Ovarian Cycle, the Biological Minimum Size and Artificial Spawning Frequency in Female *Meretrix petechialis* (Bivalvia: Veneridae) in Western Korea

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ABSTRACT : The ovarian cycle, the biological minimum size, and artificial spawning frequency by artificial spawning induction of the female hard clam, *Meretrix petechialis*, were investigated by histological observations and morphometric data. The ovarian cycle of this species can be classified into five successive stages: early active stage, late active stage, ripe stage, partially spawned stage, and spent/inactive stage. The spawning period was from June to September, and the main spawning occurred between July and August when the seawater temperature exceeds over 20℃. The biological minimum size (shell length at 50% of first sexual maturity) in females were 40.39 mm in shell length (considered to be two years of age), and all clams over 50.1 mm in shell length sexually matured. In this study, the mean number of the spawned eggs by spawning induction increased with the increase of size (shell length) classes. In case of artificial spawning induction for the clams > 40.39 mm, 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 spawning). In the experiments of artificial spawning induction during the spawning season, the interval of each spawning of this species was estimated to be 15-18 days (approximately 17 days).

Key words : *Meretrix petechialis*, Ovarian cycle, First sexual maturity, Biological minimum size, Artificial spawning, Spawning interval

INTRODUCTION

The hard clam, *Meretrix petechialis* (Pelecypoda: Veneridae) is one of the commercially important edible clams in East Asian countries, including Korea, China, and Japan (Kwon et al., 1993; Min et al., 2004). The species is mainly found in the intertidal and subtidal zones of the south and west coasts of Korea (Min et al., 2004). More specifically, in Korea, *M. petechialis* has been called "the hard clam, *M. lusoria*" because the morphology of *M. petechialis* is very similar to that of *M. lusoria*. Therefore, two species is one of the most important marine resources for human consumption.

As a consequence of reckless over-harvesting and parasitic infections, the standing stock of this hard clam has dramatically declined as a target organism and declined in recent years and the species has been denoted as a target organism and fisheries resource that should be managed using a more reasonable fishing regime (Kim, 2005). For propagation and management, it is important to understand the population characteristics with regard to the ovarian cycle and size at first sexual maturity, and the size at the rate (50%) of group sexual maturity (RM_{50}).

Previously, regarding *M. lusoria,* there have been several studies on reproductive aspects, including artificial fertilization

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and development (Choi and Song, 1974), early embryonic development and growth (Choi, 1975; Hur, 1994), reproductive cycle (Lee, 1997; Chung, 2007), oogenesis (Chung, 2007) on ecological aspects, including production (Chun et al., 1981). However, regarding *M. peteichialis,* previously, there have been a few studies on reproductive aspects, including reproductive cycle (Chung, 2006), spermatogenesis (Chung 2006), on morphology and ecology (Kwon et al., 1993) and on genetic aspect, including karyotypes (Park et al., 2011). However, there are still gaps in our knowledge regarding reproductive biology of this clam. No information on artificial spawning amounts by the individual size and spawning intervals are available. Understanding the ovarian 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 frequencies (intervals) of a population are very useful information for aquaculture, natural resource management and reproductive potential in the conservating this species. In particular, information on the size at which individuals reach first sexual maturity could be useful in determining a prohibitory measure for adequate natural resource management and reproductive potential in the conservating of this species.

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

MATERIALS AND METHODS

1. Sampling

Specimens of *M. petechialis* were collected monthly from the intertidal and subtidal zones in Simpo coastal waters of Korea (Fig. 1), for one year, from January through December, 2006. 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 am. by Kunsan Regional Maritime Affairs and Fisheries office were used for the present study.

