Reproductive Cycle and First Sexual Maturity of *Sinonovacula constricta* (Lamarck, 1818) (Bivalvia: Pharidae) in Western Korea

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ABSTRACT

The gonad index, reproductive cycle and first sexual maturity of *Sinonovacula constricta* collected from Simpo, Kimje-gun, Korea were investigated by histological analysis. The gonad index (GI) in both sexes of *S. constricta* increased from April and reached a maximum in July when the water temperature rapidly increased. And then, the GI values gradually decreased by spawning from August through October. Monthly variations in the GI showed a close relationship with ovarian development. The reproductive cycle in females and males can be classified into five successive stages: early active stage (March to June), late active stage (May to July), ripe stage (July to September), partially spawned stage (August to October), spent/inactive stage (October to March). The percentage of first sexual maturations in female and male clams of 50.1-60.0 mm in shell length was over 50%, and for clams over 70.1 mm in shell length, it was 100%. Because harvesting clams < 50.1 mm in shell length could potentially cause a drastic reduction in recruitment, a measure including a prohibitory fishing size should be taken for adequate improved fisheries resource management.

Key words: *Sinonovacula constricta*, reproductive cycle, first sexual maturity.

INTRODUCTION

The razor clam, *Sinonovacular constricta*, (Bivalvia: Pharidae) is distributed along the coasts of Korea, China and Japan. More specifically for Korea, this clam is found in silty sand in the intertidal zone of the south and west coasts of Korea (Yoo 1976; Kwon *et al.*, 1993), and it is one of the important edible bivalves. However, on account of the recent sharp reduction in the standing stock by reclamation works, water pollution, and reckless overharvesting of this clam, it has been denoted as a target organism and fisheries resources that should be managed using a more reasonable fishing regimen. For the propagation and management of a living natural resource, it is important to understand some population characteristics with regard to germ cell differentiation during oogenesis and sexual maturation of this species.

So far, Several studies have been conducted on the aspect of reproduction, including reproductive cycle and biochemical components (Han *et al.*, 2005), aspects of ecological studies, including investigation of distribution (Yoo 1976, Kwon *et al.*, 1993), the survival rate of young shells (Wu and Lin, 1987), growth and survival of cultured clam (Yoshimoto and Shutou, 1989), fluctuation of physiological indicator and morphology with growth and maturity (Yoshimoto, 1989), growth and survival of natural clam (Yoshimoto and Shutou 1990), experiment of natural spat collection (Yoshimoto *et al.*, 1990), suvival, growth and development of the larvae and spat (Lin and Tianming 1990), growth and production (Ko *et al.*, 1997), and larval swimming and post larval drifting behavior (Wang and Xu, 1997), as well as aspects of physiological studies, including natural

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variation of tissue metal concentrations (Lee, 2002) of *S. constricta* in Korea, Japan, and other countries. There are still gaps in our knowledge regarding reproductive biology. Little information is available on the reproductive cycle and the size at first sexual maturity of *S, constricta.*

Understanding the reproductive cycle and spawning period of this species will provide information needed for the determination of age and recruitment period of this population. In addition, data on size at first sexual maturity and reproductive strategy of this population can determine a prohibitory size (50% of size at first sexual maturity) for adequate natural resource management. Therefore, the purpose of the present study is to understand the reproductive cycle, spawning season and the size at first sexual maturity in order to get a prohibitory size for improved fisheries resource management of this clam based on cytological, histological, and morphometric analysis.

MATERIALS AND METHODS

1. Sampling

Specimens of *S. constricta* were collected monthly from the intertidal zone of Simpo, Kimje-gun, on the west coast of Korea, from January 2006 to December 2007. 40-50 clams, ranging from 25.6-94.7 mm in shell length, were used for the study. After alive clams were transported to the laboratory, the sizes of the specimens were recorded using a Vernier caliper.

