Natural Science Report, Ochanomizu University, Vol. 6, No. 2, 1956
お茶の水女子大学 自然科学報告 第6巻 第2号

The Spawning of *Lingula*

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Lingula unguis, formerly identified as *L. anatina*, once occurred in great abundance near the Biological Station at Misaki, and its development was splendidly worked out by Yatsu in 1902. At present, it has become rather scarce owing to unknown reasons, yet concerning its embryology there still remain many problems which require further examinations such as those about its spawning habit, its cleavage pattern and the axial relationship between eggs and larvae. The followings are the observations on the spawning habit of *Lingula* made at Misaki in 1954 and 1955.

Shedding

According to Yatsu, breeding season of *Lingula* in Misaki lasts from the middle of July to the end of August. His decision came from the plankton survey of its larvae; but, it is almost in accord with my two year's experiences, which also suggest the peek being present somewhere around the first half of August. Yatsu's observation of spawnings had been made on July 28th, 1899 and August 7th, 1901, and mine was from 6th to 10th, August, when they occurred most frequently.

The animals were cultured according to Yatsu's method with minor modifications. To imitate natural conditions, they were kept in the mud which was brought from the mud flat where they were collected. The water in the container was siphoned out once or twice a day, and the mud was exposed about one hour to direct sunlight which often raised the temperature to more than 35°C, but the animals could endure. After such exposure, fresh water was filled and it was kept standing still in most cases.

As was described by Yatsu, germ-cells are emitted through the median one of the funnels which are formed of the setae surrounding the anterior margin of shells. The funnels are widely opened then, and the emission takes place rather powerfully in a stream looking like a small fountain or, as Yatsu described it, like an eruption of miniature volcano. The stream first rises high up in the water and gradually falls toward the surface of the mud, over which it then expands. When the funnels lie obliquely, the stream marks out a fine parabolic curve, and can sometimes rise even to the height of 8 cm and reaches to a distance of 10 cm a head. At first sight, the stream gives a picture of one

simple uniform line, but many cases are found in which its proximal or basal part consists of a pair of lines, each passing side by side through the funnel, and particularly in the cases of sperm-shedding, the frontal end of the stream is often observed to be bifurcated. This fact shows that the stream is not a simple one, but is a composite of a pair of streams which come from the right and left nephridia (Fig. 1).

