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Maturation and Spawning of the Japanese Dosinia, *Dosinorbis* (*Phacosoma*) *japonicus* in the Coastal Waters of Western Korea

Ee-Yung Chung, Byung-Gyun Kim* and Young-Ho Seo

Department of Marine Living Resources, Kunsan National University, Kunsan 573-701, Korea
**Puan Hatchery, National Fisheries Research and Development Agency, Puan 579-850, Korea*

= 국문요약 =

한국 서해산 떡조개, *Dosinorbis* (*Phacosoma*) *japonicus*의 성숙과 산란

정의영 · 김병균* · 서영호

군산대학교 자연과학대학 해양자원육성학과
*국립수산진흥원 부안수산종묘배양장

1994년 1월부터 12월까지 1년간에 걸쳐 우리 나라 서해안 비인만의 조간대에서 채집된 떡조개, *Dosinorbis* (*Phacosoma*) *japonicus* (Reeve)를 대상으로 성숙 및 산란시기를 규명하기 위해 생식소 여포지수(GFI) 및 생식소지수(GI)의 월별변화, 생식주기, 월별 난경 조성 및 성비를 조사하였다.

본 종은 자웅이체이고, 생식소는 중장선 하부와 소화맹낭 그리고 섬유성 결체조직과 근섬유에 의해 치밀하게 구성된 외측 근섬유층 사이에 위치하고 있다.

산란기 추정을 위해 조사된 GFI와 GI의 월별변화 양상은 생식주기와 일치하는 경향을 보였다.

난경 55 - 65 μm인 성숙란들은 젤라틴성 막에 의해 둘러싸여 있다. 본 종의 생식주기는 초기활성기(12 - 2월), 후기활성기(2 - 5월), 성숙기(5 - 8월), 부분산란기(6 - 8월) 그리고 퇴화 및 비활성기(8 - 12월)의 연속적인 5단계로 나눌 수 있었다.

산란기는 수온 및 먹이이용 가능성과 밀접하게 관련되어 있는 것으로 보이며, 산란은 수온이 20 ℃ 이상인 6월부터 8월까지 일어났고, 산란성기는 6월과 7월이었다. 산란기는 단지 그 해 하계에만 일어남으로써 1년에 1회의 산란기를 가지나 그 산란 빈도 횟수는 1년에 1회의 산란계절 중 2회 이상 일어나는 것으로 추정된다. 암 · 수 개체들의 성비는 통계 처리한 결과 1:1 (χ^2 = 0.08, P > 0.05)로 나타나 암 · 수간 성비에 차이가 없었다.

Key words : Maturation, Spawning and Sex Ratio, *Dosrsinorbis* (*Phacosoma*) *jponicus*

INTRODUCTION

The Japanese Dosinia, *Dosinorbis* (*Phacosoma*) *japonicus*, distribute along the coastal areas of Korea, Japan and China. More specifically, in Korea, it has been mainly found in the sandbed or silty sand in the intertidal zone of south and west coast of Korea (Yoo, 1976; Kwon *et al.*, 1993), and it is one of the edible clams. Ten years ago from now, this clam was easily found and produced at the sandbed in the intertidal zone. However, in connection with the recent sharp reduction in standing stock by reckless overcatching, marine reclamation works and environmental pollutions, it has been noted as one of the target organisms which should be managed for the conservation of natural living resources, especially, in the coastal waters of western Korea.

So far, however, there has been no study on reproductive ecology using histological method in detail. We could not find out any report on the reproductive cycle of this species in Korea, except for a work dealt with its classification and distribution (Lee, 1956).

Therefore, to get its reproductive information in detail, investigations on gonadal development, spawning and sex ratio associated with population characteristics etc. were planned. Particularly, studies on reproductive cycle and strategy of this species are supposed to be one of the most important tasks for the propagation of natural resources.

The main purposes of the present study are to understand gonadal development, reproductive cycle and sex ratio of this species in terms of providing the basic information for the propagation of a natural living resources.

MATERIALS AND METHODS

Specimens of the Japanese Dosina, *Dosinorbis* (*Phacosoma*) *japonicus* were collected at the intertidal zone of Piin Bay, west coast of Korea from January to December 1994 (Fig. 1). A total of 498 clams were collected for the study.

After the live clams were transported to the laboratory, each part of the body was measured. Seawater temperatures measured at 10:00 a.m. by the Poryong Hatchery, National Fisheries Research and Development Agency were used for this study (unpublished data).

1. Gonad follicle index (GFI)

To estimate the spawning period, the mean gonad follicle index (GFI) was calculated. In each microscopic preparation, representing an anterior posterior section through the clam, the percent gonad tissue, relative to the non-gonadal tissue, and the average gonad density [ratio of area in follicle to standard area (1 mm) in the gonad] were determined in three serial sections, using the count point technique (Tinsman *et al.*, 1976). To provide a measure of gonadal development, the GFI (percent gonad × gonad density) was calculated for each clam from these determinations.