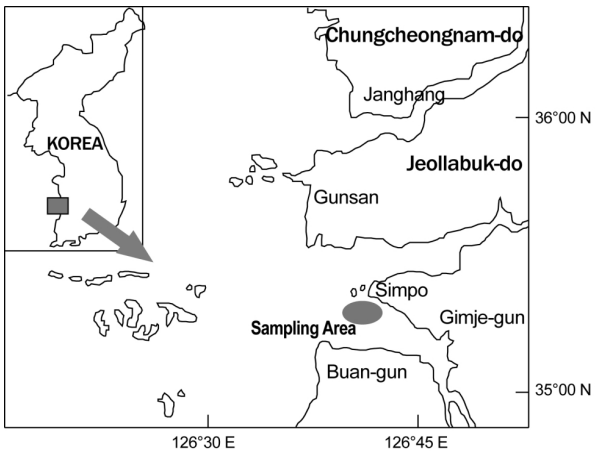

Fig. 1. Map showing the sampling area.

2. Gonad Index (GI)

To explore the spawning season by qualitative analysis (histological observations), the mean GI of the hard clam was calculated using a modification of Mann's method (1979). Each histological section of gonadal tissues was also examined in details to assess the stage of gonadal development. Staging criteria of 1 to 5 were employed for Spent / inactive (S1 = 1), Partially Spawned (S2 = 2), Early active (S3 = 3), Late Active (S4 = 4) Ripe (S5 = 5), these categories are only approximations of gonadal development because it is a continuous process and distinctions between stages are not always clear. The monthly GI for both sexes was determined by multiplying the number of specimens ascribed to each category score, summing all those values and dividing this figure by the total number of clams analyzed.

$$GI = \frac{(N \times RVSI) + (N \times RVS2) + (N \times RVS3) + (N \times RVS4) + (N \times RVS5).}{\text{Total N observed by month}}$$

Where, N: number of individuals, RVS: ranking value by stage.

3. 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 both 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.

4. Size at First Sexual Maturity by Light Microscopical Observation

For determination of the size at 50% of first sexual maturity, a total of 206 ovarian histological preparations (26.3-95.7 mm in shell length) were examined the size at 50% first sexual maturity (=biological minimum size) by histological observations from January to December 2006. The percentage (%) of first sexual maturity = No. of mature individuals × 100 / No. of total individuals investigated.

5. Biological Minimum Size (= Size at the Rate (50%) of Sexual Maturity (RM_{50})

To calculate the size at the rate (50%) of sexual maturity after fitting the rate of sexual maturity to an exponential equation, the size equivalent to the size at 50% of sexual maturity was estimated to be the sexually mature length of the population (Chung and Ryou, 2000). The exponential equation of the rate of sexual maturity is as follows: $RM = 100/1+\exp^{(a-bx)}$, where, RM: rate of sexual maturity; a, b: constants, x: shell length.

6. Induction of Artificial Spawning

1) Preparations Before the Spawning Experiment

The hard clams of 40.4-79.9 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 × 40 cm × 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 (*Isochrysis galbana, Nitzschia* sp., *Chaetoceros gracilis, Chlorella ellipsoidea, and Nannochloris oculata*, *Tetraselmis tetrathele*) were supplied as food (approximately 4-6 × 10^8 cells · G^{-1} · day^{-1} were ingested) before artificial spawning experiment. Cell densities 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 was 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 × 1.5 m × 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:

(1) 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.

(2) Thermal Shock (Water Temperature Stimulus)

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

(3) 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.

(4) 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 numbers of spawned eggs were repeatedly counted from 5 fields using a particle counter (TA. Coulter Electronics Ltd.) and a light project (Nikon V12).

2) First and Second Spawning Experiments by Artificial Inductions

To estimate the number of the first spawned eggs and spawning interval, a total of 225 clams (107 females and 118 males) which were already first spawned on June 11-12, 2006 were used for the second spawning on June 25-July 1, 2006 (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).

To estimate the number of the second spawned eggs and spawning interval, a total of 107 female clams (which were already first spawned on June 11-12, 2006) were used for the second spawning on June 25-July 1, 2006 by the same method (at intervals of 14-19 days by the modified method of Toba and Miyama (1994) as shown in Table 3.) of the first spawning experiment. 57 of 107 female clams were second spawned. And then the total numbers of the second spawned eggs/per individual by the shell size class were counted using the same methods for the first spawning experiment.

