Seasonal Changes in Reproductive Condition of the Pacific Oysters, *Crassostrea gigas* (Thunberg) from Suspended Culture in Gosung Bay, Korea

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Abstract - Seasonal variation in reproductive condition of the Pacific oyster, *Crassostrea gigas* was investigated from a suspended cultured oyster population in Gosung Bay, South Korea using histological techniques. Gametogenesis of oysters initiated in February when water temperature reached 11 to 13°C. Increase in oocyte size and the number resulting in follicle expansion was observed from March to May. First spawning of oysters observed in mid Jun when the surface water temperature reached 22 to 25°C. Spawning activity of oysters extended from mid June to late September with two marked spawning peaks in June and August. Most oysters collected from October to December exhibited few residual eggs in packed follicles exhibiting a typical spent condition. No gametes were observed from December to February from oysters collected in the Bay. Gonadal development of oysters in the Bay seemed to follow a seasonal fluctuation in environmental conditions such as water temperature and food availability in the water column. Spawning of oysters in late June was in part associated with sudden drop in salinity due to vast amount of freshwater input in the Bay after the summer flooding. Sex ratio of oysters was 59.5% male and 39.8% female. Less than 1 percent (0.6%) of the oysters examined were hermaphrodite; few eggs were observed in testis.

Key words : *Crassostrea gigas*, gametogenesis, gonadal development, reproduction, Korea

Introduction

Crassostrea gigas, the Pacific oyster is commonly distributed all along the west and south coast of Korea. They are commercially cultured using suspended hanging method in small bays on the south coast (Choi *et al.* 1997). Among the several oyster culture grounds on the south, Gosung Bay is considered to be one of the biggest culture ground. The bay located between 128° 20′N 34° 55′E and has $2,165 \times 10^4$ m^2 surface area with an average depth of 7 m (Fig. 1). In year 2000, 16,230 metric tons of oysters including shells were produced from Gosung Bay, which accounts for approximately 10% of the total oyster production in Korea (MOMAF, 2001). The oyster lands in this bay have been declining for the past few years. Several reasons including decrease in oyster growth rate, oligo-trophic condition of the culture ground and failure in healthy spat harvest for the culture has been blamed for the current decrease in oyster production (Yoo *et al.* 1980; NFRDI Report 1997; Park *et al.* 1998, 1999). Insufficient natural spat supply is also considered to be one of the major obstacles for oyster culture industry in this area. For future

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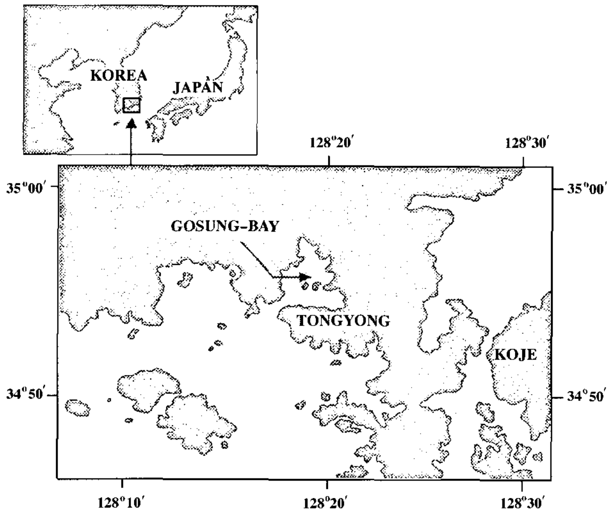

Fig. 1. Map showing the location of sampling area, Gosung Bay, Korea.

development of oyster industry, detailed study on oyster reproduction including an annual gonadal cycle, spawning timing and intensity would be valuable not just for research interest but also for the oyster aquaculture industry.

Many studies conducted on oyster reproduction have stressed that water temperature and available foods in the water column are the two major factors that control the reproductive process (Hayes and Menzel 1981; Soniat and Ray 1985; Gallager and Mann 1986; Arakawa 1990; Ruiz *et al.* 1992). In general, changes in water temperature accelerate or retard rate of gonadal development while food availability mainly determines quality and quantity of reproductive output (Loosanoff 1965; Sastry 1979; Choi *et al.* 1993; Hofmann *et al.* 1992, 1994; Kang *et al.* 2000). Kang *et al.* (2000) also reported spatial variation in spawning timing of oysters between two different localities on the south coast of Korea. Other studies reported that differences in the cycles of energy storage and reproduction of a species among different locations could be explained by differences in environmental conditions, in particular food availability (Newell *et al.* 1982; Rodhouse *et al.* 1984; Bricelj *et al.* 1987; Harvey and Vincent 1989; Navarro *et al.* 1989).

