HISTOLOGICAL OBSERVATIONS OF THE ANNUAL CHANGE IN THE GONAD OF THE STARFISH, *ASTERIAS AMURENSIS LÜKEN*

Yong Shik KIM*

The starfish, *Asterias amurensis*, the common species inhabiting the inshore waters has been generally regarded as one of the most destructive enemies for shellfishes. For example, the damage on the marketable shellfishes caused by the starfish in Tokyo Bay in 1954 amounted to as much as 400 million yen, according to the available data furnished by the Fisheries Experimental Station of Tokyo (1954). However, an actual damage inflicted by them may exceed this estimation for it should include the potential damage of the lost young shellfishes adding to the loss of the marketable shellfishes.

The growth and reproduction of the shellfishes are a sort of the important problems from the standpoint of shellfish culture. Consequently it is hoped to clarify the ecology of strafishes, which are the enemy of shellfish culture. Ecological studies on starfishes in Japan, however, have not been published except the works by Shoji (1939), Tamura & Fuji (1954), Ino *et al* (1955), and Hatanaka & Kosaka (1958). Although the above reports gave the many valuable informations on the biology of the starfish, they are not complete enough to understand the entire ecology of the starfishes.

For this reason the writer has commenced the study on its ecology throughout the life-span of the starfish, *Asterias amurensis*, inhiabiting in Mutsu Bay. An intensive accumulation of the informations on the reproductive ecology of starfish may be the most important study among many other ecological problems. For the detailed spawning habit of this animal supplies not only interesting informations on its life-history from the biological viewpoint, but also an important part of the basic knowledge to control this enemy.

The present paper deals with the gametogenetic cycle, seasonal change of gonad weight and the biological minimum size of the starfish, *Asterias amurensis*.

Before proceeding further, the writer wishes to express his cordial thanks to Emer. Prof. T. Tamura of Hokkaido University for his constant guidance and invaluable advice during the course of the work and for his kindness in reading the original manuscript. The writer also desires to acknowledge his great indebtedeness to Drs. H. Ohmi and A. Fuji of the same university for their many helpful suggestions. Thanks are offered likewisely to the staff members of Mutsu Bay

* *Laboratory of Marine Culture, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部鹹水増殖学講座)

Aquicultural Research Laboratory of Aomori Prefecture for the supply of animals studied.

Materials and Methods

The starfish, *Asterias amurensis*, was used for these studies on the development of the gametes and the seasonal change in gonad weight. Most of the starfish used in this study were from collections made from Mutsu Bay (Aomori Prefecture) once each month from January to December (1964), and an additional sample was collected in February 1966.

Thirty starfishes (arm length 40–110 mm) were dredged from a depth of about 10 m each month in 1964 and 150 individuals (arm length 30–100 mm) in February 1966. Thus approximately 500 individuals of starfish were examined throughout the course of this study. Each specimen was dissected by cuts extending from the center of the aboral surface towards the apex of each arm. After removal of the body fluid, measurements were made of body weight and arm length. The gonads were removed carefully and their wet weight taken. A small piece (ca. $2\times3\times2$ mm) was removed from the central part of the gonad and fixed in Bouin's solution for histological examination. The material was embedded in paraffin, cut into sections 6–8 μ in thickness, and stained with Delafield's haematoxylin and eosin.

Table 1. The number and sex ratio of *Asterias amurensis* examined

Months	No. of starfishes examined	Male (%)	Female (%)
Jan.	28	36.2	63.8
Feb.	29	71.6	28.4
Mar.	33	65.8	34.2
Apr.	34	48.9	51.1
May	30	40.3	59.7
June	32	38.1	61.9
July	35	45.5	54.5
Aug.	35	62.3	37.7
Sept.	31	57.4	42.6
Oct.	32	37.2	62.8
Nov.	33	75.6	24.4
Dec.	32	41.1	58.9
Average	32	51.66	48.34

Observation

General structure of the gonad: The starfish is dioecious with fertilization of the eggs taking place ectosomaticly. Distinction between male and female cannot usually be made by external appearance alone. In early autumn the gonad forms racemose branches surrounding the gonoduct on both sides of each ray extending from the base to the tip. As the gonads mature, they grow in size by January or February, they fill the entire body cavity. At this time the gonad cysts are well

developed with the largest ones nearest the base of the ray. It is apparently impossible to distinguish between gonads of males or females when their wet weight is below about 2 gr. and the color still slightly yellowish. However, as they develop the gonads of either sex are easily distinguished by the color; the overies become yellowish orange and the testes yellowish white. The sexual products are discharged from paired genital opening at the base of each ray. Soon after having discharged gametes, the gonads recede towards the base of the ray becoming semi-transparent and slightly yellowish in color.

