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Disappearance of centrioles derived from female gametes in zygotes of *Colpomenia bullosa* (Phaeophyceae)*

Taizo Motomura

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

Motomura, T. 1992. Disappearance of centrioles derived from female gametes in zygotes of *Colpomenia bullosa* (Phaeophyceae). Jpn. J. Phycol. 40: 207–214.

Isogamous fertilization and zygote development of *Colpomenia bullosa* Yamada were studied with electron microscopy. Chloroplasts and mitochondria from male and female gametes remained in zygotes. Whereas two pairs of centrioles (=flagellar basal bodies) are derived from both male and female gametes, only one pair remains within about four hours after plasmogamy. Disappearance of one pair of centrioles occurred irrespective of karyogamy. Since morphological differences between male and female gametes of *C. bullosa* could not be detected, it was impossible to determine which one pair of centrioles disappeared. But observations on polyspermic zygotes (two or three male gametes to one female gamete) showed that only one pair of centrioles disappeared even in these zygotes. As a result, it was strongly suggested that centrioles from the female gamete disappeared.

Key Index Words: *brown algae—centrioles*—Colpomenia bullosa—*fertilization—isogamy—paternal inheritance.*

Sexual reproduction in almost all algal groups (with the exception of the red algae) is conducted by motile female and male gametes. In the brown algae, three types of sexual reproduction have been confirmed, i.e., isogamy, anisogamy and oogamy (Wynne and Roiseaux 1976). Ultrastructural studies on brown algal fertilization have been carried out in detail on *Fucus* and *Laminaria* (Brawley *et al.* 1976a, b, Motomura 1990). But sexual reproduction in these genera is oogamous, so there have not been any similar studies on the fertilization of isogamous and anisogamous groups in the brown algae.

Motomura (1990) reported in detail the fertilization of *Laminaria angustata* using complete serial sections. The results indicated that chloroplasts and mitochondria in zygotes were originated from eggs, while centrioles were originated from sperms by egg centrioles disappearing after plasmogamy. Paternal inheritance of centrioles has been well known in animal fertilization (oogamy) (Schatten *et al.* 1988, Sluder *et al.* 1989, Luykx 1991). Subsequently, by comparing the development of zygotes and parthenogenotes using immunofluorescence microscopy, it became clear that this paternal inheritance of centrioles had a crucial role in normal development of zygotes, especially in normal spindle formation (Motomura 1991).

In this study, it was found that the selective disappearance of centrioles from female gametes occurs even in isogamous brown alga.

Materials and Methods

Culture. Mature gametophytes of *Colpomenia bullosa* Yamada were collected in January–April, 1988–1990, at Charatsunai, Muroran, Hokkaido, Japan. The plants were washed with autoclaved seawater, wiped with paper towels, put in Petri dishes one by one and incubated in a refrigerator overnight. The next day, cold PESI medium (Tatewaki 1966) was poured into these Petri dishes under illumination. After several minutes, many

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gametes were liberated and sexuality was determined by mixing gametes derived from different individuals. Firstly, only female gametes were inoculated into Petri dishes. Many female gametes settled down within 20-30 min and female gametes still swimming were washed out with the medium. Next, male gametes were inoculated into these dishes, and plasmogamy was synchronous. Cultures of zygotes were maintained in PESI medium, 10°C or 14°C, under continuous illumination with fluorescent lamps (55 $\mu Em^{-2}s^{-1}$ photon flux density).

TEM preparation. Zygotes were fixed at regular intervals of time. They were fixed with a solution containing 3% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.2), 2% NaCl, 1% caffeine and 0.1% $CaCl_2$ for 2 hr at 4°C. Samples were detached from Petri dishes with a soft paint brush, and the fixative solution containing samples was transferred into centrifuge tubes. Fixed zygotes were washed with 0.1 M cacodylate buffer (pH 7.2), 2% NaCl, 1% caffeine and 0.1% $CaCl_2$, pelleted by centrifugation and finally embedded in 1% agar. They were post-fixed for 2 hr in 2% OsO_4 or overnight in 1% OsO_4, 0.1 M cacodylate buffer (pH 7.2), 2% NaCl and 0.1% $CaCl_2$. Samples were stained *en-bloc* with 0.5% uranyl acetate for 15 min at 4°C, dehydrated gradually with acetone and finally embedded in Spurr's resin (Spurr 1969). Serial sections were cut with a diamond knife on a Porter-Blum MT-1 ultramicrotome and mounted on formvar-coated slot grids. Sections were stained with uranyl acetate and lead citrate or only with lead citrate, and observed with a Hitachi H-300 electron microscope. Results in this paper were obtained from zygotes which were completely serial-sectioned.

Results and Discussion

Shape and structure of female and male gametes were typical of brown algal swarmers (Clayton 1989, O'Kelly 1989) and it was difficult to distinguish gametes from their ultrastructure. They have a long, mastigoneme-bearing anterior flagellum and a short, non-decorated, posterior flagellum. A nucleus is located above a cup-shaped chloroplast which has an eyespot near the base of the posterior flagellum. One pyrenoid protrudes from the chloroplast at the opposite side of the eyespot.

Plasmogamy in *Colpomenia bullosa* proceeds as in other brown algae (Maier and Müller 1986, Peters and Müller 1986); female gametes first settle down and secrete pheromones, and then male gametes are attracted to them and plasmogamy occurs. Frequently, two or three male gametes fertilize a female gamete (polyspermy), especially when many more male gametes were inoculated than female gametes.

Both nuclei fused (karyogamy) after plasmogamy, but the timing of karyogamy was not constant. When female and male nuclei are close to each other after plasmogamy, karyogamy occurs soon afterward (Figs. 2-4). Karyogamy is delayed when one or two chloroplasts are situated between both nuclei (Figs. 11, 12).

After plasmogamy, zygotes started to develop, and germinate after about 12 hr. The zygote development was examined by serial sections till 24 hr in culture. Cellular organelles, such as chloroplasts and mitochondria from both gametes, continued to exist in the zygote development. On the contrary, one pair of centrioles disappeared during zygote development.

Figures 1-6 show six sections of a one-hour-old zygote after plasmogamy. There are two chloroplasts, each having a pyrenoid and an eyespot. Therefore this zygote was produced by normal plasmogamy, not polyspermy. The outer membranes of both nuclei have just fused (Figs. 2-4). Two pairs of centrioles (Figs. 2, 6) derived from both female and male gametes could be detected in one-hour-old zygote after plasmogamy. Basal plates were observed at the distal end of centrioles but axonemes were detached from them (Fig. 6).

One pair of centrioles disappeared in four-hour-old zygotes. Figures 7-9 show three

sections of a four-hour-old zygote after plasmogamy. This zygote contained one nucleus and two chloroplasts, each containing a pyrenoid and an eyespot. Therefore, clearly the zygote was formed after normal plasmogamy and karyogamy had occurred. In this zygote, only one pair of centrioles could be detected (Fig. 7). Also, Figures 10-12 show three sections of another four-hour-old zygote after normal plasmogamy. The nuclei (N1 and N2) were not fused yet. Similar to the previous example, only one pair of centrioles existed near the nucleus (N1) (Fig. 11), the other pair of centrioles had disappeared. Therefore, it became clear that the presence or absence of nuclear fusion did not affect the disappearance of one pair of centrioles.

Because of the identical ultrastructure of flagellar basal bodies of female and male gametes, it was difficult to determine which pair of centrioles disappeared. However observations on polyspermic zygotes suggest the female gamete's centrioles disappear. Figures 13-18 show six sections of a four-hour-old zygote of polyspermy. This zygote resulted from polyspermy (two male gametes to one female gamete) because three chloroplasts could be detected, each having a pyrenoid and an eyespot. Three nuclei were not fused yet with one another. In this polyspermic zygote, two pairs of centrioles remained (Figs. 16, 17), one pair have disappeared. Disappearance of only one pair of centrioles was not affected by the presence or absence of karyogamy, like normally fertilized zygotes. Disappearance of one pair of centrioles was also observed in polyspermic zygotes which resulted from one female gamete and three male gametes (not shown). Therefore, I believe that centrioles which were derived from the female gamete disappeared and ones derived from the male gamete remained. It means that centrioles in diploid thallus (=sporophyte) cells are originated from basal bodies (=centrioles) of flagella of male gametes. Similar results were obtained from *Scytosiphon lomentaria* (Scytosiphonales) and *Analipus japonicus* (Ralfsiales) in the brown algae (Motomura unpublished data).

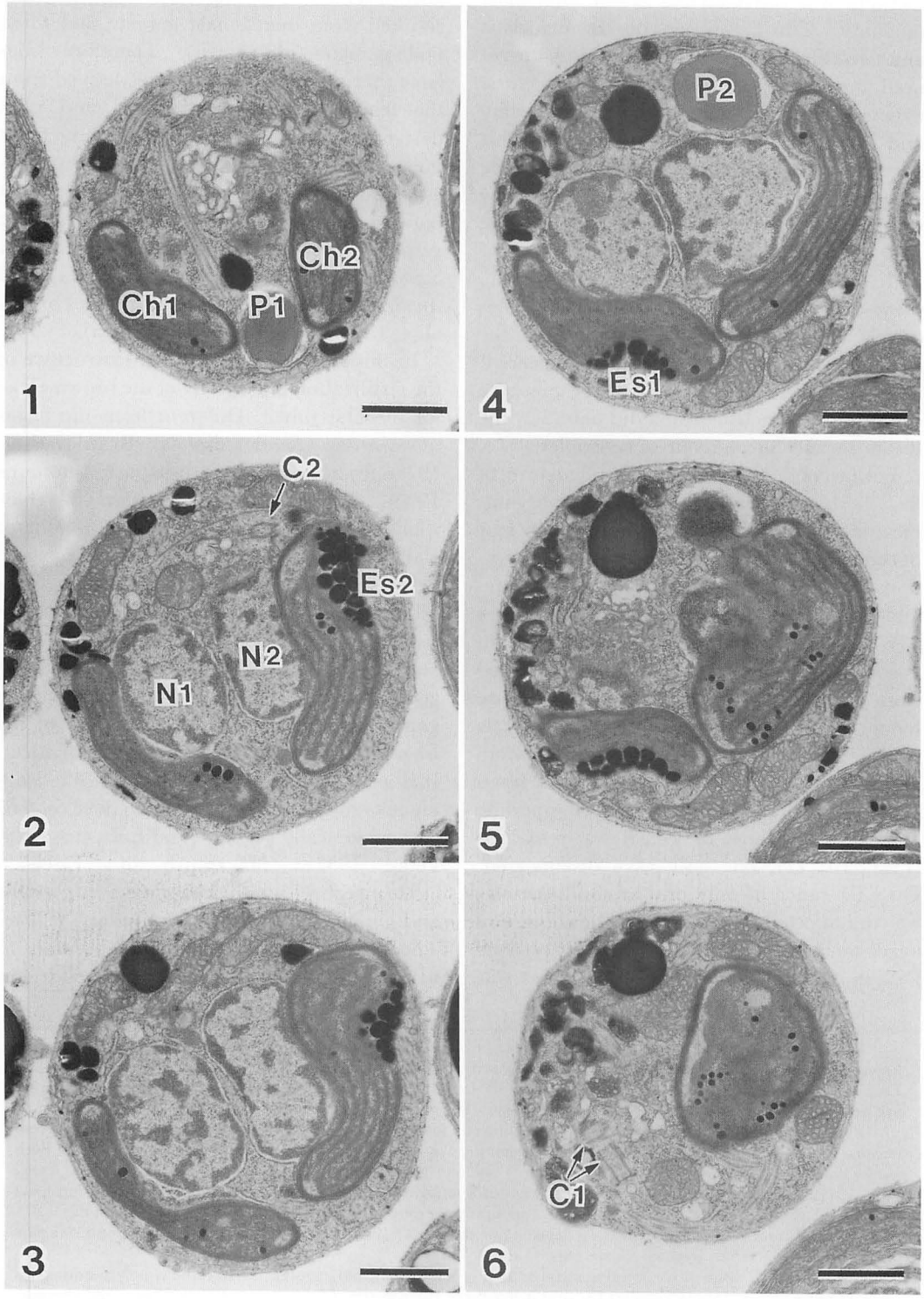
In this study, I report the ultrastructure of the fertilization in isogamy of the brown algae for the first time. Different from the oogamous group, *Fucus vesiculosus* (Brawley *et al.* 1976a, b) and *Laminaria angustata* (Motomura 1990), degradation or digestion of chloroplasts and mitochondria of male gametes was not observed in *Colpomenia bullosa* zygote development. In the oogamous groups, these cellular organelles of sperms, especially chloroplasts, are smaller than those of the eggs, and the sperms can not develop parthenogenetically. On the contrary, cellular organelles in female and male gametes of the isogamous brown algae are almost identical. It is well known that the gametes of the isogamous group of brown algae can develop parthenogenetically (Wynne and Loiseaux 1976, Peters 1987). Nakamura and Tatewaki (1976) reported parthenogenesis of female and male gametes of *Colpomenia bullosa*. Therefore, monoparental or biparental inheritance of chloroplasts and mitochondria in

Figs. 1–6. Six non-consecutive serial sections of an one-hour-old zygote. Both nuclei (N1 and N2) are just fusing their outer nuclear membranes. There are two chloroplasts (Ch1 and Ch2) containing eyespots (Es1 and Es2) and pyrenoids (P1 and P2). Two pairs of centrioles (C1 and C2) exist in this zygote and note that axonemes are detached from the centrioles having a basal plate (Fig. 6 C1). Scale bars=1 μm.

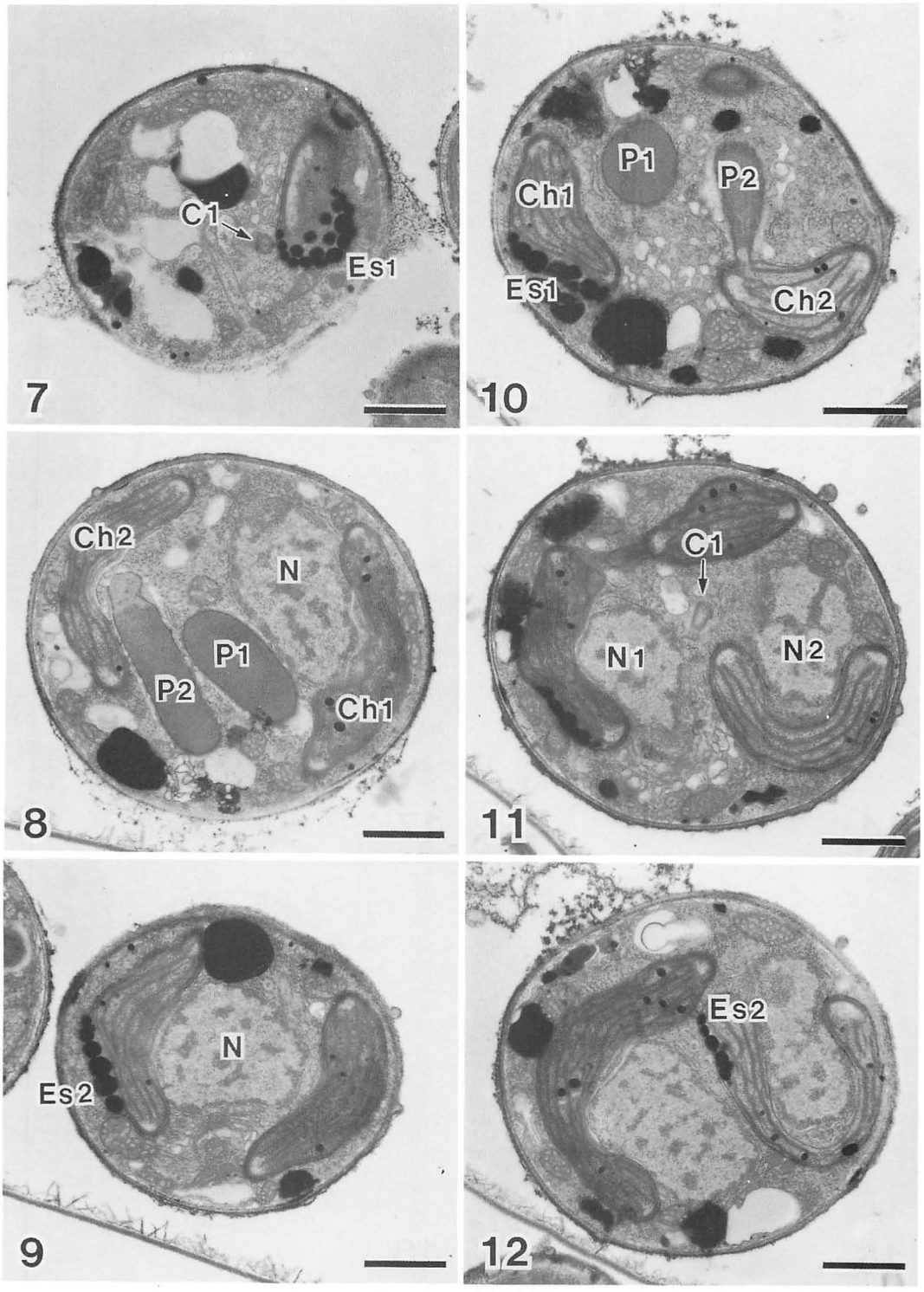
Figs. 7–9. Three non-consecutive serial section of a four-hour-old zygote. This zygote is normally fertilized because it has two chloroplasts (Ch1 and Ch2) containing eyespots (Es1 and Es2) and pyrenoids (P1 and P2). Both nuclei had already fused into one (N). Note only one pair of centrioles (C1) in this zygote. Scale bars=1 μm.

Figs. 10–12. Three non-consecutive serial section of a four-hour-old zygote. The zygote is normally fertilized because it has two chloroplasts (Ch1 and Ch2) containing eyespots (Es1 and Es2) and pyrenoids (P1 and P2). But two nuclei (N1 and N2) have not fused yet because one chloroplast (CH2) exists between them. Note only one pair of centrioles (C1) in this zygote. Scale bars=1 μm.

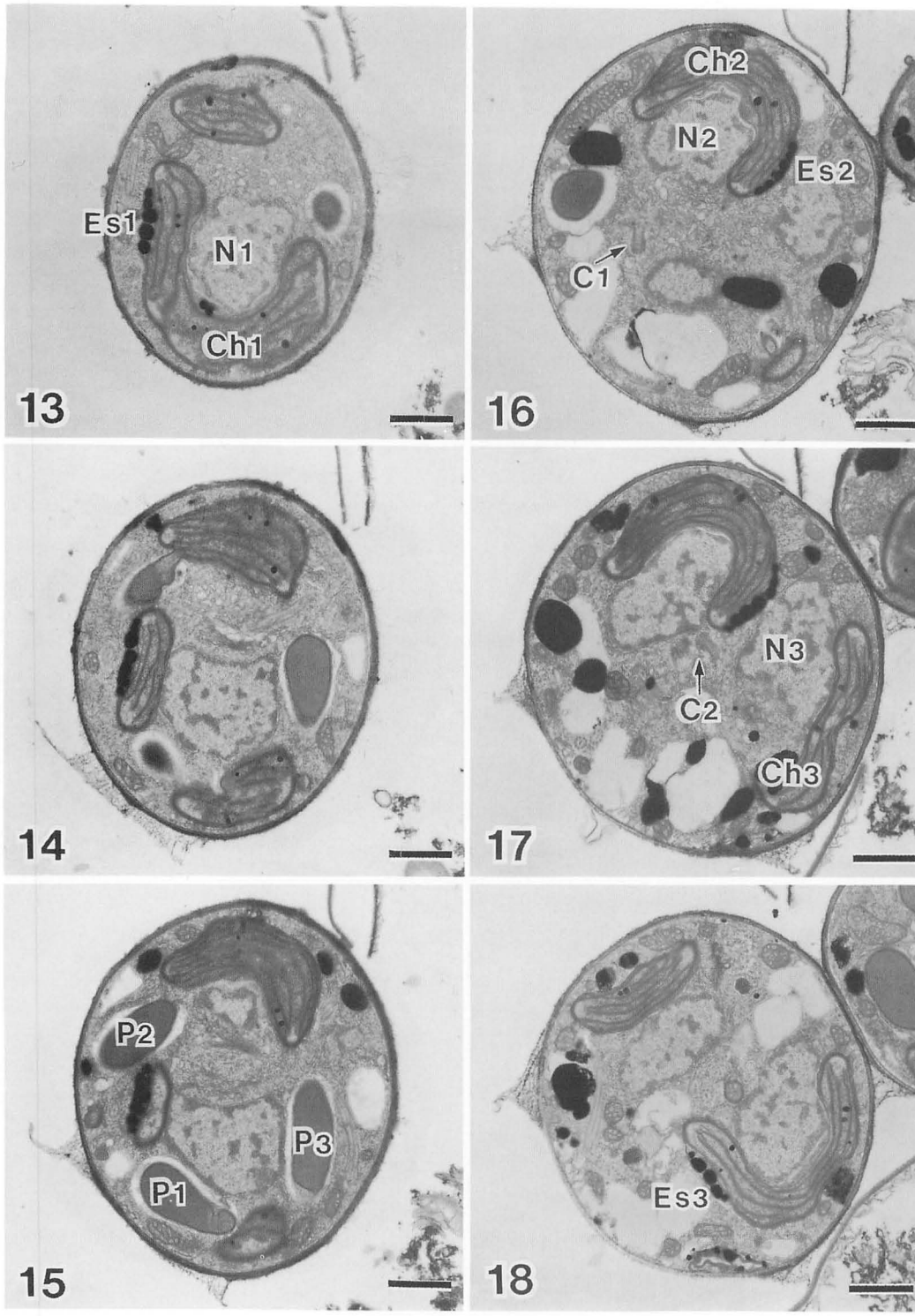
Figs. 13–18. Six non-consecutive serial section of a four-hour-old zygote. The zygote is polyspermic (two male gametes to one female gamete) because it has three chloroplasts (Ch1, Ch2 and Ch3) containing eyespots (Es1, Es2 and Es3) and pyrenoids (P1, P2 and P3). Three nuclei (N1, N2 and N3) have not fused yet. Note two pairs of centrioles (C1 and C2) in the zygote. Scale bars=1 μm.



Figs. 1–6.


C1
Es1
7
P1
P2
Ch1
Es1
Ch2
10
Ch2
N
P1
P2
Ch1
8
C1
N1
N2
11
N
Es2
9
Es2
12

Figs. 7–12.



Figs. 13–18.

zygotes might be related to the ability of both female and male gametes to grow parthenogenetically.

Even though flagellar apparatuses of female and male gametes of *Colpomenia bullosa* were identical, one pair of centrioles disappeared during the development of zygotes. Based on the observations on polyspermic zygotes, it would be proper to conclude that centrioles which were introduced from female gametes disappeared. In the case of *Laminaria angustata*, it was possible to distinguish female from male basal bodies (=centrioles) by their arrangement and connecting structures (Motomura and Sakai 1988, Motomura 1989). Motomura (1990) reported that sperm centrioles remained but egg centrioles disappeared in the zygote development of *L. angustata*. Also, in *Fucus evanescens*, liberated unfertilized eggs do not have centrioles, and sperm centrioles are introduced into the egg after plasmogamy (Motomura unpublished data). Therefore, irrespective of isogamy and oogamy, it could be considered that centrioles of the female gamete disappear and ones of the male gamete remain during brown algal fertilization and zygote development. Afterward, the centrioles from male gametes will begin to function as a component of centrosomes in vegetative cells of the diploid sporophytic generation.

Paternal inheritance of centrioles might be universal in brown algal fertilization, based on this study and previous work (Motomura 1990). The occurrence of paternal inheritance of centrioles is well known in animal fertilization (Schatten *et al.* 1988, Sluder *et al.* 1989, Luykx 1991). It is significant that paternal inheritance of centrioles, in other words, regulation of mitotic spindle pole formation in the first division of zygotes, would be a common phenomenon between the brown algal and animal fertilization.

In this experiment, I report the behavior of centrioles, which are one component of the centrosome, using electron microscopy of *Colpomenia bullosa* fertilization. However the behavior of centrosomal material (pericentriolar material), which is the actual microtubule organizing center (Robbins *et al.* 1968, Gould and Borisy 1977), in the isogamous brown algal fertilization is still obscure. Motomura (1991) reported that centrioles in *Laminaria angustata* were derived from the sperm but centrosomal material might be present already or synthesized *de novo* in the egg.

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本村泰三：褐藻ワタモ受精過程での雌性配偶子由来セントリオールの消失

同型配偶子接合を行う褐藻ワタモ（*Colpomenia bullosa*）の受精・発生過程を電子顕微鏡を用いて観察した。受精後約4時間経過した接合子では，雌・雄性配偶子から持ち込まれた二組のセントリオールのうち一組が消失していた。多精した接合子においても一組のセントリオールだけが消失することから，雌性配偶子由来のセントリオールが消失すると結論した。受精時にセントリオールが父性遺伝することは広く動物細胞（卵生殖）において知られており，また褐藻ミツイシコンブ（卵生殖）の受精過程においても同様な現象が最近明らかになった。今回の観察から同型配偶子接合を行う褐藻類においてもセントリオールは父性遺伝することが確かめられた。褐藻植物ではセントリオールは核分裂時において紡錘体の両極に一組ずつ存在することから，同型配偶子接合・卵生殖において接合子の第一回目の核分裂の分裂装置形成に共通した制御機構が存在している可能性を強く示唆する。（051 室蘭市母恋南町1-13　北海道大学理学部附属海藻研究施設）

Jpn. J. Phycol. (Sôrui) **40**: 215–227, September 20, 1992

Toxicities of pH buffer solutions to *Chara* internodal cells

Keitaro Kiyosawa

Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka, 560 Japan

Kiyosawa, K. 1992. Toxicities of pH buffer solutions to *Chara* internodal cells. Jpn. J. Phycol. **40**: 215–227.

Tris-HCl (pH 7.0), Tris-maleate (pH 7.0) and HEPES-KOH (pH 6.8–8.2), CHES (pH 8.6–10.0) and CAPS (pH 9.7–11.1) pH buffer solutions are widely used in physiological, biophysical and biochemical experiments. However, their effect on cells has not been thoroughly examined. The results of this study show that these pH buffer solutions can stop the protoplasmic streaming of *Chara* internodal cells and kill them within one to several days at 10 mol m^{-3}, probably by destruction of the membrane functions. However, the cells can be kept alive by the addition of 0.5 mol m^{-3} Ca^{2+}; if this is done, the velocity of the protoplasmic streaming remains normal for more than 10 days in 10 mol m^{-3} Tris pH buffer solutions. The same toxic phenomenon was observed in 10 mol m^{-3} HEPES pH buffer solutions, probably due to the liberation of calcium bound to the cell membrane by the K^+ added as KOH to adjust the pH value.

Key Index Words: calcium ions—Chara australis—*Charophyta—cytoplasmic streaming—Good pH buffer—HEPES—protoplasmic streaming—salt (electrolyte) tolerance—Tris-HCl—Tris-maleate—Tris pH buffer.*

The pH buffer solutions of potassium phosphate, Tris-HCl, Tris-maleate and HEPES-KOH are often used in studies in cell physiology and biophysics as well as in biochemistry. However, the toxicity of potassium phosphate pH buffer solution at pH 7.0 to the *Chara* internodal cells was pointed out by Kiyosawa and Adachi (1990). This toxic effect on cells, cell membranes and membrane fragments needs to be examined in detail.

Recently, Kiyosawa and Adachi (1990) found that *Chara* internodal cells were killed even when exposed to 10-50 mol m^{-3} KCl, 10.0 mol m^{-3} $MgCl_2$ or $Mg(NO_3)_2$, 1.0 mol m^{-3} $BaCl_2$ or $Ba(NO_3)_2$, as found with NaCl by Katsuhara and Tazawa (1986). However, these cells could survive in 80 mol m^{-3} $CaCl_2$, $Ca(NO_3)_2$, $SrCl_2$ or $Sr(NO_3)_2$ for more than ten days. Furthermore, addition of Ca^{2+} or Sr^{2+} to the KCl, $MgCl_2$ and $BaCl_2$ enabled the *Chara* internodal cells to survive for more than a week.

These studies using calcium buffer solutions showed that the minimum effective concentration of the Ca^{2+} in the surrounding solution of the *Chara* internodal cell was between pCa 5 (1.0×10^{-5} mol m^{-3}) and pCa 6 (1.0×10^{-6} mol m^{-3}). However, the calcium buffer solution needed a pH buffer to stabilize its pH during dissociation and/or binding of the calcium ions and protons from/to EGTA (Ogawa 1968). Such pH buffers as Tris and one of the Good buffers, HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), themselves may be the cause of death of *Chara* internodal cells, independently of the calcium concentration.

The Good buffers are said to be not very permeable to the cell membrane and considered to be suitable for use in biochemical, biophysical and cell physiological experiments. These buffers have one or two sulfonic groups or one or two carboxyl groups in their molecules. Usually KOH or NaOH is added when these buffers are to be used as a pH buffer solution. Although the buffers themselves should be harmless to cells, the K^+ (Kiyosawa and Adachi 1990) and Na^+ (Katsuhara and Tazawa 1986) externally added to the bathing solution of Characean

cells can kill the internodal cells. Therefore, Good pH buffer solutions containing K^+ or Na^+ of considerably high concentrations might be harmful to *Chara* internodal cells.

Tunnicliff and Smith (1981) have reported that HEPES competitively inhibits Na-independent binding or γ-aminobutyric acid (GABA) binding to its receptor, and Hanrahan and Tabcharani (1990) have shown that HEPES supplied internally blocks the anion channel of PANC-1 cells. Thus, the present study examined the toxicities of Tris pH buffers and some Good pH buffers, focusing on Tris and HEPES which are most widely used in biochemical, biophysical and cell physiological experiments. The present experiments were done to test whether or not an externally supplied Tris or Good pH buffer can kill *Chara* internodal cells as a result of biophysical and biochemical interactions with the cell membrane, as found in the case of KCl, $MgCl_2$ and $BaCl_2$ (Kiyosawa and Adachi 1990).

In this study, the toxicities of Tris and HEPES-KOH pH buffers were examined. *Chara* internodal cells in toxic electrolyte solutions, Tris and HEPES pH buffer solutions were found to show a gradual decrease in the velocity of their protoplasmic streaming with time, followed by its stopping. After this, the turgor pressure was lost, signifying plant cell death, at 1 or sometimes 2 days after the protoplasmic streaming had stopped. The velocity of the protoplasmic streaming of the *Chara* cells in Tris-HCl pH buffer solution was examined as a function of time and in relatioin to the loss of turgor pressure.

Also studied were the antagonistic effects of externally added Ca^{2+} and Sr^{2+} on the survival of *Chara* internodal cells in Tris pH buffer solutions and the antagonistic effects of externally supplied Ca^{2+} on the survival of *Chara* internodal cells in HEPES, one of the Good pH buffers.

These studies suggested that Tris and HEPES pH buffers, and other Good pH buffers, CHES (2-cyclohexylamino-ethanesulfonic acid) and CAPS (3-cyclohexylamino-1-propanesulfonic acid), disturb the normal membrane transport processes. This can be prevented by externally supplied Ca^{2+}. Therefore, the leakage of K^+, Ca^{2+} and Mg^{2+} from *Chara* internodal cells in 10 mol m^{-3} Tris-HCl (pH 6.9) and Tris-maleate (pH 7.1) solutions was also examined. The effects of CHES and CAPS on *Chara* internodal cells were studied to clarify the effect of HEPES on *Chara* internodal cells.

Materials and Methods

Uncalcified internodal cells of *Chara australis* were used. They were cultured in polyethylene buckets containing tap water and soil several centimeters thick at the bottom. Some of the buckets were exposed to the sun, and others were kept out of the sun with covers which permitted a little sunlight to pass through. Internodal cells were isolated from adjacent cells one or a few days before the experiments and incubated in artificial pond water (APW: 0.400 mol m^{-3} KCl, 0.100 mol m^{-3} NaCl, 0.300 mol m^{-3} $CaSO_4$ and 0.100 mol m^{-3} $MgSO_4$; pH ca. 5.3).

Each internodal cell (n=10) was put in a plastic vessel containing 30-40 cm^3 of the test Tris, HEPES, CHES or CAPS pH buffer solutions or deionized water of 17-18 MΩ cm^{-1} specific resistance. The cells were incubated in test solutions without agitation and observed every day for a week or 10 days. Cell death was judged from the loss of turgor pressure. This was done by slowly raising the *Chara* internodal cell with forceps from the test solution after confirming that the protoplasmic streaming had stopped. The changes in arrangement, shape and color of the chloroplasts were also observed with a microscope. If the *Chara* internodal cell bent easily on the forceps, the cell was regarded as being dead. The test solutions in plastic vessels were exchanged for newly prepared ones at 3-day intervals. The percentages of survival of the *Chara* internodal cells in Tris buffer solutions differed between those grown in the shade and in the sun, with those grown in the shade generally showing weaker tolerance to Tris buffer solutions. Thus, the per-

centages of survival of the *Chara* internodal cells for the respective test solutions were obtained from at least two experiments: one from *Chara* internodal cells (n=10) grown in the shade and the other from those (n=10) kept in the shade for one or a few weeks after having been exposed to the sun for more than several weeks or a month.

Since Tris buffers seemed to disturb the normal membrane transport processes, we examined whether or not leakage of K^+, Ca^{2+} and Mg^{2+} from the *Chara* internodal cell occurs in 10 mol m^{-3} Tris-HCl (pH 6.9) and Tris-maleate (pH 7.1) solutions. Apparent leakages of K^+, Ca^{2+} and Mg^{2+} from the *Chara* internodal cell to 10 cm^3 of 10 mol m^{-3} Tris-HCl, 10 mol m^{-3} Tris-maleate and deionized water of 10 cm^3 by 14 h after transfer of the internodal cell from APW to the respective solutions were measured by the atomic absorption method (Jarrell-Ash AA-845). The amounts of leakage of the respective ions were expressed in terms of averaged decrease in the ion concentrations of the internodal cell calculated from the volume of the internodal cell and the measured changes in the ion concentrations in the 10 cm^3 solutions. The volume of the internodal cell was calculated from the diameter measured with an optical microscope equipped with an eyepiece micrometer calibrated with an objective one, and the length was measured with a ruler. Ion leakage from *Chara* internodal cells in HEPES pH buffer solution was not measured because the HEPES pH buffer solution contained a large amount of K^+ which would have disturbed the determination of ion leakage from the *Chara* internodal cells by the atomic absorption method.

Experiments and incubation were conducted at 25±0.2°C and, unless otherwise stated, under a 12 h-12 h light-dark cycle. The light intensity was 3.4 W m^{-2}.

The velocity of the protoplasmic streaming, which is sensitive to the Ca^{2+} concentration in the cytoplasm of the *Chara* internodal cells (Williamson 1975, Tominaga and Tazawa 1981, Williamson and Ashley 1982, Tominaga *et al.* 1983), in Tris pH-buffer solutions was measured with an optical microscope equipped with an eyepiece micrometer calibrated with an objective one in continuous light because the velocity gradually decreased and attained almost equal values in a few days irrespective of differences in the types and concentrations of calcium salt solutions used, such as APW, 10 mol m^{-3} $CaSO_4$, 10 and 80 mol m^{-3} $CaCl_2$, or 10 and 80 mol m^{-3} $Ca(NO_3)_2$ solutions in the dark. However, the protoplasmic streaming did not stop in such concentrated calcium salt solutions in continuous light or in the dark (data not shown).

Results

Percentages of survival in Tris pH buffer solutions—All of the *Chara* internodal cells immersed in 5.0, 10.0 or 20.0 mol m^{-3} Tris-HCl pH buffer solution (pH 7.1) died within a few or several days (Fig. 1). The higher the concentration, the faster the drop to zero percent survival. The same results were obtained with Tris-maleate buffer solutions (pH 7.1; Fig. 2).

K^+, Mg^{2+} and Ba^{2+} tolerance of the *Chara* internodal cells increased with addition of Ca^{2+} or Sr^{2+} (Kiyosawa and Adachi 1990). Na^+ tolerance of *Nitellopsis* also increased on addition of Ca^{2+} (Katsuhara and Tazawa 1986). Figs. 3 and 4 show similar increased Tris tolerance of *Chara* internodal cells on addition of 0.5 mol m^{-3} Ca^{2+}. Addition of Sr^{2+} to Tris pH buffer solutions did not significantly increase the tolerance at a final concentration of 5.0 mol m^{-3} (Figs. 3 and 4).

The velocity of the protoplasmic streaming of the *Chara* internodal cells gradually decreased with time in 10.0 mol m^{-3} Tris-HCl buffer solution (pH 7.1), followed by the stopping of the protoplasmic streaming and death of the cell (Fig. 5). In the experiment of Fig. 5, all of the *Chara* internodal cells (n=5) in 10.0 mol m^{-3} Tris-HCl buffer solution (pH 7.1) died within a day. On the other hand, the velocity of the protoplasmic streaming of the *Chara* internodal cells in 10.0 mol m^{-3} Tris-HCl+0.5 mol m^{-3} $CaSO_4$

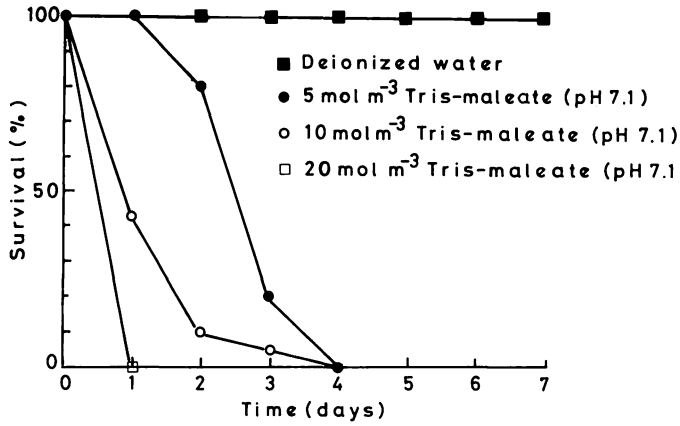

Fig. 1. Survival percentage of *Chara* internodal cells in 5.0 mol m^{-3}, 10.0 mol m^{-3} and 20.0 mol m^{-3} Tris-HCl as a function of time.

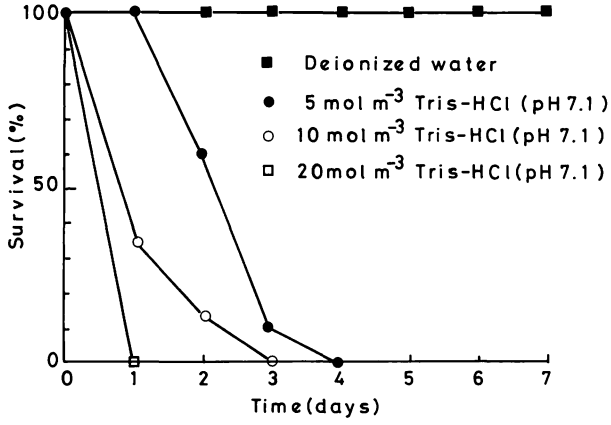

Fig. 2. Survival percentage of *Chara* internodal cells in 5.0 mol m^{-3}, 10.0 mol m^{-3} and 20.0 mol m^{-3} Tris-maleate as a function of time.

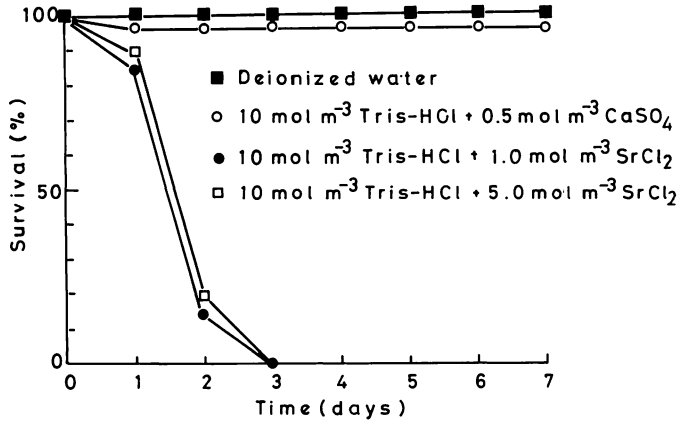

Fig. 3. Effects on survival percentage of *Chara* internodal cells of addition of 0.5 mol m^{-3} Ca^{2+} or 1.0 mol m^{-3} or 5.0 mol m^{-3} Sr^{2+} to 10.0 mol m^{-3} Tris-HCl.

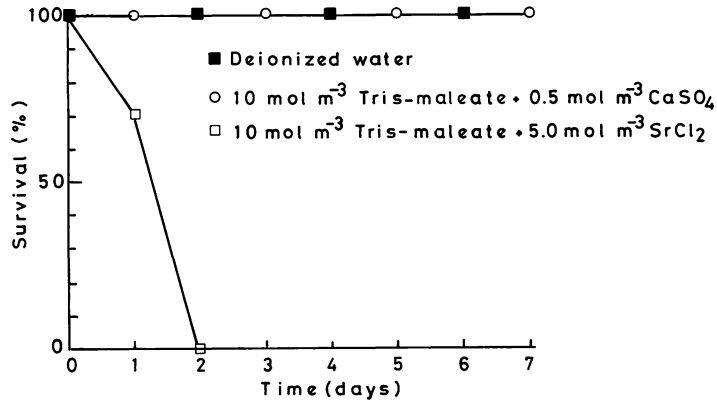

Fig. 4. Effects on survival percentage of *Chara* internodal cells of addition of 0.5 mol m^{-3} Ca^{2+} or 5.0 mol m^{-3} Sr^{-2} to 10.0 mol m^{-3} Tris-maleate.

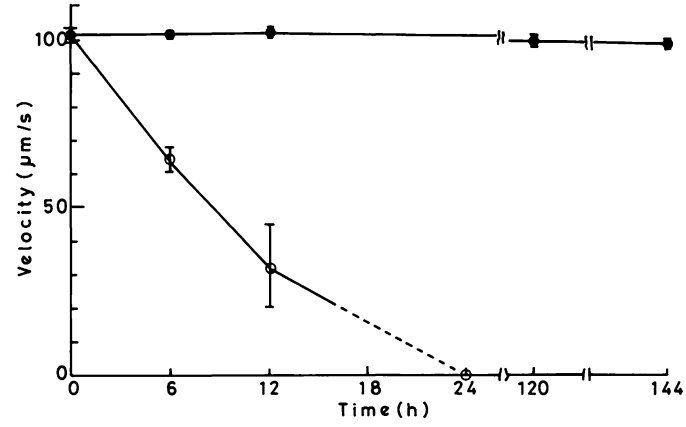

Fig. 5. Averaged values of velocity of the protoplasmic streaming of the *Chara* internodal cells in 10.0 mol m^{-3} Tris-HCl (pH 7.1) (○) and in 10.0 mol m^{-3} Tris-HCl + 0.5 mol m^{-3} $CaSO_4$ (●) as a function of time. The velocity of the protoplasmic streaming of the *Chara* internodal cells in 10.0 mol m^{-3} Tris-HCl (pH 7.1) decreased gradually with time, while that of the protoplasmic streaming of the *Chara* internodal cells in 10.0 mol m^{-3} Tris-HCl (pH 7.1) + 0.5 mol m^{-3} $CaSO_4$ remained constant for at least 144 h. All *Chara* internodal cells (n = 5) died by 24 h after immersion in 10.0 mol m^{-3} Tris-HCl, while all *Chara* internodal cells in 10.0 mol m^{-3} Tris-HCl + 0.5 mol m^{-3} $CaSO_4$ remained alive for at least 144 h. Velocities of the protoplasmic streaming are indicated with the mean ± standard error. Standard errors are shown with bars.

remained constant for at least 144 h (Fig. 5). The same results were observed with Tris-maleate and HEPES pH buffer solutions (data not shown).

The apparent leakages of K^+, Ca^{2+} and Mg^{2+} from the *Chara* internodal cell to 10.0 mol m^{-3} Tris-HCl (pH 7.1), 10.0 mol m^{-3} Tris-maleate (pH 7.1) solutions and deionized water are tabulated in Table 1. Apparently, K^+, Ca^{2+} and Mg^{2+} leaked from the *Chara* internodal cell in Tris pH buffer solutions, resulting in apparent decrease in their intracellular concentrations amounting to 6.5–8.8 mol m^{-3}, 3.9–4.6 mol m^{-3} and 0.71–0.64 mol m^{-3}, respectively.

Percentages of survival in simple HEPES solution—Until the 4th or 5th day after transfer from APW to the simple HEPES solutions of 10, 20 and 50 mol m^{-3} (pH 5.26), high percentages of survival were maintained irrespective of differences in HEPES concentrations, but were followed by a steep decrease in the

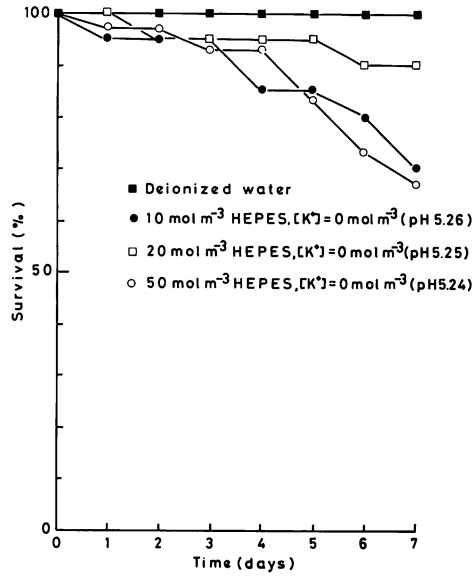

Fig. 6. Survival percentage of *Chara* internodal cells in simple 10, 20 and 50 mol m^{-3} HEPES solutions.

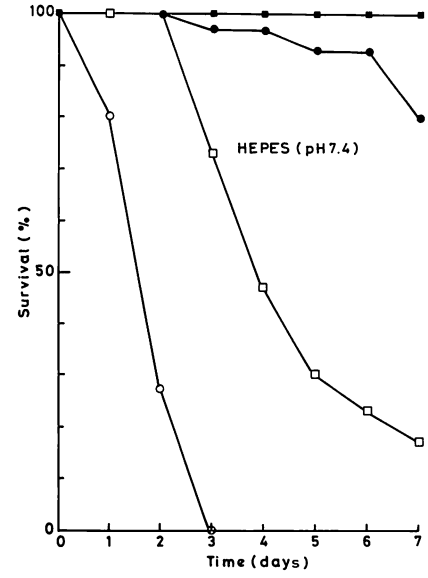

Fig. 7. Survival percentage of *Chara* internodal cells in 10 mol m^{-3} ($[K^+]$=5.0 mol m^{-3}; ●), 20 mol m^{-3} ($[K^+]$=10.0 mol m^{-3}; □) and 50 mol m^{-3} ($[K^+]$=25.0 mol m^{-3}; ○) HEPES pH buffer solutions at pH 7.4, and deionized water (■).

