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Studies on diurnal photosynthetic performance of *Sargassum thunbergii* II. Explanation of diurnal photosynthesis patterns from examinations in the laboratory

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Higher morning photosynthetic rates and afternoon photosynthetic depression of *Sargassum thunbergii* on morderately bright days reported in a previous paper has been also observed in the laboratory. A photosynthesis-light curve obtained in morning showed values much higher than that obtained in afternoon on a fine day in November. Such afternoon photosynthetic depression could not be attributed to variations in chlorophyll concentration. Photosynthesis showed constant value under a constant lower (unsaturated) photon flux density, while it showed highest values at the beginning of the illumination period and then decreased with time under a higher (saturated) photon flux density. The afternoon depression in the diurnal photosynthesis may be explained by postulating the existence of photosynthate pool size regulation.

Key Index Words: Chlorophyll—dark respiration—photosynthesis—Sargassum thunbergii.

In a previous paper (GAO and UMEZAKI 1989), diurnal photosynthesis of *Sargassum thunbergii* showed higher morning values and afternoon depression on moderately bright days in October and December. Such diurnal photosynthetic variation in *S. thunbergii* is similar to that found in higher plants (ITO *et al.* 1973, GOULDER 1970, HOUGH 1974) and phytoplankton (DOTY and OGURI 1957, YENTSCH and RYTHER 1957, LORENZEN 1963, HARRIS 1973, HARRIS and LOTT 1973, SOURNIA 1974, MARRA 1978).

From results of the previous study (GAO and UMEZAKI 1989), it can be expected that P_{max}, or the initial slope, of a photosynthesis-light curve of *S. thunbergii* differs between morning and afternoon on a day when afternoon photosynthetic depression exists. The reason for diurnal photosynthetic variatioin in *S. thunbergii* is unknown. The main purpose of this study is to make clear whether the difference in photosynthesis of *S. thunbergii* between morning and afternoon on bright days can be also observed in the laboratory and be related to any fluctuations in chlorophyll content.

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Materials and Methods

Jeveniles of *S. thunbergii* about 2 cm long were collected in November 1987 from the Maizuru Bay, one of the branch bays of Wakasa Bay facing the Sea of Japan. Following collection, samples were cleaned of obvious epiphytes before use.

Measurements of photosynthesis and respiration were carried out in the laboratory with a differential gas-volumeter (Productmeter, Nikko Kagaku Ltd.), which has eight pairs of reaction and compensation vessels kept in a water bath equipped with a motor to shake the vessels. Photosynthetic, or respiratory, rate was determined from volumes of oxygen evolved, or consumed, by samples, as reported by YOKOHAMA *et al.* (1986). For photosynthesis-light curves, various photon flux densities were attained in a stepwise fashion from the lowest to the highest by changing paper filters. Light was supplied with halogen lamps of slide projectors (Twin Cabin Super, Cabin Industry). Dark respiration was measured by covering the reaction vessel completely with opaque plastic.

On the basis of the previous study (GAO and UMEZAKI 1989), we determined to carry out measurements for the photosynthesis-light curves from 9:00 in the morning and from 16:00 in the afternoon to see if any difference can be found between morning and afternoon. It took two to three hours to finish measurements in morning or in afternoon. Samples were maintained under natural sunlight in flowing seawater (filtered through sand and coal particles) in a flow-through water box from evening of the day before the experiment. On the experiment day, as soon as measurements had been finished in morning, samples were returned to the water box and exposed to sunlight until afternoon measurements. Water temperature in the water box was similar to that in the sea.

For samples exposed to two constant light regimes of 150 and 1000 $\mu E\ m^{-2}\ s^{-1}$ supplied with incandescent lamps (National, 110V 150W), photosynthesis and dark respiration were measured with the differential gas-volumeter every hour or two in the laboratory. Different samples were used for photosynthesis and dark respiration measurements. Samples were maintained in flowing seawater during the intervals of measurements under the same light and temperature conditions as for photosynthesis measurements. Photosynthesis and dark respiration were measured at 16°C.

