

Reproductive Ecology of a Shad, *Konosirus punctatus* in Ariake Sound-II Development and Fate of the Ovarian Egg*

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In order to know the process of the maturation of a shad, *Konosirus punctatus*, observations and measurements of the ovaries and ovarian eggs were done, and the following results were obtained.

Eggs of the yolk globule stage and younger stages were observed together in an ovary during the spawning season. In some of such ovaries there were eggs older than yolk globule stage.

In egg-diameter composition of an ovary taken in the spawning season, 1~4 modes were recognized, showing the existence of some batches in an ovary.

The largest mode (the largest batch) in ovarian egg diameter composition is formed in more than 0.4 mm. The largest batch passes the stage where the mode is in 0.41~0.70 mm in 2 days of more. This batch passes the stage of 0.71~1.00 mm in about half a day from 3 a.m. to 3 p.m., and becomes ripe. The spawning is carried out in the time from 3 p.m. to midnight.

Individual shad spawns twice or more in a spawning season of one month or one month and half. Spawning interval of the individual is estimated to be 3 days or more.

Distribution, condition of the adult, and maturation of a shad, *Konosirus punctatus* have been reported as a part of the study about reproductive ecology of the shad in Ariake Sound in the previous paper (Takita, 1978). In order to know the process of the maturation in detail, observations and measurements of the ovaries and ovarian eggs were done, and some results about change of the aspect in ovary with time and development and fate of ovarian egg were obtained.

Materials and methods

The shad specimens including mature ones were collected with pound nets and gill nets on fishing boats in the same area

as explained in the previous paper from March to June in 1973, 74, and 75. The gill nets were operated from early in the afternoon to the next early morning. Sampling with gill nets were carried out from 3 to 8 p. m. The pound nets were usually operated at low tide during night and early in the morning. Samples were gotten at various time during night and early morning through the regular operations of the pound nets. Some samples were gotten with pound nets in day time by irregular operations through the courtesy of the fishermen. The samples were measured as fast as possible, and the gonads were fixed with 20 % formalin within 2 hours after the catch. After confirming no partial development of eggs in an ovary, the intermediate part of each

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ovary was used for the examinations. The usual celloidin method and stain with Meyer's haematoxylin-eosin were employed for the histological study. PAS reaction was also used on some materials.

Results and discussion

Examinations of ovarian egg size and histological observations of ovaries were carried out for 81 of 1-age shad and 122 of 2-3-age shad collected from March to June in 1974. All individuals of one sample were used in this examination when they were large of their variance of gonad indexes or small of their specimen number. When the variance of gonad indexes was small and the individual number was large, a part of them were selected to be used.

Diameter was measured for about 300 eggs larger than 0.1 mm in diameter for every specimen, then egg-diameter composition of each shad was graphed. No mode was recognized in egg-diameter composition of some individuals collected early or late in the period when the gonad index was comparatively high (Takita, 1978). Most of the ovarian eggs of these individuals were smaller than 0.4 mm. Most of the individuals collected from late March to late May have 1-4 distinct modes, showing the existence of several size groups of egg in an ovary. Frequency distributions of egg-diameter for 6 females are shown in Fig. 1. These females are; a, one collected on Mar. 19, showing no mode in its frequency distribution of egg-diameter; b, one collected on May 15, showing one distinct mode; c and d, one collected on May 15 and April 18, respectively, showing three distinct modes; e, one with two modes, which was collected on June 10 and recognized histologically to have eggs in bad condition; f, one with no

