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Reproductive Cycle of the Ark Shell, *Scapharca subcrenata*, on the West Coast of Korea[1]

Sun-Man Kwun* and Ee-Yung Chung

Department of Marine Living Resources, Kunsan National University, Kunsan, 573-701, Korea

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Monthly changes in the gonad index (GI), egg-diameter composition, gonadal development, reproductive cycle of the ark shell, *Scapharca subcrenata*, were investigated by histological method and morphometric data. This species is dioecious and oviparous. The gonad is located among the subregion of mid-intestinal gland, digestive diverticula and the outer fibromuscular layers compacted by the fibrous connective tissues and muscle fibers. The gonad index sharply increased in May, reached the maximum value in June, and then gradually decreased from July to December. The reproductive cycle of this species can be divided into six successive stages: early active stage (January to May), late active stage (June to July), ripe stage (June to August), partially spawned stage (July to September), degenerative stage (August to December), and resting stage (January to April). *S. subcrenata* spawns once a year between July and early September, and the main spawning occurred between July and August when the water temperatures were above 20℃. This evidence suggest that timings of maturation and spawning are closely related to water temperatures. Even though the spawning period was once a year, it is assumed that the number of spawning frequencies (broods) may occur more than twice during the spawning season.

Key words: *Scapharca subcrenata*, gonadal development, reproductive cycle

Introduction

The ark shell, *Scapharca subcrenata* (Pelecypoda: Arcidae), one of the commercially important clams, inhabits the intertidal and subtidal zones of mud or silty sand shores of the south and west coasts of Korea (Yoo, 1964). The yields of the family Arcidae group in Korea (*S. subcrenata, S. broughtonii*, and *Tegillarca granosa*) have decreased (Ministry of Agriculture, Forestry and Fisheries, Republic of Korea, 1996) due to marine reclamation works, reckless overcatching and environmental pollutions.

The population of this species should be restored, and it is needed to study propagation and mariculture in terms of the reproductive cycle of this species. Previously there have been some studies on aspects of reproductive ecology and propagation of the ark shell, *S. subcrenata* (Yoshida, 1953; Yoo, 1977). Hata (1948) reported the spawning season, natural occurrence of planktonic larvae, and habitats of young shells of the ark shell in Japan. Tanaka (1954) also described its reproductive ecology including fatness, the spawning season, and occurrence of planktonic larvae of the ark shell in Japan. Yoo (1964) reported growth and size, and the spawning season of the ark shell in Korea. Although the spawning season of this species have been investigated experimentally by authors mentioned above, their results are still unclear because the informations associated with spawning have been obtained through visual or dissecting microscopic observations of gonadal smears. Accordingly, there are still gaps in our knowledge with reference to reproductive ecology and propagation, and little information is available on the gonad index (GI), germ cell development, reproductive cycle, and the spawning period by histological method. Therefore, the objective of the present study is to understand gonadal development and the reproductive cycle.

[1] To whom correspondence should be addressed.
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Materials and Methods

Specimens of the ark shell, *Scapharca subcrenata* were collected monthly by dredging in the subtidal zone of Paengnyongdo, Kyonggi-do, Korea, from March 1997 to February 1998 (Fig. 1). Two hundred sixteen clams (34.00~71.90 mm in shell length) were used for the histological study. After the clams were transported alive to laboratory, shell lengths and heights were measured by a Vernier caliper, and total weight was determined using a chemical balance. Data of seawater temperatures, measured at 10:00 a.m. by the Paengnyongdo branch, Seohae Fisheries Institute, National Fisheries Research and Development Institute (unpublished data), were used for this study.

Egg diameters, the diameter of more than 1,000 eggs, were measured every month after their nuclei being centrally cut, and then the results were graphed by the frequency curve method of Pearse (1965).