Gametogenesis: In order to follow the development of the gonads throughout the year, it seemed most convenient to establish a series of arbitrary but easily recognizable stages in the reproductive cycle based on the shapes of the gametes and the space that the gametes occupy within the follicle. Seven stages covering the entire course of development of the gonads were chosen: Juvenile (Stage 0), Follicle (Stage 1), Early-growth (Stage 2), Later-growth (Stage 3), Pre-mature (Stage 4), Mature (Stage 5) and Spent (Stage 6). The characteristic features of each stage are described as follows:

Stage 0: This gonad stage is found in starfish with an arm length less than 50 mm. The gonad is semi-transparent and looks like a narrow silk thread with a length of 2–5 mm. At this stage it is impossible to recognize the existence of gametes in the transverse section of the goand stain with haematoxylin and eosin (Pl. 1, Fig. 1). In relatively advanced specimens the follicular cavity is filled with mesenchyme (Pl. 1, Fig. 2) which disappears with development, perhaps being absorbed by the gametes.

Stage 1: The follicular cavity is entirely empty, and the gonia can be recognized along the follicular wall. However, it is still impossible to determine sex (Pl. 1, Figs. 3 & 4). The size of the gonium is 1–2 μ in diameter and the gametocytes can not be recognized. Specimens belonging to this stage may be classified into two forms; one which has spawned in the preceding year and the other which is yet to spawn for the first time. The sections of these two forms are very similar in appearance except that larger empty spaces are found in the follicle of the former than in the latter. Moreover, sperms or oocytes may sometimes be observed in the follicular cavity of specimens which have spawned during the preceding year because of incomplete discharge.

Stage 2: An important feature at this stage is the appearance of oocytes and spermatocytes making it easy to differentiate between sexes.

Male: A section of the testis shows a layer of spermatocytes becoming thicker towards the center of the lumen, and spermatogonia attached to the follicular wall. It is easy to distinguish the spermatogonia from the spermatocytes when stained. A spermatocyte stains heavily with haematoxylin; the amount of cytoplasm is small and the nucleus large. The spermatogonium is surrounded

by a comparatively large amount of cytoplasm (Pl. 1, Figs. 5 & 7). The spermatogonium is 2–3 μ and the spermatocyte 3–4 μ in diameter.

Female: A section of the ovary in this stage of development can be easily recognized since it will contain a very large number of oogonia with a few young oocytes attached to the inside of the follicular wall (Pl. 1, Figs. 6 & 8). The size of the oogonium is 2–3 μ in diameter and that of the oocyte is 7–8 μ. Although the young oocytes contain a relatively small amount of cytoplasm, as the oocyte develops, it becomes richer in cytoplasm and extends towards the follicular cavity.

Stage 3: At this stage male and female starfish can be distinguished by the color of the gonads; the testes are yellowish white while the ovaries yellowish orange. The inside of the follicular wall is densely covered by a large number of cells and only the central part of the follicle is empty.

Male: The spermatocytes, which are attached to the follicular wall, increase markedly in number by fission, and form a thick layer leaving only the central part of the lumen. Spermiogenesis probably occurs at the inner surface of this thick layer; the spermatozoa is then thrust into the lumen (Pl. 2, Fig. 1). There is not much difference in the size of the spermatocytes between Stages 2 and 3.

Female: The oocytes which have increased in number while attached to the follicular wall, tend to free themselves from the wall and appear in the follicular cavity pear-shaped in form (ca. 7 μ by 12 μ in dimension). Some of free oocytes can be already seen in the lumen (Pl. 2, Fig. 2). In this stage the diameter of oocyte, which has become separated from the follicular wall, varies between 10 μ and 20 μ.

Stage 4: The gametes in both the ovaries and testes develop rapidly causing the gonads to increase markedly in size.

Male: The mature spermatocytes or spermatogonia fill the lumen in the center of the follicle (Pl. 2, Fig. 3). The spermatocytes form a cluster surrounding the spermatozoa, and spermatogenesis is actively in progress.

