Fig. 3A).

In males, testis proliferation was characterized by the increase of the acini in number and size. Spermatogonia, spermatocytes and spermatids were present in the acini, while no spermatozoa were present (Fig. 4A). Individuals in the early active stage appeared from January through March.

2) Late active stage

In females, connective tissues decreased developing oocytes and a few free oocytes were present in the

Table 1. Number of female *Meretrix lusoria* at each gonadal stage in their first sexual maturing period from April to October, 2002.

Shell length (mm)	No. clam	Number of individuals by gonadal stage*					Percentage of matured clam
		EA	LA	RI	PS	SP/IA	
26.3-30.0	21	21					0.0
30.0-35.0	23	18	3	2			21.7
35.0-40.0	26	16	3	6	1		38.5
40.0-45.0	31	14	4	10	3		54.8
45.0-50.0	29	4	5	16	3	1	86.2
50.0-55.0	23		2	14	5	2	100.0
55.0-60.0	21		1	12	4	4	100.0
60.0-70.0	16			12	2	2	100.0
70.0-80.0	12			9	2	1	100.0
80.0-90.0	11			7	2	2	100.0
90.0-95.4	8			6	2		100.0
sum	221	73	18	94	24	12	

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

Table 2. Number of male *Meretrix lusoria* at each gonadal stage in their first sexual maturing period from April to October, 2002.

Shell length (mm)	No. clam	Number of individuals by gonadal stage*					Percentage of matured clam
		EA	LA	RI	PS	SP/IA	
26.3-30.0	14	14					0.0
30.0-35.0	26	20	4	2			23.1
35.0-40.0	25	15	2	7	1		40.0
40.0-45.0	30	13	3	12	2		56.7
45.0-50.0	29	4	4	14	5	2	86.7
50.0-55.0	24		3	12	5	4	100.0
55.0-60.0	20		2	10	5	3	100.0
60.0-70.0	21			14	4	3	100.0
70.0-80.0	11			8	2	1	100.0
80.0-90.0	12			5	5	2	100.0
90.0-95.4	7			4	2	1	100.0
sum	219	66	18	88	31	16	

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

lumen, but account for less than half of the total oocytes in the follicles. More than half of the oocytes were attached to the follicle wall; their mean oocyte diameter was 50-60 μm (Fig. 3B).

In males, connective tissue gradually decreased, and spermatogonia, spermatocytes, spermatids and spermatozoa in the acini were the major cell types. But a few spermatozoa were present in the acini (Fig. 4B). The individuals in the late active stage were observed from February through May.

3) Ripe stage

In females, the ripe ovary exhibited distended follicles with detached mature and fully ripe oocytes

