Jpn. J. Phycol. (Sôrui) 37: 213–220, September 20, 1989

Photosynthetic characteristics of several species of Rhodophyceae from different depths in the coastal area of Shima Peninsula, central Japan

Noboru Murase, Miyuki Maegawa and Washiro Kida

Laboratory of Phycology, Faculty of Bioresources, Mie University, Edobashi 2–80, Tsu, Mie, 514 Japan

Murase, N., Maegawa, M. and Kida, W. 1989. Photosynthetic characteristics of several species of Rhodophyceae from different depths in the coastal area of Shima Peninsula, central Japan. Jpn. J. Phycol. 37: 213–220.

In order to elucidate vertical distribution of Rhodophyceae with reference to the photosynthetic characteristics and photosynthetic pigments, several species of Rhodophyceae were collected from shallow water (near the low water level) and deep water (about 25 m depth) in the coastal area of Shima Peninsula, Mie Prefecture, and used for measuring photosynthesis and respiration as well as for analyzing photosynthetic pigments. Photosynthesis measurements were carried out under white light similar to sunlight and under green light close to the light condition in coastal deep waters. Shallow-water species (*Gracilaria incurvata* and *Pachymeniopsis elliptica*) had higher photosynthetic efficiency for white light than for green light, whereas deep-water species (*Meristotheca papulosa, Beckerella subcostata* and *Peyssonnelia caulifera*) had higher photosynthetic efficiency for green light than for white light. The amounts of chlorophyll *a* and phycoerythrin were measured in nine shallow-water species and five deep-water species. The ratio of phycoerythrin to chlorophyll *a* contents was clearly higher in deep-water species (4.2–9.3, average 6.4) than in shallow-water species (0.5–4.3, average 2.6). It is concluded that the high photosynthetic efficiency of deep-water species for green light is due to a high ratio of phycoerythrin to chlorophyll *a* contents, and that shallow-water species have adapted to white light and deep-water species to green light. This is a kind of chromatic adaptation of Rhodophyceae by changing phycoerythrin content under natural conditions.

Key Index Words: chlorophyll a*—photosynthesis—photosynthetic pigments—phycoerythrin—Rhodophyceae—seaweed.*

Photosynthesis of algae is an important basis of the production in the coastal ecosystem. Thus, much attention has been focused on studies of photosynthesis of algae from the viewpoint of production ecology (Aruga 1986). In general, the depth to which seaweeds grow is determined by the amount of available light for photosynthesis (Duncan and Lobban 1985). Thus, the photosynthetic study with reference to photosynthetic pigments is of great importance not only for production ecology but also for physiological ecology.

Yokohama (1973a, b) and Kageyama and Yokohama (1974) reported that photosynthetic properties of seaweeds from different depths depended on light quantity and quality. Photosynthesis-light curves of seaweeds from shallow water were of the sun type, and those of seaweeds from deep water were of the shade type. Moreover, photosynthesis-light curves under white light and green light showed a remarkable difference in Chlorophyceae and Rhodophyceae. As for Rhodophyceae, Yokohama (1973b) suggested that the ratio of phycoerythrin to chlorophyll *a* had an important role in characterizing the photosynthetic properties of the species growing in shallow and deep water. Rhodophyceae containing phycobilin pigments would be expected to have a greater vertical range of growth in the sea. Furthermore, Yokohama and his coworkers reported that most of the Chlorophyceae in deep water or shade sites have siphonaxanthin as a photosynthetic pigment for collecting green light (Yokohama *et* *al.* 1977, KAGEYAMA *et al.* 1977, KAGEYAMA and YOKOHAMA 1978). MAEGAWA *et al.* (1987, 1988) reported that the difference in daily compensation point between young fronds of *Eisenia bicyclis* and *Ecklonia cava* is one of the most important factors in determining the difference in their vertical distribution. Thus, photosynthetic characteristics and pigment contents are the most important factors determining vertical distribution of seaweeds.

