Some Observations on the Development of the Sea-star, *Leptasterias ochotensis similispinis* (Clark)[1]

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(*With 9 Text-figures*)

There have been published several reports on the development of sea-stars belonging to the genus *Leptasterias*: e.g. Osterud (1918) on the breeding habit and development in *L. hexactis*, Lieberkind and Mortensen (1928) on the breeding habit of *L. mülleri* and also Gordon (1929) on the skeletal development in *L. aequalis*. It is very noticeable that these sea-stars are remarkable in their habits of brood-fostering. According to Fisher (1930) all or nearly all the species of *Leptasterias* may have the similar habit. In Japan Hayashi (1943) gave a brief note on the brood-fostering habit of *L. ochotensis similispinis* from Akkeshi. The studies on the development of brood-caring sea-stars being rather meagre, the present writer, at the suggestion of Prof. T. Uchida, has been engaged in the investigation on the development of the species for the past two years at the Akkeshi Marine Biological Station.

Before going further, the writer wishes to express his cordial thanks to Prof. T. Uchida for his kind guidance.

Spawning habit

The present species is commonly found on the coasts about Akkeshi. The breeding of the species begins generally in the end of April and lasts until the middle of May. During this season the temperature of sea-water is approximately 7.5°C. When the season begins, the sea-stars come together under large stones, from two to five for a stone. Under stones the eggs are laid mainly in a mass and grow into the youngs under the mother's protection.

In order to observe the breeding habit the writer brought fully grown animals to the laboratory. They were kept in a poorly lighted place, five individuals being put at random in each glass-vat. The males were found to move freely

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on the lateral wall of the vat, while the females remained still. The sexes above mentioned were made sure by the dissection. A few days later the males approached the females, and the arms of the former were twisted and clung to those of the latter after several trials. When the female was single in the vat in which five individuals were put, two or three males usually were in contact with the female, but rarely all the four with the latter. Soon after the "copulation" the female used to begin to lay the eggs quietly. The spawning began in the morning or evening in some cases, while in midday or midnight in others. The spawning was continued for seven hours or more in the laboratory. The copulation in the present species seems to fairly resemble that of *Asterina gibbosa* observed by Ludwig ('82). The eggs are laid in an irregular mass, forming neither ribbon-like clusters nor regular layers, but sometimes in a single layer on the substratum. The eggs newly-laid can not adhere by themselves to the wall of the vat. Some of them, therefore, fell to the bottom though the parents often catched them by means of their tube-feet. In due course of time, however, the eggs become to attach themselves to the substratum. Even after the spawning, the copulation is still maintained for several hours. The eggs laid by a female were quite various in number, from 653 to 1512 in seven individuals ranging from 27 mm to 42 mm in R. The number is seemingly related to the size of mother sea-stars as is shown in

Table I. Relations between mothers and embryos.

Size of mother (R)	Under parental protection		Under no protection
	Number of newly laid eggs in culture.	Number of embryos at blastula or gastrula stage in nature (± 50).	Number of embryos at blastula or gastrula stage in nature (± 50).
42mm	923	—	
40	1226	—	
39	1042	—	
38	875	—	
37	1512	—	
34	—	800, 650	
32	635	—	
30	—	650	
29	—	750	
28	—	600	
27	664	150, 200, 300, 600	
26	—	200	
25	—	400	
23	—	150, 500,	
21	—	100	
			250, 250, 300
mean number	985	432	266

Table II. Relations between the size of adult (R) and its gonadal condition just before the breeding season.

R(±0.5mm)	Frequency of individuals		
	With mature gonads		With immature gonads
	Female	Male	
35mm	2	0	0
34	2	0	0
33	0	0	0
32	0	1	0
31	0	0	0
30	2	1	0
29	2	0	0
28	2	0	0
27	5	1	0
26	2	1	1
25	1	1	0
24	0	3	3
23	4	4	5
22	1	1	2
21	4	1	8
20	0	3	3
19	0	4	3
18	0	4	7
17	0	4	4
16	0	0	4
15	0	0	2
14	0	0	0
13	0	0	3
12	0	0	1
11	0	0	1
Mean R	26.7mm	21.4mm	18.9mm

Table I. In natural conditions, however, the embryos in a mass are certainly fewer than in culture. In 14 individuals (21 mm to 34 mm in R) 100 to 800 embryos were counted by the writer for each mass. The mean number is 432. The embryonal stages examined were blastulae or gastrulae. The loss of eggs seems to be due to various unfavorable conditions in nature. When the mother is absent and the embryos are naked, the loss of them seems to be much conspicuous ; only 250 to 300 embryos were found in a mass. In culture such a loss of embryos did not occur as in nature. To investigate the relation between the size of sea-stars and the state of gonads, 103 individuals were examined before the breeding. The results in detail are given in Table II. From the table we can see that there are a little differences in size between the male and female in nature ; the smallest females sexually matured measuring 21 mm in R, but the males 17 mm in R. The immature specimens measure 18.9 mm in R on the average.