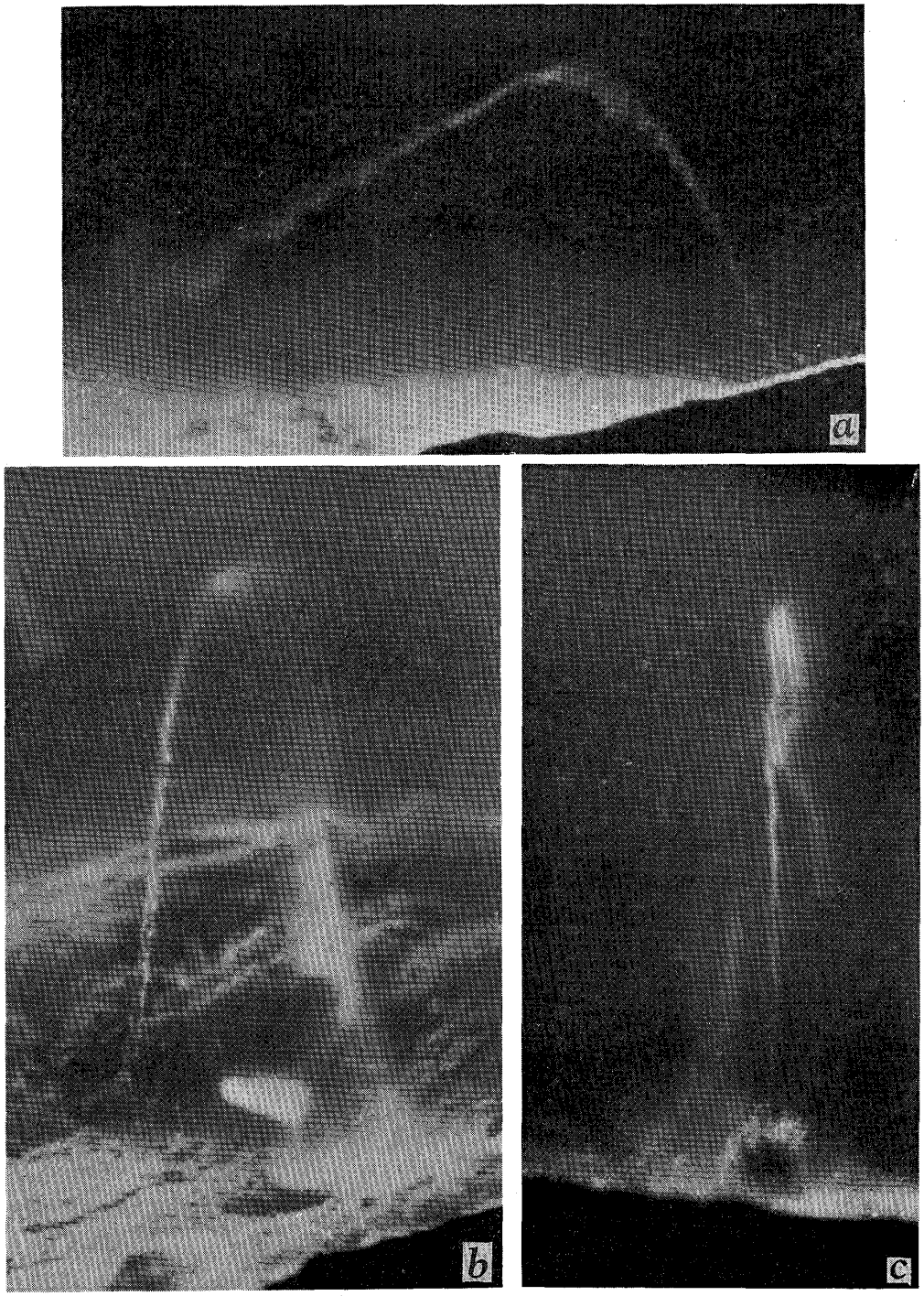

Fig. 1. Three examples of sperm-shedding. In (*a*), the shedding animal is hidden in the mud at the right-hand corner. In (*b*), the proximal part of the sperm-stream consists of two lines.

Once the emission begins, it continues uninterruptedly in most cases. Its duration, however, varies. Sometimes it is as short as for a few minutes, but usually ends within an hour. The largest emission I have ever met was that of the sperm which took place in the evening, Aug. 8th, 1955, and lasted for 2 hours and 55 minutes from 7:15 to 10:10. Generally speaking, sperm emissions continued somewhat longer than those of the egg. And the largest egg-emission witnessed lasted for 2 hours in the morning, Aug. 7th, 1955 from 7:10 to 9:10. When sperms are emitted, the stream looks milky in colour; but in the case of the female, the yellowish eggs are emitted as egg-culsters surrounded by an amount of mucus, so that the stream represents itself as an yellowish dotted line, a dot being an individual egg-cluster (Fig. 2).

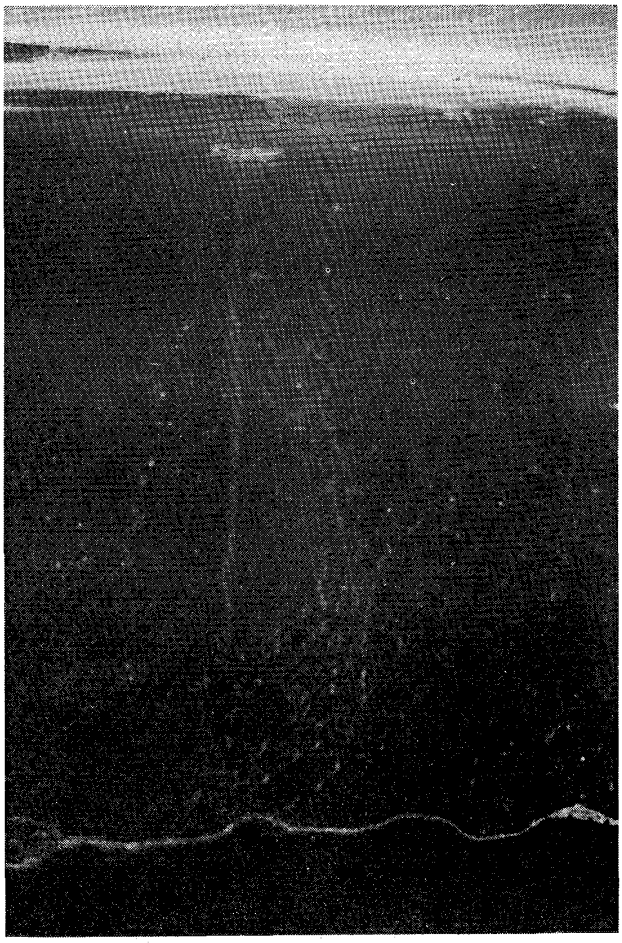

Fig. 2. An example of egg-shedding. A stream of egg-clusters is rising straight upwards to the surface of the water, and then gradually falling down towards the mud surface.