The present study deals with the factors governing the difference in vertical distribution of several species of Rhodophyceae with reference to photosynthesis and photosynthetic pigments. The aim of the present study is to measure accurately photosynthetic rates under various light conditions and conduct quantitative analysis of phycoerythrin to obtain the phycoerythrin/chlorophyll *a* ratio in relation to the vertical distribution of Rhodophyceae.

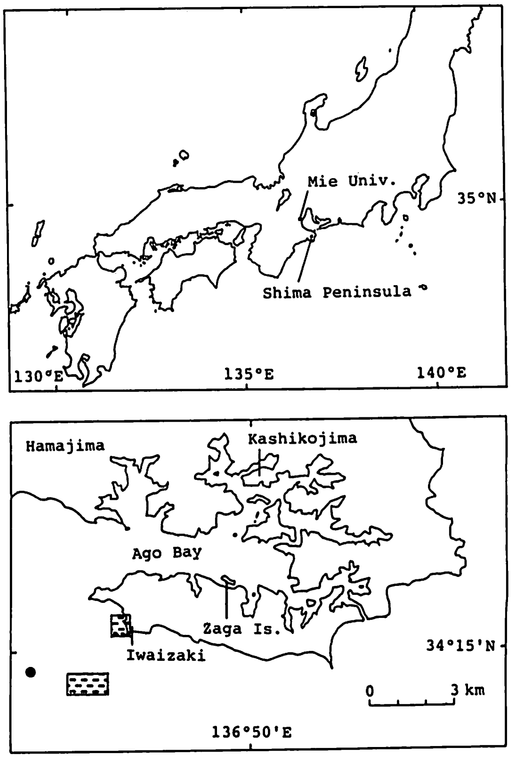

Fig. 1. Maps showing the location of study area at Shima Peninsula, central Japan. sampling area; ● station for measuring water temperature.

Materials and Methods

Photosynthesis study

Several species of Rhodophyceae were collected from different depths around the coast of Shima Peninsula, Mie Prefecture, from May to September 1987 (Fig. 1). These species were classified into two groups, "shallow-water species" and "deep-water species", according to the sampling depth. As shallow-water species, *Gracilaria incurvata* OKAMURA was collected just below the sea surface from floating buoy used for pearl oyster cultivation near Zaga Island in Ago Bay, and *Pachymeniopsis elliptica* (HOLMES) YAMADA was collected from near the low water level at the coast of Iwaizaki. Deep-water species, *Meristotheca papulosa* (MONTAGNE) J. AGARDH, *Beckerella subcostata* (OKAMURA) KYLIN and *Peyssonnelia caulifera* OKAMURA, were collected from a depth of about 25 m off Iwaizaki by SCUBA diving.

Fig. 2 shows the seasonal variations of seawater temperature at the depths of 0, 20 and 30 m (average for 8 years, 1981–1988) near the sampling area (solid circle in Fig. 1). During the period of measuring photosynthesis and respiration (May to September), the seawater temperature varied from 18.3°C in May to 26.3°C in September in surface water, from 17.8°C in May to 23.4°C in August at a depth of 20 m, and from 17.7°C in May to 20.8°C in August at a depth of 30 m.

Collected samples were transported to the Fisheries Research Laboratory of Mie University in Zaga Island and were rinsed with filtered seawater to make them free of obvious epiphytes with careful handling not to wound the fronds and were protected from direct sunlight. Sample pieces of 10–20 cm^2 were cut out from fronds, and were kept in running seawater overnight to avoid abnormal results caused by cutting (SAKANISHI *et al.* 1988). Photosynthesis and respiration were measured with Productmeter, an improved differential gas-volumeter (YOKOHAMA *et al.*