The mean gonad index (GI) was calculated using a modification of Mann's method (1979). Each histological section of gonadal tissue was also examined in detail to assess the stages of gonadal development and was scored on 0~5 scales to describe six stages of gonadal development or maturity: 0=resting stage; 1=degenerative stage; 2=early active stage; 3=late active stage; 4=partially spawned stage; 5=ripe stage. The arithmetic mean of the individual scores of the whole samples was recorded as the gonad index for each month as follows:

$$GI = \frac{(N\times RVS0)+(N\times RVS1)+(N\times RVS2)+(N\times RVS3)+(N\times RVS4)+(N\times RVS5)}{\text{Total individuals by month}}$$

where, N, number of individuals; RVS, ranking value by stage.

To analyze gonadal phases the dissected tissues were subjected to standard histological procedures (dehydrated in alcohol and embedded in paraffin) and sectioned at 3~5 μm using a rotary microtome. The sections were mounted on glass slides, stained with Harris's hematoxylin and eosin, and Mallory's triple stain.

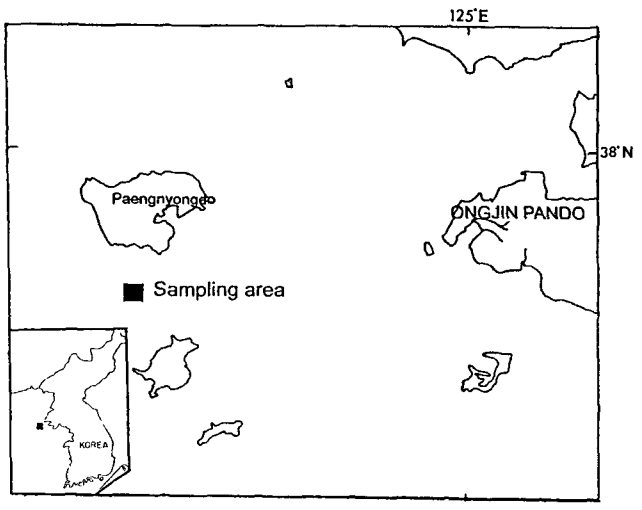

Fig. 1. Map showing the sampling area.

Results

Position and structure of the gonads

The ark shell, *S. subcrenata* is dioecious and oviparous. The gonad is located between the subregion of the mid-intestinal glands in the visceral cavity, digestive diverticula and the outer fibromuscular layers compacted by the fibrous connective tissues and muscle fibers. The ovary is composed of a number of oogenic follicles, and the testis comprises several spermatogenic follicles (Fig. 5).

Even when maturation progressed, the color of the mature ovary and testis were almost same, therefore, the sex of the clams could not be distinguished by the external feature. At this time, if they are slightly scratched with a razor, ripe eggs and milky-white sperm readily flow out. Therefore, the sex of the clams could be distinguished easily by a simple anatomical method.

Monthly changes in relative frequency distributions of the egg diameter

There are monthly changes in relative frequency distributions of the ovarian egg diameters found in sectioned ovaries (Fig. 2). Between January and February, 70% or more oocytes were 11~20 μm in diameter. From March to May, the eggs of 21~40 μm were dominant. Percentages of the eggs over 41~65 μm in diameter were about 75% in June. From July to September when spawning occurs, the number of ripe oocytes of 65~70 μm began to decrease markedly because of the discharge. A few large oocytes remain undischarged and degenerated from September to December.

