A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Annual Gametogenesis of Breeding Oyster, *Ostrea circumpicta* (Bivalvia;Mollusca) Distributed around Munseom, Jeju, Korea

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GRADUATE SCHOOL

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Table of Contents

국문초록

이 연구는 조직학적 관찰을 통한 태생굴 *Ostrea circumpicta*의 연중 생식소 발달과 보육낭 내의 유생 출현시기 및 환경인자와의 관계 등을 관찰한 결과이다. 태생굴은 2003년 9월부터 2004년 9월 사이 제주도 문섬 직벽에서 월별로 채집되었다. 조사지역의 수온은 연중 14 ℃ 에서 24 ℃의 분포를, 염분은 24.2-32.0 ppt의 변화폭을 보였다. 조직학적 관찰은 Davidson 고정액에 고정된 시료를 탈수한 뒤, 파라핀 블록을 제작, 6 ㎛ 두께로 박절하였다. Harris' hematoxylin 과 Eosin Y로 염색된 시료는 광학현미경을 이용하여 생식소의 발달 정도와 난경의 크기를 월별로 측정하였다. 보육낭에 분포하는 유생은 주사전자현미경을 이용하여 관찰하였다. 생식소 발달단계는 휴지기, 초기 발달기, 후기 발달기, 성숙기, 산란기 와 소모기로 등, 6단계로 구분하였다. 태생굴의 생식소는 수온이 24.0 ℃인 8월부터 초기/후기 발달기가 관찰되었으며, 수온이 17.3 ℃ 인 5월에 방란이 관찰되었다. 초기발달 과정의 난경은 평균 39 ㎛이었으며, 산란 직전인 5월의 경우 105 ㎛으로 측정되었다. 태생굴의 생식소에서는 연중 미성숙한 알이 관찰되었으며, 유생이 보육낭에 출연한 시기는 6월과 7월로 담륜자와 피면자 유생이 광학현미경과 전자현미경 하에서 관찰되었다. 유생의 방출시기는 6월과 7월 이후

로 추정되었다. 태생굴의 연중생식소발달과정은 계절에 따른 수온의 변화와 춘계와 추계에 일어나는 플랑크톤의 대증식과 같은 문섬 주변의 해양환경과 밀접환 관계를 갖는 것으로 추정되었다

I. Introduction

Jeju Island locates on the southern end of Korean peninsula, 33.10° to 33.50° N. Lat., 126.10° to 127.0° E. Long., and is a typical volcanic island with well developed rocky shore. The ocean around Jeju Island is a complex region, where three major water masses from the north and northwest are mixed. The warm Tsushima Current branches from the northeastward flowing warm Kuroshio Current and washes the southern coastal area of the island, giving this area somewhat higher water temperatures. The prevailing winds from the Pacific Ocean are southeasterly in summer, and also help to raise the water temperature. Due to blending of warm and cool currents around the Island, coastal Jeju has high diversity of marine fauna and flora (Oh et al., 1994; Lee, 1999; Kang et al., 2005)

Munseom, an uninhabited island located off the southern coast of Jeju (33°13′25″N, 126°33′58″E), is well known for its high species diversity and richness of marine fauna and flora (Je et al., 2002). The island characterized by volcanic rocky intertidal and subtidal zones that are subjected to strong wave action. The rocky substrata are enriched with sessile fauna such as oysters, barnacles and numerous species of

anthozoans. In particular, *Ostrea circumpicta* heavily encrusts the subtidal cliff at depths between 3 and 6 m. Surface water temperatures and salinity in this area vary from 16-22ºC and from 32.2-34.4 ppt annually (Choa & Lee, 2000; Lee et al., 2000). Due to the influence of the warm Kuroshio Current, surface water temperatures during winter are several degrees higher than in northern Jeju.

Ostrea circumpicta is a brooding oyster widely distributed in southern Japan, China and Korea (Hirase, 1930; Bernard et al., 1993; Kwon et al., 1993). Despite its abundance, studies on reproductive biology of *O. circumpicta* are extremely rare, although a few studies have investigated the ecology and reproductive biology of other brooding oysters (Bae & Bae, 1972; Yang, 1999, 2001). Kang et al. (2004) for the first time reported microscopic features of larvae and gonads of *O. circumpicta* collected from Munseom Island. They reported that the female oysters brooded larvae at the early veliger stage (115-135 μm) in the infrabranchial chamber during early summer. Although their study reports the spawning and larval appearance of *O. circumpicta*, complete gametogenic process of *O. circumpicta* is yet to be illustrated.

For understanding annual gametogenesis of *O. circumpicta*, the

oysters have been collected from Munseom Island on a monthly basis. Objectives of this study includes, 1) to understand a complete annual gametogenesis of *O. circumpicta*, 2) to observe external structures of the larvae by scanning electron microscope, and 3) to understand the influence of environmental parameters on the reproduction.

