SEASONAL CHANGES OCCURRING IN THE GONAD OF *STICHOPUS JAPONICUS*

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Introduction

In the breeding season of Holothurioidea, Kinosita (1936a, b, 1938), employing *Stichopus japonicus* in March to September, presumed the spawning season by classifying the gonad into 11 stages according to ripeness of its gametes, which is determined by microscopical examination of a pipette sample taken from the gonad. In Miyagi Prefecture, Murachi reported in his personal communication the gonadal development of *Stichopus japonicus* by means mainly of the anatomical method to the author basing on his own observation (1950). Colwin (1948) reported by the observation of shedding of the animal in the aquarium that the spawning period of *Thyone briareus* is June. Inaba (1930) stated in *Caudina chilensis* on the basis of investigation of frequency of appearance of the eggs and larvae in sea water that the breeding season begins in about the middle of May and ends in late June.

The gonad of *Stichopus japonicus* is enlarged in the first half of a year including the breeding season, but the gonad is so small that a careful microscopical examination is necessary to determine the sex in the later half of the year, as it is difficult to recognize the gonad itself under spent condition owing to extreme shrinking. Most studies concerning the breeding of Holothurioidea have employed the anatomical method in some period including breeding season. Accordingly, a systematic histological description of the development process of the gonad throughout the year is needed. It may be necessary to observe seasonal changes of the gonads to understand the sexual phenomena.

This study deals with the changes occurring in the gonad of adult *Stichopus japonicus* at different seasons of the annual cycle. It was carried out from June 1956 to November 1957. Materials for this study were collected nearly every month. The development process of its gonad was observed by anatomical and histological methods.

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Materials and Method

The materials collected from Ishiya along the coast of Funka Bay in southern Hokkaido were each more than 100 gr in body weight; the total number of animals empolyed are shown in Table 1. Gonad index means 100 times the gonad weight per

body weight. This is the most convenient unit to show the relative gonad weight. The gonad was removed from body cavity after dissection, and the sample used in histological observation, which were taken out of the gonadal tubules at the part nearest the genital pore, were fixed by Bouin. Sections were cut 10 microns thick, and are stained in Delafield's haematoxylin followed by eosin.

Results

(1) Annual development process of the gonad

The gonad of all animals collected were observed by means of the sections. To understand the development process of the gonad, it may be convenient to classify it into some stages according to the maturation. In the present study the following five stages were determined according to their development by histological observation.

(*a*) *Resting stage* (Pl. I, Figs. 1. 6)

Females: In resting condition the gonadal wall is remarkably shrunk, and the wall of follicle is very thin. The ovarian follicle contains the unspent eggs, which are comparatively small in size, during the development process. These eggs are absorbed; in some eggs are noted indistinct profiles to be destroyed by phagocytic cell.

Males: The follicle of males, like the females, has a residue of unspent spermatozoa, which show mature appearance.

(*b*) *Recovery stage* (Pl. I, Figs. 2, 7)

Females: In this stage, there are no unspawned ova in follicle. Though the ovarian wall is still in a contracted state having many rumples, the follicle layer begins to develop as a concentric circle along the inner part of the gonadal wall. Young oocytes, which are attached to a follicle, have one nucleus and chromatin-nucleolus, and are less than 30 μ in diameter. Follicle center is empty.

Males: Although the wall of the follicle is very thin in resting stage, it becomes thick in accordance with the development in this stage. Spermatogenesis has begun.

(*c*) *Growing stage* (pl. I, Figs. 3, 8)

Famales: The wall of the follicle in this stage is seen growing and protruding star-shaped towards the lumen of the tubules owing to active oogenesis, though such growth is along the gonadal wall in the former stage. Many oocytes which appear in sections are still connected with the wall of the follicle. Nucleus and Chromatin-nucleolus are clearly seen in all oocytes. By this time, there are no oocytes surrounded by follicular epithelium. A young oocyte, having small yolk, grows large in this stage: a young oocyte is less than 60 μ in diameter.

Males: In this stage, the follicle wall possesses more remarkably star-shaped appearance than that of females. There are mature spermatozoa in lumen of follicle, and active spermatozoa can be obtained from the male gonad in this stage.

(*d*) *Mature stage* (Pl. I, Figes. 4, 9)

Famales: The ovary in this stage is filled with ova, which have one nucleus and chromatin-nucleolus, and gonadal wall is enlarged. It is one of the leading chracteristics that all ova are surrounded by follicular epithelium. The ova in the gonadal tubules are polygonal in shape on microscopic examination of sections, and they are primary oocytes after growth. Large oocytes, constituting the majority of the cells in follicle, measures ca 130 μ in diameter. A few immature ova which are of ca 90 μ diameter are still to be found along the wall of follicle. The ova in earlier stage of oogenesis are practically non-existent.

