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Gametogenesis and Reproductive Cycle of the Limpet *Collisella heroldi* (Dunker, 1861)

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Abstract

The sex ratio, gametogenesis, reproductive cycle and the size at first maturity of the limpet *Collisella heroldi* were studied using the samples taken monthly from the intertidal rocky shore in Usujiri, Hokkaido. Anatomical or histological distinguishing of the sex revealed a minimum sex differentiation size of 3 mm and a sex ratio of about 1 : 1 in all 1 mm size groups of the larger animals, suggesting that there is no sex change in *C. heroldi*. The developmental process of germ cells was divided into six stages as the following: In female, there were oogonia, synaptic oocytes, previtellogenic oocytes, oil globule oocytes, vitellogenic oocytes and mature oocytes while in male, there were primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. On this base, the developmental process of the gonads was examined and revealed an annual breeding cycle which was composed of resting period: November-March: developing period: April-July; spawning period: August-October. The distribution of gonadal stages in each 1 mm size group during mature period of July 1988, showed a minimum reproduction size of 5 mm in shell length.

Introduction

The small limpet *Collisella heroldi* which is mainly distributed from southern Hokkaido to Taiwan, is a common member found in the intertidal rocky shore organic community. By now, several works have been made on the reproductive cycle of the limpets (Orton, 1928, 1956, 1961; Balaparameswara Rao, 1973; Blackmore, 1969; Picken, 1980; Fletcher, 1987). But little is known about *C. heroldi*. The studies about the gametogenesis of the limpets are very few, too. This paper deals with sex phenomenon, gametogenesis, reproductive cycle and the size at first reproduction of *C. heroldi*. This work will give more data to the studies of the reproductive ecology of the limpets. As *C. heroldi* plays a important role in the intertidal rocky shore community in Japan as a dominant spices and grazer, the present paper will also contribute useful data to the studies of the intertidal rocky shore community in Japan.

Materials and Methods

Samples were collected monthly from May 1987 to December 1988 along the intertidal rocky shore Benten-jima at Usujiri, Hokkaido (42°21′N; 140°57′E). About 900 individuals involving every size group were examined sex mostly from gonad colour, and for those which could not be distinguished sex from their gonad

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colour, the sexes were identifled from histological sections to obtain the sex ratio and the size of sex differentiation. In each month, about 50 individuals measuring over 6 mm in shell length were fixed in Bouin's fluid for 24 hours, and then dehydrated in series of alcohol. Normal paraffin embedding method was followed. The whole soft body was cut into 5-6 μm cross-sections, and stained in hematoxylin followed by eosin as a counterstain for histological observation. In July and August 1988 when the gonads of the samples were observed to be well developed, about 200 individuals under 6 mm and 100 individuals over 6 mm in shell length were examined histologically to understand the size at first maturity. Monthly, about 30 samples were fixed in 10% seawater formalin for at least one month so as to separate the gonad from the whole tissue easily, and then dried separately at 60°C for 48 h. The GSI was calculated as the relative amount (%) of gonadal material, compared to the total weight of somatic and gonad.

Results

The gonad of *C. heroldi* lies on the ventral side of the visceral mass between this and the foot. Therefore it was observed by cutting open the foot. During the breeding season, the female gonads had a reddish violet colour while the male gonads had a cream colour. Both female and male gonads were well developed in thickness and covered the whole ventral side of the visceral mass. In other seasons, it was very difficult to distinguish the sex based on gonad colour. The size of gonad was also very small, just like a layer of membrane lying along the ventral side.

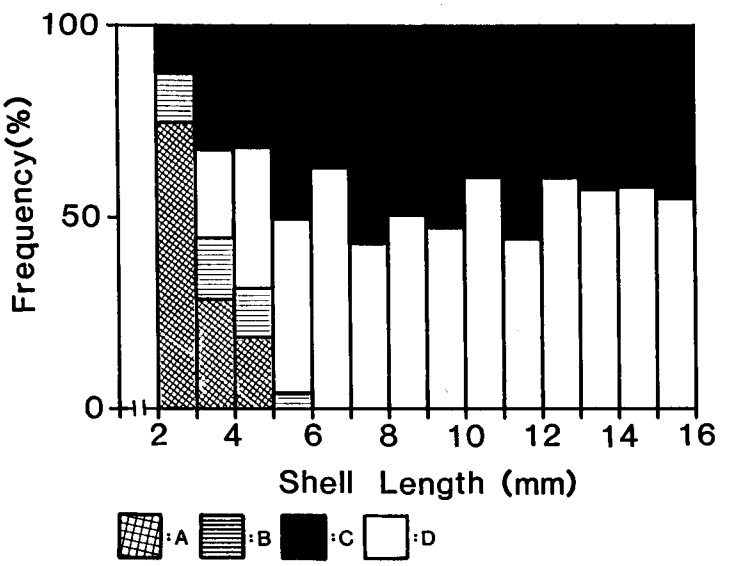

Fig. 1. Sex distribution in each 1 mm size group.
A: Limpets with no gonad, B: Neuter, C: Male, D: Female.