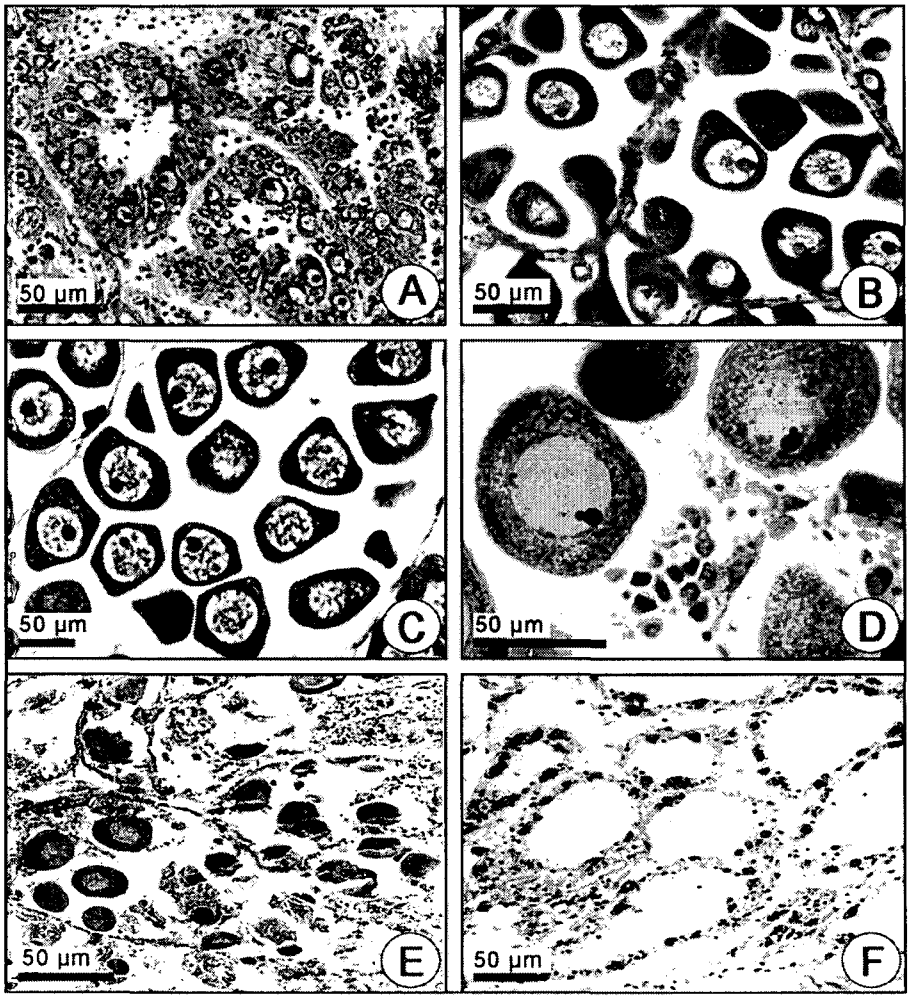

Fig. 3. Photomicrographs of gonadal phases in female *Meretrix lusoria* (A-F). **A**: Section of the follicles in the early active stage. Note oogonia and early developing oocytes attached to follicle walls (germinal epithelium); **B**: section of follicles in the late active stage. Note a number of late developing oocytes in the follicle; **C**: section of the follicles in the ripe stage. Note mature and ripe oocytes in the lumen of the follicle; **D**: a fully mature oocyte in the same stage. Note the germinal vesicle and a number of granules in the cytoplasm; **E**: section of the follicles in the partially spawned stage. Note undischarged oocytes in the lumen of the follicle after spawning; **F**: section of the follicles in the spent/inactive stage. Note newly formed oogonia on follicle walls and the connective tissues in the follicles after degeneration of the follicles.

were free in the lumen. The mean oocyte diameter is > 70 μm . Follicle size increased, while the follicle wall was thin (Fig. 3C, D).

In males, the acini in the testis were distended, the lumen was filled with a number of mature spermatozoa. Spermatids, spermatocytes and a few spermatogonia were found in the acini (Fig. 4C, D). The individuals in the ripe stage were found from April through August.

4) Partially spawned stage

In females, number of free oocytes in the follicle decreased, and empty and ruptured follicles appeared. Some oocytes underwent cytolysis. An