Female: Almost all of the pear-shaped oocytes, which had been attached to the follicular wall in the previous stage, are thrust into the lumen; the amount of cytoplasm in the oocytes also shows a marked increase. The oocytes at this stage of development attain a size of ca. 70 μ in diameter and each oocyte contains a germinal vesicle 20 μ in diameter (pl. 2, Fig. 4).

Stage 5: Well developed reproductive cell, spermatozoa or ova, fill the follicular cavity while a small number of young oocytes and spermatocytes are still present near the follicular wall. The racemose gonads attain their maximum growth in this stage and are tightly packed in the body cavity.

Male: The entire space of the follicular lumen is filled with a large number of spermatozoa although a few spermatocytes and spermatogonia are still found on the wall of the follicle. Because of the compact aggregation of the spermatozoa,

the central part of the follicular cavity stains deeply with haematoxylin; the number of spermatozoa is sparse near the follicular wall (Pl. 2, Fig. 5). Spermatogenesis has nearly stopped.

Female: All the available space in the follicular lumen is packed with circular-shaped ova 100–150μ in diameter; a few young oocytes persist near the wall. The germinal vesicles of these ova disappear at this stage (Pl. 2, Fig. 6).

Stage 6: After discharge of ova and spermatozoa the follicular cavity becomes almost empty although some young oocytes and spermatozoa may remain in the follicular lumen. The weight of the gonads decrease rapidly.

Male: The follicular wall shrinks although some relict sperm may remain within the follicular cavity (Pl. 2, Fig. 7).

Female: The ovary at this stage of development is characterized by the appearance of an empty space in the center of the follicle and by the presence of a small number of young oocytes attached to the follicular wall (Pl. 2, Fig. 8). These unspawned young oocytes will probably be absorbed. The follicular wall then shrinks rapidly.

Development of the gonads: The percentage of each of the six stages (excluding Stage 0 described above) is plotted by successive months in Fig. 1.

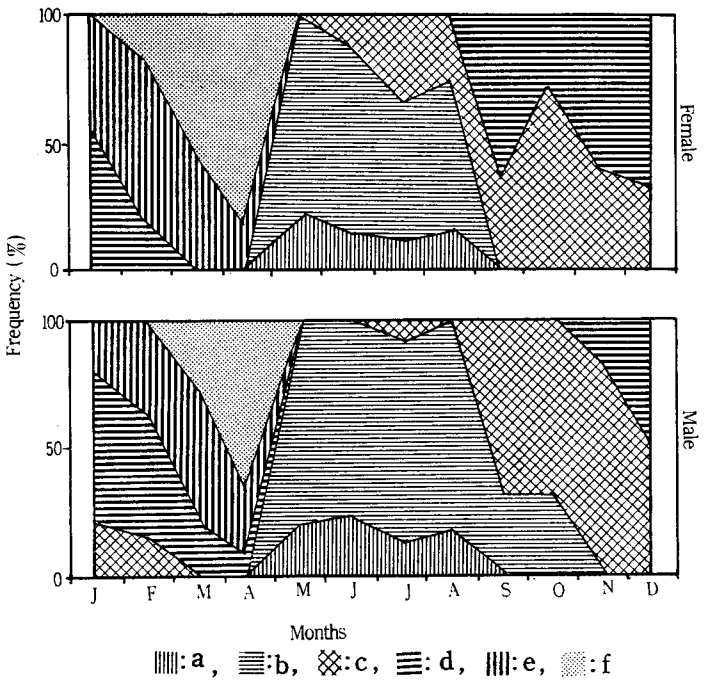

Fig. 1. The relative percentage of *Asterias amurensis* in each stage of gonad development
a: Stage 1, b: Stage 2, c: Stage 3, d: Stage 4, e: Stage 5, f: Stage 6.

The sex composition of specimens in Stage 1 could not be ascertained directly but was assumed to be 1:1 since nearly the same sex ratio was also found for adult starfish (cf. Table 1). It is evident from Fig. 1 that the occurrence of the successive stages of gonad development is closely correlated with the season. The general trend of seasonal change is summarized as follows:

In January 60% of the males and 55% of the females were in Stage 4; 20% of the males and 45% of the females were in Stage 5. In February, however, the proportion of both males and females in Stage 5 increased (39% males and 79% females); 11% of the females were in Stage 6. Thus, in January and Februrary more than 80% of the males and females were either in Stage 4 or Stage 5 of development. The number of individuals in Stages 4 and 5 decreased in frequency until early April; by mid-April the frequency of individuals in these two stages of development had dropped to a comparatively low proportion (27% for males and 16 % for females). The relative proportion of individuals in Stage 6 of development, on the other hand, increased markedly from March through April. The highest number of individuals in this stage of development was found in mid-April, 72% males and 83% females. Among the starfish collected from June to August, 94–100% of the males and 62–100% of the females were in either Stage 1 or 2. However, 10% of the females in June and 10% of the males in July were in Stage 3. Benginning in September, when 33% of the females and 67% of the males were found to be in Stage 3, the growth of the gametes progressed rapidly. Females in Stage 4 appeared in September and males in November. Females in Stage 2 disappeared from the collections by mid-September.