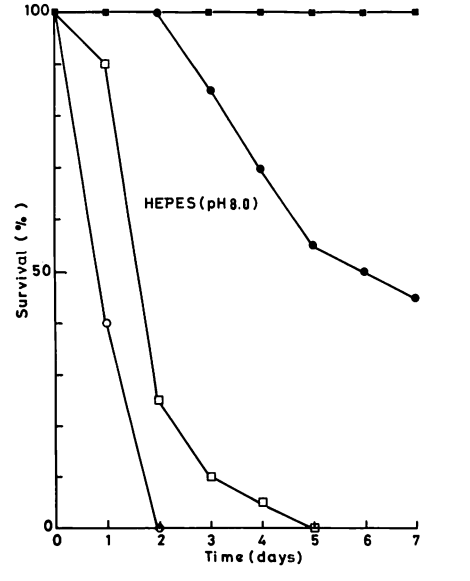

Fig. 8. Survival percentage of *Chara* internodal cells in 10 mol m^{-3} ($[K^+]$=8.9 mol m^{-3}; ●), 20 mol m^{-3} ($[K^+]$=17.8 mol m^{-3}; □) and 50 mol m^{-3} HEPES ($[K^+]$=44.5 mol m^{-3}; ○) pH buffer solutions at pH 8.0, and deionized water (■).

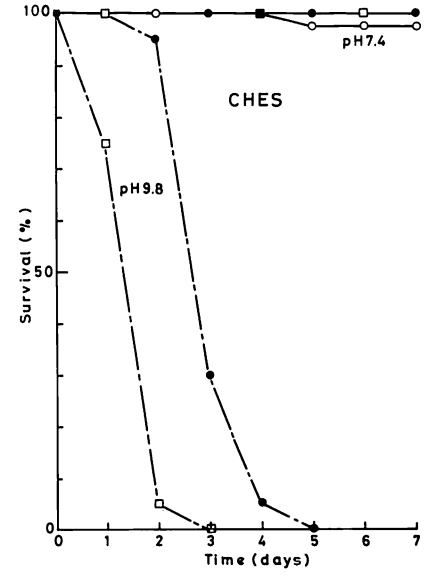

Fig. 9. Survival percentage of *Chara* internodal cells in 10 mol m^{-3} ($[K^+]$=0.1 mol m^{-3}; —●—), 20 mol m^{-3} ($[K^+]$=0.2 mol m^{-3}; —□—) and 50 mol m^{-3} ($[K^+]$=0.5 mol m^{-3}; ○) CHES pH buffer solutions at pH 7.4, and those in 10 mol m^{-3} ($[K^+]$=8.4 mol m^{-3}; ——●——) and 20 mol m^{-3} ($[K^+]$=16.8 mol m^{-3}; ——□——) CHES pH buffer solutions at pH 9.8, and deionized water (■).

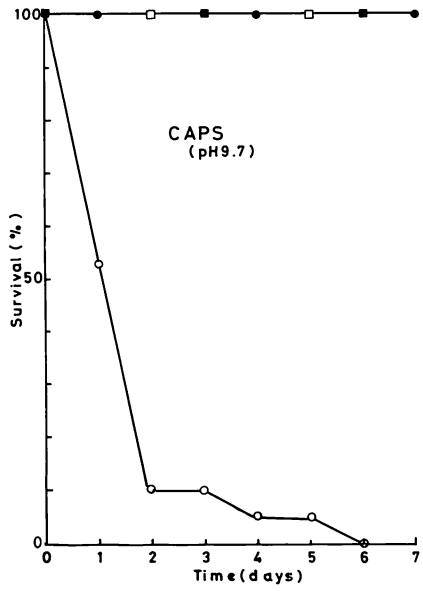

Fig. 10. Survival percentage of *Chara* internodal cells in 10 mol m^{-3} ($[K^+]=1.8$ mol m^{-3}; ●), 20 mol m^{-3} ($[K^+]=3.6$ mol m^{-3}; □) and 50 mol m^{-3} ($[K^+]=9.0$ mol m^{-3}; ○) CAPS pH buffer solutions at pH 9.7, and deionized water (■).

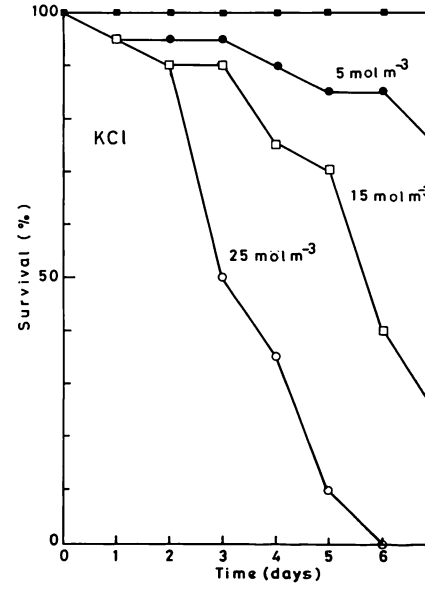

Fig. 11. Survival percentage of *Chara* internodal cells in 5.0 mol m^{-3} (●), 15.0 mol m^{-3} (□) and 25.0 mol m^{-3} (○) KCl solutions (pH ca. 5.3), and deionized water (■).

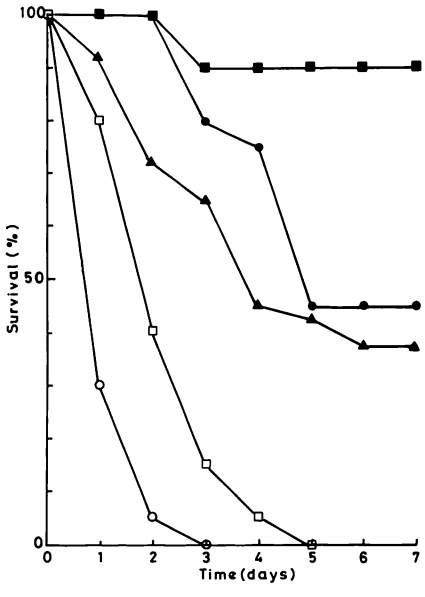

Fig. 12. Effects on survival percentage of *Chara* internodal cells in 20 mol m^{-3} HEPES (pH 8.0, □) and 20 mol m^{-3} CHES (pH 9.8, ○) pH buffer solutions on addition of 0.5 or 1.0 mol m^{-3} Ca^{2+} to their solutions; ■: 20 mol m^{-3} HEPES+0.5 mol m^{-3} $CaSO_4$; ●: 20 mol m^{-3} CHES+0.5 mol m^{-3} $CaSO_4$ and ▲: 20 mol m^{-3} CHES+1.0 mol m^{-3} $CaSO_4$.

percentage of survival after 4 days (Fig. 6).

Percentage of survival in HEPES pH buffer solution (pH 7.4)—The decrease in the percentage of survival of the *Chara* internodal cells in 10, 20 and 50 mol m^{-3} HEPES pH buffer solutions at pH 7.4 became steeper as a function of time with increase in the concentration of the HEPES pH buffer solution (Fig. 7). The K^+ concentrations from the KOH used to adjust the pH of the respective HEPES pH buffer solutions were 5.0, 10.0 and 25.0 mol m^{-3}.

Percentage of survival in HEPES pH buffer solution (pH 8.0)—The percentage of survival of the *Chara* internodal cells in 10, 20 and 50 mol m^{-3} HEPES pH buffer solutions at pH 8.0 decreased more steeply with time than those in the HEPES pH buffer solutions at pH 7.4 of the corresponding concentrations (Fig. 8; cf. Fig. 7). The K^+ concentrations from the KOH used to adjust the pH of the respective HEPES pH buffer solutions at pH 8.0 were 8.9, 17.8 and 44.5 mol m^{-3}. The higher the pH and the K^+ concentration, the steeper was the decrease in the percentage of survival as a function of time.

Table 1. Average leakages of K^+, Ca^{2+} and Mg^{2+} from *Chara* internodal cells (ΔC in mol m^{-3}) 14 h after transfer of the internodal cells from artificial pond water (pH ca. 5.3) to 10 mol m^{-3} Tris-HCl pH buffer solution (pH 7.1), 10 mol m^{-3} Tris-maleate pH buffer solution (pH 7.1) or deionized water.

Solution	ΔC^*		
	K^+	Ca^{2+}	Mg^{2+}
Tris-HCl	6.5±3.0	3.9±0.2	0.71±0.06
Tris-maleate	8.8±2.7	4.6±0.2	0.64±0.00
Deionized water	0.0±0.0	0.0±0.0	0.00±0.00

ΔC^*: Averaged decrease in the concentration of respective ion in *Chara* internodal cells (n=6) due to leakage in terms of mol m^{-3}.

Percentages of survival in CHES and CAPS—Fig. 9 shows the percentage of survival of the *Chara* internodal cells in 10, 20 and 50 mol m^{-3} CHES pH buffer solutions at pH 7.4, and those in 10 and 20 mol m^{-3} CHES pH buffer solutions at pH 9.8. The K^+ concentrations of 10, 20 and 50 mol m^{-3} CHES pH buffer solutions at pH 7.4 were only 0.1, 0.2 and 0.5 mol m^{-3}, respectively, and the CHES pH buffer solutions of such concentrations were almost nontoxic to the *Chara* internodal cells. CHES pH buffer solutions of 10 and 20 mol m^{-3} at pH 9.8 contained 8.4 and 16.8 mol m^{-3} K^+, respectively. At pH 9.8, the survival percentage of the *Chara* internodal cells decreased more steeply with time with an increase in the concentration of the CHES pH buffer solution. Also, the survival percentage of the cells at pH 9.8 decreased much more steeply as a function of time than those in CHES pH buffer solutions at pH 7.4 of the same concentrations.

In CAPS pH buffer solutions of 10 and 20 mol m^{-3}, all of the *Chara* internodal cells survived at pH 9.7 for more than a week (Fig. 10). This indicates that CAPS itself is not strongly toxic and a high pH of 9.7 is not one of the main causes of *Chara* internodal cell death. However, 50 mol m^{-3} CAPS containing 9.0 mol m^{-3} K^+ was very toxic.

Percentage of survival in KCl solutions—KCl solutions of 5.0, 15.0 and 25.0 mol m^{-3} (pH ca. 5.3) could kill the *Chara* internodal cells (Fig. 11). The higher the concentration of KCl, the more steeply the percentage of survival decreased with time.

Effects of Ca^{2+} on the percentages of survival in HEPES (pH 8.0) and CHES (pH 9.8) pH buffer solutions—All of the *Chara* internodal cells immersed in 20 mol m^{-3} HEPES pH buffer solution (pH 8.0), which was much more toxic than 10 mol m^{-3} HEPES pH buffer solution (pH 8.0), died within several days. However, addition of 0.5 mol m^{-3} Ca^{2+} to 20 mol m^{-3} HEPES pH buffer solution (pH 8.0) increased the percentage of survival of the *Chara* internodal cells to 90% (Fig. 12). Addition of 0.5 or 1.0 mol m^{-3} Ca^{2+} to CHES pH buffer solution (pH 9.8) also delayed the decrease in the survival percentage of the *Chara* internodal cells as a function of time. However, the effect of Ca^{2+} on the suvival percentage of the *Chara* internodal cells in CHES pH buffer solution was weaker than that on the survival percentage in 20 mol m^{-3} HEPES pH buffer solution (Fig. 12).

Discussion

Tris and Good pH buffers have been widely used in physiological, biophysical and biochemical studies. However, this has been done without checking their direct effects on the biochemical molecules in question or their toxic effects on the cells used. The present experiments showed that Tris and one of the Good pH buffers, HEPES, are toxic to the *Chara* internodal cells even at 10 mol m^{-3} when the cells are externally exposed to them. This indicates that Tris and HEPES pH buffers can affect the cell membrane.

The protoplasmic streaming of *Chara* internodal cells did not stop in calcium salt solutions of high concentrations such as 10 mol m^{-3} $CaSO_4$, 10 and 80 mol m^{-3} $CaCl_2$, and 10 and 80 mol m^{-3} $Ca(NO_3)_2$, and even in long-term plasmolysed *Chara Braunii* cells in $Ca(NO_3)_2$ or $CaCl_2$ solution of high concentrations (Hayashi and Kamitsubo 1959) kept under continuous light as well as in the dark. The concentration of the Ca^{2+} in the cytoplasm of *Chara* internodal cells is thought

to be as low as below 10^{-6} mol m^{-3}, but as high as ca. 10 mol m^{-3} when measured by a direct chemical method using the atomic absorption method (Okihara and Kiyosawa 1988). The electrochemical potential difference across the *Chara* cell membrane for Ca^{2+} calculated from the measured Ca^{2+} concentrations inside and outside the *Chara* internodal cell, and the electrical membrane potential difference indicates that the Ca^{2+} in APW should be forced to enter the internodal cell and stop the protoplasmic streaming of the cell. The fact that protoplasmic streaming of intact *Chara* cells in APW and calcium salt solutions of various types at high concentrations continues for a long time indicates that the normal function of the *Chara* cell membrane is to prevent a large amount of Ca^{2+} from entering the cell, which would stop the protoplasmic streaming, but to allow enough Ca^{2+} to enter to instantaneously stop the protoplasmic streaming on excitation by some stimulus, such as an electrical current (Barry 1968, Hayama *et al.* 1979, Kikuyama and Tazawa 1983, Lunevsky *et al.* 1983). The gradual decrease in the velocity of protoplasmic streaming of *Chara* internodal cells, followed by its stopping, in Tris buffer solutions or HEPES pH buffer solutions without any special stimulus indicates that the normal functions of the *Chara* cell membrane are disturbed by Tris and HEPES pH buffers.

Recently, Katsuhara and Tazawa (1987) showed that internal ATP at 1 mol m^{-3} was necessary for the cell membrane of tonoplast-free *Nitellopsis* cells to maintain salt (NaCl) tolerance in 100 mol m^{-3} NaCl in the presence of external 10 mol m^{-3} Ca^{2+}. The velocity of the protoplasmic streaming of *Chara* internodal cells immersed in a Tris pH buffer solution (pH 7.1) gradually decreased with time, followed by cell death (Fig. 5). These phenomena were observed in the HEPES solutions as well. Externally added 0.5 mol m^{-3} $CaSO_4$ could keep the survival percentage of the internodal cells at 100% and maintain the normal velocity of the protoplasmic streaming (Fig. 5). This fact suggests that Tris pH buffers cause disturbance of the normal membrane transport processes, and induce leakage of ions and some biochemical components including ATP (Williamson 1975, Shimmen 1978) responsible for maintaining the normal protoplasmic streaming and keeping the *Chara* cell alive, and that externally supplied Ca^{2+} and intracellular ATP prevent Tris pH buffers from inducing the leakages of ions and some biochemical components including ATP (cf. Katsuhara and Tazawa 1987).

From the viewpoint stated above, we examined whether or not the leakage of K^+, Ca^{2+} and Mg^{2+} from the *Chara* internodal cell occurs in 10.0 mol m^{-3} Tris-HCl (pH 7.1) and Tris-maleate (pH 7.1) solutions. Our findings (Table 1) together with those of previous work (Kiyosawa and Adachi 1990) show that the leakage of K^+ is from the cytoplasm (Katsuhara and Tazawa 1986) which contains much K^+ (MacRobbie 1962, Spanswick and Williamson 1964, Kishimoto and Tazawa 1965, Tazawa *et al.* 1974, Okihara and Kiyosawa 1988). However, most of the calcium ions liberated will be from the cell wall, to which a considerably large amount of calcium is bound (Kiyosawa and Adachi 1990, Reid and Smith 1992), and/or the cytoplasm. The liberated magnesium ions are thought to come from the cell wall and/or the cytoplasm. Leakage of the intracellular ATP has not been measured yet.

When *Nitellopsis* cells are transferred from artificial pond water (APW′: 0.1 mol m^{-3} KCl, 0.1 mol m^{-3} NaCl, 0.1 mol m^{-3} $CaCl_2$; pH ca. 5.3) to 100 mol m^{-3} NaCl+APW′, the concentration of K^+ in the cytoplasm decreases while that of Na^+ increases immediately after the transfer. This effect of the external NaCl of 100 mol m^{-3} on the cytoplasmic K^+ and Na^+ concentrations can be nullified by addition of 10 mol m^{-3} $CaCl_2$ to the external 100 mol m^{-3} NaCl (Katsuhara and Tazawa 1986). The same effects of the external 70 mol m^{-3} NaCl on the survival of *Chara corallina* internodal cells have been reported together with a decrease in the K^+ concentration and an increase in the Na^+ concentration in the vacuole occurring a few days or several

days after transfer from artificial pond water (APW″: 1.0 mol m^{-3} NaCl, 0.05 mol m^{-3} K_2SO_4, 0.1 mol m^{-3} $CaSO_4$, 5.0 mol m^{-3} HEPES titrated to pH 7.0 with NaOH) to 70 mol m^{-3} NaCl+APW″ (Tufariello *et al.* 1988). In this case, the coexistence of 7.1 mol m^{-3} Ca^{2+} is enough to prevent an increase in the vacuolar Na^+ concentration and a simultaneous decrease in the vacuolar K^+ concentration.

Cramer *et al.* (1985) and Lynch *et al.* (1987), measuring the fluorescence of Ca^{2+}-chlorotetracycline from intact cotton root hairs and protoplast suspension of corn roots, reported that externally supplied Na^+ reduced the amount of calcium binding to the plasmalemma of cotton root cells and of corn root protoplasts, respectively.

Although we have no direct and clear evidence as to whether the externally supplied alkali metal and alkali earth metal ions affect the calcium bound only to the outer surface of the cell membrane, or even the calcium inside the cell membrane, the available experimental results (cf. Katsuhara and Tazawa 1986, Tufariello *et al.* 1988, Kiyosawa and Adachi 1990) including the present ones indicate that externally supplied Ca^{2+} can prevent the disturbance of membrane integrity by externally supplied alkali metal ions, some of the alkali earth metal ions and Tris ion. These observations suggest that the externally supplied Ca^{2+} affects the cell membrane itself and can help maintain normal membrane functions by suppressing calcium liberation from the cell membrane in electrolyte solutions of alkali metal ions, some alkali earth metal ions (cf. Kiyosawa and Adachi 1990) and Tris ion.

Kiyosawa and Adachi (1990) have shown that KCl, $MgCl_2$ and $BaCl_2$, which killed the *Chara* internodal cells even at considerably low concentrations, caused liberation of almost all of the calcium bound to the *Chara* cell wall within an hour (cf. also Reid and Smith 1992). This also occurred with $SrCl_2$ added at 80 mol m^{-3}, which could keep the *Chara* internodal cells alive for more than two weeks, but did not occur with externally supplied Ca^{2+} at 80 mol m^{-3}. Also, externally supplied Ca^{2+} of 0.5-1.0 mol m^{-3} more or less inhibited liberation of the bound calcium in KCl, $MgCl_2$ and $BaCl_2$ solutions. The findings suggested that Sr^{2+} could maintain membrane integrity in a manner different from that of Ca^{2+} (Kiyosawa and Adachi 1990) or that Sr^{2+} could suppress the calcium release from the cell membrane by K^+, Mg^{2+} or Ba^{2+} but its action differed from that of externally supplied Ca^{2+}.

Externally supplied Sr^{2+}, which can maintain *Chara* cell membrane integrity in KCl, NaCl, $MgCl_2$ and $BaCl_2$, is not effective at 5 mol m^{-3} in Tris pH-buffer solutions (Fig. 3). Thus, if the viewpoint is taken that externally supplied Sr^{2+} can suppress the calcium release from the cell membrane caused by K^+, Mg^{2+} or Ba^{2+}, this observation can be simply and reasonably explained. Tris pH buffers liberate the calcium bound to the *Chara* cell membrane so effectively that it cannot be suppressed by externally supplied 5 mol m^{-3} Sr^{2+}, but can be by externally supplied 0.5 mol m^{-3} Ca^{2+}, as shown in Fig. 3. Further studies using *Chara* cell membrane or cell membrane fragments of other plants are needed to verify this.

On interpreting the effects of Good pH buffer solutions on *Chara* internodal cells obtained in the present study, together with those of previous work, the following can be considered to be important: (1) pH value, (2) K^+ concentration, and (3) the concentration of the Good buffer itself.

Chara internodal cells can survive in an acidic APW at pH 4.72 (Kiyosawa 1990). Thus, the pH of 5.24 of a simple HEPES buffer solution should not be low enough to kill *Chara* internodal cells (Fig. 6). It must be the action of the HEPES itself that kills that *Chara* internodal cells (cf. Fig. 6). As shown in Fig. 6, an increase in the number of dead *Chara* internodal cells in simple HEPES solutions after the 5th day suggests that HEPES interacts directly with the cell membrane components or channels, not mainly via lowering of the pH of the bathing solution, as reported for the isolated semicircular canal of the frog (Norris and Guth 1985), *Helix* neurons (Witte *et al.*

1985), and cultured *Drosophila* neurons (Yamamoto and Suzuki 1987).

Fig. 10 shows that the *Chara* internodal cells can survive in 10 mol m^{-3} and 20 mol m^{-3} CAPS pH buffer solutions at pH 9.7. These findings together with an earlier one (Kiyosawa 1990) indicate that *Chara* internodal cells can survive in solutions where the pH is as low as 4.72 to as high as 9.7. Therefore, in the present experiments, the pH of the test solutions probably was not the main determinant of the percentage of survival of the *Chara* internodal cells, although it may have been a contributing factor. Compared with the results of Fig. 11, the fact that the percentage of the survival of the *Chara* internodal cells decreased at a more rapid rate with time in the Good pH buffer solutions of higher concentrations seems to be explainable mainly in terms of the higher K^+ concentration in the Good pH buffer solutions. One of the main actions of the K^+ in HEPES and CHES seems to be liberation of the calcium bound to the *Chara* cell wall (cf. Kiyosawa and Adachi 1990, Reid and Smith 1992) and the cell membrane. From this point of view, the survival percentage of the *Chara* internodal cells was examined in 20 mol m^{-3} HEPES (pH 8.0)+0.5 mol m^{-3} $CaSO_4$, 20 mol m^{-3} CHES (pH 9.8)+0.5 mol m^{-3} $CaSO_4$ and 20 mol m^{-3} CHES (pH 9.8)+1.0 mol m^{-3} $CaSO_4$ in comparison with those in 20 mol m^{-3} HEPES alone (pH 8.0) and 20 mol m^{-3} CHES alone (pH 9.8) (Fig. 12).

The results of Fig. 12 clearly show that the toxicity of 20 mol m^{-3} HEPES (pH 8.0) pH buffer alone, which was more toxic than 10 mol m^{-3} HEPES pH buffer solution alone, was almost nullified when 0.5 mol m^{-3} Ca^+ was present for more than 7 days, as shown with Tris pH buffers (Fig. 3). These observations further suggest that the blocking effects of HEPES on the ion channels themselves (cf. Witte *et al.* 1985, Yamamoto and Suzuki 1987, Hanrahan and Tabcharani 1990) may have been reduced by addition of Ca^{2+} to the solution as well. These observations lead to another important conclusion that all of the pH buffers which contain K^+ (KOH), Na^+ (NaOH) or ions capable of liberating the calcium binding to the cell membrane may be toxic to cells and modify the membrane fragments.

However, the toxicity of a simple 20 mol m^{-3} CHES (pH 9.8) pH buffer, which was very toxic to *Chara* internodal cells, could not be nullified, although it was slightly reduced by addition of 0.5 or 1.0 mol m^{-3} Ca^{2+}. In the case of 20 mol m^{-3} CHES pH buffer solution to which 0.5 mol m^{-3} or 1.0 mol m^{-3} $CaSO_4$ was added, many crystals of rectangular prisms, flower-like hexagons, dumb-bells, and ellipsoid and indefinite forms, were observed at the bottom of the vessel, on the internodal cell, and on the surface of the solution. These crystals were also observed in solutions of 20 mol m^{-3} CHES (adjusted at pH 9.8 by KOH or NAOH)+1.0 mol m^{-3} $CaCl_2$ or $Ca(NO_3)_2$. However, no crystals were observed in 20 mol m^{-3} CHES (to which no KOH or NaOH was added)+5.0 mol m^{-3} $CaSO_4$ solution. In other words, even when 0.5 mol m^{-3} or 1.0 mol m^{-3} Ca^{2+} was added to 20 mol m^{-3} CHES pH buffer solution, the concentration of the Ca^{2+} in the CHES solution was lower than calculated, due to the formation of CHES-Ca crystals.

A previous paper (Kiyosawa and Adachi 1990) reported that *Chara* internodal cells kept in 10 mol m^{-3} HEPES pH buffer solution remained alive for more than 10 days. This may have resulted from the use of *Chara* internodal cells grown in the sun which had calcium-rich cell walls and also because the internodal cells had been immersed in the same HEPES solution for a week. These findings and considerations led to the conclusion that Tris ions and HEPES-KOH interact with the *Chara* cell membrane and the cell wall to induce leakage of K^+, and probably Ca^{2+} and Mg^{2+}, from inside the cell and also liberation of Ca^{2+} and Mg^{2+} bound to the cell wall and probably to the cell membrane. This finally leads to stopping of the protoplasmic streaming and death of the *Chara* internodal cell. Leakages of such biochemical components as ATP-Mg, some inorganic and organic ions

responsible for keeping the cytoplasm and the cell membrane normal and maintaining the normal protoplasmic streaming may also occur from inside the cell through the cell membrane in HEPES as well as Tris pH buffer solutions. Thus, when Tris or HEPES pH buffer solution is used with or without other electrolyte(s), a moderate amount of Ca^{2+} should be added to the solution to prevent modification of the biomembranes in such solutions.

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清沢桂太郎：車軸藻節間細胞に対する pH 緩衝液の毒性

これまで膜生理学，生物物理学及び，生化学でかなり頻繁に用いられてきた Tris-HCl (pH 7.0), Tris-maleate (pH 7.0), 及び，Good の pH 緩衝液である HEPES (pH 6.8–8.2) の車軸藻節間細胞に対する毒性を調べた。各 pH 緩衝液は，10–20 mol m^{-3} の濃度で，1 日から 4 ～ 5 日の間に車軸藻節間細胞の原形質流動を徐々に減速させ，停止させた後，同節間細胞を死に至らしめた。しかし，0.5–1.0 mol m^{-3} の Ca^{2+} を加えると，上記 pH 緩衝液の車軸藻節間細胞に対する毒性をなくした。Good 緩衝液の毒性は，同緩衝液の pH を合わせるために加える KOH の K^+ が，Tris 緩衝液の場合は Tris イオンが，細胞膜に結合している Ca^{2+} を遊離させて細胞膜の機能を損なうためと推定された。(560 豊中市待兼山町1–1　大阪大学基礎工学部生物工学科)

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Intracellular cadmium sequestration by the heavy metal-tolerant green algae *Chlorella vulgaris* and *Uronema confervicolum*

Adam T. Wilczok, Makoto M. Watanabe[1], Sanae Kawahara, Kazuo T. Suzuki[2] and Kioshi Sugahara

National Institute for Environmental Studies, Onogawa 16–2, Tsukuba, Ibaraki, 305 Japan

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Chlorella vulgaris and *Uronema confervicolum* isolated from a metal polluted river were examined for induction of metal-binding peptide formation by exposing to 20 μM of cadmium under laboratory conditions. After three weeks of cultivation 608 mg kg^{-1} and 597 mg kg^{-1} of Cd were found in dried cells of *C. vulgaris* and *U. confervicolum*, respectively, when analyzed by the atomic absorption method. About 50% of intracellular Cd in both species was associated with the 170000 *g* cell supernatant. Distributions of cadmium in the cell-soluble fractions were determined by high-performance liquid chromatography (HPLC) with detecting by atomic absorption (AAS) or inductively coupled argon plasma-atomic emission spectrometry (ICP). Significant changes in HPLC-ICP profiles of sulfur and metals in algal cytosolic fractions were induced by the exposure to cadmium. Only one metal-binding peak was observed in *U. confervicolum*, while *C. vulgaris* induced formation of three cadmium-binding peaks on a gel filtration column. High sulfur content, heat stability and high 254 : 280 asbsorbance ratio of the induced peaks suggest similarity of the isolated Cd-binding compounds to metallothioneins found in other algae and higher plants.

Key Index Words: Cd-binding compounds—Cd-tolerance—Chlorella vulgaris—Uronema confervicolum.

When exposed to heavy metals many organisms can synthesize metallothioneins (MTs)—proteins, which play a key role in metal detoxification as well as in metal ions homeostasis (Reddy and Prasad 1990, Robinson 1989). Metallothioneins are low molecular weight heat-stable proteins characterized by high contents of heavy metals and cysteine, absence of aromatic amino acids, high 254 : 280 absorbance ratio typical for thiolate complexes, and high affinity toward anion exchangers (Kagi and Kojima 1987).

Metal-binding proteins or peptides are present or inducible in various kinds of non-mammalian species (Hamer 1986) and plants (Grill *et al.* 1987, Rauser 1990). In plants they are no primary gene products and are synthesized enzymatically from glutathione by the specific enzyme γ-glutamylcysteine dipeptydyl transpeptidase (Grill *et al.* 1989). Algal metallothioneins, most often called phytochelatins, are defined as class III MTs: nontranslationally synthesized metal-thiolate polypeptides (Fowler *et al.* 1987). Metallothionein-like metal-binding proteins, phytochelatins or other less precisely defined proteins/peptides have been found in different algae: *Anacystis nidulans, Bumilleriopsis filiformis, Chlamydomonas reinhardtii, Chlorella ellipsoidea, Chlorella fusca, Chlorella pyrenoidosa, Dunaliella bioculata, Euglena gracilis, Fragilaria crotonensis, Monoraphidium minutum, Navicula pelliculosa, Phaeodactylum tricornutum, Porphirydium cruentum, Sargassum muticum, Scenedesmus quadricauda, Stichococcus bacillaris*, and *Synechococcus* sp. (Gekeler *et al.* 1988, Hart and Bertram 1980, Heuillet *et al.* 1988, Howe and Merchant 1992, Kawaguchi and Maita 1990, Nagano *et al.* 1984, Olafson *et al.* 1980, Reddy

[1] Address for reprint requests.

[2] Present address: Faculty of Pharmaceutical Sciences, Chiba University, Yayoi, Chiba, 263 Japan.

and Prasad 1989, Weber *et al.* 1987). Thus, the ability to synthesize metal-binding proteins or peptides seems common in the whole division of algae. While these compounds may be functionally analogous to animal MTs, their structure and biosynthesis are fundamentally different. Metal-binding compounds isolated from algae are supposed to be of identical structure to phytochelatins isolated from higher plants and described as $(\gamma$-Glu-Cys$)_n$-Gly (n=2 to 11) (Gekeler *et al.* 1988). Amino acid composition of *C. ellipsoidea* MTs consists of mainly glutamic acid or glutamine, arginine, glycine, and half-cysteine (Nagano *et al.* 1984), while in the other algae only glutamic acid, cysteine and glycine were found (Gekeler *et al.* 1988, Maita and Kawaguchi 1989). Molecular weight of different algal metal-binding proteins (or peptides) determined by gel filtration or SDS-electrophoresis is in the range of 1.8-20 kDa and markedly depends on the ionic strength applied as well as on the species tested (Grill *et al.* 1987, Hart and Bertram 1980, Lue-Kim and Rauser 1986, Murasugi *et al.* 1981, Nagano *et al.* 1984, Olafson *et al.* 1980).

Algal tolerance to heavy metals is correlated with the metal concentration in the environment where the algae were isolated. The isolates of Bacillariophyceae, Chlorophyceae, and Charophyceae from metal-polluted sites are mostly tolerant to the pollutant metal and retain their tolerance even for 2 years of subculture in the normal cultivation medium (Takamura *et al.* 1989, 1990). In particular, the chlorophycean algae, *C. vulgaris* and *U. confervicolum* can grow in high concentrations on Zn, Cu, and Cd. When tested for photosynthetic activity decrease, the concentrations of Cd equal to 25.0 mg l^{-1} for *C. vulgaris* and 16.6 mg l^{-1} for *U. confervicolum* caused 50% inhibition of photosynthesis (Takamura *et al.* 1989).

Recently, simultaneous determination of multielements including heavy metals and sulfur in different biological samples by HPLC-AAS and HPLC-ICP was proven as the useful tool in metal-binding proteins investigation (Sunaga *et al.* 1987, Suzuki 1991, Suzuki *et al.* 1987, 1988). In the present study, as a first step in our studies on characterization of metallothionein-like metal-binding compounds induced in *C. vulgaris* and *U. confervicolum*, we tried to determine distribution profiles of Cd and other elements in the supernatant derived from the algae by using both the HPLC-AAS and HPLC-ICP methods.

Materials and Methods

Unialgal cultures of *Chlorella vulgaris* Beij. (strain NIES PS-511) and *Uronema confervicolum* Lagerh. (NIES PS-526) were obtained from the Microbial Culture Collection at the National Institute for Environmental Studies (NIES). The strains were originally isolated from heavy metal polluted Miyata river in 1987 and deposited at the NIES-Collection (Takamura *et al.* 1989, Watanabe and Satake 1991).

Cells were cultured axenically for three weeks in the "C" medium composed of $Ca(NO_3)_2 \cdot 4H_2O$-150 mg l^{-1}, KNO_3-100 mg l^{-1}, β-Na_2-glycerophosphate-50 mg l^{-1}, $MgSO_4 \cdot 7H_2O$-40 mg l^{-1}, vitamin B_{12}-0.1 μg l^{-1}, biotin-0.1 μg l^{-1}, thiamine·HCl-10 μg l^{-1}, $FeCl_3$-588 μg l^{-1}, $MnCl_2 \cdot 4H_2O$-108 μg l^{-1}, $ZnSO_4 \cdot 7H_2O$-66 μg l^{-1}, $CoCl_2 \cdot 6H_2O$-12 μg l^{-1}, $Na_2MoO_4 \cdot 2H_2O$-7.5 μg l^{-1}, Na_2EDTA$\cdot 2H_2O$-3 mg l^{-1}, and tris (hydroxymethyl) aminomethane (Tris)-500 mg l^{-1} (pH 7.5) (Watanabe and Satake 1991) in the foam stopped 2 l Erlenmeyer flasks under illumination of ca. 100 μmol photon m^{-2} s^{-1} with a photoperiod of 12 h light: 12 h dark from the daylight fluorescent tubes at 20°C. For Cd-treatment $CdCl_2$ was added at a concentration of 20 μM at the beginning of each experiment.

Algae were harvested by filtration through a 1.0 μm Nucleopore filter under reduced pressure, washed with 0.1 M Tris-HCl buffer (pH 7.4) and homogenized in 10 ml of the same buffer using a VR 200 P homogenizer (Tomy-Seiko, Tokyo) in an atmosphere of nitrogen gas under ice-water cooling. Dry weight was determined by drying samples to a constant weight as recommended by Sorokin

(Sorokin 1973). Three 0.5 ml aliquots of each homogenate were wet-digested with 0.5 ml of mixed acids (HNO_3 : $HClO_4$, 5 : 1). The remaining portions of homogenates were diluted with Tris-HCl buffer (0.1 M, pH 7.4) to the dry mass concentration 20 mg ml^{-1}. The 7 ml aliquots were centrifuged at 170000 *g* for 60 min at 2°C. An atomic absorption spectrometer equipped with graphite furnace (Shimadzu AA 640-12) was used to measure metal concentration in the cultivation medium, digested homogenate, and crude supernatant.

The separation of algal Cd-binding compounds was performed on two kinds of columns and elution conditions; the GS column (a gel filtration column with low interactions between column coating and substrates by elution at neutral buffer conditions) and the SW column (a gel filtration column with stronger interactions of metals between column material and substrates by elution at slightly basic pH, which better separates rat metallothioneins into isoforms) (Suzuki *et al.* 1980). Aliquots (0.2 ml) of the 170000 *g* supernatant were applied on an Asahipak GS-320 column (7.6 × 500 mm; Asahi Chemical Industry, Kawasaki, Japan) and an SW column (TSK gel G3000SW, 7.5 × 600 mm with a guard column of 7.5 × 75 mm; Tosoh Co. Ltd., Tokyo, Japan). A Tris-HCl buffer solution (10 mM, pH 8.0 containing 0.1% NaN_3) was used as the mobile phase for the SW column, while 0.9% NaCl solution containing 0.05% NaN_3 was used for the GS column. The mobile phases were degassed with a Shodex Degas degasser (Showa Denko Co., Tokyo, Japan). The flow rate was maintained at 1.0 ml min^{-1} by a Gasukuro Kogyo HPLC Model 576 (Gasukuro Kogyo Inc., Tokyo, Japan). The eluate absorbances at 254 and 280 nm were measured with a programmable Spectra 200 detector (Spectraphysics) and the eluate was subsequently introduced directly into an atomic absorption spectrometer with an acetylene flame (Hitachi 170-50 A) or into a nebulizer tube of a Daini Seikosha 2500 ICP spectrometer (Seiko Instruments and Electronics Ltd., Tokyo, Japan). All the concentrations of elements were determined simultaneously according to the method described elsewhere (Sunaga *et al.* 1987, Suzuki *et al.* 1988, Suzuki 1991). The stored data were processed and converted into distribution profiles using a self-developed software and a personal computer (PC 9801, NEC, Tokyo) and XY-plotter (FP 5301R, Graphtec, Tokyo). The SW column was precalibrated with the previously described Cd-exposed rat liver supernatant (Suzuki *et al.* 1987) and aprotinin, cytochrome c, carbonic anhydrase, and albumin—gel filtration molecular weight markers (Sigma, St. Louis, USA). To determine a heat-stability of the isolated metal-binding compounds, the cell supernatants were heat-treated (70°C, 10 min) under nitrogen gas, centrifuged (5000 *g*, 10 min) and analyzed by the HPLC-ICP on the SW column as described above.

Results and Discussion

Cadmium added into the cultivation medium was easily incorporated into the algal cells. After three weeks of cultivation 608 mg kg^{-1} and 597 mg kg^{-1} of Cd were found in dried cells of *C. vulgaris* and *U. confervicolum*, when analyzed by the atomic absorption method. 49.5% of intracellular Cd in *C. vulgaris* and 51.4% in *U. confervicolum* was associated with the 170000 *g* cell supernatant subjected for HPLC separation. Distribution of metal bound to cytosolic fraction was determined by HPLC-AAS and for more detailed characterization by HPLC-ICP. The elution profiles of Cd and absorbance recorded at 254 and 280 nm during separation of Cd-exposed *C. vulgaris* and *U. confervicolum* supernatants on the GS column are presented in Fig. 1. Both analyzed strains synthesized Cd-binding compounds. Cd-peak followed by the high absorbance at 254 nm was eluted at a retention time of 10.5 min on a GS-320 column in both species. The Cd-distribution profile in the supernatant obtained from Cd-treated *C. vulgaris* suggests the presence of isoforms or three successive metal-binding components of reten-

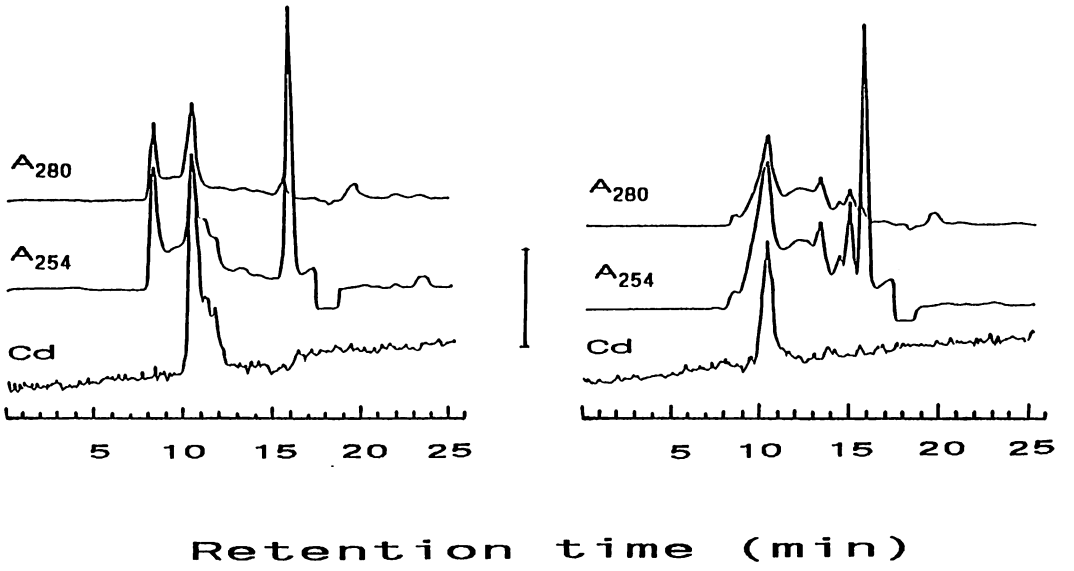

Fig. 1. Elution profiles of supernatants from Cd-exposed *C. vulgaris* (left) and *U. confervicolum* (right) on an Asahipak GS-320 column. Absorbances at 254 and 280 nm were recorded in the time course of analysis of metal-binding compounds. The vertical bar indicates the detector level (0.1 μg Cd ml^{-1}) by AAS.

tion times 10.5, 11.4, and 11.9 min, though two latter peaks were not well separated. This phenomenon was not observed in *U. confervicolum*, which bound Cd to the single peak only.

Figure 2 shows elution profiles of Cd and absorbance recorded at 254 and 280 nm during separation of Cd-treated *C. vulgaris* and *U. confervicolum* supernatants on the SW column. Three Cd-peaks were observed in *C. vulgaris*, while again only a single Cd-peak was found in *U. confervicolum*. From these results, the SW column was found more suitable for separation of cadmium-binding compounds and therefore chosen for HPLC-ICP measurements and heat-treatment experiments.

Figure 3 shows HPLC-ICP results obtained for Cd-treated and control *C. vulgaris* supernatants separated on the SW column.

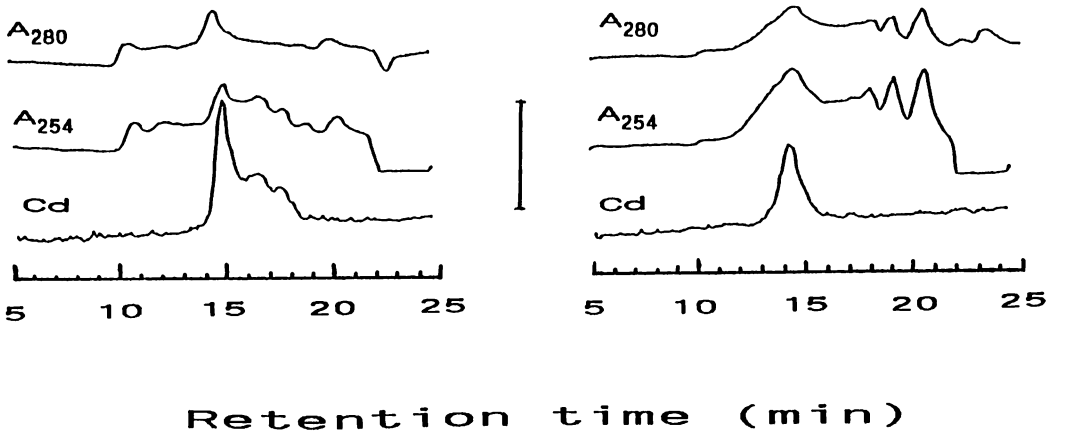

Fig. 2. Elution profiles of Cd-exposed *C. vulgaris* (left) and *U. confervicolum* (right) on a G3000SW column. The detector level (0.1 μg Cd ml^{-1}) by AAS is shown by the vertical bar.

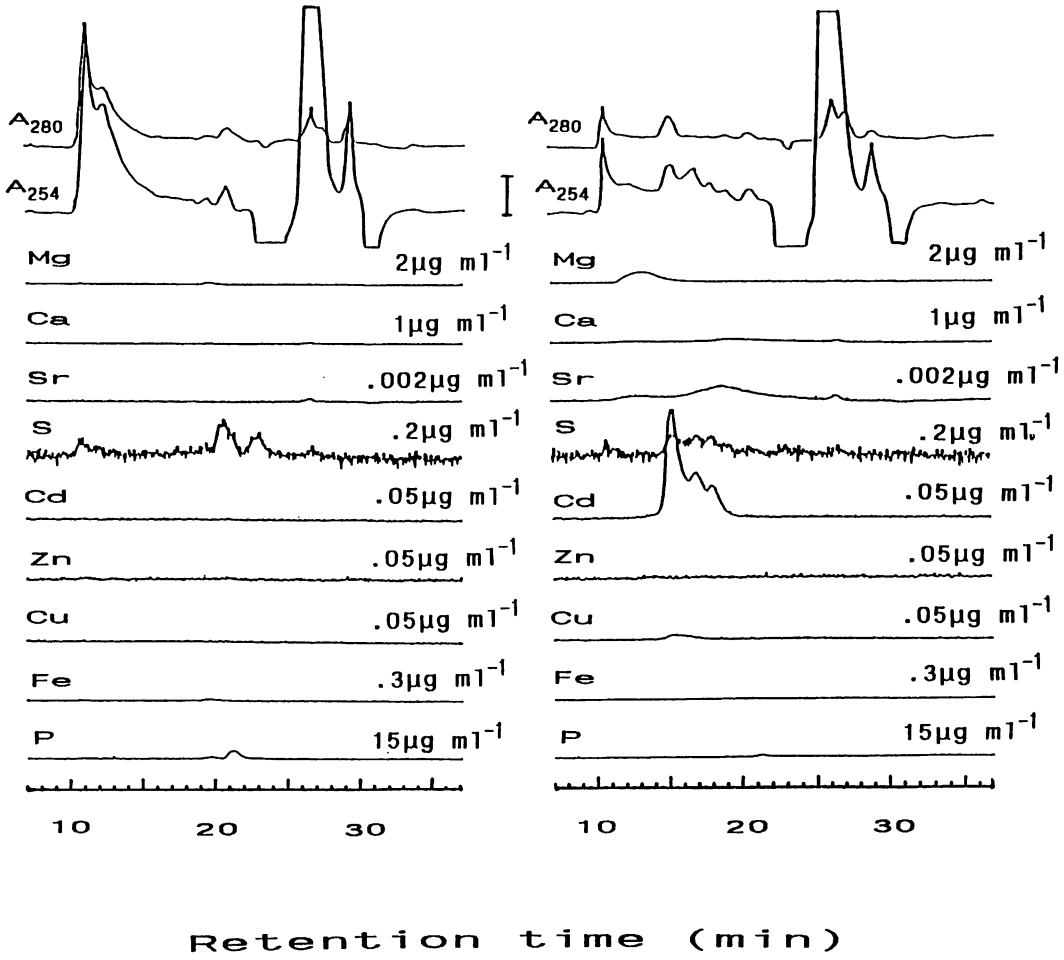

Fig. 3. HPLC-ICP profiles on a G3000SW column for the supernatants of *C. vulgaris*. Cells grown in the absence of Cd (left), cells exposed to 20 μM of Cd (right) for 3 weeks. Absorbances at 254 and 280 nm recorded in arbitrary units. The vertical bar corresponds to the detector levels of the respective elements (eg., for Cd the detector level is 0.05 $\mu g\ ml^{-1}$).

