Jap. J. Phycol. (Sôrui) 28: 219-223. December 10, 1980.

Chlorophyll *a* : *b* ratios in marine benthic green algae[1),2)]

Yasutsugu YOKOHAMA and Taku MISONOU

Shimoda Marine Research Center, The University of Tsukuba, Shimoda, Shizuoka-ken, 415 Japan

YOKOHAMA, Y. and MISONOU, T. 1980. Chlorophyll *a* : *b* ratios in marine benthic green algae. Jap. J. Phycol. 28: 219-223.

The chlorophyll *a* : *b* ratios were measured in about fifty species of marine benthic green algae over seven orders. There seemed to be a general tendency that the *a* : *b* ratios in samples collected from deeper ranges and shaded sites were lower The *a* : *b* ratio varied from 2 or above to values close to 1.0 in Ulvales, Cladophorales and Siphonocladales, while in the case of Siphonales (Codiales, Derbesiales and Caulerpales) the variability of the *a* : *b* ratio seemed to be small, and low values were generally observed.

The variation in the *a* : *b* ratio occurred not only among species but also among individuals of the same species collected from different habitats with different light field.

Key Index Words: *Caulerpales*; *Chlorophyta*; *chlorophyll* a : b *ratio*; *Cladophorales*; *Codiales*; *Dasycladales*; *Derbesiales*; *light field*; *Siphonocladales*; *Ulvales*.

JEFFREY (1965) reported chlorophyll *a* : *b* ratios below 2.0 for siphonous green algae, and KEAST & GRANT (1976) considered that the *a* : *b* ratios in siphonous green algae were characteristically lower than those in other members of the Chlorophyceae and higher plants. On the other hand NAKAMURA *et al.* (1976) concluded that the occurrence of lower *a* : *b* ratios close to 2.0 was a general characteristic of marine members of the Chlorophyceae. However, it was noticed in our previous study that the chlorophyll *a* : *b* ratio was considerably different among the algae collected from different habitats (YOKOHAMA 1973, YOKOHAMA *et al.* 1977, KAGEYAMA & YOKOHAMA 1977).

The present paper will report the chlorophyll *a* : *b* ratios measured in about 50 species including the members of Siphonales and others collected from various habitats with different light fields.

Materials and Methods

About 50 species of the Chlorophyceae of benthic type were collected from intertidal and subtidal zones at Shimoda in Central Japan and at Okinawa and Amami in the southern part of Japan. Thalli collected at Shimoda were soaked in a large volume of seawater and carried to the laboratory of the Shimoda Marine Research Center, where they were kept in running seawater for a few hours before pigment extractions. Those collected at Okinawa and Amami were frozen and carried to the laboratory, where the extractions were made.

The fresh or frozen material was extracted with cold methanol. The extract was mixed with a nearly equal volume of diethylether. The pigments were transferred to the ether layer by shaking with a 10% NaCl solution. After repeating wash with

1) Dedicated to the memory of the late Professor Tomoo MIWA.

2) Contributions from the Shimoda Marine Research Center, No. 373. This work was supported in part by Grant-in-Aids for Scientific Research Nos. 534027 and 534028 from the Ministry of Education of Japan.

Table 1. Molar ratios of chlorophyll *a* to chlorophyll *b* in marine benthic green algae of seven orders

Species	Habitat	Chl. *a* : *b*
ULVALES		
Monostroma nitidium	U	2.13
*Ulva amamiensis**	5 m	1.22
U. japonica	20 m	1.18
U. pertusa	M	1.59
Enteromorpha compressa	U	1.85
CLADOPHORALES		
Chaetomorpha sp.	U	1.89
C. crassa	0.5 m	1.45
C. spiralis	0 m	1.56
Cladophora sp.	U	2.04
C. japanica	0 m (Sh)	1.02
C. ohkuboana	17 m	1.15
C. wrightiana	2 m (Sh)	1.30
*Anadyomene wrightii**	L	1.56
Microdictyon japonicum	L (Sh)	1.14
SIPHONOCLADALES		
Cladophoropsis zollingeri	L (Sh)	1.08
Boodlea coacta (outer part)	L	2.56
(inner part)		2.04
Valonia macrophysa	18 m	1.22
Dictyosphaeria cavernosa	L	2.27
DASYCLADALES		
*Neomeris annulata**	M	1.54
*Halicoryne wrightii**	M	1.61
*Acetabularia ryukyuensis**	M	1.85
CODIALES		
Codium adhaerens	18 m	1.09
C. divaricatum	18 m	1.05
C. fragile	L	1.23
C. intricatum	0.5 m	1.41
C. latum	L	1.41
	18 m	1.25
C. mamillosum	18 m	1.10
DERBESIALES		
Bryopsis sp.	L	1.37
*Pseudobryopsis hainanensis**	Cultured**	1.37
Derbesia marina	0.5 m (Sh)	1.19
	Cultured**	0.88
Halicystis ovalis	Cultured**	0.99
Pedobesia lamourouxii	5 m (Sh)	1.11

