ISSN 0038-1578

Vol. 32 No. 4 10 December 1984.

The Japanese Journal of PHYCOLOGY

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The new Editor of the Japanese Journal of Phycology for 1985-1986 is Hiromu Kobayasi of Tokyo Gakugei University. Starting in January 1985, manuscripts should be submitted directly to **Editorial Office, The Japanese Society of Phycology, c/o Department of Biology, Tokyo Gakugei University, Koganei-shi, Tokyo, 184 Japan.**

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Jap. J. Phycol. (Sôrui) 32: 293-299. December 10, 1984

In vitro Digestibility of Algal Proteins

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FUJIWARA-ARASAKI, T. 1984. *In vitro* digestibility of algal proteins. Jap. J. Phycol. 32: 293-299.

Experiments were carried out to complement previously gained basic knowledge of algal proteins as food sources. After five hours' digestion of the alkali soluble proteins of algae *in vitro*, the digestibility with pepsin was found lower (15-56%) than these with two other enzymes, pancreatin (37-68%) and pronase (59-94%), when studies using eight edible marine algae, *Codium fragile, Ulva pertusa, Eisenia bicyclis, Undaria pinnatifida, Laminaria japonica, Analipus japonicus, Grateloupia turuturu,* and *Porphyra tenera.* Under the same condition, and when incubated for twenty-four hours, the protein digestibility became 42.4 to 90.9% with pepsin, 60.2 to 100% with pancreatin and 81.0 to 100% with pronase, respectively. The digestibility of alkali soluble proteins was particularly better than that of dried pulverized algae.

Key Index Words: *Algal proteins, alkali soluble protein,* in vitro *digestibility, marine algae.*

The value of marine algae as food has recently been re-evaluated in the hope that it may solve future food shortages. In Japan, more than one hundred species of marine algae have been used traditionally as food. Even today, the Japanese consume large quantities of marine algae such as *Laminaria, Undaria, Eisenia, Hijikia, Analipus, Monostroma, Enteromorpha, Ulva, Porphyra, Meristotheca* and *Gelidium.* Among these, the four species, *Porphyra tenera, Laminaria japonica, Undaria pinnatifida* and *Monostroma* sp. are artificially cultivated at present on a commercial scale in Japan.

It is well known that marine algae are a good source of carbohydrates, vitamins and minerals, and are a relatively high source of protein in human nutrition. Carbohydrates of marine algae are not digested by human intestinal enzymes. For this reason, they do not provide the human system with a source of calories. Moreover, they are low in fats. The edible marine algae, however, are not only predominant sources of such vitamins as A (β-carotene), B_1, B_2, B_6, B_{12}, C and niacin, but also more important sources of calcium and iron than vegetables and fruits found in the traditional human food. These facts indicate that algae have a good potential to promote health by way of nutritional and weight-reducing effects, and of such medicinal effects such as anti-lipaemic, blood-hypocholesterolaemic, and anti-tumoral etc.

The protein quality and digestibility of algae have not yet been elucidated in contrast to that of other higher plants. We have already succeeded in extracting, with dilute alkali solution, major proteins from algae with relatively high protein values (ARASAKI and MINO 1973).

These experiments were fundamental studies on the value of algal proteins as a food source. In this paper, emphasis will be placed on the digestibility of algal proteins extracted from eight species of edible marine algae in Japan, namely, *Codium fragile, Ulva pertusa, Eisenia bicyclis, Undaria pinnatifida, Laminaria japonica, Analipus japonicus, Grateloupia turuturu,* and *Porphyra tenera.* The proteolytic enzymes used were pepsin,

pancreatin and a bacterial protease, pronase.

Materials and Methods

1. Materials: Fronds were collected along the coast of the central part of Japan, washed with water, and then frozen and stored at −20°C.

2. Preparation of proteins from algae: The preparation of the alkali soluble protein was carried out with dilute alkali solution as reported previously (ARASAKI and MINO 1973).

Frozen fronds were pretreated with acetone and ether-methanol mixture (1 : 1) and further extracted with 5% NaOH. The proteins were precipitated by adjusting the pH of the solution to 4.0 with acetic acid. This procedure was repeated three times, and the purified protein, as a pale, greenish-white powder, was isolated.

3. Pulverized algae: Frozen algae were dried at 105-110°C and then pulverized for the later experiments described below.

4. *In vitro* digestion: The *in vitro* digestion study of alkali soluble proteins was performed by the method reported previously (ARASAKI and MINO 1976).

Table 1. Amino acid compositions of the alkali soluble proteins in the various marine algae*.

(g of amino acid-N/100 g of protein-N)

Amino acid	Green		Brown				Red		
	Ulva pertusa	*Codium fragile*	*Eisenia bicyclis*	*Undaria pinnatifida*	*Analipus japonicus*	*Laminaria japonica*	*Porphyra tenera*	*Grateloupia turuturu*	*Ovalbumin***
Trp	0.3	1.2	1.3	0.8	1.6	0.6	1.3	0.7	1.0
Lys	4.5	4.1	7.8	4.3	6.9	6.6	4.5	4.3	7.7
His	4.0	1.5	4.0	2.7	3.3	3.9	1.4	1.8	4.1
NH_3	1.9	3.4	3.2	2.5	3.1	3.1	5.1	1.9	5.3
Arg	14.9	12.3	18.6	7.5	12.5	9.4	16.4	15.8	11.7
Asp	6.5	6.4	5.0	5.6	6.1	6.6	7.0	5.7	6.2
Thr	3.1	2.9	2.3	2.4	3.0	3.7	4.0	3.0	3.0
Ser	3.0	2.8	2.3	2.8	3.1	3.6	2.9	2.8	6.8
Glu	6.9	6.1	7.6	5.1	6.0	5.7	7.2	6.3	9.9
Pro	4.0	3.6	4.5	2.8	3.4	3.3	6.4	5.1	2.8
Gly	5.2	5.1	6.5	4.4	5.2	6.2	7.2	5.0	3.4
Ala	6.1	6.6	7.0	4.8	5.9	7.5	7.4	5.5	6.7
**Cys	1.2	0.6	0.7	0.5	1.1	1.9	0.3	0.7	1.4
Val	4.9	5.8	5.9	4.1	4.5	5.1	6.4	4.9	5.4
Met	1.6	2.0	1.7	2.0	1.6	1.4	1.1	2.0	3.1
Ile	3.5	3.4	4.4	2.9	3.2	3.5	4.0	4.4	4.8
Leu	6.9	6.6	7.3	5.1	5.9	6.0	8.7	6.3	6.2
Tyr	1.4	1.2	2.1	1.6	1.9	1.6	2.4	0.9	1.8
Phe	3.9	3.3	4.0	3.7	3.2	3.8	3.9	3.7	4.1
Total	83.8	78.9	96.9	65.8	81.5	83.5	98.2	81.2	95.4
N %	13.2	13.7	10.6	11.6	11.7	10.4	13.6	14.4	15.8

* FUJIWARA-ARASAKI *et al.* (1984)

** Cys Performic acid oxidation

*** LARSEN and HAWKINS (1961)

The enzyme solutions, pepsin (1 : 10,000 pu, k/g, Nakarai Chemicals Ltd.) in N/50 HCl, pancreatin (Difco Laboratories) in M/50 phosphate buffer (pH 7.6), and pronase (45,000 pu, k/g, Riken Chemicals Co.) in M/20 tris buffer (pH 8.6), were used. Final enzyme and substrate concentrations were adjusted to 0.05-0.1% and 0.5-1%, respectively. The proteolytic digestion of milk casein (Hammersten, Merck), under the same conditions described above, was used as control. The reaction mixture (enzyme and substrate, 1 : 1) was incubated at 37°C for 0, 0.5, 2, 3, 4, 5 and 24 hours. To each 1 m*l* of the reaction mixture was then added 4 m*l* of 5% trichloroacetic acid (TCA) to stop the reaction. The mixture was then allowed to stand at room temperature for 30 min. The optical density of the filtrate was measured by a spectrophotometer (Shimadzu Model UV-180) at 280 nm.

5. Five and twenty-four hours' digestibility: The filtrate (1.0 m*l*) of the reaction mixture (hydrolysed for eight hours with 1.0 m*l* of conc. H_2SO_4) was analysed to determine the nitrogen content by the Micro-Kjeldahl method (ARASAKI *et al.* 1979). The resultant digestibility was expressed in terms of percentage based on the comparable casein digestibility.

Results and Discussion

1. Alkali soluble proteins: The nitrogen

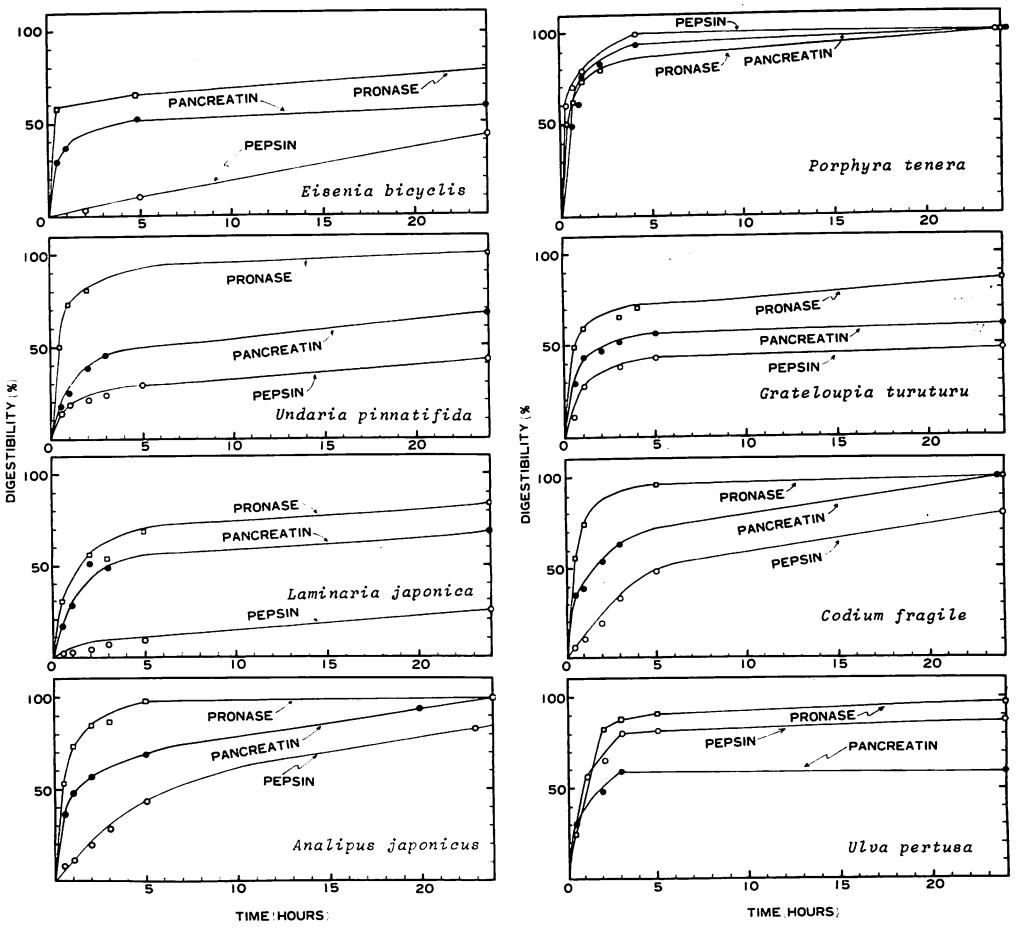

Fig. 1. Digestion curves of alkali soluble proteins with three enzymes, pepsin, pancreatin and pronase. Values of the digestibility is expressed as a percentage of casein digestibility.

content of the proteins were found to be 10-14%, which is somewhat lower than that of common proteins. The eighteen kinds of amino acids were detected in the protein and the recovery of amino acid nitrogen obtained in these experiments were of the order of 65-98% as shown in Table 1. In general, the essential amino acid content of the alkali soluble proteins was found to be present at relatively high levels, except for lysine, threonine and sulfur-containing amino acids. The amino acid compositions of the proteins in eight species were found to be similar and these results agreed well with the data obtained by LARSEN and HAWKINS (1961) from the two brown algae.

2. *In vitro* digestibility of the algal proteins: *In vitro* digestibility of the alkali soluble proteins obtained from eight species of algae was examined using three enzymes, pepsin, pancreatin and pronase. The proteolytic digestion of algal proteins was compared with that of milk casein under the same conditions described above. The percent digestion calculated proportionally against milk casein digestibility, (which was regarded as 100%), was plotted against time as shown in Fig. 1.

It was shown that the approximate maximum levels of digestion of the eight species of algae occurred after four to five hours and the digestion level then slowly increased during the next twenty-four hours. Of the three enzymes, pronase (bacterial enzyme), was shown to produce the highest level of digestion, and the next highest was pancreatin. The lowest digestion was induced by pepsin.

The results of five and twenty-four hours' digestibility experiments, performed by the Kjeldahl method (ARASAKI *et al.* 1979), where the nitrogen content in the filtrates of the reaction mixture was determined, are shown in Tables 2 and 3, respectively. The digestibility values were expressed as a percentage of digestion of casein digestibility, which was regarded as 100%. As shown in Table 2, five hours' digestion with pepsin was found to be lower (15-56%) than those with the two other enzymes, pancreatin (37-68%) and pronase (59-94%). The digestibility with pancreatin of three types of algae, *Ulva pertusa*, *Analipus japonicus* and *Porphyra*

Table 2. *In vitro* digestibility for five hours of alkali soluble proteins of algae*

Algae	Pepsin (%)	Pancreatin (%)	Pronase (%)
Codium fragile	22.5	37.2	70.9
Ulva pertusa	17.0	66.6	94.8
Eisenia bicyclis	18.7	53.7	59.1
Laminaria japonica	39.0	54.0	83.9
Undaria pinnatifida	23.9	48.1	87.2
Analipus japonicus	42.7	68.3	97.8
Grateloupia turuturu	15.8	34.0	59.2
Porphyra tenera	56.7	56.1	78.4

* Five hours' digestion: Nitrogen in the filtrates of the reaction mixture was determined by the Kjeldahl method as an estimate of digestion. The digestibility is expressed as a percentage based on the digestibility of casein. Casein digestibility was shown to be 64.3%, 90.1% and 92.9% by pepsin, pancreatin and pronase digestion, respectively, measured after five hours.

Table 3. *In vitro* digestibility for twenty-four hours of alkali soluble proteins of algae*

Algae	Pepsin (%)	Pancreatin (%)	Pronase (%)
Codium fragile	80.4	100.0	100.0
Ulva pertusa	86.2	66.2	96.2
Eisenia bicyclis	43.4	60.2	81.0
Laminaria japonica	48.5	71.2	86.3
Undaria pinnatifida	42.4	68.0	90.7
Analipus japonicus	85.0	100.0	100.0
Grateloupia turuturu	46.6	66.6	88.5
Porphyra tenera	90.9	70.5	98.3

* Twenty-four hours' digestion: Nitrogen in the filtrates of the reaction mixture was determined by the Kjeldahl method as an estimate of digestion. The digestibility is expressed as a percentage based on the digestibility of casein. Casein digestibility was shown to be 68.6%, 100.9% and 100.6% by pepsin, pancreatin and pronase digestion, respectively, measured after twenty-four hours.

tenera, was found to be 66.6, 68.3, and 56.1%, respectively, which was appreciably higher than those of the other species. Digestion with pronase was, however, found to be higher than those with the two other enzymes. The digestibility with pronase of two species, *Ulva pertusa* and *Analipus japonicus*, were also found to be 94.8 and 97.8% respectively, at the highest level. Normal digestibility with pronase was found to be about 70% in other species except in *Grateloupia turuturu* and *Eisenia bicyclis* (Table 2). As shown in Table 3, twenty-four hours' digestibility of algal proteins was found to be 42.4-90.9% with pepsin, 60.2-100% with pancreatin and 81.0-100% with pronase, and was higher than those after five hours' digestion. In particular, the digestibility with pancreatin of *Codium fragile* and *Analipus japonicus* was as high as that of casein. Six other species were also found to give the values of about 40 to 60% based on casein digestibility. Digestibility with pepsin, however, was as low as 43.4%, 48.5%, 42.4% and 46.6% in *Eisenia bicyclis*, *Laminaria japonica*, *Undaria pinnatifida* and *Grateloupia turuturu*, respectively. Digestibility of algae by pronase was found to be highest when compared with those by others enzymes. However, *Eisenia bicyclis* (81.0%), *Laminaria japonica* (86.3%) and *Grateloupia turuturu* (88.5%) were incompletely digested even after twenty-four hours' digestion.

In animal feed studies on dried algal meals some cases of low digestibility have been reported by previous investigators (BENDER *et al.* 1953, KIMURA 1952, MATSUKI 1960, and MORI *et al.* 1948).

KIMURA (1952) reported that the protein availability was 57.0% in *Laminaria japonica*, 64.1% in *Undaria pinnatifida* and 72.6% in *Porphyra tenera*, and MATSUKI (1960) also reported that it was 16.4% in *Laminaria*, 44.1% in *Undaria*, 70.8% in *Porphyra* and 44.9% in *Hijikia fusiforme* in human diets. According to MATSUKI (1960), the protein digestibility of algae was found to be higher than that of fungi and somewhat lower than that of the leaf portion. MORI *et al.* (1948) reported that the protein digestibility of eleven species of marine algae was low (15.1-71.5%). LARSEN and HAWKINS (1961) reported that values as high as 75% often observed with egg albumin could be observed in feeding tests for rats, using protein extracted with 20% sodium carbonate from two species of algae, *Chondrus crispus* and *Laminaria digitata*. Previous investigators reported that, in general, the lower digestibility of algal proteins was observed using raw materials (BENDER *et al.* 1953, KIMURA 1952, MATSUKI 1960, MORI *et al.* 1948).

In an attempt to clarify the low digestibility of the raw materials, we carried out on *in vitro* digestion of proteins by comparing the pulverized algae with the extracted proteins using *Porphyra tenera* and *Grateloupia turuturu*. The results showed without doubt that the digestibility of the pulverized algae was very low (Table 4).

In vivo experiments, however, the digestibility of *Porphyra tenera* in the raw materials was found to be 70.8% by MATSUKI (1960), 72.6% by KIMURA (1952) and 54.3-71.5% by MORI *et al.* (1948) where it was found to be higher than that of the other species tested. The result of the *in vitro* digestibility of extracted protein of *Porphyra tenera* obtained in the present experiment agreed well with the results of these investigators mentioned above. Hence, it appears that the protein

Table 4. Digestibility of pulverized algae, *Porphyra tenera* and *Grateloupia turuturu**

Algae	Enzyme	Digestibility (%)
Porphyra tenera	Pepsin	1.6
	Pancreatin	0.7
	Pronase	4.7
Grateloupia turuturu	Pepsin	6.1
	Pancreatin	9.1
	Pronase	18.6

* The digestibility is expressed as a percentage based on casein digestion. The casein digestibility was determined as 63.0%, 89.1% and 97.8% in pepsin, pancreatin and pronase digestions, respectively, measured after five hours.

in *Porphyra tenera* was easily solublized from the cell than in the case of the other species.

Recently, the similarity of protein digestibility of mono-cellular algae, *Chlorella*, and blue-green algae, *Nostoc muscorm* to human food has received attention and studies were also conducted to investigate their digestibility (COOK 1962, SUBBA *et al.* 1972, MITSUDA *et al.* 1977 and ISHII *et al.* 1974).

MITSUDA *et al.* (1977 a, b) reported that the *in vitro* digestibility in *Nostoc muscorm* is 74.4% with pepsin and 63.8% with trypsin and that of *Chlorella* with trypsin is 44.6% for dried cell and 70.9% for broken cells. MITSUDA *et al.* (1977b) also reported that the *in vivo* digestibility in *Chlorella* by rats is 59.7% for dried cells and 79.5% for broken cells (by Dyno-Mill), respectively. It was concluded by these authors that broken cells offer a good substrate for the utilization of *Chlorella* protein.

ISHII *et al.* (1974) also reported that the protein digestibility of *Chlorella* with trypsin was 69.3% for frozen cells and 86.1% for extracted protein. These results were similar to those of animal tests described above.

COOK (1962) reported that the protein digestibility of *Chlorella* by rat *in vivo* was 65.4% for dried cells and 73.0% for cells heated at 100°C for 30 min.

According to both IGARASHI *et al.* (1978) and ISHIHARA *et al.* (1968), carbohydrates and tannins i. e. phenolic compounds contained as impurities in the algae inhibited the protein digestion *in vivo*, and the digestion of *Chlorella* proteins with trypsin was inhibited not only by lipids but also by some pigments present in *Chlorella*. The alkali soluble proteins obtained in the present experiment were contaminated with small amounts carbohydrates and pigments. In view of the results from *Chrorella* the inhibition of protein digestion obtained in the present work may be due to contaminating substances.

In conclusion, the digestibility of eight species of Japanese edible marine algae used in the present experiment was found to be lower after five hours' digestion than those of animal proteins, most of them being as low as 50% of casein. However, the percentages of *in vitro* digestibility by emzymes could be improved to give the values of 42.4 to 90.9% with pepsin and to those of 60.2 to 100% with pancreatin, respectively, over twenty-four hours' digestion. This observation suggests that algal proteins may be a good source of human nutrition by further improvement of the protein digestibility.

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新崎輝子：海藻タンパク質の消化性

本研究は食糧資源としての海藻の基礎研究として，8種の日本産海藻について，著者の方法により分離したアルカリ可溶タンパク質を用い，ペプシン (1)，パンクレアチン (2)，プロナーゼ (3) のタンパク分解酵素による経時的人工消化を，ミルクカゼインを対照として行った。

その結果5時間消化では，(1) は 15～56%，(2) は 37～68%，(3) は15～56% でやや低い分解率を得たが，24時間では (1) は 42～91%，(2) は 60～100%，(3) は 81～100% でかなりよく，海藻によってはカゼインと同程度のものもあった。比較のために藻体の粉末物を用いて同様の消化実験を行ったが，その結果は非常に悪く，抽出タンパク質がすぐれていることを示した。(560 神戸市中央区諏訪山町 3-1　神戸山手女子短期大学)

Jap. J. Phycol. (Sôrui) 32: 300-306. December 10, 1984

Contribution of glycerol to osmoregulation in *Dunaliella tertiolecta* under magnesium hypertonicity

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FUJII, S., YAMAMOTO, R. and TAKADA, H. 1984. Contribution of glycerol to osmoregulation in *Dunaliella tertiolecta* under magnesium hypertonicity. Jap. J. Phycol. 32: 300-306.

The intracellular concentration of glycerol as an osmotic substance was sufficient to osmotically balance the external concentration of NaCl. Glycerol was the main osmoticum for osmotic adjustment in cells of *D. tertiolecta* cultured in a hypertonic medium with $MgSO_4$ and/or Na_2SO_4 as well as NaCl. Glycerol formation seemed to depend on the osmotic pressure in the medium, irrespective of the kind of cation or anion present. However, contents of amino acids, reducing sugars and potassium ions in the cells did not change in response to the increase in the osmotic concentrations of the media.

Key Index Words: Dunaliella; *glycerol*; *magnesium hypertonicity*; *osmoregulation*.

Unicellular green algae of the genus *Dunaliella* show outstanding adaptability and tolerance towards a very wide range of salinities from seawater to saturated salt solutions. Therefore, *Dunaliella* cells must have the osmoregulatory mechanisms to maintain suitable osmotic pressure against environmental pressure. To maintain osmotic pressure in a cell, various osmotica, which are active as osmotic substances, should be needed. The glycerol content in *Dunaliella* cells cultured in NaCl media of various concentrations has been reported to increase linearly with increasing extracellular NaCl concentration (BEN-AMOTZ and AVRON 1973 and 1981, FRANK and WEGMANN 1974). The osmotic pressure supported by intracellular glycerol in cells cultured over a wide range of NaCl concentrations has not been well examined. Thus, it is not clear whether or not glycerol is the main osmoticum in the osmoregulation of *Dunaliella*. To elucidate the role of the glycerol, the osmotic pressures derived from the intracellular glycerol content in the cells should be estimated and compared with the osmotic pressure of the medium.

Previously, we reported that *D. tertiolecta* could grow in $MgSO_4$-hypertonic medium as well as in NaCl-hypertonic medium but not in a hypertonic medium with $MgCl_2$ or $Mg(NO_3)_2$ (FUJII *et al.* 1983). In this study, we first checked whether the cells of *D. tertiolecta* cultured in a hypertonic medium with a salt other than NaCl could produce glycerol. We also estimated the osmotic pressures derived from the intracellular glycerol content in the cells and compared them with the osmotic pressures of the medium to evaluate the contribution of intracellular glycerol to osmoregulation.

Materials and Methods

Material: Cells of the green alga *Dunaliella tertiolecta*, LB 999, were cultured in a basal medium with a hypertonic concentration of $MgSO_4$, NaCl or Na_2SO_4 as described previously (FUJII *et al.* 1983). In all cultures, the initial cell number was 10^4 cells per m*l* of medium. Although the cultures were not axenic, no bacteria were observed microscopically. The cell number was determined using a microscope with a haemacytometer.

Determinations of glycerol, amino acids, reducing sugars and potassium ion: To obtain the cell extract, the cells were spun down by centrifugation at 3000 r.p.m. for 10 min resuspended in the same volume of distilled water and heated at 100°C for 5 min to inactivate the enzymes. The cell suspension was then treated with a sonicator (Tomy Seiko Co., Ltd., Model UR-200P) at 5°C for 3 min and centrifuged at 15000 r.p.m. for 10 min.

The glycerol concentration in the extract was determined enzymatically using the Biochemical Test Combination "glycerol and neutral fat" (Toyobo Co., Ltd.). A 0.02 m*l* portion of the extract was added to 3 m*l* of the enzyme solution. After the mixture had been incubated for 15 min at 37°C, the optical density was measured at 545 nm at room temperature (Hitachi, Ltd., spectrophotometer Model 220A).

The amino acids in the extract were measured according to the method of GARREL *et al.* (1972), and calculated as the L-leucine equivalent. Ninhydrin reagent contained 0.4 g ninhydrin, 80 m*l* 95% ethanol, 1 g $CdCl_2$ and 5 m*l* acetic acid in 20 m*l* water. The extract of 0.2 m*l* was incubated with 2.5 m*l* the ninhydrin reagent at 80°C for 10 min, and the optical density was measured at 506 nm.

The reducing sugars in the extract were measured by the method of Somogyi and Nelson (SOMOGYI 1952). The extract of 1 m*l* was mixed with 1 m*l* of a copper solution, heated in boiling water for 10 min, and cooled quickly. After addition of 1 m*l* the Nelson reagent, the mixture was made up to 25 m*l* with distilled water, its optical density at 500 nm was measured, and the glucose equivalent was calculated.

To measure the amount of potassium ion in the extract, 10 m*l* of it was mixed with 40 m*l* of distilled water and 5 m*l* of the ionic strength adjustor. Then the K^+ content in the sample was measured electronically with an ion meter (Toa Electronics Ltd., Model IM-20E).

Determination of osmotic pressure: The cells of *D. tertiolecta* cultured in a medium with one of the three salts, were photographed through a microscope. Their mean cell volume was calculated assuming that all cells were ellipsoidal. The osmotic pressure of the medium after removal of the cells by centrifugation was measured using a vapor pressure osmometer (Wescor Model 5100C).

Results

a) Growth of *D. tertiolecta* in hypertonic media containing various salts.

Growth experiments of *D. tertiolecta* cultured in hypertonic media with different salts showed that no growth occurred in $MgCl_2$- and $Mg(NO_3)_2$-hypertonic media, as previously reported (FUJII *et al.* 1983), but good growth occurred in NaCl-, Na_2SO_4-, $NaNO_3$- and $MgSO_4$-hypertonic media (Table 1).

b) Effect of salts on contents of var-

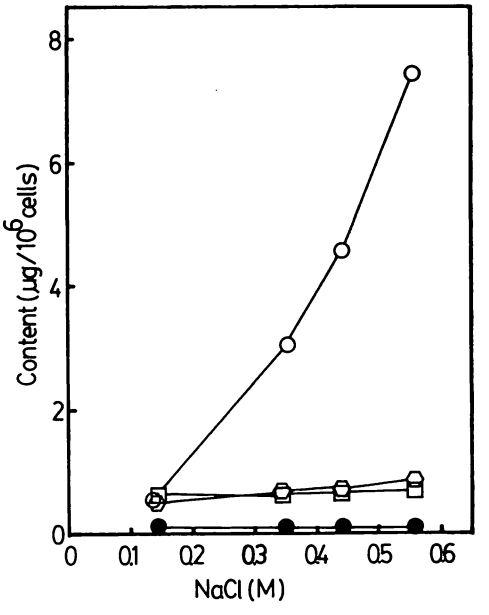

Fig. 1. Contents of various osmotica in *Dunaliella* cells maintained at different NaCl concentrations. Algae were cultured for 4 days in NaCl media of the indicated concentrations, and then contents of various osmotica in its cell extract were analyzed. Data represent the averages of three experiments. (○) glycerol; (□) amino acids; (○) reducing sugars; (●) K^+.

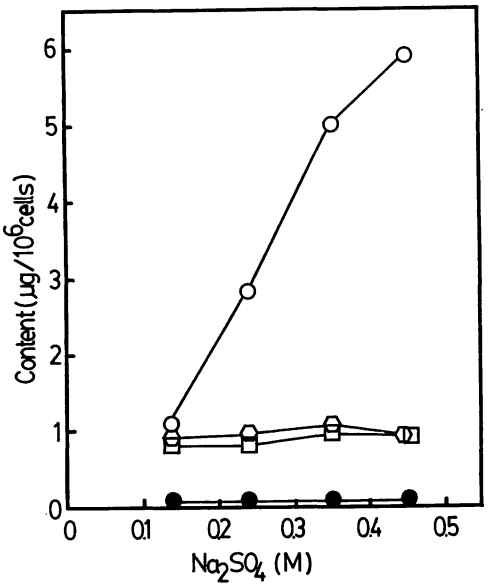

Fig. 2. Contents of various osmotica in *Dunaliella* cells maintained at different Na_2SO_4 concentrations. Algae were cultured for 4 days in Na_2SO_4 media of the indicated concentrations and then contents of various osmotica in its cell extract were analyzed. Data represent the averages of three experiments. (○) glycerol; (□) amino acids; (○) reducing sugars; (●) K^+.

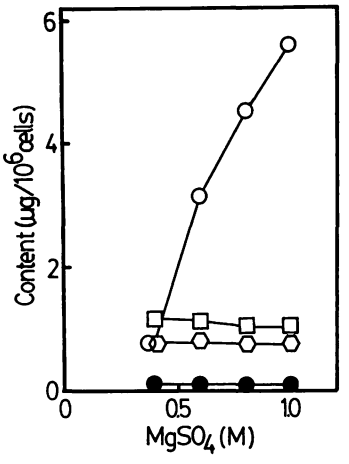

Fig. 3. Contents of various osmotica in *Dunaliella* cells maintained at different $MgSO_4$ concentrations. Algae were cultured for 4 days in $MgSO_4$ media of the indicated concentrations and then contents of various osmotica in its cell extract were analyzed. Data represent the averages of three experiments. (○) glycerol; (□) amino acids; (○) reducing sugars; (●) K^+.

ious osmotica in cells.

D. tertiolecta cells were cultured in a hypertonic medium with NaCl, Na_2SO_4 or $MgSO_4$ and contents of intracellular glycerol, reducing sugars, amino acids and K^+ per 10^6 cells were determined. As shown in Figs. 1, 2 and 3, the intracellular glycerol increased in approximately a linear relationship to the external salt concentration, while amino acids, reducing sugars and K^+ content of cells were almost constant in spite of the increasing salt concentration. Thus, glycerol seems to be a major, and probably the sole, osmoregulating agent in the cells. Proof of this requires that the osmotic pressure due to intracellular glycerol and that of ambient solution are determined.

c) Osmotic pressures of NaCl, Na_2SO_4, $MgSO_4$ and glycerol solutions.

Since the osmotic pressure of an electrolyte depends on its extent of dissociation, the osmotic pressure of each medium with NaCl. Na_2SO_4 or $MgSO_4$ was measured with the vapor pressure osmometer (Fig. 4). A linear relationship was found between the osmotic pressure and the concentration of each salt.

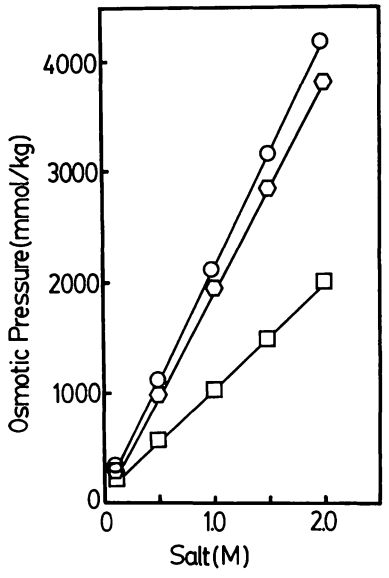

Fig. 4. Osmotic pressures of media with NaCl, Na_2SO_4 or $MgSO_4$. The osmotic pressure was measured with a vapor pressure osmometer at room temperature. (○) NaCl; (○) Na_2SO_4; (□) $MgSO_4$.

At the same concentration, the highest osmotic pressure was obtained from Na_2SO_4 and the lowest one from $MgSO_4$. The medium with 0.5 M NaCl was approximately isotonic with that of 0.45 M Na_2SO_4 or 0.94 M $MgSO_4$.

To estimate the osmotic pressure due to intracellular glycerol, that of glycerol aqueous solution at various concentrations was measured with the vapor pressure osmometer (Fig. 5). The concentration of glycerol up to 1 M was virtually equal to the osmotic pressure. In the following experiments, the values of osmotic pressure thus obtained were used.

d) Changes in the mean cell volume of cells cultured in NaCl-, Na_2SO_4- and $MgSO_4$-hypertonic media.

As the intracellular concentrations of glycerol were estimated by dividing the intracellular content of glycerol by the cell volumes, we determined the cell volume. Figure 6 shows the changes in the cell volume of *D. tertiolecta* cultured in media of different osmotic pressures containing NaCl, Na_2SO_4 or $MgSO_4$.

e) Contribution of glycerol to osmotic adjustment.

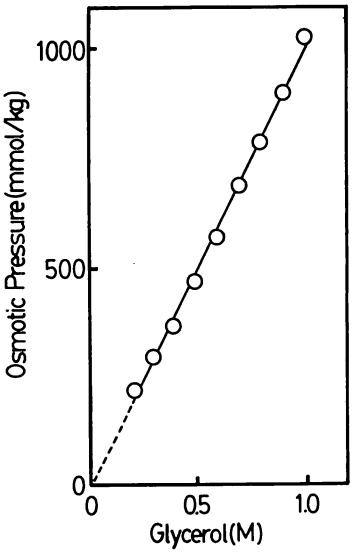

Fig. 5. Osmotic pressures of glycerol aqueous solutions of different concentrations. The osmotic pressure was measured with a vapor pressure osmometer at room temperature.

To elucidate the contribution of intracellular glycerol to osmotic adjustment, the osmotic pressures arising from the concentrations of

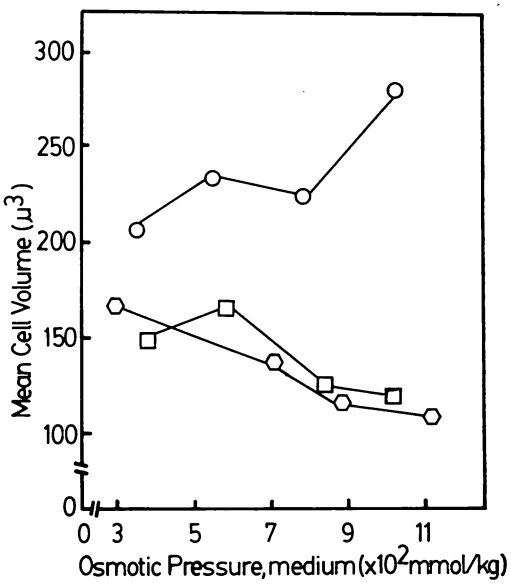

Fig. 6. Mean cell volumes of *D. tertiolecta* cells cultured for 4 days in media of different osmotic pressures containing NaCl, Na_2SO_4 or $MgSO_4$. The cells were photographed through a microscope, and then the mean cell volume was calculated, assuming that the cells had ellipsoidal shapes. (○) NaCl; (□) Na_2SO_4; (○) $MgSO_4$.

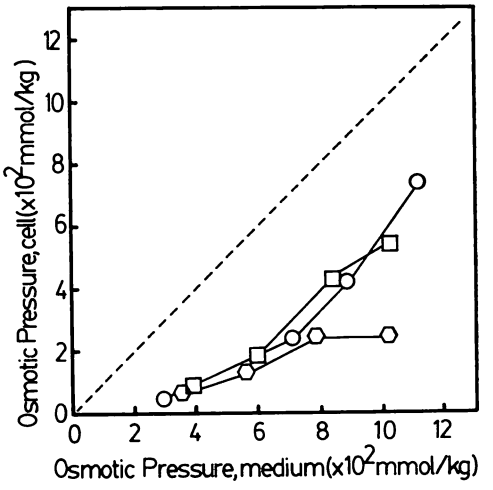

Fig. 7. Contribution of glycerol to osmotic adjustment estimated on the basis of the data shown in Fig. 5. The dotted line represents the osmotic pressure of intracellular glycerol which is equal to that of the culture medium. (○) NaCl; (□) Na_2SO_4; (○) $MgSO_4$.

intracellular glycerol were obtained on the basis of the data in shown in Fig. 5 and compared with the osmotic pressures of the culture medium. The estimated osmotic pressure due to intracellular glycerol was plotted against the osmotic pressure of the culture medium, as shown in Fig. 7, where the dotted line represents the osmotic pressure of intracellular glycerol equal to that of culture medium.

f) Contribution of glycerol to osmoregulation in cells cultured in media with higher concentrations of NaCl.

As seen in Figs. 1, 2 and 3, only intracellular glycerol increased in relation to the concentrations of salts in media. However, the contribution of intracellular glycerol was not 100% as shown in Fig. 7, indicating that other soluble components contribute as osmotica.

The contents of various components in cells cultured in media with concentrations of NaCl higher than those in Fig. 1 were measured by the methods described above, because the solubilities of Na_2SO_4 and $MgSO_4$ were not larger that that of NaCl. The results are shown in Fig. 8. The changes in

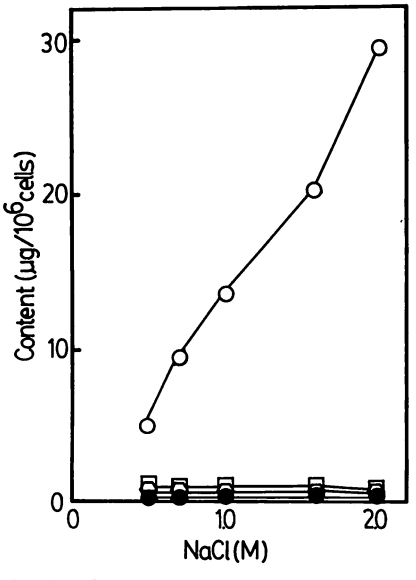

Fig. 8. Contents of various osmotica in *Dunalielia* cells cultured for 4 days in medium with higher concentrations of NaCl than those in Fig. 1. Data represent the averages of three experiments. (○) glycerol; (□) amino acids; (◇) reducing sugars; (●) K^+.

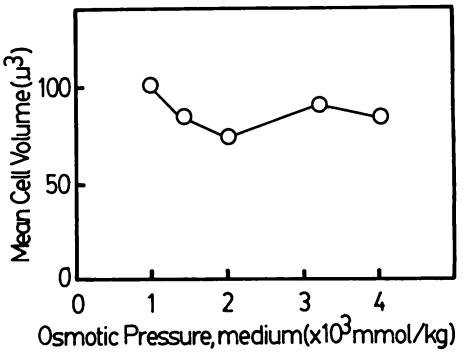

Fig. 9. Mean cell volume of *Dunaliella tertiolecta* cells cultured for 4 days in NaCl media of different osmotic pressures. The cells were photographed through a microscope, and then the mean cell volume was calculated, assuming that the cells had ellipsoidal shapes.

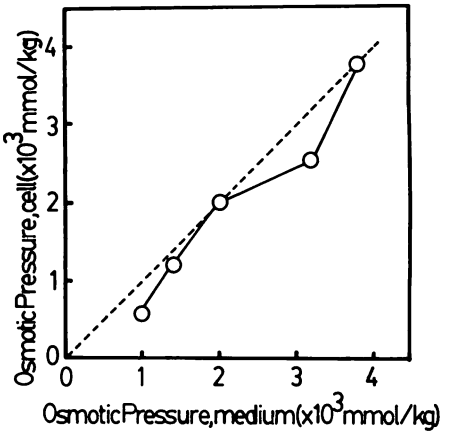

Fig. 10. Contribution of glycerol to osmotic adjustment estimated on the basis of the data in Fig. 5. The dotted line represents the osmotic pressure of intracellular glycerol which is equal to that of the culture medium.

Table 1. Effect of hypertonicity with various salts on the growth of *Dunaliella tertiolecta*.

NaCl	$NaNO_3$	Na_2SO_4	$MgCl_2$	$Mg(NO_3)_2$	$MgSO_4$
++	++	++	−	−	++

D. tertiolecta was cultured for 4 days in 0.5 M NaCl, 0.5 M $NaNO_3$ 0.45 M Na_2SO_4, 0.35 M $MgCl_2$, 0.36 M $Mg(NO_3)_2$ or 0.94 M $MgSO_4$ containing the basal culture medium. The initial cell number was 10^4 per ml (++ : good growth; − : no growth).

cell volume with increasing NaCl concentration were measured in the same manner (Fig. 9) and the contribution of glycerol to osmoregulation was also evaluated (Fig. 10).

Discussion

Little has been reported on the effects of salt, except for NaCl, on the growth of *Dunaliella* and on osmoregulation in *Dunaliella* cells cultured in a hypertonic medium of salt other than NaCl. Previously, we reported that *D. tertiolecta* could grow in $MgSO_4$-hypertonic medium as well as NaCl-hypertonic medium (FUJII *et al.* 1983). Good growth was also observed in Na_2SO_4- or $NaNO_3$-hypertonic medium, but not in $MgCl_2$- or $Mg(NO_3)_2$-hypertonic medium. Therefore, when Mg salt was used, the growth pattern differed from that of Na salt.

For this strain to grow in a hypertonic medium of salt other than NaCl, osmoregulatory mechanisms are necessary to maintain suitable osmotic pressure within the organisms. As shown in Figs. 1, 2 and 3, glycerol may be produced within the cells to counterbalance the osmotic pressures of the medium with Na_2SO_4 or $MgSO_4$ as with NaCl. According to WEGMANN (1971), *D. tertiolecta* can grow in a hypertonic medium with sucrose or 2-deoxy-D-glucose as well as NaCl, with the cells producing glycerol to maintain the osmotic pressure within them. From these results, he suggested that this glycerol formation occurred in response to osmotic pressure rather than ionic strength. We also ascertained that the changes in glycerol were caused by changes in the osmotic pressure in the medium with $MgSO_4$ or Na_2SO_4 as well as NaCl as shown in Figs. 1, 2 and 3, respectively. This means that glycerol formation depends on the osmotic pressures in a medium, irrespective of the kind of cation or anion present.

Figure 7 shows the contribution of intracellular glycerol to osmotic adjustment of *D. tertiolecta* cultured in a medium with NaCl, Na_2SO_4 or $MgSO_4$. The osmotic pressure in cells derived from the intracellular glycerol content increased with an increase in the osmotic pressure of the medium. These results indicate that glycerol plays the leading role as the major osmoticum responsible for osmoregulation of *D. tertiolecta*, irrespective of the kind of salt. However, the extent of contribution by the intracellular glycerol was not 100% as shown in Fig. 7. Especially in the case of the $MgSO_4$-hypertonic medium, its contribution was lower than in that in the NaCl- or Na_2SO_4-hypertonic medium. This may be due to differences in the cell volume, because the cell volume of *D. tertiolecta* cultured in $MgSO_4$-hypertonic medium was about twice that in the NaCl- or Na_2SO_4-medium, although the glycerol contents per 10^6 cells were almost same in all cases. Study of the instantaneous shrinkage of *D. maria* cells transferred to hypertonic media containing copper showed that the shrinkage becomes smaller, the initial rate of volume readjustment becomes faster and the new steady-state volume progressively increases as the copper concentrations become higher (RIISGARD 1979, RIISGARD *et al.* 1980). Our result may indicate that Mg-ion hypertonicity has a different effect on the volume regulation of *D. tertiolecta* compared with Na-ion hypertonicity.