Metal-binding components were again eluted as three successive fractions of retention times 15.2, 16.9, and 17.8 min respectively. The low amounts of Cu found in the Cd-binding components in both algae (see also Fig. 4) suggest that the induced compounds could bind and concentrate Cu despite the very low concentration of Cu in the medium. It must be noticed, that the cultivation medium used in the present experiment did not contain Cu added as a microelement and its concentration was below the detection limit by AAS. Nagano *et al.* (1984) observed that Cu co-eluted with Cd-binding peptides, when the algae were supplied with both metals. On the other hand Zn, known phytochelatin formation inducer in *Chlorella* and *Scenedesmus* (Gekeler *et al.* 1988), which was present as a trace element in the cultivation medium, was not co-eluted with Cd-binding fractions. Probably, the higher Zn concentration is required or some antagonisms exist between Cd and Zn affinity to the induced Cd-binding compounds. Cadmium-binding peaks were never detected in control cultures. Results obtained for *U. confervicolum* on the SW column (Fig. 4) again confirmed the induction of only one Cd-binding compound. Its

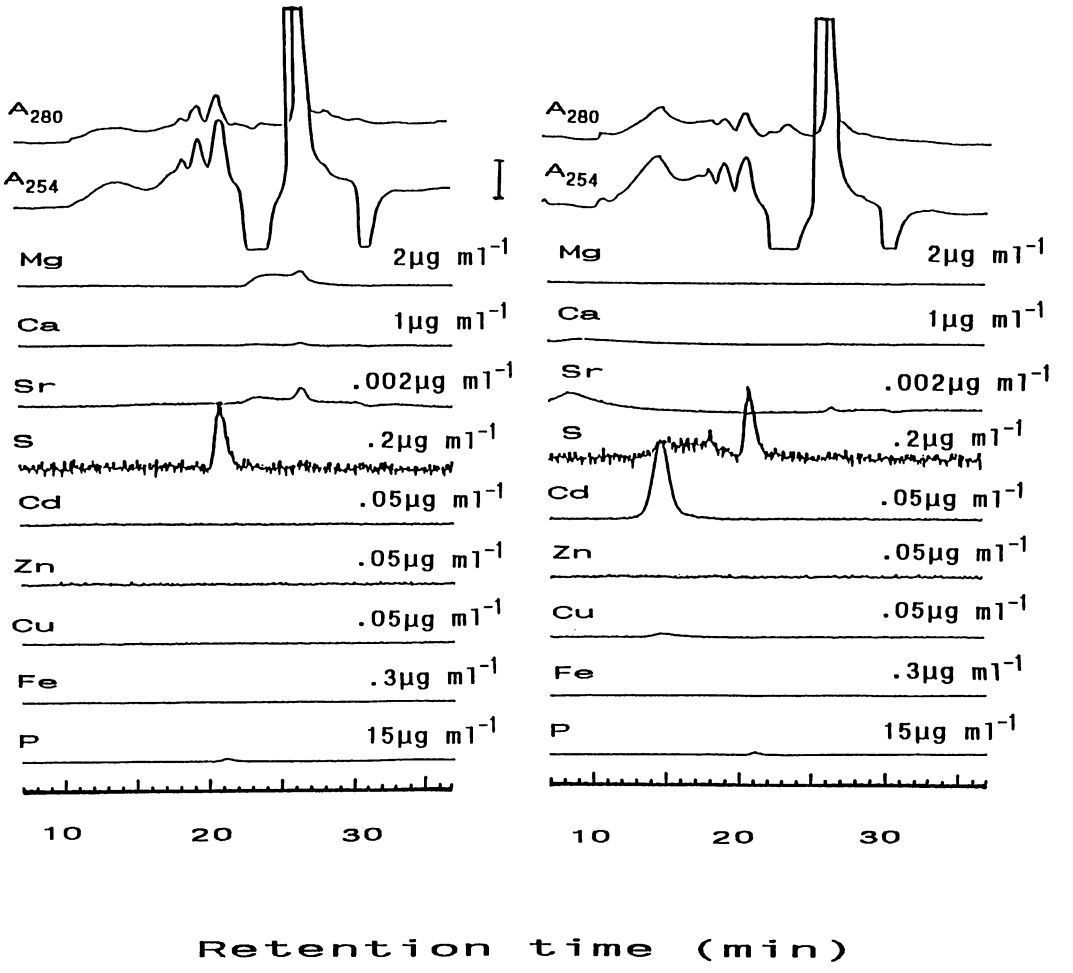

Fig. 4. HPLC-ICP profiles on a G3000SW column for the supernatants of *U. confervicolum*. Control cells (left) and cells exposed to 20 μM of Cd (right) for 3 weeks. Detector levels as in Fig. 3.

retention time (14.8 min) was shorter than that of the three Cd-binding fractions found in *C. vulgaris*.

Steffens (1990), analyzing the data on the occurrence of phytochelatins, concluded that the ability to synthesize phytochelatin in response to heavy metals is conserved from Orchidales, the most advanced group of higher plants, to the red, green, and brown algae. No other thiol-rich, heavy metal-binding compounds were detectable in the assayed plants, and phytochelatins synthesis was suggested as a generalized plant response to stress caused by heavy metals. Based on such assumption, we should not exclude that both strains examined in the present experiment formed phytochelatins with different numbers of γ-glutamyl-cysteine subunits, although the isolated Cd-binding compounds from *C. vulgaris* and *U. confervicolum* could have different characteristics. Wikfors *et al.* (1992) have reported recently that among five different Cd-tolerant algal species tested for Cd-binding polypeptides induction, only two of them, *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* produced such compounds. Cd-tolerant strains of *Isochrysis galbana, Pavlova lutheri*, and *Tetraselmis maculata* did not produce detectable amounts of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, what implies that other adaptive mechan-

isms may occur in some algae to ameliorate Cd stress. When influence of Cd on *Phaeodactylum tricornutum* was earlier analyzed by Kawaguchi and Maita (1990), two different Cd-binding peptides composed of glutamic acid, cysteine, and glycine were isolated. The chemical structure of these compounds was identical with phytochelatins induced in other algae and higher plants (Gekeler *et al.* 1988, Grill *et al.* 1987). The fact that *U. confervicolum* synthesized only one Cd-binding peak, while three Cd-peaks were found in *C. vulgaris*, suggests that the induction can be species-specific. The likelihood that metal stress in different algal species induces different specific adaptive mechanisms was earlier considered by Robinson (1989).

Shorter retention times of the Cd-binding compounds found in the Cd-treated algae compared to rat liver metallothionein-I and -II (Suzuki *et al.* 1987) obviously reflected their different chemical structure and composition. Metal-binding complexes isolated from plants are aggregates of heterogenous polypeptides and often behave like entities of 10–13.8 kDa in gel filtration media (Rauser 1990). Based on the determined structure and amino acid composition of phytochelatin isolated from *Rauvolfia serpentina*, Grill *et al.* (1987) concluded that the molecular weight of the native metal-containing phytochelatin complex was 2–4 kDa, rather than the 10 kDa often observed at low ionic strength.

As amino acid composition was not measured in the present study, the answer whether the isolated Cd-binding complexes should be classified as class II metallothioneins or phytochelatins remains too ambiguous, although some data support the latter possibility. Plants, opposite to animal species, always synthesize phytochelatins in response to heavy metals. However, Mehra *et al.* (1988) found that yeast *Torulopsis glabrata* exposed to Cu and Cd, formed both, metallothioneins and γ-glutamyl peptides for metal detoxification, and each system was regulated in metal-specific manner. Upon exposure to Cd, the cells synthesized only γ-glutamyl peptides. The coincidental synthesis of both above mentioned classes of compounds was never reported in algae or higher plants, but neither the technique applied in the present study nor methods recommended by Rauser (1991) or Grill *et al.* (1991) can resolve Cd-induced γ-glutamyl peptides and metallothioneins of the type found in *Torulopsis* (Mehra *et al.* 1988), *Saccharomyces cerevisiae* (Inouhe *et al.* 1991) or *Synechococcus* (Olafson *et al.* 1980).

A class II metallothionein isolated from metal tolerant aquatic insect, *Baetis thermicus* larvae was the heat-stable protein and most of other proteins in the supernatant were removable by heat-treatment without spoiling the metal binding capacity of metallothionein (Suzuki *et al.* 1988). Also the pea root (*Pisum sativum*) metallothionein produced in *E. coli* (Kille *et al.* 1991) seems to be a heat-stable protein. Heat-treatment to remove other "contaminating" proteins is commonly used in metallothionein purification procedures not only from animals but also from plant tissues (Rauser 1984, Rauser and Glover 1984). However, in the literature survey, we could not find any data on heat-stability of isolated metallothionein-like metal-binding complexes induced either in algae or in higher plants. In the present experiment, supernatants of Cd-exposed algae were heat-treated and the stability of metal-binding components was examined. Figure 5 illustrates HPLC-ICP profiles for heat-treated Cd-exposed algae obtained on the SW column. The isolated Cd-binding fractions were heat-stable components. The Cd, sulfur, and absorbance profiles did not change significantly after heat treatment (cf. Figs. 3-5). Minor changes in UV-profiles of Cd-exposed heat-treated supernatants were more likely observed in *C. vulgaris*. These results suggest a higher resistance to denaturation of the single Cd-binding component isolated from *U. confervicolum* compared with Cd-binding complex inducible in *C. vulgaris* and once more indicate different properties of metal-binding compounds induced in both algae observed.

Determination of amino acid composition of isolated Cd-binding compounds after their subsequent purification by reverse-phase

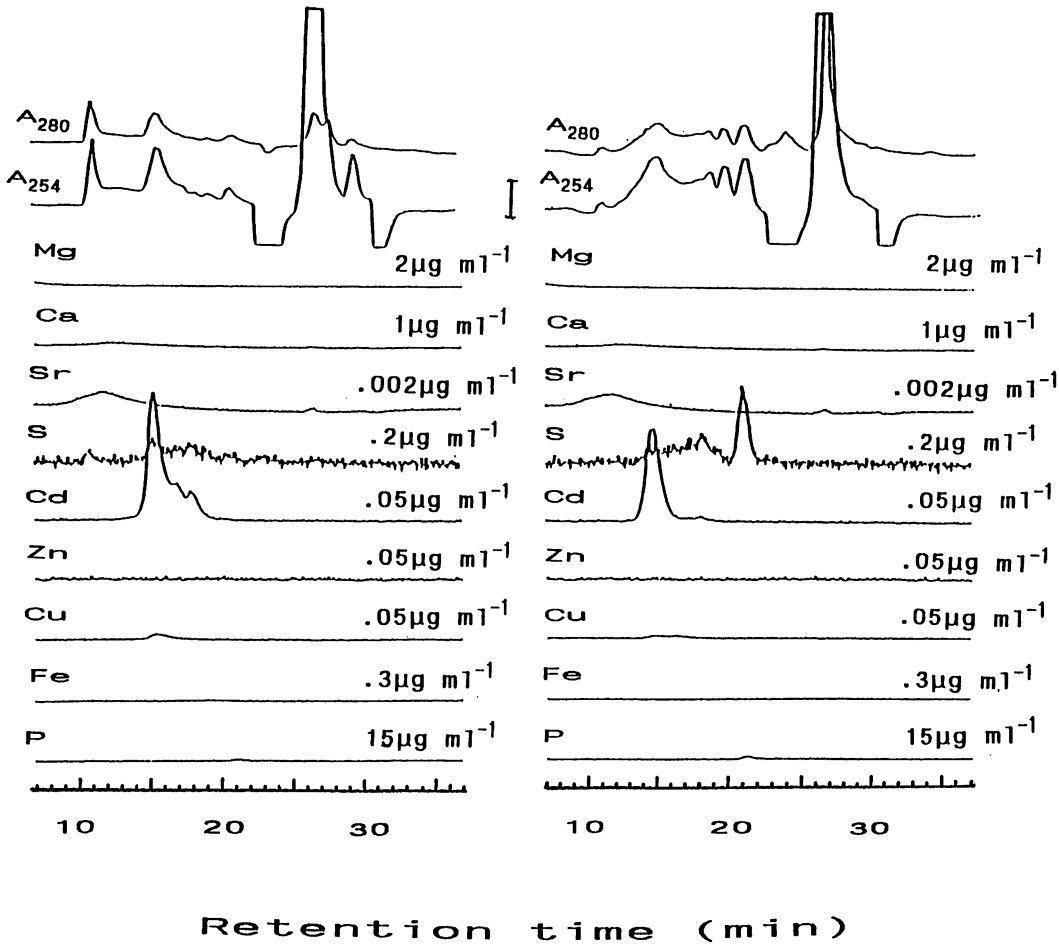

Fig. 5. Effect of heat-treatment on the distributions of elements in the supernatants of Cd-exposed algae. *C. vulgaris* (left) and *U. confervicolum* (right). Distribution profiles determined as in Fig. 3.

HPLC combined with thiol-rich compounds detection by Ellman's reagent will be a subject of our further experiments.

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Adam T. Wilczok・渡辺　信・川原早苗・鈴木和夫・菅原　淳：重金属耐性緑藻 *Chlorella vulgaris* と *Uronema confervicolum* による細胞内カドミウムの不活性化

重金属汚染河川から分離培養された緑藻 *Chlorella vulgaris* と *Uronema confervicolum* がカドミウムの存在下で誘導する重金属結合ペプチドの分析を，高速液体クロマトグラフィー (HPLC)，原子吸光装置 (AAS) 及び誘導結合プラズマ発光分析計 (ICP) を使って行った。*C. vulgaris* と *U. confervicolum* を 20 μM の塩化カドミウムが添加された培地で 3 週間培養し，細胞内のカドミウムを AAS で分析した結果，各々の細胞内には 608 mg kg^{-1} 及び 597 mg kg^{-1} のカドミウムが蓄積されていた。双方とも蓄積されたカドミウムの約50%は，170000 g の遠心で上清の画分に存在していた。この画分について，HPLC-ICP のシステムで分析した結果，*C. vulgaris* には 3 種類のカドミウム結合ペプチドが，*U. confervicolum* には 1 種類のカドミウム結合ペプチドが確認された。これらの誘導されたペプチドは，いずれもイオウを多く含有していること，熱安定性であること，254 nm と 280 nm での吸収率比が高いことから，藻類や高等植物で誘導されているメタロチオネインと類似のペプチドであると思われる。

(305 茨城県つくば市小野川16-2　国立環境研究所)

Jpn. J. Phycol. (Sôrui) 40: 239–244, September 20, 1992

Growth rates of *Gracilaria* species (Gracilariales, Rhodophyta) from Tosa Bay, southern Japan

Christine A. Orosco and Masao Ohno

Usa Marine Biological Institute, Kochi University, Usa, Tosa, Kochi, 781–11 Japan

Orosco, C. A. and Ohno, M. 1992. Growth rates of *Gracilaria* species (Gracilariales, Rhodophyta) from Tosa Bay, southern Japan. Jpn. J. Phycol. 40: 239–244.

The daily growth rates (DGR, % increase in wet weight day^{-1}) of *Gracilaria chorda, G. gigas, G. "verrucosa", G. incurvata* and *G. textorii* from Tosa Bay, southern Japan were measured. Intact, young unbranched fronds arising from new discoid basal discs were collected from natural attached populations of *Gracilaria* and grown for two months in net cages suspended in the bay. DGRs of these fronds varied during the culture period but were generally 2–4% day^{-1}, and grazers were a problem. Growth response to seawater temperature (10–33°C) was investigated by growing segments of thalli of each species for two weeks in a closed-circulating system (aquatron). *G. chorda* showed its maximum DGR at 15°C (3.82±1.00% day^{-1}), while *G. gigas* (4.74±1.02% day^{-1}), *G. incurvata* (4.19±1.16% day^{-1}) and *G. textorii* (2.91±0.70% day^{-1}) showed their maximum DGR at 20°C. *G. "verrucosa"* showed its maximum DGR (1.54±0.63% day^{-1}) at 18°C, being lowest among the species investigated, and did not exhibit a clear response to temperature. *G. "verrucosa"* showed growth at 30 (0.64±0.30% day^{-1}) and 33°C (0.79±0.33% day^{-1}).

Key Index Words: agarophytes—Gracilaria—*growth rate—seasonality—southern Japan—temperature.*

Species of *Gracilaria* are major sources of the phycocolloid, agar (Santelices and Doty 1989, McLachlan and Bird 1986). They are distributed world-wide, and 16 species have been reported from Japan (Yamamoto 1978).

Six species of *Gracilaria* occur in Uranouchi Inlet of Tosa Bay in Shikoku, southern Japan. Five of them, *Gracilaria chorda* Holmes, *G. gigas* Harvey, *G. "verrucosa"**, *G. incurvata* Okamura and *G. textorii* (Sur.) DeToni, represented more than 90% of the total macroalgal standing stock in Uranouchi Inlet during the growing season in January 1987 to December 1988, reaching a maximum value of 1,296.5 g (dry wt) m^{-2} in May 1987 (Orosco and Ohno, in press).

We are presently doing ecophysiological and biochemical studies on these *Gracilaria* species. In this paper, we report on growth rates of the five species when cultured in net cages in the field and in a temperature-controlled, closed-circulating system (aquatron).

Materials and Methods

Two growth-rate experiments using the three terete species (*G. chorda, G. gigas* and *G. "verrucosa"*) and the two flabellate species (*G. incurvata* and *G. textorii*) were carried out. All samples were collected from natural populations of *Gracilaria* in Uranouchi Inlet of Tosa Bay. Attached thalli were collected, transported to the laboratory in seawater-filled buckets and kept in running seawater.

Outdoor cage culture. For each species, five to eight healthy, intact, young unbranched fronds arising from new discoid basal discs were cleaned of sediments and epiphytes, tagged and placed in two nylon net-covered

* The taxonomic status of different populations referred to as *G. verrucosa* is uncertain (Abbott *et al.* 1985). We use this name based on the recommendation of Yamamoto and Sasaki (1988) to continue using this name for the Japanese taxon until the status of this species is resolved by crossing experiments with other Japanese populations.

cages ($10 \times 20 \times 20$ cm). The cages were hung from a raft in the harbor to a depth of 0.5 m. Wet weight of individual thalli was measured weekly. Thalli were cleaned of epiphytes and debris during each measurement. This experiment was carried out for a period of 56 days (Jan. 31, 1987 *et seq.*). Growth-rate data are expressed as the mean±S.D. of five to eight thalli. Seawater temperature, salinity, and nutrients (phosphate-phosphorus, nitrate-, nitrite- and ammonium-nitrogen) were measured during each sampling period. Nitrogen is expressed as dissolved inorganic nitrogen (DIN=nitrate+nitrite+ammonium). Salinity was measured using a conductivity meter, while nutrients were determined by colorimetric methods (Meteorological Agency 1970).

Indoor culture. This experiment was done to study the effect of seawater temperature on the growth rate.

One- to two-gram cuttings of apical portions were prepared from the collected samples and were kept in flowing seawater for 24 h before start of the experiment. Fifteen cuttings for each species, except for *G. textorii*, were inserted between the braids of rope hanging from a glass rod. These ropes were weighed down by another glass rod. *G. textorii*, because of its brittle and wide, flat thallus, was tied to the ropes by a thread passing through the thallus near its cut end. Samples were cultured in a temperature-controlled closed-circulating system, aquatron (Fig. 1, Ohno 1977). Medium used was plankton net-filtered seawater (450 *l*) from the bay. Light was provided by white fluorescent tubes at a 12 : 12 h light : dark cycle at a photon fluence rate of 65 μE $m^{-2}sec^{-1}$. The system was run for 24 h at the set temperature before the start of the experiment. Temperatures tested were 10, 12, 15, 18, 20, 22, 25, and 28°C. Growth rates of *G. "verrucosa"* were measured also at 30 and 33°C. Wet weight of each sample was taken after the 14-day incubation period. Data represent the mean±S.D. for 15 samples. This experiment was carried out from April 19 to July 8, 1987.

Daily growth rate (DGR, % day^{-1}) was calculated using the formula of Rosenberg and Ramus (1981): $DGR = 100 (\ln n_t/n_o)t^{-1}$,

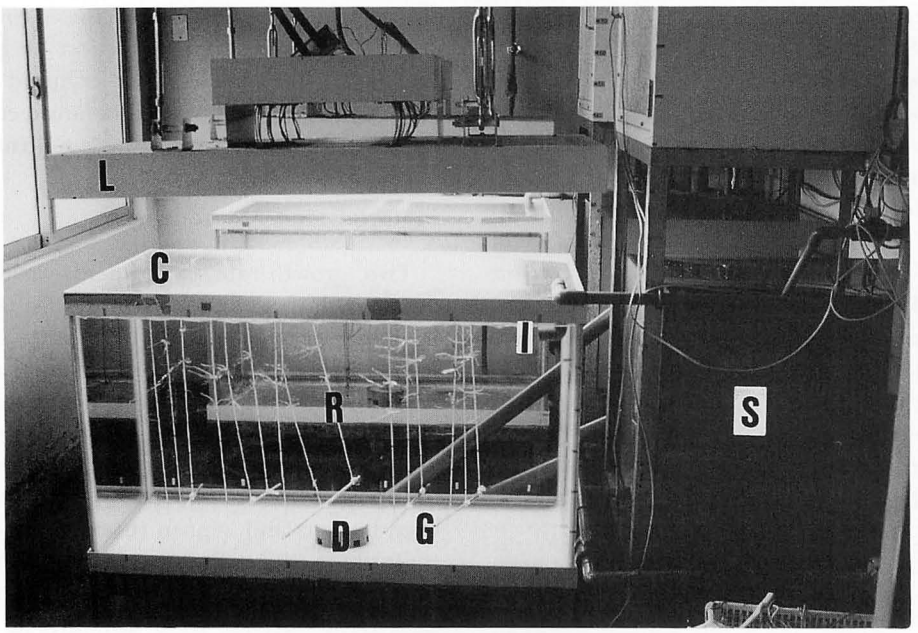

Fig. 1. Closed-circulating system (aquatron) used in the temperature-growth study. C, plexiglass cover; D, drain; G, glass rod; L, light panel; I, water inlet; R, rope; S, sand filter (recirculation tank).

where n_o is weight at the beginning of each period, n_t is weight after t days, and t is the number of days.

Results and Discussion

Ambient seawater temperature, salinity and phosphate-phosphorus varied little during the experimental period. DIN had slightly higher values in March owing to increase in the three nitrogen species measured (Fig. 2).

In the outdoor cage-culture experiment, *G. textorii* showed the highest long-term DGR over the 56-day culture period, 4.47±0.51% day^{-1}, followed by DGR of *G. "verrucosa"*, 3.86±0.85% day^{-1}. *G. chorda* and *G. incurvata* had similar DGRs, 2.97±1.36 and 2.96±0.67% day^{-1}, respectively, while *G. gigas* had the lowest DGR, 2.07±0.32% day^{-1} (Figs. 3 & 4).

However, daily growth rates varied from week to week during the 56-day culture period. *G. textorii* exhibited the highest short-term DGR of 7.90±1.26% day^{-1} at the beginning, which, however, continually decreased to a lowest DGR of 2.87±0.80% day^{-1} at the end of the culture period. *G. incurvata* had a DGR of 5.32±0.89% day^{-1} during the first week but the rate decreased to about 60% of the initial rate (3.20±1.84% day^{-1}) in February, and in March the rate was only 26-37% of the initial DGR. *G. "verrucosa"* had a high DGR during the first week of culture, but the rate decreased and remained at about 50% of the initial DGR until the beginning of March; then the rate decreased further. The DGR of this species ranged from 0.76±0.91 to 6.86±4.76% day^{-1}. *G. gigas* had the lowest DGR, 1.76±0.86 to 2.56±0.52% day^{-1}, among the five species. Over the two-month period, however, the rate was never less than 80% of the initial DGR. *G. chorda* had growth rates, equal to or slightly higher than the initial DGR (3.37±1.98% day^{-1}) in February, but in March DGR was only 62-87% of the initial rate. DGR ranged from 2.04±0.77 to 3.70±2.01% day^{-1}.

One problem encountered during the field growth study was grazing of the samples by

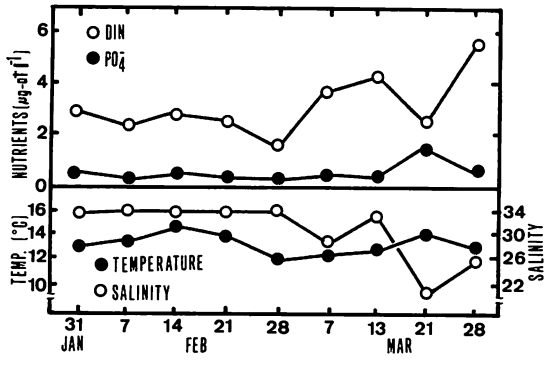

Fig. 2. Temperature (°C), salinity, DIN and phosphate (μg-at l^{-1}) of ambient seawater during the outdoor cage culture experiment.

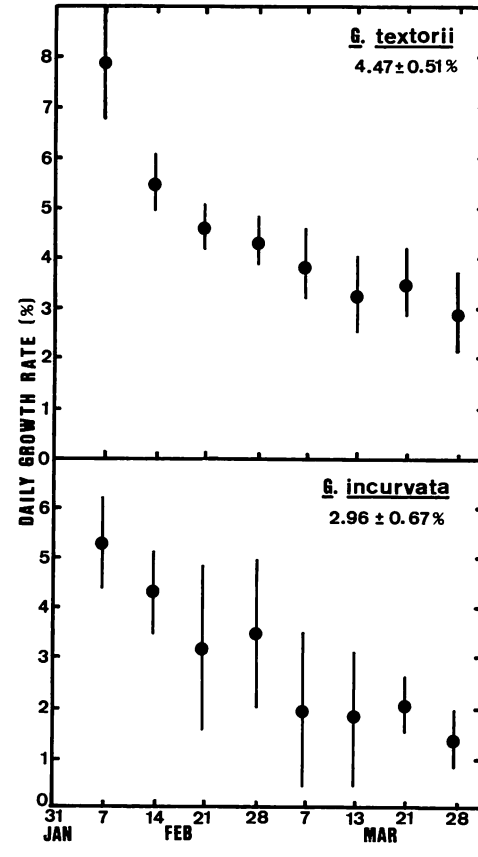

Fig. 3. Daily growth rates (DGR, % day^{-1}) of the flabellate species (*G. textorii* and *G. incurvata*) grown in net cages suspended in the bay. Plotted values represent the mean±S.D. at weekly intervals (t=7). Daily growth rate over the whole culture period (t=56) is given in the upper right-hand corner of each graph.

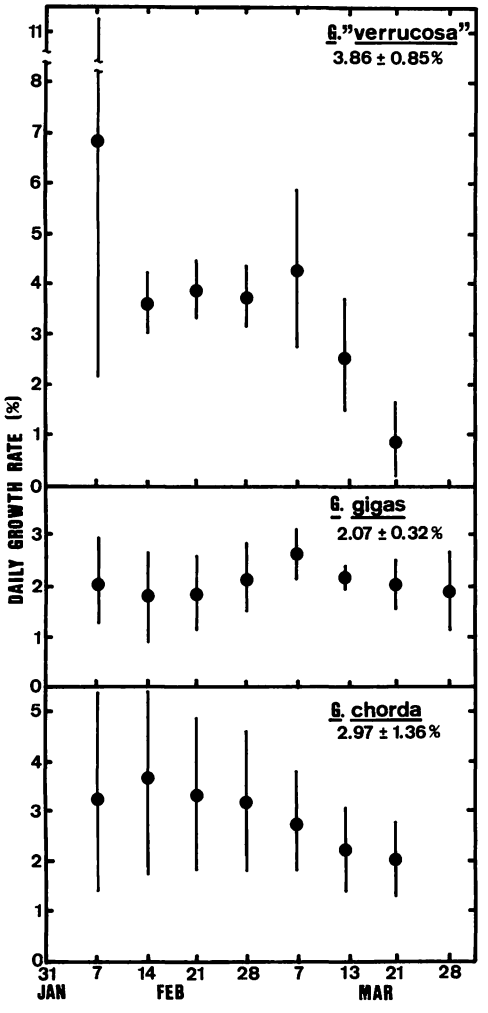

Fig. 4. Daily growth rates (DGR, % day^{-1}) of the terete species (*G. chorda, G. gigas* and *G. "verrucosa"*) grown in net cages suspended in the bay. Details are the same as in Fig. 3.

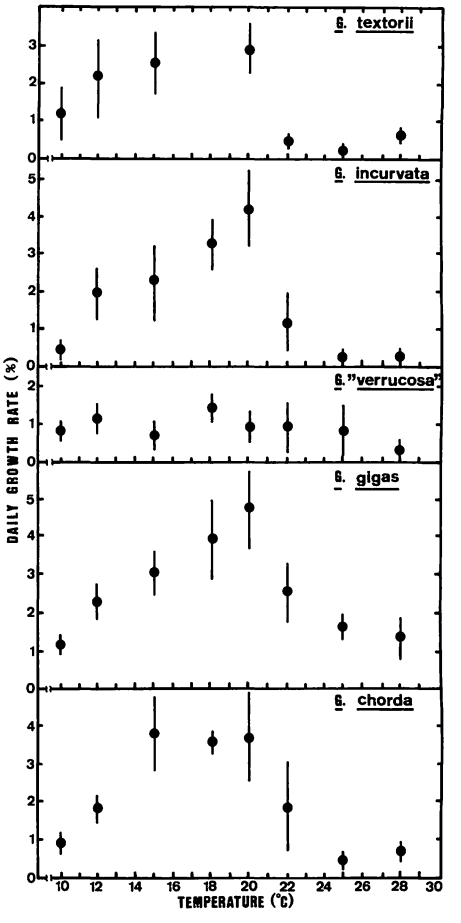

Fig. 5. Daily growth rates (DGR, % day^{-1}) of *Gracilaria* species in response to seawater temperature when grown in an aquatron for 14 days. Plotted values represent the mean±S.D.

copepods. Van Dover and Kirby-Smith (1979 in Rosenberg and Ramus 1981) noted that the amphipod *Caprella penantis* occurred among the branches of *Gracilaria* but it did not consume the host seaweed. During our field growth experiments, some copepods built their "homes" on the thalli by cementing silt around the main axis with a fibrous material and lived in the space between the thallus and the silt. Removal of these homes by forceps revealed white grazing marks on the thallus where the medulla had been exposed. Most susceptible to this attack was *G. "verrucosa"*. Some animals were also found on *G. gigas* and the fewest number was found on *G. chorda*. When these animals were found on *G. textorii* and *G. incurvata*, they formed patches of silt on the upper surface of the thallus. These structures were well-attached to the thallus and could not be removed by washing with seawater; they had to be forcefully removed by forceps. Heavy infestation resulted in fragmentation of the thallus, although in some instances the broken branches were cemented together by the silt structures.

In the aquatron experiment, growth rates increased with increase in temperature from 10 to 20°C and decreased when temperature exceeded 20°C (Fig. 5). *G. chorda* had its

highest DGR at 15°C (3.82±1.00% day^{-1}), while *G. gigas* (4.74±1.02% day^{-1}), *G. incurvata* (4.19±1.16% day^{-1}) and *G. textorii* (2.91±0.70% day^{-1}) had their maximum DGR at 20°C. *G. gigas* was the most responsive to temperature, while *G. "verrucosa"* did not show a clear response to temperature although the maximum DGR occurred at 18°C (1.54±0.63% day^{-1}). The maximum DGR was lowest in *G. "verrucosa"* among the species investigated. The growth of *G. "verrucosa"* was also measured at 30 and 33°C as its thalli were found throughout the year in the intertidal zone where the temperature attains 30°C or more. DGR of this species was 0.64±0.30% day^{-1} at 30°C and 0.79±0.33% day^{-1} at 33°C.

Our previous study on the seasonal abundance of natural populations of the same *Gracilaria* species in Uranouchi Inlet (Orosco and Ohno, in press) showed changes in biomass corresponding to changes in seawater temperature. Sporelings and new growth from perennating holdfasts or stumps are observed in late autumn to early spring when seawater temperature is 13-15°C. There is a large biomass from March to June-July, when temperature increases to about 25°C; however, biomass peaks in April-May (15-18°C). Senescence occurs after the reproductive season when water temperatures are above 23°C. Plants pass the summer as holdfasts, stumps, or spores attached to substrata covered by sand; *G. "verrucosa"* in the upper intertidal area, however, continues to grow even when water temperature is 28-30°C or slightly higher (Orosco and Ohno, in press). The present results from the aquatron experiment on *G. "verrucosa"* showed that growth is possible at 30 and 33°C. In the natural habitat, temperatures as high as these values may be reached as the sites are located in intertidal areas which are often exposed during low tides.

Thus, the growth response of the five *Gracilaria* species to temperature in the indoor culture experiment coincides well with the natural seasonal growth cycle of *Gracilaria* species in Uranouchi Inlet of Tosa Bay. Except for *G. "verrucosa"*, growth rates increase as temperature increases towards the optimum at 15-20°C above which growth rates decrease and senescence occurs.

Growth rates of *Gracilaria* species in this study are slightly lower than the general values of 5-10% day^{-1} compiled by McLachlan and Bird (1986). Short-term DGRs in the outdoor cage culture were lower than the maximum growth rates obtained in the aquatron for *G. chorda, G. incurvata* and *G. gigas*, although growth rates in the outdoor cage culture were expected to increase in the later months as the optimum temperature for growth had just started at the termination of the experiment.

G. textorii is the least tolerant to high temperature. In the aquatron, there was a drastic decrease in growth rate at temperatures higher than 20°C. It has the shortest growth period in Uranouchi Inlet; it is usually found only until June when seawater temperature reaches 22°C (Orosco and Ohno, in press).

Temperature for optimum growth of *G. "verrucosa"* in these experiments is generally lower than that of *G. verrucosa* from the Philippines (optimum: 25-30°C) grown in the laboratory between 15 and 30°C (Hurtado-Ponce and Umezaki 1987). Growth rates of *G. verrucosa* in the intertidal (5.0-16.4% day^{-1}) and in an abandoned fishpond (1.5-8.4% day^{-1}) at temperatures of 28-32°C in the Visayas, Philippines (Largo *et al.* 1989) were relatively higher than the growth rates we obtained at temperatures above 20°C. Further comparison of growth rates of *G. verrucosa* is difficult because of the uncertain status of the species from other localities (Abbott *et al.* 1985).

Based on the experimental assessment of the geographic distribution of *Gracilaria* species in relation to temperature (McLachlan and Bird 1984), the species from Tosa Bay, southern Japan still seem to belong to the temperate-water species which showed maximum growth rates at 15 or 20°C, although none of the species reported in this paper was included.

Acknowledgments

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C. A. Orosco・大野正夫：日本南岸土佐湾産オゴノリ属海藻の成長速度

日本南岸の高知県土佐湾に生育するオゴノリ属のツルシラモ，オオオゴノリ，オゴノリ，ミゾオゴノリ，カバノリについて，浦の内湾および室内培養によって日成長率 (daily growth rate, DGR) を測定した。湾内では試料をカゴに入れて水深 0.5 m につるして 2 ケ月実験を行ったが，小形甲殻類による食害の影響がみられ，すべての種類において，DGR は，2－4％の範囲であった。室内では循環式恒温水槽（アクアトロン）により水温 10–33°C の範囲で 2 週間培養を行い，成長速度を求めた。ツルシラモでは最大 DGR は 15°C でみられ，3.82±1.00% であった。オオオゴノリ，ミゾオゴノリ，カバノリでは，最大 DRG は 20°C でみられ，それぞれ 4.74±1.02%, 4.19±1.16%, 2.91±0.70% であった。オゴノリの最大 DGR は 18°C でみられ，その値は低かった (1.54±0.63%) が，DGR の温度による際は明瞭でなく，30°C では 0.64±0.30%, 33°C では 0.79±0.33% であった。(781-11 土佐市宇佐町井尻194　高知大学海洋生物教育研究センター)

Jpn. J. Phycol. (Sôrui) 40: 245–259, September 20, 1992

Diatom assemblages of sediments from the estuary of Fukuda River in Kobe along the northwestern coast of Osaka Bay with special reference to the Holocene sedimentary history

Shigeru Kumano*, Masao Nishiumi*, Goh Okuizumi* and Hiroshi Sato**

*Department of Biology, Faculty of Science, Kobe University, Rokko-dai, Nada-ku, Kobe, 657 Japan
**Division of Earth Science, Museum of Nature and Human Activities, Hyogo, Yayoiga-oka, Sanda, 669–13 Japan

Kumano, S., Nishiumi, M., Okuizumi, G. and Sato, H. 1992. Diatom assemblages of sediments from the estuary of Fukuda River in Kobe along the northwestern coast of Osaka Bay with special reference to the Holocene sedimentary history. Jpn. J. Phycol. 40: 245–259.

Diatom assemblages of sediments obtained from the estuary (Tarumi site) of Fukuda River in Kobe were analyzed in order to clarify the local Holocene sedimentary history. The results were as follows: 1) the lowermost sediment was a brackish environment at around 7000 yr B. P. 2) the first marine diatom zone (MD_1 Zone) was alternated three times by three transitional zones ($Tr_{1-1, 1-2, 1-3}$ Zone) probably caused by the developments of three sand bars across the estuary of paleo-Fukuda River during the period between 7000 and 6000 yr B. P.

Key Index Words: diatom assemblages—Fukuda River estuary—Holocene transgression—sand bar development.

The diatoms occur in virtually all bodies of water exposed to light and contain easily recognized taxa characteristic of many different environments between truly marine conditions and potable freshwater and at widely varying temperatures, salinities, pH, and chemical composition. Similar fossil forms are found in sediments that were deposited under such environmental conditions. So the diatoms have many advantages as microfossils to clarify the local paleoecological factors.

Previously we have analyzed the diatom assemblages of sediments obtained from the estuary along the Osaka Bay (Kumano and Miyahara, 1981; Kumano and Fujimoto, 1982; Sato et al., 1983), Kutcharo Lake (Kumano et al., 1984, Sekiya and Kumano, 1983) and Kushu Lake (Kumano et al., 1990a).

In Osaka Bay area, the Marine Diatom Zone (MD_1 Zone) coincided with the peak of the first Holocene transgression at about 6000 yr B.P. at several sites along the coast of Osaka Bay.

In Kutcharo Lake and Kushu Lake, deposition of the Marine Diatom Zone (MD_1 Zone) and the Transitional Zone (Tr_2 Zone) finished at about 6000 yr B.P. and 5000 yr B.P., respectively, because of the sand bar development prior to the first Holocene regression at about 4500 yr B.P., the "Middle Jomon minor regression" named by Ota et al. (1982).

In the present study, diatom assemblages of sediments obtained from the estuary (Tarumi site) of Fukuda River in Kobe were analyzed in order to clarify the local Holocene sedimentary history.

Materials and Methods

Sampling Sites

Fukuda River is about 7 km in length and Tarumi site is located at the estuary in Kobe along the northwestern coast of the Osaka Bay, at altitude about 4 m, latitude 34°35′38″ N and longitude 135°3′40″E (Fig. 1).

The excavation for the construction of

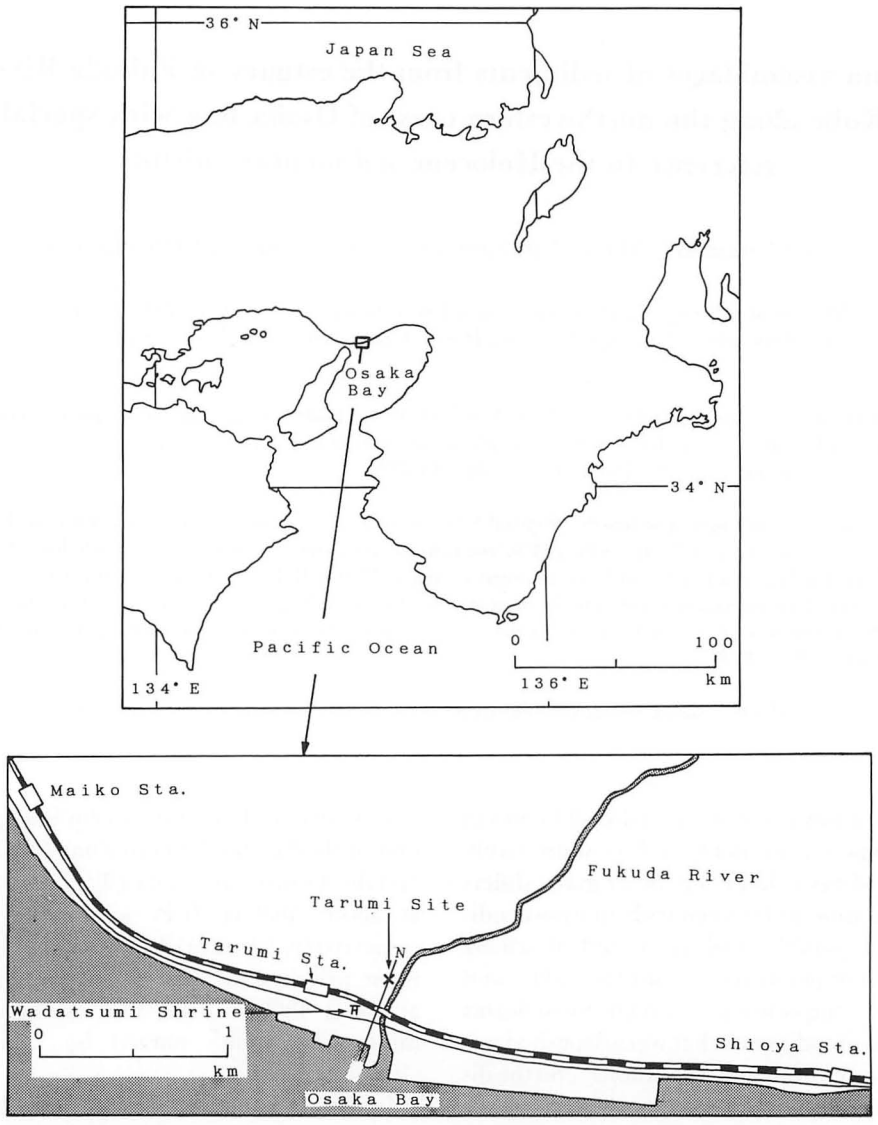

Fig. 1. The estuary (Tarumi site) of Fukuda River at altitude about 4 m is located at latitude 34°35′38″N and longitude 135°3′40″E in Kobe along the northwestern coast of Osaka Bay, central Japan.

buildings at the estuary (Tarumi site) of Fukuda River offered us outcrops of Holocene deposits, from which samples of Site A (−0.2 m to +1.4 m) and Site B (+0.8 m to +2.7 m) were collected in 1988, those of Site C (+1.0 m to +2.2 m) were collected in 1988 and those of Site D (+2.4 m to +3.7 m), were collected in 1990, respectively (Fig. 2).

^{14}C Dates and Akahoya Tephra

The ^{14}C dates were measured by Dr. Kigoshi (1992) and Akahoya tephra was identified by Dr. Danhara (1992). The ^{14}C and tephra dates from Site A to Site C are shown in the second column from Fig. 3 to 5, respectively. The plant remains at the −0.1 m horizon of Site A gave a ^{14}C age of 7220±110 yr B.P., those at the +0.1 m horizon gave a ^{14}C age of 7210±120 yr B.P., those at the +1.6 m horizon of Site C gave a ^{14}C age of 6340±110 yr B.P. and Akahoya tephra at the +1.8 m horizon showed about

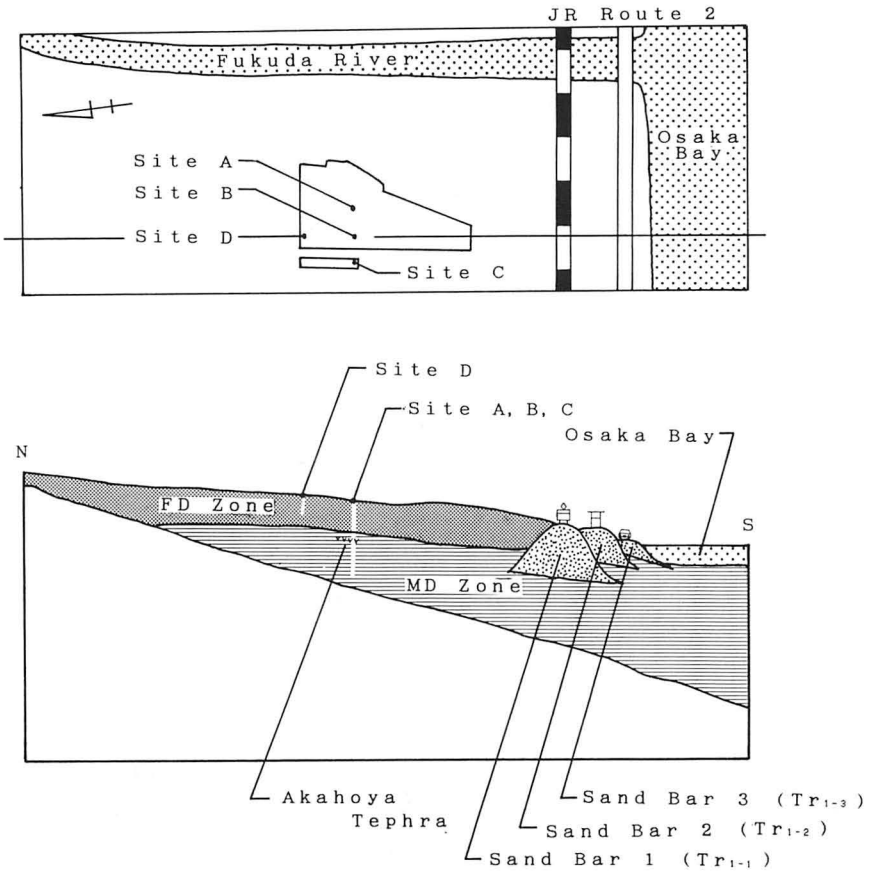

Fig. 2. Four sampling sites (site A, site B, site C and site D) and three rows of sand bars are shown (Takahashi, 1992). Sand bar 1: the innermost row of sand bars located on the track of Japanese Railway. Sand bar 2: the middle sand bar on the grounds of Wadazumi Shrine. Sand bar 3: the outermost sand bar on the national road of Rout No. 2.

