ISSN 0038-1578

Vol. 37 No. 2 20 June 1989

The Japanese Journal of PHYCOLOGY

CONTENTS

THE JAPANESE SOCIETY OF PHYCOLOGY

日 本 藻 類 学 会

日本藻類学会は1952年に設立され，藻学に関心をもち，本会の趣旨に賛同する個人及び団体の会員からなる。本会は定期刊行物「藻類」を年4回刊行し，会員に無料で頒布する。普通会員は本年度の年会費7,000円（学生は5,000円）を前納するものとする。団体会員の会費は12,000円，賛助会員の会費は1口20,000円とする。

入会，退会，会費の納入および住所変更等についての通信は **113 東京都文京区弥生2-4-16 学会センタービル内 日本学会事務センター**宛に，原稿の送付は **108 東京都港区港南4-5-7 東京水産大学 有賀祐勝気付 日本藻類学会編集委員会**宛に，また，庶務一般およびバックナンバー等については **184 東京都小金井市貫井北町4-1-1 東京学芸大学生物学教室内 日本藻類学会**宛にされたい。

The Japanese Society of Phycology

The Japanese Society of Phycology, founded in 1952, is open to all who are interested in any aspect of phycology. Either individuals or organizations may become members of the Society. The Japanese Journal of Phycology (SÔRUI) is published quarterly and distributed to members free of charge. The annual dues (1989) for overseas members are 8,500 Yen (send the remittance to the **Business Center for Academic Societies Japan, 4-16, Yayoi 2-chome, Bunkyo-ku, Tokyo, 113 Japan).**

Manuscript for publication should be submitted directly to the Editor-in-Chief, **Prof. Y. Aruga, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan.** Inquiries and other information regarding the society should be addressed to the **Japanese Society of Phycology, c/o Department of Biology, Tokyo Gakugei University, Nukuikitamachi, Koganei-shi, Tokyo, 184 Japan.**

1989-1990年役員

会　　長：	小林　弘	（東京学芸大学）
庶務幹事：	片山　舒康	（東京学芸大学）
会計幹事：	真山　茂樹	（東京学芸大学）
評 議 員：		
	秋山　優	（島根大学）
	有賀　祐勝	（東京水産大学）
	千原　光雄	（筑波大学）
	原　慶明	（筑波大学）
	石川依久子	（大阪大学）
	岩崎　英雄	（三重大学）
	増田　道夫	（北海道大学）
	三浦　昭雄	（東京水産大学）
	野沢　洽治	（鹿児島大学）
	小河　久朗	（東北大学）
	大森　長朗	（山陽学園短期大学）
	奥田　武男	（九州大学）
	舘脇　正和	（北海道大学）
	坪　由宏	（神戸大学）
	渡辺　信	（国立公害研究所）
	横浜　康継	（筑波大学）
編集委員会：		
委 員 長：	有賀　祐勝	（東京水産大学）
幹　　事：	能登谷正浩	（東京水産大学）
実行委員：	堀　輝三	（筑波大学）
	田中　次郎	（国立科学博物館）
	横浜　康継	（筑波大学）
委　　員：	榎本　幸人	（神戸大学）
	石川依久子	（大阪大学）
	加藤　哲也	（京都大学）
	鬼頭　鈞	（西海区水産研究所）
	野田　宏行	（三重大学）
	奥田　武男	（九州大学）
	舘脇　正和	（北海道大学）
	渡辺　信	（国立公害研究所）
	籔　熙	（北海道大学）
	吉田　忠生	（北海道大学）

Officers for 1989-1990

President:	Hiromu KOBAYASI	(Tokyo Gakugei University)
Secretary:	Nobuyasu KATAYAMA	(Tokyo Gakugei University)
Treasurer:	Shigeki MAYAMA	(Tokyo Gakugei University)
Members of Executive Council:		
	Masaru AKIYAMA	(Shimane University)
	Yusho ARUGA	(Tokyo University of Fisheries)
	Mitsuo CHIHARA	(University of Tsukuba)
	Yoshiaki HARA	(University of Tsukuba)
	Ikuko SHIHIRA ISHIKAWA	(Osaka University)
	Hideo IWASAKI	(Mie University)
	Michio MASUDA	(Hokkaido University)
	Akio MIURA	(Tokyo University of Fisheries)
	Koji NOZAWA	(Kagoshima University)
	Hisao OGAWA	(Tohoku University)
	Takeo OHMORI	(Sanyo Gakuen Junior College)
	Takeo OKUDA	(Kyushu University)
	Masakazu TATEWAKI	(Hokkaido University)
	Yoshihiro TSUBO	(Kobe University)
	Makoto M. WATANABE	(National Institute for Environmental Studies)
	Yasutsugu YOKOHAMA	(University of Tsukuba)
Editorial Board:		
	Yusho ARUGA	(Tokyo University of Fisheries), Editor-in-Chief
	Masahiro NOTOYA	(Tokyo University of Fisheries), Secretary
	Terumitsu HORI	(University of Tsukuba), Associate Editor
	Jiro TANAKA	(National Science Museum), Associate Editor
	Yasutsugu YOKOHAMA	(University of Tsukuba), Associate Editor
	Sachito ENOMOTO	(Kobe University)
	Ikuko SHIHIRA ISHIKAWA	(Osaka University)
	Tetzuya KATO	(Kyoto University)
	Hitoshi KITO	(Seikai Regional Fisheries Research Laboratory)
	Hiroyuki NODA	(Mie University)
	Takeo OKUDA	(Kyushu University)
	Masakazu TATEWAKI	(Hokkaido University)
	Makoto M. WATANABE	(National Institute for Eviromental Studies)
	Hiroshi YABU	(Hokkaido University)
	Tadao YOSHIDA	(Hokkaido University)

日本藻類学会秋季シンポジウムのお知らせ

日本藻類学会では，日本植物学会第54回大会（仙台，東北大学）の前日に，秋季シンポジウムおよび懇親会を下記の通り開催します。

日　　時：1989年9月26日（火）
シンポジウム　15:30～17:30
懇　親　会　　18:00～20:00

シンポジウム会場：東北大学教養部講義棟（仙台市川内），参加無料
東北大学教養部への交通案内等については，「生物科学ニュース」No. 208, p. 14–16（1989年3月号）をご参照下さい。タクシーですと，JR 仙台駅から10分（700円程度）です。

演者・演題：安部　守（山形大学）：褐藻卵の受精と発生
川嶋昭二（前北海道水産試験場）：
漁場におけるコンブの生活研究と漁場造成技術への展開

座　　長：谷口和也（東北区水産研究所）

懇親会会場・会費：東北大学教養部構内　川内厚生会館軽食堂
3,000円（当日シンポジウム受付でお支払い下さい。）
出席ご希望の方は，会場準備の都合がありますので，**8月末日までに**下記宛に葉書でお申込み願います。

工場見学：
仙台市近郊に大規模なワカメ加工工場があります。見学をご希望の方は，**8月末日までに**下記宛にお申込み下さい。なお，人数に制限（30名）がありますので，定員に達し次第締切らせていただきます。工場見学は，9月26日午後1時から約1時間です。時間に遅れた方は見学できないことがありますのでご注意下さい。
交通は，JR 仙石線で多賀城駅下車（仙台から約20分），駅前よりタクシーを利用（工場まで約10分，700円程度）することになります。
当日，工場見学をされても午後3時30分からのシンポジウムには充分間に合います。

工場：〒985 多賀城市宮内2丁目5番60号
理研食品株式会社
電話 022–365–6446

シンポジウム・懇親会・工場見学　申込先：
〒980 仙台市青葉区堤通雨宮町1–1
東北大学農学部水産学科
小　河　久　朗
電話　022–272–4321　内線 304

Jpn. J. Phycol. (Sôrui) 37: 89–98, June 20, 1989

Studies on diurnal photosynthetic performance of *Sargassum thunbergii* I. Changes in photosynthesis under natural sunlight

Kunshan GAO* and Isamu UMEZAKI

Laboratory of Fishery Resources, Division of Tropical Agriculture, Graduate School of Agriculture, Kyoto University, Kyoto, 606 Japan

GAO, K. and UMEZAKI. I. 1989. Studies on diurnal photosynthetic performance of *Sargassum thunbergii* I. Changes in photosynthesis under natural sunlight. Jpn. J. Phycol. 37: 89–98.

A flow-through system was established to carry out photosynthetic measurements of macroalgae under natural sunlight. Changes in photosynthesis of *Sargassum thunbergii* with changes in solar radiation under different weather conditions were measured with the flow-through system. It was found that photosynthetic rate was higher in the morning than in the afternoon on fine days and showed little difference between the morning and the afternoon on a rainy day. Midday, or afternoon, photosynthetic depression observed on fine days could not be accounted for by an increase in dark respiration. Despite midday or afternoon photosynthetic depression, daily net and gross productions were found to increase linearly with an increase of daily photosynthetically active solar radiation.

Key Index Words: Photosynthesis—dark respiration—PAR—Sargassum thunbergii.

In coastal areas, seaweeds play an important role as primary producers. Estimations of the primary production of seaweeds often rely on photosynthesis-light and solar radiation curves. Seaweeds are usually subjected to dramatic changes of solar radiation throughout a day. Are the changes in photosynthesis with the solar radiation independent of light histories? Is the estimated photosynthetic production close to the actual diurnal production? It is possible that P_{max} and initial slope of a photosynthesis-light curve differ when a seaweed has been exposed to different light histories, or at different times of day under natural sunlight. Diurnal photosynthesis has been found to show higher morning photosynthetic rates and afternoon depression in tropical grasses (ITO *et al.* 1973), freshwater submerged angiosperms (GOULDER 1970, HOUGH 1974) and phytoplankton (DOTY and OGURI 1957, YENTSCH and RYTHER 1957, HARRIS 1973, SOURNIA 1974, MARRA 1978). But there are few reports on the diurnal photosynthesis of seaweeds. RAMUS and ROSENBERG (1980) measured the diurnal photosynthesis of seaweeds and reported that photosynthesis-light curves measured in the laboratory showed a great difference from natural diurnal photosynthesis. However, SMITH (1981) reported no depression of diurnal photosynthetic pattern in *Codium carolinianum* and *Lodophora variegata* calculated from an *in situ* study. MAEGAWA and ARUGA (1983), studying on a cultivated *Monostroma latissimum* population, also reported that no midday or afternoon depression of photosynthesis was observed, even on fine days. Therefore, it is important to follow photosynthetic changes under natural sunlight to make clear whether seaweeds exhibit a pattern of diurnal photosynthetic variation similar to higher plants and phytoplankton, or not.

Sargassum, a genus in the Phaeophyceae, constitutes an important part of the marine flora, forming an extensive vegetation in the coastal areas of Japan and playing an impor-

* Present address: Kansai Environmental Engineering Center, Nakazaki-nishi 2–3–39, Kita-ku, Osaka, 530 Japan

tant role in the coastal ecosystem. *Sargassum thunbergii* is a perennial species growing on rocks at middle to lower level in the intertidal zone, forming a conspicuous belt in places not exposed to strong wave action, and is distributed on the coasts of both the Sea of Japan and the Pacific Ocean throughout the Archipelago of Japan. The knowledge of photosynthesis is important for the estimation of productivity of *Sargassum*. Although there are several reports on photosynthesis in *Sargassum* up to now (THOMAS and TREGUNNA 1968, YOKOHAMA 1977, MIZUSAWA *et al.* 1978, GAO and UMEZAKI 1988), nothing has been reported on diurnal photosynthesis and daily photosynthetic production of plants in this genus. In the present study it was intended to follow the photosynthetic changes of *Sargassum thunbergii* under natural sunlight to make clear its diurnal photosynthesis pattern.

Materials and Methods

The present study was carried out at the Fisheries Research Station of Kyoto University situated at the head of Maizuru Bay, one of the branch bays of Wakasa Bay facing the Sea of Japan. Here, *S. thunbergii* forms a conspicuous zone at approximately −10 to +15 cm tide level and matures in July. After maturation, the main branches decay away and newly germinated main branches and juveniles appear about two months later.

The measurements of diurnal photosynthesis were carried out in October and December 1986. Whole juveniles or young plants derived from naturally-occurring germlings were collected and cleaned of obvious epiphytes before use. Samples used for measurements of diurnal photosynthesis or dark respiration were about 1.5 and 3 cm long in October and December, respectively. Five to ten samples were used for each measurement of diurnal photosynthesis or dark respiration.

Figure 1 shows the flow-through system for measuring photosynthesis and respiration. The ends of the pipe are cone-shaped, which gives a uniform water flow through the pipe (P). Transparent and opaque pipes, 70 cm long and 3 cm in inner diameter, were used for measurements of photosynthesis and dark respiration, respectively. Samples (Th) were fixed by inserting their holdfasts into a cord and kept erect in the pipe. Water flow through the pipe was tested with uranine dye. The flow rate of the filtered seawater (filtered through sand and coal particles) was controlled with a flow meter (Kojima, RK 1200).

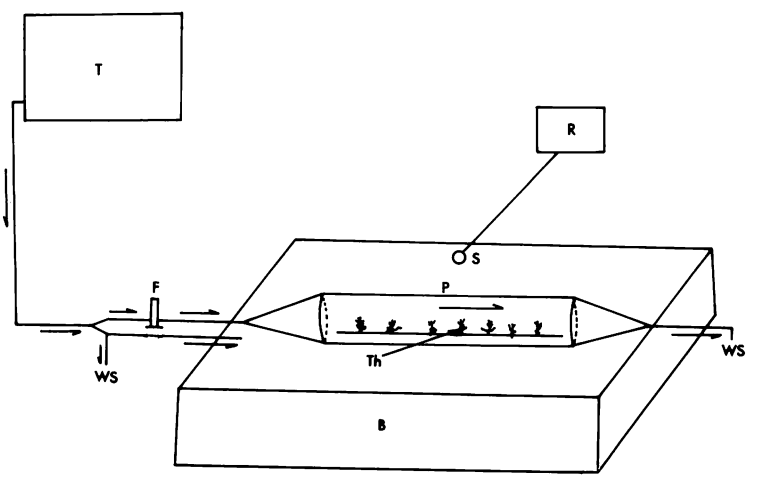


Fig. 1. Outline of the system for measuring diurnal photosynthesis and dark respiration. T, water tank for supplying filtered seawater; F, flow meter; P, assimilation pipe; Th, thallus; S, underwater quantum sensor; R, recorder; WS, water sampling tube; B, water box.

Inflowing and outflowing seawater were sampled almost simultaneously in D. O. bottles of about 100 m*l* in duplicate each time. Water sampling was made once every hour or every two hours for photosynthetic or respiratory measurements, respectively. The dissolved oxygen concentration of seawater was determined by the Winkler titration technique. The titration was made within 3 to 4 hours after water sampling. Photosynthesis (P) or respiration (R) rate [m*l* O_2 g(d.w.)$^{-1}$h^{-1}] was calculated by the following equation:

$$P \text{ or } R=(A-B)\times F\times 60\times 1/W,$$

where A and B represent D. O. concentrations (m*l* l^{-1}, 0°C and 760 mmHg) of outflowing and inflowing seawater, respectively; F, flow rate (l min^{-1}) of filtered seawater supplied into the pipe; W, dry weight (g) of samples determined after drying at 85°C for 20 hours. Different samples were used for the measurements of photosynthesis and dark respiration. During the daytime, dark respiration was measured by placing samples in an opaque pipe for about 20 minutes. After the dark respiration measurement, samples were taken out of the pipe and exposed to solar radiation under the same conditions as those for photosynthetic measurements.

Photosynthetically active radiation (PAR, 400–700 nm) of incident solar light was measured continuously from sunrise to sunset with an underwater quantum sensor (LI-COR, LI-192S) linked with a recorder (TOA Electronics Ltd., FBR-253A). Water temperature in the pipe, which was maintained in a water tank, was in harmony with that in the sea. Water temperature was in ranges of 21–23°C and 13–14°C during the expemriment periods in October and December, respectively.

In order to value diurnal photosynthesis measured under different temperatures in October and December, it was necessary to know the relationships of photosynthesis and respiration to temperature. Photosynthesis and respiration versus temperature curves were obtained in March and September in the laboratory with a differential gas-volumeter, "Productmeter" (Nikko Kagaku Ltd.), devised by YOKOHAMA and ICHIMURA (1969) and improved by YOKOHAMA *et al.* (1986). Light was supplied by incandescent "high beam bulbs" (National, 110V 150W). The water temperature was controlled by using a Taiyo Coolnit (CL-30). Dark respiration was measured by covering the reaction vessel completely with opaque plastic.

Results

The system for measuring photosynthesis and respiration in the present study kept samples erect in flowing seawater, and this made it possible to measure photosynthesis or respiration under conditions close to those in the sea. However, the time lag for detecting oxygen evolved or consumed by samples kept in the pipe, which may be related to the flow rate, the inner diameter and length of the pipe, should be considered. In preliminary experiments, changes of D. O. concentration of outflowing seawater were measured in the laboratory under constant light and temperature conditions at different flow rates after samples had been put into the pipe. As shown in Figure 2, it took about 8, 6, 5 and 2

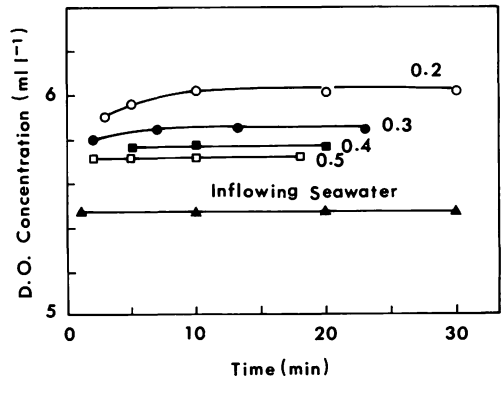


Fig. 2. Time courses of D. O. concentrations in the outflowing seawater at different flow rates (0.2, 0.3, 0.4 and 0.5 l min^{-1}) after 8 individuals of *S. thunbergii* were put into the transparent assimilation pipe at 1000 μE m^{-2}s^{-1} and 16°C in the laboratory. Solid triangles show D. O. concentrations in the inflowing seawater.

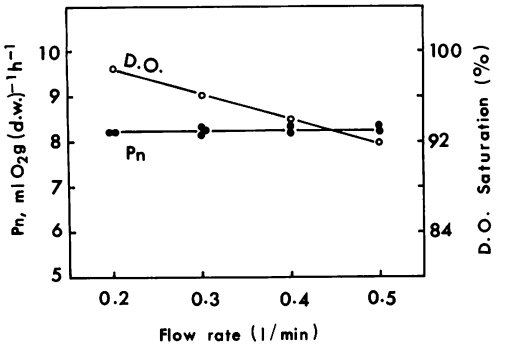


Fig. 3. Relationships of net photosynthesis (P_n) of *S. thunbergii* and D. O. saturation of outflowing seawater to flow rates. Calculated from Fig. 2.

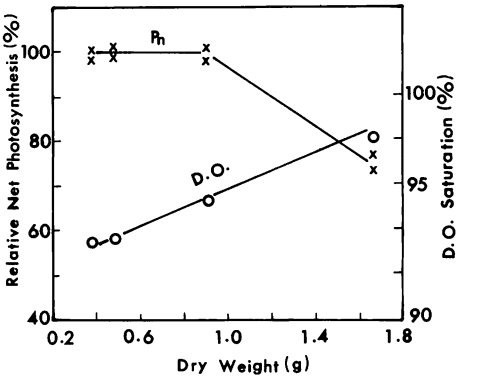


Fig. 4. Relationships of relative net photosynthesis (P_n) in *S. thunbergii* and D. O. saturation to dry weight of samples. Measured at 1000 μE m^{-2}s^{-1} and 10°C with a flow rate of 0.3 l min^{-1}.

minutes for D. O. concentrations to level off at flow rate of 0.2, 0.3, 0.4 and 0.5 l min^{-1}, respectively. The D. O. concentration was levelled in a shorter time as flow rate increased. Photosynthetic rates at different flow rates were calculated from data of Figure 2. As indicated in Figure 3, photosynthetic rate was not influenced by flow rate although the D. O. saturation decreased as flow rate increased within the range investigated. Fresh weight and dry weight of the samples used for these measurements were 5.394 and 0.794 g, respectively. Influences of flow rate on photosynthetic rate might have occurred if more samples were used at any one time.

A relationship of dry weight of samples to relative net photosynthesis is shown in Figure 4. Relative net photosynthesis showed constant values till 0.9 g as the dry weight of samples increased, and was reduced at 1.6 g dry weight of samples, while D. O. saturation increased with an increase of weight of samples.

Based on the results of Figures 2–4, the diurnal photosynthesis and dark respiration measurements of *S. thunbergii* were carried out on condition that the weight of samples was 4–5 g fresh weight (0.59–0.74 g dry weight) and the flow rate was 0.2 l min^{-1} for respiratory measurement and within a range of 0.2–0.4 l min^{-1} for photosynthetic measurement.

Figure 5 shows the diurnal net photosynthetic performance and the daily dark respiration of *S. thunbergii* juveniles on a fine day in October (A), and a fine day (B), a cloudy day (D) and a rainy day (C) in December. As indicated in Figure 5(A), net photosynthesis increased in early morning to reach a maximum at about 8 o'clock, decreased in late morning as solar radiation increased, increased again at noon and finally declined as solar radiation decreased in late afternoon. Dark respiration, which was measured with different samples kept under almost the same conditions as those for photosynthetic measurement, was found to be higher in daytime than at night, with the former about twice the latter on average. Daytime dark respiration was lower in morning and highest near midday when photosynthetic depression occurred (Fig. 5A). On a fine day in December (Fig. 5B), net photosynthesis increased to reach a maximum at about 10 o'clock in morning as solar radiation increased, and decreased in late afternoon as solar radiation decreased. Maximum of net photosynthesis was maintained for about 4 hours from late morning to early afternoon. Little difference in dark respiration was found between morning and afternoon. On a cloudy day (Fig. 5D), net photosynthesis increased with an increase in solar radiation in morning and decreased with a decrease in solar radiation in afternoon despite fluctuations in solar radiation. Dark respiration measured in early morning was

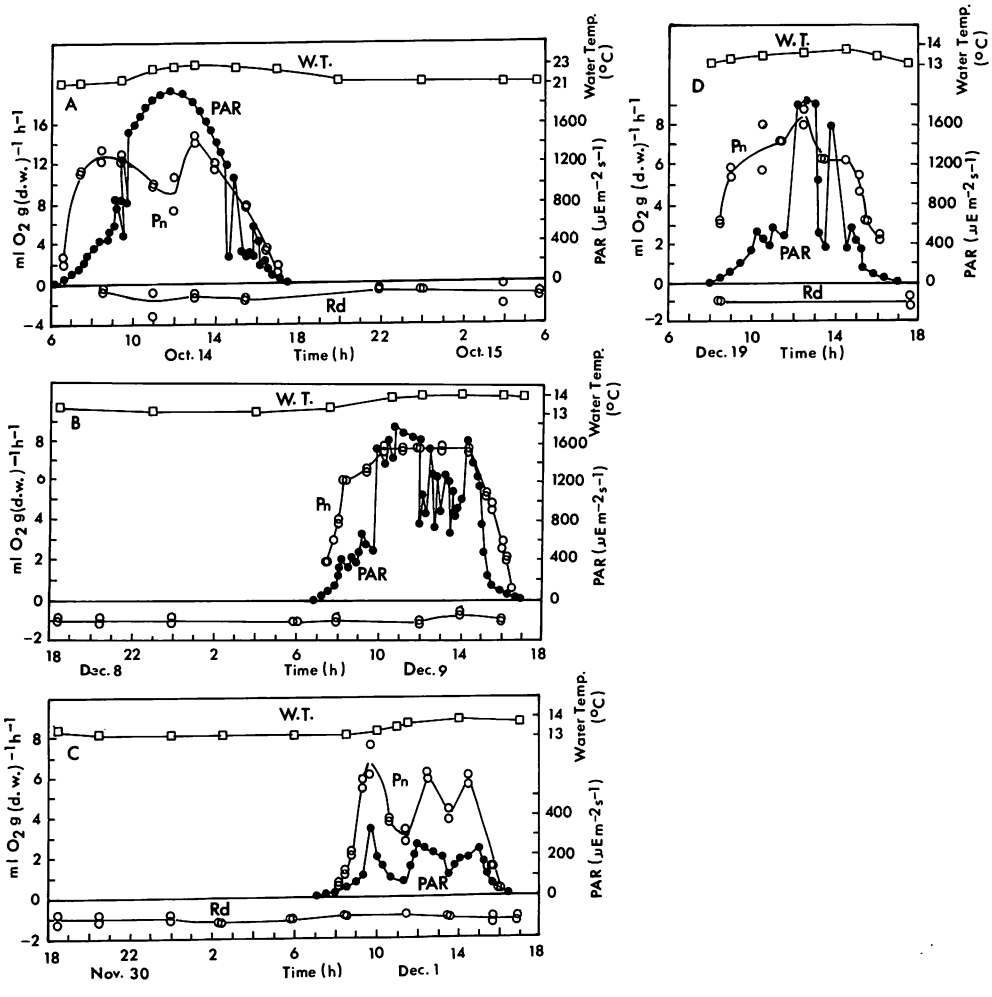


Fig. 5. Diurnal changes in net photosynthetic rate (P_n) and dark respiration rate (R_d) of *S. thunbergii* on a fine day in October (A) and December (B), and on a rainy day (C) and on a cloudy day (D) in December. Solid circles are photosynthetically active solar radiation (PAR) measured with a sensor set close to the assimilation pipe. W.T. is water temperature in the pipe.

almost the same as that in evening. This is in agreement with that on a fine day (Fig. 5B). On a rainy day in December (Fig. 5C), net photosynthesis varied with solar radiation. Dark respiration varied little throughout the day, which was in accordance with those on a fine day (Fig. 5B) and a cloudy day (Fig. 5D).

For detailed analysis of the diurnal photosynthesis of *S. thunbergii*, photosynthesis-light curves were derived from the data in Figure 5. PAR for constructing photosynthesis-light curves were determined from averaged values for 8, 6 and 5 minutes at flow rates of 0.2, 0.3 and 0.4 $l\ min^{-1}$, respectively, taking into account time lags for detecting evolved oxygen in outflowing seawater (Fig. 2). Dark respiration rates measured in early morning after sunrise and in late afternoon before sunset were used for construction of photosynthesis-light curves. As indicated in Figure 6, two photosynthesis-light curves resulted from each curve of diurnal photosynthesis; one for increasing solar radiation preceding local noon and the other for decreasing solar radiation after noon. Net photosynthesis was higher in morning than in afternoon, even for the same photosynthetically active solar radiation (PAR), on fine or cloudy-

fine days in December. Net photosynthesis increased, saturated at about 300 μE $m^{-2}s^{-1}$ and then decreased as the solar radiation increased in the morning on a fine day in October (Fig. 6A). Soon after noon, a recovery from the midday photosynthetic depression was observed. A considerable difference was found in the initial slopes of the curves derived from morning and afternoon photosynthesis, with slope much higher in morning than in afternoon. On a fine day in December (Fig. 6B), net photosynthesis increased as solar radiation increased and saturated at irradiances above 600 μE $m^{-2}s^{-1}$ in the morning, with the rate in morning higher compared with that in afternoon. On a cloudy-fine day in December (Fig. 6C), net photosynthesis was saturated at almost the same PAR as that on the fine day (Fig. 6B). Similarly, net photosynthetic rate was higher in the morning than in the afternoon. However, net photosynthesis was not saturated on the rainy day because of the much lower solar radiation, and little difference was found between morning and afternoon (Fig. 6D). Initial slope equations and P_{max} values for the curves of Figure 6 are shown in Table 1. Light compensation points of morning and afternoon curves were respectively 8 and 34 μE $m^{-2}s^{-1}$ on October 14, 21 and 22 μE $m^{-2}s^{-1}$ on December 1, 22 and 29 μE $m^{-2}s^{-1}$ on December 9, and 14 and 23 μE $m^{-2}s^{-1}$ on December 19. When

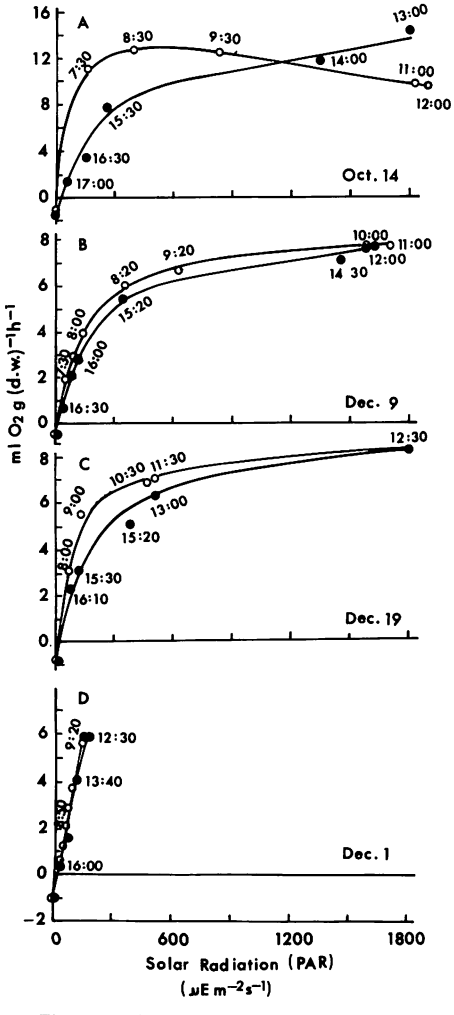


Fig. 6. Photosynthesis vs. solar radiation (PAR) curves of *S. thunbergii* obtained from Fig. 5. Open circles are for data before noon and filled circles are for data after noon. Numbers along the lines indicate the time of the day.

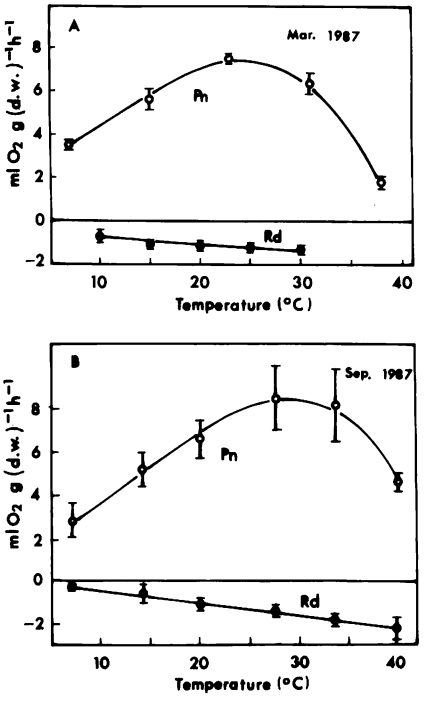


Fig. 7. Photosynthesis-temperature curves at 600 μE $m^{-2}s^{-1}$ and respiration-temperature curves in dark of *S. thunbergii*. Measurements were made with four young plants about 3 cm long (A) and with six young plants 1–2 cm long (B). Mean of three measurements with SD.

Table 1. Initial slope equations and P_{max} for photosynthesis-light curves in Figure 6. P in m*l* O_2 g(d.w.)$^{-1}$h^{-1} and I in μ E m^{-2}s^{-1}.

Date	Initial slope equation		P_{max}
	Morning	Afternoon	m*l* O_2 g(d.w.)$^{-1}$h^{-1}
Oct. 14	P=0.116I–0.93	P=0.043I–1.48	12.6
Dec. 1	P=0.045I–1.03	P=0.043I–1.01	—
Dec. 9	P=0.045I–1.00	P=0.035I–1.00	7.8
Dec. 19	P=0.069I–0.97	P=0.043I–0.97	8.3

afternoon photosynthetic depressions were observed, light compensation point of morning curves was higher than that of afternoon ones.

Daily gross and net production of *S. thunbergii* were calculated from diurnal net photosynthesis and dark respiration in daytime and nighttime, as summarized in Table 2. Daytime net photosynthesis, gross and net production and respiration expressed in m*l* O_2 were converted to those in dry weight as 1 mg O_2 (0.7 m*l* O_2) is equivalent to 0.84 mg dry matter (IKUSIMA 1967). Although PAR values were similar (36.6 and 33.2 E m^{-2} day^{-1}) on October 14 and December 9, daytime net photosynthesis, daily net production and daily gross production on the former day were 1.8, 1.7 and 2.1 times the values on the latter, respectively.

Photosynthesis-temperature curves were determined to see how photosynthesis of *S. thunbergii* was influenced by temperature. Light-saturated net photosynthesis for the young plants in March (Fig. 7A) increased to reach a maximum near 25°C and decreased at higher temperatures. Photosynthesis in juveniles with primary leaves in September (Fig. 7B) increased to reach a maximum near 28°C and decreased at higher temperatures as well. Net photosynthesis at 22°C was 1.39 and 1.46 times that at 14°C in March and September, respectively. Light-saturated net photosynthetic rate in the morning on October 14 was about 1.64 times that on December 9; it is a little higher than the rate in Figure 7. Dark respiration increased linearly with an increase in temperature in both months. Q_{10} was about 1.42 and 1.67 in March and September, respectively.

The great difference in daytime net photosynthesis, daily net production and daily gross production between October 14 and December 9 might be attributed to the differences of temperature and growth stage. Juvenile plants used for photosynthesis and respiration measurements had primary leaves in October but only filiform ones in December. Total daily dark respiration on October 14 was about 5–10% higher compared with those on days in December (Table

Table 2. Daytime net photosynthesis (P_{nd}), daytime dark respiration (R_{dd}), nighttime respiration (R_{dn}), daily gross production (P_g) and daily net production (P_n) of *S. thunbergii*, with temperature and PAR.

Date	Temp. (°C)	PAR (E m^{-2}day^{-1})	[mg(d.w.) g(d.w.)$^{-1}$]				
			P_{nd}	R_{dd}	R_{dn}	P_g	P_n
Oct 14.	22	36.6	125.3	14.8	14.9	140.1	110.4
			(87.6)*	(11.2)*	(11.3)*	(98.8)*	(76.3)*
Dec. 1	14	4.2	38.8	10.1	17.3	48.9	21.5
Dec. 9	14	33.2	69.7	11.6	16.8	81.3	52.9
Dec. 19	14	17.9	57.6	9.7	16.2	67.3	41.4

* Estimated values at 14°C in consideration of a temperature factor on the basis of the curves in Fig. 7.