2. Gonad index (GI)

To estimate the spawning period, the mean gonad index (GI) was calculated using a modification of Hillmann's methods (1993). Each histological section of gonadal tissues was also examined in detail to assess the stage of gonadal development and was scored on a 0 - 5 scale, by modified Hillman's method (1993) with minor modifications. To describe six stages of gonadal development or maturity, the following numbers were loaded in each stages: 0

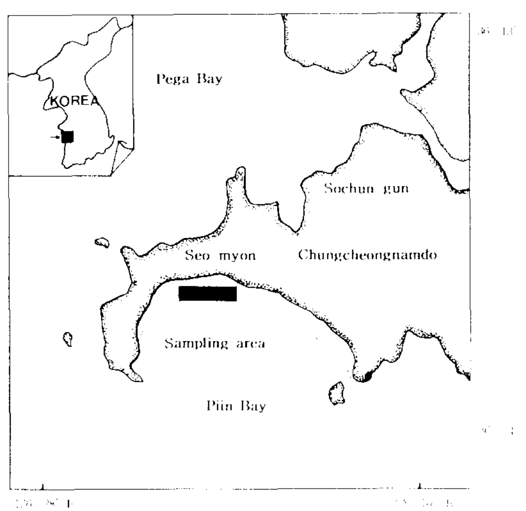

Fig. 1. A map showing the sampling area.

inactive; 1 = early active; 2 = middle active; 3 = late active; 4 = mature; 5 = ripe; 3 = partially spawned; 1 = spent. The arithmetic mean of the individual scores of the whole samples was recorded as the gonad index (GI) for each month.

3. Relative frequency distributions of the egg diameters

To investigate monthly relative frequency distributions of egg diameters, about one thousand eggs of which nuclei were centrally cut were monthly measured, and then we expressed them by the frequency histogram according to modification of Pears (1965).

4. Identification of gonadal development stage

A piece of gonad near the visceral cavity of each specimen was prepared for histological examination by fixation in Bouin's fluid, dehydration in ethanol and embedding in paraffin. Sections were cut at 5 - 6 μm on thickness and stained with Hansen's haematoxylin 0.5% eosin, Mallory's triple stain, and PAS stain. Based on morphological features and the sizes of the germ cells and tissue cells around them, the gonadal phases can be classified into five successive stages. The criteria used in defining the categories are as follows:

Early active stage :

In females, oogonia propagate on the follicular wall and early active oocytes having developed cytoplasm appear in the oogenic follicle (Fig. 2A).

In males, a number of spermatogonia and spermatocytes appear in the spermatogenic follicle (Fig. 3A). At this stage, undifferentiated mesenchymal tissues and eosinophilic cells appear in both gonads.

Late active stage :

In females, each of the oocytes makes an egg stalk attached to the follicular wall (germinal epithelium), and its nucleus enlarged to be a germinal vesicle having a small nucleolus (Fig. 2B).

In males, as the further development of the gonadal advances, each of spermatogenic follicle composes of spermatogonia, spermatocytes and spermatids in groups (Figs. 3B and 3C).

Ripe stage :

In females, the majority of oocytes in the oogenic follicle are becoming round or oval (Fig. 2C). Ripe oocyte ranging 55 - 65 μm in diameter has a large germinal vesicle. They are surrounded by the gelatinous membrane and contain their cytoplams with a large number of yolk granules (Fig. 2D).

In males, spermatids undergo transformation into spermatozoa in the center of the lumen, and spermatozoa occupy the center of the lumen. Ripe testis is characterized by the formation of streams of spermatozoa in the spermatogenic follicle (Fig. 3D).

Partially spent stage :

In females, the lumen of oogenic follicle becomes considerably empty, since about 50% of oocytes in a oogenic follicle are discharged. After spawning, the a few remained ripe oocytes are undischarged as well as young oocytes (Fig. 2E).

In males, the lumen of the spermatogenic follicle becomes empty because about 60% of spermatozoa are discharged into the surrounding environment (Fig. 3E).

Spent / Inactive stage :

In females, after spawning, the undischarged oocytes in the lumen undergo cytolysis and each oogenic follicle is contracted and degenerated. After degeneration, rearrangement of the newly formed oogenic follicle (Fig. 2F).

In males, undischarged spermatozoa scattered in the lumen begin to degenerate. After degeneration, rearrangement of the newly formed spermatogenic follicle appear for a long time (Fig. 3F).

