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Reproduction of Ulvaceous Algae with Special Reference to the Periodic Fruiting

II. Fruiting of *Ulva pertusa* during the Neap Tides in Okayama

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Fruiting of *U. pertusa* was confirmed to occur during the neap tides in Shibukawa, on the Seto Inland Sea near Okayama City, Japan. Gametophytes first become fertile towards the beginning of a neap tide series, contrary to the case in Tsuyazaki, Kyushu. They rapidly increase in number and abundant fertile gametophytes can be found on about 5 successive days. Following gametophytes, sporophytes become fertile more rapidly than the former and increasing fertile sporophytes outnumber fertile gametophytes towards the quarter of the moon. This dominancy reversal took place 3 times in this study and each happened to fall on the following day of such a quarter. Dominant fertile sporophytes are only ephemeral by the second reversal in 1 or 2 days. Thus, fruiting is at fortnightly intervals, and corresponds to the phase of the moon. Illumination seems to play an important role in fruiting, because fertile thalli were remarkably small in number after 2 days of rain. Even in the water, as well as out of the water, fertile sporophytes can not be separated from gametophytes. As the body length of male and female gametes averages 5.5 μ and 7.0 μ, respectively, this alga seems to be anisogamous.

INTRODUCTION

One of the most outstanding phenomena which some marine organisms show is a semilunar rhythmicity. In *Ulva* and *Enteromorpha,* Chlorophyceae, this phenomenon is visible as periodic fruiting under natural conditions. When these algae are fully ripened and are ready to liberate swarmers, fertile areas at blade margin look yellowish or brownish and are easily distinguished from green vegetative areas. This color change is so conspicuous or drastic in these algae that the fruiting can never be overlooked even with the naked eye, and such fruiting has been supposed to occur during the spring tides. The reports by Smith (1947), Christie and Evans (1962), and by Chihara (1969) refer to the biweekly fruiting of these algae during the spring tides.

As were reported in the previous paper (Sawada and Watanabe, 1974), however, the results we obtained were different from those described in the reports mentioned above in the fact that *Ulva pertusa* in Tsuyazaki, near Fukuoka City, fruited during the very neap tides, though at fortnightly intervals. After find-

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ing this rhythm in the summer of 1967, I was much interested to see whether this was also the case with this alga growing both in each season and in each locality where the water level changes with a certain time lag and an indigenous tidal range compared with that in Tsuyazaki. So, in the following year, I visited Shibukawa, near Okayama City, expecting to find the same development as in Tsuyazaki and also to endorse the results of the preceding year. The same research was then resumed at the same place during one critical period in June of 1971, that proved to confirm the first observations in Shibukawa. In this paper, the results will be given to show that the fertile plants of ***U. pertusa*** there appear also during the neap tides as in Tsuyazaki, and that, however, there is a second pattern pertaining to the fruiting of this alga.

MATERIAL AND METHODS

All of the material was collected in Shibukawa where the Tamano Marine Laboratory of Okayama University is located. Shibukawa, on the Seto Inland Sea about 320 km to the east of Tsuyazaki, has tides similar to those in Tsuyazaki, but with some greater tidal range particularly during the neap tides, The time when lower low water of the spring tides occurs in May is 5–7 in the evening, about 30 minutes later than that in Tsuyazaki. I stayed at the Tamano Marine Laboratory about 3 weeks, from May 15 to June 7, 1968, when this alga seemed to be conspicuously abundant. Daily observations were made around the breakwaters, and only when fertile plants were recognized, they were collected at three spots on this site (Fig. 1). The amounts of fertile plants observed under natural conditions were not always equal day by day, not because of the heavy sea but even under close observations. I attempted my best to make the same effort in collecting material so as to follow the quantitative changes of the fertile plants through this term. Observations were made when *Ulva* colonies were still in the water on the ebb tide, because it was much easier to look for fertile plants. Accordingly, they were collected at about 4 on the evening of May 31, and at about 8 on the morning of June 1, exceptionally out of a regular time interval.