(1) Size at sex differentiation and sex ratio

The results of sex differentiation and sex ratio are shown in Fig. 1. The sexes of some samples could not be distinguished both from the external appearance and from histological sections of their gonads. These were classified as neuter. Approximately 87.5% of the individuals in 2-3 mm size group were found to have no gonad or were classified as neuter. In 3-4 mm size group, the sex of more than 50% individuals could already be distinguished. Based from these, the minimum size of sex differentiation was 3 mm in shell length. In size groups measuring over 3 mm which belong to the 1 mm size interval, the ratio of female and male was uniform and about 1 : 1.

(2) Developmental process of germ cells

The developmental process of germ cells was divided into six stages following the classification of Tomita (1967) and Peredo et al. (1987).

A. Female germ cells

Oogonia: The nucleus of an oogonium was slightly bigger than that of a spermatogonium with a diameter of about 4-4.5 μm. It was slightly basophilic and the chromatin were scanty. A heavily stained nucleolus was present in the nucleus. The cytoplasm was also slightly basophilic and could not be seen clearly. The oogonia were usually gathered near the germinal epithelium (Plate I, Fig. 1).

Synaptic oocytes: The synaptic oocytes were formed after the pairing of the chromosomes of the oogonia. The diameter of the oocytes in this stage was about 7.5-10 μm. A large spherical nucleus of about 6.6 μm in diameter could be observed. One or more nucleoli measuring about 2 μm in diameter could also be seen within the nucleus. The whole cell was slightly basophilic and could not be seen clearly (Plate I, Fig. 2).

Previtellogenic oocytes: Previtellogenic oocytes were pear shape measuring 10-25 μm $\times$ 20-60 μm (short axis $\times$ long axis) and basophilic. It had a large and negatively stained nuclueus (5-20 μm in diameter) with one or two intensely stained nucleoli. The other side of the cell which had a stalk-like appearance was connected to the follicle wall (Plate I, Fig. 3).

Oil globule oocytes: With the development of previtellogenic oocytes, some spherical globules which were negatively stained, occurred in the cytoplasm. Tomita (1967) reported that these were oil globules. In the present study, the oocytes in this stage were called oil globule oocytes. During the early stages, the oil globules in a cell were few in number but large in size. As the development of the cells advanced, the oil globules increased in number but became smaller. Yolk granules could be observed within the basophilic cytoplasm. The cells were approximately 18-32 μm $\times$ 32-125 μm (short axis $\times$ long axis) in size. The diameter of the single nucleus in a cell was 18-32 μm. There were one or more nucleoli in the nucleus (Plate I, Fig. 4).

Vitellogenic oocytes: Vitellogenic oocytes were oval or round in shape measuring about 103 μm in diameter. The cell had a large negatively stained nucleus (50 μm in diameter) with a single nucleolus. Yolk granules were scattered evenly in the cytoplasm, and were acidophilic. A glue-like belt could be seen clearly around the cell (Plate I, Fig. 5).

Mature oocytes: The main difference between mature oocytes and vitellogenic oocytes was that the nuclei of the former could not be seen clearly. The cells were approximately round measuring 120-140 μm in diameter (Plate I, Fig. 6).

B. Male germ cells

Primary spermatogonia: A spermatogonium was composed of a thin layer of cytoplasm and a spherical or slightly oval nucleus measuring about 3.5-4 μm in diameter. Since the cytoplasm was very difficult to stain, the outline of the cell could not be distinguished. Inside the nucleus, the scanty and finely granular chromatin were distributed evenly and there were one or two nucleoli in it (Plate II, Fig. 1).

Secondary spermatogonia: These germ cells were formed after the primary spermatogonia underwent mitosis. The nuclei of secondary spermatogonia were about 3-3.5 μm in diameter, slightly smaller and more basophilic than the primary spermatogonia and lie close to the latter (Plate II, Fig. 2).