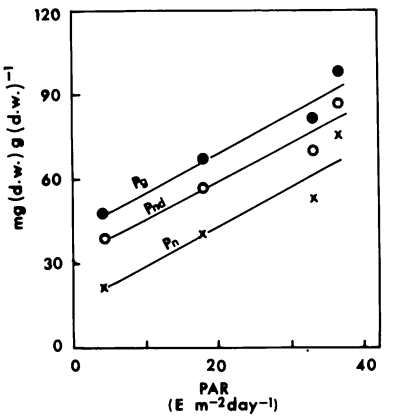


Fig. 8. Relationships of daytime net photosynthesis (P_{nd}), daily net production (P_n) and daily gross production (P_g) to PAR in *S. thunbergii* at 14°C.

2), and this must be due to higher temperature on the former than on the latter (Fig. 7). Daytime photosynthesis, daily net production and daily gross production on October 14 at 22°C were converted to those at 14°C by averaged factors (1.43 for net photosynthesis and 1.32 for respiration) from the photosynthesis-temperature and respiration-temperature curves determined in March (Fig. 7A) and September (Fig. 7B). Converted values for October 14 are higher in daytime net photosynthesis, daily gross and net production but lower in daytime and nighttime respiration compared to data for December 9. Disregarding the differences in growth stage, daytime net photosynthesis, daily net production and daily gross production were plotted as functions of PAR in Figure 8. As shown, these all increased almost linearly with increases in PAR during the period of investigation.

Discussion

Diurnal photosynthetic performance of *S. thunbergii* showed asymmetrical variation: that is, diurnal photosynthesis was found to show higher values in the morning and reduced rates in the afternoon, even for the same PAR on fine or cloudy-fine days (Fig. 6A, B & C). This is similar to patterns which were found in higher land plants (*Paspalum dilatatum* and *P. notatum*) in summer (ITO *et al.* 1973), freshwater submerged angiosperms (GOULDER 1970, HOUGH 1974) and phytoplankton populations (HARRIS and LOTT 1973, JASSBY 1978, MARRA, 1978). However, MARRA (1978) showed in the laboratory with three different light regimes (constant, simulated diurnal variation, and fluctuating) that the afternoon depression can be largely removed by superimposing a light fluctuation on the diurnal regime, and suggested that purported endogenous rhythms can not be solely responsible for the afternoon photosynthetic depression. In the present study, the magnitude of the increase in dark respiration was not great enough to account for the photosynthetic depression. This is similar to the results reported for phytoplankton by LORENZEN (1963) and HARRIS (1973). Furthermore, afternoon depressions in net photosynthesis of *S. thunbergii* were found in December on moderately bright days (Fig. 6B & C), but the dark respiration showed almost no change (Fig. 5B & D). Therefore, the afternoon photosynthetic depression of *S. thunbergii* can not be due to an increase in dark respiration.

In the present study, when light saturation did not occur on a rainy day, little difference in the photosynthetic response to light was found between morning and afternoon (Fig. 6D). RAMUS and ROSENBERG (1980) reported that diurnal photosynthesis of two green algae, *Ulva curvata* and *Codium decorticatum,* a brown alga, *Dictyota dichotoma,* and a red alga, *Gracilaria foliifera,* showed asymmetrical variation on fine days but symmetrical variation on a cloudy day. This is in agreement with results for *S. thunbergii* in the present study. However, the midday or afternoon photosynthetic depression was much smaller in the present study compared with that reported by RAMUS and ROSENBERG (1980). On October 14 the increase in the dark respiration in midday can be considered as being stimulated by the build-up of photosynthates as suggested by JASSBY (1978). Dark respiration of *S. thunbergii* maintained almost constant values in the day and night even on bright days in

December (Fig. 5B & D), although an afternoon photosynthetic depression was observed (Fig. 6B & C). This could be caused by the lower solar radiation and lower water temperature in December compared to October. Therefore, midday or afternoon photosynthetic depressions do not always coincide with increases in dark respiration.

MAEGAWA (1980) and MAEGAWA and ARUGA (1983) reported that *Monostroma latissimum* population photosynthesis showed no midday or afternoon depression even on fine days. In a natural population of seaweed, shading effects must be considered. In the community of *Sargassum thunbergii,* where plants shade each other, light conditions within the population will determine the diurnal photosynthesis of the plant. In the present study, plants maitained in the pipe were not shaded.

Estimation of daily primary production of seaweeds often depends on integration of photosynthesis -light curves determined in the laboratory with records of ambient solar radiation (e.g. BRINKHUIS 1977). However, data estimated in such a way may give rise to significant errors because of midday or afternoon photosynthetic depression. Whether photosynthesis-light curves determined in the laboratory at different times in a day are different or not, and to what degree daytime photosynthesis measured under natural sunlight is different from that estimated by the photosynthesis-light curves determined in the laboratory, should be considered in order to establish accurate estimates of primary production by seaweeds.

In the present study, differences in photosynthetic performance of *S. thunbergii* between morning and afternoon were confirmed on fine or cloudy-fine days. Such different diurnal photosynthetic performance patterns in *S. thunbergii* are difficult to explain without further investigations.

Acknowledgements

The authors would like to thank Professor Y. ARUGA, Laboratory of Phycology, Tokyo University of Fisheries, for his kind advice, and Dr. H. NAKAHARA, Fisheries Research Station of Kyoto University, for his helpful advice and discussions. The authors are also grateful to Dr. Boudewijn H. BRINKHUIS, Marine Sciences Research Center, State University of New York, and Dr. David E. JAMES, Enviromental Engineering Science, California Institute of Technology, for their kind reading of the manuscript.

References

BRINKHUIS, B.H. 1977. Comparisons of salt-marsh fucoid production estimated from three different indices. J. Phycol. **13:** 328–335.

DOTY, M.S. and OGURI, M. 1957. Evidence for a photosynthetic daily periodicity. Limnol. Oceanogr. **2:** 37–40.

GAO, K. and UMEZAKI, I. 1988. Comparative photosynthetic capacities of the leaves of upper and lower parts of *Sargassum* plants. Bot. Mar. **31:** 231–236.

GOULDER, R. 1970. Day-time variations in the rates of production by two natural communities of submerged freshwater macrophytes. J. Ecol. **58:** 521–528.

HARRIS, G.P. 1973. Diel and annual cycles of net plankton photosynthesis in Lake Ontario. J. Fish. Res. Bd Can. **30:** 1779–1787.

HARRIS, G.P. and LOTT, J.N.A. 1973. Light intensity and photosynthetic rates in phytoplankton. J. Fish. Res. Bd. Can. **30:** 1771–1778.

HOUGH, R.A. 1974. Photorespiration and productivity in submersed aquatic vascular plants. Limnol. Oceanogr. **19:** 912–927.

IKUSIMA, I. 1965. Ecological studies on the productivity of aquatic plant communities I. Measurement of photosynthetic activity. Bot. Mag. Tokyo. **78:** 202–211.

ITO, K., INOSAKA, M., NUMAGUCHI, H. and HIRAKAWA, S. 1973. Studies on winter survival of tropical grasses--VII. Diurnal variations in photosynthesis of autumn seeded dallisgrass and bahiagrass. J. Japan. Grassl. Sci. **19:** 40–41.

JASSBY, A.D. 1978. Polarographic measurements of photosynthesis and respiration. p. 285–296. *In* J.A. HELLEBUST and J.S. CRAIGIE [ed.], Handbook of Phycological Methods: Physiological and Biochemical Methods. Cambridge Univ. Press, London.

MAEGAWA, M. 1980. Measurements of photosynthesis and productivity of the cultivated *Monostroma* population. La mer, **18:** 116–124.

MAEGAWA, M. and ARUGA, Y. 1983. Photosynthesis and productivity of the cultivated *Monostroma latissimum* population. La mer, **21:** 164–172.

LORENZEN, C.J. 1963. Diurnal variation in photosyn-

thetic activity of natural phytoplankton populations. Limnol. Oceanogr. **8:** 56–62.

MARRA, J. 1978. Effect of short-term variation in light intensity on photosynthesis of a marine phytoplankter: A laboratory simulation study. Mar. Biol. **46:** 191–202.

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.

RAMUS, J. and ROSENBERG, G. 1980. Diurnal photosynthetic performance of seaweeds measured under natural conditions. Mar. Biol. **56:** 21–28.

SMITH, Jr., W.O. 1981. Photosynthesis and productivity of benthic macroalgae on the North Carolina continental shelf. Bot. Mar. **24:** 279–284.

SOURNIA, A. 1974. Circadian periodicities in natural populations of marine phytoplankton. Adv. Mar. Biol. **12:** 325–389.

THOMAS, E.A. and TREGUNNA, E.B. 1968. Bicarbonate ion assimilation in photosynthesis by *Sargassum muticum*. Can. J. Bot. **46:** 411–415.

YENTSCH, C.S. and RYTHER. J.H. 1957. Short-term variations in phytoplankton chlorophyll and their significance. Limnol. Oceangr. **2:** 140–142.

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. 1977. Productivity of seaweeds. p. 119–127. *In* K. HOGETSU, M. HATANAKA, T. HANAOKA and T. KAWAMURA [ed.] Productivity of Biocenoses in Coastal Regions of Japan. Univ. of Tokyo Press, Tokyo.

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)

高　坤山・梅崎　勇：ウミトラノオの光合成に関する研究Ⅰ．太陽光下での光合成の日変化

大型海藻の光合成を流水中で測定する流水測定法を検討し，ウミトラノオの光合成が日射の変化に伴いどのように変化するかを，同一藻体群を用いて継続的に測定した。ウミトラノオの光合成は晴天下では同じ強さの日射に対して，午前は高い速度を示し，午後は低下を示した。しかし，雨の日にはこのような差が認められなかった。晴天下に認められた午後の光合成の低下は，暗呼吸の増加によるものではない。午後の低下にもかかわらず，ウミトラノオの日生産力は日射量の増加とともに直線的に増加した。(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)

Jpn. J. Phycol. (Sôrui) 37: 99–104, June 20, 1989

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

Kunshan GAO* and Isamu UMEZAKI

Laboratory of Fishery Resources, Division of Tropical Agriculture, Graduate School of Agriculture, Kyoto University, Kyoto, 606 Japan

GAO, K. and UMEZAKI, I. 1989. Studies on diurnal photosynthetic performance of *Sargassum thunbergii* II. Explanation of diurnal photosynthesis patterns from examinations in the laboratory. Jpn. J. Phycol. 37: 99-104.

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.

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

* Present address: Kansai Environmental Engineering Center, Nakazaki-nishi 2-3-39, Kita-ku, Osaka, 530 Japan

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 μE 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

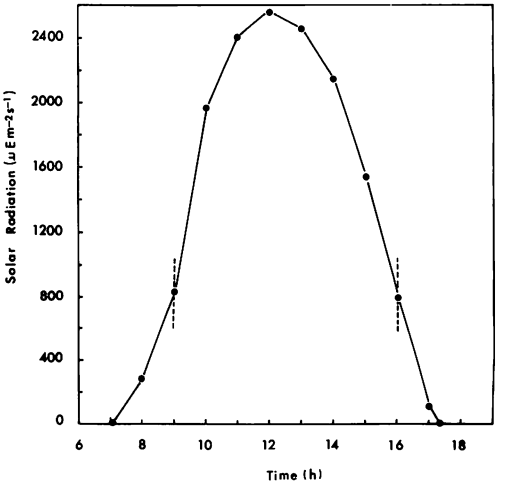


Fig. 1. Diurnal curve of solar radiation on November 21, 1987, a fine day. The vertical dotted lines show the beginning of photosynthesis measurements.

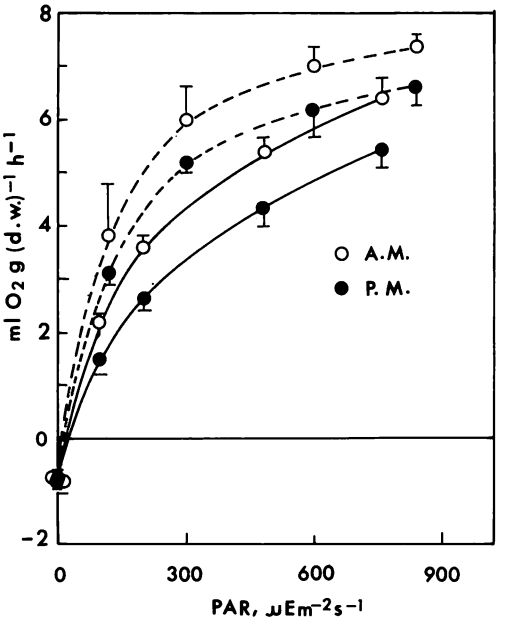


Fig. 2. Photosynthesis-light curves of *S. thunbergii*. Solid lines are curves in morning (9:00–11:00) and in afternoon (16:00–18:00) on a fine day (see Fig. 1), November 21, 1987, measured in the laboratory at 16°C with an artificial light source. Each value is the mean of five samples ± standard deviation. 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).

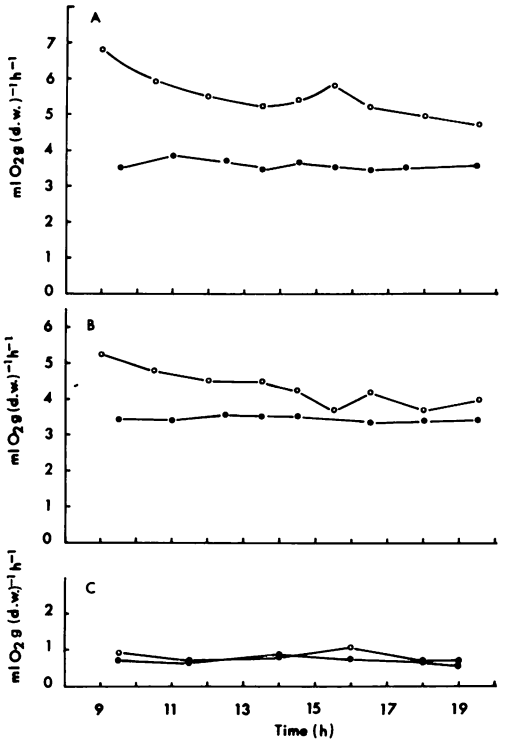


Fig. 3. Changes in net photosynthesis (A & B) and dark respiration (C) of *S. thunbergii* with time under 1000 μE m^{-2} S^{-1} (○) and 150 μE m^{-2} s^{-1} (●). (B) is a repeat of (A). Measured at 16°C. Each value represents the mean of two to three samples.

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	—	—	—
$\bar{X}$	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 μE m^{-2} s^{-1} showed much higher values and a gradual decrease with time. In contrast, the response to 150 μE 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.

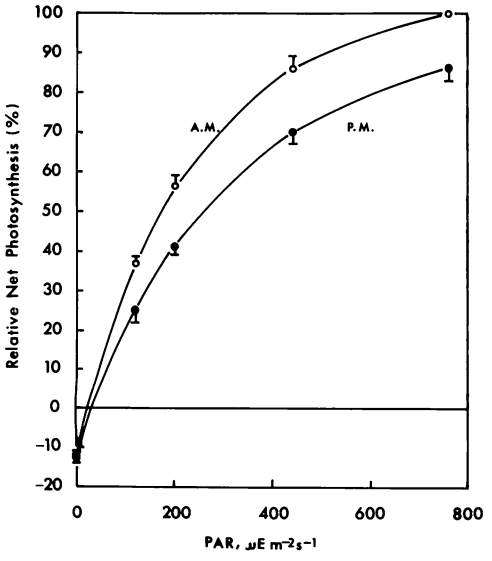


Fig. 4. Relative net photosynthesis-light curves measured in the laboratory, replotted from data obtained on November 21, 1987.

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.

Acknowledgements

The authors are indebted to Professor Y. ARUGA, Laboratory of Phycology, Tokyo University of Fisheries, for his advice, and Dr. H. NAKAHARA, Fisheries Research Station of Kyoto University, for his useful discussions. The authors are also grateful to Dr. Boudewijn H. BRINKHUIS, Marine Sciences Research Center, State University of New York, for his kind reading of the manuscript.

References

DOTY, M. S. and OGURI, M. 1957. Evidence for a photosynthetic daily periodicity. Limnol. Oceanogr. **2**: 37–40.

GAO, K. and UMEZAKI, I. 1989. Studies on diurnal photosynthetic performance of *Sargassum thunbergii* I. Changes in photosynthesis under natural sunlight. Jpn. J. Phycol. **37**: 89–98.

GOULDER, R. 1970. Day-time variations in the rates of production by two natural communities of submerged freshwater macrophytes. J. Ecol. **58**: 521–528.

HARRIS, G. P. 1973. Diel and annual cycles of net plankton photosynthesis in Lake Ontario. J. Fish. Res. Bd. Can. **30**: 1779–1787.

HARRIS, G. P. and LOTT, J. N. A. 1973. Light intensity and photosynthetic rates in phytoplankton. J. Fish. Res. Bd. Can. **30**: 1771–1778.

HOUGH, R. A. 1974. Photorespiration and productivity in submersed aquatic vascular plants. Limnol. Oceanogr. **19**: 912–927.

ITO, K., INOSAKA, M., NUMAGUCHI, H. and HIRAKAWA, S. 1973. Studies on winter survival of tropical grasses-VII. Diurnal variations in photosynthesis of autumn seeded dallisgrass and bahiagrass. J. Japan. Grassl. Sci. **19**: 40–41.

JEFFREY, S. W. and HUMPHREY, G. F. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pfl. **167**: 191–194.

LORENZEN, C. J. 1963. Diurnal variation in photosynthetic activity of natural phytoplankton populations. Limnol. Oceanogr. **8**: 56–62.

MARRA, J. 1978. Effect of short-term variation in light intensity on photosynthesis of a marine phytoplankter: A laboratory simulation study. Mar. Biol. **46**: 191–202.

MISHKIND, M., MAUZERALL, D. and BEALE, S. I. 1979. Diurnal variation *in situ* of photosynthetic capacity in *Ulva* is caused by a dark reaction. Plant Physiol. **64**: 896–899.

PALMER, J. D., LIVINGSTON, L. and ZUSY, F. D. 1964. A persistent diurnal rhythm in photosynthetic capacity. Nature, Lond. **203**: 1087–1088.

SOURINA, A. 1974. Circadian periodicities in natural populations of marine phytoplankton. Adv. Mar. Biol. **12**: 325–389.

YENTSCH, C. S. and RYTHER, J. H. 1957. Short-term variations in phytoplankton chlorophyll and their significance. Limnol. Oceanogr. **2**: 140–142.

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)

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）

Jpn. J. Phycol. (Sôrui) 37: 105–116, June 20, 1989

Ultrastructural study of sperm in *Laminaria angustata* (Laminariales, Phaeophyta), especially on the flagellar apparatus*

Taizo MOTOMURA

Institute of Algological Research, Faculty of Science, Hokkaido University, Muroran, 051 Japan

MOTOMURA, T. 1989. Ultrastructural study of sperm in *Laminaria angustata* (Laminariales, Phaeophyta), especially on the flagellar apparatus. Jpn. J. Phycol. 37: 105–116.

The ultrastructure of sperm in *Laminaria angustata* KJELLMAN, especially its flagellar apparatus, was observed by SEM and TEM, and compared with *L. angustata* zoospore and zoospores of the Laminariales previously examined. In addition to a long posterior flagellum, the sperm has the characteristic feature in that the both flagella protrude through an anterior gullet and a posterior one respectively. Two or three chloroplasts are located in the cell periphery. They are not associated with the flagellar basal bodies. Four flagellar rootlets exist; major anterior rootlet **(MAR)**, minor anterior rootlet **(mar)**, minor posterior rootlet **(mpr)** and bypassing rootlet **(BR)**. The number of microtubules consisting the **MAR** and the **BR** is five and four respectively. The **mar** and the **mpr** respectively consist of a single microtubule. The **BR** extends posteriorly along the nuclear membrane. Both flagellar basal bodies are connected to each other by an electron-dense, connecting band. These features are unique among the brown algal motile cells and different from zoospores of this species.

Key Index Words: *Flagellar apparatus*—Laminaria angustata—*Phaeophyta*—*sperm*—*ultrastructure.*

Almost all swarmers in the Phaeophyta have common features; 1) a longer, mastigonemes-bearing anterior flagellum and a shorter, undecorated posterior one with a lateral, ventral insertion of the flagella and 2) one bilobed chloroplast containing an eyespot. However, sperms of several groups in the Phaeophyta in which oogamy is prevalent (Dictyotales, Sporochnales, Desmarestiales, Laminariales and Fucales) are different from these typical characteristics. HENRY and COLE (1982a, b) observed zoospores of 17 species and sperms of 13 species belonging to the Laminariaceae, Alariaceae and Lessoniaceae of the Laminariales. They showed that the characteristic aspects of laminarialean sperms (except for *Chorda* sperm) are as follows: 1) a very long and tapering posterior flagellum (the anterior flagellum of laminarialean sperm appears identical to that of the zoospore), 2) two or three chloroplasts per sperm, and 3) the lack of an eyespot (zoospores of most laminarialean plants also lack eyespots). Recently, CLAYTON (1984b) reviewed phylogenetically close relationships between the Sporochnales, Desmarestiales and Laminariales based on the physiological characteristics of fertilization (their sexual pheromones secreted from eggs have a sperm-releasing function as well as a sperm-attracting one), the manner of gametogenesis which is influenced by blue and red light (LÜNING and DRING 1972, MÜLLER and LÜTHE 1981) or by the iron concentration in the medium (MOTOMURA and SAKAI 1984a), and especially, characteristic features of their sperms as mentioned above.

Recently, O'KELLY and FLOYD (1984) determined the absolute configuration of the

* Dedicated to the memory of the late Dr. Munenao KUROGI (1921–1988), Professor Emeritus of Hokkaido University. This work was partially supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Nos. 60480013 and 61790189).

flagellar apparatus of zoospores of *Laminaria digitata* and *Nereocystis luetkeana*. They suggested that the flagellar apparatus in brown algal motile cells might possess basically an absolute configuration with minor variations in component array which could be contributed to phylogenetic studies on the Phaeophyta.

In this study, the ultrastructure of *Laminaria angustata* sperm, especially its flagellar apparatus is investigated, and the differences in flagellar apparatus features between zoospores and sperms are discussed.

Materials and methods

Culture

Sporophytes of *Laminaria angustata* KJELLMAN were collected at Charatsunai, Muroran, Hokkaido, Japan in November and December of 1984 and 1985. Cultured specimens of female and male gametophytes were obtained from germlings of zoospores and maintained in Fe-free $ASP_{12}NTA$ medium (MOTOMURA and SAKAI 1984a, b). To induce gametogenesis, small tufts (ca. 0.5–1.0 mm diam.) of female and male vegetative gametophytes were inoculated into PESI medium (TATEWAKI 1966) separately. Cultures were incubated at 10°C, 14:10 LD cycle and 55 μmol $m^{-2}s^{-1}$ from cool-white fluorescent lamps.

Electron microscopy

Preparation of liberated sperms for electron microscopy was conducted as follows: About one week after the separate inoculation of the male and female gametophytes into PESI medium, eggs and oogonia of female gametophytes and antheridia of male gametophytes were matured in respective cultures. At this time, many sperms were liberated within seconds by the addition of one or two drops of conditioned medium from the female gametophyte culture to the mature male gametophytes (the effect of this phenomenon is presumed to be by "lamoxirene", LÜNING and MÜLLER 1978, MÜLLER *et al.* 1979, MARNER *et al.* 1984). Liberated sperms around the mature male gametophytes were immediately fixed for about 15 min. in 1% glutaraldehyde and 0.5% OsO_4 in PESI medium. After block staining in 0.5% uranyl acetate solution for 15 min. at 4°C, specimens were dehydrated in an acetone series and embedded in Spurr's epoxy resin. Serial sectionings were done with a diamond knife on a Porter-Blum MT-1 ultramicrotome, and sections were mounted on formvar-coated slot grids with careful monitoring of the orientation and sequence of the sections. Sections were stained with uranyl acetate and lead citrate, or only lead citrate, and observed with a Hitachi H-300 electron microscope. Following O'KELLY and FLOYD (1983), analysis of basal body triplet implication in micrograph of serial sections confirmed the absolute orientations derived from section mapping.

For whole-mount preparations, a drop of suspension containing liberated sperms was placed on formvar-coated grids, and fixed in a vapor of OsO_4 for 30–60 sec. After rinsing and drying, the grids were shadowed with platinum.

For SEM specimens, liberated zoospores and sperms fixed as above were placed on TOYO membrane filters (regenerated cellulose type, with 1.0 μm and 0.2 μm pore sizes), and dehydrated in an acetone series. They were critical-point-dried using dry ice, coated with gold, and observed with a Hitachi S-510 electron microscope.

Fig. 1. TEM micrograph of a liberated sperm of *Laminaria angustata* by whole-mount preparation. Note an anterior flagellum (AF) decorated with mastigonemes and a long posterior flagellum (PF).

Fig. 2. SEM micrograph of a zoospore of *L. angustata* bearing AF and PF. Arrow shows the cytoplasmic hollow along the AF.

Fig. 3. SEM micrograph of a sperm of *L. angustata* bearing AF and PF. The bases of both flagella are surrounded by the cytoplasm (arrows). Mastigonemes on an anterior flagellum are wavy or detached.

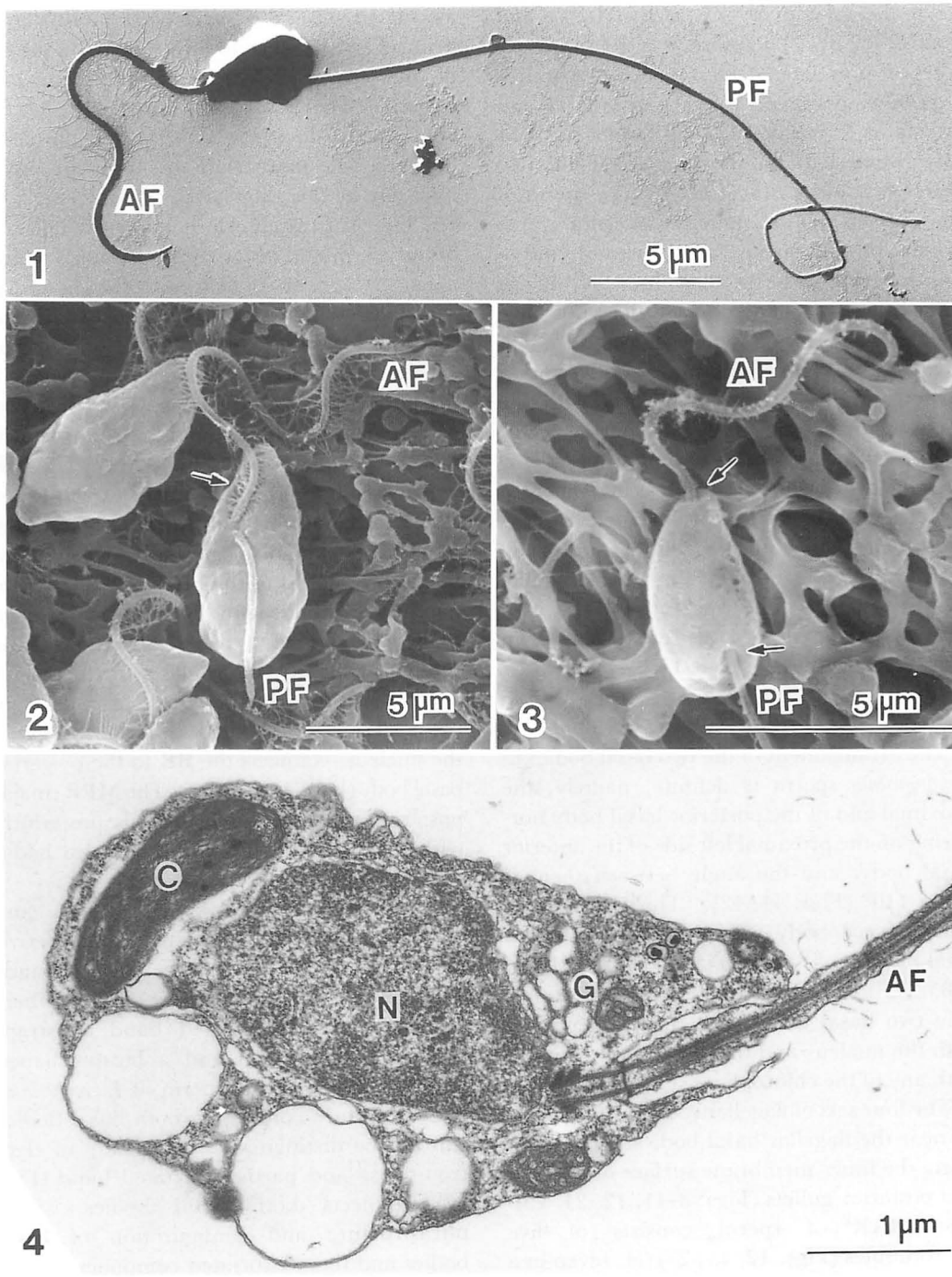
Fig. 4. TEM micrograph of a sperm of *L. angustata*. The nucleus (N) and Golgi body (G) exist near the basal body of anterior flagellum (AF) but the chloroplast (C) does not. Note that the AF protrudes through the anterior gullet.

Flagellar apparatus absolute configuration conventions and terminology are those of O'Kelly and Floyd (1984), and Barr and Allan (1985).

Results

General ultrastructure of liberated sperm

General ultrastructure (Fig. 4) of liberated



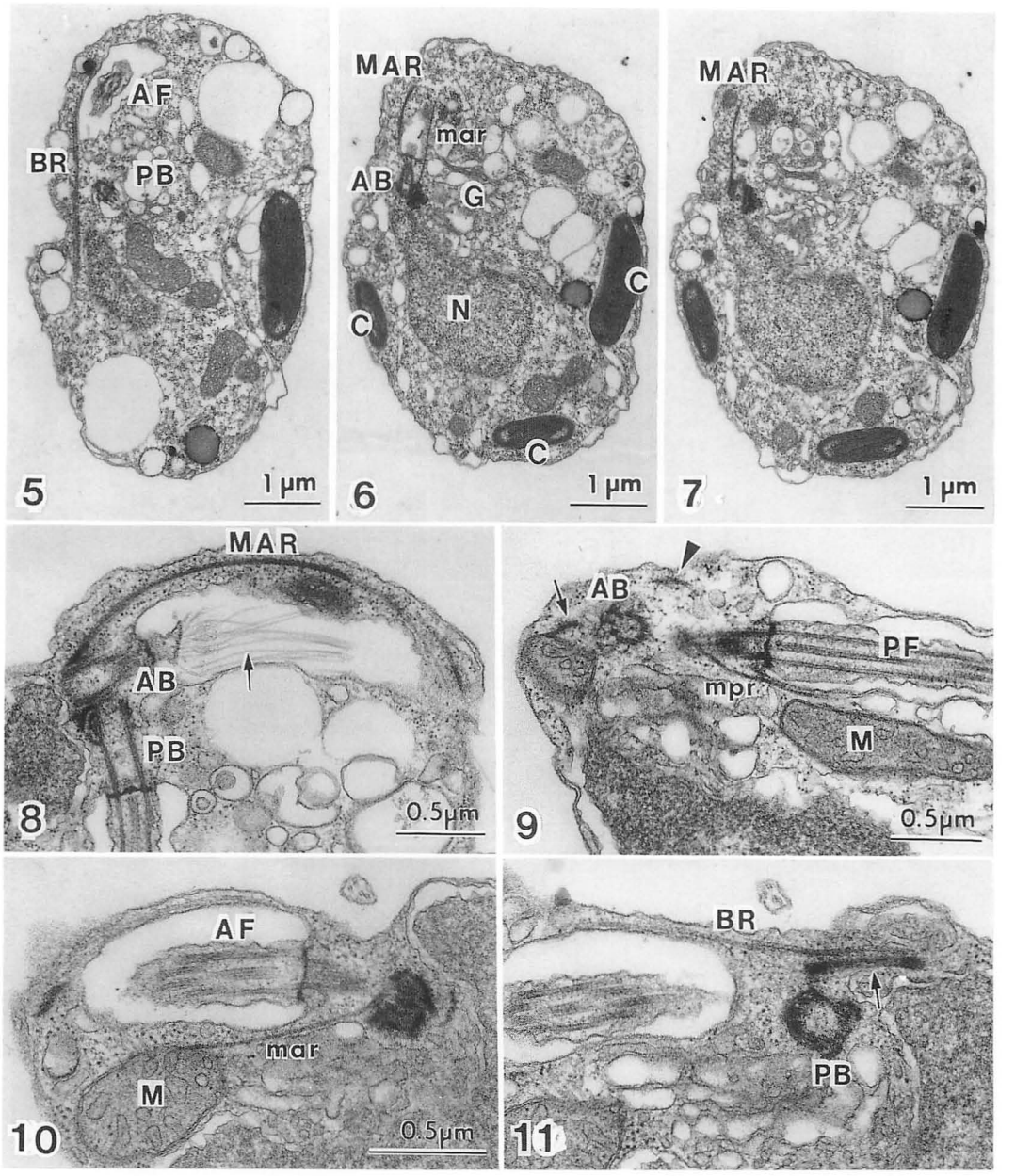
sperm (ca. 5 μm × 3 μm) of *Laminaria angustata,* including a heterochromatin-rich nucleus, several small chloroplasts, mitochondria and the Golgi body, is fundamentally consistent with those of previous investigations about the related organisms by HENRY and COLE (1982 b). Therefore, it is not necessary to report it in detail. However, differences of external appearance between zoospores and sperms of *L. angustata* newly found by SEM observation will be shown. As mentioned by HENRY and COLE (1982 b), the sperm of this species has an anterior flagellum (ca. 15 μm long) bearing mastigonemes and a long tapering posterior flagellum (ca. 40 μm long) (Fig. 1). The zoospore does not have such a long posterior flagellum (Fig. 2). Both flagella are inserted asymmetrically and a shallow groove extends anteriorly with the anterior flagellum. On the contrary, this groove can not be observed on the sperm surface. The appearance of sperm is not so uniform as that of zoospore. However, it is confirmed that both flagella of almost all sperms protrude through an anterior gullet and a posterior gullet respectively (Fig. 3). This arrangement can also be observed in thin sections (Figs. 4, 5, 8–11, 24, 25).

The flagellar apparatus

The arrangement of the two basal bodies in *L. angustata* sperm is definite, namely, the proximal end of the posterior basal body bordering on the proximal left side of the anterior basal body, and the angle between them is about 110° (Figs. 41, 42). This is one of the typical basal body configurations in brown algal motile cells (O'KELLY and FLOYD 1984, 1985, O'KELLY, personal communication). The two basal bodies are closely associated with the nucleus and the Golgi body, but not with any of the chloroplasts (Figs. 4, 6, 9–11).

