Korean J. Malacol. 26(3): 245-254, 2010

Gametogenic Cycle and the Number of Spawning Seasons by Quantitative Reproductive Analysis in Female *Ruditapes philippinarum* in Western Korea

Ee-Yung Chung[1], Chang Hoon Lee[2], Ki-Ho Choi[3], Moon Sul Choi[4] and Ki-Young Lee[4]

[1]*Korea Marine Environment & Ecosystem Institute, Dive Korea, Bucheon 420-857, Korea;*
[2]*Neoenbiz Co., Bucheon 420-806, Korea;*
[3]*West Sea Fisheries Research Institute, National Fisheries Research and Development Institute, Incheon 400-420, Korea;*
[4]*Department of Marine Biotechnology, Kunsan National University, Gunsan 573-701, Korea*

ABSTRACT

For the studies of germ cell development and maturation in the ovary, the gametogenic cycle and the number of spawning seasons per year in female *Ruditapes philippinarum* were investigated by quantitative statistical analysis using an Image Analyzer System. Compared with the results by qualitative and quantitative analyses, monthly variations in female gonad indice by qualitative histological analysis showed a pattern similar to that of the female gametogenic cycle calculated by quantitative statistical analysis. The number of spawning seasons occurred once per year, from June to October. In quantitative statistical analysis using an image analyzer system, monthly changes in the portions (%) of the ovary area to total tissue areas in females increased in March and reached a maximum in May, and then showed a rapid decrease from June to October when spawning occurred. And also monthly changes in portions (%) of follicle areas to the ovary area and in portions of oocyte areas to ovarian tissue areas in females began to increase in March and reached a maximum in May, and then. rapidly dropped from June to October when spawnig occurred. From these data, it is apparent that the number of spawning seasons occurred once per year, from June to October. Monthly changes in the number of the oocyte per mm^2 and in mean diameter of the oocyte in captured image which were calculated for each female slide showed a maximum in May and reached the minimum from December to February. Therefore, female *R. philippinarum* showed a unimodal gametogenic cycle during the year.

Key words: *Ruditapes philippinarum*, gametogenic cycle, spawning season, quantitative analysis

INTRODUCTION

The Manila clam, *Ruditapes philippinarum* (Bivalvia: Veneridae), is widely distributed along the coasts of Korea, China, Japan (Kwon *et al*., 1993) and the northwestern coast of the United States (Loosanoff and Davis, 1963; Anderson, 1982). More specifically, in Korea, it is found in the intertidal and subtidal zones of the south and west coasts of Korea (Min *et al*., 2004), and is one of the most commercially important edible clams. Recently, for the propagation of a living natural resource, it has been noted as a target organism for the development of aquaculture techniques. Therefore, it is important to study the gametogenic cycle and number of spawning seasons per year for aquaculture of this species. In particular, understanding the gametogenic cycle and spawning period of this species will provide information needed for the determination of age and recruitment period (Chung *et al*., 1994; Chung, 2008). However, exceptionally, in the case of *R. philippinarum*, the spawning seasons of this species vary with local populations throughout the world. Regarding the unimodal or bimodal gametogenic

Received August 25, 2010; Revised September 5, 2010; Accepted September 15, 2010
Corresponding author: Choi, Ki-Ho
Tel: +82 (32) 745-0734 e-mail: badaboy@nfrdi.go.kr
1225-3480/24360

cycles per year concluded by quantitative statistical analysis using an Image Analyzer System, it is known that in particular, *Mya arenaria* and *Mercennaria mercennaria* in Bivalve mollusc exhibited a change from a unimodal to a bimodal cycle with decrease in latitude (Ropes and Stickney, 1965; Brousseau, 1978; Heffernan *et al*., 1989a; Kanti *et al*., 1993).

For that reason, it is hard to perform age determination and assess population dynamics of this species because the number of spawning seasons per year has not yet been determined.

To date, there have been many studies on the number of spawning seasons by the qualitative reproductive analysis (histological observations) of *R. philippinarum* throughout the world. In different local populations of this species, in particular, there are some differences in the number of spawning seasons per year in other areas of the world: there is one spawning season during the year in British Columbia, Canada (Quayle and Bourne, 1972), Hood Canal, Washington, USA (Holland and Chew, 1974), northern Japan (Yoshida, 1953), Vostok Bay, and in the northwestern part of the Sea of Japan (Pourovsky and Yakovlev, 1992). There are two spawning seasons during the year in southern parts of Tokyo Bay (Tanaka, 1954; Ohba, 1959) and three spawning seasons during the year in the Adriatic Sea and southwestern Spain (Sarasquaete *et al*. 1991) where the first was rather weak (spring), the second was strong (summer) and the third was of variable intensity (autumn). In general, it is well-known that the number of spawning seasons per year of this species vary with latitudinal gradients (locations) of the world.

