STUDIES ON THE BIOLOGY OF THE SEA URCHIN

I. Superficial and Histological Gonadal Changes in Gametogenic Process of Two Sea Urchins, *Strongylocentrotus nudus* and *S. intermedius*

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Two sea urchins, *Strongylocentrotus nudus* and *S. intermedius* are recently being caught as animals of economical importance in Hokkaido. According to Kinoshita (1958), their animal has amounted to as much as about 7,000 tons in average from 1953 to 1957.

To know the spawning habits of these animals supplies not only one sort of interesting information on their life-history from the biological viewpoint, but also an important part of the basic information on the sea urchin fishery. Such information includes such items as the gametogenic cycle, spawning season and size at first maturity.

Among the studies dealing with the spawning habits of Echinoidea are those by Miller & Smith (1931), Tennent, Gardiner & Smith (1931), who illustrated the cytology of the egg formation of *Echinometra lucunter*, Moore (1934, 1935a), who reported the profiles of the seasonal variation in the relative gonad volume and gonad maturity of *Echinus esculentus* and *Echinocardium cordatum*, Thorson (1946), who presumed the spawning season for eight Danish sea urchins by the trace of the frequency of appearance of larvae in Danish waters, Lasker & Giese (1954), and Bennett & Giese (1955), who investigated the reproductive cycle of *Strongylocentrotus franciscanus* and *S. purpuratus*. Kawana (1938) gave an outline of various items of information on the gonad development of the common Japanese sea urchin, *Strongylocentrotus pulcherrimus*, Tennent & Ito (1941) presented detailed cytological information on the oogenesis of *Mespilia globulus* with special reference to the morphology of the chromosomes during the course of growth in the primary oocytes and meiotic divisions. However, there are virtually no published observations on the changes occurring in the gonads of the two sea urchins, *S. intermedius* and *S. nudus*, during an entire year nor also many other items of information concerned with their ecology.

The present paper describes mainly how the gametes are formed.

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Material and Method

Regular and arbitrary samples of sea urchins from the adult population (over 40 mm in test diameter) were obtained at regular intervals from Sinori, Ishiya, Muroran and Setana in southern Hokkaido, within the period from June 1956 to November 1958. The collections were usually made by means of a landing net from a depth of 3–5 m. Samples of smaller urchins (under 30 mm in test diameter) were picked up by hand at times from June 1957 to November 1958, on the rocky shores of Ishiya and Muroran. Figure 1 shows the areas and the localities at which collections were made. Approximately 1,500 urchins were examined in this work.

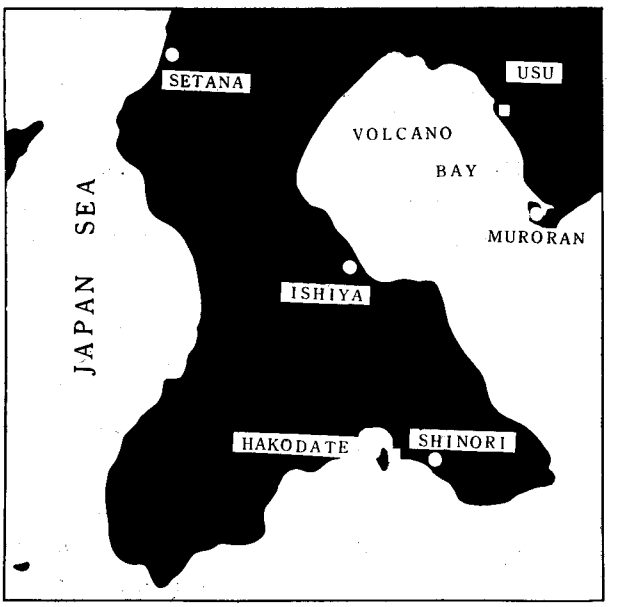


Fig. 1. Sketch-map of southern Hokkaido, showing the localities at which collections were made

Each specimen of sea urchin, after measurement of its test diameter, was immersed into a small vessel (15 × 15 × 10 cm in volume) filled with sea water. The water which overflowed from the vessel was measured as the total volume of the sea urchin. After this treatment, Aristotle's lantern of each specimen was removed, five gonads were removed with care so as to avoid any damage and adhesive water of the gonad surface was blotted with paper. These gonads were examined macroscopically and were then fixed in Bouin's fluid to provide material for histological observation.

There is always some loss of reproductive cells from the cut surface of the gonad, moreover fixation is apt to result in a slight contraction of the gonadal tissue. So all pieces to be fixed were cut large enough to allow for this loss. A small piece was cut off

the central part of the fixed gonad. After normal paraffine embedding, serial sections were prepared between 6μ to 10μ thick, and stained with Delafield's haematoxylin or Heidenhain's iron-alum haematoxylin followed by eosin or light-green as a counterstain.

Observation

General structure of the gonad

The gonads of the Echinoidea are suspended by folds of perivisceral epithelium from the inter-ambulacral plates in the apical half of the body cavity. Each of the gonads possesses a single gonoduct which opens to the exterior through an aperture in the genital plate. The gonoducts are round in cross-section, 800-1,000μ in diameter, and they lack any muscle layer, consequently are composed of an inner and outer layer of epithelium with thick connective tissue fibers between.

