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Reproduction of Ulvaceous Algae with Special Reference to the Periodic Fruiting

I. Semilunar Rhythmicity of *Ulva pertusa* in Northern Kyushu

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Fruiting of *U. pertusa* occurs during the neap tides at biweekly intervals in Tsuyazaki, northern Kyushu, Japan. Towards the beginning of a neap tide series, sporophytes first become fertile, and they alone can be found for 1 or 2 days. But fertile gametophytes rapidly increase in number 1 or 2 days after sporophytes have become fertile, and dwindling fertile sporophytes are outnumbered by increasing gametophytes. Thus, there is a dominancy reversal between sporophytic and gametophytic fertile plants in each neap tide series. Such a reversal takes place towards the first or the last quarter of the moon. Fertile plants are recognized through almost a week. Fertile areas of sporophytes are fluorescent grayish green or dull green in water, but out of water the fluorescence disappears and it is sometimes difficult to distinguish between fertile and vegetative areas without having close observations. This alga seems to be anisogamous, and there is little difference in the flagellum length of three kinds of swarmers.

INTRODUCTION

Along the coast of Japan, ulvaceous algae have been supposed to liberate swarmers during the spring tides, and this has been endorced by Chihara (1969). Not only in Japan but also in California (Smith, 1947) and in the Menai Straits (Christie and Evans, 1962), such a biweekly periodicity was previously reported. Although, in respect of the detailed fruiting process, there can be found some apparent differences between these reports, they all agree to that the liberation of swarmers occurs during the spring tides.

Since the senior author was convinced that the fruiting would occur in every spring tide series all over the Japanese coast, he did not make any consecutive observations. However, in his field trips around northern Kyushu, it would rather be so difficult for him to obtain fertile fronds in supposed period that he attempted to follow the fruiting of these algae. In 1967, the authors had an opportunity to carry out a four-month research project on the fruiting of *U. pertusa* following the occasional observations in the preceding year. The purpose of the present study was to probe whether or not the fruiting of this alga actually occurs during the spring tides in this part of Japan. The same observations were made by the senior author for three weeks in the May-June period of 1969 which proved to be quite the same as those of the preceding results. In this report, observations are presented which indicate that the fertile fronds of both sporophytes and gametophytes appear during every neap tide series, in regular sequence with a overlap of a few days.

MATERIAL AND METHODS

All of the material for the present research was collected in Tsuyazaki, about 20 km to the northeast of Fukuoka City, northern Kyushu. *U. pertusa* is one of the most thriving species among ulvaceous algae there, appearing from December to August at the latest. But the heaps of this alga are sometimes so heavy along the shores in May that the vegetative growth of this alga seems to be at its maximum in this season. Tsuyazaki was visited each day throughout May and June straightly followed with not continuous but intensive observations in July and August of 1967 until the fronds faded away. As color change is quite remarkable in fertile fronds, it is easy to distinguish fertile ones from sterile ones. Only when the fertile fronds were recognized, about 100 of them were collected and brought to the laboratory in Fukuoka City. The amounts of fertile fronds in the field were not always equal day by day, so the authors tried to make the same effort in collecting the material so as to follow the quantitative changes under natural conditions. Since daily collections were made when the material was still in water on the ebb tide, every time interval between two successive collections was variable within about 25 and 15 hours.

After return to the laboratory, each blade, usually a piece of fertile area, was placed in a separate Petri dish of sea water. Swarmers were generally ready to be released by this time, 2 to 4 hours after collecting the material. One drop of sea water with dense swarmers was placed on a slide, that was inverted for about 1 minute over a vial containing a 2 per cent solution of osmic acid. Swarmers were then stained with a solution of crystal violet for the microscopic examination to ascertain whether they were gametes or zoospores.