6300 yr B.P..

Preparation of Samples

For diatom analysis, approximately 1 g d.w. of each sample was dispersed with 10% H_2O_2 and $Na_4P_2O_7$ and the clay fraction removed by decanting. The fraction containing diatom frustules was boiled with conc. HCl and then cleaned and washed about 5 times with distilled water by centrifugation. An appropriate amount of each washed sample was then mounted with Pleurax. About 200 diatom frustules were identified and counted along a transect chosen at random on each sample slide.

The marine diatom zone (MD Zone) is considered as the zone which comprised more than 80% marine and brackish-water diatoms. The transitional zone (Tr Zone) is considered as the zone, in which marine diatoms accounted for less than 30% of the total count.

Results

The successive changes in the ecological spectra are shown in Fig. 3-5 and the successive changes in the predominant diatoms in Fig. 6-8.

Site A (−0.1 m to +1.4 m):

1. The first Transitional Zone (Tr_{1-1} Zone) (−0.1 m to +0.18 m)

a) Tr_{1-1}-a subzone

Brackish-water diatoms were dominated in

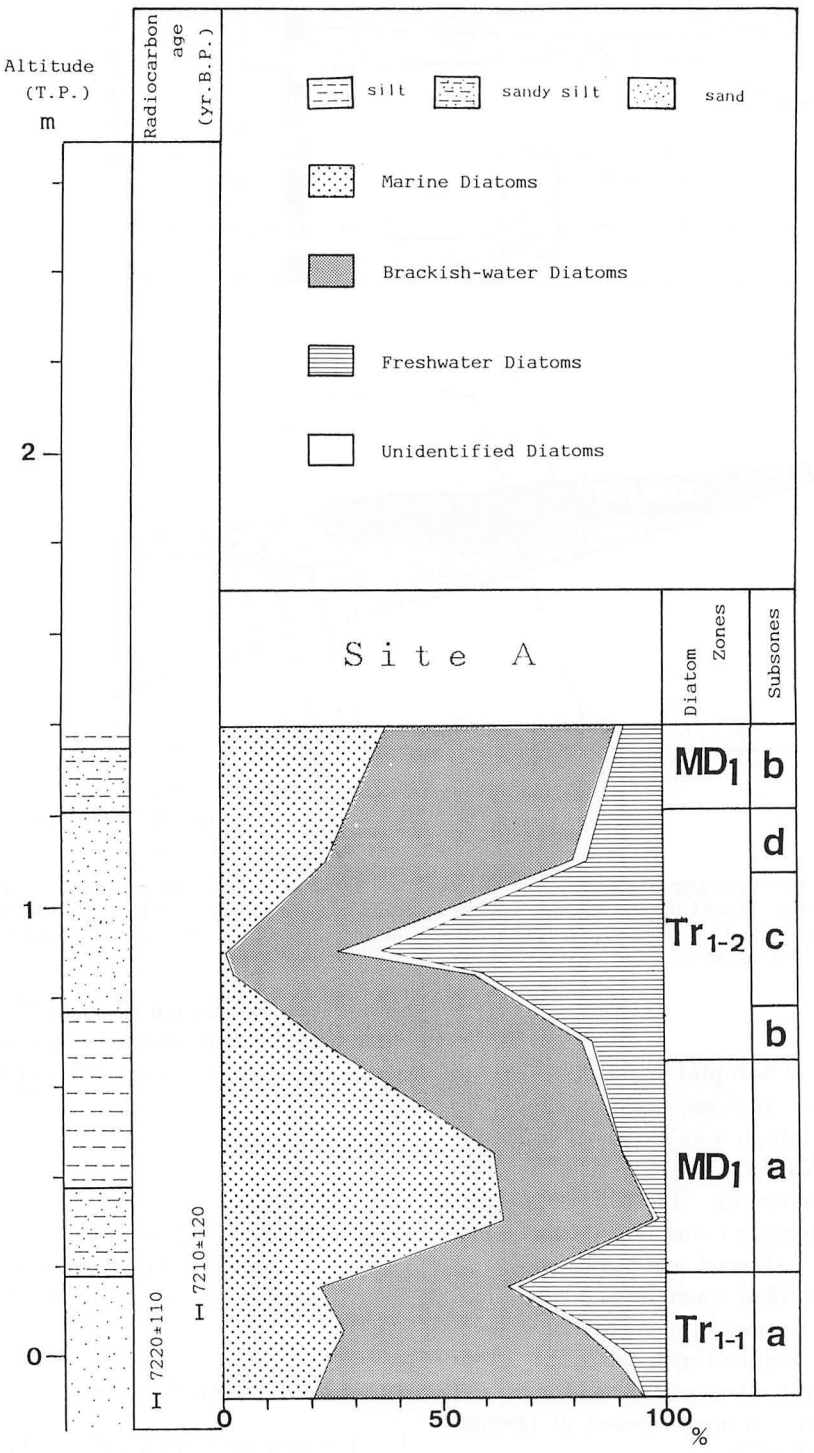

Fig. 3. Stratigraphic profile in Site A, the Fukuda River and the successive changes in the ecological spectrum. Facies of the sediments are shown in the first column, the ^{14}C dates in the second column, proportions of marine, brackish-water and freshwater diatoms in the third column, diatom zones in the fourth column and subzones in the fifth column.

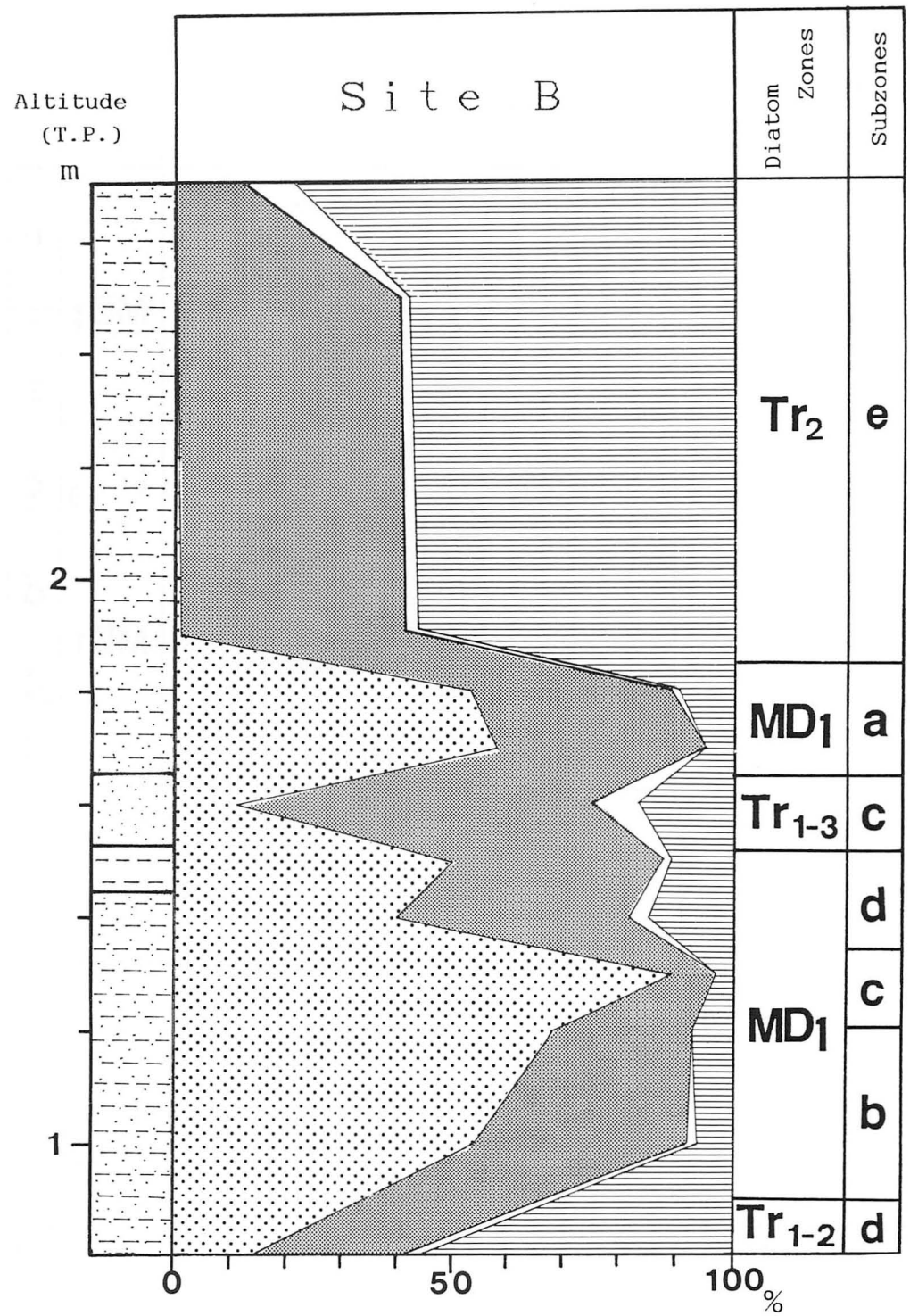

Fig. 4. Stratigraphic profile in Site B, the Fukuda River and the successive changes in the ecological spectrum. Facies of the sediments are shown in the first column, proportions of marine, brackish-water and freshwater diatoms in the second column, diatom zones in the third column and subzones in the fourth column. The other marks and symbols are the same as those in Fig. 3.

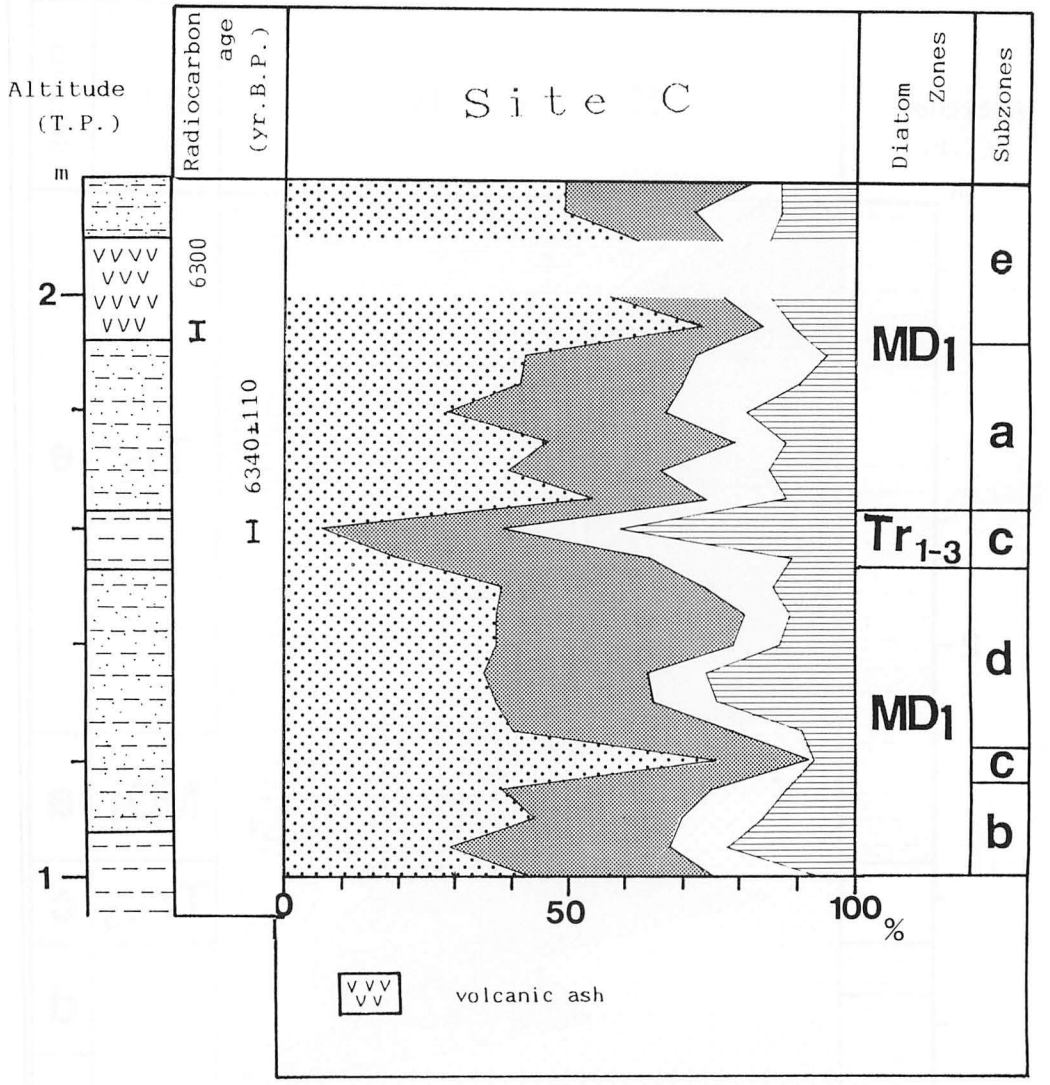

Fig. 5. Stratigraphic profile in Site C, the Fukuda River and the successive changes in the ecological spectrum. Facies of the sediments are shown in the first column, the ^{14}C and tephra dates in the second column, proportions of marine, brackish-water and freshwater diatoms in the third column, diatom zones in the fourth column and subzones in the fifth column. The other marks and symbols are the same as those in Fig. 3.

this subzone (Fig. 3): the dominant diatom at the lower horizon of this subzone was the brackish-water *Achnanthes hauckiana*, while those at the upper horizon were the brackish-water *Bacillaria paradoxa* in addition to the brackish-water *Rhopalodia gibberula*, the marine *Nitzschia granulata* and *Nitzschia punctata* (Fig. 6).

2. The Marine Diatom Zone (MD_1 Zone) (+0.18 m to +0.65 m)

a) MD_1-a subzone

In the MD_1-a subzone, marine diatoms were increased and occupied 30-60% of the total count, and brackish-water diatoms occupied 30-50% of the total throughout this subzone. Freshwater diatoms occupied less than 30% of the total (Fig. 3).

The MD_1-a subzone was dominated by the littoral *Nitzschia granulata* (about 15-50%) accompanied with the littoral *Nitzschia punctata* (about 5-20%) and the brackish-water

Achnanthes hauckiana (about 5-15%) (Fig. 6). Toward the upper horizon of this subzone, the marine diatoms were decreased, while the brackish *Rhopalodia gibberula* was increased.

3. The second Transitional Zone (Tr_{1-2} Zone) (+0.65 m to 1.21 m)

The Tr_{1-2} Zone is divided into three subzones according to the dominant diatoms.

a) Tr_{1-2}-b subzone

Marine diatoms decreased up to less than 30%, and brackish-water and marine ones counted for more than 70% of the total count (Fig. 3). The dominant diatom of this subzone was the brackish *Rhopalodia gibberula* (Fig. 6).

b) Tr_{1-2}-c subzone

At the middle horizon of this subzone, brackish-water diatoms decreased to less than 30% and only a few marine diatoms were counted, while freshwater diatoms increased up to about 60% of the total count (Fig. 3). Dominant diatoms in this subzone were the freshwater *Navicula contenta* and *Achnanthes lanceolata* accompanied with the brackish-water *Nitzschia hungarica* and *Achnanthes hauckiana* (Fig. 6).

c) Tr_{1-2}-d subzone

In the Tr_{1-2}-d subzone, marine and brackish diatoms increased up to 20-30% and about 50%, respectively, whereas freshwater ones decreased to about 20% (Fig. 3). The dominant diatoms were the brackish *Rhopalodia gibberula* and the marine *Nitzschia granulata*, and a few freshwater diatoms such as *Navicula contenta* were counted (Fig. 6).

4. The Marine Diatom Zone (MD_1 Zone) (+1.21 m to +1.4 m)

a) MD_1-b subzone

In the MD_1-b subzone, marine diatoms increased and occupied about 30-40% of the total count and brackish ones about 50% of the total count, whereas freshwater ones decreased to about 10% (Fig. 3). In this subzone, the dominant diatom was the brackish-water *Rhopalodia gibberula* accompanied with the littoral *Nitzschia granulata* and *Nitzschia punctata* (Fig. 6).

Site B (−0.8 m to +2.7 m):

1. The second Transitional Zone (Tr_{1-2} Zone) (−0.8 m to −0.9 m)

a) Tr_{-1-2}-d subzone

The Tr_{-1-2}-d subzone was occupied by about 40-70% of marine and brackish-water diatoms among which marine diatoms occupied about 15-40% of the total count (Fig. 4). Dominant diatom in this subzone was the littoral *Nitzschia granulata* and *Nitzschia punctata* accompanied with the brackish-water *Rhopadoria gibberula* and *Achnanthes hauckiana* (Fig. 7).

2. The Marine Diatom Zone (MD_1 Zone) (+0.9 m to +1.54 m)

The MD_1 Zone is divided into three subzones according to the dominant diatoms.

a) MD_1-b subzone

In the MD_1-b subzone, marine diatoms occupied about 40-70% of the total count, and brackish-water diatoms occupied about 30%. Freshwater diatoms occupied about 10-30% of the total throughout this subzone (Fig. 4). The lower horizon of this subzone was dominated by the littoral *Nitzschia punctata* accompanied with the brackish-water *Rhopalodia gibberula* and *Achnanthes hauckiana*, while at the upper horizon the dominant diatom was changed to the littoral *Nitzschia granulata* (Fig. 7).

b) MD_1-c subzone

In the MD_1-c subzone, marine diatoms increased and occupied about 70-90% of the total count, whereas brackish-water and freshwater ones occupied about 10-30% (Fig. 4). In this subzone, the dominant diatom was the littoral *Nitzschia granulata* (Fig. 7).

c) MD_1-d subzone

In the MD_1-d subzone, marine and brackish diatoms occupied about 40-70% and 20-40% of the total count, respectively, while freshwater ones less than 20% (Fig. 4). At the lower horizon of this subzone the dominant diatoms were the marine *Nitzschia punctata* and the brackish-water *Rhopalodia*

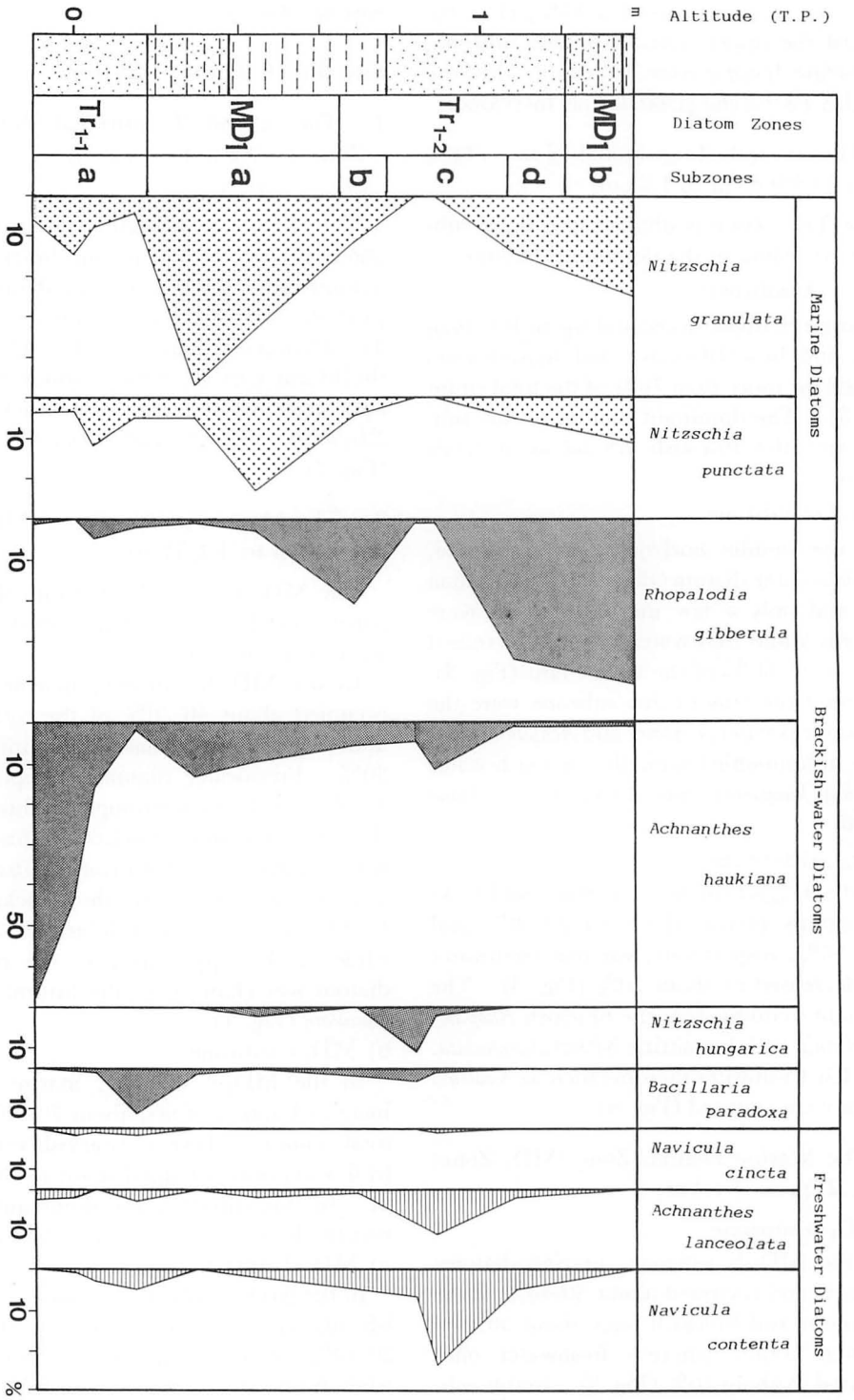

Fig. 6. Diatom diagrams of Site A showing the occurrence of the prominent taxa. Facies of the sediments are shown in the first column, diatom zones in the second column, subzones in the third column, marine diatoms in the fourth column, brackish-water ones in the fifth column, and freshwater ones in the sixth column.

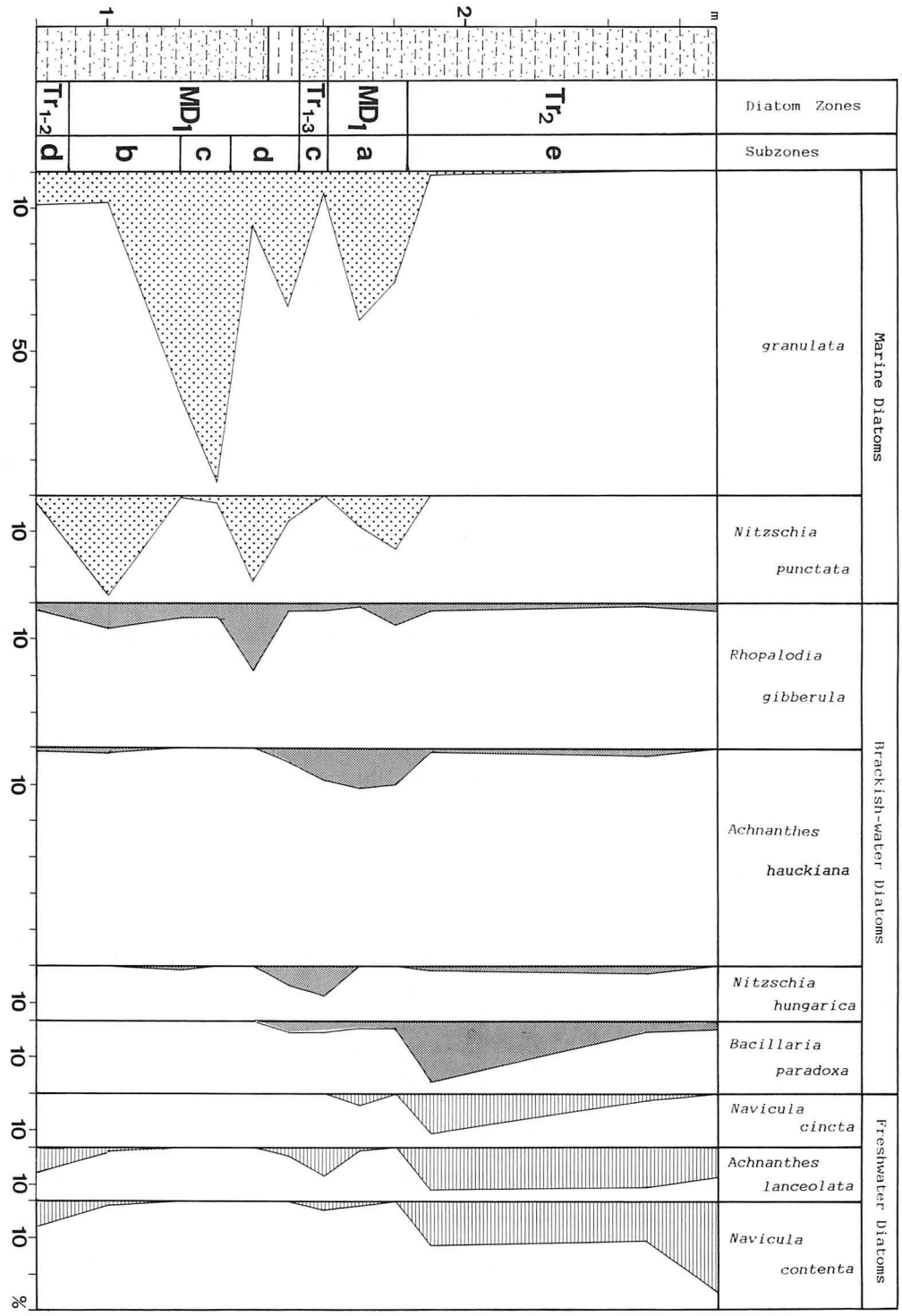

Fig. 7. Diatom diagrams of Site B showing the occurrence of the prominent taxa. Facies of the sediments are shown in the first column, diatom zones in the second column, subzones in the third column, marine diatoms in the fourth column, brackish-water ones in the fifth column, and freshwater ones in the sixth column.

gibberula, while at the upper horizon it changed to the littoral *Nitzschia granulata* (Fig. 7).

3. The third Transitional Zone (Tr_{1-3} Zone) (+1.54 m to +1.63 m)

a) Tr_{1-3}-c subzone

At the middle horizon of this subzone, marine diatoms decreased to less than 20%, brackish-water ones increased up to about 60% and freshwater ones slightly increased (Fig. 4). Dominant diatoms in this subzone were the brackish-water *Achnanthes hauckiana* and the marine *Nitzschia granulata* accompanied with the brackish-water *Nitzschia hungarica* and the freshwater *Achnanthes lanceolata*.

4. The Marine Diatom Zone (MD_1 Zone) (+1.63 m to +1.84 m)

a) MD_1-a subzone

In the MD_1-a subzone, marine diatoms increased up to about 60% of the total count, whereas brackish one and freshwater ones decreased to less than 40% and 10%, respectively (Fig. 4). The dominant diatom was the littoral *Nitzschia granulata* at the lower horizon of this subzone, while at the upper horizon the littoral *Nitzschia punctata* was dominant and accompanied with the brackish-water *Rhopalodia gibberula* and *Achnanthes hauckiana* (Fig. 7).

5. The Transitional Zone (Tr_2 Zone) (+1.84 m to +2.7 m)

a) Tr_2-e subzone

At the lower horizon of this subzone brackish-water diatoms decreased to less than 40%, a few marine ones were counted, while freshwater ones increased up to 50% (Fig. 4): various diatoms such as the brackish-water *Bacillaria paradoxa*, the freshwater *Achnanthes lanceolata, Navicula contenta* and *Navicula cincta* (Fig. 8) were found.

At the upper horizon of this subzone, brackish-water diatoms decreased less than 20%, no marine ones were counted, while freshwater one increased more than 80% (Fig. 4). Dominant diatoms in this subzone were the freshwater *Navicula contenta* and *Achnanthes lanceolata* accompanied with a few brackish-water diatoms (Fig. 7).

It is considered that the Tr_2 Zone at the horizon (from +1.84 m to +2.7 m) finished at about 6000 yr B.P., because the plant remains at the −0.1 m horizon of Site C gave a ^{14}C age of 6340±110 yr B.P. and Akahoya tephra at the +1.8 m horizon showed about 6300 yr B.P.

Site C (+1.0 m to +2.2 m):

1. The Marine Diatom Zone (MD_1 Zone) (+1.0 m to +1.53 m)

About 70% of diatoms of the MD_1 Zone was occupied by marine and brackish-water diatoms (Fig. 5). The MD_1 Zone is divided into three subzones according to the dominant diatoms.

a) MD_1-b subzone

In the MD_1-b subzone, marine and brackish diatoms occupied about 30-40% of the total of diatoms, respectively, while freshwater ones occupied about 10-20% (Fig. 5). This subzone was dominated by the littoral *Nitzschia granulata* and the brackish-water *Rhopalodia gibberula* accompanied with the littoral *Amphora acutiuscula* and *Nitzschia punctata* (Fig. 8).

b) MD_1-c subzone

In the MD_1-c subzone, marine diatoms increased up to 80% of the total, while brackish-water and freshwater ones decreased to less than 20% and less than 10%, respectively (Fig. 5). In this subzone, the dominant diatoms were the littoral *Amphora acutiscula, Nitzschia granulata* accompanied with the brackish *Rhopalodia gibberula* (Fig. 7).

c) MD_1-d subzone

In the MD_1-d subzone, marine and brackish diatoms occupied about 20-60% and about 30-40% of the total of diatoms, respectively, while freshwater ones about 10-20% (Fig. 5). At the lower horizon of this subzone various diatoms such as the littoral *Nitzschia granulata, Nitzschia punctata* and the brackish-water *Rhopalodia gibberula* were found. While in the upper horizon of this subzone the

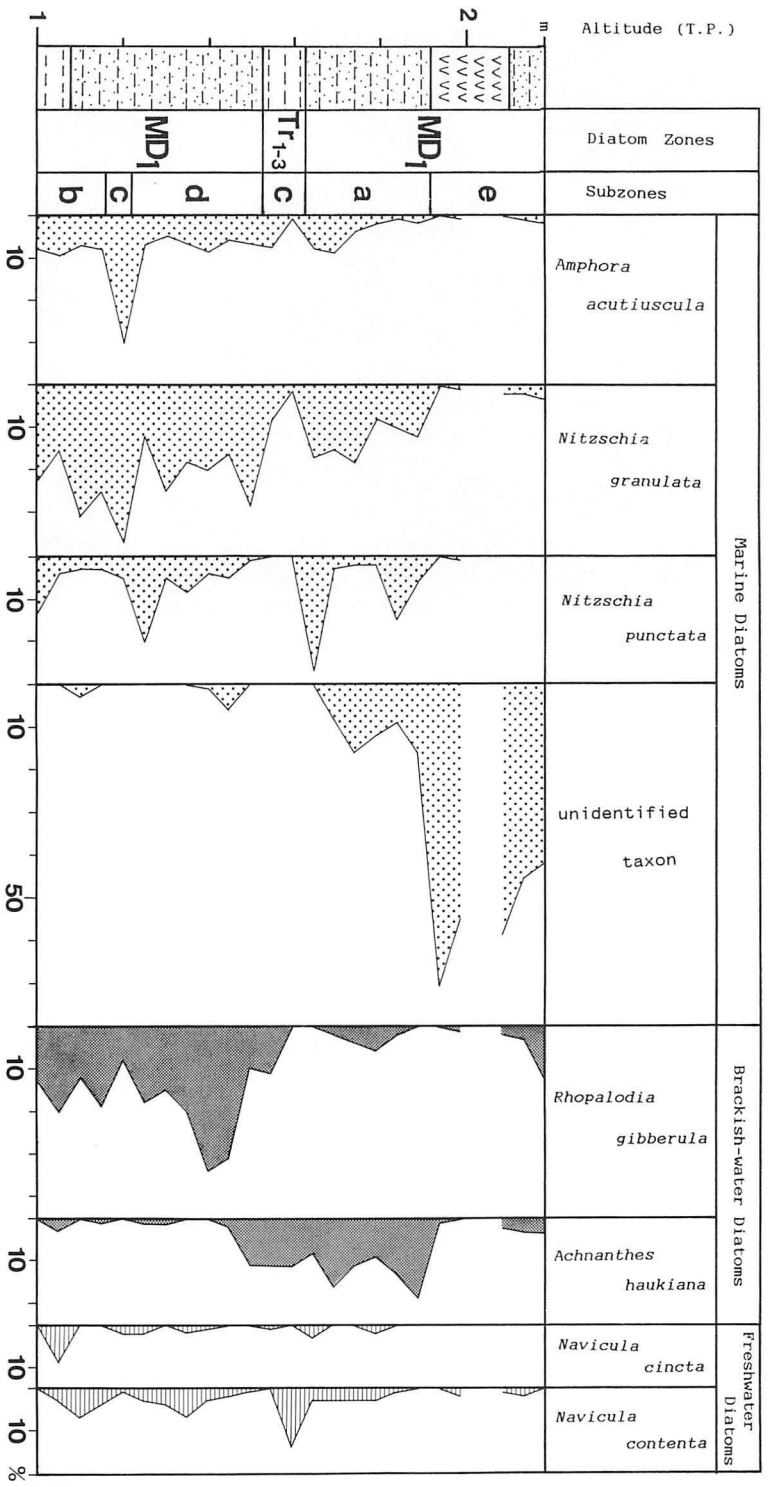

Fig. 8. Diatom diagrams of Site C showing the occurrence of the prominent taxa. Facies of the sediments are shown in the first column, diatom zones in the second column, subzones in the third column, marine diatoms in the fourth column, brackish-water ones in the fifth column, and freshwater ones in the sixth column.

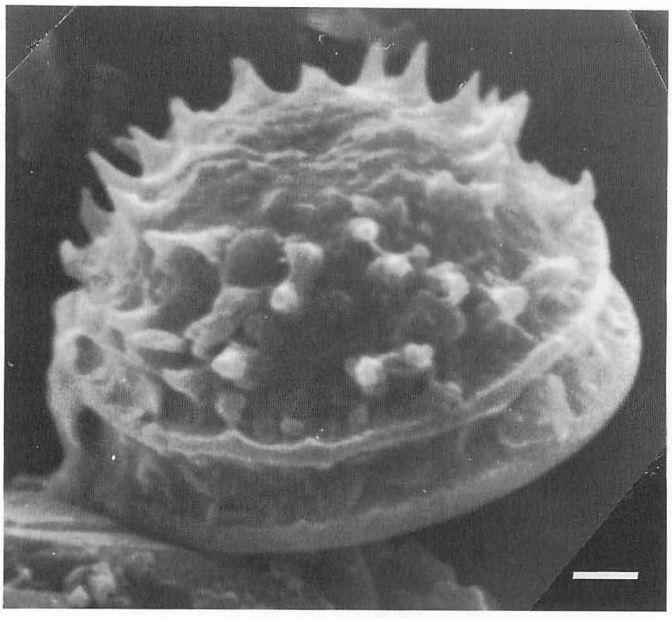

Fig. 9. A photomicrograph of an unidentified taxon assigned to the order Centrales occurred in the MD_1 Zone of Site C. This unidentified taxon assigned to the order Centrales was also abundantly found in the marine diatom zone at the Tamatsu site near Akashi River (Sato, unpublished). Scale bars indicate 1 μm.

dominant diatoms were the brackish-water *Rhopalodia gibberula* accompanied with the littoral *Nitzschia granulata* (Fig. 8).

2. The third Transitional Zone (Tr_{1-3} Zone) (+1.53 m to +1.63 m)

a) Tr_{1-3}-c subzone

Marine diatoms decreased to less than 10% and brackish-water ones occupied about 30% of the total diatoms, while freshwater ones increased up to 40% (Fig. 5). Generally, dominant diatom in this subzone was the freshwater *Navicula contenta* accompanied with the brackish-water *Achnanthes hauckiana* (Fig. 8). Dominant diatom was *Nitzschia granulata* at the lower horizon of this subzone and it was *Nitzschia punctata* at the upper horizon of this subzone.

3. The Marine Diatom Zone (MD_1 Zone) (+1.63 m to +2.2 m)

In the MD_1 Zone, marine diatoms increased and occupied 30-70% of the total of diatoms, while brackish and freshwater diatoms occupied about 10-35% and 10-20%, respectively (Fig. 5). The MD_1 Zone is divided into two subzones according to the dominant diatoms.

a) MD_1-a subzone

As shown in Fig. 8, in this subzone, the dominant diatoms were the brackish-water *Achnanthees hauckiana* and the littoral *Nitzschia granulata* accompanied with *Nitzschia punctata, Amphora acutiuscula* and an unidentified taxon.

The last taxon, which was assigned to the order Centrales (Fig. 9), can not be identified, not only at the species level but also at the genus level. This unidentified taxon is regarded as one of marine diatoms, because this taxon was also found dominated in the marine diatom zone at the Tamatsu site near Akashi River (Sato, unpublished).

b) MD_1-e subzone

The dominant diatom of this subzone was the above-mentioned identified taxon (Fig. 9) accompanied with the littoral *Nitzschia granulata*, the brackish-water *Rhopalodia gibberula* and *Achnanthes hauckiana* (Fig. 8).

Site D (+2.4 m to +3.7 m):

Diatom frustules in the sediments obtained

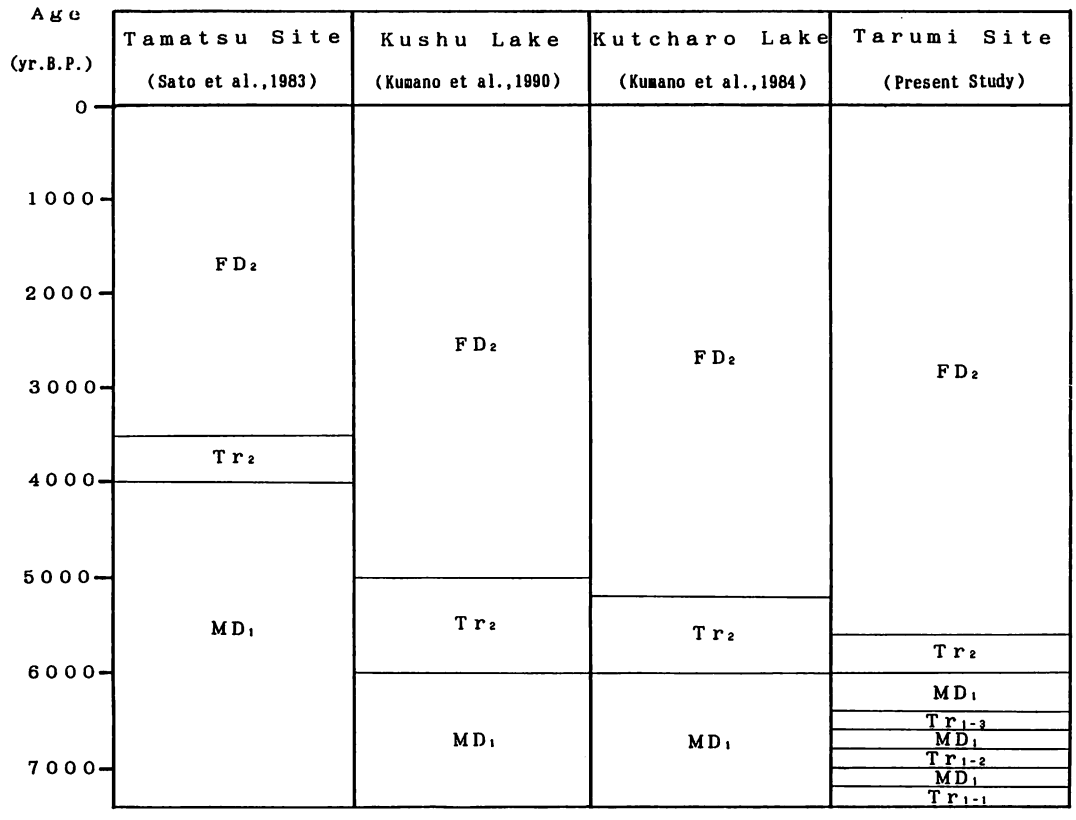

Fig. 10. Comparison of the Tamatsu site (Sato et al. 1983), the Kushu Lake core (Kumano et al. 1990a), the Kutcharo Lake site (Kumano et al. 1984) and the estuary (Tarumi site) of Fukuda River, with reference to diatom zone and subzones.

from this site were too few to count them. Freshwater and brackish diatoms were occurred, however, no marine diatom was found. For example, the lowest horizon of this site was occupied by 26 freshwater taxa and 9 brackish-water taxa, but no marine taxon was occurred. The freshwater taxa of the genus *Pinnularia* were dominated, so that these horizons might be regarded as the freshwater diatom zone (FD Zone).

Discussion

Our previous studies at several sites along the coast of Osaka Bay (Kumano and Miyahara, 1981; Kumano and Fujimoto, 1982; Sato et al., 1983), at Kamo Lake site in Sado Island (Sato and Kumano, 1985, 1986) and at Tokoro site in Hokkaido (Hamano et al., 1985) revealed that the peak of the deposition of the MD_1 Zone occurred at about 6000 yr B.P. and coincided with the peak of the first Holocene transgression at about 6000 yr B.P., and that the deposition of the MD_1 Zone and the Tr_2 Zone finished at about 5000 yr B.P. at Toya River site, Hokkaido (Ihira et al. 1985) and at 4000 yr B.P. at the Takkobu site in Kushiro Moor, Hokkaido (Kumano et al. 1990b) when the first Holocene regression occurred.

While, as shown in Fig. 10, at Kutcharo Lake site in Hokkaido (Kumano et al., 1984, Sekiya and Kumano, 1983), deposition of the MD_1 Zone and Tr_2 Zone already finished at about 6000 yr B.P., namely, the development of the lagoon or brackish lake took place at 6000 yr B.P. At Kushu Lake site in Rebun Island (Kumano et al., 1990a) deposition of the MD_1 Zone and the Tr_2 Zone already finished at about 5000 yr B.P., namely, the de-

velopment of the lagoon or brackish lake took place at 5000 yr B.P., although many authors have reported that the peak of the first Holocene transgression occurred at about 6000 yr B.P. as mentioned above. It is suggested that prior to the first Holocene regression, the "Middle Jomon minor regression" at about 4500 yr B.P. named by Ota et al. (1982), the bay-mouth sand bars were completely developed across paleo-Kutcharo Bay from Okhotsk sea at Kutcharo Lake site and paleo-Kushu Bay from Japan Sea at Kushu Lake site, respectively.

In the present study at the estuary (Tarumi site) of Fukuda River along the coast of Osaka Bay, the MD_1 Zone between 7200 and 6300 yr B.P. was alternated three times by three layers of the Tr Zones.

As shown in Fig. 2, Takahashi (1992) recognized the occurrence of three rows of sand bars across the estuary of paleo-Fukuda River developed by the coastal tidal current along the northwestern coast of Osaka bay during the first Holocene transgression. Namely, the innermost row of sand bars firstly developed is located on the tracks of Japanese Railway, the middle row of sand bars secondary developed on the grounds of Wadazumi Shrine, and the outermost sand bar tertiary developed on the national road of Root No. 2. Hence, it is likely that the first Transitional Zone (Tr_{1-1} Zone, 7200 yr B.P.) was caused by the development of the innermost (first) row of sand bar; the second Transitional Zone (Tr_{1-2}), on which many foot-prints of human being were found, was corresponded with the development of the middle row of sand bar; and the third Transitional Zone (Tr_{1-3} Zone, 6340 yr B.P.) was caused by the development of the outermost row of sand bar.

In the present study at the estuary (Tarumi site) of Fukuda River, it is considered that the Tr_2 Zone at the horizon (from +1.84 m to +2.7 m) finished at about 6000 yr B.P., because the plant remains at the −0.1 m horizon of Site C gave a ^{14}C age of 6340± 110 yr B.P. and Akahoya tephra at the +1.8 m horizon showed about 6300 yr B.P. So that, at the Kutcharo Lake site, the Kushu Lake site and Tarumi site of Fukuda River the initiations of the Tr_2 Zone are considered to have been caused by the development of sand bar, prior to the first Holocene regression, the "Middle Jomon minor regression" at about 4500 yr B.P. named by Ota et al. (1982).

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熊野　茂*・西海將雄*・奥泉　剛*・佐藤裕司：大阪湾北西沿岸・福田川河口（神戸市垂水）に於ける珪藻遺骸群集の遷移，特に完新世堆積環境の変遷について**

1)第1海産珪藻帯 MD_1 から遷移帯 Tr_2 への移行時期：木片の ^{14}C 年代値，アカホヤ火山灰の存在から，本調査地に於ける第1海産珪藻帯 MD_1 から遷移帯 Tr_2 への移行時期は，およそ6000年前であると考えられる。

2)3列の砂堆列の形成と珪藻帯との関連：およそ7000年前から6000年前の約1000年間に，第1海産珪藻帯 MD_1 中に3つの遷移帯 (Tr_{1-1}, Tr_{1-2}, Tr_{1-3}) が存在する。この3つの遷移帯の存在は，大阪湾と本調査地とを隔離するように形成された3列の砂堆列の影響を受けた古環境の変遷を反映した結果であると考えられる。(*667 神戸市灘区六甲台1丁目　神戸大学理学部生物学教室，**669-13 三田市弥生ヶ岡8丁目　兵庫県立人と自然の博物館)

Jpn. J. Phycol. (Sôrui) 40: 261–266, September 20, 1992

Regeneration of protoplasts isolated from the sporophyte of *Cladosiphon okamuranus* Tokida (Chordariaceae, Phaeophyta)

Takuji Uchida and Satoshi Arima

Nansei National Fisheries Research Laboratory, Ohno-cho, Hiroshima, 739–04 Japan

Uchida, T. and Arima, S. 1992. Regeneration of protoplasts isolated from the sporophyte of *Cladosiphon okamuranus* (Chordariaceae, Phaeophyte). Jpn. J. Phycol. **40**: 261–266.