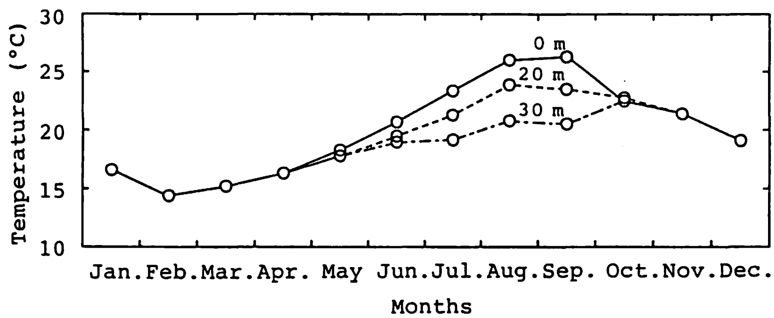

Fig. 2. Seasonal changes of seawater temperature at the depths of 0 m (—), 20 m (----) and 30 m (-·-·-) off Iwaizaki (34°14'N, 136°43'E). Average of 8 years (1981–1988).

1986, YOKOHAMA and MAEGAWA 1988). Measurements of photosynthesis and respiration were carried out at 8 different light intensities from 0 to 400μE/m^2/s under white light and green light. A projector lamp (Kondo 100 V–300 W) was used as the white light source, and the green light was obtained by penetrating the white light through a 0.4 M nickel sulfate solution 10 mm thick. Spectral distributions of white light and green light were measured with a Techtum Quantaspectrometer QSM-2500 as shown in Fig. 3. White light from the projector lamp is similar to the sunlight, although quanta of short wave band from 400 to 500 nm are slightly less than those of the sunlight. Green light is approximated to the light condition at a depth of around 20 m in coastal waters. The light intensity was controlled with neutral density filters (Toshiba TND-50, -25, -12.5). Photon flux density was measured with a quantum meter system (LI-COR LI-192SB, LI-1000).

Culture flasks of about 100 m*l* capacity were used as the reaction and reference vessels of Productmeter, and filtered seawater (30 m*l*) was poured into both vessels, with a cut frond in the reaction vessel. After pre-incubation for 30 minutes at 400 μE/m^2/s, the seawater in both vessels was renewed, and the measurement was carried out from high to low light intensity with the same frond. Respiration was measured after the photosynthesis measurement. Each measurement took about 25 minutes and the seawater was renewed each time. It took 5–6 h for a series of measurements starting from 09:00 h. Photosynthesis and respiration were measured at 20°C which was nearly the same as *in situ* seawater temperature of sampling area (cf. Fig. 2). After the measurements, fronds were rinsed with freshwater and used for area measurement. At the same time, small frond discs (0.332 cm^2) were taken for quantitative analysis of photosynthetic pigments.

Absorption spectra

Fronds used for measuring photosynthesis and respiration were transported to the Laboratory of Phycology, Faculty of Bioresources, Mie University. *In vivo* absorption spectra of the fronds were measured with a Hitachi 330 Spectrophotometer equipped with an end-on type photomultiplier.

Measurement of photosynthetic pigments

Nine species collected from intertidal zone

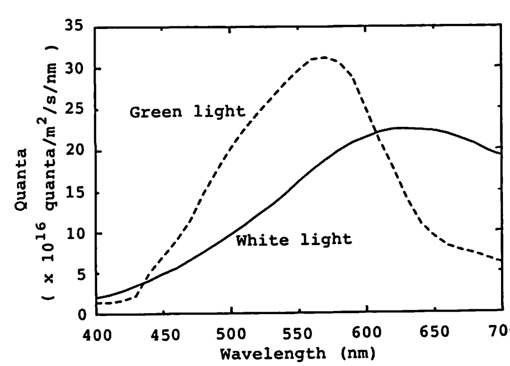

Fig. 3. Spectral distributions of white light and green light used for the measurements of photosynthesis.

to a depth of 2 m, and five species from a depth of about 25 m were used for quantitative measurement of photosynthetic pigments. A disc of 0.332 cm^2 or a piece of 0.1–0.2 g were cut out of samples. Fresh or frozen (−20°C) samples were used for extraction of photosynthetic pigments.