The four sets of flagellar rootlets are observed near the flagellar basal bodies. They run along the inner membrane surface of anterior and posterior gullets (Figs. 8–11, 12, 24, 25). The **MAR** of sperm consists of five microtubules (Figs. 12, 13, 23) (*vs.* seven in a zoospore of *L. angustata* (MOTOMURA, unpublished data.) and other Laminariales (O'KELLY and FLOYD 1984) and runs anteriorly through the right side of sperm from the proximal part of the anterior basal body (Figs. 5–8, 21–23, 41, 42). The **MAR** is closely associated with the surface of a mitochondrion (Figs. 19–21) and forms an arc at the tip of the sperm before terminating in this region. The **mar** and **mpr** rootlets run respectively along the anterior and posterior gullets. The **mar** originates from the proximal part of the anterior basal body (Figs. 6, 10, 39). It runs anteriorly along the left side of the sperm and terminates posteriorly to the apex. It consists of one microtubule and is associated with a mitochondrion located at the anterior cytoplasm (Fig. 24). The **mpr** extends posteriorly from the proximal part of the posterior basal body (Fig. 9). It consists of a single microtubule, and is also associated with a mitochondrion (Fig. 25). The **BR** which is composed of four microtubules (Figs. 12–14) originates near the anterior end of the **MAR** and extends posteriorly, passing immediately ventral to the basal bodies and apparently terminating in the cytoplasm ventral to the nucleus (Fig. 5). No associations of the rootlets with any chloroplasts are seen. A posterior fibrous band, which extends from the posterior basal body to the anterior end of the nucleus, connects the **BR** to the posterior basal body (Figs. 11, 15–17). The **MPR** (major posterior rootlet), which extends posteriorly with the **BR** from the posterior basal body, does not exist (Figs. 12–18).

In zoospores of *Laminaria digitata* and *Nereocystis luetkeana,* O'KELLY and FLOYD (1984) reported a set of three striated bands that connect the basal bodies to each other, namely a deltoid striated band, a strap-shaped striated band and a button-shaped striated band. In the sperm of *L. angustata,* these structure connecting both basal bodies can not be distinguished. Instead, an electron-dense and partially striated band (Fig. 22) connects both basal bodies. The ultrastructure and configuration of basal bodies and their associated components were observed by serial section in the views from

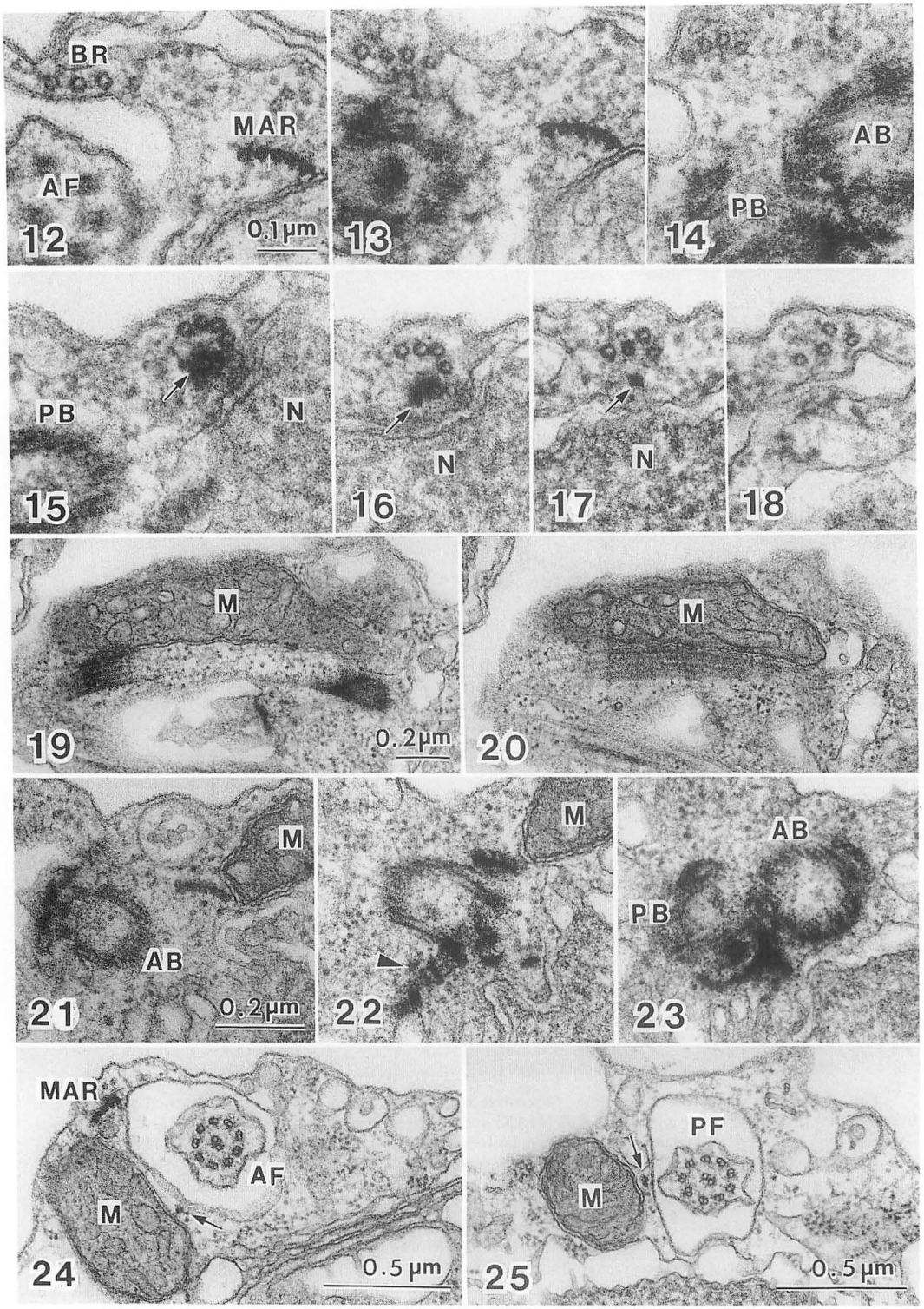


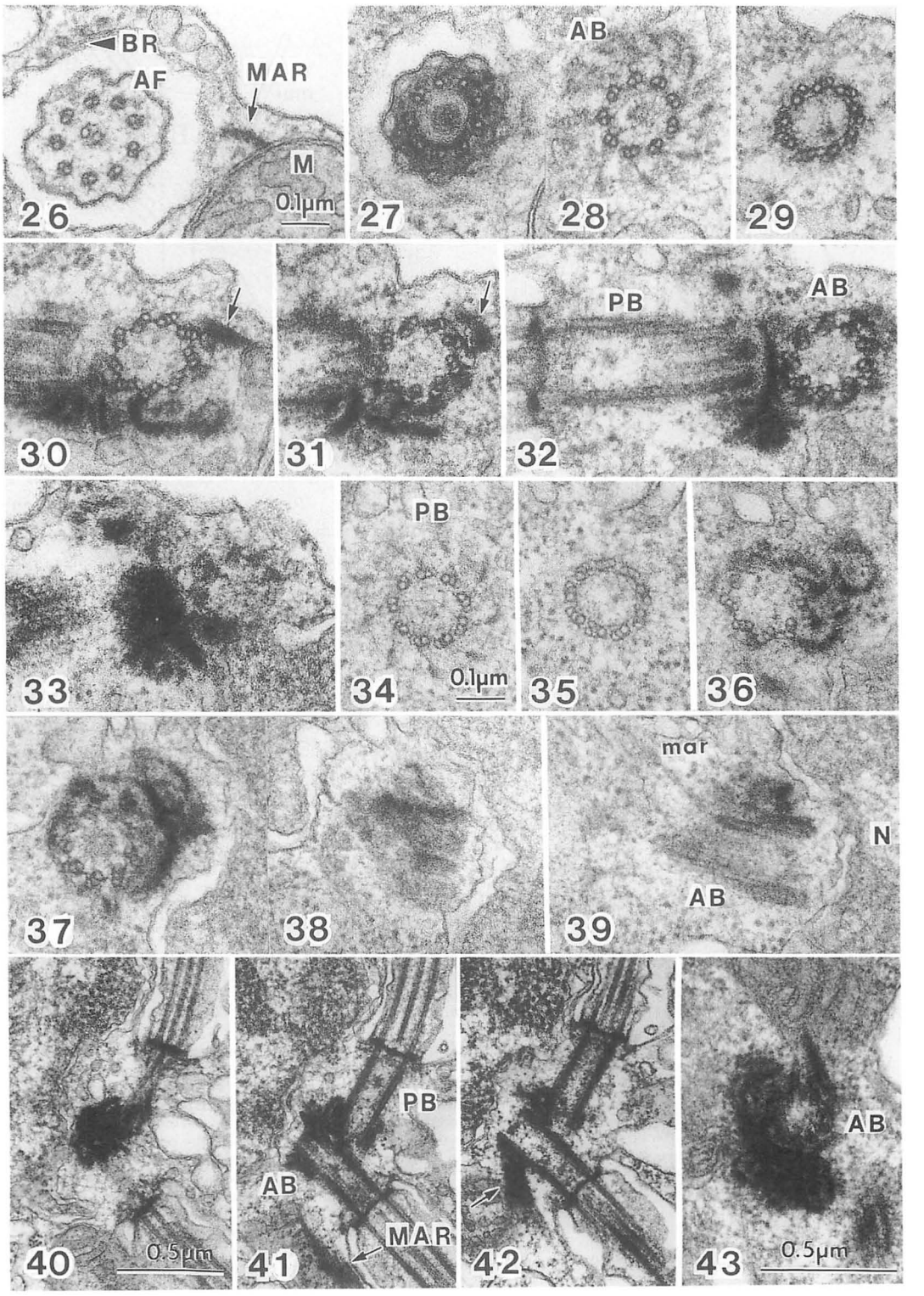
Figs. 5–7. TEM micrograph of non-consecutive serial longitudinal sections of a liberated sperm. The bypassing rootlet **(BR),** major anterior rootlet **(MAR)** and minor anterior rootlet **(mar)** are visible. The nucleus (N) and Golgi body (G) exist near the anterior flagellar basal body (AB) and posterior flagellar basal body (PB). In this case, three chloroplasts (C) are observed in the cytoplasm, but they do not exist near the basal bodies.

Fig. 8. **MAR** runs anteriorly from the proximal side of the anterior basal body. Arrow shows mastigonemes on the anterior flagellum.

Fig. 9. Note that minor posterior rootlet **(mpr)** runs from the proximal part of the posterior basal body and it is associated with the mitochondria (M). Arrow shows the **MAR** and arrowhead shows the **BR.**

Figs. 10 and 11. Non-consecutive serial sections. In Fig. 10, the minor anterior rootlet **(mar)** runs anteriorly from the proximal part of the anterior basal body and is associated with the mitochondria. In Fig. 11, the **BR** and the posterior fibrous band (arrow) are visible. Note that the posterior fibrous band is associated with the nuclear membrane.


BR
MAR
AF
12
0.1μm
13
AB
PB
14
PB
N
15
N
16
N
17
18
M
19
0.2μm
M
20
M
AB
21
0.2μm
M
22
AB
PB
23
MAR
AF
M
24
0.5 μm
PF
M
25
0.5 μm


BR
AF
MAR
M
0.1μm
26
27
AB
28
29
30
31
PB
AB
32
33
PB
34
0.1μm
35
36
37
38
mar
N
AB
39
40
0.5μm
PB
AB
MAR
41
42
AB
43
0.5μm

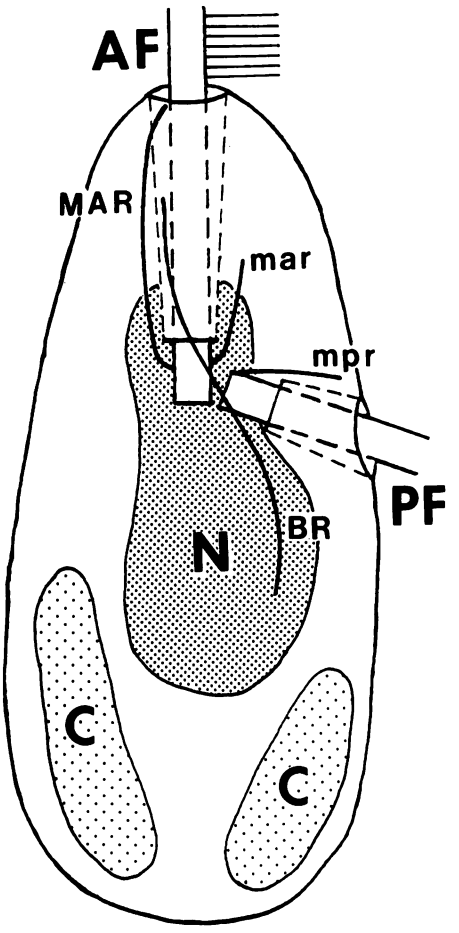


Fig. 44. Diagrammatic representation of a sperm in ventral view.

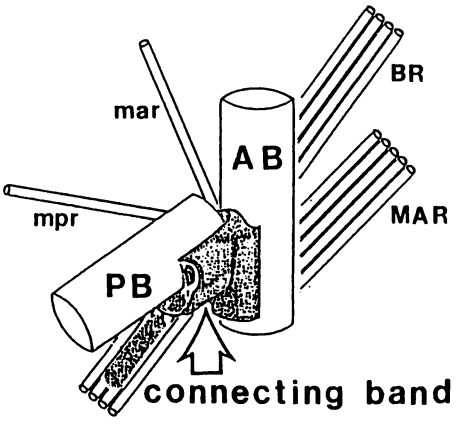


Fig. 45. Diagrammatic representation of the basal bodies and associated flagellar apparatus components.

the top of the anterior basal body to the bottom (Figs. 26–33), from the top of the posterior basal body to the bottom (Figs. 34–39) and from one side of both basal bodies (Figs. 40–42). Figure 27 shows the transverse section of the basal plate at the top of the anterior flagellum. The curved fibrils typically found at the distal end of basal bodies are shown in Figures 28 and 34. The connecting band covers the half round of the proximal side of the posterior basal body (Figs. 31–33, 36, 37, 41) and is three-lobed (Figs. 36, 37, 43). Electron dense material

Figs. 12–18. Non-consecutive serial sections of the **BR.** In Figs. 12 and 13, the **BR** consisted of four microtubules and the **MAR** consisted of five microtubules are visible. Arrow indicates the posterior fibrous band which is associated with the nuclear membrane. It is also noted that **MPR** does not occur.

Figs. 19 and 20. Consecutive serial sections grazing the **MAR.** It is noted that the **MAR** is associated with the mitochondria.

Figs. 21–23. Non-consecutive serial sections of the **MAR.** The **MAR** composed of five microtubules is deviated from the proximal side of the anterior basal body (Fig. 23) and associated with the mitochondria. In Fig. 22, striation of the connecting structure is visible (arrowhead).

Fig. 24. Transverse section of the anterior flagellum. The **MAR** and **mpr** (arrow) are visible and they are associated with the mitochondria.

Fig. 25. Transverse section of the posterior flagellum. It is noted that the **mpr** (arrow) is associated with the mitochondria.

Figs. 26–33. Non-consecutive (Figs. 26–28, 30–33 are consecutive) cross sections through the anterior flagellum (AF) and basal body (AB). Arrow shows the **MAR** and arrowhead shows the **BR.** Fig. 27 shows the curved fibrils. Profiles of the connecting band are observed in Figs. 30–33.

Figs. 34–39. Non-consecutive (Figs. 36–39 are consecutive) cross sections through the posterior basal body (PB), as seen from the distal end to the proximal end of the posterior basal body, indicated by counterclockwise implications of the nine triplets. Fig. 34 shows the curved fibrils at the distal end of the posterior basal body. In Figs. 36 and 37, the profiles of the connecting band are observed. In Fig. 39, the anterior basal body (AB) and **mar** appear.

Figs. 40–42. Consecutive serial sections through the flagellar apparatus. Arrow shows the **MAR.** The proximal end of the posterior basal body (PB) arranges as bordering on the proximal side of the anterior flagellar basal body (AB), and the angle between PB and AB is about 110°. Note that the connecting band attaches to the nuclear membrane (Fig. 40).

Fig. 43. Connecting band between the anterior basal body (AB) and the posterior basal body is visible as three-lobed. Electron-dense material is seen in each lobe of connecting structure.

can be observed in each lobe between the connecting band and the posterior basal body (Figs. 36, 37, 41, 43). This band also covers the proximal end of the posterior basal body (Fig. 32) and the side of the anterior basal body adjacent to the posterior basal body (Figs. 30–32). The nuclear protrusion extends to the basal bodies and attaches to the connecting band (Figs. 6, 40, 41). Based on the results, schematic diagrams showing the configuration of flagellar apparatus of *Laminaria angustata* sperms and its position in the cell were presented in Figures 44 and 45, respectively.

Discussion

In zoospores of *Laminaria angustata,* a shallow groove extends with the anterior flagellum from the flagellar base to the anterior top of the cell. Similar grooves have been found in TEM photographs of zoospores of *Laminaria digitata* (O'Kelly and Floyd 1984), gametes of *Scytosiphon* sp. (Clayton 1984a), zoospores of *Desmarestia viridis, D. ligulata* and several species of the Laminariales (Motomura, unpublished). It seems that these structures exist in many phaeophycean swarmers which have a typical flagellation. On the contrary, sperms of this species have no such groove, but both flagella protrude through an anterior gullet and a posterior one respectively. These structures have not been reported in the brown algal swarmers. It is probably an important characteristic of *Laminaria* that the structural differences of flagellar insertion between zoospore and sperm are possessed.

It has been demonstrated that a chloroplast with or without an eyespot always exists near the basal bodies in many swarmers of phaeophycean algae: zoospores and gametes of *Ectocarpus* and *Pylaiella* (Baker and Evans 1973a, b, Lofthouse and Capon 1975, Markey and Wilce 1976a, b), gametes of *Scytosiphon* (Manton 1964, Clayton 1984a), male gametes of *Cutleria* (La Claire and West 1979), and zoospores of the Laminariales (Toth 1976, Henry and Cole 1982a, O'Kelly and Floyd 1984). Female gametes of *Cutleria* have many chloroplasts, but one of them is close to the basal bodies (La Claire and West 1978). Even in the Fucales, the sperm has one small chloroplast near the basal bodies (Manton and Clarke 1956, Manton 1964, Bouck 1969, Berkaloff and Rousseau 1979). A sperm of *L. angustata* has two or three small chloroplasts which are located in the cell periphery. No connection between the nuclear envelope and the chloroplast endoplasmic reticulum could be detected. Despite the existence of a nucleus and a Golgi body near the flagellar basal bodies, intimate relationship between chloroplasts and the flagellar basal bodies is not present. Therefore, it is a very unique character that no chloroplast is associated with the basal bodies in *Laminaria* sperm.

There have been few reports of detailed observations on the flagellar apparatus in the brown algal swarmers. But it is now clear that five flagellar rootlets **(MAR, mar, MPR, mpr** and **BR)** compose the flagellar apparatus and their configurations are nearly identical (O'Kelly and Floyd 1984, 1985). Among them, the **MPR** is observed in many phaeophycean swarmers having an eyespot (O'Kelly, personal communication) and sperms of several species of the Fucales, *Halidrys, Cystoseira* and *Bifurcaria* (Manton 1964) and *Phyllospora* (Moestrup 1982). However, O'Kelly and Floyd (1985) reported that there are several variations in the number of microtubules of flagellar rootlets. They suggested that the number of microtubules in both the **MAR** and **BR** of almost all brown algal motile cells is basically seven and variations from "seven and seven" in sperms of the Fucales and zoospores of the Laminariales probably represent a derived status. Therefore, such variations may be useful in determining the phylogenetic relationships within and among the more advanced brown algal orders. Additionally, it must be noticed that especially in the oogamous groups, for example in the Laminariales in this experiment, gamete ultrastructure differs considerably from

almost typical zoospore ultrastructure. Therefore, it is also necessary to observe reproductive cells derived from both the gametophytic and the sporophytic generations throughout the life history of the species.

Compared with ultrastructures of phaeophycean reproductive cells examined so far, it has become clear that those of *Laminaria* have several unique characters as follows: 1) **MPR** does not exist in zoospore and sperm of *Laminaria angustata*; 2) the rootlet formula of **MAR** and **BR** of *Laminaria* sperm is "five and four", differing from "seven and five" of laminarialean zoospores and "seven and seven" of most other phaeophycean swarmers; 3) sperm has two or three small chloroplasts, which do not exist near the flagellar basal bodies; 4) the **BR** of *Laminaria* sperm extends posteriorly along the nuclear envelope, even though in almost all brown algal swarmers, including laminarialean zoospores, it extends posteriorly along the ventral surface of the chloroplast; 5) an electron-dense band with partial striation connects the basal bodies to each other in *Laminaria angustata* sperms like other laminarialean sperms (HENRY 1982b), while in zoospore, a set of three striated bands connects the basal bodies (O'KELLY and FLOYD 1984); 6) sperm has a long tapering posterior flagellum; 7) both flagella of sperm protrude through the gullets respectively; 8) **mar** and **mpr** of sperms are associated with mitochondria; and 9) egg has the vestigial flagella and unique flagellar apparatus (MOTOMURA and SAKAI 1988). These characters of reproductive cells of *Laminaria* would be important to consider the phylogenetic position of the members of the Laminariales within the brown algae.

CLAYTON (1984b) hypothesized a close phylogenetic relationships among the Sporochnales, Desmarestiales and Laminariales based on possessing their common features in that their sperms have several chloroplasts and no eyespot. Actually, it is also obvious that sperms of *Perithalia caudata* (Sporochnales) (MÜLLER *et al.* 1985) and *Desmarestia* spp. (RAMIREZ *et al.* 1986) and *Arthrocladia villosa* (Desmarestiales) (MÜLLER and MEEL 1982) have a long, tapering posterior flagellum like sperms of the Laminariales. Then, it might be possible to be more precise about the phylogenetic relationships among these three orders by examining the detailed ultrastructures of sperms and eggs on the above-mentioned features.

Acknowledgements

The present author wishes to express his gratitude to Dr. C. J. O'KELLY, Massey University, for valuable advice and critical reading of the manuscript. He is also indebted to the Japan Society for the Promotion of Science for JSPS Fellowships for this study.

References

BAKER, J.R.J. and EVANS, L.V. 1973a. The ship-fouling alga *Ectocarpus*. I. Ultrastructure and cytochemistry of the plurilocular reproductive stages. Protoplasma **77:** 1–13.

BAKER, J.R.J. and EVANS, L.V. 1973b. The ship-fouling alga *Ectocarpus*. II. Ultrastructure of the unilocular reproductive stage. Protoplasma **77:** 181–189.

BARR, D.J.S. and ALLAN, P.M.E. 1984. A comparison of the flagellar apparatus in *Phytophthora, Saprolegnia, Thraustochytrium,* and *Rhizidiomyces*. Can. J. Bot. **63:** 138–154.

BERKALOFF, C. and ROUSSEAU, B. 1979. Ultrastructure of male gametogenesis in *Fucus serratus* (Phaeophyceae). J. Phycol. **15:** 163–173.

BOUCK, G.B. 1969. Extracellular microtubules. The origin, structure, and attachment of flagellar hairs in *Fucus* and *Ascophyllum* antherozoids. J. Cell Biol. **40:** 446–460.

CLAYTON, M.N. 1984a. An electron microscope study of gamete release and settling in the complanate form of *Scytosiphon* (Scytosiphonaceae, Phaeophyta). J. Phycol. **20:** 276–285.

CLAYTON, M.N. 1984b. Evolution of the Phaeophyta with particular reference to the Fucales. p. 11–46. *In* F.E. ROUND and D.J. CHAPMAN [eds.], Progress in Phycological Research, Vol. 3. Biopress Ltd, Bristol.

HENRY, E.C. and COLE, K.M. 1982a. Ultrastructure of swarmers in the Laminariales (Phaeophyceae). I. Zoospores. J. Phycol. **18:** 550–569.

HENRY, E.C. and COLE, K.M. 1982b. Ultrastructure of swarmers in the Laminariales (Phaeophyceae). II. Sperm. J. Phycol. **18:** 570–579.

LA CLAIRE, J.W. II and WEST J.A. 1978. Light- and electron-microscopic studies of growth and reproduction in *Cutleria* (Phaeophyta). I. Gametogenesis in

the female plant of *C. hancockii*. Protoplasma **97:** 93–110.

La Claire, J.W. II and West J.A. 1979. Light- and electron-microscopic studies of growth and reproduction in *Cutleria* (Phaeophyta). II. Gametogenesis in the male plant of *C. hancockii*. Protoplasma **101:** 247–269.

Lofthouse, P.F. and Capon, B. 1975. Ultrastructural changes accompanying mitosporogenesis in *Ectocarpus parvus*. Protoplasma **84:** 83–99.

Lüning, K. and Dring. M.J. 1972. Reproduction induced by blue light in female gametophytes of *Laminaria saccharina*. Planta **104:** 252–256.

Lüning, K. and Müller, D.G. 1978. Chemical interaction in sexual reproduction of several Laminariales (Phaeophyceae): release and attraction on spermatozoids. Z. Pflanzenphysiol. **89:** 333–341.

Manton, I. 1964. A contribution towards understanding of "the primitive fucoid". New Phytol. **63:** 244–254.

Manton, I. and Clarke, B. 1956. Observations with the electron microscope on the internal structure of the spermatozoid of *Fucus*. J. Exp. Bot. **7:** 416–432.

Markey, D. and Wilce, R. 1976a. The ultrastructure of reproduction in the brown alga *Pylaiella littoralis*. II. Zoosporogenesis in the unilocular sporangium. Protoplasma **88:** 147–173.

Markey, D. and Wilce, R. 1976b. The ultrastructure of reproduction in the brown alga *Pylaiella littoralis*. III. Later stages of gametogenesis in the plurilocular gametangia. Protoplasma **88:** 175–186.

Marner, F.-J., Müller, D.G. and Jaenicke, L. 1984. Lamoxirene. Structural proof of the spermatozoid releasing and attracting pheromone of Laminariales. Z. Naturforsch. **39:** 689–691.

Moestrup, Ø. 1982. Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae, and *Reckertia*. Phycologia **21:** 427–528.

Motomura, T. and Sakai, Y. 1984a. Regulation of gametogenesis of *Laminaria* and *Desmarestia* (Phaeophyta) by iron and boron. Jpn. J. Phycol. **32:** 209–215.

Motomura, T. and Sakai, Y. 1984b. Ultrastructural studies of gametogenesis in *Laminaria angustata* (Laminariales, Phaeophyta) regulated by iron concentration in the medium. Phycologia **23:** 331–343.

Motomura, T. and Sakai, Y. 1988. The occurrence of flagellated eggs in *Laminaria angustata* (Phaeophyta, Laminariales). J. Phycol. **24:** 282–285.

Müller, D.G. and Lüthe, N.M. 1981. Hormonal interaction in sexual reproduction of *Desmarestia aculeata* (Phaeophyceae). Br. Phycol. J. **16:** 351–356.

Müller, D.G. and Meel, H. 1982. Culture studies on the life history of *Arthrocladia villosa* (Desmarestiales, Phaeophyceae). Br. Phycol. J. **17:** 419–425.

Müller, D.G., Clayton, M.N. and Germann, I. 1985. Sexual reproduction and life history of *Perithalia caudata* (Sporochnales, Phaeophyta). Phycologia **24:** 467–473.

Müller, D.G., Gassmann, G. and Lüning, K. 1979. Isolation of a spermatozoid-releasing and -attracting substance from female gametophytes of *Laminaria digitata*. Nature **279:** 430–431.

O'Kelly, C.J. and Floyd, G. L. 1983. The flagellar apparatus of *Entocladia viridis* motile cells, and the taxonomic position of the resurrected family Ulvellaceae (Ulvales, Chlorophyta). J. Phycol. **19:** 153–164.

O'Kelly, C.J. and Floyd, G.L. 1984. The absolute configuration of the flagellar apparatus in zoospores from two species of Laminariales (Phaeophyceae). Protoplasma **123:** 18–25.

O'Kelly, C.J. and Floyd, G.L. 1985. Absolute configuration analysis of the flagellar apparatus in *Giraudyopsis stellifera* (Chrysophyceae, Sarcinochrysidales) zoospores and its significance in the evolution of the Phaeophyceae. Phycologia **24:** 263–274.

Ramirez, M.E., Müller, D.G. and Peters, A.F. 1986. Life history and taxonomy of two populations of ligulate *Desmarestia* (Phaeophyceae) from Chile. Can. J. Bot. **64:** 2948–2954.

Tatewaki, M. 1966. Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. Phycologia **6:** 62–66.

Toth, R. 1976. The release, settlement and germination of zoospores in *Chorda tomentosa* (Phaeophyceae, Laminariales). J. Phycol. **12:** 222–233.

本村泰三：ミツイシコンブ（褐藻，コンブ目）の精子の微細構造，特に鞭毛基部構造について

ミツイシコンブの精子の微細構造，特にその鞭毛基部構造を透過型・走査型電子顕微鏡を用いて明らかにし，その遊走子および今までに報告されている褐藻類の遊走細胞との比較を行った。精子は長い後鞭毛を有しており，両鞭毛の付け根付近を細胞質が襟状となって取り囲んでいる。細胞質中には2–3個の葉緑体が存在しているが，それらと鞭毛基部構造との密接な関係は見られない。4組の鞭毛根 (MAR, mar, mpr, BR) が観察でき，それぞれ5, 1, 1, 4本の微小管からなる。BRは精子前方より核膜に沿って後方に伸び，MAR, mar, mprはそれぞれミトコンドリアとの連結が認められた。2本の鞭毛基部は特徴的な形態をした電子密度の高い1枚の帯状構造物でつながれている。上記の微細構造のいくつかは他の褐藻類の遊走細胞では報告されていない特徴であり，今後，褐藻植物内でのコンブ類植物の系統関係を考える上で重要であると考えられる。(051 北海道室蘭市母恋南町1–13　北海道大学理学部附属海藻研究施設)

Jpn. J. Phycol. (Sôrui) 37: 117–122, June 20, 1989

Seasonal fluctuation of *Chrysochromulina parva* (Prymnesiophyceae) in four ponds and lakes in the Kinki district, Japan

Hiroyuki Ito

Water Quality Laboratory, Kobe City Waterworks Bureau, 37–1, Kusutani-cho, Hyogo-ku, Kobe, 652 Japan

Ito, H. 1989. Seasonal fluctuation of *Chrysochromulina parva* (Prymnesiophyceae) in four ponds and lakes in the Kinki district, Japan. Jpn. J. Phycol. 37: 117–122.

Chrysochromulina parva Lackey (1939) was collected in spring from Sengari Reservoir, Karasuhara Reservoir and Yasuba-ike Pond in Hyogo Prefecture, while in winter and spring from Lake Biwa in Shiga Prefecture, Japan. It appeared at high density of 21,000 cells/m*l* on 13 April 1982 in Sengari Reservoir and 59,000 cells/m*l* on 14 March 1983 in Yasuba-ike Pond. The distinctive feature of this species is its rapid increase and subsequent decrease in a short time. *C. parva* seems to prefer eutrophic waters and is classified as eurythermal.

Key Index Words: Cell number—Chrysochromulina parva—*lake—pond—Prymnesiophyceae—reservoir—seasonal fluctuation.*

Chrysochromulina parva is a small organism with three appendages, two equal acronematic flagella and a haptonema. This species was first recorded from the Scioto River, U.S.A. (Lackey 1939). Thereafter the worldwide distribution of this species has been proved by many workers (Parke, Lund and Manton 1962, Heynig 1963, Thompson and Halicki 1965, Meyer and Brook 1969, Kristiansen 1971, Cronberg 1982, Bailey-Watts 1986, Duthie and Stout 1986, Munawer and Munawer 1986, Pollingher 1986, Reynolds 1986). In Japan it is distributed in three localities (Inouye and Chihara 1987), but its seasonal fluctuation has not been studied. The purpose of this paper is to make clear the seasonal fluctuation of *C. parva* and its character in four ponds and lakes in the Kinki district.

Materials and Methods

Study sites were: Sengari Reservoir situated over three cities, Kobe, Sanda and Takarazuka, Hyogo Prefecture; Karasuhara Reservoir, Kobe City; Yasuba-ike Pond, an irrigation pond in Takarazuka City; and Lake Biwa, Shiga Prefecture, the largest lake in Japan. Physicochemical characteristics of these ponds and lakes are summarized in Table 1. Total phosphorus and total nitrogen were determined according to procedures recommended by the Japanese Environmental Agency. Other chemical analyses were done by the methods of Japan Water Works Association (1985).

Investigations were made once or twice a week in Sengari Reservoir (March 1979-June 1987) and once a month in Yasuba-ike Pond (March 1980-June 1983), Karasuhara Reservoir (April 1978-March 1988) and the south basin of Lake Biwa (January 1981, May 1986-April 1987). Sampling stations in Sengari Reservoir are shown in Fig. 5. In Yasuba-ike Pond, water samples were taken at a station 1 m off the shore where the depth was 50 cm and in Karasuhara Reservoir at a station 3 m off the dam where the depth was about 17 m. In the south basin of Lake Biwa, water samples were taken at a station about 200 m off Yamada Habor and at a station about 2 km off the intake of the Lake Biwa Waterway No. 2 of Kyoto City.

Samples were collected in 1 *l* bottles from

Table 1. Physicochemical characteristics (mean values) of Sengari Reservoir, Karasuhara Reservoir, Yasuba-ike Pond and the south basin of Lake Biwa in the Kinki district, Japan.

Locality		Sengari Reservoir	Karasuhara Reservoir	Yasuba-ike Pond	South basin of Lake Biwa
Prefecture		Hyogo	Hyogo	Hyogo	Shiga
Area	(m^2)	1.12×10^6	1.15×10^5	2.50×10^3	5.68×10^7
Volume	(m^3)	1.16×10^7	1.32×10^6	4.00×10^3	2.00×10^8
Maximum depth	(m)	31.0	19.0	2.0	7.6
Period of investigation		Apr. 1979–Mar. 1987	Apr. 1979–Mar. 1987	Jan. 1979–Jan. 1988	May 1986–Apr. 1987
Chlorophyll *a*	($\mu g/l$)	8.7	18.4	24.5	10.1*
Total phosphorus	(mg/*l*)	0.019	0.037	0.086	0.017*
Total nitrogen	(mg/*l*)	0.54	0.81	0.99	
Total hardness	(mg/*l*)	22.8	64.7	29.7	
Alkalinity	(mg/*l*)	19.5	60.3	25.7	
COD	(mg/*l*)	2.7	4.3	9.2	
Conductivity	(μS/cm)	86.2	223	125	
pH		7.6	8.5	6.7	7.7

* Data from TEZUKA (1984).