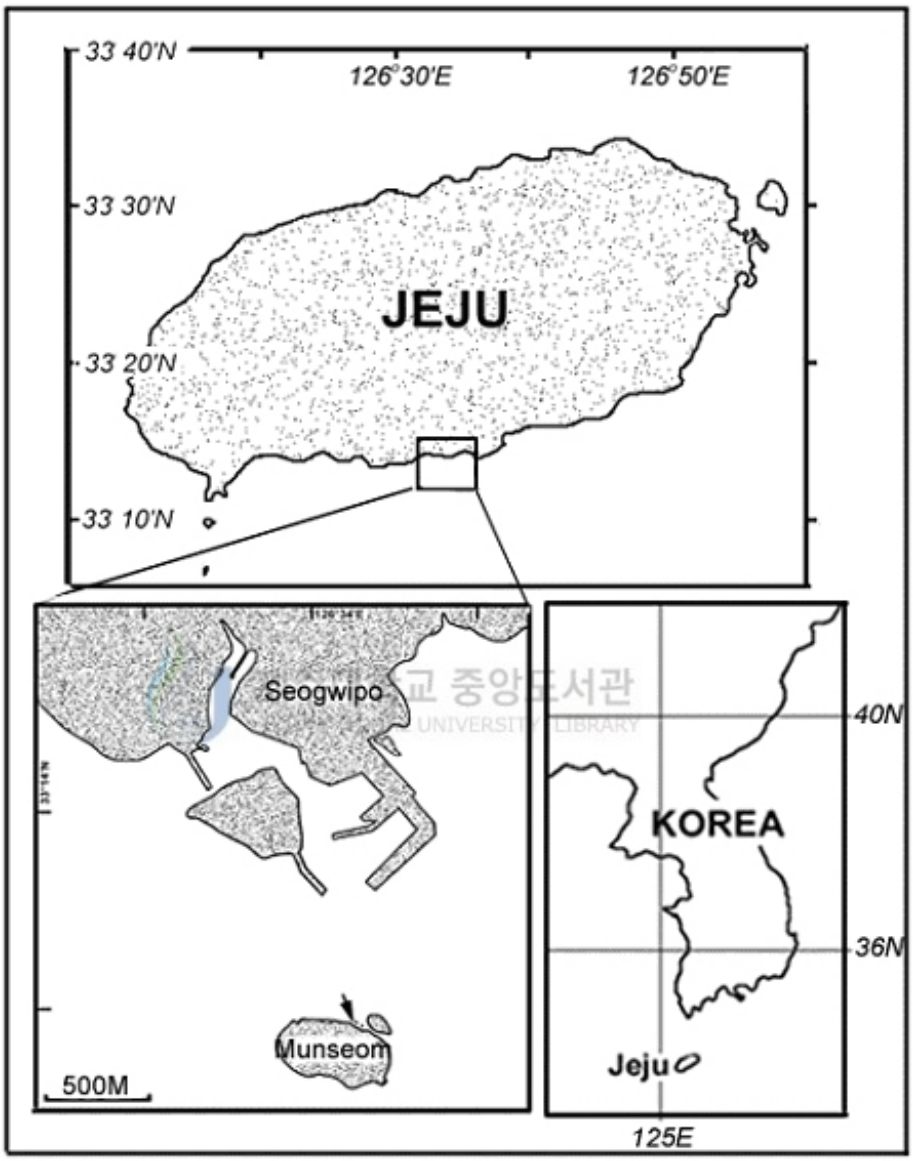

Figure 1. Map showing the location of the study site where *O. circumpicta* was collected for reproductive analysis from September 2003 to September 2004.

Figure 2. Anatomy of *O. circumpicta*. ▲, Gonad; ▲, Gill

II. Materials and Methods

1. Sampling efforts

O. circumpicta was collected by SCUBA diving at depth of 3-10 m from September 2003 to September 2004 along the coast of Munseom Island, Jeju, Korea (Fig. 1). Upon arriving at the laboratory, biometric data including shell length, shell height and tissue wet weight of each specimen were measured to the nearest 0.1 mm and to the nearest 10 mg by using Vernier calipers and electronic balance, respectively. Seasonal changes in the water temperature and salinity during sampling period were obtained from the National Oceanographic Research Institute of Korea (NORI).

2. Histology and image analysis

Figure 2 shows external feature of *O. circumpicta*. For histology, tissues including the gill and gonad were fixed in Davidson's solution for 48 hours and dehydrated in ethanol series. The tissues were then embedded in paraffin wax, sectioned to 6 μm and finally stained with Harris' hematoxylin and eosin Y. For determination of gonadal development, five follicles were randomly selected under a

light microscope then assigned one of the six developmental categories (inactive, early active, late active, ripe, spawning and spent) according to Man (1979) and Siddiqui & Ahmed (2002). Each gonadal stage was also assigned a numerical code described in the Table 1. Maturity index (MI) was then established by multiplying the number of oysters in each stage by the numerical code of the stage and dividing the sum of these products by the total number of sample (Kang et al., 2004).

$$\text{MI} = \sum_{i=0}^{n} \frac{numerical\ score \times the\ number\ of\ oyster}{total\ number\ of\ oyster}$$

where i=the numerical score of each oyster.

Size of the egg as a diameter was also determined from the randomly selected microscopic fields using an image analyzing software, to follow the seasonal changes in reproductive conditions.

3. Scanning Electron Microscopy

The oyster larvae in the gill chamber were examined by histology and scanning electron microscope (SEM). From the histological slides, oysters exhibiting the larvae in their gill chambers

were selected for the analysis. Morphology of the larvae was then observed under 400 to 1,000 magnification to illustrate internal structure such as level of digestive system development. For SEM, the larvae were collected from the infrabranchial chamber of female oyster during June 2004, fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3 at 4°C), then washed for 30 min in the same buffer. Fixed larvae were finally dehydrated in an ethanol series. The samples were freeze-dried using JED-310 Freeze-dryer and coated with platinum. The coated larvae were finally examined under JEM 1200EX II SEM. Level of shell development during larval period was categorized according to the criteria described by Marin and Luquet (2004).

Table 1. Description of gonad development of *O. circumpicta* (modified from Mann, 1979; Siddiqui & Ahmed, 2002).

Developmental Stages	Numerical Score	Description
Inactive	0	No traces of sexual activity present.
Early active	2	Male: many follicles; spermatogonia and spermatocytes numerous, no sprermatozoa. Female: oogonia arising from stem cells along the follicle; no free oocytes.
Late active	3	Male: some sperm balls, characterized by the presence of tails of spermatozoa, present in the middle of the follicles; periphery of follicle occupied by spermatids and few spermatocytes. Female: both free and attached oocytes present with distinct nuclei that stain lighter than cytoplasm.
Ripe	4	Male: sperm balls fill follicles. Female: predominantly free oocytes with distinct nucleus and nucleolus.
Spawning	5	Male: follicles partially empty, few sperm balls present Female: follicles partially empty, few free oocytes present
Spent	1	Male: half of more than half follicles appear empty of broken or both, sperm balls present in ducts; some phagocytes present. Female: half of more than half follicles walls appear broken and follicles empty; ripe ova fill genital canal; some phagocytes present.