Early development

The sperm is similar in form to that of common Echinoderms. It measures about 43 μ in total length. The head is 3 μ long, spherical in form, the middle piece cylindrical in form, about 1 to 1.5 μ in length and the tail about thirteen times as long as the head. The egg is very large, nearly spherical in form, about 0.7 mm in diameter, but rarely slightly elliptical. The colour is of yellowish, occasionally reddish orange. The egg is covered with a rather thick membrane, on the surface of which numerous minute pores penetrate the membrane toward the centre of egg. When the egg was fertilized, this membrane was separated from the surface, transforming into the fertilization membrane. It was observed that the eggs in a mass were attached one another by the fertilization membrane, so the membrane seems to be sticky. Another gelatinous membrane is newly formed beneath the fertilization membrane. The segmentation of the egg is of a holoblastic-radial type as in other sea-stars, as is shown in Textfigs. 1-3. At the early

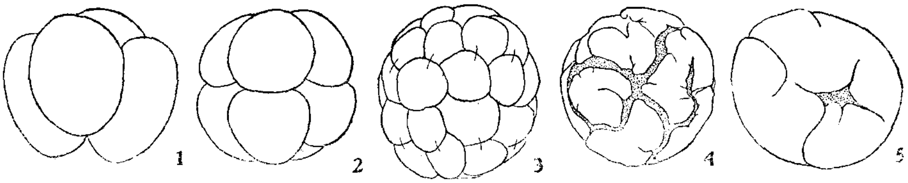

Fig. 1. 4-celled stage, viewed from side. ×35. Fig. 2. 8-celled stage, viewed from side. ×35. Fig. 3. 32-celled stage, viewed from side. ×35. Fig. 4. Wrinkled blastula stage, viewed from lower pole. ×35. Fig. 5. Gastrula, showing the cross-furrow and blastopore. ×35.

stage of the blastula, the embryo is of the wrinkled type, the blastomeres being grouped in a large number of small areas, along which the surface-furrows appear. As the embryo grows, the furrows become fewer, simpler and narrower, and consequently disappear from the surface. Near the poles alone, however, the furrows still remain in these embryos. In external appearance, the advanced blastulae recall 4- or 8-celled stages in cleavage. Then the furrows on one of the both poles become shallower and finally disappear, while the furrows on the other pole become deeper to form a small polygonal blastopore. In the more advanced embryo the blastopore and the furrows near it disappear and the surface of embryo becomes again smooth. The peculiar developmental process as stated above has been seen in some asterids ; *Solaster endeca, Henricia sanguinolenta, Asterina gibbosa* and *Astropecten irregularis.*

The times for the developmental process of each stage is shown as follows :

Stages	Times between the stages and the succeeding ones (Temp. 5.5-6.0°C)	
1-cell (Insemination)		
2-cell	19.5	hours
4-cell	9	hours
8-cell	3	hours
16-cell	3	hours
32-cell	8	hours
morula	24	hours
blastula	12	hours
gastrula	3	days
early brachiolaria	12	days

It is noticeable from the table that the stages in the early development go on very slowly.

Metamorphosis

As soon as the embryonal form becomes spherically reconstructed, a T-shaped shallow groove appears on the surface of embryo and it grows gradually deep, transforming into three brachiolar arms. In the median portion surrounded by these arms, a fixing disc makes its appearance some later on. With the growth of the larva, the brachiolar arms increase in size and come in contact with the fertilization membrane. The membrane is soon ruptured by the arms, and the larva is liberated slowly from the membrane. The hatched larva is immediately settled on the substratum by its larval organ. Soon after the settlement the fixing disc changes into a sucker with a shallow depression at the centre, by which the larva is firmly fixed. In the present species the larval mouth, anus and madreporic pore are not present in the embryonal stage, as Osterud ('18) reported in *L. hexactis*.

