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Gonadal Maturation and Artificial Spawning of the Manila Clam, *Ruditapes philippinarum* (Bivalvia: Veneridae), in Komso Bay, Korea

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We have investigated the gonad index (GI), gonadal development, reproductive cycle, first sexual maturity, sex ratio, the number of spawned eggs and spawning frequency of the Manila clam, *Ruditapes philippinarum*. Samples were collected from the intertidal zone of Komso Bay, Korea from January to December in 1999. Monthly changes in the gonad index (GI) and condition index showed a similar pattern in the reproductive cycle. The spawning period was once a year between early June and early October, there was a spawning peak between July and August when seawater temperature was over 20℃. The reproductive cycle of this species can be categorized into five successive stages; early active (February to March), late active (April to May), ripe (April to August), partially spawned (June to October), and spent/inactive stage (August to March). Percentages of first sexual maturity of female and male clams of 15.1~20.0 mm in shell length were 56.3% and 60.0%, respectively, and 100% for the clams >25.1 mm. The sex ratio of individuals >15.1 mm in shell length was about 1:1 ($\chi^2=0.02$, $p>0.05$). Number of the eggs released from each clam by the induction increased as the size of clam in terms of shell length increased. Mean number of the eggs from the second induction of the spawning was 75.35~84.30% (average 79.81%) of the number of the eggs released in the first spawning. Our data indicated that *R. philippinarum* in Komso Bay has one major spawning peak with over two minor spawning, and the interval of each spawning was estimated to be approximately 15~17 (average 16.5) days.

Key words: *Ruditapes philippinarum*, Gonadal maturation, Artificial spawning, Spawning interval, First sexual maturity, Sex ratio

Introduction

The Manila clam, *Ruditapes philippinarum* (Pelecypoda: Veneridae) is distributed along the coasts of Korea, China and Japan. In particular, it is abundant in the intertidal area of the south and west coasts of Korea where tidal flats are well developed (Yoo, 1976; Kwon et al., 1993; Chung et al., 1994). In Korea, *R. philippinarum* is one of the most important marine resources for human consumption. Recently, due to reclamation of tidal areas along the west coast, marine pollution, and reckless overharvesting of this clam, its standing stock has been declining for the past decade (Ministry of Agriculture and Forestry Republic of Korea, 1997). Therefore, it is necessary to manage the resources of the clam with a proper fishing regime that can be maintained an optimal population size.

So far, regarding reproductive ecology of the Manila clam in Korea and Japan, a lot of studies have been carried out on the growth (Choi, 1964; Hur, 1994; Goshima et al., 1996), population dynamics and secondary production (Ohba, 1959; Choi, 1987; Yoon, 1992), reproduction including maturation

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(Toba and Miyama, 1995), artificial discharge (Sagara, 1958), and the spawning season (Yoshida, 1953; Tanaka, 1954; Ohba, 1959; Holland and Chew, 1974; Ponurovsky and Yakovlev, 1992) and reproductive cycle (Toba et al., 1993; Toba and Miyama, 1994; Chung et al., 1994; Tsuji et al., 1994; Goshima et al., 1996).

Although there have been several studies on reproductive ecology of the Manila clam, in particular, only a few informations on the number of the eggs released from each clam by individual size through artificial induction and spawning intervals of this species can be available (Toba et al., 1993; Toba and Miyama, 1995). Understanding the reproductive cycle and the spawning period of *R. philippinarum* will be useful informations for age determination and the recruitment period of this population which is crucial for the management of the clam population in Komso Bay. In addition, data for the sex ratio, first sexual maturity, artificial spawning, and spawning frequency (interval) of this population would be very useful informations for aquaculture, reproductive potential and the management of natural resource.

The present study provides the reproductive ecological data of the Manila clam in Komso Bay including the reproductive cycle, first sexual maturity, sex ratio, the number of eggs produced by size, spawning interval and some basic informations useful for propagation and management for the maximum sustainable yield (MSY) of the clam.

Materials and Methods

Sampling

Specimens of the clams were collected monthly from the clam bed on the intertidal zone of Komso Bay, west coast of Korea from January to December, 1999 (Fig. 1). Clam sizes from 8.4 mm to 54.6 mm in shell length were used for the histological analysis. After the alive clams were transported to the laboratory, shell length and height were measured by a Vernier caliper, and total weight was measured using a top-loading electronic balance (Casbee MW-120).

Water temperatures during sampling period were obtained from Kochang Regional Maritime Affairs and Fisheries Office.

