Jpn. J. Phycol. (Sôrui) **36**: 24–28, March 10, 1988

Photosynthesis measurement of blade segments of brown algae *Ecklonia cava* KJELLMAN and *Eisenia bicyclis* SETCHELL

Yoshihiko SAKANISHI*, Yasutsugu YOKOHAMA** and Yusho ARUGA***

**Hokkaido Regional Fisheries Research Laboratory, Katsurakoi, Kushiro, Hokkaido, 085 Japan*
***Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka, 415 Japan*
****Laboratory of Phycology, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan*

SAKANISHI, Y., YOKOHAMA, Y. and ARUGA, Y. 1988. Photosynthesis measurements of blade segments of brown algae *Ecklonia cava* KJELLMAN and *Eisenia bicyclis* SETCHELL. Jpn. J. Phycol. **36**: 24–28.

As the measurement of photosynthesis of tissue segments of *Ecklonia* and *Eisenia* with oxygen technique had been disturbed by cutting a thallus, the present study was carried out to develop a suitable treatment of tissue segments from the thalli of these algae. It was found that photosynthetic and respiratory rates can be determined without disturbance with blade pieces of these algae after being kept in running seawater for a period longer than 3 hrs after cutting.

Blade discs of 4.1 cm^2 were cut off from grown-up bladelets of *Ecklonia cava* and *Eisenia bicyclis*. A disc was placed in a reaction vessel of a differential gas-volumeter with a capacity of about 35 ml together with 10 ml of filtered seawater, and oxygen evolution or consumption in the vessel was measured under illumination or in darkness. The rates of oxygen evolution under illumination were at almost constant high level in the discs kept in advance in the vessels in running seawater for more than 3 hrs after cutting, while the values were extremely low when the measurements were made just after cutting. In case of measuring the oxygen consumption in darkness, slightly high rates were observed just after cutting.

Comparison of photosynthetic and respiratory rates was made on an area basis between a young whole plant and a disc cut out of it. The rates of net photosynthesis and respiration of a disc were respectively 1.18 and 1.85 times higher than those of a whole plant on the average. These differences seem to be reasonable since the whole plant has portions such as stipe, holdfast and margins.

Photosynthesis-light curves were obtained without disturbance using the discs which were cut out of grown-up bladelets of *Ecklonia cava* and *Eisenia bicyclis* and kept overnight in running seawater. These curves were similar to those determined with young whole plants.

Key Index Words: Blade disc—differential gas-volumeter—Ecklonia cava—Eisenia bicyclis—*photosynthesis—productmeter—respiration.*

The brown algae *Ecklonia cava* and *Eisenia bicyclis* are widely distributed along the Pacific coast of central Japan, and form submarine forests there. Measuring the photosynthetic or respiratory activity in these species has been, however, difficult because cutting the thallus of these algae disturbs the measurements with oxygen technique (ARASAKI and TOKUDA 1967). We suppose this to be due to the consumption of oxygen in water by some substance(s) released from the wounded portion of the thallus piece. In preliminary measurements, however, the photosynthesis and respiration were successfully measured with young whole plants of *Ec. cava* and *Ei.*

Contribution No. 479 from the Shimoda Marine Research Center, University of Tsukuba.

This study was partly supported by a Grant-in-Aid for Scientific Research No. 5812410 from the Ministry of Education, Science and Culture, Japan and by Marine Ranching Program of Agriculture, Forestry and Fisheries Research Council Secretary, the Ministry of Agriculture, Forestry and Fisheries, Japan.

bicyclis, which were 10 cm in blade length, even though they had wounded parts presumably caused by grazing. In this case, many hours might have passed since the plant was grazed. Thus, it is considered that measurements of photosynthesis and respiration can probably be successfully done if a piece cut off from a thallus of *Ec. cava* or *Ei. bicyclis* is kept in running seawater for several hours after cutting.

The present study was carried out to develop a suitable treatment of blade pieces from the thalli of *Ec. cava* and *Ei. bicyclis* before measuring photosynthesis and respiration in order to avoid any unreliability similar to that observed by ARASAKI and TOKUDA (1967).

Materials and Methods

Young and adult plants of *Ecklonia cava* and *Eisenia bicyclis* used in this study were collected from a depth of about 5 m and 1 m, respectively, in Nabeta Bay, Shimoda, Shizuoka Pref., and brought to the Shimoda Marine Research Center, University of Tsukuba. The young plants of about 10 cm in blade length were kept in running seawater in the laboratory before the measurements of photosynthesis or respiration, while the adult plants were kept in an outdoor water tank supplied with flowing seawater before discs of 4.1 cm^2 were cut out of their bladelets. The discs were then kept in running seawater in the laboratory for different lengths of time before use.