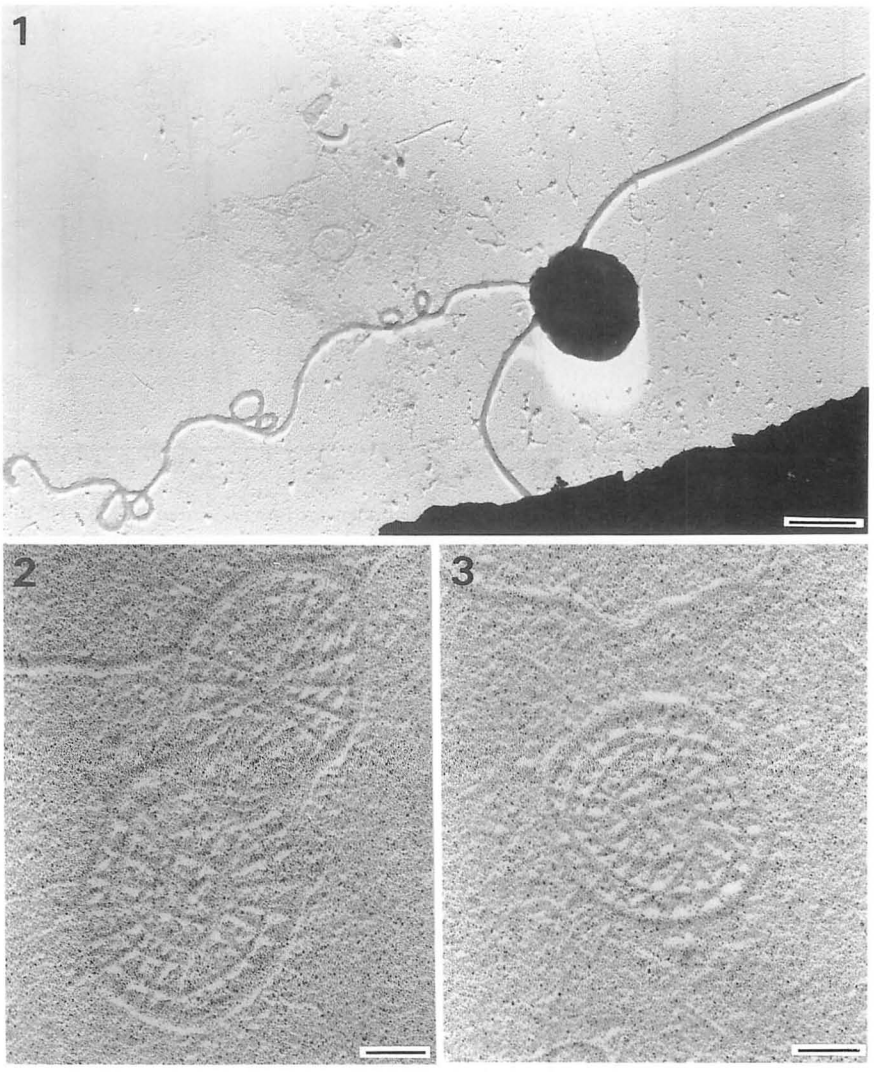
the surface at all stations. Water sample of 0.5 *l* each was fixed immediately with 0.5% Lugol's solution and subsequently with 2% formalin. The fixed sample was settled for a week and concentrated to 10 m*l*. Unfixed sample of 0.5 *l* each was centrifuged at 3,000 r.p.m. for 10 min. and concentrated to 1 m*l*. Both fixed and unfixed concentrated samples were used for examinations of *C. parva* with optical and electron microscopes. The cell number per m*l* of *C. parva* and other phytoplankton at each sampling time was estimated under an optical microscope by counting whole cells in 1/1000 m*l* of the fixed concentrated samples using a Fuchs Rosental hemacytometer. The method used to observe *C. parva* with a transmission electron microscope (JEM 100B) is described in a previous paper (ITO 1988).

Results

A very small organism with three appendages and two brown chloroplasts was collected from four ponds and lakes in the Kinki district, Japan. It was observed by electron microscopy and identified as *Chrysochromulina parva* by its very long haptonema and structure of scales lying above the cell surface. The haptonema is 7–15 times as long as the cell length. Scales are circular or oval with concentric striations and widely spaced radiating ridges (Figs. 1–3).

Sengari Reservoir

C. parva appeared from March to May (Fig. 4). The maximum peak in each year was observed on 27 April 1979, 13 April 1982, 2 May 1983, 7 May 1984, 7 May 1985, 21 April 1986 and 18 May 1987 at St. 5. The density of each peak was 6,800, 21,000, 3,700, 5,800, 2,800, 7,700 and 6,200 cells/m*l*, respectively, and their percentage to the total phytoplankton cell number was 57.1, 87.1, 45.4, 82.3, 42.4, 88.5 and 31.5%, respectively. *C. parva* was dominant at each time except for 1987 when it was subdominant next to *Uroglena americana*. It occurred at a temperature range from 7 to 23°C, and the densest population was encountered at 12–17°C. High density lasted for a short time. It appeared for a month or less except in 1983 and 1986. In 1982, for example, the maximum density observed on 13 April was reached in four days from a population of 840 cells/m*l* on 9 April. The high density



Figs. 1–3. *Chrysochromulina parva*. Fig. 1. A desiccated cell. Figs. 2 & 3. Scales detached from a desiccated cell. (Scale bars: 2 μm in Fig. 1; 0.1 μm in Figs. 2 & 3).

lasted for a very short time and the number fell to 150 cells/m*l* by 26 April.

The density of *C. parva* was different from station to station. In 1979, the density at St. 1 and St. 3 was 2,500 and 13,000 cells/m*l* respectively on 27 April, while on 4 May it was 13,000 and 1,500 cells/m*l* respectively. *C. parva* was not uniformly distributed in Sengari Reservoir (Fig. 5).

Yasuba-ike Pond

C. parva appeared also from March to May (Fig. 6). It was dominant on 23 April 1980, 9 April 1981, and 14 March and 13 April 1983. The density at each time was 19,000, 58,000, 59,000 and 25,000 cells/m*l* respectively, and their percentage to the total phytoplankton cell number was 56.1, 78.3, 63.9 and 31.2% respectively. The water temperature at the time when the maximum density in each year was recorded was 17.0°C in 1980, 14.7°C in 1981 and 7.8°C in 1983.

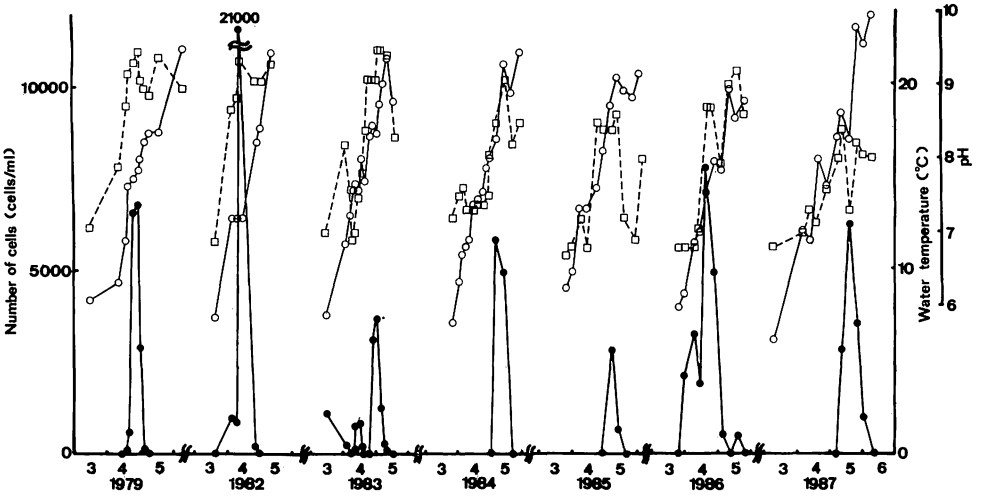


Fig. 4. Seasonal fluctuation of *Chrysochromulina parva* in Sengari Reservoir from March 1979 to June 1987 (solid circles) in relation to changes in surface water temperature (open circles) and pH (open squares).

Karasuhara Reservoir

C. parva was observed only once on 23 May 1979 during ten years from April 1978 to March 1988. The high density of this species was readily imagined because the water color changed from green to brown over the whole reservoir, although the quantitative study was not done. At that time the water temperature was 21.9°C, and pH 9.6.

The south basin of Lake Biwa

C. parva was subdominant next to *Fragilaria crotonensis* at a station about 200 m off Yamada Harbor on 23 January 1981. The density at

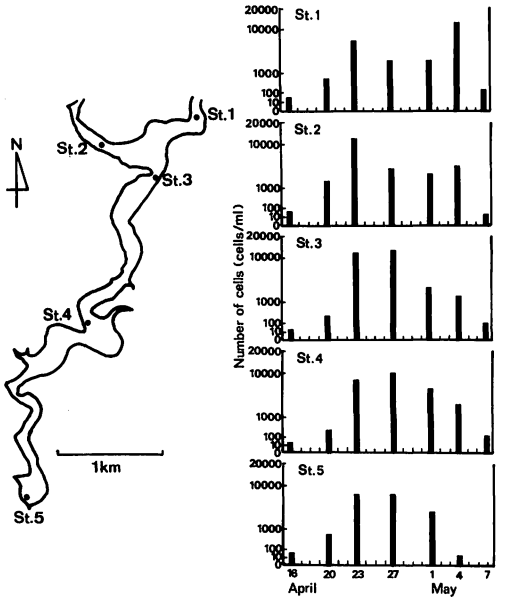


Fig. 5. Map showing five sampling stations and fluctuation in the cell number of *Chrysochromulina parva* in Sengari Reservoir from 16 April to 7 May in 1979.

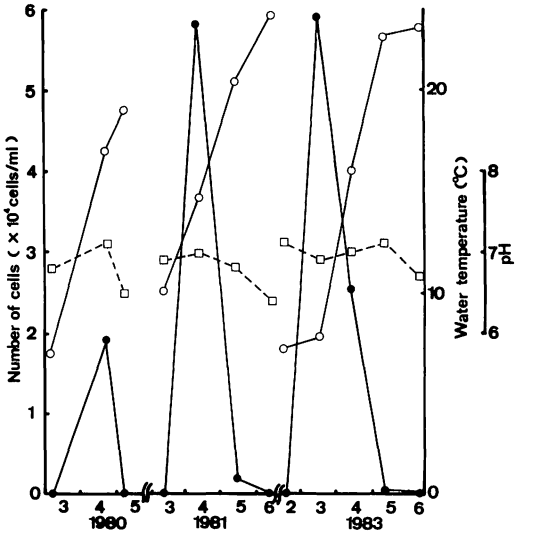


Fig. 6. Seasonal fluctuation of *Chrysochromulina parva* in Yasuba-ike Pond from March 1980 to June 1983 (solid circles) in relation to changes in surface water temperature (open circles) and pH (open squares).

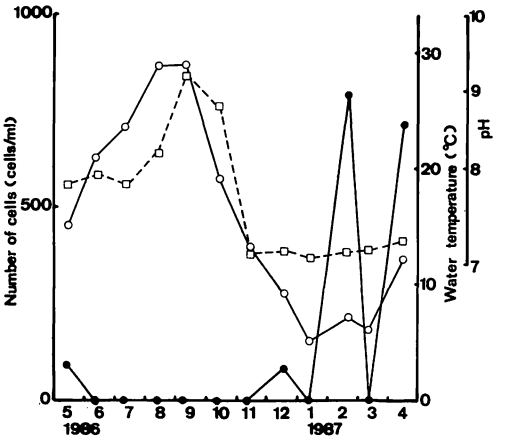


Fig. 7. Seasonal fluctuation of *Chrysochromulina parva* in the south basin of Lake Biwa from May 1986 to April 1987 (solid circles) in relation to changes in surface water temperature (open circles) and pH (open squares).

that time was 3,500 cells/m*l*, and the water temperature was 3.0°C. In the period from 13 May 1986 to 14 April 1987, *C. parva* appeared on 13 May and 17 December 1986 and on 17 February and 14 April 1987 at a station about 2 km off the intake of the Lake Biwa Waterway No. 2. The density at each time was very low, but on 17 February 1987 it was 790 cells/m*l* and *C. parva* became dominant, occupying 44.6% of the total phytoplankton cell number (Fig. 7).

Discussion

In Sengari Reservoir and Yasuba-ike Pond, *Chrysochromulina parva* rapidly increased and subsequently decreased in a short time. A similar phenomenon has been reported: The highest density of 32,000 cells/m*l* was observed in June 1960 in an English lake (Parke, Lund and Manton 1962). It was reached in two weeks from a population of 300 cells/m*l*, and two weeks later the density fell to 1,000 cells/m*l*. The high density of *C. parva* reaching 50,000 cells/m*l* was also observed in a small Danish lake in July 1969 (Kristiansen 1971). From these results it is apparent that *C. parva* rapidly increases and subsequently decreases in a short time, its characteristically high population density lasting for a very short time.

In the present study, *C. parva* occurred in a wide temperature range from 3 to 23°C and high densities above 10,000 cells/m*l* were reached at temperatures from 8 to 17°C. In two Japanese lakes, Kasumigaura and Hinuma, water blooms of this species occurred in December (Inouye and Chihara 1987). In a subtropical Israeli lake, *C. parva* appeared at high temperatures up to 30°C and maximum development was established at 14–18°C (Pollingher 1986). In a Danish lake, a high density of *C. parva* was observed at 15.8°C (Kristiansen 1971). Based on all available data, *C. parva* is classified as eurythermal with high abundance at low temperatures below 18°C. In Sengari Reservior and Yasuba-ike Pond, *C. parva* increased as water temperature became high and it decreased or disappeared at temperatures above 20°C. It is considered that water temperature is the major factor controlling seasonal fluctuation of *C. parva*.

Sengari Reservoir, Karasuhara Reservoir and Yasuba-ike Pond are eutrophic in terms of total phosphorus and total nitrogen concentrations (Sakamoto 1966), while Lake Biwa is intermediate between mesotrophic and eutrophic (Tezuka 1984). The maximum density of *C. parva* in Yasuba-ike Pond was 2.8 times higher than that in Sengari Reservoir, and 6 times higher than that in Lake Biwa. This implies that the *C. parva* biomass becomes larger as eutrophication progresses. *C. parva* has been reported from eutrophic ponds and lakes in England (Parke, Lund and Manton 1962, Reynold 1986), Scotland (Bailey-Watts 1986), Sweden (Cronberg 1982), Germany (Heynig 1963), Canada (Munawer and Munawer 1986) and Israel (Pollingher 1986) but not from oligotrophic lakes. It is inferred that *C. parva* prefers eutrophic waters.

In Japan *C. parva* has been recorded from Lake Kasumigaura, Lake Hinuma and Osaka Bay (Inouye and Chihara 1987). In this paper it is clearly shown that *C. parva* rapidly increases and subsequently decreases in a short time in four ponds and lakes having

different water quality. *C. parva* will be commonly collected from more localities in Japan by samplings at short intervals.

Acknowledgement

The author whishes to express his gratitude to Director K. HARIMAYA and Dr. H. YANO (Kobe City Waterworks Bureau) for their encouragement, to Mr. M. NAKAHARA (Kobe City Waterworks Bureau) for his helpful assistance in the quantitative study, and to Mr. M. ANDO, Mr. M. NISHIKAWA and Mr. T. NEGORO (Kyoto City Waterworks Bureau) for samplings in Lake Biwa. He also thanks Dr. E. TAKAHASHI (Kobe University) for his helpful advice in this study.

References

BAILEY-WATTS, A.E. 1986. Seasonal variation in size spectra of phytoplankton assemblages in Loch Leven, Scotland. Hydrobiologia **138:** 25–42.

CRONBERG, G. 1982. Phytoplankton changes in Lake Trummen induced by restoration. Folia Limnol. Scand. **18:** 1–119.

DUTHIE, H.C. and STOUT, V.M. 1986. Phytoplankton periodicity of the Waitaki Lakes, New Zealand. Hydrobiologia **138:** 221–236.

HEYNIG, H. 1963. *Chrysochromulina parva* Lackey im Plankton Mitteldeutschlands. Arch. Protistenk. **106:** 453–455.

INOUYE, I. and CHIHARA, M. 1987. Haptophytes. p. 582–617. *In* H. TAKANO, M. CHIHARA and Y. FUKUYO [ed.] A guide for studies of red tide organisms. Japan Fisheries Resource Conservation Association. (in Japanese)

ITO, H. 1988. Scale-bearing chrysophytes in the south basin of Lake Biwa. Jpn. J. Phycol. **36:** 143–153.

JAPAN WATER WORKS ASSOCIATION. 1985. Josui Shiken Hoho. Japan Water Works Association, Tokyo. (in Japanese)

KRISTIANSEN, J. 1971. A Danish find of *Chrysochromulina parva* (Haptophyceae). Bot. Tidsskr. **66:** 33–37.

LACKEY, J.B. 1939. Notes on plankton flagellates from the Scioto River. Lloydia **2:** 128–143.

MEYER, R.L. and BROOK, A.J. 1969. Freshwater algae from the Itasca State Park, Minnesota. II. Chrysophyceae and Xanthophyceae. Nov. Hedw. **17:** 105–112.

MUNAWER, M. and MUNAWER, I.F. 1986. The seasonality of phytoplankton in the North American Great Lakes, a comparative synthesis. Hydrobiologia **138:** 85–115.

PARKE, M., LUND, J.W.G. and MANTON, I. 1962. Observations on the biology and fine structure of the type species of *Chrysochromulina (C. parva* Lackey) in the English Lake District. Arch. Mikrobiol. **42:** 333–352.

POLLINGHER, U. 1986. Phytoplankton periodicity in a subtropical lake (Lake Kinneret, Israel). Hydrobiologia **138:** 127–138.

REYNOLDS, C.S. 1986. Experimental manipulations of the phytoplankton periodicity in large limnetic enclosures in Blelham Tarn, English Lake District. Hydrobiologia **138:** 43–64.

SAKAMOTO, M. 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. Arch. Hydrobiol. **62:** 1–28.

TEZUKA, Y. 1984. Seasonal variations of dominant phytoplankton, chlorophyll *a* and nutrient levels in the pelagic regions of Lake Biwa. Jap. J. Limnol. **45:** 26–37.

THOMPSON, H. and HALICKI, P.J. 1965. *Chrysochromulina parva* in Eastern Kansas. Trans. Am. Microsc. Soc. **84:** 14–17.

伊藤裕之：近畿地方の4湖沼における *Chrysochromulina parva* LACKEY（プリムネシウム藻綱）の季節的消長

Chrysochromulina parva（プリムネシウム藻綱）が兵庫県の千苅貯水池，鳥原貯水池，安場池から春期に，滋賀県の琵琶湖から冬～春期に採集された。千苅貯水池では1982年4月13日に最大 1 m*l* 当たり，21,000細胞，安場池では1983年3月14日に59,000細胞といった高密度で出現した。本種は短期間に速く増減し，また富栄養の水界を好み広温性である。（652 神戸市兵庫区楠谷町37–1　神戸市水道局水質試験所）

Jpn. J. Phycol. (Sôrui) 37: 123–128, June 20, 1989

Notes on *Urospora penicilliformis* ARESCHOUG from Hakodate, Hokkaido

Hiroshi YABU

Faculty of Fisheries, Hokkaido University, Hakodate, 041 Japan

YABU, H. 1989. Notes on *Urospora penicilliformis* ARESCHOUG from Hakodate, Hokkaido. Jpn. J. Phycol. 37: 123–128.

Observations were made with reference to the chromosomes of *Urospora penicilliformis* Areschoug (Chlorophyta, Cladophorales) from Hakodate, Hokkaido. The chromosome counts were 12 in the zygote-germlings, and 6 or 12 in the zoospore-germlings, indicating to have haploid and diploid zoospores released from the filaments.

Key Index Words: *Chromosome—Cladophorales—cytology*—Urospora penicilliformis.

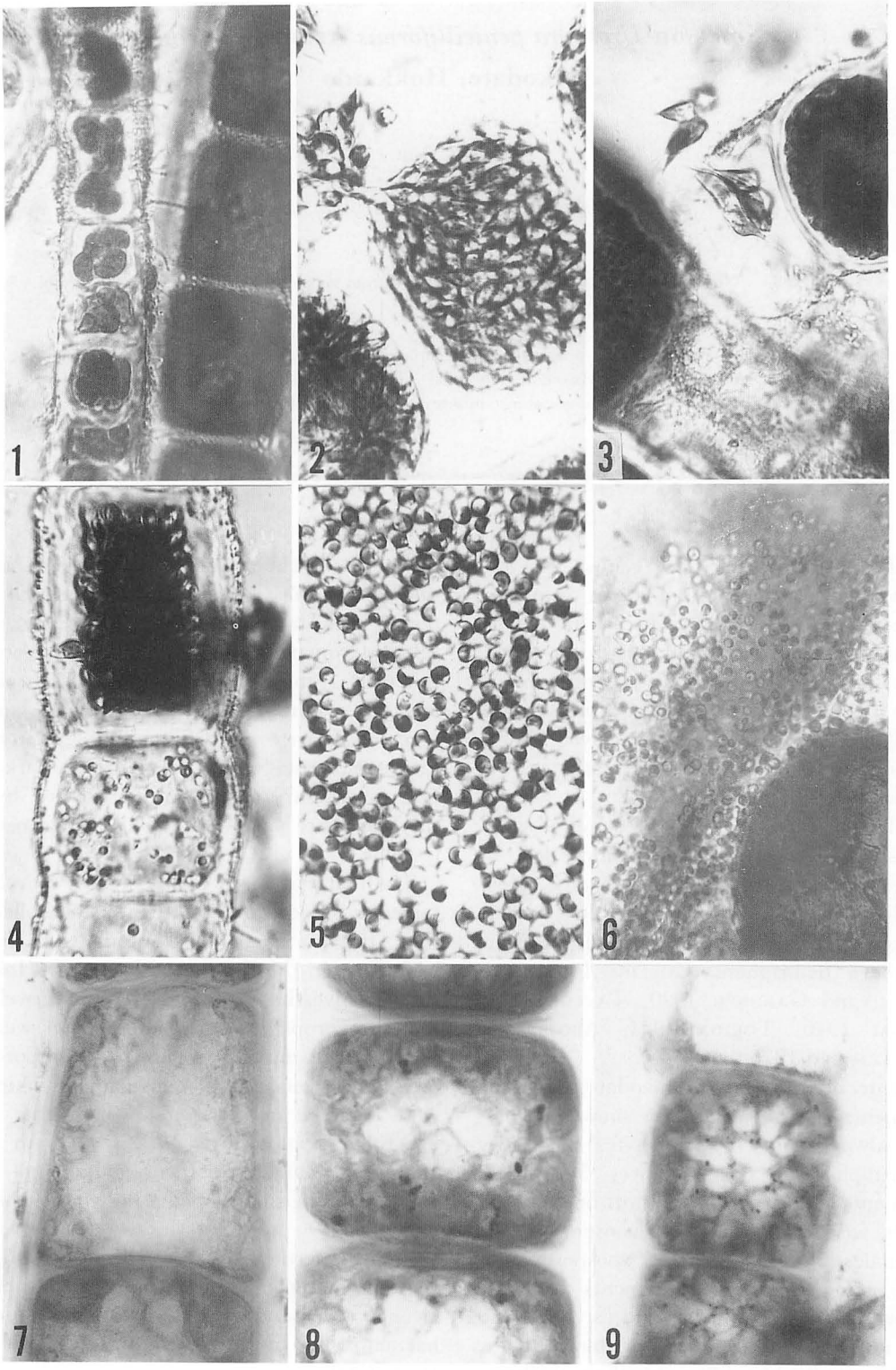
The species in the genus *Urospora* (Cladophoraceae, Chlorophyta) is of interest in having Codiolum stage in the life history (JORDE 1933, KORNMANN 1961a, b, 1966, NAGATA 1971). This genus comprises more than 10 species in the world, but none of the cytological events have been given except some mitotic figures in the vegetative cells of *U. incrassa* by KANNO (1936).

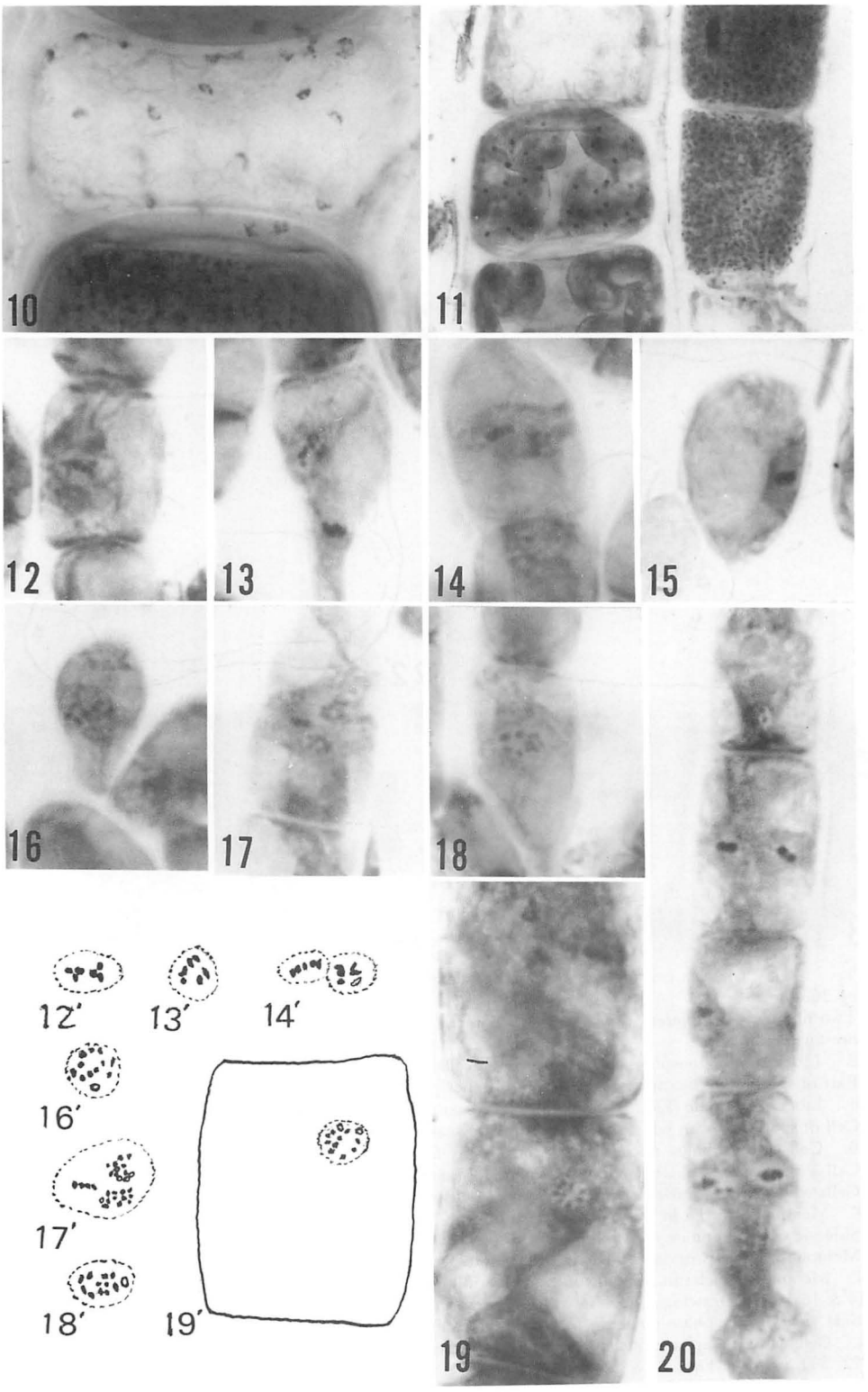
The present paper informs some observations with reference to cytology for *U. penicilliformis* ARESCHOUG at Hakodate, Hokkaido. This species is distributed widely along the coasts generally in the north of the northern hemisphere (COLLINS 1909, SETCHELL and GARDNER 1920, TAYLOR 1937, NAGAI 1940, TOKIDA 1954, ABOTT and HOLLENBERG 1976, etc.).

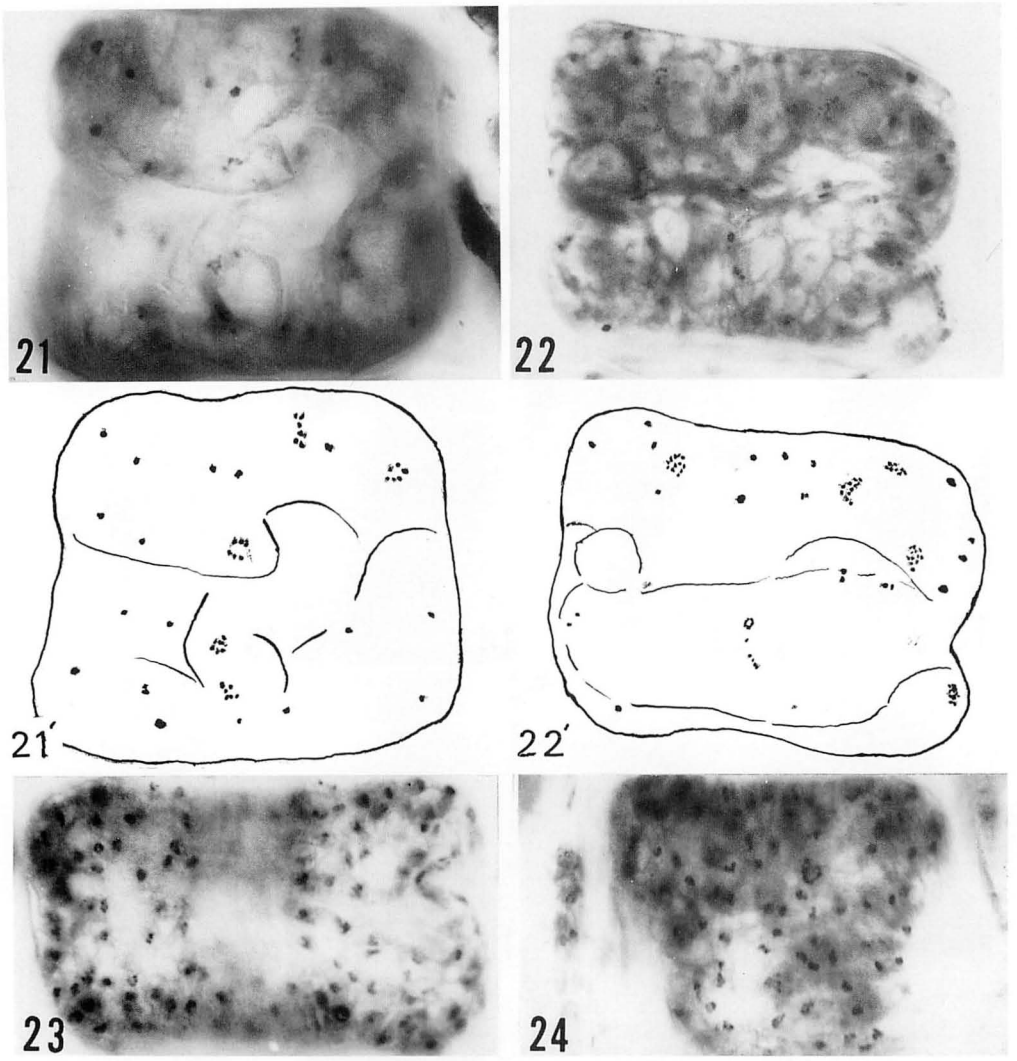
In my observations at Hakodate, the fertile filaments of *U. penicilliformis* showed to present always plenty of sporophytes, but scanty gametophytes. For instance, 500 fertile filaments collected from Shinori on May 28, 1979, were made up of 412 zoosporophytes, 26 males, 30 females and 32 zoosporophytes with gametangia. The filaments are quite varied in thickness not beyond ca. 100 μ and the number of zoospores or gametes in the cells considerably differs by their thickness (Figs. 1–4). The zoospores and gametes are released en mass or one by one through a pore. In the latter case, zoospores are always released from portion of the tail, and on liberation (Fig. 3) they turn round quickly to begin active swimming. Zoosporangia and gametangia are easily distinguishable even in the juvenile stage; the formers are darker green in colour and coarse in structure, but the latters are yellowish green and more or less fine.

The materials used for cytology were obtained at Shinori in May 1979. After collection, the materials were brought to the laboratory in the Faculty of Fisheries, Hokkaido University. In the laboratory, well-mature sporophytes and gametophytes were selected for fixing and for culture. Zoospores or zygotes of male and female gametes which were obtained from those shown in Figs. 5 and 6 had been cultured in ESP medium at 10°C under 3000 lux. In my culture, all of their germlings developed into young thin filaments.

Both the fertile filaments collected and the spore-germlings in culture were fixed in aceto alcohol (1:3) and stained with aceto-iron-haematoxylin-chloral hydrate solution (WITTMANN 1965).


1
2
3
4
5
6
7
8
9


10
11
12
13
14
15
16
17
18
19
20
12′
13′
14′
16′
17′
18′
19′



Figs. 1–24. *Urospora penicilliformis* ARESCHOUG collected from Hakodate, Hokkaido. Fig. 1, ×310; Figs. 2–24, ×560.

1. Two filaments in different thickness. The filament in the left side is a sporophyte bearing cells leading to zoospore-formation.
2 & 3. Liberation of zoospores through a pore of cells.
4. Part of sporophyte bearing zoosporangium (upper portion) and male gametangium (lower portion).
5 & 6. Liberated female (Fig. 5) and male (Fig. 6) gametes. Zygotes from those gametes were used for culture.
7. Cell of sporophyte, in which pyrenoids turn to weak in staining at the beginning of nuclear divisions.
8 & 9. Cell with prophase nuclei leading to zoospore-formation.
10. Cell with prophase nuclei leading to gamete-formation.
11. Cells with dividing nuclei leading to zoospore (left in the figure) and gamete-formation (right in the figure).
12–14. Metaphase nuclei in the zoospore-germlings.
15. Side view of metaphase nucleus in one-celled stage of zoospore-germling.
16. Metaphase nucleus in one-celled stage of zygote-germling.
17–19. Metaphase nuclei in the cells of zygote-germlings.
12′–14′ & 16′–19′. Drawings of 12–14 & 16–19, respectively.
20. Side view of metaphase nuclei in the young filament of zygote-germling.
21–24. Cells with metaphase nuclei leading to zoospore-formation.
21′ & 22′. Drawing of Figs. 21 & 22, respectively.