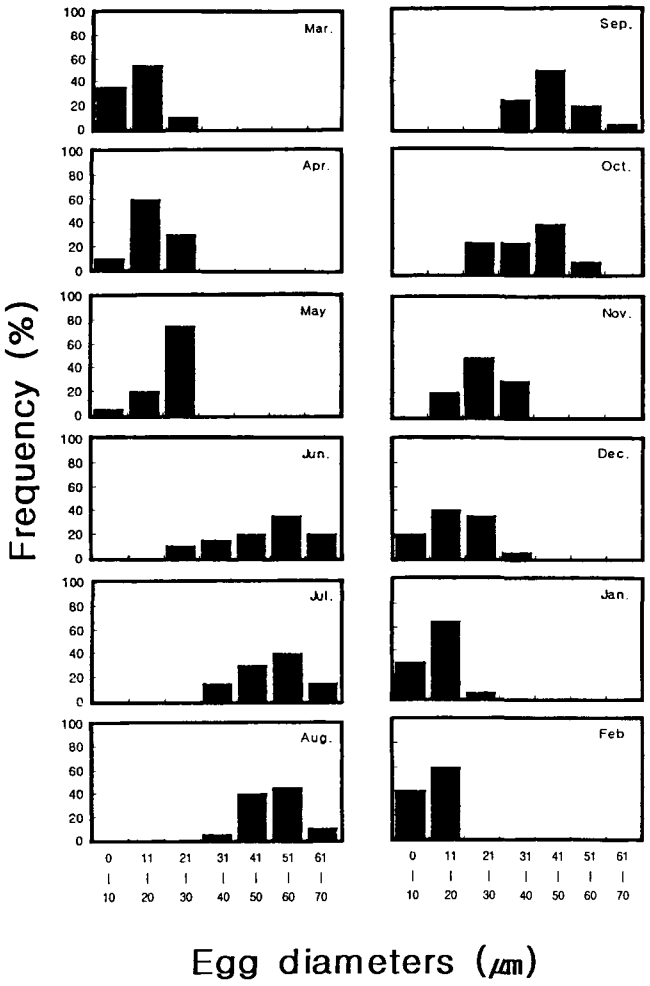

Fig. 2. Relative frequency distributions of the ovarian egg diameters.

Monthly changes in the gonad index (GI)

The GI at different gonadal phases from March 1997 to February 1998 were calculated by ranking values obtained every month (Fig. 3). The GI slightly increased from January to May, and reached the maximum (GI, 4.4) in June. However, the values rapidly decreased from July to September, and gradually decreased from October to December. Thus, variations in the GI showed a periodicity.

Gonadal phases and reproductive cycle

Based on the morphological features and sizes of the germ cells and the tissue cells around them, the gonadal phases can be categorized into six successive stages (Fig. 4).

Early active stage

The follicles of the gonad occupy about 15~20% of whole gonad. In females, oogonia and oocytes propagate along the oogenic follicular wall near

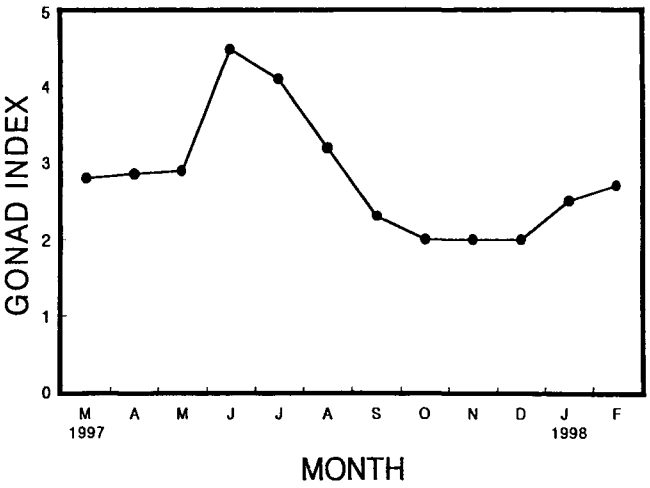

Fig. 3. Monthly changes in the gonad index of *Scapharca subcrenata*.