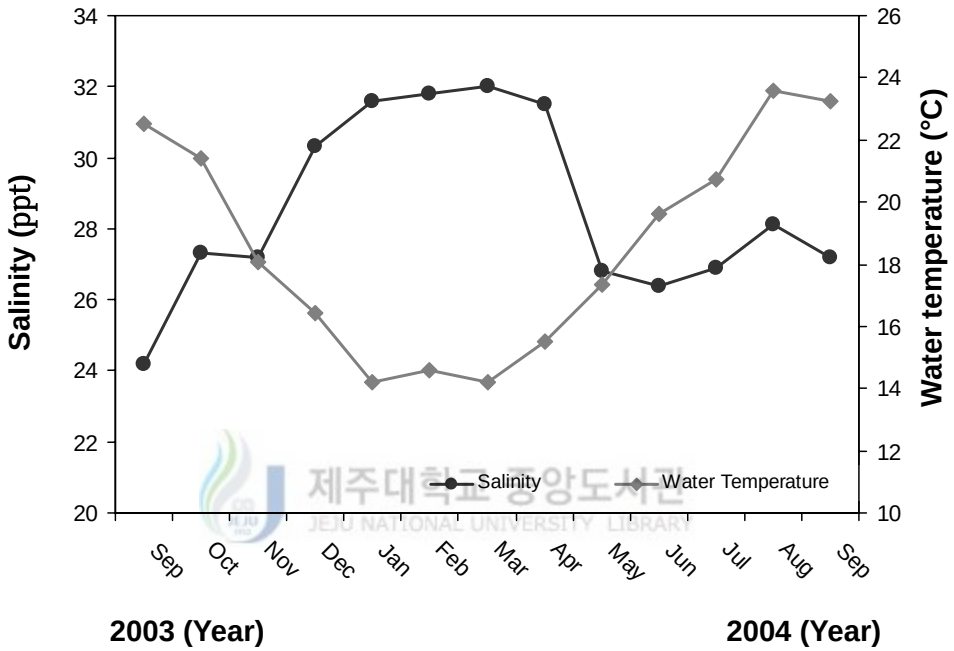

Figure 3. Seasonal changes in surface water salinity and temperature from September 2003 to September 2004 in Munseom Island (provided from NORI).

III. Results

1. Water temperature and salinity

Seasonal variations of the surface water temperature and salinity are plotted in Fig. 3. The water temperature varied from 14°C in January 2004 to 25°C in July 2004, and the salinity ranged from 24.2 ppt in September 2003 to 32 ppt in March 2004.

2. Description of gonadal development

Figure 4 shows morphological features of the gonad development of female oysters. Oogonia and oocytes proliferation along the germinal ephithelium is clearly seen during early and late active stages (Fig. 4A and B). During the late active stage, follicles are greatly expanded and coalesced, and the cytoplasm becomes less basophilic than the previous stage (Fig. 4B). Fully ripe oocytes containing large nucleus with nucleolus are also visible during late active stage (Fig. 4C). In this stage follicles are greatly expanded. Free oocytes are visible in the lumina of follicles during spawning stage, and the lumina become empty, resulting in decreasing the follicle area in the gonad (Fig. 4D). A few numbers of relict oocytes were

observed in the follicles in spent stage (Fig. 4E).

Different gonad development phases of the male oysters are listed in Figures 5. It is noticeable that male gonad often exhibited different stages of gonad development within a same follicle. In early active stage, spermatogonia and spermatocytes could be found in a follicle (Fig. 5A). In late active stage, spermatocytes developed into spermatid and several groups of spermatid are distributed in the middle of the follicle, while a number of spermatocyte lumps are arranged along the spermatogenic follicles (Fig. 5B). In ripe stage, the follicles containing a number of lumps of spermatid and spermatozoa are greatly expanded (Fig. 5C). In spawning stage, a large number of spermatozoa in the spermatogenic follicle are discharged, and the lumen become empty (Fig. 5D). In this phase, groups of spermatid are located along the follicle walls, while spermatozoa appear in the middle of the follicle. In spent stage, most follicles become empty and the remaining spermatozoa and spermatids become degenerated (Fig. 5E).

Figure 6 shows the undifferentiated stage. This stage is characterized by thinning and collapsing of the follicle wall and it is impossible to distinguish the sex due to non-existence of the gametes.

3. Annual reproductive cycle of O. circumpicta

Table 2 summarizes monthly changes in frequency distribution of the developmental stages. Analysis of the histological preparation indicated that gonad development of the male and female is rather synchronous all year round and is associated with the seasonal surface temperatures and salinity changes (Fig. 3). In September 2003, oysters in early active stage were initially observed and the early active oyster occurred until March 2004. Late active oysters were first observed in November 2003 and continued to occur in April 2004. In March 2004, some ripe oysters appeared and occurrence of the ripe oysters peaked in April, indicating that they are ready for spawn during this period. The surface water temperature increased from 14 (in November) 17°C in April. Spawning initiated in May and continued until July 2004 when water temperature increased from 17°C to over 20°C while salinity dropped from 32 to 27 ppt. Spent oysters occurred mainly in July and August 2004 when the surface temperature showed its annual maximum. Sexually undifferentiated oysters appeared in late summer during August and September of 2003 and 2004.

Seasonal change in gonad development was also inferred from

the monthly changes in the egg size (Fig. 7). In early active stage, young oocytes averaging 60 μm in diameter were dominant during September and October of 2003. Fully ripe eggs initially appeared in March and peaked in May were recorded to 104 μm in diameter. The proportion of ripe eggs in the follicle decreased in June, as a consequence of discharging the egg through spawning. Figure 8 plots seasonal changes in MI. MI increased slowly from September 2003 to January 2004. MI increased at a faster rate from winter to early spring in 2004 and reached the maximum in May when the egg diameter was also in its annual maximum. During June and August, MI dropped when most oysters were in spent stage.

In conclusion, cyclic changes in gonad development of *O. circumpicta* in Munseom Island can be summarized as follows; 1) initiation of oogenesis and spermatogenesis in early fall (August to September), 2) growth of the germ cells from late fall to early Spring (November to March), 3) ripe and ready for spawning in late Spring to early summer (April to May), 4) spawning in summer (May to July) and 5) spent and inactive in late summer (from July to August, Fig. 9).

4. The brooding larvae

Trochophore and veliger larvae of *O. circumpicta* could be identified in the infrabranchial chambers of the female during June and July (Fig. 10). Rudiments of shell were overlaid dorso-lateral edge of the late trochophore (Fig. 11). Ectoderms embayed to constitute foregut. Frontal section of veliger showed well-developed digestive system (Fig. 12). The veliger also displays locomotive cilia on the surface of velum. Esophagus can also be identifiable at the interior part of velum. Intestine, stomach and digestive gland were observed in the larval digestive system. Retractor muscle is also identifiable near the shell rudiment. Histological section of the larvae at the infrabranchial chambers suggested that the veliger larvae uptake food particles from waters supplied to the chamber by the mother oyster and digest.