Following to these stages stated above, the larval disc becomes markedly prolonged antero-posteriorly and flattened on both the right and left sides. On the left side the hydrocoele lobes 1 and 2 are simultaneously developed. Successively the lobes 3 and 4 are formed, and then the lobe 5 appears. These lobes are arranged in a U-shaped form. The hydrocoelic gap in the present species is formed between the lobes 1 and 5, such as in *Asterina gibbosa*, *Asterias rubens* and *Solaster endeca*, though not so markedly in those species. The arm-rudiments I to IV arise from the dorsal side of the lobes 1 to 4, corresponding in position to the latter. They occur nearly at the same time. There remains a separation between arm-rudiments I and IV for a while. The hydrocoele lobes develop further on to become a complete ring by means of confusiong both the ends of the slightly opened hydrocoele ring. The arm V is added between the arms I and IV, on the outer side of the hydrocoele ring.

There occurs no change of the relative position between rings, hydrocoele and arm, as are reported in *Asterias* and *Asterina*. The present type of the rings belongs to the quinque-radiate forms with abbreviated ontogeny proposed by Gemmill ('16). Accordingly the numbering of the arm-rudiments adopted by the writer is as follows; the arm corresponding in position to the hydrocoele lobe 1 is designated as arm I, and the remnants are counted anticlockwise in aboral view.

On the other hand, the brachiolar arm becomes degenerated gradually, and the madrepore opens in interradius I/II, near the base of arm II. On the oral side of larva the first pair of tube-feet is sprung out from the proximal portion of hydrocoele lobe. The tube-feet on the lobes 3 and 4 are differentiated more quickly than those on the rest; the tube-feet on the lobes 2 and 5 are next in order; while these on the lobe 1 are the slowest. In the present species, accordingly, the sequence in formation of tube-feet is not related to that of hydrocoele lobes. It is to be noted, however, that the quite similar mode in the formation of tube-feet is known in *Henricia sanguinolenta* and also in *Asterina gibbosa*. The second pair of tube-feet succeeds the first in the same sequence of formation as mentioned above. When two pairs of the organ are formed, the unpaired tube-foot, azygous tentacle, is formed. In the present stage the larval organ becomes slightly elastic, and numerous fine cilia are found on the aboral surface. When the larva at this stage was removed from the substratum by needle, the larva could not adhere again to the ground any longer. And also when the larva were turned upside down, the animal could not right the body and crept merely counter-clockwise by dorsal cilia.

Concerning the spines, a central spine and five interradials develop at first; the former being situated in the centre of disc, and the latter in interradii, one for each. Among five interradial spines, the spines located in three interradii, III/IV, IV/V and V/I, become prominent first and the spines in interradii, I/II and II/III, appear slightly later on. After the appearance of these spines, the terminal spines become formed in pairs on each arm-rudiment. The spines located on arms III and IV develop more quickly than those on the other arms. The spines on arms II and V are next in order, and the spines on arm I are the slowest in appearance. The formation of the terminal spines seems to cor-

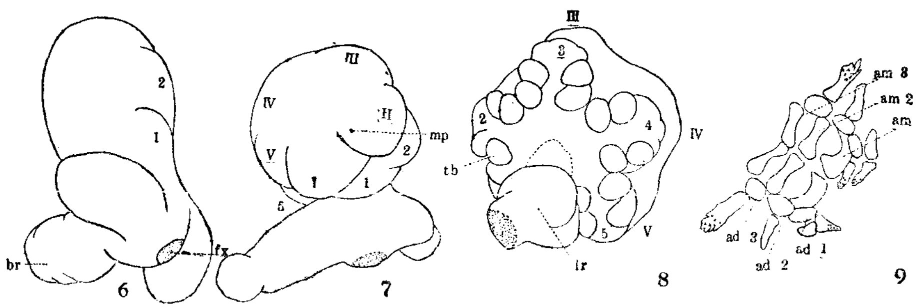

Fig. 6. Ventral view of early brachiolaria, showing the budding of hydrocoele lobes 1 and 2: br, brachiolar arm; fx, fixing disc. ×35. Fig. 7. Avoral view of brachiolaria: I-V, rays; 1-5, hydrocoele lobes; mp, madrepore. ×35. Fig. 8. Oral view of brachiolaria at metamorphosis, showing the rudimental tube-feet and degenerated larval organ: lr, larval organ; tb, tube-foot. For explanation of the number I-V and 1-5 see Fig. 7. ×35. Fig. 9. Aboral skeleton: ad, adambulacral ossicle; am, ambulacral ossicle. ×25.