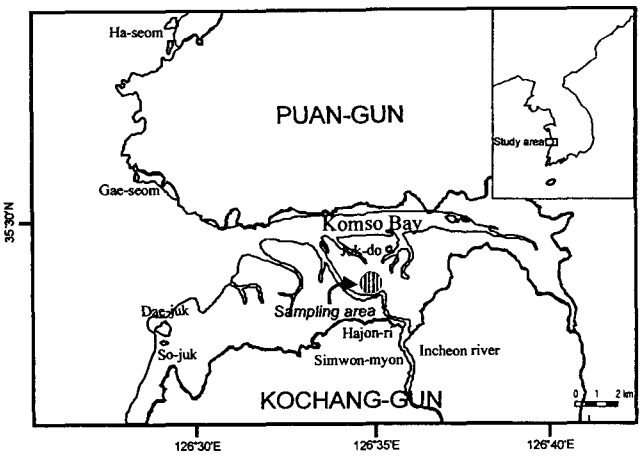

Fig. 1. Map showing the sampling area.

Gonad index (GI) analysis

A total of 364 histololgical sections were used in the calculation for GI according to a modification of Mann (Mann, 1979). Each histological section was also examined to assess the gonadal development and was scored on 0 to 5 scale; 0, inactive stage (S0); 1, spent stage (S1); 2, early active stage (S2); 3, late active stage (S3); 4, partially spawned stage (S4); 5, ripe stage (S5). The mean GI was obtained by multiplying the number of the clams at each gonadal stage by the numerical ranking of the stage, and the value divided by the total number of the clams analyzed.

$$GI = \frac{(NRVS0)+(NRVS1)+(NRVS2)+(NRVS3)+(NRVS4)+(NRVS5)}{\text{Total N observed by month}}$$

Where, N is the number of individual analyzed and RVS is a ranked value on each stage.

Microscopic examination of gonadal tissues

For histology, gonadal tissues were removed from shells and preserved in Bouins fixative for 24 hours and washed in running tap water for 24 hours. The tissues were then dehydrated in alcohol, embedded in paraffin and sectioned at 5 to 7 μm using a rotary microtome. The sections were then mounted on glass slides, and stained with either Hansen's hematoxylin-0.5% eosin, Mallorys triple stain or PAS stain. Based upon microscopic observation, the sections were assigned to be one of 5 stages; 1) early active, 2) late active, 3) ripe, 4) partially spawned, and 5) spent/inactive stage, two or more

different reproductive stages often occurred simultaneously within a section, assigning gonadal stage on the section was made based upon the conditions of the majority of the gonadal maturation in the section.

Size of first sexual maturity

The percentage of first sexual maturity was investigated from the histological preparations to certify shell lengths of specimens that reached the maturity and participated in reproduction (spawning) in the reproductive cycle. A total of 216 clams ranging from 8.4 to 54.6 mm in shell length were used for the analysis.

Sex ratio

The sex ratios of the sexually mature clams (over 15.1 mm in shell length), collected monthly from January to December, 1999, were determined monthly. Four hundred twenty two clams were sexually identified by light microscopic examination of histological preparations. A Chi square test for goodness-of-fit was applied to test the hypothesis of equal representation of female and male clams.

Induction of spawning

A total of 377 Manila clams of 20.2~46.7 mm in shell length, collected from Komso Bay, were grouped by every 5.0 mm, as group A (1 year old) to group F (4 years old). For acclimation of adult clams in the laboratory environment, clams were placed in rearing mesh containers (40 cm×40 cm×10 cm) with a 10 cm-deep layer of sand substrate. Sand substrates were collected from the shellfish bed in Komso Bay, sieved to remove any course particles (particle size >1.0 mm diameter) and silt, washed with tap water, and dried before use. A total of 377 individuals (180 females and 197 males confirmed by anatomical and histological analyses after the first spawning experiment) ranging from 20.2 to 46.7 mm in shell length had been reared for 3 days in two FRP rearing tanks (1.0 m×1.5 m×0.5 m) without food supply until the beginning of the experiment. For the first spawning experiment between 23 and 24 July 1999, several 20 mL beakers were placed in the waterbath with an 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, Nannochloris oculata*) were supplied as food (approximately $4\sim6\times10^8$ cells $\cdot g^{-1}\cdot days^{-1}$ were ingested daily) before artificial spawning experiment. Density of phytoplankton were measured using a particle counter (TA-II, Coulter Electronics Ltd.). The salinity of natural filtered seawater, the velocity of running seawater and initial seawater temperature in the FRP rearing tank during artificial spawning experiment were 31.5, 0.5 L/min., and 25±0.5℃, respectively. Seawater in the aquarium replaced daily during the experiment.