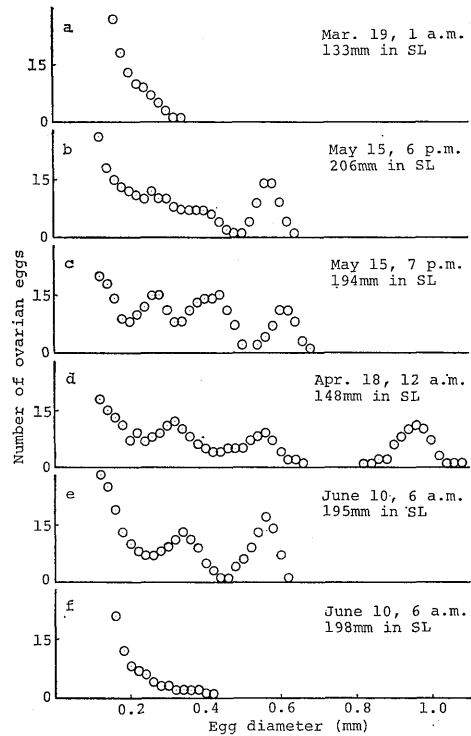


Fig. 1. Frequency distributions of egg-diameter of six shad ovaries.

mode collected on June 10 and regarded to have finished the spawning. The histologically bad condition is going to be explained later in this paper.

Changes of the mode number with time of collection are presented in Table 1. It shows the appearance of the individuals with many modes in the period when the gonad index is high in value.

In order to know development and fate of the ovarian eggs in spawning period, the position of modes in egg-diameter composition were compared among specimens examined. The individuals of each age-group with any mode in egg-diameter composition were combined through the period investigated, and their modes were plotted being arranged in the order of their position. The result of 1-age group is shown in Fig. 2. The largest mode (the mode of the largest position in egg-diameter) of each individual is situated in more than 0.4

Table 1. Changes of mode number in ovarian egg diameter composition.
Numbers show the number of females showing clear mode of each number.

Age	1					2·3				
	0	1	2	3	4	0	1	2	3	4
Mar. 19		2				2	2	3		
30		1					5	3		
Apr. 10		1	5	1		1	1	4	2	
18			3	6	4	1	1	4	7	6
30			3	3			1	8	3	
May 8				15	16	1		10	3	
15				1	5	4	1	7	14	3
28				1	4	1		1	4	4
June 10		-	-	-	-	-	1	2	5	

mm. Eggs larger than 1.01 mm are ripe or nearly ripe, and most of the eggs larger than 1.08 mm had already been ovulated. The next largest mode becomes larger with development of the batch forming the largest mode (the largest batch). The next largest mode in the ovary with ovulated eggs is at about 0.5 mm. Since bias of group maturation with respect to sampling time could not be removed (Takita, 1978), and the sampling was not at random, the developmental speed of each mode can not be estimated from the slope of the arranged modes. However, it can be acceptable that

an abrupt increase in egg size occurs just before the ovulation as presumed before (Kamiya, 1916; Kuwatani et al., 1956), from the difference of gradients between the rows of the largest and the next largest modes. The same result as in 1-age group was obtained for 2·3-age group.

Histological process of the ovarian egg development of the shad is almost the same as known about many fishes. There were eggs in the yolk vesicle stage and earlier stages in immature ovaries occurred late in March. In almost all occurred in April and May, there were eggs in the yolk globule stage and earlier stages. In some of the ovaries, well grown eggs such as ones in the migratory nucleus stage or the ripe stage, were found with eggs in the yolk globule stage and earlier stages (Fig. 3).

Thickness of zona radiata and size of yolk globule have been said to indicate the degree of egg development in the yolk globule stage (Takano et al., 1973; Yamamoto, 1953). However, neither any change in zona radiata thickness nor any difference in situation of yolk vesicle was recognizable in ovarian eggs in this stage of the shad. Besides, yolk globule showed changes of its size according to time when the shad was caught, in addition to developmental stage of the egg. Histological photographs of