3) Third Spawning Experiment by Artificial Induction

To estimate the number of the third spawned eggs and spawning intervals, a total of 57 females which were second spawned on 10-17 July 2006 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. Monthly Changes in the Gonad Index (GI)

Monthly changes in the GI of *M. petechiales* are shown in Fig. 2. The GI in females and males began to gradually increase in February and reached a maximum (4.7) in June, and then the GI values gradually decreased from June to September when spawning occurred and relatively high water temperatures were maintained. Thereafter, the GI values temporarily reached a minimum in October (1.1). Monthly changes in the GI in female in 2006 showed a peak in May during the year, and which indicates that the number of spawning period of Korean population of *M. petechialis* is once a year.

2. Ovarian Cycle with the Gonad Developmental Stage

Based on morphological features and sizes of the germ cells and surrounding tissues, frequency of gonadal phases of the *M. petechialis* was shown in Fig. 2. The ovarian cycle with gonadal stages of this species can be classified into five successive stages: 1) early active stage, 2) late active, 3) ripe stage, 4) partially spawned stage, 5) spent and inactive stage. Gonad developmental stages showed a periodicity. The stages and the criteria used to define them are as follows:

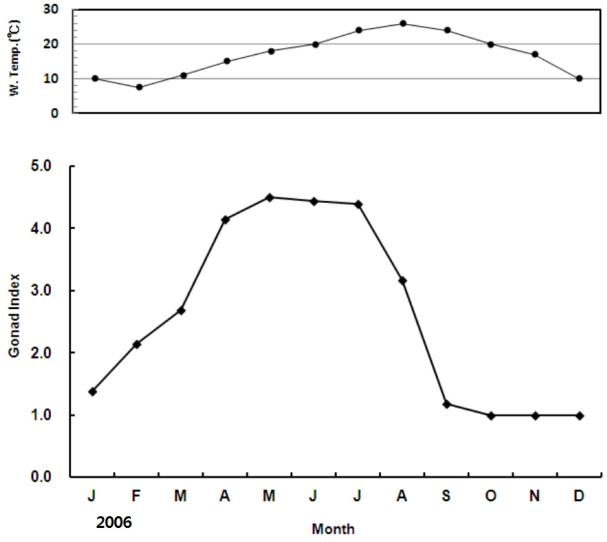

Fig. 2. Monthly changes in the mean gonad index and water temperatures from January to December, 2006.

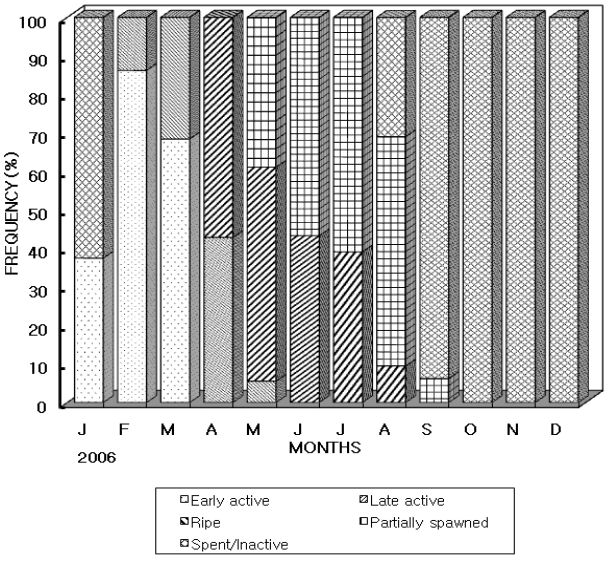

Fig. 3. Frequency of the gonad development phases in female *Meretrix petechialis* from January through December, 2006.

