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Reproductive Biology of the Pen Shell, *Atrina* (*Servatrina*) *pectinata* on the Boryeong Coastal Waters of Korea

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ABSTRACT

The gonad index, condition index, reproductive cycle and spawning of the pen shell *Atrina* (*Servatrina*) *pectinata* were investigated using samples from the subtidal zone of Nokdo on the Boryeong coastal waters of Korea. Samples were collected monthly by SCUBA divers for one year from January to December, 2001. *A.* (*Servatrina*) *pectinata* is dioecious and oviparous. The spawning season of this species occurred once a year from June to August, with the main spawning occurring between June and July when the seawater temperature was around 20℃. Ripe oocytes were about 60–65 μm in diameter. The reproductive cycle of this species could be classified into five successive stages; early active stage (November to March), late active stage (February to May), ripe stage (April to July), partially spawned stage (June to August), and spent/inactive stage (August to October). Monthly changes in the gonad index reached a maximum (4.6) in May (ripe stage), thereafter, the GI values gradually decreased from June to August when spawning occurred continuously. Therefore, monthly changes in the GI values showed a similar pattern to the gonadal phase. The condition index (CI) of the meat part without the posterior adductor muscle reached the maximum in June (ripe and partially spawned stage) and the minimum in September (spent/inactive stage), Accordingly, monthly changes in the condition indice of the meat part without the posterior adductor muscle coincided with the gonadal phases.

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INTRODUCTION

The pen shell *Atrina* (*Servatrina*) *pectinata* is one of the commercially important edible bivalves in East Asian countries, including Korea, China, and Japan (Yoo, 1976; Kwon *et al.,* 1993). The species is mainly found in the subtidal zone of Nokdo along the Boryeong coastal waters of Korea, inhabiting silty sand bottoms up to 15-20 m depth. As a consequence of reckless over-harvesting, the standing stock of this species has dramatically reduced in recent years, and it has been denoted as a target organism and fisheries resource that should be managed with a more reasonable fishing regime. For propagation and management, it is important to understand its population characteristics with regard to the reproductive cycle and the spawning period.

Many studies have examined on aspects of the ecology, including age and growth (Ryu *et al.,* 2001), on the aspects of aquaculture, including technique development of aquaculture (Yang *et al.,* 1995; Yoo *et al.,* 1998), on the aspects of physiology, including nutrient components (Choe, 1993; Baik *et al.,* 2001) and on genetic divergence (Yokogawa, 1996) of *Atrina* (*Servatrina*) *pectinata*. Regarding this species, several studies have focused on reproduction, aquaculture, ecology and physiology. Despite these studies referred above, little information on the reproductive cycle of this species is available. The knowledge of the

reproductive cycle will provide necessary information for the spawning period and the recruitment period of this species would be very useful for propagation, aquaculture and resource management. Therefore, the main aim of the present study is to understand the reproductive cycle and the spawning period of this species.

MATERIALS AND METHODS

1. Sampling

Samples of the pen shell, *Atrina* (*Servatrina*) *pectinata*, were collected monthly in the period of January to December 2001 by dredging in the subtidal zone of Nokdo, Korea (Fig. 1). A total of 492 clams ranging from 13.2 to 15.1 cm length was collected during the study. Pen shells were transported alive to the laboratory, and shell length and total weight were measured. Unpublished surface seawater temperature, measured at 10:00 a.m. at the Boryeong Fisheries Hatchery in Boryeong, were used for this study.

2. Gonad index (GI)

To explore the spawning period indirectly, the mean gonad index (GI) of the pen shell was calculated using a modification of Mann's method (1979). Each section of gonadal tissue for histological observation was also examined in detail to assess the stage of gonadal development and was scored on 0-5 scale to describe six stages of gonadal development or maturity: 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 arithmetic mean of the individual scores of the whole samples was recorded as the gonad index (GI) for each month.