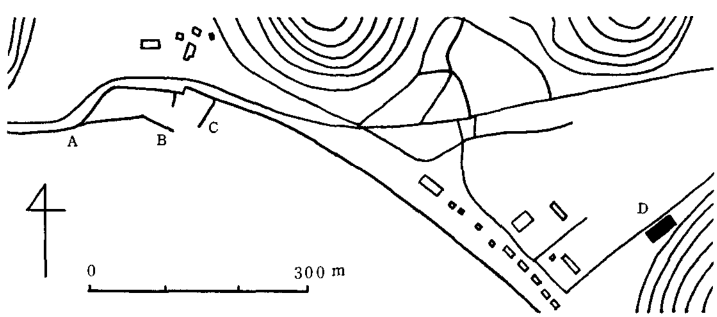

Fig. 1. A map of Shibukawa, near Okayama City, showing the Tamano Marine Laboratory of Okayama University (D) and the breakwaters where the daily observations were made (A, B, C).

After return to the laboratory, a piece of fertile area of each material was placed in a separate Petri dish of seawater. Swarmers thus liberated were fixed over a vial containing *2* per cent solution of osmic acid. They were then stained with the solution of crystal violet for the microscopic examination to see if they were biflagellate gametes or quadriflagellate zoospores.

In 1971, I made the second visit there during the presumed critical period in June to confirm the foregoing results.

RESULTS

Development of fertile plants

Daily observations were successfully completed without 'fail chiefly because of the abundance of this alga and also because of a calm sea during this term. When profuse fertile plants spread over the breakwaters, a little over 100 samples were collected and as many as 100 of them were examined in the laboratory. The figure 100 in two tables represents the abundant, innumerable fertile plants in the field, while those under 100 represent inconsiderable fertile ones, collected in proportion to the amount of them which was visually judged.

Table 1 shows the results obtained in two periods : one, from May 16 to June 6, 1968, sufficiently covering two successive neap tides ; the other, one critical period from June 15 to 18, 1971. *Ulva* colonies were dotted with only a few yellowish-brown fertile plants during the spring tides, and numerous fertile ones appeared on the breakwaters during the neap tides. With two exceptions on May 25 and 26, occasional fruiting occurred everyday even during the spring tides, but the amount was so small that they could not bear comparison with that during the neap tides. As far as the rise and fall of fertile plants is concerned, it corresponds to that in Tsuyazaki in general.

Apart from the occasional fruiting during the spring tides, however, gametophytes, instead of sporophytes, dominantly began fruiting towards the beginning of a neap tide series, contrary to my expectation and to the case in Tsuyazaki. Fertile plants rapidly increased in number up to "100" and continued swarmer-releasing for 5 consecutive days. The fertile gametophytes dominated fertile sporophytes in the early stage of this development. Fertile sporophytes, on the other hand, more rapidly increased in number until they outnumbered fertile gametophytes on the third or on the fourth day in such a "100"-day term. In Tsuyazaki, such reversed dominancy was, though opposite between gametophytes and sporophytes, retained until the cessation of fruiting towards the beginning of a following spring tide series. The dominancy of sporophytes in Shibukawa was, however, only ephemeral by the second reversal, and the fruiting of this alga came to an end with dominant fertile gametophytes 1 or 2 days after this second reversal. It was not until the fertile plants had disappeared on May 25 that a fruiting pattern of this alga in brief was found out.

Because fertile sporophytes outnumbered fertile gametophytes on May 20, the following day of the last quarter of the moon, I presumed that these fertile sporophytes would again outnumber fertile gametophytes around June 5, following day of the first quarter of the moon. In the second neap tides, the same

Table 1. Rise and fall of fertile plants of *Ulva pertusa* in Shibukawa, on the Seto Inland Sea near Okayama City.