In Korea, the number of spawning season during the year was once a year by qualitative analysis (histological observation, Chung *et al*., 1994, 2005). However, in fact, the number of spawning seasons during the year by qualitative reproductive analysis is not correct because two or more stages often occurred simultaneously within each tissue section. In this case, therefore, the staging criteria decisions were made according to conditions of majority of tissue sections by researcher's individual subjectivity.

Thus, sometimes the qualitative analysis of gonad developmental stages by individual subjectivity is not correct. Therefore, the number of spawning seasons needs to be studied by quantitative reproductive analysis (statistical analysis) for the confirmation of the unimodel or bimodel cycle of gonads per year.

Henceforce, in case of the southern districts of Tokyo Bay, Japan, it is supposed that the number of spawning seasons per year should be confirmed by quantitative reproductive analysis through statistical analysis of histological tissue sections. Although there have been many studies on the number of spawning seasons per year for aquacuclture and management of natural living resource of *R. philippinarum*. we could not find any results associated with the number of spawning seasons per year by quantitative statistical analysis of histological tissue sections of this species in other areas of the world. Therefore, the purpose of this study is to describe the gametogenic cycle of this species with germ cell developmental stages and compare the number of spawning seasons per year by quantitative statistical analyses using an image analyzer system for aquaculture and natural living resources management.

MATERIALS AND METHODS

1. Sampling

Specimens of *R. philippinarum* were collected monthly at the intertidal and subtidal zones of Simpo, Jollabuk-do, Korea (Fig. 1) from January to December 2004. The Manila clams ranging from 45.2 to 55.2 mm in shell length were used for the present study. After the clams were transported alive to the laboratory, shell length and total weight were immediately measured.

2. Production of histological tissue section slides of the ovarian tissues

For light microscopic examination of histological tissue section slides by quantitative analysis, a total of 245 individuals were used for the production of histological tissue section slides of ovarian tissues.

Ovarian tissues were removed from shells and preserved in Bouin's fixative for 24h. They were then washed with running tap water for 24h. Tissues were

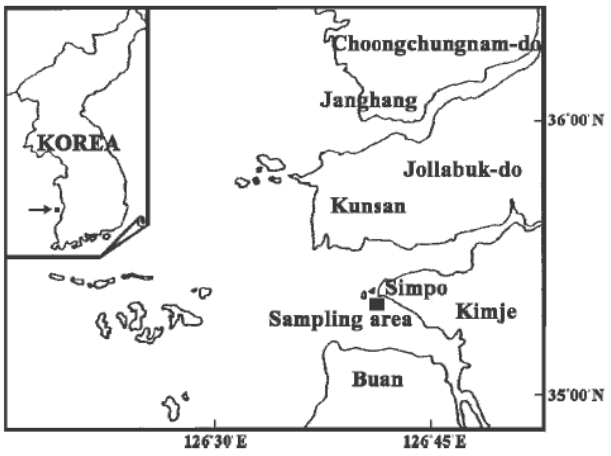

Fig. 1. Map showing the sampling area.

then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5-7 μm thickness using a rotary microtome. Sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, and examined using a light microscope (Zeiss Axiovert 10 microscope).

3. Quantitative statistical Analysis using An Image Analyzer System

Histological tissue slides were observed for quantitative analysis by an image analyzer system. Slides were viewed on a stereo-zoom microscope (Nikon, SMZ-U) from which the images were captured by a TOSHIBA Model IK-642K CCD camera and then viewed on a SAMSUNG color video monitor. The image analyzer (BMI plus, WINATech Co.) is capable of automatic measurement of area and diameter of polygons encircled by the operator, counting objects that are contrasted by background color (in black and white mode), and performing statistical analysis on numerous characteristics of objects in the captured images. Measurements on female tissue were carried out for areas of total tissue, the ovary, the follicle, the oocyte, the number of the oocytes per unit area, and the diameter of each oocyte. As for males, the areas of total tissue, the testis, and the spermatogenic stages were measured. Measurements on total tissue, the ovaries, and the testes areas were conducted at a magnification of 7.5×, at which the field area of the captured images was 60 mm^2, while the other measurements were done at a magnification of 75× (field area: 0.524 mm^2). Twenty individuals per month and two fields per slides were analyzed. Areas of total tissue, the ovaries, the follicles, the testes, and the spermatogenic stages were measured by manually tracking the margins of objects with a pointer on the captured images. Counting and measuring the diameter of the oocytes was carried out by converting captured color images to black-and-white images with the appropriate threshold, and then conducting an automatic measurement procedure provided by the software. From the measured values of image analyses, (1) the percent of field occupied by the ovary to total tissue, (2) the percent of field occupied by the follicle to total tissue, (3) the percent of field occupied by the follicle to the ovarian tissue, (4) the percent of field occupied by the oocytes to the ovarian tissue, (5) the number of the oocyte per mm^2, and (6) mean diameter of the oocyte in captured image were calculated for each female slide. A one-way ANOVA (multiple comparisons by Duncan's procedure) was applied to compare the means of monthly data. One-way t-tests were used to determine significant differences in the data of two adjacent months. All statistical analyses were done using the SPSS package.