The process of the regeneration of protoplasts was studied for the edible marine brown alga *Cladosiphon okamuranus*. The protoplasts were prepared from sporophytes by enzymatic degradation of the cell wall in the presence of EGTA, a calcium-specific chelating agent. Regeneration of the protoplasts followed three different patterns. Most of the protoplasts grew into filamentous or clumpy germlings, which matured to release plurispores. The resulting discoid germlings developed into normal, erect, sporophyte thalli. Some protoplasts divided to form cell aggregations, which did not grow further. A few protoplasts remained as single cells which gradually enlarged and became poorly pigmented. Results provide a method for the production of sporophyte thalli from protoplasts of this commercially important species.

Key Index Words: Cladosiphon—*EGTA—enzyme degradation—plurispore—protoplast—regeneration.*

The preparation and culturing of protoplasts are useful basic techniques for the breeding of marine algae. These techniques make it possible to produce many clones from seaweed strains which have valuable characteristics for mariculture, and also to attempt somatic cell fusion for breeding purposes. There have been several reports on the isolation of protoplasts from marine algae by enzymatic degradation of the cell wall (Saga and Sakai 1984, Fujita and Migita 1985, Fisher and Gibor 1987, Yamaguchi *et al.* 1988, Butler *et al.* 1989, Kloareg *et al.* 1989, Chen 1989). However, the culturing of isolated protoplasts has not always been successful. For the Phaeophyta, few studies have succeeded in regenerating a normal thallus from an isolated protoplast (Ducreux and Kloareg 1988).

The phaeophyte *Cladosiphon okamuranus* Tokida is an economically important species and is cultivated along the coasts of Japan's southwestern islands. There have been some reports on the life cycle and ecology of this species (Shinmura 1974a, 1974b, 1975), but biotechnological and morphogenetic studies have not been conducted.

In the present study, protoplasts of *C. okamuranus* were isolated, and the process of regeneration into normal thalli was investigated for future investigations in breeding and morphogenesis.

Materials and Methods

Plant material

Unialgal cultures of *Cladosiphon okamuranus* were obtained by culturing plurispores released from a parent thallus collected in May 1991 from a commercial farm for this species located in the town of Tatsugo in Kagoshima prefecture. Cultures were incubated in a photoperiod of 15L: 9D under cool-white fluorescent lamps (ca. 36 $\mu E/m^2/s$ at the surface of the culture vessels) at 20°C. ESI (Tatewaki 1966) was used as a culture medium and was replenished at intervals of 30–40 days. Cultures were grown in cylindrical glass vessels (ϕ5.5 cm × 8 cm) with a 120 ml of medium or in Erlenmeyer flasks with a 0.5 *l* of medium. Thalli grown to 1–2 cm in height were used as materials for protoplast

preparation.

Protoplast preparation

Protoplasts were prepared by enzyme degradation. To make cell walls accessible to the enzymes, EGTA (ethylene glycol-bis (β-aminoethyl ether) N,N,N′,N′-tetraacetic acid), a calcium-chelating agent, was added to the enzyme solution. According to Butler *et al.* (1989), EGTA improved the protoplast yield of *Laminaria saccharina* and *L. digitata* which may have resulted from the dissolution of the alginate gel by removal of calcium from the polygluronate linkages. The procedure for protoplast preparation is shown in Fig. 1. The cultured thalli (80-140 mg fresh weight) were maintained for 10 min in 10 ml of solution I (Table 1) which was prepared using ASP12NTA (Provasoli *et al.* 1957) as a basal solution. Then, the thalli were cut into small pieces (ca. 1 mm square) followed by the enzyme treatment. For the enzymatic degradation of cell walls and intercellular substances, the pieces of thalli were incubated with 5 ml of solution II (Table 1) for 1 hour at 20-22°C with reciprocal shaking (30 strokes min^{-1}). After incubation, the digested tissue was filtered through 20 μm nylon mesh to remove tissue fragments. The protoplast suspension thus obtained was settled for 30 min. Then the supernatant was replaced with solution I, followed by gentle shaking. In the same manner, washing was repeated 4 times with solution III (Table 1) reducing the sorbitol concentration to 0.7, 0.5, 0.2, and 0 M in the process. The number of protoplasts was counted with a haematocytometer.

Protoplast culture

Protoplasts were cultured in plastic dishes (ϕ35 mm × 10 mm) with 4 ml of medium or in multi-well plates (ϕ16 mm × 17 mm, 24 wells) with 2 ml of medium in each well. To observe the fate of individual protoplasts, they were isolated into separate wells. The culture medium was modified ASP12 NTA, in

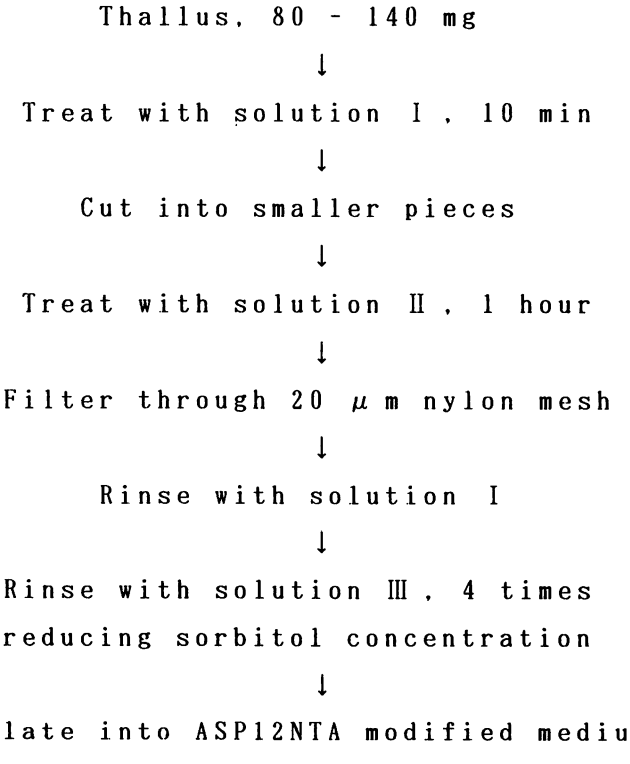


Fig. 1. Method for preparation of protoplasts of *Cladosiphon okamuranus*.

Table 1. Composition of the enzyme solution and washing solutions for protoplast preparation

Component	Solution I	Solution II	Solution III
NaCl	280 mg	280 mg	280 mg
$MgSO_4 \cdot 7H_2O$	70 mg	70 mg	70 mg
$MgCl_2 \cdot 6H_2O$	40 mg	40 mg	40 mg
KCl	7 mg	7 mg	7 mg
$CaCl_2$	—	—	11 mg
$NaNO_3$	1 mg	1 mg	1 mg
KH_2PO_4	64 μg	64 μg	64 μg
Sodium glycerophosphate	100 μg	100 μg	100 μg
P II metals*[1]	0.1 ml	0.1 ml	0.1 ml
Vitamin B_{12}	2 ng	2 ng	2 ng
Thiamine	1 μg	1 μg	1 μg
Biotin	10 ng	10 ng	10 ng
Tris*[2]	—	—	10 mg
MES*[3]	43 mg	43 mg	—
EGTA*[4]	38 mg	38 mg	—
Sorbitol	1.27 g	1.27 g	0–1.27 g
AAP*[5]	—	100 mg	—
Cellulase*[6]	—	50 mg	—
Macerozyme*[7]	—	50 mg	—
Dextran sulfate	—	100 mg	—
pH	6.5	6.5	7.5
Total	10 ml	10 ml	10 ml

*[1] Provasoli *et al.* (1957), *[2] Tris hydroxymethyl aminomethane, *[3] 2-(N-Morpholino) ethanesulfonic acid, *[4] Ethylene glycolbis (β-aminoethyl ether) N,N,N′,N′-tetraacetic acid, *[5] Abalone acetone powder (Sigma), *[6] Cellulase Onozuka RS (Yakult), *[7] Macerozyme R-200 (Yakult)

which K_3PO_4 was replaced with KH_2PO_4 keeping phosphorus at the same concentration and from which Na_2SiO_3 was omitted. Other culture conditions were the same as described under 'Plant material'.

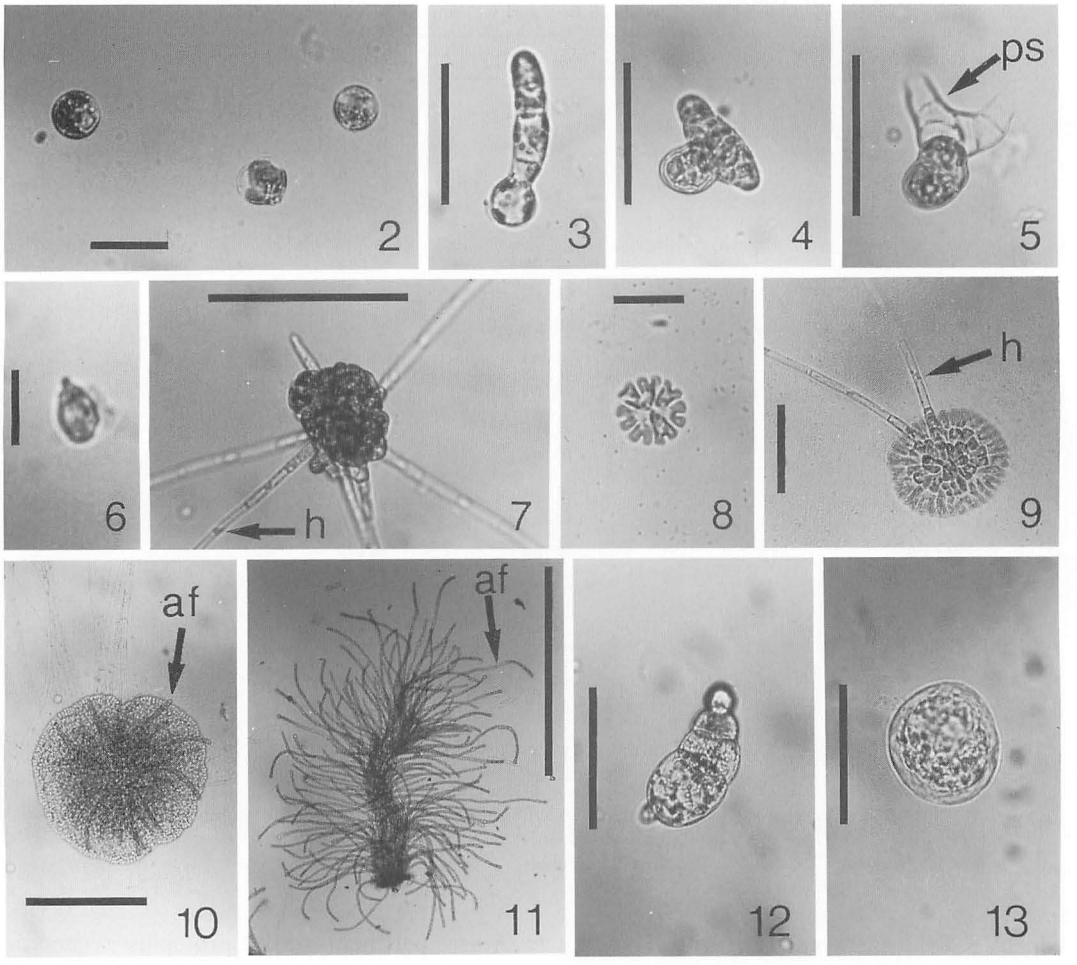
Results

Protoplast preparation

The yield of protoplasts in solution II was 9.4×10^4/100 mg fresh weight. The protoplasts were brownish and spherical; their size was 9–18 μm in diameter (Fig. 2). Judging from their color and the size, most of them had been released from the assimilatory filaments. The cells of other portions such as the subcortex, the medullary layer, and the cortex hairs were weakly pigmented and large, whereas the cells of the assimilatory filaments were densely pigmented and small. Thus, these experiments on the protoplast regeneration focused on protoplasts released from the assimilatory filaments.

Protoplast regeneration

Three regeneration patterns were observed for the protoplasts isolated from the assimilatory filaments of this species. About 30% of all protoplasts divided into two cells 4–16 days after isolation and developed into filamentous or clumpy germlings (Figs. 3, 4). These microthalli formed plurilocular sporangia after 7–20 days in culture and released plurispores (Figs. 5, 6) which were biflagellate and pear shaped. Some of the microthalli grew further to form cell aggregations with colorless hairs (Fig. 7), releasing plurispores. These cell aggregations never grew into normal thalli. After settling on the bottom of the vessels, most of the plurispores developed int-



Figs. 2–13. Protoplast regeneration of *Cladosiphon okamuranus*. Fig. 2. Protoplasts released from the assimilatory filaments. Scale bar=20 μm. Fig. 3. A filamentous germling 5 days after the isolation of a protoplast. Scale bar=40 μm. Fig. 4. A clumpy germling 11 days after the isolation of a protoplast. Scale bar=40 μm. Fig. 5. A clumpy microthallus after releasing plurispores. Scale bar=40 μm. Fig. 6. A plurispore. Scale bar=10 μm. Fig. 7. A cell aggregation developed from a germling, producing colorless hairs. Scale bar=100 μm. Fig. 8. A discoid germling from a plurispore. Scale bar=20 μm. Fig. 9. A disc producing colorless hairs. Scale bar=50 μm. Fig. 10. A disc producing assimilatory filaments 13 days after germination of a plurispore. Scale bar=200 μm. Fig. 11. An erect, sporophyte thallus 35 days after germination of a plurispore. Scale bar=1 mm. Fig. 12. A cell aggregation with large and poorly pigmented cells. Scale bar=40 μm. Fig. 13. A single large cell with poorly pigmented cell 40 days after isolation of a protoplast. Scale bar=100 μm. ps, plurilocular sporangium; h, hair; af, assimilatory filament.

discoid germlings (Fig. 8), although some of them formed cell clumps which matured to release plurispores again. In some cases, the cell contents were extruded from the cells of the microthallus and developed in the same way as the plurispores. The discs became larger through several cell divisions, producing colorless hairs (Fig. 9). Within 20 days after the settlement of the spores, the discoids began to develop erect filaments from their central areas (Fig. 10). They continued to elongate and differentiated to form the normal erect thalli of the sporophytes (Fig. 11).

A small number of protoplasts (less than 3% of the total) formed cell aggregations by successive cell division (Fig. 12). When the germlings became 6-10 celled masses, cell division ceased and the cells began to enlarge and

become poorly pigmented. The aggregations neither developed further nor formed any reproductive cells.

Unlike these two types of regeneration, a few protoplasts (less than 3% of the total) remained as single cells even 40 days after the isolation, becoming larger and poorly pigmented (Fig. 13). They resembled medullary cells in color and size.

Discussion

Most of the viable protoplasts of *Cladosiphon okamuranus* obtained in these experiments gave rise to filamentous or clumpy germlings which matured to release biflagellate plurispores. In some members of the Chlorophyta, however, the individual protoplast enlarges to form a sporangium directly without forming a multicellular germling. For example, the protoplasts of *Ulva pertusa* transform into zoosporangia, and the protoplasts of *Monostroma nitidum* develop into gametangia (Fujita and Migita 1985). Besides these regeneration patterns, it is known for various species of marine algae that protoplasts can grow directly into intact thalli (Fujita and Migita 1985, Kitoh 1985, Ducreux and Kloareg 1988, Saga and Kudo 1989). In the present case, the protoplasts of *C. okamuranus* never developed into intact thalli directly, but only through the formation of reproductive cells.

A low percentage of *C. okamuranus* protoplasts grew to form filamentous cell aggregations characterized by large and poorly pigmented cells. They formed neither intact thalli nor reproductive cells. A similar result has been reported for *Monostroma angicava* by Saga and Kudo (1989). In that case, a small number of protoplasts prepared from a female gametophyte grew into callus-like cell aggregations which never developed into leafy thalli nor produced any reproductive cells. The third pattern of the protoplast regeneration in *C. okamuranus* was that the protoplasts remained as single cells, increasing in size. Thus, three different types of protoplast regeneration were observed in this species. It has been reported for several algal species that protoplasts isolated from the same individual followed several regenerative processes. This is reasonable considering that even a simple leafy thallus such as *Porphyra* has different types of cells (Polne-Fuller and Gibor 1984). The protoplasts of *C. okamuranus* prepared in the present study were released mostly from the assimilatory filaments. Therefore, it is probable that the cells of the filaments differentiate to a certain extent, and that protoplasts from the subcortex and the medullary layer follow different regeneration processes from those demonstrated here. Chen (1989) reported that protoplasts of *Porphyra linearis* showed several regeneration patterns and that a cell-suspension culture could be established from the protoplast-derived cells which did not regenerate into thalli. Furthermore, it was shown that these cells in a cell-suspension culture regenerated into organized thalli by altering culture conditions including temperature, photoperiod and irradiance. It is worth to examine the effect of culture conditions on the protoplast regeneration of *C. okamuranus*. Another possible explanation for the variety of regeneration patterns of algal protoplasts relates to differences in coexisting bacteria and their effects on the developmental pattern of the protoplasts (Uchida *et al.* 1992). It has been reported that growth and morphogenesis of some algal species, such as *Porphyra tenera* (Tsukidate 1977), *Ulva lactuca* (Provasoli and Pintner 1980) and *Monostroma oxyspermum* (Tatewaki *et al.* 1983), are affected by bacteria. The study of protoplast regeneration using axenic cultures is important for assessing the bacterial effects on the pattern of regeneration.

The present investigation provides a method for the production of sporophyte thalli from protoplasts of *Cladosiphon okamuranus*. This may serve as the basis for future studies on breeding and morphogenesis of this species.

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内田卓志・有馬郷司：オキナワモズク胞子体から作出したプロトプラストの再生

鹿児島県竜郷町地先で得たオキナワモズク胞子体からプロトプラストを作出し，その再生を観察した。プロトプラストの再生には次のような3通りのタイプが観察された。最も高率で生じたタイプではプロトプラストが数回細胞分裂を繰り返して糸状あるいは不定型の細胞塊となった後，成熟して遊走子を放出した。遊走子は細胞分裂を繰り返して盤状体を形成し，胞子体に成長した。また別のタイプでは，プロトプラストは細胞塊を形成したが，細胞分裂は数回で停止し，細胞の肥大化及び色調の薄くなる傾向がみられた。他には，プロトプラストは細胞分裂を行わず，細胞の肥大化のみられるタイプが観察された。(739-04 広島県佐伯郡大野町丸石2-17-5 水産庁南西海区水産研究所)

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Gonium sociale (Volvocales, Chlorophyta) from Antarctica

Hisayoshi Nozaki* and Shuji Ohtani**+

*National Institute for Environmental Studies, 16–2 Onogawa, Tsukuba-shi, Ibaraki, 305 Japan
**National Institute of Polar Research, 9–10, Kaga 1-chome, Itabashi-ku, Tokyo, 173 Japan

Nozaki H. and Ohtani S. 1992. *Gonium sociale* (Volvocales, Chlorophyta) from Antarctica. Jpn. J. Phycol. 40: 267–271.

Detailed accounts of *Gonium sociale* (Dujardin) Warming originating from Antarctica were obtained, based on cultured materials isolated from a meltwater pool near Great Wall Station on King George Island. The alga exhibited vegetative colonies, which were essentially the same as those of *G. sociale* previously reported from non-antarctic regions, except for its somewhat larger size. In addition, the effects of temperature on the growth of the antarctic plant were studied at 5–25°C, in comparison with those of a Japanese strain of *G. sociale*. The antarctic strain was able to grow normally at 5, 10 and 15°C, but showed abnormal colonies at 20°C and did not grow at 25°C. In contrast, the Japanese strain produced normal vegetative colonies at 5–25°C. This is the first report on identification of antarctic colonial Volvocales at the species level.

Key Index Words: *Antarctica—Chlorophyta—culture*—Gonium sociale—*morphology—Volvocales.*

The occurrence of the colonial Volvocales in Antarctica has been reported by Thomas (1965) for *Pandorina* sp. and by Parker *et al.* (1972) for *Gonium* sp. However, their studies were not based on cultured materials, and detailed accounts and identification at the species level are lacking for these algae.

During the "Japanese-Chinese co-operative study on terrestrial biology in King George Island" (Ohtani and Nakatsubo 1992), one of the authors (S.O.) found a colonial green flagellate growing in a meltwater pool near Great Wall Station. Unialgal cultures of this alga were established from the water sample and detailed accounts were obtained. Vegetative morphology observed by light microscopy clearly indicated that the organism is referable to *Gonium sociale* (Dujardin) Warming. In addition, the effects of temperature on growth of this Antarctic alga were studied, in comparison with those of a Japanese *G. sociale* strain. Morphological details and the effects of temperature on growth of *G. sociale* originating from Antarctica are described in this report.

+ Present address: Department of Biology, Faculty of Education, Shimane University, Nishikawatsu, Matsue-shi, Shimane 690, Japan.

Materials and Methods

Water samples were collected in a meltwater pool near Great Wall Station on King George Island in December 1990. The pool was about 1 m in diameter and 20–30 cm in depth. The water was at 5.5°C and pH 10.6, and its conductivity was 845 μS/cm. During November to December of 1990, all the water in the pool often became frozen. Unialgal cultures were established by streaking the diluted sample on a Bold Basal Medium (BBM) (Nichols 1973) agar (1.5%) plate. For observation, the cultures were grown in screw-cap tubes containing 12 ml of AF-6 medium (Kato 1982), with 40 ml/l of distilled water substituted for soil-water medium (Starr and Zeikus 1987). The cultures were maintained at 15°C under an irradiance of 5000 lux, with a 14-h daylength provided by cool-white fluorescent lamps. For growth experiments, 1 ml of an actively growing culture (*c.* 1×10^4 colonies/ml) at

15°C was inoculated into 12 ml of the new growth medium. The inocula were then placed at 5, 10, 15, 20 or 25°C, under the same illumination as described above. After seven days, growth of the colonies was detected with a stereomicroscope. A strain of *G. sociale* var. *sociale* originating from Japan (Nozaki 1986a, b) was also used and treated as described above. Light microscopy was carried out using a Nikon LUR-Ke microscope equipped with phase optics or a Leitz Orthoplan Microscope with Nomarski interference optics.

Results and Discussion

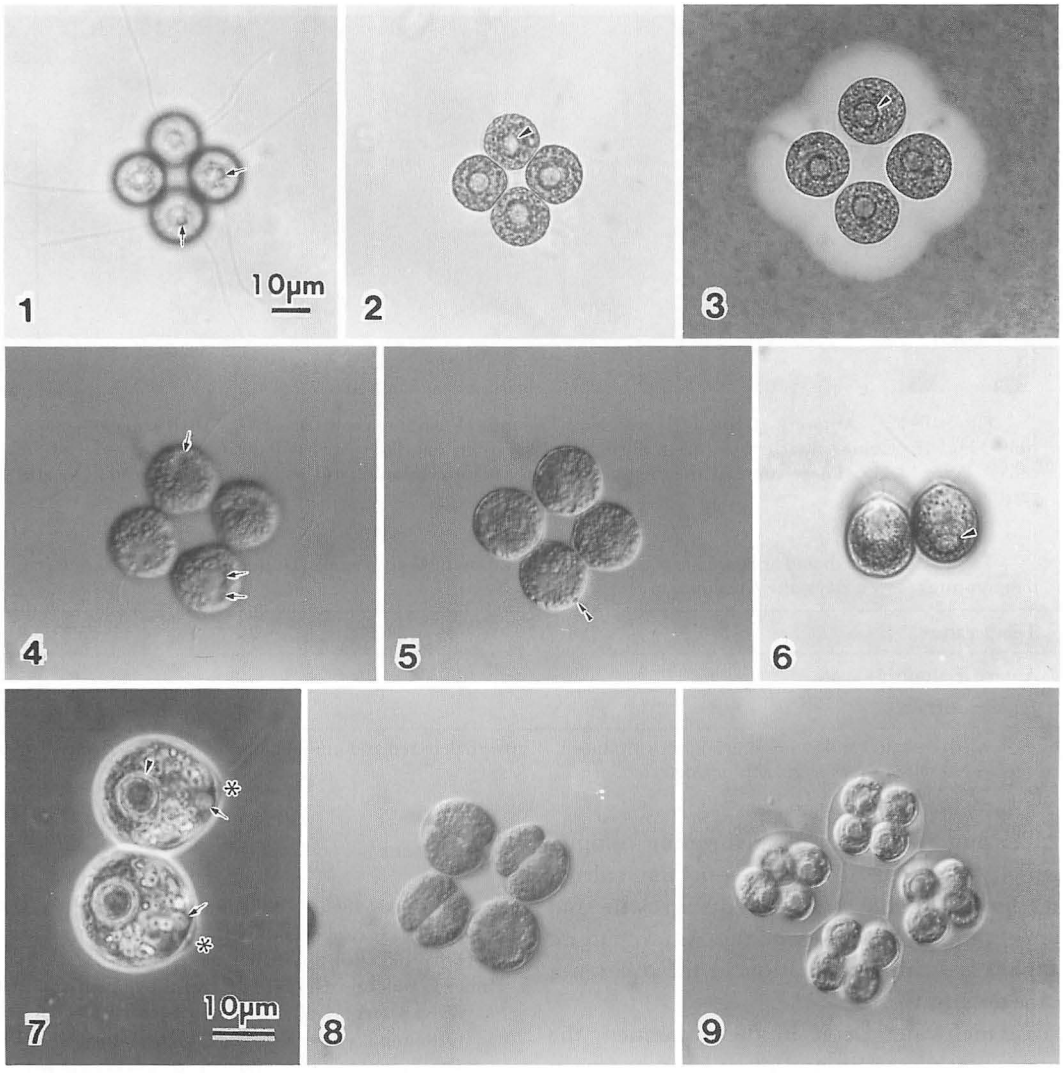
Vegetative colonies of the antarctic plant were square in shape (Figs. 1-5), measuring up to 50 μm in diameter, and each generally contained four cells, which were placed in the four corners of the square and oriented their anterior-posterior axes toward nearly the same direction (Fig. 6). The whole colony was embedded in a watery gelatinous matrix, which could be recognized clearly in an ink preparation (Fig. 3).

The cells were ovoid or nearly spherical in shape, up to 20 μm wide, and each had two equal flagella, a stigma (Fig. 5), two contractile vacuoles at the base of the filagella (Figs. 1, 4, 7) and a massive cup-shaped chloroplast. The chloroplast usually had a single large pyrenoid in the bottom (Figs. 2, 3, 6, 7). Each protoplast was enclosed by a gelatinous sheath, which exhibited a broad papilla at the base of the flagella (Fig. 7). The constitutive cells were connected by the two protuberances of each gelatinous sheath, forming a square fenestration in the center of the colony (Fig. 5).

In asexual reproduction, each protoplast within the gelatinous sheath conducted two longitudinal divisions (Figs. 8, 9). After the divisions, each daughter protoplast produced two equal flagella and developed a stigma and a single basal pyrenoid in the chloroplast. The newly formed daughter colonies measured 22-25 μm in diameter. During the daughter colony formation, the parental gelatinous sheath became expanded and the daguther colony remained for some time within the expanded sheaths (Fig. 9). The daughter colonies were then gradually liberated from their parental sheaths.

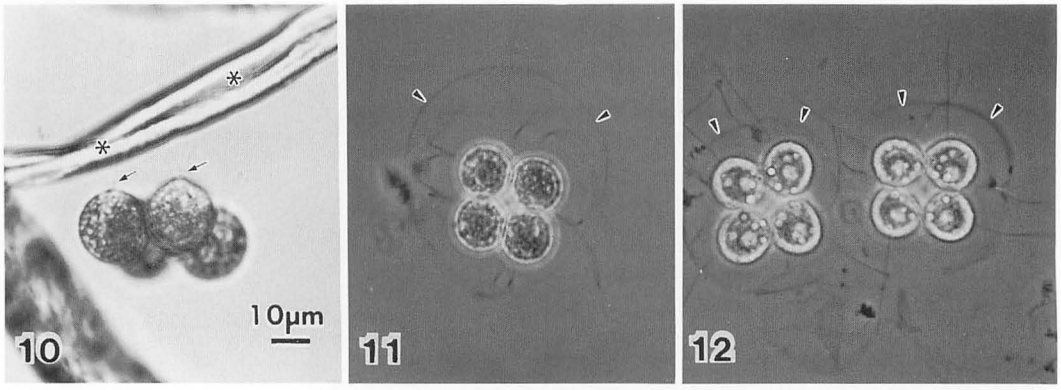
The vegetative morphology of the present organism agreed well with that of *G. sociale* collected in non-antarctic regions (Stein 1959, Huber-Pestalozzi 1961, Nozaki 1986b). Stein (1959) observed two varieties of this species, var. *sociale* and var. sacculum Stein, on the basis of her cultured materials. In lacking a "sac" (mother cellular sheath) in vegetative colonies (Fig. 6), the present antarctic alga could be assigned to *G. sociale* var. *sociale*. However, the antarctic alga produced vegetative colonies of somewhat larger size. According to Stein (1959) and Huber-Pestalozzi (1961), colonies and cells of *G. sociale* var. *sociale* measure 20-48 μm in diameter and 6-16 μm in width, respectively. Nozaki (1986b) reported the maximum diameter of the colonies of *G. sociale* var. *sociale* originating from Japan to be 32 μm. However, Hansgirg (1888) reported a larger form of *G. sociale* as *G. sociale* var. *majus* Hansgirg, which was collected in Czechoslovakia in November. The cells of this variety were 15-18 μm wide, but rarely up to 21 μm. Therefore, the present antarctic alga may be referable to this variety, which has been previously collected only once in winter (Stein 1959).

When the cultures were grown at 5, 10 or 15°C, they produced vegetative colonies which were always swimming actively. They were spread throughout the culture medium, except for some colonies gathering near the surface of the liquid by phototaxis. However, the colonies gathered and attached to the inner surface of the glass of the culture tube when they were grown at 20°C. When such colonies were observed after shaking the culture tube by hand for preparation, two types of colonies were recognized. The morphology of the first type was essentially the same as that of the normal motile colonies at low temperature. However, the motility of such colonies was very low and the colonies



Figs. 1–9. Antarctic strain of *Gonium sociale* (Dujardin) Warming. Arrow head indicates pyrenoid. Figs. 1–3, 6. Bright field. Figs. 4–6, 8, 9. Nomarski interference contrast. Fig. 7. Phase contrast. Fig. 1. Surface view of vegetative colony grown at 5°C, showing contractile vacuoles (arrows). Fig. 2. Optical section of colony in Fig. 1. Fig. 3. Colony observed in ink preparation (10°C). Note encompassing gelatinous matrix. Fig. 4. Surface view of colony grown at 5°C, showing contractile vacuoles (arrows). Fig. 5. Optical section of colony in Fig. 4. Double arrow heads indicates stimga. Fig. 6. Lateral view of colony grown at 5°C. Fig. 7. Cells showing anterior papilla (asterisk) of gelatinous sheaths. Arrow indicates contractile vacuole. Figs. 8, 9. Asexual reproduction (10°C). Fig. 8. Two-celled stage. Fig. 9. Newly formed daguther colonies within parental colony. Scale in Fig. 1 applies to Figs. 2–6, 8, 9.

became attached to the substratum with their flagella. Such behavior was clearly observed when the materials were mounted with cotton fibrils (Fig. 10). The second type includes fairly mature colonies, which measured up to 35 μm in diameter and remained within their expanded parental gelatinous sheaths with their long flagella retained within the sheaths (Fig. 11). These flagella often projected through the parental sheaths (Fig. 12). Such colonies were also immobile. Growth was not detected in the antarctic alga at 25°C. On the other hand, the Japanese strain of *G. sociale* was able to grow at 5, 10, 15, 20 and



Figs. 10–12. Antarctic strain of *Gonium sociale* (Dujardin) Warming grown at 20°C. All at same magnification. Fig. 10. Colony attached to cotton fibril (asterisks) by its flagella. Arrows indicate flagellar bases of the cells. Figs. 11, 12. Phase-contrast micrographs of fairly mature colonies still within parental cellular sheaths (arrow-heads).

Table 1. Growth and appearance of colonies in two strains of *Gonium sociale* (Dujardin) Warming at different temperatures, seven days after inoculation.

Temperature	5°C	10°C	15°C	20°C	25°C
Antarctic strain	+	+	+	#	*
Japanese strain	+	+	+	+	+

+ growth detected and swimming colonies produced; # growth detected and immobile colonies attached to the inner surface of the glass tube; * growth not detected.

25°C and exhibited only swimming colonies which were spread throughout the culture medium. Table 1 represents growth and appearance of colonies of the Antarctic and Japanese strains in relation to the difference of temperature.

In meltwater pools in the antarctica, the water was very cold and often became frozen (see Materials and Methods). It therefore seems likely that the growth of non-motile colonies at 20°C in the antarctic strain may result from its reaction to the unusual high temperatures for it.

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野崎久義*・大谷修司**：南極産の *Gonium sociale*（緑藻・オオヒゲマワリ目）

南極産の *Gonium sociale* (Dujardin) Warming をキングジョージ島の長城基地付近の水溜りより分離・培養し，その詳細を得た。本藻の栄養群体の形態は南極以外の場所から今までに報告された *G. sociale* と基本的に一致したが，ややそのサイズが大きかった。この南極産の株の温度による増殖を調べたところ，5度から15度では正常な生育をしたが，20度に於いては異常な非遊泳の群体を作り，25度では生育を示さなかった。一方，日本産の *G. sociale* の株は，5度から25度に於いて，正常な遊泳群体を作った。本報告は南極産の群体性オオヒゲマワリ目に於ける最初の種レベルの同定である。（*305 茨城県つくば市小野川16–2　国立環境研究所生物圏環境部環境微生物研究室，**173 東京都板橋区加賀1–9–10　国立極地研究所　（現）690 島根県松江市西川津町1060　島根大学教育学部生物学研究室）

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紅藻ウタスツノリの培養

能登谷正浩・菊池則雄・有賀祐勝・三浦昭雄

東京水産大学資源育成学科（108 東京都港区港南4-5-7）

Notoya, M., Kikuchi, N., Aruga, Y. and Miura, A. 1992. *Porphyra kinositae* (Yamada et Tanaka) Fukuhara (Bangiales, Rhodophyta) in culture. Jpn. J. Phycol. **40**: 273–278.

Life cycle of *Porphyra kinositae* (Yamada et Tanaka) Fukuhara was completed in culture. Growth and reproduction of both conchocelis and blade phases were examined under different temperatures and light regimes. Carpospores mostly developed into conchocelis at 10–20°C, and about 2% of carpospores developed directly into conchosporangial branches at 20°C. Conchosporangial branches were produced at 15°C under 10L : 14D within 2 months. Conchospores developed into blades, which matured at 10 and 15°C under 10L : 14D. At 20°C under 10L : 14D, blades grew very slowly and attained only 3 mm long in 72 days. Only a very small number of monospores were liberated from blades 7–10 mm long at 15°C under 10L : 14D within a month.

Key Index Words: *Bangiales—laboratory culture—life cycle*—Porphyra kinositae—*Rhodophyta.*
Masahiro Notoya, Norio Kikuchi, Yusho Aruga and Akio Miura, Laboratory of Phycology, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan

紅藻アマノリ属植物の生活環は，Drew (1949) の糸状体期の発見によって，巨視的な葉状体と微視的な糸状体の2つの異なる形態の世代から成ることが知られた。その後，室内培養による生活環の観察によって，葉状体に形成される単胞子や不動胞子による無性生殖のみの生活環をもち，世代交代のないもの (Conway *et al.* 1975) や，糸状体から直接葉状体が発達するもの (Miura 1961, Krishnamurthy 1969) などが報告されている。また，世代交代を行なう基本的な生活環のほかに，葉状体，糸状体および protothallus からの単胞子の放出や，その他のサブサイクルの存在など (Cole and Conway 1980, Kapraun and Luster 1980, Freshwater and Kapraun 1986, Kapraun and Lemus 1987)，種によってそれぞれ特有な生活環をもつことが報告されている。

これまでに日本に分布するアマノリ属植物は33種報告されている (Miura 1988) が，室内培養によって生活環が完結された種は5種にすぎない（Iwasaki 1961，鬼頭1978，右田・伊藤1987，飯間・右田1990)。そこで著者らは日本に分布するアマノリ属の生活環を明らかにする目的で，これまで数種について室内培養を試みている。本研究ではウタスツノリ *Porphyra kinositae* (Yamada et Tanaka) Fukuhara を室内培養し，その生活環を完結させるとともに，葉状体および糸状体の生長や成熟に及ぼす温度，照度，日長などの影響を調べたので以下に報告する。

材料と方法

室内培養には1989年3月7日に北海道南部日本海沿岸の歌棄で採集されたウタスツノリ葉状体 (Fig. 1, A) を母藻として用いた。成熟葉状体の果胞子形成部から約1 cm 角の葉片を切り取り，その表面を筆を用いて滅菌海水中でよく洗浄した後，滅菌海水とともにシャーレに入れ，15°C の培養庫内で照度 2000 lux（約 20 μmol m^{-2} s^{-1}）の下に約1時間放置して果胞子の放出を待った。放出された果胞子はガラスピペットで吸い取り，新たな滅菌海水へ移す操作を数回繰り返してよく洗浄した後，スライドグラス上に載せて発芽させ，単藻培養とした。

糸状体の生長や成熟の観察には，予め 20°C, 14L : 10D で糸状体を培養し，これをミキサーで長さ約 0.2 mm に細断した後スライドグラス上に付着させ，種々の条件下で培養した。糸状体の塊の長径と短径を測定すると同時に殻胞子嚢の形成についても観察した。培養は，温度 10, 15, 20°C, 照度 1000, 2000, 4000, 8000 lux（約10, 20, 40, 80 μmol m^{-2} s^{-1}), 光周期は長日 (14L : 10D), 短日 (10L : 14D) を組み合わせた合計24条

件下で行い，1週間ごとに11週目まで観察した。

葉状体の生長や成熟の観察にはクレモナ糸に付着させた殻胞子を枝付きフラスコで通気培養したものを用い，葉状体の長さと幅を5～10日目ごとに測定し，同時に単胞子の放出や雌雄生殖細胞の形成についても観察した。葉状体の培養は温度10, 15, 20°C, 照度2000～2500 lux (20–25 μmol $m^{-2}s^{-1}$) で，全て10L : 14Dの短日条件の下で行った。

培養液としてGrund改変培地 (McLachlan 1973) を用い，1週間ごとに交換した。

染色体の観察には，試料を酢酸：アルコール（1：3）で1日間固定した後，酢酸・鉄ヘマトキシリン・抱水クロラール液 (Wittmann 1965) で染色した。

結　果

1. 生活環

天然の葉状体 (Fig. 1, A) から放出された果胞子は直径12.0～18.9 μm の球形で，赤褐色を呈していた (Fig. 1, B)。果胞子は15°Cの長日条件下で培養するとスライドグラスに付着後約2日で発芽した (Fig. 1, C) が，胞子内容物の糸状体への移行は見られなかった。その後，糸状体は分枝しながら次第に生長して塊状になり，1週間後にはその直径は1 mm程度にまで生長した (Fig. 1, D)。この糸状体を15°Cの短日条件下に移すと，2か月後にはほとんどで殻胞子嚢の形成が認められた (Fig. 1, E)。

一方，20°Cの長日条件下および短日条件下ではともに果胞子は糸状体となることなく，直接殻胞子嚢枝によく似た体に発達したものが約2％あった (Fig. 1, F and G)。この場合の発芽体の生長は極端に遅く，果胞子発芽後24日目でも長さ200 μm程度であった (Fig. 1, F)。その後2か月半培養を継続したが，糸状体の発出は認められなかった (Fig. 1, G)。しかし，これらの発芽体を15°Cの短日条件下に移して培養したところ，1週間後に胞子の放出が認められ，この胞子は葉状体に生長した。

15°Cおよび20°Cで培養した殻胞子嚢から放出された殻胞子は，いずれも直径9.7～17.4 μm（平均15.3 μm）の球形で，果胞子と同様に赤褐色を呈していた (Fig. 1, H)。殻胞子は基質に付着後2日程で発芽し，15°Cの短日条件下で培養すると，4日目には3～4細胞に (Fig. 1, I)，10日目には50細胞程度になり，長さ約90 μm の葉状体に生長した (Fig. 1, J)。約2か月後には葉長，葉幅ともに約2～3 cmに達し，雌雄の成熟が認められた。成熟は精子嚢斑の形成が早く，体の先端部から始まって基部近くまで縁辺に形成され，所々，斑状に嚢果の形成が認められ，雌雄同株であった (Fig. 1, N)。嚢果は16 (a/2, b/2, c/4)，精子嚢は128 (a/4, b/4, c/8) の分裂表式であった。

染色体数については，精子形成時にn＝3が観察された (Fig. 1, O)。

葉状体からの単胞子の放出は，15°Cの短日条件下で培養約1か月後の葉長，葉幅ともに7～10 mmの時期に，葉状体30個体から約10個程度で，極く少量の放出が認められた。また，この単胞子を同条件下で培養したところ，約2か月後には葉長1.5～2 cmに達して成熟したが，この葉状体からは単胞子の放出は認められなかった。

2. 糸状体および葉状体の生長と成熟に及ぼす温度，照度および光周期の影響

糸状体の生長の比較をFig. 2に示す。糸状体の生長は20°Cで最も速く，温度が低くなるに従って遅くなる傾向が認められた。照度に関しては，20°Cの培養では1000 lux（約10 μmol $m^{-2}s^{-1}$）で最も速く，高

Fig. 1. *Porphyra kinositae* (Yamada et Tanaka) Fukuhara in culture. (A) Gametophytes collected at Utasutsu, Hokkaido, Japan on March 7, 1989. (B) Carpospore released from a natural material. (C) Carpospore germling of two days old at 15°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (14L : 10D). (D) Filamentous conchocelis thalli of three weeks old at 20°C and 3000 lux (ca. 30 μmol $m^{-2}s^{-1}$) (14L : 10D). (E) Conchosporangial branches of four weeks old at 15°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (10L : 14D). (F) Conchosporangial branch developed directly from carpospore, twenty days old at 20°C and 3000 lux (ca. 30 μmol $m^{-2}s^{-1}$) (14L : 10D). (G) Conchosporangial branches developed directly from carpospore, two months old at 20°C and 3000 lux (ca. 30 μmol $m^{-2}s^{-1}$) (14L : 10D). (H) Conchospore liberated from conchosporangium cultured at 15°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (10L : 14D). (I) Conchospore germling of four days old at 15°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (10L : 14D). (J) Young blade of ten days old at 15°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (14L : 10D). (K) Mature blades of 74 days old at 10°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (10L : 14D). (L) Mature blades of 52 days old at 15°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (10L : 14D). (M) Immature blades of 74 days old at 20°C and 2500 lux (ca. 25 μmol $m^{-2}s^{-1}$) (10L : 14D). (N) Surface view of carposporangia, antheridia and spermatia. (O) Three chromosomes in antheridium cell division. (Scale: 3 cm in A is also for K–M; 20 μm in B is also for C–F, H–J and N; 100 μm in D is also for E and G; 10 μm in O).

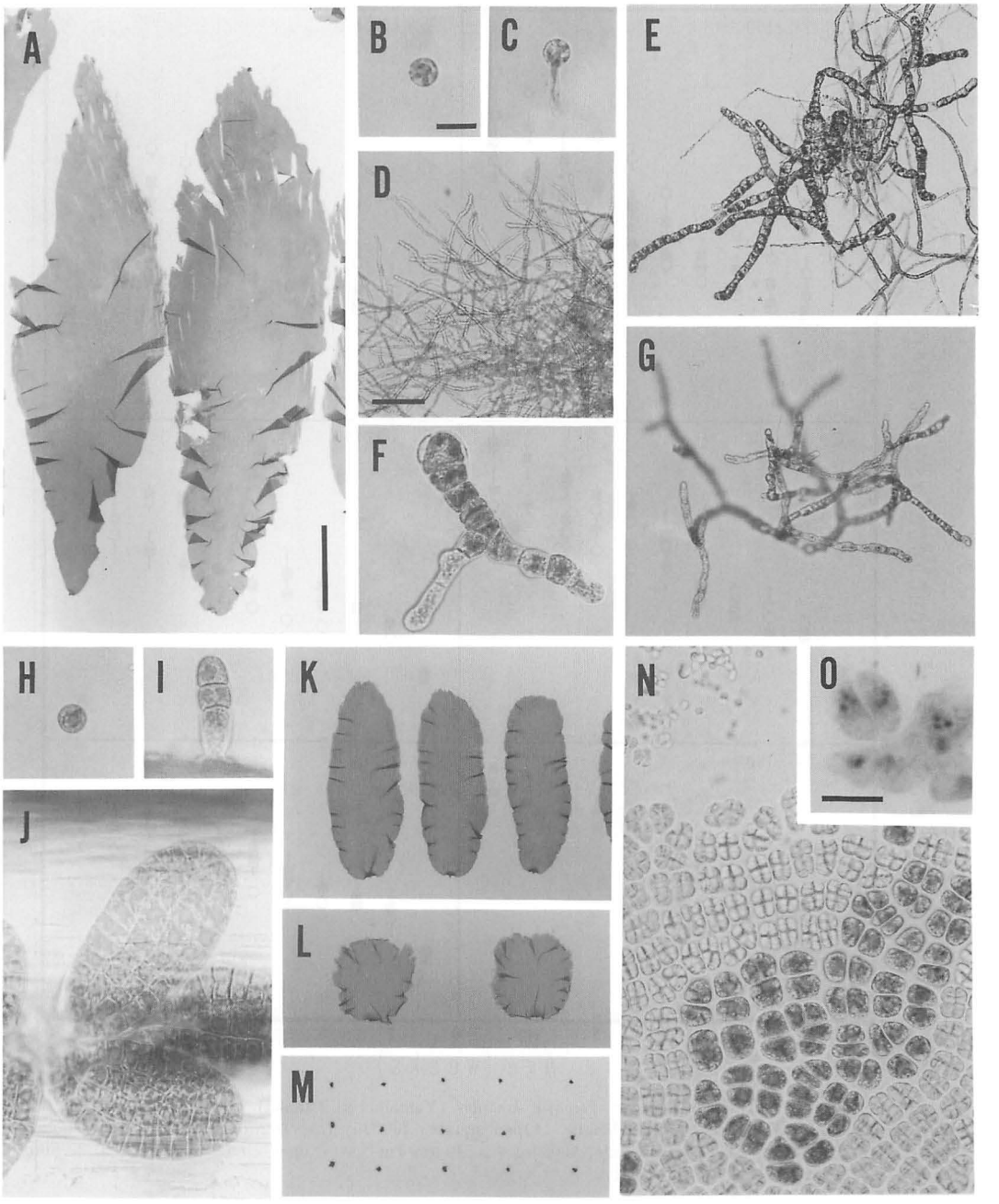

Fig. 1.