Seasonal change of gonad weight: By using an index of gonad development the variation in gonad weight for male and female starfish can be followed through-

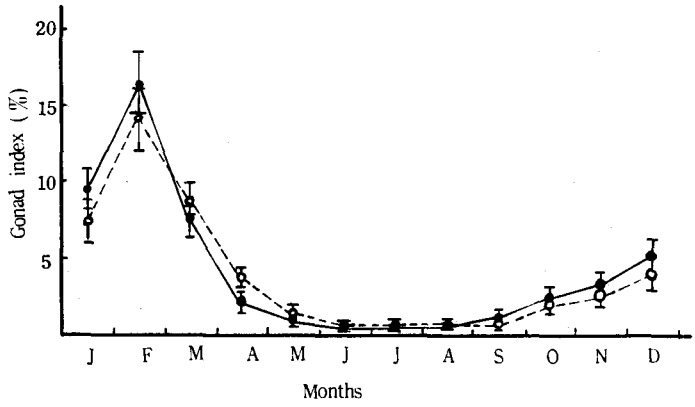

Fig. 2. Seasonal change in the gonad index for both sex of *Asterias amurensis*
Each vertical bar indicates the range (plus or minus) of the standard error of the mean.
●: Male, ○: Female.

out the year (Fig. 2). The gonad index (GI) indicates simply the percentage weight of the gonad calculated by the following formula:

$$GI = 100 \times GW/BW$$

Where GW is the gonad weight and BW the body weight. Although there is considerable difference between the values for male and female, the index values show a slowly increasing trend for both sexes during the period between September and December. However, the index begins to increase markedly in January and reaches a peak (an average of 14.2% for males and 16.7% for females) in Feburary. This abrupt change in the value of the index is caused by an increase in the amount of sexual products in the gonad. The index value begins to decrease in March and April and drops to a minimum of 1.0% for females and 1.5% for males in mid-May. These data clearly show that the period of reproduction for starfish begins in mid-February and is completed by approximately in mid-May. From June to August the minimum level of 0.3% to 0.5% is maintained and does not show any marked variation. Comparison between Fig. 1 and Fig. 2 indicates that the seasonal change of gonad index is closely associated with the development of the gametes. Namely, the values of the gonad index increase with development of the gametes and decrease with discharge of the gametes.

Annual reproductive cycle: Based upon both seasonal development of the gametes and seasonal change in gonad weight described above, the annual cyclic process of gonad growth of starfish in Mutsu Bay can be divided into the following four periods:

1) Maturing (January-February): In this period almost all individuals are either in Stage 4 or 5, and the value for the gonad index is at its peak.

2) Spawning (March-May): The number of individuals in Stage 5 decreases and the number in Stage 6 increases rapidly. The value of the goand index drops markedly.

3) Recovering (June-August): Specimens collected during this period consist mostly of individuals in Stage 1 or 2. The gonad index reaches its minimum value.

4) Growing (September-December): The number of individuals in Stage 3 increases, and that in Stage 4 begins to appear in November. The value of the gonad index increases slightly.

Relation between arm length and development of the gonad: From the information on the development of the gametes and the seasonal change in gonad weight, it is clear that the maturing period for gonads of starfish found in Mutsu Bay extends from January through February. Therefore, February was chosen as the best time to observe the relation between gonad development and size of the individual. A hundred and fifty specimens with arm lengths ranging from 30 mm to 100 mm were collected from Mutsu Bay in February 1966. The relation between

the arm length and the gonad weight of these animals is shown in Fig. 3. This Figure shows that the rate development of gonads in individuals over 55 mm in arm length differs significantly from individuals which are under 55 mm in arm length. The two exponential curves cross at an arm length of about 55 mm.