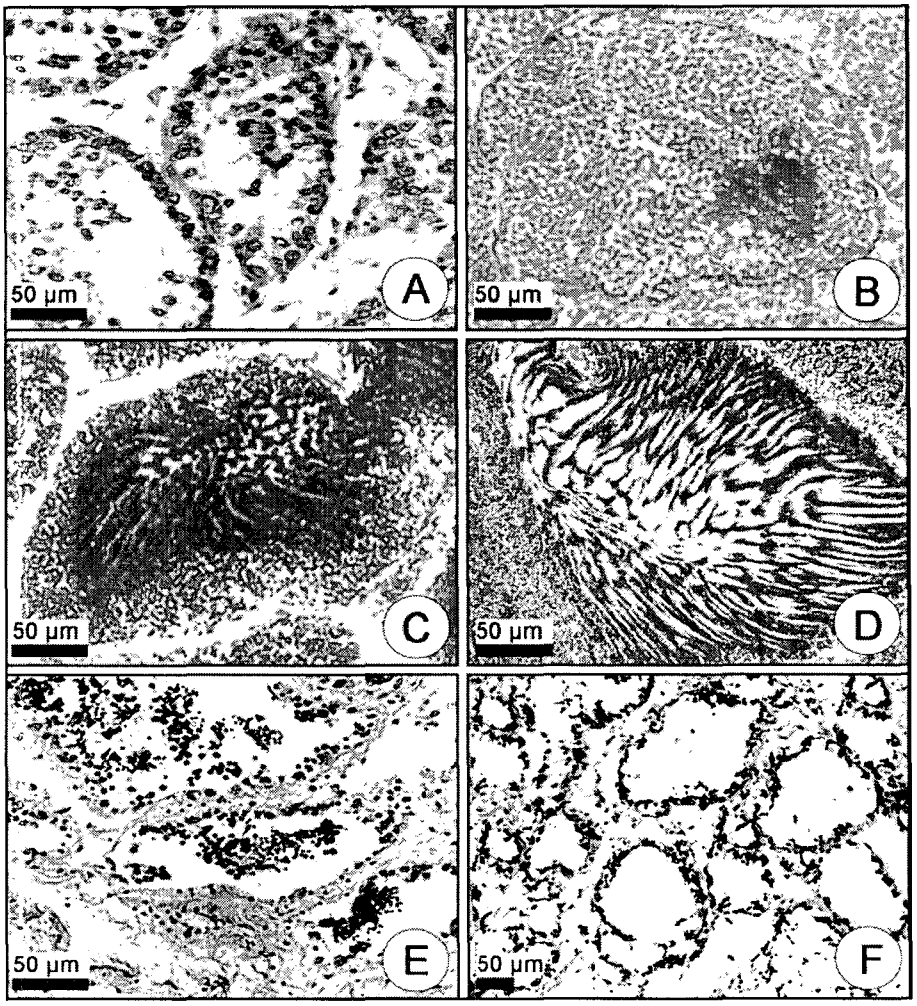

Fig. 4. Photomicrographs of gonadal phases in male *Meretrix lusoria* (A-F). **A**: Section of the acini in the early active stage. Note spermatogonia and spermatocyte; **B**: section of the acini in the late active stage. Note a number of a few spermatogonia, spermatocyte, spermatid, small number of spermatozoa during spermiogenesis; **C**: section of the acini in the ripe stage. Note a number of spermatozoa in the lumen; **D**: section of the acini in the partially spawned stage. Note a number of undischarged spermatozoa; **E**: section of the acini in the spent stage. Note undischarged spermatozoa degenerated in the lumen of the acini; **F**: section of the acini in the inactive stage. Note newly formed spermatogonia on the germinal epithelium and the connective tissues in the acini after degeneration.

empty space appeared in the center in over 25% of the follicles (Fig. 3E).

In males, the acini were collapsed or decreased in size. A small number of undischarged spermatozoa and spermatids were present in the lumen, while an empty space appeared in the center in over 30% of the acini (Fig. 4E). Spawning occurred from June to September, and the main spawning peak occurred from July through August.

5) Spent/inactive stage

In females, after spawning, half or more than half of the follicles were empty. Follicles were shrunken and the follicle wall was broken. Thereafter, newly formed oogonia appeared among the connective tissues and phagocytes (Fig. 3F).

In males, the acini were shrunk and disorganized. Only residual spermatozoa connective tissue and phagocytes can be found, thereafter, newly formed spermatogonia appeared among the connective tissues (Fig. 4F). The individuals in the spent/inactive stage appeared from September through February.

2. First sexual maturity

Female: First sexual maturity of a total of 221 individuals ranging from 26.3 to 95.4 mm in shell length was investigated histologically during before and after breeding season (from May to October). The rate of shells of different size that reached the first sexual maturity was summarized in Table 1. In case of some individuals with gonad developmental stage in the late active stage between April and May, it is supposed that they can reach maturity except for individuals in the early active stage during breeding season. First sexual maturity was 0% in female hard clams of 26.3-30.0 mm in shell length, if they were at the early active stages during the breeding season. The percentage of first sexual maturity of female clams of 30.0-35.0 mm in females was 21.7%, most of the individuals were still in the early active stage. Percentage of first sexual maturity in 40.0 to 45.0 mm in shell length was 54.8%, all female clams were in the early and late active stage, ripe stage and partially spawned stage. Sexual maturity was 100% for the clams over 50.0 mm in shell length.

Male: First sexual maturity of a total of 219 individuals ranging from 26.3 to 95.4 mm in shell length was investigated histologically during before and after the breeding season. The rate of shells of different size that reached the first sexual maturity was summarized in Table 2. In case of some individuals with gonad developmental stage in the late active stage between April and May, 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 26.3-30.0 mm in shell length, if they were at the early active stage during the breeding season. The percentage of first sexual maturity of female clams of 30.0-35.0 mm in females was 23.1%, most of the clams were still in the early active stage. Percentage of first sexual maturity of 40.0-45.0 mm-sized clams was 56.7%, all of which were in the early and late active stage, ripe stage and partially spawned stage. Sexual maturity was 100% for the clams over 50.0 mm in shell length.