照度になる程遅かったが，15°C および 10°C の培養では 2000 lux（約 20 μmol m^{-2} s^{-1}）で最も速い傾向が見られた。温度が低くなるに従って，糸状体の生長に対する照度の影響は少なくなった。

殻胞子嚢枝の形成率の比較を Fig. 3 に示す。殻胞子嚢の形成は 15°C の短日条件下で最もよく，培養 3 週目から認められ，8 週目までには各照度で100%の糸状体に形成された。15°C の長日条件下や 20°C の長日および短日条件下では，培養 4 週目から殻胞子嚢が形成され始めたが，11週目になっても20%以下であっ

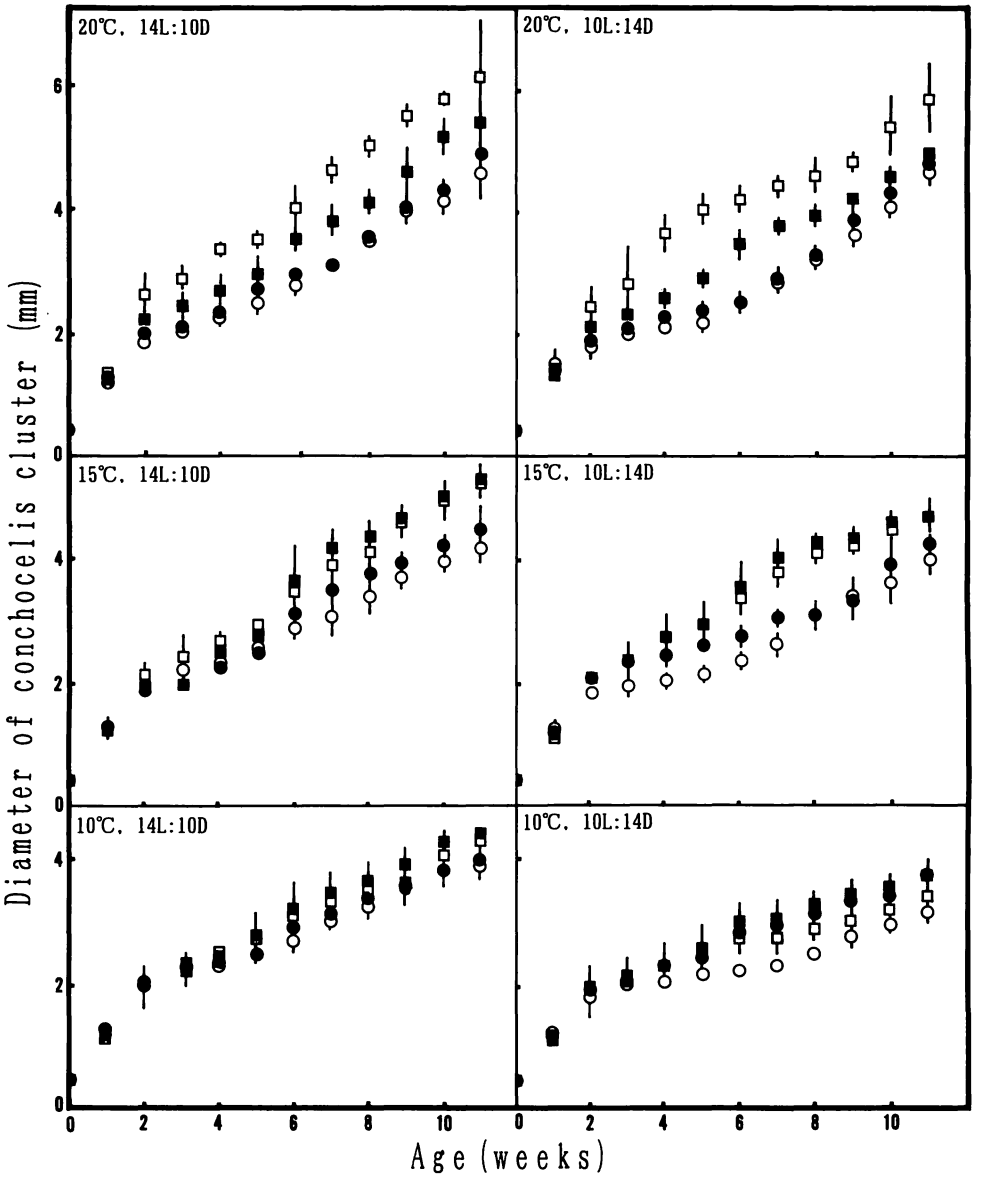

Fig. 2. Growth of conchocelis in *Porphyra kinositae* (Yamada et Tanaka) Fukuhara under different temperatures, light intensities and daylength. Open square, 1000 lux (ca. 10 μmol m^{-2} s^{-1}); solid square, 2000 lux (ca. 20 μmol m^{-2} s^{-1}); solid circle, 4000 lux (ca. 40 μmol m^{-2} s^{-1}); open circle, 8000 lux (ca. 80 μmol m^{-2} s^{-1}). Vertical bar, standard deviation.

た。また，10°C では長日条件下でも短日条件下でも殻胞子嚢の形成は認められなかった。殻胞子嚢の形成に及ぼす照度の影響は何れの温度でも大きな差は認められなかった。

殻胞子は，15°C の短日条件下では既に培養 9 週目に多量に放出されたが，15°C の長日条件下では培養11週目に極く少量認められるのみで，その他の条件下では全く認められなかった。

葉状体の生長 (Fig. 4) は 15°C で最も速く，約 7 週間で葉長，葉幅ともに 3 cm 程の円形となって成熟した (Fig. 1, L)。10°C ではこれより生長は遅かったが葉長は葉幅の約 2 倍の長楕円形となり，約 2 か月半の培養で葉長 5～6 cm，葉幅約 2 cm に達して成熟が認められた (Fig. 1, K)。しかし，20°C では約 2 か月半

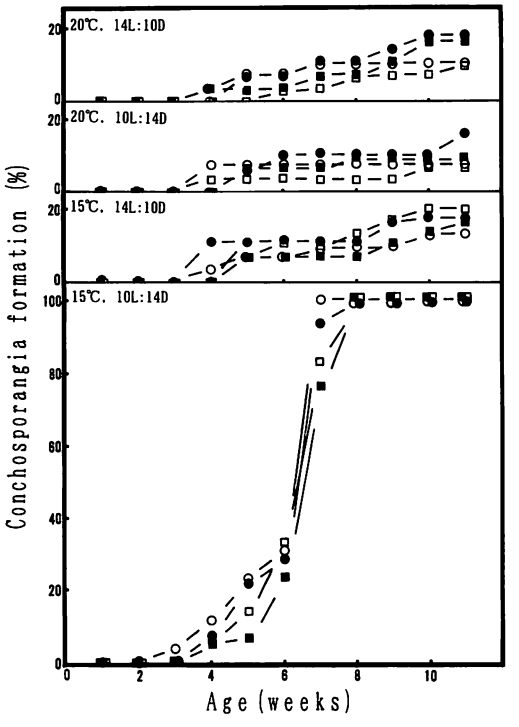

Fig. 3. Formation of conchosporangial branch in *Porphyra kinositae* (Yamada et Tanaka) Fukuhara under different temperatures, light intensities and daylength. Open square, 1000 lux (ca. 10 μmol m^{-2} s^{-1}); solid square, 2000 lux (ca. 20 μmol m^{-2} s^{-1}); solid circle, 4000 lux (ca. 40 μmol m^{-2} s^{-1}); open circle, 8000 lux (ca. 80 μmol m^{-2} s^{-1}).

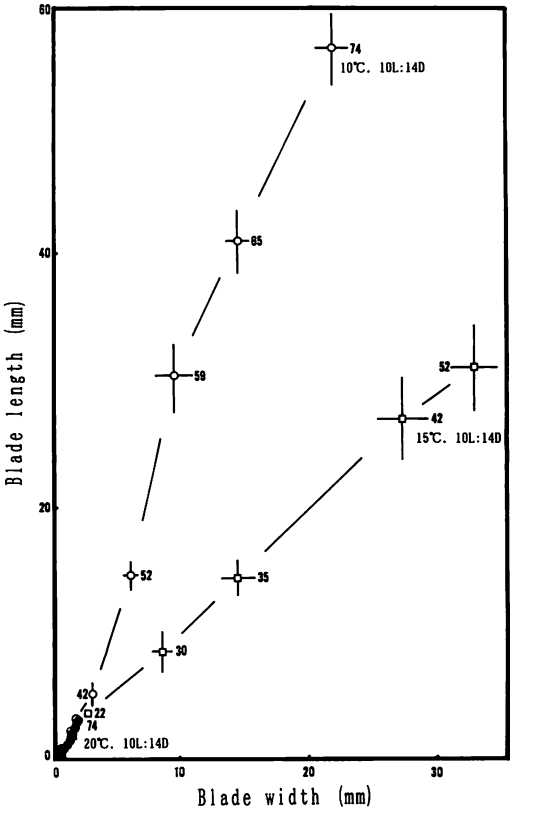

Fig. 4. Growth of blade in *Porphyra kinositae* (Yamada et Tanaka) Fukuhara under different temperatures. Numerals indicate days in culture. Open circle, 10°C and 2000–2500 lux (20–25 μmol m^{-2} s^{-1}) (10L : 14D); open square, 15°C and 2000–2500 lux (20–25 μmol m^{-2} s^{-1}) (10L : 14D); solid circle, 20°C and 2000–2500 lux (20–25 μmol m^{-2} s^{-1}) (10L : 14D). Vertical and horizontal bars, standard deviation.

の培養でも葉長約 3 mm で成熟は認められなかった (Fig. 1, M)。

考　察

ウタスツノリは北海道および青森県の日本海沿岸に分布することが知られている（福原1968)。北海道南部沿岸の歌棄から得られた葉状体を母藻とした上述の培養結果から本種は Fig. 5 に示すような生活環をもつものと考えられる。

本研究では 20°C の高温で極く少量の果胞子が直接

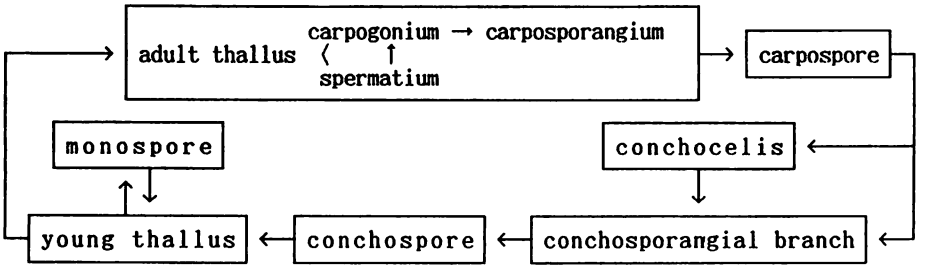

Fig. 5. Life cycle of *Porphyra kinositae* (Yamada et Tanaka) Fukuhara.

殻胞子嚢を形成するのが認められたが，これに似た事例は Cole and Conway (1980) が *P. schizophylla* の果胞子発芽体で観察している。この殻胞子嚢様の体は prothallus へ発達し，胞子を放出する場合があり，この胞子は発芽して葉状体に生長すると報告している。本研究のウタスツノリでは prothallus 様の発芽体は認められなかったが，放出された胞子は葉状体に生長したことから，果胞子から直接殻胞子嚢が形成されたものと推察される。しかし，これまで日本産の種でこのような発芽体は報告されていない。

殻胞子の放出は 15°C のみで見られたが，発芽は 10, 15, 20°C の何れの温度でも認められた。葉状体は低温の 10°C で大型の体となって成熟したが，天然では葉長 20～70 cm，葉幅 5～15 cm となり，本研究の培養藻体よりはるかに大きくなる。さらに，本種の葉状体が出現する12～3月の歌棄付近の水温は 4～8°C (福原1968)であることから，10°C 以下の低温では，これらの培養藻体より大型の体に生長するものと推察される。

ウタスツノリの単胞子についてはこれまで知られていなかったが（福原1968)，本培養によって 15°C の短日条件下で葉長 7～10 mm の葉状体から短期間にのみ極く少数放出されることが分かった。

これまでに報告のある雌雄生殖細胞形成時の分割様式（Tanaka 1952, 福原1968）や染色体数 (Yabu 1972) は，本研究の観察でも同様の結果であった。

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Donald Kaczmarczyk and Robert G. Sheath*: Pigment content and carbon to nitrogen ratios of freshwater red algae growing at different light levels

Key Index Words: carbon/nitrogen—freshwater rhodophytes—pigments—Rhode Island streams—shading.
Donald Kaczmarczyk and Robert G. Sheath, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

Rhodophyta growing in streams are frequently subjected to shading by riparian vegetation which results in significant seasonal variations in light quantity and quality. It has been suggested that there is a relationship between this photoregime and the predominance of phycocyanin in freshwater red algae (Sheath 1984). However, there is little evidence to support this suggestion (Kaczmarczyk and Sheath 1991; Raven 1992). Pigment content and phycobiliprotein to chlorophyll *a* ratios can also change in response to variations in nitrogen metabolism (Maccoll and Guard-Friar 1987). In order to examine the combined effects of light and nitrogen content on photosynthetic pigments in freshwater red algae, a survey was conducted in April of 1987. The study examined phycobiliprotein and chlorophyll *a* content as well as carbon to nitrogen (C/N) ratios in different rhodophyte taxa growing in streams which were subjected to varying degrees of shading.

Light measurements were taken at eleven stream sites in southern and western Rhode Island (U.S.A.). These readings were taken within two hours of noon at the stream surface using a LICOR quantum meter (Model LI-185B). By combining meteorological data (% cloudiness and daylength, National Weather Service—Warwick, R. I., U.S.A.) with the light measurements at the stream, it was possible to obtain estimates of the mean energy received by the plants (in mol m^{-2} d^{-1}) at each site.

The total list of Rhodophyte taxa collected included the following: *Audouinella hermannii*, (ROTH) DUBY [=*A. violacea* (KÜTZ.) HAMEL], *Batrachospermum boryanum* SIROD., *B. gelatinosum* (L.) DC. (=*B. moniliforme* ROTH), *B. sirodotii* SKUJA ex REIS [=*B. virgatum* (KÜTZ.) SIROD.], *Lemanea fluviatilis* (L.) C. AG., *Sirodotia suecica* KYLIN and *Tuomeya americana* (KÜTZ.) PAPENFUSS. Algal populations were collected in triplicate and returned to the laboratory for pigment and carbon-nitrogen analysis. Epiphytes and debris were mechanically removed from the samples upon microscopic examination. The samples were then uniformly blotted to remove excess water and divided in half. One half was used for pigment analysis and the other half was subjected to carbon-nitrogen analysis. Fresh weights were obtained for all subsamples with a Mettler AE-200 balance. Pigment analysis was performed as outlined in Kaczmarczyk and Sheath (1991). For determination of carbon and nitrogen content, algal samples were ground and then resuspended in distilled water. They were dried by boiling off the water in a microwave oven. Portions of the dried samples were then weighed on a Cahn Electro Balance and carbon to nitrogen ratios were obtained from standard curves after combustion in an Elemental Analyzer (Carl Erba Model 1106).

Differences in means among populations were calculated based on the following: total pigment, phycobiliprotein to chlorophyll *a* ratio (PBP/chl *a*), phycocyanin to phycoerythrin (PC/PE) and carbon to nitrogen (C/N). To test differences among samples, a one-way analysis of variance (ANOVA) was performed using the Minitab computing system

* Author for correspondence

Table 1. Total pigment content (mg g^{-1} fw), phycobiliprotein to chlorophyll *a* (PBP/chl *a*), phycocyanin to phycoerythrin (PC/PE) and carbon to nitrogen (C/N) ratios of freshwater red algae collected from Rhode Island streams with varying degrees of shading (Light energy estimates in mol m^{-2} d^{-1}).

Species	Energy	Total Pigment	PBP/chl *a*	PC/PE	C/N
Audouinella					
A. hermannii	21	0.510	0.304	0.494	9.4
	35	0.125	0.572	0.494	11.8
	39	0.386	0.315	0.654	9.0
Batrachospermum					
B. boryanum	15	0.110	1.520	1.433	7.5
	16	0.158	0.982	0.848	6.1
	29	0.084	0.982	0.933	7.1
	33	0.054	1.141	0.767	7.4
	35	0.111	1.192	0.764	9.0
	39	0.124	1.489	0.642	7.8
	44	0.193	1.151	0.914	6.9
B. gelatinosum	29	0.059	0.772	0.744	10.0
	35	0.099	0.782	1.000	9.3
B. sirodotii	21	0.174	0.465	1.938	8.8
	29	0.082	0.695	1.944	8.2
	33	0.066	0.616	1.389	11.5
	35	0.086	1.008	2.611	8.1
Lamanea					
L. fluviatilis	21	0.062	0.469	1.733	11.6
	39	0.070	0.839	1.162	13.5
Sirodotia					
S. suecica	15	0.053	0.441	0.914	8.3
	29	0.157	0.490	1.750	8.8
	33	0.053	0.481	3.000	8.8
Tuomeya					
T. americana	21	0.138	0.272	1.857	10.1
	33	0.092	0.335	1.286	9.1
	34	0.094	0.362	1.000	9.3

(Ryan *et al.* 1976). Pearson-product moment correlations were calculated between light energy and both pigmentation (total pigment, PBP/chl *a* and PC/PE) and the C/N ratio.

There were no significant differences among pigment amounts and ratios in populations of the seven rhodophyte species despite the variations in light energy at the stream sites (Table 1). The following trends were observed in pigment differences between species: 1) all samples of *Audouinella hermannii* had a significantly higher total pigment content than that of other species except *Batrachospermum boryanum*; and 2) samples of *B. boryanum* had a significantly higher PBP/chl *a* ratio than that of *Sirodotia suecica* and *Tuomeya americana*.

There was a significant negative correlation between mean total pigment and light energy in *T. americana*. However, no other significant correlations were observed between light energy and pigment content or ratios of the other taxa.

There were no significant differences in the C/N ratio among any of the species in the survey (Table 1). Likewise, there was no significant correlation between light energy and the C/N ratios among populations of any of the species examined. The C/N

values ranged from 6.1 (*B. boryanum* at 16 mol $m^{-2} d^{-1}$) to 13.5 (*Lemanea fluviatilis* at 39 mol $m^{-2} d^{-1}$).

All seven of the taxa analyzed in this study were found in streams with varying light energy levels and hence occurrence did not appear to be significantly affected by light regime. This agrees with the findings of Sheath and Burkholder (1985) who did not observe a relationship between freshwater rhodophyte distribution and stream shading. The results contrast with the predictive model of Vannote *et al.* (1980); namely, freshwater macroalgae are expected to be localized where light penetration is maximum.

The lack of significant differences among populations at varying light regimes was notable. The one exception was the negative correlation between total pigments in *Tuomeya americana* and light energy. The lack of correlation between total pigment and light in other species is in accord with the findings of Rider and Wagner (1972), who observed little change in the pigment content of two *Batrachospermum* species grown under different light levels.

Mean C/N ratios in this survey were in the range of 7.0 to 11.0 given for *Lemanea mamillosa* by Raven (1992) and close to the 12.0 average ratio reported for marine macroalgae (Lobban *et al.* 1985) and for autochthonous organic matter within freshwater systems (Wetzel 1983). However, for most populations, the C/N ratios fell below the latter value.

Phycobiliproteins can act as storage pools of nitrogen in red and blue-green algae (Bird *et al.* 1982, Lapointe 1985, Maccoll and Guard-Friar 1987). In the Rhodophyta, nitrogen-enriched plants of *Gracilaria tikvahiae* increased total pigment content (Bird *et al.* 1982). In this study, however, the lack of significant differences in C/N ratios among species suggested that the pigment differences did not result from nitrogen availability.

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D. Kaczmarczyk and R. G. Sheath：異なる光条件下で生育した淡水産紅藻の色素含量と C/N 比

淡水産紅藻において，光条件とフィコシアニン量との間にはある関係が既に示唆されている。また，フィコビリン蛋白量及びその Chl a 量に対する比が窒素代謝の変化に応じて変化し得ること，フィコビリン蛋白は紅藻や藍藻において，窒素の貯蔵プールの役割をもち得ることも既に示されている。

そこで，川の異なった光量下に生育する淡水産紅藻（5 属 7 種）について，平均日中光量が，フィコビリン色素 (PBP)［フィコシアニン (PC) とフィコエリスリン (PE)］の含量，PC と PE の含量比，炭素と窒素の含量比（C/N 比）及び Chl a と PBP の含量比に及ぼす影響について調べた。しかし，生育場所の光量，色素含量及び上記の比率の間には有意の相関は認められなかった。(Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9)

Jpn. J. Phycol. (Sôrui) 40: 283–285, September 20, 1992

Mitsuo Kajimura: Lectotypification of *Scinaia moniliformis* J. Agardh (Galaxauraceae, Rhodophyta)[1]

Key Index Words: Galaxauraceae—lectotypification—Rhodophyta—Scinaia moniliformis.
Mitsuo Kajimura, Marine Biological Station, Shimane University, Kamo, Saigo, Oki-gun, 685 Japan

In 1885 J. Agardh described *Scinaia moniliformis* from the two specimens ("Hab. ad Port Phillip Novae Hollandiae australis: I. Br. Wilson!") which were collected by John Bracewood Wilson from Port Phillip Heads, Victoria, Australia. No holotype specimen, however, was indicated by J. Agardh for *Scinaia moniliformis*.

These two original material specimens were each mounted on a small herbarium sheet and these two small sheets were mounted on a herbarium sheet (Fig. 1a, b). One had a reddish label of "Typus!" (Fig. 1a) which was added later by a staff of LD routinely (personal communication with Dr. Per Lassen). This specimen (LD Herb. Agardh No. 32207) was collected on February 28, 1882.

After careful examination of these two specimens the present writer has found that the one specimen (LD Herb. Agardh No. 32207) is a mature male plant with spermatangial sori which are distinguishably yellow in the present dried condition, excluding utricles and formed restrictedly to approximately upper half of the terminal or subterminal segments (Fig. 3). The spermatangial sori in this species are considered to be the 'modified apical cap-type' as seen in *Scinaia pseudo-moniliformis* Kajimura (1991), but the other specimen (LD Herb. Agardh No. 32208) is sterile.

Consequently the present writer has chosen the male mature specimen (LD Herb. Agardh No. 32207) from these two specimens as the lectotype (Fig. 2) for *Scinaia moniliformis* according to the Article 7.4 of the International Code of Botanical Nomenclature (Greuter *et al.* 1988) this time.

Scinaia moniliformis J. Agardh, Lunds Univ. Årssk. 21: 72, 1885.
Lectotype: LD Herb. Agardh No. 32207, Feb. 28, 1882, Port Phillip Heads, Victoria, Australia, Botanical Museum, Lunds University.

[1] Contribution No. 53 from Oki Marine Biological Station, Shimane University.

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The present writer wishes to thank Dr. Per Lassen of the Botanical Museum, Lunds University (LD) for his helpful suggestions as well as the loan of the original material specimens of *Scinaia moniliformis*. He also wishes to acknowledge his indebtedness to Drs. Dan H. Nicolson and James N. Norris of Smithsonian Institution, Washington D.C. for their helpful suggestions and critical reading of the manuscript.

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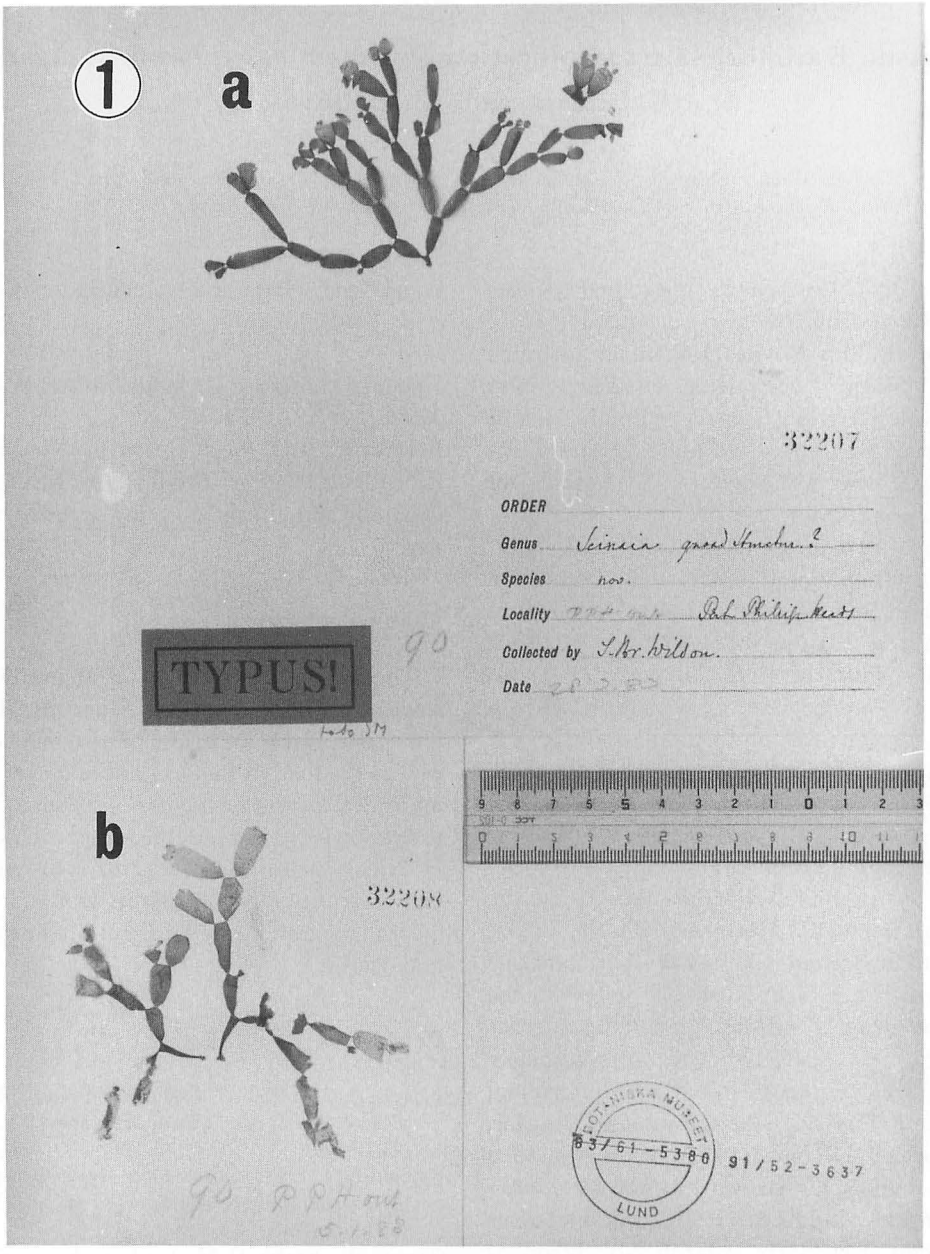


Fig. 1. *Scinaia moniliformis* J. Agardh.
Two original material specimens (a: LD Herb. Agardh No. 32207, male; b: LD Herb. Agardh No. 32208, sterile).

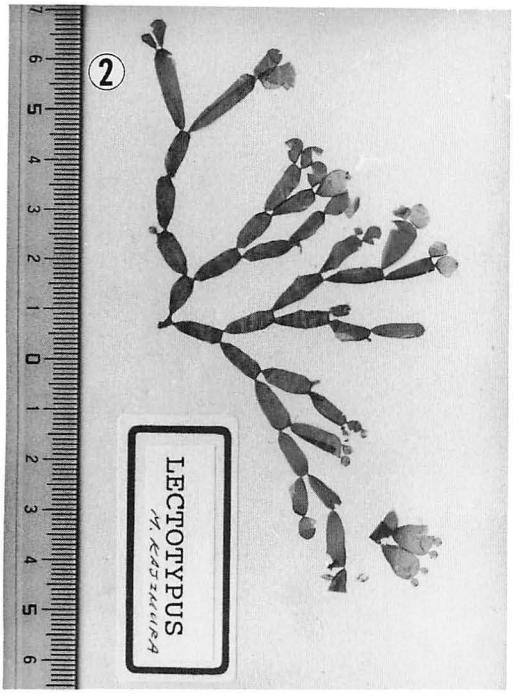


Fig. 2. *Scinaia moniliformis* J. Agardh.
Male lectotype specimen (LD Herb. Agardh No. 32207).

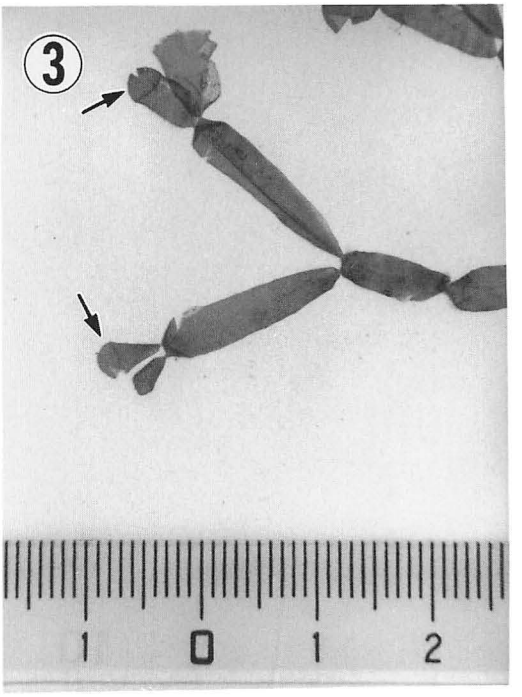


Fig. 3. *Scinaia moniliformis* J. Agardh.
Part of the male lectotype specimen showing two spermatangial sori of the 'modified apical cap-type' by arrows.

梶村光男：ジュズフサノリ（紅藻植物門，ガラガラ科）の選定基準標本の選定

紅藻植物門，ガラガラ科ジュズフサノリの選定基準標本を国際植物命名規約第7.4条に従って，上記2原資料標本から選定した。（685 島根県隠岐郡西郷町大字加茂194　島根大学理学部附属臨海実験所）

藻類 Jpn. J. Phycol. 40: 287–288, September 20, 1992

加藤季夫：プロピオンカーミン染色によるピレノイド・センターの2つの型の識別

Sueo Kato: Discrimination of two types of pyrenoid centres by staining with propionocarmine.

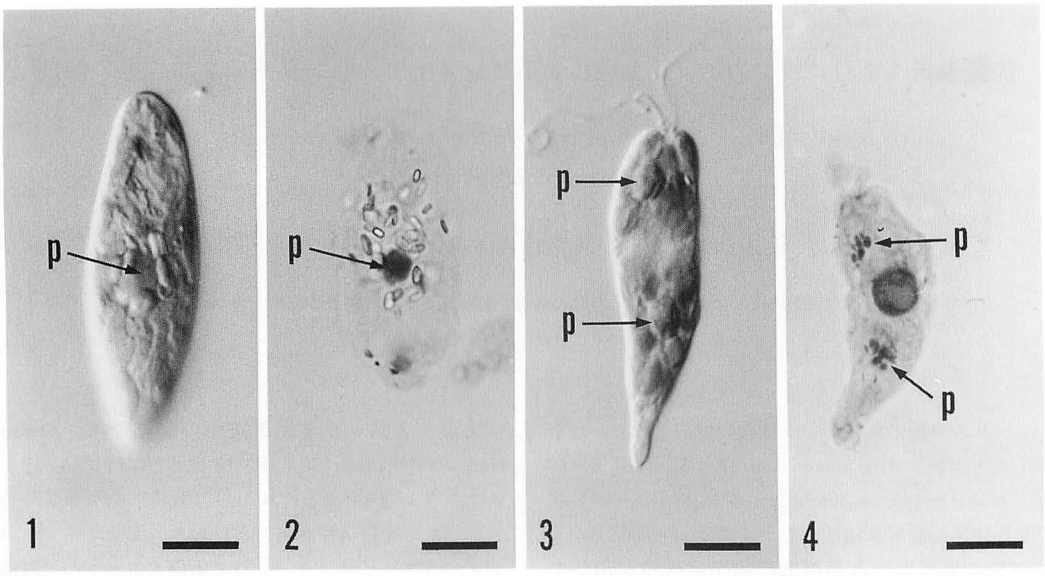
Key Index Words: Euglena viridis—Eutreptiella eupharyngea—*propionocarmine—pyrenoid centre—staining.*
Sueo Kato, Laboratory of Natural Science, Kokugakuin University, Higashi 4–10–28, Shibuya-ku, Tokyo, 150 Japan

ミドリムシ類の葉緑体の5種類の型の1つに，ピレノイド・センター (pyrenoid centre) あるいはパラミロン・センター (paramylon centre) から多くのリボン状の葉緑体片が放射状に拡がる型がある (Leedale 1967)。この型の葉緑体の微細構造は電子顕微鏡を用いて *Euglena viridis* Ehr. で調べられ (Leedale 1982), そのピレノイド・センターは1つのピレノイドからできていると報告されている。ところが，Walne *et al.* (1986) は *Eutreptiella eupharyngea* Walne *et al.* の葉緑体を電子顕微鏡で観察し，そのピレノイド・センターは *Euglena viridis* のものとは異なり，多くのリボン状の葉緑体片の先端にあるピレノイドが集まって出来ていると報告している。このことから，ピレノイド・センターには2つの型があることが明らかになった。ピレノイド・センターがどちらのつくりをしているかは電子顕微鏡による観察でしか識別できないと考えられてきたが，今回，ピレノイドの染色に用いられるプロピオンカーミン (Rosowski and Hoshaw 1970) でピレノイド・センターを染色することにより，光学顕微鏡による観察でも両者を容易に識別できることが判明したので，ここで報告する。

材料と方法：実験には *Euglena viridis* のクローン培養株 E-1164（神奈川県横浜市緑区の早淵川，1991年2月28日採集）と *Eutreptiella eupharyngea* のクローン培養株 ME-64（神奈川県横須賀市佐島港，1991年4月24日採集）の2株を用いた。培養は温度 20°C, 照度 3000 lux, 12時間明期・12時間暗期の明暗周期の条件下で行い，E-1164 株には AF-6 培地（加藤1982)を，ME-64 株には PES 培地 (Provasoli 1966) をそれぞれ用いた。ピレノイド・センターの染色は対数増殖期の藻体を用い，プロピオンカーミン（固定時間10分，1/10濃度の媒染液で媒染時間10分，染色時間5分）で行った。

結果と考察：光学顕微鏡での観察では，*Euglena viridis* の葉緑体はパラミロン粒で囲まれたピレノイド・センター (Fig. 1) とそれから放射状に拡がる多くのリボン状の葉緑体片からできており，ピレノイド・センターはプロピオンカーミンで染色すると1つの暗紫色の塊となっていた (Fig. 2)。一方，*Eutreptiella eupharyngea* の葉緑体もパラミロン粒で囲まれたピレノイド・センターとそれから放射状に拡がる多くのリボン状の葉緑体片からできており，*Euglena viridis* の葉緑体と同様のつくりをしているようにみえるが，そのピレノイド・センターはプロピオンカーミンで染色すると多くの小さい暗紫色の粒に分れていた (Fig. 4)。*Eutreptiella eupharyngea* をノマルスキー式微分干渉装置を用いて観察すると，ピレノイド・センターは微かに分れているようにもみえる (Fig. 3)。しかし，このような像は *Euglena viridis* においてもみられることがあることから，染色なしにはピレノイド・センターがどちらの型かは判断が困難である。

今回のピレノイド・センターの染色による観察結果は，電子顕微鏡での *Euglena viridis* (Leedale 1982) と *Eutreptiella eupharyngea* (Walne *et al.* 1986) の観察結果と一致しており，ピレノイド・センターが1つのピレノイドからできているか，それとも，多くのピレノイドが集まってできているかは，プロピオンカーミンでピレノイド・センターを染色することにより光学顕微鏡でも容易に判断できることがわかった。ミドリムシ類のうち，*Eutreptia* 属，*Eutreptiella* 属および *Euglena* 属の *Radiatae* 亜属のものはピレノイド・センターから多数のリボン状の葉緑体片が放射状に拡がる葉緑体をもっているが，そのピレノイド・センターがどちらの型かについて明確になっているのは *Euglena viridis* と *Eutreptiella eupharyngea* の他には *Eutreptia pertyi* Pringsheim (Dawson and Walne 1991) に限られていると思われる。



Figs. 1–2. *Euglena viridis*. 1. A cell not stained. 2. A cell stained with propionocarmine. A pyrenoid centre is composed of one pyrenoid.

Figs. 3–4. *Eutreptiella eupharyngea*. 3. A cell not stained. 4. A cell stained with propionocarmine. Two pyrenoid centres are composed of many small pyrenoids. p: pyrenoid centre. Scale bars: 10 μm.

上記の3種のミドリムシ類以外のものについても，そのピレノイド・センターがどちらかのつくりをしているかについて今後調べる必要があり，それに関して，このプロピオンカーミンによる染色法は処理が簡単で確実に識別できることから，有効な手段の1つと考えられる。

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藻類 Jpn. J. Phycol. 40: 289–305, September 20, 1992

総　説

海藻・海草相とその環境条件との関連をより詰めて求める試み

須　藤　俊　造

(186 東京都国立市谷保2686–12)

Suto, S. 1992. A trial to relate marine benthic floras more precisely to their environmental conditions. Jpn. J. Phycol. **40:** 289–305.

The author tried to relate marine benthic floras in coastal waters more precisely to their environmental conditions from data in available reports.

In the present study, the 92 species of marine floras were chosen from those which occur commonly in their native waters and are easy to identify, so that more than 20 of them would be found in any open coastal waters. Their distributions in open coastal waters, in the Seto Inland Sea and in four other inland seas fully investigated are shown in 2 Tables. Floras are characterized respectively by the combination of the presence or absence of each of the 92 species. The rate of similarity between two floras is estimated by the similarity ratio, the ratio of the number of species common in these two floras to the number of all species present in them.

Surface water temperatures in February and in August, salinity, grade of water pollution, wave hight and slope of the bottom bed were selected as important and numerical environmental factors for marine floras.

Results of cluster analyses using similarity ratios for floras in 44 open coastal waters are shown, connected with water temperatures in February and in August, indicating independent effects of the two temperatures on marine floras.

Ranges of all six environmental factors for the distribution of each species can be obtained by taking the lowest and the highest values of the factors from those in the waters where the species occurs commonly, eliminating some abnormal data.

The information in 5 Tables in this manuscript will make it possible

1) to judge rates of similarity of a marine benthic flora observed in a study to those established by similarity ratios between them;

2) to estimate values of environmental factors and their changes in coastal waters from species and the changes in them found in the waters;

3) to anticipate species and changes in species occurring in a marine flora in coastal waters, from environmental factors and changes in them, as surveyed in the waters.

Key Index Words: environmental factor—geographical distribution—marine benthic flora—marine pollution—marine topography—salinity—species composition—temperature—wave.
Shunzo Suto, Yaho 2686–12, Kunitachi-shi, Tokyo, 186 Japan.

海藻・海草植生と環境について，岡村（1931）は海流との関係を論じ，瀬川（1956）は緑藻と褐藻の種数比と，中原ら（1971）はそれに生活型も加えて，水温との関連を示し，新崎（1976）はコンブ目とヒバマタ目の種数比を海域，そこの水温条件の指標として提案し，同じく新崎（1984）は大型海藻・海草主体に，動物との生態関係も加えて水温との関係を論じ，谷口（1971）は干潮線付近より上の群落を優越種主体に分けて水温・塩分との対応を報じ，さらに新崎（1975）は伊勢湾・東京湾中心に，Hirose (1978) は大阪湾等で，海水の汚染と種の消長を報じた。種別にはアマモ，ワカメ，マコンブ，マクサ，オニクサ等がとりあげられ，それらの分布と水温の関係が報告されている。

固着生育する海藻・海草の植生はその海域の既往変化も加えた環境に強く影響され，逆の見方からはその海域環境のよい生物指標と考えられる。この意味から応用面では沿岸工事等の影響判定調査にこの植生が加えられているが，結果はほとんど役立てられていない。原因は植生と環境の関係の情報がこのような目的にはなお不十分なことにあると思われる。

ここでは，大きな地理的隔離がないと考えられる国内沿岸域の中では，植生と環境の間に比較的簡単な関連があることを予想し，集積された既往資料の照合・解析から，植生と環境の関連をよりつめて求めることを試み，上記等の応用面からの要請にもある程度応じられるようにすることを目指した。

方　法

植生を，構成する海藻・海草の種組成で表し，植生と環境の関連を，植生を構成するそれぞれの種の環境反応の総合として捉えようと考えた。種組成をとると，全国にわたって多くの既往資料を活用でき，相互の異同の程度も類似比（後述）で容易に表現できる。Setchell (1920) は外海で年間の最低期および最高期水温が海藻の種別の地理的分布を制約していると報じた。これを拡張して水温に，より局域的であるが影響の大きい数ケの環境要因も加え，海藻・海草各種の水平分布はそれぞれの生育，繁殖への各環境要因要求の上・下限によって制約された結果と考えて，各種の分布と各沿岸域環境の照合から種別に出現域の各環境要因の範囲を求め，植生をそこの環境で各要因要求がすべて満足される種の集団と考えた。

なお，植生で量の要素も加えるのは，利用できる既往資料がなお少なく，植生相互の異同度の表現も容易でなく，対応する環境要因数が増え，さらに種間競争，動物による食害，病害等の影響も大きく加わり，解析が困難なので今回は見送った。

別法として，種組成と環境の関連を各2沿岸域の種組成の類似比（次項参照）と全環境要因の総合距離（差）の関係として捉える方法も考えられるが，全要因総合距離の求め方に問題があり，またその結果からは植生から環境の，また逆に環境から植生の具体的な推定ができないなど，結果の活用も限られると考えたので，種組成類似比と最低月および最高月水温距離の関係から，外海沿岸植生の大体の区分を求めるのに用いるだけにとどめた。

2ケの種組成の異同の程度は千原ら（1970）および，太田（1973）にならい，類似比 similarity ratio（共通種数／総種数，以下 R の略称も使用，％で表示，太田の rate of relationship と同じ）で表すこととした。類似比の長所は直観的にわかりやすいことで，次に述べる対象選定種数の範囲では，R≧90％で類似，≧80％で相当似ていると見当づけられる（カイ二乗法による近似検定）。欠点は対象2植生の一方であげられた種が他方での検出に漏れた場合にはそのまま類似比低下の誤差となることである。この検出漏れによる誤差を小さくするため，千原らおよび，片田（1975）にならい，大-中型で出現域では普通に現れ，かつ同定に困難が少ない種の中から，外海各域で20種以上検出されることを目標にして92種をとり，この選定種組成を全種組成に代えることとした。また2種組成の類似比を求める時，どちらか一方にでも出現が稀な種，あるいは同定に疑問のある種がある場合には，その種は類似比の算出からは除くことにした。

ここで出現が稀とは，植生資料で稀または少ないと注記されたもの，および一水域の数ケの資料中，1ケ～ごく少数のもののみに記載された種をいい，分布表では r で表した。また分布表で同定に疑問のある種は？で表した。

上述の種の選定の適否は結果に影響する。目的からは，環境要求の異なる種を均等にとりたいが，今はそれができないので，選定にあたっては，分類上の各部門にわたり，北方種や南方種も適当に含み，量的に大きい大型褐藻類，検出されやすい有用藻類は多く加えるようにした。今回の選定はいわば第一次試案で，目的によりよくあうよう今後の修正の必要も考えられる。

なお種名は海藻は吉田ら（1990），海草は田中ら（1962）によった。ただし，リシリコンブとホソメコンブは分布と分布域水温の検討から川嶋（1977）を参照してマコンブにあわせた。ナガコンブは別種との意見があるが，一応ミツイシコンブに含めた。資料のホンダワラ属の種名は Yoshida (1983) により判定した。アラメは新崎（1985）により2型に分けることが提唱されたが，既往資料との照合が困難で，それぞれの分布域の水温等の範囲が求め難い（後述の水温の項参照）ので，今回は合わせて取り扱った。また既往資料の「ヒトエグサ」について，伊勢湾とその周辺域のものは喜田1966に従いヒロハノヒトエグサとし，その他の内湾域と瀬戸内海のものは？とし，本邦中・南部域海域のものは疑問は残るが一応多くの既往資料にあげられたヒトエグサのままとした。

環境要因としては重要でかつ数値データがえられる水温，塩分，汚染度，波高，海底傾斜度をとりあげた。それぞれの指標値は後記する。上記以外で少数種の分布に局域的に強く影響している潮汐条件等は本文及び Table 5 で種別に注記した。光条件，栄養度，また種間競争，動物による食害等は種の水平分布への影響は一般的には小さいと考えて取り上げなかった。

それぞれの選定種の分布域の各環境要因値の範囲

を，分布と各沿岸域の環境要因値を対照して求めた。この際にも，種の出現が稀な場合，および種の同定に疑問がある場合は除外して求めることとした。

結果として，1) 任意の沿岸域の選定種組成は，そこの各環境要因値が分布域要因値の範囲に入るすべての種で構成され，2) 逆にある選定種組成が見られる沿岸域の各環境要因値はそれぞれ，構成各種の分布域各要因値の範囲の中の共通する部分に入り，3) また検討を省いた環境要因等による乱れは小さいことを期待した。

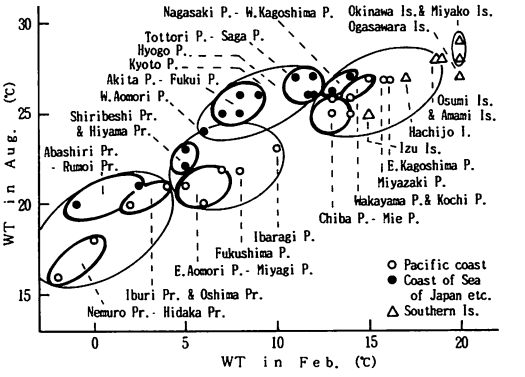

Fig. 1. Results of cluster analyses on species compositions of marine benthic floras in open coastal waters, connected with water temperatures in February and in August.
In the figure, waters are plotted by their WT in Feb. and in Aug. Next, plots of waters are enclosed by a contour line, when the floras in the waters are gathered into a cluster by cluster analyses. Thick and fine contour lines show higher and lower similarity levels of clusters, respectively.