Observations of the gonad organs show that 13-15 pairs of racemose branches extend to each side of the gonoduct, usually arranged oppositely. In the range up to 20 mm in test diameter all specimens of *S. intermedius* possessed five small gonads which appear semi-transparent to the naked eye. The apices of almost all acini branch in Y shaped form. In the specimens of 20-25 mm test diameter, the aciniformed structure increases in size and dimension; their apices show three or four branches although the superficial feature of their gonads still shows semi-transparency. The ramification of acini of gonads in the animal of over 25 mm test diameter increase greatly in number. Gonads in this stage differ from the former one in their colour; they show yellowish brown. Typical examples of a primary gonad were illustrated in Figure 2. No morphological difference is found in a primary gonad between *S. intermedius* and *S. nudus*. There is no essential difference in gross structure between male and female gonads of the specimens over 40 mm in diameter. Mature ovaries are reddish brown and mature testes are creamy white in colour. On the other hand, when completely contracted or during an early stage of gametogenesis it is extremely difficult to discriminate between the sexes, as all gonads appear reddish brown.

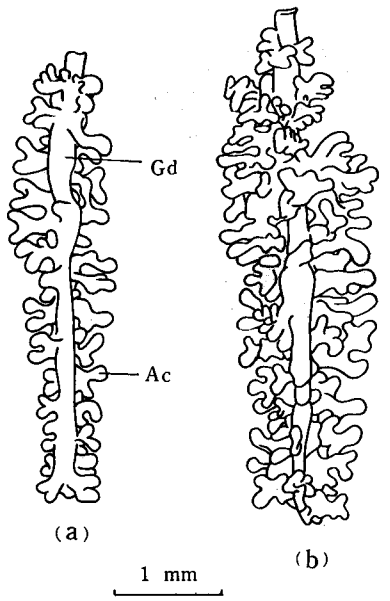


Fig. 2. Primary gonad of *Strongylocentrotus intermedius* to show general morphology
Ac., acinus; Gd., gonoduct

The gonad walls of both sexes, in respect to histology, consist of an outer epithelium layer of flat epithelial cells whose distal surface is bathed by perivisceral fluid, a middle layer of conspicuous

definite bands of smooth muscle cells and connective tissue, and an inner layer of developing germ cells. These observations coincide with the findings announced by Wilson (1937, 1940), and by Tennent & Ito (1941) on ***Arbacia punctulata*** and ***Mespilia globulus*** respectively.

The male follicle usually contains a few early germ cells near the follicle wall, but the lumen is filled with spermatozoa. The female follicle is occupied by a few young oocytes attached to the wall, while the lumen is packed with large oocytes. Secondary oocytes having a female pronucleus appear polygonal in section, and are packed tightly in the follicles ; the diameter of such oocytes in sections is about 80-100μ.

Gametogenesis in male and female urchin

In describing the gametogenesis in male and female urchin it is convenient to establish a series of arbitrary but easily recognizable stages in its cycle. Six stages including development and regression of gonads are categorized, these being determined in the main by the predominating cell type within a follicle in company with the superficial appearance of the gonads (Fig. 3 and Plates I & II) ; they are defined as follows :

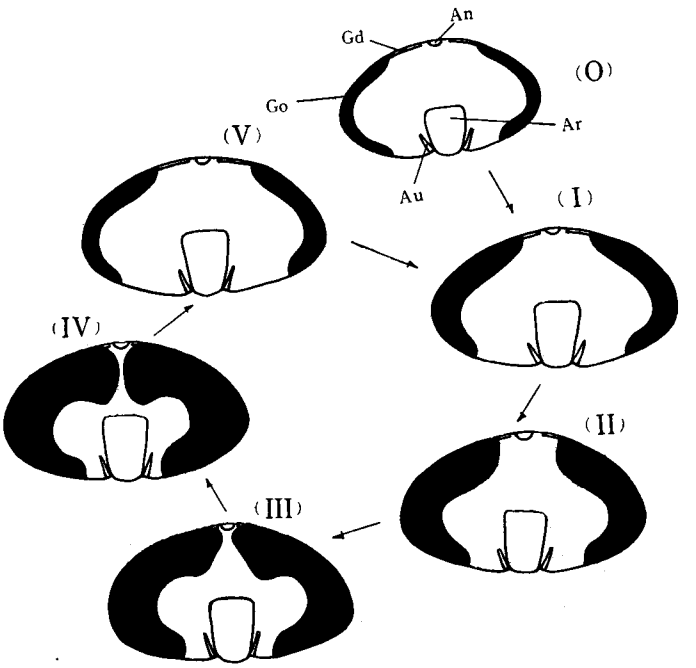


Fig. 3. Diagrammatic figure illustrating cyclic changes in the superficial appearance of the gonad
An., anus; Ar., Aristotle's lantern; Au., auricula; Gd., gonoduct; Go., gonad

Stage 0 (*Neuter*)

*S. **intermedius*** of 15-20 mm and *S. **nudus*** under 30 mm in test diameter possess neuter gonads, which are small, elongated and narrow in dimensions, semi-transparent in

external appearance.

Microscopic examination of sections shows that the lobule-wall of a gonad is very thin. In this stage, therefore, it is impossible to discern any sex difference.

Stage I (*Developing virgin and Recovering spent*)

Growth occurs in dimensions to some extent and sex is recognizable by microscopic examination. ***Developing virgin*** gonad and ***Recovering spent*** gonad become very similar to each other in the sectional feature with the exception of occurence of a larger space in the follicle of the latter than of the former.

