

Gonadal Development, First Sexual Maturity and Sex Ratio of the Sun and Moon Scallop *Amusium japonicum japonicum* on the Coastal Waters of Jeju-do, Korea

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한국 제주도산 해가리비 *Amusium japonicum japonicum*의 생식소 발달, 군성숙도 및 성비

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ABSTRACT : Reproductive cycle, gonadosomatic index(GSI), egg diameter composition, first sexual maturity, sexually matured length(50% of first sexual maturity), and sex ratio of *Amusium japonicum japonicum*, were investigated by histological observations and morphometric data. Samples were collected monthly from the subtidal zone of Sogwipo, Jeju-do, Korea, for two years. The sun and moon scallop *Amusium japonicum japonicum* is dioecious. Monthly variation in the GSI showed similar patterns with the reproductive cycle. Ripe oocytes were about 70~90 μm in diameter and had thick egg membranes. The spawning period was from November to January, and the main spawning occurred between November and December when the seawater temperature was relatively low. From monthly changes in egg diameter composition, the spawning period was once a year, although the number of spawning frequencies is assumed to occur more than twice during the spawning season. The reproductive cycle of this species could be divided into five successive stages: early active stage(April to June), late active stage(June to September), ripe stage(October to November), spawning stage(November to January), and spent/resting stage(February to April). First sexual maturities in female and male scallops ranging from 85.1 to 90.0mm in shell length were over 50% and they were 100% for scallops over 90.0mm in shell length. In this population, sexually matured shell lengths(50% of rate of group maturity) in females and males were 86.96 and 86.59mm, respectively. The female to male sex ratio among individuals over 85.1mm in shell length was not significantly different from 1:1($\chi^2=0.18$, $p>0.05$). No evidence of hermaphrodite was found in histological sections of any scallop examined.

Key words : *Amusium japonicum japonicum*, Reproductive cycle, First sexual maturity, Sex ratio.

요 약 : 한국 제주도산 해가리비(*Amusium japonicum japonicum*)를 대상으로 생식주기, 생식소중량지수(GSI), 난경조성 변화, 군성숙도, 성적 성숙체장(50% 군성숙도) 및 성비를 조직학적 관찰 및 생체 측정에 의해 조사하였다. 해가리비는 자웅이체이다. 생식소 중량지수(GSI)의 월별 변화는 생식주기와 유사한 양상을 보였다. 완숙 난모세포들은 직경이 약 70~90 μm 정도이며, 두터운 난막을 가지는 특징을 가지고 있다. 산란기는 11월부터 1월 사이이며, 주 산란은 해수 수온이 낮은 11~12월 사이에 일어난다. 월별 난경 조성 변화를 보면, 산란기 중 산란빈도 수는 2회 이상으로 추정되지만, 산란기 간은 1년에 한번이다. 본 종의 생식주기는 초기 활성기(4~6월), 후기 활성기(6~9월), 완숙기(10~11월), 산란기(11~1월), 그리고 퇴화 및 휴지기(2~4월)의 연속적인 5단계로 나눌 수 있다. 각장 85.1~90.0mm인 암·수의 해가리비 군 성숙도는 50%이었고, 각장 90.0mm 이상인 개체들은 100%이었다. 이 개체군에서 성적으로 성숙한 암·수 개체들의 각장(군 성숙도 50%) 크기는 각각 86.96mm와 86.59mm이었다. 각장 85.1mm 이상인 암·수 개체들의 성비는 1:1로 유의한 차이를 보이지 않았다($\chi^2=0.18$, $p>0.05$). 조사된 해가리비의 조직학적 절편에서 자웅동체인 개체는 발견되지 않았다.

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INTRODUCTION

is one of commercially important edible scallops in East Asian countries, including Korea, China, and Japan(Yoo, 1976; Kwon *et al.*, 1993). This species is mainly found in the subtidal sandy areas up to a depth of 20~40m along the coast of Jejudo, a major southernmost island in Korea. As a consequence of reckless over-harvesting, the standing stock of this scallop has dramatically reduced in recent years, and it has been denoted as a target organism and fisheries resource that should be managed using a more reasonable fishing regime. For propagation and management, it is important to understand its population characteristics with regard to the reproductive cycle and first sexual maturity.