5. Sex ratio

The sex ratios of the Japanese Dosinia were estimated by collection of monthly specimens of 498 sexually mature individuals from January to December 1994. The clams were sexed by photomicroscopic examination of histological preparations. A Chi square goodness-of-fit was used to test the hypothesis of equal representation of females and

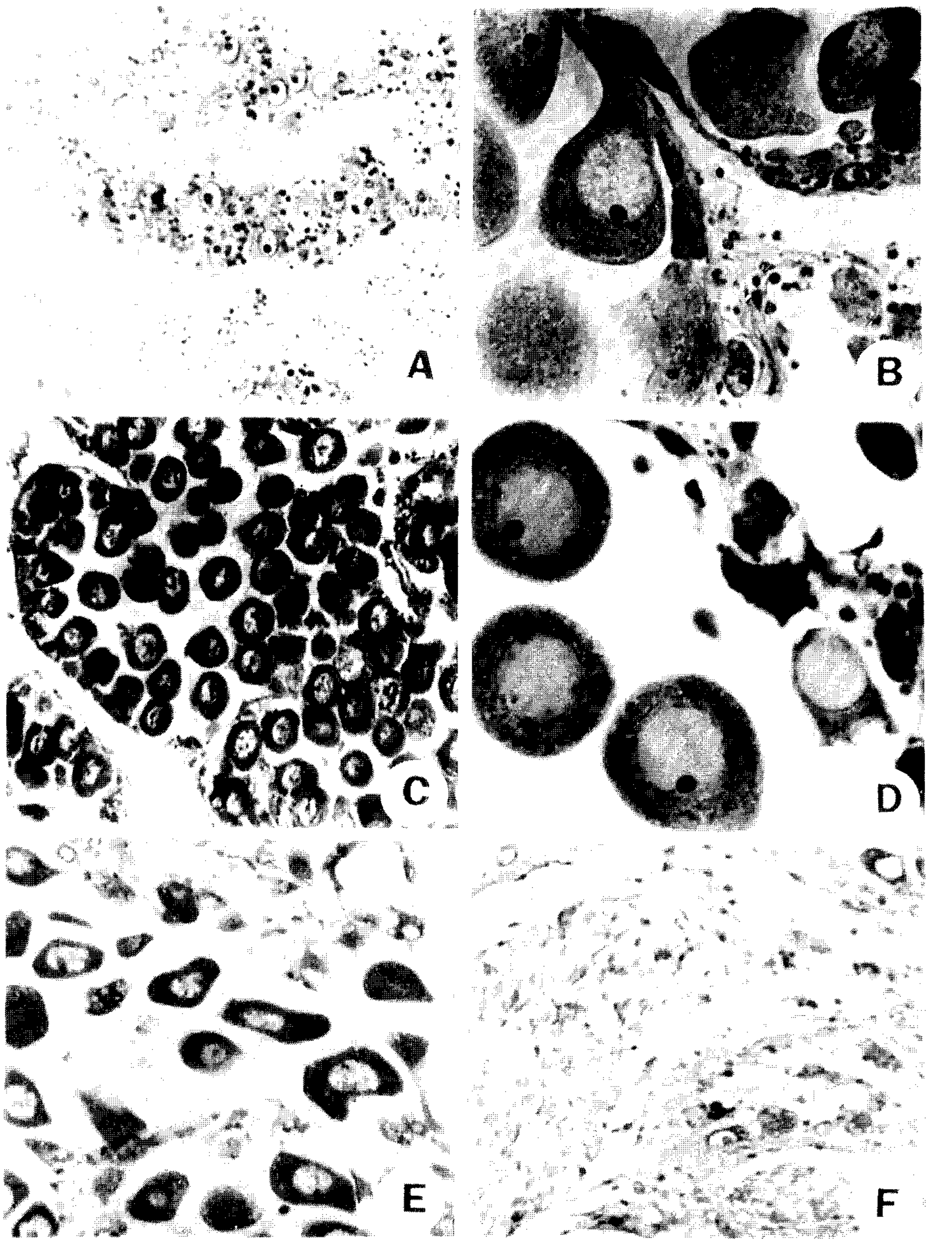

Fig. 2. Gonadal phase of female Japanese Dosinia, *Dosinorbis* (*Phacosoma*) *japonicus* as seen by light microscopy. A, Transverse section of the oogenic follicle in the early active stage; B, section of the oocytes in the late active stage; C, section of ripe oocytes in the ripe stage; D, section of fully ripe oocytes; E, section of the follicles in the partially spawned stage; F, section of follicles in the spent / inactive stage. A, E, F × 200; B, D × 430, C × 150.