For the first spawning induction, 377 female and male clams were exposed to the air, feeding stimulus, thermal shock such as rasing and falling and exposed to the sperm fluid (Loosanoff and Davis, 1963; Hur, 1994; Toba and Miyama, 1994). After exposure to the air for one hour, each clam was transferred in a 200 mL beaker, sufficient amount of cultured microalgae-supplemented seawater were supplied as food (6 kinds of phytoplankton), and then water temperatures were continously raised up to 29℃ for 40 minutes from the initial level of 25℃. One mL of the total spawned eggs per individual clam was transferred to a cell counter, and the number of spawned eggs were counted from 5 fields using a light project (Nikon V12).

To estimate the number of the second spawned eggs and spawning intervals, a total of 260 clams (119 female and 141 male individuals which were the first spawned on 24~25 July 1999) were used for the second spawning. Environmental conditions in the FRP tanks for the second induction of spawning were maintained as the conditions applied for the first induction. For the second induction of spawning after the first spawning, the first spawned individuals were exposure to the air and sufficiently cultured microalgae-supplemented seawater. After receiving thermal shock, they were exposed to the sperm fluid at intervals of 14~17 days (from 7 August to 10 August) by the method of Toba and Miyama (1994). The number of the eggs released from each clam in different size classes were counted using the particle counter.

Results

Microscopic feature of the gonads

The gonads were located between the digestive diverticula and the outer fibromuscular layers compacted by the fibrous connective tissues and muscle fibers. As the gonads become mature, they extended to the lowest part of the muscular layers around the foot. The gonads were composed of a number of follicles. Although the gonads were getting mature, both sexes were not easily distinguishable by external appearance because both mature ovary and testis were the same in color (pinkish white). But when they were slightly scratched with razor, ripe eggs and milky white sperms flow out readily. Therefore, their sexes were easily distinguishable by examing eggs or sperm under a light microscope dissection. After spawning and the gonads become degenerated, it was difficult to distinguish the sex even under microscope by dissection.

Monthly changes in the gonad index (GI)

The GI was calculated by values of ranks in accordance with gonadal phases after histological observation of the specimens by Mann's method (Mann, 1979). As shown in Fig. 2, the GI gradually increased from March to April, and reached a maximum (4.7) in May when seawater temperature rapidly increased. Thereafter, the values gradually decreased from June to October when spawning occurred.

Reproductive cycle with gonad developmental stage

Frequencies of gonadal phases of the Manila clam were shown in Fig. 3. Based on morphological characteristics of germ cells and surrounding tissues, gonadal phases of this species can be divided into 5 successive stages. The figure showed a periodicity in gonadal phase as shown in GI.

Early active stage

In females, 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 stage, the mean oogonium and oocyte diameters were 10~11 μm and <20 μm, respectively (Fig. 4A).

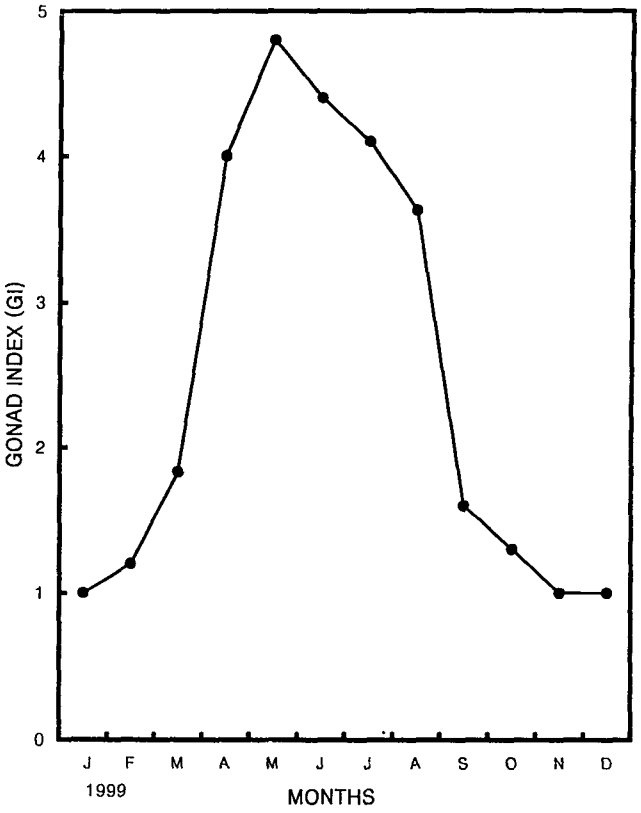

Fig. 2. Monthly changes in the gonad index of *Ruditapes philippinarum* in Komso Bay.