RESULTS

A detailed insight into the gametogenic cycle of *R. philippinarum* population was ascertained from a combination of quantitative data gathered during the study period from January to December 2004.

QUANTITATIVE RESULTS

Female *R. philippinarum* showed a unimodal gametogenic cycle in the results by quantitative analysis (Fig. 2), as seen in the results of monthly changes in the gonad index (Fig. 3). The percents of field occupied by the ovary to total tissue area began to increase in March. The ovary area greatly increased from March to May (37.2%-80.4%, $p = 0.002$), and reached a maximum in May (80.4%), and then gradually decreased from June to November (73.7%-40.2%, $p = 0.015$). During the winter period

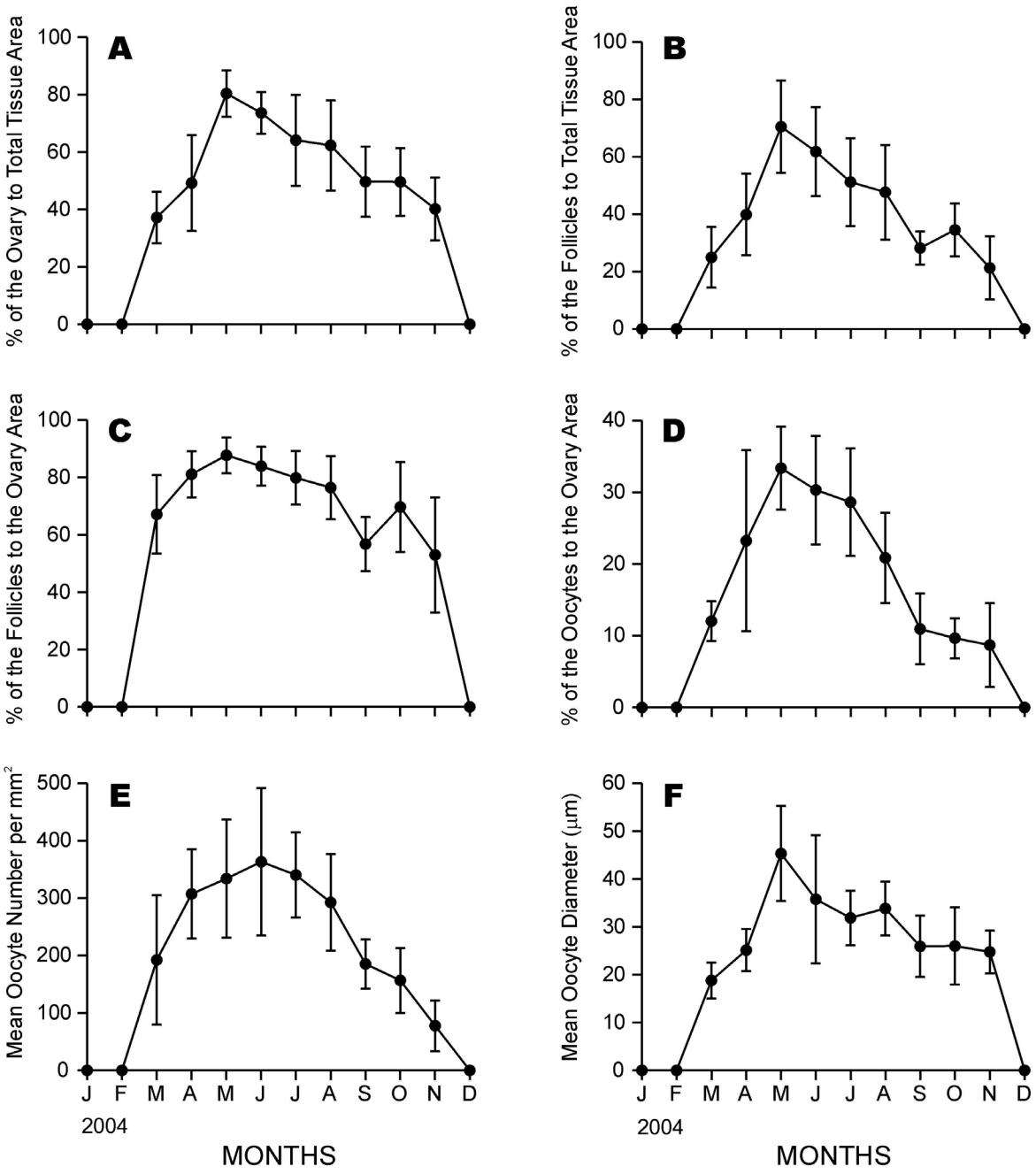

Fig. 2. Monthly changes in quantitative reproductive traits in female *Ruditapes philippinarum*. **A**: percent of area occupied by ovary to total tissue area, **B**: percent of area occupied by follicles to total tissue area, **C**: percent of area occupied by follicles to ovary area, **D**: percent of area occupied by oocytes to ovary area, **E**: mean number of oocytes per mm^2, **F**: mean diameter of oocytes (μm).

(January, February, and December), the ovarian tissue was rarely found to have a proportion of the ovary to total tissue that was less than 1%. Variations of the ovary area among individuals were so high that there were no significant differences during March-April, May-June, June-July, August-October, and October-November (one-way ANOVA: $p = 0.111$, 0.126, 0.330, 0.059, and 0.111, respectively, Fig. 2A).

The percent of field occupied by the follicle to total tissue (Fig. 2B) and the percent of field occupied by follicle to ovarian tissue showed similar patterns to the ovary area. However, higher values (more than 50%) of the follicle area in the ovary lasted longer than in the total tissue (Fig. 2C). The follicle area in the ovary increased rapidly from near 0% in February to 67.1% in March ($p = 0.005$). It increased even more to over 80% during April-June and then decreased slowly until November. Still, a high proportion of follicle area to ovary was observed in November (53.0%). During the winter period (January, February, and December), the percent of follicle area was lower than 1%. There was no significant difference in follicle area to ovary during April-August (one-way ANOVA: $p = 0.385$). The percent of ovarian tissue occupied by the oocyte, the number of the oocytesper unit ovary area, and the mean diameter of the oocyte also showed similar patterns to that of the ovary area (Fig. 2D, E, F).