The instrument used for measuring photosynthesis and respiration was 'Productmeter', a differential gas-volumeter devised by YOKOHAMA and ICHIMURA (1969) and improved by YOKOHAMA *et al.* (1986), which had been used for physiological and ecological studies of seaweeds (YOKOHAMA 1972, 1973a, 1973b, 1973c, KAGEYAMA and YOKOHAMA 1974, HATA and YOKOHAMA 1976, KAGEYAMA and YOKOHAMA 1977, MIZUSAWA *et al.* 1978, KAGEYAMA *et al.* 1979, KATAYAMA *et al.* 1985, SAITOH *et al.* 1986, MAEGAWA *et al.* 1987). Several types of reaction and compensation vessels attachable to this instrument were reported by YOKOHAMA and MAEGAWA (1988). A blade disc and 10 m*l* of filtered seawater were placed in a flask type reaction vessel with a capacity of about 35 m*l*, and a similar amount of filtered seawater was placed in a compensation vessel of the same type. A young whole plant and 50 m*l* of filtered seawater were placed in a culture flask type reaction vessel with a capacity of about 200 m*l*, and a similar amount of filtered seawater was placed in a compenastion vessel of the same type.

A slide projector (Elmo S-300) with an incandescent lamp (Kondo 100 V 300 W) was used as the light source for the photosynthesis measurements. The sample was irradiated with light of 15 klux at 20°C under shaking of 100 rpm and 3 cm in amplitude. Respiration was measured in the same manner but in complete darkness.

Results

Fig. 1 (A and B) shows the rates of oxygen evolution measured under irradiation by light of 15 klux in vessels containing blade discs of *Ecklonia cava* and *Eisenia bicyclis* kept for different lengths of time in running seawater after being cut off from the bladelets. Most of the observed values were extremely low just after cutting, but were at almost constant high level 3 hrs or more after cutting. The rate of oxygen consumption in darkness showed, however, no marked change with time, though it was slightly high just after cutting as shown in Fig. 1 (C). This suggests that the extremely low rate of oxygen evolution under illumination just after cutting is not attributable to the consumption of oxygen in water by any substance released from the wounded portion of the blade disc. However, these results may at least indicate that the photosynthetic and respiratory rates of *Ec. cava* and *Ei. bicyclis* can be measured without disturbance using blade discs kept in running seawater for a period

longer than 3 hrs after cutting.

In order to know whether the values obtained in such a way are at a normal level, the photosynthetic or respiratory rate of young whole plants of *Ei. bicyclis* was compared with that of a cut disc (Table 1). The values in case of a disc on an area basis were generally higher than those of a whole plant. This difference may be due to the presence of parts such as stipe, holdfast and margins in the whole plant (Fig. 2) which might have relatively low photosynthetic and respiratory activities.

Fig. 3 shows photosynthesis-light curves in discs cut out of grown-up bladelets of *Ec. cava* and *Ei. bicyclis* kept overnight in running seawater before use. As can be seen, it is clear that they were obtained without

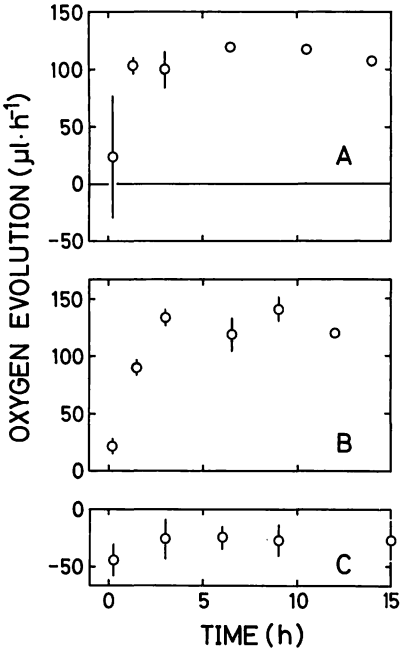

Fig. 1. Rates of oxygen evolution in vessels which contained 10 ml of filtered seawater and a disc of 4.1 cm² kept for different periods in running seawater after being cut off from a bladelet of *Ecklonia cava* (A and C) or *Eisenia bicyclis* (B) measured at 15 klux (A and B) or in darkness (C). Temperature was 20°C.

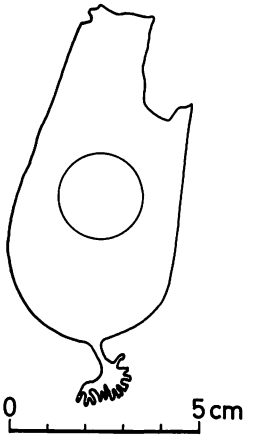

Fig. 2. A profile of a young plant of *Eisenia bicyclis* used for comparing the photosynthetic activities of the whole plant with that of a 4.1 cm² disc cut off from it. The disc was cut off from the part indicated by the circle in the blade.