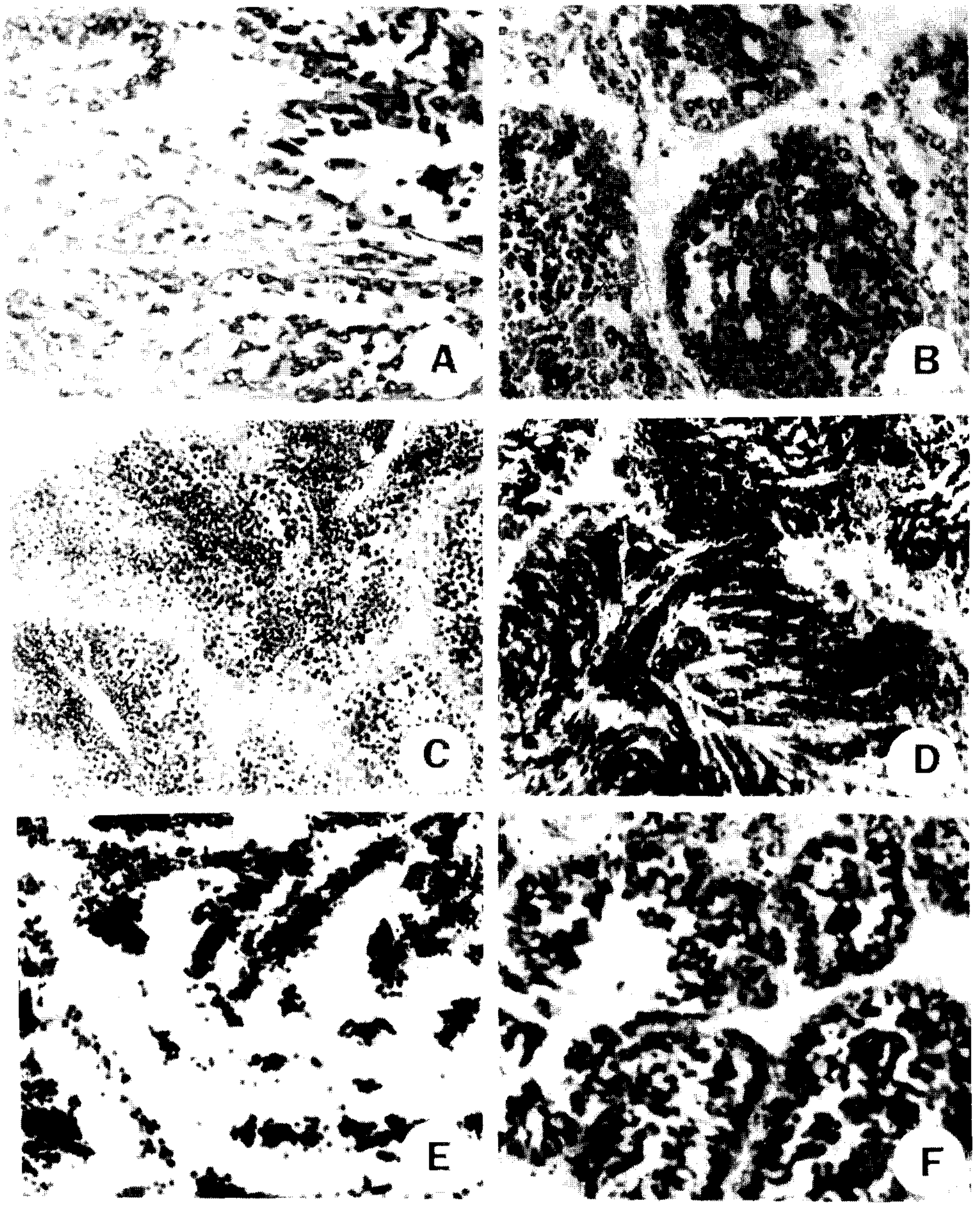

Fig. 3. Gonadal phase of male Japanese Dosinia, *Dosinorbis* (*Phacosoma*) *japonicus* as seen by light microscopy. A, transverse section of the spermatogenic follicles in the early active stage; B and C, section of the follicles in the late active stage; D, section of the follicles in the ripe stage; E, section of partially spawned testis; F, section of the follicles in the spent / inactive stage. A, B, C, D, E, F × 300.

males.

RESULTS

1. Position and structure of the gonads

Sexuality of this species is dioecious, the gonads locate among the subregion of mid-intestinal glands, 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 numerous spermatogenic follicles (Figs. 4A and 4B).

Associated with the progress of maturation, the gonads extended to the lowest part of the muscular tissue layers around the foot. As the gonadal maturation progresses, the external features of mature ovary and testis were light brown and yellow in colour, respectively. At this time, when they were slightly scratched with a razor, ripe eggs and milky white sperms were flowed out readily. Therefore, their sex can be distinguishable easily by anatomy. But, after spawning, the gonads are degenerated and it becomes difficult to distinguish their sexes.

2. Monthly changes in gonad follicle index (GFI)

To estimate the spawning period, monthly variations in the GFI were examined from January to December 1994 (Fig. 5).