In other countries several studies have examined aspects of the reproduction in *Amusium* spp., including reproductive biology required to be more specific(Dredge, 1981), larval development(Rose *et al.*, 1988), artificial spawning and larval rearing(Beldam & Del Norte, 1988; Chaitanawisuti & Menasveta, 1992), large scale hatchery production techniques(Cropp, 1993), on aspect of ecology including growth and recruitment(Williamas & Dredge 1981), daily growth ring(Joll, 1988), and swimming(Morton, 1980).

Regarding *A. japonicum japonicum*, there have been several studies on reproduction, such as reproductive ecology and early development(Ha, 1994), and on aquaculture, including age and growth(Son *et al.*, 1996) and spat production(Son *et al.*, 1998). Although there have been several studies on reproductive ecology, little information is available on the reproductive cycle, first sexual maturity, and the sex ratio of this species.

Understanding reproductive cycle and spawning period and frequency distributions of egg diameters of *A. japonicum japonicum* will provide necessary information needed for the determination of age and recruitment period and for the estimation of number of spawning frequencies during annual spawning period. Additional information on first sexual maturity(rate of group maturity) and the sex ratio of this species would be very useful for propagation, aquaculture, and resource management. In particular, understanding first sexual maturity can determine a prohibitory measure for adequate natural resource management by

individual sizes of biological minimum size(50 % of first sexual maturity). The present study aimed to understand the reproductive cycle and spawning period, first sexual maturity, and sex ratio of *A. japonicum japonicum* in order to provide basic information associated with the propagation and management of a natural living resource.

MATERIALS AND METHODS

1. Sampling

Specimens of the sun and moon scallop, *Amusium japonicum japonicum* were collected by dragging in the subtidal zone of Sogwipo, Jejudo, Korea, for two years from June 1997 to May 1999(Fig. 1). A total of 523 scallops ranging from 20.2 to 98.1mm in shell length were collected for the study. Scallops were transported alive to the laboratory and each part of the body was measured. Unpublished seawater temperature data measured at 10:00 a.m. in Sogwipo were used for this study.

2. Gonadosomatic Index(GSI)

To estimate the spawning period, monthly changes in the mean gonadosomatic index(GSI) were calculated for two years by the following formula:

$$\text{GSI} = \text{Gonad weight(g)} \times 100 / \text{Total weight(g)}$$

3. Frequency Distributions of Egg Diameters

In order to investigate monthly relative frequency distributions of egg size, in each month about a thousand eggs were centrally cut, nucleic diameter was measured, and then graphed by the frequency curve method of Pearse (1965).

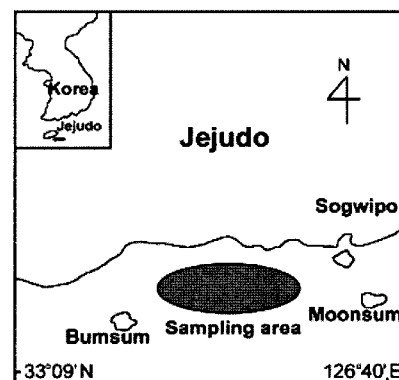


Fig. 1. Map showing the sampling area.

4. First Sexual Maturity and Sexually Mature Length

The percentages of first sexual maturity were investigated histologically in order to determine shell lengths of specimens that participated in reproduction from April to October. First sexual maturity was estimated by the ratio of mature individual to number of females. To calculate sexually mature length, after fitting the rate of group maturity first sexual maturity to an exponential equation, the size equivalent to 50% of first sexual maturity was estimated to be the sexually mature length.

5. Sex Ratio

Sex ratios of the surf scallops were estimated by collecting monthly samples of 461 sexually mature individuals (shell length 85mm in diameter) from January to December, 1996. Scallops were sexed by microscopic examination of histological slides. A Chi square, goodness-of-fit test was used to test the hypothesis of equal representation of females and males.