Figure 13 shows external features of the trochophore and veliger larvae observed by SEM. The larvae in the infrabranchial chambers could be found only in June and July and it accounted for 50 % and 29 % of the total oysters analyzed respectively (Table 3). Sizes of the trochophore ranged from 111 μm to 130 μm, and veliger ranged from 135 μm to 205 μm in shell length respectively. It was

believed that the veliger larvae observed in this study is prodissoconch I since the larvae have straight hinge and absence of the rim of prodissoconch II (Fig. 13D). No veliger or trochophore larvae were found in the infrabranchial chambers of oysters collected in August, suggesting that the larvae were released to the water column sometime between July and August. Combining with the gonad development, it is believed that *O. circumpicta* brood their larvae for up to 2 months after spawning (June and July).

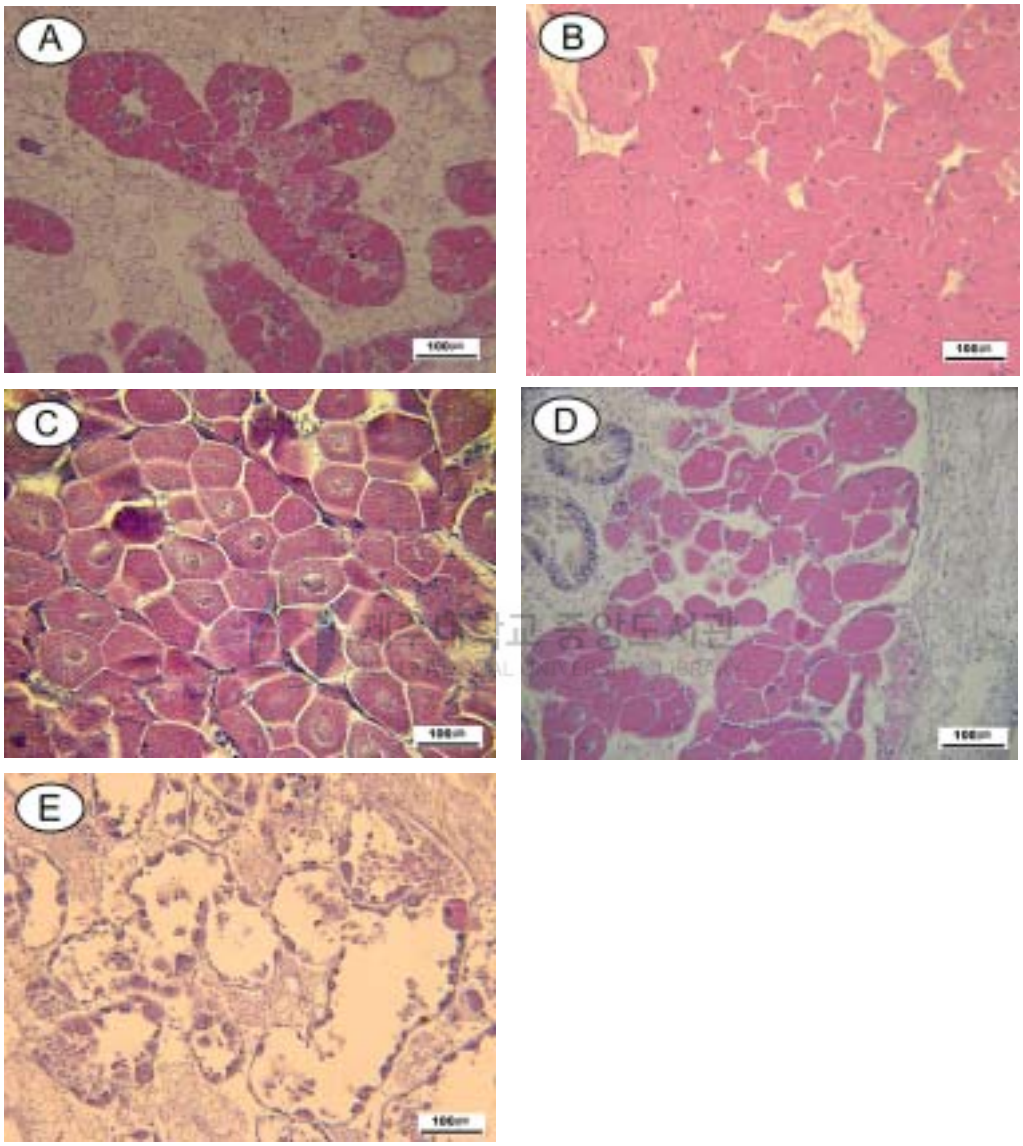

Figure 4. Photomicrographics of reproductive stages of female *O. circumpicta*. A, early active; B, late active; C, ripe; D, spawning; E, spent.