Chlorophylls were extracted with 90% acetone after grinding samples frozen at −20°C with sea sand, and extracts were filtered through absorbent cotton. Absorbances of acetone extracts were measured at 750, 664 and 630 nm with a spectrophotometer (Hitachi, Model 100-2). Concentrations of chlorophylls *a* and *c* were calculated by the formulae of JEFFREY and HUMPHREY (1975).

Results

Variation in solar radiation on the day when photosynthesis-light curves were obtained is shown in Figure 1. Photosynthesis-light curves determined in the laboratory in morning and afternoon on that day showed results (Fig. 2) similar to those derived from diurnal photosynthesis reported in a preceding paper (GAO and UMEZAKI 1989), i.e. higher morning photosynthetic rate and afternoon depression. Such a difference in photosynthesis-light curve between morning and afternoon determined in the laboratory must be related with the variation in diurnal photosynthesis. As compared in Figure 2, photosynthesis in afternoon measured in the laboratory was similarly reduced under natural sunlight, although the values measured under sunlight in flowing seawater were higher than those determined under artificial light with the differential gas-volumeter.

Samples used for determination of chlorophyll content were maintained under the same conditions as for photosynthesis

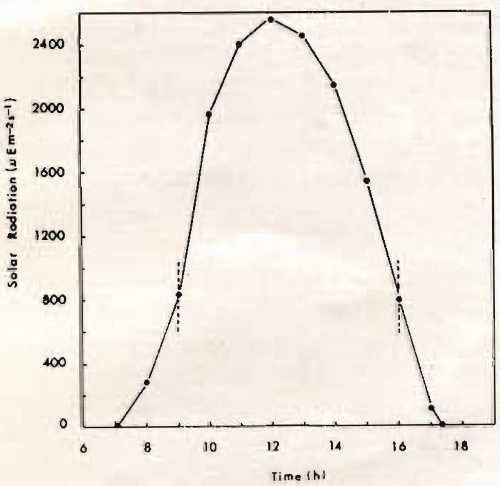

Fig. 1. Diurnal curve of solar radiation on November 21, 1987, a fine day. The vertical dotted lines show the beginning of photosynthesis measurements.

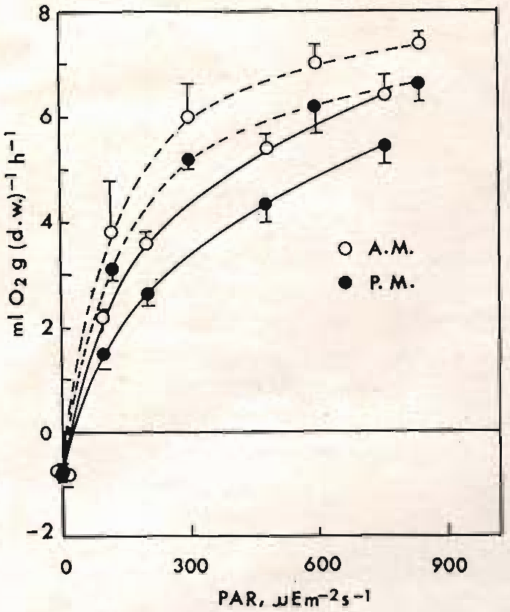

Fig. 2. Photosynthesis-light curves of *S. thunbergii*. Solid lines are curves in morning (9:00–11:00) and in afternoon (16:00–18:00) on a fine day (see Fig. 1), November 21, 1987, measured in the laboratory at 16°C with an artificial light source. Each value is the mean of five samples ± standard deviation. Dotted lines are curves measured under natural sunlight in flowing seawater at 14°C; each value is the mean of data obtained on December 9 and 19, 1987 ± standard deviation (GAO and UMEZAKI 1989).