Males: The spermary containing much quantities of ripe spermatozoa is larger than that of former stage. Spermatogenesis continues in this stage. Such spermary which contain aboundant ripe apermatozoa may be in the mature stage.

(*e*) *Shedding stage.* (Pl. I, Figs. 5, 10)

Females: The animal in this stage indicates a spawning individual. Ripe ova in follicle appear loose for shedding, though they are compactly contained in it in mature stage. In addition, they are rotund in shape and uniform in diameter, 160 × 130 μ, having one nucleus and chromatin-nucleolus. Follicular epithelium cannot be seen clearly unlike the situation in mature stage.

Males: The spermary is expanded by ripe spermatozoa, and gonadal wall becomes very thin. Spermatogenetic activities slow down. In some sections gaps are observed to occur in lumen of the follicle.

(2) Seasonal changes of gonad

Gonad index (Fig. 1) indicates seasonal variations of gonad weight. In Fig. 2 the gonad of the animal is classified into the above five stages according to its maturity, and developing degree of the gonad in the animal population is given by the proportion in each stage-numbers per total in each month. Seasonal changes in gonadal development

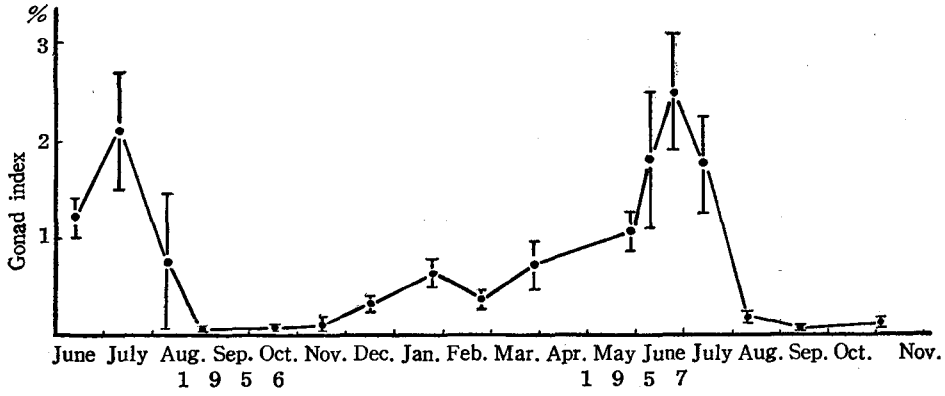

Fig. 1. Seasonal changes of the gonad weight of *Stichopus japonicus*, showing the average percentage in gonad weight per body weight
Vertical lines cover the range of the standard error.