Primary spermatocytes: The primary spermatocytes were formed as a result of mitosis of the secondary spermatogonia. Although the nuclear diameter of both cells were similar, the primary spermatocyte could be distinguished easily from the secondary spermatogonia by their thick granular chromatin scattered randomly or gathered to one side in the nuclei (Plate II, Fig. 3).

Secondary spermatocytes: Secondary spermatocytes were the result of meiotic division of the primary spermatocytes. The nuclear diameter of secondary spermatocytes was approximately 1.5-2 μm which was about half the size of the earlier stage. They often gathered together and intermingled with spermatids (Plate II, Fig. 4).

Spermatids: Spermatids were derived from the second meiotic division of secondary spermatocytes. These cells had small round nuclei (1 μm in diameter) which were heavy basophilic (Plate II, Fig. 5).

Spermatozoa: Spermatozoa were formed after the metamorphosis of spermatids. The mature spermatozoon which was heavily stained had a triangular head of approximately 2.2 μm in length and a flagella of about 13 μm long. The acrosome could not be visualized owing to the small size of the sperm (Plate II, Fig. 6).

(3) Developmental stages of gonads

The developmental stages of female gonads were classified according to the stage of germ cells dominant (over 50% of the total number of germ cells) in a section. In males it was the stage of germ cells dominant (over 50%) in a given area of a section. The developmental stages of male and female gonads were also classified into 6 stages separately.

A. Female gonads

Resting stage: The gonads were composed of connective tissues, oogonia, synaptic oocytes and a few previtellogenic oocytes (Plate III, Fig. 1).

Early active stage: The number of previtellogenic oocytes were over 50% of the total germ cells. Oogonia and synaptic oocytes were few and were seen embedded in the walls of gonadal follicles or scattered near the edge of gonad. The early oil globule oocytes appeared in some gonads (Plate III, Fig. 2).

Late active stage: The late oil globule oocytes and the acidophilic stained vitellogenic oocytes could be seen. But the germ cells in this stage were also mainly

previtellogenic oocytes. The oogonia and synaptic oocytes were scarce, and were distributed at the edge of the gonad (Plate III, Fig. 3).

Mature stage: Ripe ovary was mainly composed of vitellogenic and mature oocytes. Few previtellogenic oocytes were scattered near the edge of the ovary (Plate III, Fig. 4).

Spawning stage: The mature oocytes were separated from each other. Between them were oogonia, synaptic oocytes, and previtellogenic oocytes. The main cells were also mature oocytes (Plate III, Fig. 5).

Spent stage: Connective tissues, oogonia, synaptic oocytes, a few previtellogenic oocytes and some remaining mature oocytes were seen in this stage (Plate III, Fig. 6).

B. Male gonads

Resting stage: In this stage the gonads were composed of connective tissues, primary spermatogonia and occasionally a few spermatozoa which were remnants of the previous spawning (Plate IV, Fig. 1).