2. Gonad index

To identify the spawning period, a total of 455 ovarian histologic preparations (50.1-94.7 mm in shell length) were examined for determination of the gonad index (GI) from January 2000 to December 2001. The mean GI was calculated using a modification of Mann's methods (1979). Each histological section of ovarian tissue was also examined in detail to assess the stage of ovarian development and was scored on a 1-5 scale to describe fivestages of ovarian activity: 1=spent/ inactive stage (S1); 2=partially spawned stage (S2) 3=early active stage (S3); 4=late active stage (S4); and 5=ripe stage (S5). The mean GI was obtained by multiplying the number of female clams

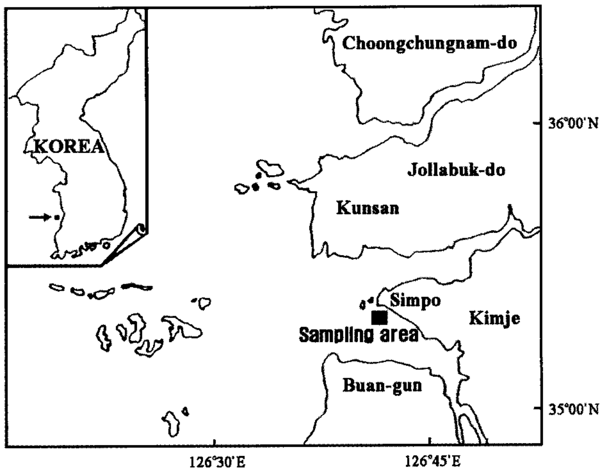

Fig. 1. Map showing the sampling area.

in each stage by the numerical ranking of that stage (NRV) and dividing the resulting value by the total number of clams in the sample. The arithmetic mean of the individual scores of the whole samples was recorded as the GI for each month. The following formula was used to determine the gonad index.

$$GI = \frac{(NRVS1)+(NRVS2)+(NRVS3)+(NRVS4)+(BRVS5)}{\text{Total N observed by month}}$$

The high average values of GI coincide with gonadal maturity. Minimal average values following high average values are considered an indication of spawning (Chung, 2007).

3. First sexual maturity by light microscopic observation

For the study of size at sexual maturity, a total of 225 ovarian preparations (25.6-94.7 mm in shell length) were histologically examined for evidence of maturation and spawning from June to November, 2006. The size equivalent to 50% of size at sexual maturity was estimated to be the biological minimum size for natural resource management.

RESULTS

1. Position and morphology of the gonad

S. constricta is dioecious organism. The gonads are located between the visceral mass and the reticula connective tissue of the foot. When the gonads are mature, mature ovary was pinkish-white color as

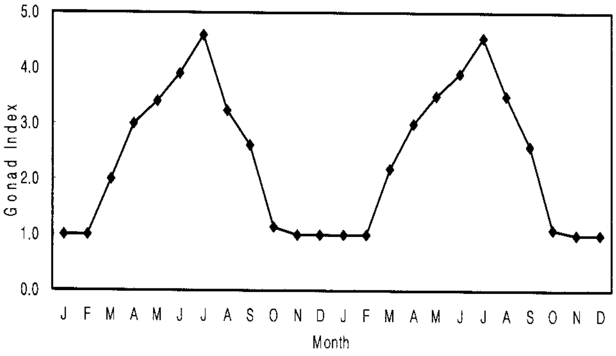

Fig. 2. Monthly changes in the mean gonad index (GI) of *Sinonovacula constricta* from 2006 to 2007.

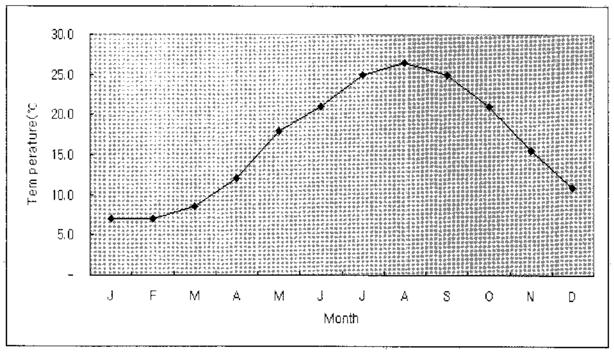

Fig. 3. Monthly changes in the mean seawater temperature at the smapling area January to December, 2006.