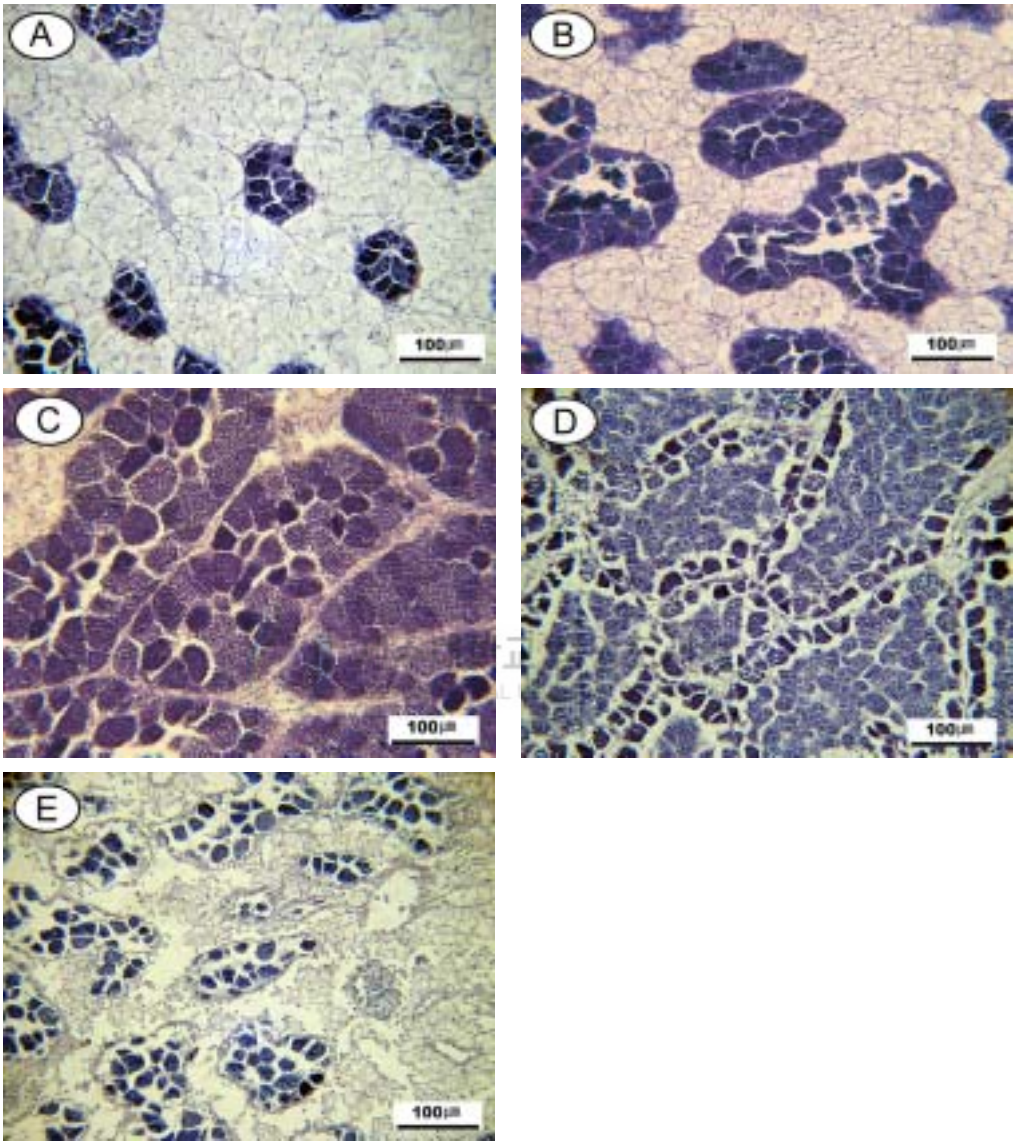

Figure 5. Photomicrographics of reproductive stages of male *O. circumpicta*. A, early active; B, late active; C, ripe; D, spawning; E, spent.

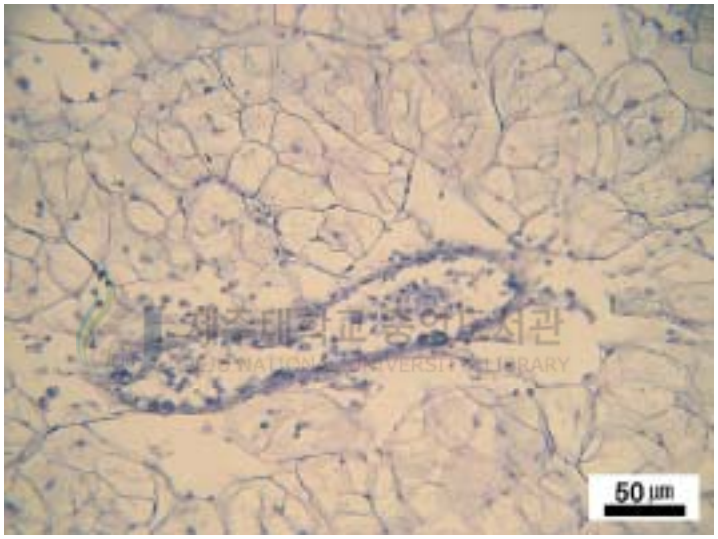

Figure 6. Undifferentiated stage of *O. circumpicta*.

Table 2. Frequency (%) of gonadal maturation of *O. circumpicta*.

Period	Inactive	early active	late active	ripe	spawning	spent
2003 Sep.	17.6	82.4	0.0	0.0	0.0	0.0
Oct.	0.0	100.0	0.0	0.0	0.0	0.0
Nov.	0.0	46.2	46.2	0.0	0.0	7.7
Dec.	0.0	65.3	34.7	0.0	0.0	0.0
2004 Jan.	0.0	85.5	14.5	0.0	0.0	0.0
Feb.	0.0	30.0	70.0	0.0	0.0	0.0
Mar.	0.0	25.3	68.0	6.7	0.0	0.0
Apr.	0.0	0.0	12.0	88.0	0.0	0.0
May	0.0	0.0	0.0	30.0	65.0	5.0
Jun.	0.0	2.9	0.0	0.0	61.4	35.7
Jul.	0.0	0.0	0.0	0.0	40.0	60.0
Aug.	11.6	31.6	0.0	0.0	5.3	51.6
Sep.	13.8	86.2	0.0	0.0	0.0	0.0