Proportions of oocyte area ranged from 8.7% to 33.4% with a peak in May. During the period of September-November, the oocyte area greatly decreased to less than half of that value during April-August, while the follicle area during the same period remained high (Fig. 2D). The number of the oocytes also decreased greatly during September-November (Fig. 2E). Mean oocyte diameter reached a maximum in May (45.3 ± 9.94 (S.E.) μm). Large sized eggs were found even in November (24.7 ± 2.23 (S.E.) μm, Fig. 2F). There was good consistence in the time of peaks in May among the ovary area, the follicle area, the oocyte area, and the oocyte diameter. Only the number of the oocytes reached its maximum in June. Especially, in quantitative reproductive analysis (statistical analysis) using an image analyzer system, the patterns of monthly changes in the portions (%) of the areas occupied by follicles to the ovary area in females showed a maximum in May and reached the minimum in December to February 2004.

DISCUSSION

1. Comparisons of the ovarian gametogenic cycle by qualitative and quatitative analyses

To compare the results of the gametogenic (reproductive) cycle by qualitative and quantitative analyses in female *R. philippinarum*, in this study, we quoted the results on gametogenic cycle by qualitative analysis already reported by Chung *et al*. (1994) and Choi *et al*. (2005). They reported that the reproductive cycle in female *R. philippinarum* can be classified into 5 successive stages: the early acive stage (January-March), late active stage (February-may), ripe stage (April-August, a maximum in May), partially spawned stage (May-October, with peak spawnig between July and August), and spent/inactive stage (August-February).

In this study, according to the results of ovarian gametogenic cycle by quantitative analysis, the results of monthly changes in portions (%) of ovary areas to total tissue areas began to rapid increase in March, and reached a maximum in May, thereafter their proportions (%) gradually decreased from June-October when spawning occurred. And monthly changes in proportions (%) of follicle areas to ovary areas also began to rapid increase in March, and reached a maximum in May, thereafter, their proportions (%) gradually decreased from June-October when spawning occurred, and the main spawning occurred between July and August. In particular, peak mature oocyte level occurred in May followed by a significant decrease from June-October which indicated spawning, and the main spawning occurred between July and August.