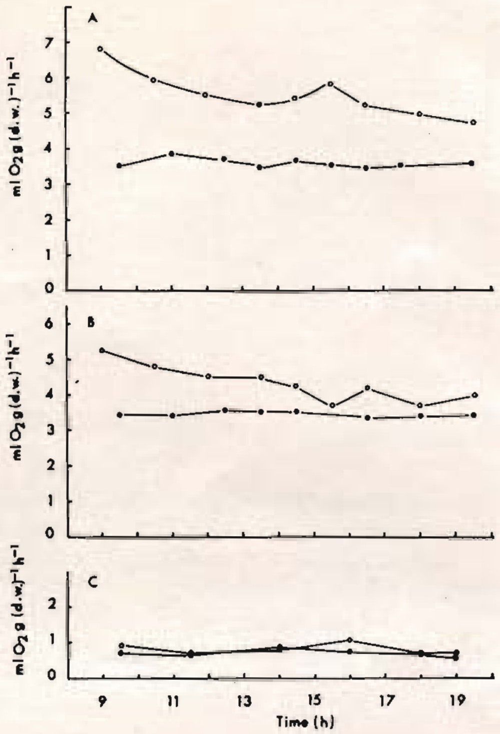

Fig. 3. Changes in net photosynthesis (A & B) and dark respiration (C) of *S. thunbergii* with time under 1000 μE m^{-2} S^{-1} (○) and 150 μE m^{-2} s^{-1} (●). (B) is a repeat of (A). Measured at 16°C. Each value represents the mean of two to three samples.

measurements. Collection of samples from the flow-through water box was carried out in morning and in afternoon on the same day, at almost the same time (Fig. 1) as determinations of photosynthesis-light curves. As indicated in Table 1, afternoon chlorophyll *a* values were slightly lower than morning ones, and *c:a* ratios showed slightly higher values in afternoon. However, as can be seen from the standard deviations or by t-test analysis, values are not significantly (P>0.2) different between morning and afternoon.

Table 1. Comparisons of chlorophyll contents [mg g(f.w.)$^{-1}$] of *S. thunbergii* between morning and afternoon on a fine day, November 21.

No.	a.m. (9:30)			p.m. (16:00)		
	Chl. *a*	Chl. *c*	*c:a*	Chl. *a*	Chl. *c*	*c:a*
1	0.668	0.085	0.127	0.556	0.065	0.117
2	0.541	0.063	0.116	0.561	0.076	0.135
3	0.611	0.076	0.124	0.605	0.079	0.131
4	0.654	0.072	0.110	0.582	0.074	0.127
5	0.702	0.077	0.110	—	—	—
X̄	0.635	0.075	0.117	0.576	0.074	0.128
SD	0.072	0.008	0.008	0.022	0.006	0.008

Figure 3 shows differences in photosynthesis and dark respiration of *S. thunbergii* maintained under low and high light conditions. At the beginning of the experiment, the response to 1000 $\mu E\,m^{-2}\,s^{-1}$ showed much higher values and a gradual decrease with time. In contrast, the response to 150 $\mu E\,m^{-2}\,s^{-1}$ showed almost constant values from the beginning to the end of the experiment (Fig. 3 A & B). Nevertheless, dark respiration of samples kept under the two constant light regimes showed almost constant values throughout the experiment, and a difference in dark respiration was not found between the two light regimes (Fig. 3 C).