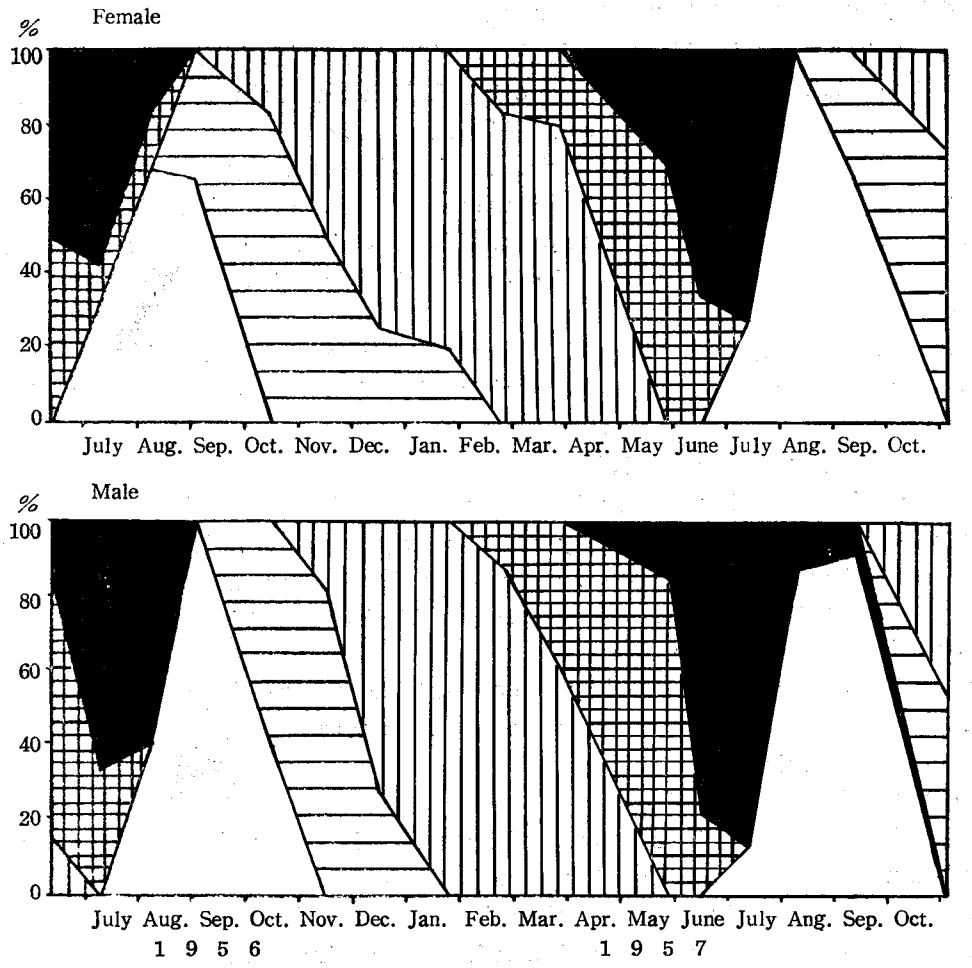

Fig. 2. The breeding cycle of the animal from June 1956 to November 1957 to show the percentage in the gonad stages of recovery, growing, mature, shedding, and resting stage

Recovery stage, Growing stage, Mature stage,
Shedding stage, Resting stage.

of *Stichopus japonicus* are precisely described as follows:

June–July: The animals collected in this period possess the largest sex gland to be found throughout the year; the gonad index rises very rapidly showing a peak in this term. Specifically, index value attains to maxima of average 2.1 % on July 10, 1956 and to 2.5 % on June 18, 1957. Afterward it declines down, as the animal passes into the resting stage. On June 18, 1957 one animal group consisted of some animals which had a small gonad of partly spent together with others which had a large gonad attaining

to 8 % of the body weight. The average gonad index showed the above value. The proportion of shedding animals was 70 –80 % of all those collected in this term, whilst the remainder showed either mature or resting stage. As shedding stage was most frequent appearance, this season answers to the spawning season in southern Hokkaido.

August–October: In this term it is not easy to find the gonad by anatomical examination as it is very small in size. Index value is approximately 0.05 %. Microscopical observation of sections reveals that 70–100 % of animals are in resting stage, which is exceedingly dominant.

November: The gonad is slightly recovered and more recognizable than in former period. Gonad index shows 0.2 % in average value (Fig. 1). Oogenetic activities commence in overies, and majority of animals are in the recovery stage.

December–March: Index value increases from 0.3 % to 0.7 % in average (Fig. 1). Simultaneously with the enlargement of the gonad, the observation of sections shows outstanding development of gametes. In the process of the development of the gonad, growing stage appears dominantly in both females and males (Fig. 2). Ten per cent of animals collected in February are in mature stage, thus oogenesis is most active. Some spermaries in this month contain mature spermatozoa, which begin to swim regularly and actively when removed from the spermary and placed in a dish containing sea water.

May: The gonads of animals collected in May enlarge more rapidly than those in former months; index value rises to 1.05 % in average. The inclusion of a number of gametes in a follicle is the most characteristic appearance. In the observation of sections, 85 % of males and 70 % of females belong to mature stage, which is dominant. The remainder are in shedding stage.

Discussion

Kinosita (1936a, b, 1938) reported that the spawning season of *Stichopus japonicus* is from July to August by observation on the materials collected from seven stations on the coast of Hokkaido. In this study is was found that spawning period extends from the middle of June over a period of two months.

The water temprature in the spawning season showed fluctuation between 12° and 22°C in this observation. Tokuhisa (1915) noted it to be from 16° to 20°C in his study of *Stichopus japonicus*. Inaba (1930) reported it to range from 13.5° to 18°C during spawning season of *Caudina chilensis*. Colwin (1948) observed the spawning of *Thyone briareus* when the water temperature of the aquarium varied between 16° and 22°C. Accordingly, the water temperature of the main spawning period of those species may be concluded to be from 15° to 20°C. The water temperature may be presumed to be a preminent factor which causes shedding.