The main objectives of this study are to investigate (1) gametogenesis and spawning period of *C. gigas* in Gosung Bay; (2) influence of environmental parameters especially temperature on gonadal development.

Materials and Methods

1. Oyster sampling and histological preparation

Adult Pacific oysters with a mean shell length over 8 cm were collected from 3 different sampling locations in Gosung Bay, Korea. From each sampling site, an oyster string was randomly taken for the analysis. Sampling continued from January to December 2000 on a monthly basis. From June to August, oysters were collected on a biweekly basis in order to follow spawning activity of oysters. Water temperature and salinity were recorded *in situ* when the sampling was made. Upon arrival at the laboratory, shell length of oysters was recorded in mm using a caliper. Flesh of oysters was then removed from the shell and wet tissue weight was measured after removing excessive water with absorbent tissue paper.

Thirty to 45 oysters were collected from each oyster string for histological preparation. A transverse section was made in the middle of the oyster body and a 3 mm-thick section was fixed in Bouin's solution. The oyster sections were then dehydrated in an ethanol series of progressive concentrations, cleared in xylene, and embedded in paraffin. Four μm-thick serial sections were cut with a rotary microtome and stained with Harris' haematoxilyn and eosin Y.

2. Gonadal development

Based upon microscopic examination of the histological preparation, gonadal development of *C. gigas* was categorized into five stages as described by Heffernan (1989). Gonadal development of each oyster was then scored on a 0 to 4 scale; 0, undifferentiated stage, 1, spent stage, 2, developing stage, 3, ripe stage, 4, spawning. The monthly gonafal index (GI) for both sexes was determined by multiplying the number of specimens ascribed to each category by the category score, summing all such values and dividing this figure by the total number (Heffernan 1989).

Results

1. Sampling effort and environmental conditions

Monthly mean salinity and temperature of water column are plotted on Fig. 2. Surface water temperatures varied seasonally with a maximum of 30.4°C in July and a minimum of 4.3°C in February. Highest salinity was recorded in May, 34.3‰. From June to August, salinity dropped abruptly and reached a minimum value of 27.6‰. From August onward, salinity fluctuated around 31‰ and increased to 32.4‰ at the end of the year. Fig. 3 summarizes size frequency distribution of oysters analyzed in this study. Size class of 80 to 90 mm in shell length was most common, accounting for 28 to 32% of total oyster analyzed. The ratio of males

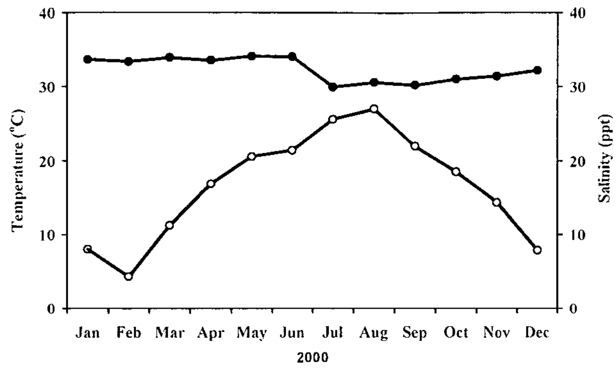

Fig. 2. Seasonal variation of water temperature and salinity in Gosung Bay during January to December 2000.

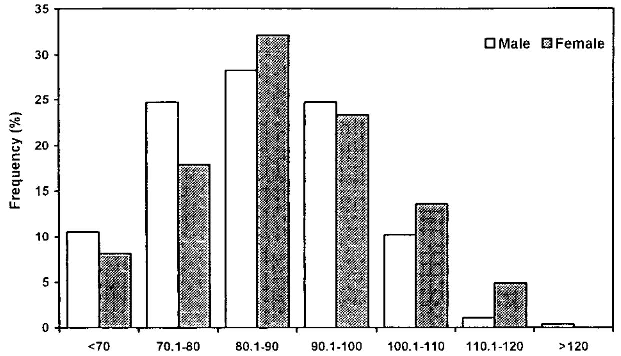

Fig. 3. Shell length frequency distribution of male and female oysters during sampling period.

(59.5%) was higher than females (39.8%) during sampling period. Hermaphroditic individuals were also found in June and November although they were only 0.6% of the total oyster examined.