In males, gonad development was characterized by the increase of the follicles in number and size. Spermatogonia and spermatocytes were present in the follicles, while no spermatozoa were present (Fig. 5A). Individuals in the early active stage appeared from February to March in both sexes.

Late active stage

In females, connective tissue in the follicle was gradually decreased, developing oocytes and a few free oocytes were present in the lumen. More than half of the oocytes attached to the follicular wall, the mean oocyte diameter was 40~50 μm (Fig. 4B).

In males, connective tissue in the follicle was gradually decreased. Spermatogonia, spermatocytes, spermatids and spermatozoa appeared in the follicle with a small number of spermatozoa (Fig. 5B).

Individuals in the late active stage was observed from April to May.

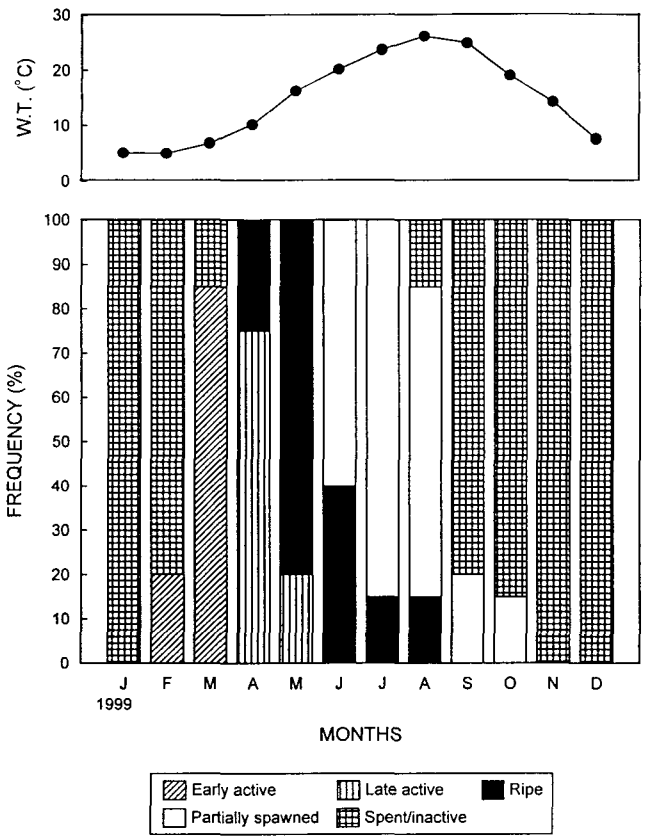

Fig. 3. Frequency of gonadal phases of *Ruditapes philippinarum* and the mean seawater temperature from January to December 1999.