The GFI values in both sexes began to increase between April and May and reached the maximum (GFI, 28.50 in females and 40.5 in males) in June. And then, the GFI values in female and male sharply decreased from June to August. Thereafter, they showed relatively lower values. Therefore, it is assumed that the spawning period is from June to August.

3. Monthly changes in gonad index (GI)

To estimate the spawning period, monthly variations of the GI from January to December 1994 were calculated by values of ranks in accordance with gonadal phases. Variations in the GI of this species are shown in Fig. 6. Between March and April when seawater temperatures gradually increase, the GI gradually increased and reached the maximum value (GI, 4.75) in May. Thereafter, the values rapidly decreased between June and July when the mean seawater temperatures relatively high. Therefore, the spawning period might be from June to August. Thereafter, the GI values gradually decreased from September to November. In the present study, monthly changes in the GI coincided with the trend of changes in the GFI values. Variations in the GI showed a periodicity with gonadal development.

4. Monthly changes in relative frequency distributions of the egg diameters

To estimate the spawning period, relative frequency distributions of the ovarian egg diameters were measured using histological preparations from January to December 1994 (Fig. 7).

Between January and February 1994, relative frequencies in egg diameter were over 60% among those oocytes in diameters of 11 - 20 μm. Egg diameter of a large group began to increase in March, and attained to the diameter of over 51 μm in 20% of late active oocytes in April. Percentage of eggs of over 61 μm (ripe oocyte) in diameter was about 30% in May. From June to August when spawning began, ripe oocytes of approximately 61～70 μm began to decrease in number because of their discharge.

A few of undischarged large oocytes were degenerated from July to September. After spawning, or degenerating, newly formed oogonia and oocytes of below 20 μm in diameter were present from October to December; however, it could be observed that a few primary oocytes remained in the follicle till December.

5. Reproductive cycle and seawater temperature

The frequencies of gonadal phases of the Japanese

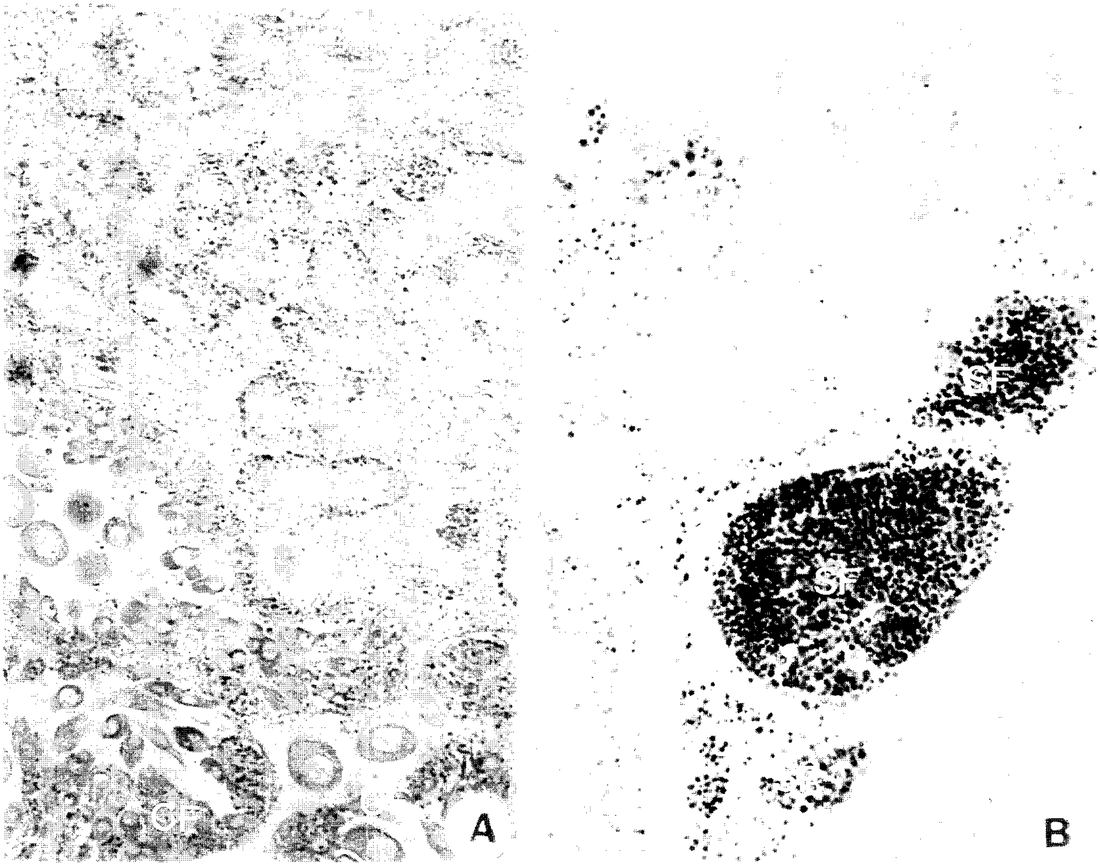

Fig. 4. Position and structure of the gonads of *Dosinorbis* (*Phacosoma*) *japonicus*. A, structure of female gonad; B, structure of male gonad. Abbreviation: DD, digestive diverticulum; MG, mid-intestinal gland; FI, fibromuscular layers; OF, oogenic follicle; SF, spermatogenic follicle.