3. Artificial spawning

1) Spawning reaction rate and spawning intervals

Spawning reaction rate by artificial induction and the number of spawned individuals were summarized in Table 3. A total of 102 female clams spawned after the first spawning induction between June 1, 2003 and June 2, 2003 in the indoor laboratory. A total of 52 clams among the first spawned 102 females (spawning reaction rate, 50.98%) spawned again after artificial induction during the period of June 16 to 21, 2003. A total of 23 clams among 52 females spawned (the third spawning reaction rate, 44.23%) showed the third spawning during the period of July 2 to June 7, 2003. The spawning interval between the first and second spawnings of this species was 15-18 days (from June 1 to June 16 and 17, and from June 2 to June 17-20, 2003); the spawning interval between the second and the third spawnings was 15-18 days under the conditions of sufficient food supply in the FRP rearing aquaria.

2) Number of spawned eggs by size

In the first spawning, the mean numbers of

Table 3. Spawning reaction rate by artificial induction and the number of spawned individuals of *Meretrix lusoria* from Simpo, Korea.

First spawning		Second spawning		Third spawning	
Date	No. spawned	Date	No. spawned	Date	No. spawned
June 1, 2003	58	June 15	0		
		June 16	13	June 30	0
				July 1	0
				July 2	3
				July 3	4
				July 4	0
		June 17	16	July 1	0
				July 2	0
				July 3	0
				July 4	2
				July 5	2
				July 6	0
June 2, 2003	44	June 16	0		
		June 17	6	July 1	0
				July 2	1
				July 3	3
				July 4	0
		June 18	7	July 2	0
				July 3	0
				July 4	2
				July 5	0
				July 6	1
		June 19	5	July 3	0
				July 4	0
				July 5	0
				July 6	2
				July 7	1
				July 8	0
		June 20	5	July 4	0
				July 5	0
				July 6	0
				July 7	2
				July 8	0
		June 21	0		
Total	102		52		23
Spawning rate			50.98% (SS/FS)*		44.23% (TS/SS)*

* FS: first spawning; SS: second spawning; TS: third spawning

spawned eggs in shell-length groups of 40.0-45.0 mm, 55.0-60.0 mm and 65.0-70.0 were 1,501,486 ± 143,512 eggs, 2,426,586 ± 242,288 eggs, and 3,165,667 ± 206,290 eggs, respectively. On the whole, the mean number of the first spawned eggs increased with the increase of shell length of female clams. As shown in Table 4, the number of the eggs released after the second induction were 83.93-88.85% of those released from the first spawning, and the number of spawned eggs after the third spawning induction ranged 88.58-97.41% (average 94.55%) of the second spawning and showed 76.86-86.55% (average 81.46%) of the first spawning, respectively. The number of spawned eggs gradually decreased with the increase of the number of spawning frequency (the first, second, and third spawnings) in each shell-length

group.

DISCUSSION

In the present study, *Meretrix lusoria* from Simpo coastal waters of Korea, initiated gonadal development during the late winter-early spring seasons when water temperatures was relatively low, while chlorophyll a levels were high during the period (Kim, 1999). The gonadal phases were in the inactive stage during the winter months (January to February) because of lower temperatures and insufficient food organisms.

Sastry (1966, 1968) contended that gonadal growth and gametogenesis in *Argopecten irradians* took place under the temperature conditions at which nutrient mobilization for the gonad occurred and temperature acted as a triggering stimulus for initiation of the oocyte growth phase. According to Chung *et al.* (2005), when gonadal maturation was artificially induced during the spawning period after supplying sufficient foods (phytoplankton) to *Ruditapes philippinarum,* most of the first spawned individuals reached the ovaries in the ripe stage within 14-19 days after spawning. while in *Meretrix lusoria,* mature ovary took 15-18 days after spawning. Therefore, our results are similar to those reported by Chung *et al.* (2005). We suggest that temperatures and food availability are required for active growth of oocytes at the beginning of oogenesis and for attaining maturity ultimately limit the annual period of ovarian development and oogenesis.

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 depends on ingested food, stored reserves, or some combination of two (Sastry, 1979; Barber, 1984). According to the report by Chung *et al.* (2005), food levels (phytoplankton) were high in mid spring (April) and

Table 4. Mean number of spawned eggs of *Meretrix lusoria* in each shell-sized group and the spawning reaction rate by the spawning frequency.