Early active stage: After the number of primary and secondary spermatogonia

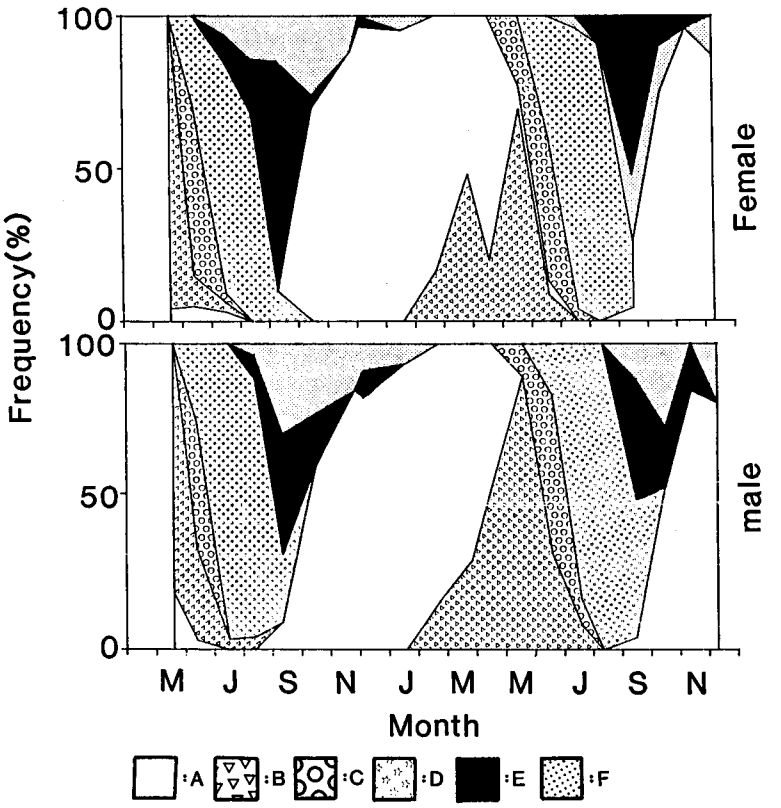

Fig. 2. Diagram showing the relative proportion (%) of animals in each gonad stage.
A: Resting stage, B: Early active stage, C: Late active stage, D: Mature stage, E: Spawning stage, F: Spent stage.

increased sharply, spermatocytes of both stages also appeared. The walls of gonadal follicles became thicker and could be seen clearly. A few spermatozoa were present at the center of the follicles. Sperms were not commonly seen in this stage (Plate IV, Fig. 2).

Late active stage: A number of sperms appeared at the center of the follicles and formed radially oriented columns with the tails toward the center of the follicles. The area covered by sperms was less than 50% of the total area in one section (Plate IV, Fig. 3).

Mature stage: Germ cells in this stage were mainly sperms. The follicular walls of the gonad were very thin and the limits between follicles were poorly defined (Plate IV, Fig. 4).

Spawning stage: Several spaces could be observed in the gonads. The walls which composed of germ cells in early stages had developed slightly. The sperms were divided into several small groups by these walls and spaces (Plate IV, Fig. 5).

Spent stage: The gonads were mainly composed of primary connective tissues, spermatogonia and some remaining sperms (Plate IV, Fig. 6).

(4) Reproductive cycle

The monthly distribution of each gonad stage is shown in Fig. 2. Both female

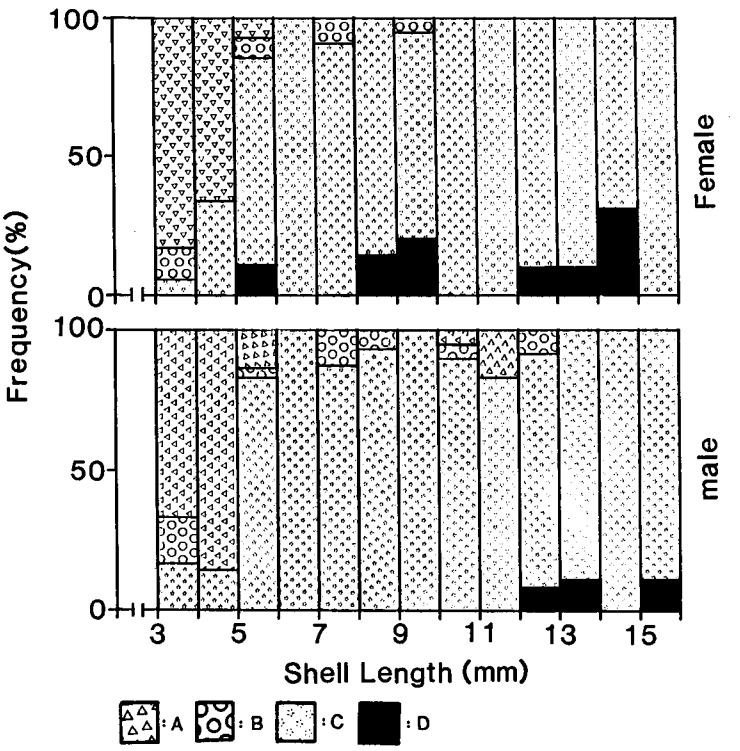

Fig. 3. Gonad stage frequency distribution.
A: Early active stage, B: Late active stage, C: Mature stage, D: Spawning stage.

and male gonads began to develop rapidly starting in April, and in May about 90% were in the early or late active stage. In June, approximately 30% of the gonads were mature and increased to more than 90% in July. The gonads in the spawning stage were observed in August. In September, more than 70% were in the spawning or spent stages and at least 70% went into resting stage in the following month. Most of the gonads were observed to remain in the resting stage until April of the next breeding cycle. This observation suggests that the spawning of *C. heroldi* occurred once yearly and the period was from August to October with the peak of spawning occurring in early September. The annual breeding cycle was described as follows: developing period, April-July: spawning period, August-October; resting period, November-March.