RESULTS

1. Position and Morphology of the Gonads

The sun and moon scallop, *A. japonicum japonicum* is dioecious composed of well-defined male and female individuals. Gonads are conical or crescent in shape and they are separated from the digestive diverticula and adductor muscle. As gonads are getting mature, they encircle the adductor muscle near digestive diverticula (Fig. 2). Ovaries are composed of a number of oogenic follicles and testes consisted of a number of the acini. When they were matured, the external color of ovary became pink and that of testis milky white or light yellow. Therefore, their sexes could be easily distinguishable by visual observations and dissection. At this time, if they are slightly scratched with a razor, ripe eggs and spermatozoa readily flow out. But after spawning, the gonads are degenerated, and then they become difficult to distinguish their sexes by external colour or dissection.

2. Monthly Changes in the Gonadosomatic Index (GSI) and Sea Water Temperatures

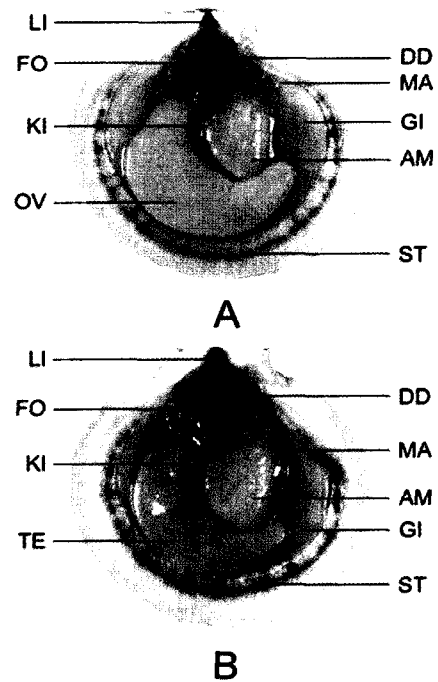


Fig. 2. Anatomy of the sun and moon scallop *Amusium japonicum japonicum*, removed from its shell. A, female; B, male. Abbreviations: AM, adductor muscle; DD, digestive diverticulum; FO, foot ; GI, gill; KI, kidney; LI, ligament ; MA, mantle; OV, ovary; ST, sensory tentacle; TE, testis.

Monthly GSI changes in females were shown in Fig. 3. In 1997, the GSI in females and males gradually began to increase in July and reached the maximum (female mean 22.17; male mean 14.98) in November when seawater temperature gradually decreased. Then, the GSI rapidly decreased from December to January when relatively low

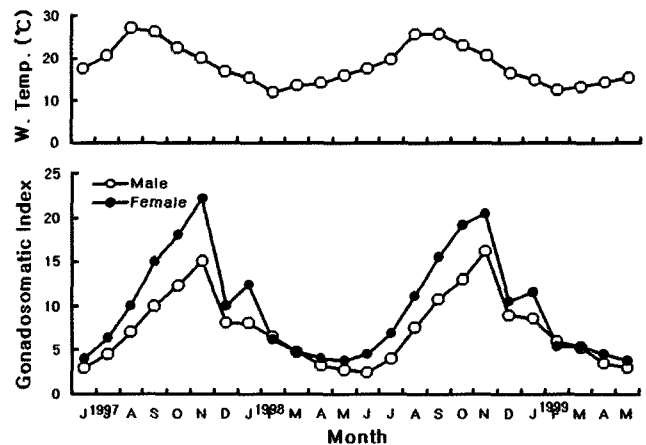


Fig. 3. Monthly variation of the gonadosomatic index (GSI) and the mean water temperatures, for two years from June 1997 to May 1999.

water temperatures were maintained and spawning occurred continuously. Thereafter, the mean value temporarily reached the minimum in May(3.44) in female and June(2.45) in male scallops. Monthly changes in the GSI in females and males in 1997 showed a similar pattern to those in 1998.

3. Monthly Changes in Relative Frequency Distributions of the Egg Diameters

To estimate the spawning period and the number of spawning frequencies, relative frequency distributions of the ovarian egg diameters were measured using histological preparations during the breeding season.