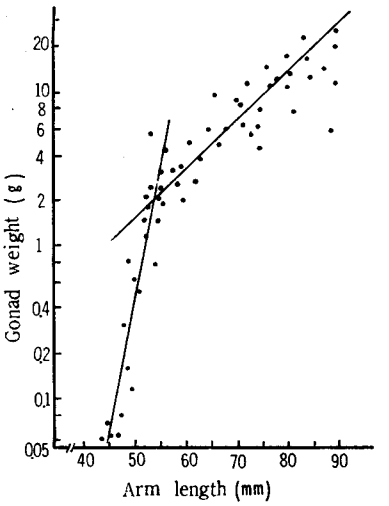

Fig. 3. Relation of gonad weight to arm length (from samples collected in February 1966)

The percentage of individuals of various arm length in each stage of gonad development is shown in Fig. 4. All individuals with arm lengths under 40 mm are in Stage 0, and individuals with arm lengths from 40 mm to 50 mm have gonads in an early stage of development (Stage 0, 1, or 2). The early stages of gonad development are also found in specimens with arm length of 50 mm to 55 mm; 50% of the females and 30% of males had gonads in either Stage 4 or 5. Mature gonads (Stage 4 or 5) are predominant specimens with arm lengths of 55 mm or more. From the results shown in Figs. 3 and 4, it is concluded that individuals of *Asterias amurensis* first reach sexual maturity when the arm length is about 55mm. Most individuals with arm lengths between 40 mm and 55 mm in February still possess immature gonads. These animals would not be able to attain full maturity in time to spawn in that smae year.

To summarize, the population of *Asterias amurensis* can be dividided into three different groups according to arm length and the degree of gonad growth;
1) Juvenile: This group consists of individuals with arm lengths under 40 mm. The sex of the individuals can not be recognzied.
2) Growing: Individuals in this group possess immature gonads. The arm lengths vary between 40 mm and 50 mm.
3) Adult: Specimens in this group have arms measuring over 55 mm in length. Their gonad growth shows an annual cyclic change.

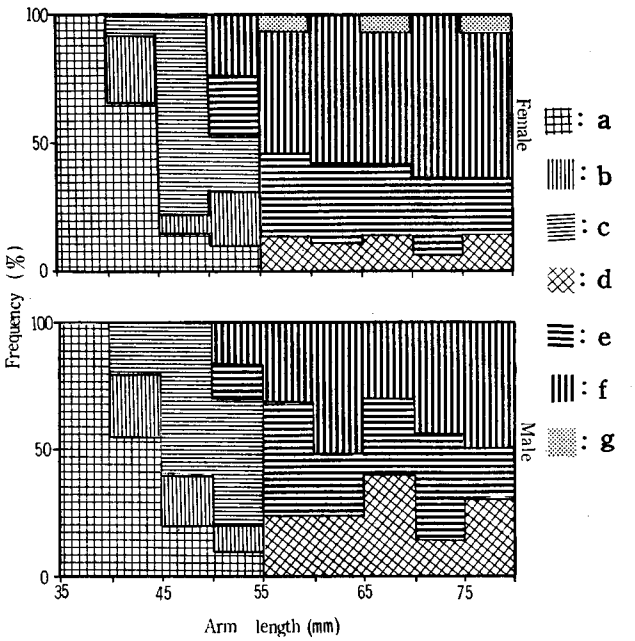

Fig. 4. Relation of each gonad stage to arm length for *Asterias amurensis*
a: Stage 0, b: Stage 1, c: Stage 2, d: Stage, 3,
e: Stage 4, f: Stage 5, g: Stage 6.

Discussion

Several different systems of subdividing the entire developmental process of echinoderm gametes have been reported. Vevers (1949) divided the developmental process of gametes of *Asterias rubens* into six stages. Fuji (1960) described six stages for *Strongylocentrotus intermedius*. Tanaka (1958) proposed five stages for *Stichopus japonicas* from Hokkaido, but Choe (1963) divided the gametes development for the same species from Aichi Prefecture into only three stages. In the present study development of the gametes of *Asterias amurensis* has been divided into seven stages. These subdivisions are, in principle, arbitrary and depend upon the species as well as on the author's definition. The subdivisions described above are considered to be a practical system that divides the entire process into a reasonable number of stages which are easily recognized by examination with an ordinary microscope.

