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Breeding and Settlement of *Chthamalus challengeri* Hoek on the Southern Coast of Hokkaido

Toshiaki IWAKI*

Abstract

A sessile barnacle *Chthamalus challengeri* Hoek, sampled in the southern part of Hokkaido, had its gonad development studied histologically and the seasonal settlements of its cyprids and young barnacles were observed. The progressive development of their gonads was classified into the following five arbitrary stages: Recovering, Growing, Pre-mature, Mature and Spent. There is no evidence to suggest that, in each individual as well as in the population, either the ovary or testis preceded the development of the other. Such developments were followed by the appearance of egg masses in the mantle cavity and the growth of seminal vesicles. The breeding cycle occurred only once a year without a definite cessation, and the full annual cycle of the gonad development was divided into three periods, i.e., Resting (June-September), Growing (October-February) and Spawning (March-May). These cyclic changes occur at two other periods such as Embracing (April-June) and Settling season (July-September). The present investigation represents its breeding habits in regions near the northern limits of its geographical distribution.

Barnacles show extreme varieties in their breeding activities, so it may be possible to sort them into several types of breeding. For example, *Elminius modestus* Darwin, the warm temperate form, produces about ten broods a year over a wide range of temperature, and *Chthamalus stellatus* (Poli) is characterized by producing several broods over a limited range of temperature during the breeding season. On the contrary, the boreo-arctic species, such as *Balanus porcatus* (da Costa), *B. balanoides* (Linnaeus) and *B. hameri* (Ascanius), yield only a single brood a year and require low temperature. The detailed descriptions of their breeding habits have been published by Barnes[1], Crisp,[2–5] Moore[6][7] and other authors.[8–15] As to *Chthamalus challengeri* Hoek, Luckens[15][16] classified the developmental states of ovaries and embryos in the mantle cavity into several stages based on their external appearance. Similar methods have been employed by Crisp[3] and Crisp & Davies[4] in their observations on the breeding of *B. porcatus* and *E. modestus*.

The small sized sessile barnacle, *C. challengeri*, distributes along the rocky shore of Hokkaido as far east as Akkeshi, and as far north as Rishiri Island.[16] Although this species is a dominant barnacle along the protected- and the open-coast of Hakodate located at the southernmost part of Hokkaido, no pieces of information

* *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)

have been hitherto published on its gonad development during a whole year. The present study aims to follow the cycle of gonad changes which occur throughout the year in the population of *C. challengeri* collected in Hakodate.

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Materials and Methods

Regular monthly samples, each containing 40 to 60 individuals, were provided for the examination of their gonad condition. They were collected from the break-water at Nanaehama in Hakodate Bay, over the period from June 1968 to July 1969. The samples removed from the rock were fixed immediately in Bouin's fluid or formaline, and then decalcified in 3 % formic acid. After a normal paraffin embedding, serial sections were prepared at 8 μ in thickness, and stained with Delafield's haematoxylin followed by eosin as a counter stain.

In order to examine the settlement of cyprid larvae and young barnacles of this animal, 16 black panels of plastic, each measuring 18×25 cm, were hung on the round piers at the levels ranging from 80 cm above the chart datum to 28 cm below from October 1968 to October 1969. After each immersion of ten days or so, the number of cyprids and metamorphosed barnacles settled on them were counted. The location of experimental panels set on the pier lay near the station where the samplings for the observation of gonad development were made.

Results

Gametogenesis

Two ovaries lie in the connective tissue between the mantle cavity and the basal membrane. If the ovaries develop moderately or well, the branched ovarian tubules can be recognized clearly in reddish yellow color through the transparent basis. The ripe ova move from the oviducts into the oviducal gland,[17] where they are enclosed by a thin membrane, and then paired egg masses discharge into the mantle cavity. On the other hand, testis follicles are buried under the cuticle of the prosoma and are connected with the seminal vesicles. The paired seminal vesicles unite within the pedicel of the penis (articulated organ) to give a single ductus.

It is convenient to divide the gonad development into several stages for microscopic examination. In the present study it is classified into the following five stages.

1. Recovering stage

Female: The ovary is still very small (ca. 30 μ in inside diameter), but it is firmer and more substantial than that immediately after spawning. The ova unspawned during a previous spawning period still remain. The clusters of oogonia occur in the center of the ovarian tubule, and a number of young oocytes surround these oogonia. The oogonium, spherical or oval in shape, has a sharply outlined nuclear membrane and a scanty cytoplasm which does not stain specifically. Young oocytes just beginning their growth are characterized by a large nucleus and a nucleolus. The concentration of yolk globules in their cytoplasm is thinner than that of further growing oocytes (PL. I-1 & 3).