In addition, monthly changes in proportions (%) of the oocyte areas to the ovary areas, the number of the oocytes per mm^2, and mean diameter of the oocytes showed the same or similar patterns: the maximum in May, and then rapidly dropped from June-October which indicated spawning.

Therefore, compared female gametogenic cycle by qualitative analysis (gonad index) with those by quantitative statistical analysis, the results of female gametogenic cycle and gonadal maturation by qualitative histological analysis coincided with those studied by quantitative analysis. Judging from the results confirmed by quatitative statistical analyses using an Image Analyzer System, testicular

gametogenic cycle was confirmed to be a unimodal gametogenic cycles showing a maximum maturity and one spawning season per year from June-October.

Giese (1959) and Sastry (1979) reported that in general, latitudinal differences in timing of the reproductive cycles of marine molluscs. In particular, some authors (Ropes and Stickney, 1965; Brousseau, 1978; Heffernan *et al*., 1989a) reported that *Mya arenaria* and *Mercennaria mercennaria* in Bivalve mollusc exhibited a change from a unimodal to a bimodal cycle with decrease in latitude. However, some authors (Heffernan *et al*., 1989b; Heffernan *et al*., 1989c) reported that several other bivalves (i.e, *Geukensia demisa*, *Crassostrea virginica* and *Spisular solidissima similis*) showed unimodal gametogenic cycle in the southeastern U.S. waters (Kanti *et al*., 1993). In this study, the gametogenic cycle in female *R. philippinarum* by quantitative statistical analysis showed a unimodal gametogenic cycle.

2. Number of spawning seasons per year by quantitative analysis

From the results of the number of spawning seasons investigated by quantitative statistical analysis using an Image Analyzer System, the testicular gametogenic cycle was clarified to be a unimodal gametogenic cycles showing a maximum maturity in May and one spawning season per year, from June-October.

Regarding the spawning season of different local populations of *R. philippinarum*, it is well-known that the number of spawning seasons by qualitative analysis (histological observations) varied with latitudinal gradients. In case of the southern districts of Tokyo Bay, Japan, Ko (1957) described that the number of spawning seasons in Sasebo Bay, Nagasaki, Japan were twice per year: the first spawning season (April-July) and the second spaning season (September-November). And Tanaka (1954) also reported that from the southern part area of Kando district to Kumamoto distict, Japan, the number of spawning seasons of *R. philippinarum* were twice per year: the first spawning season (spring) and the second spawning season (autumn). Although the number of spawning seasons of *R. philippinarum* in southern districts of Tokyo Bay, Japan showed twice per year by qualitative analysis, their analysis were not correct because the results were not confirm by quantitative statistical analysis using an Image Analyzer System. Especially, in case of the results showing two spawning seasons per year, it needs to confirm to get an accurate results by quantitative statistical analysis. However, in the northern districts of Tokyo Bay, Japan, Momoyana and Iwamoto (1973) reported that the number of spawning seasons of *R. philippinarum* in Hokkaido, Japan was once a year during the summer season.

Regarding the Korean Manila clam, Kurashige (1943) described that the number of spawning season of *R. philippinarum* in two different districts of Korea was once a year: from May to early early October in Taeya, Chungcheongnam-do, Korea, and from mid May to late October in Dadepo, Busan, Korea. In this study, the number of spawning seasons of this species by qualitative analysis (histological observations) was once a year from early June to early October in Simpo, Jeollabuk-do, Korea (Choi *et al*., 2005).

Accordingly, the results on the spawning periods by quantitative analysis coinsided with those studied by qualitative analysis. In consequence, the spawning periods by qualitative and quantitative analyses in Simpo, Korea were about one month later than the rsults reported by Kurashige (1943). Thererfore, it is assumed that some local variations and timing of spawning of this clam might be related to the geographical differences in the water temperatures, time of the food production (phytoplanktons), and some other environmental factors (Ko, 1957; Momoyama and Iwamoto, 1979).

3. Ovarian development and maturation

A wide range of exogenous factors has recently been suggested as controls for gonadal development and maturation in marine bivalves. Of various factors, water temperature and food availability seem to be particularly important. Sastry (1966, 1968) stated that these and other factors (salinity, day length, etc) probably interact with endogenous factors (neuroendocrine activity) in a complex manner to control the initiation of gametogenesis. Sastry (1968)

stated that sea water temperature acts as a triggering stimulus for the initiation of the germ cell growth phase. The water temperatures required for activating the growth of germ cells at the beginning of oogenesis and spermatogenesis and for attaing maturity ultimately limit the annual period of gonad activity and gametogenesis in the natural environment. In this study, gamete differentiation of *R. philippinarum* began in the winter-early spring seasons, and reached maturity in the population from April to August when water temperatures were increased. After basic metabolic requirements are satisfied, gonad activity and gametogenesis of this species occur under temperature conditions that allow nutrients mobilization to the gonads (Sastry, 1966).