In both the vegetative and reproductive cells, the nuclear divisions occurred simultaneously (Figs. 7–11 & 21–24). In the germlings composed of less than about 20 cells, the same nuclear phases appeared occasionally in all of the cells (Fig. 20). When the nuclear divisions set in, pyrenoids came to weak in staining and nuclei began to grow larger (Figs. 7 & 8). With advance of the divisions, the pyrenoids completely disappeared, and the nuclei turned to be more and more stained well (Fig. 10). The metaphase nuclei in which chromosomes were countable were encounterd in the vegetative cells of the spore-germlings and in the zoosporangial cells. Each chromosome was uniform, minute, subspherical with diameter of ca. 1.5 μm in vegetative cells, but smaller in zoosporangia. Of the materials with metaphase nuclei, I found either 6 or 12 chromosomes in both the zoospore-germlings and zoosporangia, but 12 chromosomes in the zygote-germlings (Figs. 12–24).

Thus, the filaments of *U. penicilliformis* growing at Hakodate are obvious to have two kinds of zoospores, diploid or haploid. The chromosome numbers could not be ascertained in the gametangia, but it was suggested that the diploid filaments would be derived from either zoospores or zygotes. From the tables of 1.6 shown by GODWARD (1966) it is recognized that *U. penicilliformis* (n=6; 2n=12) has the smallest chromosome number in the species of Cladophorales hitherto reported, which is identical to *Cladophora flexuosa* HARV., *Cl. sericea* KÜTZ., *Cl. rupestris* KÜTZ., *Spongomorpha lanosa* KÜTZ., *Acrosiphonia traillei* BATT., *Chaetomorpha area* KÜTZ., and *Ch. metagonium* KÜTZ. In the present study, I could not succeed to obtain Codiolum stage in the culture of zoospores and zygotes perhaps due to the unsuitable culture conditions, and also to ascertain the occurrence of meiosis in any zoosporangial cell in the filaments. It is thinkable that the meiosis of this alga would take place at the formation of zoospores within the Codiolum stage. This is an interesting problem to be solved.

References

ABOTT, L.A. and HOLLENBERG, G.J. 1976. Marine algae of California. Stanford Univ. Press, Stanford.

COLLINS, F.S. 1909. The green algae of North America. Tufts College Studies **2**: 79–480.

GODWARD, M.B.E. 1966. 1. The Chrolophyceae. p. 1–77. *In* Godward, M.B.E. (ed.), The Chromosomes of the Algae. Edward Arnold Ltd., London.

JORDE, I. 1933. Untersuchungen über den Lebenzyklus von *Urospora* ARESCH. und *Codiolum* A. BRAUN. Nyt Mag. f. Naturv. **73**: 1–19.

KANNO, R. 1936. On the zoospores of the genus (Hormiscia, Urospora) Bull. Japan. Soc. Sci. Fish. **5**: 177–182.

KORNMANN, P. 1961a. Die Entwicklung von *Codiolum gregarium* A. BRAUN. Helgoländer wiss. Meeresunters. **7**: 252–259.

KORNMANN, P. 1961b. Über *Codiolum* und *Urospora*. Helgoländer wiss. Meeresunters. **8**: 42–47.

KORNMANN, P. 1966. Hormiscia neu definiert. Helgoländer wiss. Meeresunters. **13**: 408–425.

NAGAI, M. 1940. Marine algae of the Kurile Islands. I. Jour. Fac. Agr., Hokkaido Imp. Univ. **46**: 1–138.

NAGATA, K. 1971. On the life history of *Urospora mirabilis* ARESCHOUG from Muroran. Bull. Jap. Soc. Phycol. **19**: 97–103.

SETCHELL, W.A. and GARDNER, N.L. 1920. The marine algae of the Pacific coast of North America II. Chlorophyceae. Univ. Calif. Publ. Bot. **8**: 139–381.

TAYLOR, W.R. 1937. Marine algae of the northeastern coast of North America. Univ. Michigan Studies Sci. Ser. **13**: 1–427.

TOKIDA, J. 1954. Marine algae of southern Saghalien. Mem. Fac. Fish. Hokkaido Univ. **2**: 1–264.

WITTMANN, W. 1965. Aceto-iron-haematoxylin-chloral hydrate for chromosome staining. Stain Tech. **40**: 161–164.

籔　凞：北海道函館産のシリオミドロについて

北海道函館産の緑藻シリオミドロについて主として細胞学的研究によって得た知見を記述した。採集した藻体並びに遊走子と接合子を培養して生じた発生体とを醋酸・アルコールで固定し，醋酸・鉄・ヘマトキシリン・抱水クロラール液で染色し，藻体の遊走子嚢内核分裂で6又は12個，遊走子発芽体で同じく6又は12個の染色体を，接合子発芽体では12個の染色体を観察し，遊走子には n 又は 2n の核を有する2種類があることを確かめた。
(041 函館市港町3-1-1　北海道大学水産学部)

Jpn. J. Phycol. (Sôrui) 37: 129–136, June 20, 1989

Method for quick evaluation of cell viability in marine macroalgae*

Naotsune SAGA, Yoshihiko SAKANISHI and Takashi OGISHIMA

Hokkaido Regional Fisheries Research Laboratory, Fisheries Agency, Kushiro, Hokkaido, 085 Japan

SAGA, N., SAKANISHI, Y. and OGISHIMA, T. 1989. Method for quick evaluation of cell viability in marine macroalgae. Jpn. J. Phycol. 37: 129–136.

Various staining-dyes were tested with cultured cells of the economically valuable marine macroalgae, *Enteromorpha intestinalis, Porphyra yezoensis* and *Macrocystis pyrifera* in order to develop a rapid, simple and reliable technique for determining algal cell viability. Pieces of the tissue were immersed in the dye solutions of the cell viability checking kit and observed with a microscope after 10 min. The most suitable dyes tested were FDA for detection of viable cells and phenosafranin for dead cells in *Enteromorpha intestinalis* gametophyte, and neutral red for viable cells and Evans blue for dead cells in *Macrocystis pyrifera* gametophyte. The most suitable dye tested was Evans blue for detection of dead cells, and autofluorescence of phycoerythrin was useful for detection of viable cells in *Porphyra yezoensis* conchocelis.

Key Index Words: Cell viability—Chlorophyta—Enteromorpha intestinalis—Macrocystis pyrifera—*Phaeophyta*—Porphyra yezoensis—*Rhodophyta—staining—tissue culture.*

Accurate evaluation of cell viability is a very important step in studies on algal tissue culture in many ways. Determining cell viability is essential to development of efficient methods for mutagenesis and freeze preservation in cultured algal cells. Usually cell viability is determined by microscopic assessment of cytoplasmic features or plasmolysis capacity in our laboratory. However, sometimes these methods are insufficient to clear-cut evaluation of cell viability. Various staining methods have been used to detect live or dead higher plant cells (WHITHERS 1980), and a few staining methods have been used to detect live or dead macroalgal cells. TTC (OGATA 1956) and neutral red (TERUMOTO 1960, SAKAI and SUGAWARA 1978) were used to detect live algal cells, while erythrosine (MIGITA 1967) was used to dead algal cells.

The purpose of the present paper is to examine various staining dyes in order to develop a rapid, simple and reliable technique for determining cell viability in marine macroalgae.

Materials and Methods

The materials used in this study were an axenic strain of *Enteromorpha intestinalis* (gametophyte), HRFRL culture collection no. EI-001, an axenic strain of *Porphyra yezoensis* (conchocelis), HRFRL culture collection no. PY-401 and axenic male and female strains of *Macrocystis pyrifera* (gametophyte), HRFRL culture collection no. MP-101 (male strain) and no. MP-201 (female strain). They were obtained by the one step antibiotic disk method described previously (SAGA and SAKAI 1982). Ten colonies of the organisms which developed into a mass, 1.0 mm in diameter, were inoculated into plastic vessels (Tissue Culture Flask # 25100, Corning) containing 30 m*l* of ASS 1 medium (Table 1). The cultures were kept for 1 month on a horizontal reciprocal shaker (Thermoshaker Model XY-11, Thermonics Co.; amplitude

Abbreviations: FDA, fluorescein diacetate; TTC, triphenyl tetrazolium chloride.

* This study was partly supported by a Grant-in-Aid for the Prior Basic Research Project from Science and Technology Agency of Japan.

Table 1. Composition of ASS 1 per 100 m*l*, pH 8.0.

NaCl	2.5 g	Na_2-glycerophosphate	2 mg
$MgSO_4 \cdot 7H_2O$	1.0 g	$NaHCO_3$	10 mg
KCl	70 mg	Vitamin mix ASS*	0.1 m*l*
$CaCl_2 \cdot 2H_2O$	110 mg	Metal mix ASS**	1 m*l*
$NaNO_3$	10 mg	HEPES	100 mg

* 1 m*l* vitamin mix ASS contains: thiamine HCl 100 μg, nicotinic acid 100 μg, putrescine·2HCl 10μg, Ca·pantothenate 100μg, riboflavin 10 μg, pyridoxine·2HCl 10 μg, pyridoxamine·2HCl 10 μg, *p*-aminobenzoic acid 10 μg, biotin 1 μg, inositol 1 mg, choline-H_2-citrate 100 μg, thymine 100 μg, orotic acid 100 μg, cyanocobalamine 1 μg, folic acid 1 μg, folinic acid 0.1 μg.

** 1 m*l* of metal mix ASS contains: Fe (as Fe-sequestren) 100 μg, B (as H_3BO_3) 100 μg, Mn (as Cl^-) 100 μg, Zn (as Cl^-) 10 μg, Co (as Cl^-) 1 μg, Mo (as Na_2MoO_4) 10 μg, Cu (as Cl^-) 1 μg, Br (as K^+) 1 mg, Sr (as Cl^-) 100 μg, Rb (as Cl^-) 10 μg, Li (as Cl^-) 10 μg, I (as K^+) 1 μg.

40 mm, 40 strokes/min) in an incubator (Cultivation Chamber Model CU-250, Tomy Seiko Co.) which was regulated at 15°C, 14:10 hr L:D cycle and ca. 60 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent lamps. The dead organisms were prepared by heat-killing at 70°C for 5 min.

The live and dead organisms were transferred into a CVC-kit (cell viability checking kit) and immersed in the dye solutions for 10 min. The CVC-kit was prepared by the following procedures: the staining-dyes were dissolved, in a concentration of 0.01%, in autoclaved seawater; adjusted the pH to 8.0 and put into each well of a plastic vessel (Cell Wells # 25860, Corning) up to 0.25 m*l*. The compounds tested were Evans blue (Merk Co.), phenosafranin (Katayama Chemical Co.), eosin Y (Wako Pure Chemical Industries), erythrosine (Tokushu Chemical Co.), neutral red (Kanto Chemical Co.) and FDA (Aldrich Chemical Co.). After immersion, the organisms were rinsed several times with autoclaved seawater and transferred onto a glass slide. Then, a coverglass was lowered over the organisms and the slide was observed with a microscope (Vanox T Model-AHBT, Olympus). The FDA-treated organisms were observed with the same microscope using a fluorescence system (Vanox T Model-AH2RFL, Olympus). A mercury-vapor lamp (HBO-200W/2, Osram) was used in combination with a dichroic mirror (DM-500, Olympus), exciter filter (BP-490, Olympus) and absorber filter (AFC & O-515, Olympus).

Results

Evans blue, phenosafranin, eosin Y and

Table 2. Staining of live and dead gametophyte cells with various dyes in *Enteromorpha intestinalis*.

Staining dyes	Live cells		Dead cells	
	PTS	CW	PTS	CW
Evans blue	−	++	+	++
Phenosafranin	−	±	++	±
Eosin Y	−	±	+	±
Erythrosine	−	±	++	±
Neutral red	++	±	−	±
FDA	++	−	−	−

++ stained deeply; + stained moderately; + stained lightly; ± stained infrequently; − not stained; PTS=protoplasts; CW=cell walls.

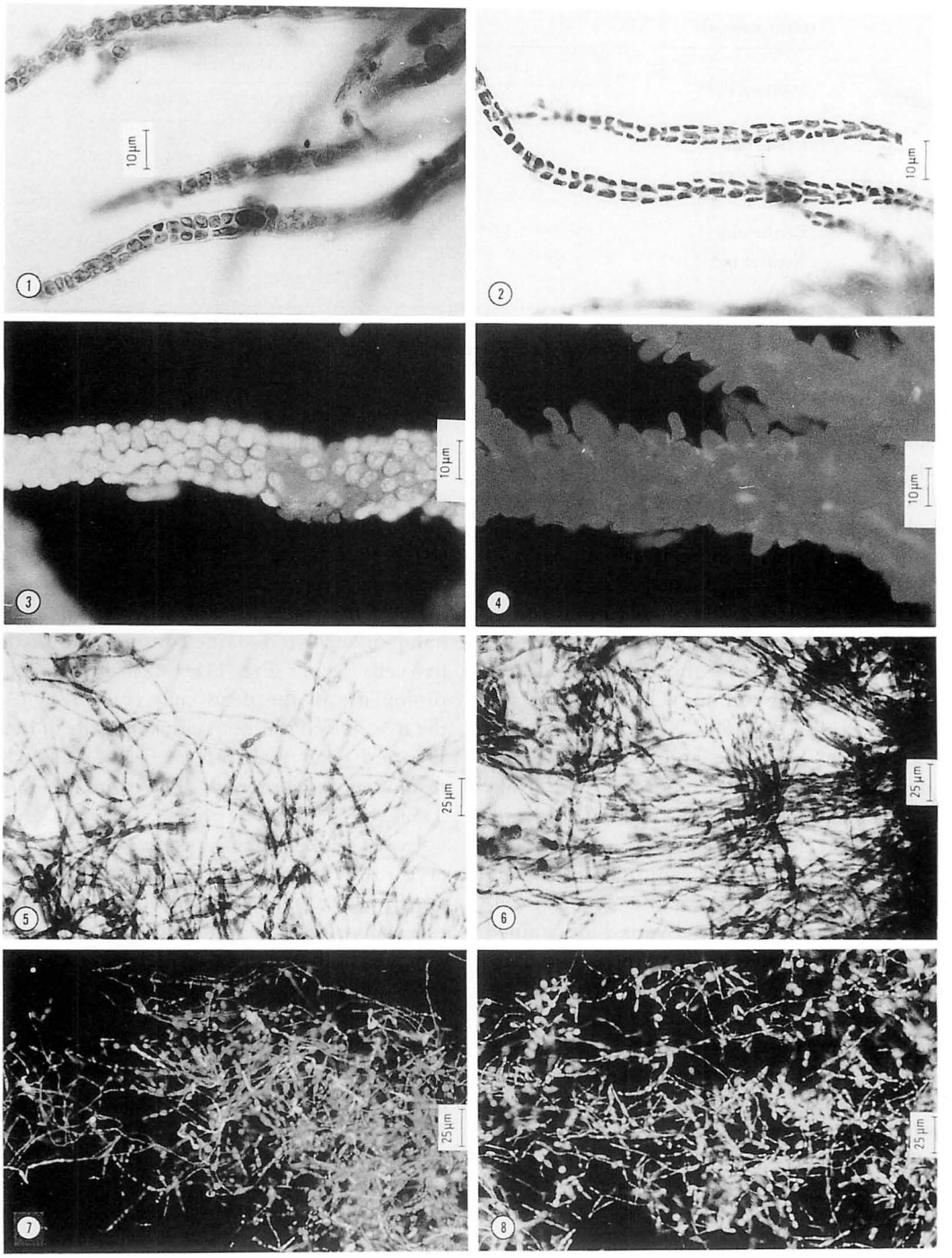


Fig. 1. Live *Enteromorpha intestinalis* gametophyte cells treated with phenosafranin. Fig. 2. Dead *Enteromorpha intestinalis* gametophyte cells treated with phenosafranin. Fig. 3. Live *Enteromorpha intestinalis* gametophyte cells treated with FDA. Fig. 4. Dead *Enteromorpha intestinalis* gametophyte cells treated with FDA. Fig. 5. Live *Porphyra yezoensis* conchocelis cells treated with Evans blue. Fig. 6. Dead *Porphyra yezoensis* conchocelis cells treated with Evans blue. Fig. 7. Autofluorescence of live *Porphyra yezoensis* conchocelis cells. Fig. 8. Autofluorescence of dead *Porphyra yezoensis* conchocelis cells.

Table 3. Staining of live and dead conchocelis cells with various dyes in *Porphyra yezoensis*.

Staining dyes	Live cells		Dead cells	
	PTS	CW	PTS	CW
Evans blue	−	±	++	±
Phenosafranin	−	−	+	−
Eosin Y	−	−	++	−
Erythrosine	−	−	++	−
Neutral red	++	−	−	−
FDA	−	−	−	−

++ stained deeply; ++ stained moderately; + stained lightly; ± stained infrequently; − not stained; PTS=protoplasts; CW=cell walls.

erythrosine stained protoplasts of dead *Enteromorpha intestinalis* cells, and phenosafranin stained them especially deeply (Fig. 2). But these dyes did not stain protoplasts of live *E. intestinalis* cells (Fig. 1). Evans blue stained cell walls of both live and dead cells deeply, and phenosafranin, eosin Y and erythrosine stained them infrequently. On the other hand, neutral red and FDA stained protoplasts of the live cells deeply (Fig. 3), but they did not stain protoplasts of the dead cells (Fig. 4). Neutral red stained cell walls of both live and dead cells infrequently, but FDA did not stain them at all. The results obtained by the present study are summarized in Table 2.

Evans blue, phenosafranin, eosin Y and erythrosine stained protoplasts of dead *Porphyra yezoensis* cells, and Evans blue stained them especially deeply (Fig. 6). But these dyes did not stain protoplasts of live *P. yezoensis* cells (Fig. 5). On the other hand, neutral red stained protoplasts of the live cells. The color of autofluorescence of live *P. yezoensis* cells is orange (Fig. 7), and the color of autofluorescence of dead *P. yezoensis* cells is yellow (Fig. 8). All the dyes tested did not stain cell walls of both live and dead *P. yezoensis* cells except Evans blue. The results aforementioned are summarized in Table 3.

Evans blue, phenosafranin, eosin Y and erythrosine stained protoplasts of dead male *Macrocystis pyrifera* cells, and Evans blue stained them especially deeply (Fig. 10). But these dyes did not stain protoplasts of live male *M. pyrifera* cells (Fig. 9). On the other hand, neutral red stained protoplasts of the live cells deeply (Fig. 11), but it did not stain protoplasts of the dead cells (Fig. 12). All the dyes tested did not stain cell walls of both live and dead male *M. pyrifera* cells. The results aforementioned are summarized in Table 4.

Evans blue, phenosafranin, eosin Y and erythrosine stained protoplasts of dead female *M. pyrifera* cells, and Evans blue stained them especially deeply (Fig. 14). But these dyes did not stain protoplasts of live female *M. pyrifera* cells (Fig. 13). On the other hand, neutral red stained protoplasts of the live cells deeply (Fig. 15), but it did not stain protoplasts of the dead cells (Fig. 16). All the dyes tested did not stain cell walls of both live and dead female *M. pyrifera* cells. The results aforementioned are summarized in Table 5.

Fig. 9. Live *Macrocystis pyrifera* gametophyte cells treated with Evans blue (male strain). Fig. 10. Dead *Macrocystis pyrifera* gametophyte cells treated with Evans blue (male strain). Fig. 11. Live *Macrocystis pyrifera* gametophyte cells treated with neutral red (male strain). Fig. 12. Dead *Macrocystis pyrifera* gametophyte cells treated with neutral red (male strain). Fig. 13. Live *Macrocystis pyrifera* gametophyte cells treated with Evans blue (female strain). Fig. 14. Dead *Macrocystis pyrifera* gametophyte cells treated with Evans blue (female strain). Fig. 15. Live *Macrocystis pyrifera* gametophyte cells treated with neutral red (female strain). Fig. 16. Dead *Macrocystis pyrifera* gametophyte cells treated with neutral red (female strain).

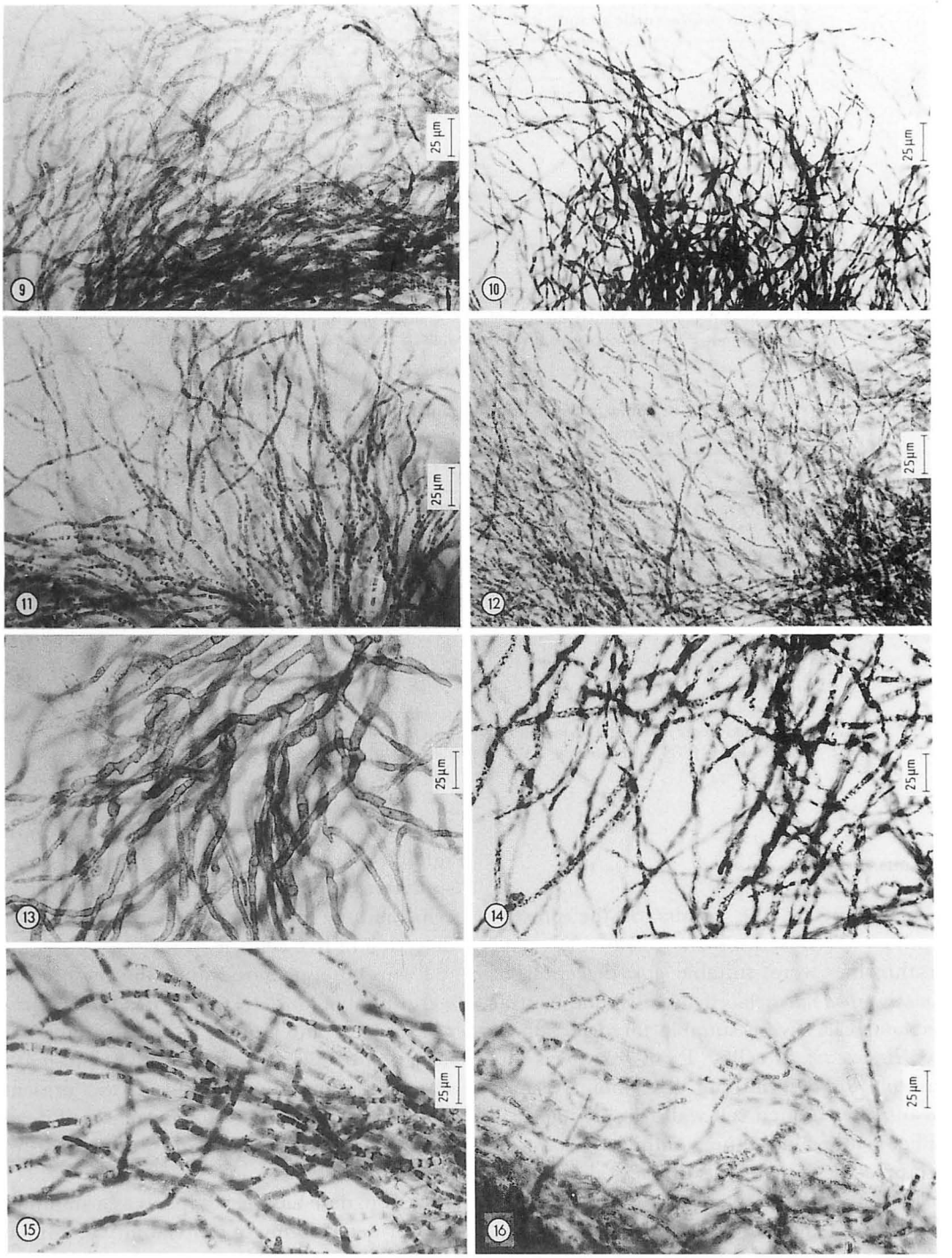

9
25 µm
10
25 µm
11
25 µm
12
25 µm
13
25 µm
14
25 µm
15
25 µm
16
25 µm

Table 4. Staining of live and dead gametophyte cells with various dyes in *Macrocystis pyrifera* (male strain).

Staining dyes	Live cells		Dead cells	
	PTS	CW	PTS	CW
Evans blue	−	−	++	−
Phenosafranin	−	−	+	−
Eosin Y	−	−	±	−
Erythrosine	−	−	+	−
Neutral red	++	−	−	−
FDA	−	−	−	−

++ stained deeply; + stained moderately; + stained lightly; ± stained infrequently; − not stained; PTS=protoplasts; CW=cell walls.

Table 5. Staining of live and dead gametophyte cells with various dyes in *Macrocystis pyrifera* (female strain).

Staining dyes	Live cells		Dead cells	
	PTS	CW	PTS	CW
Evans blue	−	−	++	−
Phenosafranin	−	−	+	−
Eosin Y	−	−	±	−
Erythrosine	−	−	+	−
Neutral red	++	−	−	−
FDA	−	−	−	−

++ stained deeply; + stained moderately; + stained lightly; ± stained infrequently; − not stained; PTS=protoplasts; CW=cell walls.

Discussion

According to the results of the present study, phenosafranin, eosin Y and erythrosine were suitable for detection of *Enteromorpha intestinalis* dead cells, and neutral red and FDA were suitable for detection of live *E. intestinalis* cells. Evans blue was unsuitable for determining cell viability, since Evans blue stained cell walls deeply and it is difficult to evaluate the staining condition of protoplasts. The most suitable staining-dyes tested in the present study were FDA for detection of the live cells and phenosafranin for detection of the dead cells in *E. intestinalis*.

Evans blue, phenosafranin, eosin Y and erythrosine were suitable for detection of dead *Porphyra yezoensis* cells, and neutral red and autofluorescence of the cells were suitable for detection of live *P. yezoensis* cells. The most suitable staining-dye tested in the present study was Evans blue for detection of dead cells, and the autofluorescence was useful for detection of live cells in *P. yezoensis*.

Evans blue, phenosafranin and erythrosine were suitable for detection of dead *Macrocystis pyrifera* cells, and neutral red was suitable for detection of live *M. pyrifera* cells. The most suitable staining-dyes tested in the present study were Evans blue for detection of dead cells and neutral red for detection live cells in *M. pyrifera*.

FDA to date has been applied as a staining-dye for determining of cell viability in animal cells (ROTMAN and PAPERMASTER 1966) and plant cells (WIDHOLM 1972, NAG and STREET 1973, WITHERS and KING 1979, SIMINOVITCH 1979). FDA probably stains live algal cells in the same manner that it does animal cells

(ROTMAN and PAPERMASTER 1966) and plant cells (WIDHOLM 1972). FDA, the nonpolar molecule, enters into the live cells where esterase cleaves off the acetate residues leaving fluorescein which then accumulates. FDA itself can not fluoresce but these fluorescein molecules can fluoresce. Neutral red has been applied as staining-dye for determining cell viability in animal cells (ETOH 1978) and plant cells (SIMINOVITCH 1979), and it probably stains live algal cells in the following manner: the live cells take up neutral red and concentrate it in their vacuoles (SIMINOVITCH 1979).

Evans blue, phenosafranin, eosin Y and erythrosine have been applied as staining-dyes for determining cell viability in many kinds of plant cells (GAFF and OKONG'O-OGOLA 1971, WIDHOLM 1972, HAUPTMANN and WIDHOLM 1982). These staining-dyes stain only dead protoplasts and are probably excluded from live protoplasts by the intact plasma membrane (WIDHOLM 1972).

The color change of the autofluorescence in *Porphyra* cells probably represents fluorescence from the red algal pigment phycoerythrin (POLNE and GIBOR 1982).

In the present study, a rapid, simple and reliable technique for determining cell viability was established employing the CVC-kit in the marine macroalgae. Although we have applied this method to a few species of economically valuable marine algae, it should be useful with other marine macroalgae.

Acknowledgement

We wish to express our thanks to Dr. M. KAILL, Department of Fish and Game, State of Alaska, U.S.A., for his critical reading of the manuscript.

References

ETOH, K. 1978. Cell culture of fishes. Heredity **32(7)**: 64–71. (in Japanese)

GAFF, D. F. and OKONG'O-OGOLA, O. 1971. The use of non-permeating pigments for testing survival of cells. J. Exp. Bot. **22**: 756–758.

HAUPTMANN, R. M. and WIDHOLM, J. M. 1982. Cryostorage of cloned amino acid analog-resistant carrot and tobacco suspension cultures. Plant Physiol. **70**: 30–34.

MIGITA, S. 1967. Viability and spore-liberation of *Conchocelis*-phase, *Porphyra tenera*, freeze-preserved in seawater. Bull. Fac. Fish. Nagasaki Univ. **22**: 33–43.

NAG, K. K. and STREET, H. E. 1973. Carrot embryogenesis from frozen cultured cells. Nature **245**: 270–272.

OGATA, E. 1956. On the use of tetrazolium salt as a viability indicator for *Conchocelis* filaments-I. Investigation of conditions affecting the TTC reaction. Bull. Japan. Soc. Sci. Fish. **22**: 94–98.

POLNE, M. and GIBOR, A. 1982. The effect of high intensity U.V. radiation on benthic marine algae. p. 573–579. *In* J. CALKINS [ed.], The role of solar ultraviolet radiation in marine ecosystems. Plenum Publ., New York.

ROTMAN, B. and PAPERMASTER, B. W. 1966. Membrane properties of living mammalian cell as studied by enzymatic hydrolysis of fluorogenic esters. Proc. Natl. Acad. Sci. U.S. **55**: 134–141.

SAGA, N. and SAKAI, Y. 1982. A new method for pure culture of macroscopic algae, one step selection method. Jpn. J. Phycol. **30**: 40–43.

SAKAI, A. and SUGAWARA, Y. 1978. Survival of plant germplasm in liquid nitrogen. p. 345–359. *In* P. H. LI and A. SAKAI [eds.], Plant cold hardiness and freezing stress-mechanisms and crop implications. Academic Press, New York.

SIMINOVITCH, D. 1979. Protoplasts surviving freezing to −196°C and osmotic dehydration in 5 molar salt solutions prepared from the bark of winter black locust trees. Plant Physiol. **63**: 722–725.

TERUMOTO, I. 1960. Frost-resistance in a marine alga *Ulva pertusa* KJELLMAN. Low Temp. Sci. Ser. B **18**: 35–38.

WIDHOLM, J. M. 1972. The use of fluorescein diacetate and phenosafranin for determining cell viability of cultured plant cells. Stain Technol. **47**: 189–194.

WITHERS, L. A. 1980. Preservation of germplasm. Intl. Rev. Cytol. **11B** (Suppl.): 101–136.

WITHERS, L. A. and KING, P. J. 1979. Proline: A novel cryo-protectant for the freeze preservation of cultured cells of *Zea mays* L. Plant Physiol. **64**: 675–678.

嵯峨直恒・坂西芳彦・荻島　隆：大型海産藻類の細胞生存率の簡易評価法

細胞の生存率を正確かつ簡便に評価することは，海藻の組織培養の種々の局面において必要な技術である。ボウアオノリ，マクロシスティス・ピリフェラ，スサビノリの緑褐紅にわたる有用海藻の無菌培養細胞を材料として，種々の染色剤による生細胞と死細胞の染め分けを検討した。ボウアオノリ（配偶体）では生細胞の検出にはフルオレセインジアセテートが，死細胞の検出にはフェノサフラニンが有効であった。マクロシスティス・ピリフェラ（配偶体）では生細胞の検出にはニュートラルレッドが，死細胞の検出にはエバンスブルーが有効であった。スサビノリ（コンコセリス）では生細胞の検出にはフィコエリスリンの自家蛍光が，死細胞の検出にはエバンスブルーが有効であった。また，染め分けに有効な数種の染色剤の入ったマルチプレート CVC-kit（細胞生存率評価キット）を作製し，大型海産藻類の細胞生存率の簡易評価法を開発した。(085 北海道釧路市桂恋116　水産庁北海道区水産研究所)

Jpn. J. Phycol. (Sôrui) 37: 137–143, June 20, 1989

Examination of the type material of *Diploneis boldtiana* Cl. (Bacillariophyceae)

Masahiko Idei* and Hiromu Kobayasi**

*Institute of Biological Sciences, University of Tsukuba, Tsukuba-shi, Ibaraki, 305 Japan
**Department of Biology, Tokyo Gakugei University, Koganei-shi, Tokyo, 184 Japan

Idei, M. and Kobayasi, H. 1989. Examination of the type material of *Diploneis boldtiana* Cl. (Bacillariophyceae). Jpn. J. Phycol. 37: 137–143.

The type material of *Diploneis boldtiana* Cl. in the P. T. Cleve Collection was examined by LM and SEM together with recent material from Finnish lakes. *D. boldtiana* has following features; the outer wall of the alveolus is composed of double rows of areolae, but near the margin it has three rows of areolae; the last two alveoli are wide, and form a triangular shape; a small round opening is present at the margin of each alveolus.