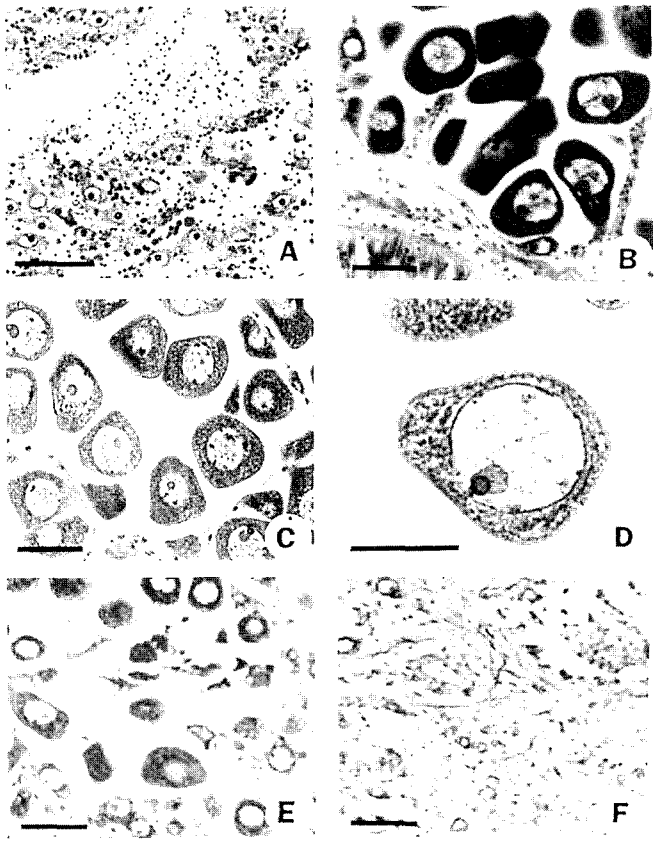

Fig. 4. Photomicrographs of gonadal phases of the female Manila clam, *Ruditapes philippinarum* (A~F). A, Section of oogenic follicles in the early active stage. Note oogonia and early developing oocytes attached to follicular walls (germinal epithelium). Scale bar=50 μm; B, section of follicles in the late active stage. Note a number of late developing oocytes in the follicle. Scale bar=50 μm; C, section of the follicles in the ripe stage. Note mature and ripe oocytes in the lumen of the follicle. Scale bar=50 μm; D, a fully mature oocyte in the same stage. Note the germinal vesicle and a number of granules in the cytoplasm. Scale bar=50 μm; E, section of the follicles in the partially spawned stage. Note undischarged oocytes in the lumen of the follicle after spawning. Scale bar=50 μm; 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 bar=50 μm.

Ripe stage

In females, the ripe ovary exhibited expanded 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 also increased, while follicular wall was thin (Figs. 4C and 4D).

In males, the follicles in the testis were expanded, the lumen was filled with a number of mature spermatozoa. Ripe testis is characterized by the formation of streams of spermatozoa in the lumina of the follicles (Figs. 5C and 5D). Sexually matured females and males appeared from April to August.

Partially spawned stage

In females, number of free oocytes in the follicle decreased, and empty and ruptured follicles appeared. Some oocytes undergo cytolysis (Fig. 4E).

In males, the follicles 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 the follicle (Fig. 5E).

Spawning occurred from early June (60%) to October (15%), and one spawning peak of both sexes occurred between July and August.

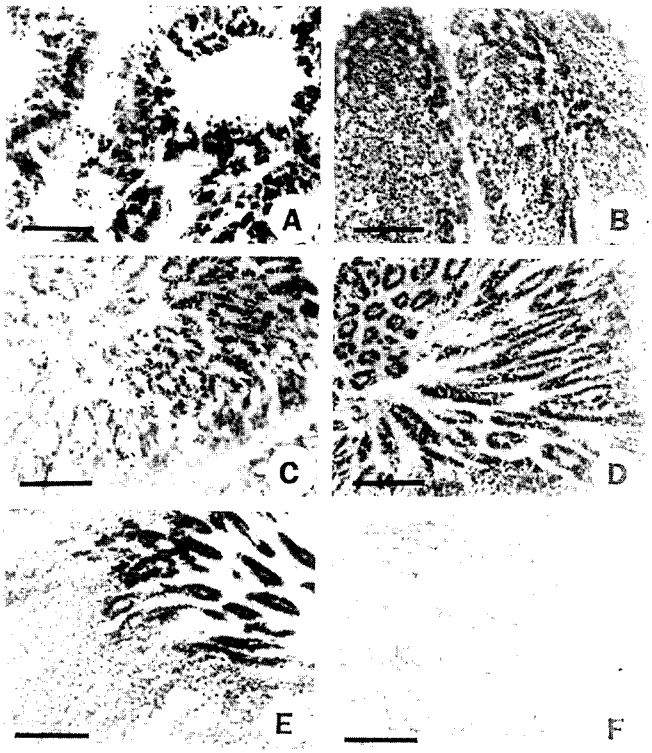

Fig. 5. Photomicrographs of gonadal phases of the male Manila clam, *Ruditapes philippinarum* (A~F). A, Section of spermatogenic follicles in the early active stage. Note spermatogonia and spermatocytes near the germinal epithelium. Scale bar=50 μm; B, section of follicles in the late active stage. Note spermatocytes, spermatids and spermatozoa during spermiogenesis. Scale bar=50 μm; C, section of the follicles in the ripe stage. Note spermatids and numerous spermatozoa in the lumen of the follicle. Scale bar=50 μm; D, fully ripe spermatozoa in the same stage. Note numerous spermatozoa with their tails in the lumen. Scale bar=50 μm; E, section of the follicles in the partially spawned stage. Note undischarged spermatozoa and spermatids in the lumen after release of sperms. Scale bar=50 μm; F, section of the follicles in the spent/inactive stage. Note newly formed spermatogonia on the germinal epithelium and the connective tissues in the follicle after degeneration of the follicles. Scale bar=50 μm.