選定種の分布と水温

選定種の分布と水温との関係は生物，水温の長年の豊富な情報の蓄積がある外海沿岸について求めた。

外海沿岸の単位水域は県などの沿岸域とやや広くとり，波当たり，海底傾斜などが異なる水域を含むようにした。ただし環境差から，青森と鹿児島両県は東西に分け，宗谷支庁管区（以下支庁管区を省略）は東は網走，西は留萌に併せ，渡島の函館以西は檜山に含めた。また南方諸島域では諸島を単位としたが，八丈島は伊豆七島から分けた。

各単位水域での各選定種の出現如何をそれぞれの中部域主体に資料から，必要に応じ現地情報も加えて判定して Table 1 に示した。

次に各域の年間最低および最高月水温 °C（以下 2，8 月水温という）を県内沿岸定点観測（九州西・北岸は定点が不足のため浅海定線観測で補足）の長年平均から求めて Table 2 に示し，Fig. 1 にプロットした。図で 2，8 月水温は太平洋沿岸ではほぼ一直線上に乗っている。それに対して日本海等（日本海，オホーツク海および東支那海をいう，以下同じ）沿岸では上に外れ，特に新潟～兵庫で著しい。

Table 1に みられる各 2 沿岸域の種組成類似比 R は平均的にはその 2 域の 2，8 月水温距離（2，8 月水温差を d_1, d_2 として $(d_1^2+d_2^2)$ の平方根，図上での 2 点間の距離）が小さいほど大きく，水温距離＜1.5 (d_1, $d_2 \leqq 1$) では R 平均値は90％と高い。ただし南方諸島間およびそれらと他の沿岸域間の R のみは70％以下と低いが，これはおそらく珊瑚礁の状況，その他沿岸地勢の単純さなどの影響に，多少の地理的隔離，調査不十分も加わった結果と思われ，南方種の選定不適当もあるかもしれない。

各選定種別に Table 1 の分布と Table 2 の各域 2，8 月水温を対照し，また Fig. 1 の各域 2，8 月水温プロットにそこでの出現の有無を印して，その種分布域の 2 および 8 月水温の範囲を求めた。多くの種では判定が容易（Fig. 2 にジョロモクの場合を例示）で，

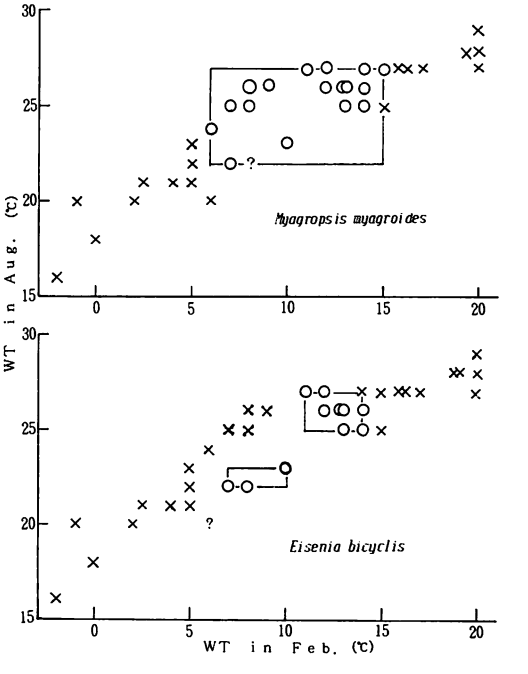

Fig. 2. Ranges of water temperatures in February and in August found in each distribution of *Myagropsis myagroides*, a common pattern, and of *Eisenia bicyclis*, a rare one.

Table 1-1. Geographical distributions of 91 common species in marine benthic floras in open coastal waters.

Species	Pacific Coast																Southern Is.							Coast of Sea of Japan, etc.																				
	Nemuro–Tokachi Pr.	Hidaka Pr.	Iburi Pr.	Oshima Pr.	E. Aomori P.	Iwate P.	Miyagi P.	Fukushima P.	Ibaragi P.	Chiba & Kanagawa P.	Pen. Izu	Mie P.	Wakayama P.	Kochi P.	Miyazaki P.	E. Kagoshima P.	Osumi Is.	Amami Is.	Okinawa Is.	Miyako Is.	Izu Is.	Hachijo Is.	Ogasawara Is.	W. Kagoshima P.	Kumamoto P.	Nagasaki P.	Saga P.	Fukuoka P.	Yamaguchi P.	Shimane P.	Tottori P.	Hyogo P.	Kyoto P.	Fukui P.	Ishikawa P.	Toyama P.	Niigata P.	Yamagata P.	Akita P.	W. Aomori P.	Hiyama Pr.	Shiribeshi Pr.	Rumoi-W. Soya Pr.	E. Soya-Abashiri Pr.
Monostroma nitidum							r			c	c	c	c	c		c	c	c	c	c			c	c	c	c	c	c	c		r													
Ulva pertusa	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Dictyosphaeria cavernosa										r	c	c	c	c			c	c	c	c	c	c	c	r	c	r		r																
Halicoryne wrightii														r				c	c																									
Neomeris annulata															c	c	c	c	c	c			c																					
Bryopsis plumosa		c	c	c		c	c		c	c	c	r					c	c			c			r		c		c	c	c	c	c	c	c	r	r	r	c	c	c	c	c	c	r
Caulerpa cupressoides												r	r				r	c	c	r		c																						
C. okamurae										c	c	c	c	c	c	c					c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r			
C. racemosa										r	r	r	c	c	c		c	c	c	c	c	c	c		r	r																		
Halimeda opuntia												r					c	c	c	c			c																					
Analipus japonicus	c	c	c	c	c	c	c	c	c																										r					r	c	c	c	c
Chordaria flagelliformis	c	c	c																																		r		r			r	c	c
Cladosiphon okamuranus																		c	c	c																								
Ishige okamurae							r	r	c	c	c	c	c	r	c	c	c	c			c	c		c	c	c	c	c	c	c	c	c	c	c	r		r	r	r	r	r			
Nemacystus decipiens										c	c	c	r	c			c	c						c	r	c	c	c	c	c	c	c	c	c	c	c	c	c	r					
Colpomenia sinuosa	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Scytosiphon lomentaria	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c	c	c	c					c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Desmarestia viridis	c	c	c	c	c	c	c	c	c	r		r	r													r		c	c		c		c	c	c	c	c	c	c	c	c	c	c	c
Alaria crassifolia		c	c	c	c	c	r																																					
A. praelonga	c																																											c
Undaria pinnatifida		r	c	c	c	c	c	c	c	c	c	c	c		r	r								c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
Costaria costata	c	c	c	c	c	c	c																																	c	c	c	c	c
Ecklonia cava									c	c	c	c	c	r	c											?	?	?	?															
E. kurome										r	r	r	c	r	r										c	c	c	c	c	c	c	c	c	c	c	c	c							
E. stolonifera																										c		c	c		c		c	c	c	c	c	c	c	c	r			
Eckloniopsis radicosa										c	c	c	c	c	c	r	c				c			c	c																			
Eisenia bicyclis						r	c	c	c	c	c	c	c											r		c	c	c	c	c	c	c												
Laminaria angustata	c	c	c	r																																								
L. japonica			c	c	c	c	ç	c																															r	c	c	c	c	c
Dictyota dichotoma			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Padina arborescens				r			r			c	c	c	c	c	c	c	c	c			c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c				
P. minor												c	c	c	c	c		c	c	c		c	c	c	c	c	c	c	c		c	r												
Cystoseira hakodatensis	c	c	c	c	c	c			r																															r	r	c	c	c
Hormophysa cuneiformis																r	r	c	c	c																								
Myagropsis myagroides							c		c	c	c	c	c	c			r									c	c	c	c	c	c	c	c	c	c	c	c	c	c	c				
Fucus distichus	c	c	c	c	r	r																																						c
Pelvetia wrightii	c	c	c	c	c	c	r	r	r																																		c	c
Hizikia fusiformis				r	c	c	c	c	c	c	c	c	c	r	c	c	r	r	c		c			c	c	c	c	c	c	c	c	c	r	r	r						c			
Sargassum confusum		r	c	c	c	c	c	r																	r	c	c	c	c	c	c	c	c	c	c		c	c	c	c	c	c	c	c
S. duplicatum											r	c	c	c	c	c		c	c		c	c		c																				
S. hemiphyllum							r		r	c	c	c	c	c	c	c	c	c			c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r				
S. horneri			r	c	c	c	c	c	c	c	c	c	c	r	r	r	r	c			c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r	
S. macrocarpum						r			c	c	c	c	c	c			c	c	r		c			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c				
S. okamurae									r	c	c	c	c	c	c																													
S. patens							r		r	c	c	c	c	c	c	c	c	r	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c				

Coast of Sea of Japan, etc.: Coast of Sea of Japan, of Sea of Okhotsk and of East China Sea.

Table 1-2. (Continued).

Species	Pacific Coast																Southern Is.							Coast of Sea of Japan, etc.																				
	Nemuro–Tokachi Pr.	Hidaka Pr.	Iburi Pr.	Oshima Pr.	E. Aomori P.	Iwate P.	Miyagi P.	Fukushima P.	Ibaragi P.	Chiba & Kanagawa P.	Pen. Izu	Mie P.	Wakayama P.	Kochi P.	Miyazaki P.	E. Kagoshima P.	Osumi Is.	Amami Is.	Okinawa Is.	Miyako Is.	Izu Is.	Hachijo Is.	Ogasawara Is.	W. Kagoshima P.	Kumamoto P.	Nagasaki P.	Saga P.	Fukuoka P.	Yamaguchi P.	Shimane P.	Tottori P.	Hyogo P.	Kyoto P.	Fukui P.	Ishikawa P.	Toyama P.	Niigata P.	Yamagata P.	Akita P.	W. Aomori P.	Hiyama Pr.	Shiribeshi Pr.	Rumoi-W. Soya Pr.	E. Soya-Abashiri Pr.
Sargassum piluliferum					c	c	c			c	c	c	c	c	c	c	r		r		c			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c				
S. ringgoldianum							c		c	c	c	c	c	c	c	r								r	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r					
S. sandei												r	c	c	c	c		c	c		c	c		c																				
S. siliquastrum			r		r	c	c	c	c	c	c	c	c	r	r	r	r				c			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		r	
S. thunbergii	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
S. yezoense				r	r	c	c																			c	c	c		c			c	c	c			r	c		c	c	c	
Turbinaria ornata												r		r		r	r	c	c	c			c																					
Porphyra pseudolinearis	c	c	c	c	c	c	c	c	c																			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
P. variegata	c	c	c	c																																					c	c	c	c
P. yezoensis	c	c	c	c	c	c	c	c	c																	c		c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	
Galaxaura fastigiata										c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c		c	c	c	c	c							
Acanthopeltis japonica										c	c	c	c	c	c	r	c	c			c					r																		
Gelidium elegans		r		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c			c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
G. japonicum									c	c	c	c	c	c	c	c	c	c			c	c		r	r	r		c	c	c	c													
G. pacificum									r	c	c	c	c	c							c	c																						
Pterocladia capillacea			r		c	c	c	c	c	c	c	c	c	c	c	c	c	c			c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
Amphiroa dilatata					r					c	c	c	c	c	c	c	c	c			c	c		c	c	r	c	c	c	c	c	c	c	c	c	c	r	c	c	c				
Corallina pilulifera	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Constantinea subulifera	c	c																																										
Neodilsea yendoana	c	c	c	c	c	c	c	c	c																														r	r	c	c	c	c
Gloiopeltis furcata	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c	c	c	r		c	c		c	c	c	c	c	c		c		c	c	c	c	c	c	c	c	c	c	c	c
G. tenax										r	r	c	c	c		c	c	c			c			c	c	c		c	c		c	c	r											
Grateloupia filicina			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
G. turuturu			r	c	c	c	c	c	c	c		c	r	r							c			r		r	r		c		c	c			c	c		c	c	c	c	c	c	c
Pachymeniopsis elliptica					c	c	c	c	c	c	c	c	c	c	c	c					c	c		c			r				c		c	c		c	c	c	c					
Prionitis angusta						r				c	c	c	c	c	c	c	c	c	c		c			c	c	c						r												
Chondrus yendoi	c	c	c	c	c	c	c																																	c	c	c	c	c
Gigartina intermedia					c	c	c	c	c	c	c	c	c	c	c	c	r	r			c	c		c	c	c	c	c	c	c	c	c	c	c	c	r					c	c		
Rhodoglossum japonicum	c	c	c	c	c	c	c																																	c	c	c	c	c
Gracilaria asiatica	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c			c			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c
Gymnogongrus paradoxus					c	c	c	c	c	c	c	c	c	c	c						c	c	c																	r				
Plocamium telfairiae						c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		r			
Eucheuma denticulatum														r	r		r	c	c	c																								
Meristotheca papulosa										c	c	c	c	c	c	c	c	c			c	c		c	c	c	c	c			r			r										
Solieria pacifica						r				c	c	c	c	c		r	c	c			c			c	c	c	c	c	c		c					r								
Turnerella mertensiana	c	c						r																																				
Lomentaria catenata				c	c	c	c	c	c	c	c	c	c	c	c	c	c	c			c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r	
Campylaephora hypnaeoides		c	c	c	c	c	c	c	c	c	c	c	r	c			r				c			r	r	r	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Ceramium kondoi	c	c	c	c	c	c	c	c	c	c	r	c		c	c		r	r						r			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Dasya sessilis			r		c	c	c	c	c																				c	c	c	c	c	r			c	c	c	c	c	c	c	r
Chondria crassicaulis			c	c	c	c	c	c	c	c	c	c	c	c		r					c	c		r	r	r	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
Digenea simplex												r	r	r	c		c	c	c	c			c	c	r																			
Neorhodomela aculeata	c	c	c	c	c	c	r																									r				r		c	c	c	c	c	c	c
Thalassia hemprichii																		c	c	c																								
Phyllospadix iwatensis	c	c	c	c	c	c	c	c	c	r																									?	?	c	c	c	c	c	c	c	c
Zostera marina	c	c	c	c	c	c	c	c	c	c	c	c	c		c	r								c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c			c

P.: Prefecture. Pr.: Province in Hokkaido.

Table 2. Surface water temperatures in February and in August in open coastal waters.

Coastal waters	WT (°C)		Coastal waters	WT (°C)	
	Feb.	Aug.		Feb.	Aug.
PACIFIC COAST			COAST OF SEA		
Nemuro Pr.			OF JAPAN, ETC.		
-Tokachi Pr.	−2	16	W. Kagoshima P.		
Hidaka Pr.	0	18	Kumamoto P.	14	27
Iburi Pr.	2	20	Nagasaki P.	13	26
Oshima Pr.	4	21	Saga P.		
E. Aomori P.	5	21	Fukuoka P.	12	26
Iwate P.	6	20	Yamaguchi P.	12	26
Miyagi P.	7	22	Shimane P.	12	27
Fukushima P.	8	22	Tottori P.	11	27
Ibaragi P.	10	23	Hyogo P.		
Chiba P. &			Kyoto P.	9	26
Kanagawa P.	13	25	Fukui P.		
Pen. Izu	14	25	Ishikawa P.	8	26
Mie P.	13	26	Toyama P.		
Wakayama P.	14	26	Niigata P.	9	26
Kochi P.	15	27	Yamagata P.	8	25
Miyazaki P.	16	27	Akita P.	7	25
E. Kagoshima P.	16	27	W. Aomori P.	6	24
SOUTHERN IS.			Hiyama Pr.	5	23
Osumi Is.	19	28	Shiribeshi Pr.	5	22
Amami Is.	19	28	Rumoi Pr. &		
Okinawa Is.	20	28	W. Soya Pr.	2	21
Miyako Is.	20	29	E. Soya Pr. &		
Izu Is.	15	25	Abashiri Pr.	−1	20
Hachijo I.	17	27			
Ogasawara Is.	20	27			

P., Pr. and "Coast of Sea of Japan, etc.": see footnotes for Table 1.
WT in Feb. and in Aug.: means of many years' data at one to several stations on each coast.

Table 5 に結果を示した。

しかし，フシスジモク，エゾノネジモクでは 1) 太平洋北部・日本海北部と 2) 日本海中・南部とに分かれた 2 ケの水温範囲が判定され，いづれも水温要求の異なる 2 群を含むと考えた方がよいと思われた。またアラメでは宮城～茨城と太平洋・日本海両中・南部とに分かれた 2 ケの水温範囲が判定された (Fig. 2) が，それらは新崎 (1985) によるアラメの 2 型の分布とは一致しない。以上は Table 5 では一応 1 種として合わせた水温範囲をあげ，その範囲内で出現のない沿岸域を注記するという表現法で示した。

オオブサ等は日本海沿岸には，反対にツルアラメ等は太平洋沿岸にはみられない。またヒジキ等の潮間帯種は本州北部の日本海沿岸では潮汐条件から適水温域でも生育していない。これらについても不出現域を注記した。

なお日本海等沿岸のクロメの分布域中，山口～長崎の資料・情報にカジメが散見されるが，その分布域水温範囲に太平洋沿岸でのそれと差がみられるなどもあってクロメの誤認との疑問を感じ，Table 1 での表示を？とした。またワカメ等少数種では内湾域資料により水温上，下限値に修正を加えた。

国内では，年間の最低月水温がウミヒルモ等 (Miki 1934)，マクサ（木下1942)，ワカメ（新崎1958)，オニクサ（遠藤ら1960）などの，最高月水温がアマモ (Miki 1933, 川崎ら1990) の分布を制約すると報告さ

れている。2，8月水温に代えて年平均水温をとると，太平洋，日本海等両沿岸の水温変動の差が表せなくなり，水温差と類似比の関係は乱れが大きくなり，後述する水温と植生のクラスター分析結果との照合も困難になる。海域による水温変動の違いが種の分布，植生の差に現れているので，水温条件としては少なくも最低期と最高期水温をともにとることが必要と考える。

Table 5 にあげたのは国内の外海域主体での海藻・海草各種の分布域水温値の範囲であり，2月，8月水温間の相関の影響も加わっているが，国外のより低い・高温域にも分布する種を除いて，多くの場合，それぞれの種の分布を制約する水温値に近いものと思われよう。なお上記国内報告での分布域水温上・下限値はTable 5 のそれらの数値とほぼ一致している。

別に，外海各域間の種組成類似比を最長距離法中心に，重心法，メジアン法も併用して，クラスター分析した。結果から Fig. 1 で，種組成が3方法でほぼ共通して同じクラスターにまとめられた海域の2，8月水温プロットを，類似レベルが高いほど太い線で囲んだ。これからも一般的には2，8月水温が近い海域の種組成が似ているのがみられる。沿岸域を種組成の類似度から大きく分けると，1）根室～日高，網走～留萌，胆振・渡島，2）後志・檜山，青森東～宮城，福島，茨城，3）青森西，秋田～福井，兵庫，京都，鳥取～佐賀，4）千葉～三重，和歌山・高知，宮崎，鹿児島東，同西，熊本・長崎，伊豆七島，八丈島，大隅・奄美諸島，5)沖縄・宮古諸島，小笠原諸島となった。類似比からこの結果は岡村（1936），瀬川（1956），新崎（1976）らの海藻分布の区分と大差はないが，ただ金華山を境とする差が小さく，代わって犬吠岬での差が大きく出ている。これは両者を境とする沿岸域の水温距離差の反映でむしろ妥当ではないかと思われる。

選定種の分布と塩分，汚染度，波高，海底傾斜度

小沿岸域別に種組成と表記に2月水温も加えた5環境要因値のデータをほぼ共に入手できた大阪湾東岸，英虞湾，伊勢湾，東京湾口の計27小域での選定種組成 (Table 3) と5環境要因値 (Table 4) を照合して，種別に分布域要因値の範囲を求めた。なお8月の水温はこれらの海域ではほとんどの出現種の分布を制約していないと認めて省いた。

2月水温，塩分，汚染度の指標値には浅海定線観測などから植生調査域に近い測点の2月 WT °C, 全年 Cl ‰，全年 COD_{OH}（アルカリ性での $KMnO_4$, 100°C, 20 min による COD (JIS K 0102)，以下 COD という）mg/l の5～20年平均値をとった。波高は域内または至近漁港の設計沖波波高 m（以下で $H_{1/3}$ の略称も使用）を指標値とした。ただし，大阪湾内は関西空港環境影響調査の最大有義波高で代え，（ ）をつけて示した。海底傾斜度は海図で水深 10 m 線の距岸距離（湾奥では 5 m 線のそれの2倍，埋め立てなどがある時はそれ以前の推定距離）を km 単位で求めて指標値とし，D で示した。各沿岸域のこれらを Table 4 にあげた。ただし一部は推定値により，（ ）を付して示した。

ここで，定線観測は2（または1）ヶ月に1回だけなので，WT, Cl, COD 各指標値の精度は高いとはいえないし，Cl 値は植生に影響が大きい出水時の低塩分値と，COD 値は同じく夏の高 COD 値と相関はあるが十分とはいえない。設計沖波波高は水深や地形で複雑に変化する生育現場の波高と違うし，最荒天時の有義波高でふだんの波高の数倍にもなる，などの問題があるが，データ入手の制約から今回はこれらの指標値で我慢せざるをえなかった。なお波と流れを合わせた海水流動の指標値として半球形石膏の減重速度が用いられ，小域内で海藻種別分布とのよい対応が見られた（川井ら1982，太田ら1990）が，その広域的適用は困難と思われ，また流れは資料不十分なので，今回は見送った。海底傾斜度はふつう急であれば岩底，緩やかであれば砂底と底質の大略の指標でもある。

なお，Cl は種別分布域の下限値のみを，COD は上限値のみを求め，Cl の上限値，COD の下限値はふつう外海での値なので省いた。$H_{1/3}$ は下限値を求めた。波高がその種の要求する波の強さ以下の所にはその種は分布しないからである。生育域では沖波が水深，地形により弱められるため，沖波が強くても，波の蔭になる所には弱い波を要求する種も生育しうるので，上限値はデータからは求まらない。なお外海開放域の $H_{1/3}$ はふつう 8～11 m である。D は上限値のみを求め，下限値はデータからは求まらなかった。

各選定種別に，その分布域の各環境要因の上・下限値を前記4内湾での分布 (Table 3) と各域環境要因指標値 (Table 4) を照合して求めて Table 5 に示した。4内湾を併せることにより，要因値のより範囲の広い組み合わせが多数得られて上・下限値をよりつめて求められると共に，要因間の相関の影響を著しく低められることを期待した。しかしなお資料数の不足と，要因間にまだ残された相関から，表示の上・下限値は今後

Table 3. Geographical distributions of 52 common species in marine benthic floras in the four inland seas fully investigated and those in the Seto Inland Sea.

Species	Osaka Bay					Ago Bay					Tokyo Bay				Ise Bay														Seto Inland Sea								
	Kada	Kojima	Tan-no-wa	Ozaki	Kaizuka	Area A, mouth	Area B	Area C	Area D	Area D, bottom	Jo-ga-shima I.	Matsuwa	Kamoi	Hashirimizu	Suga-shima I.	Ise	Matsusaka & Tsu	Yokkaichi	Shin-maiko	Toyohama	Shino-jima I.	Pt. Irago	Saku-shima I.	Ooi	Nishio	Isshiki	Oki-no-shima I.	Kii Channel	Osaka Bay	Sea of Harima	Bisan Channel	Sea of Hiuchi	Sea of Aki	Sea of Suo	Sea of Iyo	Bungo Channel
Monostroma latissimum	?					c	c	c	c	c	?	?	?		c					c	c		c					?	?	?	?	?	?	?	?	?
Ulva pertusa	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Bryopsis plumosa	c	c	c	c	c						c	c	c		c	r	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r	c	r		
Caulerpa okamurae						c	c				c	c										c							r	r	r	r	c	c	c	
Ishige okamurae	c					c	c	c			c	c	c	r	c					c	c	c	c					r	r	r			r	r	c	c
Nemacystus decipiens											r	r	r		c	c			c	c	r												r	c	c	
Colpomenia sinuosa	c	c	c			c	c	c	c	c	c	c	c	c	c		c		c	c	c	c	c	c		c	c	c	c	c	c	c	c	c	c	r
Scytosiphon lomentaria	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c			c	c	c	c	c	c				c	c	c	c	c	r		c	
Desmarestia viridis	c	c	c	c											c		c		c		r		r				r	c	c	c	c	c	r	c	c	
Undaria pinnatifida	c	c	c	c		c					c	c	c	c	c	c	c	r	c	c	c	c	c	c			r	c	c	c	c	c	r	c	c	c
Ecklonia cava	c	?	?			c					c	c	c	r	c													c	?	?		?	?	?	?	c
E. kurome	c	c																										c	c		c	c	c	c	c	
Eisenia bicyclis			r			c	c				c	c	c	c	c					c	c	c	c				r	c	r	c	c	c	c	c	c	c
Dictyota dichotoma	c					c	c	c	c		c	c	c		c						c	c	c				r	c	c	c	c		c	c	c	r
Padina arborescens	c	c				c	c	c	c		c	c	c	c	c					c								c	c	r			r	r	c	c
Myagropsis myagroides	c	c	c			c					c	c	c	c	c					c	c	c	c				c	c	c	r	r	c	c		c	r
Hizikia fusiformis	c	c				c	c	c			c	c	c	c	c					c	c	c	c				r	c	c	r		c	c	c	c	c
Sargassum confusum	c					c					c	c	c															r	r	r	r	c	c	c	c	c
S. hemiphyllum	c					c	c				c	c	c	r	c					c	c	c	c				r	c	r	r		c		r	c	c
S. horneri	c	c	c	c	c	c	c	c	c		c	c	c	c	c	c	c			c	c		c	c				c	c	c	c	c	c	c	c	c
S. macrocarpum	c	c				c					c	c	c		c								r					c	c	c	r	c	c	c	c	c
S. okamurae						c					c	c			c													r						c	c	
S. patens	c					c	c	c	c	c	c	c	c	r	c					c	c		c					c	r	c	c	c	c	c	c	c
S. piluliferum	c					c	c	c	c	c	c	c	c		c					c	c		c				r	c	r	c	r	c	c	r	c	c
S. ringgoldianum	c					c	c				c	c	c	r	c					r		c						c	r						c	c
S. siliquastrum	c	c				c	c				c	c	c	r	c						c		c				r	c	c	c	c	c	c	c	c	c
S. thunbergii	c	c	c			c	c	c	c		c	c	c	r	c	c	r		r	c	c	c	c	c			c	c	c	c	c	c	c	c	c	c
Galaxaura fastigiata						c	c				c	c			r						r	r						c				r		r	r
Acanthopeltis japonica	c										c	c	r		c							c						c				r		r	
Gelidium elegans	c	c	c			c	c	c	c	r	c	c	c	c	c	c	r			c	c	c	c	c			c	c	c	c	c	c	c	c	c	c
G. japonicum	c					c	c				c	c			c						c	c						c						r	
G. pacificum											c	c	r		c																				r
Pterocladia capillacea	c					c	c	c	c	r	c	c	c		c						c	r	c				r	c	r	r	c	c	c	r	c
Amphiroa dilatata	c					c					c	c	r	r	c						c	r	r					c	r	c	c	r		c	c
Corallina pilulifera	c	c				c	c	c			c	c	c	c	c					c	c	c	c	c			c	c	c	c	c	c	r	r	c	c
Gloiopeltis furcata	c	c	c			c	c	c	c		c	c	c	c	c	c				c	c	c	c	c			c	c	c	c	r			c	c	
G. tenax	?	?	?	?	?										c						c	c	r					?	?	?	?		?		c	c
Grateloupia filicina	c	c	c	c	c	c	c	c			c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c		c	c	c	c	c	r	c	c	c
G. turuturu	c	c	c								c	c	c	c	c	c	c	c	c	c	c	r	c	c		c		c	c	c	r			c	c	
Pachymeniopsis elliptica	c					c	c				c	c	c	c	c					c	c	c	c				c	c		r	c				r	
Prionitis angusta	c					c					c	c	r		c							r	r					c				r		c	c
Gigartina intermedia	c					c	c				c	c	c	c	c	c				c	c	c	c				c	c	c	r	r	c	r	c	r	r
Gracilaria asiatica	c	c	c	c	c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	r	c	c	c
Gymnogongrus paradoxus											c	c	c	c	c	r					c	c	c					r						r	
Plocamium telfairiae	c	c	c			c	c				c	c	c	c	c					c	c	c	c				r	c	c	c	c	c	c	c	c	c
Meristotheca papulosa	c										c	c			c							r	r					c						c	r
Solieria pacifica						c					c	c	c	c	r						c	r							r	r	r	c	c		c	
Lomentaria catenata	c	c	c				c	c	c		c	c	c	c	c	c				c	c	c	c					c	c	r		c	r	c	c	
Campylaephora hypnaeoides	c					c					c	c	r	r	c					c	c	c	c					r	r	r	r	r		c	c	
Ceramium kondoi											c	c	c	r	c						c		c					c	r	r	c	c	c	r	r	
Chondria crassicaulis	c										c	c	c	r	c				r	c	c	c	c				c	r	r	r	c	c	r	c	c	
Zostera marina	c	c	c	c	c	c	c	c	c	c	c	c	c	r	c	c	c		c	c	c		c	c	c	c	c	c	c	c	c	c	c	c	c	c

Area A to D′ in Ago Bay: see Maegawa *et al.* 1982.

Table 4. Five environmental factors for marine benthic floras in four inland seas fully investigated and those in the Seto Inland Sea.

Coastal waters	WT (°C) in Feb.	Cl (‰)	COD_{OH} (mg/l)	$H_{1/3}$ (m)	D (km)
OSAKA BAY					
Kada	10.5	17.8	1.1	11	0.25
Kojima	9.9	17.6	1.4	(2.1)	0.3
Tan-no-wa	8.8	17.4	1.5	(2.2)	0.9
Ozaki	9.0	17.3	1.9	(2.3)	0.9
Kaizuka	8.7	17.0	2.2	(2.3)	2.2
AGO BAY					
Area A, mouth	13.5	18.0	1.1		0.2
Area B	13.0	17.8	1.2		0.3
Area C	12.3	17.8	1.2	1.4	0.25
Area D	11.1	17.3	1.3	(<1.0)	0.15
Area D′, bottom	10.6	16.5	1.3	(<1.0)	0.8
TOKYO BAY					
Jo-ga-shima I.	13.0	18.7	1.0	9	0.25
Matsuwa	13.1	18.5	1.2		0.3
Kamoi	10.6	18.2	1.3		0.4
Hashirimizu	9.8	17.8	1.6		0.4
ISE BAY					
Suga-shima I.	10.2	17.1	0.7	6	0.3
Ise	6.8	15.8	1.6	2.7	1.6
Matsusaka & Tsu	7.1	14.7	2.2	2.8	0.8
Yokkaichi	8.4	13.1	2.0	2.8	1.6
Shin-maiko	8.4	13.1	2.1	2.0	1.2
Toyohama	8.9	16.6	1.1	2.5	0.3
Shino-jima I.	7.3	16.8	1.2	2.6	0.4
Pt. Irago	9.5	17.3	0.8	8	0.2
Saku-shima I.	7.3	16.9	1.2	2.5	0.4
Ooi	(8.0)	16.7	1.7	1.8	0.4
Nishio	(8.0)	15.0	1.8	1.5	1.9
Isshiki	8.0	16.0	1.8	1.7	2.4
Oki-no-shima I.	6.0	16.8	1.7	2.0	0.8
SETO INLAND SEA					
Kii Channel	11	18	0.9	5–6	
Osaka Bay	9	17	1.8	2.5	
Sea of Harima	8.5	17.5	2.1	3	
Bisan Channel	8.5	17.5	1.3	2	
Sea of Hiuchi	8.5	17.5	1.5	3	
Sea of Aki	10	18	1.0	2	
Sea of Suo	8	18	0.9	(3)	
Sea of Iyo	10	18	1.2	3–4	
Bungo Channel				5–6	

Area A to D′ in Ago Bay: see Maegawa *et al.* 1982.

WT, Cl and COD_{OH}: means of 5–20 years' data at the nearest station in oceanographical investigations.

COD_{OH}: COD by alkaline $KMnO_4$, 100°C, 20 min (JAS K 0102, 1986).

$H_{1/3}$: the max. significant offshore wave height set in planning fishing ports on each coast.

D: offshore distance (km) of the 10 m depth.

Numbers in ( ) are estimated values.

Table 5-1. Ranges of six environmental factors in coastal waters for each distribution of 92 common species in marine benthic floras.

Species	Ranges of environmental factors						Remarks
	WT (°C)		Cl (‰)	COD_{OH} (mg/l)	$H_{1/3}$ (m)	D (km)	
	Feb. L–U	Aug. L–U	L	U	L	U	
Monostroma latissimum	7– 14		16.5	1.3	(1)	.8	A) in and around Ise Bay
M. nitidum	12– 20	25–29					
Ulva pertusa	−2– 20	16–29	13.1	2.2	(1)	2.4	S) on sandy bed also
Dictyosphaeria cavernosa	13– 20	25–29					
Halicoryne wrightii	19– 20	28–28					
Neomeris annulata	16– 20	27–29					
Bryopsis plumosa	0– 19	18–28	13.1	2.2	1.5	2.4	
Caulerpa cupressoides	17– 20	27–28					
C. okamurae	6– 20	24–27	17.3	1.2	(3)	.3	
C. racemosa	14– 20	25–29					
Halimeda opuntia	19– 20	27–29					
Analipus japonicus	−2– 10	16–23			(2)		
Chordaria flagelliformis	2– 3	16–21			(3.5)		
Cladosiphon okamuranus	19– 20	28–29					
Ishige okamurae	7– 19	23–28	16.8	1.3	1.4	.4	T) scarce in IK–AT in JC
Nemacystus decipiens	8– 19	25–28	13.1	2.1	2.0	1.6	S) on sargasso plants
Colpomenia sinuosa	−2– 20	16–29	13.1	2.2	(1)	2.4	
Scytosiphon lomentaria	−2– 20	16–28	13.1	2.2	(1)	2.2	
Desmarestia viridis	−2– 12	16–27	13.1	2.2	2.0	1.2	
Alaria crassifolia	0– 6	18–21			(3.5)		U) not found in JC
A. praelonga	−2–−1	16–20					
Undaria pinnatifida	2– 14	20–27	13.1	2.2	1.8	1.6	
Costaria costata	−2– 7	16–24			(2.5)		
Ecklonia cava	10– 16	23–27	17.1	1.3	6	.4	
E. kurome	8– 14	26–27	17.6	1.4	(2.1)	.3	
E. stolonifera	6– 13	24–27					U) not found in PC
Eckloniopsis radicosa	13– 19	25–28					
Eisenia bicyclis	7– 14	22–27	16.6	1.6	2.5	.4	U) missing in KT–AT in JC
Laminaria angustata	−2– 2	16–20					U) not found in JC
L. japonica	−1– 8	20–24			(2.5)		
Dictyota dichotoma	−1– 20	20–29	16.8	1.3	(1)	.4	
Padina arborescens	6– 19	24–28	16.6	1.6	(1)	.4	
P. minor	11– 20	26–29					
Cystoseira hakodatensis	−2– 7	16–24					
Hormophysa cuneiformis	19– 20	28–29					
Myagropsis myagroides	6– 15	22–27	16.6	1.7	2.0	.9	
Fucus distichus	−2– 4	16–21			(1.5)		
Pelvetia wrightii	−2– 6	16–21			(2.5)		
Hizikia fusiformis	5– 20	20–28	16.6	1.6	1.4	.4	T) missing in TY–AM in JC
Sargassum confusum	−1– 13	20–27			(1.8)		U) missing in IG–ME in PC
S. duplicatum	13– 20	25–28					U) missing in KM–NS in JC
S. hemiphyllum	7– 19	25–28	16.6	1.3	2.5	.4	
S. horneri	4– 19	20–28	14.7	2.2	(1)	2.2	
S. macrocarpum	6– 19	24–28	17.1	1.4	(2.1)	.4	
S. okamurae	13– 16	25–27	17.1	1.2	6	.3	U) not found in JC
S. patens	6– 20	24–28	16.5	1.3	(1)	.8	

WT: see footnote for Table 2. Cl, COD_{OH}, $H_{1/3}$, and D: see footnotes for Table 4. L and U: lower and upper limits. Numerals in ( ): uncertain values.

A): area where the alga was found by Kida (1966). S): substrata except rocky bed. T): missing locally due to unsuitable tidal conditions. U): missing locally due to indefinite reasons.

Table 5-2. (Continued).

Species	Ranges of environmental factors						Remarks
	WT (°C)		Cl (‰)	COD_{OH} (mg/l)	$H_{1/3}$ (m)	D (km)	
	Feb. L–U	Aug. L–U	L	U	L	U	
Sargassum piluliferum	5–16	20–27	16.5	1.3	(1)	.8	
S. ringgoldianum	7–16	22–27	17.1	1.3	(4)	.4	
S. sandei	14–20	25–28					U) not found in JC
S. siliquastrum	5–15	20–27	16.8	1.4	(2.1)	.4	
S. thunbergii	−2–20	16–28	15.8	1.7	(1)	1.6	
S. yezoense	2–13	20–27			(2)		U) missing in FS–ME in PC
Turbinaria ornata	19–20	27–29					
Porphyra pseudolinearis	−2–12	16–27			(2.5)		
P. variegata	−2– 5	16–23					
P. yezoensis	−2–13	16–27			(2.5)		
Galaxaura fastigiata	8–20	25–29	17.8	1.2	(4)	.3	
Acanthopeltis japonica	13–19	25–28	17.1	1.2	6	.3	U) not common in JC
Gelidium elegans	2–19	20–28	15.8	1.7	(1)	1.6	
G. japonicum	10–19	23–28	16.8	1.2	2.6	.4	
G. pacificum	13–17	25–27	17.1	1.2	6	.3	U) not found in JC
Pterocladia capillacea	2–19	20–28	16.8	1.3	(1)	.4	
Amphiroa dilatata	6–19	24–28	16.8	1.2	2.6	.4	
Corallina pilulifera	−2–20	16–29	16.6	1.7	1.4	.8	
Constantinea subulifera	−2– 0	16–18					
Neodilsea yendoana	−2–10	16–23			(1)		
Gloiopeltis furcata	−2–19	16–28	15.8	1.7	(1)	1.6	
G. tenax	10–19	25–28	16.8	1.2	2.6	.4	
Grateloupia filicina	2–20	20–28	13.1	2.2	1.4	2.4	
G. turuturu	−1–15	20–27	13.1	2.2	1.7	2.4	
Pachymeniopsis elliptica	5–17	20–27	16.6	1.7	2.0	.8	
Prionitis angusta	13–20	25–28	17.1	1.2	(4)	.3	
Chondrus yendoi	−2– 7	16–24			(2)		
Gigartina intermedia	5–17	20–27	15.8	1.7	2.0	1.6	T) missing in MG–AM in JC
Rhodoglossum japonicum	−2– 7	16–24			(1.5)		
Gracilaria asiatica	−2–19	16–28	13.1	2.2	(1)	2.4	
Gymnogongrus paradoxus	5–20	20–27	16.8	1.6	2.5	.4	U) not found in JC
Plocamium telfairiae	6–20	20–29	16.6	1.6	(2.1)	.9	
Eucheuma denticulatum	19–20	28–29					
Meristotheca papulosa	12–19	25–28	17.1	1.2	(4)	.3	
Solieria pacifica	11–19	25–28	16.8	1.6	2.6	.4	
Turnerella mertensiana	−2– 0	16–18					
Lomentaria catenata	4–19	20–28	15.8	1.6	(1)	1.6	
Campylaephora hypnaeoides	−1–15	18–27	16.6	1.2	2.5	.4	S) on sargasso plants
Geramium kondoi	−2–16	16–27	16.8	1.3	2.5	.4	
Dasya sessilis	2–12	20–27					
Chondria crassicaulis	2–17	20–27	16.6	1.7	2.0	.8	
Digenea simplex	16–20	27–29					
Neorhodomela aculeata	−2– 8	16–25			(1)		
Thalassia hemprichii	19–20	28–29					S) on coral reef and sand
Phyllospadix iwatensis	−2–10	16–26					
Zostera marina	−2–16	16–28	13.1	2.2	(1)	2.4	S) on sandy mud

PC: Pacific coast. JC: Coast of Sea of Japan, of Sea of Okhotsk and of East China Sea.

AB: Abashiri Pr., AM: Aomori P., AT: Akita P., FS: Fukushima P., IG: Ibaragi P., IK: Ishikawa P., KM: Kumamoto P., KT: Kyoto P., ME: Mie P., NG: Niigata P., NS: Nagasaki P., TY: Toyama P., (P.: Prefecture, Pr.: Province in Hokkaido).

情報の充足によりある程度修正される可能性を残していると考えられる。なお，上・下限値にTable 4中の推定値を用いた場合，および他域の情報で補足したフサイワヅタの$H_{1/3}$などの少数の要因推定値もTable 5に（ ）を付して加えた。

ヒロハノヒトエグサの要因値は分布が確認された伊勢湾およびその近傍域のみで求めた。また大阪湾内の資料に散見されるマフノリおよびカジメは同定に疑問を感じ，さらにそれらの分布域の2月水温，塩分，$H_{1/3}$などが，同定が確実な他の分布域でのそれらの範囲から外れると見られたことから，Table 3での表示を？とし，Table 5の要因値はこれらを除いて求めた。

伊勢湾など4内湾に分布しない種については情報不足から分布域の4要因値の範囲を求められなかった。ただ一部北方種の$H_{1/3}$の概要だけを次の方法で推定し，（ ）を付して補足した。それは，近くで波の強さのみが異なる2小域での種組成を北海道および三陸の資料から抽出し，結果を整理して種を分布域の波の強さで順序付け，その中の$H_{1/3}$が既知の種と比べて，陸奥湾での$H_{1/3}$と種の分布の情報も参照して，北方種の$H_{1/3}$を見当付けるという方法である。

Table 3, 4に瀬戸内海の灘等別の種の分布と環境要因平均値も付記したが，種の分布域の環境要因値の参考とするにとどめた。

結果と考察および結果の活用

日本の沿岸の中では大きな地理的隔離は見られないので，各沿岸域を通して海藻・海草植生と環境との間に比較的簡単な関連があることを予想し，その関連を，既往情報の再検討から，従来よりつめて求めることを試みた。

各沿岸域の海藻・海草植生の指標として，豊富な分布資料の蓄積があり，検出，同定が容易と思われた92の普通種を選定してその種組成をとった。県等の中部域とやや広くとった外海各沿岸域の種組成をTable 1に，小域別にほぼ充分な資料が得られた伊勢湾等4内湾各小域の種組成に，参考として瀬戸内海の各灘等とやや広域の平均的なそれも付加して，Table 3に示した。

2-種組成の異同度の判定には両者の類似比を用いた。類似比の算出にあたっては，2-種組成の何れか一方にでも出現が稀，または同定に疑問のある種がある時はその種を除いて行った。

種の分布，従って種組成に関係する重要でかつ数値データがえられる環境要因として，水温，塩分，汚染度，波高および海底傾斜度をとりあげ，それぞれの指標値として，年間の最低および最高月水温°C（以下2，8月水温という），年平均Cl‰，同COD_{OH}（前章参照，以下CODという）mg/l, 域内または至近漁港の設計沖波波高$H_{1/3}$ m, 水深10 m線の距岸距離D_{km}を用い，種組成表示域でのそれらの数値をTable 2, 4に示した。

各選定種の分布域の2，8月水温の範囲は主として外海沿岸で，他の4要因の範囲は伊勢湾等の4内湾で，分布と分布域要因値を対照して求め，Table 5にまとめた。この際，出現が稀な場合，あるいは同定に疑問がある場合は除外して行った。Cl‰の上限，CODの下限は一般に外海での値なので省略した。$H_{1/3}$の上限，Dの下限はこの資料からは求まらず，表記できなかった。また4内湾に出現しない種の水温以外の4要因値は資料が得られず，一部の北方種の$H_{1/3}$の下限だけを不十分な推定値で加えた以外は空欄として残し，今後の資料の追加による充足に期待することとした。

大多数の種は表示の条件を満足する沿岸域のほとんど全部に出現しているが，少数の種ではその一部，あるいは相当数の沿岸域群に分布しない場合が見られた。外海域での顕著な例として，ツルアラメなどは太平洋沿岸には分布しない，ヒジキなどは潮汐条件から本州北部日本海沿岸では出現しない（斉藤1972），アマモは内湾砂泥地がないと生育しない，などが挙げられる。これらとは別に，フシスジモク，エゾノネジモク，アラメなどはおそらく温度要求の異なる2（以上）ケの群が含まれていると思われ，それを1種としてまとめた水温範囲内の沿岸域では分布しない部分が見られる。こうした少数の例外はTable 5に種別に注記を加えて示した。

なお資料で九州北岸および大阪湾等のクロメ分布域中に散見される「カジメ」，また大阪湾等の「マフノリ」は同定に疑問を感じ，各環境要因の範囲を求める際にはこれらは除外した。ヒロハノヒトエグサの要因値は分布が確認されている伊勢湾及びその近傍域のみから求めたが，その他の沿岸域に見られる既往資料の「ヒトエグサ」はなお分類上の検討を要するように思われ，その結果によっては両種の分布，したがって要因値の修正を要することも考えられよう。

Table 5のチェックもかねて，逆にその種別の2，8月水温範囲とTable 2の水温を照合し，注記で必要な一部修正をして，外海沿岸各域の種組成を推定し，

それらのTable 1の種組成の再現度を類似比で試算すると平均で95%と高い。各県等の中部域と沿岸域をやや広く取ると，低塩分，高汚染度の影響はほとんどなく，波の不足もなく，種々の地形が含まれるので海底傾斜度の制約もなく，その種組成は主として水温に左右されていると見られる。例外的に八丈島，小笠原諸島では70%台と低いが，その原因としては単調な地形等の影響が，小笠原諸島ではさらに植生の調査不十分も考えられる。

またTable 5とTable 4を照合して伊勢湾等4内湾各小域の種組成を推定し，それらのTable 3の種組成の再現度を類似比で試算したが，平均で86%とやや低かった。主因は湾奥数域で類似比が著しく低いことで，ここで用いた手法の湾奥の局域的な植生・環境の変化への対応不十分が認められた。なお水温以外の4要因を1ケづつ除いた試算では類似比は何れでもほぼ10%低下し，各要因の寄与が認められた。

これらの結果を一般化して，海藻・海草の各選定種は，各環境要因値がTable 5に表示した範囲内の沿岸域には，注記した少数の例外を除いて，大多数の場合にはふつうに出現し，範囲を外れた環境の沿岸域には多くの場合出現しないか，出現しても稀であると考えてよいであろう。表示した数値は既往資料から各種が普通に出現すると見られた沿岸域の各環境要因値の上・下限であり，各環境要因間の相関が，2，8月水温では太平洋，日本海等のそれらを併せ，Cl等4要因では4内湾の資料を併せるなどで軽減させる努力をしたにも関わらず，なお残って影響している可能性もあるが，国外のより北方，南方域にも分布する種の水温値を除いて，多くの場合にその種の生育，繁殖のための環境要因要求の上・下限値に近いものと思われよう。はじめに植生と環境の間に比較的簡単な関連を予想したが，それは国内沿岸域間では，そこで生育，繁殖のための各環境要因要求が共通に満足させられる種が共通に出現すると表現できるであろう。

ただし，$H_{1/3}$の上限とDの下限は求まらず，Table 5に表示できなかった。このため地形の単調な小沿岸域では，波が強すぎて，または傾斜が急すぎて生育しない種があることの情報が欠けている。また伊勢湾等の4内湾に分布しない種について，水温以外の要因の分布域上・下限値のほとんどが空欄で残され，これらの要因の制約による不出現の情報も欠けていることになる。

以上とは別の環境と種組成の関連を求める方法として，水温については，外海沿岸域で2域の2，8月水温距離が小さいほど，種組成の類似比が平均的に高いこと，また各域の2，8月水温の分布と各域種組成の類似比によるクラスター分析の結果がよく対応することが見られた。クラスター分析の結果は岡村（1931），瀬川（1965），新崎ら（1976）による海藻分布の区分と，金華山を境とする差より犬吠岬でのそれが，両者を境とする沿岸域の水温距離の差を反映してより大きい(Fig. 1)こと以外は，ほぼ類似している。なお各2沿岸域の種組成の差と環境差の関係を，種組成類似比と水温以外の要因も加えた環境要因総合距離（差）との関係として捉えるのは，各要因指標値の合目的的な変換が困難であることなどから今はできなかった。

Table 5の種分布域の各環境要因値の範囲，Table 1 and 3の各沿岸域の種組成，類似比による種組成の異同度の判定を併せると，植生の，また環境の調査結果から次のような判定，推定等ができる場合が多いと考える。ただし上述したTable 5の情報不足から一部の不出現種を出現種に加えてしまうなどの誤りが入る可能性が残されている。

a）調査種組成の位置づけ：調査で得られた種組成を既知の各沿岸域種組成と対比し，大きい類似比を与

Table 6. Two examples of similarity ratios (R%) between species composition of marine benthic flora on a coast surveyed and that in its adjacent waters, established in Tables 1 and 3.