Fresh samples were used for the measurement of chlorophyll *a* content. Chlorophyll *a* was extracted in 90% acetone in a mortar. The extract was centrifuged for 5 minutes at 3000 rpm, and absorbances at 750, 663, 645 and 630 nm were measured with a Hitachi 101-01 Spectrophotometer. The amount of chlorophyll *a* was calculated by the equation of SCOR-UNESCO (1966).

Frozen samples were transported to the Laboratory of Phycology, Faculty of Bioresources, Mie University and were used for measurement of phycobilin content. Phycobilins were extracted in phosphate buffer solution (pH 6.5) with 0.2 g of quartz sand in a mortar. The extract was centrifuged for 30 minutes at 3000 rpm, and the supernatant was ultracentrifuged for 30 minutes at 35000 rpm. Absorbances of the supernatant at 750, 650, 620 and 565 nm were measured with a Hitachi 100-20 Spectrophotometer, and the amount of phycoerythrin was calculated by the equation of FUJITA (1979).

Results

Photosynthesis-light curves

Fig. 4 shows photosynthesis-light curves of two shallow-water species, *Gracilaria incurvata* and *Pacymeniopsis elliptica,* under white light and green light. Relative gross photosynthetic rates on a frond area basis are illustrated in the figure. In both species, the photosynthetic rate increased linearly with increase in light intensity in the range lower than 50 $\mu E/m^2/s$, and it increased slowly with further increase in light intensity. The photosynthetic rate was almost saturated at 200 $\mu E/m^2/s$ under both green light and white light. In the light intensity range lower than 200 $\mu E/m^2/s$, the relative photosynthesis of both species was higher under white light than under green light.

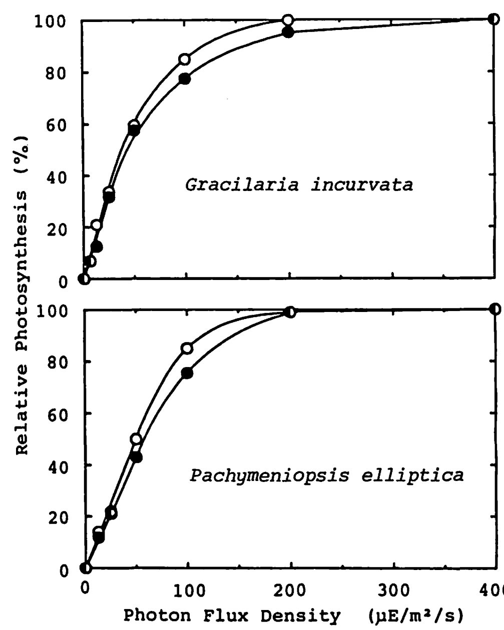

Fig. 4. Photosynthesis-light curves of two shallow-water species under white light (○) and green light (●) at 20°C.

Fig. 5 shows the relative photosynthesis-light curves of three deep-water species, *Meristotheca papulosa, Beckerella subcostata* and *Peyssonnelia caulifera,* under white light and green light. In all the three species, the photosynthetic rate increased linearly with increase in light intensity in the range lower than 50 $\mu E/m^2/s$, and it increased slowly with further increase in light intensity. Light saturation points of *Meristotheca papulosa* and *Beckerella subcostata* were 200 $\mu E/m^2/s$ under white light and 100 $\mu E/m^2/s$ under green light. As for *Peyssonnelia caulifera,* light saturation point was 100 $\mu E/m^2/s$ under white light and 70 $\mu E/m^2/s$ under green light. The relative photosynthesis was higher under green light than under white light in the light intensity range lower than 200 $\mu E/m^2/s$ in *Meristotheca papulosa* and *Beckerella subcostata,* and in the light intensity range lower than 100 $\mu E/m^2/s$ in *Peyssonnelia caulifera.*