Monthly changes in relative frequency distributions of the ovarian egg diameters can be seen in sectioned ovaries from June to May(Fig. 4). percentages of oocytes measuring more than $50\mu\text{m}$ in diameter increased between June and July 1997. Between August and September, mature and ripe oocytes measuring approximately $70\mu\text{m}$ in diameter began to increase.

In October the number of ripe oocytes with larger diameter began to increase in number, and relative frequencies of ripe eggs ranging from $80\mu\text{m}$ to $90\mu\text{m}$ in diameter were over 60%. In November, when the spawning

period began, the number of ripe oocytes measuring about $70\sim 90\mu\text{m}$ began to decrease. However, at least two or three groups of remaining developing eggs grew to larger egg diameters step by step. Spawning of ripe oocytes occurred continuously from December 1997 to January 1998, thereafter, a number of eggs with large egg diameters decreased considerably in number because of their discharge. From February to April, small number of large remaining oocytes of $< 60\sim 70\mu\text{m}$ in egg diameter began to degenerate, while many oogonia and oocytes measuring $10\sim 20\mu\text{m}$ in egg diameter remained in the oogenic follicles. Thereafter, the number of developing oocytes measuring $40\sim 50\mu\text{m}$ in egg diameter began to increase in May.

4. Reproductive Cycle with Gonad Developmental Stages

Based on morphological features and sizes of the germ cells and the tissue cells around them, the reproductive cycle with gonadal phases could be classified into five successive stages(Fig. 4). The stages and the criteria used in defining them are as follows:

Early active stage: In females, oogonia and early developing oocytes propagated along the follicular wall(germinal epithelium) and the undifferentiated mesenchymal tissues of the ovary. The oogonia and early developing oocytes were about $9\sim 11\mu\text{m}$ and $15\sim 20\mu\text{m}$ in size, respectively. At this time, the total volume of the ovary was small, and the follicular wall was thick(Fig. 5A).

In males, spermatogenesis occurs in the acini of the testis. The spermatogonia and spermatocytes were $8\sim 9$ and $6\sim 7\mu\text{m}$ in diameter, respectively, and appeared in a layer along the acinus wall(Fig. 5G). In 1997 and 1998, the individuals in the early active stage appeared from April to June when seawater temperatures were gradually increased.

Late active stage: In females, there is a large amount of ovogenic activity. A number of developing oocytes, ranging from $30\sim 40\mu\text{m}$ in diameter, appears in the oogenic follicles. Each oocyte has an egg-stalk attached to the follicular wall, and its nucleus enlarge to be a germinal vesicle having a small nucleolus. When late developing oocytes grew to $45\sim 60\mu\text{m}$ in diameter, the follicular wall

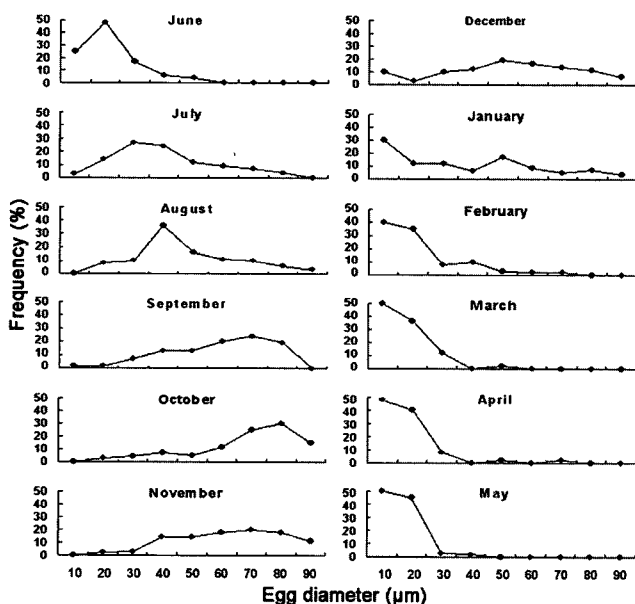


Fig. 4. Monthly changes in relative frequency distribution of the ovarian egg diameter of *Amusium japonicum* from June 1997 to May 1998.