(5) Size at first maturity

The result is shown in Fig. 3. It is clear that both in female and male samples over 5 mm size groups, approximately more than 90% of the gonads were in the mature or spawning stage, whereas in under 5 mm size groups, at least 60% individuals were in the early active stage. It was reported that the early active stage gonads during a mature or spawning period could not reach maturity in the same year in the sea urchin (Fuji, 1967). Therefore, the size at first maturity was about 5 mm in *C. heroldi*.

Discussion

The sex ratio of *C. heroldi* was uniform and about 1 : 1 in the present study. This is different with the well known common European limpet *Patella vulgata*, which is typified by the predominance of males in the smaller size group and females in the larger size group (Orton, 1928; Orton et al., 1956; Thompson, 1980). Similar result was found by Balaparameswara Rao (1973) in the Indo-pacific limpet *Cellana radiata*. For the reason suggested by Balaparameswara Rao, there is no sex change in *C. heroldi*.

Although the gametogenesis of *C. heroldi* is followed almost the same process found in other mollusks such as the clam *Mesodesma donacium* (Peredo, 1987) and the abalone *Haliotis discus hannai* INO (Tomita, 1967), there is a little difference between the former and the later. While there is no free eggs observed during spawning stage and the mature oocytes have intact germinal vesicle in the later, mature oocytes in the ovary of *C. heroldi* was found free from each other during spawning period. Nuclei of the mature oocytes were indistinct. Fertilized eggs could be got with artificial fertilization, indicating that the mature oocytes have finished both morphological and physiological maturity.

The monthly histological and quantitative examination (GSI) of the gonads and the sea water temperature showed a close relationship comparing Figs. 2, 4 and 5. From October to April when the sea water temperature went down to the lowest value in February and remained a low level, most of the limpets (more than 50%) were in the resting stage. Parallel to that, a GSI value of 5% was observed in October and the low level was remained. Recovery gonads occurred in March when the sea temperature began to rise. From May to August accompanying the quick rising of sea water temperature, most of the gonads of the limpets showed a active

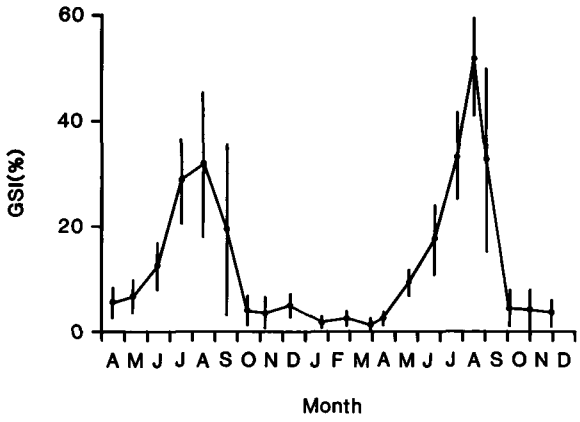

Fig. 4. Seasonal changes of gonadosomatic index. Each vertical bar shows the range of standard deviation.

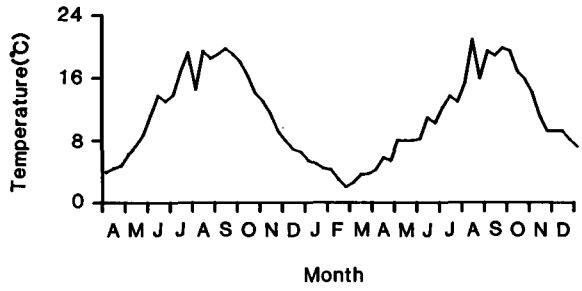

Fig. 5. Sea water surface temperature at Usujiri during the study period.

and synchronous development both histologically and quantitatively. More than 90% individuals were in ripe stage and the rest came into spawning stage in August while the GSI reached a peak of 32% and 51.9%, in 1987 and 1988 respectively. This development may be because of the increase of the algal food in spring. The reason for the difference of the peak value of GSI between 1987 and 1988 has not been known. There needs more study on the effects of both organic and inorganic factors on the reproduction of the limpet. Spawning began in August and continued until January, but most of the individuals spawned within a short period which was between late August and early September when the seawater temperature began to decrease. The GSI also began to decrease corresponding to the spawning stage. In October most of the gonads came to rest stage and the GSI decreased sharply to the low value of about 5%, and a distinct reproductive cycle finished. This pattern is similar with the European limpet *Patella vulgata* (Orton et al., 1956). But many other limpets spawn twice or more during one year such as the limpet *Patelloidi alticostata* (Fletcher, 1987) and the Indo-pacific limpet *Cellana radia* (Balaparameswara Rao, 1973). Balaparameswara Rao (1973) suggested that the