Male: The testis in this stage is also small, and is filled with cells which are similar to those existing outside the germinal epithelium. The spermatogonia arranged in a lateral band on the follicular wall have a very little cytoplasm and an ovoid nucleus occupying almost the entire cell; the nucleolus is situated in the center of the nucleus. A small number of various synaptic spermatocytes occur in this stage, but no spermatogenesis progressed further (PL. I-2 & 4).

2. Growing stage

Female: Ovarian tubules increase in their width owing to the growth of oocytes. This stage shows an actual oogenesis, and is characterized by a successive growth of oocytes. Yet a number of young oocytes and more abundant growing oocytes are observable. The latter measuring ca. 40×30 μ in diameter have a round germinal vesicle and a densely stained nucleolus (PL. I-5).

Male: The swelling of the testis follicles is well advanced as a result of the multiplication of spermatogonia. Spermatocytes migrate centripetally inward from the periphery of the follicle. No spermatid and no spermatozoa are found in follicles. Seminal vesicles still remain scanty (PL. I-6).

3. Pre-mature stage

Female: In this stage an ovary can be seen distinctly through the basal membrane; a ramose ovary is reddish yellow in color. It considerably increases its dimension as compared with that in the above stage. The oocytes elongate remarkably and their dimension attains ca. 70×40 μ, although a few young oocytes also persist near the wall of the ovarian tubule. The germinal vesicle stains rather densely. In the cytoplasm of these elongated oocytes are contained numerous yolk globules (PL. I-7).

Male: Testis follicles attain almost their maximum size. A great number of spermatocytes distribute uniformly in the follicle, although a very few spermatids and spermatozoa can be seen near the center (PL. I-8).

4. Mature stage

Female: Oocytes now reach their maximum dimension of ca. 80×110 μ. Almost all the available space of the follicIar lobes is packed with these oocytes, so that the surface of the ovarian tubules becomes rugged. The cytoplasm of these mature oocytes stains rather deeply with eosin; the nucleus can be seen near the periphery of the egg membrane staining very deeply with haematoxylin (PL. II–1).

Male: Testes in mature stage occupy the greater part of the prosoma. In these follicles there appears a swirling mass consisting of fully developed spermatozoa, although a few spermatogonia, a small amount of spermatocytes and spermatids are found in the periphery of the follicles. Seminal vesicles begin to bloat and contain a considerable amount of sperm (PL. II–2).

5. Spent stage

Female: The ovary in spent stage is histologically characterized by the appearance of an empty space in the central region of the follicle, by the presence of a small amount of residual eggs, and also by the existence of developing oocytes which are not sufficient to produce the following brood (PL. II–3).

Male: With the extrusion of the spermatozoa from testes follicles the testis reduces its size markedly. Then seminal vesicles occupy the dimension of more than one half of the prosoma (PL. II–4 & 6).

Seasonal changes in the development of the gonad

A relationship of the developmental stage between female and male follicles in each individual as observed during June 1968 and July 1969 is shown in Table 1. It is generally recognized that the spent and recovering stages in male follicles are in agreement with those in female ones, though a slight time lag is seen between female and male follicles in the growing, pre-mature and mature stages. The percentage of a given stage of an ovarian development in fertilized individuals is shown at the righthand column of the same table. The result shows that most parts of fertilized individuals have an ovary in the spent stage.

Figure 1 shows monthly percentages of each developmental stage in male and female follicles. In December and January, animals of mature stage appear in the population, and attain their maximum proportion in March and April (ca. 30 % in female and ca. 60 % in male, respectively). From April the spent individuals

Table 1. *Development of testis and ovary in each individual, and the ovarian development in animals bearing egg masses.*

(Ovary)	No. of individuals (Testis)						Ovary in fertilized individuals (%)
	Recovering	Gwoing	Pre-mature	Mature	Spent	Total	
Recovering	126	59	1	1	22	212	4.8
Growing	6	2	13	18	6	45	4.8
Pre-mature	0	0	2	50	15	67	3.6
Mature	0	0	0	44	25	69	14.4
Spent	32	0	1	66	206	305	72.4
Total	167	61	17	179	274	668	100.0

increase abruptly in frequency and show the highest proportion in May (ca. 80 % in male and ca. 90 % in female). In both sexes the spent individuals disappear followed by the recovering stage in October maintaining considerable proportions from October to December in males, and from October to February in females (50–70 %, both). The growing and pre-mature stage individuals increase in proportion with the decrease of recovering stage individuals.