Factors in Shedding

1) *Time of Day*

Yatsu could observe the spawning of *Lingula* three times. The first was at 6:00 A.M. on July 25th, 1899 and the other two were also on the morning (the time unknown) of Aug. 7th, 1901. All were several days after the collection. But, my experiences in 1954 were all had in the evening. The first occurred as late as 11:00 P.M., on Aug. 6th and the second at 7:30 P.M., on the 8th of the same month. They occurred several days after the collection as Yatsu's had. Various factors which might influence the process, such as the time of day, or the time of high or low tides was suspected, but no decisive evidence was then obtained.

In the summer of 1955, I was favored by a fairly lot of chances for observing the spawning, and the results obtained were briefly summarized as in Table I. As can be seen from the table, about a half

Table I

List of Sheddings Occurred in the Summer of 1955

		Morning				Afternoon				
Group	Date of shedding	Time of shedding	Time of sunrise (Tokyo)	Time of high tide	Succession of sheddings	Time of shedding	Time of sunset (Tokyo)	Time of high tide	Succession of sheddings	Date of collection
A	July 20	6:30	4:39	4:46	♂-♀	3:30	6:55	6:21	♂	July 19
B	July 21 July 25					2:45 9:00	6:54 6:54	6:51 8:46	♂ ♂♀	July 19
C	Aug. 5 Aug. 5 Aug. 5 Aug. 5 Aug. 6 Aug. 8 Aug. 9 Aug. 10	5:30 7:15 7:15 10:00 5:00 before 9:00 6:30	4:51 4:51 4:51 4:51 4:52 4:54 4:55	5:21 5:21 5:21 5:21 5:56 8:01 8:51	♀-♀ ♂ ♂-♂ ♀ ♂♀ ♀♀♀♀ ♀♀♀♀	 8:40 5:10* 9:00	 6:40 6:39 6:38	 7:41 7:01 8:31	 ♀-♀-♀-♀ ♀-♀-♀-♀-♀-♀ ♀	Aug. 4
D	Aug. 6 Aug. 7 Aug. 8	 5:30 4:30	 4:53 4:53	 6:41 7:11	 ♂-♂-♂-♀-♀ ♂♀♀♀♀	9:00 8:30 7:15	6:42 6:41 6:40	7:01 7:16 7:41	♂♀-♀ ♂-♀ ♀♀-♂-♀-♂-♀♀♀	Aug. 6
E	Aug. 6 Aug. 7 Aug. 8	 6:40 4:30	 4:53 4:53	 6:41 7:11	 ♂ ♂♂♀	9:00 8:10 7:15	6:42 6:41 6:40	7:01 7:16 7:41	♂-♀ ♂-♂-♂-♂ ♂♂-♀	Aug. 6
F						4:30 9:20	6:26 6:23	6:41 7:11	♀♀ ♂-♀♀♀♀♀♀♀♀♀	Aug. 20

Time of sunrise or sunset at Misaki is almost the same with that at Tokyo. * Shedding of this case was experimentally induced.

number of the cases listed (12 out of 26 cases) spawned in the morning, while the remaining 14 cases in the afternoon, and mostly after sunset. But any correlation between the time of spawning and that of flowing or ebbing of the tide could be found, for no indication of the shifting in the former was noticed along with that in the latter.

Among the cases of spawning in the morning, 2 cases that occurred on Aug. 9th and 10th lack their exact records of starting time, since these were used for another experimental purpose as will be explained later. Of the remaining 10 cases, in 9 were found spawnings taking place between 4:30 and 7:30 o'clock. The one case of spawning as late as at 10:00 A.M., Aug. 5th was a female. The vessel containing this individual had been overnight run by sea water together with other two vessels which had a few males. These males, however, spawned at 7:15 o'clock in the same morning in spite of continual cooling by the running water.