In the ripe ova of *Stichopus japonicus*, Inaba (1937) stated that they are uniformly elliptical in shape, 165.5 × 142.8 μ in dimentions. They were measured similarly by

Kinosita (1938), 165 × 143 μ. In the present study they measure 160 × 135 microns. In mature stage, as already noted, a few immature ova are observed to exist along the inside of the wall of the follicles. Perhaps they seem to remain under resting condition in the follicle after shedding of the ripe ova.

It is observed in lamellibranchial bivalves such as *Ostrea virginica* (Loosanoff, 1942) and *Pinctada martensii* (Tateishi & Adachi, 1957) that may unspent gametes, which are mature, remain in their follicles in spent animas. Uuspawned ova are absorbed, but unspent spermatozoa remain till the next breeding season. As for Holothurians, Ohsima (1925) reported that unspent immature ova degenerate and collapse. In the present study microscopic examination of the sections of unspawned ova of *Stichopus japonicus* substantiated the above mentioned fact announced by Ohshima, but no spermatozoa were found in the spermary in the recovery stage. It seems probable, therefore, that unspent spermatozoa are absorbed. Although it may be unsuitable to compare the animal under question with Mollusca, the fate of unspent spermatozoa shows the difference between Holothurian and bivalves. Coexistence of unspent gametes and sexual cells newly formed in one follicle was not observed in the present study.

As for breeding of limpet (Orton et al., 1956) the process is classified into developing, spawning and resting stages. Development stage is short, and spawning season extends for the most part of the year. The ripe ova remain in the ovaries for a long time until water temperature becomes suitable for spawning. In *Cucumaria japonica* (Kinosita & Sibuya, 1941) the breeding season is a long period from early March to middle November, and the ripe gametes are in the gonad throughout the year. In amongst animals, the process of gonadal development varies between animals. However, it is observed in the present study that the gonadal development in an animal group changes in uniformity in each season, and that some dominant stage occurs present in each season. Comparing the present animal with other animals, it is a characteristic that the recovery and growing stages are long. With the enlargement of the gonad beginning in the later part of May, the gametes develop to sheddable condition following mature stage. It is difficult to obtain fertilizable ova in *Stichopus japouicus* which has the process of development described above, and the artificial fertilization is difficult to be applied. In males, different from females, the ripe spermatozoa occasionally used in artificial fertilization can be easily obtained.

In oyster and clam (Loosanoff, 1937, 1942), the gonad remains quiescent showing little gametogenetic activity when the temperature reaches to the critical hibernation point. Similarly, the gonad of *Stichopus japonicus* examined in September and October is small in size, showing resting stage. After November the gonad begins to develop. According to an ecological observation (Tanaka, 1958), the animal is under estivation during these two months. Therefore, it is suggested that the growth of the gonad

begins with the recovery of feeding in November.

As the sexual cycle is treated in this study using adult *Stichopus japonicus* only, the life cycle in its young stage remains yet unknown as seen from the view point of the reproduction of the animal.

Summary

1. Seanoal changes occurring in the gonad of *Stichopus japonicus* are observed by anatomical and histological methods.

2. The process of development of the gonad of the animal is classified into five stages according to the degree of development.

3. The gonad develops in November to the following June; the peak of gonad index occurs between June and July.

4. The spawning season of *Stichopus japonicus* in southern Hokkaido extends through two months from the middle of June.

5. In the gonad, fertilizable ova are observed for a short period, while the ripe spermatozoa are present in spermaries from February to the spawning season.