1) Early Active Stage

Oogonia and previtellogenic oocytes proliferate along the follicular wall (germinal epithelium) in the ovary. The oogonia and previtellogenic oocytes are 9-11 μm and 15-25 μm in diameter, respectively. Also some auxiliary cells, which are attached to the previtellogenic oocyte, appear near the follicular wall of the oogenic follicle (Fig. 4A). Female individuals in the early active stage appear from Januarybruary to March when seawater temperatures are gradually increasing (Figs. 2, 3).

2) Late Active Stage

A number of the early vitellogenic oocytes, ranging from 30 to 45 μm in diameter, appear in the oogenic follicles. Each oocyte has an egg-stalk attached to the follicular wall and the nucleus is enlarged, having a small nucleolus. At this time, some auxiliary cells, which were attached to the stalk region of an early vitellogenic oocyte, appear near the follicular wall. When late vitellogenic oocytes grow to 45-50 μm in diameter, the cytoplasm of the oocytes are filled with a number of yolk granules (Fig. 4B). Female individuals in the late active stage are found from February to May when seawater temperatures are relatively high (Figs. 2, 3).

3) Ripe Stage

The ripe ova are 65-70 μm in diameter, and are round or oval in shape. They are located in the centre of the lumen. At this time, ripe ova have a large germinal vesicle; are surrounded by a thick egg envelope. The cytoplasm of the ripe ovum is filled with a number of yolk granules (Fig. 4C). Ripe ovaries are found from April to August when seawater temperatures are gradually decreasing (Fig. 2).

4) Partially Spawned Stage

50-60% of the ripe ova in the oogenic follicles have been discharged. The lumen is largely empty. Spawned ovaries are characterized by the presence of a few ripe, undischarged and previtellogenic oocytes in the lumen (Fig. 4D). Female individuals in the partially spawned stage appear from May through September, with the main spawning event occurring between December and January when seawater temperatures are relatively low (Figs. 2, 3).

5) Spent/Inactive Stage

After spawning, the undischarged oocytes in the lumen undergo cytolysis and the products of gamete atresia are resorbed (Fig. 4E). Thereafter, rearrangement of some new oogonia and connective tissue appeared in the oogenic follicles (Fig. 4F). Female and male individuals in this stage are found from February to April when seawater temperatures are relatively low (Fig. 2).