A total of individuals was used to calculate the gonad index (GI). Monthly changes in the mean GI were calculated using the following equation:

GI = (N x RVS0 + N x RVS1 + N x RVS2 + N x RVS3 + N x RVS4 + N x RVS5) / Total number of clam observed by month

Where, N: number of individuals at a gonadal development stage, RVS: ranking value by stage.

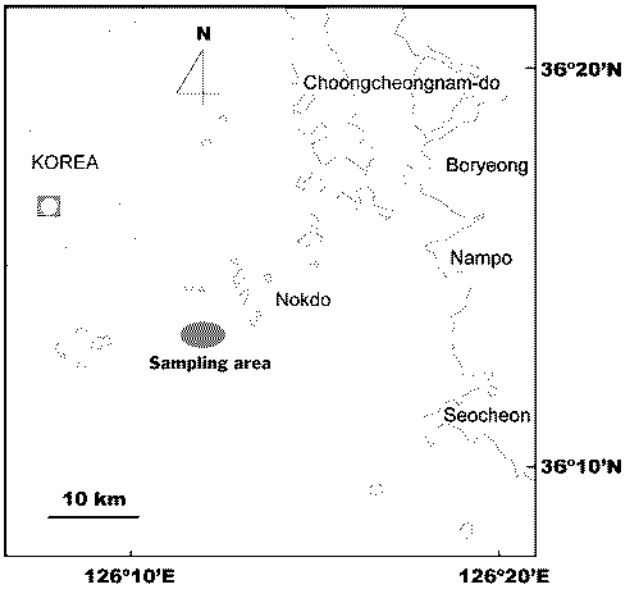

Fig. 1. Map showing the sampling area.

3. Condition Index (CI)

To explore the spawning period indirectly, monthly changes in the condition index (CI) of *Atrina* (*Servatrina*) *pectinata*, was calculated using the following equation:

CI = MAM (g) / SH^3 (cm) X 1,000

Where, MAM: the meat part weight (g) without the posterior adductor muscle, SH: shell height (cm).

4. Histological analysis

For light microscopic examination of the histological preparations, a total of 492 gonad tissues was

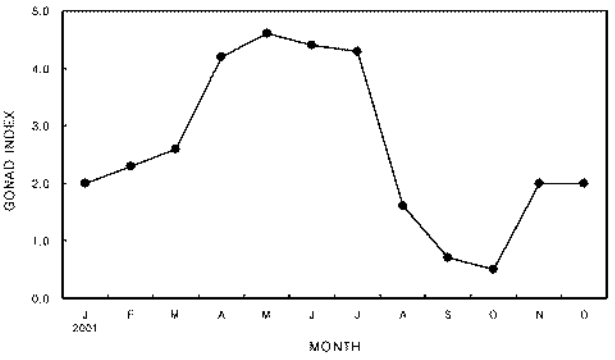

Fig. 2. Monthly changes in the gonad index of *Atrina* (*Servatrina*) *pectinata*, for one year from January to December, 2001.

removed from shells and preserved in Bouin's fixative for 24 hrs and then washed with running tap water for 24 hrs. The tissues were then dehydrated in alcohol and embedded in paraffin molds. The embedded tissues were sectioned at 5-7 μm thickness using a rotary microtome. The sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, Mallory's triple stain and PAS stain, and examined using a light microscope.

RESULTS

1. Gonad index (GI)

Monthly changes in the gonad index were showed in Fig. 2. The GI increased rapidly from February and reached the maximum (4.6) in May when seawater temperature rapidly increased. Then, the GI gradually decreased from June to August when relatively high water temperatures were maintained and spawned continuously. Thereafter, the GI values temporally reached the minimum (0.5) in October when spawning had completely finished.

2. Condition index (CI)

Monthly changes in the condition index (CI) of the meat part without the posterior adductor muscle were showed in Fig. 3. The CI increased gradually from February and reached the maximum in June. Then, the CI values gradually decreased from July to September and reached the minimum in October.