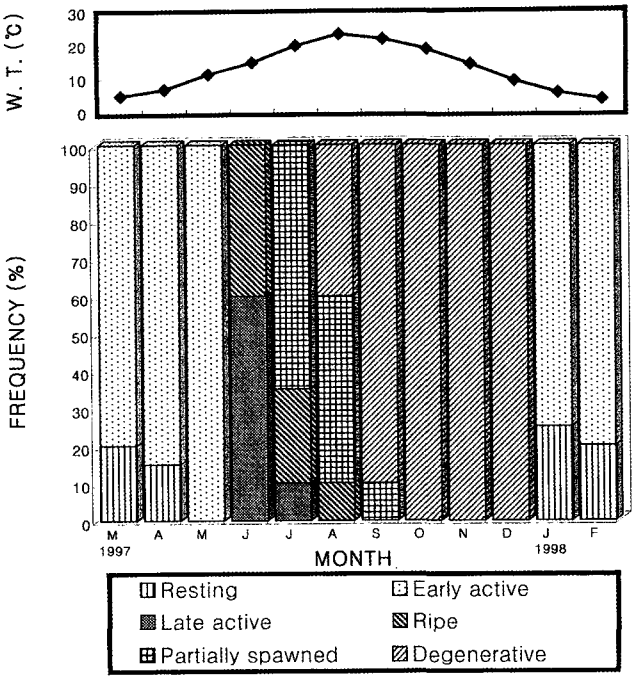

Fig. 4. Frequency of gonadal phases of *Scapharca subcrenata*, and monthly changes in seawater temperatures from March 1997 to February 1998.

the mesenchymal tissues of the ovary. The diameters of oogonia and oocytes were approximately 10 μm and 20~30 μm, respectively. At this time, the total volume of the ovary was small, and the follicular wall thick (Fig. 5A).

In males, spermatogenesis occurs in the follicles of the testis. The diameters of spermatogonia and spermatocytes were 8~9 μm and 6~7 μm, respectively,