RESULTS

Periodicity of reproduction

Tsuyazaki itself is on the open sea, but the station where the field observations and collections were made is located at the head of a long comma-shaped beach, facing south. According to such topographical conditions, daily observations were possible even in the rough sea all through the term. The present alga grows on the breakwater and scattered stones around it, in the middle to low intertidal zone, In Tsuyazaki, as elsewhere along the western coast of Japan, the water level changes within a small range during the neap tides, and the highest low water through a lunar month does not fall low enough to expose the present alga to the air, leaving most of its colonies in water, as far as day time concerned. Such was the case when fertile fronds were collected every morning during the neap tides.

Table 1 shows the results obtained from June 12 to July 1 of 1967, covering

Table 1. Rise and fall of fertile plants of *Ulva pertusa* in Tsuyazaki, northern Kyushu, 1967.

Date	Moon's age	Number of plants examined			Time of low water	Water level at low water
		Total	Gametophyte	Sporophyte		
June 12	3.9	24	0	24	556	78
13	4.9	50	0	50	642	81
14	5.9	99	6	93	740	82
15	6.9◐	93	75	18	853	81
16	7.9	44	44	0	1021	74
17	8.9	69	69	0	1145	62
18	9.9	88	88	0	12.51	47
19	10.9	57	51	6	1347	32
20	11.9	38	16	2	1438	19
21	12.9	3	2	1	1526	11
22	13.9○	0	0	0	3613	9
23	14.9	0	0	0	1658	11
24		0	0	0	1743	18
25	16.9	0	0	0	517	73
26	17.9	62	0	62	558	75
27	18.9	103	27	76	644	77
28		55	55	0	733	81
29	20.9	44	42	2	834	84
30	21.9◑	85	83	2	948	84
July 1	22.9	39	39	0	1111	80

two successive neap tides. Only a few fertile plants were recognized during the spring tides, and numerous fertile ones spread over the substrata during the neap tides. The rise and fall of fertile plants represents a fundamental pattern of fertility in this alga there. Towards the beginning of a neap tide series, the fertile sporophytes came first to appear and rapidly increased in number. Then, they gradually or rather abruptly ceased fruiting, releasing zoospores on straight 3 or 4 days. The gametophytes, on the other hand, began fruiting 1 or 2 days after the sporophytes had become fertile. The fertile gametophytes increased in number as rapidly as the sporophytes did, but the cessation of fruiting was not as sharp as that of sporophytes, dwindling away into nothing in 6 days or more. As are shown on June 15 and 28, dominant fertile sporophytes on previous days were replaced with increasing fertile gametophytes. Because such reversals in number had been taking place since May when they began the research, it was not impossible to presume when the next reversal would occur. Consequently, not straight but intensive observations were continued until *U. pertusa* faded out late in August, with special attention being given to supposed critical periods. Three dates confirmed during this term are listed in Table 3 together with other such ones during this season of the year.

In 1969, the senior author resumed the same investigation to confirm two striking facts revealed the year before last : One, occurrence of fertile plants during the neap tides ; the other, dominancy reversal between sporophytic and gametophytic fertile plants. Collections were made at the same place in the same way. But this time, as much as 100 samples were examined in the laboratory when fertile plants were numerous, and in case of not numerous, appropriate samples were examined in proportion to the amount of fertile plants which were visually judged. Although no lines for the ecological survey were employed in collecting the material, some clues on the quantitative changes of fertile plants have been found out. Table 2 shows the results obtained from May *20* to June 8, including two critical periods covering two successive neap tides. This indicates that the same happened as in 1967. For example, there were thousands of fertile plants on May 24, the day of the first quarter of the moon, and no fertile plants were recognized on May 31, the day of full moon. Furthermore, dominancy reversal between sporophytic and gametophytic fertile plants took place on May *23* and on June 6, exactly reproducing that of 1967. According to the additional observations made about two weeks later of the same month, the next reversal occurred on June *22* after a 16-day interval.

Table *2.* Rise and fall of fertile plants of *Ulva pertusa* in Tsuyazaki, northern Kyushu, 1969.