Dosinia are shown in Fig. 8. Early developing female and male gametes in the early active stage took place from December to February when sea water temperatures were very low. The individuals in the late active stage appeared from February to August when the seawater temperatures were rapidly rising. As a result of the increase in number and growth of gametes, the gonad size gradually increased. The first ripe female and male individuals (approximately 70%) appeared in May, and then, mature and ripe oocytes (over 60 μm in size) were found from May to August when the seawater temperatures were very high, and large number of spermatozoa appeared to be full in the follicles of the gonads. A number of fully ripe oocytes and spermatozoa began to discharge from June (about 65% in both sexes). The partially spawning period of this species appeared from June to early August when the seawater temperatures reached above 20℃, and the one spawning peaks occurred between June and July during the spawning period of the year. After partially spawning, undischarged oocytes or spermatozoa in the follicles were degenerated in most follicles. In the spent/inactive stage, there was no sign of gonadal activity from August to December when the

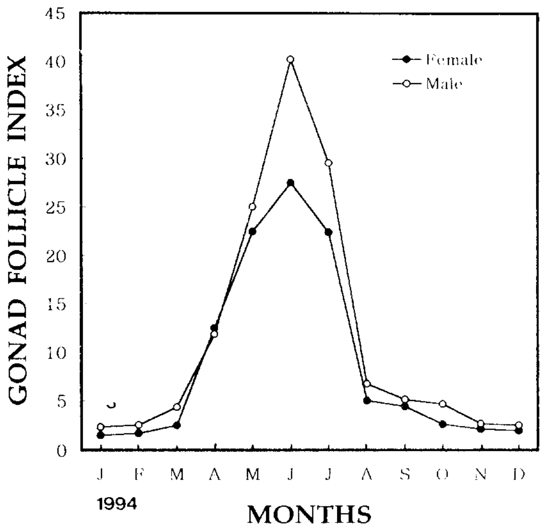

Fig. 5. Monthly changes in gonad follicle index (GFI) from January to December 1994.

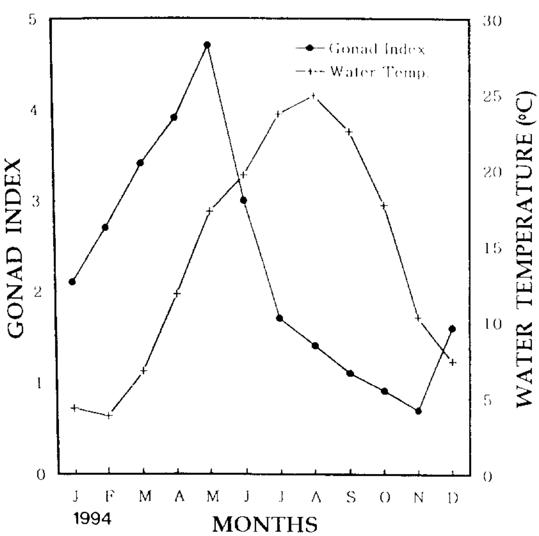

Fig. 6. Monthly changes in gonad index (GI) and the mean seawater temperature from January to December 1994.

seawater temperatures gradually decreased.

6. Sex ratio

A total of 243 and 237 out of 498 clams were females and males, respectively (Table 1). The sex

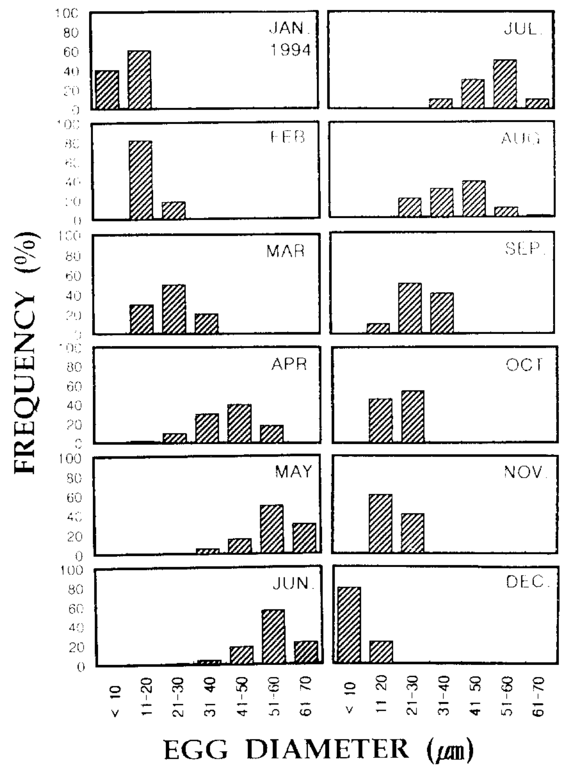

Fig. 7. Relative frequency distributions of the ovarian egg diameters.