Other soluble components, that is amino acids, reducing sugars and cations, also had roles as osmotic agents. In fact, as pointed out by GINZBURG *et al.* (1983), glycerol was clearly not the only osmotic agent within the cells. And as reported by GIMMLER and SCHERLING (1978), Na or K cation in *D. parva* may have been partially responsible for the compensation of the external osmotic pressure. However, the K^+ content in *D. tertiolecta* did not increase in proportion to the salt concentration increase in the medium as shown in Figs. 1, 2 and 3. This difference may be due to a difference in species.

We evaluated the contribution of intracellular glycerol to the osmotic adjustment in cells cultured in media with higher NaCl concentrations than those as shown in Fig. 1. As seen in Fig. 10, the sum of the intracellular concentration of glycerol in cells

cultured in the medium with an osmotic pressure greater than 1000 mmol/kg (equivalent to 0.5 M NaCl medium) is sufficient to balance the external NaCl concentration. These results also show that intracellular glycerol is the major osmoticum and other soluble components are minor osmotic agents.

We evaluated the contribution of intracellular glycerol to the osmotic adjustment, assuming that the intracellular glycerol is homogeneously distributed within a *D. tertiolecta* cell. However, no definite evidence exists to verify this assumption. If glycerol is localized within an organelle, for example, a vacuole, the content of intracellular glycerol may be sufficient to the compensate for the external osmotic pressure.

Acknowledgement

The authors wish to express their thanks to Dr. S. MANTANI of Tezukayama College for his advice.

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藤井修平・山本良一・高田英夫：マグネシウム塩高張環境下における *Dunaliella tertiolecta* の浸透圧調節に対するグリセロールの寄与

D. tertiolecta の硫酸マグネシウム高張環境下における浸透圧調節を調べた。その結果，グリセロールのみが浸透圧調節物質として寄与していることを確かめた。塩化ナトリウムまたは硫酸ナトリウムの高張環境下についても同様に調べたところ，硫酸マグネシウム高張下の場合と同じ程度に，グリセロールが浸透圧調節物質として寄与していることがわかった。このことより，*D. tertiolecta* における浸透圧調節物質としてのグリセロール合成は，塩の種類ではなく，培地の浸透圧に依存していると考えられる。(631 奈良市学園南3丁目 帝塚山短期大学)

Jap. J. Phycol. (Sôrui) 32: 307-318. December 10, 1984

The ultrastructure of gametes of *Spongomorpha duriuscula* (Acrosiphoniales, Chlorophyta), with special reference to the flagellar apparatus

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MIYAJI, K. and HORI, T. 1984. The ultrastructure of gametes of *Spongomorpha duriuscula* (Acrosiphoniales, Chlorophyta), with special reference to the flagellar apparatus. Jap. J. Phycol. 32: 307-318.

The fine structure of the biflagellate gametes of the multinucleate, septate siphonous green alga, *Spongomorpha duriuscula* (RUPRECHT) COLLINS, was investigated with particular reference to the flagellar apparatus. The gametes have features believed to be most reliable for the taxonomic definition of the Ulvophyceae sensu STEWART and MATTOX, namely the 11/5 o'clock position (counterclockwise) of basal bodies and their associated microtubular rootlets, and the presence of terminal caps. In addition, they have other characters which are presumably shared with some members of the Ulvophyceae; 180° rotational symmetry, basal body overlap, the absence of scales and rhizoplasts, the presence of a one-piece, non-striated capping plate, electron dense material inside basal bodies, striated X-membered rootlet associated bands, the tiny, second proximal connecting fibre, proximal sheaths and a mating structure.

Additional features which have not been reported so far in other green algae are the presence of the third, indistinct proximal fibre and a gap between two anterior triplets of basal body when viewed in cross section.

Key Index Words: *Acrosiphoniales*; *flagellar apparatus*; *green alga*; Spongomorpha duriuscula; *ultrastructure*; *Ulvophyceae*.

Studies on cell division and the flagellar apparatus of the green algae by electron microscopy have provided the ultrastructural criteria useful for the modern taxonomy of green algae (e.g. STEWART and MATTOX 1975 1978, STEWART *et al.* 1973, SLUIMAN *et al.* 1983, O'KELLY and FLOYD 1983). However, these features have been investigated less in the septate siphonous green algae (species of the Cladophorales and Acrosiphoniales).

The ultrastructure of mitosis and cytokinesis has been studied in *Cladophora glomerata* (L.) KÜTZING (MCDONALD and PICKETT-HEAPS 1976), *C. flexuosa* (GRIFFITHS) HARVEY (SCOTT and BULLOCK 1976), *Acrosiphonia spinescens* (KÜTZ.) KJELLM. (HUDSON and WAALAND 1974), and that of the flagellar apparatus in *Urospora penicilliformis* (ROTH) ARESCHOUG (KRISTIANSEN 1974, SLUIMAN *et al.* 1982), *Cladophora* sp. (FLOYD 1981) and *Chaetomorpha spiralis* OKAMURA (HIRAYAMA and HORI 1984).

The order Acrosiphoniales is a group of filamentous, branched or unbranched, uninucleate or multinucleate green algae. The order is small and composed of three genera, *Acrosiphonia* J. AGARDH, *Spongomorpha* KÜTZING and *Urospora* ARESCHOUG. On the basis of the quadriflagellate zoospore ultrastructure of *U. penicilliformis*, it has been suggested that *Urospora* should be assigned

to the Ulvophyceae (SLUIMAN *et al.* 1982), in spite of its unusual microanatomy. Based on the ultrastructural study of the mitosis-cytokinesis in *U. neglecta* and *U. wormskioldii*, LOKHORST and STAR (1983) suggested that *Urospora* can be placed in the Chlorophyceae, since they regarded that *Urospora* has a reduced type of phycoplast, and pose a question about the maintenance of the Ulvophyceae as a separate class.

Study on life history of *Spongomorpha duriuscula* (RUPRECHT) COLLINS by laboratory culture (MIYAJI unpublished observation) has revealed that the filamentous gametophytes of this alga are identical in the morphology of thallus, but dioecious. They produce biflagellate isogametes. In the present study the ultrastructure of the gamete of one sex was examined.

Materials and Methods

The isolate of *Spongomorpha duriuscula* (RUPRECHT) COLLINS used in this study was originally established from zoospores released from green unicellular endophytes (*Chlorochytrium inclusum* KJELLM.) of the red alga, *Rhodophysema* sp. collected on 27 June, 1975, at the coast of Rausu, Hokkaido, Japan, by Dr. Michio MASUDA. Since then unialgal culture has been maintained at 10°C in Provasoli's Enriched Sea Water (PES) medium. Illumination at about 5000 lux is provided by cool-white fluorescent lights on a 16 : 8 (L : D) photoperiod. The release of gametes is induced by immersing the cultured thallus into fresh medium or lighting the thallus. Fresh gametes have positive phototaxis, and they gather towards the light source. After collecting by centrifugation at ca. 3,000 rpm, they were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) containing 0.25 M sucrose for 2-3 h at the room temperature. Then they were rinsed in the same buffer. During this procedure the concentration of sucrose was gradually reduced. The gametes were post-fixed in 2% OsO_4 for 3 h at the room temperature.

Specimens were dehydrated in a graded ethanol series and embedded in Epon. The materials were sectioned by LKB 8800 ultrotome using a diamond knife and double stained with uranyl acetate and lead citrate and examined in Hitachi HU-12A electron microscope.

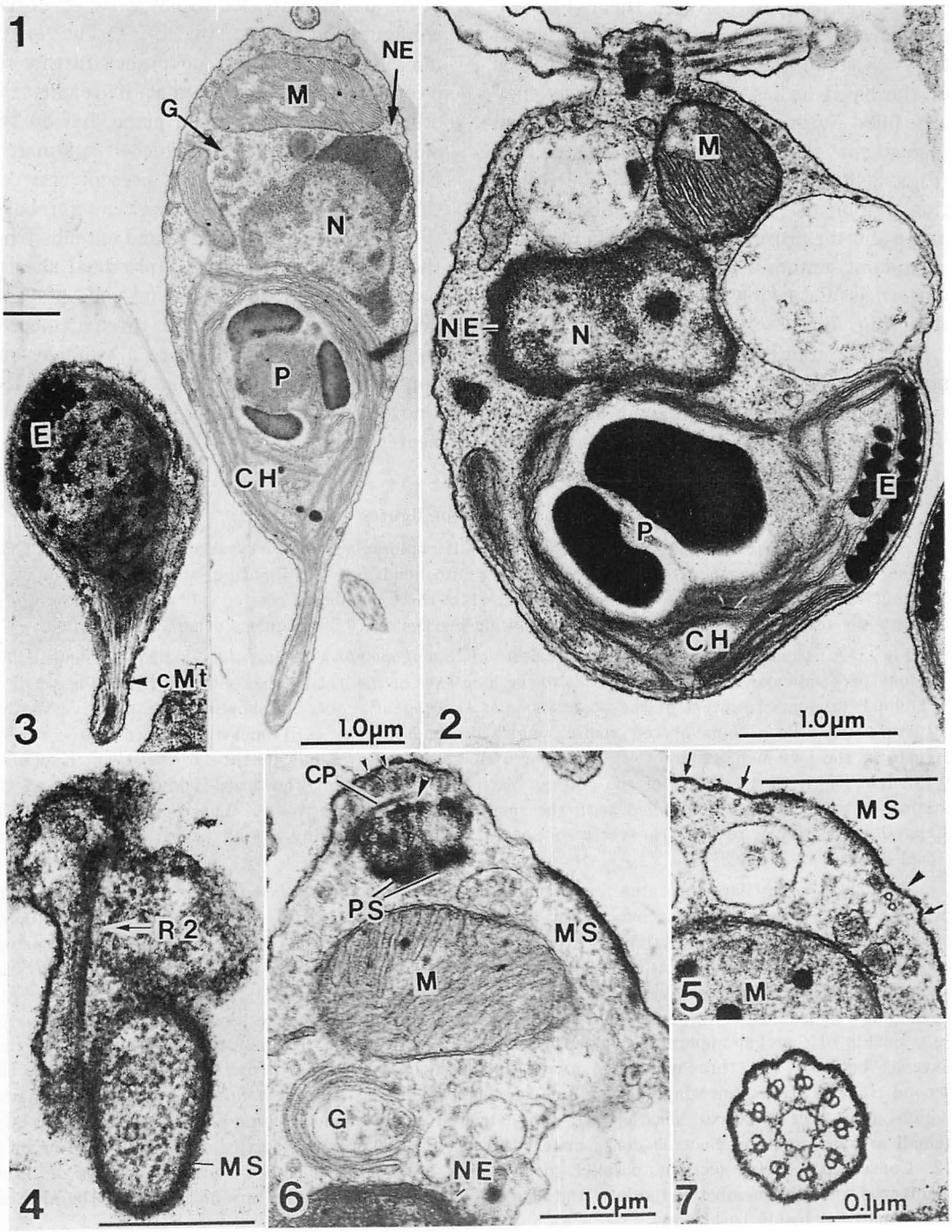
Results

General cell structure: The gametes are oblong or elliptical in shape, approximately 5-7 μm in length, 2-3 μm in width and bear a conspicuous tail (Figs. 1, 3). A papilla present at the cell anterior contains small vesicles filled with electron dense material (Fig. 6). The nucleus is located at the centre of the cell and the lumen of the nuclear envelope is filled with electron dense material (Figs. 1, 2). A single giant mitochondrion with many, well developed cristae lies between the nucleus and the basal bodies (Figs. 1, 2, 6). A golgi body is often found in the area between the nucleus and the mitochondrion (Fig. 6). The endoplasmic reticulum extends from the outer membrane of nuclear envelope, but is poorly developed (not shown). A single cup-shaped chloroplast, situated in the posterior portion of the gamete and extending into the posterior tail (Fig. 1), includes a large central pyrenoid ensheathed by polypyramidal starch plates (Fig. 1). The dense matrix of the pyrenoid is never traversed by thylakoid. The chloroplast also contains an eyespot composed of two layers of closely packed osmiophilic globules (Figs. 2, 21). The eyespot is situated on the lateral surface of the cell in the longitudinal plane passing between the two basal bodies (Fig. 2). The posterior tail of the cell body is filled with cytoskeletal microtubules (Fig. 3) which originate in the vicinity of the flagellar apparatus and extend posteriorly beneath the cell periphery.

Posterior to the basal bodies there is a uniquely differentiated portion of the plasmalemma which is underlaid by a thick layer of electron dense material, 30-50 nm in thickness (Figs. 4-6). This structure protrudes slightly to the rest of the plas-

malemma. Longitudinal and transverse sections indicate that this structure is oval in shape (Fig. 4), measuring 0.8–1.3 μm in length and 0.4–0.7 μm in breadth. This may correspond to the mating structure known in the gametes of *Ulva lactuca* L. (MELKONIAN 1980b). No scales are present on either cell or flagellar surface.

Flagellar apparatus: Two flagella emerge from a papilla, and longitudinal and transverse sections show that their basal bodies lie in the same plane perpendicular to the



longitudinal axis of the cell (Fig. 2). When viewed from the cell anterior, the basal bodies are displaced relative to each other and are oriented at the 11 and 5 o'clock positions (by terminology of ROBERTS *et al.* 1982, MELKONIAN and BERNS 1983) relative to the vertical line between them (Fig. 13) and parallel to the long axis of each basal body (Figs. 13, 14). The proximal halves of the basal bodies are unusual since they are filled with the electron dense material instead of the usual cartwheel structure (Figs. 8, 10b, c, e, 12b, 13b, c). The transition region is composed of a longer distal and a shorter proximal part and there is the prominent septum (Figs. 12b, 13b). A stellate pattern is discernible in the trrnsverse section (Fig. 7).

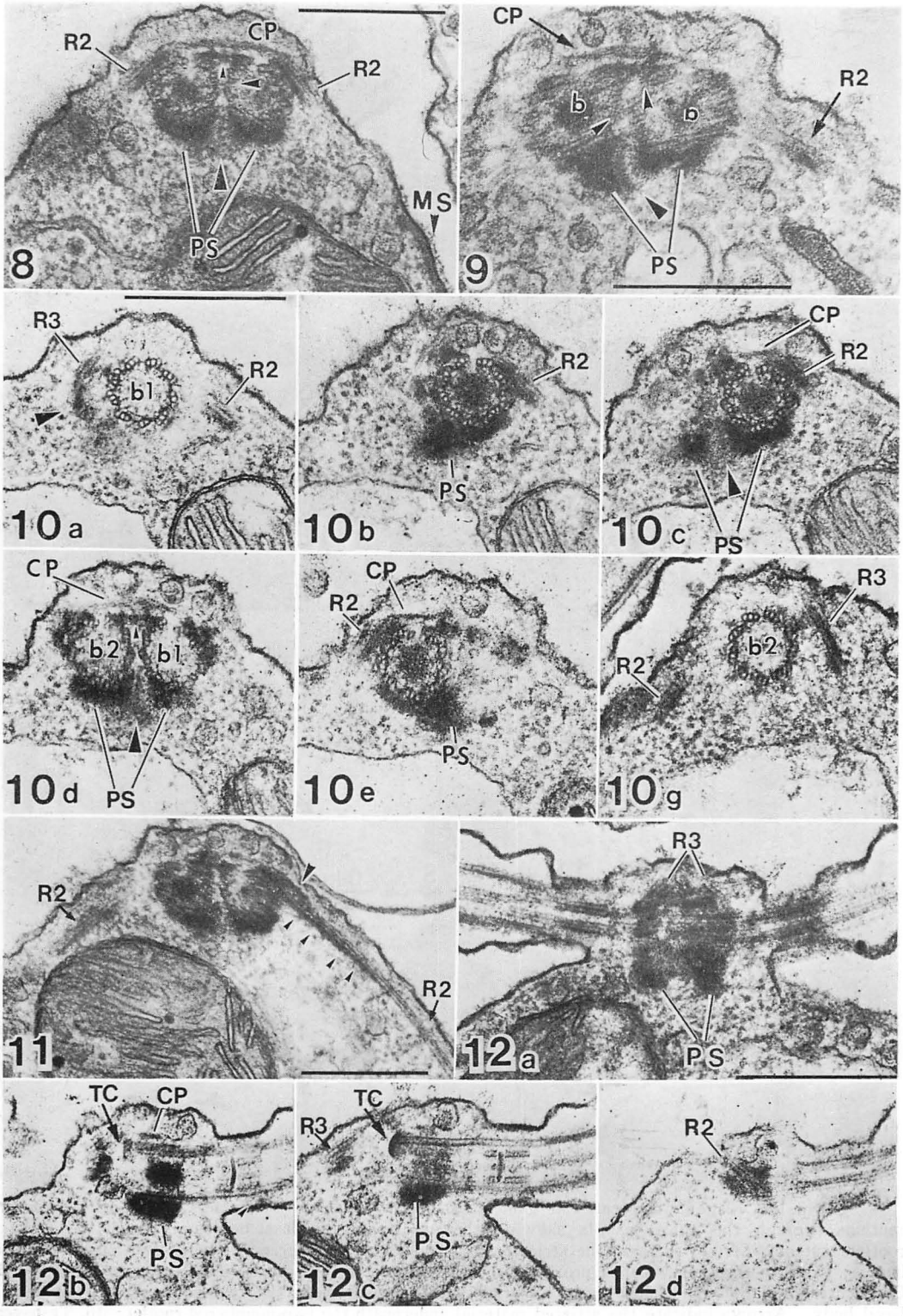
The four microtubular rootlets are cruciately arranged (Fig. 13). The three-membered rootlets originate from the inner sides of the overlapping basal bodies and are displaced in the 11/5 o'clock configuration (Fig. 14). In the immediate vicinity of the origination, rootlet microtubules lie linearly (Fig. 17), but one microtubule very soon becomes reorientated se that it lies under the other two, thus for some distance transverse sections of the flagellar rootlet display a two-over-one configuration (Figs. 18, 19). The lower microtubule, however, undergoes further reorientation (Fig. 20) so that it rejoins the other two in the original plane, but on the side opposite to that on which it originated. Electron dense material is present between the two upper and the lower microtubules (Figs. 18, 19). A striated band extends from the electron dense material (proximal sheath) present around the proximal ends of basal bodies and attaches to the three-membered rootlets at the point where a two-over-one configuration of microtubules begins (Fig. 18). One of the three-membered rootlets descends near the eyespot (Fig. 21).

Explanation of figures

Abbreviations used in figures: b=basal body; CH=chloroplast; cMt=cytoskeltal microtubule; CP=capping plate; E=eye spot; G=golgi body; M=mitochondrion; MS=mating structure; N=nuclues; NE=nuclear envelope; R2=two-membered rootlet; R3=three-membered rootlet. The alphabets in figures indicate the order of section in a series. All scale markes are 0.5 μm unless otherwise indicated.

Fig. 1-7. General features of the gamete of *Spongomorpha duriuscula*. 1 and 2. Longitudinal sections perpendicular (1) and parallel (2) to the long axes of the basal bodies showing the cell organelles; 3. Oblique tangential section of the posterior tail; 4. Tangential section showing the putative mating structure and the two-membered rootlet which descends nearly; 5. Transverse section of the mating structure, the two-membered rootlet (large arrow). Small arrows indicate the cytoskeltal microtubules; 6. Median longitudinal section of the anterior part of a cell showing the basal body complex and the mating structure. Papilla is filled with the small vesicles (small arrows). Large arrow indicates tiny striated connective fibre; 7. Transverse section of a flagellum showing a stellate pattern in the transitional region.

Fig. 8-12. Flagellar apparatus in gametes of *Spongomorpha duriuscula*. 8. Section showing the proximal ends of basal bodies connected by a non-striated capping plate, a tiny striated fibre (upper small arrow) and a fine connective fibre (middle arrow). Lower arrow indicates the striated structure connecting two proximal sheaths; 9. Oblique section showing two proximal sheaths connected by the striated structure (lower arrow). Upper arrow indicates the second tiny striated connecting fibre and middle one indicates the third tiny fibre; 10. Consecutive serial transverse sections showing the spatial relationship of flagellar apparatus components. Large arrow in Fig. 10a indicates the striated band that extends between the three-membered rootlet and basal body. Uupper arrow in Fig. 10d indicates the second tiny striated connecting fibre and lower arrows in Fig. 10c, d indicate the striated material which joins two proximal sheaths; 11. The striated material underlaying the two-membered rootlet (small arrows) and the electron dense material overlaying the proximal portion of rootlet (large arrow); 12. Consecutive serial sections parallel to the long axes of basal bodies showing insertion of three-membered and two-membered rootlets, the shape of proximal sheath, the terminal cap and the electron dense material inside basal body.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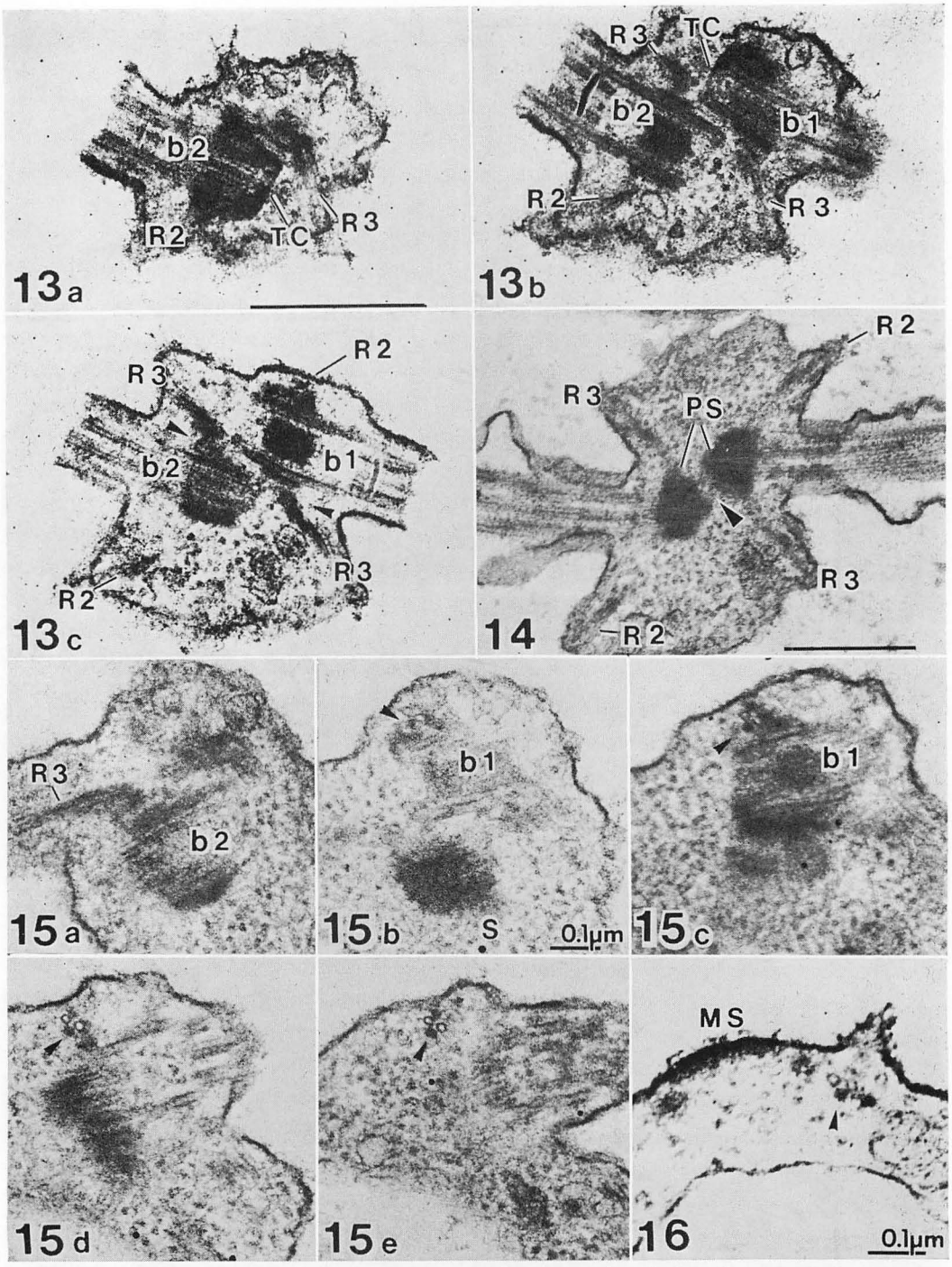
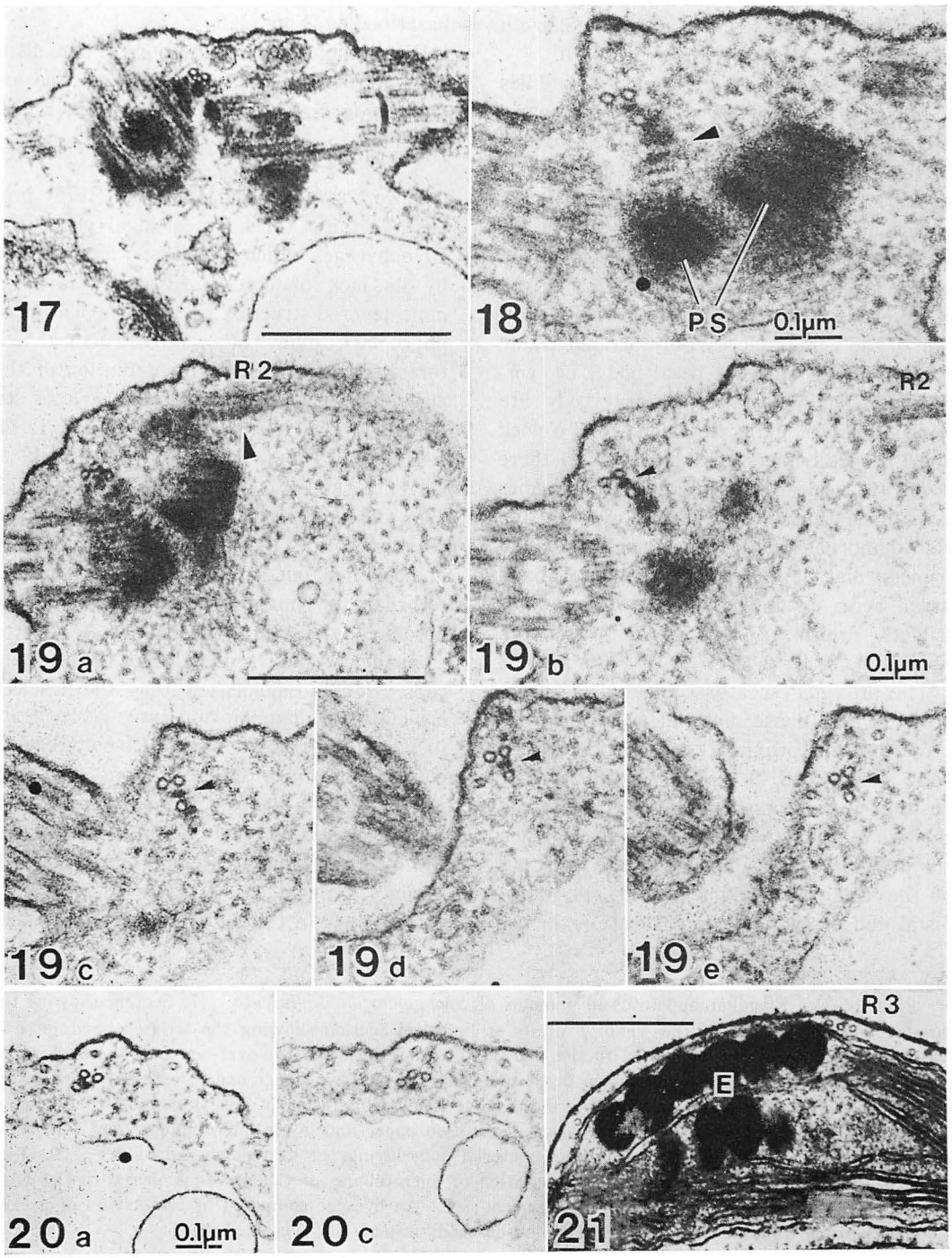


Figs. 13-16. Flagellar apparatus in gametes of *Spongomorpha duriuscula*. 13. Serial transverse sections through the apical papilla showing the overlapping of basal bodies and cruciately arranged rootlet system. Arrows indicate the striated band connecting the three-membered rootlets to the basal bodies; 14. Two triangular-shaped proximal sheaths connected by the striated material (arrow); 15. Consecutive serial sections showing the spatial relationship between the two-membered rootlet and the basal body. Arrows indicate the striated material underneath the two-membered rootlet; 16. Transverse section showing the two-membered rootlet, which descends near the mating structure. The underlaying striated material of the two-membered rootlet still remains (arrow).

The two-membered rootlets emanate from the outer sides of the basal bodies (Figs. 14, 15). Two rootlets form a straight line. Striated material underlies two-membered rootlet (Figs. 11, 19a), and ends near the anterior edge of the putative mating structure (Fig. 16). In addition the proximal part of these rootlets is covered for some distance by an electron dense material (Fig. 11). It starts at a point slightly posterior to the



point of attachment to the basal body (Fig. 15c) and soon bifurcates, part being associated with each microtubule (Fig. 15d). One of them runs for only a short distance over its microtubule, whereas the other extends further posteriorly (Fig. 15e). One of two-membered rootlets descends along the margin of the mating structure (Figs. 4, 5, 16).

A one-piece, non-striated capping plate lies to link two basal bodies at their distal parts (Figs. 8, 9, 10d). In these cells the proximal portions of basal bodies are also joined by a second, more tiny striated fibre. It is attached at one end to the triplet in the 11 o'clock position of the clockwisely arranged nine triplets of one basal body, and at the other end to the triplet in the 1 o'clock position of the counterclockwisely arranged nine triplets of the adjacent basal body, i.e. corresponding triplets in each basal body are joined (Figs. 8, 10d). Between the 11 o'clock and 1 o'clock triplets in each basal body there is always a clear gap (Fig. 10d). Sometimes it is found that the 9 o'clock triplet of the right-hand basal body (clockwise rotation of triplets) and the 3 o'clock triplet of left-hand basal body (counterclockwise rotation of triplets) are also joined by a further thin fibre (Fig. 8, 9).

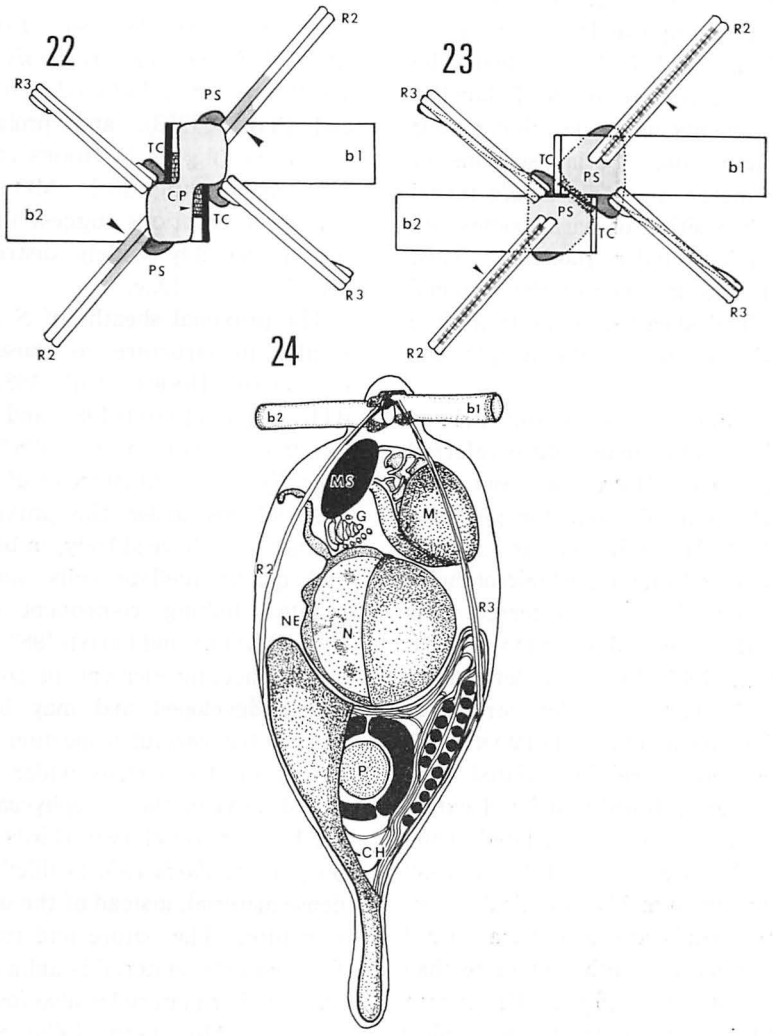
The proximal sheaths composed of unstriated electron dense material are closely appressed to the proximal portion of each basal body (Figs. 6, 8, 9, 10b-e, 12a-c). Their position in relation to the basal bodies and relative to each other, and their shape when cut in a plane perpendicular to the long axis of the cell, are shown in Fig. 14. The distal end of each proximal sheath is thicker on the inner than on the outer side and curves towards the other basal body (Figs. 10b-e). The two triangular sheaths are connected by the striated material (Figs. 8, 9, 10c, d, 14). Terminal caps are attached to the anterior side of the proximal ends of basal bodies (Figs. 12b, c, 13a, b).

Diagrammatic reconstructions of the flagellar apparatus and the gamete of *Spongomorpha duriuscula* are given in Figs. 22-24.

Discussion

Since STEWART and MATTOX (1978) proposed a new third green algal class, the Ulvophyceae, which was first characterized by its lack of certain features, that is, a multi-layered structure, a phragmoplast or a phycoplast, many other ultrastructural features presumably useful for definition of the class have been suggested (for reviews see HOOPS *et al.* 1982, MELKONIAN 1979 1980b 1981 1982, MELKONIAN and BERNS 1983, O'KELLY and FLOYD 1983, SLUIMAN *et al.* 1980 1982). O'KELLY and FLOYD (1983) have reassessed the usefulness of those features and pointed out that more reliable features for characterizing the Ulvophyceae are the absolute orientation of flagellar apparatus components, and the presence of the terminal caps. They emphasized that the absolute orientation of flagellar apparatus components in algae referable to the Ulvophyceae is counterclockwise, different from clockwise orientation in the Chlorophyceae sensu STEWART and MATTOX. Their concept of the flagellar apparatus configuration closely resembles those of ROBERTS *et al.* (1982) and MELKONIAN and BERNS (1983), in which

Figs. 17-21. Flagellar apparatus in gametes of *Spongomorpha duriuscula*. 17. Section showing the three-membered rootlet in the vicinity of its origin; 18. Section showing the striated band (arrow) which connects the three-membered rootlet and the proximal sheath. Two-over-one configuration of the three-membered rootlet, and an electron dense material between two upper and lower microtubules are seen; 19. Consecutive serial sections showing two-over-one configuration of the three-membered rootlet. The electron dense material is present between the two upper and lower microtubules (small arrows). Large arrow in Fig. 19a indicates a striated material underlaying the two-membered rootlet; 20. Two sections from a series showing the reorientation of microtubules of the three-membered rootlet from two-over-one configuration to a linear arrangement; 21. An eyespot composed of two layers of packed globules and the three-membered rootlet which descends nearby the eyespot.



Figs. 22-24. Diagramatic representations of the flagellar apparatus and the gamete of *S. duriuscula*; 22. The flagellar apparatus viewed from the anterior of the cell. Small arrow indicates the second tiny striated connecting fibre. Large arrows indicate the electron dense material over the two-membered rootlet; 23. The flagellar apparatus viewed from the anterior of the cell, but this drawing illustrating transparently the posterior part of the flagellar apparatus. Arrows indicate the striated material underneath the two-membered rootlet. For simplicity, the striated bands connecting the three-membered rootlets to the proximal sheaths have been omitted in both figures; 24. Diagramatic representation of the positional relationships among the flagellar apparatus, mating structure, eyespot and other cell organelles in the gametes.

the configuration of the basal bodies in the Ulvophyceae is described as being "the 11/5 o'clock" arrangement. The main difference between two definitions is that the former takes into consideration the arrangement of the basal bodies and associated microtubular rootlets in quadriflagellate as well as biflagellate cells, while the latter is concerned only with the biflagellate cells. HIRAYAMA and HORI (1984), however, found the different absolute orientations between two pairs of basal bodies in the quadriflagellate zoospore of *Chaetomorpha spiralis* OKAMURA, that is, 11/5 o'clock in the upper pair of basal bodies

and 1/7 o'clock in the lower pair of basal bodies. Thus, they suggested that the deignation 11/5 o'clock and 1/7 o'clock should be applied to the respective pairs of flagellar apparatus components when describing their absolute configuration. Serial sections of biflagellate gametes of *S. duriuscula* reveal the 11/5 o'clock position of basal bodies and X-membered microtubular rootlets. This, together with the presence of the terminal caps and proximal sheaths, suggests a close affinity of *S. duriuscula* with the ulvophycean algae.

It has been suggested that the tiny striated fibrous band is found in many algae referred to the Ulvophyceae (HIRAYAMA and HORI 1984). The band extends from the proximal posterior end of the basal body and specifically attaches to the X-membered microtubular rootlet. This band is usually referred to as the system II fibre (MELKONIAN 1980a, ROBERTS *et al.* 1981 1982) as defined by MELKONIAN (1980a); but the structures named as the lateral fibre (SLUIMAN *et al.* 1982), spindle-shaped straight striated fibre (HERTH *et al.* 1981), broadly striated extension (HOOPS *et al.* 1982), striated band (O'KELLY and FLOYD 1983) or "SB2" (STUESSY *et al.* 1983) are presumably identical to it. These striated bands are also characterized by their shortness in length (not more than 1 μm long, mostly 0.2-0.6 μm) (HIRAYAMA and HORI 1984). In contrast the typical system II fibre, the rhizoplast, approaches the cell surface or the nucleus (MELKONIAN 1980a) and is much longer (1.1-5.2 μm in length) (for a review see HIRAYAMA and HORI 1984). The striated band found in *S. duriuscula* agrees with the criteria summarized above.

The flagellar apparatus of *S. duriuscula* has other characteristics worth to mention, although their diagnostic and phylogenetic implications are not yet clear. The tiny basal body connecting band present in this alga is striated and links single triplets of the adjacent basal bodies at their proximal ends. Since such a connecting band was first observed in the male gametd of *Bryopsis maxima* (HORI 1977), it has been reported to occur in *Derbesia tenuissima* (ROBERTS *et al.* 1981), *Pseudobryopsis* sp. (ROBERTS *et al.* 1982), *Friedmannia israelensis* (MELKONIAN and BERNS 1983), *Entocladia viridis* (O'KELLY and FLOYD 1983), and probably *Ulvaria oxysperma* (fig. 14 in HOOPS *et al.* 1982) and *Ulva lactuca* (fig. 10 in MELKONIAN 1979). These observations suggest that this structure is possibly widely distributed in the ulvophyceaen algae.

The proximal sheaths of *S. duriuscula* are similar in structure to those of *Ulvaria oxysperma* (HOOPS *et al.* 1982), *E. viridis* (O'KELLY and FLOYD 1983) and *Enteromorpha intestinalis* (STUESSY *et al.* 1983) and *Urospora pencilliformis* (SLUIMAN *et al.* 1982). This material lies under the proximal posterior surface of each basal body, in both biflagellate and quadriflagellate cells, and there is a striated linking component (HOOPS *et al.* 1982, O'KELLY and FLOYD 1983). The sheaths and connecting element in some algae are poorly developed and may be easily overlooked, but careful inspection will probably reveal in due course wider occurrence of the sheaths in the Ulvophyceae.

About proximal two thirds of the basal body in *S. duriuscula* is filled with electron dense material, instead of the usual cartwheel structure. The nature and functional significance of the material is unknown at present, but a similar material is also found in *Ulvopsis grevillei* (MOESTRUP 1978) and *E. viridis* (O'KELLY and FLOYD 1983).

Acknowledgement

We would like to thank Dr. Michio MASUDA, Hokkaido University for providing collecting materials.

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Note added in proof,

Since this paper was prepared, an article containing relevant information on the flagellar apparatus of the gametes of *Acrosiphonia arcta* and *Urospora gregaria* has appeared (FLOYD and O'KELLY, Amer. J. Bot., 71: 111-120, 1984).

宮地和幸*・堀　輝三：緑藻類モツレグサの配偶子の微細構造，特にその鞭毛装置について**

有隔多核緑藻モツレグサ（*Spongomorpha duriuscula*）の双鞭毛性配偶子の細胞構造，特に鞭毛基部装置を調べた。この配偶子はアオサ綱（sensu STEWART and MATTOX 1978）の定義に現在最も有効と考えられている次のような形質を有していた：(1) 2個の鞭毛基部とそれに付属する2組の微小管性鞭毛根系の1組の反時計方向にずれた11/5時配列，(2) ターミナル・キャップ構造の存在。さらに，この配偶子はアオサ綱に所属すると考えられている藻のいくつかと共通する形質も有している。それらは180°回転対称性，鞭毛基部の部分的なかさなり，鱗片およびリゾプラストの欠損，無紋性鞭毛基部結合構造，鞭毛基部内電子密度物質，X本微小管性鞭毛と鞭毛基部とを結ぶ有紋繊維帯，第二鞭毛基部結合繊維，鞭毛基部鞘構造および配偶子接合構造の存在，等である。

また，本研究では他の緑藻類では従来知られていなかった特徴もいくつか明らかになった。（*274 千葉県船橋市三山 2-2-1，東邦大学理学部生物学教室　**305 茨城県新治郡桜村天王台 1-1-1　茨波大学生物科学系）

Jap. J. Phycol. (Sôrui) 32: 319-332. December 20, 1984

Studies on *Monostroma* (Monostromataceae, Chlorophyta) in British Columbia with emphasis on spore release

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GOLDEN, L. and GARBARY, D. 1984. Studies on *Monostroma* (Monostromataceae, Chlorophyta) in British Columbia with emphasis on spore release. Jap. J. Phycol. 32: 319-332.

Field and culture studies of the marine algae of Triple Island and McNichol Creek in northern British Columbia confirm the presence of three species of *Monostroma*: *M. grevillei*, *M. oxyspermum* and *M. undulatum*. *M. undulatum* is recorded for the first time in the eastern Pacific. *M. arcticum* sensu KORNMANN and SAHLING is considered conspecific with *M. grevillei*. Morphology, spore germination and early development in culture for all species were consistent with observations of these taxa from Europe and Japan. Spore release is similar in all species and this is used to argue that *Monostroma* is a natural group. *Ulvopsis*, *Protomonostroma* and *Gayralia* are synonymized with *Monostroma*.

Key Index Words: *algal systematics*; Monostroma; *British Columbia*; *Chlorophyta*; *phytogeography*; *spore release*

Monostroma was classically defined as a genus of green blade-like monostromatic algae (e.g. WITTROCK 1866, ROSENVINGE 1893). With the advent of culture and developmental studies this generic concept was shown to be inadequate, and with the work of KUNIEDA (1934), KORNMANN and SAHLING (1962), KORNMANN (1964), GAYRAL (1965), BLIDING (1968) and TATEWAKI (1969) new generic concepts have evolved based on characteristics of life history, and ontogeny (see TANNER 1981 for review). The described or redescribed genera are *Ulvaria* RUPRECHT, *Kornmannia* BLIDING, *Ulvopsis* GAYRAL, *Protomonostroma* VINOGRADOVA, *Gayralia* VINOGRADOVA and *Capsosiphon* GOBI.

Early records of monostromatic green algae in the northwestern Pacific were summarized by SETCHELL and GARDNER (1903, 1920) who described eight taxa from Alaska to northern Washington. SCAGEL (1966) provided a major literature review and included five species: *M. arcticum* WITTROCK, *M. fractum* JAO, *M. fuscum* WITTROCK, *M. oxyspermum* (KUETZING) DOTY and *M. zostericola* TILDEN. ABBOTT and HOLLENBERG (1976) referred to three species from California (*M. grevillei* (THURET) WITTROCK, *M. oxyspermum* and *M. zostericola*). In addition, LINDSTROM (1977) summarized previous records from Alaska and included five species (*M. arcticum*, *M. areolatum* SETCHELL et GARDNER, *M. fuscum*, *M. grevillei* and *M. oxyspermum*). Other than the paper of DUBE (1967) on *Ulvaria obscura* KUETZING GAYRAL (as *Monostroma fuscum*) there are no reports of life history and developmental patterns of monostromatic green algae in western North America. *Ulvaria obscura* is a member of the Ulvaceae (BLIDING 1968) and will not be considered in this paper. *Monostroma areolatum* is conspecific with *Kornmannia zostericola*

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(TILDEN) BLIDING which will be treated elsewhere. Regarding *M. fractum*, we have no information.