Key Index Words: *Diatom*—Diploneis—Diploneis boldtiana—*fine structure*—*type material.*

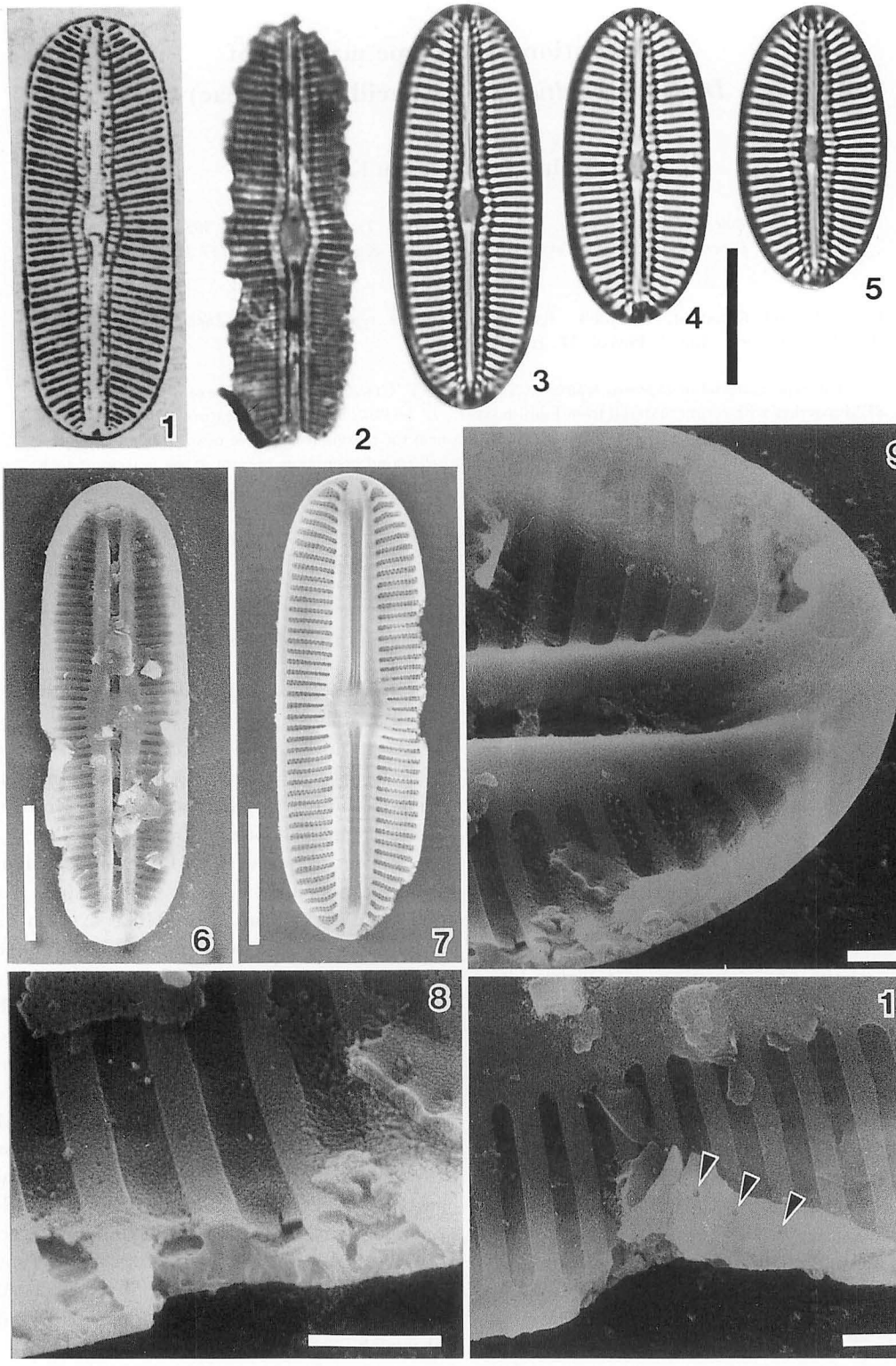
The first total systematic treatment of the genus *Diploneis* was accomplished by Cleve (1894) who described many new species and transferred many taxa to this genus, mainly from *Navicula*. The name *Diploneis* was first given by Ehrenberg (1844). *Diploneis boldtiana* was also described by Cleve (1891) from Finnish material (northern Savolaks) collected by R. Boldt. Since these early reports, information about *D. boldtiana* has come mostly from Finland (Hustedt 1937, Mölder and Tynni 1973, Krammer and Lange-Bertalot 1986). We have already reported the fine structure of *D. parma* Cl., which was described at the same time with *D. boldtiana* in Cleve's paper of 1891, based on the type material (Idei & Kobayasi 1988a). Cleve (1891) examined only a few specimens for his original description. We also examined a part of the same sample, but unfortunately found only one valve. However, we were able to get some valuable information in characterizing this taxon from this specimen. We have also found many specimens from three Finnish lakes and have been able to carry out more detailed investigations.

Material and Methods

The material used in this observation is listed as follows:
(1) Unmounted type material from Lake Viando (Sb. 4, No. 12:4, Savonia borealis, Viando) housed in the Swedish Museum of Natural History, Stockholm.
(2) Recent material from Finland: sediment from Lake Pääjärvi on 11 August 1986, K-6331; sediment from Lake Mekrijärvi on 12 August 1986, K-6338; sediment from Lake Isolampi on 12 August 1986, K-6345.

These specimens were treated with sulphuric acid and potassium nitrate to remove organic matter, and then washed in distilled water. For LM, the cleaned valves were embedded in Pleurax. Valves for SEM were prepared by air drying on glass coverslips which were then fixed to metal stubs. They were coated with gold-palladium using a Fine Coat JFC-1100 and observed with a JEOL-F15 field emission type SEM. The specimens for TEM were placed on formvar-coated 50-mesh copper grids and observed using a JEOL-100C.

The terminology follows Anonymous


1
2
3
4
5
6
7
9
8
10

(1975), Ross *et al.* (1979), von Stosch (1975) and Mann (1981).

Observations and Discussion

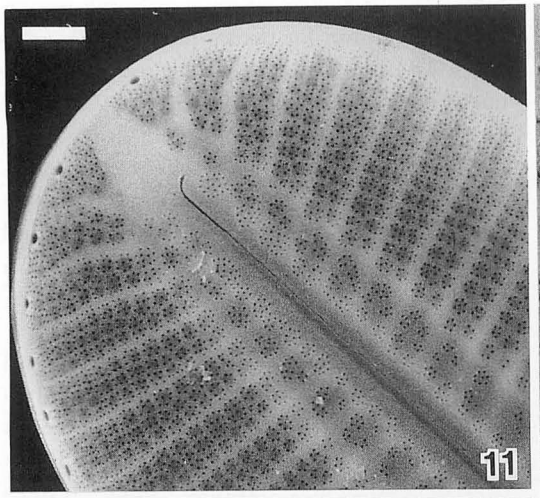
The type material of *D. boldtiana* in the Swedish Museum was very scarce and we were able to examine only a small part of it. Only one valve specimen was found (Figs. 2, 6, 8–10). It is linear-elliptic, 30 μm long and 11 μm wide. The longitudinal canals are narrow and almost straight except at the central area. The transapical striae are 14 in 10 μm. These values are fully consistent with the original description and figure (Fig. 1). Valves in the recent material from Finnish lakes are 20–30 μm long and 11–12 μm wide (Figs. 3–5). There is a small range of variation of the valve width. Valve shape is linear-elliptic in large specimens and elliptic in small ones. The striae are constantly 13 or 14 in 10 μm. They are parallel near the center and gradually radiate toward the apices, though in the original figure they are radiate throughout the valve. The last stria is parallel to the apical axis. We regard this feature as one of the criteria for this taxon. According to Hustedt (1937) the striae are composed of two rows of areolae (27–30 in 10 μm), but they are not visible by LM. However, TEM and SEM observations showed that the striae are basically two rows of areolae, as mentioned by Hustedt (1937), but the number of the areolae is denser (35–38 in 10 μm) than that of the Hustedt's description. This density is beyond the resolving power of LM.

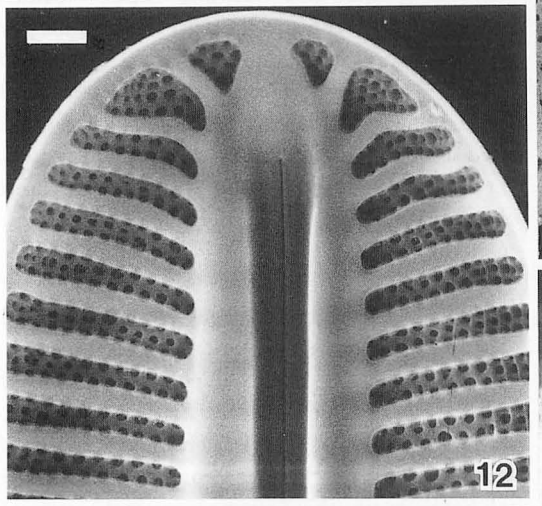
The specimen obtained from type material was observed without acid treatment, so it was covered with a lot of inorganic and organic material, and the external surface was almost entirely covered with organic material. However, it gave us some important information for taxonomy (Figs. 2, 6, 8–10). The longitudinal canals are constantly narrow over their length as seen by LM, and do not have openings on their inner walls (Figs. 6, 7). In *Diploneis* generally, transapical costae are straight and slightly curved near the poles. In *D. boldtiana* the second costa from the last is strongly curved. As a result the last two alveoli are apparently wide, and form a triangular shape (Figs. 9, 12).

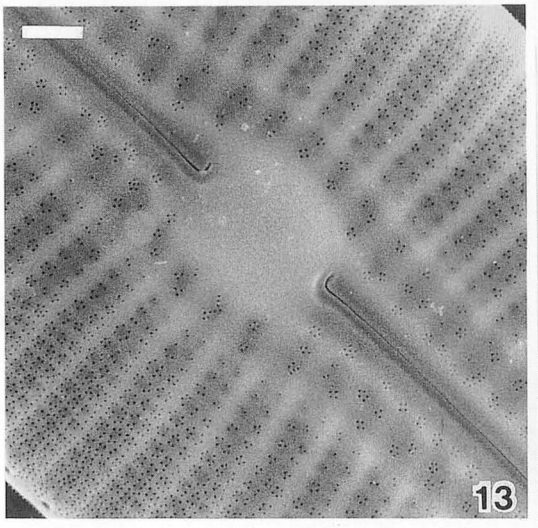
The external raphe fissure is narrow and straight. The central and terminal fissures are short and curved at nearly right angles in the same direction (Figs. 11, 13). In *Diploneis* curved central and terminal raphe endings have not been reported except marine species *D. crabro* (Sims and Paddock 1979, Navarro 1982). *D. oculata, D. minuta* (Idei and Kobayasi 1986) and *D. marginestriata* (Idei and Kobayasi 1988b) have straight fissures at both ends. *D. papula* (Gerloff and Helmcke 1975) and *D. finnica* have a straight central fissure and a curved terminal one. The internal raphe fissure is narrow straight slits which terminate simply at both central and terminal ends (Fig. 12).

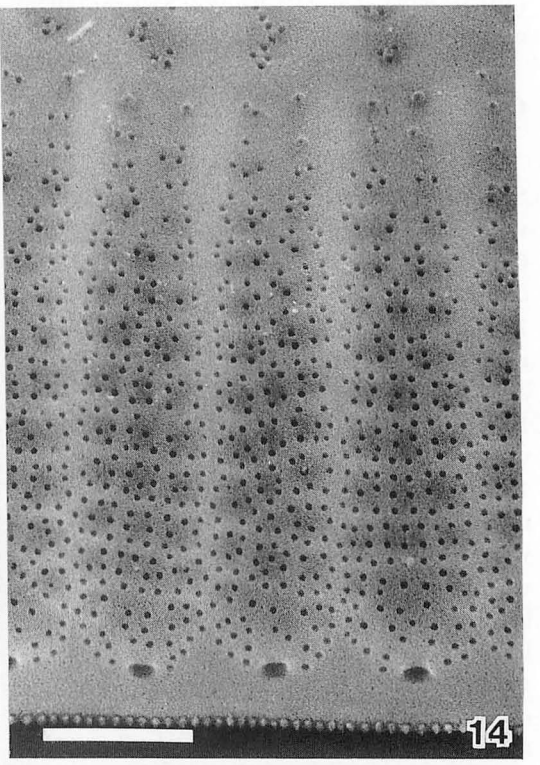
The outer wall of the alveolus is composed of double rows of areolae (Figs. 12, 15, 17, 18), but near the margin it has three rows of areolae (Fig. 12). Moreover, the second alveolus from the last has several rows of areolae (Fig. 12). The outside of each areola is occluded by a cribrum (Figs. 11, 13, 14). The perforations of the cribra are densely and regularly scattered, but those of the central region surrounding the central nodule are scarce due to the small areolae (Figs. 13, 14, 18). Externally the hyaline lines showing the boundaries of alveolus are present, even though they are not apparent at the margin (Figs. 11, 14), as seen in *D. parma* (Idei and

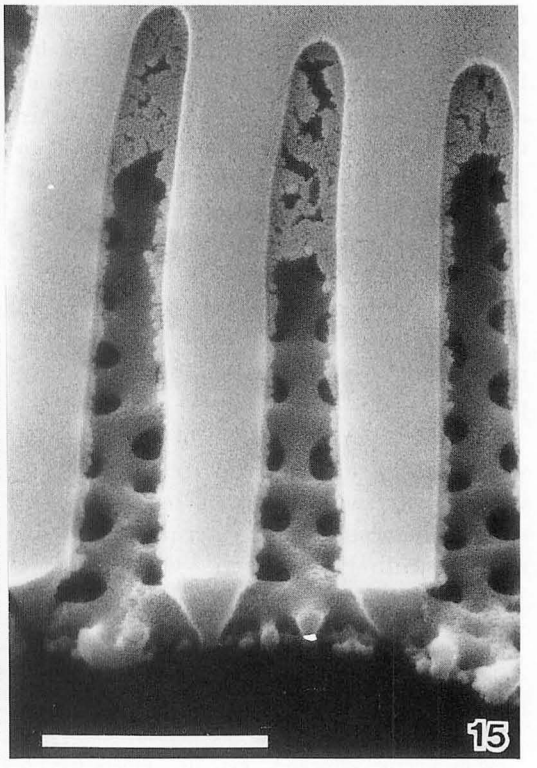
Fig. 1. P. T. Cleve's original figure of *Diploneis boldtiana*. Fig. 2. Light micrograph of the isotype specimen coated with gold-palladium used for SEM observation. Sb. 4. Fig. 3. The large linear-elliptic valve. K-6345. Fig. 4. The medium sized valve. K-6331. Fig. 5. The small elliptic valve. K-6345. Fig. 6. Internal view of the valve which is the same as Fig. 2. Fig. 7. Internal view of the valve. K-6345. Fig. 8. Enlargement of Fig. 6 showing the broken edge of the alveoli and transapical costae. Fig. 9. Enlargement of Fig. 6 showing the internal valve end. Fig. 10. Enlargement of Fig. 6 showing the openings of alveoli at the valve margin (arrowheads). Bar in Figs. 6, 7=10 μm, in Figs. 9, 10=1 μm. Figs. 6–10. SEM.


11


12


13


14


15

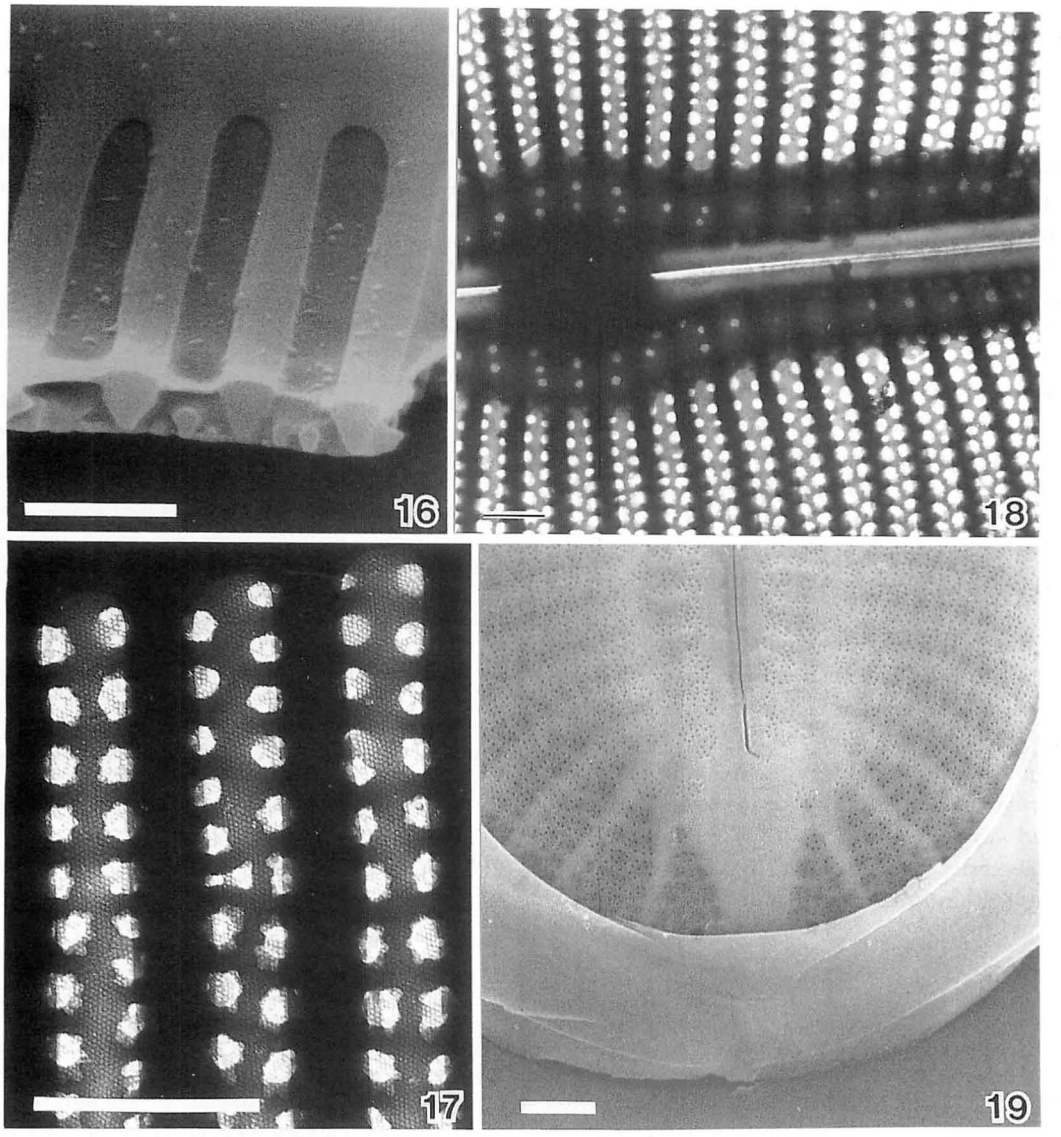


Fig. 16. Internal broken valve showing the inner occlusion of the alveoli. K-6345. Fig. 17. The inner occlusions with perforations arranged in an hexagonal array. TEM. K-6345. Fig. 18. Smaller areolae of the alveoli surround the central nodule. TEM. K-6345. Fig. 19. External valve end showing the open ends of the valvocopula and the ligula of the pleura. K-6331. Bars=1 μm.

KOBAYASI 1988a).

A small round opening is present externally at the margin of each alveolus (Fig. 10 arrowhead, Figs. 11, 14). We regard this feature as one of the important taxonomic criteria for this species. The same feature,

Fig. 11. External valve end showing the curved terminal raphe fissure. K-6331. Fig. 12. Internal valve end showing the alveoli lacking inner occlusions, and the two sets of triangular-shaped alveoli at the valve pole. K-6345. Fig. 13. External central valve showing the curved central raphe fissures. K-6331. Fig. 14. Detail of the valve showing the areolae occluded by cribra and the opening at the end of each alveolus. K-6331. Fig. 15. Internal valve showing the detail of a broken edge of the transapical costae, and the two rows of areolae and the eroded innner occlusions of the alveoli. K-6345. Bars=1 μm.

but different in shape, is present in *D. finnica* (manuscript under review).

Marginal granules are also present at the valve margin (Fig. 14). There are 12 or 13 granules in 1 μm as in *D. parma* (IDEI and KOBAYASI 1988a). The shape of the transapical costa in cross-section is obovate, being narrow externally and broadly rounded internally (Figs. 8, 15, 16). Internally each alveolus is occluded by a thin siliceous layer with perforations arranged in a hexagonal array (Figs. 10, 16, 17). This inne occlusion of the alveolus is attached to the widest portions of the transapical costae. The cingulum is composed of two bands, one valvocopula and one pleura (Fig. 19), but their details were not observed.

Acknowledgements

Special thanks are due to Prof. N. LUNDQVIST of the Swedish Museum of Natural History for his generous help in providing original material from the P. T. CLEVE Collection. We also thank Prof. M. CHIHARA of the University of Tsukuba for his encouragement and advice, and Dr. R. W. RIDGE of the University of Tsukuba for correcting our English and making helpful comments.

References

ANONYMOUS 1975. Proposals for a standardization of diatom terminology and diagnoses. Nova Hedwigia Beih. **53**: 323–354.

CLEVE, P. T. 1981. The diatoms of Finland. Acta Societatis pro Fauna et Flora fennica. **8**(2): 1–68, pl. 1–3.

CLEVE, P. T. 1894. Synopsis of the naviculoid diatoms. Kongl. Sv. Vet. Akad. Handl. **26**(2): 1–194. pl. 1–5.

EHRENBERG, C. G. 1844. Mitteilung über 2 neue Lager von Gebirgsmassen aus Infusorien als Meeres-Absatz in Nord-Amerika und eine Vergleichung derselben mit den organischen Kreide-Gebilden in Europa und Afrika. Ber. Akad. Wissensch. Berlin **1844**: 57–97.

GERLOFF, J. and HELMCKE, J. -G. 1975. Der Feinbau der Schalen von *Diploneis papula* (A.S.) CLEVE, *Diploneis smithii* (BRÉB.) CLEVE und *Diploneis parca* (A.S.) BOYER. Willdenowia **7**: 539–563.

HUSTEDT, F. 1937. Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete. *In* L. RABENHORST [ed.], Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. **7**(2): 578–718. Akad. Verlag, Leipzig.

IDEI, M. and KOBAYASI, H. 1986. Observations on the valve structure of fresh water *Diploneis* (Bacillariophyceae), *D. oculata* (BRÉB.) CLEVE and *D. minuta* PETERSEN. Jap. J. Phycol. **34**: 87–93.

IDEI, M. and KOBAYASI, H. 1988a. Examination of the type specimens of *Diploneis parma* CL. p. 397–403. *In* F. E. ROUND [ed.], Proc. 9th Internat. Diatom Symp. Biopress, Bristol.

IDEI, M. and KOBAYASI, H. 1988b. A light and electron microscopic study of the benthic diatom *Diploneis marginestriata* HUST. (Bacillariophyceae). Jpn. J. Phycol. **36**: 277–284.

KRAMMER, K. and LANGE-BERTALOT, H. 1986. Bacillariophyceae. 1 Teil. Naviculaceae. *In* H. ETTL, J. JERLOFF, H. HEYNIG, D. MOLLENHAUER [eds.], Süsswasserflora von Mitteleuropa, **2/1**. Gustav Fischer, Stuttgart. 876pp.

MANN, D. G. 1981. Sieves and flaps: siliceous minutiae in the pores of raphid diatoms. p. 279–300. *In* R. Ross [ed.], Proc. 6th Symp. Fossil and Recent Diatoms. Otto Koeltz, Koenigstein.

MÖLDER, K. and TYNNI, R. 1973. Über Finlands rezente und subfossile Diatomeen VII. Bull. Geol. Soc. Finland **1973**(45): 159–179.

NAVARRO, J. N. 1982. A survey of the marine diatoms of Puerto Rico. V. Suborder Raphidineae: Families Achnanthaceae and Naviculaceae (Excluding *Navicula* and *Mastogloia*). Botanica Marina **25**: 321–338.

ROSS, R., COX, E. J., KARAYAVA, N. I., MANN, D. G., PADDOCK, T. B. B., SIMONSEN, R. and SIMS, P. A. 1979. An amended terminology for the siliceous components of the diatom cell. Nova Hedwigia Beih. **64**: 513–533.

SIMS, P. A. and PADDOCK, T. B. B. 1979. Observations and comments on some prominent morphological features of naviculoid genera. Nova Hedwigia Beih. **64**: 169–191.

VON STOSCH, H. A. (1975). An amended terminology of the diatom girdle. Nova Hedwigia Beih. **53**: 1–35.

出井雅彦*・小林　弘**：*Diploneis boldtiana* Cl. のタイプ材料の調査

スウェーデンの自然史博物館所蔵の P. T. Cleve のコレクションの中の *Diploneis boldtiana* のタイプ材料を光顕及び走査電顕を用いて調査した。また同時にこの種の原産国であるフィンランドの現生の試料についても調査した。その結果以下のような特徴がみられた。長胞条線の外壁は 2 列の胞紋からなるが，殻縁近くでは 3 列となる。殻端の 2 つの長胞条線は特に広く三角形となる。各長胞の外側の殻縁側末端には 1 個の開口がある。(*305 茨城県つくば市天王台1-1-1　筑波大学生物科学系　**184 東京都小金井市貫井北町4-1-1　東京学芸大学生物学教室)

新刊紹介

ABBOTT, I. A. (Ed.): Taxonomy of Economic Seaweeds with Reference to Some Pacific and Caribbean Species, vol. II. xv＋264pp. California Sea Grant College Program, University of California, La Jolla, California. 1988. $10.00

亜熱帯と熱帯に生育する有用海藻の分類学に関する国際的なワークショップが開かれていることを御存じの方も多いと思われる。第1回は1984年にグアム島で開かれ，翌年に論文集が出版されている。第2回は中国の青島で1986年に開催された。本書はそのワークショップの成果を纏めたもので，昨年末に出版された。今回のワークショップにはアメリカ合衆国（6名），チリ（1名），中国（5名）及び日本（吉田忠生博士1名）から研究者が参加している。

今回は褐藻ホンダワラ属ホンダワラ亜属，紅藻テングサ目，オゴノリ属・*Polycavernosa* 属，キリンサイ属・新たに提唱された *Kappaphycus* 属及びソゾ属の5グループが扱われている。ソゾ属は第1回のワークショップでは扱われていないが，最近寒天を生成する種がこの属に見つかったことで今回加えられた。何れのグループも多くの種が記載されているが，種の把握も十分でない場合があることから，命名上と分類学上の諸問題を生じており，それらを解決する試みとしてこのワークショップが企画された。このようなグループにおいては，ある地域のみを対象にした個人的な研究では自ずから限界があり（あるいは新たな問題を生じかねないが），それぞれの専門家が標本とデータを持ち寄って討議を行うことは，そのような問題解明に向けて大変望ましいことである。その成果として，vol. I に収録された *Polycavernosa* 属の確立や様々な海域から報告されてきた *Gracilaria verrucosa* の異同の確認が挙げられよう。

本書では先に述べた5グループが5章に分かれて報じられている。第Ⅰ章では日本・台湾，中国，ミクロネシア，及びハワイ海域のホンダワラ亜属が扱われ，それぞれの海域ごとに分かりやすい総説的な論文として発表されている。*Zygocarpicae* 節に限って報告した TSENG & LU の論文は力作である。第Ⅱ章では中国と台湾海域のテングサ目が扱われているが，チリの SANTELICES は病気のため実際のワークショップには出席出来ずに，論文のみの参加になっている。そのためにほぼ同じ海域のテングサ目が，別々の著者によって論文として発表されている。このグループについては，今年 La Jolla で開かれる第3回のワークショップに期待しよう。RODRÍGUIZ & SANTELICES のテングサ属とオバクサ属の区別に関する論文も収録されており，今後の問題提起として興味深い内容を含んでいる。オゴノリ属と *Polycavernosa* 属に関する3篇を含む第Ⅲ章は vol. I の補遺的な意味が強い。また，vol. I のオゴノリ属に関する章は繁雑すぎて分かりにくい。この属全体についての纏まった総説をこのワークショップに期待したい。第Ⅳ章では2名の論文参加者によってキリンサイ属と *Kappaphycus* 属が扱われている。DOTY と CHENEY の2篇はいずれも優れた論文であるが（前者のアマクサキリンサイの命名上の記述には問題がある），このような参加形式はワークショップ本来の意義と矛盾する。第Ⅴ章ではハワイ諸島と中国沿岸のソゾ属が扱われている。本属の分類学に多くの貢献をされている斎藤譲博士が参加されなかったのは残念である。斎藤氏はハワイ諸島のソゾ属の論文を発表され（今回の MCDERMID の論文との間にはいくつかの異同が認められる），中国沿岸には日本との共通種も多い。この章の MCDERMID による Introduction はソゾ属の分類学的研究の簡潔な総説として優れている。

本書ではまた多くの新分類群が記載されており，文献としての価値も高い。残念ながらいくつかについては，解剖図もなく現代の新分類群の発表形式として望ましくない。折角の試みを安易な新分類群の発表の場として欲しくない。

苦言も呈したが，分かりやすい総説的な論文と優れた原著論文が含まれており，これらのグループの専攻以外の方にもご一読をお勧めする。

注文は下記宛に直接されたい。Check あるいは money order で送金可能である。

Communications Department
Californnia Sea Grant College Program
University of California, San Diego A-032
La Jolla, California 92093-0232
U. S. A.

（北海道大学理学部植物学教室　増田道夫）

Jpn. J. Phycol. (Sôrui) 37: 145–149, June 20, 1989

日本新産紅藻ヒメベニヤバネグサ（新称）*Membranoptera spinulosa* (RUPRECHT) KUNTZE（紅藻　コノハノリ科）について

松本正喜・吉田忠生

北海道大学理学部植物学教室（060　札幌市北区北10条西8丁目）

MATSUMOTO, M. and YOSHIDA, T. 1989. On *Membranoptera spinulosa* (RUPRECHT) KUNTZE (Delesseriaceae, Rhodophyta) new to Japan. Jpn. J. Phycol. 37: 145–149.

Membranoptera spinulosa (RUPRECHT) KUNTZE is reported from Nemuro Peninsula, eastern part of Hokkaido in Japan. The thalli are small, marginally and alternately branched with conspicuous midribs and with dentate and crisped margins. Intercalary cell divisions are absent in every cell row. Initials of third-order cell rows do not always reach the margin. Alae are monostromatic and provided with microscopic side-veins. Procarps are born on the primary cell rows. Each procarp is composed of a carpogonial branch and two groups of sterile cells. Cystocarps are arranged on surface of midrib. Carposporangia are formed in chain. Tetrasporangial sori extend from ultimate branchlets to main branches, except the midrib and marginal portion.

Key index words: *Delesseriaceae—Hokkaido—Japan*—Membranoptera spinulosa—*Rhodophyta.*
Masaki Matsumoto and Tadao Yoshida, Department of Botany, Faculty of Science, Hokkaido University, Sapporo, 060 Japan

北海道東部の根室半島太平洋岸において小型のコノハノリ科の一種を採集した。この藻体の生長点やプロカルプ，嚢果，四分胞子体などの形態的特徴から，コノハノリ亜科，ベニヤバネグサ群の *Membranoptera spinulosa* (RUPRECHT) KUNTZE と同定した。この種はカムチャッカ半島やアリューシャン列島など北太平洋から報告されているが (WYNNE 1970)，日本からの報告はない。また，NAGAI (1941) が，千島列島から *M. denticulata* (MONTAGNE) KYLIN として記録している藻体は，腊葉標本の比較によりこの種とは別種と認められたので，*Membranoptera spinulosa* を日本新産種とし，和名としてヒメベニヤバネグサを提案する。

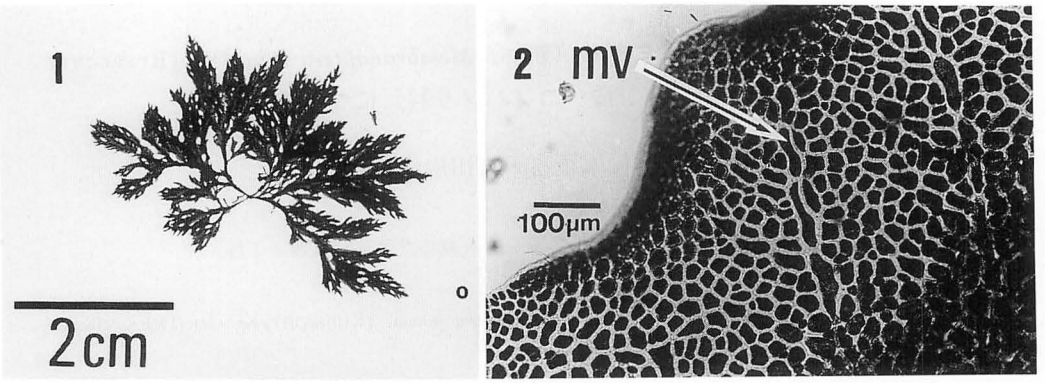
材　　料

観察には根室半島先端部の納沙布岬で1978年5月に打揚げとして得た雌性配偶体と，近接したトリトエウス沖で1988年4月に潜水により得た貝の体上に付着していた雄性配偶体，雌性配偶体，四分胞子体を用いた。外部形態の観察に用いた腊葉標本は北大理学部の標本室に保管されている (SAP 034546, SAP 051704)。

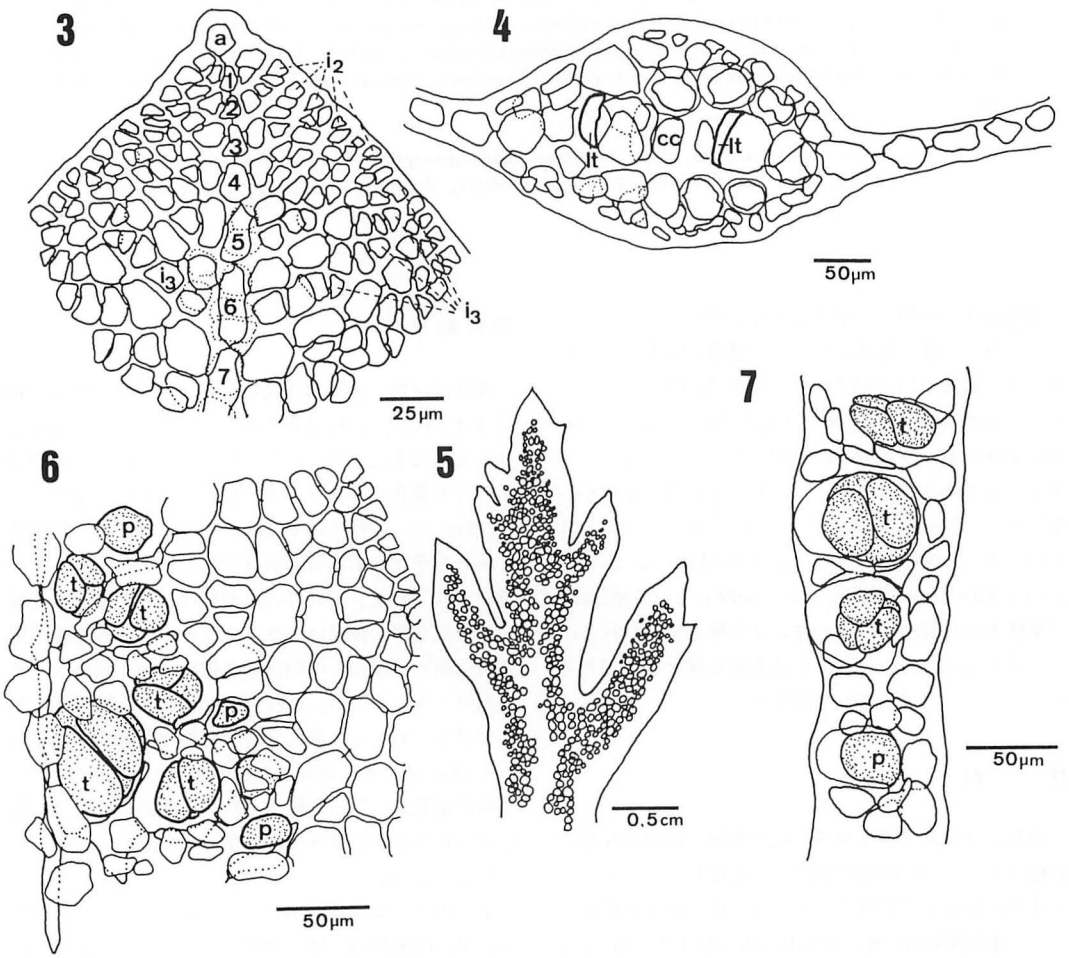
観察結果

藻体は紅色，小型で体高数 mm から 2 cm 程度，幅は 1–1.5 mm で，枝は互生的に縁辺から生じ，藻体全体に明瞭な中肋が見られる。中肋の両側には一細胞層からなる翼状部があり，その縁辺には鋭い鋸歯がみられ (Fig. 1)，藻体下部では縮れている。雌雄の配偶体と四分胞子体の末端枝の形態を比較すると，幾らかの違いがみられた。配偶体の末端枝は，その幅が広く縁辺はほぼ全縁で鋸歯は小さく少ない。四分胞子体の末端枝は細く，縁辺には明らかな鋸歯が上に向かって狭い角度で生じている。翼状部には中肋から縁辺部にかけて単条，もしくは稀に分岐する顕微鏡的側脈が見られる (Fig. 2)。中肋部分は中心細胞をとりまく髄層と，小型の細胞からなる皮層があり，髄層細胞にはソゾ属に見られるような半月型の肥厚 (lt) が認められるものがある (Fig. 4)。