Date	Moon's age	Global[1] solar radiation (cal/cm²)	Number of plants examined: Total	Gameto-phyte	Sporo-phyte	Time of low water	Water[2] level at low water (cm)
1968							
May 16	18.5	599	6	6	0	810	95
17	19.5	441	55	54	1	913	96
18	20.5	142	100	**100**	0	1022	93
19			100	96	4	1130	86
20	21. .5◑ 22. 5	148 371	33	**15**	18	1233	77
21	23.5	565	100	**34**	**66**	1332	65
22	24.5	447	100	62	38		53
23	25.5	502	62	62	0	1409	42
24	26.5	551	2	2	0	1533	34
25	27.5	385	**0**	0	0	1559	28
26	“8.5	493	0	0	0		24
27	29.5.	91	47	15	32	1642	20
“8	0.8	398	14	2	12	1714	19
29	1.8	480	10	2	8	1745	17
30	2.8	559	41	10	31	1823	17
31	3.8	5713	14	11	3	1904	2u
June 1	5.8	400	95	95	0		95
2	6.8	518	100	82	18	938	93
		423	100	68	32		90
4	7.8◐	515	100	52	48	1028	83
5	8.8	579	100	**33**	**67**	1221	73
6	9.8	548	100	57	43	1315	61
1971							
June 15	21.6	482	100	86	14	1006	84
16		522	21	21	0	1101	75
17	23.0 22.6◑		100	49	**51**	1158	64
18	24.6 23.6	504 365	100	79	21	1257	51

1) Observed in Okayama City : Month. Rep. Japan Meteorol. Agency.
2) Obtained by multiplying the hight at Uno by 0.786, the ratio according to my observations on June 16, 19, 1971: Tide tables, Vol. 1, M. S. A., Japan.

was reproduced as that in the preceding neap tides. Namely, gametophytes dominantly began fruiting towards the beginning of the neap tide series and fertile plants very soon reached "100." Following gametophytes, fertile sporophytes increased in number until they outnumbered fertile gametophytes on June 5, then they were again outunmbered by the fertile gametophytes on the following day. Though 100 fertile plants were examined in the laboratory on June 6, the amount of them on the breakwaters was not as much as that found on the preceding day, and it was clearly expected that the figure would be far less than one hundred on June 7.

Since I was not familiar with the water conditions in this area, the material was collected separately at three spots around the breakwaters in order not to fail in the consecutive observations and collections (Fig. 1). I could not lay stress on A in Fig. 1, and collected only 20 samples because of footing there and ticularly possible rough sea. I chose C for the last resort and collected 50 samples, and at B, 30 samples were collected. In Table 2 the results obtained at each of three spots did not always correspond to one another.

Table 2. Rise and fall of fertile plants of *Ulva pertusa* at three respective spots in Shibukawa, near Okayama City.

Date	Total	A Gameto-phyte	A Sporo-phyte	B Gameto-phyte	B Sporo-phyte	C Gameto-phyte	C Sporo-phyte
1968							
May 16	6	–	–	–	–	6	0
17	55	2	1	19	0	33	0
18	100	20	0	30	0	50	0
19◑	100	19	1	29	1	48	
20	33	2	2	9	14	4	
21	100	5	15	14	16	15	35
22	100	9	11	11	19	42	8
23	62	15	0	30	0	17	0
24	2	0	0	2	0	0	0
25	0	0		0	0	0	0
26	0	0	0	0	0	0	0
27●	47	1	8	3	13	12	10
28	14	0	2	2	6	0	4
29	10	0		2	3	0	5
30	41	1			3	5	16
31	14	3			0	5	0
June 1	95	17	0	30	0	48	0
2	100	13	7	27	3	42	8
3	100	14	6	20	10	34	16
4◐	100	7	13	12	18	33	17
5	100	7	13	9	21	17	33
6	100	11	9	20	10	26	24
1971							
June 15	100	15	5	26	4	45	5
16◑	21	0	0	0	0	21	0
17	100	5	15	16	14	28	22
18	100	10	10	25	5	44	6

(Number of plants examined)