extended breeding season and the absence of a resting sense in *C. radiata* was due to the uniformly high temperature in a tropical location. Fletcher (1987) found the spawning activity of the same species of *P. alticostata* was different with locations. The population of *P. alticostata* in more cold region had one period of spawning as *C. heroldi* and *P. vulgata*. It is clear that the sea water temperature plays a important role in the reproductive activity of the limpets. As *C. heroldi* has a wide distribution, the study of the reproductive activity of *C. heroldi* in other location will be expectancy.

Orton et al. (1956) and Balaparameswara Rao (1973) found that the rough seas caused by high wind speed induced spawning. In the present study there is no exact data about the factors stimulating spawning. But it seems that September is typified with rough seas. Picken (1980) found the rising of the sea water temperature could induce spawning of the limpet *Nacella concinna*. Branch (1981) suggested that spawning occurred at a time of rising or high sea surface temperature in species with planktotrophic larvae because the latter was linked with increased light intensity and production of phytoplankton on which limpet larvae feed. In the present study the spawning occurred from August when the sea temperature was high, but it was in a period following the decrease of sea temperature. More studies should be conducted on the spawning stimuli and then on the reproductive strategy of *C. heroldi*.

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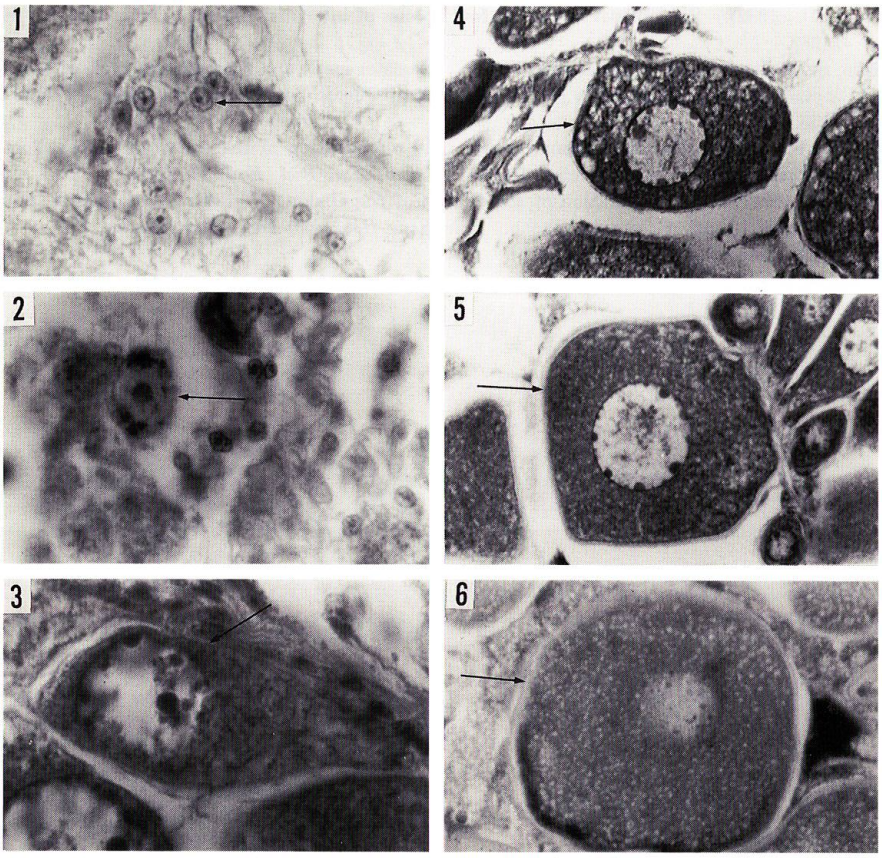

PLATE I. Histological microphotograph showing various female germ cells.