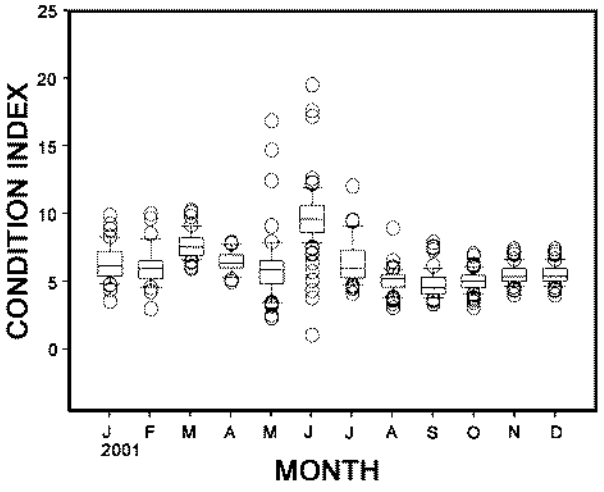

Fig. 3. Monthly changes in condition coefficient of meat part without the posterior adductor muscle of *Atrina* (*Servtrina*) *pectinata*, for one year from January to December, 2001.

3. 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 could be categorized into six successive stages (Fig. 4).

1) Early active stage

The follicles of the gonad occupied about 15-20% of the whole gonad. In females, oogenesis occurred in the oogenic follicles of the ovary. Oogonia and oocytes propagated along the follicular wall of the ovary. The diameters of oogonia and oocytes were approximately 10 μm and 20-30 μm, respectively. At that time, the total volume of the ovary was small, and the follicular wall was thick (Fig. 5A). In males, spermatogenesis occurred in the acini of the testis. The diameters of spermatogonia and spermatocytes were 8-9 μm and 6-7 μm, respectively, and appeared in a layer along the acinus wall (Fig. 6A). Individuals in the early active stage appeared from November to March when seawater temperatures were about 11℃.

2) Late active stage

In females, 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 had a large germinal vesicle and an egg-stalk attached to the follicular wall. At that time, the follicular wall became thin gradually (Fig. 5B).

In males, the spermatids (3-4 μm in diameter) moved toward the center of the acinus lumen. As the testis developed, a dense area of spermatocytes and spermatids occupied approximately one-third to a half of the lumina in the acini. Spermatozoa appeared in part of the acini (Fig. 6B). Individuals in the late active stage were found from February to May when seawater temperature were over 15℃.

3) Ripe stage

In females, the majority of maturing oocytes grew to

50-60 μm in diameter, becoming round or oval in shape, and were located in the center of the lumen. There was an increase in the ratio of cytoplasm to the nucleus. At that time, the follicular wall become very thin, the ripe eggs (60-65 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. 5C). In males, a few spermatids began to undergo transformation into the differentiated spermatozoa in the center of the lumina of the acini. The ripe testis was characterized by the formation of a number of spermatozoa (Fig. 6C). Mature and ripe gonads in both sexes were found from April to July when seawater temperature was 15.2-22.4℃.

4) Partially spawned stage

In females, the lumina of the follicles became considerably empty after 40-60% of the oocytes in the follicles being discharged. Spawned ovaries were