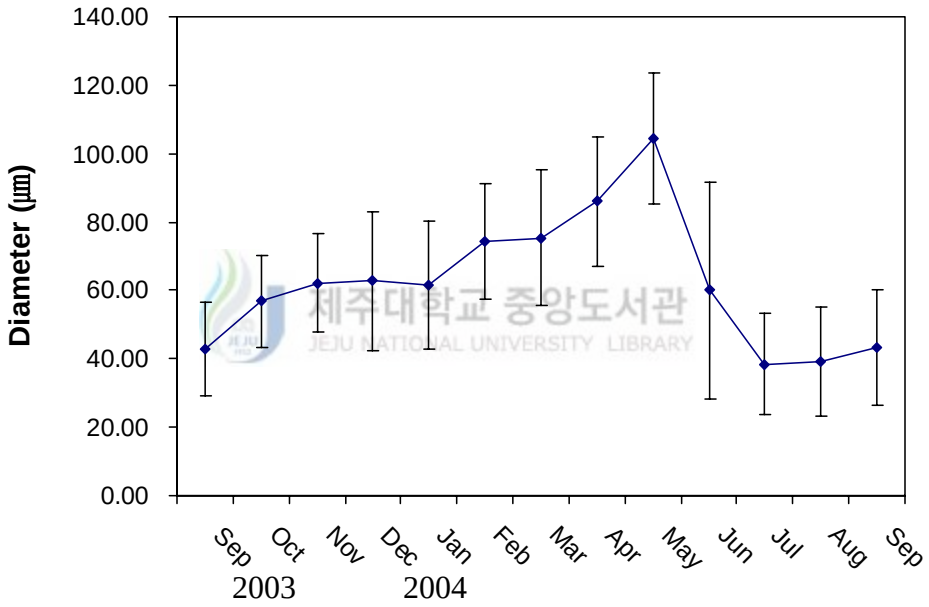

Figure 7. Monthly mean changes in egg diameters of *O. circumpicta.*

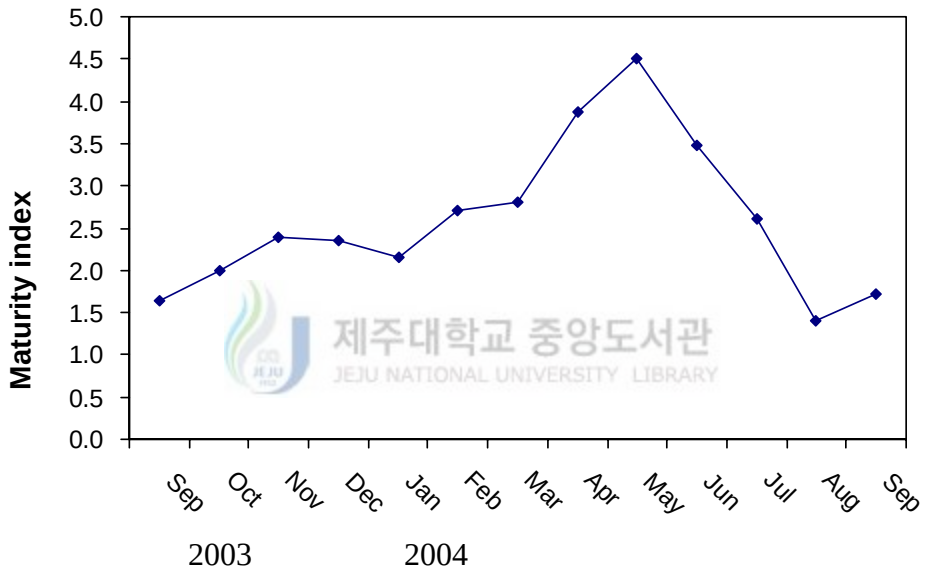

Figure 8. Monthly mean changes in gonadal maturity index of *O. circumpicta.*

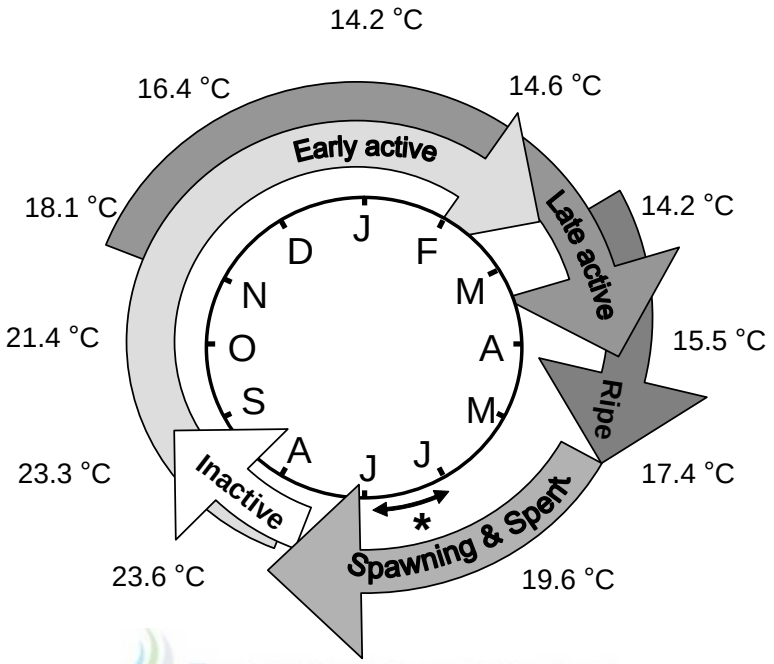

Figure 9. Cyclic change in gametogenesis of oysters distributed around Munseom. *=Periods of larvae were observed in infrabranchial chamber.

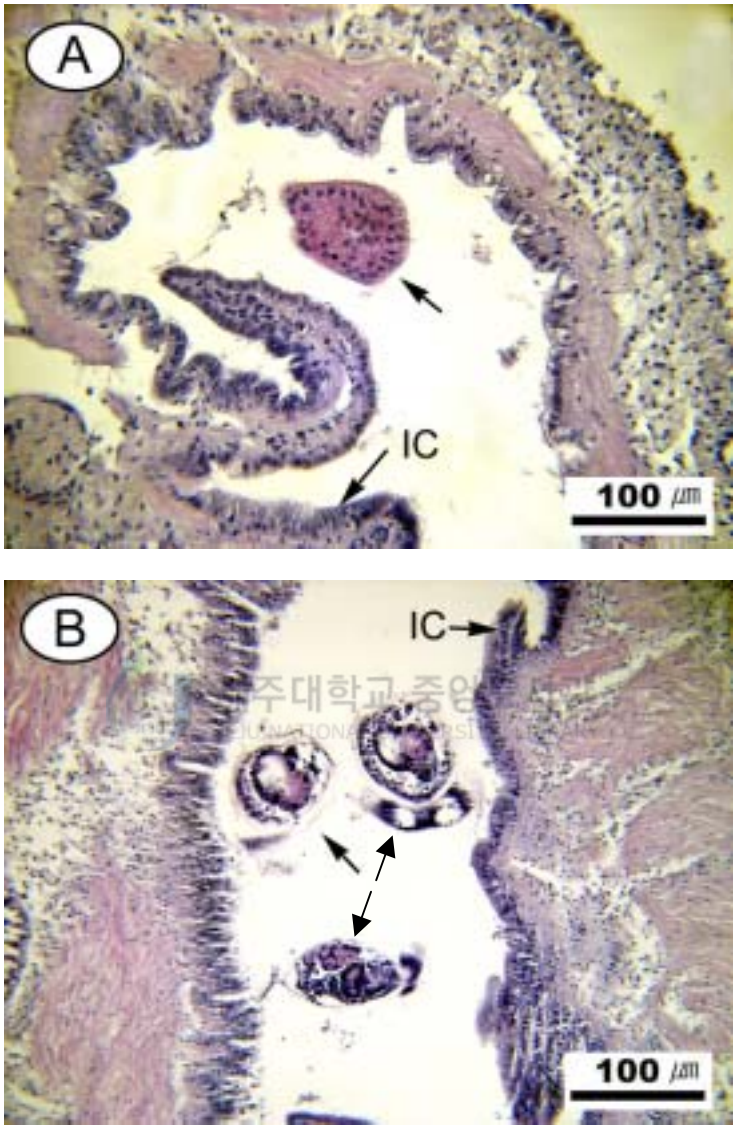

Figure 10. Light microscopic images of *O. circumpicta* larvae in infrabranchial chamber. A, trochophore; B, veliger, IC, infrabranchial chamber.