Species	Habitat	Chl. $a:b$
CAULERPALES		
Caulerpa ambigua	L (Sh)	1.67
C. okamurai	L	1.52
C. peltata var. *peltata**	L (Sh)	1.33
C. racemosa var. *clavifera* f. *macrophysa**	L	1.54
C. racemosa var. *laete-virens*	L	1.67
*Chlorodesmis comosa**	L	1.67
	2 m	1.61
*Udotea javensis**	L	1.67
*U. orientalis**	L	1.64
*Avrainvillea erecta**	L	1.69
*Tydemania expeditionis**	2 m (Sh)	1.23
Halimeda discoidea	0.5 m	1.52
H. incrassata f. *incrassata**	L	1.47
*H. macroloba**	L	1.30
	2 m (Sh)	1.23
H. opuntia f. *opuntia**	L	1.54

Number in m denote depth from low water mark. U=upper intertidal zone; M=middle intertidal zone; L=lower intertidal zone. Sh denotes shaded site. * Collected at Okinawa or Amami. ** Cultured for more than one month under 2 klux of fluorescent illumination.

the NaCl solution, the ether layer was dried up under reduced pressure, and the residue was redissolved in a small volume of ether.

The absorption spectra of the ether solutions of pigment were determined with a Shimadzu Spectrophotometer UV-200. The concentrations of chlorophyll *a* and *b* were calculated from the absorbances at 642.5 nm and 660 nm, known from the absorption spectra, using the equation of COMAR & ZSHEILE (1942).

Results

Table 1 presents the chlorophyll $a:b$ ratios measured in about 50 species over seven orders of the Chlorophyceae collected from various habitats. As can be seen in the table, the chlorophyll $a:b$ ratios are different among the materials. There seems to be a general tendency that the samples collected from deeper ranges or shaded sites possess lower values of chlorophyll $a:b$ ratio. The samples of each order collected from deeper ranges or shaded sites possess the $a:b$ ratios close to 1.0. However, the $a:b$ ratios in the samples collected from sunny sites at shallow waters are remarkably different among the orders. Those in Codiales, Derbesiales and Caulerpales are generally lower than those in Ulvales, Cladophorales, Siphonocladales and Dasycladales. The correlation between the chlorophyll $a:b$ ratio and the habitat in each order is more noticeable in Fig. 1 made from the data presented in Table 1.

Difference in the chlorophyll $a:b$ ratio is also remarkable between individuals of the same species collected from different habitats. *Codium latum* was collected from both lower intertidal zone and the depth of 18 m. The $a:b$ ratio observed with the sample from the intertidal zone was 1.41, while that with the sample from the deeper range was 1.25. A similar difference can be seen between the samples of *Halimeda*

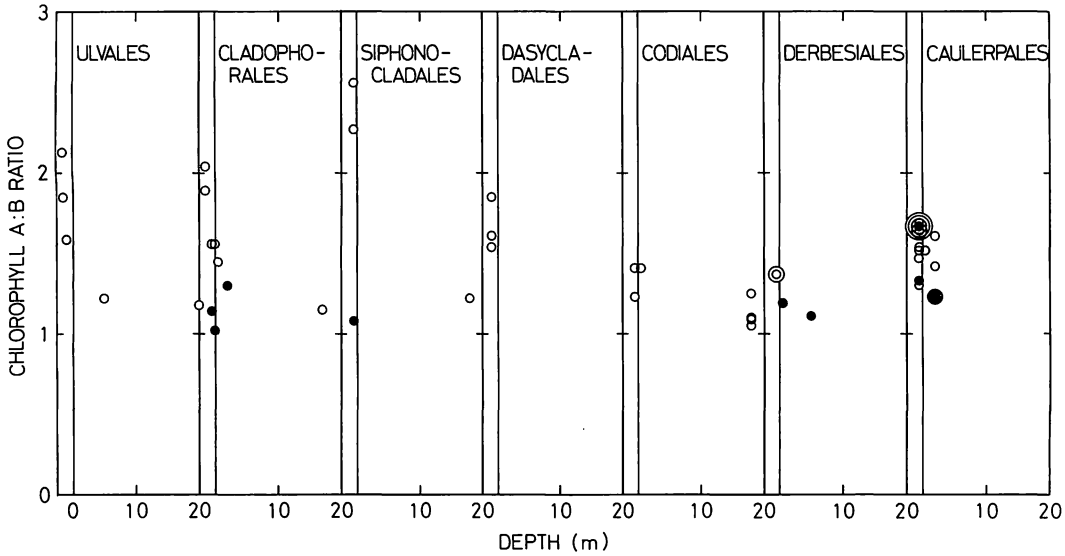

Fig. 1. Interrelation between chlorophyll *a* : *b* ratio and habitat. Depth means that from low water mark. Closed circle denotes shaded site.