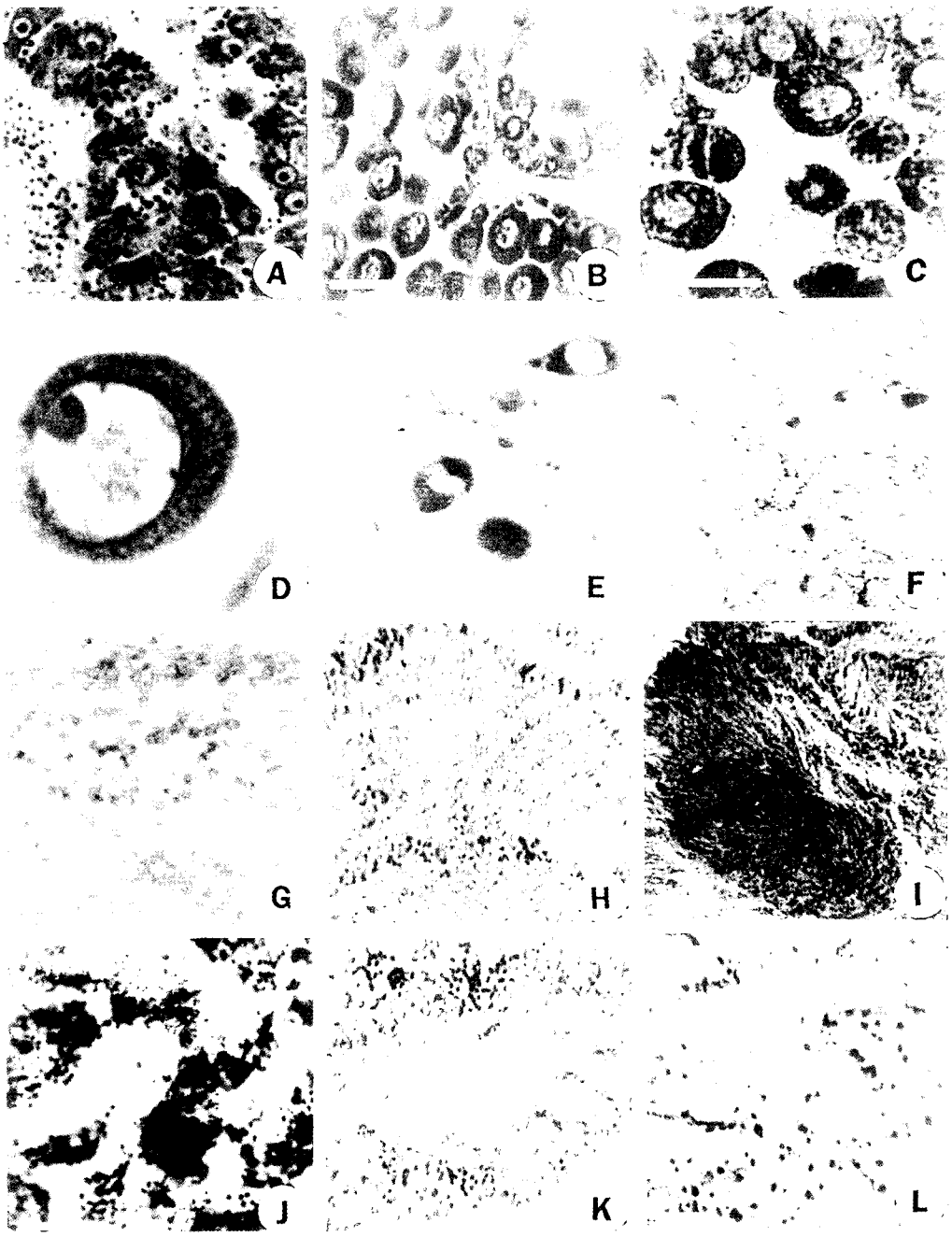

Fig. 5. Photomicrographs of the gonadal phases of female *Scapharca subcrenata*. A, transverse section of the oogenic follicle in the early active stage, scale bar=50 μm; B, section of the oocytes in the late active stage, scale bar=50 μm; C, section of the oocytes in the ripe stage, scale bar=50 μm; D, a ripe oocyte, scale bar=50 μm; E, section of follicles in the partially spawned stage, scale bar=50 μm; F, section of follicles in the degenerative/resting stage, scale bar=50 μm; G, transverse section of spermatogenic follicles in the early active stage, scale bar=50 μm; H, section of the follicles in the late active stage, scale bar=50 μm; I, section of the follicles in the ripe stage, scale bar=50 μm; J, section of follicles in the partially spawned stage, scale bar=50 μm; K, section of the follicle in the degenerative stage, scale bar=50 μm; L, section of the follicles in the resting stage, scale bar=50 μm.

and appeared in a layer along the follicular wall (Fig. 5G).

Individuals in the early active stage appeared from January to May when seawater temperatures were <11℃.

Late active stage

In females, there was a large amount of ovogenic activity. A number of the oocytes of 31~40 μm in

diameter appeared in the follicles. When the oocytes grew to 51~60 μm in diameter, each oocyte has a large germinal vesicle and an egg-stalk attached to the follicular wall. At this time, the follicular wall became thin (Fig. 5B).

In males, spermatocytes develop into spermatids. The spermatids (3~4 μm in diameter) moved toward the center of the lumen having layers. As the testis developed, a dense area of spermatocytes and spermatids occupied approximately one-third to a half of the lumina in the follicles. Spermatozoa appeared in part of the follicles (Fig. 5H).

Individuals in the late active stage were found from June to July when seawater temperature were >15℃.

Ripe stage

In females, the majority of maturing oocytes grew to 51~60 μm in diameter, becoming round or oval in shape, and were located in the center of the lumen. There is an increase in the ratio of cytoplasm to the nucleus (Fig. 5C). At this time, the follicular wall became very thin, the ripe eggs (61~70 μm in diameter) were surrounded by the gelatinous membranes. The cytoplasm of the oocytes contained a large number of yolk granules, while the follicular wall was very thin (Fig. 5D).

In males, a few spermatids began to undergo transformation into the differentiated spermatozoa in the center of the lumen. The ripe testis was characterized by the formation of a number of spermatozoa (Fig. 5I).

Mature and ripe gonads were found from June to August when seawater temperature was 15.2~22.4℃ (Fig. 4).

Partially spawned stage

In females, the lumina of the oogenic follicles became considerably empty after 50~60% of the oocytes in the oogenic follicles being discharged. Spawned ovaries were characterized by the presence of a few ripe undischarged oocytes and very young oocytes in the lumen (Fig. 5E).

In males, after a large number of spermatozoa in the follicles were discharged into the surrounding water, the lumen became empty. However, a number of spermatozoa, spermatids, and spermatocytes still remained in the lumen (Fig. 5J).