As shown in Figure 1 the annual cycle of the gonad development may be divided into three periods as follows;

(a) Resting season (June-September): In this period most individuals of a population show the spent stage.

(b) Growing season (October-February): The greater part of animals shows the recovering stage, and early growing gametes are actively produced.

(c) Spawning season (March-May): This period is characterized by spawning activity and the population is in a reproductive condition.

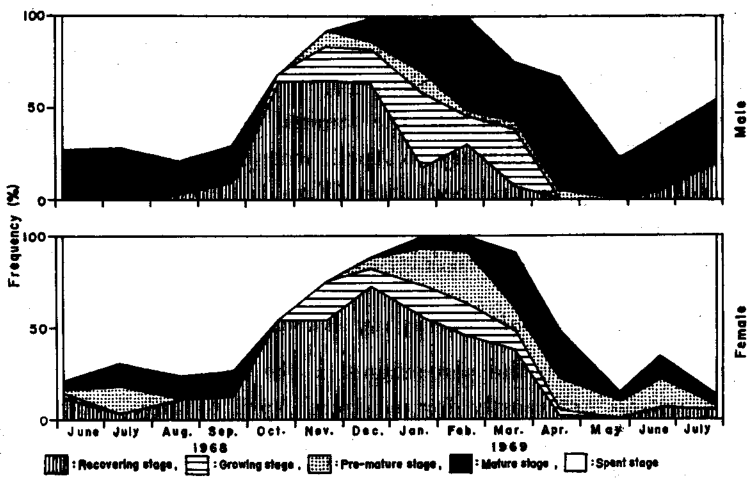

Fig. 1. Seasonal changes in the gonad showing the percentages of each developmental stage.

Development of the seminal vesicle

The follicles communicate directly with the paired seminal vesicles and ripe spermatozoa are discharged into them. Therefore, the progress of swelling in the seminal vesicle follows the decline of the testis, and then this vesicle occupies the greater part of the body as shown in Plate II–6. At insemination a mass of sperms passed through the penis is deposited in the mantle cavity of the functional female. After fertilization there appears a marked regression in this organ. Figure 2 illustrates the seasonal development of the seminal vesicles. Their development in each individual is assessed by the proportion occupying the

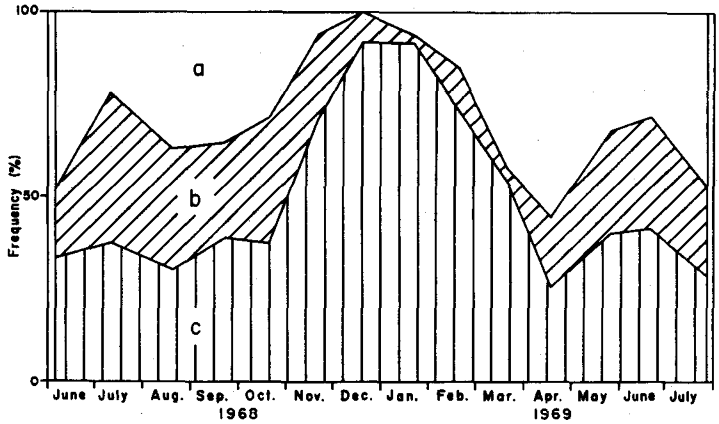

Fig. 2. Development of seminal vesicles occupying the prosoma; a: >1/2, b: 1/4–1/2, c: <1/4.

prosoma, as observed in a section parallel with the basal membrane, and the developmental assessment is assigned to one of the following classes: (a) more than 1/2, (b) from 1/4 to 1/2, (c) less than 1/4.

The spermatozoa discharged into the seminal vesicle from the testis is most active in the months from February to April, when the mature stage of the male follicle is replaced mostly by the spent stage (Fig. 1). In April the seminal vesicles attain their full development. After this month they shrink abruptly with the successive discharge of sperms, and later gradually untill the end of November.

Seasonal changes in animals bearing egg masses and in settlements

In Figure 3 are shown the percentages of the barnacles bearing egg masses. Fertilized egg masses are mostly formed from April to July, when the seminal vesicles shrink abruptly. From this the copulation of the contiguous individuals in the natural population is understood as having been performed successfully.