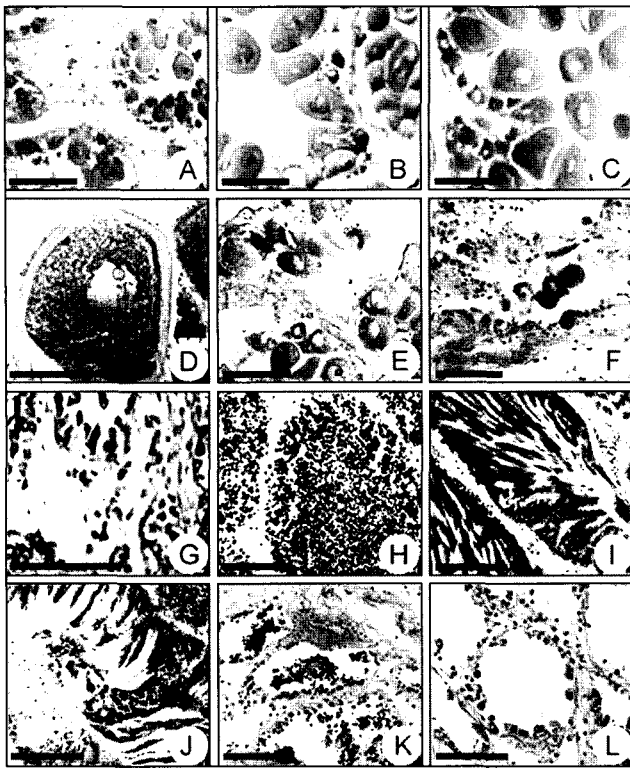


Fig. 5. Gonadal phase of female *Amusium japonicum japonicum* as seen by light microscopy. A: section of follicles in the early active stage; B: section of follicles in the late active stage; C: section of follicles in the ripe stage; D: section of a fully ripe oocyte; E: section of follicles in the partially spawned stage; F: section of follicles in the spent/resting stage; G: transverse section of acini in the early active stage; H: section of the acini in the late active stage; I: section of the acini in the ripe stage; J: section of the acinus in the partially spawned stage; K and L: sections of the acini in the spent/resting stage. Scale bars= 70 μ m.

was thin and undifferentiated mesenchymal cells gradually disappeared(Fig. 5B).

In males, spermatocytes develop into spermatids by meiosis. Spermatids moved toward the center of the lumen, measuring 3~4 μ m in diameter, and showed layers. As the testis develops, a number of spermatocytes, spermatids, and a few spermatozoa occupied approximately one-third to one-half of the lumina in the acini(Fig. 5H). Female and male individuals in the late active stage were found from June to September when seawater temperatures rose rapidly in 1997 and 1998.

Ripe stage: In females, the majority of oocytes grew to 60~70 μ m in diameter, becoming round or oval in shape,

and was located in the center of the lumen. There was an increase in the ratio of cytoplasm to the nucleus. At this time, the follicular wall became very thin and ripe eggs measuring about 70~90 μ m in diameter had a large germinal vesicle. Each oocyte had an egg-stalk attached to the follicular wall and its nucleus enlarged to be a germinal vesicle having a small nucleolus. Thick egg membrane of a ripe oocyte was surrounded by gelatinous membrane. The cytoplasm of oocytes contained a number of yolk granules. At this time, the follicular wall was very thin(Figs. 5C, D). In males, a small number of spermatids began to undergo transformation into 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 October to November when seawater temperatures gradually decreased in 1998.

Spawning stage: In females, since about 50~60% of oocytes in the oogenic follicles were discharged, the lumen of the oogenic follicles became considerably empty. Spawning ovaries are characterized by the presence of a few ripe undischarged oocytes and very young oocytes in the lumen(Fig. 5E).

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

The spawning period in females and males occurred once a year from November to January, and the main spawning occurred between November and December when seawater temperatures were relatively low between 1997 and 1998.

Spent/resting 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 re-sorbed. Thereafter, the rearrangement of newly formed oogonia and connective tissues occurred in the follicles (Fig. 7F).