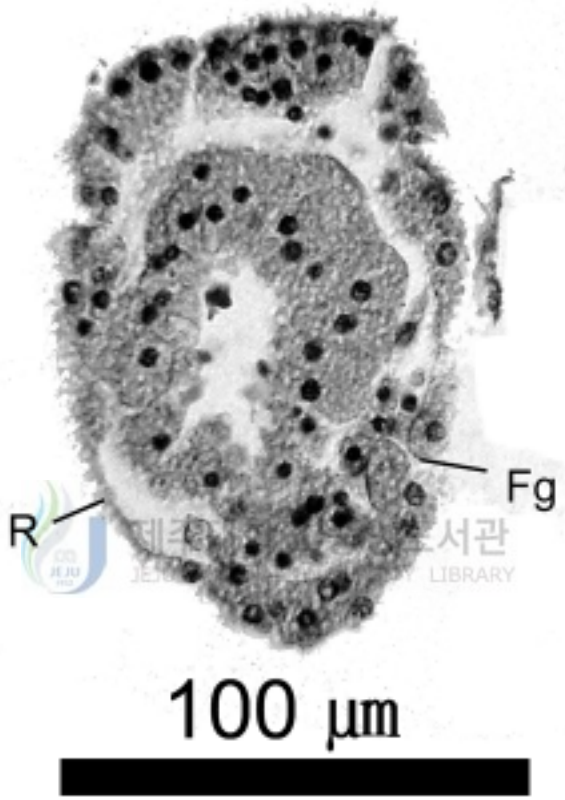

Figure 11. Trochophore of *O. circumpicta* stained with Hemotoxylin an Eosin. Rudiments of shell are overlaid circumference of trochophore. Fg, foregut; R, rudiment of shell.

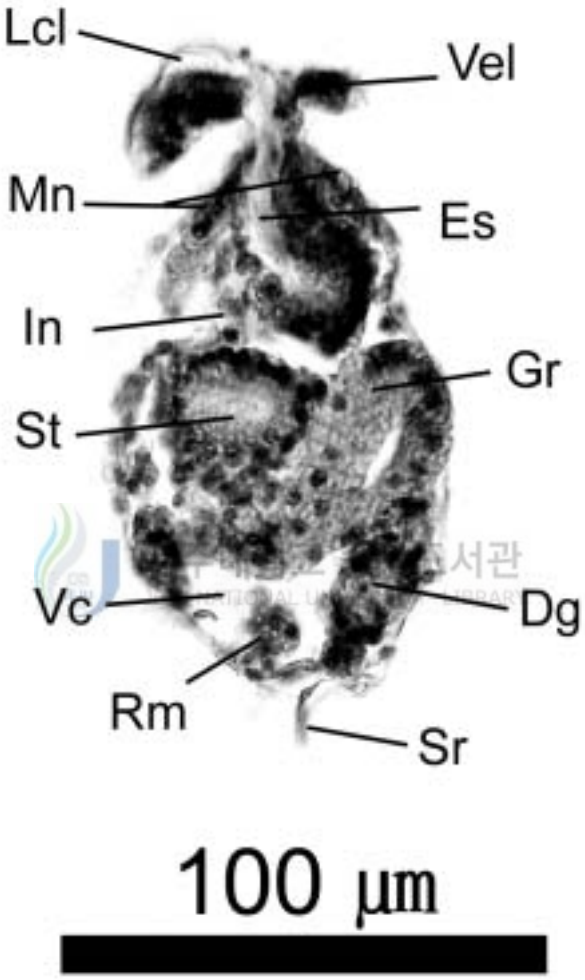

Figure 12. Frontal section of larval *O. circumpicta* stained with Hemotoxylin an Eosin. Dg, digestive gland; Es, esophagus; Gr, gill rudiment; In, intestine; Lcl, locomotory cilia; Mn, Mantle; Rm, retractor muscle; Sr, shell rudiment; St, stomach; Vel, velum; Vc, visceral cavity.

Table 3. Percentage of oysters bearing larvae (trochophore and veliger) in the infrabranchial chamber.

Period	Percentage
June 2003	50
July 2003	29

Table 4. Sizes of trochophore and veliger larvae of *O. circumpicta*. SD=standard deviation

Stage	Max. (μm)	Min. (μm)	Mean (μm) ± SD	N
Trochophore	130	111	121±6	6
Veliger	205	136	175±16	66

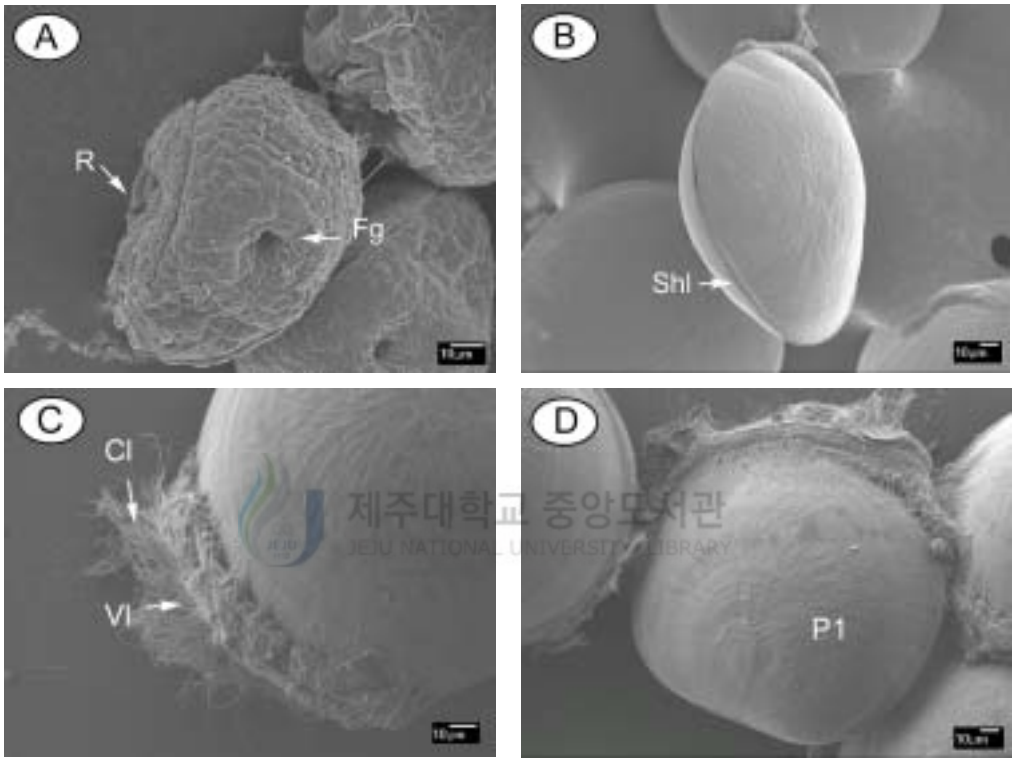

Figure 13. Larval development of *O. circumpicta*. A, Ventral view of late trochophore; B, Posterior view of veliger; C, Ventral view of veliger; D, Lateral view of veliger. Cl, cilium; Fg, foregut; Ic, infrabranchial chamber; P1, prodissoconch I; R, rudiment of shell; Shl, straight hinge line; Vl, velum.