In macroscopic observation, however, ***Developing virgin*** gonad differs from ***Recovering spent*** one in having smaller dimensions. Moreover, ***Developing virgin*** gonad is still whitish in colour, while ***Recovering spent*** gonad is reddish brown on account of its developing stage.

Female: Sections of the overy in this stage can be easily recognized, since a very large number of oogonia and a few young oocytes have become attached along the inside of follicle wall. The oogonium is more or less spindle-shaped in form. Its cytoplasm surrounds a prominent nucleus as a thin and homogeneous layer. A young oocyte is irregularly spherical and has one relatively large nucleus surrounded by a thin accumulation of basophilic cytoplasm, which although smooth and homogeneous in apparance is more clearly defined than in the oogonium. The average size of an oocyte is about 15μ in diameter, but some of the youngest ones measure only about 5μ. The nucleus is spherical and sharply limited by its membrane. The nucleolus is situated eccentrically in the reticulum as a chromatin spherule.

Male: The characteristic feature of Stage I testis in section is the presence of numerous spermatogonia and spermatocytes along the follicle wall. It is easily possible to differentiate a spermatocyte from a spermatogonium, as the former is smaller in size and less stainable with haematoxylin than the latter. The spermary of this stage shows low spermatogenic activity. The wall of the male follicle, like the female one, is in a contracted state having many rumples.

Stage II (*Growing*)

Gonads are uniformly reddish brown in colour, showing no differentiation between testis and ovary.

Female: In the periphery of lobules small oogonia are still found, but they are less in number than in the former stage. Various synaptic young oocytes, which connect with one another along the inside of lobules, are arranged in lateral bands. Their diameters now attain 40-60μ. A round germinal vesicle lies in the central region of the oocyte, measuring 20-30μ in size. Further advanced oocytes protrude inward into the follicle adhering to the wall, although they are restricted to a small number. The ovary expands simultaneously with the growth of the oocytes.

Male: The spermary shows vigorous spermatogenesis; production of spermatogonia and spermatocytes advances rapidly along the periphery of the follicles. Consequently, male follicles in section are prominently margined with numerous gametes. No spermatozoa are found in follicles.

Stage III (*Pre-mature*)

Both male and female gonads show a considerable increase in dimensions as compared with Stage II. The sexes are roughly recognizable in this stage by the colour of the gonad; the female gonad is yellowish brown in *S. nudus* and reddish brown in *S. intermedius*, while the male gonad is whitish yellow or creamy white in both species.

Female: A Stage III gonad displays active oogenesis, and is characterized by a great increase in the size of individual advanced oocytes. Numerous large oocytes show an oval appearance, projecting markedly toward the center of the follicle; their dimension is about 80–140μ × 40–80μ. The large germinal vesicle shifts toward the pole of the oocytes and becomes round with a smooth contour. Such ovarian eggs become free from the wall of the follicles with growth, showing spherical or ellipsoidal in shape; their diameter now attains about 80–100μ. They have a large germinal vesicle measuring about 40μ in diameter. Ovarian follicles of this stage possess various oocytes ranging 10–70μ in size, and bear ripe ova in the center of the follicle lumen. In general, however, almost all of the available area in the follicle is occupied by primary oocytes reaching their maximum size.

Male: The spermary in this stage shows very vigorous spermatogenesis as well as in former stage. Spermatocytes and spermatids increase considerably in number; a few spermatozoa migrate centripetally inward from the periphery of the follicle. In more advanced male follicles small sperm patches have been already formed in the center of the follicle, although their area is still limited. Sperm patches are common in Stage III testis; these patches probably consist of the earlier-formed cells.

Stage IV (*Mature*)

Gonads of both sexes reach their peak growth showing maximum dimensions and volume. The gonad acquires a fully matured appearance. The sex of the urchin can be easily distinguished by the colour of the gonads.

Female: Almost available space of the follicular lumen is packed with circular-shaped secondary oocytes measuring about 80–100μ in diameter, although a very few young oocytes persist near the wall. The mature oocytes in this stage do not differ much from those of the Stage III ovary in size, but their contents show a marked change; the germinal vesicle of these eggs shows signs of breaking down or disappearing, the cytoplasm is homogenous and stains very faintly with haematoxylin.

Male: The spermary is expanded by the ripe spermatozoa, while in the thin membranous wall of the follicle spermatogenesis continues steadily, although the activity slows

down. Almost the entire space of the follicular lumen is fully occupied by mature spermatozoa and shows marked volution in shape owing to aggregation of the spermatozoa.

Stage V (*Spent*)

The spent gonad is thin and of small dimensions in superficial appearance. Both male and female gonads are evenly full whitish-brown in colour. No visible differentiation is notable between an ovary and a testis.

Female: Although microscopic examination of sections shows that the features of the spent gonad vary with time after spawning, in general, the ovary of this stage is characterized by the appearance of an empty space in the center of the follicles, and by the presence of a few unspawned, but apparently ripe, ova. The follicle wall is remarkably shrunk and the middle layer, which consists of muscle fiber and connective tissue, is markedly visible.

The relict ova in the follicle are gradually resorbed by phagocytes, and the gaps of the follicular center are gradually filled with connective tissue. Oogonia and young oocytes increase in number progressively, the ovary again resumes similarity in shape and appearance is that observed in a Stage I gonad.

Male: The most noticeable histological change, additional to the great reduction of spermatozoa, is the presence of gaps in the lumen of the follicle. In some sections a small pactch of relict sperm is observed near the wall or in the lumen.