2. Seasonal changes in reproduction condition of oysters

1) Microscopic appearance of gonadal development

Photomicrographs of various developmental stages of male and female oysters are presented on Fig. 4 and 5. In undifferentiated stage, little or no gonadal tissue was visible and sex of oyster cannot be distinguished at this period (Fig. 4A & 5A). During developing stage, the number of follicles increased and the follicles became expanded (Fig. 4B & 5B). As the developing stage progressed, the follicles continued to expand and coalesce. No mature gametes were observed during this period (Fig. 4C & 5C). Late gonadal developmental stage was characterized with greatly expanded and coalesced follicles with some connective tissues remained unfilled. During this period, the lumen was filling with growing and ripe oocytes. Fig. 4D shows spawning female gonad, exhibiting free mature oocytes filling the follicles. Free gametes were also visible in gonoduct during this period. In spawning male, the follicles were almost filled with spermatozoa (Fig. 5D). After completion of spawning, gonads were considerably shrunken in volume and contain a small number of residual gametes (Fig. 4E & 5E). Fig. 4F shows a typical female spent gonad, displaying empty follicles with a few number of free oocytes. For males, the follicles are collapsed and decreased in size. A few follicles contain a small amount of residual unspent spermatozoa (Fig. 5F). Hermaphroditic oysters are shown on Fig. 6, exhibiting developing testis and ovary in a follicle (Fig. 6A & B).

2) Reproductive pattern

Fig. 7 shows changes in GI of oysters in the Bay during the course of study. Microscopic observation of the histological slides indicated that gametogenesis of oysters started as early as in December or January. Monthly mean GI increased at a fast rate from February to April as the surface water temperature elevated. In mid June, mean GI of oysters indicated that most oysters were ready for spawning or in partial spawning.

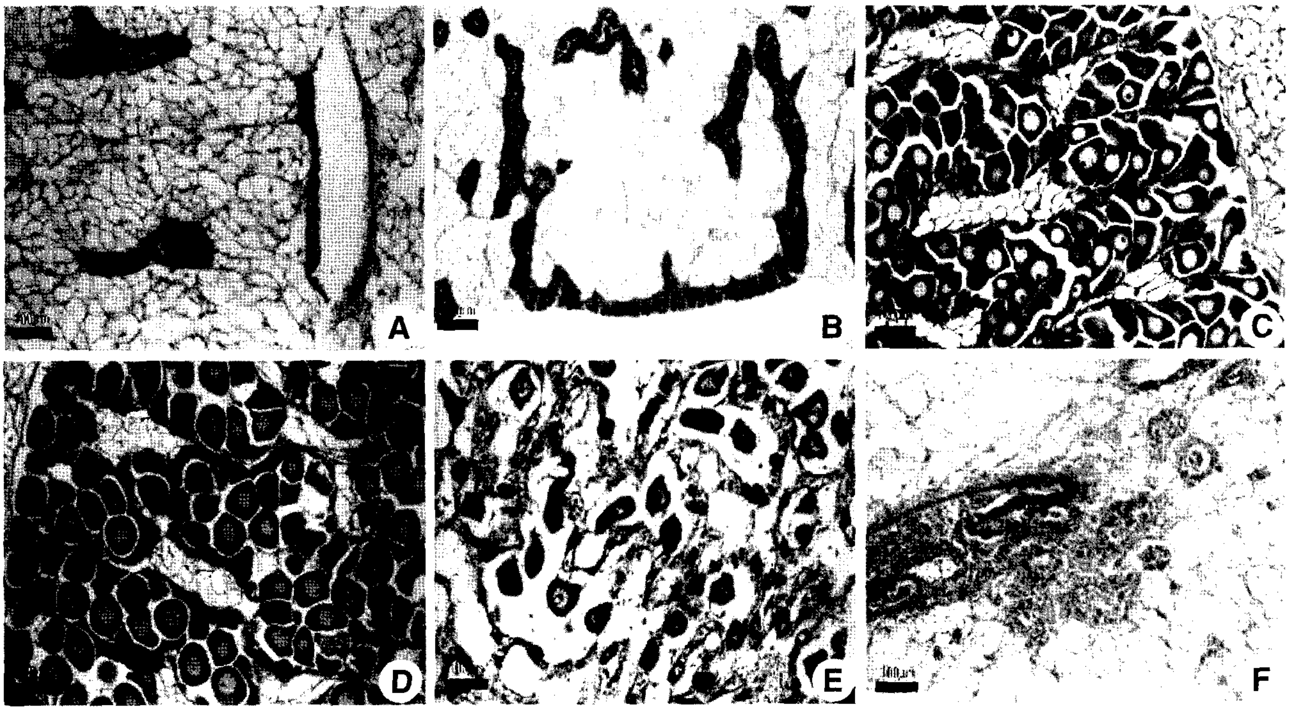

Fig. 4. Gonadal development in female oysters. (A) Sexually undifferentiated stage, (B) Early development stage, (C) Late development stage, (D) Ripe stage, (E) Spawned stage, (F) Gonadal tissue atrophy

