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Reproductive Biology of the Female Manila Clam, *Ruditapes philippinarum* (Bivalvia: Veneridae) on the West Coast of Korea

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ABSTRACT

Reproductive cycle, first sexual maturity, spawning amount related with the size and spawning interval in female *Ruditapes philippinarum* were investigated by histological observation and the analysis of morphometric data during artificial spawning induction. *Ruditapes philippinarum* is dioecious and oviparous. The reproductive cycle of this species can be subdivided into five successive stages: early active stage (January to March), late active stage (February to May), ripe stage (April to August), partially spawned stage (May to October), and spent/inactive stage (August to February). The spawning period was once a year between May and early October, and the main spawning occurred between July and August when seawater temperature was approximately 20ºC. Percentages of first sexual maturity of female clam of 15.1-20.0 mm in shell length were 56.3%, and 100% for the clams > 25.1 mm. The mean number of the spawned eggs increased with the increase of size classes (shell length). In case of spawning induction by the same size class, the number of spawned eggs were gradually decreased with the increase of 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 15-17 days (average 16.5 days).

Keywords: *Ruditapes philippinarum*, Gonadal maturation, First sexual maturity, Artificial spawning amount, Spawning interval.

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INTRODUCTION

The Manila clam, *Ruditapes philippinarum* (Pelecypoda: Veneridae) is distributed along the coasts of Korea, China and Japan. More specifically, it is found in the intertidal and subtidal zones of the south and west coasts of Korea (Yoo, 1976; Kwon *et al.*, 1993; Chung *et al.*, 1994). In Korea, the Manila 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 (Ministry of Agriculture and Forestry Republic of Korea, 1997). Therefore, it is necessary to manage the population of the clam with a proper harvesting regime that will maintain an optimal population size in aquafarm.

In Korea and Japan, there have been many previous studies on aspects of ecology including growth (Choi, 1964; Hur, 1994; Goshima *et al.*, 1996), population dynamics and secondary production (Ohba, 1959; Choi, 1987; Yoon, 1992), on aspects of reproduction including maturation (Toba and Miyama, 1995), artificial discharging (Sagara, 1958), the spawning season (Yoshida, 1953; Tanaka, 1954; Ohba, 1959; Holland and Chew, 1974; Ponurovsky and Yakovlev, 1992) and reproductive cycle (Toba *et al.*, 1993; Chung *et al.*, 1994; Toba and Miyama, 1994; Tsuji *et al.*,

1994; Goshima *et al.*, 1996).

Although there have been several studies on reproductive ecology of the Manila clam, especially, 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, sex ratio, 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 conservation of this species.

The purpose of the present study is to understand reproductive ecological data on the Manila clam including the reproductive cycle, first sexual maturity, the sex ratio, the number of spawned eggs, spawning interval and some basic acquaculture information for propagation and management in a shellfish farm.

MATERIALS AND METHODS

1. Sampling

Specimens of the Manila clam, *Ruditapes philippinarum* were collected monthly from the intertidal zone (shellfish farms) in Gomso Bay, western coast of Korea from January through December, 2000. 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 Gochang Regional Maritime Affairs and Fisheries office were used for the present study.

2. Histological analysis and histological staging

A total of 364 clams over 15.1 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 hours and then washed with running tap water for 24 hours. The tissues were then dehydrated in alcohol, embedded in paraffin and sectioned at 5-7 μm using a rotary microtome. Sections were then mounted on glass slides, stained with either Hansen's hematoxylin-0.5% eosin and PAS stain, and were analyzed using a light microscope. Examination of gonad variability in *Ruditapes philippinarum* showed no significant differences in reproductive state between 7 random sections taken from different positions in the ovary. Sections were assigned to one of 5 stages: 1) early active, 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 percentages of first sexual maturity were investigated from the histologically prepared preparations to certify shell lengths of specimens that reached maturity and participated in reproduction during the ripe and breeding seasons from May through October 2000. A total of 123 clams ranging from 8.1 to 54.9 mm in shell length were used for the study of first sexual maturity.

4. Induction of artificial spawning

1) Preparations before the spawning experiment

A. Specimen collection and group by the size class: The Manila clams of 20.2-46.7 mm in shell length, which were collected from Gomso Bay, were used for artificial spawning experiment. Adult clams were sorted into 6 size classes with a 5.0 mm interval. The first size class represented individuals that were one year of age, while the last class consisted of four year olds.

B. Acclimation conditions of the adult clams: For acclimation of adult Manila 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 Gomso Bay, they were sieved to remove any coarser particles (particle size > 1.0 mm diameter) and were

put into rearing containers after washing with tap water and drying.