Relation between gonadal histological stage and gonad weight

The following described experiment was designed to learn how the gonad weight changes with progressive development of gametes and what relationship exists between gonad weight and test diameter or volume of the sea urchin. Figure 4 illustrates the relations between gonad weight and the size or the total volume in the animal belonging to each histological gonad stage as above described. It may be presumed from the above illustration that an exponential relation is found between the gonad weight and the test diameter, while the relationship between test volume and gonad weight is represented by a straight line, within the limit of the data used in this representation.

Correlation coefficient (γ) and correlation ratio (η) are computed from each relation. If the relation between two dimensions (test diameter—gonad weight or total volume—gonad weight relation), is a completly linear regression, it must be expected that the value of correlation coefficient is equal to the correlation ratio. The significance of the discrepancies between γ and η is tested by the analysis of covariance. Results of analysis obtained are summarized in Table 1. From the above table, it is clearly demonstrated that the relation between the gonad weight and the test volume may be represented by a linear regression extending over all gonad stages, while a linear relation between the gonad weight and the test diameter is calculated in some urchins. Such ratio obtained from smaller individual will be on a lower level than that of larger one. On the other hand,

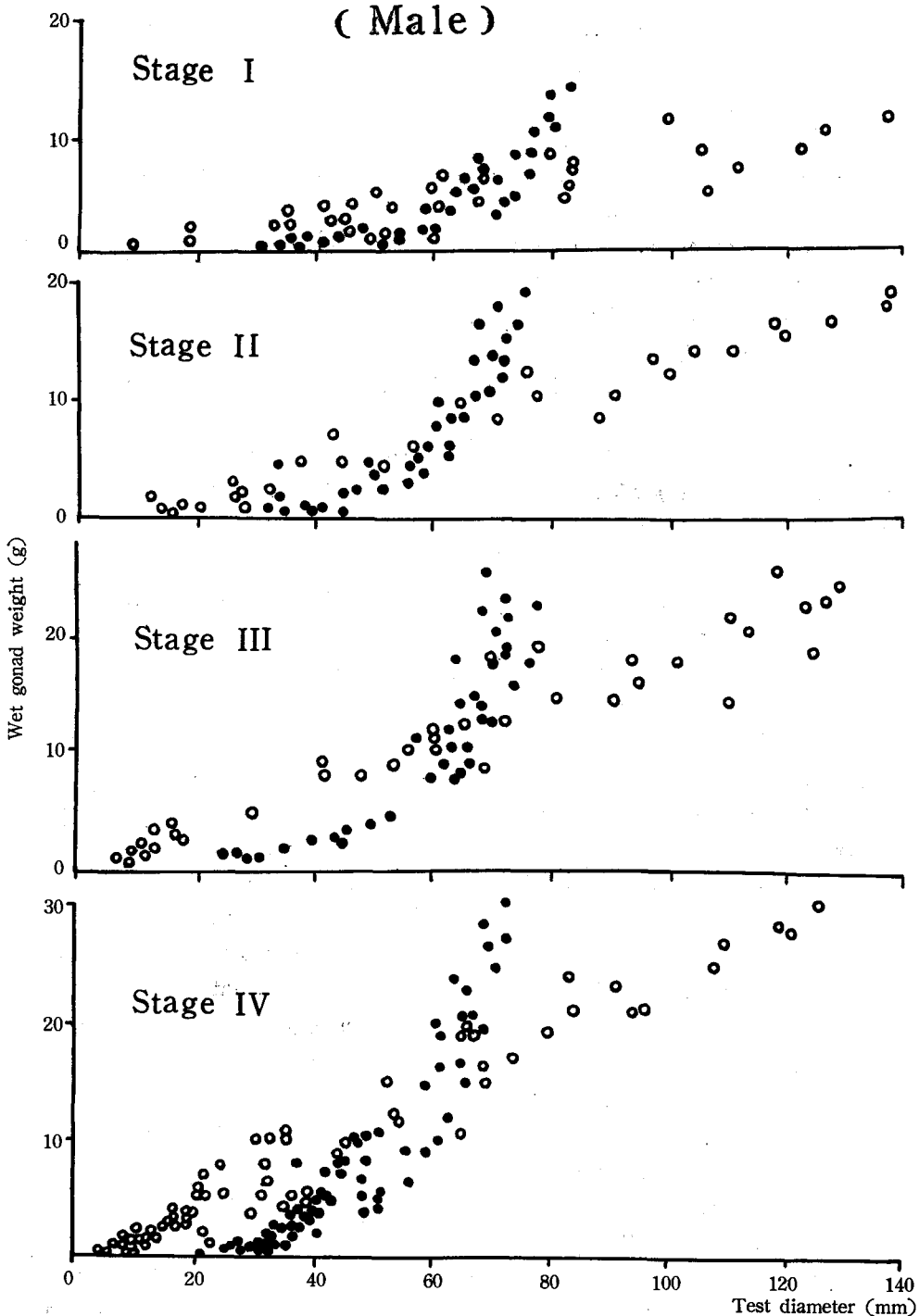
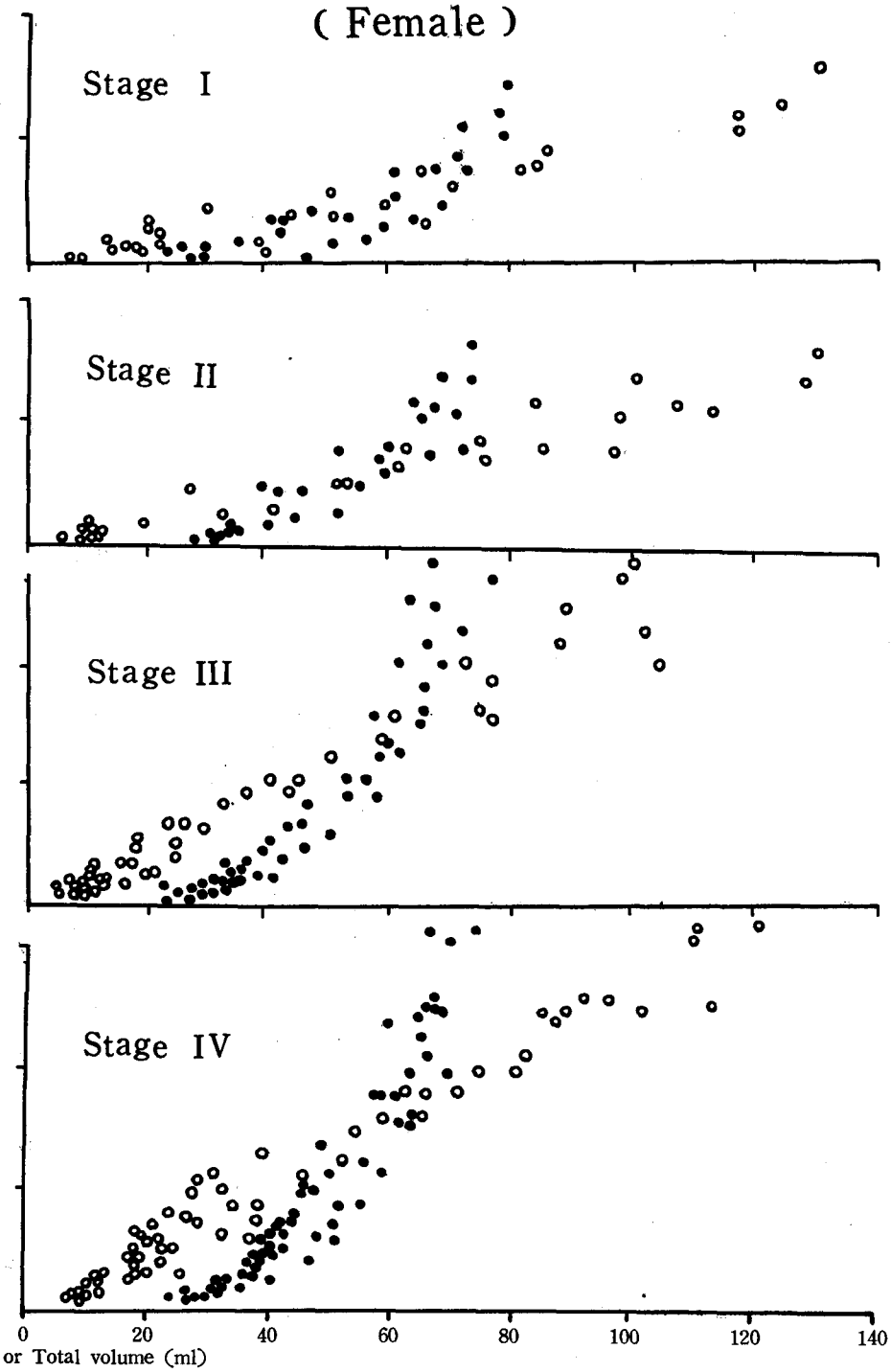