Almost all of the cyprid larvae and the young barnacles which settled on

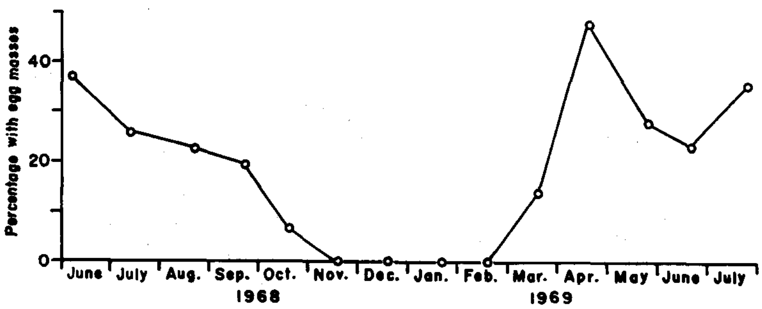

Fig. 3. Seasonal changes in percentage of the fertilized individuals.

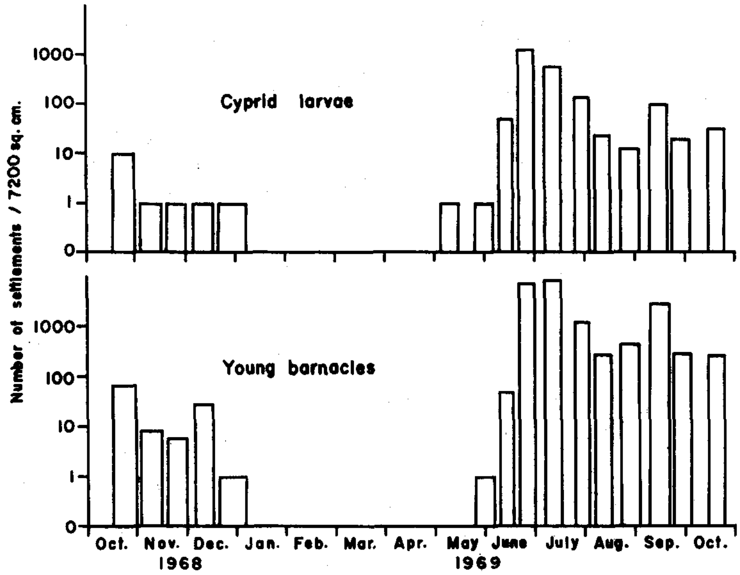

Fig. 4. Seasonal changes in the settlement of cyprids and metamorphosed barnacles.

experimental panels are identified to be *C. challengeri*, except for a small number of settlements of other species. The cyprid larvae of this species are colorless, their oval form measuring 596±46 μ in length and 315±18 μ in width (PL. II–7). Figure 4 shows the number of the cyprids and metamorphosed barnacles which attached to 16 panels during about every ten days. The settling area of each period is 16 $(25\times18)=7{,}200$ cm^2. With the onset of the cyprid attachment, the young spats begin to set in May, and attain their maximum settlement from late June to mid-July. From late July to mid-September, their appearance decreases gradually, but continues until the next January. In the present study all panels were fastened on the piers, so the settled animals were influenced by the oceanographic condition.

Discussion

In the reproductive ecology of the barnacle it has been customary to judge the approximate state of the gonad from its external appearance, but this method is only adaptable when the external features of the gonad change with the growth of gametes. However, an abnormal gonad condition in barnacles, such as senile condition[6], and an anatomical change of the body induced by crowding have been reported.[20] In *C. challengeri* or other species by a histological method a detailed observation of the gonad development can be made, even if there may exist obstructions.