of remaining 18 clams was not determined because of having only a few indistinguishable sex cells. There was no significant difference in the numbers of females and males present ($\chi^2 = 0.08$, $p > 0.05$), and monthly comparisons showed no statistical differences in the numbers of female and male clams for the twelve-month period. Accordingly, the sex ratios of individuals were not statistically different from 1:1.

DISCUSSION

Most marine molluscs have their own special characteristics of breeding habits, and their reproductions change with seasonal variations (Blootian *et al.*, 1962). The breeding period of marine bivalves occur during the period of abundant food (Griffiths, 1977). Bivalves tend to spawn when

Table 1. Monthly variations in sex ratios of *Dosinorbis* (*Phacosoma*) *japonicus* (Reeve)

Data	Female (inds.)	Male (inds.)	Undetermined (inds.)	Total (inds.)	Sex ratio [F/(F+M)]	χ^2*
Jan. 1994	14	18	6	38	0.44	0.50
Feb.	22	18	3	43	0.55	0.40
Mar.	27	20	3	50	0.57	1.04
Apr.	19	24	0	43	0.44	0.58
May.	28	21	0	49	0.57	1.00
Jun.	16	19	0	35	0.46	0.26
July.	17	20	0	37	0.46	0.24
Aug.	25	20	0	45	0.56	0.56
Sep.	18	22	0	40	0.45	0.40
Oct.	20	25	0	45	0.44	0.56
Nov.	17	14	0	31	0.55	0.29
Dec.	20	16	6	42	0.56	0.44
Total	243	237	18	498	0.51	0.08

* The critical value of χ^2 test of equal numbers of female and male is 3.84 at 95% significance.

food availability is enough for the planktotrophic larvae and for recovering the energy consumed by adults for spawning (Bayne, 1976). In the present study, the spawning season of this species occur from June to July, the main spawning occurred between June and July when the sea water temperatures were high. Accordingly, this species belongs to the summer breeder (Blootian *et al.*, 1962), and it is assumed that this spawning period is suitable to adequate nutrition for the planktonic larvae and adult clams. The timing of spawning and the number of spawning frequencies of this species during the one spawning period per year are assumed to be more than twice in the summer spawning season during the year. Therefore, it can be considered that this clam is a multiple spawning species.

Gonad development and maturation of bivalves are closely related to water temperature (Turner and Hanks, 1960; Sastry, 1966, 1968; Chang and Lee, 1981; Chung et al., 1991), food organism (Sastry, 1966, 1968; Maru, 1976) and day length (Simpson, 1982).

Sastry and Blake (1971) contended that some environmental condition (subject to abundant food and at least 15℃ of water temperature) should be maintained for gonad development and growth of the oocytes. But, if the gonad has been kept within 5℃ of water temperature, the oocyte can be differentiated in the gonad. Compared with our experimental results, gonad development and growth of oocytes were active from the spring season because of abundant food organisms and increasing water temperature (over 15℃), while they were in the immature stage during the winter season because of insufficient food and lower temperature (below 5℃). Therefore, our results coincide with Sastry and Blake's report (1971).

In molluscs, most of the gonads are shown to be associated with some part of the digestive system. The reproductive and digestive systems must be functionally significant. Sastry (1979) described that gonad development of marine bivalve has high

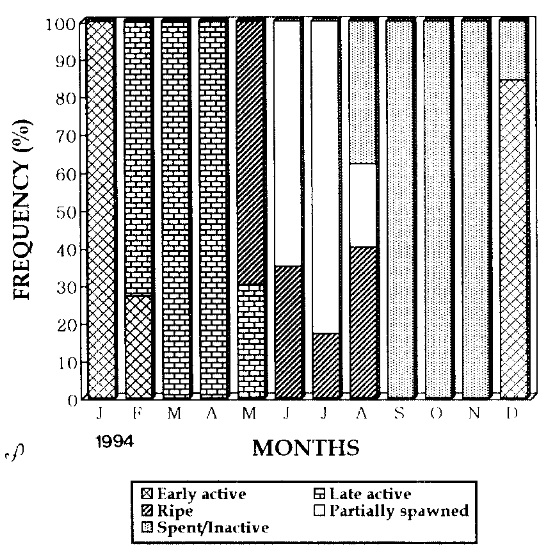

Fig. 8. Frequency of gonadal phases of *Dosinorbis* (*Phacosoma*) *japonicus* from January to December 1994

energy demands, and this energy may be derived from food ingested directly from the ambient water, or from stored reserves (nourishments) transferred directly from the digestive diverticula. Sastry (1968) stated that gonad size increased during periods of increased phytoplankton production, while digestive diverticular size are remained constant at that time.