Ino *et al.* (1955) reported that the breeding season for the starfish, *Asterias amurensis*, in Tokyo Bay was from late February to early March when the water temperature was about 11°C. From the results of observations shown in Figs. 1 and 2, the breeding season of *Asterias amurensis* in Mutsu Bay covers a period from

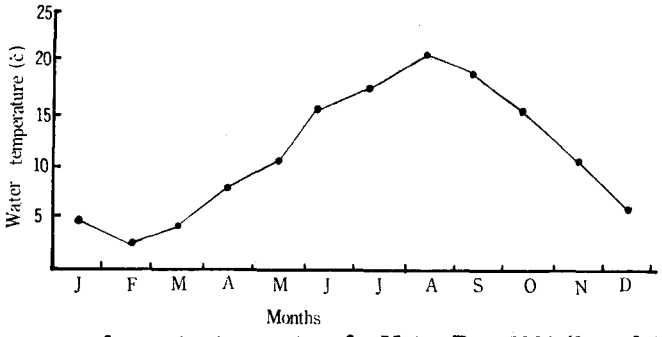

Fig. 5. Average surface water temperature for Mutsu Bay, 1964 (from data collected by Mustu Bay Aquicultural Research Laboratory)

late Feburary to early May; the most active spawning occurs in March and April. Fig. 5 summarizes the surface water temperatures obtained by members of the Mutsu Bay Aquicultural Research Laboratory, Aomori Prefecture. The temperatures during spawning season of *Asterias amurensis* in Mutsu Bay range between 5° and 10°C. Water temperature during the spawning season for *Asterias forbesi* in Long Island Sound was 15°C (Galtsoff and Loosanoff, 1939), and that for *Asterias rubens* in Plymouth Sound was 10°C (Vevers, 1949). Thus even for the same species, *Asterias amurensis*, the spawning season and the water temperature differ markedly according to geographical locality.

The limited capacity to move and the short distance that these benthic animals move during their entire life cycle make their distribution highly localized and well adapted to the environmental conditions in which they live. Giese (1958) and Pearse (1965) cite several physical and chemical environmental factors (including food) which may effect development of the gonads, but just how these factors act remain to be proven.

Ino *et al.* (1955) states that the arm length of *Asterias amurensis* in Tokyo Bay was 46 mm for female and 47 mm for male at first maturity. The present study, however, shows that for the same species in Mutsu Bay, the arm length will be more than 55 mm for both male and female individuals with mature gonads as understood in Fig. 4.

Pearse (1965) reported that young oocytes found in the mature follicle of the gonad of *Odontaster varidus* from the Antarctic Ocean remained in the follicle and began to proliferate in the succeeding year's spawning period; the total period of growth of the oocytes was between 18 and 24 months. In the present study it seems that the young oocytes found immediately after the spawning were either absorbed or discharged later because none were found in the follicular wall during the recovery period. Thus the period of oocyte growth in the gonad of *Asterias amurensis* in Mutsu Bay is about 9 months (Fig. 1).

According to Kurata and Kajimura (1954) the sex ratio of *Asterias amurensis* in Tokyo Bay was 1:1.24, and according to Ino *et al* (1955), 1:1. Fuji (1960) examined 1,748 individuals of *S. intermedius* taken from various areas around Hokkaido and found a sex ratio close to unity. The sex ratio obtained in the present study was 1:1.09 for all individuals except for the sex for the juvenile which, as mentioned above, could not be identified. Thus, the sex ratio for *Asterias amurensis* is also considered to be 1:1.

Summary

1. The development of the gonad and relation between arm length and gonad development of *Asterias amurensis* have been critically studied.

2. The diameter of the mature ovum varied between 100 and 150 μ.

3. Development of gamete can be divided into seven stages: Juvenile, Follicle, Early-growth, Later-growth, Pre-mature, Mature and Spent.

4. The index of gonad development peaks in February (14.2% for male and 16.7% for female) and drops to a minimum of 0.3% (both male and female) in June.

5. The annual cyclic process of gonad development is divided into the following four periods: Recovering (June-August), Growing (September-December), Maturing (January-February) and Spawning (March-May)

6. Individuals attaining first maturity have an arm length of about 55 mm.

7. The population of *Asterias amurensis* is divided into three separate group by the arm length to correspond to the degree of gonad growth: Juvenile, Growing and Adult. Water temperature in the spawning season ranged between 5 and 10°C.

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Explanation of Plates

PLATE I

Asterias amurensis

Photomicrographs of transverse sections through gonads in various stages of maturity.