Of 14 cases of spawning in the afternoon, the one on Aug. 9th should be excluded, as this was also used for another experimental purpose as described later. Of the remaining 13, in 10 cases spawning took place in the evening from 7:15 to 9:00, and in the other 3 it occurred before evening. The materials that spawned at 4:30 on Aug. 20th had been newly collected and just brought into the laboratory, when they began to spawn while being prepared for culturing. Some stimuli such as a sudden change of water must have had some effect. As regards the other two cases whose spawning occurred at 3:30 P.M., July 20th and at 2:45 P.M., July 21st in the materials collected on July 19th, no possible causes could be adequately ascribed. But, the spawning in these 3 cases had occurred either at the beginning or near the end of the breeding season, and it is thinkable that during these periods some conditions might be present which made the spawning rather sporadic.

In contrast to these exceptional few cases, materials collected during the first half of August provided much clearer results. They strongly showed a bent for spawning in daily rhythm; that is, they spawned regularly twice a day, the one taking place soon after sunrise and the other soon after sunset, and perhaps the best ex-

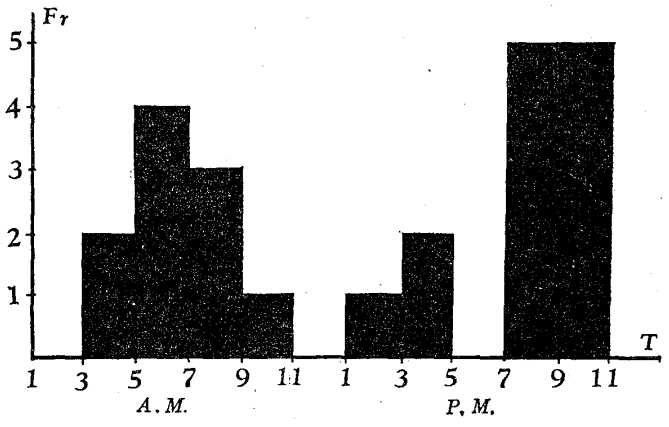

Fig. 3. Frequencies of occurrence in spawnings (*Fr*) according to the time of day (*T*).

amples of this were furnished by the materials collected on Aug. 6th. These were 21 in number and were grouped into two, D and E. The group D consisted of 13 individuals and were cultured in the vessel D, while 8 individuals of the group E in the vessel E. Both were cultured under similar conditions, whereupon spawning began in both from the very evening of the day collected and continued in a quite similar fashion until several days after.

In the vessel D, for example, a male and almost simultaneously a female began to spawn in that evening at 9:00 o'clock, and this was followed by another female at about 30 minutes later. In the next morning (Aug. 7th), spawning took place rather in a larger scale. This was begun by a male at 5:30, and then the second male, that was tangentially opposite to the first male and was being poured upon itself the sperm suspension, started its spawning at 6:30. At 70:8, when the water was much contaminated by the sperm suspension, the third male began to spawn, and this was followed by two females at 7:10 and 7:26 respectively. The spawning by one of these females continued for 2 hours, and this was the largest egg-emission ever witnessed. Unlike the morning-shedding, that in the evening completed rather shortly. It was initiated by a male at 8:30, and a female followed at 8.57.

In the next morning (Aug. 8th), when I entered into the laboratory at 5:30, sheddings were taking place in both D and E vessels. In D, these were by one male and by four females, of which one was the individual that had spawned last evening. But, from the degree of contamination of the water and from the eggs not cleaving yet, their starting time was inferred as within an hour, hence it was recorded as 4:30. After sheddings were over, the vessel was washed and filled with a fresh water. Then, the male that had already ceased its spawning at 6:00, started it again at 7:15. In that evening, spawnings took place rather in a large scale. This was initiated at 7:15 by two females, which had shedded in the morning. At 7:55 a male started its shedding, and 2 minutes later a female nearby followed as if affected by the male. At 8:00, the second male began to spawn, and this was followed at 8:10 by the fourth female and at 8:35 by two more females that had shedded in the morning. Thus, the shedding in the vessel D completed. Its culturing was continued several days more, but no more shedding was observed.

As was said before, spawnings in vessel E persisted in quite a similar fashion with those in vessel D. They also started from the evening, Aug. 6th and completed in the evening, Aug. 8th, when the largest sperm-emission above mentioned took place. Their similarities lie not only in the times of spawning, but also in the starting time of each spawning. As seen in Table I, the only exception was in the

morning, Aug. 7th, where the spawning in D started at 7:40 by a male, while in E it did at 5:30 by a male.

That the spawning of *Lingula* takes place regularly twice a day at least during the middle part of the breeding season, is clearly indicated by the foregoing descriptions. But, the problem whether this is a tendency inherent in the animal, or a result affected by the change of light condition, is difficult to ascertain at present. Some experiments were tried, but there were indications that both might be true.