In the present study, the gonad is adjacent to the digestive diverticula or joined to them in part. But, we could find easily that the number and stored reserves (components) of the tubules in the digestive diverticular per square mm somewhat changed with the gonadal phase. Therefore, it is supposed that the gonadal development is closely related to the digestive diverticular to get energy sources.

After spawning, some phenomena with reference to production and resorption of gametes were easily found in the gonads of this species, especially, it seems that the continuous production and resorption of gametes depend on environmental temperature and food availability (Morvan and Ansell, 1988; Paulet, 1990). If too much reproductive energy for the production of gametes may be required, nutritive reserves may not be enough to allow all eggs to reach the critical size for spawning. In this situation, some of hopeless gametes of them should be resorbed by way of the gamete atresia. For resorption, some of gamete atresia should occur to reallocate the reproductive energy for still-developing oocytes or to use for other metabolic purposes by the bivalves (Dorange and LePennec, 1989; Motavkine and Varaksine, 1989).

Therefore, it is assumed that this species should have a mechanism to resorb and utilize the high nutritive reserves by way of gamete atresia rather than releasing hopeless gametes.

Some authors (Rae, 1978; Sutherland, 1970) reported that breeding strategies varied with depth gradients. Sutherland (1970) stated that in the intertidal zone *Collisella scabra* spawns once a year in high-tide habitats and most of the year in low-tide habitats.

According to our histological study, the spawning period of *D.* (*Phacosoma*) *japonicus* occurs once a year as the same as in high tide and low tide habitats of the intertidal zone. Therefore, we have somewhat different opinions between Sutherland's report (1970) and our results about spawning frequency.

With reference to temperature latitude effects, Rand (1973) concluded that breeding strategies vary with latitudinal gradients. Northern climates are characterized by a single synchronous spawning per year, temperate climates by two spawnings and tropical ones by year round spawning.

So far, there are some previous studies on the spawning of perennial bivalves. Although they were the same species, in general, number of spawning frequencies per year in most bivalves are once a year in the northern districts (*e.g.*, Hokkaido and most of Korean districts) of Tokyo Bay, Japan, while twice a year in the southern districts of Tokyo Bay; for instance, in Japanese and Korean bivalves, *e.g.*, *Ruditapes philippinarum* (Kurashige, 1943; Ko, 1957; Momoyama and Iwamoto, 1979; Kim *et al.*, 1986), Meretrix lusoria (Chosen Fisheries Experiment Station, 1937; Taki, 1950; Kim *et al.*, 1986), and Mactra veneriformis (Iwata, 1948; Oshima et al., 1965). Among the same species, however, their spawning frequencies showed two kinds of spawning patterns (once or twice a year), according to the distributions. Thus, it is supposed that number of spawning frequency in perennial bivalves (iteroparous species) may vary with characteristics of local populations between intraspecies according to the temperature latitude. Therefore, on the whole, we agree with Rand's opinion (1973).

SUMMARY

To investigate monthly changes in the gonad follicle index (GFI), gonad index (GI), reproductive cycle, egg-diameter composition and sex ratio of the

Japanese Dosinia, *Dosinorbis* (*Phacosoma*) *japonicus* (Reeve), the specimens were collected from the intertidal zone of Piin Bay, Poryong city, Chungcheongnamdo, west coast of Korea, from

Sexuality of this species is dioecious. The gonads are located among the subregion of mid-intestinal glands, digestive diverticula, and the outer fibromuscular layers compacted by the fibrous connective tissues and muscle fibers. The gonads of mature individuals extend toward the reticular connective tissue of the foot.

Monthly variations of the GFI and GI values calculated for estimation of the spawning period, are coincided with the reproductive cycle.

The ripe eggs ranging from 55 to 65 μm in diameter are surrounded by gelatinous membrane. Reproductive cycle of this species can be divided into five successive stages: early active (December to February), late active (February to May), ripe (May to August), partially spawned (June to August), and spent/inactive (August to December) stages. It seems that the spawning period was closely related to the water temperature and food availability, and spawning occurred from June to August when the seawater temperature was above 20℃. The peak spawning occurred between June and July. The spawning period was once a year (only summer season during the year), while it is assumed that number of spawning frequencies might occur more than twice during the spawning season per year. The sex ratios of individuals were not statistically different from 1: 1 (χ^2 = 0.08, $p > 0.05$).

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