In this paper we present a field and culture study of the remaining monostromatic green algae known from British Columbia. The species treated are *M. grevillei* (including *M. arcticum*), *M. undulatum* WITTROCK and *M. oxyspermum*.

Study sites

Triple Island (54°17′ N 130°53′ W) was the primary study site. It is a small group of exposed granitic islets 40 km west of Prince Rupert, northern British Columbia. Tides are mixed semi-diurnal with an amplitude of 7.5 m. Water temperature varies from 5°C in January to about 13°C in August. Salinity is ca. 32‰ through most of the year with slightly lower values in July/August. McNichol Creek (54°20′ N 130°21′ W), 2 km northwest of Prince Rupert is a protected, nutrient rich, estuarine habitat. Additional sites included Barkley Sound, Vancouver Island and Vancouver harbour in southern British Columbia.

Materials and methods

From each field sample plants were processed as follows: 1) 25–50 separate blades were placed singly in 300 m*l* plastic glasses to release spores, 2) samples of spores were subsequently cultured in filtered seawater and/or PES medium (PROVASOLI 1968) in a north facing window during 1983 or in a growth chamber in 1984, at ca. 11°C and 16–8 h, 3) spore release was followed by examining transverse sections and surface views of actively releasing material, and 4) samples were stained with 1% aqueous aniline blue and/or preserved on permanent slides in 30% Karo. Some plants were fixed in 5% formalin/seawater. Populations were sampled sporadically through the growing season (November to June) on a daily, weekly or monthly basis.

Voucher specimens are deposited in the Herbarium at the University of British Columbia (UBC).

Results

Monostroma grevillei (THURET) WITTROCK

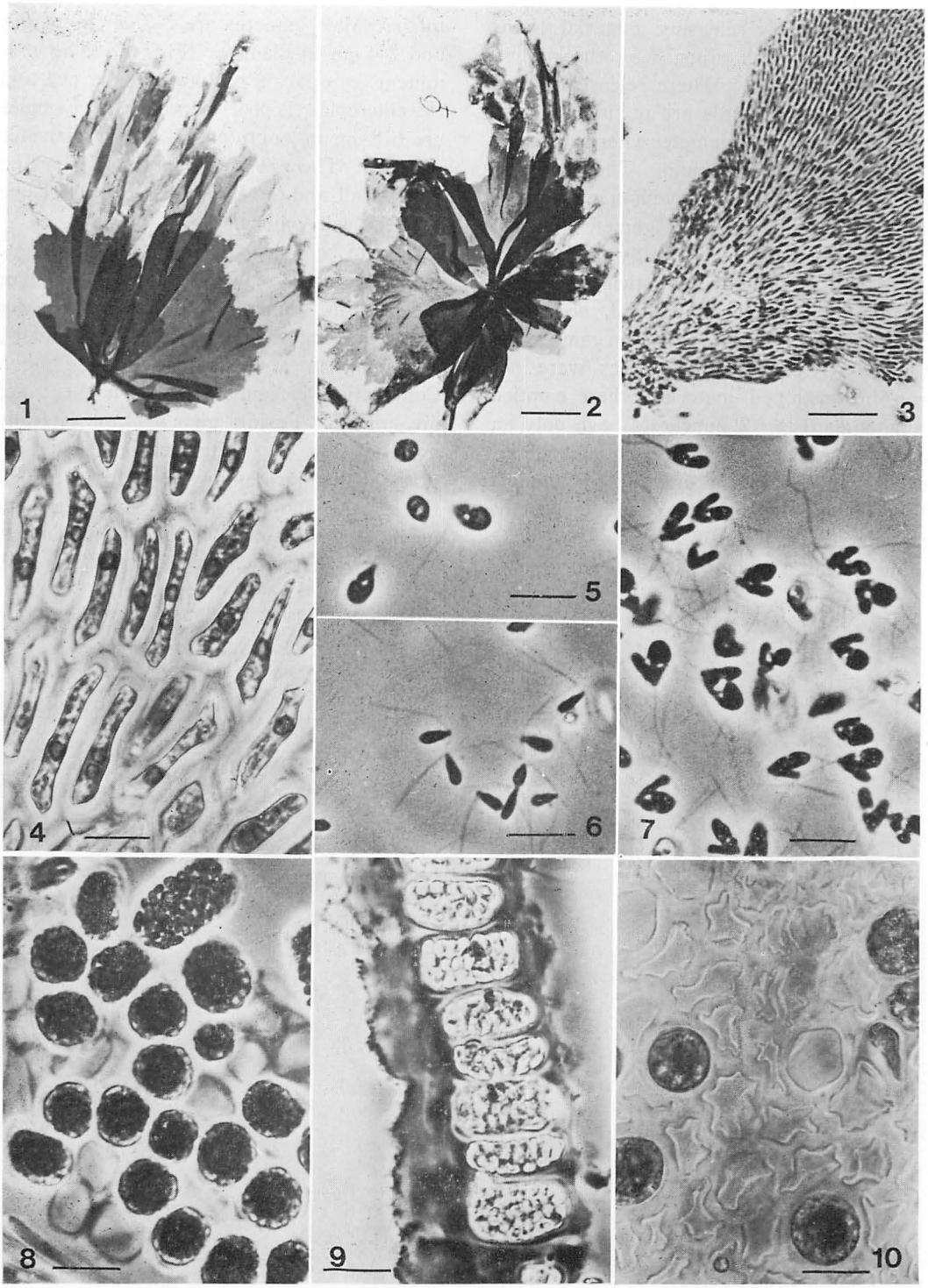
Habitat and seasonality: *Monostroma grevillei* is usually present at Triple Island from late November until mid June. Early in the season populations are limited to shallow, upper intertidal rock pools (ca. 7 m), which become dominated with small plants (ca. 1 cm in height) by January. As days lengthen, deeper and lower pools (at ca. 4–6 m) are colonized. By April, *M. grevillei* is common in the low intertidal zone and attains a size of 15–30 cm. Plants found in June are subtidal at ca. 5 m depth when intertidal plants are rare.

Monostroma grevillei is usually epilithic early in the season but often becomes epiphytic in the late spring. The sac stage is small and transient in intertidal plants, however, subtidal plants may remain sac-like up to 20–30 cm in length. Careful collection and observation of basal portions from most plants will show the remains of the saccate morphology.

Anatomy: Thalli are differentiated into basal, vegetative and reproductive zones, each with its characteristic cell types. Cells near the base are double-ended and have one to several pyrenoids. They are up to 100 μm in length (Fig. 4) and tend to be in linear files. Distally there is a transition zone (Fig. 3) where cell length decreases and cells are more rounded as in typical vegetative portions. Frond thickness varies between individuals and parts of the same thallus. Small, reproductive winter plants may be only 12 μm thick whereas subtidal thalli are >50 μm thick.

Reproduction: *Monostroma grevillei* is dioecious; macroscopically the gametangial zone of the male plant (Fig. 1) is lighter than that of the female plant (Fig. 2). Plants are usually reproductive for several days preceding spring tides.

Gamete release was observed on numerous

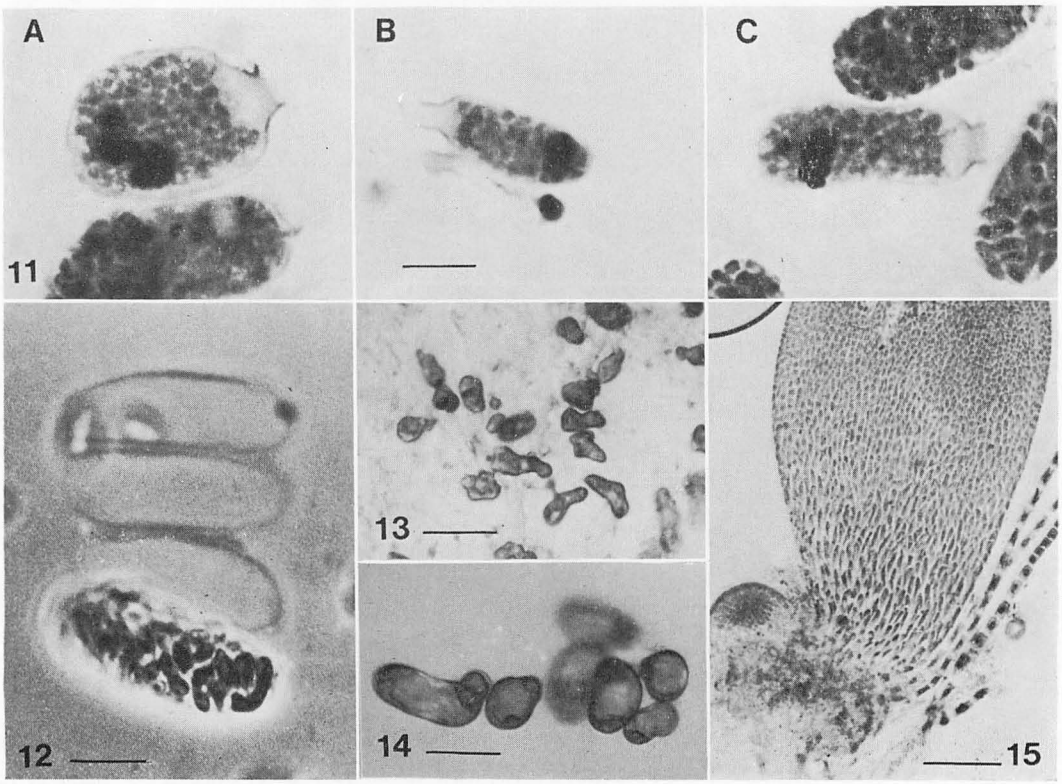


Figs. 1-10. *Monostroma grevillei*. 1. Habit of releasing male plant. Scale=10 mm; 2. Habit of releasing female plant. Scale=10 mm; 3. Lower portion of thallus. Scale=100 μm; 4. Double-ended cells stained with IKI from lower portion of thallus. Scale=25 μm; 5. Female gametes. Scale=10 μm; 6. Male gametes. Scale=10 μm; 7. Fusing gametes. Scale=10 μm; 8. Surface view of release area with upper wall absent. Scale=20 μm; 9. Transverse section through gametangial area of female plant with one wall nearly dissolved. Scale=20 μm; 10. Surface view of released area showing retained gametangia with apparent irregular openings. Scale=20 μm.

thalli. In actively releasing material there is no external wall (Figs. 8) or the wall is only locally present. Where release has not yet occured, both walls are of similar thickness. During release gametes emerge through a pore in the gametangium. They may or may not be enclosed in a common membrane; the latter bursts and the gametes stream though the wall matrix upwardly and laterally until a break in the external cuticle is reached. In aniline blue stained material, occasional flared-necked pores are present on gametangia (Fig. 11 A-C). These structures were not seen in unstained material where emptied gametangia (Fig. 12) appear to have only an unraised circular pore up to 8 μm in diameter. In surface view of the emptied gametangia in a released thallus, the pores appear as irregular rents (Fig. 10).

The released gametes are positively phototactic. Male gametes are 5-7 μm in length and 2-4 μm in diameter (Fig. 6). The prominent eyespot is posterior in the cell and the chloroplast is poorly developed. Females are 6-8 μm in length and 3-5 μm in diameter (Fig. 5). The eyespot is in the midportion of the cell and the chloroplast comprises one half the cell volume.

Gamete behaviour differs in the two sexes. Females swim slowly with frequent changes in direction, remaining in a field of view for 20 sec or longer. Males 'flip-flop' rapidly, remaining in a general area indefinitely. Conjugation is immediate when the sexes are mixed. Fusing gametes are laterally apposed (Fig. 7). After fusion the eyespot of the male migrates to the posterior end of the zygote, and flagella are either shed immediately or retained for up to ten minutes. When zygotes are placed in cultures with



Figs. 11-15. *Monostroma grevillei*. 11A-C. Aniline blue stained gametangia with flared-necked exit pore. Scale=10 μm; 12. Three empty and one nonreleased gametangia. Scale=10 μm; 13. Codiolum cells removed from barnacle shell. Scale=15 μm; 14. Free living codiolum cells in culture. Scale=20 μm; 15. Field-collected disc with upheaval and sac stages found on utricle of *Codium fragile*. Scale=150 μm.

barnacle shell fragments, settlement is random with no apparent substrate preference. Zygotes and unfertilized females developed into codiolum cells (Figs. 13-14) and no survival of male gametes was observed. One month old codiolums removed from decalcified barnacle shells (Fig. 13) had similar gross morphology to the free living codiolums (Fig. 14).

The above observations were repeated with plants from Barkley Sound in April 1982. Similar results were obtained except that unfertilized male and female gametes were both nonviable.

Discussion: *Monostroma grevillei* has been cultured many times (GAYRAL 1965, KORNMANN and SAHLING 1962, JÓNSSON 1968, BLIDING 1968, TATEWAKI 1969 (as *M. angicava* KJELLMAN), and this paper) with only slight deviations in results; dioecious gametophytic blades alternate with a shell boring sporophytic codiolum stage. During ontogeny a prostrate disc is produced which forms the sack stage through a central upheaval. The retention of the emptied gametangia on the vegetative thallus after their release was previously noted by GAYRAL (1965) and TATEWAKI (1969) who emphasized spore release as a taxonomic feature. We consider this character diagnostic for *M. grevillei*.

Monostroma grevillei in British Columbia agrees with literature accounts in most aspects; although, it differs in that a sac-like adult form reported in European literature (e.g. BLIDING 1968) is rarely seen. Although we did not observe development of the zoospores in culture, their ontogeny is evident in field material, especially epiphytic plants on *Codium fragile* (SUHR) HARIOT (Fig. 15).

Distribution: *Monostroma grevillei* is a circumboreal species. We consider the Japanese account by TATEWAKI (1969) under the name *M. angicava* to refer to *M. grevillei*. TATEWAKI describes both bi- and quadriflagellate spores being produced by the codiolum stage, whereas other authors only refer to quadriflagellate spores. TATEWAKI (1972) also noted a different chromosome number (n=ca. 8) from that given by JÓNSSON (1968) (n=6) for plants from Roscoff. Chromosome numbers are needed from more localities before this feature can be used to segregate species.

Monostroma arcticum WITTROCK

Habitat and ecology: *Monostroma arcticum* was found once in April 1983 mixed with intertidal *M. grevillei*. An intensive search in May 1984 located two additional thalli in the subtidal region. These were reproductive several days before the majority of the *M. grevillei* population. Plants were recognized when swarmers became phototactically negative and settled immediately after release.

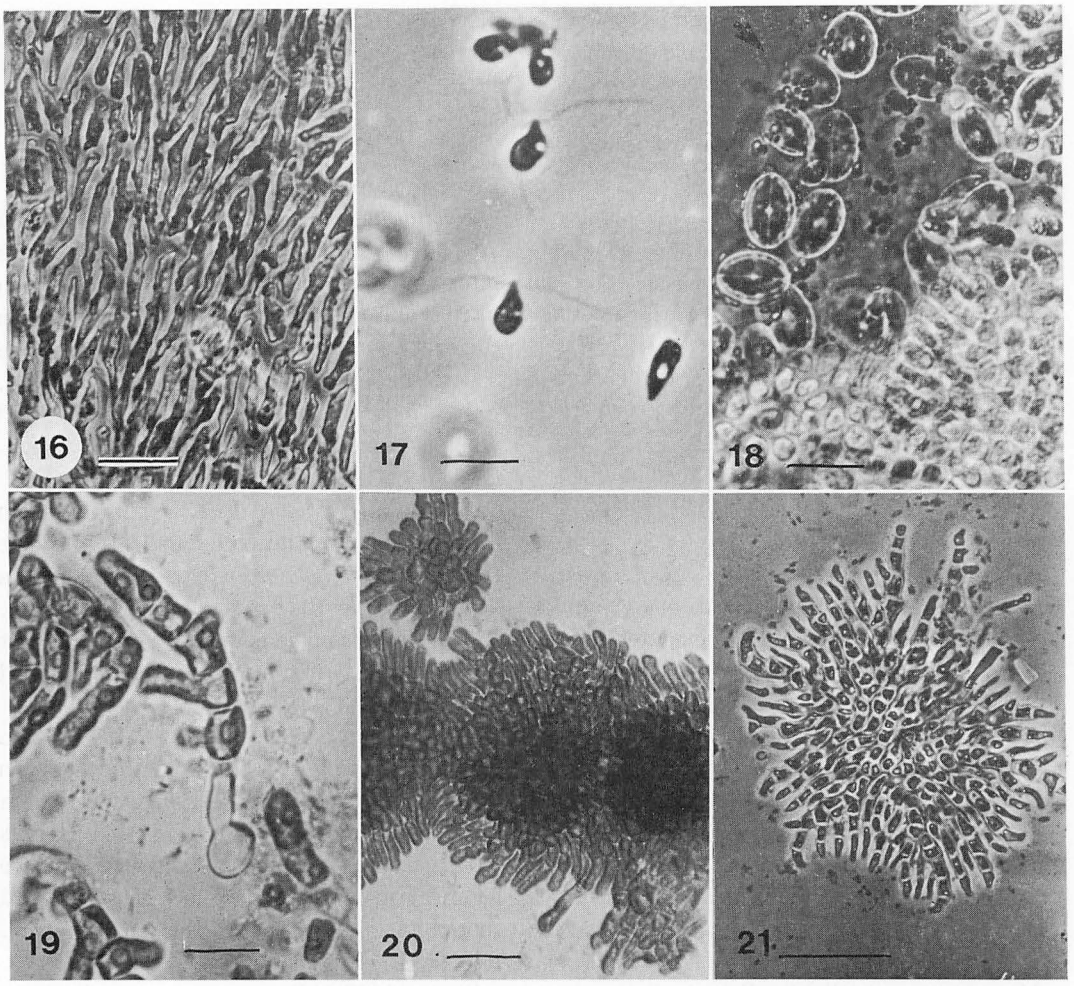
Anatomy: *Monostroma arcticum* from Triple I. is vegetatively indistinguishable from *M. grevillei* (see that species for a description of the vegetative morphology). However, the double-ended cells at the plant bases (Fig. 16) are more irregular than typical *M. grevillei*.

Reproduction: In the two years *Monostroma arcticum* was collected, different mechanisms of spore release were observed. In 1983 a cleared margin was present (Fig. 18), whereas plants collected in 1984 showed spore release identical to *M. grevillei*, i.e. with emptied sporangia remaining on the thallus (Fig. 10).

Released biflagellate swarmers are 8-10 μm in length (Fig. 17) with a prominent eyespot. They are negatively phototactic and settle immediately. No interaction was noted when male gametes of *M. grevillei* were introduced into the release vessels containing active swarmers of *M. arcticum*.

Settled zoospores occasionally showed empty spore germination (Fig. 19). After several weeks prostrate discs were formed (Figs. 20-21) with upheaval occurring in four weeks.

Discussion: Since the culture work of KORNMANN and SAHLING (1962), *Monostroma arcticum* has come to refer to a *M. grevillei*-like plant that recycles asexually by means of biflagellate swarmers. These germinate directly into a prostrate disc which then becomes saccate. Their plants were not



Figs. 16-21. *Monostroma arcticum*. 16. Irregular pattern of double ended cells from based of plant. Scale=50 μm; 17. Biflagellate swarmers. Scale=10 μm; 18. Cleared release area from 1983 thallus indicating wall limit (arrow). Scale=35 μm; 19. Empty spore germination. Scale=15 μm; 20. Prostrate discs from 1984 plants beginning upheaval. Scale=50 μm; 21. Prostrate disc from 1983 plant. Scale=50 μm.

distinguished on vegetative features from *M. grevillei*. This species concept was endorsed by BLIDING (1968) who cultured plants from Sweden and from the type locality in northern Norway. Only six thalli were found by BLIDING in Sweden, all being from the subtidal zone and agreeing "almost completely" with *M. grevillei* from the same location. The Norwegian plants were from the upper intertidal region and differed from *M. grevillei* in having a distinctive layering of the thallus wall observed in transverse section (BLIDING 1968) as previously noted by JAASUND (1965).

In summary, *Monostroma arcticum* differs from *M. grevillei* only in its life history. We question the recognition of any entity at the species level based exclusively on such grounds. In addition, in British Columbia and Sweden only a few individuals have been found with the appropriate life history. JÓNSSON (1968) also notes occasional development of gametes of *M. grevillei* directly into the gametophytic stage without an intervening codiolum. In recognition of the complexities in the life history of *Ulva mutabilis* (see review by FJELD and LØVLIE 1976), we do not consider it suitable to recognize *M.*

arcticum sensu KORNMANN and SAHLING (1962) in British Columbia or elsewhere unless large, self-propagating populations can be found. If such populations can be found then varietal rank would be warranted, as previously suggested by ROSENVINGE (1893).

Monostroma undulatum WITTROCK

Habitat and seasonality: Blades of *Monostroma undulatum* are present at Triple I. from late March when thalli are predominantly epilithic, until early June when all surviving plants are epiphytic on *Fucus gardneri* SILVA. In the field this species may be distinguished from *M. grevillei* when either is reproductive.

Habit: The plants are typically smaller (to 10 cm) (Fig. 22) and a lighter green than *M. grevillei*. Many blades arise from a proliferous base. When epiphytic, rhizoids may penetrate deeply into the host (Fig. 23). Blades are lanceolate as juveniles, becoming more ovate with age. Fronds are delicate and fragile.

Anatomy: Thalli of *Monostroma undulatum* are differentiated into rhizoidal, vegetative and marginal cell types (Figs. 23-25). In surface view, the central basal area has long, narrow, more-or-less double-ended cells that may be 100 μm in length (Fig. 25), and usually have one pyrenoid. Distally, cells become more quadrate and in mid-blade cells are ca. 15×15 μm. At apices and in marginal regions near the base cells are much smaller and ca. 5×5 μm. Thallus thickness is also variable with blades being ca. 50 μm thick near the base, ca. 35 μm in mid portions and ca. 20 μm or less at the margins.

The strongly differentiated rhizoidal and marginal cell types at the base of *M. undulatum* (Fig. 24) provide a diagnostic vegetative feature to distinguish it from *M. grevillei*. The juxtaposition of these cell types is absent at the base of *M. grevillei* (see Figs. 3, 24).

Reproduction: At Triple Island *Monostroma undulatum* is reproductive over several days during neap tides. As the tide returns and covers the plants, the apical margin of the blade is covered with small air bubbles which aggregate into a green froth or scum. This material is comprised of small pieces of thalli and many sporangia which have become dissociated from the plants. From the apices of these sporangia, quadriflagellate swarmers are released one at a time. In blocks of sporangial cells still contained within the thallus wall, the spores move through the mucilaginous wall material to the edge of the fragment where they are liberated.

Swarmers of *Monostroma undulatum* have a distinctive clumping behaviour in which they aggregate with posterior ends touching and flagellated ends free (Fig. 26). This clumping continues for several minutes after which the spores disperse.

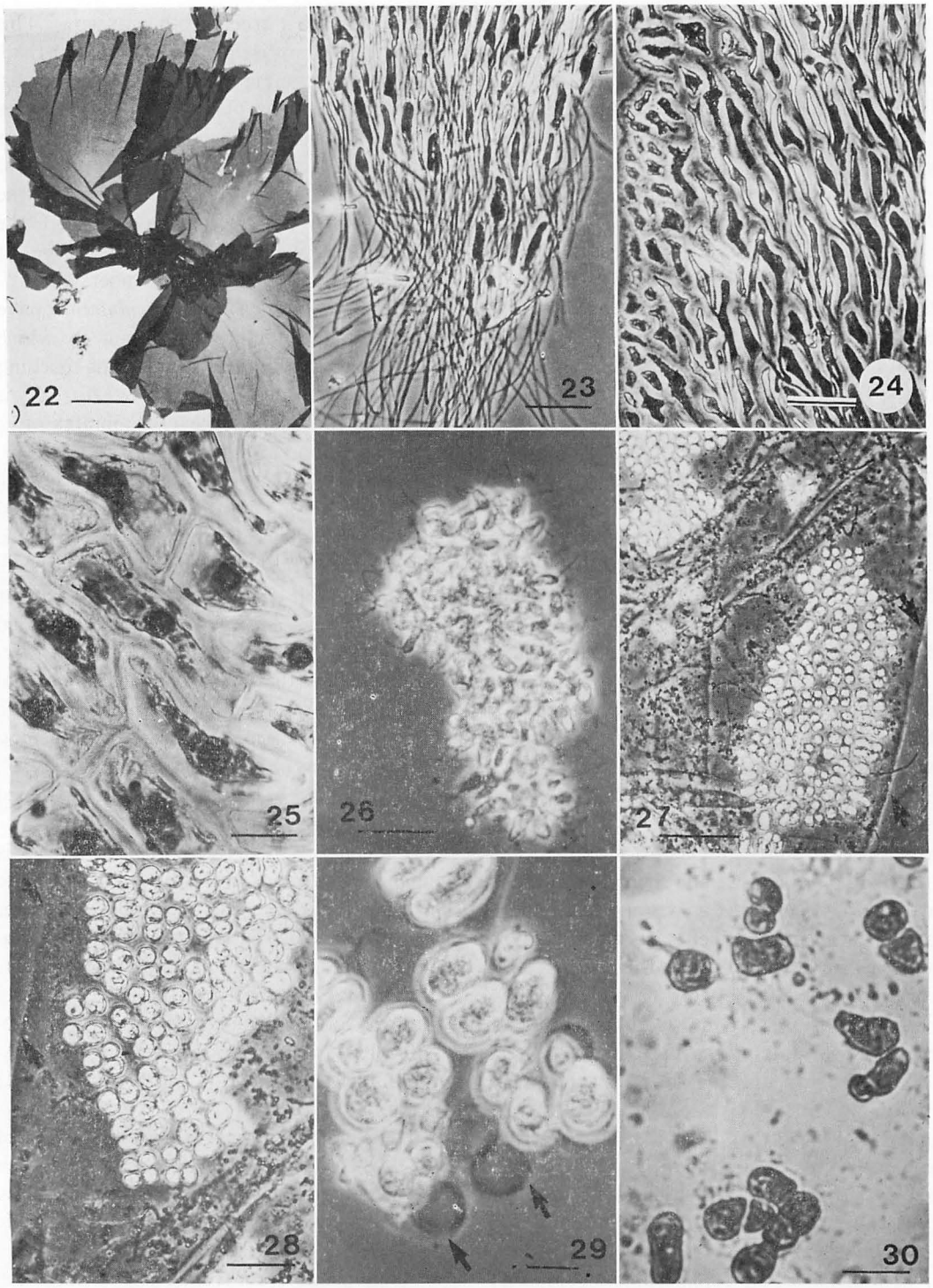
Herbarium sheets of reproductive *Monostroma undulatum* will often show a cleared margin. Examination of formalin preserved material of actively releasing blades shows a thallus wall with geometrically regular blocks of unreleased sporangia intermingled with cleared areas (Figs. 27-28). Cross sections confirm that the external thallus wall is no longer present. Emptied sporangia were infrequent and a small circular pore, opening externally, was observed occasionally (Fig. 29).

Germinated swarmers eventually developed into a squat codiolum (Fig. 30).

Discussion: *Monostroma undulatum* is unique in the genus in having quadriflagellate swarmers that give rise to a codiolum stage. The codiolum also has quadriflagellate swarmers that germinate into a uniseriate filament. This forms a monostromatic blade directly without an intervening saccate phase (YOSHIDA 1964, as *M. pulchrum* FARLOW; TATEWAKI 1969; KORNMANN and SAHLING 1962; BORASO 1977).

The clumping zoospores were first reported by YAMADA and SAITO (1938) and have been consistently reported by subsequent workers. This behaviour may be considered diagnostic for *Monostroma undulatum*.

Adult plants from Triple Island lack the lanceolate blades with undulating margins typical of the species. However, the characteristic shape is usually present in juvenile plants.



Figs. 22-30. *Monostroma undulatum*. 22. Habit. Scale=10 mm; 23. Basal portion of plant with extensive penetrating rhizoids. Scale=70 μm; 24. Margin of plant near base with small cells juxtaposed to rhizoidal cells. Scale=50 μm; 25. Double-ended cells in lower thallus stained with IKI. Scale=15 μm; 26. Large cluster of quadriflagellate swarmers. Scale=15 μm; 27. Partially released area of thallus. Note straight margins of remaining sporangia. Arrows indicate margin of thallus. Scale=50 μm; 28. Detail of sporangial area with one wall absent. Arrows indicate thallus margin. Scale=20 μm; 29. Group of freed sporangia. Note empty sporangia with pores (arrows). Scale=10 μm; 30. Codiolums resulting from germination of quadriflagellate spores. Scale=20 μm.

Distribution: *Monostroma undulatum* is present on both sides of the Atlantic Ocean from Newfoundland (SOUTH and HOOPER 1980) and Helgoland (KORNMANN and SAHLING 1962 1977). It is also known from Japan (YOSHIDA 1964, TATEWAKI 1969) and Saghalien (TOKIDA 1954) in the western Pacific and from Argentina (BORASO 1977) in the south Atlantic. In western North America we have identified specimens from the Pribilof and Aleutian Islands in Alaska and northern British Columbia. These are the first records of *M. undulatum* from the eastern Pacific.

Monostroma oxyspermum (KUETZING) DOTY

Habitat and seasonality: *Monostroma oxyspermum* is a brackish water species not found at Triple Island, although it is common in the Prince Rupert area. This species shows a wide variety of habits from small, *Prasiola*-like plants on high intertidal pilings (Fig. 31) to saltmarsh plants that may exceed a meter in length. At McNichol Creek on the northwest side of Prince Rupert harbour, a large, dense population extends about 30 m up the stream. The species is present throughout the year.

Anatomy: The thallus is differentiated into two regions, an upper blade portion and a lower rhizoidal zone (Figs. 31-35). Rhizoidal cells (ca. 40 μm) have an irregular arrangement (Figs. 34, 35), rounded apices and an extended, narrow process (Figs. 34, 35). In some cases thalli produced secondary rhizoidal regions at the blade margins (Fig. 32). Usually there is an abrupt transition between rhizoidal and vegetative cells (Fig. 34). In living material vegetative cells are closely adpressed, polygonal to rounded, and 10-20 μm in diameter. In dried material, cells may become plasmolyzed and appear in groups of 2-4 (Fig. 33). In transverse section plants vary from 10 μm in winter juveniles to about 50 μm in large plants.

Reproduction: *Monostroma oxyspermum* is reproductive for several days prior to spring tides. Sporangial areas have a slight orange tint which facilitates recognition of reproductive plants in the field.

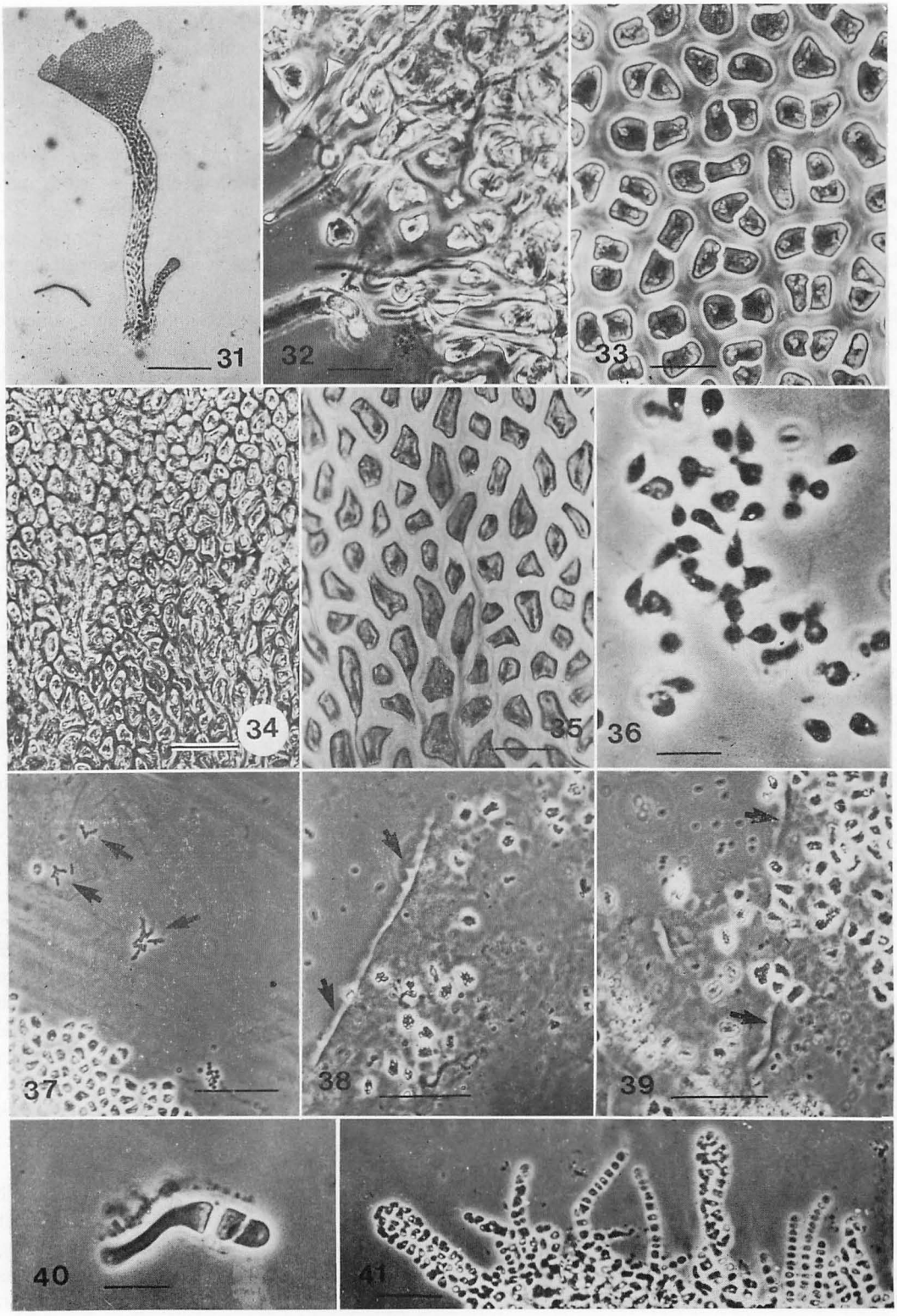
A released thallus may show a cleared margin up to 250 μm wide (Fig. 37). When fresh, the cleared thallus wall has cell impressions remaining from the released sprorangia (as in Fig. 8). However, this feature diminishes with preservation. When thalli are folded, jagged cell impressions may be discerned along the fold line (Figs. 38-39). In released areas of plants only one thallus wall is present; where spore release has not yet occurred, two walls of similar thickness are present. Swarmers emerge rapidly from sporangia though a pore, and move through the wall matrix until a break in the cuticle is found. Emptied sporangia were not observed.

The biflagellate swarmers (Fig. 36) are 8-10 μm in length and have prominent eyespots. They are negatively phototactic and swim for up to 60 min, after which they settle and attach to available substrata. Released zoospores are active, crossing a 400× field of view within several seconds and tend to swim in one direction. Mixing spores from several thalli has no apparent effect on behaviour and no indication of cell fusion was found. Rare quadriflagellate spores were also present.

Spores germinate into a uniseriate filament (Figs. 40-41) which in turn becomes saccate (Fig. 41). The above features were also observed in plants from Vancouver harbour.

Discussion: *Monostroma oxyspermum* refers to an asexual species that recycles blades with biflagellate zoospores. The spores germinate into a uniseriate, filamentous stage which becomes tube-like, then saccate and finally opens into a monostromatic blade (BLIDING 1935, as *M. wittrockii* BORNET; IWAMOTO 1960, as *M. tubiforma* IWAMOTO; KIDA 1964, as *M. wittrockii*; KORNMANN 1964; GAYRAL 1965; BLIDING 1968, as *Ulvaria*; TATEWAKI 1969).

Monostroma oxyspermum is a polymorphic species of brackish waters which led to it being described as several different species. Culture studies from areas as widespread as Japan (KIDA 1964) and Scandinavia (BLIDING 1968) and British Columbia have given similar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results, showing the conspecificity of several taxa. Material from Prince Rupert differed from other descriptions in becoming saccate almost immediately with the *Enteromorpha*-like stage being transitory.

The account of spore release given here differs markedly from that given in most accounts, where *Monostroma oxyspermum* is considered to have a dissolution-type of release (GAYRAL 1965, TATEWAKI 1969, VINOGRADOVA 1969).

Distribution: *Monostroma oxyspermum* is widely distributed in the northern hemisphere from the tropics to the arctic. The species is reported from the southern hemisphere, but the necessary culture studies to confirm this distribution have not been carried out. In Pacific North America it is present in brackish areas from California to Alaska.

Discussion

Monostroma sensu WITTROCK (1866) and the broader concept of ROSENVINGE (1893) has been recognized as a heterogeneous assemblage of species for about fifty years, based upon culture studies. Thus the segregation of *Ulvaria* with its type species *U. obscura* (GAYRAL 1965, BLIDING 1968), *Kornmannia* with its type species *K. leptoderma* (KJELLMAN) BLIDING (BLIDING 1968), and the tentative placement of *M. groenlandica* J. AGARDH in *Capsosiphon* (VINOGRADOVA 1969, see GARBARY *et al.* 1982 for discussion) have received general acceptance. However, there is no concensus among systematists as to the classification of the taxa remaining in *Monostroma*. For example, *M. oxyspermum* has been treated by various workers as belonging to three genera (*Monostroma*, *Ulvaria*, *Gayralia*), three families (Monostromataceae, Ulvaceae, Gayraliaceae) and two orders (Ulvales, Ulotrichales). Such divergences of opinion are the result of the differential weight given to specific morphological, developmental and life history features.

Monostromataceae sensu KUNIEDA (1934) has a life history characterized by an alternation of codiolum and blade phases. Further divisions utilized the ontogeny of the codiolum zoospores as generic or family criteria (GAYRAL 1965, KORNMANN 1964, VINOGRADOVA 1969). More recently, ultrastructure of pyrenoids (HORI 1972) and flagellated cells (e.g. O'KELLY *et al.* 1984) has been used in the search for natural groupings. Mechanisms of spore release are usually claimed to support such classifications.

This paper examines details of spore release and, contrary to previous reports (e.g. GAYRAL 1965, TATEWAKI 1969), we find the process to be similar in the species studied. Thus all taxa show release of spores through a pore in the sporangial wall, spore movement through the thallus wall matrix, and liberation through regions where the cuticle is absent. In all cases this process is followed by disappearance of the thallus wall on the releasing side, and its retention on the opposite side. In *M. grevillei* emptied gametangia remain on the thallus wall, whereas in other species they are shed (or not resolved).

Anatomical details of releasing sporangia are difficult to resolve. In the rare instances when released sporangia have been found, the morphology is similar (i.e. *M. grevillei* and *M. undulatum*). The previously unreported flared necks on gametangia of *M. grevillei* (c.f. DUBE 1967, Fig. 9, for *Ulvaria*)

Figs. 31-41. *Monostroma oxyspermum*. 31. Field collected plants with saccate juvenile. Scale=150 μm; 32. Secondary rhizoids from perennating prostrate plant. Scale=40 μm; 33. Grouping of cells on dried thallus. Scale=40 μm; 34. Transition area between normal vegetative and rhizoidal cells. Scale=80 μm; 35. Detail of cells in rhizoidal region. Scale=40 μm; 36. Biflagellate swarmers. Scale=10 μm; 37. Cleared margin of released thallus with *Microthamnion kuetzingianum* NAEG. (arrow) Scale=100 μm; 38. Portion of actively releasing thallus with some sporangia on surface of remaining wall. Arrows indicate remains of sporangial impressions in wall. Scale=100 μm; 39. As in previous figure. Note outer wall absent. Scale=100 μm; 40. Three celled sporeling. Scale=10 μm; 41. Cluster of cultured plants with various developmental stages. Scale=50 μm.

were only seen using material stained with aniline blue. Such structures are of potential phylogenetic importance and may reflect common ancestry with the Ulvaceae.

It is our opinion that the residual *Monostroma* group should be treated as a single genus in which three subgenera (*Monostroma, Protomonostroma, Gayralia*) may be recognized based on the ontogeny of the blade. The subgenus *Monostroma* has a disc-sac ontogeny as described for *M. grevillei*; the subgenus *Gayralia* has a filament-tube-sac ontogeny as described for *M. oxyspermum*; the subgenus *Protomonostroma* has a filament-blade ontogeny as described for *M. undulatum*. If additional, correlating features are found, these subgenera may warrant generic rank. We feel that too few species have been examined for flagellar ultrastructure (i.e. only *M. grevillei*. *M. bullosum* (ROTH) THURET and *M. oxyspermum*) (review in FLOYD and O'KELLY 1984) to base generic segregation on such features. The taxa examined only include representatives of two of the three subgenera.

If *Monostroma* is monophyletic, then ontogenetic patterns are not as conservative in evolutionary terms as is generally assumed. Laboratory culture of various green algae has demonstrated a range of developmental patterns that are controlled by environmental or apparently stochastic factors. Thus *Urospora* spp. may develope a prostrate habit at high temperatures (KORNMANN and SAHLING 1977, HANIC 1965). TANNER (1979) also reports that *Ulva californica* WILLE in COLLINS *et al.* develops a prostrate system first when grown at high temperatures, and an erect uniseriate filament first when grown under lower temperatures. In addition, TATEWAKI (1969) described the development of three different ontogenies in *Kornmannia zostericola* for plants grown under similar culture conditions. This variation suggests that developmental features may have functional significance, and not provide adequate foundation for segregating higher taxonomic levels (i.e. genera or families).

Given the present absence of data on hybridization and comparative cytology, we contend that wide species concepts are appropriate. By using anatomical and culturing criteria, *Monostroma grevillei*, *M. undulatum* and *M. oxyspermum* are indistinguishable in Europe, |Pacific North America and Pacific Asia. Furthermore, given BORASO's (1977) results for *M. undulatum* from Argentina, we predict that many South American and Antarctic species will be conspecific with boreal ones. Such taxa may include *Protomonostroma rosulatum* VINOGRADOVA (1983), *M. hariotii* GAIN (1912), *M. ecuadoreanum* TAYLOR, and *M. dactyliferum* TAYLOR (1945).

Acknowledgements

The authors express sincere thanks to Dr. L. HANIC for comments on an earlier draft of the manuscript. This work was supported by a Natural Sciences and Engineering Research Council of Canada Grant U-0014 to D. G.

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L. ゴールデン*・D. ガーベリイ**: ブリチッシュコロンビア産 *Monostroma*（緑藻ヒトエグサ科）の胞子放出様式を特性とした分類研究

ブリチッシュコロンビア北部のトリプル島及びマックニコール入江における海藻類の野外観察と培養実験から，*Monostroma grevillei*, *M. oxyspermum* と *M. undulatum* の3種類の生育が確認された。*M. undulatum* は東部太平洋域では，初めての記載である。*M. arcticum* sensu KORNMANN et SAHLING は *M. grevillei* と同種であると思われる。凡ての種類の培養による形態，胞子発芽及び初期発生は，ヨーロッパと日本産のこれらの種での観察と一致した。胞子放出は凡ての種で共通しているが，これは *Monostroma* が一つの自然グループであること示す根拠となり，*Ulvopsis*, *Protomonostroma* 及び *Gayralia* は *Monostroma* としてまとめられる。
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Jap. J. Phycol. (Sôrui) 32: 333-347. December 10, 1984

Comparative studies on the growth and photosynthesis of the pigmentation mutants of *Porphyra yezoensis* in laboratory culture*

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KATO, M. and ARUGA, Y. 1984. Comparative studies on the growth and photosynthesis of the pigmentation mutants of *Porphyra yezoensis* in culture. Jap. J. Phycol., 32: 333-347.

Growth, photosynthesis and respiration were studied with the wild, red and green type fronds of *Porphyra yezoensis* under laboratory conditions. The mean length of the wild (C-13), red (C-22) and green type (C-32) fronds was 4.5, 2.8 and 1.8 mm respectively in 25 days old populations. The mean relative growth rate per day was highest (0.24, 0.22 and 0.18 in the wild, red and green type fronds, respectively) during the earliest growth period and became lower with frond age in all the three strains, and the differences among the strains were almost negligible during the period later than 25 days old. In fronds of the three strains younger than 30-40 days old, the photosynthetic rate was nearly saturated at 15-20 klux but continued to increase slightly up to 90 klux. The saturation light intensity became lower with frond age. The photosynthetic rate on a frond area basis was higher both at low and high light intensity in the wild type fronds than in the red and green type fronds, but the photosynthetic rate on a chlorophyll *a* basis was almost the same in the three types of fronds. The maximum light-saturated photosynthetic rates were observed at 20-25°C in photosynthesis-temperature curves of all the types of fronds. The photosynthetic rate on a frond area basis became lower with age at each temperature in all the three strains. Little difference was obtained in the respiratory rate among the three types of fronds. The light-limited photosynthetic rate was lower in green light than in white light in all the three types of fronds, and the difference was remarkable especially in the green type frond which has very low phycoerythrin content. In another green type strain (C-0), the relative growth rate was slightly lower than that of the wild type strain (C-13) but equal to the red type strain (C-22) at the early growth period, and slightly higher than that of the above three strains at the later stage of growth. The photosynthetic rate on a frond area basis was relatively low in the green type strain (C-0).