C. Installed apparatus: Several beakers (20 ml) were placed in the water-bath equipped with automatic water temperature control system, and several aeration apparatus were installed.

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

E. Natural filtered seawater: 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°C, 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 20.2 to 46.7 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 before the beginning of the experiment. A design for the first spawning experiment on July 1 to 2, 2000 is 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 hours.

B. Thermal shock (water temperature stimulus): After exposure stimulus and food supply, water temperatures were continuously raised upto 29°C for 40 minutes from the initial level of 25°C (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 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 89 female clams which were already first spawned on July 1 to 2, 2000, were used for the second spawning on July 15 to 19, 2000 (at intervals of 14-17 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 14-17 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 was 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 54 female clams which were the second spawned on July 15-19, 2000) were used for the 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 ovarian developmental stage

Frequency of gonadal phases of the Manila clam was shown in Fig. 1. Based on morphological characteristics of germ cells and surrounding tissues, gonadal phases of this species can be divided into 5 successive stages. Ovarian developmental stages showed a periodicity.

1) Early active stage

This stage was characterized by the expansion of the follicle and the appearance of oogonia and early developing oocytes along the follicular wall. No free oocytes were present in the lumen. At this time, the mean oogonium and oocyte diameters were 10-11 μm and < 20 μm, respectively (Fig. 2A).

Individuals in the early active stage appeared from January through March.

2) Late active stage

At this stage, the connective tissues in the follicle were gradually decreased, developing oocytes and a few free oocytes were present in the lumen. More than half of the oocytes were attached to the follicular

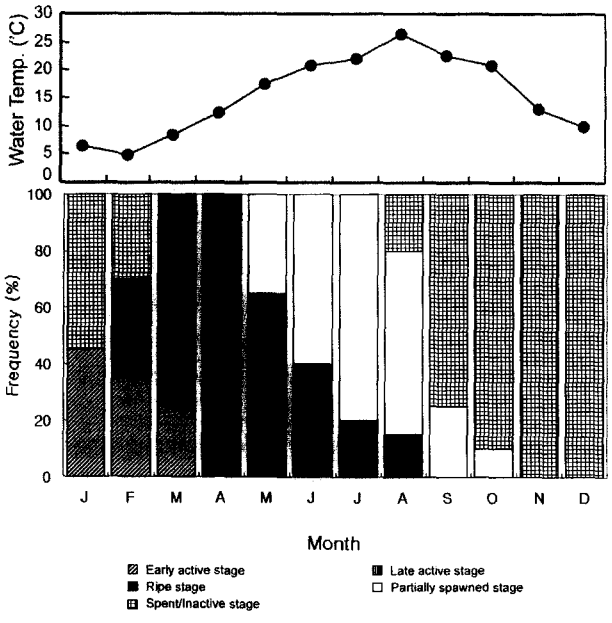

Fig. 1. Frequency of the ovarian developmental phases of *Ruditapes philippinarum* and the mean seawater temperature from January through December, 2000.

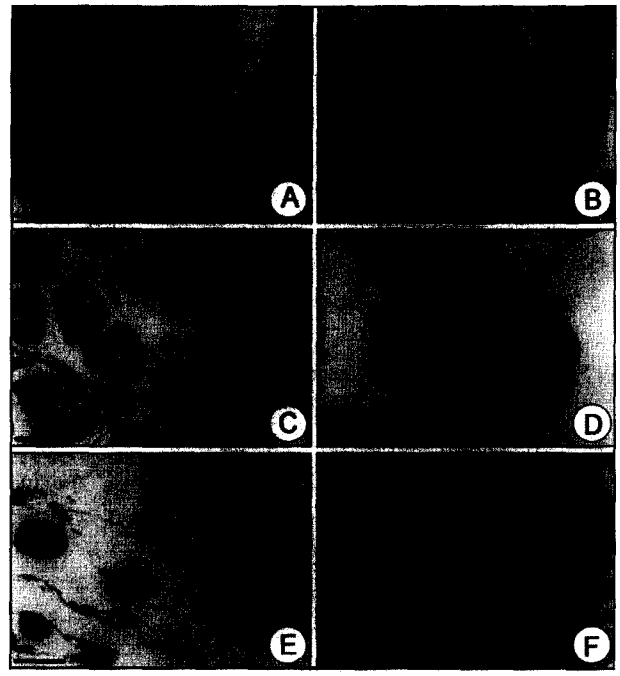

Fig. 2. Photomicrographs of the ovarian developmental phases of *Ruditapes philippinarum* (A-F). **A**, Section of the follicles in the early active stage. Note oogonia and early developing oocytes attached to follicular walls (germinal epithelium); **B**, section of follicles in the late active stage. 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 follicular walls and the connective tissues in the follicles after degeneration of the follicles. Scale bars = 50 μm.

wall, and the mean oocyte diameter was 40-50 μm (Fig. 2B). The individuals in the late active stage were observed from February through May.