To test the second alternative, the light condition was artificially altered by covering with a black cloth the vessel whose individuals were spawning regularly twice a day. The group C collected on Aug. 4th was first used for this experiment. It was covered soon after the evening shedding on Aug. 8th was over, and left so until the next morning. But, when uncovered at 90:0 A.M., it was found that the spawning had already begun by four females. The same experiment was repeated with the same materials in the following morning, but the result was the same and the spawning occurred at 6:30 A.M. in spite of the darkness. Group D and E were also spawning regularly twice a day. Group D was placed in the dark at 3:00 P.M., on Aug. 8th, and E was left as control. But spawnings took place at 7:15 in both.

These results showed that the spawnings persisted to take place at regular time of day notwithstanding the light condition being changed artificially or remained unchanged. But there was one case in which the change of light condition was able to induce the initiation of spawning. This occurred when the Group C was used as material and was covered at 4:00 P.M., Aug. 9th, when the spawning was found initiated at 5:10 P.M. instead of at the regular time after sunset.

2) *Influence of Sexes*

Yatsu had an opinion that the female could only spawn after it was influenced by the sperm shedding from nearby males. But, my experiences taught that this was not the case, although the sheddings initiated by female individuals were comparatively few in number. Among 28 cases so far observed, 5 cases were not ascertained as to which sex had initiated, and among the remaining 23, 14 (60.8%) were begun by the male, while 9 (39.2%) by the female. This showed that although both sexes were capable of initiating shedding, it was far easier for the male than for the female.

As described several times before, when the shedding was started by some individuals, this was generally followed successively by other individuals in the same container. This fact suggested the possibility of the initiation of shedding being induced by sheddings from other individuals. And perhaps, this view was also favored by often obser-

vable cases in which the shedding by some individual begun soon after this individual was covered by the sperm suspension emitted by other individuals. Sperm suspension or the emitted egg-masses had been tried to stimulate shedding, but so far the experiments were not successful.

3) *Population in the Vessel*

When a number of individuals are cultured in the same vessel, there are chances to have the sperm-sheddings by more individuals than desired. In such cases, the water in the vessel, much contaminated by the emitted sperm suspension, becomes inadequate for further observation and for the collection of eggs. Therefore, in the materials collected on Aug. 4th, it was tried to culture two or three individuals in each smaller vessels. But, the spawnings in these vessels did not occur so frequently, and they occurred only in the morning, Aug. 5th in 4 vessels out of 10, thus prepared, and in one in the morning, Aug. 6th until the morning, Aug. 8th (Table I).

In the afternoon, Aug. 8th, all individuals in these vessels were gathered and cultured in one container. Then, the spawning took place at 8:40 in the evening of that day, and this continued quite regularly twice a day until the evening, Aug. 10th.

This result suggests that a certain condition, under which the individuals in the same container could get some influences from each other, is important for the shedding to initiate. We have no knowledge at present about what kind of influences they were exerting to each other, but something effective must be secreted into the water from the mature individuals.

Summary

1) The breeding season of *Lingula unguis* lasts in Misaki from the middle of July to the end of August, and there is the peek around the first half of August.

2) Germ-cells are emitted through the median funnel of setae at the anterior margin of shells, and this takes place rather powerfully in a stream looking like a small fountain. Generally speaking, sperm-emission continues somewhat longer than the egg-emission, and the largest emission of sperm witnessed continued for 2 hours and 55 minutes, while the egg-emission was for 2 hours.

3) Among the factors in shedding, time of day was considered to be the most important. Sheddings occurred quite regularly twice a day at least during the middle part of the breeding season, the first one taking place in the morning soon after sunrise and the second one in the evening soon after sunset. But, the problem whether this per-

sistent tendency was inherent in the animals, or it was a result affected by the change of light condition was not settled. There were evidences that both might be true.

4) When shedding was initiated by some individuals, it was generally followed successively by other individuals in the same vessel. This fact suggested that possibilies of some effect in the shedding of some individuals that might induce other individuals to shed. Some experiments were tried, but no experimental evidence of it was obtained.

5) Aggregation of animals seemed to provide a certain condition that might be important for the animals to shed, since the frequency of shedding was greatly reduced in the vessel in which only a small number of individuals were cultured.

Literature

Yatsu, N. 1902. On the development of *Lingula anatina*. Jour. Coll. Sci. Imp. Univ., Tokyo. 17.

(*Received Jan. 31, 1956*)