Key Index Words: *chlorophyll* a; *culture*; *growth*; *photosynthesis*; *phycocyanin*; *phycoerythrin*; *pigmentation mutants*; Porphyra yezoensis; *respiration*.

The color of *Porphyra* fronds is dependent on the contents and the ratios of such pigments as chlorophyll *a*, phycoerythrin, phycocyanin and carotenoids, and is an important factor which controls the commercial value of the dried "Nori". There is a high correlation between the pigment contents and amino acid contents which flavor the dried Nori (MIURA 1976, SAITO *et al.* 1975). Furthermore, these pigments play a very important role in trapping light energy for photosynthesis. Especially, the existence of phycoerythrin and phycocyanin is very important for the light-harvesting capabilities of

* Financially supported in part by Nori Zoshoku Shinkokai (Nori Cultivation Promotion Association).

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red algae. Phyccerythrin and phycccyanin fill in, or at least narrow, much of the light energy gap left by chlorophyll *a* and carctenoids, allowing the algae to use the solar radiation much more efficiently in photosynthesis, in a manner much like that of fucoxanthin in the brown algae (GOVINDJEE and BRAUN 1974).

In recent years, color mutants (the red and the green type mutants in reference to the wild type) have been confirmed or established in cultivated populations and in laboratory cultures of *Porphyra yezoensis* and *P. tenera* (ARUGA and MIURA 1984). Various types of variegated chimeral fronds composed of two or more sectors of different colors have been found in cultivated populations and in laboratory cultures of *P. yezoensis* (MIURA 1976 1984). On the other hand, KOBARA *et al.* (1976) have obtained the green type individuals from the green type sector of a variegated chimeral frond of *P. yezoensis* found in a cultivated population, and have succeeded in completing the life cycle of the green type mutant in laboratory culture. The distinction of the wild, red and green type fronds is possible by naked eyes. ARUGA and MIURA (1984) have made clear their characteristics by comparing the *in vivo* absorption spectra. The red type is distinguished from the wild type by clear two absorption maxima due to phycoerythrin and a shift of absorption maximum due to phycocyanin. The green type has remarkably lower absorbance in the wavelength range mainly due to phycoerythrin than the wild and red types. These characteristics are consistently found in each type fronds, in each type sectors of chimeral fronds and in each type conchocelis. In addition to these strains, later, the yellow type strain was newly established by cross breeding of the red and the green type mutant strains under laboratory conditions, the details of which will be published elsewhere.

MIURA (1976) reported the patterns and frequency of occurrence of variegated chimeral fronds in *P. yezoensis* populations both under field and laboratory conditions. He also suggested the possibility and importance of utilizing the color mutants as markers in breeding of *Porphyra* for making clear the genetic pattern. MIURA and KUNIFUJI (1980) summarized their genetic study of *P. yezoensis* utilizing the color mutants. Comparative physiclogical and biochemical studies with these color mutants were reported only by KIKUCHI *et al.* (1979) and MERRILL *et al.* (1983).

The present study deals with the growth, photosynthesis and respiration of the wild, red and green type fronds of *P. yezoensis* under laboratory conditions, and will give a clue which contributes to make clear the role of phycobilin pigments in *Porphyra*.

Material and Methods

The wild type (W, strain number C-13), red type (R, C-22) and green type (G, C-32) strains used in the present study are the strains isolated through carpospore collection from a variegated chimeral frond which was found in a cultivated population of *Porphyra yezoensis* at Shitazu, Futtsu, Chiba Prefecture, in March 1975 and composed of the wild, red and green type sectors. Another green type strain (G, C-0) of *P. yezoensis* used is the strain isolated by KOBARA *et al.* (1976). These strains are kept as free-living conchocelis in laboratory cultures at 20°C under a 14 : 10 LD cycle.

By transferring from stock cultures to the lower temperature and short-day conditions (15°C and 10 : 14 LD), the conchocelis matured and released conchospores, which immediately attached to synthetic fibers (Cremona monofilaments) of about 4 cm long and developed into fronds (leafy thalli). The day of the conchospore attachment was assigned to zero-day for the age of fronds. The culture medium was the natural seawater, which was collected from the Kuroshio off the Izu Oshima Island and filtered through a glass fiber filter (Whatman GF/C), enriched with modified ESP (PROVASOLI 1966) as shown in Table 1. Until 30 days old, the frond cultures were maintained in 1 *l* flat-bottom flasks

Table 1. Composition of the modified ESP medium used in the present study. To obtain ESP add 2 m*l* of ES enrichment to 100 m*l* of filtered seawater.

(A) ES Enrichment	
H_2O	100 m*l*
$NaNO_3$	350 mg
Na_2-glycerophosphate	50 mg
Fe (as EDTA, 1 : 1 molar)	2.5 mg
P II metal mix*	25 m*l*
Vitamin B_{12}	10 μg
Thiamine	0.5 mg
Tris buffer	500 mg
pH	7.8
*(B) P II metal mix	
H_2O	100 m*l*
H_3BO_3	114 mg
$FeCl_3 \cdot 6H_2O$	4.9 mg
$MnCl_2 \cdot 4H_2O$	14.4 mg
$ZnSO_4 \cdot 7H_2O$	2.2 mg
$CoCl_2 \cdot 6H_2O$	0.4 mg
Na_2-EDTA	100 mg

with a branch for aeration at the bottom corner and were kept in the incubator at 15°C and 10 klux with a photoperiod of 10 : 14 LD. The fronds were removed from the synthetic fibers and cultured in 25 *l* culture tanks (Nihon Chisei Sangyo Co., Ltd.) with 2 cool-white fluorescent lamps which supplied illumination of 3, 5 and 10 klux to the lower, middle and upper part of the tanks, respectively. The cultures were aerated with an air pump throughout the experiment. The culture medium was renewed every 5 days.

The growth was determined by measuring of the frond length. During the initial 25 days, a few Cremona monofilaments with fronds were used to measure the length of about 100 fronds at intervals of 5 days. After 25 days old, 30 fronds were used for the growth measurements. The fronds less than 2 mm long were measured under the microscope with a screw micrometer, and those larger than 2 mm long with a slide calipers. Although the release of monospores was observed after 15 days old, the fronds from monospores were not used for the measurements.

The light-and-dark bottle method was employed for the measurements of photosynthesis and respiration. One or more fronds were placed in a D.O. bottle of about 100 m*l* filled with filtered seawater, and incubated for 20 or 40 min for photosynthesis or respiration measurement, respectively. The oxygen concentration in seawater was determined by the Winkler titration technique before and after the incubation. Photosynthesis was measured at 15°C and various light intensities by changing the distance of D.O. bottles from the light source to obtain photosynthesis-light curves. Photosynthesis-temperature curves were obtained at 25 klux by changing temperature at intervals of 5°C in the range 5–30°C. Respiration-temperature curves were obtained in the same temperature range in the dark. A photoreflector lamp (Toshiba 100 V 500 W, Spot) was used for photosynthesis measurements. The light intensity was measured with a Toshiba SPI-5 photometer. In the measurements with fronds of 48 days old, the basal and mature marginal parts of fronds were cut off to exclude the marginal effects partly related to the sexual maturation (cf. OGATA and MATSUI 1963). The measurements were started in the middle of light period to exclude the effect of diurnal rhythm (OOHUSA *et al.* 1977). The photosynthesis under green light was measured by using a colored cellophane filter with a transmittance spectrum as shown in Fig. 1.

Immediately after the end of each experiment, sample fronds were estimated for their area and preserved in a desiccator for the measurements of dry weight and pigment contents. After weighing with a chemical balance, the dry samples were smashed in a mortar with 90% acetone or distilled water to obtain the extracts of pigments. The absorbances of the extracts were measured with a Shimadzu QV-50 spectrophotometer. Chlorophyll *a* concentration of the 90% acetone extract was calculated by the formula of SCOR-UNESCO (1966). Phycoerythrin and phycocyanin contents of the water extract

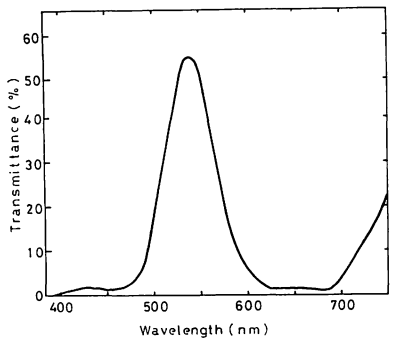

Fig. 1. Transmittance spectrum of a colored cellophane filter used for the measurement of photosynthesis under green light.

were calculated by using the extinction coefficients reported by Ó hEOCHA (1965). *In vivo* absorption spectra of the fronds were obtained with a Shimadzu MPS-50L recording spectrophotometer with the air as reference.

Results

1) Growth

The frond length compositions of the wild (C-13), red (C-22) and green (C-32 and C-0) type fronds of 10, 15, 20 and 25 days old are shown in Fig. 2. The mean lengths (M), standard deviations (SD) and coefficients of variability (CV) were calculated (Table 2). The frond length compositions were almost the same in the wild, red and green type populations of 10 days old. A difference was, however, found among the populations of 15 days old; the wild type population was composed of a great number of fronds 0.4-0.8 mm long, while the red and green type (C-32 and C-0) populations 0.2-0.6 mm long. The wild type population of 20 days old was composed of many fronds larger than 1.5 mm long while the red and green type (C-32

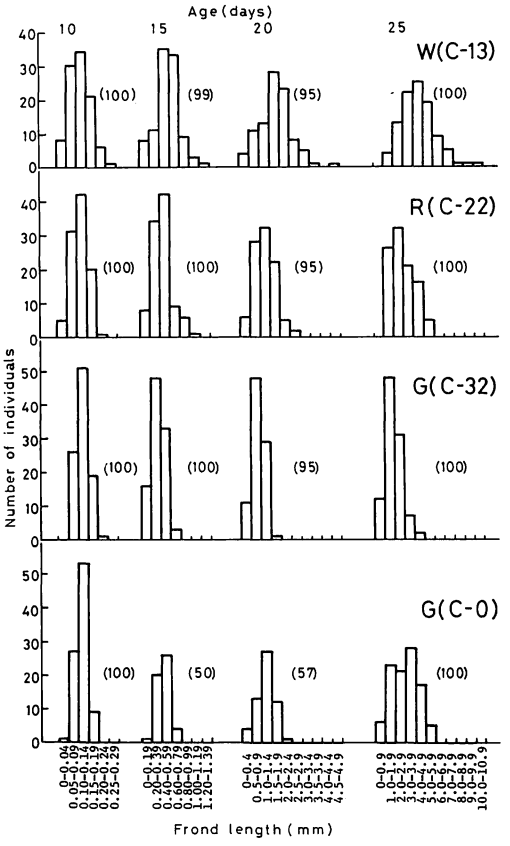

Fig. 2. Fond length compositions of the wild (W), red (R) and green type (G) fronds (10, 15, 20 and 25 days old) of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the number of sample fronds at each measurement.

Table 2. Mean frond length (M, μm), standard deviation (SD) and coefficient of variability (CV) of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laborarory.

Strain		Frond age (days) 10			15			20			25		
		M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
W	(C-13)	117	53	45	567	321	41	1825	774	42	4.5×10^3	1.6×10^3	37
R	(C-22)	112	39	35	434	181	42	1240	528	43	2.8×10^3	1.2×10^3	41
G	(C-32)	125	36	29	358	123	34	923	410	44	1.8×10^3	0.7×10^3	44
G	(C-0)	109	27	25	425	118	28	1150	405	35	2.9×10^3	1.3×10^3	44

and C-0) populations many fronds less than 1.5 mm long; the red and green type (C-32) populations were composed of many fronds 1.0-1.5 mm long and the green type (C-32) populations many fronds 0.5-1.0 mm long. Among the populations of 25 days old, the wild type population was composed of many fronds 3-6 mm long, some fronds larger than 6 mm long and a few fronds less than 2 mm long; the red and green type (C-0) populations were composed of many fronds 1-5 mm long and more fronds 1-2 mm long than the wild type; the green type (C-32) population was composed of many fronds 1-3 mm long, of which fronds 1-2 mm long occupied 50% of all the fronds.

The mean frond length was almost the same, about 0.12 mm, in all the populations of 10 days old. However, the difference of the mean length became clear as the fronds aged. The mean frond length of the wild, red and green type (C-32 and C-0) populations of 25 days old was 4.5, 2.8, 1.8 and 2.9 mm, respectively (Table 2).

It is presumed that the differences of the frond length composition and the mean frond length are due to the difference of the growth rate among the strains of different types. Therefore, at each age the fronds were arranged according to the frond length and classified in four groups. The relative growth rate per day was calculated with the mean frond length of each group and the total mean frond length (Table 3) using the following formula:

Table 3. Mean frond length (μm) and relative growth rate per day of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Fronds were classified in four classes according to their frond length. (M) is the relative growth rate per day for the mean frond length in Table 2.

Strain	Age (days)	Frond length 10	15	20	25		Relative growth rate 10-15	15-20	20-25	19-25
W (C-13)		54	280	877	2.6×10^3		0.33	0.23	0.22	0.26
		96	495	1613	3.8×10^3		0.33	0.24	0.17	0.24
		131	645	2058	4.9×10^3		0.32	0.23	0.17	0.24
		188	859	2836	6.7×10^3		0.30	0.24	0.17	0.24
						(M)	0.32	0.23	0.18	0.24
R (C-22)		67	226	587	1.5×10^3		0.24	0.19	0.19	0.21
		99	376	1048	2.3×10^3		0.27	0.20	0.16	0.21
		122	486	1394	3.1×10^3		0.28	0.21	0.16	0.22
		166	676	1961	4.5×10^3		0.28	0.21	0.17	0.22
						(M)	0.27	0.21	0.16	0.22
G (C-32)		82	186	471	0.9×10^3		0.16	0.19	0.13	0.16
		110	333	725	1.5×10^3		0.22	0.16	0.14	0.17
		134	406	1031	2.0×10^3		0.22	0.19	0.13	0.18
		173	511	1489	2.9×10^3		0.22	0.21	0.13	0.19
						(M)	0.21	0.19	0.13	0.18
G (C-0)		76	273	595	1.3×10^3		0.26	0.16	0.15	0.19
		98	397	1043	2.3×10^3		0.28	0.19	0.16	0.21
		117	471	1295	3.5×10^3		0.28	0.20	0.20	0.23
		147	571	1933	4.5×10^3		0.27	0.21	0.20	0.23
						(M)	0.27	0.20	0.18	0.22

$$\text{Relative growth rate} = \frac{\ln l_2 - \ln l_1}{t_2 - t_1},$$

where l_1 and l_2 are the length of frond of t_1 and t_2 days old, respectively. There was little difference in the relative growth rate between the results from the total mean frond length and from the mean frond length of the four groups. Although clear differences were found in the relative growth rate for the total mean frond length among the wild, red and green type (C-32 and C-0) populations at 10-15 days, the differences became smaller as the fronds aged. The relative growth rates of the wild, red and green type (C-0) populations of 20-25 days old were almost the same, whereas that of the green type (C-32) population was lower. The relative growth rate for the total mean frond length was 0.24, 0.22, 0.18 and 0.22 in the wild, red and green type (C-32 and C-0) populations, respectively, for the period of 10-25 days. Thus, there were significant differences in the relative growth rate among the wild, red and green type populations, even though the relative growth rate became lower as the fronds aged in each type population. In order to follow furthermore the growth of each type populations, 30 fronds of 25 days old from each population were classified in 5 groups according to the frond length; <3.0, 3.0-3.9, 4.0-4.9, 5.0-5.9 and >6.0 mm. The relative growth rate for the mean frond length of each group was calculated (Table 4). There was little difference in the relative growth rate for 25-66 days among the wild, red and green type (C-32) populations, whereas the green type (C-0) population showed a slightly higher relative growth rate than the other populations. The reproductive maturation was observed in the wild, red and green type (C-32) fronds of about 45 days old, while in the green type (C-0) fronds it was not observed

Table 4. Mean frond length (mm) and relative growth rate per day of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Selected 30 individuals of 25 days old were classified in 5 classes according to their frond length.

Strain	Age (days)	Frond length							Relative growth rate						
		25	30	37	45	54	66	80	25-30	30-37	37-45	45-54	54-66	66-80	25-66
		2.1	4.8	9.6	—	—	—	—	0.17	0.10	—	—	—	—	—
		3.5	10.5	22.2	45	66	73	—	0.22	0.11	0.09	0.04	0.01	—	0.07
W (C-13)		4.4	13.2	30.4	59	76	98	—	0.22	0.12	0.08	0.03	0.02	—	0.08
		5.4	15.9	35.8	70	90	105	111	0.22	0.12	0.08	0.03	0.01	0.01	0.07
		7.6	22.4	51.9	100	151	189	221	0.22	0.12	0.08	0.05	0.02	0.01	0.08
		2.3	6.1	12.4	—	—	—	—	0.20	0.10	—	—	—	—	—
R (C-22)		3.5	9.4	20.7	40	66	87	100	0.20	0.11	0.08	0.06	0.02	0.01	0.08
		4.5	11.7	27.4	58	90	112	125	0.19	0.12	0.09	0.05	0.02	0.01	0.08
		5.4	15.3	36.0	73	119	156	189	0.21	0.12	0.09	0.05	0.02	0.01	0.08
		2.4	6.1	11.2	—	—	—	—	0.19	0.09	—	—	—	—	—
G (C-32)		3.4	9.6	17.8	35	57	75	78	0.21	0.09	0.08	0.05	0.02	0.01	0.08
		4.8	12.7	26.1	54	73	99	113	0.19	0.10	0.09	0.03	0.03	0.01	0.07
		1.7	4.4	9.0	—	—	—	—	0.19	0.10	—	—	—	—	—
		3.4	10.7	23.5	55	92	122	165	0.23	0.11	0.11	0.06	0.02	0.02	0.09
G (C-0)		4.5	14.0	33.0	70	115	153	196	0.23	0.12	0.09	0.06	0.02	0.02	0.09
		5.4	16.0	36.9	75	123	178	220	0.23	0.12	0.09	0.05	0.03	0.02	0.09
		6.6	19.3	38.9	79	149	211	286	0.23	0.10	0.09	0.07	0.03	0.02	0.08

even at 88 days old. The relative growth rate became successively lower as the fronds aged from 25 to 80 days old. The relative growth rate for 25-30 days was a little higher than that for 20-25 days (Tables 3 and 4). This seems to be related to the removal of fronds from Cremona monofilaments resulting in the decrease of frond density in culture.

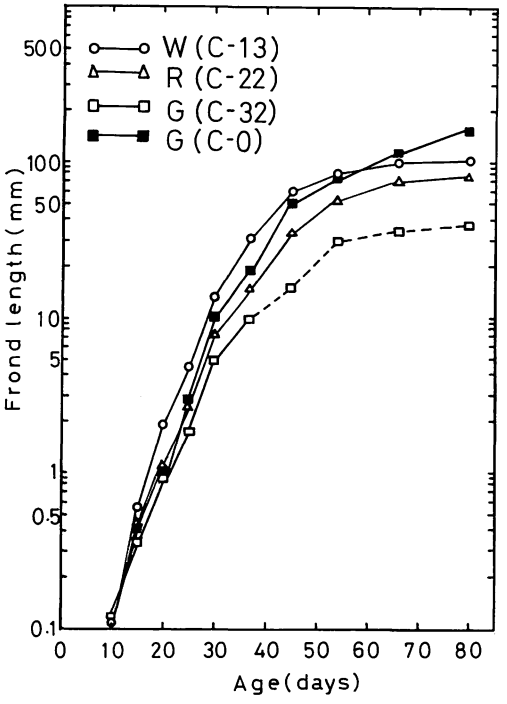

Fig. 3. Growth curves of the wild (W), red (R) and green type (G) fronds of cultured in laboratory.

The growth curves of the wild, red and green type fronds are shown in Fig. 3 as based on the mean frond length. The mean frond length was about 100, 80, 40 and 160 mm in the wild, red and green type (C-32 and C-0) populations, respectively, at the end of culture. These differences could be resulted largely from the differences of the growth rate in younger stage. It is possibly due to the fastest growth rate after 25 days old and the delay of reproductive maturation that the green type (C-0) fronds became longest of the four strains at the end of culture (cf. Table 4).

2) Photosynthesis and respiration

Photosynthesis-light curves of the wild, red and green type fronds obtained at 15°C and 0-90 klux are shown in Fig. 4. Fronds of 32-36 days old were the youngest used to measure photosynthesis and respiration. In these fronds the photosynthetic rates on a frond area basis were nearly saturated at 15-20 klux, but the rate continued to increase slightly with increase in light intensity up to 90 klux; i.e. the light saturation of photo-

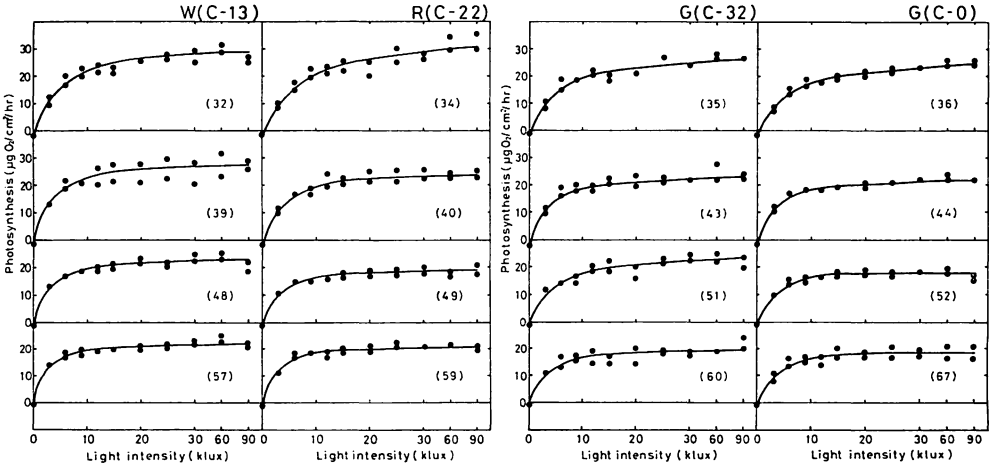

Fig. 4. Photosynthesis-light curves at 15°C of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the frond age in days.

synthesis was not clear. However, the light saturation became clear as fronds aged; the photosynthetic rates were saturated at 5-10 klux in the wild and red type fronds and at 10-15 klux in the green type fronds. No inhibition of the photosynthetic rate by high light intensity was observed in the range employed in the present experiment.

The photosynthetic rates on a frond area basis at 3, 9 and 30 klux obtained from the photosynthesis-light curves are shown in Fig. 5. At 30 and 9 klux, the photosynthetic rate decreased with frond age in the wild and green type (C-32) fronds, while in the green type (C-0) fronds it similarly decreased until 52 days old and was constant after that. In the red type fronds, on the other hand, the photosynthetic rate at 30 and 9 klux decreased rather sharply with age and increased a little at 57 days. The changes of the photosynthetic rates at 30 and 9 klux correspond well to the changes of the relative growth rate in the wild and green type (C-32 and C-0) fronds. The photosynthetic rate at 3 klux indicated no significant changes with age in all of the strains used. The photosynthetic rates at 30 and 9 klux were higher in the wild and red type fronds than in the green type fronds except for the rate of the red type fronds at 49 days. The photosynthetic rate of the green type (C-32) fronds was slightly higher than that of the green type (C-0) fronds at 30 and 9 klux. At 3 klux the photosynthetic rate of the wild type fronds was higher than that of the red and green type (C-32 and C-0) fronds which showed almost the same rates.

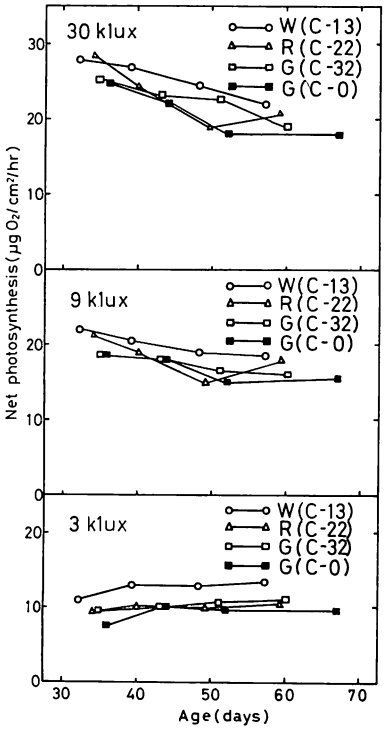

Fig. 5. Changes in the net photosynthetic rates at 3, 9 and 30 klux of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the photosynthesis-light curves in Fig. 4.

The changes of chlorophyll *a* content per cm^2 of frond area are shown in Fig. 6. The chlorophyll *a* content decreased with frond age in all the strains. The changes were especially remarkable in the wild, red and green type (C-32) fronds, but slight in the green type (C-0) fronds. The chlorophyll *a* content of the green type (C-0) fronds was lower than that of other type fronds. Although the photosynthetic rate on a frond area basis was different among the strains of different types, the photosynthetic rate on a chlorophyll *a* basis was not so different in all the types of fronds, and its changes with age were not conspicuous as a whole (Fig. 7).

The respiratory rates on a frond area basis

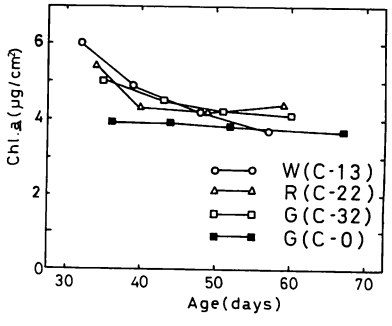

Fig. 6. Changes in chlorophyll *a* content per unit frond area of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory and used for the measurements of photosynthesis.

measured at 15°C in the dark showed great variations, but it is presumed that the rate decreased with frond age (Fig. 8). No remarkable differences were confirmed in the respiratory rate among the wild, red and green type fronds.

Photosynthesis- and respiration-temperature curves of the wild, red and green type fronds obtained at 5-30°C and 25 klux are shown in Fig. 9. The photosynthetic and respiratory rates of the three types of fronds showed the same tendency against temperature. The photosynthetic rate on a frond area basis increased with increase in temperature, attained a maximum at 20-25°C and decreased remarkably at 30°C. The respiratory rate on a frond area basis increased slightly with increase in temperature in the range of 5-30°C.

The changes with frond age of photo-

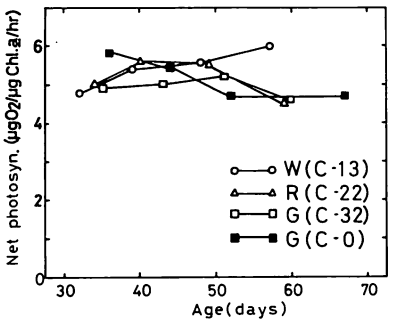

Fig. 7. Changes in the net photosynthetic rate on a chlorophyll *a* basis at 30 klux of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory.

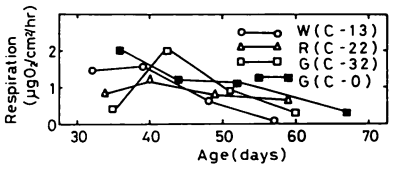

Fig. 8. Changes in respiratory rate at 15°C of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the photosynthesis-light curves in Fig. 4.

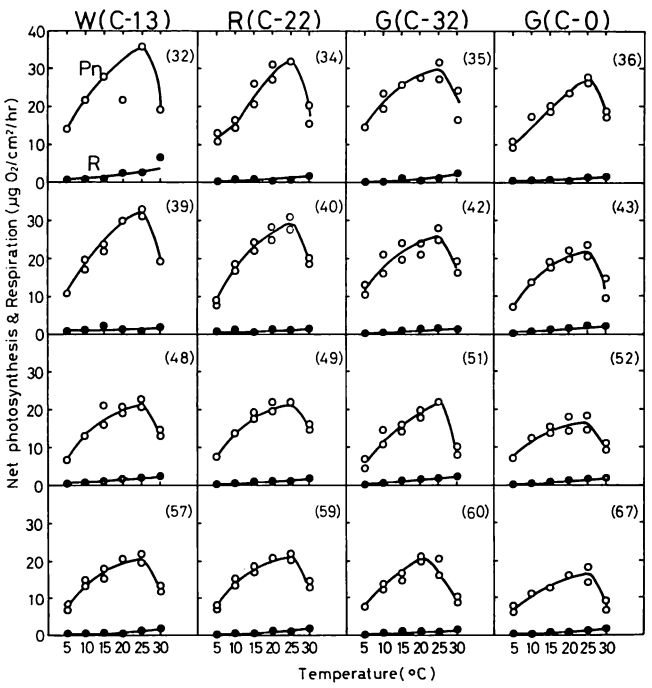

Fig. 9. Photosynthesis-temperature curves (○) at 25 klux and respiration-temperature curves (●) of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the frond age in days.

synthetic rates at 5, 10, 15, 20, 25 and 30°C are shown in Fig. 10. The photosynthetic rates decreased with frond age at each temperature in all the types of fronds, and the decrease was especially remarkable at 20 and 25°C. These changes were consistent with the changes of the photosynthetic rate at 30 and 9 klux shown in Fig. 5. The maximum photosynthetic rate attained at 25°C in most of the cases and was 35, 32, 30 and 27 μg $O_2/cm^2/hr$ in the wild, red and green type (C-32 and C-0) fronds of 32-36 days old, respectively. The maximum photosynthetic rates of the fronds of about 60 days old were almost the same, 20-21 μg $O_2/cm^2/hr$, in the wild, red and green type (C-32) fronds; but in the green type (C-0) frond it was considerably low, 16 μg $O_2/cm^2/hr$.

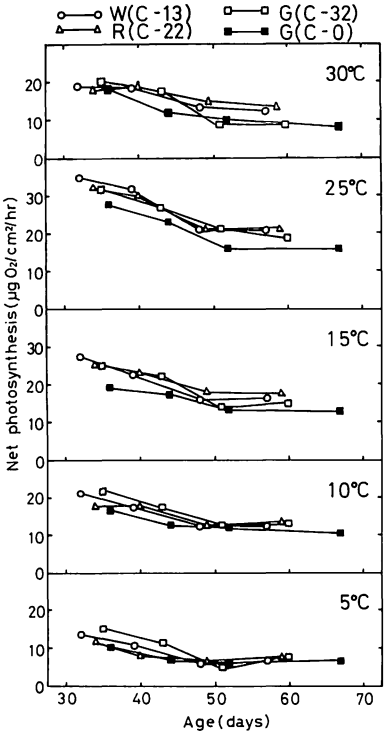

Fig. 10. Changes in the net photosynthetic rate at 5, 10, 15, 20, 25 and 30°C under 25 klux of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the photosynthesis-temperature curves in Fig. 9.

The changes of the respiratory rates at 15°C obtained from the respiration-temperature curves are shown in Fig. 11. The respiratory rates showed considerable variations until about 50 days, thereafter they gradually decreased with frond age. As an accuracy of the respiratory measurements by the present technique is not very good, the results shown in Figs. 9 and 11 would only suggest that the respiratory rate decreased gradually

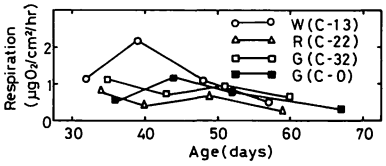

Fig. 11. Changes in the respiratory rate at 15°C of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the respiration-temperature curves in Fig. 9.

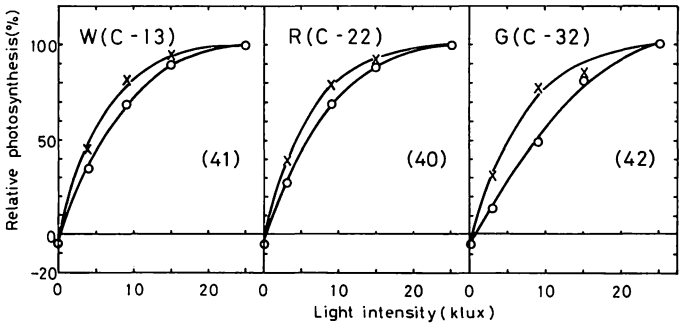

Fig. 12. Relative photosynthesis-light curves under white light (×) and green light (○) of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the frond age in days.

with frond age.

Photosynthetic rates under white light and green light were compared with the wild (C-13), red (C-22) and green type (C-32) fronds of 40-42 days old. Photosynthetic rates were measured four times at 15°C under 3, 9, 15 and 25 klux, and respiratory rates were also measured at the same temperature. The relative photosynthesis-light curves obtained are shown in Fig. 12. The photosynthetic rates of all the three types of fronds were lower in green light than in white light. The extent of lowering in green light was slight in the wild and red type fronds, but it was remarkable in the green type frond. The result thus indicates that the green type frond is inferior to the wild and red type fronds in using green light for photosynthesis.

Discussion

The four strains of *Porphyra yezoensis* used in the present study have respective characteristic colors which are quite clearly reflected to their *in vivo* absorption spectra (Fig. 13). The spectra show conspicuous differences in the wavelength range where phycoerythrin and phycocyanin mainly take part in the absorption. Detailed comparisons of the spectra and pigment contents of the strains were described by ARUGA and MIURA (1984). Fronds of the red type strain have lower phycocyanin content and higher phycoerythrin/phycocyanin ratio (Table 5), while

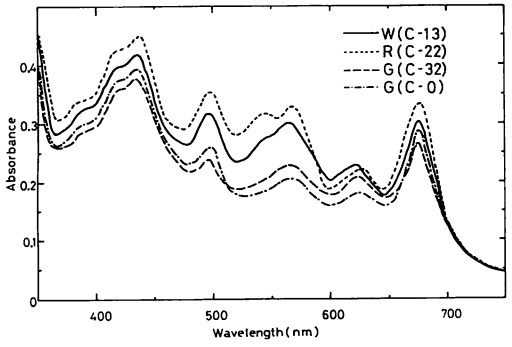

Fig. 13. *In vivo* absorption spectra of the wild (W), red (R) and green type (G) fronds (54 days old) of *P. yezoensis* cultured in laboratory.

Table 5. Phycoerythrin (PE) and phycocyanin (PC) contents of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory.

Strain	PE (μg/cm^2)	PC (μg/cm^2)	PE/PC
W (C-13)	33.2	14.0	2.4
R (C-22)	28.3	10.2	2.8
G (C-32)	16.7	9.8	1.7
G (C-0)	17.4	11.4	1.5

fronds of the two green mutant strains show especially lower absorbance in the wavelength of 460-600 nm (Fig. 13) and have lower phycoerythrin content and consequently lower phycoerythrin/phycocyanin ratio (Table 5). As the chlorophyll *a* content of the three types of fronds is almost at the same level except for the green type (C-0) frond (cf. Fig. 6), it is important to compare the growth and photosynthesis in consideration of the contents and the ratios of photosynthetic pigments in the three types of fronds.

There are several reports as to the culture conditions of *Porphyra* fronds. KINOSHITA and TERAMOTO (1958) obtained the highest growth of *P. tenera* at 15°C and 6000 lux. IWASAKI and MATSUDAIRA (1958) and IWASAKI (1965) showed that the most suitable conditions for *P. tenera* were 14-16°C and an illumination by sheltered sunlight of 9 hr/day. The natural seawater from Tokyo Bay was used by KINOSHITA and TERAMOTO (1958) for culturing *P. tenera*. IWASAKI and MATSUDAIRA (1958) and IWASAKI (1965) studied the nutritional requirements of *P. tenera*. TATEWAKI (1971) indicated that the ESP medium was suitable for culturing many marine algae. KOBARA *et al.* (1976) were successful in completing the life cycle of the green type mutant of *P. yezoensis* in the ESP medium. In the present study the modified ESP medium was used to culture the three types of fronds of *P. yezoensis* at 15°C and 3-10 klux (10 hr light/day), and the growth of the fronds was fairly good even though the conditions and the medium were not confirmed to be most suitable.

OOHUSA *et al.* (1977) investigated the

diurnal variations of photosynthesis and respiration in *P. yezoensis*. They found out that the photosynthetic rate attained its maximum in the light period and its minimum in the dark period, and the respiratory rate showed its maximum at the end of the light period and its minimum at the end of the dark period. As the measurements of photosynthesis and respiration were started usually at the same time in the middle of the light period in the present study, comparisons of the results can be properly made without consideration of the diurnal rhythms.

In the present study the highest relative growth rate, 0.32/day, was obtained in the wild type fronds of *P. yezoensis* during the period of 10-15 days old (Table 3). The relative growth rate was high in an earlier period of growth and decreased with frond age in all the three types of fronds. This trend was in agreement with that of cultivated *Monostroma latissimum* (MAEGAWA and ARUGA 1974). However, such difference as observed in *M. latissimum* of the relative growth rate according to the frond length was not obtained in the present study. It was possibly due to the fact that all the fronds used in the present study were removed from Cremona monofilaments and cultured under the same light and nutrient conditions. Although YOSHIDA (1972) reported the highest relative growth rates of 0.27 and 0.46/day in cultivated *P. tenera* and *P. pseudolinealis*, respectively, the direct comparisons cannot be made between the result of laboratory culture and that of field cultivation.

SATOMI *et al.* (1968) showed that the photosynthetic ability of cultivated *P. yezoensis* was initially low, attaining its maximum of 30-35 m*l* O_2/g (d.w.)/hr at about 40 days after the conchospore seeding, and then continuously decreased to about half the maximum rate in 3 months after the seeding. The maximum photosynthetic rate of 30 μg O_2/cm^2/hr obtained with the wild type fronds in the present study was well comparable to the values of SATOMI *et al.* (1968). The photosynthetic rates at higher light intensities and at various temperatures decreased with frond age (Figs. 5 and 10). The same trend was also obtained both in cultivated *P. yezoensis* (SATOMI *et al.* 1968) and in cultivated *M. latissimum* (MAEGAWA and ARUGA 1974) whose environmental conditions were variable. Therefore, the frond age seems to be important in controlling the growth, photosynthesis and respiration.

The pattern of photosynthesis-temperature curves was almost the same in the three types of fronds of *P. yezoensis* (Fig. 9). The photosynthetic rate showed its maximum at 20-25°C, mostly at 25°C, irrespective of the frond age. The same type of photosynthesis-temperature relationships were reported with Rhodophycean seaweeds living in winter (YOKOHAMA 1973a), and with seaweeds in the colder region of Japan (HATA and YOKOHAMA 1976) and in arctic regions (HEALEY 1972). The photosynthetic rate at 15-25°C of the green type (C-0) frond was mostly lower than that of other types of fronds (cf. Fig. 10). No special differences were found in the photosynthesis-temperature relationship among the three types of fronds.

Algae generally have considerable flexibility in responding to surrounding light conditions by changing the contents and the ratios of photosynthetic pigments. The chlorophyll *a*-biliprotein system of the blue-green algae and red algae shows much greater flexibility in changing the pigment ratio than the chlorophyll *a*-carotenoid combination (HALLDAL 1970). YOKOHAMA (1973b) showed that the higher efficiency in utilizing green light, which was observed in the red algae from deeper range, was considered to be due to a high ratio of phycoerythrin content to chlorophyll *a* or phycocyanin content. According to CALABRESE (1972) *Petroglossum nicaeense* living inside of a sea cave adapted themselves to the shade by considerably increasing the phycoerythrin formation for a more efficient utilization of light energy. CALABRESE and FELICINI (1973) showed that the massive accumulation of phycoerythrin and chlorophyll *a* in the red thallus of *Glacilaria compressa* permitted a relatively

high photosynthetic efficiency even at low light intensity. In the present study a significant difference was found in the photosynthetic rate under green light between the green type frond and the wild or red type frond (Fig. 12). The photosynthetic rate was lower in green light than in white light in all of the three types of fronds, but the difference was especially remarkable in the green type frond which had considerably lower phycoerythrin content than the wild and red type fronds. The fact that the photosynthetic rate was lower in green light than in white light is in agreement with the character of the red algae which adapt themselves to the environment in the upper region of the sea (YOKOHAMA 1973b). It is likely that the wild, red and green type fronds cultured in the present study adapted themselves to white light, but details of this point remain to be investigated. The red type frond, which is presumed to be a qualitative mutant in phycobilins, showed almost the same photosynthetic response as the wild type frond under green light. This seems to indicate that phycoerythrin of the red type frond functions to the same extent as that of the wild type in harvesting green light for photosynthesis.

Obvious differences were found of the growth rate in an early period of growth among the wild, red and green type fronds (Table 3), but the differences became small as the fronds aged. In the blue-green alga *Anacystis nidulans*, yellow-green mutants which have normal chlorophyll but only half the phycocyanin of the parent were similar to the parent in the specific growth rate and photosynthetic rate, but blue mutants with somewhat higher phycocyanin but only one-third the chlorophyll of the parents are dissimilar to the parent in the specific growth rate and photosynthesis (STEVENS and MYERS 1976). A mutant of the coccoid blue-green alga *Agmonellum quadruplicatum*, which had a higher content of chlorophyll *a* relative to phycocyanin than the wild type, showed impaired growth on the medium to which NO_3 was added as a nitrogen source (STEVENS and VAN BAALEN 1970). In the present study the photosynthetic rate on a frond area basis was higher in the wild and red type fronds than in the green type frond, but this was mainly due to the difference of chlorophyll *a* content per unit frond area. There was no significant difference in the photosynthetic rate on a chlorophyll *a* basis among the three types of fronds. It is not considered that a decrease of phycoerythrin content had any effect on the photosynthetic rate under white light. Therefore, the difference in the growth rate at an early period of growth among the three types of fronds, especially a considerably inferior growth rate of green type, is considered not to be due to a decrease of phycoerythrin content by mutation.

The green type (C-0) fronds grew up largest of all at 80 days old in spite of their lower growth rate at an early period of growth and lower photosynthetic rate. This seemed to be mainly due to the delayed reproductive maturity. Although the photosynthesis, of course, plays an important part in the growth, other factors, such as reproductive maturity, hormones, vitamins and other growth regulators, which control the growth, should be investigated in the future.

Acknowledgements

We express our cordial thanks to Dr. A. MIURA, Tokyo University of Fisheries, for his encouraging advice throughout the present study. Financial support of the study by Nori Zoshoku Shinkokai (Nori Cultivation Promotion Association) is gratefully acknowledged.

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加藤光雄・有賀祐勝：培養によるスサビノリ色彩変異体の生長と光合成に関する比較研究

スサビノリ（*Porphyra yezoensis*）の野生型，赤色型，緑色型について，室内培養条件下で葉状体の生長と光合成に関する比較研究を行なった。葉齢25日の平均葉長は，野生型（C-13）で 4.5 mm，赤色型（C-22）で2.8 mm，緑色型（C-32）で 1.8 mm であった。1日あたりの平均相対生長率は，いずれの色彩型でも生長初期には高く（野生型 0.24，赤色型 0.22，緑色型 0.18），葉齢とともに低下し，葉齢25日以後は色彩型間で差がほとんど認められなくなった。葉齢 30～40 日以前の葉状体では，その光合成速度は 15～20 klux でほとんど光飽和に達したが，90 klux までわずかながら上昇がみられた。光飽和に達する光強度は葉齢とともに低下した。葉面積あたりの光合成速度は，弱光下でも強光下でも，赤色型や緑色型より野生型で高かったが，クロロフィル *a* 量あたりの光合成速度は色彩型間でほとんど差がみられなかった。いずれの色彩型の光合成-温度曲線でも，光飽和光合成速度は20～25°C で最大値を示し，葉面積あたりの光合成速度は葉齢とともに低下した。呼吸速度については，色彩型間でほとんど差異は認められなかった。光強度制限下での光合成速度は，いずれの色彩型でも白色光下より緑色光下で低かったが，フィコエリスリン含量の著しく低い緑色型でその差は特に顕著であった。他の緑色型（C-0）では，生長初期の相対生長率は野生型（C-13）よりわずかに低く，赤色型（C-22）とほぼ同じであったが，後期には上述の3つの色彩型より若干高かった。緑色型（C-0）の葉面積あたりの光合成速度は比較的低かった。

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Jap. J. Phycol. (Sôrui) 32: 348-353. December 10, 1984

Studies on the freshwater Rhodophyta of Brazil I. Three taxa of *Batrachospermum* ROTH from the northeastern State of Serigipe

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NECCHI, O. Jr. and KUMANO, S. 1984. Studies on the freshwater Rhodophyta of Brazil I. Three taxa of *Batrachospermum* ROTH from the northeastern State of Serigipe. Jap. J. Phycol. 32: 348-353.