macroloba collected from a sunny site at lower intertidal zone and a shaded site at the depth of 2 m.

Boodlea coacta collected from lower intertidal zone possessed the *a* : *b* ratio of 2.56, the highest of all the values observed in this study. This value was, however, not that of whole plant of this sample. The collected sample was shaped into a hemispherical clump with the radius of about 8 cm. The highest value was that in the cells of its outer part, and the value in the cells of its inner part was 2.04.

The chlorophyll *a* : *b* ratio was also shifted under artificial conditions. A ratio lower than 0.9 was observed with the sample of *Derbesia marina* cultured for more than one month under 2 klux of fluorescent illumination with day length of 14 hours at 20°C, while the value in the sample collected from nature, a shaded site at the depth of 0.5 m was 1.19.

Discussion

We observed the variation in the chlorophyll *a* : *b* ratio from about 2.5 to 1.0. The variation can occur not only among species but also among individuals of the same species, and there seems to be a close correlation between the *a* : *b* ratio and the light field.

The *a* : *b* ratios observed with the shallow-water samples of Siphonales (Codiales, Derbesiales and Caulerpales) were generally lower than those with the shallow-water ones of the other orders. Therefore, as far as the shallow-water samples are concerned, members of Siphonales seem to possess chlorophyll *a* : *b* ratios considerably below those found in other species, as observed by JEFFREY (1965) and KEAST & GRANT (1976). In this study, however, exceedingly low value close to 1.0 was also observed with the deep-water samples or shade ones of Ulvales, Cladophorales and Siphonocladales.

RAMUS *et al.* (1976 a, 1976 b) also reported the adaptive change in the chlorophyll *a* : *b* ratio for *Ulva lactuca* and *Codium fragile*. The higher proportion of chlorophyll *b* can be more favorable for growth in deep waters and shaded sites since chlorophyll *b* absorbs short-wave light more effectively than chlorophyll *a* does. Another characteristic of green algae growing in deep waters or shaded sites is to contain siphonaxanthin as a photosynthetic pigment col-

lecting green light dominating underwater in the coastal area (YOKOHAMA *et al.* 1977, KAGEYAMA *et al.* 1977, KAGEYAMA & YOKOYAMA 1977).

References

COMAR, C. L. and ZSCHEILE, F. P. 1942. Analysis of plant extracts for chlorophylls *a* and *b* by a photoelectric spectrophotometric method. Plant Physiol. **17**: 198–209.

JEFFREY, S. W. 1965. Pigment composition of Siphonales algae in the brain coral Fabia. Biol. Bull. **135**: 141–148.

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., 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.

KEAST, J. F. and GRANT, B. R. 1976. Chlorophyll *a*:*b* ratios in some siphonous green algae in relation to species and environment. J. Phycol. **12**: 328–331.

NAKAMURA, K., OGAWA, T. and SHIBATA, K. 1976. Chlorophyll and peptide composition in the two photosystems of marine green algae. Biochim. Biophys. Acta **126**: 234–243.

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

YOKOHAMA, Y. 1973. 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., KAGEYAMA, A., IKAWA, T. and SHIMURA, S. 1977. A carotenoid characteristic of chlorophycean seaweeds living in deep coastal waters. Bot. Mar. **20**: 433–436.

横浜康継・御園生　拓：　海産底生緑藻のクロロフィル *a*:*b* 比

7目約50種にわたる海産緑藻について，クロロフィル *a*:*b* 比を測定したところ，深所や陰所から採集されたものほど，*a*:*b* 比は小さくなるという傾向がみられた。しかしその変異の幅は目により異なり，アオサ目・シオグサ目・ミドリゲ目では大きく，2あるいはそれ以上から1近くまでの値がみられるのに対して，ミル目・ツユノイト目・イワヅタ目などのクダモ類では全般的に低い値がみられた。

クロロフィル *a*:*b* 比の変異は，種間のみならず，生育環境を異にしていた同種個体間でもみられた。

(415 静岡県下田市5-10-1，筑波大学下田臨海実験センター)