Absorption spectra

In vivo absorption spectra of two shallow-

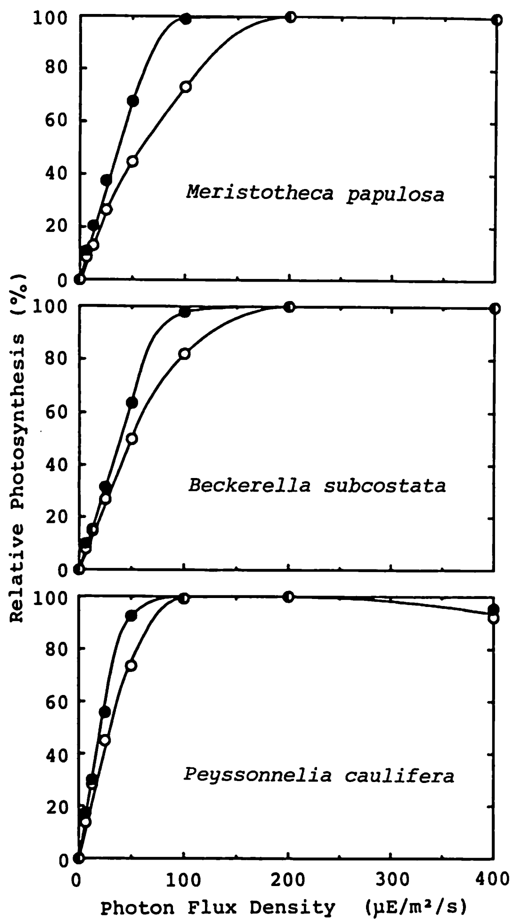

Fig. 5. Photosynthesis-light curves of three deep-water species under white light (○) and green light (●) at 20°C.

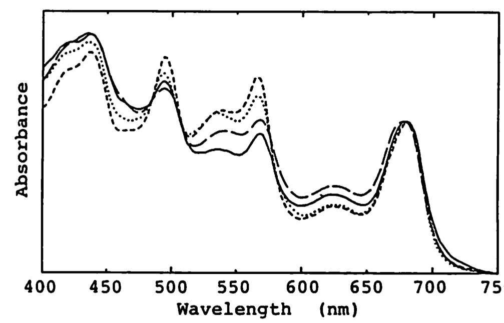

Fig. 6. *In vivo* absorption spectra normalized at 680 nm of shallow-water species, *Gracilaria incurvata* (—) and *Pachymeniopsis elliptica* (– –), and deep-water species, *Meristotheca papulosa* (----) and *Beckerella subcostata* (······), used for measurements of photosynthesis.

water species and two deep-water species, which were used for measuring photosynthesis and respiration, are shown in Fig. 6. For comparisons the spectra are normalized at 680 nm where the absorption by chlorophyll *a* dominates. There were considerable differences of absorbance in green region of 490–580 nm between shallow-water species and deep-water species. Absorbances were higher in deep-water species than in shallow-water species at the wave lengths from 490 to 580 nm, where the absorption by phycoerythrin predominates. Thus, it is expected that deep-water species are relatively rich in phycoerythrin, suggesting to have higher phycoerythrin/chlorophyll *a* ratio, as compared with shallow-water species.

Photosynthetic pigments

Chlorophyll *a* and phycoerythrin contents of nine shallow-water species and five deep-water species were estimated. Chlorophyll *a* contents were 4.2–19.4 $\mu g/cm^2$ in shallow-water species and 9.4–16.3 $\mu g/cm^2$ in deep-water species. Phycoerythrin contents were 10.4–63.5 $\mu g/cm^2$ in shallow-water species, and 49.5–90.8 $\mu g/cm^2$ in deep-water species. The level of chlorophyll *a* contents in shallow-water species was almost the same as that in deep-water species. However, the level of phycoerythrin contents in deep-water species was higher than that in shallow-water species.