In June of 1971, I made the second visit there to review the results obtained in 1968. Because there were 1 or 2 particular days when fertile sporophytes outnumbered fertile gametophytes, the purpose of this visit was to confirm the occurrence on such days around the quarter of the moon. Collections were made at the same site in the same way. Although the dominancy of fertile sporophytes over the fertile gametophytes on June 17 was not as considerable as that in 1968, the fruiting pattern took the same course following the results in the preceding investigation. Namely, fertile plants were examined on "100" on 15th and the result turned out to be of the gametophyte dominancy. On 16th, however, the amount of fertile plants was remarkably small probabiy due to the watery weather on 14th and 15th. On 17th, fertile sporophytes outnumbered fertile gametophytes, though by a narrow margin. On 18th, fertile gametophytes again dominated fertile sporophytes, and the amount of fertile plants on the following day was, though visually judged, far less than "100" as was expected, completely reproducing the results in 1968.

As for the each result at each of three spots around the breakwaters, the development of both fertile gametophytes and sporophytes was not the same as that of the counterpart in 1968. At both B and C, the fertile sporophytes did not increase in number as much as they would supposedly outnmber the fertile gametophytes on 17th, and neither did they on 18th without dominating the fertile gametophytes throughout the period. Only at A, the trend was barely retained as in 1968.

Colors of fertile areas and swarmers

As have been reported in several papers, male, female, and sporophytic plants are generally separated by their colors if they are in fruiting conditions. Every fertile frond is bordered in yellowish tan area, and male ones are usually considered to be the most yellowish. This agrees with the material in Shibukawa as well as that in Tsuyazaki. According to my observations in Tsuyazaki, the fertile areas of sporophytes were grey-green and fluorescent when they were in the water. But out of the water, the fluorescence disappeared and it used to be a little difficult to recognize those fertile areas from the vegetative. That was why the observations and collections had been made when *Ulva* colonies were still in the water on the ebb tide. In prospect of this in Shibukawa, I got to the breakwaters at the right time so that I might not miss the fertile sporophytes. However, the fertile sporophytes there looked much the same as fertile gametophytes, particularly of the typical female, and it was not until the swarmers had been examined under the microscope that each plant was ascertained whether it was gametophytic or sporophytic. In Tsuyazaki, it could have been possible to make exclusive collections of fertile sporophytes on the shores if it had been necessary for any particular purpose. But in Shibukawa, separation of fertile sporophytes from fertile gametophytes, specially from female ones, depended completely upon the microscopic examination in the laboratory. Although fertile areas of male gametophytes are most yellowisy, they are not always clearly distinguished from those of female ones, because the former varies in color from yellow to dark yellow and the latter from dark yellow to yellowish brown.

The clouds of male and female gametes usually retain the colors of fertile areas of respective gametophytes, which means that two model drops of opposite sex produced zygotes in almost all cases by mixing them together, but there were also many clouds confusingly or erroneously colored. The clouds of zoospores were generally green in spite of the tinge of their mother thalli, but there were still some which could be taken for female gametes without microscopic examinations.

Size of swarmers

As we reported in the previous paper, sexual reproduction of this alga seems to be anisogamous in Tsuyazaki. Because of the different pattern of fruiting as well as the different appearance of the fertile sporophytes from those of Tsuyazaki, I measured the size of swarmers. After the swarmers were separated into three kinds, male, female, and zoosporic, each of 20 swarmers from each of 10 plants was measured of its length, width, and flagellum length.

Male gametes are 2.3-4.3 $\times$ 4.3-7.6 μ and female ones are 2.5-5.1 $\times$ 5.3-8.1 μ in size. Mean values of their body length are, respectively, 5.5 μ and 7.0 μ. Those of width are 2.8 μ and 3.4 μ. Male and female gametes are not distinctly separated in both body length and width, and there can be some female gametes smaller than male gametes. But as the mean values show, male gametes are generally smaller than female ones by 1.5 μ. Zoospores are 7.6-13.2 μ long with mean value of 10.0 μ, and 4.6-7.8 μ wide with mean value of 6.3 μ. Though some zoospores small in body length are as long as big gametes, they are apparently more wide than the latter and it is quite easy to distinguish zoospores from gamtes under the microscope even without flagella. Flagellum length varies within a certain range between 10.6 μ and 15.7 μ. Mean values of both zoospores and female gametes are 13.1 μ and 13.3 μ, with no remarkable differences. That of male gametes is 12.5 μ. It can be said that male gametes have rather shorter flagella than others as far as the mean values are concerned. But it may also be accepted that there are no significant differences among their flagellum lengths.