Three taxa of *Batrachospermum* ROTH (Rhodophyta) from the northeastern Brazilian State of Serigipe are studied. *Batrachospermum capense* STARMACH ex NECCHI et KUMANO (Section *Contorta*) is lectotypfied, and *B. capense* STARMACH ex NECCHI et KUMANO var. *breviarticulatum* NECCHI et KUMANO, var. nov., is described based on the number of cells of primary branchlets and on the size of gonimoblasts. *B. orthostichum* SKUJA (Section *Setacea*) is recorded for the second time in the literature, and *B. cayennense* MONTAGNE (Section *Aristatae*) for the first time for Brazil.

Key Index Words: Batrachospermum capense *var.* breviarticulatum, *var. nov.*; Batrachospermum cayennense; Batrachospermum orthostichum; *Brazil*; *freshwater Rhodophyta*; *taxonomy.*

Brazilian frashwater Rhodophyta, especially those of genus *Bartrachospermum* ROTH, have received up today a little attention of specialists. Only 13 taxa of the genus were already reported for Brazil. However, most of them are included in general lists of taxa of other groups of algae, and some contain only brief descriptions and usually no illustrations. Among the papers exclusively dealing with Brazilian freshwater Rhodophyta, it should be mentioned that of SKUJA (1931), in which two new species of *Batrachospermum, B. orthostichum* and *B. procarpum,* from Santa Teresa, Satate of Espirito Santo, are described. *Batrachospermum vagum* (ROTH) C. AGARDH var. *periplocum* is described by SKUJA (1969) based on the material from Rio Negro, in the northern State of Amazonas.

The present authors are starting a series of papers aiming to contribute towards the knowledge of Brazilian freshwater Rhodophyta. The present paper deals with three taxa of *Batrachospermum* from the north-

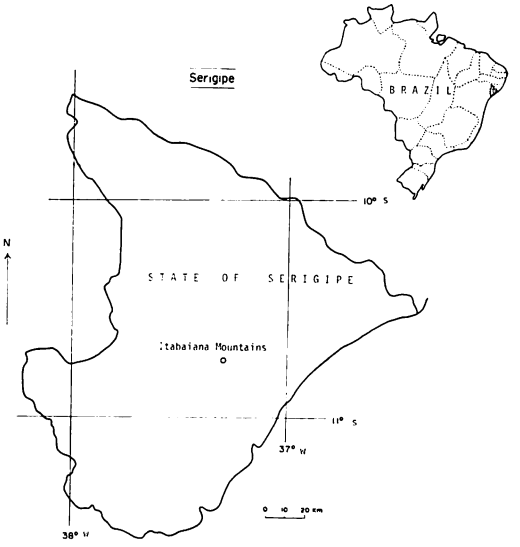


Fig. 1. Map of the State of Serigipe showing the locality from which specimens were collected.

eastern State of Serigipe. All materials studied were collected from two rivulets at Itabaiana Mountains, located at Município of Areia Branca, situated at 10°46′ lat. S. 37° 18′ long. W. as shown in Fig. 1. All specimens studied are deposited at the Herbarium of Institute of Botany, São Paulo, Brazil (SP).

Lectotypification of *Batrachospermum capense*[1] STARMACH

Bartrachospermum capense was described by STARMACH (1975) but no holotype was designated. This species is not validly published according to Art. 37.1 of the International Code of Botanical Nomenclature. Thus, based on the Guide for determination of types (item 3 and 4), the present authors designate the lectotype as follows:

Lectotypus: leg. J. RZOSKA, det. K. STARMACH, KRA, SP 187186, rivulo Du Cap, insula Mahé, insulis Seychelles.

Descriptions of Taxa and Discussions

1. *Batrachospermum capense* STARMACH ex NECCHI et KUMANO var. *breviarticulatum* NECCHI et KUMANO, var. nov. (Figs. 2-11).

Frons monoica, 7 cm alta, 350-550 μm crassa, abundanter irregulariteque ramosa, modice mucosa. Cellulae axiales cylindricae, 50-150 μm crassae, 330-530 μm longae. Verticilli cylindrici, contigui et plus minusve compressi. Fasciculi duo vel tres e cellulis basi ramulorum primariorum orientes. Ramuli primarii di- vel trichotome ramificantes, ex 4-7 cellulis constantes; cellulae fasciculorum lanceoloto-ellipticae; pili numerosi, in quoque cellula unus vel duo, longitudine varientes. Ramuli secundarii numerosi, totum internodium obtegentes. Spermatangia globosa, 6-7 μm diametro, in ramulis primariis et secundariis terminalia vel lateralia. Ramuli carpogoniferi e cellulis basi ramulorum orientes, ex cellulis 5-8 disci- vel doliiformibus constantes, tortuosi, carpogonium 40-72 μm longum; trichogyne cylindrica, indistincte pedicellata. Bracteae numerosae, breves. Gonimoblasti singuli, globosi vel semiglobosi, 400-550 μm crassi, 190-270 μm alti, verticilli crassior, in centro verticilli inserti; fila gonimoblastorum laxe agglomeratia. Carposporangia obovoidea, 7-10 μm crassa, 12-16 μm longa.

Frond monoecious, 7 cm high, 350-550 μm wide, abundantly and irregularly branched, moderately mucilaginous. Axial cells cylindrical, 50-150 μm wide, 330-530 μm long. Whorls cylindrical, more or less compressed, touching each other. 2-3 fascicles arising from the ovoidal basal cell of primary branchlet. Primary branchlets di- or trichotomously branched, consisting of 4-7 cell-stories; cells of fascicles lanceolato-elliptical; hairs numerous, 1-2 in each terminal cell, varying in length with an inflated base. Secondary branchlets numerous, well-developed, straight or slightly curved, covering all the internodes. Spermatangia globular, 6-7 μm in diameter, terminal or lateral on primary and secondary branchlets. Carpogonium-bearing branch arising from the basal cell of primary branchlet, consisting of 5-8 disc- or barrel-shaped cells, twisted; carpogonium 40-72 μm long; trichogyne cylindrical, indistinctly stalked. Bracts numerous, short, forming a glomerule with the carpogonium-bearing branch. Gonimoblasts single, globular or semiglobular, 400-550 μm wide, 190-270 μm high, higher than the whorls; gonimoblast filaments loosely aggregated. Carposporangia obovoidal, 7-10 μm wide, 12-16 μm long.

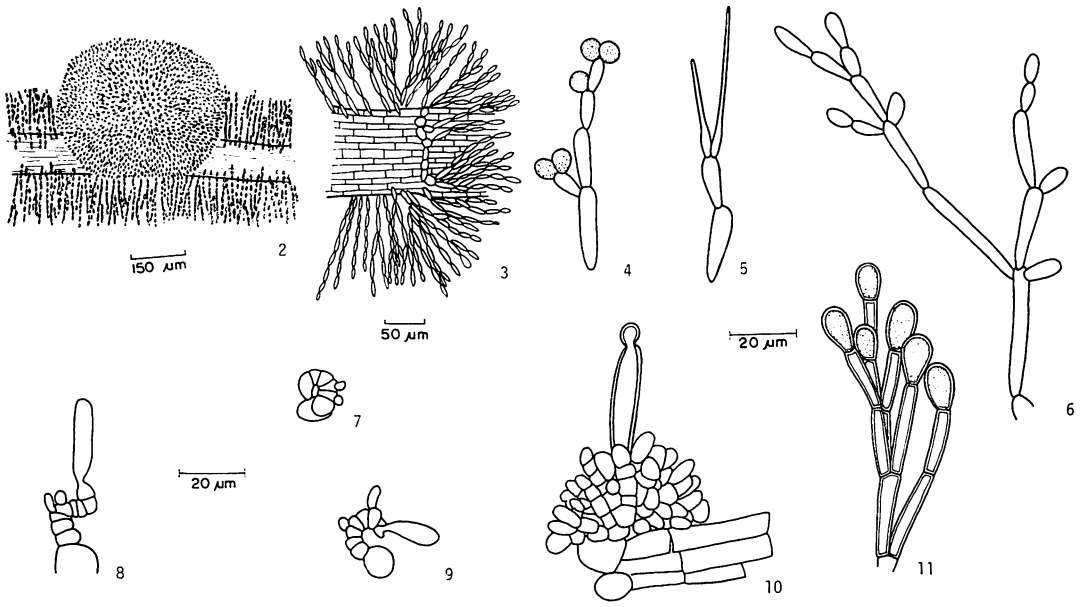
Holotype: E. C. Oliveira FILHO, SP-187102, 05/XII, 1974, Herbarium of Insitute of Botany, São Paulo, Brazil (SP).

Type Locality: Município of Areia Branca, Itabaiana Mountains, Serigipe State, Brazil.

Habitat: This variety grows epilithic in a mountain rivulet associated with *Batrachospermum cayennense* MONTAGNE.

This variety differs from *Batrachospermum capense* STARMACH ex NECCHI et KU-

1) The ending of the epithet was changed to agree with the neuter gender of the generic name.



Figs. 2-11. *Batrachosperum capense* STARMACH ex NECCHI et KUMANO var. *breviarticulatum* NECCHI et KUMANO, var. nov. 2. Part of a thallus showing a mature gonimoblast; 3. Strucutre of a whorl; 4. Spermatangia terminal and lateral on a primary branchlet; 5. An apex of a primary branchlet; 6. Detail of a primary branchlet; 7-9; Early stages in the development of the carpogonium with twisted carpogonium-bearing branches; 10. Early stage in the development of gonimoblast filaments; 11. Carposporangia terminal on gonimoblast filaments.

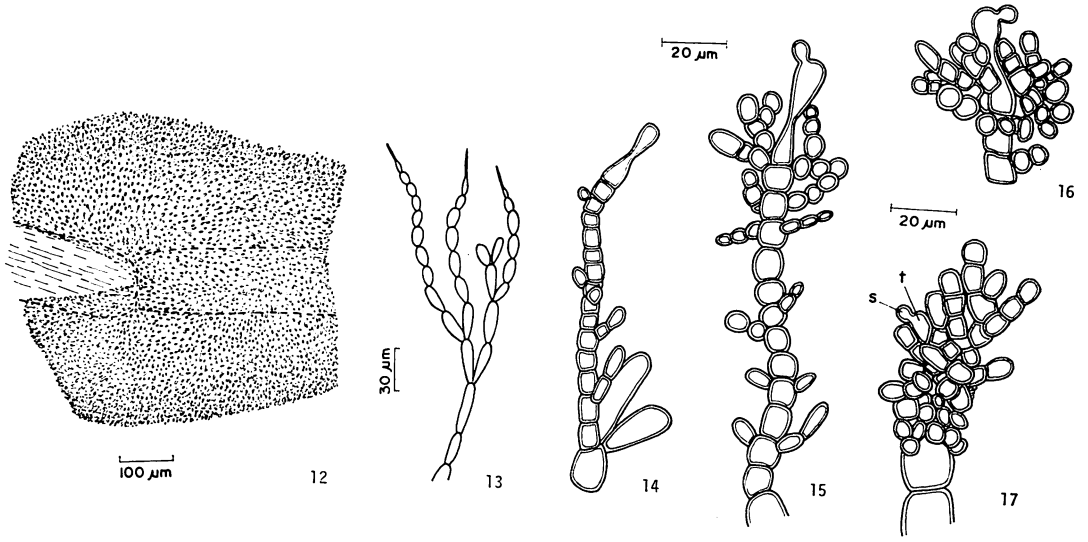
MANO in the number of cells of primary branchlets and in the size of gonimoblasts. In the type variety, the primary branchlets consists of 7-13 cell-stories and the whorls are 400-580 μm wide (STARMACH 1975) or 400-620 μm wide (STARMACH 1977). While, in the present new variety, they consist of 4-7 cell-stories and the whorls are slightly narrower (350-550 μm wide). The gonimoblasts in the type variety are 600-850 μm wide (STARMACH 1975 1977), while they are 400-550 μm wide in the present new variety.

STARMACH (1975) at the time of its original description considered *B. capense* belonging to section *Viridia*. However, the carpogonium-bearing branch in this species is spirally twisted, thus permiting the species inclusion in the section *Contorta* as it was already made by KUMANO (1982).

Batrachospermum capense is only known from its type locality, Seychelles, Mahé Island. The present study is, therefore, the second record for the species. This fact suggests that it most probably has a more extense geographical distribution. Also, the morphological discrepancies between the Brazilian and the original specimens are obviously due to the virtual absence of knowledge of the species today.

2. *Batrachospermum cayennense* MONTAGNE (Figs. 12-17)

Frond dioecious, 2 cm high, 400-650 μm wide, irregularly branched, moderately mucilaginous. Axial cells cylindrical, 95-160 μm wide, 400-950 μm long. Whorls pear-shaped or sphaerical, touching each other. 2-3 fascicles arising from the ovoidal basal cell of primary branchlet. Primary branchlets with 3-6 branches, di- or trichotomously branched, consisting of 7-12 cell-stories; proximal cells of fascicles ellipsoidal, distal cells obovoidal or spherical; hairs numerous, short, with an inflated base. Secondary branchlets numerous, straight, oblique, covering all the internodes. Male plants not yet observed. Carpogonium-bearing branch



Figs. 12-17. *Batrachospermum cayennense* MONTAGNE 12. Structure of a whorl; 13. Detail of a primary branchlet; 14. Young carpogonium-bearing branch with a carpogonium initial; 15. Fertilized carpogonium; 16-17. Early stages in the development of gonimoblast filaments. (s. spermatium, t. trichogyne).

arising from the basal cell and from the first cell of primary branchlet, very long, consisting of 12-30 barrel-shaped cells; carpogonium 27-37 μm long; trichogyne club-shaped, indistinctly stalked. Bracts sparse, short, composed of 1-3 cells. Gonimoblasts single or double, inserted at periphery of whorl, immature. Carposporangia obovoidal, immature.

Specimen examined: E.C. Oliveira FILHO, SP-187100, 05/XII, 1974, Município of Areia Branca, Itabaiana Mountains, Serigipe State, Brazil.

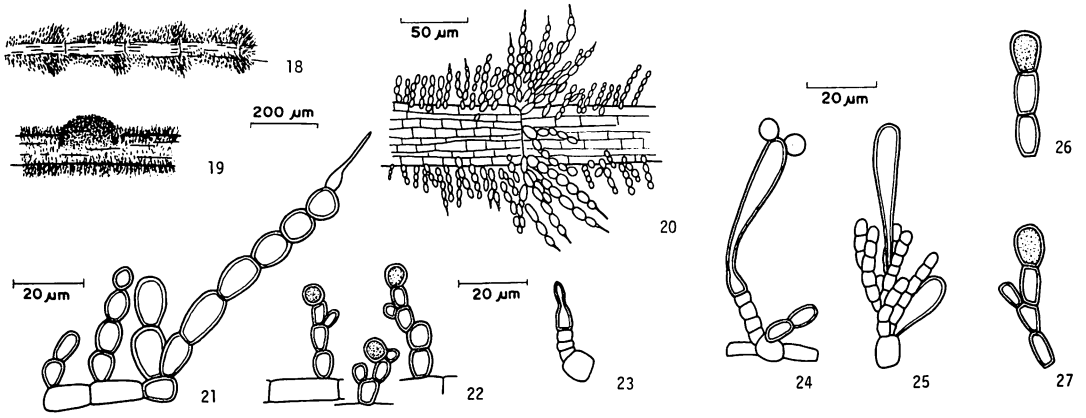
Habitat: In a mountain rivulet associated with *B. capense* STARMACH ex NECCHI et KUMANO var. *breviarticulatum* NECCHI et KUMANO.

Distribution: French Guiana, Madagascar, Malaysia and Brazil.

Batrachospermum cayennense MONTAGNE belongs to the section *Aristatae* SKUJA, and it was originally described based on the material from Cayenne, French Guiana. Later on, BOURRELLY (1964) reported this species from Madagarscar, and KUMANO and RATNASABAPATHY (1982) studied the development of carposporophyte based on materials collected from Malaysia. The section *Aristatae* is characteristic by possessing very long carpogonium-bearing branches. In the species we examined, the carpogonium-bearing branch is composed of 12-30 barrel-shaped cells, and longer than those reported by previous authors.

3. *Batrachospermum orthostichum* SKUJA (Figs. 18-27)

Frond monoecious, 7 cm high, 150-250 μm wide, abundantly and irregularly branched, poorly mucilaginous. Axial cells cylindrical, 30-65 μm wide, 200-350 μm long. Whorls pear-shaped at the apex, cylindrical, compressed, touching each other at the middle and basal portions. 2-3 fascicles arising from the globose basal cell of primary branchlets. Primary branchlets dichotomously branched, consisting of 3-7 cell-stories; proximal cells of fascicles obovoid or barrel-shaped, distal cells spherical; hairs abundant, 1-2 in each terminal cell, short, with an inflated base. Secondary branchlets numerous, slightly curved, covering all the internodes. Spermatangia globular, 6-8 μm in diameter, terminal or lateral on secondary



Figs. 18-27. *Batrachospermum orthosticum* SKUJA 18. Structure of whorls; 19. Part of a thallus showing a mature gonimoblast; 20. Structure of a whorl showing primary and secondary branchlets; 21. Detail of primary and secondary branchlets; 22. Spermatangia terminal on secondary branchlets; 23. Carpogonium-bearing branch with a young carpogonium; 24. Mature carpogonium with club-shaped trichogyne; 25. Fertilized carpogonium with spermatia; 26-27. Carposporangia terminal on gonimoblast filaments.

branchlets, rarely on primary branchlets. Carpogonium-bearing branch arising from the basal cell of primary branchlet, consisting of 3-5 barrel-shaped cells; carpogonium 36-47 μm long; trichogyne club-shaped, indistinctly stalked. Bracts more or less numerous, short, composed of 1-5 barrel-shaped cells. Gonimoblast single, semiglobular, 60-110 μm high, 140-220 μm wide, higher than the whorl. Carposporangia obovoid, 8-11 μm wide, 11-14 μm long.

Specimen examed: E.C. Oliveira FILHO. SP-187101, 05/XII, 1974, Município of Areia Branca, Itabaiana Mountains, Serigipe State, Brazil.

Habitat: Epilithic in a mountain rivulet.

Distribution: Brazil.

SKUJA (1931) described and propose *B. orthostichum* as a new species based on the material collected from Santa Teresa in the State of Espirito Santo, and classified it as a member of the section *Setacea*. Since that time, this species was never reported again in the literature. The present information is, therefore, the second record for the species. *Batrachospermum orthostichum* is only known from Brazil, and it has been collected in several other localities, this fact suggests a more extense geographical distribution in Brazil than it was thought formerly, and this species probably occur in other countries in the world. The specimen examined is very similar to that described and illustrated by SKUJA (1931).

Acknowledgements

The authors wish to express their sincere appreciations to Dr. Carlos E. de M. BICUDO of the 'Instituto de Botânica' São Paulo, and to Mr. Tarciso S. FILQUEIRAS of the 'Instituto Brasileiro de Geografia e Estatistica', Brasilia for valuable criticisms, to Dr. Eurico C. de Oliveira FILHO of the University of São Paulo for collection of the specimens, and to Dr. Karol STARMACH of the Polish Academy of Sciences, Institute of Botany, Krakow for sending the original material of *B. capense*. This study was supported in part by a 'FAPESP, Fundação de Amparo á Pesquisa do Estado de São Paulo' Grant nr 82/1071-9 given to the senior author.

Resmo

Três táxons de *Batrachospermum* ROTH (Rhodophyta) do Município de Areia Branca, Estado de Serigipe, são estudados. *Bartrachospermum capense* STARMACH ex NECCHI

et KUMANO (Seção *Contorta*) é lectotipificado e *B. capense* STARMACH et NECCHI et KUMANO var. *breviarticulatum* NECCHI et KUMANO, var. nov., descrito com base no número de células do ramo verticilar e no tamanho do gonimoblasto. *B. orthostichum* SKUJA (Seção *Setacea*) é registrado pela segunda vez na literatura e *B. cayennense* MONTAGNE (Seção *Aristatae*) pela primeira vez no Brasil.

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ネッシィ O. Jr.* ・熊野 茂: ブラジルの淡水産紅藻 I. ブラジル北東部, セリジペ州のカワモヅク属2種1新変種について**

STARMACH (1975) が *Batrachospermum capense* を記載する際，基準として用いた複数の標本のうちの1つが選定基準標本として選定された。

ブラジル北東部のセリジペ州，アレイア・ブランカ郡のイタバイアナ山地中の小流中から，カワモヅク属コントルタ節の1新変種 *B. capense* var. *braviarticulatum* が記載された。本新変種は基本変種に比較して，1次輪生枝を構成する細胞数が少く，嚢果が小型であることで区別できる。現在までブラジル以外から報告のないセタケア節の *B. orthcstichum* が上記の同じ小流中から発見され，これは本種の第2の報告である。また，アリスタタエ節の *B. cayennense* がブラジル新産種として報告された。(*01000 ブラジル サンパウロ 私書箱4005 植物研究所藻類部門，**657 神戸市灘区六甲台町 神戸大学理学部生物学教室)

Jap. J. Phycol. (Sôrui) 32: 354-363. December 10, 1984

Observations on *Trentepohlia lagenifera* (HILD.) WILLE (Chlorophyceae, Trentepohliaceae)[1)]

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NAKANO, T. and HANDA, S. 1984. Observations on *Trentepohlia lagenifera* (HILD.) WILLE, (Chlorophyceae, Trentepohliaceae). Jap. J. Phycol. 23: 354-363.

Trentepohlia lagenifera (HILD.) WILLE, an alga of the Chlorophyceae, was investigated in wild and cultured specimens. Specimens used were collected and isolated from tree trunks in Miyajima Island and adjacent areas, Hiroshima Prefecture. In wild specimens, morphological observation was made in detail and its results were discussed. In cultured specimens, two morphologically different forms were observed. The form A formed small spot colonies on agar plate and its cell shape and size were similar to those of the wild specimen. On the other hand, the form B formed coarse, broadly expanding, fluffy colonies on agar plate. Its cells were much longer than those of the wild specimen. These two forms were described as cultured forms.

Key Index Words: *aerial algae*; *Chlorophyceae*; *morphological forms*; Trentepohlia lagenifera.

Trentepohlia lagenifera (HILD.) WILLE, known as an alga of the aerial Chlorophyceae, is widely distributed in the world, occurring mainly in tropical and subtropical regions (HILDEBRAND 1861, HARIOT 1889, DE WILDEMAN 1891, PRINTZ 1939, CRIBB 1968, etc.). It is in Japan, commonly found in south and southwestern parts (HIROSE *et al.* 1977). This alga is growing on barks and leaves of trees as well as on surface of stones and ground. The algal colony is usually yellow to reddish-orange or olive-green to yellow-green.

During our study of epiphytic algae on tree trunks in Miyajima Island and its adjacent areas, Hiroshima Prefecture, we frequently found reddish-orange to orange-colored colonies of the alga. They agreed well with the descriptions of *T. lagenifera* by HILDEBRAND (1861) and PRINTZ (1939). Moreover, there were two morphological forms observed in the culture of this alga.

The purpose of this paper is to describe in detail the morphology of both the wild and cultured specimens, and to compare the two forms observed in culture.

Materials and Methods

Colonies of *T. lagenifera* were collected with barks from tree trunks by striping it with knife in Miyajima Island and adjacent areas, Hiroshima Prefecture. Specimens used in this study are listed in Table 1 with their sample numbers, collection dates, localities and names of host trees.

In the laboratory, a portion of the colony carefuly scraped off from bark surface was used for observation with light microscope. Another portion of the colony scraped was

1) Contribution from the Phytotaxonomical & Geobotanical Laboratory, Hiroshima University, N. Ser. No. 289.

Table 1. Summary of data on the specimens studied.

Specimen No. (HIRO)	Collection Date	Locality	Host tree
sh-164	Jan. '81	Miyajima-cho, Miyajima Isl.	*Eurya japonica*
sh-165	Jan. '81	Miyajima-cho, Miyajima Isl.	*Ilex pedunculosa*
sh-166	Jan. '81	Miyajima-cho, Miyajima Isl.	*Symplocos theophrastaefolia*
sh-184	Apr. '81	Ogauchi-cho, Hiroshima City	*Cryptomeria japonica*
sh-212	June '81	Miyajima-cho, Miyajima Isl.	*Juniperus rigida*
sh-219	June '81	Miyajima-cho, Miyajima Isl.	*Eurya japonica*
sh-221	June '81	Miyajima-cho, Miyajima Isl.	*Myrica rubra*
tn-223	Oct. '81	Okimi-cho, Nomishima Isl.	*Symplocos lucida*

sonicated for about one minute to obtain a more uniform suspension of small pieces of algal filaments. The suspension was aspirated onto a sterile agar plate which had been prepared by adding 1.5% agar to Bold's Basal Medium (1N BBM) as modified by BISCHOFF and BOLD (1963). Aspiration was acomplished in the manner described by WIEDEMAN *et al.* (1964). Petri dishes were placed under standard conditions (about 3000 lux light intensity on a diurnal light cycle of 12 hr light and 12 hr darkness at 22±1°C). After about one month, distinguishable colonies selected under stereoscopic binocular microscope were removed from the agar plate and inoculated on 1N BBM agar slants as unialgal cultures.

Some of these unialgal cultures were purified to the axenic state by the following method before being studied in detail. Short portions near the apex of algal filaments projecting into air on agar slant were picked off and transferred onto proteose agar medium (STARR 1964). After two to three weeks, algal filament which were still axenic were transferred to agar slant of 3N BBM (BROWN and BOLD 1964) and maintained as stock cultures.

Unialgal and axenic cultures are deposited in the Botanical Institute, Hiroshima University (CCHU).

Results

1. Observation on wild specimens

1) Colony

The colonies of *Trentepohlia lagenifera* collected formed fine cushion-like expansions of filaments closely placed on tree trunks. They were orange to orange-yellow by the presence of orange-red haematochrome pigments in their cells. On the shaded side of tree trunks, however, colonies were usually green to yellow-green, which was due to a little amount of haematochrome pigments.

2) Morphology of filaments and cells

Filaments were irregularly branched, mostly short and torulose, and erect and

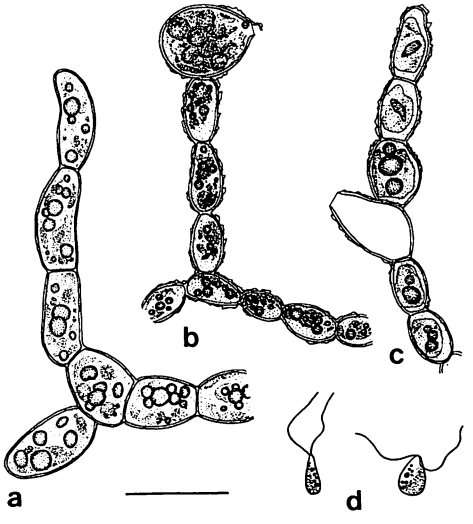

Fig. 1. *Trentepohlia lagenifera.* Wild specimens. a. Filament without granular secretions b. Sessile, terminal sporangium on filament with granular secretions; c. Sessile, intercalary empty sporangium; d. Biflagellate swarmers. Scale bar: 20 μm.

prostrate filaments were not sharply distinct from one another. Cells were ellipsoidal to barrel-shaped, and most of those at the surface portion of a colony had granular secretions on the outer cell wall (Fig. 1b, 2a). However, cells at the under portion of the colony or those of the filaments growing on the shaded side of tree trunks did not have such secretions usually (Fig. la).

Cell size was measured on filaments selected randomly from some small portions of a colony. The size of cells was variable in each

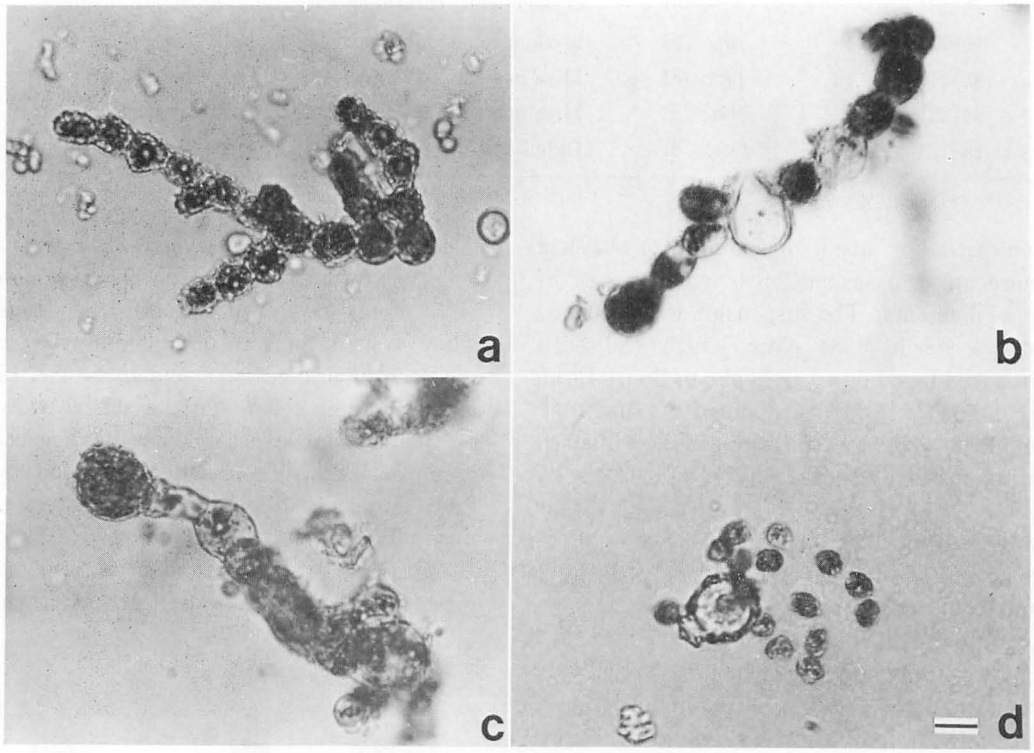

Fig. 2. *Trentepohlia lagenifera*. Wild specimens. a. Filament; b. Sessile, intercalary empty sporangium; c. Stalked sporangium; d. Liberation of four-flagellate swarmers from sporangium detached from stalk cell. Scale bar (10 μm) in d applies also to a-c.

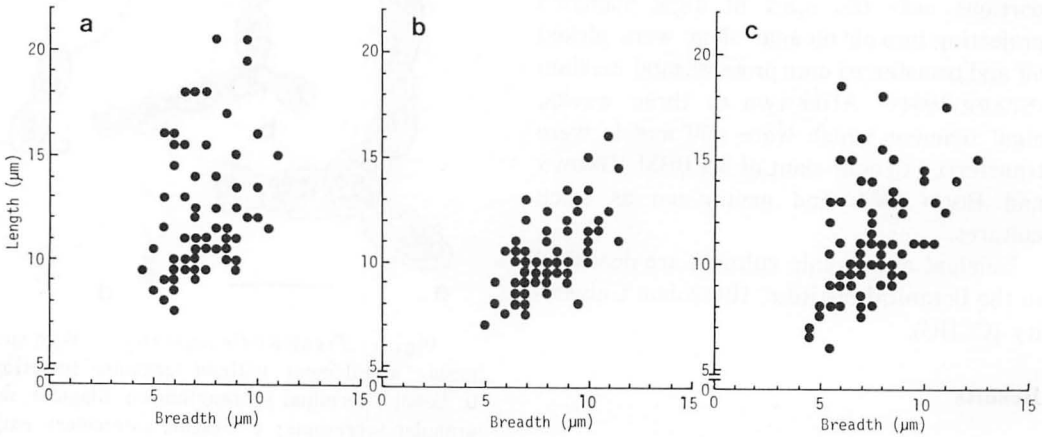

Fig. 3. Scatter diagram showing the cell size of *T. lagenifera* from wild specimens. a. Specimen no. sh-164; b. sh-219; c. tn-223.

sample, as can be seen in the scatter diagrams presented in Fig. 3, but it usually showed a small variation within a given filament. Cell size was 6.0–20.5 μm (average 12.3 μm) in length and 4.5–12.5 μm (average 7.7 μm) in breadth.

Chloroplasts were usually difficult to observe due to the presence of many haematochrome pigments. However, in cells of the filament at the under portion of a colony and on the shaded side of tree trunks, chloroplasts were visible because haematochrome pigments were scarce or absent. In these cells, parietal chloroplasts were ribbon-like or broken up into small discs (Fig. 1a). Pyrenoids and starches could not be observed in our specimens.

3) Reproductive cells

Both sessile and stalked sporangia were found in our specimens. Sessile sporangia occupied very diverse positions and were formed singly in terminal or intercalary on filaments (Fig. 1b, c, 2b). Sessile sporangia were usually flask-shaped with a short ostiole, 10.0–15.0 μm in diameter and contain 4, 8 or usually 16 swarmers. Swarmers escaped one by one from the ostiole of sporangium within about one minute after adding the water. They were flattened ovoid or pear-shaped, with two anterior flagella of equal length which were longer than the body length (Fig. 1d) and had several minute haematochrome grains and a single cup-shaped chloroplast, but no eye-spot. When liberated swarmers swam very actively and rapidly, and after few minutes they stopped movement, becoming round and shedding the flagella. They sometimes behaved as isogametes, although they were not successful in fusing with each other.

Stalked sporangia were also observed in some wild specimens (Fig. 2c, d). Those sporangia appeared only as terminal appendages, which were usually subspheroidal and were easily detached from a stalk-cell. The stalk-cell consists of a broad and subspheroidal proximal portion and a narrow and cylindrical distal portion. The stalked sporangia produced a considerable number of swarmers with four flagella of equal length, which were similar in both shape and size to those of the swarmer with two flagella. The liberation of swarmers was frequently observed in wild specimens collected in summer season.

2. Observations on cultured specimens

1) Colony

When the suspension of algal filaments was spread onto 3N BBM agar plates and inoculated under standard conditions, many colonies became visible to the naked eye after about one month. Two forms of colony, which were called form A and form B, respectively, or either of the two forms were observed on agar plates. The appearance of the two forms in cultures is summarized in Table 2. The form A formed orange, small spot colonies which developed more slowly on 3N BBM agar plate (Fig. 5a). On the other hand, the form B formed orange, coarse, broadly expanding fluffy colonies which developed more luxuriantly on agar plate containing 3N BBM (Fig. 5b).

2) Morphology of fiaments and cells

The two forms of this alga differ from each other in the morphology of both filaments and cells in cultures. A part of the results of cell size measurement in some isolates is shown in Fig. 4.

Filaments of the form A spread onto agar plate were richly and irregularly branched and formed many short branches, showing no differentiation of prostrate and erect systems (Fig. 5c, 6a). The branches were usually raised alternately to one another and usually originated from near the upper end of the parent cells. Young branches at first appeared as thin-walled protrusions, which swelled and gradually increased in size.

Individual cells were cylindrical to ellipsoidal, and showed some variation in both shape and size, ranging from 7.0 to 18.5 μm (average 10.8 μm) in length and from 4.5 to 12.0 μm (average 7.2 μm) in breadth. The size and shape of cells were similar to those of the wild specimens (Fig. 4), but the wall was smooth and did not show such grannular

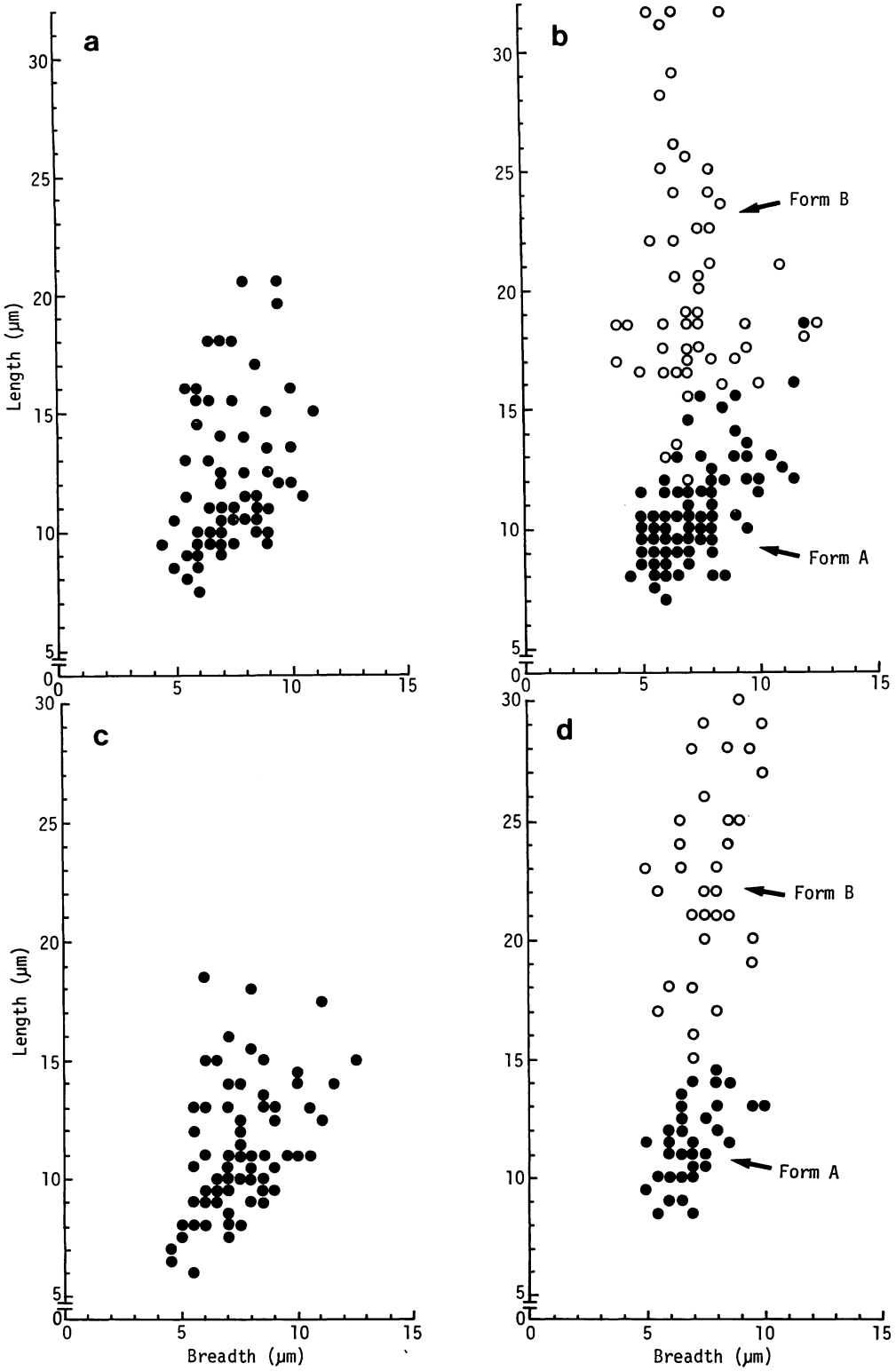

Fig. 4. Scatter diagram showing the cell size of *Trentepohlia lagenifera* from wild and cultured specimens. a. Specimen no. sh-164; b. Culture no. CCHU 2102 (Form A) and 2111 (From B); c. Specimen no. tn-223; d. Culture no. CCHU 2163 (Form A) and 2152 (Form B).

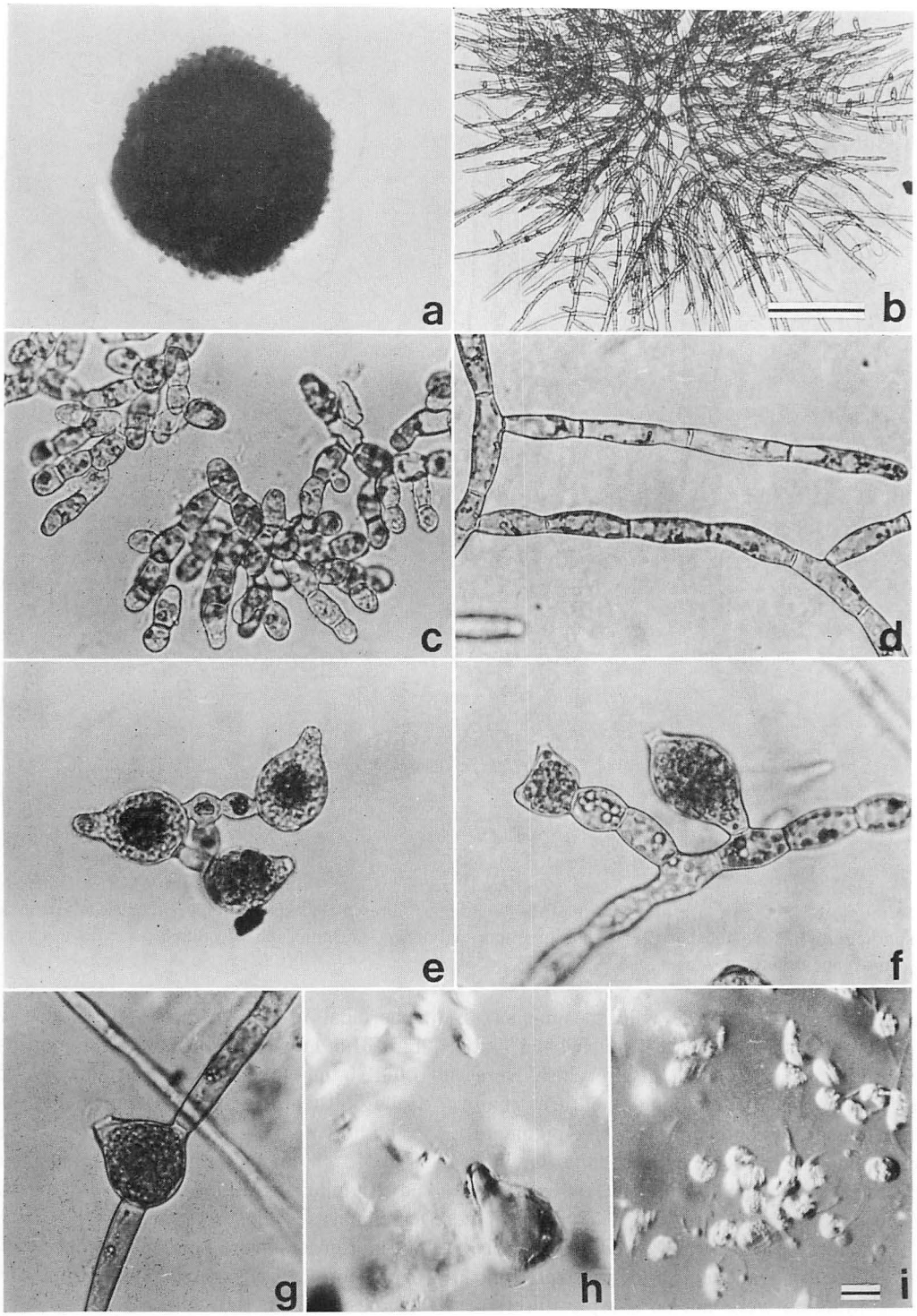

Fig. 5. *Trentepohlia lagenifera.* Cultured specimens. a, b. Colonies on agar plate (a. Form A; b. Form B); c, d. Filaments (c. Form A; d. Form B); e, f. Sessile sporangia (e. Form A; f. Form B); g. Mucilage secretion from ostiole of sporangium; h. Liberation of swarmers from sporangium; i. Biflagellate swarmers. Scale bar (100 μm) in b applies also to a; scale bar (10 μm) in i applies also to c-h.