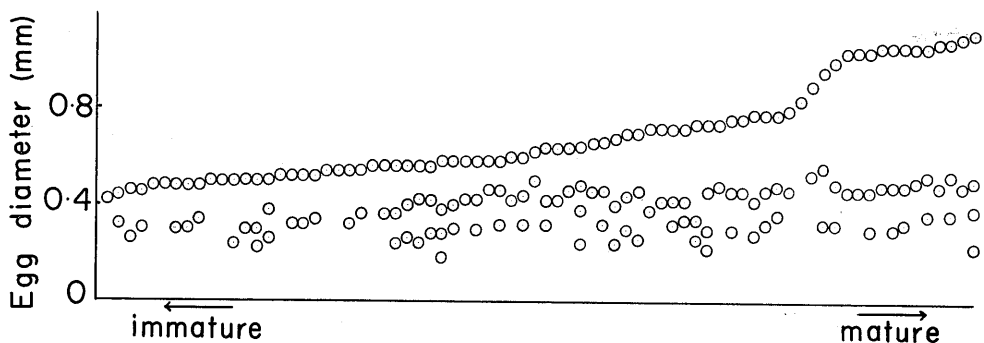


Fig. 2. Arrangement of modes in egg-diameter compositions of 1-age shad.

1-age shads with any mode were combined through the period investigated, and their modes were plotted being arranged in the order of their position.

ovaries of the individuals collected in 1974 spawning season are presented in Fig. 3. As shown in the figure, the egg sizes and the histological status show that the largest batch of G is in the same stage as that of B, and the largest batch of I is in the intermediate stage between those of A and B. However, the yolk globules of G and I are much smaller than those of A and B. Diameter of the yolk globule in A and B are no less than twice of those of G and I. Yolk globules of the largest batch in C and F are much larger than those in G and I too, though developmental stages of the formers seem to have a little more advanced than those of the latter. The largest batch of E is transparent. The next largest batch in this ovary having a mode at 0.52 mm is regarded to be in the same stage as the largest one of A. The yolk globules of the next largest batch in E is much smaller than those in A. Nucleus in the egg with small yolk globules is surrounded with a material which is stained well with haematoxylin. The eggs with large yolk globules are filled with yolk globules around the nucleus, and the above mentioned material is obscure in it. Size of yolk globule can not indicate clearly the degree of ovarian egg development in the yolk globule stage. The degree was estimated according to distribution and shape of oil globules, movement of nucleus, and fusion of yolk globules in this study.

Experience of spawning and relative length of time after the spawning are proved

on the basis of appearance of empty follicle cells. The cells in various conditions were recognized in ovaries of the shad too (Fig. 3, G, H). In some ovaries, new and old empty follicle cells were observed to exist together. Examining the relation between condition of the empty follicle cells and size of the yolk globules, it was found that the yolk globules in the ovaries with empty follicle cells were always small. Among the shads without empty follicle cells, ones caught earlier than April 18 had large yolk globules, while ones caught late in April and May and June always had small yolk globules. The aspect of empty follicle cells varied from new one to old fragmentary one which is almost being absorbed. From the above observations, it is assumed that the ovary having the egg with large yolk globules is the one which has no experience of spawning in the season, and small globules indicate the experience of spawning in the season. The determination of spawning experience by size of yolk globules was impossible on the ovaries having well developed eggs with fusing yolk globules except ovaries with fragmentary empty, follicle cells.

Empty follicle cells of *Oryzias latipes* have been reported mostly to disappear in three days after spawning, and not always to prove the spawning experience (Yamamoto and Yoshioka, 1964). The variety of condition of the cells in the shad suggests rapid disappearance of the cells too, and the determination of spawning experience based

Fig. 3. Photomicrograph of the ovaries of the shad.

Date of the collection, standard length of the specimen and position of the largest mode of egg-diameter composition of each are as follows.

A, Mar. 30, 208 mm, 0.52 mm;	B, Mar. 30, 214 mm, 0.62 mm;
C, Apr. 10, 192 mm, 0.64 mm;	D, May 8, 150 mm, 0.78 mm;
E, Apr. 18, 153 mm, 0.90 mm;	F, Apr. 18, 148 mm, 0.64 mm;
G, Apr. 18, 203 mm, 0.62 mm;	H, May 16, 203 mm, 0.48 mm;
I, June 10, 195 mm, 0.56 mm;	J, June 10, 198 mm, no mode.