In males, the few remaining spermatozoa and spermatids

were degenerated. The products of gamete atresia were resorbed(Fig. 5K). Thereafter, the rearrangement of a few newly formed spermatogonia and connective tissues occurred in the acini in this stage(Fig. 5L). Female and male individuals in this stage were found from February to April when seawater temperatures were relatively low.

5. First Sexual Maturity

First sexual maturities of a total of 171(90 females and 81 males) individuals of *A. japonicum japonicum* were investigated histologically in order to certify shell length of scallops that reached maturation and participated in reproduction from October(before spawning) to late February(after spawning). As shown in Table 1, percentages of first sexual maturity for female and male scallops ranging from 85.1 to 90.0mm were 55.5 and 57.1%, respectively, and they were both 100% for scallops over 90.1mm in shell length.

Table 1. Shell length at first sexual maturity in *Anusium japonicum japonicum*

Shell length(mm)	Female		Male	
	Number	Mature(%)	Number	Mature(%)
20.2~ 30.0	4	0	4	0
30.1~ 40.0	7	0	5	0
40.1~ 50.0	7	0	4	0
50.1~ 60.0	6	0	6	0
60.1~ 70.0	7	0	7	0
70.1~ 75.0	8	0	6	0
75.1~ 80.0	8	0	8	0
80.1~ 85.0	9	11.1	6	16.7
85.1~ 90.0	9	55.5	7	57.1
90.1~ 95.0	7	100	6	100
95.1~100.0	7	100	4	100
100.1~105.0	4	100	5	100
105.1~110.0	4	100	7	100
110.1~114.8	3	100	6	100
Total	90		81	

Sexually Mature Length of the Scallop

The shell lengths of sexually mature scallops(50% of rate of group maturity) in females and males that fitted to the exponential equation were 86.96 mm in female and 86.59 mm in male in shell length(Fig. 6).

6. Sex Ratio

As shown in Table 2, of 461 scallops examined, 235 were females and 226 males. There was no significant difference in the numbers of females and males present($\chi^2=0.18, p>0.05$), and monthly comparisons showed no statistical differences in the numbers of female and male scallops. In eight out of twelve months there were more females than males and the opposite was true for the remaining four months. The sex ratios of individuals over 85.0 mm in shell length were not significantly different from 1:1. All sun and moon scallops sampled were dioecious, and no evidence of hermaphroditism was found in histological sections of any scallop examined.

DISCUSSION

Regarding germ cell development and function of the

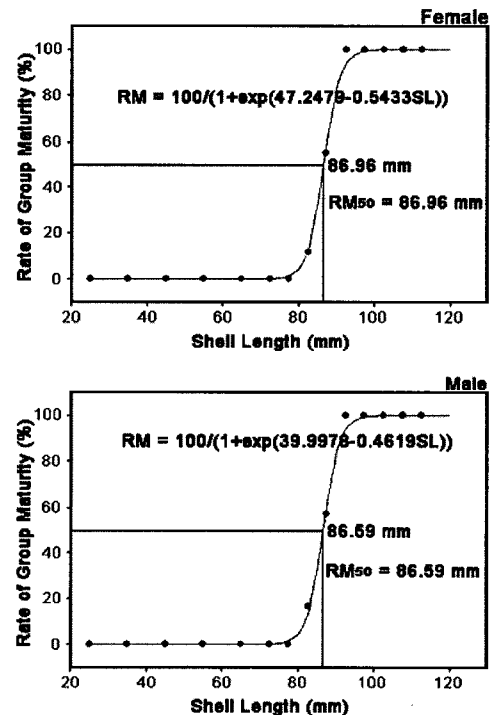


Fig. 6. Relationship between the rate of group maturity(%) and shell length(mm) of *Anusium japonicum japonicum*.