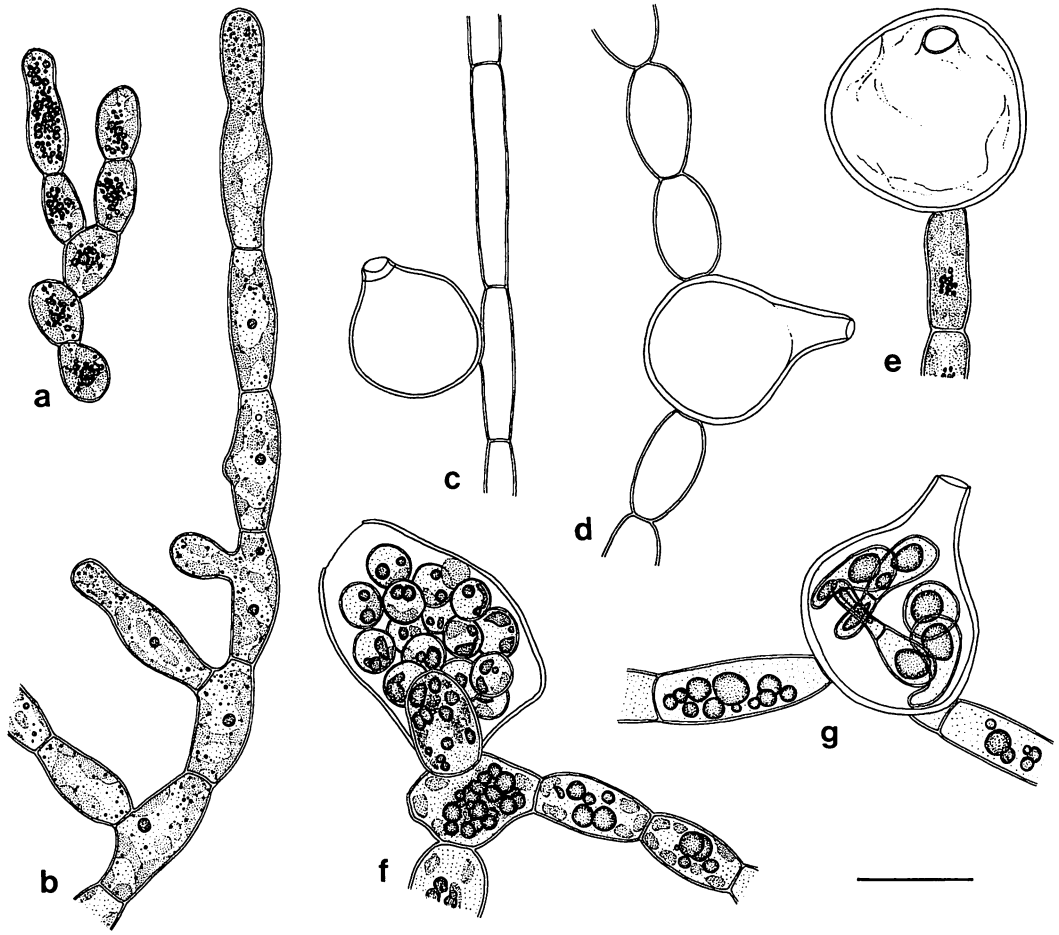

Fig. 6. *Trentepohlia lagenifera*. Cultured specimens. a. Filament of form A; b. Filament of form B; c. Sessile, lateral sporangium; d. Sessile, intercalary sporangium; e. Sessile, terminal sporangium; f. Aplanospore formation in a sporangium; g. Germination of aplanospores in a sporangium. Scale bar: 20 μm.

secretions as seen in the wild specimens.

Chloroplasts were parietal and ribbon like or broken up into small discs, which were visible more easily in younger cultures than older ones in which haematochrome pigments were abundantly produced in cells as in wild specimens. Pyrenoids and starches were not observed in these cells.

Filaments of the form B were irregularly and coarsely branched, and prostrate and erect systems were not so sharply differentiated (Fig. 5d, 6b). The process of branching was similar to that of the form A. Individual cells greatly varied in shape, being cylindrical to ellipsoidal, sometimes subspheroidal in old cultures. Cell size was 12.0-31.5 μm (average 19.8 μm) in length and 4.0-12.5 μm (average 7.5 μm) in breadth. Cells of the form B were longer than those of both the wild specimens and the form A (Fig. 4). The wall was smooth and showed no granular secretions as seen in the wild specimens.

Chloroplasts were similar in shape to those of the form A. Pyrenoids and starches were not observed in the cells.

3) Reproductive cells

Both the form A and the form B formed sessile sporangia of the same shape in culture (Fig. 5e, f, 6c, d, e). These sporangia arose

quite irregularly, being lateral, terminal or intercalary in position, and occurred usually singly and rarely in pairs. They varied in shape, being globose, subglobose and usually flask-shaped. Their size was 15.0-23.0 μm in diameter and larger than that of the wild specimen. The ostiole was colorless, longer than in the wild specimen and deliquesed at the tip to allow the swarmers to escape. The content of a sporangium was divided successively into 4, 8 and usually 16 cells in the ultimate. After the secretion of a little mucilage from the tip of the ostiole (Fig. 5g), swarmers escaped one by one from the ostiole of sporangium within about one minute after adding water (Fig. 5h-i). They contained several minute haematochrome grains with a single cup-shaped chloroplast and were similar to those of wild specimens in both shape and size.

Some swarmers unreleased from the sporangium were rarely observed. After a time they lost flagella and became globose, and developed into spheroidal aplanospores with a very thin cell wall in the sporangium (Fig. 6f). They enlarged and produced a knob-like protuberance which was later separated by a septum (Fig. 6g). The further development of these cells could not be observed in this study.

Neither stalked sporangia nor swarmers with four flagella were observed in the cultured specimens.

Discussion

As described above, the granular secretions on the cell wall were observed in wild specimens which were growing at the surface portion of a colony, but they were not observed in wild specimens from the under portion of a colony and growing at the shaded side of tree trunks and also in cultured specimens. From these results, we consider that those secretions are facultative productions under a certain growth condition.

HILDEBRAND (1861) showed a flask-shaped sporangium with a long ostiole in his description of *T. lagenifera*. PRINTZ (1939) also described a similar sporangium for this species in his monograph of the Trentepohliaceae. In our observations on wild specimens of this species, sessile, terminal and intercalary sporangia were flask-shaped and had a short ostiole (Fig. 1b, c, 2d). However, sporangia with a long ostiole were also observed though in a few cases in cultured specimens (Fig. 6d, g). CRIBB (1968) also reported the sporangium of the same shape with a long ostiole in this species which was isolated on nutrient agar from a tree trunk. The length of the sporangium ostiole in *T. lagenifera* seems to be variable with the difference of growth conditions.

In this study, two forms of sporangium, sessile and stalked (the latter only in the wild specimens), were observed. OLTMANNS (1922) has considered the sessile sporangium of the Trentepohliaceae as gametangium and SUÉMATU (1957) has reported that the intercalary sporangium was gametangium in *T. umbrina*. As to the nature of swarmers from such a sporangium, MAYER (1909) has considered them as gametes which lost sexual function and MAYER (1909) and FRITSCH (1935) have concluded that gametes were capable of germinating directly without fusion or giving rise to aplanospores. On the other hand, SUÉMATU (1951 1957) has observed a few instances of gamete fusion in *Cephaleuros virescens* and *Phycopeltis epiphyton*. SUÉMATU (1957) also reported the presence of many aplanospores in the gametangium of *Cephaleuros*. Moreover, he has reported the formation of hypnospores and filaments from the germinating aplanospores in *Trentepohlia aurea*. In cultured specimens of *Physolinum monile*, KHAN (1951) has reported that biflagellate swarmers became aplanospores and began to germinate inside the sporangium. We also observed biflagellate swarmers released from sessile sporangia. These swarmers showed the behavior as isogametes, although their fusion was not observed in this study. Some of them began to germinate in the sporangium and deveoloped a knob-like protuberance which was later separated by a septum, as reported by

KHAN (1951) for *Physolinum monile*. Unfortunately the further development was not observed in this study. Taking published data into consideration in addition to the present results, we are inclined to regard these swarmers as gametes.

Stalked sporangia of the Trentepohliaceae have been considered as zoosporangium by OLTMANNS (1922). MEYER (1909) also recognized the stalked sporangium as zoosporangium in which four-flagellate zoospores were produced. We agree with him in regarding these four-flagellate swarmers as zoospores. In this study, stalked sporangia were observed only in the wild specimens. This sporangium was easy to detach from a stalk-cell and produced four-flagellate zoospores (Fig. 2d). We suppose that easy detaching of a zoosporangium is useful for their wind-dispersal. Germination of zoospores was not observed in this study.

In the present study, two morphological forms were found in cultured specimens. The main differences between the form A and the form B were shown in colony form and cell size. The form A formed small spot colonies on agar plate and the cell size was similar to that of the wild specimens. On the other hand, the form B formed coarse, broadly expanding and fluffy colonies on agar plate. Cells of the form B increased in length and developed about two times or more longer than those of the wild specimens. The elongation of cell length in cultured specimens of *Trentepohlia* has been observed by SUÉMATU (1962) in *T. umbrina*. There were no conspicuous differences between the above-mentioned two forms in the shape and size of the sporangium. As shown in Table 2, there was found mixed state of the two forms or only either of the two forms on an agar plate. In wild specimens, however, we were not able to find any difference in the colony form and cell size. Characteristics of the two forms are considered in every respects to fall within the category of *T. lagenifera* that was circumscribed by HILDEBRAND (1861) and PRINTZ (1939) on the basis of wild specimens, but they still appear to be different taxa as far as they are recognized in our cultures. We consider that the two forms may be separated at variety level. In this study, however, we should hold off giving them any definite taxonomical position, because such differentiation into two forms was not detected in our wild specimens examined. Additional studies are needed to answer the taxonomical questions for these forms.

Table 2. Appearance of two forms in cultured specimens.

Specimen No. (HIRO)	Form A	Form B
sh-164	2102*	2111
sh-165	2100	—
sh-166	—	2064
sh-184	—	2046
sh-212	—	2098
sh-219	—	2133
sh-221	2147	—
tn-223	2163	2152

* Culture No. (CCHU).

Acknowledgements

We wish to express our thanks to Prof. Hisatsugu ANDO, Hiroshima University, who read the manuscript and gave us valuable advice. We are also very grateful to Prof. Mitsuo CHIHARA, University of Tsukuba, for his kind review of the manuscript and helpful suggestions.

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中野武登*・半田信司 *Trentepohlia lagenifera* (HILD.) WILLE (緑藻類，スミレモ科) の観察**

緑藻類，スミレモ科の *Trentepohlia lagenifera* について，野外および培養標本を基に観察を行った。野外標本のコロニーから藻体を分離，培養した結果，コロニーの形態と細胞の形態に2型のあることが明らかになった。Form A は，糸状体が密に分枝して，小塊状のコロニーを形成し，細胞が短い。Form B は，糸状体が長く伸び，分枝が少なく，粗なコロニーを形成し，細胞は前者より著しく長い。生殖器は，両型とも同じ形態を示した。これらの2型は，培養標本を基にする限りでは，変種として記載されるものと考えられるが，野外標本中にこれらの2型を見出すことができなかったため，本報告では，両型を単なる培養型として記載するにとどめた。(*730 広島市中区東千田町 1-1，広島大学理学部植物学教室，**733 広島市中区広瀬北町 9-1，広島県地区衛生組織連合会)

藻類 Jap. J. Phycol. 32: 364-372, December 10, 1984

石灰紅藻オオシコロとミヤベオコシにおけるウロン酸多糖の局在性と石灰化部位との関連について（藻類の $CaCO_3$ 沈着に関する研究 VI）

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OKAZAKI, M., SHIROTO, C. and FURUYA, K. 1984. Relationship between the location of polyuronides and calcification sites in the calcareous red algae *Serraticardia maxima* and *Lithothamnion japonica* (Rhodophyta, Corallinaceae) (Studies on the calcium carbonate deposition of algae-VI). Jap. J. Phycol. 32: 364-372.

Histochemical and scanning electron microscopic (SEM) observations have been carried out to examine possible relationship between the location of polyuronides such as alginate and $CaCO_3$ deposition in the tissues of the calcareous red algae *Serraticardia maxima* (Corallinoideae) and *Lithothamnion japonica* (Melobesioideae). Acid polysaccharides such as pectin, alginate and carrageenan are stained by alcian blue, but cellulose, a neutral polysaccharide is not. Methylation of polyuronides such as pectin and alginate blocks their affinity for alcian blue, but saponification of the methylated polyuronides restores the affinity. On the other hand, sulphated polysaccharide (carrageenan) can not be stained by alcian blue after methylation and subsequent saponification treatment. Thus, alginate or alginate-like polysaccharides are histochemically distinguishable from sulphated polysaccharides. This technique has revealed that the acid-insoluble polyuronides such as alginate are localized in cuticles and cell walls of epithallial, cortical and medullary cells of decalcified intergenicular tissue of *S. maxima*. In decalcified protuberance of *L. japonica*, cell walls of perithallial cells contain similar acid polysaccharides. It is not possible to determine the intercellular location of polysaccharide because the intercellular space is almost lacking in *S. maxima*, and because this space becomes dilated extensively by decalcification in *L. japonica*. SEM observations of calcified and decalcified specimens show that $CaCO_3$ is deposited in cell walls of epithallial, cortical and medullary cells of *S. maxima*. In perithallum of *L. japonica* a heavy calcification occurs in cell walls and intercellular spaces. These results suggest that at least some relationships exist between polyuronides such as alginate and cell wall calcification in these algae.

Key Index Words: *Acid polysaccharides*; *Alcian blue-staining*; *Alginate*; *Calcareous red algae*; *Calcification*; *$CaCO_3$ deposition*; *Corallinaceae*; *Polyuronides*; *Rhodophyta*.

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紅藻サンゴモ科石灰藻は方解石型の $CaCO_3$ を細胞壁及び細胞間隙に沈着することが知られている（BOROWITZKA and VESK 1978 1979）。この藻の石灰化に関する生理学的研究（BÖHM 1978, BOROWITZKA 1979）及び石灰化部位の形態学的研究（BAILY and BISALPUTRA 1970, GIRAUD and CABIOCH 1979, MIYATA *et al.* 1980）はこれらの藻の石灰化機構と石灰化部位における Ca 結合能を持つ多糖類との密接な関連を強く暗示している。これらの藻の細胞壁及び細胞間隙において，この様な能力を備えた多糖類として酸性多糖が考え易いが，既に YENDO (1904), MATTY and JOHANSEN (1981) はルテニウムレッドを用いて組織化学的な手法で細胞壁にペクチン様物質が存在することを報告している。しかしルテニウムレッドは酸性多糖の一般的な染色剤であり，硫酸多糖とカルボキシル基を有する含ウロン酸多糖とを識別するには適当ではなく，また上述の研究はその様な目的で行われたものでもない。最近，OKAZAKI *et al.* (1982) は今回

用いた実験材料のオオシコロ及びミヤベオコシからCa結合物質を単離し，これをウロン酸多糖のアルギン酸と同定した。さらにこのアルギン酸がCaを特異的に結合することに注目して，アルギン酸のCa塩をCa供与体とする石灰化機構を提唱した。しかしアルギン酸の組織内分布については不明であり，この仮説は充分なものとは言えない。

この論文では塩基性色素のアルシアンブルーを用いて硫酸多糖とウロン酸多糖を組織化学的に識別する方法を検討し，さらにオオシコロとミヤベオコシの組織におけるウロン酸多糖の分布と石灰化部位との関連を検討した。

材料と方法

1. 材料　実験には紅藻サンゴモ科有節サンゴモ亜科のオオシコロ (*Serraticardia maxima*) と無節サンゴモ亜科のミヤベオコシ (*Lithothamnion japonica*) の2種を使用した。いずれも風乾藻体を実験に用いたが，ミヤベオコシは北海道大学水産学部の正置富太郎教授並びに宮田昌彦氏の御厚意により譲り受けた。

2. 光顕観察

1) 組織切片の染色，メチル化及び鹸化

オオシコロは藻体先端部を，またミヤベオコシは藻体表面の突起 (Protuberance) を切り取り，まず10%ホルマリン海水 (pH 7.8) にて室温下で48時間固定した。次にこれらの試料を2N塩酸で一昼夜脱灰し，常法に従ってパラフィン切片を作製した。切片の染色は酸性多糖に特異的でかつ鋭敏に反応することが知られている (Steedman 1950, Lison 1960) アルシアンブルー (MCB Manufacturing Chemists 社, Alcian blue 8GX) を使用した。また硫酸多糖と含ウロン酸多糖を区別するために Fisher and Lillie (1954), Lillie (1958) の方法に従って試料のメチル化 (Methylation) 及びその後の鹸化 (Saponification) 処理を行い，その後アルシアンブルーで染色した。次に染色液及びその使用法について簡単に述べる。

〔染色液〕 1% アルシアンブルー液 (蒸留水 97 m*l* に氷酢酸 3 m*l* を加え，これにアルシアンブルー 8GX 1 g を溶解したもの)。脱パラフィンした切片を室温で30分間染色する。染色後水洗して観察する。

〔メチル化のための反応液〕 100 m*l* のメタノールに 0.8 m*l* の濃塩酸を加えたもの。これに脱パラフィンした切片を浸し約 60°C で6時間反応させる。反応後水洗して染色する。反応の対照として，塩酸を含まないメタノール中で同条件下で処理したものを作製する。

〔鹸化のための反応液〕 1% 水酸化カリウム・エタノール溶液 (70% エタノールに 1% になる様に水酸化カリウムを溶解したもの)。これにメチル化処理した切片を浸し，室温で20分間反応させる。反応後水洗して染色する。

2) 標準多糖の染色，メチル化及び鹸化

紅藻で知られている数種の多糖類(前田・西澤 1974, 西澤 1977, Okazaki *et al.* 1982)，褐藻アルギン酸及びミカンのペクチンについてアルシアンブルーに対する染色性を検討し，組織切片の染色性と比較した。

〔標準多糖〕 カラゲナン (Iwai Kagaku Yakuhin 社, Carrageenan 402, 硫酸多糖)，セルロース (東洋沪紙社, Cellulose powder B, 200～300 mesh, 中性多糖)，紅藻アルギン酸 (Okazaki *et al.* 1982 の方法で紅藻オオシコロから単離したもの，ウロン酸多糖)，褐藻アルギン酸 (Kelco 社，精製アルギン酸ソーダ，ウロン酸多糖)，ペクチン (Waco Pure Chemical 社, Pectin from citrus, ウロン酸多糖)。これらの標準多糖の1% 水溶液を卵白アルブミンの塗布してあるスライドグラス上に滴下し，これを乾固した。これを前述の組織切片の場合と同様にして染色または処理（メチル化及び鹸化）して観察した。

3. 走査型電顕観察

石灰化部位を検討する目的で，脱灰及び未脱灰試料の2種類を作成した。まず常法に従って乾燥試料を5% グルタルアルデヒドと2% オスミウム酸で2重固定した。これを Shimada *et al.* (1976) の方法で凍結割断し，臨界点乾燥後，走査型電子顕微鏡 (JSM-F15, 15 KV) で観察した。また脱灰試料は2重固定後の藻体を 2N 塩酸で処理して完全に $CaCO_3$ を溶解除去したものから前述と同様の方法で作製した。

結　果

1. 標準多糖のアルシアンブルー染色

一部の紅藻に特有の細胞間多糖のカラゲナン (Car)，セルロース (Cel)，ペクチン (Pc)，紅藻アルギン酸 (Al_1) 及び褐藻アルギン酸 (Al_2) の計5種の標準多糖について，アルシアンブルーに対する染色性を調べた (Fig. 1)。図中にはそれぞれの物質を直接染色したもの (図中最左列)，またはメチル化 (M) 及び鹸化 (S) 後，染色したものを示してある。MC はメチル化処理の対照を示す。

中性多糖であるセルロースは酸性基をもたないので，アルシアンブルーでは全く染色されなかった (Cel)。セルロースの微顆粒が観察されるのは，顆粒自身が幾

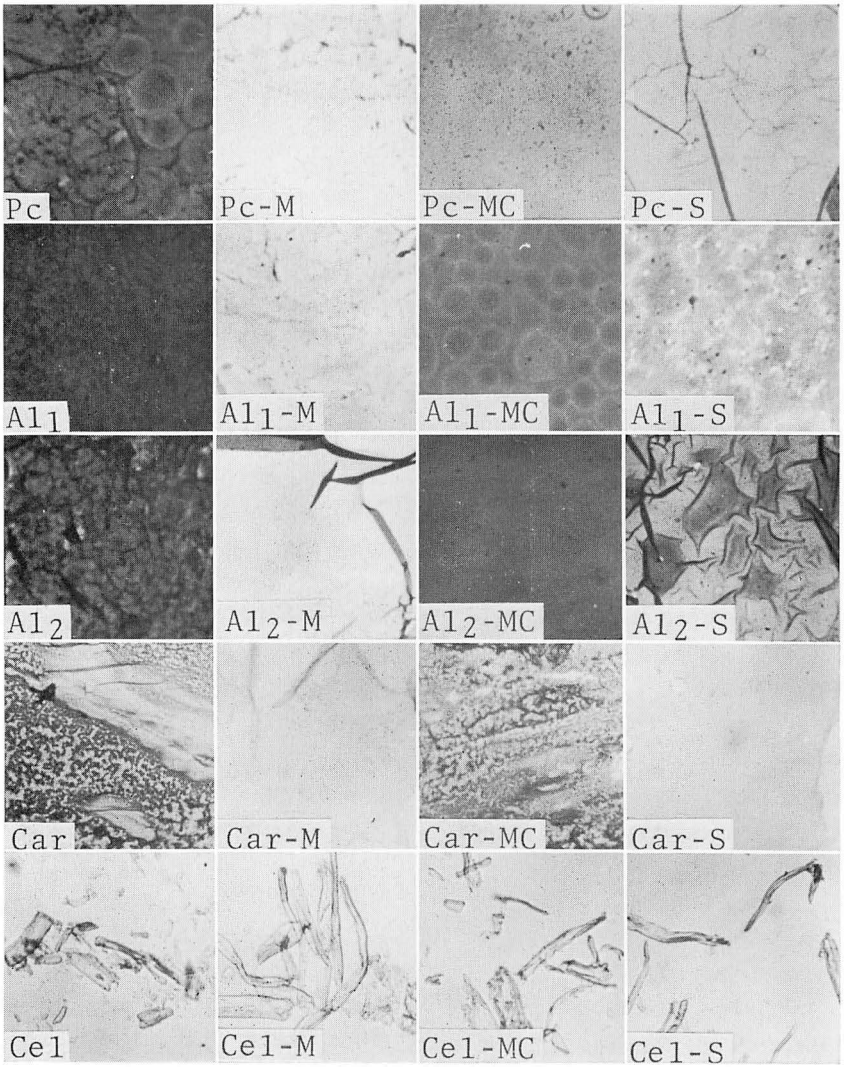


Fig. 1. Staining of various polysaccharides by alcian blue. The following polysaccharides were used as standard; pectin (Pc), alginate from coralline red alga (Al_1), alginate from brown alga (Al_2), carrageenan (Car) and cellulose (Cel). Series of left column and a symbol MC show staining without any treatment of the sections and staining of the sections after methylation in a medium omitted HCl (control for methylation treatment), respectively.

分コントラストを示すためで，色素で染色されたためではない。酸性多糖はアルシアンブルーにより強く染色されたが (Pc, Al_1, Al_2, Car)，いずれもメチル化するとその染色性は著しく低下した (Pc-M, Al_1-M, Al_2-M, Car-M)。一方メチル化の対照では，メチル化を触媒する塩酸が反応液中に含まれていないため，未処理の場合と同じ様に強く染色されている (Pc-MC, Al_1-MC, Al_2-MC, Car-MC)。メチル化処理したウロン酸多糖に鹸化処理をほどこすと，ペクチン，アルギン酸はアルシアンブルーに対する染色性をほぼ回復した (Pc-S, Al_1-S, Al_2-S)。これに対して，硫酸多糖のカラゲナンの場合には，メチル化処理に続く鹸化後もアルシアンブルーに対する染色性は回復せず低下したままであった (Car-S)。この様にウロン酸多糖と硫酸多糖が鹸化処理後にアルシアンブルー色素に対して異なった反応を示すのは次の様に説明されている。(1) ウロン酸多糖はメチル化処理によりカルボキシル基がメチル化されて封鎖されるのに対して，硫酸多糖

はその硫酸基が加水分解により脱離する (FISHER and LILLIE 1954)。(2) メチル化処理したものに鹸化処理をほどこすと，ウロン酸多糖から脱メチル化が起こり再び塩基性色素に対する染色性を回復するが，硫酸多糖は既に硫酸基を失っているため染色性の回復は起こらない (SPICER and LILLIE 1957)。この染色法は動物組織中の酸性多糖を組織化学的に識別するために確立されたものであるが，KANTOR and SCHUBERT (1957)は単離したコンドロイチン硫酸を用いてLILLIEら（前述）の組織化学で適用されたとほぼ同じ条件下で上記 (1), (2) の反応が起きることを証明している。今回，著者らは酸性多糖に特異的でかつ鋭敏に作用し，さらに染色液の pH を調整する必要のないアルシアンブルー（塩基性色素の一種）(STEEDMAN 1950) を用いたが，Fig. 1 の結果からウロン酸多糖を硫酸多糖から組織化学的に比較的明瞭に区別出来ることが明らかになった。今回はウロン酸多糖であるアルギン酸の石灰藻組織中の分布を知ることを目的にしているが，この物質は酸不溶性であるのでパラフィン切片作製時の酸による脱灰処理でも溶出することはあり得ないことも指摘しておきたい。

2. 組織切片のアルシアンブルー染色

オオシコロの先端節間部の縦断切片に見られる構造を Fig. 2A に示した。藻体は多軸構造を示し，藻体中心部の髄 (Me, Medulla) と藻体外層の皮層 (Cx, Cortex) から成る。Fig. 2B はその皮層部分の拡大像で，皮層細胞の外側には一層の表皮細胞 (E, Epithallial cell) が存在し，クチクラ (Cu, Cuticle) がそれを被っている。Fig. 2C はミヤベオコシの突起 (Protuberance) 周辺部の構造を示す。数層の表皮細胞が重なった表層 (Epithallium) とそれによって被われた中層 (P, Perithallium) から成り，中層では細胞の融合も観察された。クチクラは観察されなかった。

次に 2 種の藻体組織のアルシアンブルー染色の結果について述べる。オオシコロの皮層（縦断切片）と髄（横断切片）については，それぞれ Fig. 3A 及び 3B に示した。藻体組織を直接アルシアンブルーで染色すると，クチクラと表皮細胞，皮層細胞及び髄細胞の細胞壁が良く染色された (A-No, B-No)。またこれらの細胞の細胞間隙はほとんど存在しなかった。組織切片をメチル化すると，クチクラ及び前述の各細胞の細胞壁に見られたアルシアンブルーに対する染色性は著しく低下することがわかった (A-M, B-M)。しかしこのメチル化した切片を鹸化して染色すると，これらの部分の染色性に明らかな回復が認められた (A-S, B-S)。この様な結果は，これらの細胞壁にウロン酸多糖，例えば紅藻のアルギン酸の存在を示すものである。

Fig. 3C, D はミヤベオコシについての染色結果で，Cは突起周辺部の中層細胞の縦断面像を，Dは突起中央部における中層細胞の横断面像を示している。いずれの細胞もその細胞壁がアルシアンブルーによって良く染色されていることがわかる (C-No, D-No)。脱灰により糸状の細胞列が互いに分離し，間隙が異常に拡大しており，この部位には好アルシアンブルーの物質

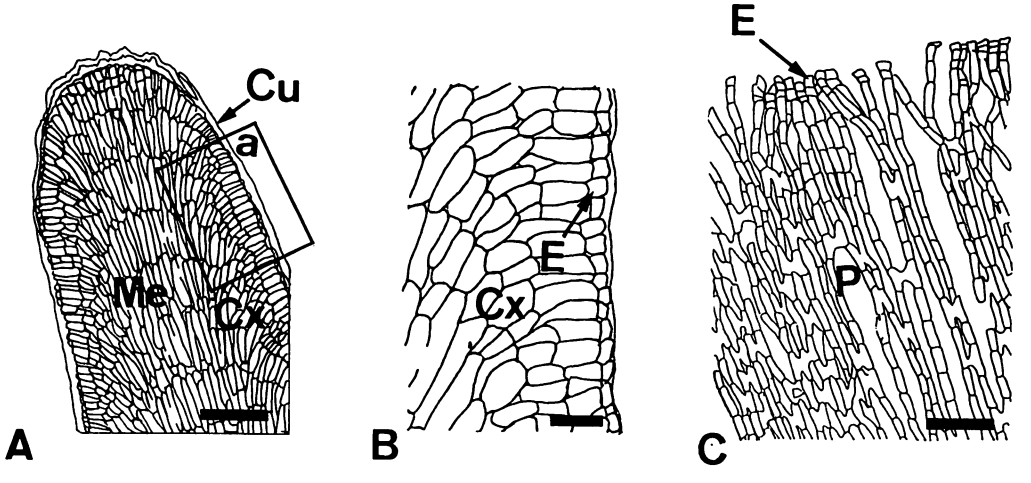


Fig. 2. Diagrams of tissue sections. A. organization at the apex of *Serraticardia maxima*; B. detailed structure of region "a" shown in A; C. detailed structure of perithallum in protuberance of *Lithothamnion japonica*. Cu. cuticle; Cx. cortex; E. epithallial cell; Me. medulla; P. perithallum Scale=100 μm (A), 25 μm (B, C).

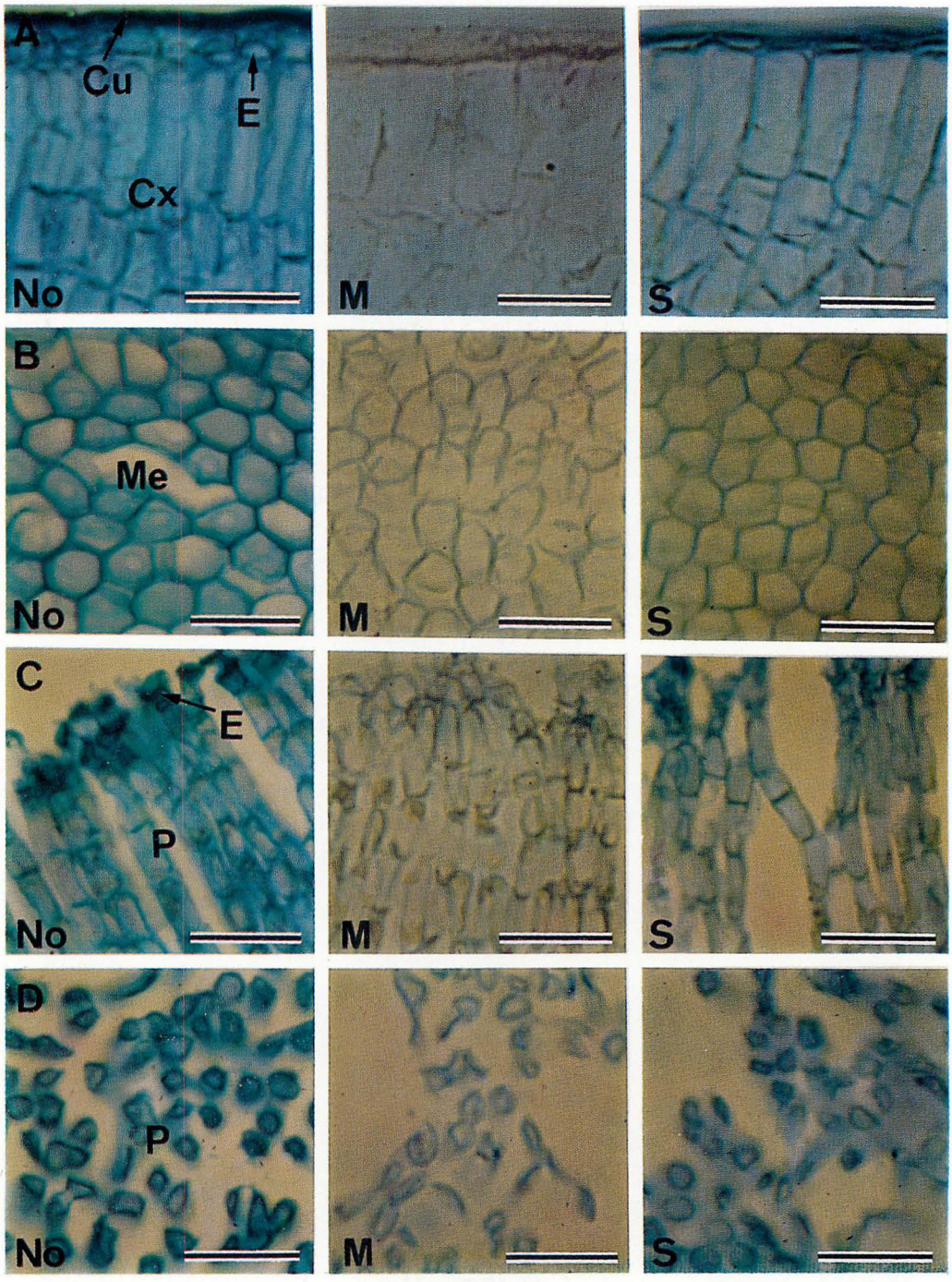
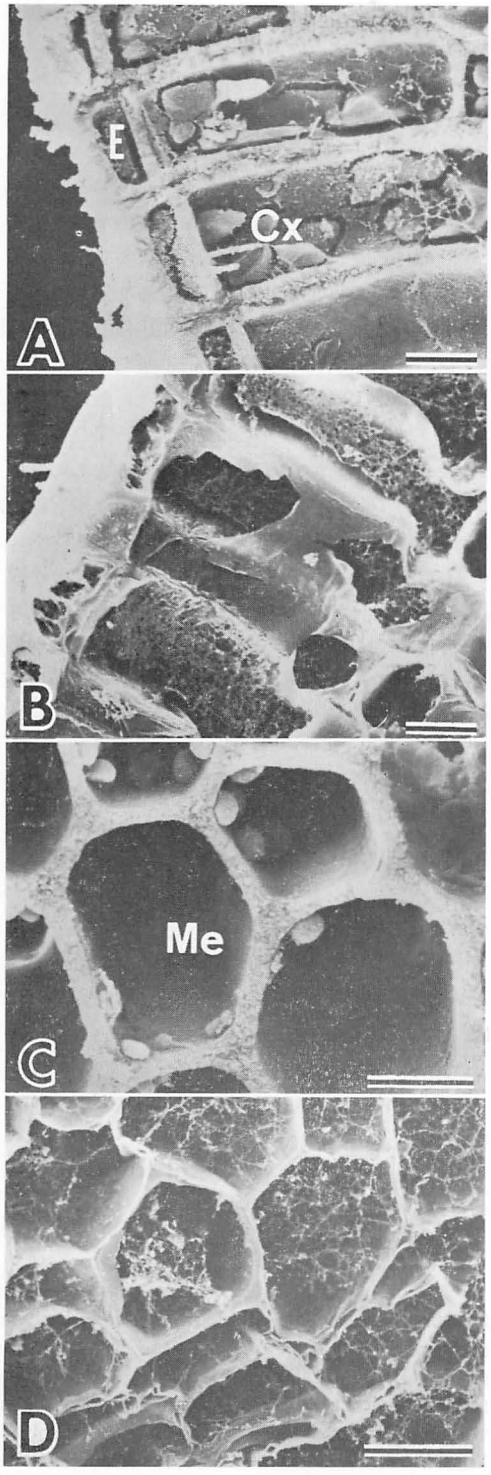


Fig. 3. Alcian blue-staining of sections of *S. maxima* and *L. japonica*. A-B. sections of intergeniculum of *S. maxima*; C-D. sections of perithallum of *L. japonica*; A-C. longitudinal sections; B-D. transverse sections; A symbol No shows staining without any treatment of tissue sections. For other symbols see Fig. 2. Note blockade of basophilia by methylation (M) and restoration of it by saponification (S). Scale=20 μm.

は認められなかった。またオオシコロの細胞壁と同様に，メチル化処理により細胞壁のアルシアンブルーに対する染色性は著しく低下した（C-M, D-M)。さらにメチル化した切片を鹸化すると，明らかに染色性の回復が見られること (C-S, D-S) から，オオシコロの場合と同様ウロン酸多糖の存在を示している。しかし両種においてメチル化切片の鹸化後の染色性は未処理の切片と比較して幾分劣り，鹸化処理で染色性が完全に回復しているとは言えない。これは標準物質の結果 (Fig. 1) についても言えるが，組織切片の場合には硫酸多糖の共存を示すとも考えられる。



3. 走査型電顕観察

藻体の石灰化部位を知る目的で，酸による脱灰試料と未脱灰試料について走査型電顕で観察した。Fig. 4 はオオシコロの表皮，皮層及び髄部の電顕像である。Fig. 4A（未脱灰像）と 4B（脱灰像）の比較から，脱灰像での表皮及び皮層細胞の細胞壁及び細胞間隙が著しく収縮し $CaCO_3$ の消失をうかがわせる。同様に髄においても髄細胞壁及び細胞間隙が石灰化していることがわかる (Fig. 4C, 4D)。脱灰像 (4D) において，細胞壁または間隙から剝離したと思われる繊維状物質が確認された。

次にミヤベオコシについての観察結果を Fig. 5 に示した。Fig. 5A は突起周辺部の未脱灰中層細胞の電顕像（縦断面像）で 5B はその脱灰像，また Fig. 5C は突起中央部の未脱灰中層細胞（横断面像）を示し，5D はその脱灰像である。これらの未脱灰，脱灰像を比較すると，オオシコロの場合と同様に脱灰像 (5B, 5D) では細胞間隙及び壁が著しく収縮しており，これらの部位が石灰化していたことを示している。また壁中の $CaCO_3$ 結晶は両部位とも細胞表面に対して垂直で放射状に配列していると思われる (Fig. 5A, 5C)。これらの 2 種の石灰藻で得られた石灰化部位の知見は，既に報告されている他のサンゴモ科石灰藻の知見と良く一致するものである (Borowitzka and Vesk 1978 1979)。

考　　察

紅藻植物の細胞壁及び細胞間隙の多糖類としては，今回の実験に標準物質として用いたカラゲナン，セルロースの他に寒天，キシラン，マンナン，フノラン，

Fig. 4. Scanning electron micrographs of the fractured surface of calcified and decalcified tip segments of *S. maxima*. A, C. calcified specimens; B, D. decalcified specimens; A, B. longitudinally fractured epithallum and cortex; C, D. transversely fractured medulla. For symbols see Fig. 2. Scale=5 μm.

ポルフィラン型ガラクタン等が知られている（前田・西澤 1974, 西澤 1977）。今回は後者の5物質についてはアルシアンブルーに対する染色性を検討していないが，酸性基をもたぬキシラン，マンナン及びほとんど硫酸基をもたず(1% 以下)，酸性多糖とは言い難い寒天は今回の実験対象から除いた。またフノランとポルフィラン型ガラクタンは，それぞれ硫酸基を約19%及び8%を含有する硫酸多糖であるため，アルシアンブルー染色に陽性であると考えられるが，メチル化処理後の鹸化処理でも染色性の回復は認められないはずである。

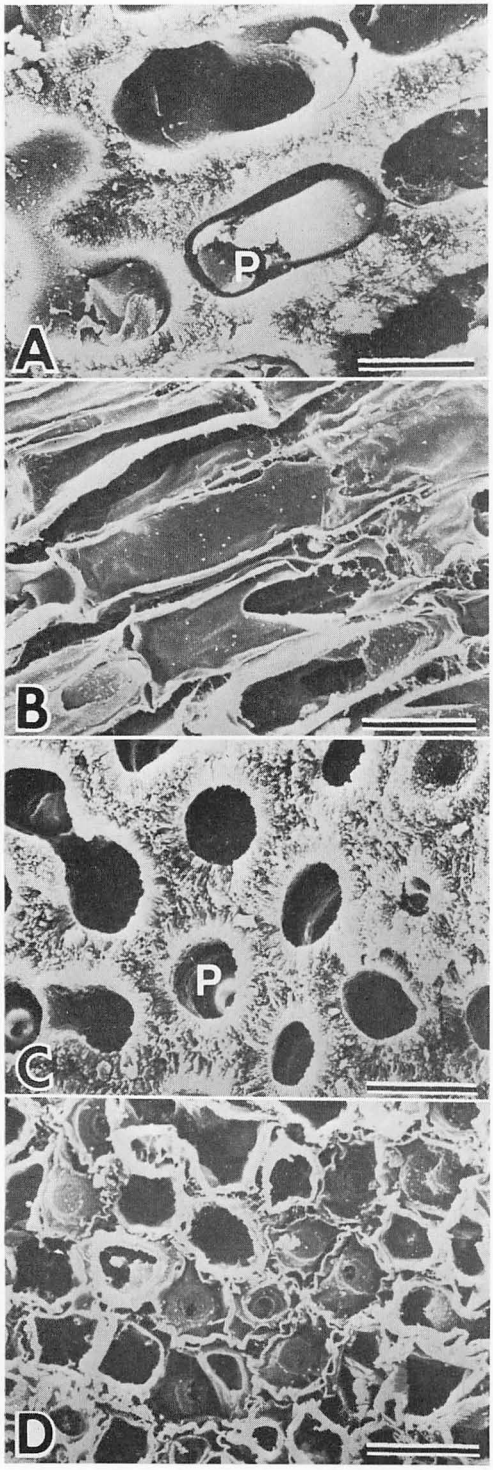


Fig. 5. Scanning electron micrographs of the fractured surface of calcified and decalcified protuberance of *L. japonica*. A, C. calcified specimens; B, D. decalcified specimens; A, B. longitudinally fractured perithallum; C, D. transversely fractured perithallum. For symbols see Fig. 2. Scale=5 μm.

今回の実験から2種のサンゴモ科石灰藻の組織にはアルシアンブルー染色に陽性の酸性多糖が存在すること，またメチル化処理後鹸化処理によりアルシアンブルーに対する染色性を著しく回復する物質が存在することが明らかになった。この様な染色傾向を示す多糖をサンゴモ科石灰藻の中で探すと，現時点では OKAZAKI *et al.* (1982) が単離したアルギン酸の存在が最も有力である。今回のアルシアンブルー染色法によって，このアルギン酸あるいはそれを主体とした酸性多糖は，オオシコロでは藻体表面のクチクラ層と節間部を構成する各種細胞の細胞壁，またミヤベオコシにおいては中層細胞の細胞壁に分布することが強く示唆された。既に MATTY and JOHANSEN (1981) はサンゴモ (*Corallina officinalis*) のクチクラ層及び上述の細胞壁にルテニウムレッドに陽性のペクチン様物質の存在を，また YENDO (1904) もカニノテ属の一種 (*Amphiroa tuberculosa*) の皮層及び髄部の細胞壁が同色素で染色されることを報告している。これらの研究で，ルテニウムレッドで染色される物質はペクチン様物質と呼ばれているが，サンゴモ科にペクチンが存在することは未だ証明されていない。OKAZAKI *et al.* (1982) がサンゴモ科石灰藻で発見したアルギン酸はウロン酸多糖という点ではペクチンと似ており，従って従来からサンゴモ科の組織化学的研究でペクチン様物質と呼ばれている物質はこのアルギン酸である可能性が高い。

次にこのアルシアンブルーで染まるアルギン酸もしくはそれに類似した酸性多糖の組織における分布と石灰化部位との関連について考察したい。既に結果の項で述べた様に，走査型電顕による脱灰及び未脱灰試料の比較観察から，2種の石灰藻において節間細胞（表皮，皮層，髄の各細胞を含む）の細胞壁（オオシコ

ロ），または中層細胞の細胞壁，細胞間隙（ミヤベオコシ）に $CaCO_3$ が沈着していることが明らかになった。この2つの石灰化部位のうち，細胞壁にはウロン酸多糖が局在していることは上述の通り明らかである。またクチクラ層にもこの酸性多糖が含まれていることがわかったが，GIRAUD and CABIOCH (1979) はこの部位も石灰化していることを報告しており大変興味深い。ただ今回の研究では石灰藻の主要な石灰化部位の1つである細胞間隙には酸性多糖の存在を認めることができなかった。これはオオシコロの細胞間隙は光顕切片では認め難い程狭く，またミヤベオコシにおいては脱灰処理によって細胞間隙が異常に拡張するので，この部位での物質の存在を組織化学的に検出し難いためと，脱灰に伴う剝離に対する疑問が残るためである。しかし今回得られた結果は少なくとも，アルギン酸を主体とした酸性多糖が存在する部位では必ず石灰化が見られることを示すもので，OKAZAKI *et al.* (1982) が指摘した様に紅藻のアルギン酸と石灰化機構の間には何らかの関係があることを暗示するものである。OKAZAKI *et al.* (1982) はサンゴモ科石灰藻から抽出したアルギン酸はCaを特異的に結合するので，Caの供与体として $CaCO_3$ 沈着に関与するか，また同時に $CaCO_3$ 結晶の核としてその結晶型を決定する鋳型として働くものと考えている。褐藻にアルギン酸が存在することは周知であるが，褐藻ではウミウチワ属の数種のみが石灰化することが知られているに過ぎない (BOROWITZKA 1982)。これは石灰化機構にはアルギン酸等の有機基質 (Organic matrix) 以外の要因，例えば藻の構造，炭酸イオンの供給及び $CaCO_3$ 結晶成長の阻害物質（フエノール化合物等）の有無等が密接に関連していることを意味する。

最後に，稿を終わるにあたり，英文要旨の御校閲を賜わった南カロライナ大学電子顕微鏡センターの渡部哲光教授に厚くお礼申し上げる。

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藻類 Jap. J. Phycol. 32: 373-378, December 10, 1984

スサビノリ殻胞子とその発芽体における核分裂の観察

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Porphyra yezoensis UEDA used as a material in this study was isolated and preserved as free-living conchocelis filaments in the Laboratory of Algal Cultivation of Tokyo University of Fisheries. The conchosporangial cells, released conchospores, conchospore germlings and monospores were fixed in Carnoy's fluid and stained with Wittmann's method. The conchosporangial cells have a single nucleus and 6 chromosomes; the conchospores have also a single nucleus which are somewhat different from the ordinary resting nucleus in appearance. In the conchospore germlings, the diplonema stage, diakinesis stage and metaphase in meiosis were observed. The conchospore germlings showed three ring-shaped chromosomes in the metaphase. Two nucleus were observed in the two-cell germlings. The monospore germlings have three chromosomes. It has been considered that the meiotic division in this species possibly takes place in the course of development from conchosporangial cell to conchospore germlings.