Confusing colors of swarmers between female gametes and zoospores led me to compare the size of swarmers. The presumed correlation that the bigger ones show more green tint was not true with the female gametes, and the cloud of female gametes with mean value of 6.0 μ in body length was about to be taken for a zoosporic one.

DISCUSSION

In our previous paper (Sawada and Watanabe, 1974), two facts were clarified on the fruiting of ***U. pertusa*** in Tsuyazaki, northern Kyushu. The observations reported in this paper strongly agree to one of those facts in that the fruiting of this alga occurs during the neap tides. The investigators, who have dealt with the periodic fruiting or liberation in ulvaceous algae (Smith, 1947 ; Christie and Evans, 1962; Chihara, 1969), admit that it has occurred during the spring tides. In their reports, however, there are some instances to show that those algae could also fruit during the neap tides. According to Smith, fertile sporophytes of ***U. lobata*** appeared on July 11-13, 1944, the moon's age of 22.4, last quarter, on 13th. Christie and Evans (1962) noted that maximum liberation of swarmers from ***E. intestinalis*** occurred about three-five days before the highest tide of each lunar period in the fall season. If liberation occurs five days before the highest tide, it may be considered that the liberation is closer to the neap tides. Even with such cases, fruiting of these algae can generally be supposed to occur during the spring tides. Accordingly, ***U. pertusa*** in Shibukawa follows this alga in Tsuyazaki on its appearance of fertile plants,

However, the rise and fall of respective fertile plants, sporophytes and gametophytes, was qiute different in Shibukawa from that in Tsuyazaki and there has been no such a development reported. Tidal condition may be referred as one of factors to causing this difference. Both in Shbukawa and Tsuyazaki, the tides show a considerable diurnal inequality as elsewhere along the Pacific coast of Japan, and the lower low water during the neap tides attains water level close to the lowest through a lunar month. This should not be the only referable factor for that difference, but it may well be taken into consideratavion.

On May ***20,*** 1968, fertile plants were unexpectedly small in number as is shown in two tables. It seems that this followed the watery weather on two days previous to May 20. Such a decrease was also reported to occur in our previous paper. So, there is a strong assumption that if it had not rained on the preceding days, fertile plants would have been examined as many as "100." The same happened on June 16, 1971, during my second staying there. Though solar radiation measured 482 cal/cm^2 in Okayama City on June 15, it drizzled all day long in Shibukawa ; moreover, 1 was caught in occasional showers on my way from Kyushu on June 14.

While illumination seems to be influential on the fruiting of these algae, it is sometimes correlated and sometimes not with the liberaton of swarmers. Smith recognized the gamete discharge in his field observation a half hour before sunrise as well as in the experiments conducted in a dark room. According to Schiller (1907), on the other hand, discharge of gametes from *U. lactuca* generally occurs early in the morning, 5.30-6.30, but if it is dark because of a mist, discharge finally begins at about noon, about 2 in the afternoon in many cases. Föyn (1955) noted in his specific study of *U. lactuca* that the gametes were liberated immediately after the light was switched on, the zoospores 6 to 8 hours later. In Shibukawa, liberation did not always occur in the morning and I was able to collect fertile plants even in the afternoon for their marked color change. They used to liberate swarmers when they were reflooded by the incoming tide or, as Yamada and Saito (1938) reported, at any time of a day when they were put into the seawater in the laboratory. Thus, some culture examinations are desirable to assess the influence of illumination on the fruiting and liberation of reproductive cells in these algae.

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