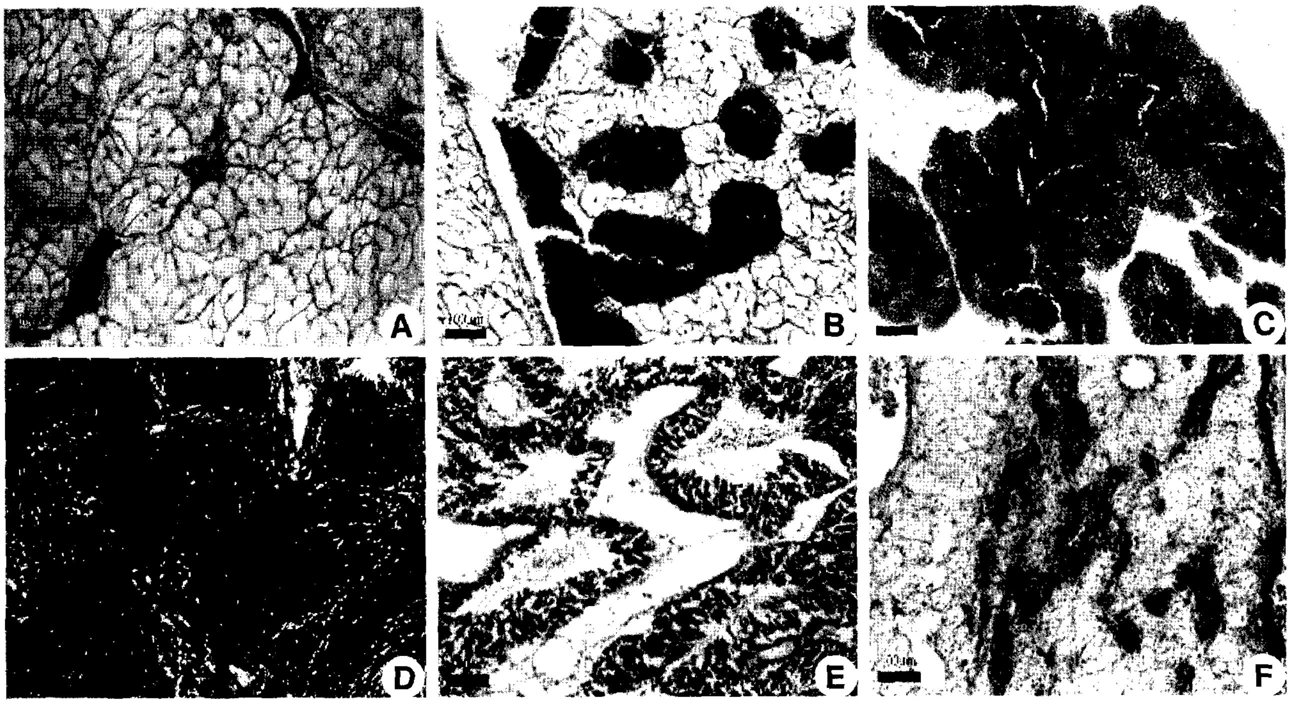

Fig. 5. Gonadal development in male oysters. (A) Sexually undifferentiated stage, (B) Early development stage, (C) Late development stage, (D) Ripe stage, (E) Spawned stage, (F) Gonadal tissue atrophy.