Fig. 4. Scatter diagrams showing the relation between gonad weight
● ; Gonad weight—test diameter relation,



or Total volume (ml)
and test diameter or total volume of *Strongylocentrotus intermedius*
○ ; Gonad weight—total volume relation

Table 1. Test for significance of the discrepancies between γ and η
When the result of the test has significance ($F_0 > F$), linear regression is rejected in the confidence limit of 95 %

a) Wet gonad weight—test diameter relation

Gonad stage	Male			Female		
	γ	η	F_0 F	γ	η	F_0 F
I	0.693	0.808	2.24<2.64	0.918	0.954	2.59<2.85
II	0.572	0.588	9.09>8.76	0.921	0.976	9.72>2.93
III	0.845	0.899	4.76>2.87	0.901	0.932	4.16>2.62
IV	0.850	0.893	5.27>2.52	0.910	0.932	3.76>2.56

b) Wet gonad weight—total volume relation

I	0.851	0.912	2.24<3.02	0.962	0.984	1.41<2.85
II	0.843	0.867	2.44<3.51	0.976	0.992	1.92<3.13
III	0.906	0.954	1.21<2.63	0.979	0.983	1.43<2.86
IV	0.964	0.959	1.41<2.08	0.955	0.972	1.66<2.67

if the ratio of the gonad weight to the total test volume is computed, it will be found that it shows a constant value irrespectively of the test volume of urchin.

In this paper, ***gonad coefficient*** is derived from the following formula as a unit of the grade of maturity of a gonad. This is:

$$GC = G_w/V_t \times 100$$

in which GC is the value of gonad coefficient, G_w is wet weight of the gonad in gr. and V_t is total test volume of sea urchin in ml.