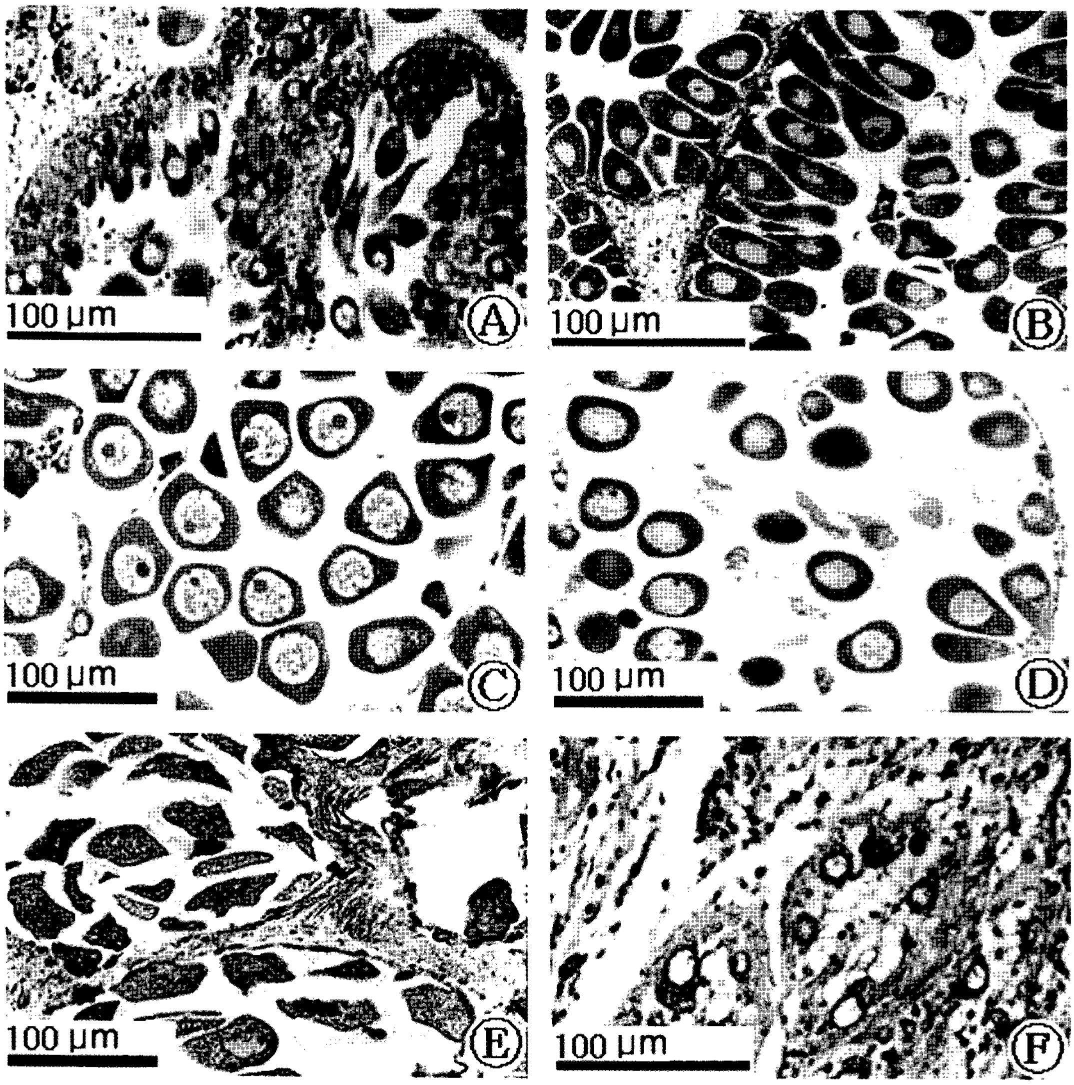

Fig. 4. Photomicrographs of the gonadal phases in female *Sinonovacula constricta*. **A**, Section of oogenic follicles in the early active stage; **B**, Section of follicles in the late active stage; **C**, Section of follicles in the ripe stage; **D**, Section of follicles in the partially spawned stage; **E**, Section of follicles in the spent stage; **F**, Section of follicles in the inactive stage.

mature testis. Therefore, the sex of individuals could not be easily distinguished by external features. However, if the ripe ovary and testis were slightly scratched with a razor, a number of ripe yellowish brown eggs and spermatozoa flowed out readilly. Therefore, sex could be easily distinguished by dissection. After spawning, undischarged germ cells degenerated, and then it becomes difficult to distinguish the sexes by external color or dissection.

2. Monthly changes in the gonad index

The gonad index (GI) gradually increased between April and May and reached a maximum (mean 4.6) in July when seawater temperature sharply increased. The GI then gradually decreased from August to October when spawning occured. Monthly changes in the GI in 2007 showed a similar trend to those in 2006. Monthly variations in the GI showed a close relationship with ovarian development (Fig. 2).