All photographs are made to scale.

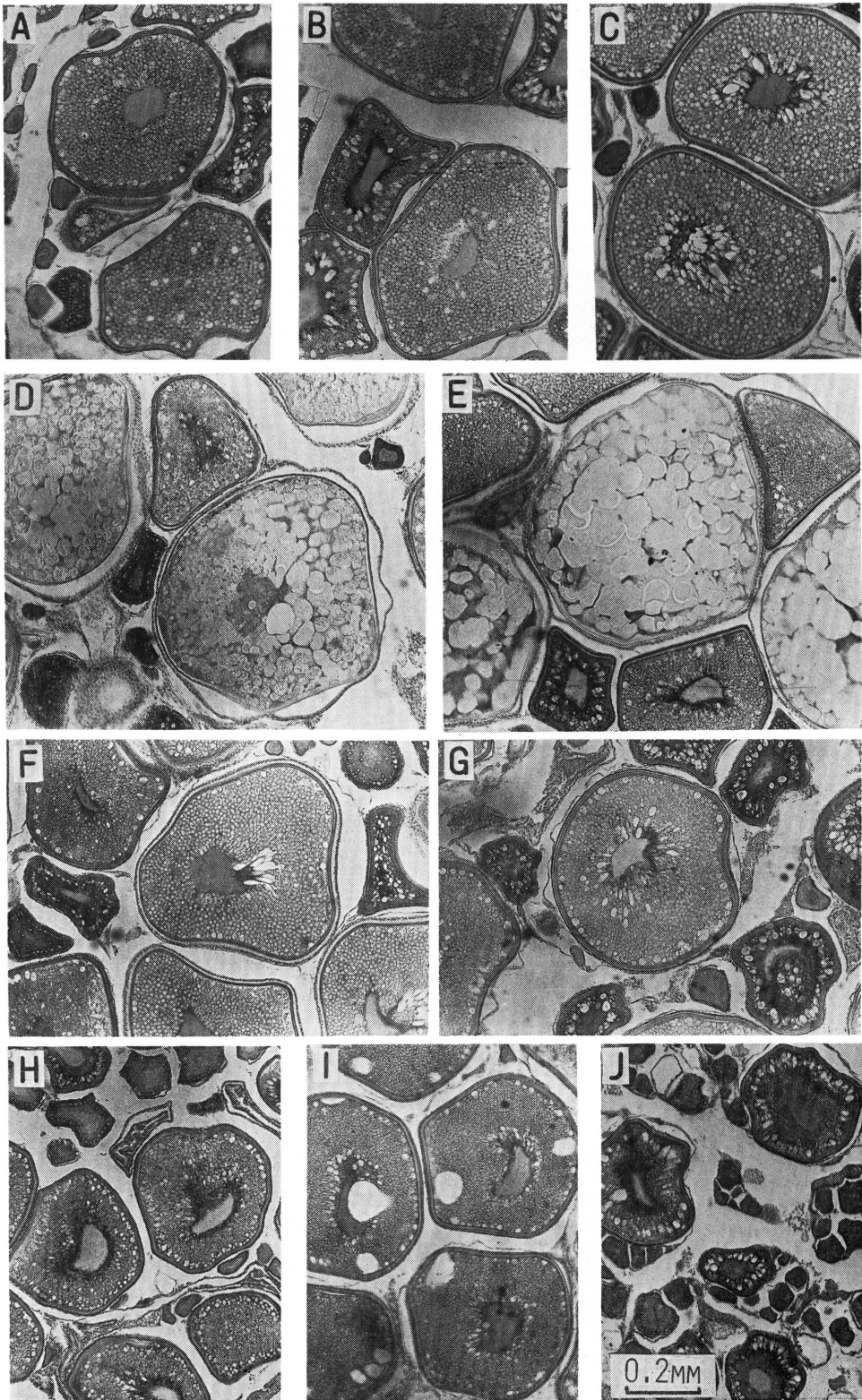


Fig. 3. Photomicrograph of the ovaries of the shad.