3) Ripe stage

The ripe ovary exhibits distended follicles with mature and fully ripe oocytes. Half or more than half of oocytes were free in the lumen, and the mean ripe oocyte diameter was 55-62 μm in diameter. Follicle size increased, while follicular wall was thin (Fig. 2C, 2D). Sexually mature females appeared from April through August.

4) Partially spawned stage

The follicles were collapsed or decreased in size and the number of undischarged free oocytes in the follicle. Empty and ruptured follicles appeared. Some oocytes underwent cytolysis (Fig. 2E). Spawning occurred from May to October, and one spawning peak occurred between July and August.

5) Spent/inactive stage

After spawning, at the spent stage, the oocytes in most follicles of the ovary were degenerated. There was no sign of ovarian activity, half or more than half of the follicles were empty. Follicles became contracted and degenerated undischarged oocytes in the lumen underwent cytolysis. Thereafter, newly formed oogonia appear among the connective tissues and phagocytes (Fig. 2F). Individuals in the spent/inactive stage appeared from August through February.

2. First sexual maturity

First sexual maturity of a total of 123 clams ranging from 8.1 to 54.9 mm in shell length (Table 1) was investigated histologically during the breeding season. The breeding season of *Ruditapes philippinarum* was found to be from May to October. 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 Manila clams in 8.1-10.0 mm-sized group, if they were at the early active stages during the breeding season. The percentage of first sexual maturity of female clams of 10.1-15.0 mm-sized group was 14.3%, most of the clams were still in the early active stage. Percentage of first sexual maturity in 15.1-20.0 mm-sized group was more than 50%, all of which were in the late active, ripe or partially spawned stages. Sexual maturity of the clams over 25.1 mm in shell length was 100%.

3. Artificial spawning

1) Spawning reaction rate and spawning intervals

Spawning reaction rate by artificial induction and the number of spawned individuals are summarized in

Table 1. Number of Manila clams at each gonadal stage in a shell-sized group during the breeding season.

Shell length (mm)	No. of clam at the gonadal stage of					Total	Maturity (%)
	EA	LA	RI	PS	SP/IA		
8.1-10.0	12					12	0
10.1-15.0	12	1	1			14	14.3
15.1-20.0	7	3	3	3		16	56.3
20.1-25.0	3	2	4	3		12	75.0
25.1-30.0		2	5	4	2	13	100.0
30.1-35.0		3	5	5	3	16	100.0
35.1-40.0		3	4	6	2	15	100.0
40.1-45.0			8	4	1	13	100.0
45.1-50.0			6	2	1	9	100.0
50.1-54.6				2	1	3	100.0

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

Table 2. A total of 89 female clams spawned after the first spawning induction between July 1, 2000 and July 2, 2000 in the indoor laboratory. A total of 54 clams among the first spawned 89 females (spawning reaction rate, 63.39%) spawned again after artificial induction during the period of July 17 to 19, 2000. A total of 40 clams among 54 female spawned twice (spawning reaction rate, 74.07%) showed the third spawning between 2 August and 5 August, 2000. The spawning interval between the first and second spawnings of this species was 15-17 days (from July 1 to July 17 and 18, and from July 2 to July 17 to 19, 2000); the spawning interval between the second and the third spawnings was 16-17 days (Table 2) under the conditions of sufficient food supply in the FRP rearing aquarium.

2) Number of spawned eggs by shell size

During the first spawning, the mean number of spawned eggs in shell-length groups of 20.0-25.0 mm, 25.1-30.0 mm and 40.1-45.0 were 286,606 ± 30,413 eggs, 448,268 ± 25,664 eggs, and 1,540,154 ± 58,359 eggs, respectively. In general, the mean number of the first spawned eggs increased with the increase of the shell length of female clams. As shown in Table 3, the number of the eggs released after the second induction were 76.69-86.06% of those released from the first spawning, and eggs released during the third spawning ranged 87.75-94.87% and 72.76-77.22% of those released from the second and first spawnings, respectively. The number of spawned eggs gradually decreased with the increase of the number of spawnings (the first, second, and third spawnings) in each shell-length group.