respond in sequence to that of tube-feet; the faster the hydrocoele lobes are provided with the tube-feet, the more rapidly the spine grows in the radius. As the terminal spines on each arm increase in number, the inter-ambulacral spine is newly formed between the terminal and interradial spines. The radial spines, one for each radius, are developed lastly among these five kinds of the primary aboral spines. The rudimental crossed pedicellariae also occur on inter-ambulacral ossicles and the terminals. In the stage mentioned above, the oral spines appear around the water-vascular ring, two or, rarely, one for each interradius. The mouth portion becomes to have a small opening, and the rudiment of eye-spot is formed as a small slightly reddish bulb in each tentacle. The embryo is of petaloid form with a short and thick stalk, and the disc of the sea-star is distinguished from the blund rays. In the later stage, the tube-feet become functional and the larva begins to move more freely around the larval organ fixed to the bottom. The organ is elastic and sensitive to stimuli. If the larval body or the organ are touched slightly with needle, the muscles of the organ are retracted to draw the body toward the fixing point. The youngs measuring 0.8 mm in R are furnished with fundamental plates and four pairs of tube-feet. The larval organ is afterwards abandoned, and the anus opens in the centre of disc. Thus, the youngs disperse from the habitat of their mothers about in the middle of June at Akkeshi.

The skeleton of the young sea-star

1) *Aboral skeleton*: The dorso-central plate is thin, perforated, nearly pentagonal in form, each corner being located in radius. There are three spines on the plate; one of them stands in the centre of disc, and each of the other two is found between the centre of plate and the corners in radii I and V. The anus is located in radius I near the central spine. The interradial plates are perforated, tetrapezoid in form, similar to the dorso-central in size. The plates are bent toward the oral side of body. They are arranged in each interradius around the dorso-central, the upper base being opposite to the latter. The interradials are each armed with three spines, one standing in the middle of the upper base, the rest in each corner of the lower base. The former spine appears preceding the latter. The terminals are also perforated, wider than long and sub-hexagonal in form. The plate is provided with eleven spines, seven of which are located in the dorsal surface, and the remainders in the ventral surface. The six spines on the dorsal surface are arranged in pairs along the dorso-lateral margins of the plate, and a spine is situated near the centre of the plate. The terminal spines on the ventral side are arranged in a series along the terminal margin of the plate. A pair of rudimental crossed pedicellariae are found on the plates, each situated between the proximal first and second spines in the dorso-lateral series. It measures 0.08 mm in length. A small interambulacral plate is added in each space enclosed by the three plates; radial, interradial and terminal plates. The inter-ambulacrals are longer than wide, rectangular in form, each being beset with a crossed pedicellaria at the proximal end and with a spine at the distal.

The crossed pedicellariae are larger than those on the terminal plates, measuring about 0.11 mm in length. The radial plates developed later are small, roundish in form, then gradually become sub-pentagonal, about half as large as the inter-radials. The radials are each armed with a single spine.

These aboral plates of five kinds described just above grow further on, increasing in size, to form a primary meshed skeleton with smaller secondary plates developed later. It is noteworthy that the straight pedicellariae are absent in the young stage mentioned above, though two crossed ones are present. The specimens are perhaps too young to have them.

2) *Ventral skeleton*: The ambulacrals are arranged in pairs on each side of the middle line of ray, where they are in contact with each other at their ends. The first ambulacral ossicle (A_1 plate according to the numbering of Ludwig), is stouter and broader than the succeeding ones The adambulacral plates are arranged in longiseries, at the outer ends of the ambulacrals, but the former do not correspond in position to the latter. The first adambulacrals (Ad_1, Ludwig) are in contact with the first ambulacral plates (A_1, Ludwig). The adambulacrals are oval in form, each with a single spine. The first tube-foot is boarded with three ossicles; the second adambulacral plate, and first and second ambulacrals. The buccal ring of the young is composed of the first ambulacral plates (A_1, Ludwig) and first adambulacrals (Ad_1, Ludwig). In the present species the mouth-angle plates (Ad_1, Ludwig) have not a calcified connection with the corresponding ambulacral plates (A_1, Ludwig) but in *Solaster endeca* and *Crossaster papposus* observed by Gemmill ('16), the former are usually continuous with the latter by calcification.

Judging from Fisher's paper ('30) the manner of the brood-protection of the genus *Leptasterias* seems not to be the same in different species. However, so far as the development of the present species has been examined by the writer, the embryonal form of the species in each stage bears in general resemblance to that of *L. hexactis* reported by Osterud ('18). It is surmised, therefore, that the developmental course of *Leptasterias* would be, as a rule, similar in each species, although the parental care may have some influences on the development of the broods.

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