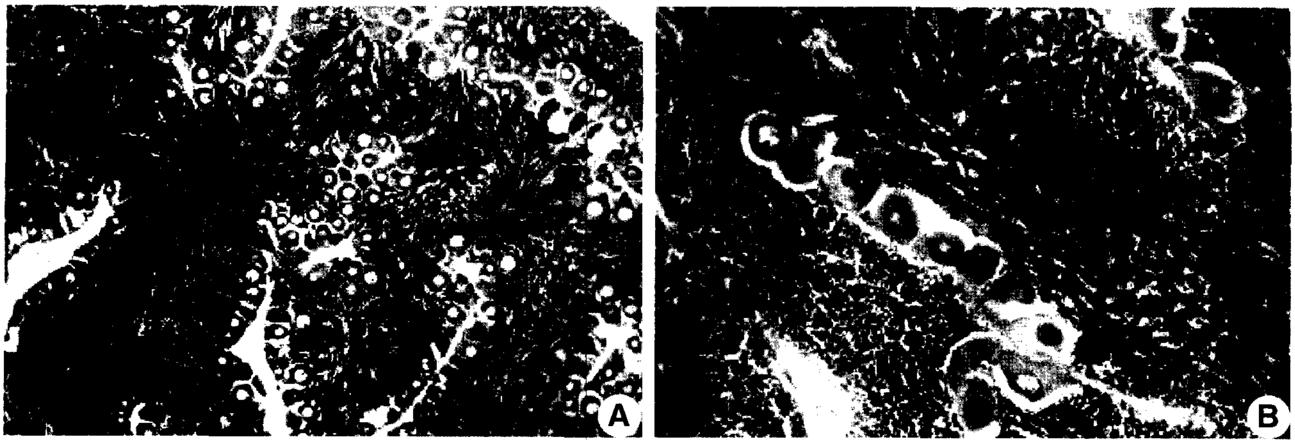

Fig. 6. Hermaphroditic oysters. (A) Developing oocytes in periphery of follicle wall with sperm in follicular lumen, (B) In the same follicles, both sexes are developed (bar 50 μm).

Table 1. Percentage of oysters at each gonadal development stage during sampling period

	Sampling period (from January to December 2000)														
	Jan.	Feb.	Mar.	Apr.	May	14-Jun.	28-Jun.	28-Jul.	14-Aug.	28-Aug.	Aug.	Sep.	Oct.	Nov.	Dec.
Gonadal stages															
Indifferent	100	75.6	8.9												51.1
Developing		24.4	77.4	60.6	14.4			13.8		2.2	4.9				
Ripe			13.7	39.4	78.9	31.9	29.9	47.6	48.9	33.3	25.7	2.4			
Spawning					6.7	68.1	52.6	31.9	39.0	53.9	38.2	33.5	26.3	11.3	
Spent							17.6	6.7	12.1	10.6	31.1	64.2	73.7	88.7	48.9

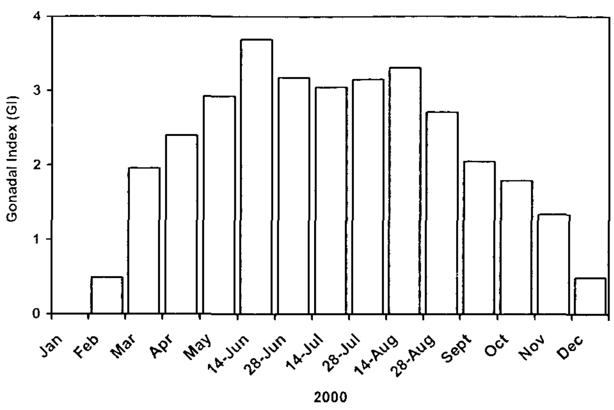

Fig. 7. Seasonal variation of gonadal index of oysters during year 2000.

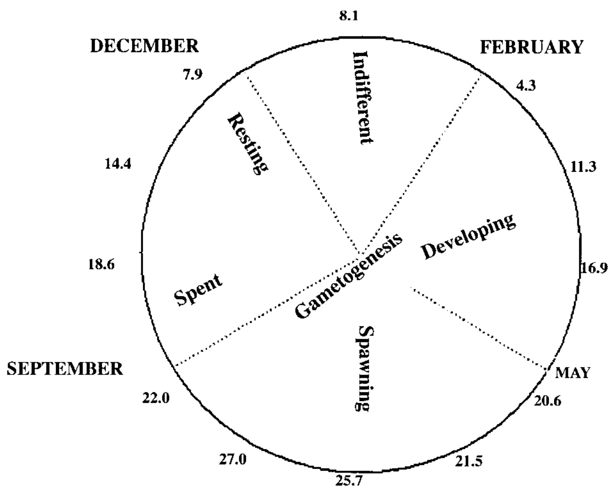

Fig. 8. Cyclic change in gametogenesis of oysters in Gosung Bay. The numbers represent monthly mean value water temperature (°C).