DISCUSSION

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

Table 2. Spawning rate of *Ruditapes philippinarum* after artificial induction.

First spawning		Second spawning		Third spawning	
Date	No. spawned	Date	No. spawned	Date	No. spawned
July 1, 2000	61	July 15	0		
		July 16	0		
		July 17	17	July 31	0
				August 1	0
				August 2	6
				August 3	6
		July 18	16	August 1	0
				August 2	0
				August 3	6
				August 4	6
July 2, 2000	28	July 16	0		
		July 17	0	July 31	0
		July 18	11	August 1	0
				August 2	0
				August 3	5
				August 4	4
		July 19	10	August 2	0
				August 3	0
				August 4	4
				August 5	3
Total	89		54		40
Spawning rate	-		60.67%		74.07%

factors (neuronal and hormonal) within an organism. In the present study, *Ruditapes philippinarum* from Gomso Bay, the west coast of Korea, initiated gonadal development during the late winter-early spring seasons when water temperatures were relatively low, while chlorophyll a levels were high during the period (Kim, 1999). The gonadal phases were in the inactive stage during the winter (January to February) because of lower temperatures of 5.0-5.5℃ and insufficient food organisms as shown in Fig. 3 (Ministry of Maritime Affairs and Fisheries, Republic of Korea, 2001).

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 our report (Ministry of Maritime Affairs and Fisheries, Republic of Korea, 2001) on artificial induction of gonadal maturation of *Ruditapes philippinarum* during the spawning period in Gomso Bay, ovarian developments with rearing conditions of feeding (sufficient food supply) and non-feeding (starvation) at intervals of 14-17 days after the first artificial spawning showed considerable differences between the feeding and non-feeding groups. In case of feeding groups, most of the first spawned individuals reached the ovaries in the ripe stage within 14-17 days after spawning. However, in non-feeding groups, most of individuals appeared the ovaries in the early or late active stages only within the same period. Accordingly, we suggest

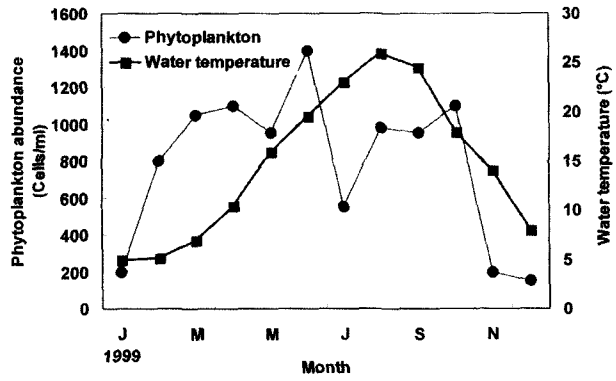

Fig. 3. Monthly changes of phytoplankton abundance and water temperature in Gomso Bay (National Fisheries Research and Development Institute, 1999).

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 our results (Ministry of Maritime Affairs and Fisheries, Republic of Korea, 2001), in Gomso Bay, food levels (phytoplankton) were high in mid spring (April) and 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

Table 3. Mean number of spawned eggs of *Ruditapes philippinarum* in each shell-sized group.

Shell length (mm)	First spawning		Second spawning		Third spawning	
	No. spawned clam	Mean ± SD	No. spawned clam	Mean ± SD	No. spawned clam	Mean ± SD
20.2-25.0	5	286,606 ± 30,413				
25.1-30.0	19	448,268 ± 25,664	11	369,544 ± 16,521	9	340,653 ± 24,823
30.1-35.0	30	950,404 ± 34,625	22	728,899 ± 26,510	18	691,487 ± 23,456
35.1-40.0	22	1,450,638 ± 43,852	12	1,236,689 ± 24,544	9	1,120,167 ± 37,884
40.1-45.0	7	1,540,154 ± 58,359	5	1,323,400 ± 43,436	4	1,161,250 ± 64,486
45.1-46.9	6	1,442,626 ± 94,578	4	1,241,485 ± 14,438		

maturity and spawning in *Ruditapes philippinarum*.

Regarding seawater temperature, Toba and Miyama (1995) stated that "the environmental temperature is probably not a limiting factor to reproductive activity for *Ruditapes philippinarum*", and gonadal development occurs between seawater temperature of 10℃ (early active stage) to 27℃ (late active stage) regardless of the initial gonadal condition, accordingly, "this species is considered to be more strongly influenced by food availability than by water temperature (min. 8℃, max. 27℃)". We agree with this opinion, and we assume that in case of this species, food supply is more important factor than seawater temperature for gonadal development and maturation in Gomso Bay.