The gonad cofficient in the samples collected from various locations in southern Hokkaido is compared with each gonadal stage by sex and species (Table 2). Generally, it is assumed from above table that the change in the gonad coefficient with the development of gametes in two sexes runs a slightly different course, though the gonad coefficients of urchins taken at various locations represent a considerable fluctuation even in the creatures of the same gonad stage. In female urchins, a comparatively rapid increase takes place in the gonad coefficients between Stage I and Stage III, and the difference of the gonad coefficients between Stage III and Stage IV has no significance. However, in male gonad, the gonad coefficient shows a gradual augmentation with advance in gonad stages when compared with female urchins.

As already shown in the former section, in female gonads the gametes in the mature ovary do not differ much from these of the pre-mature ovary in size, number and the degree of occupancy within the follicle space, while the female gonads between ***Recovering***

Table 2. Comparison of the gonad coefficients in two sea urchins collected from different localities in southern Hokkaido

Gonad stage	Mean gonad coefficient ± standard error						
	S. intermedius				*S. nudus*		
	Muroran	Setana	Ishiya	Shinori	Setana	Ishiya	Shinori
Male I	10.4±2.1	9.8±2.0	7.0±1.1	7.0±1.0	15.9±2.0	10.6±1.3	11.9±1.1
Male II	19.2±1.8	13.1±1.6	13.1±2.0	16.3±2.0	18.6±1.1	12.6±2.1	16.9±2.0
Male III	22.6±0.6	16.9±2.7	18.9±2.1	22.0±1.9	23.3±2.8	16.5±2.1	20.1±1.6
Male IV	25.7±1.9	20.7±2.0	22.4±3.0	25.1±2.8	25.6±2.0	19.5±1.8	24.9±1.9
Male V	7.2±1.1	3.5±1.0	4.2±0.6	6.2±0.9	3.9±0.7	9.7±0.3	6.3±0.9
Female I	12.2±0.9	10.6±1.0	6.5±0.8	8.7±0.9	14.1±1.9	8.7±0.8	13.3±1.6
Female II	20.6±0.8	14.7±2.4	11.8±2.3	17.7±1.8	22.3±2.1	13.0±1.0	18.1±2.1
Female III	24.5±2.4	17.8±1.1	21.8±1.2	24.8±2.0	27.1±1.6	18.2±2.6	24.3±2.4
Female IV	28.6±1.8	20.7±1.7	25.3±2.9	28.3±2.6	27.9±3.5	24.0±2.1	26.3±2.8
Female V	4.8±0.8	4.3±0.6	4.3±0.6	5.3±1.0	5.2±1.2	11.5±2.2	5.7±1.0

spent and ***Pre-mature*** stage show a considerable difference in size and number of the gametes therein. The male gonad continuously produces germ cells in various developmental stages during the course of advancement in the gonad stages; resultant change is a gradual increment in size and number of the gametes.

Discussion

The most striking feature about the gametogenic cycle of these two urchins is the preponderance of phagocytic cells, stained strongly with haematoxylin, within the follicles after spawning. The resorption of residual germ cells by phagocytosis is well known to occur in bivalve molluscus (Loosanoff, 1937, 1942 ; Tateishi & Adachi, 1957 ; Tranter, 1958 ; Marson, 1958), and such an activity displayed by holothuria phagocytes has already been described by Oshima (1925) and Tanaka (1958). In the present observation, some relict ova showing degeneracy of cytoplasm are noted within the spent follicles ; it may be assumed that such indistinct appearance of residual ova are caused by destruction by phagocytic cells.

The question concerned in the origin of germ cells is one of basic and interesting information in respect to the gametogenic process of echinoids. Tennent, Gardiner & Smith (1931) on ***Echinometra lucunter*** suggested that the cells of the outer epithelium layer of the gonad wall contributed to the visible supply of germ cells by migrating through the

layer of muscle fibers to the inner epithelium. In their paper dealing with the morphology of oogonesis in *Mespilia globulus*, Tennent & Ito (1941) concluded that the outer epithelial layer of the ovary was the true germinal layer supplying the mother cells of the oogonia, which migrate through the muscular layer toward the lumen of the ovary where they divide, differentiate and enlarge. In the present investigation no attempt was made to trace the origin of the germ cells, but it seems that the smallest cells formed on the inner layer of walls resemble closely the cells of the outer epithelial layer.

At the present time a few records of hermaphrodite echinoids are available. From the table by Harvey (1956), the hermaphrodite echinoids are characterized, as a rule, by entire gonads of an individual, testes and ovaries in the same animal. More rarely ovarian and testicular tissue develop side by side in the same gonad, as displayed in the detailed histological descriptions by Moore (1935 b), Harvey (1939), Neefs (1952) and Boolootian & Moore (1956). No hermaphrodic individuals were found among the about 1,500 freshed specimens of the two sea urchins, *S. intermedius* and *S. nudus*.

In general, shedding of gametes within one spawning season may be roughly divided into two types; the first one shows several waves of spawning and the other occurs once during the spawning season. In the former case, various gametogenic processes are observed at the different parts of the gonadal tissue, such as clam, oyster (Loosanoff, 1937, 1942) and pearl oyster (Tateishi & Adachi, 1957 ; Tranter, 1958). In other type, it is observed that the gametes developing in the similar degree are shown by entire gonads of an individual. For testing the effect of partial differences in the degree of development of gametes, histological sections were prepared from the margin and the inside of anterior, middle, and posterior portions of the gonad. In the histological aspects of such preparations, so far as the materials employed in the present investigation are concerned, no significant difference is recognized in any of these portion in the five gonad segments. From this observation, it is strongly suggested that the development of gametes progresses dissimilarly over all the gonads in the same specimen.