Date	Moon's age	Number of plants examined			Time of low water	Water level at low water
		Total	Gametophyte	Sporophyte		
May *20*	3.8	0	0	0	1819	36
21	4.8	80	1	79	559	76
22	5.8	100	5	95	642	83
23	6.8	100	**75**	**25**	736	87
24	7.8◐	100	86	14	850	90
25	8.8	**100**	99	1	1018	85
26	9.8	72	70	2	3 140	74
27	10.8	100	96	4	1241	58
28	11.8	11	11	0	1330	40
2Y	12.8	16	8	8	1417	24
30	13.8	18	18	0	1503	11
31	14.8○	0	0	0	1549	2
June 1	15.8	0	0	0	1637	−1
2	16.8	1	0	1	1727	0
3	17.8	0	0	0	1819	6
4	18.8	62	0	62	607	70
5	19.8	100	6	94	702	76
6	20.8	12	9	3	803	79
7	21.8◑	28	26	2	918	79
8	22.8	100	88	12	3 039	75

During the second neap tide series in the research of 1969, fertile plants were unusually few on June 6 and 7, and so were they on May ***26.*** If it had been essential to obtain 100 samples, he might have collected them with some efforts, As he tried to make the same effort throughout the term, these figures mean that there were only a few fertile plants in the field. Such irregularity in amount of fertile plants on each of neap tide days has been noticed since 1967 in the preceding research. One of the most probable factors that control the fruiting of ***U. pertusa*** seems to be light condition. For, it rained 1 or 2 successive days before the fall of fruiting. Some referable factors including weather conditions will be dealt with in following papers.

Table 3 shows 11 dates and related references when fertile sporophytes were outnumbered by fertile gametophytes. Of 11 dates confirmed during the period from May to August, 4 fell on either the first or the last quarter of the moon, and 5 fell on the previous day to these quarters, and even the rest 2 fell within 2 days of the quarters. The amplitude of water level in northern Kyushu is the least sometimes on the days of the first or the last quarter, or sometimes on the following days of these quarters during the season concerned. Fruiting is accordingly centered during the neap tides, and occurs periodically with a mean time interval, as marked by the day when fertile gametophytes outnumber sporophytes, of 14.7 days. Considering that this interval is close to one half of a lunar month of 29.5 days, it can be accepted that the fruiting of *U. pertusa* corresponds to the phase of the moon.

Table 3. Dates and conditions when fertile gametophytes outnumbered fertile sporophytes in *Ulva pertusa.*

Date		Interval (days)	Moon's age	Before (−) or after (+) quarters (◐,◑)	Time of low water	Water level at low water
1967						
May	3		23.2	+2	1207	83
		14				
	17		7.5	◐	906	87
		14				
	31		21.5	◑	923	89
		15				
June	15		6.9	◐	853	83
		13				
	28		19.9	−2	7333	81
		16				
July	14		5.9	−1	833	7%
		14				
	28		20.4	−1	741	77
		16				
Aug	13		7.0	◐	925	66
1969						
May	23		6.8	-1	736	87
		14				
June	6		20.8	-1	803	79
		16				
	22		7.2	-1	811	84

Colors of fertile areas and swarmers

As Arasaki (1946) and Smith (1947) have reported the color change in fertile areas, three plants of *Ulva*, i.e., male, female, and asexual, are supposed to be distinguished with the naked eyes if they are in fruiting conditions. Although there are some small differences in color between their expressions, it seems that they agree with each other in general. In *U. pertusa* around Tsuyazaki, colors of male and female fertile areas are not always distinctly separated, but fertile areas of male gametophytes are yellow to dark yellow and the most yellowish of all as have been described by the two authors. Those of female are dark yellow to yellowish brown. Clouds of male and female gametes also retain the colors of fertile areas of respective gametophytes. Thus, gametic union has been able to be observed in almost all cases by mixing 2 drops of male and female gametes each of which showed the most probable tint.