Spent/Inactive stage

After spawning, at the spent stage, the gametes in most follicles of both sexes were degenerated. In females, 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 appeared among the connective tissues and phagocytes (Fig. 4F).

In males, follicles were shrunk and disorganized. Only residual spermatozoa, connective tissue and phagocytes could be found, thereafter, newly formed spermatogonia appeared among the connective tissues (Fig. 5F).

Individuals in the spent/inactive stage appeared from August to March.

First sexual maturity

First sexual maturity of a total of 216 individuals (114 females and 102 males ranging from 8.4 to 54.6 mm in shell length) was investigated histologically in order to certify shell length of the individuals that reached maturation and participated in reproduction between June and October (the spawning period)(Table 1).

Percentages of first sexual maturity of female and male clams of 10.1~15.0 mm in shell length were 14.3% and 17.6%, respectively, >50% for 15.1~20.0 mm and 100% for those of >25.1 mm.

Table 1. Shell length of first sexual maturity of *Ruditapes phillippinarum* from Komso Bay

Shell length (mm)	Female		Male	
	Number	Maturity (%)	Number	Maturity (%)
8.4~10.0	12	0	14	0
10.1~15.0	14	14.3	17	17.6
15.1~20.0	16	56.3	15	60.0
20.1~25.0	12	75.0	18	83.3
25.1~30.0	13	100.0	12	100.0
30.1~35.0	16	100.0	11	100.0
35.1~40.0	15	100.0	8	100.0
40.1~45.0	8	100.0	4	100.0
45.1~50.0	5	100.0	2	100.0
50.1~54.6	3	100.0	1	100.0
Total	114		102	

Sex ratio

A total of 422 Manila clams (200 females, 197 males and 25 indeterminate), over 15.1 mm in shell length, were examined to determine sex ratio by histological preparations. The sex of the remaining 33 individuals could not be identified because they had some parasites or a few indistinguishable sex cells in various stage (Table 2). There was no significant difference in the number of females and males present ($\chi^2=0.02$, $p>0.05$), and monthly comparisons showed no statistical difference in the number of female and male clams. The sex ratios

Table 2. Monthly variaions in sex ratios of the adult clam, *Ruditapes phillippinarum*

Date	Female (ind.)	male (ind.)	Indeterminate (ind.)	Total (ind.)	Sex ratio (F/F+M)	χ^2 (Chi squared)
Jan. 1999	16	17	4	37	0.48	0.03
Feb. 1999	20	14	4	38	0.59	1.06
Mar. 1999	12	19	5	36	0.39	1.58
Apr. 1999	16	22	3	41	0.42	0.95
May. 1999	18	12	4	34	0.60	1.20
Jun. 1999	18	22	5	45	0.45	0.40
Aug. 1999	15	19	0	34	0.44	0.47
Sep. 1999	19	24	0	43	0.44	0.58
Oct. 1999	22	16	0	38	0.58	0.95
Nov. 1999	16	14	0	30	0.53	0.13
Dec. 1999	28	18	0	46	0.61	2.17
Total	200	197	25	422	0.50	0.02

The critical value for Chi square for goodness-of-fit test of equal numbers of females and males, at 95% significance were 3.84.

of individuals over 15.1 mm in shell length were not statistically different from 1:1.

Artificially induced spawning

Results of the number of eggs spawned from spawning induction by size class were summarized in Table 3. Two hundred sixty of 377 female and male individuals were spawned by the first induction of spawning, and then 195 of 260 female and male clams were spawned in the second spawning experiment, their spawning rates by the first and second inductions of spawning showed 68.96% and 75.00%, respectively. The spawning rates of the first and second spawning of female clams were 66.11% and 75.63%, respectively. Those of the first and the second spawnings of male clams were 71.57% and 74.47%, respectively.