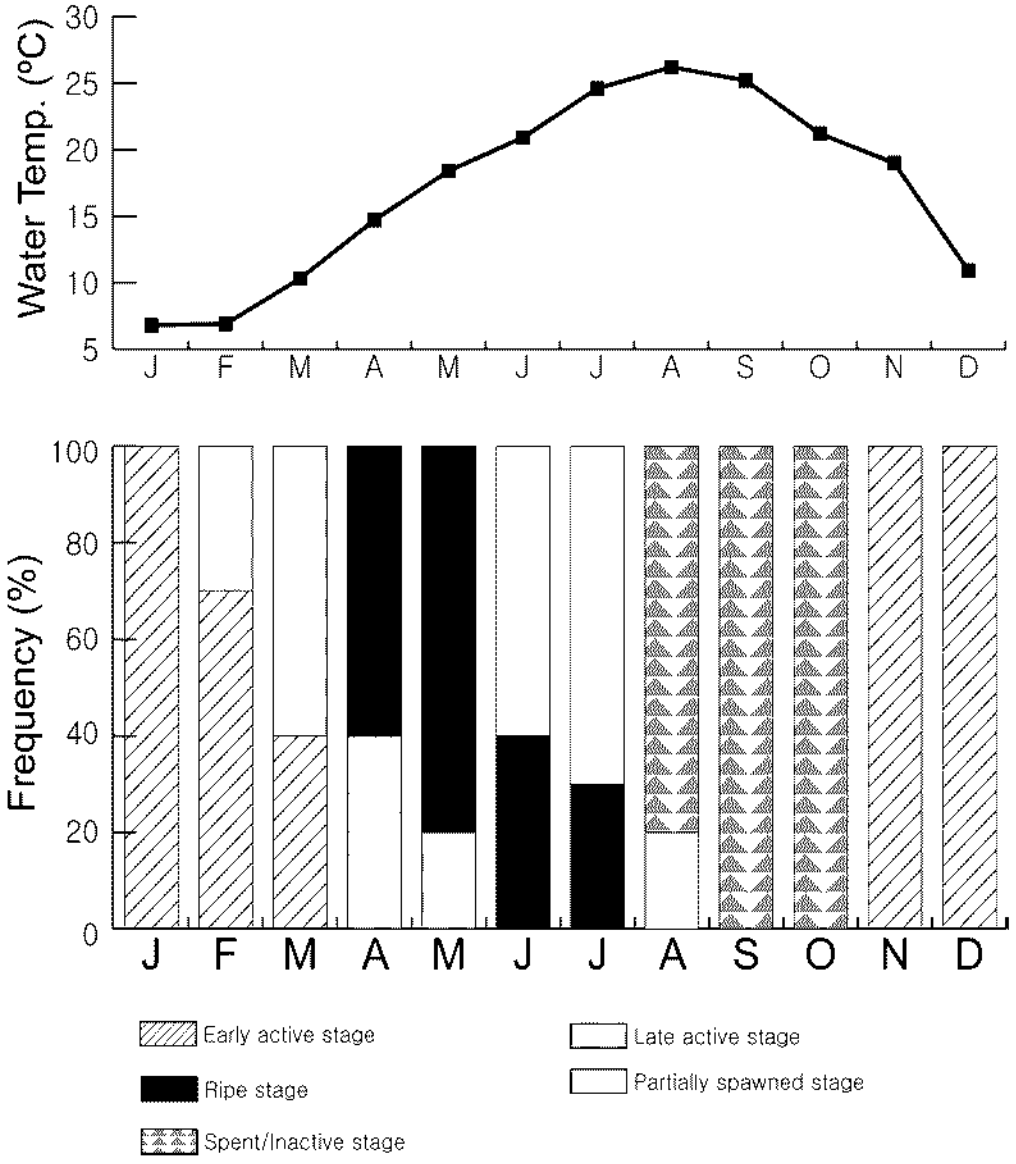

Fig. 4. Frequency of gonadal phases *Atrina* (*Servatrina*) *pectinata* and the mean seawater temperatures, for one year from January to December, 2001.

characterized by the presence of a few undischarged oocytes and very young oocytes in the lumina of the follicles (Fig. 5D). In males, after a large number of spermatozoa in the acini were discharged into the surrounding water, the lumen became empty. However, a number of spermatozoa, spermatids, and spermatocytes ever remained in the acinus lumen (Fig. 6D).

The spawning period of this species occurred once a year from early June to August and the main spawning occurred between June and July when seawater temperatures were higher than 20℃.