According to Arasaki, fertile areas of sporophytes are yellowish deep green, which are difficult to be distinguished from vegetative parts, and they are dark green by Smith. Those of sporophytes of ***U. pertusa*** in Tsuyazaki look grayish green or dull green and rather fluorescent when they are in water, so they can never be overlooked. But out of water, the fluorescence disappears and it is hard to discern between fertile and vegetative without having a close observation. This led the authors to reach Tsuyazaki when water is high enough to cover the *Ulva* colonies. Fertile sporophytes are abundant under such conditions as fronds are hardly emerged out of water at the ebb tide during the neap tides, which favored the authors to perceive them in the very early days of this research. Clouds of zoospores from sporophytes are green with little yellow or brown tint.

Size of swarmers

Isogamous as well as anisogamous sexual reproduction has been reported on ***U. pertusa*** at some localities in Japan. But since no reports on this subject are available with the material obtained in western Japan, the authors measured the size of swarmers. After they were confirmed as male gametes, female ones or zoospores, each of 20 swarmers from each of ***20*** plants was measured in body length, width, and flagellum length. Because the authors had been able to recognize fertile gametophytes on the breakwater even late in the morning, the liberation of swarmers might not always take place early in the morning, and it had been quite easy to obtain swarmers in the laboratory at any time of a day. Freshly liberated swarmers were fixed, stained, and microscopically measured.

The body length of male gametes varies between 5.1 and 7.6 μ, but many of them are 5.6–6.1 μ long. That of female gametes varies between 5.3 and 8.4 μ, and many of them are 7.0-7.6 μ long. The ranges apparently overlap with each other, but female gametes are generally a little longer than male ones by about 1.5 μ. On the width of gametes, male and female gametes measure, respectively, 2.5-3.8 μ and 2.5-4.5 μ, and many of female gametes are more wide than male ones by about ***0.5*** μ. Zoospores measure 8.1-12.7 μ long and 4.3-7.6 μ wide, most of which are 9.9–10.4 μ long and 5.1-5.6 μ wide, being the biggest of the three kinds of swarmers. Although there are some differences in the size of three kinds of swarmers, there are little remarkable differences in the length of flagella, measuring 10.1–15.2 μ long, and about one half of swarmers have flagella of 12.7-13.2 μ long.

DISCUSSION

In their culture studies on ulvaceous algae, Miyake and Kunieda (1931) reported that only 8 plants out of several hundreds of *Ulva* gave gametes at Misaki. Such a considerable unbalance in the amount between gametophytes and sporophytes was again reported by Yamada and Saito (1938) on ***U. pertusa*** and ***E. linza*** at Muroran. They examined 75 plants of ***U. pertusa*** and found that 72 of them produced gametes and the rest produced zoospores. On ***E. linza,*** they examined 151 plants of which 140 produced zoospores and 11 plants 2-ciliated swarmers. According to Smith (1947), these conflicting reports seemed to be well explained with the fact that they did not fruit simultaneously. In Tsuyazaki, however, the results were quite different from those of others on the facts that both sporophytes and gametophytes fruit during the neap tides and that sporophytes first become fertile which are then outnumbered by fertile gametophytes.

Miyake and Kunieda observed a large number of gametes from 6 plants of *Ulva* on the early morning of July 3, 1930, one of the neap tide days at the moon's age of 6.6. Since there is occasional fruiting during the spring tides in Tsuyazaki, it may be possible to observe fertile plants during the neap tides at Misaki where the fruiting is supposed to occur during the spring tides. During the spring tide series of a spring-summer season, tide is the lowest at about 11 o'clock, as far as day time concerned, in the central Pacific coast of Japan including Misaki, while it is at about 5 o'clock in the afternoon in Tsuyazaki. Field conditions may be different by such a time lag causing differences in illumination or desiccation. The influential environmental factors, however, may be left unknown until some researches are carried out on *U. pertusa* in Misaki region.

Chihara (1969) reported that big male gametes as well as small female ones measure 6.5 μ in body length in *U. pertusa.* According to the present authors, both male and female gametes considerably vary in size and they are not clearly separated each other. But, as mean values of each show, it can be said that this species is anisogamous, while in an anisogamous species of *U. lobata,* male and female gametes are, respectively, 5.3-7.2 μ and 6.0-8.8 μ, the ranges being overlapping to some extent.

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