In the first spawning experiment, 119 of 180 female individuals spawned on 24 July 1999 in the indoor laboratory. The number of the first spawned eggs by size class (shell length) of females ranging from 20.0 to 25.0 mm in shell length (one year of age) was 201,000~253,000 eggs (231,206±18,082 eggs),

Table 3. Number of spawned eggs by size of *Ruditapes phillippinarum* from Komso Bay

Shell length (mm)	First spawning					Second spawning					
	N_0	N_1	Range of spawned eggs	Average ± SE	% of N_1 to N_0	N_1	N_2	Range of spawned eggs	Average ± SE	% of N_2 to N_1	R(%)= SS× 100/FS
20.2~25.0	24	15	201,000 ~253,000	231,206 ± 18,082	62.50	15	0	0	0	0	0
25.1~30.0	30	21	261,000 ~546,000	428,571 ± 40,418	70.00	21	12	323,000 ~403,000	361,305 ± 15,817	57.14	84.30
30.1~35.0	35	24	864,000 ~1,102,000	948,375 ± 30,412	68.57	24	15	656,310 ~1,149,000	714,608 ± 17,799	62.50	75.35
35.1~40.0	36	24	1,413,000 ~1,719,000	1,560,680 ± 42,514	66.67	24	13	1,156,000 ~1,315,000	1,230,102 ± 25,977	54.17	78.82
40.1~45.0	32	21	1,456,000 ~1,792,000	1,655,060 ± 45,736	65.63	21	13	1,276,000 ~1,356,000	1,319,513 ± 1,239,569	61.90	79.73
45.0~46.9	23	14	1,374,000 ~1,782,000	1,533,606 ± 66,118	60.87	14	7	1,286,000 ~1,72,000	1,239,569 ± 11,123	50.00	80.83
Female Spawnng rate (%)	180	119			*65.71 66.11	119	90			*57.14 75.63	*79.81
Male Spawning rate (%)	197	141			71.57	141	105			74.47	
Total Spawning rate (%)	377	260			68.97	260	195			75.00	

*mark represents the mean number of subtotal; FS represents the mean number of the eggs spawned by the first induction of spawning; N_0 represents the number of individuals used for the initial experiment; N_1 represents the number of the first spawned individuals at the intervals of 15 to 17 days after the initial experiment; N_2 represents the number of the second spawned individuals at the intervals of 15 to 17 days after the first spawning of N_1; SE represents standard error; SS represents the mean number of the eggs spawned by the second induction of spawning.

while that of females ranging from 25.0 to 30.0 mm in shell length (two years of age) was 261,000~546,000 eggs (428,571 ± 40,418 eggs). Individuals of 35.1~40.0 mm in shell length, considered to be three years old, spawned 1,413000~1,719,000 eggs (1,560,680 ± 42,514 eggs). Accordingly, the number of the first spawned eggs increased with the increase of size class (shell length) and age of female clams. Ninety of 119 firstly spawned individuals in the indoor laboratory were spawned on 7~9 August 1999.

On the whole, the number of the second spawned eggs increased with the increase of shell length as seen in the first spawning. The range of the mean number of the second spawned eggs was 75.35~84.30% (average 79.81%) of the first spawned eggs. In the investigation of spawning intervals, the second spawning were not induced on the 14th days (7 August) after the first spawning on 24~25 July, while the induction of the second spawning occurred successfuly from the 15th days after the first spawning. Spawning intervals between the first and second spawnings of this species showed 15~17 days (average 16.5 days) under the conditions of sufficient food supply in the FRP rearing tank at the laboratory.

Discussion

Gonad Index (GI)

In general, the high average values of GI were coincident with gonadal maturity, while minimal average values following the high average values were considered as an indicator of spawning (Jaramillo et al., 1993; Kanti et al., 1993; Chung, 1997). In the present study, the gonad index of *R. philippinarum* was increased in spring season when gonadal development occurred, and it reached to the maximum in May when gonadal maturation was completed. Thereafter, the values rapidly decreased from June to November when spawning and resorption occurred. The GI showed a highly positive correlation with gonadal development, maturation, spawning and degeneration of the gonad.

Gonadal Development and Maturation

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 were generally affected by the environmental conditions, and by interactions of exogeneous factors (water temperature, food organism and day length) and endogenous factors (neuronal and hormonal) within an organism. In the present study, *R. philippinarum* from Komso Bay, the west coast of Korea initiated gonadal development during the late winter-early spring when water temperatures were relatively low, while chlorophyll-*a* level was high (Kim, 1999). The gonadal phases were in the inactive stage during the winter season because of lower temperatures and insufficient food organisms.

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 National Fisheries Research and Development Institute (1999) in Komso Bay, food level (phytoplankton) was high in mid spring (April) and early summer (June). In the present study, gonadal development and maturation occured in mid April and June when seawater temperatures were relatively high and food level was high.