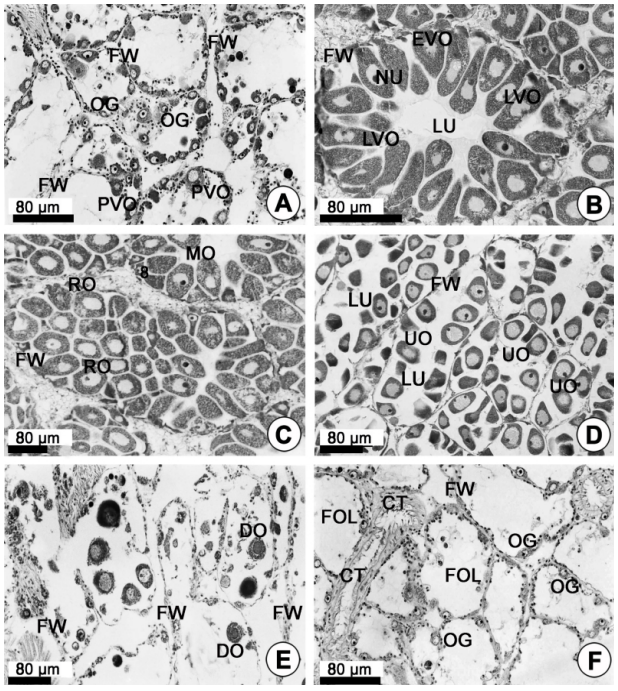

Fig. 4. Photomicrographs of gonadal phases of the female *Meretrix petechialis* (A-F). Photomicrographs of gonadal phases of the female *Meretrix petechialis*. A, Section of the follicles in the early active stage. Note oogonia and previtellogenic oocytes attached to follicular walls (germinal epithelium); B, Section of follicles in the late active stage. Note a number of early vitellogenic and late vitellogenic oocytes in the follicle; C, Section of the follicles in the ripe stage. Note maturing oocytes and ripe ova in the lumen of the follicle; D, Section of the follicles in the partially spawned stage. Note a number of undischarged oocytes in the lumen of the follicle after spawning; E: Section of the follicles in the spent stage. E, Section of the follicles in the spent stage. Note residual undischarged oocytes and a number of degenerating oocytes in the lumen of the follicles; F, Section of the follicles in inactive stage. Note a few oogonia on well-developed follicular walls and the connective in the follicles. Abbreviations: CT, connective tissue; DO, degenerating oocyte; EVO, early vitellogenic oocyte; FOL, follicle; FW, follicular wall; LU, lumen; Mo, maturing oocyte; NU, nucleolus; OG, oogonia; PVO, previtellogenic oocyte; RO, ripe ova; UO, undischarged oocyte.

3. Size at First Sexual Maturity in Females

A total of 206 females individuals of *M. petechialis* were investigated histologically to determine the shell lengths of hard clams that reach maturation and participate in reproduction from May (before spawning) to late August (after spawning).

As shown in Table 1, it was found that the percentage of first sexual maturity of smaller individuals ranging from 26.3-30.0 mm in shell length was 0%, and that those individuals were in the early active stage, characterized by a small number of oogonia and the appearance of previtellogenic oocytes. It is supposed that their sizes at sexual maturity could not have been reached until late August when spawning was completed. In addition, the percentage of first sexual maturity of female clams ranging from 30.1-35.0 mm shell length was 20.8%, and that those individuals were in the early active, late active and ripe stages during the period between June and August, when spawning was observed among older individuals. However, younger animals had a small number of oogonia and a number of previtellogenic oocytes. A number of vitellogenic oocytes and a small number of mature oocytes were present in the follicles of the ovary. It is supposed that their sizes at sexual maturity could not have been reached until late August when the spawning of a few mature individuals was completed. In addition, the percentage of first sexual maturity of female clams ranging from 50.1-55.0 mm in shell length was 51.6%, and those individuals were in the early active, late active, ripe, and partially spawned stages during the breeding season. In contrast, the percentage of first sexual maturity of all individuals of shell length greater than 25.1 mm is 100%, and that those individuals were in the late, ripe, partially spawned, and spent / inactive stages. Accordingly, it is assumed that most individuals can reach full maturity by late August if they are larger than 50.1 mm in shell length.

4. Biological Minimum Size (= Size at the Rate (50%) of Group Maturity; RM_{50})

As shown in Fig. 5, shell lengths of sexually mature Manila clams (sizes at the rate (50%) of group maturity, RM_{50}) that were fitted to an exponential equation were

Table 1. Shell lengths of first sexual maturity in female *Meretrix petechialis* from April to October, 2006

Shell length (mm)	Number of individuals by gonadal stage*					Total ind.	Mature(%)
	EA	LA	RI	PS	SP/IA		
26.3-30.0	19					19	0.0
30.1-35.0	19	3	2			24	20.8
35.1-40.0	16	5	4	1		27	37.0
40.1-45.0	15	4	9	3		31	51.6
45.1-50.0	5	5	13	3	1	27	85.2
50.1-55.0		2	12	4	2	20	100.0
55.1-60.0		2	10	4	2	18	100.0
60.1-70.0			10	3	1	14	100.0
70.1-80.0			6	2	2	10	100.0
80.1-90.0			5	3	1	9	100.0
90.1-95.7			4	2	1	7	100.0
Total						206	