In Figure 5 are shown two seasons after oviposition, Embracing and Settling

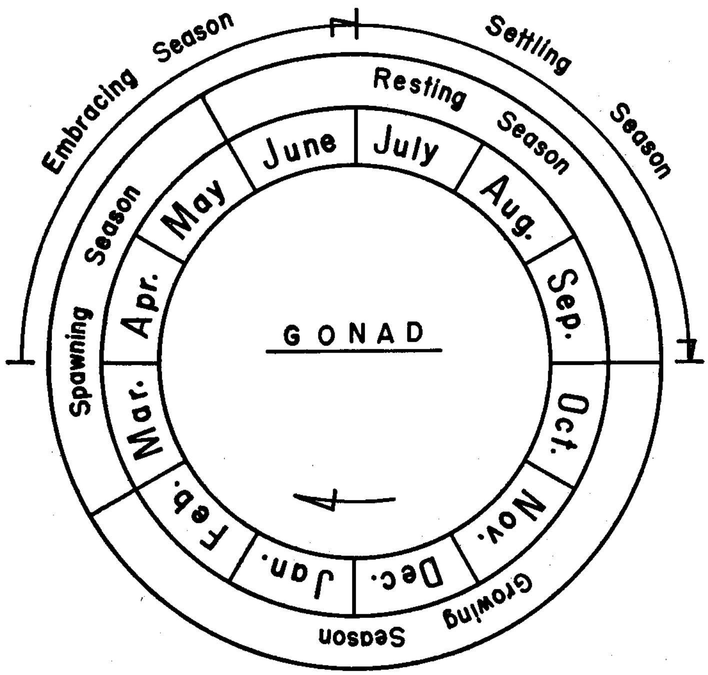

Fig. 5. Diagram showing the cyclic change of the breeding in *C. challengeri*.

seasons, beside the above mentioned three seasons in the gonad span, and a schematic representation is given on the cycle of these periods. Hirai[21] reproted the breeding season of *C. challengeri* to be found in May and June at Asamushi, but Luckens[15][16] mentioned that the lower level barnacle* may breed from late March to October, while in the upper level one** two distinct breeding seasons were observed, one from April to early June, and the other from late August to early October. As known from Figure 3, the period of fertilization of this species at Hakodate extends from March to October, quite the same as at Asamushi. It is also evident that this species collected in Hakodate Bay has no definite cessation of breeding as shown in Figures 1 & 3. This appears to be due to the author's method in which two levels were not separated, and these different breeding patterns may be caused by the characteristic age distribution found in the different shore levels as Kato et al. reported.[18][19]

In *C. challengeri* at both levels the individuals produce at least two broods and at the lower level probably three broods.[15] Compared with other species, the breeding behaviour of *C. challengeri* is not so active as in *E. modestus*,[9–11] of which the ovarian development is always ahead of the embryonic development in

* Indicates the barnacles that inhabit the lower half zone, and ** those that inhabit the upper half zone.

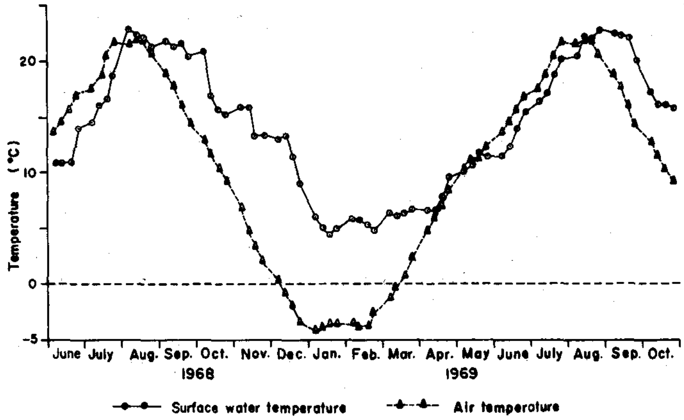

Fig. 6. Weekly water, and air, temperatures in Hakodate Bay during the investigation.

its breeding season, and *B. pacificus*,[22] which would produce surprisingly about thirty broods a year. In the latter two species the gonads are reported to be found in all stages between the immature and spent conditions when embryos are still retained in their mantle cavity, whereas in *C. challengeri* most gravid individuals are restricted within the spent stage individuals (Table 1).

The development of the gonads may be influenced by a number of factors, such as temperature, food, light and others. As to *C. challengeri*, it is not known how far it is directly controlled by these factors. From Figures 1, 3 and 6, a direct stimulus due to change of temperature is, however, improbable in this species since the animals bearing egg masses and showing their gonads in all stages of their development were observed when the air and sea water temperatures were extremely low. In the shore water of southern Hokkaido regular diatom outbursts are observed from April to June and from October to December.[23][24] These two periods correspond to Free-swimming and Growing seasons respectively and then foods are available to the larvae and the gonad growth of adult barnacles.