Discussion

From the present study it is clear that the difference in the photosynthetic response of *S. thunbergii* to light between morning and afternoon observed in the laboratory was similar to that derived from diurnal photosynthesis measured under natural sunlight. However, net photosynthetic rate under natural sunlight measured with the flow-through system was higher than that in the laboratory with a gas-volumeter. This may be due to differences in the two kinds of photosynthesis measurements and in light sources. The flow-through system keeps samples in flowing fresh seawater, which may enhance photosynthesis of *S. thunbergii*. Spectrum differences between sunlight and artificial light of incandescent lamps may also contribute to the results. As indicated in Fig. 4, the photosynthesis-light curve obtained in morning from 9:00 to 11:00 was about 30% (at low light levels) and 15–20% (at high light levels) higher than that in afternoon from 16:00 to 18:00. However, no difference was found in chlorophyll contents between morning and afternoon (Table 1). Therefore, it can be concluded that the higher morning rate and afternoon depression of photosynthesis of *S. thunbergii* did not result from diurnal variation of chlorophyll contents. PALMER *et al.* (1964), working on photosynthetic rhythms in the marine diatom *Phaeodactylum tricornutum*, found no change in chlorophyll content in the cell. MARRA (1978) reported that afternoon depression of photosynthesis in the marine diatom *Lauderia borealis* was not related to chlorophyll content in the cell. It seems that there are no variations in chlorophyll content coincident with diurnal photosynthetic variation in both micro- and macroalgae.

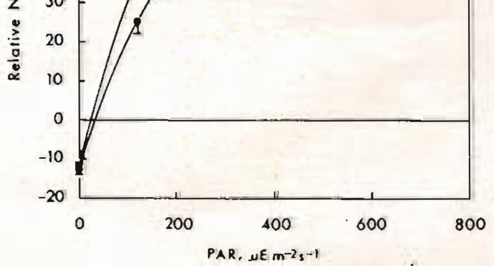

Fig. 4. Relative net photosynthesis-light curves measured in the laboratory, replotted from data obtained on November 21, 1987.

The afternoon photosynthetic depression of *S. thunbergii* may be related, in the same way as suggested by MARRA (1978) for phytoplankton, with saturation of the pool of Calvin cycle intermediate on moderately bright days when photosynthesis is very active. Regulation of photosynthesis should be expected to match biosynthetic activity of the plant. MISHKIND *et al.* (1979) reported that diurnal variation of photosynthetic capacity in *Ulva lactuca* was not attributable to changes in chloroplast orientation, in photosynthetic unit size and in the turnover time of the reaction center; they suggested that it might be caused by a dark reaction. In the present study, photosynthesis of *S. thunbergii* was at its maximum at the beginning of the exposure to a saturating photon flux density and gradually decreased with time; however, it maintained almost constant values when exposed to an unsaturating photon flux density (Fig. 3). This agrees with results reported for a marine diatom *Lauderia borealis* grown in axenic continuous culture by MARRA (1978). These results might be explained by Calvin cycle intermediate pools getting saturated fast when photosynthesis is performed actively under a saturating photon flux density and the dark reaction which empties the pools remaining unchanged (without changes of temperature). Under a non-saturating photon flux density, photosynthesis proceeds slowly and is not sufficient to saturate the pool of photosynthates. Therefore, photosynthetic depression could not be observed. This also is supported by the fact that afternoon depression was not found on a rainy day, as shown in the previous paper (GAO and UMEZAKI 1989).

Since relative net photosynthesis of *Sargassum thunbergii* was 15-30% higher in morning than in afternoon, estimated values for its daily productivity will be equivalently higher (within the phton flux density range investigated) if the morning photosynthesis-light curve is used for calculations. Therefore, more attention should be paid to diurnal variation in P_{max} and initial slopes of a photosynthesis-light curve for the estimation of the primary production based on photosynthesis.

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高　坤山・梅崎　勇：ウミトラノオの光合成に関する研究 II. 実験室内での結果に基づく考察

前報のウミトラノオの光合成に見られた晴天下における午後の低下はクロロフィル含量の日変化によるものではないことが分かった。11月の晴れの日に室内で午前中に測定した光合成–光曲線は午後に測定したものより高かった。また，室内において一定の光強度下にウミノトラノオを維持すると，弱い光強度（150 $\mu Em^{-2}s^{-1}$）の場合には光合成は一定の速度を維持したが，強い光強度（1000 $\mu Em^{-2}s^{-1}$）の場合には最初高い速度を示した後に低下していった。このような低下は光合成産物の蓄積が原因と考えられる。（606 京都市左京区北白川追分町 京都大学大学院農学研究科熱帯農学専攻水産資源学研究室）