The periods of food abundance and of gonad development of *R. philippinarum* are nearly coincident gonad growth and gametogenesis in spring coincided with peak food levels, although food concentrations remained high throughout the summer months (Kim, 2005). Therefore, it is assume that if food and temperature criteria are met, growth of germ cells is initiated in conjunction with the transfer of nutrients from digestive diverticular to the gonad. However, it is assumed that the amount of nutrients mobilized for the gonad maturation depends not only on the food level, but also on the water temperature and the basic metabolic requirements of the clams.

In Korean coastal waters, growth and production of bivalves is relatively high from spring to early summer seasons (Chung *et al*., 1994; Kim, 2005) due to the abundance in phytoplankton. Thus, abundant food supply (e.g., bivalves) is available to *R. philippinarum* during the period of gonadal development and maturation. Therefore, it is suggested that gonadal development and maturation of the Korean *R. philippinarum* is closely related to temperature change and food availability. Fretter (1984) observed that in temperate zones, the seasonal temperature fluctation associated with changing illumination is a controlling factor in gametogenesis. In consequence, gonadal development and maturation of this species may be retarded under low illumination, due to the decrease in food availability caused by diminished primary production of phytoplankton.

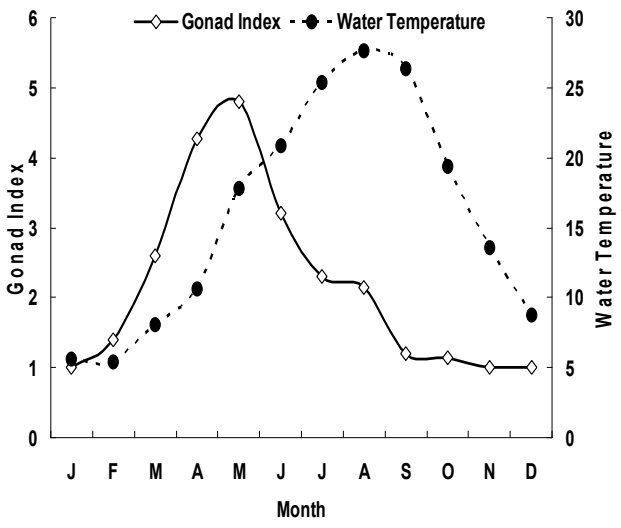

Fig. 3. Monthly changes in the gonad indice (GI) by qualitative analysis and seawater temperatures from January to December, 2004. (National Fisheries Research and Development Institute, 2004).

4. Quantitative analysis

The Manila clam, *R. philippinarum*, showed a unimodal gametogenic cycle as found in other clams found on the Korean coast. Quantitative results by an image analysis of *R. philippinarum* showed the peaks of maturity in May (Fig. 2). If we compare quantitative results with that by qualitative analysis (the gonad index) of the Manila clams studied in previous works, as shown in Fig. 3, the period of maturation of *R. philippinarum* by qualitative results and qualitative results were similar patterns between May-June. On the whole, the periods of maximum maturity during the year were quite similar to the May-June results of *Cyclina sinensis* (Chung *et al*., 1991), *Mactra veneriformis* (Chung and Ryou, 2000), *Meretrix lusoria* (Chung, 2007), and *Saxidomus purpuratus*. The peak of oocyte area to ovarian tissue in May implies the readiness of maturation (Fig. 2D). The spawning seemed to be initiated in June with a decrease of oocyte area. A significant decrease in oocyte area was found during June-September, indicating the major period of spawning. The

spawning period of *R. philippinarum*was also similar to other clams mentioned above (June-September for *C. sinensis*, *M. veneriformis*, and *M. lusoria* May-October for *S. purpuratus*). The follicle area to the ovarian tissue rapidly increased in March (67.1%), but the increase in the oocyte area to the ovarian tissue at this period was relatively low (12.0%). During and after spawning, the follicle area did not decrease in proportion to the oocyte area, rather it remained high until November (53.0%) when the oocyte area was decreased to less than 10%. The follicular tissue seemed to be formed earlier (in March) than the period of maturation (April-May) and to degenerate later (in November) than the period of spawning (June-September). In observation of slides after the period of spawning (September-November), a large portion of empty space was found in most of the follicles, which indicated the post-release of oocytes. However, during this period, follicles were not entirely empty. Parts of follicles were occupied by oocytes and eggs. Eggs found in this period had a somewhat different shape than those during the spawning period. They seemed to be remnant eggs unreleased during the spawning period and were being reabsorbed to the body. From this, it can be explained that the number of eggs and mean diameter of oocytes during Septembe-November was not zero (Fig. 5E, F). In conclusion, *R. philippinarum* does not seem to release eggs entirely during the spawning period. Kanti *et al*. (1993) reported the egg number per follicle in their works with image analysis on the southern surfclam, *Spisula solidissima similis*. In this study, egg number per follicle was not considered as an index to express the gametogenic cycle. In the case of *R. philippinarum*, the size of follicle tended to increase toward the spawning period and was highly variable among individuals. So, variability in the number of follicles per field was great and heterogeneous even within an individual. In addition, the size of eggs also was different between months and variation in egg size in a field was not homogeneous among months, especially during the spawning period. Therefore, the number of eggs per unit area of ovary was applied instead of the number of eggs per follicle in this study.