Fig. 7 shows the ratio of phycoerythrin (PE) to chlorophyll *a* (Chl.*a*) contents in nine shallow-water species and five deep-water species. The PE/Chl.*a* ratios in deep-water species (4.2–9.3, average 6.4) were clearly higher than those in shallow-water species (0.5–4.3, average 2.6)

Discussion

The present study was attempted to elucidate vertical distribution of Rhodophyceae with reference to the photosynthetic characteristic and the amount of photosynthetic pigments. Several samples collected from shallow water (near the low water level) and from deep water (about 25 m)

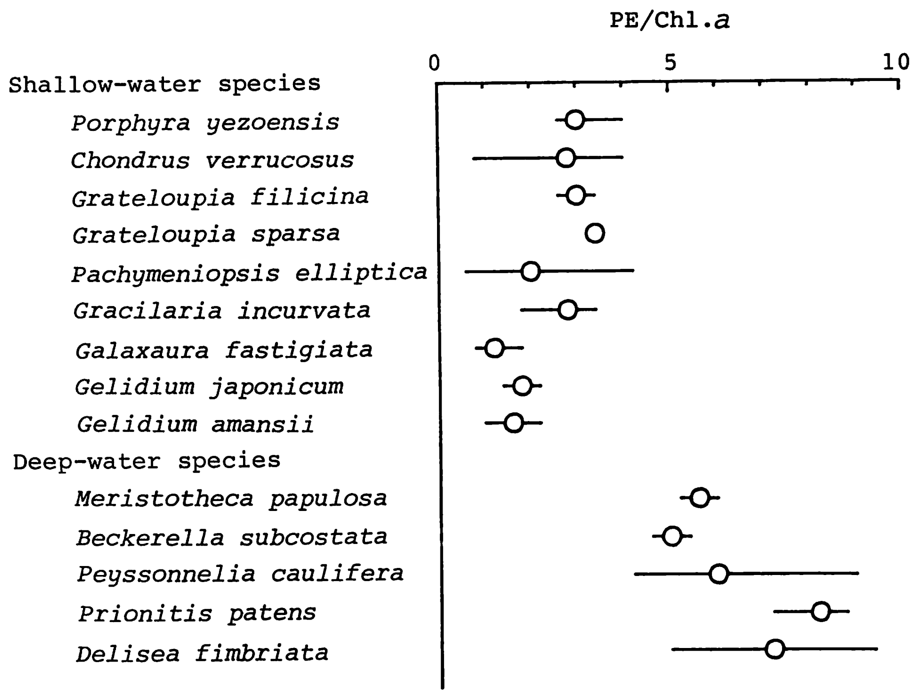

Fig. 7. The ratio of phycoerythrin to chlorophyll *a* contents of shallow-water species and deep-water species. Average (○) and the range for 4–15 samples are illustrated in each species.

near the lower limit of algal vegetation in a coastal region were compared with respect to their photosynthetic rates under white light (similar to sunlight) and green light (similar to the light condition in coastal deep water) and to their PE/Chl.*a* ratios.

Shallow-water species (*Gracilaria incurvata* and *Pachymeniopsis elliptica*) had higher photosynthetic efficiency for white light than for green light (Fig. 4). Conversely, deep-water species (*Meristotheca papulosa, Beckerella subcostata* and *Peyssonnelia caulifera*) had higher photosynthetic efficiency for green light than for white light (Fig. 5). These results are almost similar to those by YOKOHAMA (1973b). As for shallow-water species, the difference of photosynthetic efficiency between white light and green light obtained in the present study was well in agreement with that reported by YOKOHAMA (1973b). As for deep-water species, however, there were remarkable differences between the present result and YOKOHAMA's (1973b) result. This discordance may be due to the difference in the depth of sampling; 8–10 m in YOKOHAMA (1973b) and 25 m in the present study.