Relatively lower GI observed in late June indicated that first spawning of oysters in the bay occurred from mid June to late June. Increase in GI from mid July to mid August then dropped in late August suggested that another mass spawning of oyster population occurred from mid to late August. Table 1 also summarizes monthly changes in percent composition of various developmental stages. The data indicated that spawning was quite synchronous in the first spawning peak in June (64.3~68.1%) relative to those in August (53.3~58.3%). Sexually indifferent oysters were dominant during December and February. Fig. 8 also illustrates cyclic changes in gonadal development of oysters in the bay.

Discussion

Several studies have reported that reproductive process of marine bivalves is mainly governed by two major external parameters, temperature and food availability (Bayne 1976; Ruiz *et al.* 1992). In general, temperature accelerates or decelerates the rate of gamete production, while food availability determines quantity as well as quality of the reproductive output (Galtsoff 1964; Thompson *et al.* 1996). In temperate area, cyclic changes of gonadal development in bivalves often matches with seasonal changes in water temperate and phytoplankton blooms which provide sufficient food for both broodstock and larvae (See review of Galtsoff 1964). Studies previously conducted on gonadal development of *C. gigas* in small bays on the south coast of Korea also confirmed that seasonal changes in temperature and food supply in the water column are coincided with cyclic changes in reproductive condition of oysters (Bae and Han 1998; Park *et al.* 1998; Hyun *et al.* 2001; Kang *et al.* 2000).

Oysters in Gosung Bay begin oogenesis in March when water temperature reaches 10 to 12°C. The oocytes grow at a faster rate from April to early June as surface water temperature increase also at a faster rate. Histological data indicate that spawning occurs as early as May and it continues until the end of October while water temperature fluctuates from 20 to 27°C. During this period two major spawning peaks are observed, one in late June and the other in late August. Most oysters complete spawning from September to October and become reproductively inactive in November when water temperature drops below 17°C. Spawning of oysters often occurs at a certain water temperature (Galtsoff 1964; Kang *et al.* 2000; Thompson 1996). Mann (1979) suggested that a minimum temperature of 18 to 20°C is necessary to induce spawning in *C. gigas*. In contrast, Kennedy and Krantz (1982) reported that some oysters initiate spawning even when water temperature is below 20°C. In Gosung Bay, oysters started spawning when the surface water temperature reached 18 to 20 °C, indicating that 18 to 20°C is a minimum temperature to initiate spawning. Kang *et al.* (2000) also reported that oysters on the south coast of Korea may start spawning when water temperature rise to 18 to 20°C.

Continuous or multiple spawning of oysters has been reported from oysters distributed on the south coast of Korea. Bae and Han (1998) reported that oysters in Gosung Bay initiate spawning in late June when water temperature reached 23 to 25°C. Park *et al.* (1999) also investigated gametogenic cycle of *C. gigas* at two oyster culture grounds near Gosung Bay. In their study, suspended culture oysters in Tongyoung and Koje started spawning in early May and the spawning continued to October. The oysters in those areas also exhibited two distinct spawning peaks, one in late June to early July and the other in late August. Kang *et al.* (2000) observed two major spawning peaks of oyster populations at Osu and Hansan-Koje Bay in southern coast of Korea. Such a continuous spawning in *C. gigas* also has been reported in Japan. Spawning activity of oysters in Hiroshima continues from May to September with a peak in July-August (Arakawa, 1990). Arakawa (1990) reported that *C. gigas* in Hiroshima spawn when water temperatures are 21 to 30°C although an optimal temperature for spawning is considered to be 25°C. Kobayashi *et al.* (1997) also reported similar pattern of oyster spawning in Hinase water near Hiroshima; gonadal development occurs at water temperature above 23°C and spawning occurs at water temperature above 27~28°C.

According to Bayne (1976), *C. gigas* is classified as an opportunistic species whose gametogenesis occurs concurrently with the accumulation of energy reserves, resulting in spawning in the summer after spring phytoplankton bloom. For oysters, it is important to coordinate the time of spawning with the phytoplankton bloom so that the larvae and adults can access the abundant food supplies. Successful spawning and consequent larval development would maximize the probability of successful recruitment. During the course of study in Gosung Bay, the D-shaped oyster larvae first observed in late June and the presence of larvae in the water column continued until the early September (MOMAF 2001). However, the larval abundance in the water column was highest in mid July and the second highest in late August (MOMAF 2001). It is believed that the first peak of larval occurrence was a consequence of the first spawning peak occurred in late June.

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