It is well known that the gametogenic cycle is related to the seasonal change of water temperature. The effect of water temperature on the maturation of *R. philippinarum* was also evident in this study. Rapid increases in the ovary area and the oocyte area were found when the water temperature was gradually increasing (Fig. 3). The water temperature during the spawning period (June-September) was always higher than 20℃. When water temperature decreased to less than 20℃, spawning stopped. So, we can conclude that the lower limit of water temperature required for the spawning of *R. philippinarum* is about 20℃. During the spawning period, food condition is very important to the successful growth and recruitment of larvae to the population for the next year. The period of larval appearance in overlying water was from June to September with the density of 724-5,001 ind. m^{-3} (NFRDI 1999), the same as the period of spawning (Fig. 2). During this period, the cell density of phytoplankton as a food source of the larvae was 560-1, 553 cells ml^{-1}, which is sufficiently high enough to nourish the released larvae of *R. philippinarum*. Therefore, food availability for the larvae of *R. philippinarum* during the spawning period does not seem to be so critical.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. William Heard, the Florida State University for helpful comments on the manuscript, and also thank English editor, Mr. Amy Lynne Paine of HARRISCO, Korea, for proofreading and correction the manuscript.

This research was supported in part by the funds from the Research Projects of the Fisheries Science Institute, Kunsan National University.

REFERENCES

Anderson, G.J. (1982) Comments on the settlement of Manila clam spats (*Tapes philippinaum*) at Filucy Bay. Washington, USA. *Journal of Shellfish Reseach,* **2**: 115.

Brousseau, D.J. (1978) Spawniong cycle. fecundity and recruitment in a pupulation of soft-shell clam. *Mya*

arenaria from Cape ann, Massachusetts. *Fisheries Buletin*. **76**: 155-166.

Choi, K.H., Park, G.M. and Chung, E.Y. (2005) Ovarian maturation in female *Ruditapes philippinarum* on the west coast of Korea

Chung, E.Y, Lee, T.Y and An C.M. (1991) Sexual maturation of the venus clam, *Cyclina sinensis*, on the west coast of Korea. *Journal of Medical & Applied Malacology,* **3**: 125-136.

Chung, E.Y, Ryou D.K, and Lee J.H. (1994) Gonadal development, age and growth of the shortnecked clam, *Ruditapes philippinarum* (Pelecypoda: Veneridae), on the coast of Kimje. *Korean Journal of Malacology*, **19**: 38-54.

Chung, E.Y and Ryou D.K. (2000) Gametogenesis and sexual maturation of the surf clam, *Mactra veneriformis* on the west coast of Korea. *Malacologia,* **42**: 149-163.

Chung, E.Y., Park, YJ, Lee, J.Y and Ryu, D.K. (2005) Germ cell differentiation and sexual maturation of the hanging Cultured female scallop *Pationpecten yessoensis* on the East coast of Korea. *Journal of Shellfish Research,* **24**: 913-921.

Chung, E.Y (2007) Oogenesis and sexual maturation in *Meretrix lusoria* (Roding 1978, Bivalvia: Veneridae) in western Korea. *Journal of Shellfish Research,* **26**: 71-80.

Chung, E.Y (2008) Ultrastructural studies of oofenesis and sexual maturation in female *Chalmys* (*Azumapecten*) *farreri farreri* (Jones & Preston, 1904, Pteriomorphia: Pectinidae) on the western coast of Korea. *Malacologia*, **50**: 279-292.

Fretter, V. (1984) *Prosobranchs*. pp.1-45. ***In***: Laar, eds., *The Mollusca*, Vol. 7, Academic Press, New York 352 pp.

Giese, A.C. 1959. Compartative Physiology: Annual reproductive cycles of marine invertebrates. *Review of. Physiology,* **21**:547-576.