In *in vivo* absorption spectra (Fig. 6), it is clear that the deep-water species absorb green light (490–580 nm) more effectively than the shallow-water species do. The wavelengths of green region corresponds to the absorption band of phycoerythrin, a photosynthetic accessory pigment in Rhodophyceae. Therefore, the difference of absorption spectra in green region between shallow-water species and deep-water species is due to the difference of phycoerythrin content. Higher content of phycoerythrin in deep-water species is quite convenient for utilizing green light which occupies a greater part of irradiance at depths around 20 m in the coastal water.

Chlorophyll *a* contents were not so greatly different between shallow-water species and deep-water species. Phycoerythrin contents were appreciably higher in deep-water species than in shallow-water species. As a result, PE/Chl.*a* ratios were clearly higher in deep-water species than in shallow-water species (Fig. 7) as expected from the difference in absorption spectra in Fig. 6. Thus, the high photosynthetic efficiency of deep-water species for green light is due to their high ratio of phycoerythrin to chlorophyll *a* contents.

It was reported that there is a considerable change in phycoerythrin content of Rhodophyceae under natural conditions depending on the depth of water (RAMUS *et al.* 1976a, b, MOON and DAWES 1976) and under culture conditions depending on the light quality (BRODY and EMERSON 1959). There was a clear difference in phycoerythrin content between shallow-water species and deep-water species, even though the range of variation was not small in several species (Fig. 7).

In the present study, it is clearly shown that the difference of photosynthetic characteristics between shallow-water species and deep-water species is dependent on phycoerythrin content which is closely related to the light conditions in growing sites, and that the shallow-water species have adapted to white light and the deep-water species to green light. They are examples of chromatic adaptation of Rhodophyceae by changing their phycoerythrin contents according to the light conditions at growing depths.

Acknowledgement

We sincerely wish to thank Dr. H. AMANO of the University of Mie for his advice on the analyses of photosynthetic pigments. We wish to express our cordial gratitude to Dr. Y. YOKOHAMA of the Shimoda Marine Research Center, the University of Tsukuba, for reading the manuscript. We are indebted to Fisheries Research Institute of Mie Prefecture for seawater temperature data.

References

ARUGA, Y. 1986. Roles of algae in aquatic ecosystem. p. 113. *In* M. AKIYAMA, Y. ARUGA, M. SAKAMOTO and Y. YOKOHAMA [eds.] Ecology of algae. Uchida-Roukakuho, Tokyo (in Japanese).

BRODY, M. and EMERSON, R. 1959. The effect of wavelength and intensity of light on the proportion of pigments in *Porphyridium cruentum*. Amer. J. Bot. **46:** 433–440.

DUNCAN, M.J. and LOBBAN, C.S. 1985. Light and photosynthesis. p. 4–34. *In* C.S. LOBBAN, P.J. HARRISON and M.J. DUNCAN [eds.] The physiological ecology of seaweeds. Cambridge University Press, Cambridge.

FUJITA, Y. 1979. Methods in biochemical studies of algae. p. 503–505. *In* K. NISIZAWA and M. CHIHARA [eds.] Methods in phycological studies. Kyoritsu Shuppan, Tokyo (in Japanese).

KAGEYAMA, A. and YOKOHAMA, Y. 1974. Photosynthetic properties of marine benthic brown algae from different depths in the coastal area. Bull. Jap. Soc. Phycol. **22:** 119–123 (in Japanese).

KAGEYAMA, A. and YOKOHAMA, Y. 1978. The function of siphonein in a siphonous green alga *Dichotomosiphon tuberosus*. Jap. J. Phycol. **26:** 151–155.