IV. Discussion

Oysters are either larviparous or oviparous: oysters in the Genus *Ostrea* are larviparous while oysters in other Genus such as *Crassostrea* or *Saccostrea* are oviparous. The larviparous brooding oyster produces relatively small number of large oocytes and localizes fertilization at the brood chambers. In contrast, oviparous oysters broadcast relative large number of small eggs into the water column (see reviews by Buroker, 1985 and Ó. Foighil & Taylor, 2000). The brooding oysters in the Genus *Ostrea* are also oceanic and prefer to distribute in stable saline environment while other broadcasting oysters prefers to occur in estuarine habitat. Up to now, two brooding oyster, *O. denselamellosa* and *O. circumpicta,* have been reported in Korean water (Yang et al., 1999; Kang et al. 2004).

The present study first attempts to investigate an annual gametogenic cycle of *O. circumpicta* distributed in Munseom Island. The maturation index (MI) indicated that the gametogenesis initiated during early fall, spawning occurred in late spring through early summer and terminated in the middle summer. The annual reproductive cycle of *O. circumpicta* in Munseom Island is somewhat

comparable to those of *C. gigas* occurring along the south coast of Korea. According to Kang et al. (2003), *C. gigas* in Goseong Bay (190 km north east of Munseom) initiated gametogenesis as early as late February and spawned mostly in late June to early July. The early active gametes observed during September 2003 and March 2004 suggests that relatively high water temperature along Munseom Island enables *O. circumpicta* to continue gametogenesis even in winter period. Indeed, the surface water temperature in Munseom Island during winter is several degrees higher than that in Gosung Bay due to lower latitude and effects of the warm Kuroshio currents (NFRDI, 2005).

Water temperature is one of the crucial factors governing gonad maturation of marine bivalves (Giese, 1959). For example, spawning and gametogenesis of oysters begins earlier and last longer in warmer areas while the duration of spawning period is rather short and gametogenic process is slower and take longer period in colder waters (Thompson et al., 1996). Water temperature also varies with latitude and reproductive process of oyster often shows a latitudinal gradient. According to Cranfield and Michael (1989), breeding period of *Tiostrea*, a brooding oyster varies with latitude. Westerskov (1980) also

reported that water temperature regulates triggering the gametogenesis of *Tiostrea*. Walker and Power (2001) reported annual gametogenesis of the Chilean brooding oyster, *O. chilensis*. They observed that the oyster spawns all year round in warmer water, while a population of oyster in colder areas exhibits a single spawning peak during an annual reproductive cycle.

In addition, food availability appears to determine the duration of gametogenic process including spawning and subsequent larval development (Holland, 1973; Bayne et al., 1976; Hofman, 1992; Pazos et al., 1997). Level of food in the water column often accelerates or retards growth and gonad maturation of oysters (Wilson et al., 1996; Labarta et al., 1999). Several studies carried out adjacent to Munseom Island reported that available food in the water column for suspension feeders along Munseom Island is somewhat lower than south or west coast of Korea (NFRDI; Lee et al., 1989; Yoon, 1993). Phytoplankton blooming around Munseom Island often occurs in late spring or in late summer or autumn (Choa et al., 2000; Lee et al., 2000; Shynn & Lee, 2002). The single spawning pulse of *O. circumpicta* observed in this study area may be associated with the low level of food supply, although further study needs to be carried out to verify this hypothesis.

SEM and histology show that trochophore and veliger larvae are incubated in the infrabranchial chamber. Female brooding oyster discharges mature oocytes via gonopores into the exhalent chambers of the mantle cavity. The larvae are forced into inhalant chamber by highly characteristic adductor muscle spawning contraction, called trans-ctenidial ovulation (Ó Foighil & Taylor, 2000). Chaparro (1993) observed larvae in brood sac using endoscope connected to a video monitor. According to his study, the brooded larvae of *O. chilensis* showed a circulating pattern within the mantle cavity; water currents ejected larvae from the swarm and transport them between the demibranchs to the posterior region. Larval *O. circumpicta* was observed from June to July 2004 in this study, and their shell lengths of trochophore and veliger larvae averaged 121 and 175 μm, respectively. Brooding period and shell size of larvae vary depending on species and water temperature; larval size varies inversely with water temperature and larval period is somewhat longer in colder water (Jeffs et al., 1997; Castaños et al., 2005). Compared to the larval period reported by other studies, larval period of *O. circumpicta* is consider to be shorter and it is closely associate with water temperature and food condition in Munseom Island, although further study of those two environmental

parameters on oyster reproduction needs to be carried out.

VI. Summery

This study investigated the annual reproductive cycle and larval period of the brooding oyster *O. circumpicta* in relation to environmental conditions. Oysters were collected from Munseom Island, Seogwipo, Korea from September 2003 to September 2004. Surface water temperature and salinity varied from 14 to 24°C and from 24.2 to 32. ppt, respectively during the study period. Histology and SEM were applied to investigate seasonal variation in gametogenesis and larvae. Gonad development of the male and female was synchronous. Early-late active oysters were observed from September 2003 to April 2004. Ripe gametes appeared when water temperature was elevated from 14 to 17°C. Fully mature eggs averaged 104 μm in diameter. Spawning initiated in May and lasted until July 2004 when water temperature increased from 17°C to above 20°C. Larvae of *O. circumpicta* in the infrabranchial chambers were observed under light microscope and SEM in June and July. Finally, this study indicates that gametogenesis of *O. circumpicta* distributed in Munseom, Jeju, Korea is associated with seasonal changes in water temperature and plankton blooming in spring and autumn.

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