Table 1. Comparisons of the photosynthetic rates ($\mu l\ O_2 \cdot cm^{-2} \cdot h^{-1}$) at 20°C and 15 klux and the respiratory rates at 20°C in darkness between the young whole plant and a disc cut out of it in *Eisenia bicyclis*.

Photosynthesis			Respiration		
Whole Plant	Disc	Disc/Whole P.	Whole Plant	Disc	Disc/Whole P.
22.7	28.5	1.26	7.0	10.0	1.43
29.3	32.1	1.10	7.3	4.9	0.67
36.7	30.0	0.82	3.0	9.3	3.10
17.3	28.1	1.62	9.4	8.4	0.89
30.6	36.7	1.20	6.8	11.6	1.71
27.5	30.5	1.11	5.7	5.1	0.89
23.0	28.4	1.23	3.5	11.1	3.17
24.5	27.3	1.11	2.9	8.4	2.90
	Mean	1.18±0.21		Mean	1.85±1.06

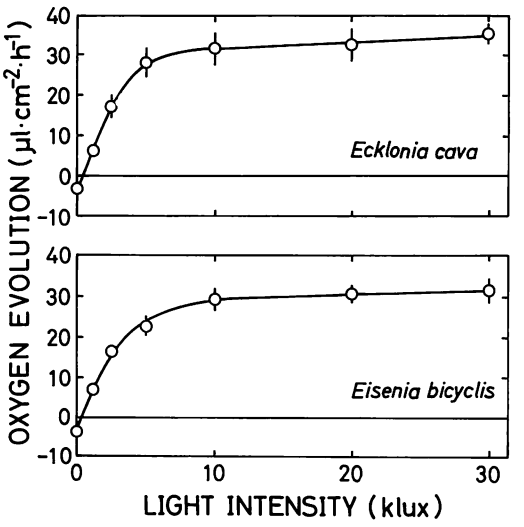

Fig. 3. Photosynthesis-light curves at 20°C of the discs cut off from bladelets of *Ecklonia cava* and *Eisenia bicyclis* kept overnight in running seawater after cutting.

disturbance.

Discussion

The results of the present study indicate that the blade discs of *Ecklonia cava* and *Eisenia bicyclis* should be kept in running seawater for more than 3 hrs after cutting in order to measure their photosynthetic and respiratory rates without disturbance (Fig. 1).

The propriety of values thus obtained is supported by HATCHER (1977) who reported that in *Laminaria longicruris* the photosynthetic and respiratory rates on an area basis of an algal piece were respectively 1.2 and 1.8 times higher than those of a whole plant. In the present study, the photosynthetic and respiratory rates of discs cut out of young plants of *Ei. bicyclis* were respectively 1.18 and 1.85 times higher than those of whole plants on an average (Table 1).

Photosynthesis-light curves obtained with the discs cut out of grown-up bladelets of *Ec. cava* and *Ei. bicyclis* (Fig. 3) are similar to those of young whole plants reported by MAEGAWA *et al.* (1987). This is an additional support for the propriety of the values obtained with the blade discs.

The mechanism disturbing the measurements of photosynthesis and respiration of discs of *Ec. cava* and *Ei. bicyclis* just after cutting still remains unknown. Although we presumed that the disturbance is due to the consumption of oxygen in water by some substance(s) released from the wounded portion of the blade disc, the rate of oxygen consumption in the vessel containing a disc measured in darkness just after cutting was not high enough to explain the extremely low rate of oxygen evolution in the vessel in the same conditions but under illumination.

References

ARASAKI, S. and TOKUDA, H. 1967. Productivity of seaweeds. p. 2–4. *In* HOGETSU, K. [ed.], Studies on the Productivity of Marine Biocoenoses. JIBP/PM Section. (in Japanese)

HATA, M. and YOKOHAMA, Y. 1976. Photosynthesis-temperature relationships in seaweeds and their seasonal changes in the colder region of Japan. Bull. Jap. Soc. Phycol. **24**: 1–7 (in Japanese with English summary).

HATCHER, B.G. 1977. An apparatus for measuring photosynthesis and respiration of intact marine algae and comparison of results with those from experiments with tissue segments. Mar. Biol. **43**: 381–385.

KAGEYAMA, A. and YOKOHAMA, Y. 1974. Photosynthetic properties of marine benthic brown algae from different depths in coastal area. Bull. Jap. Soc. Phycol. **22**: 110–123 (in Japanese with English summary).