Heffernan, P.B., Walker, R.L. and Carr, J.L. (1989a) Gametogenic cycles of theree bivalves in Wassaw Sound, Georgia Ⅰ: *Mercenaria mercenaria* (Linnaeus, 1758). *Journal of Shellfish Research,* **8**: 51-60.

Heffernan, P.B., Walker, R.L. and Carr, J.L. (1989b) Gametogenic cycles of theree bivalves in Wassaw Sound, Georgia II: *Crassostrea virginica* (Gmelin, 1971). *Journal of Shellfish Research,* **8**: 61-70.

Heffernan, P.B., Walker, R.L.and Carr, J.L. (1989c) Gametogenic cycles of three bivalves in Wassaw Sound, Georgia Ⅲ: *Geukensia demissa* (Dillwyn) *Journal of Shellfish Research,* **8**: 327-334.

Holland, D.A. and Chew KK (1974). Reproductive cycle of the manila clam Washington. *Proceedings of National Shellfish Research Association,* **64**: 53-58.

Kanti, A, Heffernan PB, and Walker RL (1993) Gametogenic cycle of the southern surfclam, *Spisula solidissimasimilis* (Say, 1822), from St. Catherine Sound, Georgia. *Journal of Shellfish Research,* **12**: 255-261.

Kim, Y.M. (2005) A Study on reproductive ecology of the hard clam, *Meretrix lusoria*, on the west coast of Korea. Ph. D. Thesis, *Kunsan National University* 123 pp.

,Ko, Y. (1957) Some histological note on the gonads of *Tapes japonica Deshayes*. *Bulletin of Japanese Society Fisheries,* **23**: 394-399.

Kurashige, H. (1943) Seasonal variation in the weight and volume as well as the chemical composition of the soft body of *Tapes philippinarum* with special reference to its spawning. *Bulletin of Korean Fisheries Experimental Station* **8**: 115-140.

Kwon, O..K, Park, G.M, and Lee, J.S. (1993) *Coloured shells of Korea*. Academy Pub Co. Seoul 288 pp.

Loosanoff, V.L, and Davis, H.C. (1963) Rearing of bivalve mollusks. Advance *Marine Biology, Academic press* **1**: 110-112.

Min, D.K., Lee, J.S, Ko, D.B, Je, J.G. (2004) *Mollusks in Korea*. Hanguel Graphics, Busan, Korea 566 pp.

National Fisheries Research and Development Institute (1999) *Development of optimal technology for sustaining production in a shellfish farm*. NFRDI 208 pp.

Momoyana, G. and Iwamoto, T. (1979) On the spawning season of the short necked clam in Yamaguch and Okai Bay. *Bulletin of yamaguchi Prefecture Fisheries Experimental Sttation,* **7**:19-28.

Ohba, S. (1959) Ecological studies in the natural population of a clam, *Tapes japonica*, with special reference to seasonal variations in the size and structure of population and to individuals growth. *Biological Journal of Okayama University,* **5**: 13-42.

Ponurovsky, S.J. and Yakovlev, Y.M. (1992) The reproductive biology of the Japanese littleneck. *Tapes philippinarum* (A. Adams & Reeve, 1850) (Bivalvia: Venerida). *Journal of Shellfish Research,* **11**: 265-277.

Quayle, D.B.. and Bourne, N. (1972) The clam fisheries of British Columbia. *Fisheries Research Board Canadian Bulletin,* **179**: 70.

Ropes, J.W. and Stickney, A..P. (1965) Reproductive cycle of *Mya arenaria* in New England. *Bioogical Buletin,* **128**:315-327.

Sastry, A.N. (1966) Temperature effects in reproduction of the bay scallop, *Aquipecten irradians* Lamarck. *Biological Buletin,* **130**: 118-134.

Sastry, A.N. (1968) Relationship among food, temperature and gonad development of the bay scallop, *Aquipecten irradians* Lamarck. *Physiol Zool,* **41**: 44-53.

Sastry. A.N. (1979) Pelecypoda (Excluding Ostreidae). pp 113-292. ***In***: *Reproduction in marine invertebrates*. Vol. 5: Pelecypods and lesser classes. A. C. Giese and J. S. Pearse (eds.) Academic Press, New York.

Tanaka, Y. (1954) Spawning season of important bivalves in Ariake Bay Ⅲ. *Tapes philippinarum*. *Bull Jap Soc Sci Fish,* **19**: 1165-1167.

Yoshida, H. (1953) Studies on larvae and young shells

of industrial bivalves in Japan. *J Shimonoseki Fisheries College,* **3**: 1-106.