Table 2. Monthly variations in sex ratios of the adult clam *Amusium japonicum japonicum* (>85.1mm in shell length)

Month	Female (ind.)	Male (ind.)	Total (ind.)	Sex ratio (F/(F+M))	Chi squared
Jun. 1997	17	22	39	0.44	0.64
Jul. 1997	16	20	36	0.44	0.44
Aug. 1997	20	18	38	0.53	0.11
Sep. 1997	22	20	42	0.52	0.10
Oct. 1997	21	19	40	0.53	0.10
Nov. 1997	22	18	40	0.55	0.40
Dec. 1997	20	17	37	0.54	0.24
Jan. 1998	24	18	42	0.57	0.86
Feb. 1998	20	17	37	0.54	0.24
Mar. 1998	21	16	37	0.57	0.68
Apr. 1998	15	21	36	0.42	1.00
May 1998	17	20	37	0.46	0.24
Total	235	226	461	0.51	0.18

The critical value for χ^2 goodness-of-fittest of equal numbers of females and males, (1 df) at 95% significance is 3.84. Ind means number of individuals.

undifferentiated mesenchymal cells, several authors (Lee, 1972; Chang & Lee, 1982; Chung *et al.*, 1986, 1987, 1988; Chung & Kim, 1989) reported that the undifferentiated mesenchymal cells and eosinophilic granular cells, which were abundant in the germinal epithelium in the early and late active stage and which gradually disappeared with gonadal maturation, can be considered as a kind of nutritive cells associated with the formation and development of the germ cells in their early stages. In this study, we found the same results and came to the same conclusion.

In marine bivalves, the continuous production and resorption of gametes may be regarded as an adaptation to environmental temperature and food availability (Morvan & Ansell, 1988; Paulet, 1990). If the reproductive energy allocated to the production of gametes is too large, nutritive reserves may not be enough to allow all eggs to reach the critical size for spawning and fertilization. In this case, the products of gamete atresia may be resorbed and

the energy reallocated to still developing oocytes or used for other metabolic purpose by the bivalves (Dorange & Lepennec, 1989; Mortavkine & Varaksine, 1989). Therefore, it is assumed that as in other bivalves, *A. japonicum japonicum* should have a reproductive mechanism to resorb and utilize the high nutritive substances rather than releasing hopeless gametes.

Of the spawning periods of *Amusium* spp., our histological observations on the sun and moon scallop showed that spawning of *A. japonicum japonicum* in Jeju, Korea occurred from November to January and the spawning peak was observed in November. Judging from monthly changes in relative frequency distribution of the ovarian egg diameter and artificial spawning experiment in the laboratory (Son *et al.*, 1998), the spawning period was once a year, however, we assumed that spawning occurred twice or more times during a spawning period.

The spawning period of *A. balloti* in the Shark Bay, Australia is between December and January (Heald & Caputi, 1981) and that of *A. pleuronectes* in Thailand is from November to February (Chaitanawisuti & Mennasveta, 1992). Therefore, we suppose that on the whole, spawning of *Amusium* spp. may occur during the period from November through February. Breeding may occur seasonally or year-round, and as in the other marine invertebrate groups (Webber & Giese, 1969), breeding season is affected by local environmental conditions as well as genetic differences among populations (Chung *et al.*, 1991, 2002). Regarding breeding habits of marine mollusks, Boolootian *et al.* (1962) placed mollusks into three large categories: (1) year-round breeders, (2) winter breeders, and (3) summer breeders. We found that *A. japonicum japonicum* belongs to the winter breeder class.

Son *et al.* (1996) found that *A. japonicum japonicum* reached a shell length of 90.7mm after one year. In the present study, percentages of first sexual maturity of female and male scallops were 55.5 and 57.1%, respectively among those individuals ranging from 85.5mm to 90.0mm in shell length, and 100% in those over 95.0mm.

Sexually mature shell lengths of this population (50% of rate of group maturity) in females and males were 86.96mm and 86.59mm in shell length, respectively.

According to the growth curves for the mean shell length fitted to von Bertalanffy's equation by Son *et al.* (1996), ages and shell length are as follows:

Age(years)	Mean shell length(mm)
1	62.8
2	90.7
3	107.4
4	117.4
5	123.4

Therefore, individuals ranging from 85.0~89.9mm in shell length were considered to be about two years old. We assume that both sexes of this population begin reproduction about two years of age.

In the aspect of natural resources management, the present study suggests that catching scallops < 85.0mm in shell length or < 2 years old can potentially lead into a drastic reduction in its recruitment. A prohibitory measure should be taken for adequate natural resources management.

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