3. Reproductive cycle with gonad developmental stages

Based on the morphological features of the germ cells and other surrounding cells, the gonad developmental stages were classified into five successive stages. The criteria in defining each stage are as follows:

4. Early active stage

In females, the ovary was composed of a number of oogenic follicle, The early active stage was characterized by gradual expansion of the oogenic follicle, and the follicular walls in this stage are relatively thick. The oogonia and early developing oocytes, which is propagating along the follicular wall, were about 10-11 μm and 15-20 μm in size, respectively. At this time, the total volume of the ovary was small (Fig. 4A).

In males, spermatogenesis occurred in acini. The spermatogonia and spermatocytes were 8-9 μm and 6-7 μm in diameter, respectively, and they appeared along the acinus wall (Fig. 5A). In 2006, the individuals in the early active stage appeared from March to June when sea water temperatures gradually increased (Fig. 3).

5. Late active stage

In females, this stage was characterized by the presence of late developing oocytes. A number of these oocytes, ranging from 25-30 μm in diameter, appeared in the oogenic follicles. When the oocytes grow to 45-50 μm in diameter, each oocyte had an egg-stalk attached to the follicular wall (germinal epithelium). At this time, the follicular walls were thin (Fig. 4B).

In males, a few spermatogonia and spermatocytes were present near the acinus wall. However, a number of spermatids, measuring 3-4 μm in diameter were also found in the lumen of the acinus. At this time a few spermatids began to transform into differentiated spermatozoa in the centre of the lumen. As spermatogenesis progresses, the number of spermatocytes, spermatids and the few spermatozoa occupied approximately one-third to one-half of the lumina of the acini (Fig. 5B). In 2006, the individuals in the late active stage are found from May to July when sea water temperatures sharply increase.

6. Ripe stage

In females, at this stage, the follicular wall of the follicle became very thin. The majority of oocytes grew to 50-60 μm in diameter and were located in the center of the lumen of the follicle. The ripe eggs were about 60-65 μm in diameter, and they were surrounded by the gelatinous membranes. The cytoplasms of the eggs contained a large number of yolk granules (Fig. 4C).

In males, the acinus wall in this stage was very thin. Numerous spermatozoa, a number of spermatids, and small number of spermatocytes were present in the acini. Especially, lumina of the acini were filled with numerous spermatoza (Fig. 5C, D). In 2006, the individuals in the ripe stage are found from July to September when seawater temperatures are relatively high (over 20°C, Fig. 3).

7. Partially spawned stage

In females, since about 50-65% of the oocytes in the follicles were discharged, the lumina of the follicles became considerably empty. Spawned ovaries were characterized by the presence of a few undischarged oocytes and early developing oocytes in the lumen (Fig. 4D).

In males, the lumina of the acini were empty because over 50% of the spermatozoa have been discharged, but undischarged spermatozoa as well as spermatids remained in the lumen of the acinus (Fig. 5E). In 2006, the individuals in the partially spawned stage continued from early August to October, and the main spawning occurred between July and August when sea water temperatures were greater than 20°C.

8. Spent / Inactive stage

In females, after spawning, the undischarged oocytes in the lumen of the follicle undergo cytolysis, and each follicle was contracted and degenerated. The products of gamete atresia were resorbed (Fig. 4E). Thereafter, the rearrangement of newly formed oogonia and the connective tissues occurred in the follicles (Fig. 4F).

In males, after discharge of spermatozoa, undischarged spermatozoa were degenerated. The