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

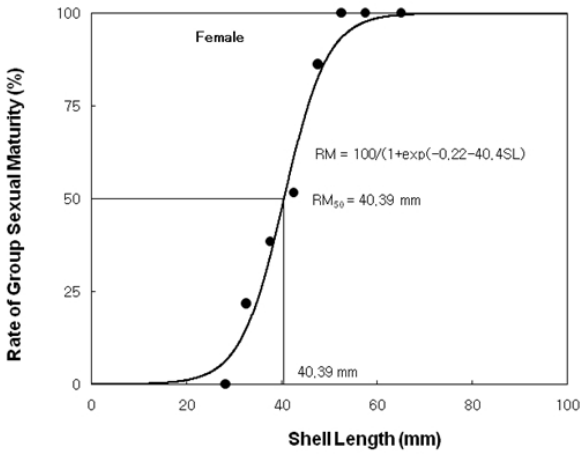

Fig. 5. Relationship between the rate of group sexual maturity (%) and shell length (mm) in female *Meretrix petechialis*.

40.39 mm in females.

5. Artificial Spawning

1) Number of Spawned Eggs by Size

The mean numbers of spawned eggs of *M. petechialis* in each shell size group were showed in Table 2. In particular, we can confirm the number of spawned clams and the mean number of eggs spawned in shell length groups of 40.1-45.0 mm (considered to be the size at 50% of first sexual maturity) and 50.1-55.0 mm (considered to be the size at 100% of first sexual maturity) after the first, second, and third artificial spawning inductions.

In case of the first spawning, the number of spawned clams and the mean numbers of spawned eggs in shell length group of 40.1-45.0 mm were 9 clams and 1,611,586± 154,616 eggs, respectively, however, those in 50.1-55.0 mm in shell length group were 18 clams and 2,067,472± 212,362, respectively. Accordingly, the number of spawned clams and the mean number of eggs spawned in shell length groups increased with the increase of shell length except for shell length groups of relatively older individuals.

In the shell length group of 40.4-45.0 mm, the number of spawned clams and the mean number of spawned egg by the first and second artificial spawning inductions were 9 clams and the mean 1,611,586±154,616 eggs, in the second spawning, those were 3 clams and 1,318,704± 168,272 eggs. In shell length group of 50.1-55.0 mm, the number of spawned clams and the mean number of spawned egg were 18 clams and the mean 2,067,472±

Table 2. Mean number of spawned eggs of *Meretirx petechialis* in each shell size group.

Shell length	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 (%)
40.4-45.0	9	1,611,586±154,616	3	1,318,704±168,272	81.83			
45.1-50.0	13	1,803,157±186,525	8	1,536,323±224,352	85.20	4	1,079,384±194,786	70.26
50.1-55.0	18	2,067,472±212,362	12	1,841,401±204,186	89.07	5	1,402,326±207,592	76.16
55.1-60.0	21	2,504,575±355,378	11	2,192,357±293,374	87.53	6	1,704,148±209,478	77.73
60.1-65.0	20	2,863,764±237,306	9	2,404,572±208,562	83.97	4	2,029,897±207,214	84.42
65.0-70.0	10	3,067,768±248,362	6	2,541,652±169,583	82.85	4	2,102,345±169,723	82.71
70.1-75.0	9	2,868,689±228,215	4	2,473,552±159,739	86.23	2	1,831,645±178,838	74.05
75.1-79.9	7	2,779,865±205,476	4	2,435,246±187,764	87.60			
Mean %					85.54			77.56
Total No. of spawned female clams	107		57 (53.27%)			25 (43.86%)		

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

212,362 eggs by the first spawning induction, however, 12 clams and 1,841,401±204,186 eggs occurred by the second spawning induction, and 5 clams and 1,402,326±207,592 eggs occurred by the third artificial spawning inductions. The percentage of the mean number of spawned egg by the second spawning was 89.07% of the first spawned eggs, and that by the third spawning induction was considered to be 76.16% of that of the mean number of the spawned egg by the second spawning induction.