5) Spent/inactive stage

In females after spawning, the undischarged oocytes in the lumen of the follicle underwent cytolysis, and each follicle was contracted and degenerated. The products of gamete atresia were resorbed. Thereafter, the rearrangement of newly formed connective tissues, a few oognia appeared on the newly formed follicular walls (Fig. 5E, F). In males, undischarged spermatozoa and spermatids were degenerated in the

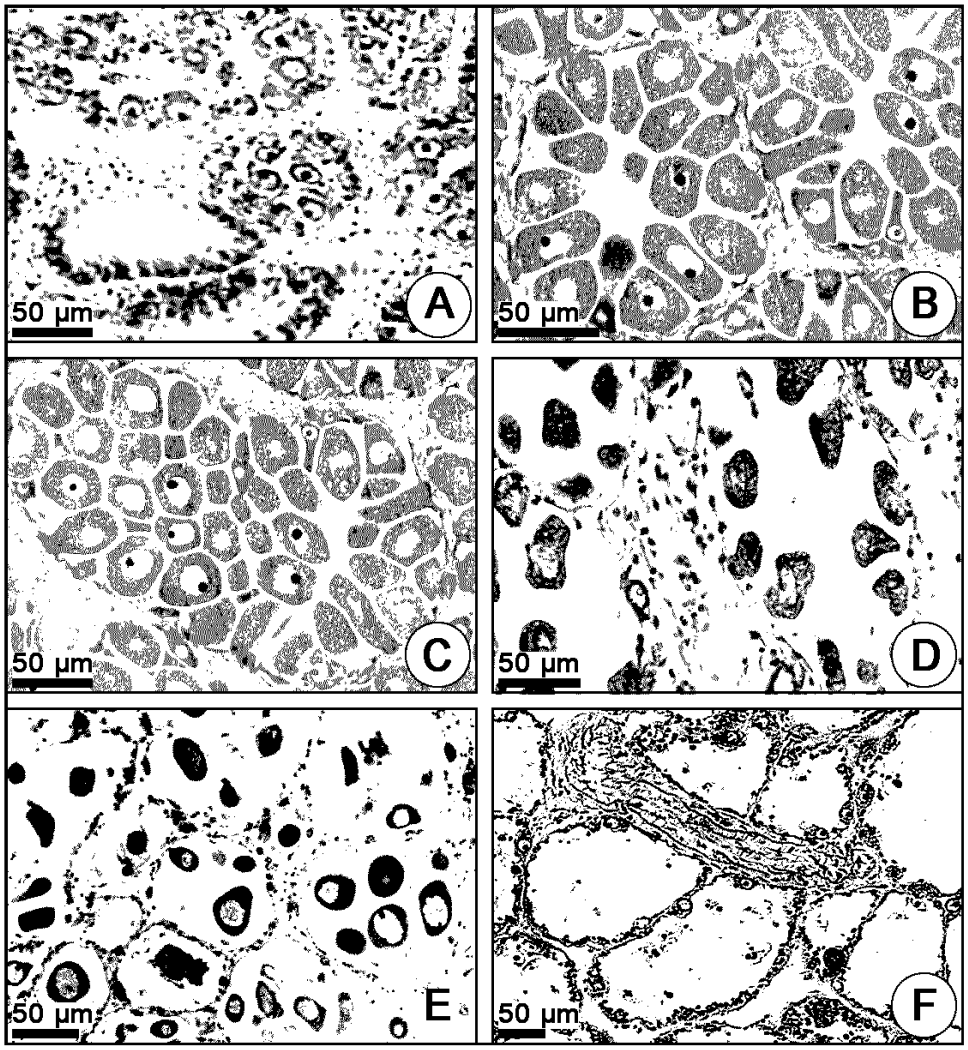

Fig. 5. Photomicrographs of the gonadal phases in female pen shell. *Atrina* (*Servatrina*) *pectinata*. **A**, transverse section of the follicles in the early active stage; **B**, section of the follicles in the late active stage; **C**, section of the follicles in the ripe stage; **D**, section of the follicle in the partially spawned stage; **E**, section of the follicles in spent stage; **F**, section of the follicles in the inactive stage.

acini. Thereafter, the rearrangement of newly formed connective tissues and a few spermatogonia appeared on the newly formed acinus walls (Fig. 6E, F). Individuals in the spent/inactive stage were found from August to November.