Gonadal development and maturation were coincided with the periods when food was abundant in Komso Bay. Accordingly, it is assumed that gonadal development and maturation of *R. philippinarum* are closely related with the increase of seawater temperature and high food availability.

Breeding Pattern

Studing natural reproductive cycle or spawning cycle are essential for not only to studies of population dynamics (i.e., age determination and the recruitment period) but also to our understanding of biogeography and speciation. The reproductive cycle comprises the entire sequence of events from activation of the gonad to spawning and the subsequent recession of the gonad (Chung, 1997). In nature there are considerable variations in the reproductive cycle of *R. philippinarum*. Intraspecific 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 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 the same species, there are some difference between the reproductive cycles of *R. philippinarum* in the other areas of the world; 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 East Sea of Korea (Ponurovsky and Yakovlev, 1992); while two in southern Japan (Tanaka, 1954; Ohba, 1959). In the present study, this species in Komso Bay, Korea has one spawning period as in the northern districts of Tokyo Bay, Japan. Therefore, it is assumed that the number of spawning seasons during the year in the same species of bivalves varied with temperature-latitude.

First Sexual Maturity

Sexual maturity in this study was assessed as a function of age and shell length. Age or length can be used as a convenient indicator. According to the results of percentages of first sexual maturity, those of female and male of 15.1~20.0 mm in shell length were 56.3% and 60.0% and 100% in those >25.1 mm. Ko (1957) reported that the sizes of first sexual maturity ranged 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 Bertalanffys equation by Chung et al. (1994), ages and shell lengths are as follows:

Therefore, individuals 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 harvesting Manila clam <15 mm in shell length or <1 year old would be caused a drastic reduction of its recruitment.

Table 4. Ages and shell lengths of *Ruditapes philippinarum* on Kimje coastal area, Korea (Chung et al., 1994)

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

Measurement of the number of the eggs spawned and spawning interval

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 eggs spawned in the first spawning by the artificial spawning experiment of this species ranged from 201,000 eggs (minimum) to 1,792,000 eggs/individual (maximum), while Toba and Miyama (1994) reported that those of artificially spawned eggs of this species in Tokyo Bay, Japan ranged from 244,000 (minimum) to 1,345,000 eggs/individual (maximum). Accordingly, those of artificially spawned eggs showed slightly differences between both populations of this species.

On the whole, the number of the eggs of the first and the second spawnings showed the increase with the increase of size classes (shell length) and ages. The spawning rates of the second spawning in both sexes, females and males were higher than those of the first spawning. The mean number of the second spawned eggs by size classes was 75.35~84.30% (average 79.81%) of the number of the first spawned eggs.

Beside this species, there are some reports on the artificial spawning of other species of bivalves. 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 contrasted sites on the English and Welsh coasts. Accordingly, it is assumed that the number of eggs spawned by size class are influenced by natural environmental variables such as temperature, food supply, and tidal exposure since these factors broadly determine levels of net production.

From the indoor experimental results of the present study, the mean of spawning interval (23~25 July to 7~10 August, 1999) between the first spawning and second spawning of this species was 15~17 days (average 16.5 days) under the conditions of sufficient food supply in the FRP rearing tank at the laboratory. Regarding artificial spawning inductions, Toba and Miyama (1994) reported that the mean spawning interval of the Japanese *R. philippinarum* was 14~15 days under the indoor experimental condition, and several spawnings occurred during the two spawning seasons in Tokyo Bay.

According to the number of eggs spawned per individual and spawning intervals of this species, we assume that the number of eggs produced vary with size classes and spawning broods. According to the results of our indoor spawning experiment induced between 14~17 days after the first artificial spawning, the second spawning reactions successfully occurred between the 15th and 17th days except for no spawning reaction on the 14th days under the indoor conditions.

Accordingly, our data indicated that *R. philippinarum* in Komso Bay has one major spawning peak with over one minor spawning, and the interval of each spawning was estimated to be approximately 15~17 (average 16.5) days. The number of spawning frequencies (broods) are assumed to be more than two times or several times during a spawning period as in the results reported by Toba and Miyama (1994).

The informations on spawning intervals and the number of eggs spawned by size class of this species are very important for the study of reproductive potential and amount of recruitment of natural living resources in the future. Henceforth, their studies should be carried out continuously for artificial seedling production in the indoor rearing laboratory for propagation and management of living resources.

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