on it is difficult. However, size of yolk globules is able to help the determination. In 1974, the shad with spawning experience had not been caught until April 18. Of nineteen 2-3-age individuals, only one, and of fourteen 1-age individuals, five were regarded to have no spawning experience as of April 18. On and after April 25, all individuals examined except the ones with ripe eggs were regarded to have the experience. That is, the shad in Ariake Sound begins to spawn one year after the birth, and the spawning in 1974 began in about the middle of April.

According to the position of the largest mode of egg-diameter composition, the largest batches in ovaries were divided into three stages. Histological feature of each is as follows.

0.41~0.60 mm: Earlier, many vacuoles are scattered in gaps between yolk globules (Fig. 3, A, H). Later, comparatively large oil globules are situated closely around the nucleus (Fig. 3, C).

0.61~0.80 mm: This stage includes the features from the same appearance as that of the grown one of the previous stage to the completion of the nucleus migration (Fig. 3, D).

0.81~1.00 mm: In this stage, egg shows the same feature as shown in Fig. 3, D early, and afterward, it gets near to the ripe stage (Fig. 3, E).

In order to know the spawning frequency of the individual shad, the relation between the largest mode of egg-diameter composition and condition of the empty follicle cells was examined on individuals with spawning experience (Table 2). Most of the modes of the ovaries with new follicle cells are in 0.41~0.50 mm, and modes of the ovaries with old follicle cells are in 0.41~0.80 mm. Most of the modes of the ovaries

Table 2. Relation between condition of the empty follicle cells and the largest mode of diameters of the ovarian eggs observed on 1-age shad with spawning experience in the season.

Numbers show the individual number examined.

Mode of the ovarian eggs (mm)	New empty follicle	Old empty follicle	No empty follicle
0.41~0.50	4	7	
0.51~0.60	1	14	5
0.61~0.70		5	4
0.71~0.80		7	6
0.81~0.90			1
0.91~1.00			1

in which no follicle cell was recognized are in 0.51~0.80 mm.

The larger the time after the spawning shown by the condition of empty follicle cell is, the larger the ovarian egg is. In some ovaries with almost transparent large eggs, some fragmentary empty follicle cells were observed. Some ovaries with both new and old follicle cells had the eggs of more than 0.41 mm with much yolk in it. These facts and the development of the next largest batch following the largest one (Fig. 2) demonstrate that the individual spawns two or three times or more in one season.

The largest mode of the egg-diameter composition in the ovary with new empty follicle cells is almost in the same position as the next largest mode of the ovary with ripe eggs. In order to see the development of ovarian egg from a spawning to the next, the relation between the largest mode of each ovary and the time of the capture was examined (Table 3). The shad with ripe or nearly ripe eggs of more than 1.00 mm were caught from 3 p. m. to 6 a. m. However, the major number of the shad

Table 3. Relation between position of the largest mode in ovarian egg size composition and the time of the capture.

Time	0.41- 0.60 mm	0.61- 0.70 mm	0.71- 0.80 mm	0.81- 1.00 mm	1.01- 1.12 mm
0 a. m.	○				
3	○	○	○		
4	○				
5	○				
6	○	○	○		
11	○	○		○	
0 p. m.	○	○		○	
1	○	○			
3	○	○			○
4	○	○			○
5	○	○			○
6	○	○			○
7	○	○			○

with ripe eggs caught from 0 to 6 a. m. at least had already released a part of or most of the ripe eggs. They were regarded not to complete their spawning after the ovulation owing to the confinement in the pound net. Those with ripe eggs caught from 0 to 6 a. m. are eliminated from this table.