DISCUSSION

Monthly changes in the gonad index increased rapidly from February (early and late active stages) and reached the maximum in May (late and ripe stages) when the seawater temperature rapidly increase. Then, the GI values gradually decreased from June to August (partially spawning stage). Accordingly, variations in the GI showed a close relationship with gonadal activity.

Monthly changes in the condition index of the meat part without the posterior adductor muscle increased from February (early and late active stage) and reached the maximum in June (ripe stage). Thereafter, the their values gradually decreased from July to

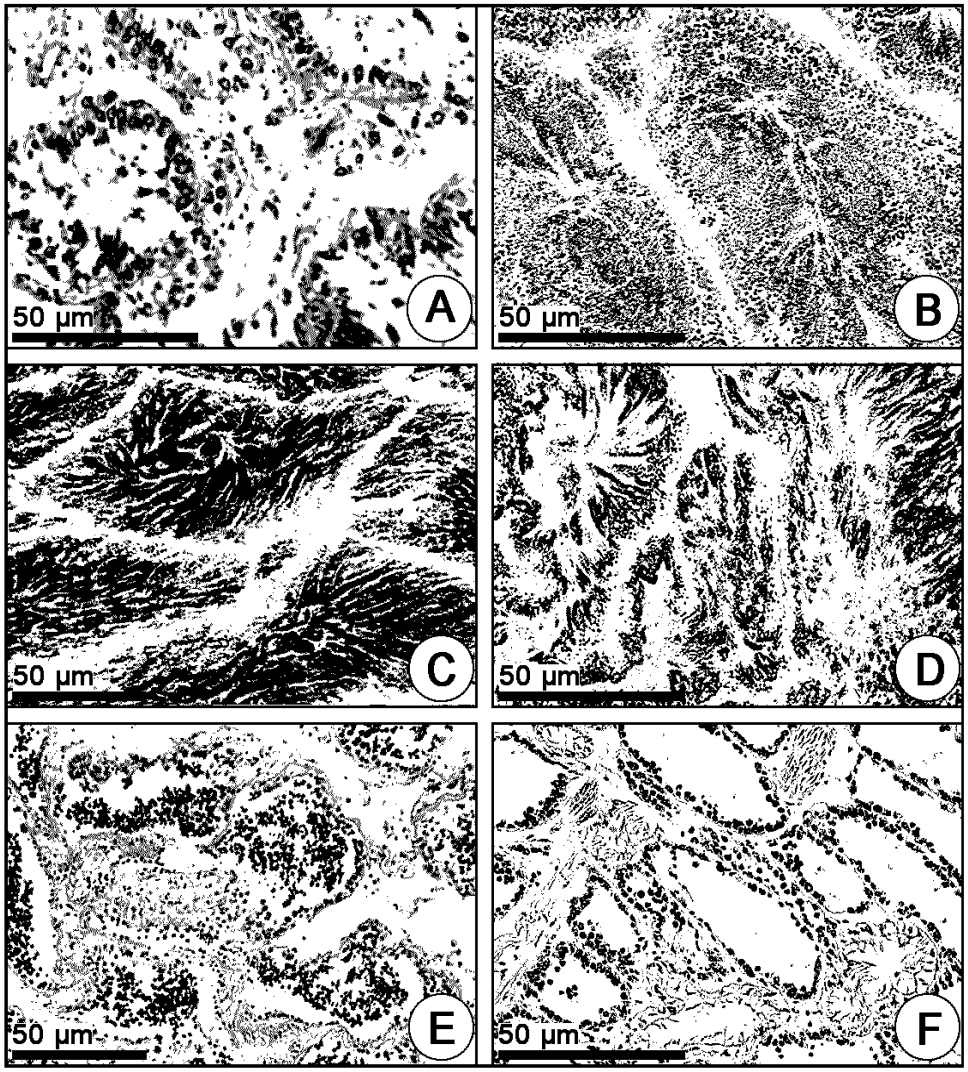

Fig. 6. Photomicrographs of the gonadal phases in male pen shell, *Atrina* (*Servatrina*) *pectinata*. **A**, transverse section of the acini in the early active stage; **B**, section of the acini in the late active stage; **C**, section of the acini in the ripe stage; **D**, section of the acini in the partially spawned stage; **E**, section of the acini in the spent stage; **F**, section of the acini in the inactive stage.