KAGEYAMA, A., YOKOHAMA, Y., SHIMURA, S. and IKAWA, T. 1977. An efficient excitation energy transfer from a carotenoid, siphonaxanthin to chlorophyll *a* observed in a deep-water species of chlorophycean seaweed. Plant Cell Physiol. **18:** 477–480.

MAEGAWA, M., YOKOHAMA, Y. and ARUGA, Y. 1987. Critical light conditions for young *Ecklonia cava* and *Eisenia bicyclis* with reference to photosynthesis. Hydrobiol. **151/152:** 447–455.

MAEGAWA, M., KIDA, W., YOKOHAMA, Y. and ARUGA, Y. 1988. Comparative studies on critical light conditions for young *Eisenia bicyclis* and *Ecklonia cava*. Jpn. J. Phycol. **36:** 166–174.

MOON, R.E. and DAWES, C.J. 1976. Pigment changes and photosynthetic rates under selected wavelengths in the growing tips of *Eucheuma isiforme* (C. AGARDH) J. AGARDH var. *denudatum* CHENEY during vegetative growth. Br. Phycol. J. **11:** 165–174.

RAMUS, J., BEALE, S.I., MAUZERALL, D. and HOWARD, K.L. 1976a. Changes in photosynthetic pigment concentration in seaweeds as a function of water depth. Mar. Biol. **37:** 223–229.

RAMUS, J., BEALE, S.I. and MAUZERALL, D. 1976b. Correlation of changes in pigment content with photosynthetic capacity of seaweeds as a function of water depth. Mar. Biol. **37:** 231–238.

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.

SCOR-UNESCO W.G. 17. 1966. Determination of photosynthetic pigments. UNESCO Monogr. Oceanogr. Methodol. **1:** 9–18.

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

YOKOHAMA, Y. 1973b. Photosynthetic properties of

marine benthic red algae from different depths in the coastal area. Bull. Jap. Soc. Phycol. **21**: 119–124 (in Japanese).
YOKOHAMA, Y., KAGEYAMA, A., IKAWA, T. and SHIMURA, S. 1977. A carotenoid characteristic of chlorophycean seaweeds living in deep coastal waters. Bot. Mar. **20**: 433–436.
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).
YOKOHAMA, Y. and MAEGAWA, M. 1988. Measurements of photosynthesis and respiration of large samples by 'Productmeter', a differential gas-volumeter. Jpn. J. Phycol. **36**: 29–36 (in Japanese).

村瀬　昇・前川行幸・喜田和四郎：三重県志摩半島沿岸における生育水深の異なる紅藻数種の光合成特性

三重県志摩半島沿岸の低潮線付近の浅所および水深 25 m 付近の深所から採取した紅藻数種について光合成測定と色素分析を行い，得られた光合成活性および色素組成から，紅藻の垂直分布特性を解明しようと試みた。

太陽光に近い白色光と水深 20 m 付近の光の波長組成に近似させた緑色光の下で，光合成—光曲線を求めたところ，浅所産のものは白色光を，深所産のものは緑色光を効率よく光合成に利用することが明らかになった。そこで、クロロフィル *a* と緑色域の光を吸収する紅藻特有の光合成色素フィコエリスリンを定量し，クロロフィル *a* に対するフィコエリスリンの含有比 (PE/Chl. *a*) を求めた。その結果，PE/Chl. *a* は浅所産のものでは 1～4 であるのに対し，深所産のものでは 4～9 と高かった。これらのことから，深所産紅藻は，浅所産のものに比べてフィコエリスリンの含有比が高いために，緑色域の光を中心とする沿岸深所の光環境下で効率よく光合成を行っていることが明らかとなった。

以上のことから，浅所産および深所産紅藻は，それぞれの生育水深の光環境によく適応した光合成特性と色素組成をもっていることが明らかとなった。これは，紅藻類におけるフィコエリスリン含有比を生育水深によって変えることによる色適応の結果である。(514 三重県津市江戸橋2-80　三重大学生物資源学部藻類増殖学研究室)