Fig. 1. Oogonia (arrow). × ca. 1000
Fig. 2. Synaptic oocyte (arrow). × ca. 1000
Fig. 3. Previtellogenic oocyte (arrow). × ca. 1000
Fig. 4. Oil globule oocyte (arrow). × ca. 400
Fig. 5. Vitellogenic oocyte (arrow). × ca. 400
Fig. 6. Mature oocyte (arrow). × ca. 400

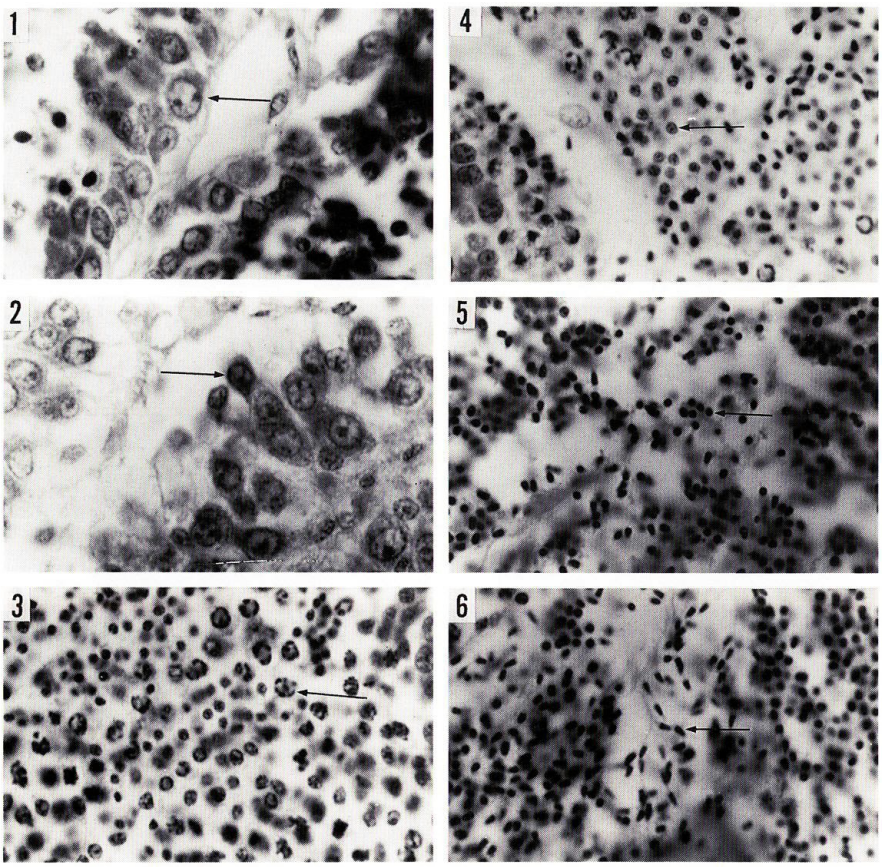

PLATE II. Histological microphotograph showing various male germ cells.

Fig. 1.	Primary spermatogonia (arrow).	× ca. 1000
Fig. 2.	Secondary spermatogonia (arrow).	× ca. 1000
Fig. 3.	Primary spermatocytes (arrow).	× ca. 1000
Fig. 4.	Secondary spermatocytes (arrow).	× ca. 1000
Fig. 5.	Spermatids (arrow).	× ca. 1000
Fig. 6.	Spermatozoa (arrow).	× ca. 1000