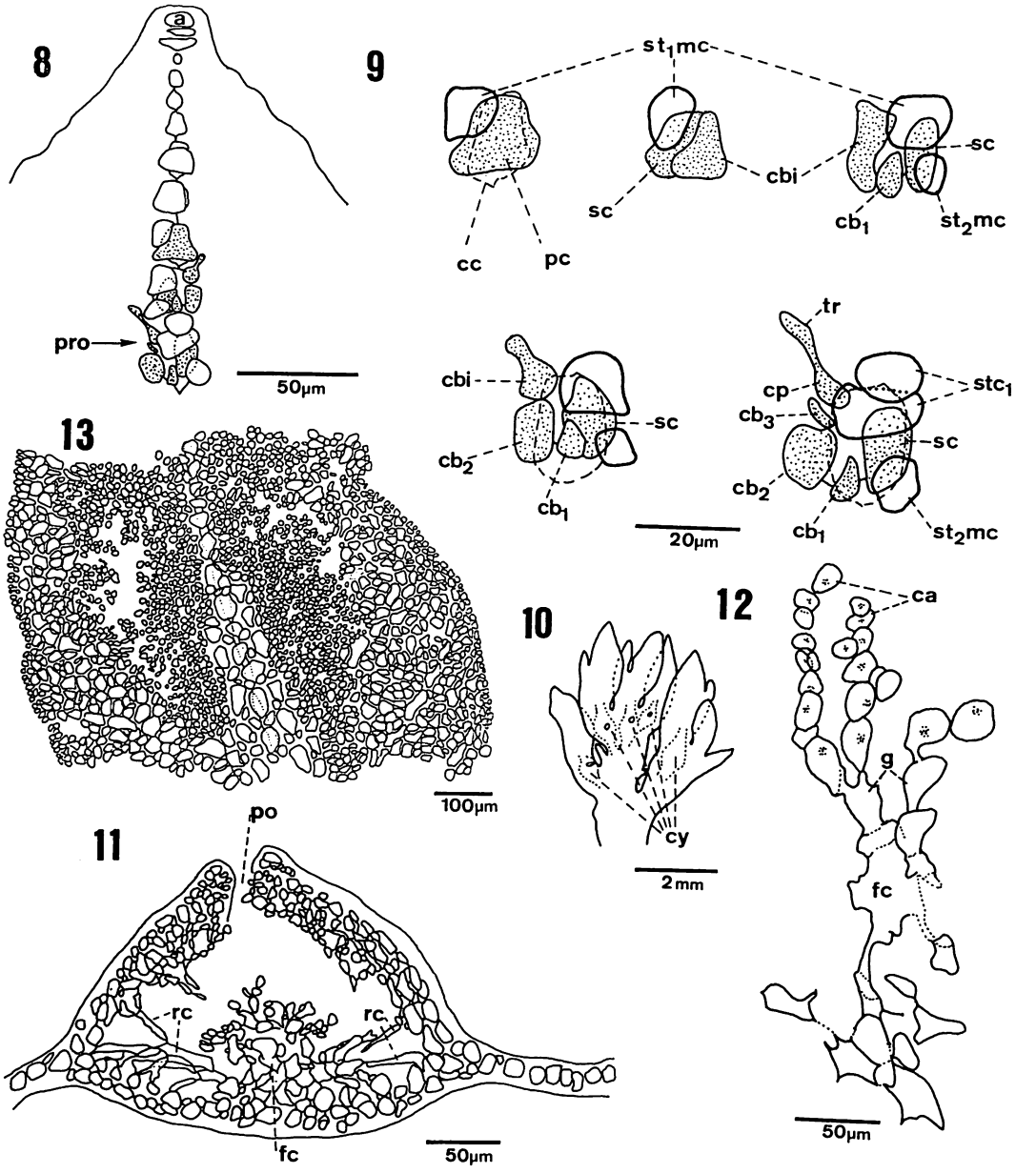
生長点では頂端細胞 (a) が横に関節し，第一位細胞列，第二位細胞列，第三位細胞列のいずれの細胞列にも介生分裂は見られない。そして，第二位細胞列の initial cell (i_2) はその全てが縁辺に達するが，第三位細胞列の initial cell (i_3) は，必ずしも縁辺に達するとは



Figs. 1–2. *Membranoptera spinulosa* (RUPRECHT) KUNTZE. 1. Tetrasporophyte (SAP 051704) collected from Nemuro Peninsula. 2. Microscopic side-vein on alae. mv, microscopic side-vein.



Figs. 3–7. *Membranoptera spinulosa*. 3. Apex of thllus showing apical segmentation. 4. Cross section of thallus. 5. Apical part of thallus with tetrasporangial sorus. 6. Surface view of tetrasporangial sorus. 7. Longitudinal section of thallus with tetrasporangia. a, apical cell; cc, central cell; i_2, i_3, initials of second- and third-order cell rows; lt, lenticular thickening; p, tetrasporangial primordium; t, tetrasporangium.



Figs. 8–13. *Membranoptera spinulosa*. 8. The procarps arranged on the primary cell row. 9. Stages in development of procarps. 10. Apical portion of female thallus with cystocarps. 11. Cross section of young cystocarp. 12. Carposporangia formed in chain. 13. Surface view of young spermatangial sorus. a, apical cell; ca, carposporangium; cb_{1-3}, first, second and third cell of carpogonial branch; cbi, initial cell of carpogonial branch; cc, central cell; cp, carpogonium; cy, cystocarp; fc, fusion cell; g, gonimoblast; pc, pericentral cell; po, ostiole; pro, procarp; rc, rhizoidal cell; sc, supporting cell; st_1mc, st_2mc, mother cells of first and second groups of sterile cells; stc_1, first group of sterile cells; tr, trichogyne.

限らない (Fig. 3)。

四分胞子嚢斑は線状で，末端枝から主枝にかけて縁辺を除く中肋の両側に，求頂的に密な状態で形成される (Fig. 5)。四分胞子嚢とその原基は第二位細胞列，第三位細胞列の primary cell から切り出され，皮層細胞と同等の位置を占めるため皮層細胞に覆われること

なく形成される。四分胞子嚢の分裂様式は原則的に三角錐型である (Figs. 5, 6)。

プロカルプは第一位細胞列上に形成される (Fig. 8)。その発達過程を Fig. 9 に示した。第一位細胞列上に生じた周心細胞 (pc) から，まず，第一次中性母細胞 (st_1mc) が切り出され，その後，周心細胞は支持細胞 (sc) と造果枝母細胞 (cbi) に分裂する。支持細胞からはさらに第二次中性母細胞 (st_2mc) が生ずる。造果枝母細胞は，造果枝の細胞を cb_1, cb_2 の順に切り出し，最後に cb_3 と造果器 (cp) を作り，4 細胞性の造果枝を完成する。第一次中性母細胞は受精前に分裂し，プロカルプは 2 群 3 個の中性細胞を伴うことが多い。嚢果は末端枝の中肋上にできる (Fig. 10)。中央部に融合細胞 (fc) があり，この細胞から出る造果枝 (g) から，果胞子嚢 (ca) が鎖状に連なって形成される (Figs. 11, 12)。融合細胞をささえる細胞からは仮根状細胞 (rc) が伸び，皮層細胞に連絡している (Fig. 11)。

雄性配偶体は雌性配偶体に比べて小さく，体高は 5 mm, 幅 0.5 mm 程度しかない。精子嚢斑は藻体中部に中肋と縁辺を除いて生じる。今回得られた材料では精子嚢の原基は出来ていたが，成熟したものは非常に少なかった (Fig. 13)。

考　察

プロカルプを第一位細胞列上に生じ，いずれの細胞列にも介生分裂がなく，第三位細胞列の initial cell が必ずしも縁辺に達しないなどの特徴から，この藻はコノハノリ亜科，ベニヤバネグサ群に属する。さらに嚢果を直接体上に生じ，一細胞層の翼状部をもつのでベニヤバネグサ *(Membranoptera)* 属であることが認められた (WYNNE 1983)。ベニヤバネグサ属は，北半球北部より12種類報告されており，種のレベルで区別する形質として，縁辺の形態，鋸歯の形状，顕微鏡的側脈の有無，藻体のサイズ，中肋の状態，四分胞子嚢斑の形態などが用いられる (WYNNE 1970)。このうち，縁辺の鋸歯と顕微鏡的側脈のあるものとしては，*M. spinulosa, M. serrata* (POSTELS et RUPRECHT) ZINOVA, *M. denticulata* の 3 種類がある (MONTAGNE 1849, ZINOVA 1965, WYNNE 1970)。*M. denticulata* は，藻体のサイズが大型で，縁辺も縮れず，四分胞子嚢斑は，末端枝にのみ形成され，根室産 *Membranoptera* とは異なる (RUPRECHT 1851, HARVEY 1853, KÜTZING 1866) (Table 1)。ZINOVA (1965) は，*M. spinulosa* を *M. serrata* から，藻体のサイズが小型であることと，中肋が細いことで区別し，四分胞子嚢斑の位置については，*M. serrata* で全ての順位の枝に形成されると記載している。一方，RUPRECHT (1851) は，*M. spinulosa* の四分胞子嚢斑が先端から離れて形成されることを指摘している。根室産 *Membranoptera* は，藻体が極めて小型であり，細い中肋をもちながら，四分胞子嚢斑が末端枝にも形成されるなど，*M. spinulosa* と *M. serrata* との中間的な位置にある。WYNNE (1970) は，両種の中間的な形質を持つ標本を得ていることと，*M. spinulosa* の中肋の状態が，藻体の発達程度や世代によって変化することから，2 種を同一種ではないかと提案しているが，ZINOVA

Table 1. Comparisons of the species of genus *Membranoptera* provided with dentate margins and microscopic side-veins on the alae.

	Height (cm)	Breadth (mm)	Position of tetrasporangial sori	Nature of margins	Shape of dentations	Midrib
M. spinulosa from Nemuro	0.5–2	1–1.5	whole length of branches	crisped in lower portion	small and sharp	conspicuous narrow
M. spinulosa (RUPERCHT 1851)	—	<2	farther down from apex	crisped	small and sharp	—
M. spinulosa (WYNNE 1970)	3–7	1–3	disappear where marginal branches occur	crisped or smooth	small and sharp	conspicuous narrow
M. serrata (ZINOVA 1965)	15	3–5	whole length of branches	—	large	very broad
M. denticulata (NAGAI 1941)	3.5–12	1–4	within branchlets*1	often more or less crisped	small and sharp*2	conspicuous broad*3
M. denticulata (HARVEY 1853)	10–12.5	6.4–13	near apex of small branchlets	—	small or large linear, acute	conspicuous

*1 Our observation on Nagai's specimens, tetrasporangial sori also developed in main and secondary branches, but sparser than those of the specimens from Nemuro. *2, 3 Results of our observation.

(1965) の概念に基づき，別々の分類群にとどめている。筆者等もこの扱いに従い，*M. spinulosa* と *M. serrata* を別種とみなし，そのうえで，根室産 *Membranoptera* は藻体が極めて小型で，細い中肋を持ち，鋸歯が小さく鋭利であることから *M. spinulosa* と同定した (Table 1)。そして，*M. spinulosa* を日本新産種とし，その和名としてヒメベニヤバネグサを提案する。

Nagai (1941) が千島列島から *M. denticulata* を報告しているので，根室産 *M. spinulosa* との関係を調べるため北大農学部に保管されている腊葉標本を観察した。その結果，この藻体は大型であり，四分胞子嚢は，末端枝から主枝にかけて形成されるが密度が低く，特に主枝の下部ではほとんど形成されず，根室産 *M. spinulosa* とは別種と認められた (Table 1)。また，*M. denticulata* において，末端枝以外に四分胞子嚢斑が形成されるという報告はなく，さらに，縁辺が縮れることなどから，千島産 *Membranoptera* は *M. denticulata* とは別種と思われる。ただし，標本が不足しており，種については特定できない。

最後に，助言をくださった札幌大学　三上日出夫教授，永井氏の標本閲覧を許可してくださった北大農学部　四方英四郎教授，並びに採集に協力してくださった釧路水産試験場　鳥居茂樹氏に深く感謝致します。

文　献

Harvey, W.H. 1853. Nereis Boreali-Americana. Pt. 2. Smiths. Contr. Knowledge. **4**(5): 1–258. 36 pls.

Kützing, F.T. 1866. Tabulae Phycologicae, oder Abbildungen der Tange. **16:** 1–35. 100 pls. Nordhausen.

Montagne, C. 1849. Sixième centurie de plantes cellulaires nouvelles, tant indigènes qu'exotiques. Ann. Sc. Nat. Bot. Sér. 3, **11:** 33–66.

Nagai, M. 1941. Marine algae of the Kurile Islands. 2. Jour. Fac. Agr. Hokkaido Imp. Univ. **46:** 139–310. pls 4–6.

Ruprecht, F.J. 1851. Tange des ochotskischen Meeres. Middendorff's sibirische Reise, 1 lief. **2:** 193–435. pls 9–18.

Wynne, M.J. 1970. Marine algae of Amchitka Island (Aleutian Islands). 1. Delesseriaceae. Syesis **3:** 95–144.

Wynne, M.J. 1983. The current status of genera in the Delesseriaceae (Rhodophyta). Botanica Marina **26:** 437–450.

Zinova, A.D. 1965. Species familiae Delesseriacearum (Rhodophyta) in parte Septentrionali Oceani Pacifici. Novitates Systematicae Plantarum non Vascularium, 1965: 78–97.

Jpn. J. Phycol. (Sôrui) 37: 150–152, June 20, 1989

Masakazu TATEWAKI, WANG Xiao-Yang and Isamu WAKANA: A simple method of red seaweed axenic culture by spore-washing*

Key Index Words: Antibiotic mixture—axenic culture—red seaweed—spore-washing medium
Masakazu Tatewaki, Wang Xiao-Yang and Isamu Wakana, Institute of Algological Research, Faculty of Science, Hokkaido University, Muroran, Hokkaido, 051 Japan

The axenic culture is an essential of biological research including tissue culture, protoplast isolation and fusion, and gene transfer. In green and brown seaweeds, it is generally possible to obtain axenic cultures by washing the motile reproductive cells in a series of sterile medium (CHAPMAN 1973). However, establishing of axenic cultures in red seaweeds is hard because of the absence of motile cells throughout the life cycles. Earlier methods usually used small pieces of vegetative thalli for sterilization. The wounded part, resulting from cutting into small pieces, is contaminated by microorganisms, which also makes it difficult to obtain axenic cultures. Exceptionally, the sterilization, using the dip and drag on agar plates containing antibiotics (TATEWAKI and PROVASOLI 1964), is only successful for thalli with smooth surfaces or simple thallus organization, but not with rough and complex thalli.

Although the surface of red seaweeds have luxuriant microbial growth, the spores are generally produced under aseptic conditions in tetrasporangia and carposporangia. Thus, if freshly liberated spores are axenic while mechanically isolating by capillary pipettes, it should be possible to obtain axenic cultures. However, normally spores of red seaweeds settle and attach to the substratum soon after liberation, and a few times of serial washing of spores in plain seawater lower the percentage of spore germination. In this paper, a new and simple method of axenic culture, in conjunction with impeding spore settlement, the mechanical washing and the antibiotic treatment of spores in red seaweeds is reported.

Neorhodomela aculeata (PEREST.) MASUDA, *Symphyocladia latiuscula* (HARV.) YAMADA, *Ceramium japonicum* OKAMURA, *Ptilota serrata* KEUTZING, *Palmaria palmata* (L.) KUNTZE, *Chondrus yendoi* YAMADA *et* MIKAMI and *Dumontia simplex* COTTON were used in this study.

Small pieces of fertile branches (2–4 cm) or blades (2–4 cm^2) bearing tetrasporangia or carposporangia were excised from freshly collected thalli. They were cleaned by brushing with a soft paint-brush, rinsed several times in sterile (autoclaved) seawater, and placed in a Petri dish (9 × 2 cm) containing 30 m*l* of sterile seawater. Most pieces liberated spores within 30 min (Fig. 1A). Newly liberated spores were taken up with a capillary pipette under a stereo-microscope (× 40–80) and washed 3–5 times in a depression glass slide containing 1–2 m*l* of the spore-washing medium (Fig. 1B). This medium, containing extracellular substances of spore-producing thalli, was prepared as follows: 1) 10 g of fresh thalli was soaked in 50–100 m*l* of seawater for 1 hr and then the seawater was filter-sterilized through a 0.22 μm pore size membrane filter, or 2) 100 mg of fresh thalli was placed in a test tube containing 10 m*l* of seawater and autoclaved for a few minutes, then 1–2 drops of the decoction were diluted with 10 m*l* of sterile seawater. In the spore-washing medium, the rapid settling and attachment to substratum of spores in most species were delayed for a few hours without any adverse effects by washing and isolation. However, normal attachment occurred soon after

* This research was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (58540442 and 60480013).

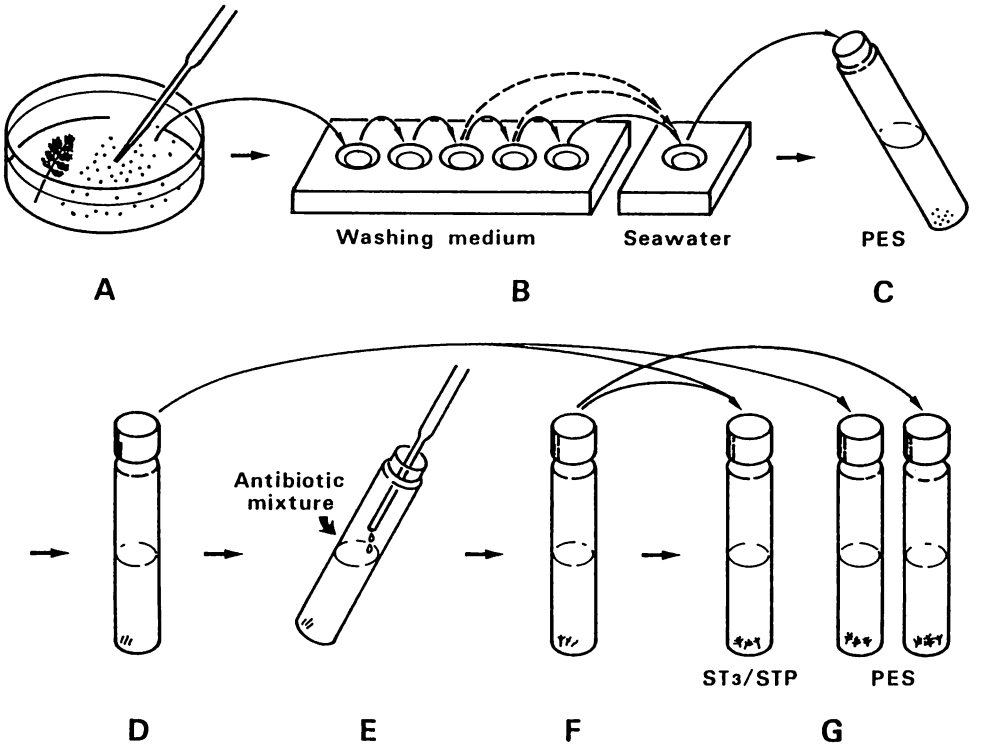


Fig. 1. Simple method of obtaining red seaweed axenic culture. A. Place a piece of cleaned fertile thallus in sterile seawater to get newly liberated spores. B. Wash spores 3–5 times in the spore-washing medium containing extracellular substances of the spore-producing thallus and once in seawater. C. Inoculate 10–20 spores to culture medium (PES). D. Culture under the optimum condition for 10–15 days until sporelings recognized with the naked eyes. E. Add 4–5 drops of antibiotic mixture to the medium. F. Culture for 7–14 days in the medium with antibiotics (replace with new culture medium). G. Inoculate young sporelings into sterility-test media and maintain them 20°C for 20 days—When confirmed axenic culture, inoculate them into desired culture media.

transfer to plain seawater or culture media.

Following the wash with the spore-washing medium, the spores were rinsed in plain seawater and then 10–20 spores were introduced into a screw cap test tube containing 10 m*l* of PES medium (Provasoli 1966) to establish unialgal culture. The above-mentioned isolation and washing procedures were done at low temperatures; sterile media or seawater used for washing were kept at 10–14°C. The isolated algae were maintained under optimum culture conditions for each species (usually 14–18°C, 12–18 W/m^2, 14 L: 10 D cycle) (Fig. 1D). Some of the sporelings which were developed from the serial washed spores, were axenic. For example, with tetraspores of *Palmaria palmata* and *Ptilota serrata,* axenic cultures were obtained at 10–20% of the trials. Sterility was tested by transferring a few sporelings into ST3 or STP sterility-test medium (Tatewaki and Provasoli 1964) and maintaining them for at least 20 days at approximately 20°C. Confirmed axenic sporelings were transferred into the desired culture media (Fig. 1G).

In addition, 10–15 days after establishing unialgal cultures, when the sporelings first become visible to the naked eyes, 4–5 drops (75–100 μl) of the antibiotic mixture were added aseptically to each culture tube containing 10 m*l* of PES medium (Fig. 1E). The antibiotic mixture contained 100,000 units penicillin G potassium, 250 mg streptomycin sulfate, 25,000 units polymyxin B sulfate and 1 mg chloramphenicol per 10 m*l* of distilled water (modifying the formula of the mixture developed by Provasoli 1958, Tatewaki and Provasoli 1964, and Tatewaki 1981). The

mixture was filter-sterilized and could be preserved by freezing in 1–2 m*l* aliquots for one week, although it is best to prepare the mixture just before using. The sporelings were left in the medium with antibiotics for 7–14 days under optimal culture conditions and then the medium was replaced with fresh PES (Fig. 1F). Axenic cultures were thus obtained with high percentage (>90%) of success for the species examined. When spores and sporelings younger than 5 days were dosed with the aitibiotic mixture, they were killed or stunted even by 3–5 days antibiotic treatment. Thus, we recommend that 10-day or older sporelings are treated by antibiotics after the spore-washing procedure. Such sporelings which are visible to the naked eyes, survive and develop into healthy axenic cultures.

The spore-washing media presumably contain extracellular substances originating from spore-producing plants (tetrasporophytes or female gametophytes). These substances which delay spore settlement, may assure spore dispersal from the site of the sporulating plants. We are presently investigating the biological and chemical nature of these substances.

Acknowledgements

We wish to express our sincere thanks to Dr. L.D. DRUEHL (Simon Fraser University) for his kind reading and correcting of the manuscript. Anonymous reviewers also provided constructive criticism.

References

CHAPMAN, A.R.O. 1973. Methods for macroscopic algae. p. 87–104. *In* STEIN, J.R. [Ed.] Handbook of phycological method. Culture methods and growth measurements. Cambridge Univ. Press, Cambridge.

PROVASOLI, L. 1958. Effect of plant hormones on *Ulva*. Biol. Bull. **114:** 375–384.

PROVASOLI, L. 1966. Media and prospects for the cultivation of marine algae. p. 63–75. *In* WATANABE, A. and HATTORI, A. [Eds.] Cultures and Collections of Algae. Jap. Soc. Plant Physiol., Tokyo.

TATEWAKI, M. 1981. Culture collection of seaweeds. A report of Grant-in-Aid no. 448018 from Sci. Res. Fund of the Ministry of Education, Sci. and Cul., Japan p. 1–19. (in Japanese)

TATEWAKI, M. and PROVASOLI, L. 1964. Vitamin requirements of three species of *Antithamnion*. Bot. Mar. **6:** 193–203.

舘脇正和・王　暁　陽・若菜　勇：胞子洗浄を利用した簡易な紅藻の無菌培養法

胞子の機械的洗浄と，それに続く発芽体の抗生物質処理の併用による，簡単かつ確実な紅藻の無菌化法を報告する。まず，放出直後の四分胞子及び果胞子を，胞子放出藻体の溶出液からなる無菌化済みの胞子洗浄液中で洗浄する。この洗浄液は胞子の基質着生時期を遅延させる働きを示し，洗浄操作に伴う胞子の損傷を軽減するのに役立つ。数回の洗浄によって，胞子は正常な発芽能を備えたまま単藻培養株として得られ，その一部はこの洗浄処理だけで無菌となる。さらに，生長した発芽体が肉眼で認められるようになる培養開始10–15日後に，洗浄だけでは無菌化されなかった培養に抗生物質を添加することによって，効果的に無菌株が得られた。(051 室蘭市母恋南町1–13　北海道大学理学部附属海藻研究施設)

Jpn. J. Phycol. (Sôrui) 37: 153–155, June 20, 1989

Mitsuo KAJIMURA : Lectotypification of *Predaea tokidae, Predaea bisporifera* (Nemastomataceae, Rhodophyta) and *Antithamnion okiense* (Ceramiaceae, Rhodophyta)*

Key Index Words: Antithamnion okiense—*lectotypification*—Predaea bisporifera—Predaea tokidae.
Mitsuo Kajimura, Marine Biological Station, Shimane University, Kamo, Saigo, Oki-gun, 685 Japan

Holotypes of the three red algal species *Predaea tokidae* KAJIMURA (KAJIMURA 1987a), *Predaea bisporifera* KAJIMURA (KAJIMURA 1987a) and *Antithamnion okiense* KAJIMURA (KAJIMURA 1987b) have been destroyed by mail accident. Lectotypification of each species with isotype specimens therefore has been made by the present author in accordance with Article 7.4 of the International Code of Botanical Nomenclature (GREUTER *et al.* 1988) as follows:

Predaea tokidae KAJIMURA, Phycologia 26: 419, figs. 1–12, 1987.

Lectotype (Fig. 1): OS 9876 (male), deposited in the Phycological Herbarium of the National Science Museum, Tokyo (TNS-AL-37403).

Predaea bisporifera KAJIMURA, Phycologia 26: 421, figs. 13–23, 1987.

Lectotype (Fig. 2): OS 9836 (male), deposited in the Phycological Herbarium of the National Science Museum, Tokyo (TNS-AL-37402).

Antithamnion okiense KAJIMURA, Bot. Mar. 30: 178, figs. 7–19, 22, 23, 1987.

Lectotype (Figs. 3, 4): OS 9938 (sterile), deposited in the Phycological Herbarium of the National Science Museum, Tokyo (TNS-AL-37401).

Acknowledgements

The present author wishes to acknowledge his indebtedness to Dr. P.C. SILVA, Department of Botany, University of California and Dr. Wm. J. WOELKERLING, Department of Botany, La Trobe University, for their helpful suggestions. The present author also wishes to acknowledge his indebtedness to Dr. G.T. KRAFT, School of Botany, University of Melbourne, for his helpful suggestions and critical reading of the manuscript.

References

KAJIMURA, M. 1987a. Two new species of *Predaea* (Nemastomataceae, Rhodophyta) from the Sea of Japan. Phycologia **26**: 419–428.

KAJIMURA, M. 1987b. *Cladophoropsis corallinicola* sp. nov. and *Antithamnion okiense* sp. nov.: Two deep-water algae from the Sea of Japan. Bot. Mar. **30**: 177–186.

GREUTER, W., BURDET, H.M., CHALONER, W.G., DEMOULIN, V., GROLLE, R., HAWKS-WORTH, D.L., NICOLSON, D.H., SILVA, P.C., STAFLEU, F.A., VOSS, E.G. and MCNEILL, J. (Eds.) 1988. International Code of Botanical Nomenclature, adopted by the 14th International Botanical Congress, Berlin, July-August 1987. Regnum Veg. 118, xiv+328 pp. Koeltz Scientific Books, Königstein.

* Contribution No. 43 from Oki Marine Biological Station, Shimane University.

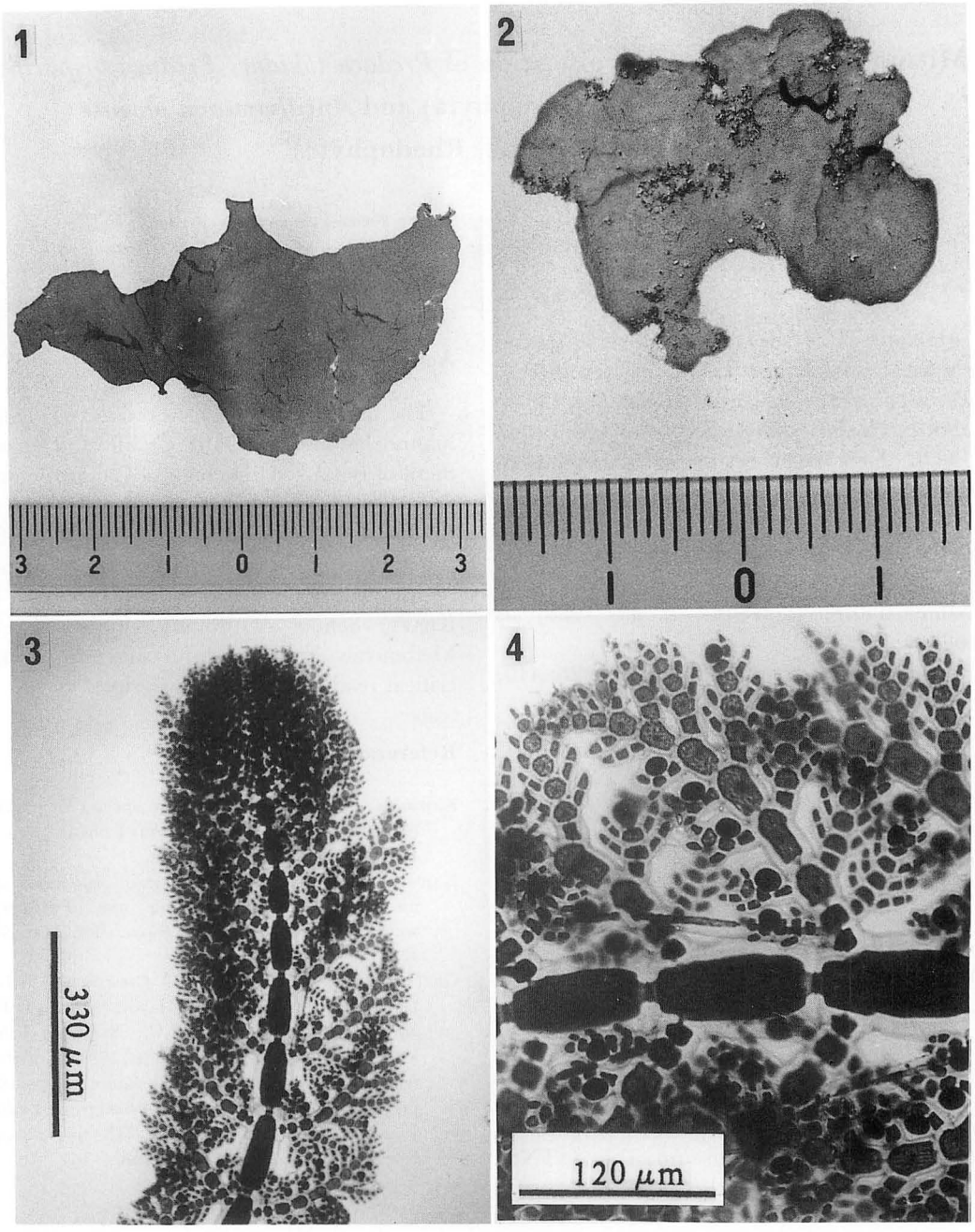


Fig. 1. *Predaea tokidae* KAJIMURA. Lectotype, collected at 40 m depth off Tsudo, the Oki Islands on 10 June, 1985.

Fig. 2. *Predaea bisporifera* KAJIMURA. Lectotype, collected at 20 m depth off Tsudo, the Oki Islands on 18 April, 1979.

Figs. 3 & 4. *Antithamnion okiense* KAJIMURA.