Key Index Words: *Bangiales*; *Meiotic division*; *Nuclear division*; Porphyra yezoensis; *Rhodophyta*.

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ISHIKAWA (1921), TSENG and CHANG (1955) はアサクサノリ *Porphyra tenera* KJELLMAN について減数分裂は造果器内で起こり，従って果胞子は単相であると報告した。これに対し，*Giraud et Magne* (1963) は *P. umbilicalis* (L.) J. AG. var. *laciniata* (LIGHTF) THURET. の殻胞子嚢細胞において，減数分裂の特徴であるディアキネシス期，チゴネマ期，ティプロネマ期を観察し，殻胞子嚢の頂端又は中間細胞で減数分裂は起こることを報告した。MIGITA (1967) および鬼頭 (1978) はスサビノリ *P. yezoensis* UEDA について，葉状体細胞はnで，糸状体細胞は2nである，更に殻胞子嚢および殻胞子発芽体の細胞分裂の観察結果から，減数分裂は殻胞子嚢形成時に行なわれるとし，また単胞子発芽体も単相であることを報告した。

また右田 (1974) は殻胞子嚢内の細胞質が未分裂のまま放出されることもあるのでそれらの細胞は減数分裂の機会を失ない，放出後発芽時に染色体数が減数されることも全く否定はできないと述べている。

そこで筆者らは上述の右田 (1974) の推察を確めるために，スサビノリの殻胞子嚢と殻胞子とその発芽体および単胞子発芽体の核分裂について観察した。その結果スサビノリでは減数分裂は殻胞子の発芽時に起こることを示唆する観察結果を得たので以下にその結果を述べる。

材料と方法

本研究では東京水産大学藻類増殖学教室に分離保存されているスサビノリ *Porphyra yezoensis* UEDA の無基質糸状体を材料として用いた。

糸状体はあらかじめ温度 20°C，照度 2000 lux, 10時間明期と14時間暗期の条件下で培養し，その後，15°C, 6000-7000 lux, 10時間明期，14時間暗期の条件下に移し，通気培養を行って殻胞子を放出させた。殻胞子はその容器中の培養液をナイロン布地で濾過した後，遠心分離（1500 G，4000 rpm，10 min）して採集した。この殻胞子は殻胞子とその発芽体の核分裂を観察するために，スライドグラスに滴下し，乾燥しないようにシャーレ中に収容静置して固着させて，上述の短日低温条件下で静置培養した，殻胞子の静置培養開始から，

その発芽体が2細胞に達するまで固定を続けた，特に発芽体が1細胞から2細胞期となるまでは30分間おきにその発芽体を固定した。その殻胞子嚢及び殻胞子の一部は懸濁状態でも固定した。

殻胞子のさらに他の一部は同様の短日低温条件下で幼芽期から幼葉期にいたるまで培養を続けた，葉長約7-10 cmの幼葉は多量の単胞子を放出した，この単胞子も殻胞子の採集の場合と同様に遠心分離して採集し発芽させて1細胞期で固定した。

上述の材料の固定はカルノア液（アルコール3：酢酸1）で行ない，WITTMANN (1961) の酢酸鉄ヘマトキシリン抱水クロラール液で染色し，それをスライドグラス上で押しつぶしてから，光学顕微鏡により核および染色体の観察をした。培養液にはPES (PROVASOLI 1968) を用いた。PES調製用の海水は黒潮流域で採水し数ヶ月保存した海水をもちい，グラスファイバーフィルター (Whatman G, F/C) で濾過し，塩分を33‰に調整した後，加熱加圧滅菌 (120°C, 1気圧, 20分) して用いた。

結果と論議

スサビノリにおける殻胞子嚢，殻胞子および単胞子とそれらの発芽体についての細胞学的研究結果はMIGITA (1967)，鬼頭 (1974 1978) によって，すでに報告されている。筆者等の核と染色体の数および形状についての観察結果はMIGITA (1967)，鬼頭 (1978) の研究結果によく一致していた。すなわち殻胞子嚢の細胞には核が1個だけ観察され，また，染色体は6本数えられた (Figs. 1a, b, 4A, B)，糸状体から放出された殻胞子の大部分 (99.9%) は単核であることが確認された (Figs. 1c, 4C)，殻胞子内の核では多数の染色顆粒が集まった状態を示し，通常の休止期の核と異なっていた。

放出された殻胞子は基物に付着後すぐその外部に細胞壁を形成し，発芽体となる。その時期には染色性顆粒の変化はあまり著しくなかった。分裂前期の前半では，染色顆粒の集まった状態を示す場合が多い，殻胞子では放出されて約2日後，発芽体が形成されてから，核内の小さな染色性顆粒は漸時大きさを増し，やがて染色糸になる。この分裂前期では細糸期，合糸期，太糸期の3期を区別することはできなかった (Figs. 1d-f, 4D-F)。

染色糸は次第に太くなり，やがて染色体が形成され，分裂前期の後半の染色体が認められた，対をなした3組のディプロネマ期（複糸期）が観察された (Figs. 1g-i, 4G-I)，さらにこれらの染色体は凝縮し，染色体間の距離は増して，V型あるいはX型等を示すディアキネシス期（移動期）を示した (Figs. 2j-m, 4J-M)，次いで，染色体は第一分裂中期に移行し，さらに短縮し，3本の太い環状の染色体像を呈した。この環状の染色体の大きさは不均一であり，径約0.5～1.5 μmである (Figs. 2n,o; 4N, O)。

分裂後期には染色体がそれぞれ3本ずつに分かれている。それは二つの染色体群として徐々に分離し始め (Figs. 2p, 4P)，その時に染色体群の間には紡錘糸が明らかに認められる (Figs. 2q,r; 4Q, R)，互いに細胞の相反する極に向かって移動する，その中に太い環状の3本の染色体群が二つ分離する像もみられる (Figs. 3s,t; 4S, T)，その後更に細胞中央部にて，細胞は2分される，2細胞期の発芽体には1個単相性間期核が観察された (Figs. 3u, 4U)。減数分裂の結果必ず4細胞が形成されないので，MIGITA (1967) は減数分裂の第2分裂が省略される場合があるとし，鬼頭 (1974) は減数分裂は必ずしも細胞がこのように数個に分割する部位でのみ行なわれるとはいえないと述べている。同様に今回の観察で1細胞を起源として4個の核が同時に観察された例は認められなかった。

同様の方法で単胞子の1細胞期の発芽体をしらべた，単胞子の1細胞期の発芽体の染色体は棒状を呈し，その数は3本であった (Figs. 3v, 4V)，殻胞子の1細胞期の発芽体にみられる環状の染色体とは著しく異なっていた。

MIGITA (1967)，鬼頭 (1974 1978) はスサビノリにおいて，殻胞子嚢内で殻胞子嚢成熟過程において減数分裂がみられた。しかし殻胞子の発芽体で減数分裂の特徴としてのディプロネマ期とディアキネシス期などは観察していない。殻胞子の1細胞期の発芽体の染色体と単胞子の1細胞期の発芽体の染色体とは著しく異なっていた。

以上のことから，筆者らは今回の観察では殻胞子嚢内で減数分裂像は観察しなかったが，放出された殻胞子の核はいずれも染色糸の集合体として認められたこと，この形状には例外はみられなかったことなどは殻胞子嚢の内容が減数分裂を経ることなく，複相のまま放出されて，殻胞子になることを示唆していると考えられる。成熟した殻胞子嚢から放出された殻胞子は複相であり，減数分裂は殻胞子の発芽時に行なわれることによって，葉状体が形成されるものと考えざるを得ない。右田 (1974) はまれには殻胞子嚢内での減数分

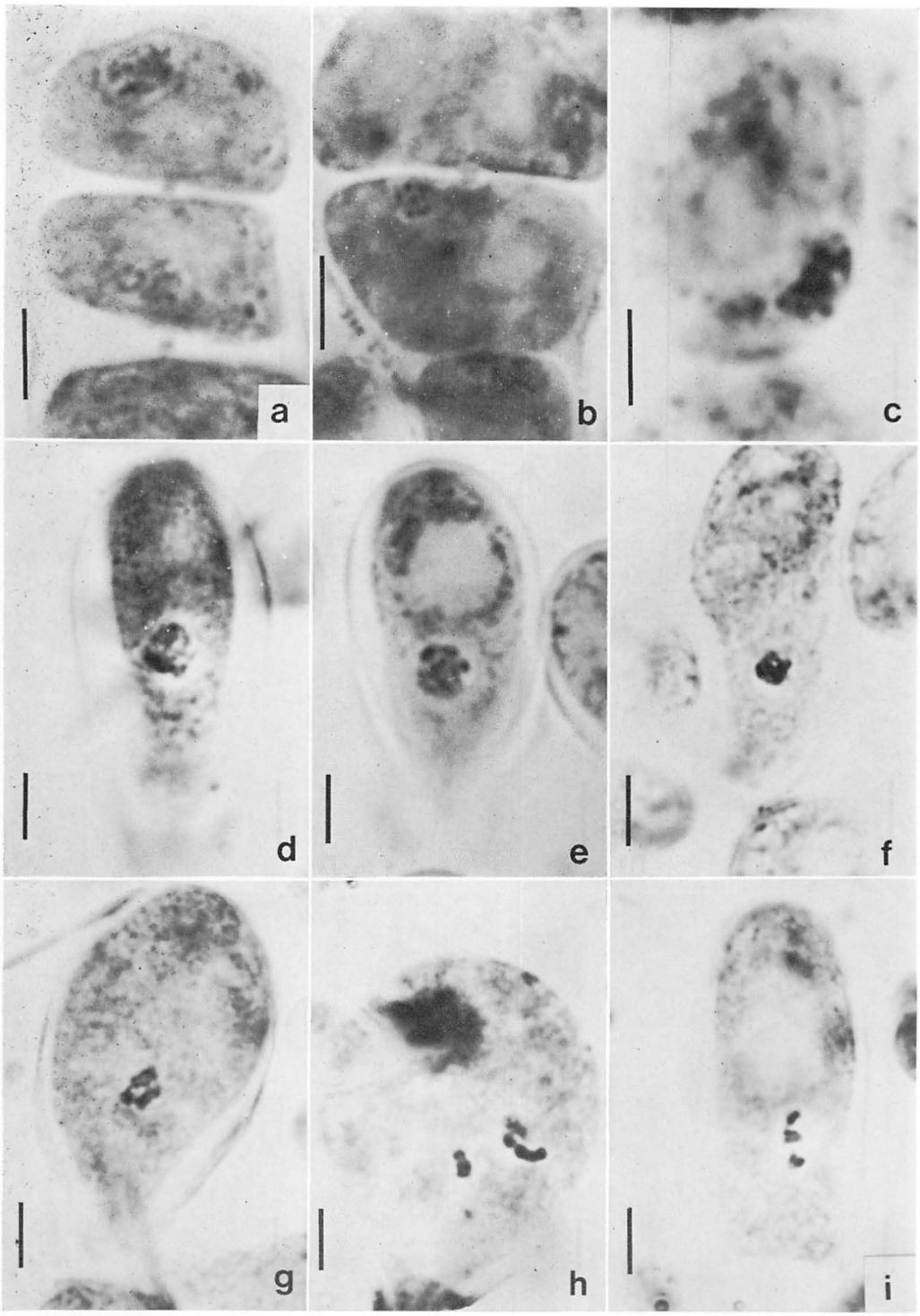

Fig. 1. Photomicrographs of meiotic division in germination of conchospores in *Porphyra yezoensis*. See the explanatory diagram, Fig. 4A-I. Figs. a, b. Metaphase showing six chromosomes in conchosporangial cells; c. A conchospore showing a resting nucleus; d-f. Prophase showing chromatin threads; g-i. Diplonema stage. Scale (a-i) = 5 μm.

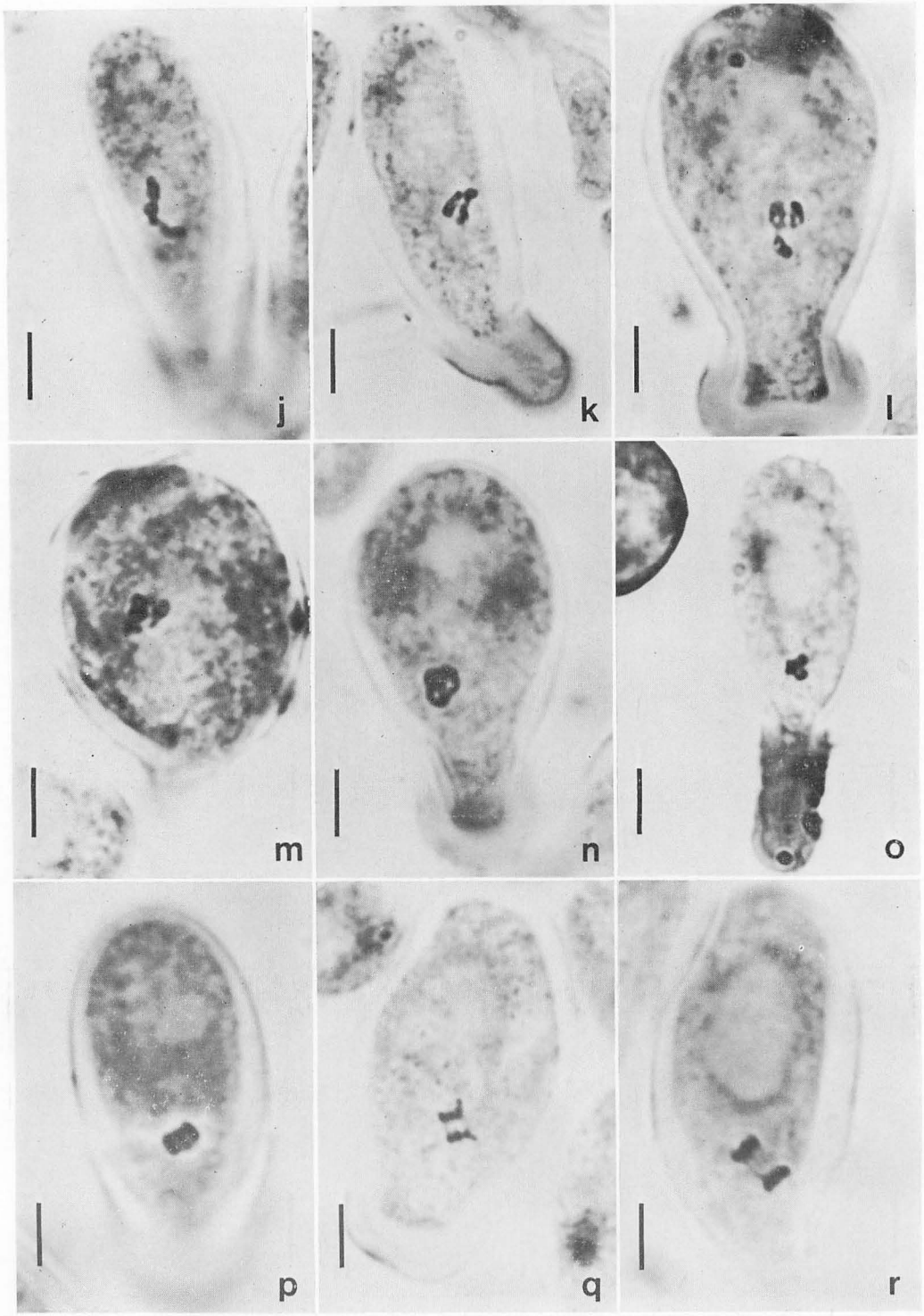

Fig. 2. Photomicrographs of meiotic division in germination of conchospores in *Porphyra yezoensis*. See the explanatory diagram, Fig. 4J-R. Figs j-m. Diakinesis stage; n, o. Metaphase; p-r. Anaphase. Scale (j-r) =5 μm.

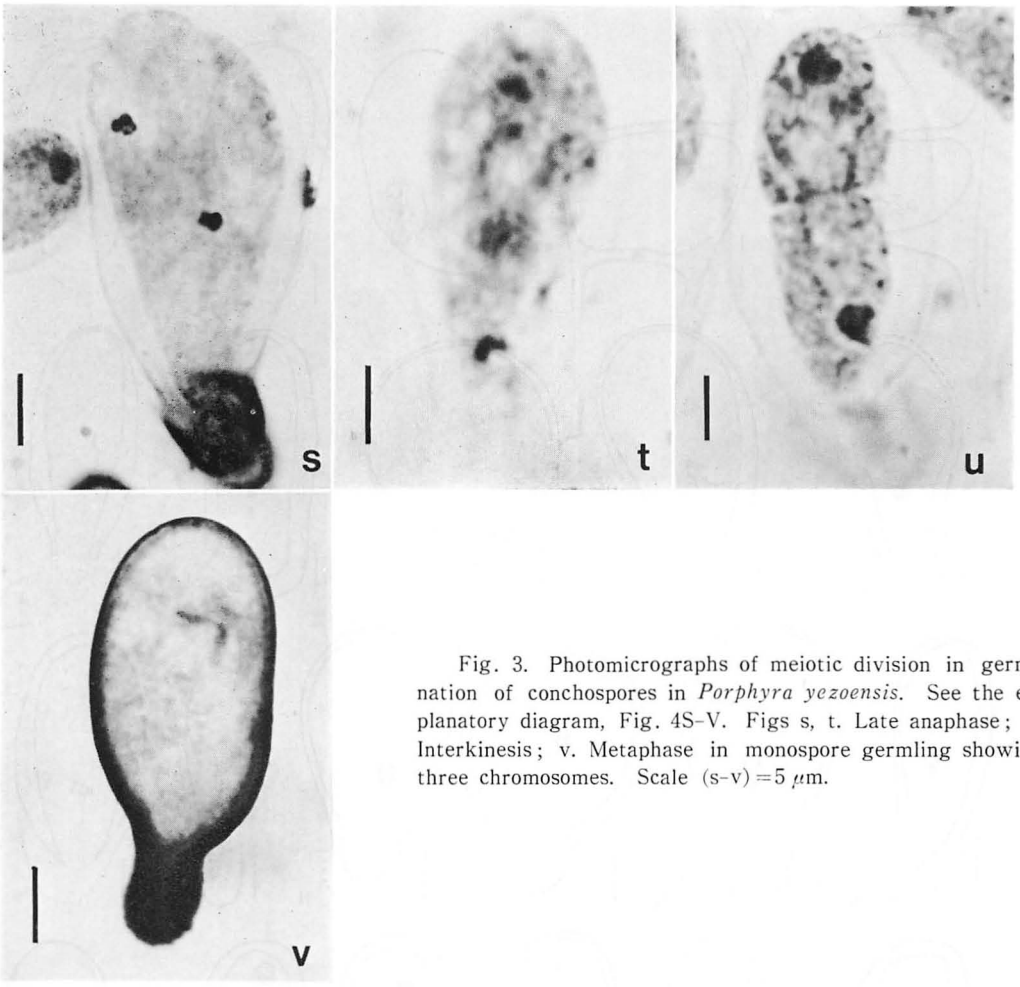

Fig. 3. Photomicrographs of meiotic division in germination of conchospores in *Porphyra yezoensis*. See the explanatory diagram, Fig. 4S-V. Figs s, t. Late anaphase; u. Interkinesis; v. Metaphase in monospore germling showing three chromosomes. Scale (s-v) = 5 μm.

裂の機会を失し未分裂のまま放出されることもあり得るので，それらの胞子は発芽時に染色体数が減数されることも否定はできないと述べている，本観察では葉状体発芽期に複糸期，移動期，中期における環状の染色体などの染色体像が観察された。従ってスサビノリでは殻胞子の発芽時に減数分裂が起こり，葉状体はこの減数分裂の過程を通じて形成されるものと考えられる。

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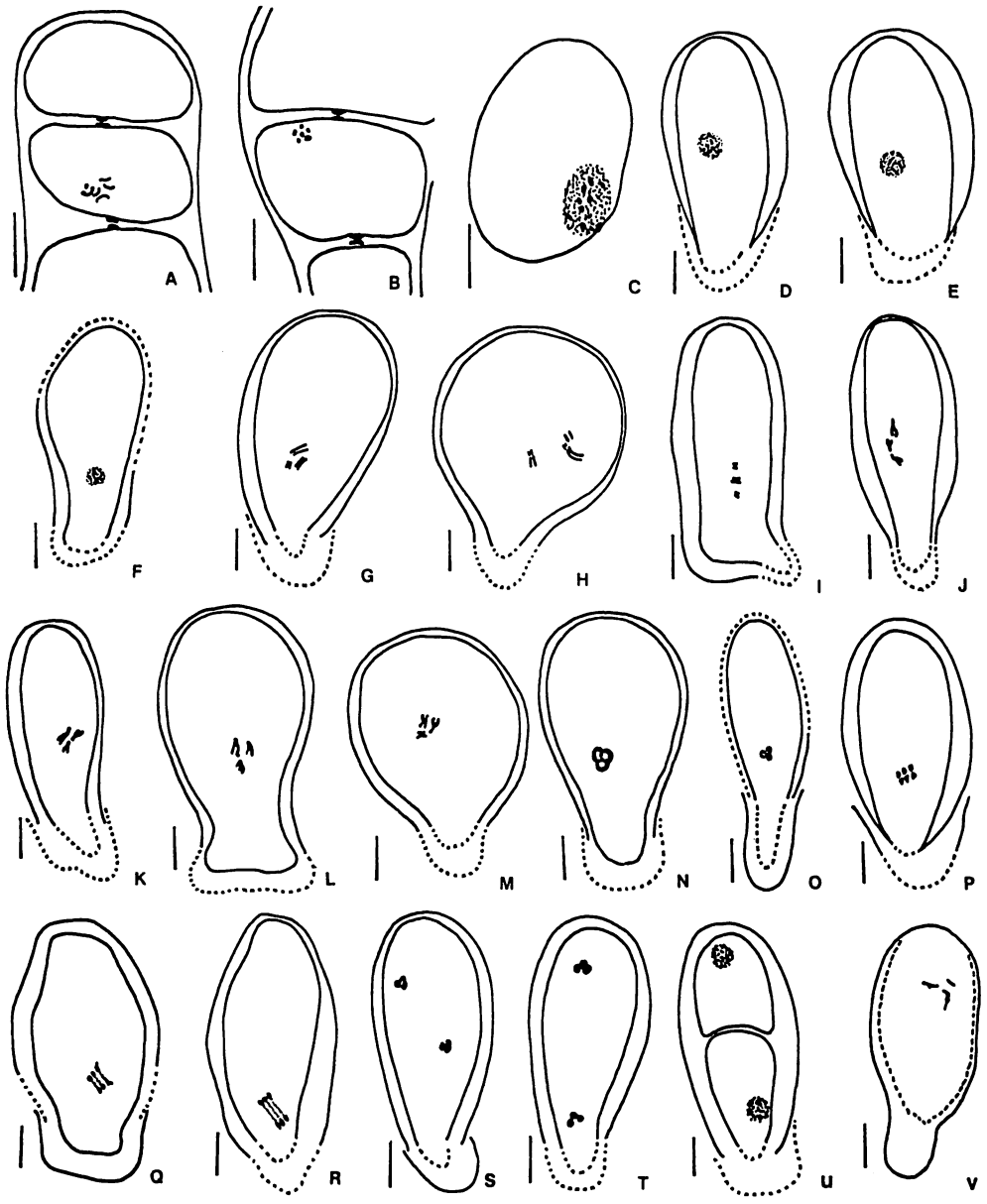

Fig. 4. The explanatory diagram of Figs. 1, 2 and 3. A, B. Metaphase showing six chromosomes in conchosporangial cells; C. A conchospore showing a resting nucleus; D-F. Prophase showing chromatin threads; G-I. Diplonema stage; J-M. Diakinesis stage; N, O. Metaphase. P-R. Anaphase; S-T. Late anaphase; U. Interkinesis; V. Metaphase in monospore germling showing three chromosomes. Scale (A-V) = 5 μm.

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Review

Kombu cultivation in Japan for human foodstuff

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With the advance of seaweed culture technology, modern Kombu (*Laminaria*) cultivation has been expanding so rapidly compared to the traditional Kombu fishing of Japan. However, most production of cultivated Kombu is limited to the four prefectures, Hokkaido, Aomori, Iwate and Miyagi, of northern Japan. In Hokkaido, the largest Kombu production area, three representative cultivation methods are adopted, e. g. Two Year Cultivation, Cultivation by Transplanting and Forced Cultivation, using high quality species as *Laminaria japonica, L. ochotensis, L. diabolica* and *L. angustata*. The two year cultivation requires more than twenty months to produce good quality for consumption, whereas in the forced cultivation Kombu of almost the same quality is produced within eleven months by severely controlled seedling production on land and subsequent regular cultivation in the sea as based on scientific surveys and long experience. The transplanting method is widespread among fishermen, often using jointly the other cultivation methods. The annual production of cultivated Kombu in Japan jumped from 284 tons wet weight in 1970 to 44,220 tons in 1981 and this accounts for about 28% of the total Kombu production.

Key Index Words: *Cultivation districts*; *cultivation methods*; *growth*; *Kombu cultivation*; Laminaria; *productivity*.

Kombu is the common Japanese name for the edible species of *Laminaria* and some closely related genera such as *Kjellmaniella*, *Cymathaere* and *Arthrothamnus*, which all belong to the Laminariales.

Kombu has long been a part of the Japanese diet and the Japanese people have a special liking for the seaweed. According to some ancient documents, Kombu was gathered in the beginning of the eight century by the Ainu, a native people who lived on Yezo Island, the old name for Hokkaido, and in other northern districts of Japan. As the northern areas became more developed, the output of Kombu increased gradually through the efforts of many Japanese colonists. At present, Kombu harvested from natural reefs consists of fourteen species, all of which are found on the coasts of Hokkaido. Only two species, *Laminaria japonica* and *L. religiosa*, grow around the prefectures of Aomori, Iwate and Miyagi in the northern part of Honshu, the main island of Japan.

In the beginning, because the output of Kombu was small, the product was considered a delicacy and was eaten only by the privileged classes in Kyoto, the old capital of Japan. Its use expanded gradually to the *Samurai* cast and then to the general public. Thus, the custom of using Kombu spread over the entire country and has continued over a great period of time.

From early times, the Kombu plants gathered from Yezo Island were transported to Kyoto and other cities in western Japan by ships sailing the Sea of Japan along the so-

called "Kombu Road". By the middle of the seventeenth century, this sea route had extended to Osaka by way of the Inland Sea. Since that time, Osaka has become the major market for Kombu in Japan. The traditional food processing industry for Kombu also developed in Osaka and in neighbouring cities of the western district. The Kombu road of the Sea of Japan no longer exists, having given way to transport by land. Osaka, however, remains the major Kombu market and processing area.

For more than two hundred years, fishermen have attempted to increase Kombu resources by means of various propagation techniques as planting stones or concrete blocks, blasting reefs and weeding out of competitors on the natural Kombu grounds (HASEGAWA 1971a 1975, KAWASHIMA 1972). In spite of these efforts, however, recent annual yields of natural Kombu in Japan have stayed at approximately 150,000 tons wet weight. The production of some species of good quality, such as *Laminaria japonica*, *L. ochotensis*, *L. diabolica* and *L. angustata*, is failing to meet consumer demand; because production is very unstable and is in fact gradually decreasing overall, the market price fluctuates greatly.

To increase the output of good quality Kombu, an artificial cultivation technique was first tested from 1955 to 1956 in Hokkaido (KAWAI and HASHIBA 1958). Four years after this, the first full-scale experiment of Kombu cultivation was started by research workers and fishermen in Hokkaido. At the beginning of Kombu cultivation in Hokkaido, the cultivation technique for Wakame (*Undaria*) was adopted. This technique had already spread widely among fishermen. However, the anticipated results were not obtained because, whereas Wakame is an annual plant, Kombu usually requires two winters to attain commercial size and quality.

Through study, the technique has been improved gradually and now Kombu cultivation is widespread among fishermen not only in Hokkaido but also in Aomori, Iwate and Miyagi Prefectures. Furthermore, there have been a few recent attempts to cultivate Kombu outside of its natural area of distribution in Tokyo Bay, the Inland Sea and Ariake Bay. Through these efforts, in recent years, the production of cultivated Kombu in Japan reached more than 40,000 tons wet weight which corresponds to about 28% of the total production of Kombu in Japan.

As representative macroalgae of the Phaeophyceae in Japan, the study of the Laminariales as pure botanical research would be interesting. However, practical research on how to protect this resource and to increase production of the commercially valuable Kombu is thought to be more important. The following description deals in general with the current artificial cultivation of Kombu mainly in Hokkaido, the chief producing district of Japan.

Cultivation Districts and Species

Although Kombu grows extensively along the coasts of northern Japan, the distribution range of each species is divided into fairly well defined areas as shown in Fig. 1. On the other hand, the cultivation of Kombu is carried out only in those areas where Kombu of the highest quality and price can be grown so that the greatest profit can be made. For this reason, both cultivation districts and species are severely limited at present. The following is a list of those districts in which Kombu cultivation occurs on a commercial scale and of the main species which are utilized.

I. Hokkaido
   1. The southwestern district (Oshima Province)
      Laminaria japonica (Ma-Kombu)
      Laminaria angustata (Mitsuishi-Kombu)
   2. The northern district (Soya Province)
      Laminaria ochotensis (Rishiri-Kombu)
   3. The eastern district (Nemuro and Kushiro Provinces)
      Laminaria diabolica (Oni-Kombu)

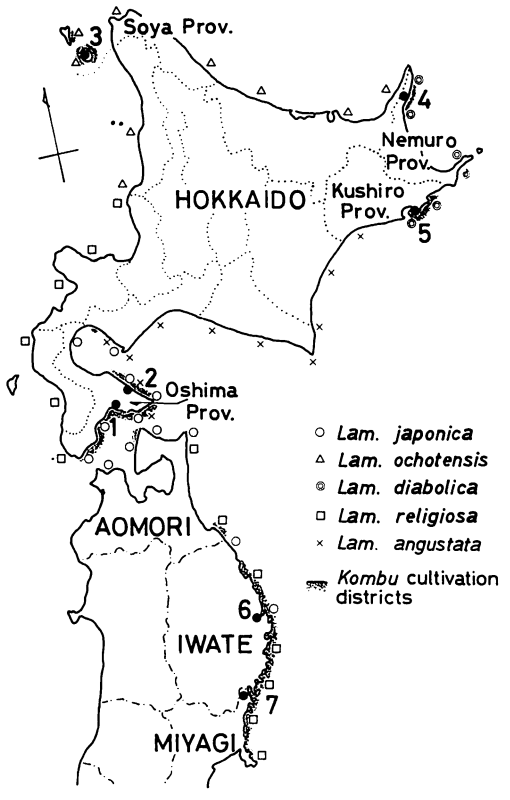

Fig. 1. Distribution of five *Laminaria* species and their cultivation districts dotted along the coast of northern Japan. The numbers 1-7 correspond to those of the locations in Fig. 2.

II. Honshu, in the prefectures of Aomori, Iwate and Miyagi
Laminaria japonica
Laminaria religiosa (Hosome-Kombu)

Of these cultivation districts, Oshima Province in southwestern Hokkaido is Japan's leading Kombu cultivation center where 70% of the cultivated Kombu is produced. Originally, this province was recognized as the most famous natural Kombu producing district and *Laminaria japonica*, the main species gathered there, is the best in terms of quality and price. However, the annual yield of this Kombu is not as great as that of other lower ranked species such as *Laminaria longissima* which grows on the coasts of Kushiro and Nemuro Provinces. *Laminaria japonica* production is less than 15% of the total production of Kombu in Hokkaido. In order to increase this percentage, farmers and reserchers have devoted a great deal of time to the development and improvment of Kombu cultivation.

Water Temperature of the Cultivation Grounds

Figure 2 shows the seasonal change of water temperature at seven representative locations where Kombu cultivation is currently carried out. As shown in this figure, the water temperature differs about 5°C between the northern and the southern locations, and this difference in water temperature shows the specific temperature range for the species of Kombu cultured in each location. The water temperature range which is suitable for the growth of all cultivated Kombu species seems to be between 5 and 20°C. In the northern locations such as Rausu and Akkeshi where ice floes occur in winter, the temperature range is lower for such hardy species as *Laminaria diabolica*.

The Fundamental Procedure for Kombu Cultivation

Kombu has the *Laminaria* type of life history which consists of an alternation in life forms between the microscopic gametophyte (n) and macroscopic sporophyte (2n). The success of Kombu cultivation depends on the scientific control of the growth and maturation of the plant throughout its entire life cycle. Kombu cultivation is divided into two steps: artificial seedling production in special facility on land and farming on cultivation apparatus in the sea.

(1) *Seedling production*

Seedling production consists of the control of spore release and the subsequent culture of zoospore germlings on an artificial substratum. This work is carried out in a facility provided with seeding and culture room which has equipments to control water temperature, light, nutrient and other conditions.

Synthetic strings of about 3 mm in diameter are used as the substratum for seeding. These strings consist of three strands of

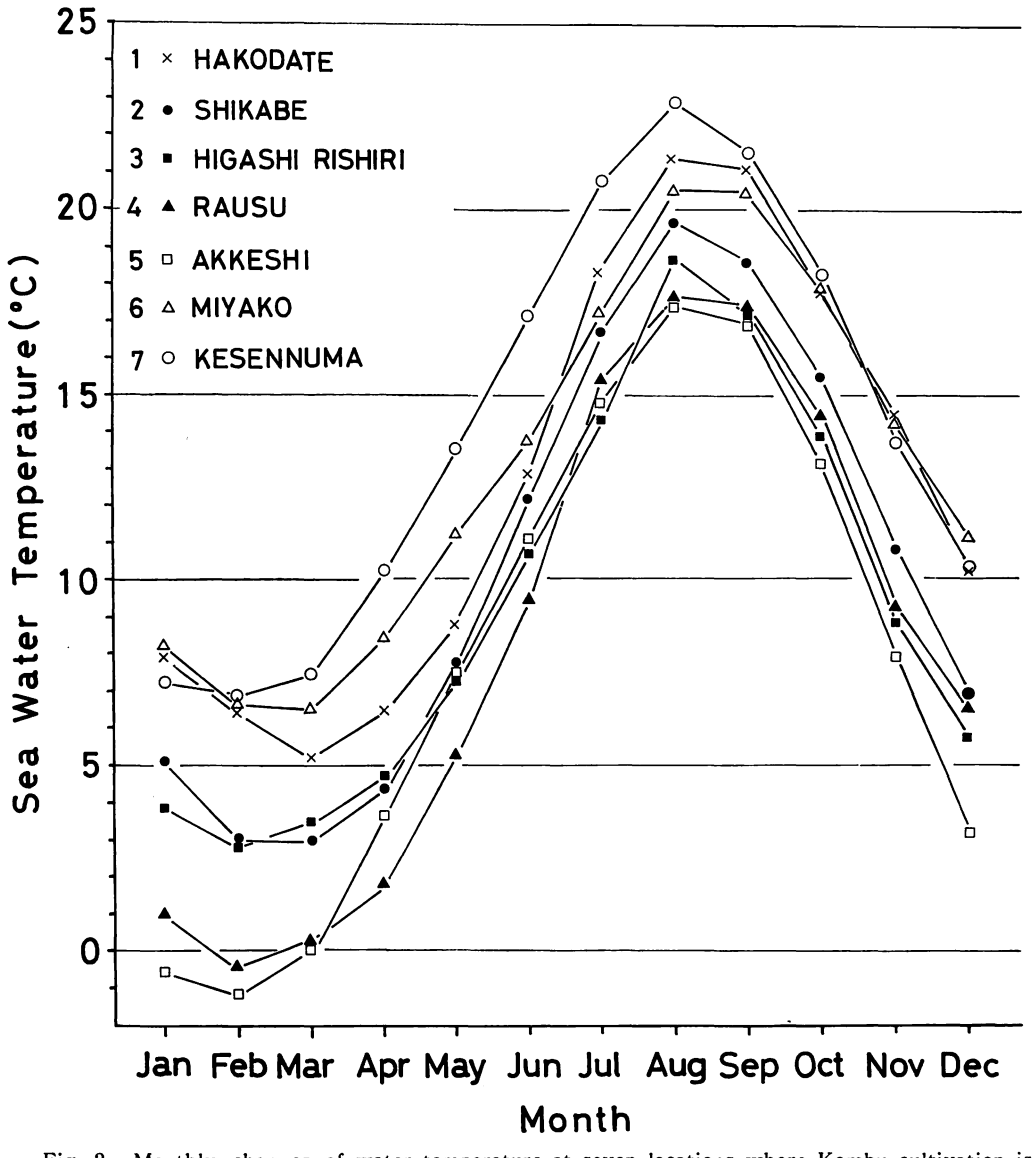

Fig. 2. Monthly changes of water temperature at seven locations where Kombu cultivation is carried out. The location numbers correspond to the numbers in Fig. 1.

different thickness which are twisted together and treated with an artificial resin to prevent unravelling. The strings are rinsed in running freshwater for several days, dried and then wound around a plastic frame to make a spore collector as shown in Fig. 3.

Before seeding occurs, a tank is filled with sterilized seawater which is maintained at about 15°C, and 1.023-1.025 in specific gravity. Well matured plants, usually collected from natural Kombu grounds the day before, are dried in a dark and cool place for about a half day. The plants are then placed into the tanks and within a half hour the zoospores are released in great numbers. This spore suspension is poured into the seeding tank filled with sterilized seawater (Fig. 4). At this point the concentration of the zoospores in the seeding tank is important in order to allow for adequate

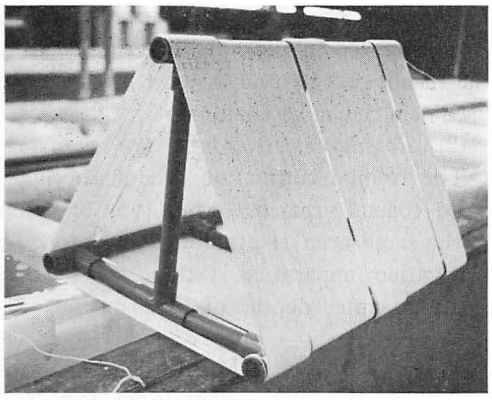

Fig. 3. Spore collector. Synthetic string used as substrate for the seeding is wound on a triangular frame made from plastic pipe. One collector contains about 300 m of string, of which 200 m is used in practice.

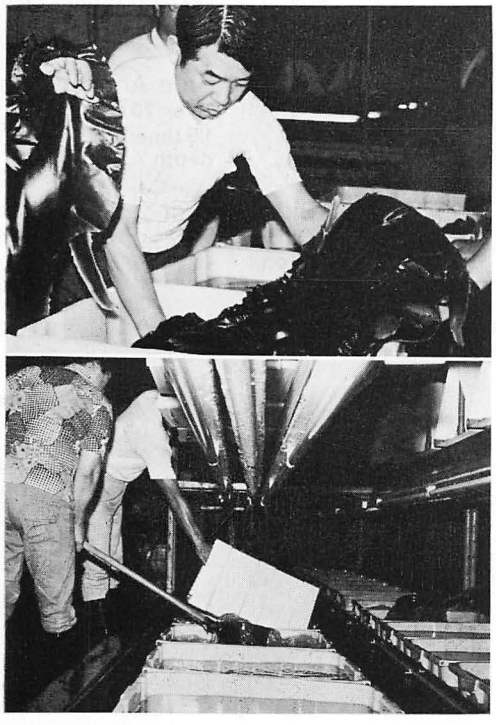

Fig. 4. Artificial seeding work on land.
The upper picture: mother blades with mature zoosporangial sorus are soaked in seawater, then, zoospores are released in great numbers in tank. The lower picture: zoospore suspension is poured into clean sea water in seeding tanks and immediately thereafter spore collector is submerged in the tank.

germling growth on the strings. This concentration should be about 5-10 zoospores in a field when one drop of the seawater is applied to a slideglass and viewed under the microscope at a magnification of 150. The spore collector is immersed into the tank immediately after adding the spores. The time required for the spores to settle on the strings varies with water temperature, but it is usually completed in about 24 hours when the water temperature is around 15°C.

After the seeding, the spores on the strings grow into gametophytes. These gametophytes in turn produce young sporophytes.

This culture of germlings is generally carried out from the end of August until the end of November. During this period the temperature of the seas around Hokkaido decreases from about 20 to 10°C. The temperature required for the gametophytes to produce young sporophytes is about 13-15°C. Therefore, in order to accelerate the seedling production, the culture water must be cooled to less than 15°C if the culture starts in summer or early autumn season. In the culture room, the culture tanks are arranged in large, shallow concrete pools ($8\times2\times0.5$ m) into which is pumped freshwater cooled at a temperature of 13-15°C by cooling device. But, in order to keep costs down, a simple cooling method, fresh spring water at a temperature of about 15°C, is used in some facilities.

White fluorescent lamps are used for the culture of germlings as the conditions vary too much according to the daily change in weather. Generally, the light intensity required is 3,000 to 6,000 lux with a light period between 12 to 16 hours.

Prior to 1970 the nutritional requirements for seedlings were poorly understood, only nitrogen and phosphorus being added to the culture seawater in accordance with the Schreiber's prescription. As a result of work by TATEWAKI (1966) PROVASOLI's ES solution with the addition of iodine was used, resulting in dramatic improvement in seedling growth. The seawater is changed every 10 or 15 days and, at the same time, nutri-

ents are added. During the culture period light aeration is applied.

When the conditions are right, the gametophytes will mature in about 7 days after the culture started and in about 3-8 days after that, embryonic sporophytes start to grow. In about 45 days, the thalli have grown up to 5 mm in length. However, depending on the culture conditions, this period can be longer. The time when seedling production occurs and the length of the culture period in tank are determined as to the needs of the cultivation method to be used (cf. the next chapter).

(2) *Regular cultivation in the sea*

The Kombu cultivation grounds occupy a fixed coastal area and other type of fishing in the same area is prohibited by law. The cultivation apparatus is then set in these areas at water depths of 15-25 m.

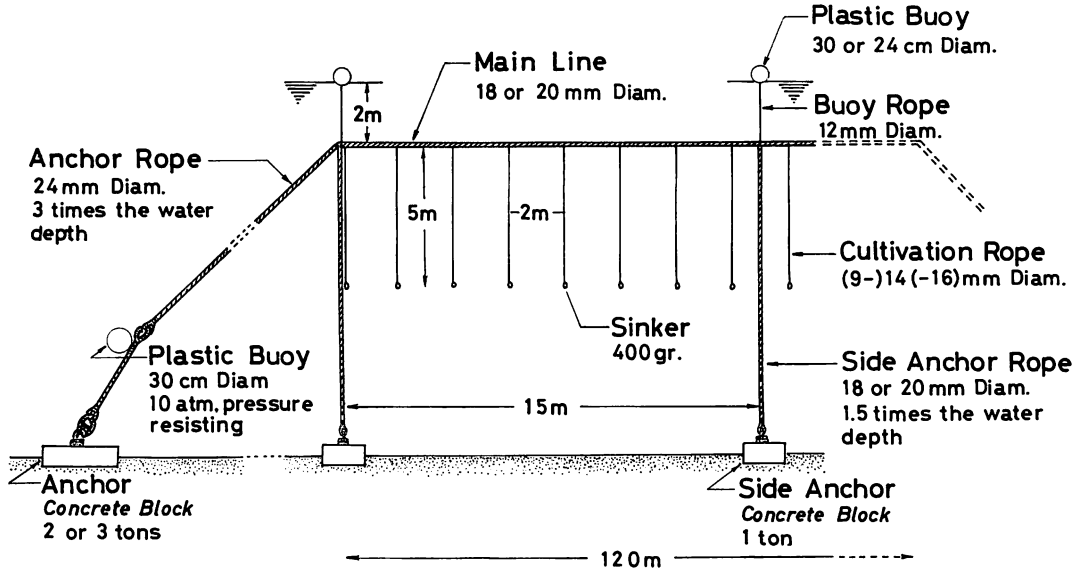

Fig. 5. Profile of a standard Kombu cultivation apparatus (*Noren Shiki*) used in southwestern Hokkaido.