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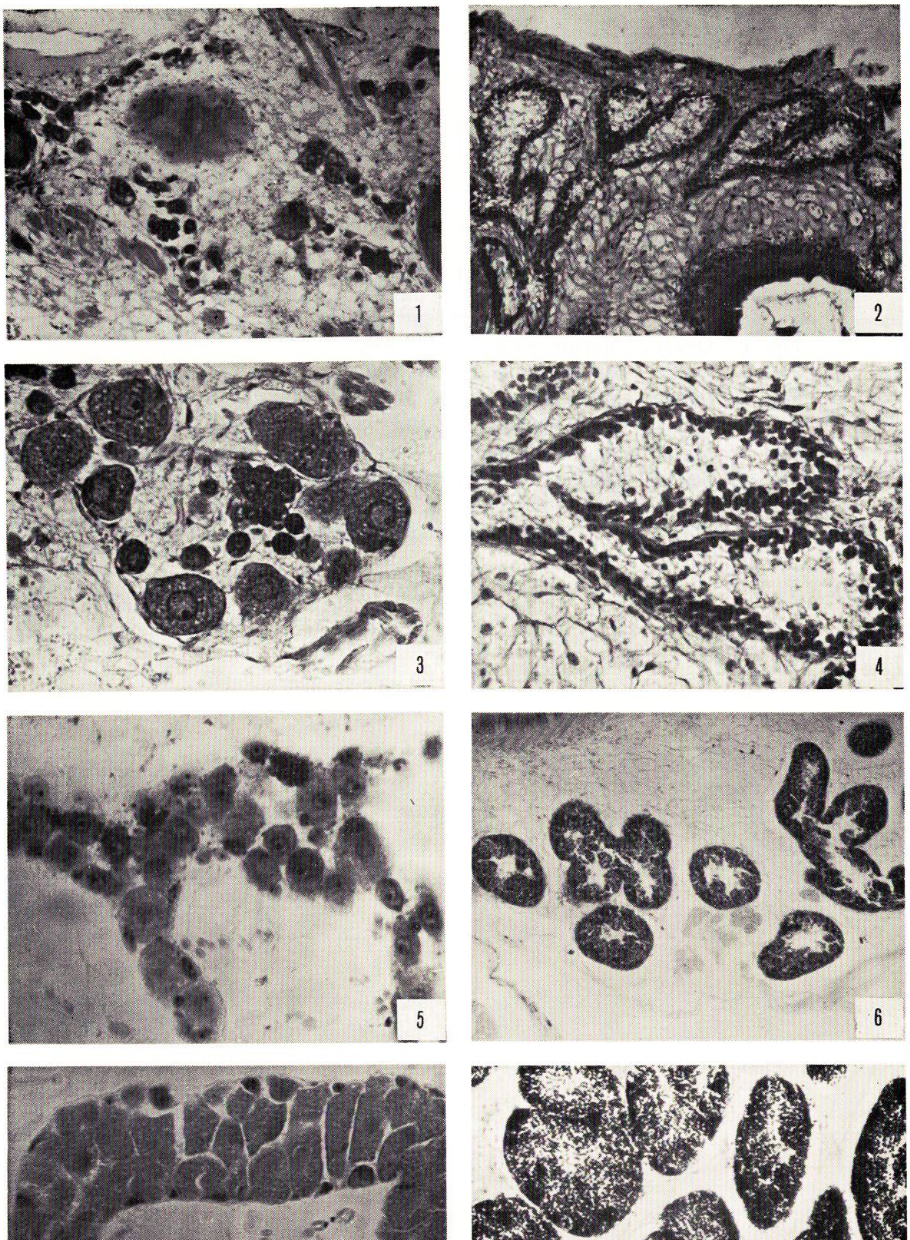
Explanation of Plates

PLATE I

Chthamalus challengeri Hoek

Photomicrographs showing the developmental stages of the gonad.

Fig. 1. Ovary: Recovering stage, × ca. 100.
Fig. 2. Testis: Recovering stage, × ca. 100.
Fig. 3. Ovary: Recovering stage, × ca. 400.
Fig. 4. Testis: Recovering stage, × ca. 400.
Fig. 5. Ovary: Growing stage, × ca. 150.
Fig. 6. Testis: Growing stage, × ca. 100.
Fig. 7. Ovary: Pre-mature stage, × ca. 150.
Fig. 8. Testis: Pre-mature stage, × ca. 100.