Accordingly, in case of the same shell length group, the number of spawned clams and the number of spawned eggs by the first, second, third spawning inductions increased with the increase of shell length, respectively, ecept for shell length groups of relatively older individual. In this study, the mean total number of eggs spawned by the second spawning was considered to be 85.54% of that of the first spawning, and also that of eggs spawned by the third spawning induction was considered to be 77.56% of that of the second spawning. Accordingly, 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) (Table 2).

2) Spawning Reaction Rate and Spawning Intervals

The spawning reaction rate by the artificial induction and the number of spawned individuals were summarized in Table 3. A total of 107 female clams reacted and spawned to the first spawning induction: 56 female clams reacted to first spawning induction in June 11, 2006, and 51 female clams spawned in June 12, 2006 in the indoor laboratory. Of the first spawned 107 female clams, a total of 57 female clams (spawning reaction rate, 53.27%) spawned again after artificial induction during the period of June 25-27, 2006 and from June 26 to July 1, 2006. A total of 25 clams of 57 females clams (the second spawning) in the third spawning during the period of July 10 to July 13, 2006. The spawning interval between the first and second spawning of this species was 15-18 days (from June 26 to July 10-July 13, from June 12 to June 27-30, 2006); the spawning interval between the second and the third spawning was 15-18 days (Average 17 days) under the conditions of sufficient food supply in the FRP rearing aquaria.

DISCUSSION

The gonad index (GI) of this species began to increase in spring months and reached a maximum in June when the water temperature rapidly increased. Thereafter, the GI values then showed a gradual decrease because of spawning, with the increase of water temperatures. Chung (2007) reported that the high average values of the GI coincided with gonadal maturity, and the minimal average value following high average valueswere considered an indication of spawning. Accordingly, variations in the GI showed a close relationship with gonadal development and gonadal activity. In this study, monthly variations in the GI by qualitative analysis showed a maximum showing an uni-modal cycle during the year. Therefore, monthly changes in the GI in both sexes in 2006 showed a similar pattern with gonadal development and the spawning period of this species in Korea showed once a year, indicating a uni-modal cycle. In the present study, *M. petechialis* 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 (phyto-

Table 3. Spawning rates of *Meretrix petechialis* by the artificial spawning inductions in the indoor experiment

First spawning		Second spawning		Third spawning	
Date	No. spawned female clams	Date	No. spawned female clams	Date	No. spawned female clams
June 11, 2006	56	June 25, 2006	0		
		June 26	15	July 10	1
				July 11	2
				July 12	1
				July 13	1
		June 27	17	July 11	1
				July 12	1
				July 13	1
				July 14	1
June 12, 2006	51	June 26, 2006	0		
		June 27	5	July 11	1
				July 12	1
				July 13	1
				July 14	1
		June 28	8	July 12	1
				July 13	1
				July 14	1
				July 15	1
		June 29	6	July 13	1
				July 14	1
				July 15	1
				July 16	1
		June 30	6	July 14	1
				July 15	1
				July 16	1
				July 17	1
		July 1 2006	0		
Total No. of spawned clams	107 (FS)		57 (SS)		25 (TS)
Spawning rate	100%		53.27% (SS/FS)		43.86% (TS/SS)

* FS, first spawning; SS, second spawning; TS, third spawning.

plankton) to *Ruditapes philippinarum,* most of the first spawned individuals reached the ovaries in the ripe stage within 14-19 days after spawning. While in *M. petechialis*, 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) in western Korea.

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 *M. petechialis*.

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 *M. petechialis*. 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, *M. petechialis* in western Korea 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 the percentage of first sexual maturity, those of females of 40.1-45.0 mm in shell length was 51.6%, and 100% in those of females> 50.1 mm in shell length.

According to the growth curves for the mean shell length fitted to von Bertalanffy's equation used by Kim (2005), individuals ranging from 40.1 to 45.0 mm in shell length are considered to be two years old. We assume that this clams begin to reproduce, and they are participated in 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 about 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 spawning 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 spawning 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 *R. 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 numbers 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 40.1-45.0 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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