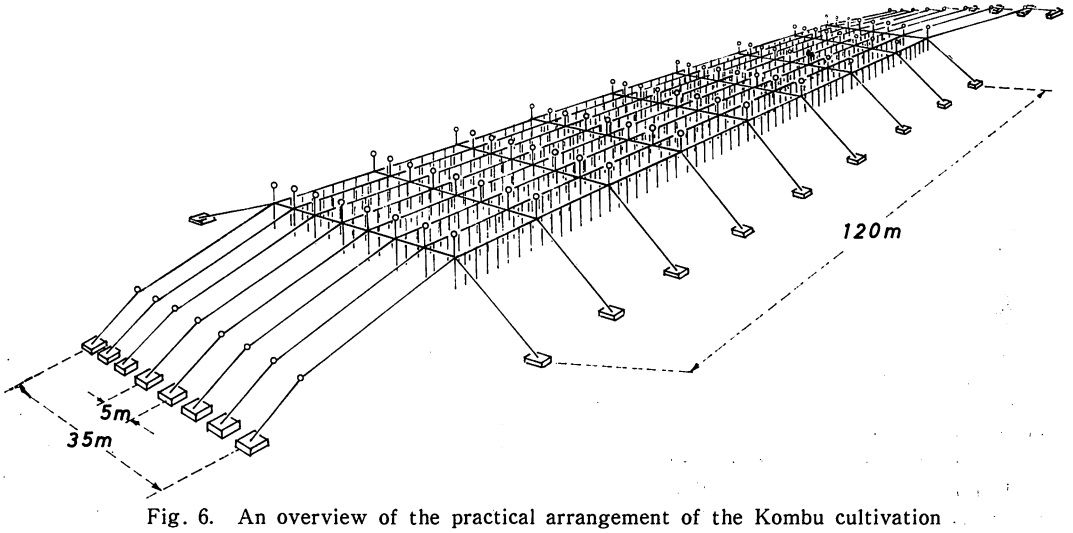

Fig. 6. An overview of the practical arrangement of the Kombu cultivation apparatus set in the cultivation ground.

Standard cultivation apparatus is a long line style of 120 m in length which is made of synthetic fiber ropes, synthetic buoys and concrete block anchors to make a large scale set. The sets are fixed firmly so as to prevent damage from large waves (Figs. 5 and 6).

The next step in the sequence of cultivation in the sea is provisional outplanting of the previously cultivated germlings (sporophyte). The seedling strings are first distributed to the fishermen, who then hang the strings in the sea under natural conditions so that healthy sporophytes will grow and adapt to these conditions (Fig.7-A). This provisional outplanting lasts 7-10 days and during this time weak sporophytes germlings will fall off naturally from the strings.

After that, the strings are cut in 5 cm lengths and are inserted into the cultivation ropes at 30 cm intervals for the regular cultivation (Figs. 5 and 7-B). Then, these ropes are hung vertically from the main line of the cultivation apparatus at 2 m intervals

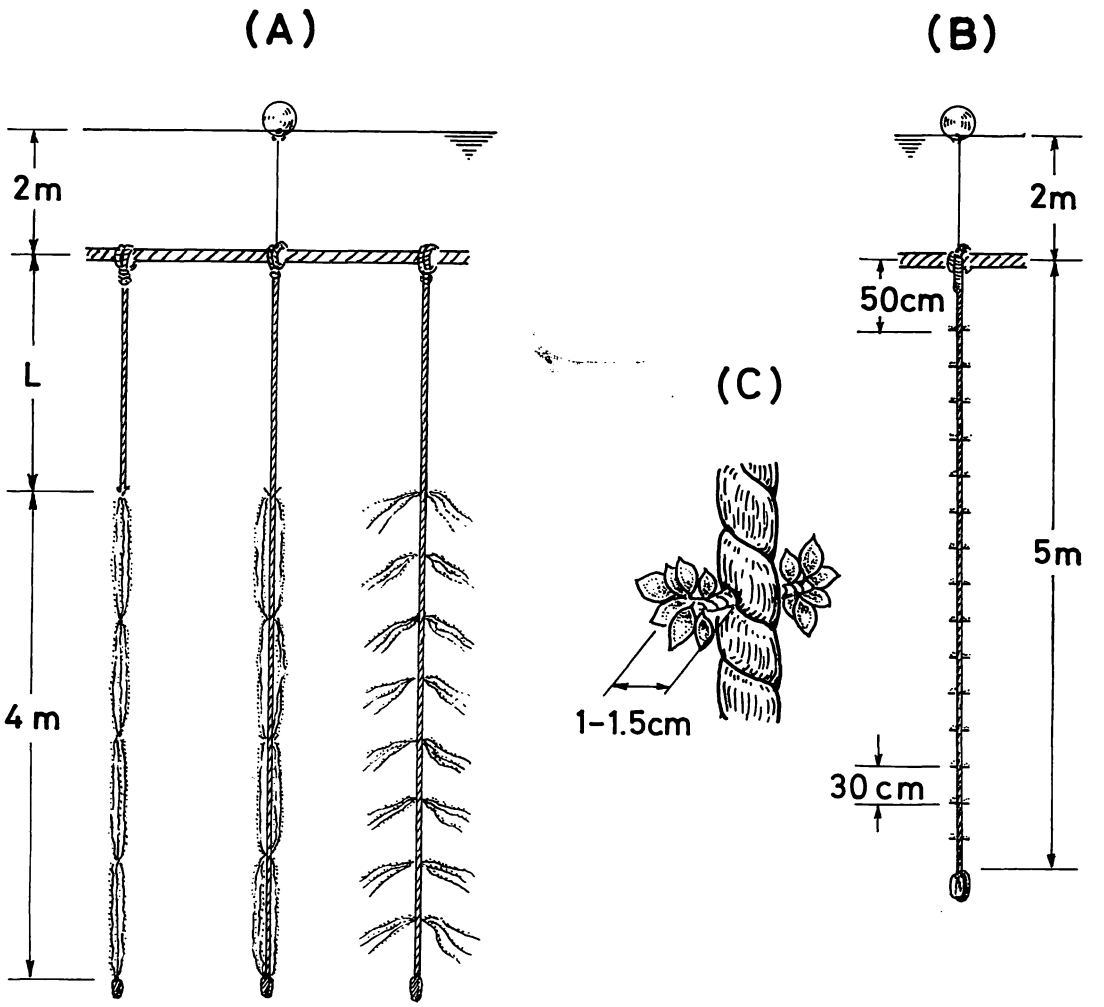

Fig. 7. Processes for the outplanting of seedlings. (A). Three examples of hanging seedling strings for provisional outplanting. L is 5-7 m at the beginning, but is gradually shortened to 1.5 m; (B). Fixation of seedling strings to cultivation rope by the insertion method for regular cultivation; (C). Detail of inserted seedling string.

(Figs. 5 and 7-B), or, alternatively, the ropes are stretched horizontally and fixed into the main line directly. The former is called *Noren Shiki* in Japanese (Vertically Hanging Method) and is used widely in the southwestern district of Hokkaido, and the latter, *Hae-nawa Shiki* (Longline Method) or *Suihei Shiki* (Horizontally Stretching Method), is used in the eastern and northern districts of Hokkaido and Honshu.

The depth at which the main lines are set varies according to such environmental conditions as transparency of water and wave action at each location. For example, in southwestern Hokkaido this depth is 2 m but in the northern and eastern districts it is about 5-7 m. However, in the eastern district where there are ice floes from January until April, the entire cultivation apparatus sinks below the ice close to the bottom which is more than 20 m in depth. This way the apparatus is protected from damage by large floes.

From spring to summer of the year of harvest, during the season when growth is good, the main lines are raised gradually until they are almost to the water surface so that the Kombu is well exposed to sunshine. In this way the quality of the plants improves due to an increase in photosynthetic production.

In addition to controlling the cultivation depth, it is very important to thin out excessive plants from the cultivation ropes to prevent overgrowth and to promote Kombu of good quality. Of the plants remaining on the ropes, those which are not firmly attached are bound lightly with soft synthetic tape to prevent them from being washed away. Daily maintenance of the cultivation apparatus including removal of all epiphytes is also important during the entire cultivation period.

Representative Methods of Kombu Cultivation

The three representative methods of Kombu cultivation which are actively used in Hokkaido are diagramed in Fig. 8.

(1) *Two Year Cultivation*

All the cultivated Kombu in Hokkaido are biennial plants. The two year cultivation is a very orthodox method to reproduce faithfully the life cycle of the natural biennial Kombu. It was the first cultivation method attemped in Hokkaido and is even now widely used as a basic cultivation method in the eastern and northern districts as well as in a part of southwestern district of Hokkaido.

In this method, seedling production starts from October. After that, plants are left to grow through two winter seasons and harvested in July and August after three calender years. Consequently, this method produces two year Kombu which is almost of the same quality as the natural plant. However, the biggest problem with this method is that it takes more than twenty months from seeding to harvest and it results in a comparatively high price of the products.

(2) *Cultivation by Transplanting*

Kombu is able to attach not only to rocks but also to ropes, wooden posts, piles and other substrata in the sea. This ability is most pronounced from winter to spring when the activity of the meristem increases and new holdfasts are formed. During this time the fishermen can successfully thin out the excess fronds completely with holdfast from the cultivation ropes and transplant by fastening them onto a new rope with thin, soft tape.

In about ten to fifteen days new haptera grow and fix the frond firmly to the new cultivation rope. Natural Kombu which has been washed up on land by storms is also used sometimes.

The advantage of this method is that it conserves manpower and materials. It is often combined with the other cultivation methods in order to increase production as shown in Fig. 8.

(3) *Forced Cultivation*

Because the two year cultivation method takes over twenty months to complete, there can be only one harvest every two years at one fishing ground. To try to solve this

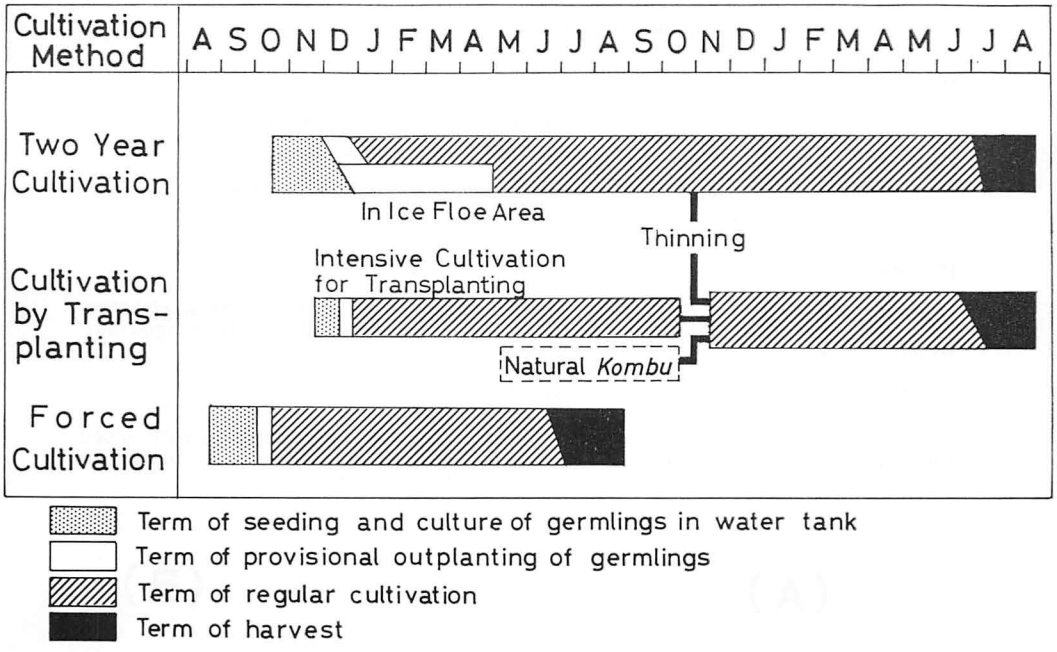

Fig. 8. Diagramatic flow chart of three representative Kombu cultivation methods in Hokkaido.

problem, HASEGAWA (1971b 1975) and his coworkers worked from 1966 to 1970 to create a method that would cut the production time in half, but yet would produce plants of the same quality as the two year Kombu at Minamikayabe, the most famous Ma-Kombu producing district in Oshima Province, Hokkaido. The epochmaking new cultivation technique that resulted from this research is called "Forced Cultivation". This method has since spread widely to southwestern Hokkaido and now the production by this method accounts for 92% of the total cultivated Kombu produced in this district.

There are four basic technological features concerning the forced cultivation method.

Firstly, seedling production is done as early as possible. Usually, in southwestern Hokkaido, the seeding is carried out from the end of August to the middle of September. This seeding period is about 30-45 days earlier than that of two year cultivation. The subsequent culture of zoospore germlings in tanks is continued for about 45 days under the conditions as shown in Table 1 (Fig. 9).

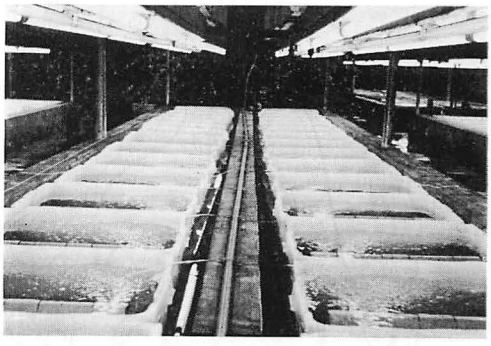

Fig. 9. The seedling production facility for forced cultivation. Seedlings are cultured in tanks under severely controlled conditions of water and light.

Secondly, regular cultivation in the sea begins immediately after the water temperature of fishing ground reaches 18°C or less. This is around the middle of October in southwestern Hokkaido.

Thirdly, the Kombu must be grown as quickly as possible when the water temperature is lowest. This is in winter up to the end of February when the water temperature is 3-5°C. Because the Kombu grows quickly and densely on the

Table 1. Culture conditions of seedlings in the forced cultivation of Ma-Kombu (*Laminaria japonica*)

Water temperature	13-15°C
Light intensity	For the first week: 2,000 lux For the second week: 4,000 lux Thereafter: 6,000 lux (With white fluorescent lighting)
Photoperiod	12(-16) hours light
Changing of culture water	All the medium is changed first two weeks after cultur bigins, thereafter the half is changed once a week.
Aeration	Aerate slowly
Nutrients	ESI medium (Provasoli's ES medium with added iodine of 1 mg per *l*) is added (2%) to each renewed culture water.

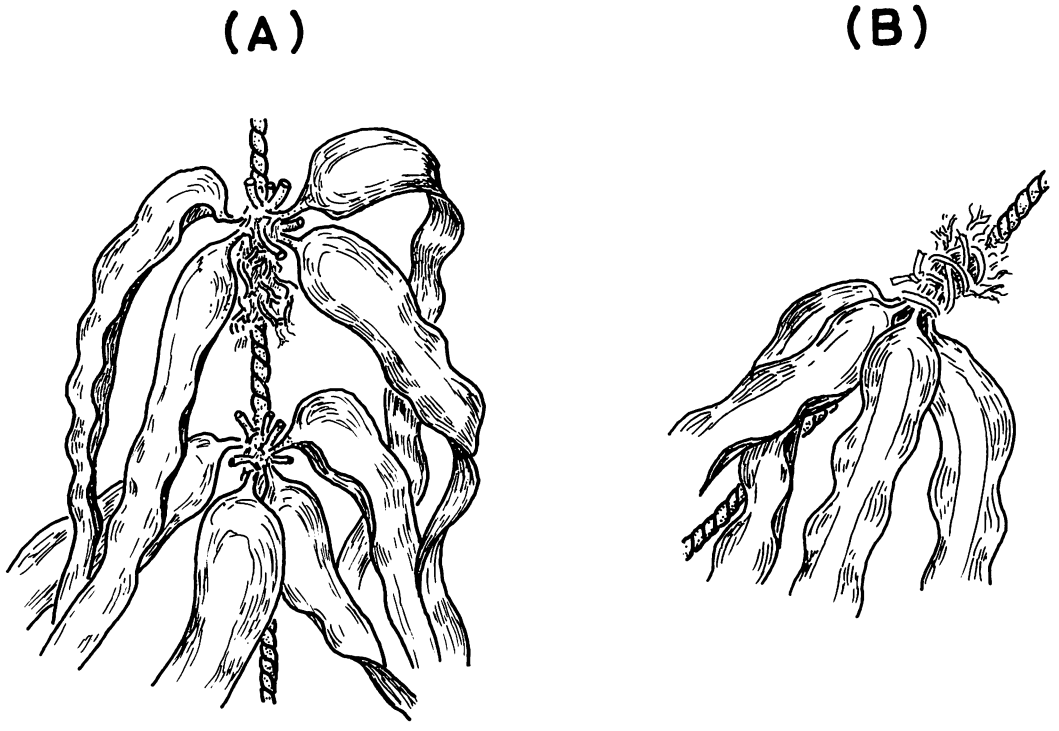

Fig. 10. Treatment to accelerate growth and prevent loss of plants.
(A). Thinning of excessive plants; (B). Binding of unstable holdfats with tape.

cultivation ropes, the fishermen must drastically thin them out during midwinter, from the end of December to the beginning of March. Finally 4-5 plants remain where each piece of seedling string was originally inserted in the cultivation rope (Fig. 10-A). This corresponds to 12-15 plants per meter of cultivation rope. At the same time, since

the sea is very rough during winter, it is important to secure weak haptera to the ropes with thin, soft synthetic tape in order to prevent the plants from being washed away (Fig. 10-B). At present, the forced cultivation method does not adapt well to the fishing grounds in northern and eastern Hokkaido because such exacting work as thinning and fastening the plants is very difficult to do under the much harsher winter conditions found in those areas.

Fourthly, it is to insured that the Kombu receives proper exposure to the light. Beginning from March the weight and length of the blade increase with increasing photosynthetic activity. To increase light exposure, the vertically hanging cultivation rope is raised gradually to within 2 meters of the water surface. At this point the rope is secured horizontally with its lower end attached to the neighbouring main line. This should be completed by the end of May and, as a result, all the plants should be exposed to uniform light conditions. In the last stage of regular cultivation, the horizontally secured ropes are raised further to within half a meter of the water surface (Fig. 11).

Up to the present, the theoretical basis of forced cultivation has not yet been solved satisfactorily from the physiological point of view; but a great deal of interest is taken in ecological phenomena by practical cultivation experiments. The force cultivated biennial plant grows very rapidly for four months around March when the water temperature is at its lowest. On those plants which grow especially rapidly, distinct zoosporangia begin to form near the upper end of the blade around February. After this, as the blade continues to grow, its apical portion withers and is washed away. Then, in late summer when the plant has attained a sufficient size and weight, the blade produces zoosporangia for the second time on the basal portion. This process of producing zoosporangia two different times is in common with typical biennial Kombu which produces zoosporangia once on the first year blade and then again on the second year one. Namely, it can be seen that the force cultivated Kombu com-

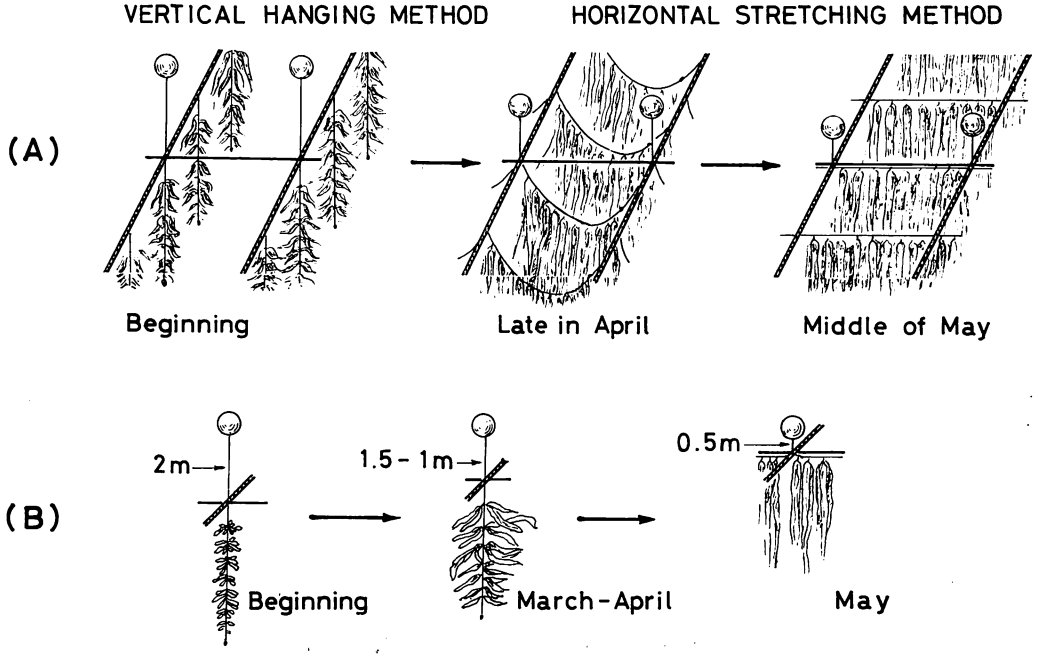

Fig. 11. Methods for growing high quality Kombu. (A). Alteration from vertical hanging method to horizontal stretching method; (B). Raising the cultivation ropes to increase light exposure.

pressed the life span of the natural biennial Kombu into only one year and is quite different from natural annual Kombu which produces zoosporangia only once in a lifetime (Fig. 12).

Growth and Quality of Force Cultivated Kombu

(1) *Growth*

An investigation into the forced cultivation techniques using *Laminaria japonica*, Ma-Kombu, was carried out by FUNANO and ISHIKAWA (1974) from November 1972 to August, 1973. Fig. 13 shows the seasonal changes of blade length, width and wet weight, as well as the substantiality value which is the ratio of blade weight to area. Fig. 14 shows the seasonal changes in apparent daily increase for the same parameters. There are four distinctive features which can be seen in these figures as follows:

(i) As determined from an average of forty-five plants taken at harvest time, the blade length is 500 cm, the blade width is 18 cm and the blade wet weight is 1,250 g.

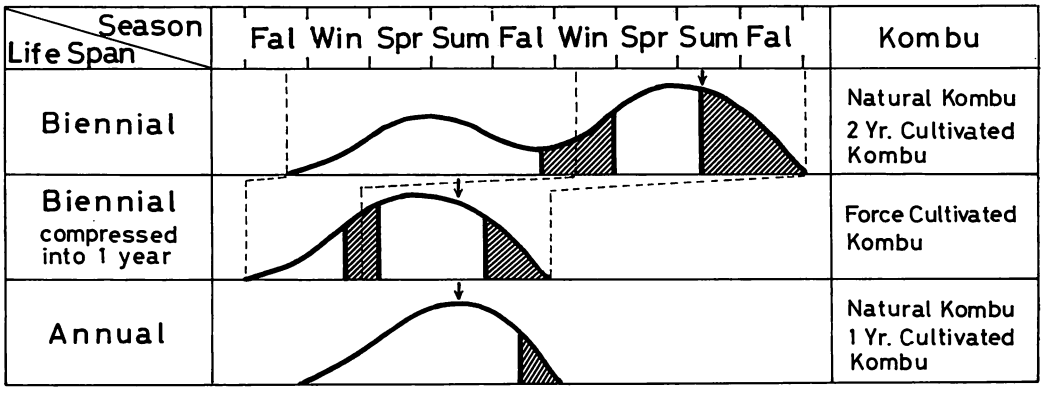

Fig. 12. Diagramatic representation of the life history of Kombu having three different life spans (sporophyte stage). The ordinate approximately indicates the relative rate of sporophyte length. The shaded portion denotes the reproductive season and the arrow the beginning of harvest season.

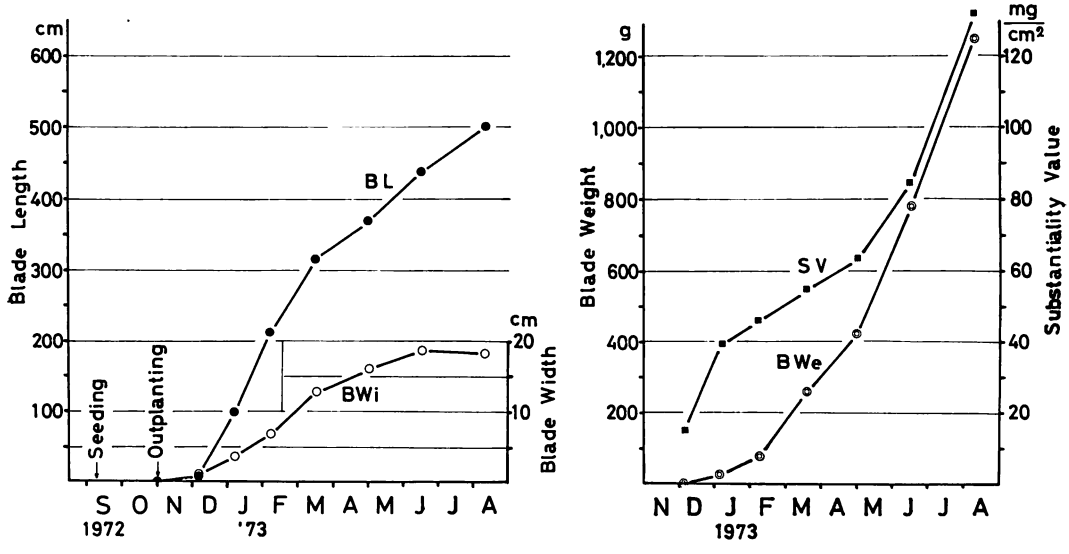

Fig. 13. Monthly changes in the average blade length, width, wet weight and substantiality value of *Laminaria japonica* during forced cultivation. Redrawn from FUNANO and ISHIKAWA (1974).

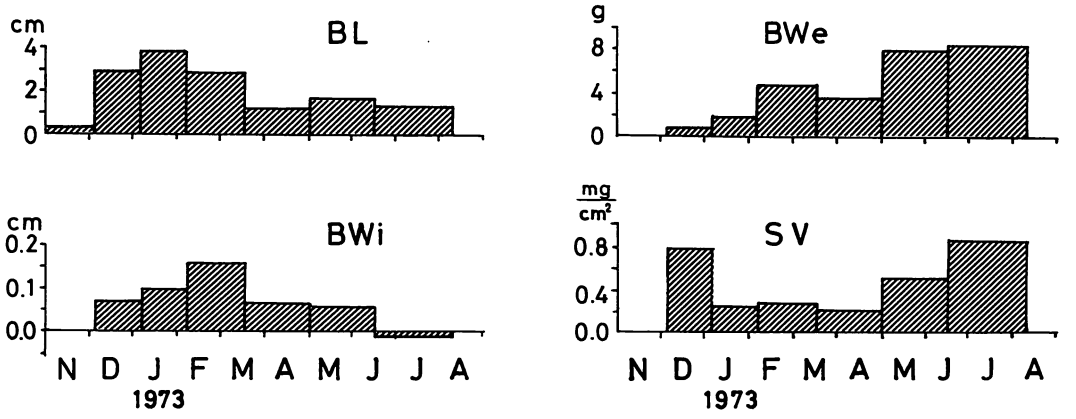

Fig. 14. Monthly changes in the daily apparent increase in blade length, width, wet weight and substantiality value of *Laminaria japonica* during forced cultivation. Redrawn from FUNANO and ISHIKAWA (1974).

(ii) The blade length increases rapidly during the winter season from December to March. After March, from spring to summer, growth slows down. The peak of apparent daily growth is from the beginning of January until the beginning of February and is about 3.6 cm per day.

(iii) The increase in blade width follows the increase in blade length. The peak of daily growth in blade width is from the beginning of February until the middle of March and is about 0.16 cm per day. However, from the middle of June, there is a slight decrease in blade width.

(iv) Whereas the increase in blade length begins to slow in March, the blade wet weight increases rapidly, producing Kombu of high quality. The apparent daily increase in wet weight peaks at 8 to 8.5 grams per day during this period.

(2) *Quality*

To determine instantly whether the Kombu is high enough in quality for consumption before harvesting, a method which utilizes blade length and width in centimeters, and blade wet weight in grams is used. Using these three factors in a formula of weight times 1,000 divided by length times width, gives the substantiality value in a unit of milligram per square centimeter as shown below (KAWASHIMA 1972):

$$\frac{\text{Weight (g)} \times 1{,}000}{\text{Length (cm)} \times \text{Width (cm)}} = \text{Substantiality Value (mg/sq·cm)}.$$

It is known from long experience that at the start of the harvest at about the end of July the substantiality value of natural Kombu is usually about 100-120. As the season progresses, the value further increases to 150 or more. As the graph in Fig. 13 shows, the value for force cultivated Kombu is 137 at harvest time which is of a sufficiently high quality (Fig. 15).

Studies on the chemical components of the force cultivated Ma-Kombu have been conducted by OHISHI and KUNISAKI (1970) and FUNAOKA *et al.* (1974). In force cultivated Kombu, glutamic and aspartic acids, proline and alanine changed most remarkably through the culture period. The content of glutamic acid increased considerably from April to July and proline and alanine increased in May and June, but then decreased in July. Consequently, in summer, the free amino acid composition of force cultivated Ma-Kombu becomes similar to that of natural two year Kombu (OHISHI and KUNISAKI 1970). On the other hand, the former plant contains more amino-nitrogen, total reducing sugar and crude fat than the latter (FUNAOKA *et al.* 1974).

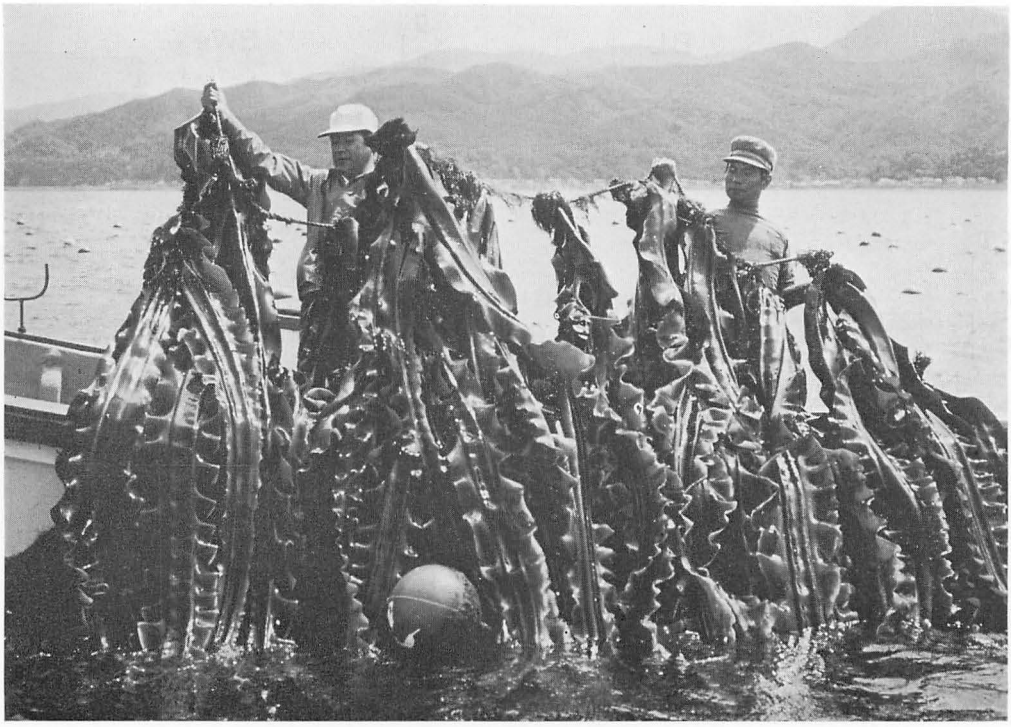

Fig. 15. The harvest of force cultivated Ma-Kombu (*Laminaria japonica*) at Minamikayabe, Hokkaido.

In practical terms, force cultivated Kombu tends to deteriorate in quality more than natural Kombu even if they are handled in the same way. According to HASEGAWA (1978), this deterioration is probably due to changes in the quantities of intercellular substances during forced growth. At present, however, the quality of the Kombu has been improved by improving cultivation and processing techniques.

Productivity of Cultivated Kombu

In Japan, average annual yield of natural Kombu in 1970–1981 has been about 133,000 tons wet weight although the yield varies greatly each year according to the change in natural conditions (Fig. 16). In Hokkado, it has reached about 119,700 tons during the same period and account for about 90% of all the natural production in Japan.

On the other hand, full scale Kombu cultivation has been carried out since 1970 and the productivity has increased rapidly with the passing years, jumping from a total of 284 tons wet weight in 1970 to 44,220 tons in 1981, which accounts for about 28% of the total production of both natural and cultivated Kombu in Japan (Fig. 16). Hokkaido produced 73% of the total of cultivated Kombu from 1977 to 1981, while Iwate Prefecture was the second with 23% and Aomori and Miyagi Prefectures produced only 4% in total.

The objective of the Kombu industry in Japan has been traditionally to produce good quality Kombu for human foodstuff. The expectations of the Japanese people for cultivated Kombu are that it should be of the same quality as natural Kombu. Kombu is enjoyed by the Japanese but it is not a part of the staple diet. Instead it is used as an accompanying dish to meals and as a health food. Therefore, excessive production would lead to chaos in the market.

Until today, most of the processed foods

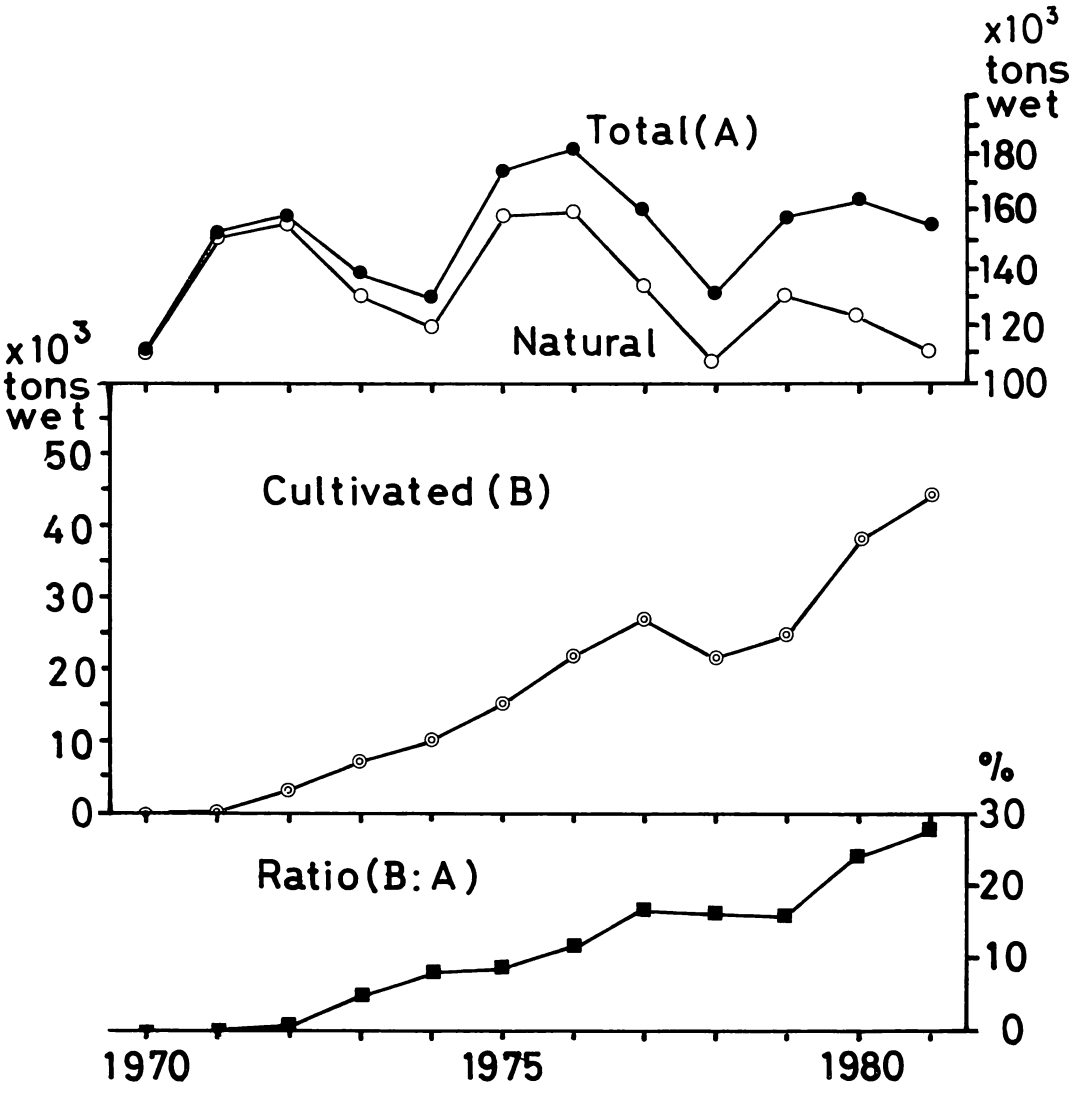

Fig. 16. Changes of Kombu production in Japan from 1970 to 1981, showing the total amount (A), natural, and cultivated Kombu (B), and the ratio of B to A. Data from Annual Report of Catch Statistics on Fishery and Aquaculture, 1970-1981 (Ministry of Agriculture, Forestry and Fishery, Japanese Government).

which contain Kombu have been manufactured entirely from dried Kombu. However, with recent improvements in processing techniques the changing tastes, new processed foods made from raw or salted Kombu have been developed and are highly favored. If new uses for Kombu as food develop, the productivity of cultivated Kombu could be still more raised in future.

Acknowledgements

I wish to express my special thanks to Professor Elise WOLLASTON, University of Adelaide, and Emeritus Professor Munenao KUROGI, Hokkaido University, who gave me an opportunity to present the original paper of this review at the 13th International Botanical Congress, Sydney, 1981.

I express my gratitude to Dr. Akio MIURA and Dr. Yusho ARUGA, Tokyo University of Fisheries, for many invaluable suggestions for rewriting of the original paper as well as for their critical reading of the manuscript. I am also grateful to Miss Kathleen Carroll TORKKO, Tokyo University of Fisheries, for her kindly reading and correcting the manuscript. I should like to thank Dr. Yoshio HASEGAWA, ex-Director of Hokkaido Regional Fisheries Research Laboratory, and Mr. Takashi FUNANO, Hokkaido Institute of Mariculture, for their kind advice.

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川嶋昭二： 日本における食用コンブの養殖

日本人の嗜好的副食品や健康食品として広く親しまれているコンブは古くから天然資源に依存してきたが，近年技術の発達に伴って北海道，青森，岩手，宮城各県で養殖生産が盛んになってきた。主産地北海道ではマコンブ，リシリコンブ，オニコンブ，ミツイシコンブを用い，2年養殖，移植養殖，促成養殖が行われる。生産の主体である促成養殖は北海道南西部のマコンブ地帯に広く普及し，科学的に管理された人工種苗生産と長年の調査，経験に基づく本養成管理により約11か月で良品質のコンブを生産する。日本の養殖生産は1970年の284トン(生重)から1981年には44,220トンへと飛躍的に増加し，乾燥品の利用のほか，生鮮，塩蔵品の利用も進んできた。

(042 函館市湯川町1丁目，北海道立函館水産試験場)

——学 会 録 事——

1. 第3回国際藻類学会議の検討

この件についてのワーキンググループ設定とその人選は，本年8月の臨時総会で会長一任とされ，その結果，このグループは，岩本会長のほか，千原光雄，堀輝三，徳田 廣，小林 弘，有賀祐勝，吉田忠生，梅崎 勇，榎本幸人で構成され，第1回検討会が10月19日午後東京水産大学植物学教室で開催された。遠隔地の吉田，梅崎，榎本の3氏は欠席されたが，最近の国際会議開催要件などをうかがうため，日本学術会議国際会議係長安達氏と山本係員のご出席を得た。

検討会では次のことが話しあわれた。

1) 第1回のカナダ，1971年札幌での国際海藻学会議の様子から，日本で開催した場合の参加者数は最高400人，外国人は半分はいくまい。

2) 会議開催を賄う経費は参加費，国の助成金，財団等の助成金，展示費，一般の寄附金が考えられる。本学会議のように小規模なものでは，国の助成金をもらうことと，大蔵省から免税募金の認可を得ることが極めて困難な現状である。したがって，これらが無いつもりで検討を進める必要がある。

3) 参加費は5万円では高過ぎる。3万5千円が限度であろう。来年コペンハーゲンでの参加費は3万6千円位，1971年札幌での国際海藻学会議は参加費30米ドル（当時約10,800円）であった。

4) 開催する場合の会場候補として，札幌市教育文化会館，筑波学園都市，京都国際会議場，神戸国際会議場などの公的機関があげられ，それらの資料に基づいて検討が行われた。

5) 免税の裏付けのない募金で，どの程度の寄附金などが集められるか次回までに会長などで心当りをあたることとし，次回の会合は11月下旬とした。

（岩本記）

新 入 会

住 所 変 更

訃 報

下記の会員が逝去された旨，事務局に連絡がありました。ここに慎んで哀悼の意を表し，ご通知申し上げます。

正会員 三重県 佐 藤 忠 勇氏

THOMAS

デイライトタイプ
レシプロシェーキング インキュベーター

光源として20W螢光灯、プラントルクス等が10本取付け可能で、最大10,000ルクスの照度が得られ、スイッチにより半分の点灯も可能です。さらに、24時間タイマーと連動させて、最小15分から最大24時間まで自由な照射条件が作れます。

レシプロの振盪機構はつまみひとつで自由に速度が可変でき、回転数もデジタル表示します。振盪パネルはワンタッチで交換可能、オプションとして御要望に合せたどのようなパネルも作成いたします。

恒温機構も10℃から60℃の広帯域で使用でき、恒温振盪培養機としての使用はもちろんのこと、陽光恒温器としても使用でき、藻類の増殖試験等に最適です。長時間試験にも充分使用できるようデジタル設定の運転用タイマーを備え、経時後自動OFF、または自動ONが可能です。さらに高温防止器などの安全装置も装備していますので無人運転等多様な運転操作が安心して行えます。

※この外にも各種振盪培養機があります。カタログ御請求ください。

温度範囲10℃～60℃

仕様

外　　法	W900×D780×H1,520mm
器内有効内法	W720×D650×H　520mm（ランプ無し 660mm）
振盪パネル	600×600mm（500mℓ坂口フラスコ25本掛、その他試験管、フラスコ、パネル等任意取付可）
振　盪　巾	70mm
振　盪　数	30～200R.P.M.（回転計付）
温度範囲	10℃～60℃
温度分布	±1℃　　温度精度：±0.5℃
安全装置	ヒーター断線、センサートラブル、異常高温を警報加熱・冷却装置　自動カット機構付

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)

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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5,500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3,000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700円.

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

2. **Index of the Bulletin of Japanese Society of Phycology.** Vol. 1 (1953)-Vol. 10 (1962) Price 1,500 Yen for member, 2,000 Yen for non member, Vol. 11 (1963)-Vol. 20 (1972), Price 2,000 Yen for member, 2,500 Yen for non member. Vol. 1 (1953)—Vol. 30 (1982). Price 3,000 Yen for member, 3,500 Yen for non member (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 photographies and list of publications of the late Professor Yukio YAMADA. ¥6,000 (incl. postage, surface mail).

4. **Contributions 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. ¥4,000 (incl. postage, surface mail).

5. **Recent Studies on the Cultivation of *Laminaria* in Hokkaido** (in Japanese). 1977. 65 pages. Four papers followed by discussions are included, which were presented in a symposium on *Laminaria*, sponsored by the Society, held in Sapporo, September 1974. ¥700 (incl. postage, surface mail).

昭和59年12月5日　印刷
昭和59年12月10日　発行

編集兼発行者　三　浦　昭　雄
〒108 東京都港区港南4—5—7
東京水産大学　植物学教室内

印　刷　所　学術図書印刷株式会社
〒176 東京都練馬区豊玉北2丁目13番地

発　行　所　日本藻類学会
〒108 東京都港区港南4—5—7
東京水産大学　植物学教室内
振替　東京 4 1 3 9 1 7 6

Printed by GAKUJUTSU TOSHO Printing Co.

本誌の出版費の一部は文部省科学研究費補助金（研究成果刊行費）による。

第 32 巻　第 4 号　昭和 59 年 12 月 10 日

藻　類

目　次

日　本　藻　類　学　会