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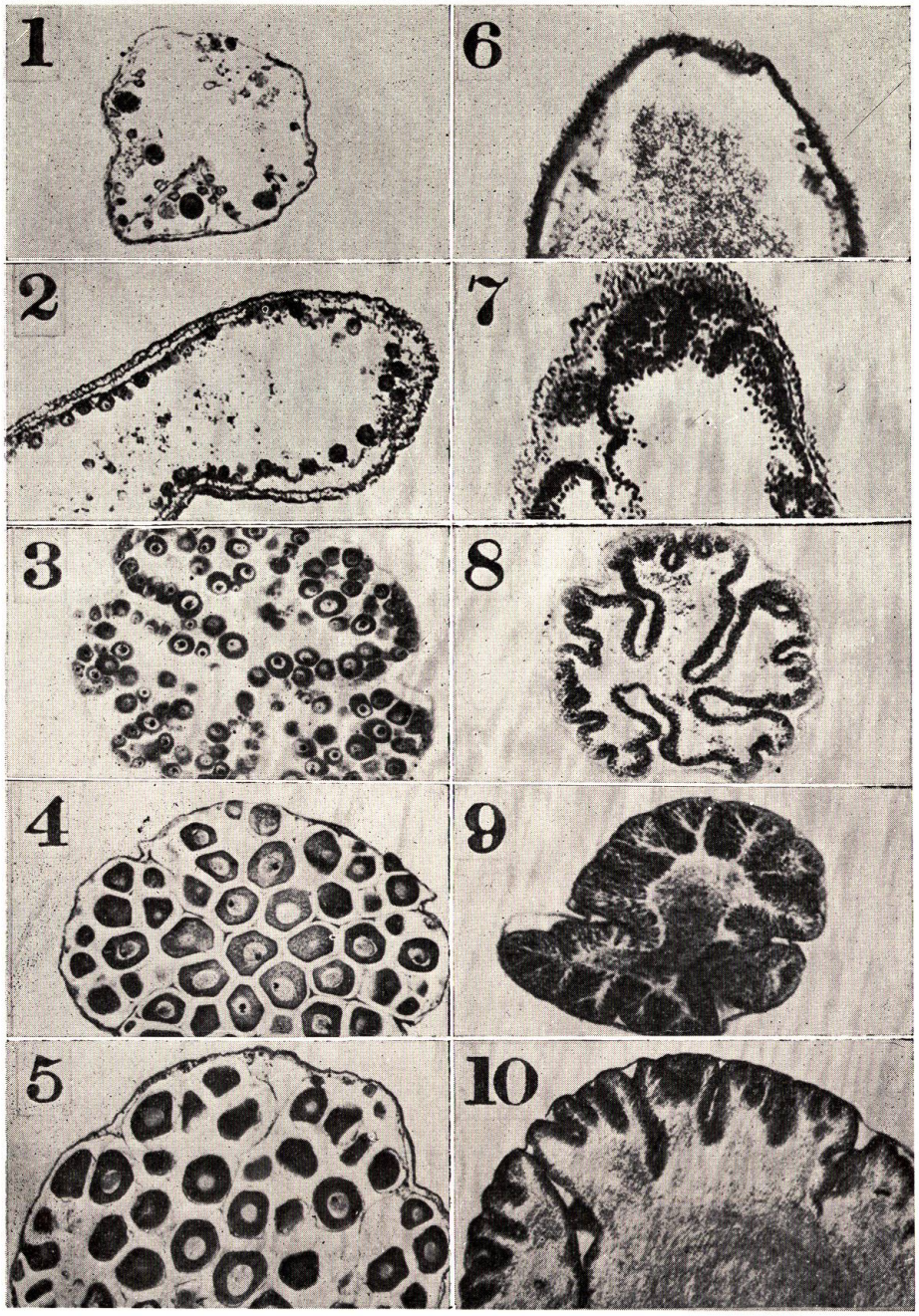
Appendix Seasonal changes of maturity of *Stichopus japonicus*

Date		Resting stage ♂	Resting stage ♀	Recovery stage ♂	Recovery stage ♀	Growing stage ♂	Growing stage ♀	Mature stage ♂	Mature stage ♀	Shedding stage ♂	Shedding stage ♀
1956	6–11					2	1	9	4	2	5
	7– 8		2					2	1	4	4
	8–11	3	4						2	4	4
	9– 1	3	2		1						
	10–15	6		4	5		1				
	11–15			6	8	2	4				
	12–14			2	3	5	9				
1957	1–25				1	10	4				
	2–25				2	7	8	1	2		
	3–27					8	4	5	1		
	5–25					2		9	4	2	2
	6–7,18							2	2	7	4
	7– 5	1	3							7	8
	8–10	13	4							2	
	9–11	9	2	1	1						
	11– 5	5	6	4	2						

Number of individuals examined is indicated in the table.

PLATE I. Microphotographs showing the stages in development and depletion of the gonad of *Stichopus japonicus*

Fig. 1. Female gonad; Resting stage. ×ca 54
Fig. 2. Female gonad; Recovery stage. ×ca 90
Fig. 3. Female gonad; Growing stage. ×ca 72
Fig. 4. Female gonad; Mature stage. ×ca 54
Fig. 5. Female gonad; Shedding stage. ×ca 54
Fig. 6. Male gonad; Resting stage. ×ca 80
Fig. 7. Male gonad; Recovery stage. ×ca 72
Fig. 8. Male gonad; Growing stage. ×ca 72
Fig. 9. Male gonad; Mature stage. ×ca 54
Fig. 10. Male gonad; Shedding stage. ×ca 54



Y. TANAKA: Gonad of *Stichopus japonicus*