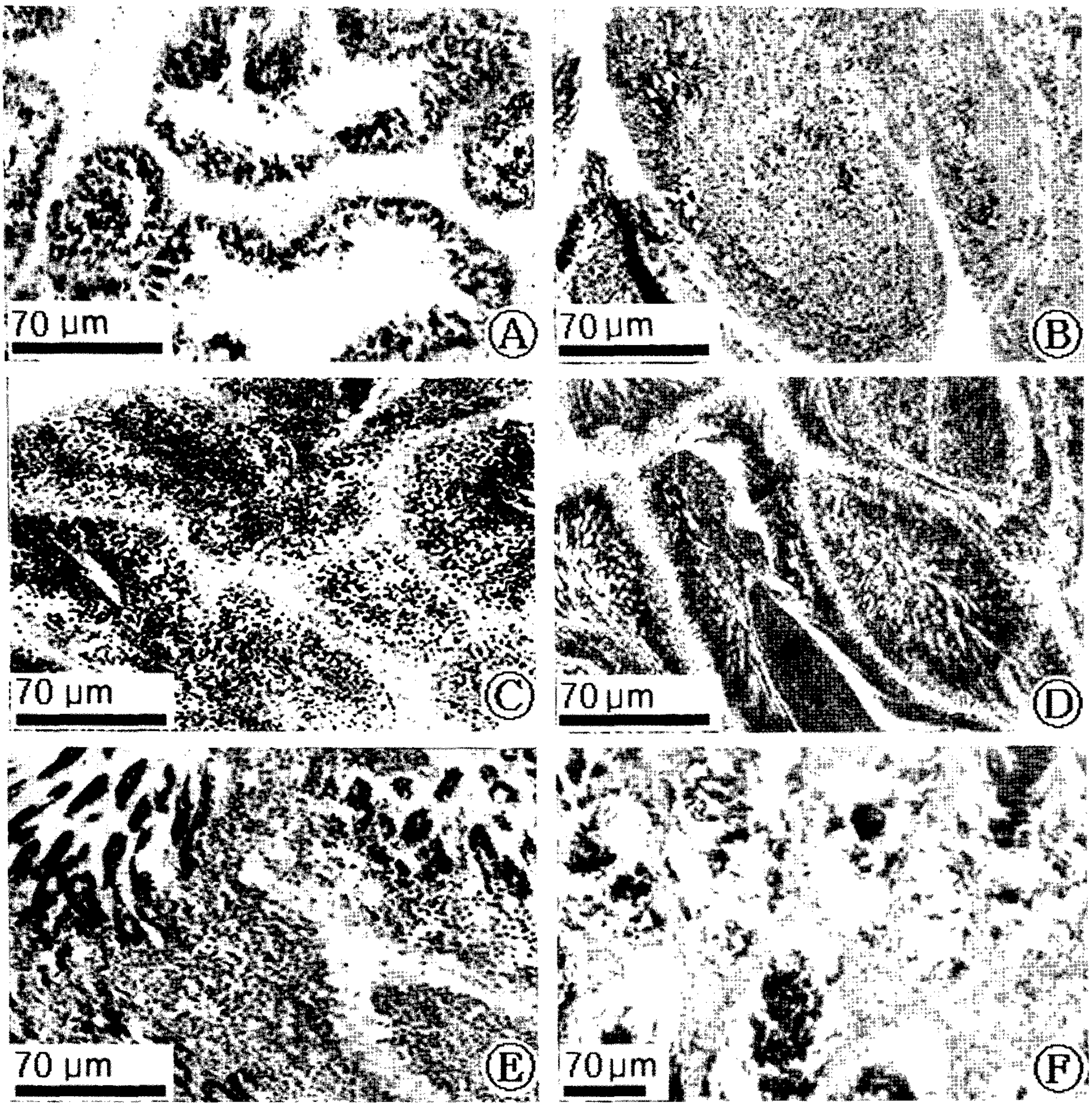

Fig. 5. Photomicrographs of the gonadal phases in male *Sinonovacula constricta*. **A**, Section of the acini in the early stage; **B**, Section of the acini in the late active stage; **C**, **D**, Section of the acini in the ripe stage; **E**, Section of the acini in the partially spawned stage; **F**, Section of the acini in the inactive stage.

rearrangement of a few newly formed spermatogonia and connective tissue occurrd in the acini (Fig. 5F). The individuals in this stage appeared from October to March when seawater temperatures gradually decreased.

9. First sexual maturity

For the study of size at first sexual maturities, a total of 446 (225 females and 221 males) individuals of *Sinonovacular constricta* were investigated histologically in order to certify the shell lengths of clams participating in reproduction from July (before spawning) to November (after spawning). In females, as shown in Table 1, the percentage of first sexual maturity of female clams ranging from 50.1 to 60.0 mm was 51.2%, and it was 100% for clams over 70.1 mm in shell length. In males, the percentage of first sexual maturity of female clams ranging from 50.1 to 60.0 mm is 56.1%, and it was 100% for clams over 70.1 mm in shell length (Table 2). Therefore, the percentages of first sexual maturity of female and male clams of 50.1 to 60.0 mm were over 50%, and it was 100% for both clams over 70.1 mm in shell length.