Shell length (mm)	First spawning		Second spawning			Third spawning			
	No. of spawned clam	Mean ± SD	No. of spawned clam	Mean ± SD	SS/FS (%)	No. of spawned clam	Mean ± SD	TS/SS (%)	TS/FS (%)
40.0-45.0	8	1,501,486 ± 143,512	4	1,273,642 ± 142,350	84.83				
45.0-50.0	11	1,686,167 ± 179,235	6	1,463,128 ± 167,012	86.77	3	1,296,034 ± 124,258	88.58	76.86
50.0-55.0	19	1,954,250 ± 220,209	9	1,736,394 ± 212,316	88.85	4	1,691,448 ± 186,296	97.41	86.55
55.0-60.0	23	2,426,586 ± 242,288	11	2,081,248 ± 236,262	85.77	6	1,965,239 ± 224,368	94.43	80.99
60.0-65.0	18	2,974,460 ± 256,017	8	2,584,771 ± 267,458	86.90	4	2,492,316 ± 198,122	69.42	83.79
65.0-70.0	9	3,165,667 ± 206,290	5	2,680,937 ± 173,495	84.68	3	2,591,234 ± 188,657	96.54	81.85
70.0-75.0	8	2,785,468 ± 176,216	5	2,337,745 ± 148,727	83.93	3	2,192,864 ± 164,529	93.80	87.73
75.0-79.4	6	2,668,750 ± 184,338	4	2,294,483 ± 198,656	85.98				
Total. (average spawning rate)	102		52		(85.96)	23		(94.55)	(81.46)

* FS, first spawning; SS, second spawning; TS, third spawning.
* SD means the standard deviation.

early summer (June). In the present study, ovarian growth and oogenesis in mid spring (April) coincide with high food level. The highest food level that occurred in early summer is necessary for oocyte maturity and spawning in *Meretrix lusoria*.

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 (Chung *et al.*, 2000; 2002). 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 *Meretrix lusoria*. Intra-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, 1997).

Rand (1973) stated that breeding strategy vary with latitudinal gradient: *i.e.*, Northern climates are 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 of the same species, there are some differences between the reproductive cycles of Veneridae bivalve, *Ruditapes philippinarum* in the other areas of the world; one spawning period in northern districts of Tokyo Bay (Yoshida, 1953) and two spawning periods in southern Japan (Tanaka, 1954; Ohba, 1959). In the present study, *Meretrix lusoria* has one spawning period as in *R. philippinarum* in the northern districts of Tokyo Bay, Japan. Therefore, it is assumed that the number of spawning frequencies in the same species vary with temperature-latitude.

First sexual maturity is assessed as a function of age and shell length. Age or shell length can be used as a convenient indicator. According to the results of percentages of first sexual maturity, those of females and males of 40.0-45.0 mm in shell length were 54.8% and 56.7%, respectively, and 100% in those of females and males > 50.0 mm in shell length.

According to the growth curves for the mean shell length fitted to von Bertalanffy's equation on by Kim (2005), individuals ranging from 40.0 to 45.0 mm in shell length are considered to be two years old. We assume that both sexes begin reproduction at two years of age.

For natural resources management of this species, the present study suggests that catching the hard clam < 40.0 mm in shell length or < 2 years old can potentially cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate natural resources management.

As shown in Table 3, the second spawning intervals were 15-18 days after the first spawning, and the third spawning intervals showed 15-18 days after the second spawning. Accordingly, the spawning intervals of this species were approximately 17 days under the conditions of sufficient food supply in the FRP aquarium in the laboratory. According to the number of spawned eggs per clam, it is assumed that the number of spawned eggs vary with size classes, spawning frequency and food supply. Even though the spawning season of this species occurs once a year in Korea, judging from these results of our indoor rearing experiment, it is assumed that the number of spawnings will be several times during the spawning period.

The number of eggs spawned artificially showed differences between shell-length groups of this species. On the whole, the first, second and third spawnings showed the increasing number of eggs as increase the size (shell length) and age, and in case of the same sized class, the mean number of the second and third spawned eggs varied with the spawning frequencies, as seen in *Ruditapes philippinarum* (Chung *et al.*, 2005).

Bayne *et al.* (1983) reported a ten-fold difference between the maximum and minimum values in egg production, reproductive value in *Mytilus edulis* from six sites on the English and Welsh coasts. Accordingly, it is assumed that the number of spawned eggs by the size class are influenced by natural environmental variables such as temperature,

food supply and tidal exposure.

From overall results mentioned above, we could get some basic information as follows; The spawning season of this species was from June to September. From this result, we confirmed that 50% of first sexual maturity could be seen in the group of 40.0-45.0 mm in shell length, and clams with these sizes are considered to be two years old (Kim, 2005). Therefore, a prohibitory measure and fishing prohibit period should be taken for adequate natural resources management.

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