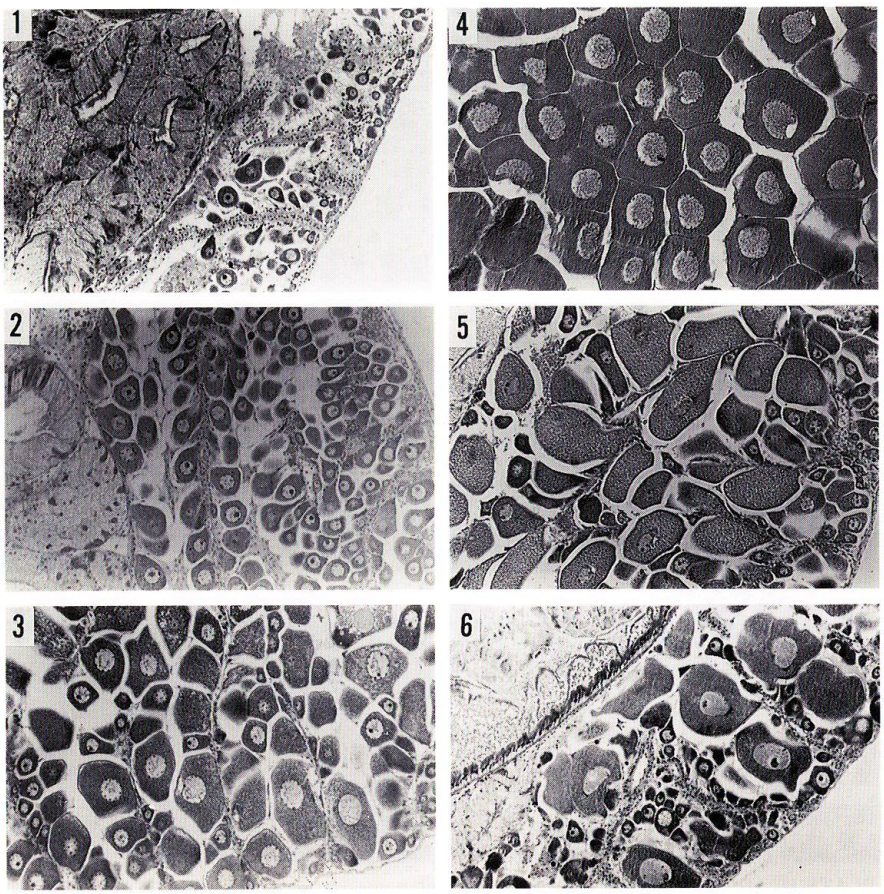

PLATE III. Histological photomicrographs showing ovary in different stages.

Fig. 1. Resting stage. × ca. 100
Fig. 2. Early active stage. × ca. 100
Fig. 3. Late active stage. × ca. 100
Fig. 4. Mature stage. × ca. 100
Fig. 5. Spawning stage. × ca. 100
Fig. 6. Spent stage. × ca. 100

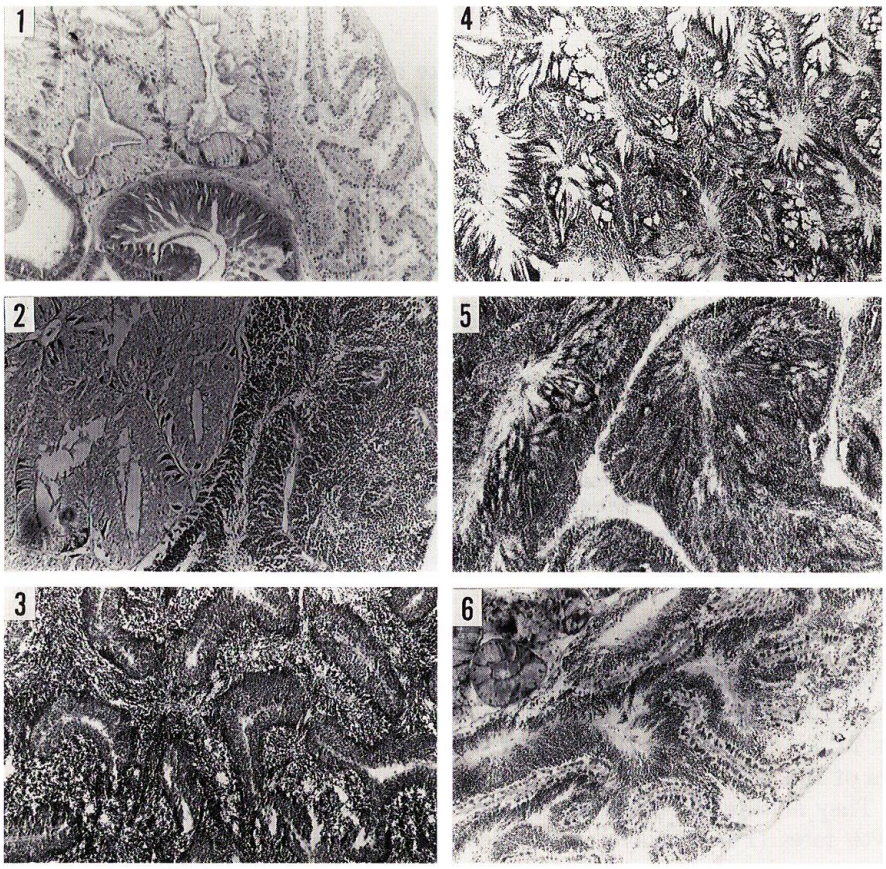

PLATE IV. Histological photomicrographs showing testis in different stages.

Fig. 1.	Resting stage.	× ca. 100
Fig. 2.	Early active stage.	× ca. 100
Fig. 3.	Late active stage.	× ca. 100
Fig. 4.	Mature stage.	× ca. 100
Fig. 5.	Spawning stage.	× ca. 100
Fig. 6.	Spent stage.	× ca. 100