Figs. 1 and 2. Stage 0 goad (Juvenile). × 300

Fig. 3. Stage 1 gonad (Follicle). × 100

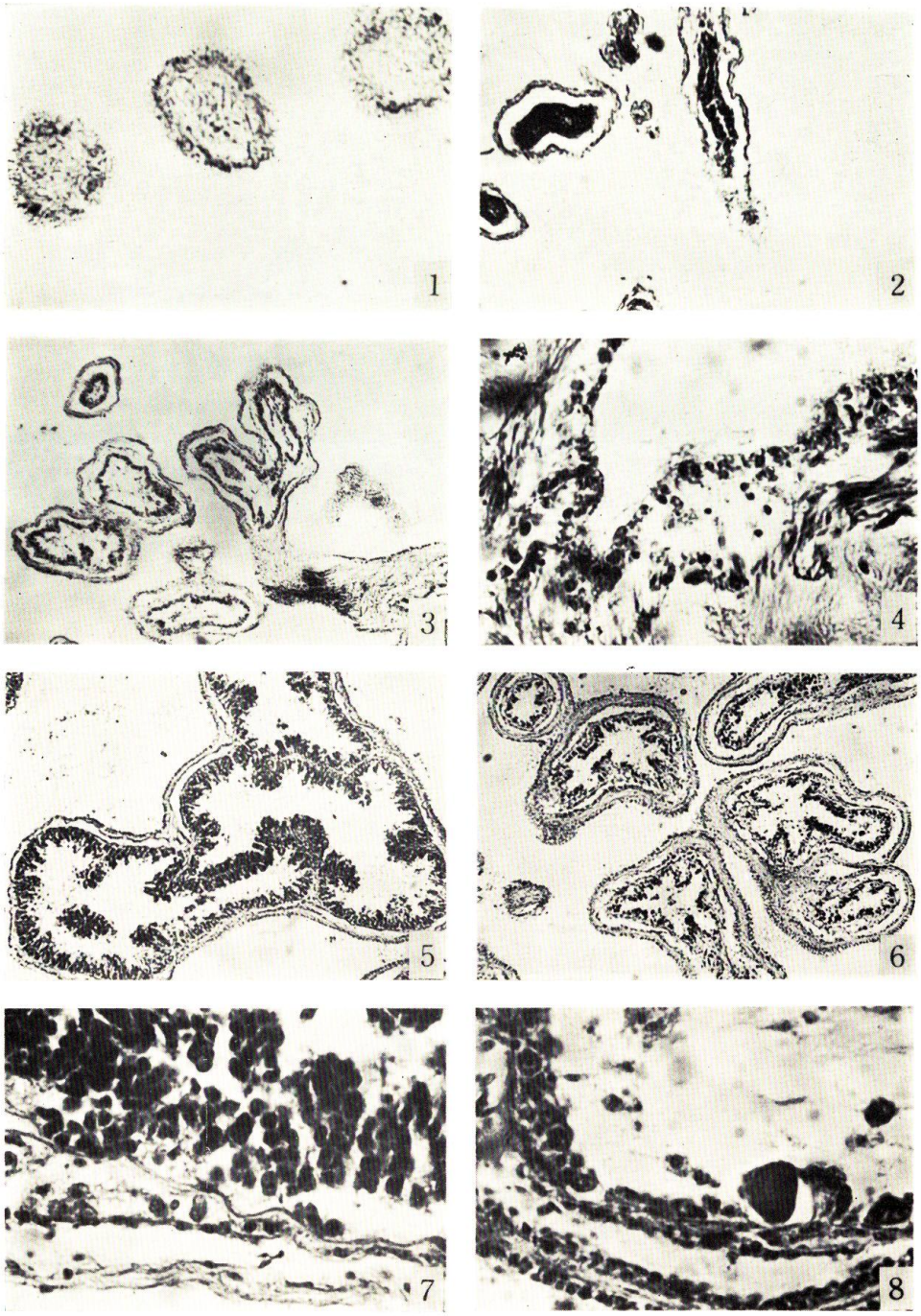
Fig. 4. Stage 1 gonad (Follicle). × 600

Fig. 5. Stage 2 testis (Early-growth). × 100

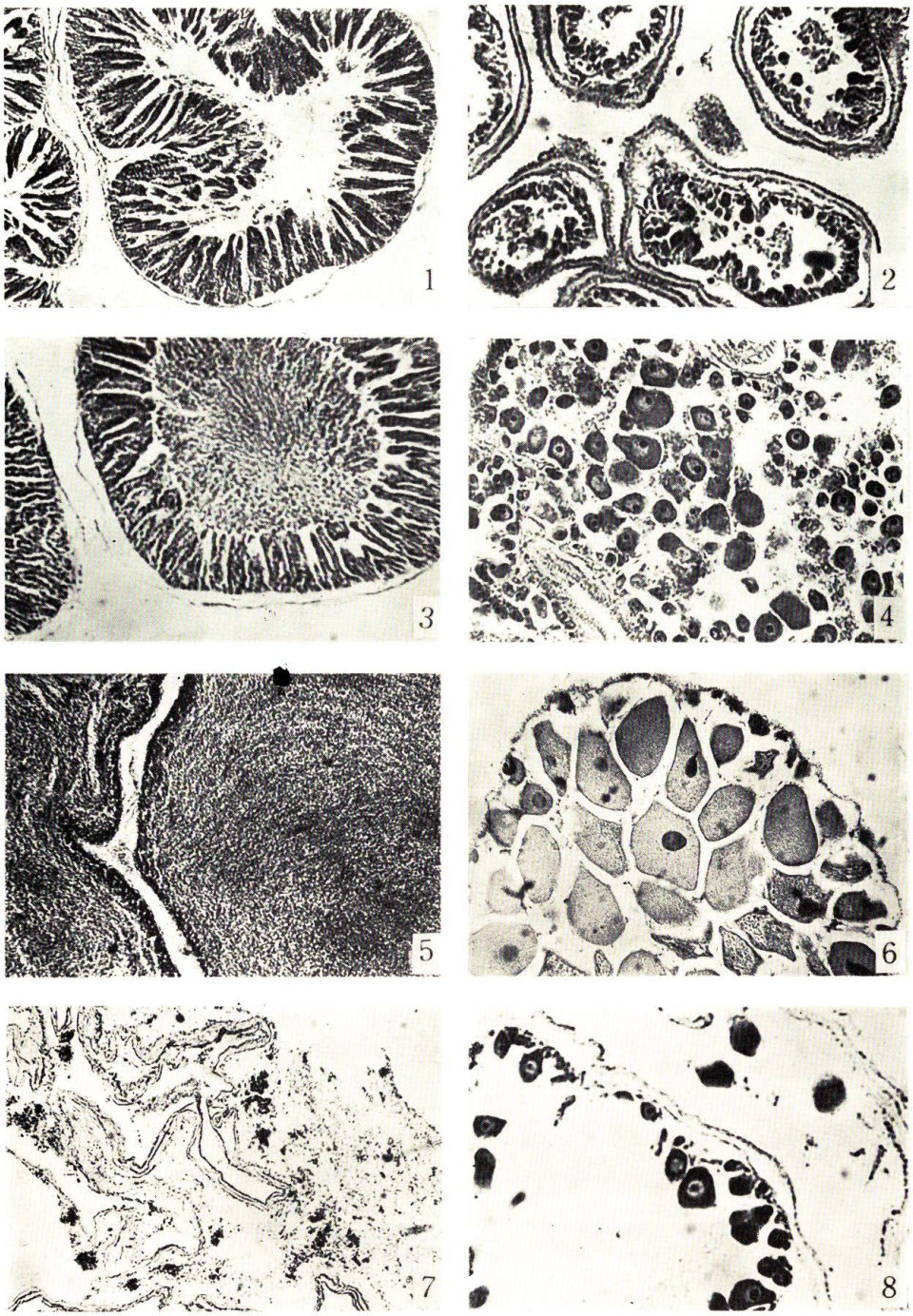
Fig. 6. Stage 2 ovary (Early-growth). × 100

Fig. 7. Stage 2 testis (Early-growth). × 600

Fig. 8. Stage 2 ovary (Early-growth). × 600



Y. S. KIM. : Annual change in the gonad of the starfish.



Y. S. KIM. : Annual change in the gonad of the starfish.

PLATE II

Same as in Plate I.

Fig. 1. Stage 3 testis (Later-growth). × 100
Fig. 2. Stage 3 ovary (Later-growth). × 100
Fig. 3. Stage 4 testis (Pre-mature). × 100
Fig. 4. Stage 4 ovary (Pre-mature). × 100
Fig. 5. Stage 5 testis (Mature). × 100
Fig. 6. Stage 5 ovary (Mature). × 100
Fig. 7. Stage 6 testis (Spent). × 100
Fig. 8. Stage 6 ovary (Spent). × 100