It is inferred from Table 2 that the values of the gonad coefficient in the two sea urchins here studied show some considerable differences even in the similar histological stages when compared with one another from specimens collected from various habitats. This fact suggests that there may be a discrepancy between the fecundity of the sea urchins collected from the various localities. It is reasonably presumed that these differences may be due to local variation in the standing crop of algae taken as a food by the urchin, although the cause of such differences is not certain at present.

Summary

1. Observations have been made on some superficial and histological features of the gonads of *Strongylocentrotus nudus* and *S. intermedius*, by examination of samples

from four different localities in southern Hokkaido.

2. The primary gonad of two sea urchins consists of 13 to 15 pairs of racemose branches extended on each side of a single gonoduct, usually arranged oppositely. There is no difference in superficial appearances, such as colouration, gross structure and overall dimensions, between the primary gonads of male and female animals.

3. The development of the gonads is categorized into six arbitrary stages by histological and anatomical examination ; they are defined as follow : Stage 0 (*Neuter*), Stage I (*Developing virgin* and *Recovering spent*), Stage II (*Growing*), Stage III (*Premature*), Stage IV (*Mature*), and Stage V (*Spent*).

4. The gonad coefficient, which is the ratio of the wet weight of gonadal tissue to the total test volume, is proposed as a unit for measurement of the maturity grade of the gonad. This is believed to be one of the most convenient units to show the relative gonad weight. The value of the gonad coefficient is closely correlated with the gametogenic development.

5. It is noticed that the gonad coefficient, even in the same histological stage, shows a considerable difference in specimens from various localities.

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EXPLANATION OF PLATES

PLATE I

Strongylocentrotus intermedius

Photomicrographs of transverse sections through gonads in various stages of maturity. Magnification of the figures is about 60 times.

Fig. 1. Stage 0 gonad (*Neuter*).

Fig. 2. Stage I testis (*Developing virgin*).

Fig. 3. Stage I testis (*Recovering spent*).

Fig. 4. Stage II testis (*Growing*).

Fig. 5. Stage II testis (*Growing*). Another aspect, showing a slightly advanced testis.

Fig. 6. Stage III testis (*Pre-mature*).

Fig. 7. Stage IV testis (*Mature*).

Fig. 8. Stage V testis (*Spent*). Note small groups of unshedding sperms in lower side.