The spawning period of this species occurred once a year from early July to September and the main spawning occurred from July to September when seawater temperatures were >20℃.

Degenerative stage

In females after spawning, the undischarged oocytes in the lumen of the oogenic follicle underwent cytolysis, and each follicle was contracted and degenerated. The products of gamete atresia were resorbed (Fig. 5F).

In males, a few undischarged spermatozoa and spermatids were degenerated (Fig. 5K). Individuals in the degenerative stage were found from August to December.

Resting stage

The rearrangement of newly formed connective tissues occurred in the follicles of the ovary and testis (Figs. 5F, 5L). The individuals in this stage appeared from January to April when the seawater temperatures were >5℃.

Discussion

Gonadal development and maturation

Studies of natural reproductive cycle are one of the most valuable things since the onset and duration of both gametogenesis and maturation in most bivalves can exhibit considerable temporal and spatial variations (Raymond and Thomas, 1992).

In the present study, gonadal development and maturation of *S. subcrenata* occurred relatively late from the late spring to the autumn when seawater temperatures gradually increased, various food organisms (phytoplankton) began to be abundant, and various nutrient reserves were stored in the digestive diverticula. During the periods of lower temperatures and insufficient food organisms, the gonadal phases were in the immature stage.

So far, several authors stated that gonadal development and maturation of bivalves are related to exogenous factors, e.g. water temperature (Sastry, 1966, 1968), food availability (Sastry, 1966, 1968; Griffiths, 1977), and day length (Simpson, 1982), and endogenous factors e.g., nutrient reserves, hormonal cycles and genotype (Seed and Suchanek, 1992) that determine the initiation and duration of gametogenesis and spawning (Newell et al., 1982). The effect of water temperature on active gonad development and maturation of *S. subcrenata* was evident. Therefore, the periods of gonadal development and maturation almost coincide with the periods of favorable water temperature. Accordingly, it is supposed that gonadal development and maturation of this species are closely related to exogenous factor (water temperature).

Breeding pattern and the number of spawning periods

Most marine molluscs have their own special breeding habits. According to Boolootian et al. (1962), the breeding habit can classify molluscs into three large categories: (1) year-round breeders, (2) winter breeders, and (3) summer breeders. In the present study, spawning of this species occurs from July to September. Accordingly, this species belongs to the summer breeders.

Rand (1973) stated that breeding strategy varies with latitudinal gradient. If the same species inhabits different latitudes, the same species in northern climates is characterized by a single synchronous spawning per year, temperate climates by two spawning seasons, and tropical ones by year-round spawning. According to several reports (Kurashige, 1943; Ko, 1957; Momoyama and Iwamoto, 1979), the effect of different temperatures with latitudinal gradient is evident, especially, with reference to the number of the spawning seasons per year of *Ruditapes philippinarum*, compared with the reports obtained from other area.

For instance, a comparison can be made between the reproductive cycle of *R. philippinarum* with latitudinal gradient. This species has been reported to have one spawning period in Korea (Chung et al., 1994), British Columbia, Canada (Quayle and Bourne, 1972), and Hood Canal, Washington, U.S.A. (Holland and Chew, 1974) and Northern Japan (Yoshida, 1953), however, two spawning periods in southern Japan (Tanaka, 1954) and three spawning periods in the Adriatic Sea, southwest Spain (Sarasquete et al., 1990).

In the present study, the ark shell (*S. subcrenata*) from Paengnyongdo, Korea has one spawning period from July to September. And the ark shell in Japan has also one spawning period: from the end of June to September in Nakano-umi (Hata, 1948) and from the middle of June to the middle of October in Ariake Bay (Tanaka, 1954). Thus, the spawning period of the ark shell in Korea is similar to those of the ark shell in Japan. Therefore, it is assumed that the number of spawning periods per year in the same species are associated with temperature-latitudinal gradient. Even though the spawning period was once a year, it is assumed that the number of spawning frequencies (broods) may occur more than twice during the spawning season.

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