Table 1. Shell length at first sexual maturity in female *Sinonovacula constricta* from June to November, 2006

Shell length (mm)	Number of individuals by gonadal stage*					Total	Mature
	EA	LA	RI	PS	SP/IA	ind.	(%)
25.6-30.0	12					16	0
30.1-40.0	18					18	0
40.1-50.0	33	2	4	2		41	19.5
50.1-60.0	21	6	10	6		43	51.2
60.1-70.0	5		20	12	8	45	88.9
70.1-80.0			10	8	4	22	100.0
80.1-90.0			8	10	6	24	100.0
90.1-94.7			5	8	3	16	100.0
Total						225	

*Gonadal stage: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage SP/IA, spent/inactive stage

Table 2. Shell length at first sexual maturity in male *Sinonovacula constricta* from June to November, 2006

Shell length (mm)	Number of individuals by gonadal stage*					Total	Mature
	EA	LA	RI	PS	SP/IA	ind.	(%)
27.5-30.0	10					10	0
30.1-40.0	17					17	0
40.1-50.0	19	14	5	1		39	23.1
50.1-60.0	16	6	14	5		41	56.1
60.1-70.0		2	21	16	2	41	90.2
70.1-80.0			16	10	5	31	100.0
80.1-90.0			6	12	7	25	100.0
90.1-94.7			6	7	4	17	100.0
Total						221	

*Gonadal stage: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage SP/IA, spent/inactive stage.

DISCUSSION

Gonad index

Chung *et al.* (2007) reported that the high values of GI coincide with gonadal maturity, and the minimal values following high values were considered an indication of spawning. In the present study, the GI of *S. constricta* reached a maximum in July when the gonad were matured. And then, the GI values gradually decreased because of spawning. Accordingly, variations in the GI in females and males showed a close relationship with gonadal development and activity.

Gonadal development and maturation

A wide range of exogenous factors has recently been suggested as controls for gonadal development and maturation in marine bivalves. Of these factors, water temperature (Sastry, 1966, 1968, 1970; Chung *et al.*, 2005) and food availability (Chung *et al.*, 2005; Chung, 2007) seem to be particularly important. However, Sastry (1968) and Chung (2007) stated that these and other factors (salinity, day length, etc.), probably interact with endogenous factors (neuroendocrine activity) in a complex maner to control the initiation of gametogenesis.

In the present study, gonadal development and maturation of *S. constricta*

occurred relatively late from the late spring to the autumn when sea water temperatures gradually increased, various food organisms (phytoplankton) began to be abundant, and various nutrient reserves were stored in the digestive diverticula. During the periods of lower temperatures and insufficient food organisms the gonadal phases were in the immature stage.

According to the results of the present study, development of gametes to maturity in *S. constricta* was accelerated after gametogenesis has been initiated, and the development of gametes to maturation was dependent on water temperature. However, it is assumed that the amount of nutrients mobilized for the gonad maturation depends not only on food level, but also on the water temperature and the basic metabolic requirements of the clams (Chung, 2007). And in this study, the reproductive cycle of this species coincides with the results reported by Han *et al.* (2005).

First sexual maturity

In females and males, all specimens of 25.6-30.0 mm length were in the early active stage although collected during breeding seasons, and our gonad histology indicates that none of them could fully develop: only small numbers of oogonia and early developing oocytes were present in the oogenic follicle and small number of spermatogonia and a number of spermatocytes were present in the acini. In case of the specimens of 30.1-40.0 mm in females and males, the size of the early developing oocytes indicate that they could not have reached maturity until October, when spawning was ended. In males, the testes indicate that they could not have reached maturity until October, when spawning was ended. Accordingly, the percentage of first sexual maturity of female and male clams ranging from 30.1-40.0 mm in shell length is 0%. However, individuals 50.1-60.0 mm in shell length belonged to one of the early active, late active, ripe and partially spawned stages during the breeding season. In females, twenty two individuals in the late active, ripe, and partially spawned stage underwent gonadal development, whereas 21 individuals in the early active stage did not. In males, 25 individuals in the late active, ripe, and partially spawned stage underwent gonadal development, whereas 16 individuals in the early active stage did not. It was observed that more than 50% of clams of 50.1-60.0 mm length, reached the size at first sexual maturity. However all clams in the ripe, partially spawned, and spent/inactive stages reached it if they were larger than 70.1 mm. These mean that larger individuals can reach maturity earlier than smaller individuals. These results suggest that because catching clams < 50.1 mm in shell length could potentially cause a drastic reduction in recruitment, Accordingly, a fishing prohibitory size (measure) should be taken for adequate fisheries resource management. Thus, information on size at sexual maturity is very important and can determine a fishing prohibitory size for adequate natural resources management by biological minimum size (50% of size at sexual maturity).

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