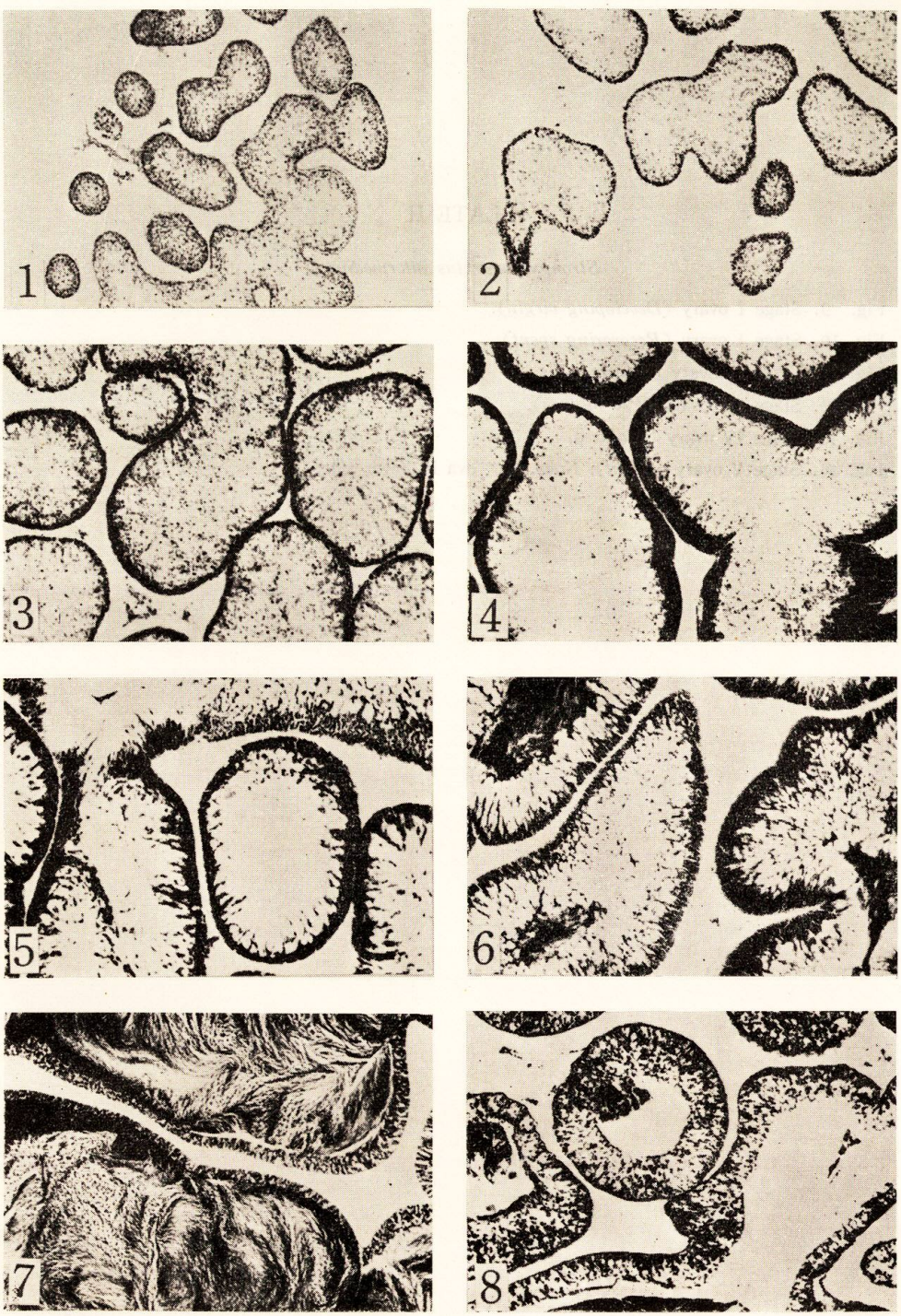

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PLATE II

Strongylocentrotus intermedius

Fig. 9. Stage I ovary (*Developing virgin*).

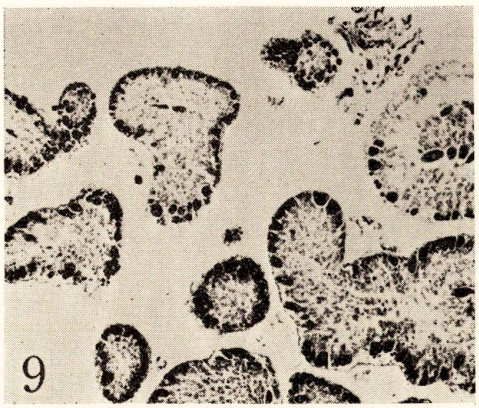
Fig. 10. Stage I ovary (*Recovering spent*).

Fig. 11. Stage II ovary (*Growing*).

Fig. 12. Stage III ovary (*Pre-mature*).

Fig. 13. Stage IV ovary (*Mature*).

Fig. 14. Stage V ovary (*Spent*). Note relict ova in lower side.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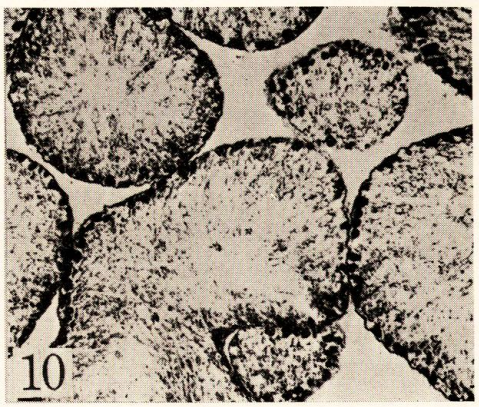
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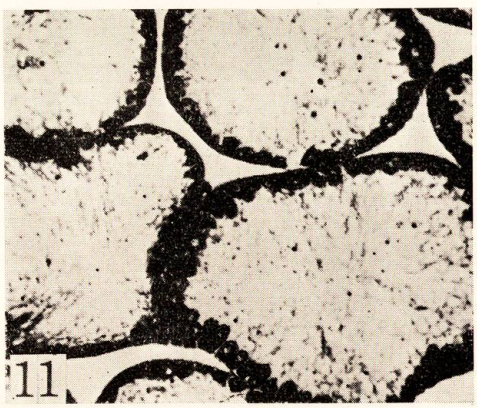
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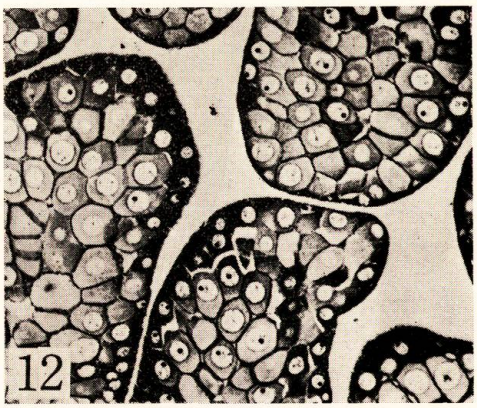
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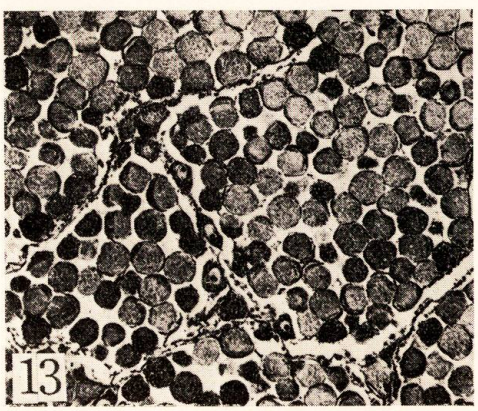
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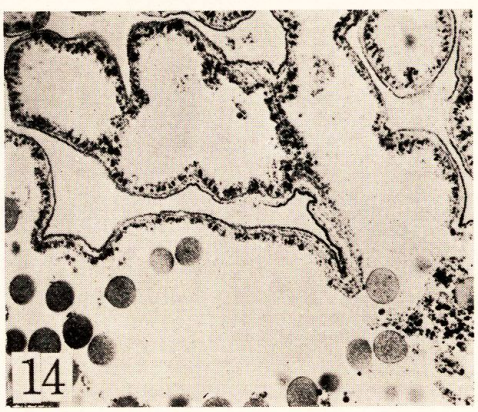
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