3. Lectotype, collected at 40 m depth off Tsudo, the Oki Islands, on 31 May, 1986.

4. Part of the lectotype showing some whorl-branches bearing gland cells commonly on the adaxial side.

梶村光男：*Predaea tokidae, Predaea bisporifera*（ヒカゲノイト科，紅藻植物門）及び *Antithamnion okiense*（イギス科，紅藻植物門）の選定基準標本の選定

Predaea tokidae, Predaea bisporifera 及び *Antithamnion okiense* の正基準標本が破損した為，国際植物命名規約第 7.4 条に従って，上記 3 種の複基準標本を選定基準標本として選定した。(685 島根県隠岐郡西郷町大字加茂 194　島根大学理学部附属臨海実験所)

—学 会 録 事—

1. 日本藻類学会第13回大会

1989年3月30日・31日の両日，東京女子体育大学2号館において，第13回大会を開催した。大会会長は福島博氏（東女体大）で，参加者は153名であった。講演は50題の一般講演（うち展示5題）と，L. J. Goff女史（米国カリフォルニア大）による特別講演“The Origins and Evolution of Red Algal Parasites”があった。

大会第1日目に同会場において総会を開催し，引続き同大学6号館食堂で約2時間にわたって懇親会を開催した。懇親会は吉武佐紀子（日女衛生短大）・大野正夫（高知大海洋生物センター）両氏の司会，印東弘玄氏（東教大名誉教授）の乾杯の音頭で始まった。宴を飾る料理は，そのすべてが福島氏と関係諸氏による心のこもった手作りで，終始和やかな雰囲気の中，盛会裡に終了した。また，大会第2日目の昼休みには，同大学7号館第3体育館にて，同大学生による新体操のエキジビジョンが約1時間にわたって行われた。その華麗なる演技に，しばし目を奪われ，心和やむ一時であった。東京女子体育大学の教官諸氏および学生諸君，ならびに横浜市立大学の小林艶子女史および学生諸君には大会運営にあたっていろいろご協力頂き，厚く御礼申し上げる。

懇親会参加者

秋山 優，鯵坂哲郎，新井章吾，有賀祐勝，飯田高明飯間雅文，石井明子，石川依久子，石田健一郎，石田勇人猪口真実，市村輝宣，出井雅彦，井上 勲，印東弘玄上杉伸子，榎本幸人，江原友子，大沢賢司，大谷修司大塚春江，大野正夫，大葉英雄，大森長朗，岡崎恵視奥田武雄，長舩哲斎，C. A. オロスコ，甲斐貴子笠井文絵，加崎英男，片山舒康，勝山志乃，川口栄男川嶋昭二，北原節子，木村憲司，北山太樹，倉島 彰栗原美香，黒田充恵，桑野和可，小亀一弘，小林艶子小林 弘，L. Goff，A. Coleman，今野敏徳斎藤 譲，坂井厚子，坂西芳彦，坂元忠明，坂本照正佐藤恵美，佐藤博雄，清水 晃，申 宗岩，杉田美成子杉原実奈子，与座功子，高橋永治，竹下俊治，立沢秀高田中次郎，田村美砂子，千原光雄，張 暁明，筒井 功寺尾公子，寺脇利信，中井末松，中嶋 泰，中山真理長島秀行，中田由和，南雲 保，名畑進一，西脇優子野崎久義，能登谷正浩，野呂忠秀，長谷栄二，濱田 仁馬場将輔，林 至宏，林田文郎，原 慶明，半田信司坂東忠司，福島 悟，福島 博，福永公平，藤田大介古角正晴，星野 淳，堀 輝三，堀口健雄，本田大輔前川行幸，増田道夫，松本正喜，真部永地，真山茂樹丸山 晃，三浦昭雄，御園生拓，峰 一朗，宮村新一D.M.Y. Yano，山岸高旺，山本秀一，山本鎔子，横浜康継吉崎 誠，吉武佐紀子，吉田忠生，渡辺 信，渡辺里香綿貫和彦，和田直也，安井 肇

2. 編集委員会・評議員会

第13回大会の前日，3月29日に東京女子体育大学2号館5階会議室において編集委員会（14:00～15:30）および評議員会（15:30～19:30）を開催し，1989年度総会に提出する報告事項・議題などの審議を行った。報告事項・議題については総会の項を参照されたい。

編集委員会出席者：有賀祐勝編集委員長，能登谷正浩編集幹事，田中次郎，堀 輝三編集実行委員，石川依久子，榎本幸人，奥田武男，鬼頭 鈞，吉田忠生の各編集委員，小林 弘会長および片山舒康，真山茂樹各幹事。

評議員会出席者：小林 弘会長，有賀祐勝（編集委員長を兼任），石川依久子，大森長朗，小河久朗，奥田武男，原 慶明，増田道夫，三浦昭雄，渡辺 信の各評議員，片山舒康，真山茂樹各幹事。

3. 1989年度総会

1989年3月30日（大会第1日目）の特別講演終了後，東京女子体育大学2号館225教室において総会を開催した。小林 弘会長の挨拶に続いて，加崎英男氏を議長に選出して議事に入った。

Ⅰ．報告事項

1. 庶務関係

(1)会員状況（1989年3月現在）：名誉会員3名，普通会員540名，学生会員45名，団体会員42名，賛助会員11名，外国会員86名，購読93件，寄贈・交換27件。(2)昭和63年度文部省科学研究費刊行助成金「研究成果公開促進費」交付額は930千円で，責任頁は300頁である。なお，昭和64年度（平成元年度）分として補助要求額1,897千円，責任頁300頁を申請した。(3)第13回大会後（4月1日）にワークショップ（藻類分類基礎講座，講師：高橋永治氏，加崎英男氏）を東京女子体育大学で実施する。(4)故黒木宗尚氏の遺志により，黒木

家から山田基金へ40万円の寄付があった。

2. 会計関係

(1)12月31日現在の昭和63年度の会費納入率は，普通会員87%，学生会員87%，賛助会員79%，団体会員100%，外国会員50%である。(2)昭和63年度一般会計と同山田幸男博士記念事業基金特別会計の決算報告は，1989年3月3日，瀬戸良三（神戸女学院大学），清水晃（奈良女子大学）の両会計幹事により適正であると承認された。

3. 編集関係

(1)昭和63年度に発行した第36巻1～4号は，総頁数341頁，掲載論文数26編，短報10編，総説2編，広告14頁であった。頁当りの平均経費は，12,576円であった。掲載論文の超過頁は32頁，カラー頁は2頁であった。(2)今年度から会誌の印刷を中西印刷㈱に依頼した。1989年3月10日に発行した第37巻1号は，掲載論文数9編，短報2編，ニュース3編，訃報，第13回大会講演要旨などを含めて88頁であった。(3)1989年3月20日現在の投稿論文数は25編で，内10編が第37巻2号に掲載予定である。

4. その他

昭和63年度秋季シンポジウムを日本植物学会大会前日の昭和63年10月12日午後に，岡山市の山陽学園短期大学で開催した。

Ⅱ．審議事項

1. 庶務関係

以下のことが審議され，承認された。(1)本年度秋季シンポジウムを日本植物学会第54回大会の前日（9月26日）に，小河久朗氏（東北大学）を世話人として仙台市で開催する。(2)日本藻類学会第14回大会は1990年3月29日・30日に坪　由宏氏（神戸大学）を世話人として神戸大学教養部で開催する。また，1991年の第15回大会は，沖縄（琉球大学）に開催をお引受け願う。(3)1989-1990年度の会計監事を岡崎恵視（東京学芸大学），加藤季夫（国学院大学）の両氏に委嘱する。(4)1990年度より学会事務委託先を(財)学会事務センターから中西印刷㈱に変更する。(5)会費・別刷代・バックナンバー代のカード支払制を来年度から導入する。(6)日本藻類学会会則第8条中，外国会員の会費8,500円を7,000円に改訂する。なお，これの施行は来年度以降とする。(7)山田幸男博士記念事業基金の運用については，昭和62年度総会（第11回大会）の決定にしたがって，学会賞を設ける。持ち回り評議員会，臨時評議員会で具体的内容をつめる。(8)寄付金の受け入れ規定を作成する。

2. 会計関係

(1)昭和63年度一般会計決算報告及び同監査報告は表-1のとおり承認された。

(2)昭和63年度山田幸男博士記念事業基金特別会計の決算報告及び同監査報告は表-2　のとおり承認された。(3)1989年度一般会計予算案は表-3　のように可決承認された。

3. 編集関係

以下のことが審議され，承認された。(1)論文などの開始頁を奇数頁に統一する。(2)大会講演要旨は次回から1頁あたり4題とし，専用の原稿用紙に書いてもらいオフセット印刷する。参加申し込み者には，プログラムをゲラの段階でコピーして配布する。(3)投稿された論文などの版権は，日本藻類学会に帰属する。これの取扱は，編集委員長が責任をもって対処する。(4)和文及び応用分野の論文の投稿を歓迎する。

4. その他

(1)韓国藻類学会李会長から，将来日韓合同大会を開催したいとの申し入れがあった。執行部で具体化を検討する。(2)今大会の世話人福島　博氏から大会の運営方法などの問題点を改善してほしい旨の発言があった。執行部で，改善策を検討する。

4. 日本藻類学会藻類分類基礎講座（第4回ワークショップ）報告

1989年4月1日(土)　日本藻類学会第13回大会の翌日，東京女子体育大学4号館生物実習室にて，藻類分類基礎講座を開催した。高橋永治氏と加崎英男氏を講師に，下記の20名が参加した。午前は高橋氏による黄金藻の分類の講義がスライドを使って行われ，午後は加崎氏による，車軸藻の講義および実体顕微鏡による観察が行われた。なお，本藻類分類基礎講座の内容と詳細は笠井文絵女史（国立公害研）の参加記（次号掲載予定）を参照されたい。

参加者：大沢啓志（横浜市大），大塚春江（神奈川公衛試），笠井文絵（国立公害研），栗原美香（横浜市大），小林艶子（横浜市大），小林　弘（東京珪藻研），坂本忠明（横浜市大），高村典子（国立公害研），根本隆夫（鹿児島大），野呂忠秀（鹿児島大），橋本達夫，坂東忠司（京都教大），福島　博（東京女体大），真山茂樹（東学大），丸山　晃（東大応微研），渡辺　信（富山大），宮地重遠（東大応微研），濱田　仁（富山医大），本田大輔（筑波大），和田直也（横浜市大）

表-1　昭和63年度　一般会計決算　(63. 1. 1-63.12.31)　　日本藻類学会

収入の部 (円)		支出の部 (円)	
会費	4,784,331	印刷費	5,187,800
普通会員	3,561,000	印刷代	4,443,370
学生会員	220,000	別刷代	744,430
外国会員	420,931	編集費	436,230
団体会員	302,400	英文校閲料	100,000
賛助会員	280,000	編集補助費	50,000
販売	676,500	通信連絡費	286,230
定期購読	513,000	会誌発送費	247,930
バックナンバー	163,500	庶務費	1,123,148
別刷代	763,822	事務用品費	4,890
超過頁負担金	348,000	会議費	43,380
広告代	170,000	通信・印刷費	272,398
利子	6,046	事務整理補助費	16,000
プログラム代	36,000	諸雑費	562,480
雑収入	172,948	幹事旅費補助	24,000
刊行助成金	930,000	幹事手当	200,000
		学会センター業務委託費	785,872
		第12回大会補助	100,000
		秋季シンポジウム会場費	50,000
小計	7,887,647	小計	7,930,980
前年度繰越金	4,457,390	次年度繰越金	4,414,057
合計	12,345,037	合計	12,345,037

貸借対照表

借方 (円)		貸方 (円)	
普通預金 (第一勧銀)	433,668	未払金	32,100
普通預金 (山梨中央銀)	1,900,000	前受会費	1,723,000
学会センター預け金	2,865,508		
郵便振替	50,290	前期繰越金	4,457,390
小口現金	17,781	当期繰越金	−43,333
未収金	801,910		
仮払い金	100,000	次期繰越金	4,414,057
合計	6,169,157	合計	6,169,157

1989年3月3日

日本藻類学会会長　梅崎勇　㊞
日本藻類学会会計幹事　鯵坂哲郎　㊞

本会計決算報告は適正である事を認める。
1989年3月3日

日本藻類学会会計監事　瀬戸良三　㊞
日本藻類学会会計監事　清水晃　㊞

表-2　昭和63年度山田幸男博士記念事業特別基金会計決算　(63. 1. 1-63.12.31)　　日本藻類学会

収入の部（円）		支出の部（円）	
山田幸男博士追悼号（4冊）	28,000		
学会出版物売上金			
コンブ論文集（3冊）	3,000		
日米セミナー（2冊）	8,000		
寄付	400,000		
利子	56,312		0
小計	495,312	小計	0
前年度繰越金	1,466,522	次年度繰越金	1,961,834
合計	1,961,834	合計	1,961,834

貸借対照表

借方（円）		貸方（円）	
定期預金（住友銀行）	1,900,000	前期繰越金	1,466,522
普通預金（住友銀行）	61,834	当期繰越金	495,312
		次期繰越金	1,961,834
合計	1,961,834	合計	1,961,834

1989年3月3日

日本藻類学会会長　梅崎勇　㊞
日本藻類学会会計幹事　鰺坂哲郎　㊞

本会計決算報告は適正であることを認める。
1989年3月3日

日本藻類学会会計監事　瀬戸良三　㊞
日本藻類学会会計監事　清水晃　㊞

表-3　1989年度　一般会計予算　　日本藻類学会

収入の部 (円)		支出の部 (円)	
会費	4,631,500	印刷費	5,200,000
普通会員 (486)	3,402,000	印刷代	4,500,000
学生会員 (40)	200,000	別刷代	700,000
外国会員 (51)	433,500	編集費	407,000
団体会員 (33)	396,000	英文校閲料	100,000
賛助会員 (10)	200,000	編集補助費	50,000
販売	361,040	通信連絡費	257,000
定期購読 (63)	311,040	会誌発送費	257,000
バックナンバー	50,000	庶務費	617,000
別刷代	700,000	事務用品費	40,000
超過頁負担金	200,000	会議費	50,000
広告代	150,000	通信・印刷費	155,000
利子	5,000	事務整理補助費	60,000
プログラム代	36,000	諸雑費	100,000
雑収入	30,000	幹事旅費補助	52,000
刊行助成金	930,000	幹事手当	160,000
		学会センター業務委託費	850,000
		第12回大会補助	120,000
		秋季シンポジウム会場費	50,000
小計	7,043,540	小計	7,501,000
前年度繰越金	4,414,057	予備費	3,956,597
合計	11,457,597	合計	11,457,597

表-4　日本藻類学会第13回大会会計報告　日本藻類学会大会準備委員会

収入の部 (円)		支出の部 (円)	
大会参加費		プログラム代	26,260
予約 (68名)	161,500	懇親会費	248,847
当日 (59名)	140,000	通信費	8,273
懇親会費		アルバイト代・謝金	208,303
予約 (62名)	155,000	雑費	188,833
当日 (33名)	82,500	学会返還金	484
学会補助金	120,000		
講座受講料 (11名)	22,000		
合計	681,000	合計	618,000

1989年4月5日　　第13回大会会計幹事　福島　博　㊞

—会 員 移 動—

新　人　会

住 所 変 更

訃　　報

本会会員　阪井與志雄氏は去る1989年4月14日逝去されました。
謹んで哀悼の意を表します。　　日本藻類学会

退　　会

佐々木　潤（北海道），須田彰一郎（茨城県），北島　　親（栃木県），窪田英夫（長野県），原田　昇（長野県），佐々木正人（東京都），篠村知子（東京都），島松秀典（東京都），下田正雄（東京都），山本海苔研究所（東京都），加藤光秋（埼玉県），倉掛武雄（愛知県），今津達夫（兵庫県）

日本藻類学会々則

第1条　本会は日本藻類学会と称する。
第2条　本会は藻学の進歩普及を図り，併せて会員相互の連絡並に親睦を図ることを目的とする。
第3条　本会は前条の目的を達するために次の事業を行なう。
1. 総会の開催（年1回）
2. 藻類に関する研究会，講習会，採集会等の開催
3. 定期刊行物の発刊
4. その他前条の目的を達するために必要な事業

第4条　本会の事務所は会長が適当と認める場所に置く。
第5条　本会の事業年度は1月1日に始まり，同年12月31日に終わる。
第6条　会員は次の4種とする。
1. 普通会員（藻類に関心をもち，本会の趣旨に賛同する個人で，役員会の承認するもの）
2. 団体会員（本会の趣旨に賛同する団体で，役員会の承認するもの）
3. 名誉会員（藻学の発達に貢献があり，本会の趣旨に賛同する個人で，役員会の推薦するもの）
4. 賛助会員（本会の趣旨に賛同し，賛助会員会費を納入する個人又は団体で，役員会の推薦するもの）

第7条　本会に入会するには，住所，氏名（団体名），職業を記入した入会申込書を会長に差出すものとする。
第8条　普通会員は毎年会費7,000円（学生は5,000円）を前納するものとする。但し，名誉会員（次条に定める名誉会長を含む）は会費を要しない。外国会員の会費は8,500円とする。会長の承認を得た外国人留学生は帰国前に学生会費の10年分を前納することができる。団体会員の会費は12,000円とする。賛助会員の会費は1口20,000円とする。
第9条　本会には次の役員を置く。
会長　1名　　幹事　若干名　　評議員　若干名　　会計監事　2名
役員の任期は2ヵ年とし重任することが出来る。但し，会長と評議員は引続き3期選出されることは出来ない。役員選出の規定は別に定める（付則第1条～第4条）。本会に名誉会長を置くことが出来る。
第10条　会長は会を代表し，会務の全体を統べる。幹事は会長の意を受けて日常の会務を行う。会計監事は前年度の決算財産の状況などを監査する。
第11条　評議員は評議員会を構成し，会の要務に関し会長の諮問にあずかる。評議員会は会長が招集し，また文書をもって，これに代えることが出来る。
第12条　1. 本会は定期刊行物「藻類」を年4回刊行し，会員に無料で頒布する。
2. 「藻類」の編集・刊行のために編集委員会を置く。
3. 編集委員会の構成・運営などについては別に定める内規による。

（付則）
第1条　会長は国内在住の全会員の投票により，会員の互選で定める（その際評議員会は参考のため若干名の候補者を推薦することが出来る）。幹事は会長が会員中よりこれを指名委嘱する。会計監事は評議員会の協議により会員中から選び総会において承認を受ける。
第2条　評議員選出は次の二方法による。
1. 各地区別に会員中より選出される。その定員は各地区1名とし，会員数が50名を越える地区では50名までごとに1名を加える。
2. 総会において会長が会員中より若干名を推薦する。但し，その数は全評議員の1/3を越えることは出来ない。
地区割は次の8地区とする。北海道地区，東北地区，関東地区，東京地区，中部地区（三重を含む），近畿地区，中国・四国地区，九州地区（沖縄を含む）。

第3条　会長，幹事及び会計監事は評議員を兼任することは出来ない。
第4条　会長および地区選出の評議員に欠員を生じた場合は，前任者の残余期間次点者をもって充当する。
第5条　会員がバックナンバーを求めるときは各号1,750円とし，非会員の予約購読料は各号3,000円とする。
第6条　本会則は昭和63年3月31日より改正施行する。

投　稿　案　内

Ⅰ．編集の方針　本誌には藻学と応用藻学に関する会員の未発表の，論文・総説・短報（短い調査報告など）・速報・雑録（採集地案内・分布資料・ニュース・所見・新刊紹介など）を掲載します。論文はデータや考察の独創性の有無に重点を置いた編集委員会の審査を経たのち受理されます。原稿の取捨，掲載順序，体裁などは編集委員会および編集幹事で決めます。原稿は和文または英文とし，論文は刷上り英文10頁，和文6頁，総説15頁，短報3頁，雑録1頁以内を無料とします。頁の超過は制限しませんが，超過頁分については1頁当たり12,000円が必要です。折り込み，色刷りなどの費用は著者負担となります。また，速報は2頁以内と制限があり，有料で1頁12,000円の掲載料が必要です。和文原稿では5枚（ワープロでは2枚）が，英文原稿では2枚が刷上り1頁となる見当です。

Ⅱ．報文の書き方　和文原稿は400字詰原稿用紙（横書きB5またはA4）に，当用漢字，新仮名使い（生物名は片仮名）を用い楷書体で書き，ワープロの場合は1行35字，28行に明瞭に印字して下さい。英文原稿は厚手タイプ用紙を用い，ダブルスペースで1行65字，28行にタイプまたはワープロで印字し，十分な英文添削または校閲を経たのち提出して下さい。新種の発表や学名の記載に当たっては国際植物命名規約に従って下さい。なお，アラビア数字・メートル法・摂氏温度を用い，学名などのイタリック体には下線1本，人名などのスモールキャピタルには下線2本，ゴシック体には波状線1本を記入して下さい。

例：Batrachospermum ectocarpum Sirod., Summary, sec, min, hr, nm, μm, mm, cm, m, μl, ml, l, μg, mg, g, N, M, ppm, lux, g (gravity), 25°C など。

原稿は，標題・英文要約（和文・英文原稿共）・本文・引用文献・和文摘要（英文原稿のみ）・表と図とその説明（英文）の順にまとめて1組とし，コピー共3組（写真は現物1組と現物または写真コピー2組，電子複写などは不可）にしてお送り下さい。

(1) **標題と要約**　英文原稿では，欄外見出し・標題・著者名・宛先・要約の順に，和文原稿では，欄外見出し（英）・標題と著者名（和と英）・要約（英）の順に記入してください。要約は著者名・標題・雑誌名・まとめ（200字・必要に応じて400字まで）・アルファベット順のキーワード（5～10語）の順に記入し，研究費に対する謝辞は脚注に入れて下さい。

(2) **本文**　標題紙に記した以外の謝辞は，なるべく本文の末尾に入れて下さい。表と図は必ず本文中に引用し（Fig. 1, Table 1のように），文献の引用は次の例にならって，著者名と出版年および必要に応じて頁（単行本の場合）を明示して下さい。

例：‥‥aquatic ecosystems (Welch 1972, 1974), Liebig's (1840 p. 23) "low of the minimum" is‥‥, ‥‥が知られている (Yamada 1949), 岡村 (1907 p. 56) は，

(3) **引用文献**　本文中で引用した文献のみを，別紙にアルファベット順に列挙して下さい。引用は，①原著の引用と，②図書目録を見て目的の書物を捜し当てるための引用の2本立てとし，それぞれが　イ）著者名　ロ）出版年　ハ）標題（巻次を含む）　ニ）対照事項（頁・図など）　ホ）出版事項（出版者・出版地）のうちの必要部分からなるよう順を追って下例にならって記入して下さい。

（単行本）　①，②共通　広瀬弘幸イ) 1959.ロ) 藻類学総説.ハ) 内田老鶴圃，東京.ホ)

（単行本中の1章）　①Drebes, G.イ) 1977.ロ) Sexuality.ハ) p. 250–283.ニ)　②*In* D. Werner [ed.]イ) The biology of diatoms.ハ) Blackwell Sci. Publ., London.ホ)

（叢書中の分冊）　①Hustedt, F.イ) 1930.ロ) Bacillariophyta.ハ)　②*In* A. Pascher [ed.]イ) Süswasser-Flora Mitteleuropas. ed. 2. No. 10.ハ) Gustav Fischer, Jena.ホ)

（雑誌の中の1論文）　①森　通保イ) 1970.ロ) *Batrachospermum ectocarpum* Sirod. の分類学的研究.ハ) ②藻類 **8**ハ) : 1–8.ニ)

①Mori, M.イ) 1975.ロ) Studies on the genus *Batrachospermum* in Japan.ハ) ②Jap. Journ. Bot. **20**ハ) : 461–485.ニ)

(4) **和文摘要**　英文原稿の場合のみ，和文で，著者名・標題・宛先も入れ400字以内にまとめて下さい。

(5) **表と図およびその説明**　英文で書き，表と図は原寸大（印刷頁の寸法は14×20.5 cm，片段のときは幅6.6 cm）またはA4版程度に仕上げ，図には倍率を示すスケールを入れ，線や記号，文字，数字はレタリング用具などを用いて鮮明に記入し，そのまま印刷に廻せるようにして下さい。なお，特に表の組版を希望の場合はその旨明記して下さい。表と図の上には割付，指定，レタリングや写真の脱落防止の必要上，必らずトレーシングペーパーを付け，その下端に著者名・番号・希望縮尺を記入して下さい。表と図の説明は別紙とし，それを入れる場所を本文原稿右欄外に明示して下さい。

Ⅲ．校正と別刷　著者校正は初校のみとし，印刷所から送りますので，3日以内に校正して同封の別刷申込書に所定の事項を記入し編集委員会宛に返送して下さい。別刷代は，論文・総説・短報に限って50部を学会で負担します。

Information for Authors (Revised March 1988)

Members of the Society are invited to contribute original research reports, short communications, review articles and rapid communications in Japanese or English on all aspects of phycology. Every research paper is read and criticized by reviewers on the basis of its originality and the discussion presented. Where appropriate, reviewers other than those on the Editorial Board are consulted. Final responsibility for selection and published order of papers rests with the Editor. Research reports not longer than 10 printed pages in English and 6 printed pages in Japanese including figures and tables, short communications within 3 printed pages and review articles within 15 printed pages will be published without excess charge (exclusive of reprints); additional published pages will be charged to the author (12,000 Yen per single printed page). Rapid communications acceptable within 2 printed pages will be published in the possible earliest issue with charge at 12,000 Yen per single printed page.

The manuscript should conform exactly to the following instructions. The **manuscript** should be typewritten, double-spaced in 65 letters per line and 28 lines, on thick paper of 21.5×28 cm or A4 size. Symbols, units and nomenclature should conform to international usage. The S. I. metric system should be used for all numerical data. Words to be printed in italics should be underlined. The original copy and two duplicates are required. The first page should have only the title, full name(s) of the author(s) and institution with address, and any necessary footnote. A short running title should be included. Acknowledgements preferably follow the text but precede the references. Tables and legends for figures should be on separate pages and be placed after the references.

An **abstract** of not more than 200 words is required. At the end of the abstract, 5–10 Key Index Words should be given alphabetically for aid in indexing. A Japanese abstract will be provided by the Editor from translation of the abstract.

References. Citations in the text should read thus: LIEBIG's (1840 p. 23) ... or ... (WELCH 1972, 1974). In the list at the end of the paper, references should be typed in alphabetical order. Each reference should be given in the following order: Name, Initials, Date, Title, Journal Volume: first page-last page. Example:

> MIKAMI, H. 1978. On *Laingia hookeri* (Rhodophyceae, Delesseriaceae) from New Zealand. Jap. J. Phycol. **26**: 65–68.

A book title should be followed by the name of publisher and place of publication. Example:

> ABBOTT, I. A and HOLLENBERG, G. J. 1976. Marine algae of California. Stanford Univ. Press, Stanford.

Tables should be numbered with Arabic numerals, have a title, and be referred to in the text.

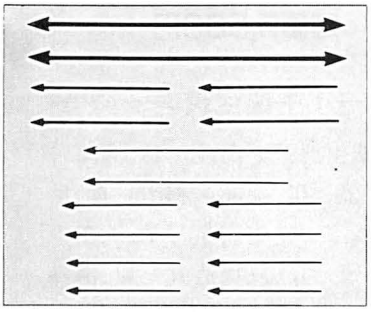
Figures, whether line drawings or photographs, should be numbered consecutively in Arabic numerals, and referred to in the text. The maximum size for a full page figure is 14×20.5 cm. Line drawings should be made with black ink on white paper or blue-lined graph paper. Letters and numerals should not be made by hand, but should be made neatly with a lettering device (not a typewriter) and be of such size that the smallest character will not be less than 1 mm high when reduced. The original drawing and two sets of clear copies are required. Photographs must be of good quality. They should be grouped to conform to the page style and format of the Journal and preferably be submitted at a size that permits reproduction without reduction. Photographs should be submitted in triplicate. Coloured plates may be printed at the expense of the author. The insertion of tables and figures in the text should be indicated on the right-hand margin of the sheet.

Proofs should be checked carefully and should be returned by airmail to the Editor within three days of receipt. The author will receive 50 offprints free of charge. Additional copies can be ordered at cost on the reprint ordering form sent with the proofs.

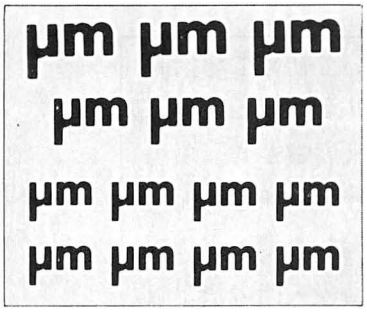
新製品ご案内!!

レタリングシート（ブラック アンド ホワイト）

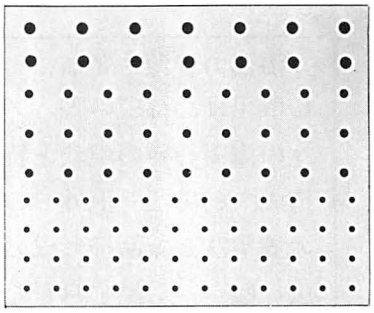
EMI NO.82014



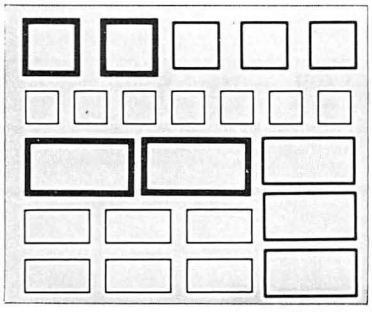
EMI NO.82016



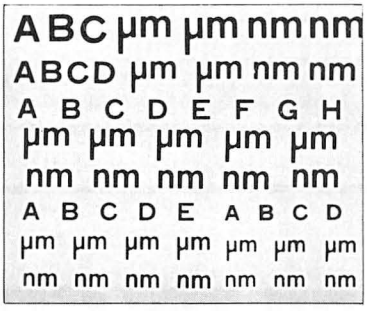
EMI NO.86626



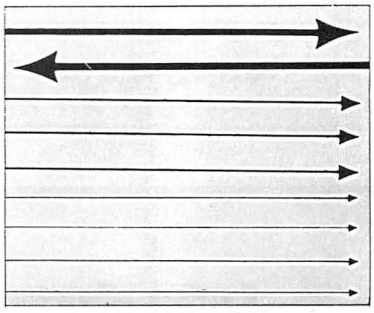
EMI NO.86627



EMI NO.86902

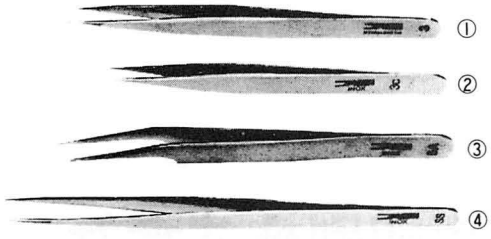


EMI NO.86916



※レタリングシートの総合カタログが出来ました。下記の住所へカタログをご請求下さい。

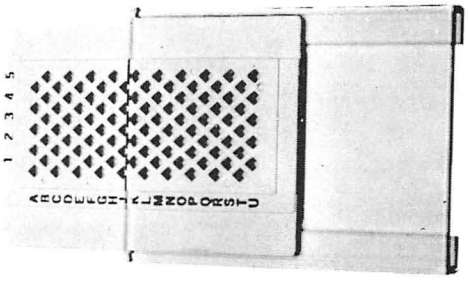
西独製 精密ピンセット



①時計ピンセット　③5型変形ピンセット
②3Cピンセット　④SS型ピンセット

各1本：￥2,200

EMグリッドボックス



1個：￥1,800　10個：￥15,000

EM資材直販センター
〒274 千葉県船橋市三山5-6-1 TEL.0474(75)5783
東京営業所：TEL.03(988)9906

学 会 出 版 物

下記の出版物をご希望の方に頒布致しますので，学会事務局までお申し込み下さい。(価格は送料を含む)

1. 「藻類」バックナンバー　価格，会員各号1,750円，非会員各号3,000円，30巻4号（創立30周年記念増大号，1—30巻索引付）のみ会員5,000円，非会員7,000円，欠号：1—2号，4巻1，3号，5巻1—2号，6—9巻全号。

2. 「藻類」索引　1—10巻，価格，会員1,500円，非会員2,000円，11—20巻，会員2,000円，非会員3,000円，創立30周年記念「藻類」索引，1—30巻，会員3,000円，非会員4,000円。

3. 山田幸男先生追悼号　藻類25巻増補．1977．A5版，xxviii＋418頁．山田先生の遺影・経歴・業績一覧・追悼文及び内外の藻類学者より寄稿された論文50編（英文26，和文24）を掲載，価格7,000円。

4. 日米科学セミナー記録　Contributions to the systematics of the benthic marine algae of the North Pacific. I. A. Abbott・黒木宗尚共編．1972．B5版，xiv＋280頁，6図版．昭和46年8月に札幌で開催された北太平洋産海藻に関する日米科学セミナーの記録で，20編の研究報告（英文）を掲載。価格4,000円。

5. 北海道周辺のコンブ類と最近の増養殖学的研究．1977．B5版，65頁。昭和49年9月に札幌で行なわれた日本藻類学会主催「コンブに関する講演会」の記録。4論文と討論の要旨。価格1,000円。

Publications of the Society

Inquiries concerning copies of the following publications should be sent to the **Japanese Society of Phycology, c/o Department of Biology, Tokyo Gakugei University, Nukuikitamachi, Koganei-shi, Tokyo, 184 Japan.**

1. **Back numbers of the Japanese Journal of Phycology** (Vols. 1–28, Bulletin of Japanese Society of Phycology). Price, 2,000 Yen per issue for member, or 3, 500 Yen per issue for nonmember; price of Vol. 30, No. 4 (30th Anniversary Issue), with cumulative index (Vols. 1–30), 6,000 Yen for member, or 7,500 Yen for nonmember (incl. postage, surface mail). Lack: Vol. 1, Nos. 1–2; Vol. 4, Nos. 1, 3; Vol. 5, Nos. 1–2; Vol. 6–Vol. 9, Nos. 1–3.

2. **Index of the Bulletin of Japanese Society of Phycology.** Vol. 1 (1953)–Vol. 10 (1962), Price 2,000 Yen for member, or 2,500 Yen for nonmember; Vol. 11 (1963)–Vol. 20 (1972), Price 3,000 Yen for member, or 4,000 Yen for nonmember. Vol. 1 (1953)–Vol. 30 (1982), Price 4,000 Yen for member, or 5,000 Yen for nonmember (incl. postage, surface mail).

3. **A Memorial Issue Honouring the late Professor Yukio Yamada** (Supplement to Volume 25, the Bulletin of Japanese Society of Phycology). 1977. xxviii＋418 pages. This issue includes 50 articles (26 in English, 24 in Japanese with English summary) on phycology, with photographs and list of publications of the late Professor Yukio Yamada. 8,500 Yen (incl. postage, surface mail).

4. **Contribution to the Systematics of the Benthic Marine Algae of the North Pacific.** Edited by I. A. Abbott and M. Kurogi, 1972. xiv＋280 pages, 6 plates. Twenty papers followed by discussions are included, which were presented in the U.S.-Japan Seminar on the North Pacific Benthic Marine Algae, held in Sapporo, Japan, August 13–16, 1971. 5,000 Yen (incl. postage, surface mail).

5. **Recent Studies on the Cultivation of *Laminaria* in Hokkaido** (in Japanese). 1977. 65 pages. Four papers followed by discussion are included, which were presented in a symposium on *Laminaria,* sponsored by the Society, held in Sapporo, September 1977. 1,200 Yen (incl. postage, surface mail).

1989年6月10日　印刷
1989年6月20日　発行

Printed by Nakanishi Printing Co., Ltd.

編集兼発行　有賀祐勝
〒108 東京都港区港南 4-5-7
東京水産大学藻類学研究室内
Tel. 03-471-1251　内線 315

印刷所　中西印刷株式会社
〒602 京都市上京区下立売通小川東入
Tel. 075-441-3155

発行所　日本藻類学会
〒184 東京都小金井市貫井北町 4-1-1
東京学芸大学生物学教室内
Tel. 0423-25-2111

本誌の出版費の一部は文部省科学研究費補助金「研究成果公開促進費」による。
Publication of The Japanese Journal of Phycology has been supported in part by a Grant-in-Aid for Publication of Scientific Research Result from the Ministry of Education, Science and Culture, Japan.

第37巻　第2号　1989年6月20日

藻　類

目　　次

日　本　藻　類　学　会