As shown in table 3, it seems to take one day or more to pass the egg size stage of 0.41~0.60 mm because of the long period of their appearance. It seems to be attributable to the bias owing to the scarcity of the samples that the individuals showing 0.61~0.70 mm of their mode were very few in night. The batches of this stage seem to appear allday, and it seems to take one day or more to pass this stage too. The batches having the mode at 0.71 mm and more first appeared at 3 a. m., and the shift of the mode was successive in time. Accordingly, the batch which has passed the stage of 0.61~0.70 mm attains to the stage of 0.71

~0.80 mm from midnight to the next morning, gets to the stage of 0.81~1.00 mm around noon, and becomes ripe from about 3 p. m. to the evening. The time when the shad with ripe eggs were gotten corresponds to the spawning time of the shad presumed before (Kamiya, 1916; Kuwatani et al., 1956). Spawning interval is estimated to be 3 days or more.

Some ovaries of the shad collected early in June had many vacuoles of various size in ovarian egg (Fig. 3, I). These ovaries still had one or two modes in egg-diameter composition (Fig. 1. e), and they had eggs in the yolk globule stage, being larger than 0.4 mm in diameter. The development of ovarian eggs in such an ovary was rather slow in comparison with the condition of the empty follicle cells. In some other ovaries, eggs were smaller than 0.4 mm and no mode was formed (Fig. 1, f; Fig. 3, J). Appearance of these ovaries corresponds with temporal decrease of gonad index, fatness (Takita, 1978), and the mode number in egg diameter composition, suggesting the end of their spawning late in May in the case of 1974. The shad of 2.3-age had finished their spawning slightly earlier than 1-age shad. Spawning duration of the shad population is estimated as one month or one month and half, and that of the individual may be a little shorter.

Conclusion

Eggs of the yolk globule stage and younger stages were observed together in an ovary during the spawning season. In some of such ovaries there were eggs older than the yolk globule stage.

In egg-diameter composition of the ovaries taken in the spawning season, 1~4 modes were recognized, showing the exist-

ence of several size groups of egg in an ovary.

The largest mode of egg-diameter composition (the mode of the largest position) is formed in more than 0.4 mm. A batch forming the largest mode passes the stage where the mode is in 0.41~0.60 mm (the yolk globule stage) in one day or more. It passes the stage of 0.61~0.70 mm (from the yolk globule stage to the migratory nucleus stage) in one day or more, and that of 0.71~0.80 mm (from the migratory nucleus stage to the fusing yolk globule stage) and that of 0.81~1.00 mm (the ripening stage) in several hours, respectively. The batch forming the largest mode attains to ripe stage from about 3 p. m. to the evening. The spawning is carried out in the time from 3 p. m. to midnight.

Individual shad spawns twice or more in a spawning season of one month or one month and half. Spawning interval of the individual is estimated to be 3 days or more.

Experience of spawning in the season can be indicated by existence of empty follicle cells and size of yolk globules in ovarian egg.

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有明海産コノシロの増殖生態—II

成熟過程

田 北 徹

コノシロ *Konosirus punctatus* の成熟過程を明らかにするため、卵巣と卵巣卵の観察と測定を行った。

産卵期の卵巣には卵黄球期の卵とそれより若い卵が混在する。そのような卵巣の中に、卵黄球期よりさらに発達した卵が混在するものもある。

産卵期には、卵巣卵卵径の頻度分布に1~4個のモードが認められ、1卵巣中に数個の卵群が存在することを示している。

卵径頻度分布中の最大のモード(最も発達した卵群)は0.4mm以上で形成される。その卵群はモードが0.41~0.70mmにある発達段階を2日かそれ以上で、0.71~1.00mmを約半日、およそ午前3時から午後3時までで経過し、完熟となる。産卵は午後3時ごろから夜中にかけて行われる。

1個体はひと月またはひと半月にわたる産卵期に2回またはそれ以上の産卵を行う。個体の産卵間隔は3日またはそれ以上である。