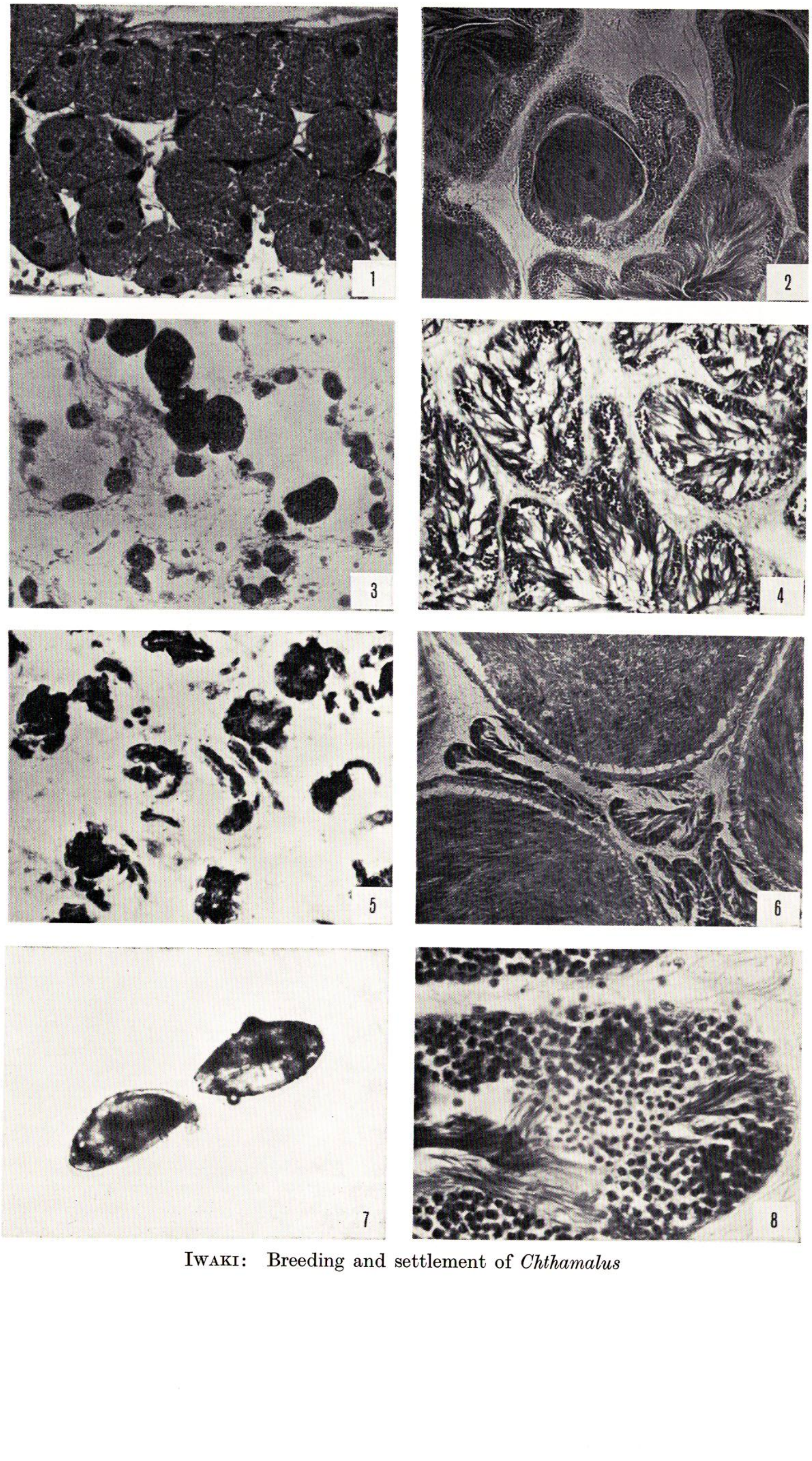


IWAKI: Breeding and settlement of *Chthamalus*

PLATE II

Chthamalus challengeri Hoek

Fig. 1. Ovary: Mature stage, × ca. 100.
Fig. 2. Testis: Mature stage, × ca. 100.
Fig. 3. Ovary: Spent stage, × ca. 100.
Fig. 4. Testis: Spent stage, × ca. 100.
Fig. 5. Embryos in mantle cavity, × ca. 100.
Fig. 6. Seminal vesicles at their maximum size.
Fig. 7. Cyprid larvae removed from experimental panels.
Fig. 8. Progressive spermatogenesis in the testis follicle.



IWAKI: Breeding and settlement of *Chthamalus*