Investigations of natural reproductive cycle or spawning cycle are important not only to studies of population dynamics (*i.e.*, age determination and the recruitment period) but also to understanding of biogeography and speciation (Chung and Ryou, 2000; Chung *et al.*, 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 *Ruditapes philippinarum*. Intra-specific variations in the timing of spawning periods and the amount of gametogenic material produced vary with years and latitudinal gradient, because variations in environmental conditions influence the reproductive process (Chung, 1997).

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

In case of different populations of *Ruditapes philippinarum*, there are some difference of the reproductive cycles; there is one spawning period in British Columbia, Canada (Quayle and Bourne, 1972), Hood Canal, Washington, USA (Holland and Chew, 1974), northern Japan (Yoshida, 1953), and Vostok Bay, northwestern part of the Sea of Japan (Ponurovsky and Yakovlev, 1992); while two in southern Japan (Tanaka, 1954; Ohba, 1959). In the present study, this species has one spawning period as in the northern districts of Tokyo Bay, Japan. Therefore, it is assumed that the number of spawning frequencies in the same species varied with temperature latitude.

Sexual maturity was assessed as a function of age and shell length in this study. Age or shell length can be used as a convenient indicator. In the present study, the first sexual maturity rate of female and male in 15.1-20.0 mm-shell length group was 56.3%, and all of the clams over the shell length of 25.1 mm showed sexual maturity. Ko (1957) reported that the first sexual maturity could be seen in group of 10.0-15.0 mm in shell length in Sasebo Bay, Japan, while Goshima *et al.* (1996) described that shell lengths at first maturity were 25 mm (2 years) and 27 mm (2 or 3 years) for males and females, respectively in Saroma Lagoon, Hokkaido, northern Japan. Therefore, it is assumed that the size of first sexual maturity of the local population of Manila clams varied with their habitat latitudes.

According to the growth curves for the mean shell length of Manila clams fitted to von Bertalanffy's equation by Chung *et al.* (1994), clams of 15.0-20.0 mm in shell length are considered to be one year old. We assume that both sexes of this population begin reproduction at one year of age. For natural resources management of this species, the present study suggests that catching Manila clam < 15 mm in shell length or < 1 year old can potentially cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate natural resources management.

From the indoor experimental results (Table 2) of the present study, the second spawning intervals were 15-17 days after the first spawning, and that of the third spawning intervals were 16-17 days after the second spawning of this species. Accordingly, the spawning intervals of this species were approximately 16.5 days under the conditions of sufficient food supply in the FRP aquarium in the laboratory. Toba and Miyama (1994) reported that the mean spawning interval of the Japanese *Ruditapes philippinarum* was

14-15 days under indoor experimental conditions. Therefore, our result was similar to that of Toba and Miyama (1994) under laboratory conditions.

According to the number of spawned eggs per individual of this species, it is assumed that the number of spawned eggs vary with shell size classes, spawning frequency and food supply. Even though the spawning season of this species occurs once a year in Korea, judging from the results of our indoor rearing experiment, it is assumed that the number of spawnings will be several times during the spawning period.

In general, estimation of spawned eggs in bivalves have usually been obtained either by directly inducing clams to spawn in the laboratory and then counting them or weighing the gametes released, or indirectly from allometric equations related to weight loss on spawning to dry body weight or shell length (Thompson, 1979; Bayne and Worrall, 1980; Kautsky, 1982; Rodhouse *et al.,* 1984).

In the present study, the number of spawned eggs in the first spawning by the artificial spawning induction of this species ranged from 256,000 eggs per individual (20.2-25.0 mm, 1 year old) to 1,598,000 eggs per individual (40.1-45.0 mm, 4 years old). However, Toba and Miyama (1994) reported that those of artificially spawned eggs of *Ruditapes philippinarumin* Tokyo Bay, Japan ranged from 244,000 per individual (minimum) to 1,345,000 eggs per individual (maximum). Accordingly, those of artificially spawned eggs showed differences between populations of this species. As shown in Table 3, on the whole, the first, second and third spawnings showed the increase with the increase of the size classes (shell length) and age, and in case of the same size class, the mean number of the second and third spawned eggs were 76.69-86.06% and 72.76-77.22% of the number of the first spawned eggs, respectively. There are reports on the artificial spawning of other bivalves. Thompson (1979) reported that a female *Mytilus edulis* (*ca.* 7 cm in shell length) was able to produce approximately 7-8 $\times$ 10^6 eggs during the spawning period, while larger individuals can produce as many as 4 $\times$ 10^7 eggs.

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.

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