August (partially spawned stage) and reached the minimum in October (spent/inactive stage). Accordingly, monthly changes in the condition index of the meat part without the posterior adductor muscle show a similar pattern with reproductive activity or gonad development.

Recently, many authors (Sastry, 1966, 1968, 1970, 1979; Sastry and Black, 1971) reported that as controls for gonad development and maturation in marine bivalves, exogeneous factors (temperature and food availability) and endogenous factors seem to be particularly important. However, Sastry (1966, 1968) stated that these and other factors (salinity and day length, *etc.*) probably will interact with endogenous factors (neuroendocrine activity) in a complex manner to control the initiation of gametogenesis. According to Sastry (1968, 1970), seawater temperature acts as a triggering stimulus for initiation of the oocyte growth phase. The temperatures required for activating growth of the oocytes at the beginning of oogenesis and for attaining maturity ultimately limit gonad activity and gametogenesis in the natural environment. In the present study, gametogenesis of *Atrina* (*Servatrina*) *pectinata* began from January to March and reached maturity from April through July when seawater temperatures gradually increased. Accordingly, gonad activity and gametogenesis of the species occurred under temperature conditions that allow nutrient mobilization to the gonads, after basic metabolic requirements are satisfied (Sastry, 1966). The periods of food abundance and of gonad development of this species nearly coincide. Gonad growth and gametogenesis from the early spring coincided with peak food levels. Therefore, it is assumed that if food and water temperature criteria are met, growth of oocytes is initiated in conjunction with the transfer of nutrients from the digestive diverticula to the gonad.

Comparisons of the spawning period of *Atrina* (*Servatrina*) *pectinata* in different localities are shown in Table 1. According to the histological observations, spawning of this species in Boryeong coastal waters occurred from June to August. The spawning period in Yeoja Bay and Jinhae Bay, south coast of Korea, occurs from June to September. Therefore, our results showed similar results to three different Bays, (Yoo and Yoo, 1984; Kim *et al.,* 1985; Yoo *et al.,* 1988). As shown in Table 1, the Japanese *A. pectinata* spawns once a year between June and July in Fukuoka coast, Japan, and spawning occurs from mid July to late August in Ariake Sea, Japan. Therefore, our results coincide with the results of Fujimori (1929) and Watanabe (1938). It is well-known that most marine invertebrate species have unique breeding season. Boolootian *et al.* (1962) described that breeding patterns of molluscs can be classified into three large categories, based on their spawning behavior or seasonality: 1) year-round breeders, 2) winter breeders, and 3) summer breeders. According to histological observations of the gonad of this species, spawning occurred from June through August. Therefore, *Atrina* (*Servatrina*) *pectinata* belongs to summer breeders.

In *Cyclina sinensis* (Chung *et al.,* 1991), *Ruditapes*

Table 1. A comparison of the spawning period of the pen shell, *Atrina* (*Servatrina*) *pectinata*, in different geographic locations.

Location	Spawning period	Sources
Fukuoka coast (Japan)	June-July	Watanabe, 1938
Ariake Sea (Japan)	mid July-late August	Fujimori, 1929
Yeoja Bay (Korea)	June-August	Kim *et al.,* 1985
Yeoja Bay (Korea)	July-August	Yoo and Yoo, 1984
Jinhae Bay (Korea)	July-September	Yoo *et al.,* 1988
Boryeong (Korea)	June-August	Present study

philippinarum (Chung *et al.,* 1994) and *Mactra veneriformis* (Chung *et al.,* 1988), spawning occurs in seawater above 22℃. The water temperature in the spawning period closely coincides with that of *Atrina* (*Servatrina*) *pectinata* found in the previous studies.

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