KAGEYAMA, A. and YOKOHAMA, Y. 1977. Pigments and photosynthesis of deep-water green algae. Bull. Jap. Soc. Phycol. **25**: 168–175 (in Japanese with English summary).

KAGEYAMA, A., YOKOHAMA, Y. and NISIZAWA, K. 1979. Diurnal rhythm of apparent photosynthesis of a brown alga, *Spatoglossum pacificum*. Bot. Mar. **22**: 199–201.

KATAYAMA, N., TOKUNAGA, Y. and YOKOHAMA, Y. 1985. Effect of growth temperature on photosynthesis-temperature relationships of a tide pool alga *Cladophora rudolphiana* (Chlorophyceae). Jap. J. Phycol. **33**: 312–316.

MAEGAWA, M., YOKOHAMA, Y. and ARUGA, Y. 1987. Critical light conditions for young *Ecklonia cava*

and *Eisenia bicyclis* with referance to photosynthesis. Hydrobiol. **151/152**: 447–455.

MIZUSAWA, M., KAGEYAMA, A. and YOKOHAMA, Y. 1978. Physiology of benthic algae in tide pools I. Photosynthesis-temperature relationships in summer. Jap. J. Phycol. **26**: 109–114.

SAITOH, M., KATAYAMA, N. and YOKOHAMA, Y. 1986. Photosynthesis-temperature relationships of marine benthic algae in Otsuchi Bay. Otsuchi Mar. Res. Cent. Rep. **12**: 9–14 (in Japanese).

YOKOHAMA, Y. 1972. Photosynthesis-temperature relationships in several benthic marine algae. p. 286–291. *In* NISIZAWA, K. [ed.], Proc. 7th Intl. Seaweed Symp. Univ. of Tokyo Press, Tokyo.

YOKOHAMA, Y. 1973a. A comparative study on photosynthesis-temperature relationships and their seasonal changes in marine benthic algae. Int. Revue ges. Hydrobiol. **58**: 463–472.

YOKOHAMA, Y. 1973b. Photosynthetic properties of marine benthic green algae from different depths in the coastal area. Bull. Jap. Soc. Phycol. **21**: 70–75 (in Japanese with English summary).

YOKOHAMA, Y. 1973c. Photosynthetic properties of marine benthic red algae from different depths in coastal area. Bull. Jap. Soc. Phycol. **21**: 119–124 (in Japanese with English summary).

YOKOHAMA, Y. and ICHIMURA, S. 1969. A new device of differential gas-volumeter for ecological studies on small aquatic organisms. J. Oceanogr. Soc. Japan **25**: 75–80.

YOKOHAMA, Y. and MAEGAWA, M. 1988. Measurements of photosynthesis and respiration of large samples by 'Productmeter', a differential gas-volumeter. Jap. J. Phycol. **36**: 29–36 (in Japanese with English summary).

YOKOHAMA, Y., KATAYAMA, N. and FURUYA, K. 1986. An improved type of 'Productmeter', a differential gas-volumeter, and its application to measuring photosynthesis of seaweeds. Jap. J. Phycol. **34**: 37–42 (in Japanese with English summary).

坂西芳彦*・横浜康継・有賀祐勝***：カジメおよびアラメの葉片を用いた光合成の測定**

大型褐藻のカジメやアラメから切出した葉片を用いて酸素法で光合成や呼吸を測定すると安定した値が得られないことが知られているが，切出した葉片を流海水中に3時間以上漬けてから測定に用いると安定した測定結果が得られることが分った。

カジメおよびアラメから面積 4.1 cm^2 の円形葉片を切出し，差働式検容計プロダクトメーターの反応容器（容量 35 m*l*）に 10 m*l* の濾過海水と共に入れて，酸素の放出または吸収を測定した。光照射下での酸素放出速度は，切出した直後の葉片では著しく低かったが，切出してから流海水中に3時間以上漬けた葉片では安定して高い値を示した。切出した直後の葉片の暗所での酸素消費速度はやや高かった。若い個体全体とそれから切出した葉片とで単位面積当りの光合成速度と呼吸速度を比較したところ，後者の純光合成速度と呼吸速度は前者のそれぞれ1.18倍と1.85倍（平均値）であった。これは，個体全体を用いた場合には代謝速度の低い茎状部，付着器，葉状部の縁片などが含まれるためであろう。カジメおよびアラメの側葉から切出し，一夜流海水に漬けた葉片を用いて信頼できる光合成—光曲線を容易に作ることができた。（*085 北海道釧路市桂恋116 北海道区水産研究所，**415 静岡県下田市5丁目10-1 筑波大学下田臨海実験センター，***108 東京都港区港南4－5－7 東京水産大学藻類学研究室）