Area searched	Coastal waters with established floras			
	Around the area searched		Neighboring the area searched	
Off Ikata power station, Ehime P.	Sea of Iyo	83	Sea of Aki	56
			Sea of Suo	55
			Bungo Channel	73
Off Kyowa-Tomari power station, Shiribeshi Pr.	Shiribeshi Pr.	64	W. Soya & Rumoi Pr.	61
			Hiyama Pr.	65
			W. Aomori P.	50

P.: Prefecture, Pr.: Province in Hokkaido.

Table 7. Estimations of environmental factors in two coastal waters from marine benthic floras found in them, compared with those observed in surveys.

Area searched		WT (°C) Feb.	WT (°C) Aug.	CL (‰)	COD_{OH} (mg/l)	$H_{1/3}$ (m)	D (km)
Off Ikata power station, Ehime P.	Est.	12–13	26–27	≧18.2	≦1.1	≧4	≦0.4
	Obs.	12.2	—	18.6	0.8	4	0.1
Off Kyowa-Tomari power station, Shiribeshi Pr.	Est.	4– 7	20–25	≧16.5	≦1.2	≧3	≦0.4
	Obs.	5.1	21.1	18.5	0.7	(8)	0.3
Off Nanao power station, Ishikawa P.	Est.	8–14	23–28	≧16.8	≦1.2	≧2.5	≦0.5
	Obs.	7.2	27.4	17.4	1.0	(≦3)	—

P.: Prefecture, Pr.: Province in Hokkaido.
Est. WT, etc.: Ranges of each factor, commonly satisfying demands (cf. Table 5) of all species in the area investigated.
Obs. WT, etc.: in surveys conducted throughout one year.
Numbers in ( ) are uncertain values.

えるそれに近いと位置づけできる。Table 6 に環境影響判定調査の 2 事例をあげた。伊方および共和・泊発電所近辺海域の種組成は周辺の伊予灘および後志・檜山沿岸域の種組成との類似比がそれぞれ83%および64～65%と最も高い。なお泊地先関連の類似比が60%台と低いのは，外海に面した単調な小沿岸域で波陰がないため，一部のホンダワラ類，ツルツルなど相当数の波に弱い種が出現しないことが主因と考えられる。別に七尾発電所の同調査での近辺域（七尾南湾）の種組成は石川県外海域の種組成から $H_{1/3}$≧3 m と波要求の強い種を除いたものとの類似比が82%と最も高い。

b）調査種組成から環境の推定：調査域で全出現種に共通な分布域環境要因値の範囲を Table 5 から抽出してそこの環境要因値を推定できる。種組成と環境要因値がともに求められた 3 調査事例について，種組成からの環境要因推定値に実測値を対比して Table 7 にあげた。

c）環境からの種組成の推定：環境調査結果と Table 5 を照合してそこでの出現種を推定できる。植生も相当よく調査された上記伊方および七尾発電所周辺海域について，Table 7 の環境値（七尾の $H_{1/3}$ は湾口も含めて <3 m とした）から種組成を推定し，調査種組成との類似比を求めてそれぞれ83%，82%がえられた。別に淡路島岩屋で浅海定線観測等による環境値（2 月 WT: 9.3°C, Cl: 17.6‰, COD: 1.1 mg/l, $H_{1/3}$: 3.1 m, D: 0.3～0.7 km）からの推定種組成と資料（広瀬ら1965）による種組成との類似比は81%であった。何れでも調査結果と推定の違いの主体は一部の出現推定種が調査では検出されていないことにあった。

d）環境変化による種組成変化の予想：環境要因の変化予測値と表 5 から消失種および新出現種を予想できる。ただし種の新規の出現，繁殖にはある程度の期間を要する場合が少なくないであろう。適切な事後調査事例が見当たらず，この予想の例示とチェックができなかった。

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藻類 Jpn. J. Phycol. 40: 307–309, September 20, 1992

有賀祐勝：髪菜 *Nostoc flagelliforme*（藍藻）の生育地と分布

Yusho Aruga: Habitat and distribution of "Facai", *Nostoc flagelliforme* (Cyanophyta)

Key Index Words: *Cyanophyta—distribution—habitat*—Nostoc flagelliforme.
Yusho Aruga, Laboratory of Phycology, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan

藍藻の髪菜(はっさい,中国語はファーツァイ"Facai") *Nostoc flagelliforme* Berk. et Curt. は中国産の食用藻類であり，日本では中華料理の材料として比較的よく知られているが，その生育地について知っている人は皆無に近いと思われる。今からおよそ80年前すでに遠藤（1912）や岡村（1913）によって髪菜は注目されていたにもかかわらず，その生育地を確かめた日本人はいなかったようである。著者は1991年7月に機会を得て訪中し，髪菜の生育現場を見ることができたので以下に報告する。

今回の訪中で髪菜を見せてもらったのは，寧夏回族自治区の青銅峡广武と賀蘭山小口子である。北京から特急列車で24時間半で寧夏回族自治区の銀川に到着。銀川市のホテルに泊り，翌朝マイクロバスでホテルを出発し，途中寧夏農学院生物系の髪菜研究グループの人達と合流し，約2時間走って銀川の南方にある青銅峡市に入り，さらに髪菜の生育地である山の方に向った。最初にバスから降ろされたのはまったく水けのない荒原（半乾荒原あるいは半乾燥草原）で广武という所，海抜1,270 mの山の上であった。すっかり乾燥した土地には中国名で草覇王というハマビシ科の小さな植物やその他の乾生植物がまばらに生えているだけの場所であった（Fig. 1）。まばらな小植物の根元や直径4〜5 cmの石の近く（いずれも裸地表面）に，ここにあると指差された土の上を眼を凝らしてよく見ると，乾燥した髪菜がまさに髪の毛のように小さな塊りをなしていた（Fig. 2）。少し離れた所を更に2か所案内してもらったが，頂上が海抜1,500 mの山の中腹の海抜1,350 mの所などで，殆ど最初の場所と同じ状況であった。

また別の日に，やはりマイクロバスで長時間走り，銀川の北方にある賀蘭山県の乾沟と西夏王墓近くの山の2か所を案内してもらい，髪菜を観察した（Fig. 3）。いずれも青銅峡の生育現場と殆ど変わりない環境の所

Fig. 1. Habitat of the blue-green alga *Nostoc flagelliforme* Berk. et Curt. in Ningxia, China.

Fig. 2. The blue-green alga *Nostoc flagelliforme* Berk. et Curt. on bare land in Ningxia, China (ca. 1.3×).

であった。ここは集落から著しく離れたところにあり，髪菜を採集する人々は山の石室のようなところに2晩3晩と野宿して髪菜を集めて持ち帰り，出荷するとのことである。1人1日の採集量は15〜50gくらいとのこと。採集した後，雑草除去を行なって保存するが，それから後は商人に売り渡すかまたは国家農産品公司に納入する。商人や国家農産品公司の段階では，水洗や雑草除去が行なわれ，自然乾燥または機械（電気）乾燥されて，最終的な商品となる。髪菜の販売価格は，銀川の百貨店では50g入，100g入，250g入などが売られていたが，それぞれ18元，36元，94元であった。また，北京では50g入が19元であった。これは，中国の人達の生活費のレベルを考慮すると，相当高価なものである。

寧夏農学院では生物系副主任の華振基副教授を中心とする研究グループが髪菜の培養実験を行なっている。最終的には養殖を目指して，液体培地や寒天培地を使って培養実験を進めており，髪菜の形態，生態，生理なども研究している。寧夏では，農業科学院がかつてドイツの研究者と共同で髪菜の増養殖に関する研究を行なっていたが，1989年から髪菜のプロジェクトは農学院に移行されて現在に至っているとのことである。

中国では，髪菜は陝西，寧夏，甘粛，内蒙古，青梅の西北5省に分布しており，海抜1,100〜1,500 mで年間の降水量が300 mm以下（6〜8月に集中）の所に分布は限られているとのことである。世界的には中国の他，モンゴル，旧ソ連邦，チェコスロバキア，フランス，モロッコ，ソマリア，メキシコ，米国などに分布することが知られているが(Li, 1991)，水の中に生育する藻類ではなく，土壌藻(soil algae)というよりはむしろ陸生藻(terrestrial algae)と呼ぶのが相応しい藻類である。すでに述べたように著しく乾燥した土壌の表面（裸地表面）を生育場所としており，ごくわずかの雨とおそらく霧などから水の供給を得ていると考えられる。中国では，髪菜が生育している所の土壌は，Ca含量が高く，NやPおよび有機物の含量が低いとのことである。また，内陸の高地であるから温度変化も非常に極端であり，−35°Cから+87°Cにも及ぶ（年平均気温5-9°C）ところである。髪菜の生育の好適温度は25〜35°Cといわれている(Li, 1991)。

寧夏で髪菜が見られるところのまばらな植生の中の代表的な植物は，シソ科の冬青叶兔唇花(*Lagochilus ilicifolius*)，アカザ科の珍珠猪毛菜(*Salsola passerina*)と白茎塩生草(*Halogeton arachnoideus*)，ハマビシ科の草霸王(*Zygophyllum mucronatum*)，およびTamaricaceaeの紅砂(*Reaumuria soogorica*)などであった。

なお，日本で発行された書物の中の髪菜に関する記

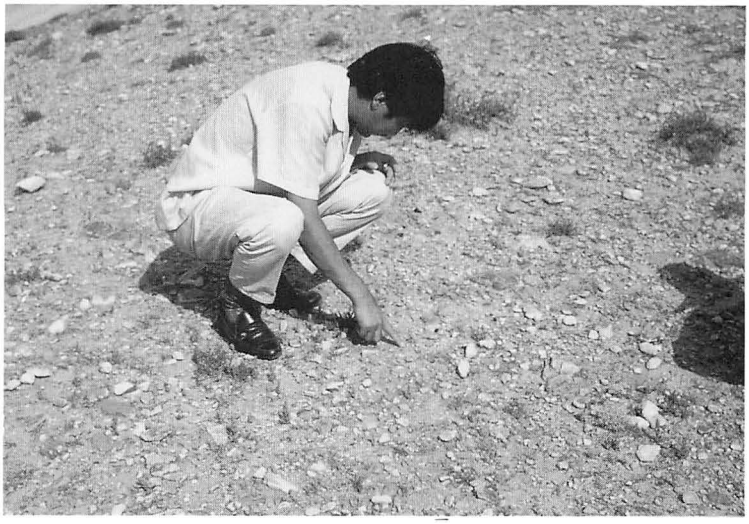

Fig. 3. Habitat of the blue-green alga *Nostoc flagelliforme* Berk. et Curt. in Ningxia, China.

述は，学術書でも一般の書物でも殆ど間違っている。例えば，「中国四川省の渓流中に生ずる」（殖田ら，1963）とか，「淡水産の藻類の一種」であり「苔類に属し，ミズゴケの一種であって渓間中に生ずる」（田中，1991）などとして，「淡水藻」として扱われているが，明らかに間違いであり，前述のように裸地表面に生育する陸生藻類である。

謝　辞

今回の訪中で，髪菜生育地を見るため多くの方々に大変お世話になった。特に見学旅行の準備をしてくださった中国水産科学研究院の李竹青さん，寧夏回族自治区農業庁の王秩宗副庁長，寧夏農学院の蘇煥蘭院長，楊桂清副教授，生物系副主任華振基副教授，王俊さんはじめ髪菜研究グループの皆さんに心から感謝申しあげたい。

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岡村金太郎　1913．髪菜ニ就テ．植雑 **27:** 177–183.

田中静一（編著）1991．中国食物事典．柴田書店，東京．

殖田三郎・岩本康三・三浦昭雄　1963．水産植物学．恒星社厚生閣，東京．

（108 東京都港区港南4–5–7　東京水産大学藻類学研究室）

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片山舒康：小・中学校理科教科書における藻類の扱われ方（1）これまでの教科書にみられる変遷

Nobuyasu Katayama: "Algae" in science education at primary and lower secondary school level.
(1) A survey of science textbooks for the last 40 years.

Nobuyasu Katayama, Department of Biology, Tokyo Gakugei University, Koganei-shi, Tokyo 184, Japan.

1. はじめに

最近，藻類関係者の集まりで，「藻類に興味を持つ者が少なくなった」「藻類を研究したいという学生がほとんどいない」といったことがよく聞かれる。この原因を探っていくと，小・中学校で藻類についてほとんど学習していないという現状が浮かび上がってくる。いつからこういった状態になったのだろうか？本報告では，この問題点を探るため昭和20年代後半からこれまでの小・中学校理科教科書における教材としての藻類の取り扱われ方を調べた。

2. 調査した教科書

わが国の初等中等教育段階の教育課程（カリキュラム）は，文部省がおよそ10年毎に改訂する学習指導要領に従って編成される。この学習指導要領は，第二次世界大戦後間もなく試案が作られ，次いで，昭和20年代後半にその試案の改訂版という形で第1回のものが出された。その後4回にわたる改訂を経て現在に至っているのだが，第2回目の改訂後，学習指導要領に法的拘束力が付加されており，教科書の内容は学習指導要領に基づかなければならなくなっている。小・中学校の教科書の場合，指導書作成協力者と文部省初等中等教育局職員によって作成される指導書を参考にして作られることが多く，さらに教科書検定官の指示にしたがって内容の変更等が行われる。教科書に取り上げられる教材は，原則的には各教科書執筆者の裁量に任されているのだが，指導書での指示や検定過程での指導等によってどの教科書も同じ様なものになる。教科書は，普通（実施に間に合うように）学習指導要領改訂後3年目または4年目に初版が発行され，その後ほぼ3年毎に改訂版が出されている。

小学校学習指導要領試案改訂版は，昭和27年に出された。ついで，昭和33年（37年度より完全実施）・昭和43年（46年度より実施）・昭和52年（55年度より実施）と改訂され，最近では平成元年の改訂があり，本年度から実施に移っている。中学校の学習指導要領は，昭和26年に試案改訂版が出された（発行は昭和27年）。ついで，昭和33年（36年度より完全実施）・昭和44年（47年度より実施）・昭和52年（56年度より実施）と改訂され，最近では平成元年の改訂があり，来年度から実施される。

そこで，昭和27年以降36年以前（以後この時期をⅠ期とよぶ）の小学校と中学校の理科教科書，及び，昭和33年（Ⅱ期）・昭和43年または昭和44年（Ⅲ期）・昭和52年（Ⅳ期）の各学習指導要領に準拠して作られた小学校と中学校の理科教科書で取り上げられている藻類の種類を調査した。調査には，現在も小学校と中学校の理科教科書を出版している大日本図書・学校図書・教育出版・新興出版社啓林館・東京書籍（Ⅰ期は教育出版を除く4社，Ⅱ期以降は5社）のものを選んだ。

3. 小学校理科教科書に出てくる藻類の種類と数

表1は，調査した各期の小学校理科教科書に名前の出てくる藻類数を植物門別に示したものである。また表2は，各期の小学校理科教科書に取り上げられている藻類名と取り上げている教科書会社数（以下教科書数とする）を示したものである。Ⅰ期とⅡ期には，海藻の学習が行われていた（磯の生物あるいは海の生き物といったもの）ので，海藻を中心として藻類の数が多かった。しかし，Ⅲ期以降は海藻が学習内容から削

この調査の一部は、昭和63・平成元年度科学研究費補助金，一般研究C「小・中・高を通した光合成学習のためのカリキュラム開発」（課題番号 63580224）によって行われた。

表1　各期の小学校理科教科書に出てくる藻類の各門ごとの種類数

門の名称*	教科書出版時期**			
	Ⅰ	Ⅱ	Ⅲ	Ⅳ
紅色植物	10	9		
渦鞭毛植物		1	1	
褐色植物　珪藻類***		1	3	
褐藻類	7	8		
ミドリムシ植物	1	1	1	1
緑色植物****	5	8	7	7
合　計	23	28	12	8

* 門の名称は岩波生物学辞典第3版による。
** 調査した教科書の出版時期は本文を参照。
*** 総称としてのケイソウは全期に出てくる。
**** すべて緑藻類。

除されたため，紅色植物（紅藻）と褐藻は全く姿を消し，わずかにメダカの育ち方を学習する際にメダカの餌に関連した「水の中の小さな生き物」として渦鞭毛藻，ケイソウ，ミドリムシ，数種の緑藻が取り上げられているだけであった。表2でも分かるように，平成3年度まで使用されていたⅣ期の教科書では，緑色の藻類のみとなっている。

さらに，教科書に取り上げられている藻類名と各期におけるそれらの取り上げられ方を詳細に調べてみた。Ⅰ期に半数以上の教科書（2社以上）に取り上げられている藻類は15種類，Ⅱ期に半数以上の教科書（3社以上）に取り上げられている藻類は12種類であった。Ⅰ期・Ⅱ期共に半数以上の教科書が取り上げていたのは，アサクサノリ（アマノリ）・ツノマタ・テングサ（マクサ）・フノリ・アラメ・カジメ・コンブ・ホンダワラ・ワカメ・アオサ・アオノリ・ミルの12種類で，全て海藻であった。ところが，Ⅲ期になると，半数以上の教科書（3社以上）に取り上げられている種類はわずかにミドリムシ・アオミドロ・ボルボックス・ミカヅキモの4種類（総称としてのケイソウを含めれば5種類）となり，Ⅳ期にはこれがさらにアオミドロ・クンショウモ・ボルボックスの3種類に減少した。

4. 中学校理科教科書に出てくる藻類の種類と数

表3は，調査した各期の中学校理科教科書に名前の出てくる藻類数を植物門別に示したものである。また表4は，各期の中学校理科教科書に取り上げられている藻類名と取り上げている教科書数を示したものであ

表2　小学校理科教科書に取り上げられている藻類名とそれを取り上げている教科書数

藻類名	教科書出版時期*			
	Ⅰ	Ⅱ	Ⅲ	Ⅳ
紅色植物				
アサクサノリ	4	5		
アマノリ	2			
アカバ	1			
オゴノリ	1	1		
カニノテ		1		
カイニンソウ(マクリ)	2			
ツノマタ	2	4		
テングサ（マクサ）	4	4		
トサカノリ	1	1		
ヒラクサ	1			
フサノリ		1		
フノリ	3	4		
渦鞭毛植物				
ツノモ		1	2	
褐色植物				
珪藻類				
（ケイソウ）	1	2	4	2
コアミケイソウ			1	
ツノケイソウ		1	2	
ハネケイソウ			1	
褐藻類				
アラメ	3	3		
ウミウチワ	1	2		
ウミトラノオ		1		
カジメ	4	4		
コンブ	3	3		
ヒジキ	1	2		
ホンダワラ	4	5		
ワカメ	4	4		
ミドリムシ植物				
ミドリムシ	2	2	5	2
緑色植物				
（リョクソウ）		1		
アオミドロ	1	1	4	4
アオサ	4	4		
アオノリ	3	5		
イカダモ			1	2
クンシュウモ		1	2	4
ジュズモ			1	
チリモ		1		
ツヅミモ			2	1
ホシミドロ				1
ボルボックス		1	3	1
ミカヅキモ	1	2	5	4
ミル	4	4		

* 調査した教科書の出版時期は本文を参照。

表3　各期の中学校理科教科書に出てくる藻類の各門ごとの種類数

門の名称*	教科書出版時期**			
	Ⅰ	Ⅱ	Ⅲ	Ⅳ
藍藻植物		1	1	2
紅色植物	8	6	7	4
渦鞭毛植物		1	1	2
褐色植物　珪藻類***		1	1	2
褐藻類	7	7	14	7
ミドリムシ植物	1	1	2	1
緑色植物　緑藻類	9	12	12	13
車軸藻類			1	1
合　計	25	29	39	32

* 門の名称は岩波生物学辞典第3版による。
** 調査した教科書の出版時期は本文を参照。
*** 総称としてのケイソウは全期に出てくる。

る。藻類が取り上げられているのは，各期で若干の違いはあるが，「植物の世界」あるいは「植物の種類と生活」といった単元の中の，水の中の植物・胞子で殖える生物・微生物などの学習項目である。取り上げられている藻類の種類数が最も多いのはⅢ期（39種類）で，ついでⅣ期（32種類），Ⅱ期（29種類），Ⅰ期（25種類）の順であった。Ⅰ期には小学校理科教科書と同様に海藻が主に取り上げられていたものが，Ⅱ期以降では広く全ての植物門を取り上げようとする方向に変わってきたことが，表3・4からわかる。ところが，表5に示すように，藻類及び海藻という用語はほとんどの教科書に出てくるにもかかわらず，紅藻・褐藻・緑藻という用語はⅢ期以降次第に用いられなくなっており，系統的な扱いは軽視される傾向がみられる。Ⅲ期以降には生態に関する学習が重視され始めた。Ⅲ期以降の教科書で，水の中の主な生産者としてケイソウがどの教科書にも大きく取り上げられており，海の中の生産者も海藻ではなく，もっぱら植物プランクトンに注目させている。このことは，表4でプランクトン性あるいは顕微鏡レベルの藻類の種類数が増加していることからも分かる。

中学校理科教科書に取り上げられている藻類名（総称としてのケイソウは含まず）と取り上げ方を，表4でさらに詳細に調べてみた。Ⅰ期に半数以上の教科書（2社以上）に取り上げられていた藻類は16種類で，その内訳は，紅藻4種類，褐藻7種類，ミドリムシ1種類，緑藻4種類であった。また，そのうち14種類が海藻であった。Ⅱ期に半数以上の教科書（3社以上）

表4　中学校理科教科書に取り上げられている藻類名とそれを取り上げている教科書数

藻類名	教科書出版時期*			
	Ⅰ	Ⅱ	Ⅲ	Ⅳ
藍色植物				
ネンジュモ				1
ユレモ		4	1	2
紅色植物				
アサクサノリ	4	5	4	3
オゴノリ	1		1	
オバクサ	1			
ツノマタ	3	3	3	2
テングサ	4	5	5	5
トサカノリ	1	1	2	2
ハナフノリ			1	
フノリ	2	3	1	
マクリ	1	1		
渦鞭毛植物				
ツノウズオビムシ	1			
ツノモ		1	1	1
褐色植物				
珪藻類				
ツノケイソウ				1
ハネケイソウ		2	1	2
褐藻類				
アラメ	2	3	2	1
イシゲ			1	
イソモク			1	
イロロ			1	
イワヒゲ			1	
ウミウチワ	3	1	1	2
ウミトラノオ			1	
カジメ	2	2	1	1
コンブ	4	5	5	5
（マコンブ・トロロコンブ）				
ツルアラメ			1	
ヒジキ	3	4	1	2
フクロノリ			1	
ホンダワラ	4	4	4	4
ワカメ	3	4	5	5
ミドリムシ植物				
ウチワヒゲムシ			1	
ミドリムシ	3	5	5	3
緑色植物				
緑藻類				
アオサ	4	5	5	4
（アナアオサ・ボタンアオサ）				
アオノリ	4	5	4	3
アオミドロ	4	5	4	5
カワノリ		1		1
クラミドモナス	1	1	2	
クロレラ		4	3	3
クンショウモ			2	3
サヤミドロ				1
ジュズモ		2		
ツヅミモ	1	5	2	
バンドリナ	1			
ヒラタヒゲマワリ				1
ホシミドロ		3	2	1
ボルボックス	1		1	1
マリモ		1	1	1
ミカヅキモ	1	4	4	3
ミル	3	3	2	1
車軸藻類				
シャジクモ			1	1

* 調査した教科書の出版時期は本文を参照。

表5　各期の中学校理科教科書が取り上げている藻類関係の用語と取り上げている教科書数

用　語	教科書出版時期*			
	Ⅰ	Ⅱ	Ⅲ	Ⅳ
ソウ類（そう類・藻類）	2	5	5	5
モ	1			
海ソウ（海そう・海草）	4	5	5	5
淡水ソウ		1		
ランソウ		2	2	2
紅ソウ（コウソウ・紅そう・紅草）	4	5	2	1
褐ソウ（カッソウ・かっそう・褐草）	4	5	2	1
ケイソウ	4	5	5	5
ベン毛ソウ			1	
接合ソウ	2			
緑ソウ（リョクソウ・緑そう・緑草）	4	5	4	2

* 調査した教科書の出版時期は本文を参照。

に取り上げられていた藻類は19種類で，藍藻1種類，紅藻4種類，褐藻5種類，ミドリムシ1種類，緑藻8種類であった。海藻は12種類と減少していた。ところが，Ⅲ期になると，半数以上の教科書（3社以上）に取り上げられている藻類は12種類となり，取り上げられている種類数の合計は各期の中で最も多いものの，各社に共通する種類は減少している。その内訳は，紅藻3種類，褐藻3種類，ミドリムシ1種類，緑藻5種類で，このうち海藻は8種類であった。Ⅳ期はⅢ期と同じ12種類で，内訳は，紅藻2種類，褐藻3種類，ミドリムシ1種類，緑藻6種類であった。そして，海藻はさらに減って7種類になっている。

上で述べたように，戦後の理科教科書を調べてみると，小学校理科では藻類の取り上げ方は，次第に軽くなってきたといえよう。また，中学校理科においては，取り上げられる種類数はさほど減らないものの，取り上げられている藻類を系統的にみると偏りが生じてきている。特に，海藻についての学習は，小学校では全く行われなくなり，中学校でも普通は3年間を通してわずか1単位時間（50分）の授業の中で終わってしまう。

5. 前学習指導要領（昭和52年改訂），及びそれに準拠した理科教科書での藻類の扱いの問題点

中学校理科における最近の藻類の扱い方には，ひとつ重大な問題があるように思う。それは，光合成の学習が陸上の高等植物中心に行われるのは致し方ないとしても，生態系の学習において水の中の生産者は植物プランクトンであるといった扱いをしていることである。中学校では，光合成をする生物＝藻類以上の（葉緑素あるいは葉緑体を持った）植物＝生態系の生産者という扱いをすべきであろう。ところが，指導書及び教科書では，系統的な扱いを無視するかのように，光合成をする生物（植物）＝緑色の植物＝緑色植物としている。光合成の学習対象を陸上の高等植物から様々な生態系の種々の植物に拡大すべき中学校段階の扱い方としては，これは非常に問題のあるところである。その点を前学習指導要領（昭和52年-昭和62年）と，それに準拠して作られた平成4年度までの教科書の内容を分析して述べてみたい。

中学校理科指導書（文部省1978)においては，（1）生物の種類と生活の中では，「植物（種子植物，シダ類，コケ類，ソウ類など）の特徴の一つは，光合成によって生活に必要な有機物を自らが合成するということである。」(p. 72) と解説されている。この扱い方に徹すればよいものを，（5）生物どうしのつながりでは，「生物は，緑色植物が合成した有機化合物をもとにして生活していること，並びに生物界は，植物，動物及び微生物が互いに関連し合って生活していることを……理解させる。」(p. 99) という表現に変わってしまう。そして，その後は，「ア（ア）緑色植物は，光を利用して二酸化炭素と水から有機化合物をつくり，また，光合成には，光，二酸化炭素の量などの条件が影響すること。」(p. 100) となり，さらに，解説の中でも，「緑色植物が光を利用して合成した有機物」(p. 101) とか「光合成を行なう緑色植物を生物界における生産者と考える……」(p. 104) といった記述になっているのである。また，（7）人間と自然の中でも，「生産者（緑色植物）……」(p. 120) という記述がみられる。

中学校理科の教科書の内容は，当然のことながら上述の指導書の内容を忠実に反映した形となっている。植物の種類と生活の単元では「種子植物・シダ類・コケ類・ソウ類は日光を受けて養分を作って生活する」と述べられている。しかし，生物どうしの働きを学習する段階では，「緑色の葉を持つ植物が光合成をする」という記述となり，「緑色の色素を持つ植物を緑色植物とよぶ」ようになり，ついには「緑色植物が光合成をする」になってしまい，「自然界の生産者の緑色植物……」となってしまうのである。その一方で，「水の中では，生産者であるソウ類……」とか，「海の植物プランクトンや海ソウが酸素を供給する」といった

記述もあり,内容が統一されていない教科書もあった。

光合成をする植物は，みな葉緑素を持っているが，紅藻や褐藻あるいは珪藻などは緑色に見えない。陸上で生活をしている我々の身の回りの植物の多くが緑色植物であるからといって，自然界の生産者が緑色植物であると言いきってしまうのは問題である。緑色植物以外の植物についても学習する中学校段階では，緑色の植物あるいは緑色植物という言葉を，緑色の種子植物・シダ類・コケ類及び緑藻類に限って用いるべきだと考える。単元間で学習内容が一致しないのでは，学習者が混乱することは目に見えている。光合成をする生物（植物）は，光合成生物（植物）とよべば全く問題がないと思う。

6. おわりに

今回は，これまでの学習指導要領に準拠した教科書に取り上げられていた藻類に関して調べた結果を報告した。すでに述べたように，小学校学習指導要領は本年度から実施されており，中学校学習指導要領も来年度から実施される。小学校理科教科書はすでに発行され，中学校理科教科書も本年秋には見本本が完成するはずである。新しい学習指導要領は，これまでのものよりも寿命が短いようであるが，これから数年間の小学校・中学校の理科教育の中で藻類はどのように扱われていくのだろうか。次回は，新しい学習指導要領とそれに準拠して作られた教科書を分析して報告する。

謝　辞

中学校理科教科書の調査を手伝ってくれた皆川富美さんに感謝する。

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（184 小金井市貫井北町4–1–1　東京学芸大学生物学科）

藻類 Jpn. J. Phycol. 40: 317–318, September 20, 1992

川嶋昭二：海藻標本で描いた絵

Shoji Kawashima: Picture painted by dried specimens of seaweed

海藻標本といえば藻類の研究者や夏休みの宿題に小・中学生が作るもので，一般には縁のないものと私たちは思いがちである。ところが，そんな学問や勉強とは無縁の仕事をしながら海藻に魅せられ，その乾燥標本で絵を「描く」趣味に熱中している人がいる。

その人は渡辺勇さん（68才）といい，北海道大学の学生寮（旧恵迪寮）のボイラーマンをしておられるが，同じキャンパスの中で世界的な海藻研究が盛んに行なわれていることなど夢にも知らず，また海藻の名もコンブ，ワカメ，ノリくらいしか知らなかったという全くの素人の方である。ある日，その渡辺さんに絵を見せて頂き，話を伺った。

渡辺さんの海藻との付き合いは函館で働いていた4年前のある日，海辺に出かけて打ち上げられた色とりどりの海藻の美しい姿，形に接し目を見張ったときから始まった。それから何度か海辺で海藻を拾い，自己流で乾燥標本作りに熱中していたが，その標本を眺めているうちに，これは風景画の素材に使えそうだと気づいたという。

2年前に札幌に帰り今の仕事についたが，1991年の夏には1か月をかけて北陸，山陰地方から北九州，四国そして東海，関東各地を車で廻りたくさんの海藻を採集してきた。その成果は何冊かのファイルに納まり，また塩蔵して保存されている。

渡辺さんの絵は30×40 cmほどの四季おりおりの風景画で，背景の山や湖あるいは空などは油絵の具で描いているが，それに用意したくさんの乾燥標本の中から樹木や草になりそうなものを選んで張り付けてある。近景の大きな木は太い幹と枝葉の部分をそれぞれ違う種類の標本を巧みに組み合わせ，木それぞれの感じや遠近による表現にも海藻の特徴をうまく利用する工夫をしている。使われている海藻はマクサ，オバクサ，ハリガネ，スギノリ，オキツノリ，ホンダワラ類のような樹枝状のものが多い。

ただ，絵には形のほかに季節にふさわしい色彩が必要であるが，標本自体の色だけでは特別な表現以外はどうしても不足する。それで渡辺さんは乾燥標本にあらかじめ緑，赤，黄，白など季節に合わせたいくつか

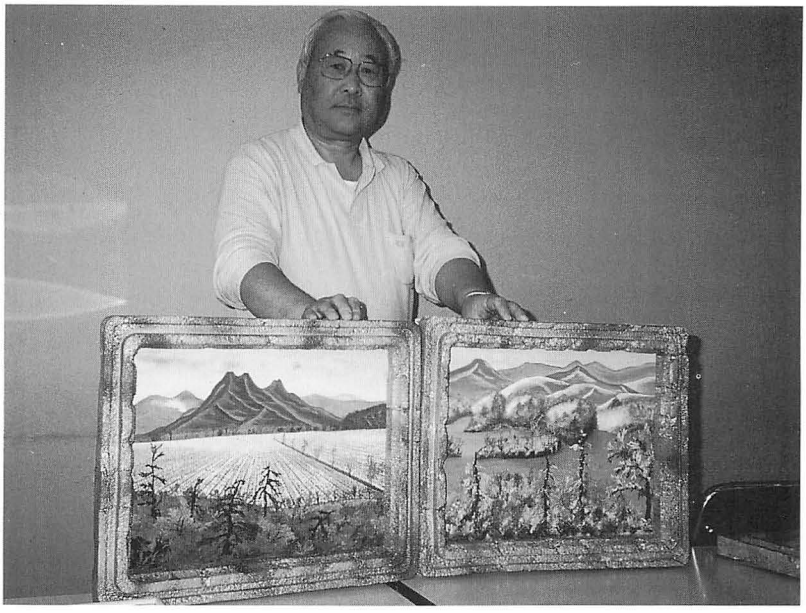

Fig. 1. Mr. Isamu Watanabe and his handmade pictures. The trees and grasses in the picture are expressed by dried and colored specimens of various kinds of seaweed.

の色を絵の具で塗ってたくさん保存しておき，状況にあわせてこれらを使う工夫をしている。

ところで，私たちの常識では海藻標本は台紙上で乾燥するものであるが，渡辺さんはそのことを知らず全くの自己流で台紙なしの標本を作っていた。このことが，かえって標本を絵を「描く」素材として自由に利用するという発想を生み出したのであって，もし常識通りの海藻標本を作っていたら単なる標本マニアで満足していたかもしれない。

渡辺さんの絵は，本人も言われるようにまだまだ習作の段階であり，もっとたくさんの海藻を利用すれば一層楽しい絵になりそうである。さらにその手法を利用すれば奥行のある芸術的作品を作ることもできるだろう。

ともあれ，その絵を見せられて私は研究者ではちょっと思い付かない海藻の楽しみ方に新鮮さを覚え，いささか虚をつかれた感じさえした。子供達の情操教育などにも取り入れられるだろうし，海藻の勉強でもこんな遊び心から入ったら楽しくなるだろう。

(041 函館市日吉町4-29-15)

新 刊 紹 介

大橋広好訳：「国際植物命名規約　1988」
津村研究所発行　2,500円

学名に含まれる情報はたくさんあります。その種のたどった歴史が凝縮されているといっても過言ではありません。一つの例として，褐藻ヘラヤハズの学名 *Dictyopteris prolifera* (Okamura in De Toni et Okamura) Okamura [Basionym: *Haliseris prolifera*] には次のような履歴が要約されています。はじめに岡村は De Toni との共著の論文の中で *Haliseris prolifera* を新種として記載しました。のちに，この属名 *Haliseris* C. Agardh 1820 が *Dictyopteris* Lamouroux 1809 の異名 (nomenclatural synonym), すなわち同じ藻類に 2 つ以上の名前があるときの非合法な方の名前 (illegitimate name) とわかったので，優先権 (priority) をもつ *Dictyopteris* に属を移して，新組合わせ (new combination; comb. nov.) としました。この際，最初に記載された種名が，基礎異名またはバシオニム (basionym) とよばれます。なおそれ以前にもこの属には *Neurocarpus* Weber et Mohr 1806 という属名が存在し，優先権がありました。でも *Dictyopteris* の方が一般に用いられる機会が多かったので，国際会議ではこちらが保存名 (conserved name; nom. cons.) として認められ，*Neurocarpus* は廃棄名 (rejected name; nom. rej.) として用いることはできなくなったのです。

さて，上の例で述べたような学名の書き方や変更の手続きは，学者によりまちまちでした。国際的に取り決めておかなくてはならないということで，20世紀初頭より国際的な植物学の会議があるたびに国際植物命名規約としてまとめられることになりました。1987年ベルリンの国際植物学会議で決まったベルリン規約 (1988) が最新のものです。ところで，この規約はほとんど法律文で書かれています。法律書はその内容を覚えておくものではなく，問題に直面したときに使いこなすものです。そのためにいちいち英語で書かれた原書を参照していたのでは，訳すことに精力を使い果たしてしまい，本当に理解したい内容がわからずじまいで終わってしまいます。かつて私自身，大学院のセミナーで「シアトル規約」「シドニー規約」の日本語訳を試みました。でもただ逐語的に訳したので，本来意味するところが伝わりません。結局，微妙な問題は原書にあたって，苦労して解決することになりました。本書はそういう状況の中で強く待ち望まれていた日本語訳といえます。しかも原書の直訳ではなくその法律文特有のニュアンスをうまく日本語でつたえています。例えば，conserved name は従来の訳語である「保留名」のかわりに「保存名」とされ，分かりやすくなっています。また diagnosis も，従来の「記相」から「判別文」となり，しっくりしました。また protologue を「初発表文」とし，synonym や homonym を単に「異名」「同名」としているのも明瞭でよいと思います。原書にはカリフォルニア大学のシルバ博士による藻類の実例がいくつも引用されており，その和訳も学名理解の手助けとなって，親近感をおぼえます。また和英ラテン語による事項索引が，新旧語訳も含めてあり，とても使いやすくなっています。本書を最初に目を通したとき，理解が十分でなかった部分がわかり，目から鱗が落ちるように感じました。私ども，多かれすくなかれ学名を使用したり，理解したりする必要がある者にとり本書は座右の書といえます。

ひとつ，原書の 3 分の 2 を占める保存名，廃棄名のリストが収録されていないのが残念です。世界的に見て，今後ますます保存名が追加される方向にあります。本書にこのリストが収録されることを期待いたします。

（国立科学博物館　田中次郎）

新刊紹介

Abbott, I. A. (Ed.): Taxonomy of Economic Seaweeds. With reference to some Pacific and Western Atlantic species. vol. III. xiv＋241 pp. California Sea Grant College, University of California, La Jolla, California. 1992. $10.00

本書は1989年8月にカリフォルニア大学サンディエゴ校スクリプス海洋研究所において行われた「第3回有用海藻の分類に関するワークショップ」の成果をまとめたものである。このワークショップはハワイ大学のI. A. Abbott教授を中心にCalifornia Sea Grant College Programの後援によって開催されているもので第1回目はグアム島で，第2回目は中国の青島で開催され，それぞれの成果はこのシリーズの第1巻，第2巻としてすでに刊行されている。第3回ワークショップは17名の研究者の参加によって行われ，日本からは吉田忠生氏，鰺坂哲朗氏の2名が参加している。このうち8名がホンダワラ類，5名がオゴノリ類，4名がテングサ類，1名がキリンサイ類を主な対象としている(一部メンバーの重複あり)。

本書は全体でインデックスを除くと4つの章から成り立っており，それぞれの章に編者のイントロダクションがつけられている。このうち，全体の約3/5がホンダワラ類に当てられており，このワークショップの今回の主要な課題であったことがわかる。ホンダワラ類の章のイントロダクションで編者のAbbott氏はホンダワラ類の詳細な分類の研究が以前の2回のワークショップの成果で大きく前進したもののまだ端緒についたばかりであることを強調している。これまでに温帯と亜熱帯域のホンダワラ類についてはかなり検討がなされたが，熱帯の大部分がまだ手つかずの状態であり，温帯，亜熱帯域ですらまだ全体を網羅したとはいえない。今回は中国のホンダワラ類のほか韓国とフィリピンの標本の分類が新たに検討されており，さらに琉球列島とカリブ海のホンダワラ類の比較研究の結果など全体で8つの論文が収められている。次のテングサ類の章には3つの論文が含まれている。編者も述べているようにこれまでのこのワークショップにおけるテングサ類の分類はどちらかというとこれまでの分類系を継承するものであったがこの巻においてはその分類形質を見直そうとする試みがみられ，*Gelidium*属と*Pterocladia*属の関係の見直しや*Gelidiella*属の形態に関する論文が含められている。一方，オゴノリ類の分類では過去のワークショップでも*Polycarvernosa*や*Gracilariopsis*の有効性など特に属レベルの取り扱いに関して多くの新しい提案がなされやや混乱した状況にあったが，今回のワークショップの結論としては古い属名である*Gracilaria*属だけを用いるということになったようである。本書のオゴノリ類の章には3つの論文が含まれている。また，最後のキリンサイ類の章には吉田忠生氏のアマクサキリンサイの選定基準標本(lectotype)の選定に関するノートが含まれている。

本ワークショップは札幌での第4回ワークショップも終了し，今後もさらに継続されることと思うが，各国の研究者の意見交換の場としてまた国際共同研究の基礎としてさらに発展することを期待する。

本書の購入をご希望の方は下記の住所に直接申し込めば入手できる。

California Sea Grant College, University of California, 9500 Gilman Drive, La Jolla, California 92093-0232, U.S.A.

(北大・理・植物　川井浩史)

―学　会　録　事―

―会　員　移　動―

新　　入　　会

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ニュース

国際シンポジウム「ハプト藻の生物学」のお知らせ

The BIOLOGY of the PRYMNESIOPHYTA

Plymouth, UK,

29th March-1st April, 1993

The aim of this international symposium is to bring together phycologists, marine and freshwater ecologists, biochemists, and all others who have an interest in this important group of organisms. Contributions on any aspect of prymnesiophyte biology, ecology, physiology or biochemistry will be welcome. Sessions will include invited and volunteered contributions, and there will be poster sessions and opportunities for informal workshops.

For further details, please contact either:

Dr. J. C. Green,
Plymouth Marine Laboratory,
Citadel Hill,
Plymouth PL1 2PB

Tel: 0752 222772
Fax: 0752 226865

or

Dr. B. S. C. Leadbeater,
School of Biological Sciences,
The University,
Birmingham B15 2TT

Tel: 021 414 5567
Fax: 021 414 5925

日本学術会議だより　No.25

学術国際貢献特別委員会設置される

平成4年5月　日本学術会議広報委員会

日本学術会議は，去る4月15日から17日まで第114回総会（第15期3回目の総会）を開催し，新たに「学術国際貢献特別委員会」を設置しました。今回の日本学術会議だよりでは，同総会の議事内容及び3月に開催されたＡＡＳＳＲＥＣ執行委員会等についてお知らせいたします。

旧ソ連邦の科学者に対する緊急の支援措置について（会長談話）

平成4年2月25日
日本学術会議
会長　近藤次郎

ソ連邦が解体したことに伴い、旧ソ連邦における多くの科学者は，研究の継続が困難となり，研究組織も崩壊の危機に直面していると伝えられており，これが事実とすれば，世界に与えるその影響は計り知れないものがあると思われる。

いうまでもなく，人類の進歩にとって科学の向上発展は不可欠のものであり，その意味で，今日の旧ソ連邦の実情は憂慮に堪えないところである。

この際，我々日本の科学者は，学協会等を通じる等の方法で，旧ソ連邦の科学者に対し，能う限りの支援を行う必要があると考える。

なお，旧ソ連邦の科学者と我が国の科学者との間の一般的な国際学術交流・協力をより一層充実するための方策等については，我が国の学術の分野における国際貢献の一環として，日本学術会議において引き続き検討することといたしたい。

（注）
本談話は，日本学術会議において国際交流・協力問題について調査・審議を行っている第6常置委員会から2月14日㈮の連合部会に問題提起され，各部会で検討され審議を経た後，2月25日㈫の第785回運営審議会に提案され審議されたものである。

日本学術会議第114回総会報告

日本学術会議第114回総会（第15期3回目の総会）は，4月15日～17日の3日間開催された。

第1日（4月15日）の午前。まず，会長からの前回総会以後の経過報告及び各部・各委員会等の報告が行われた。次いで，今回総会に提案されている2案件について，それぞれ提案説明がなされた後，質疑応答が行われた。

第1日の午後。各部会が開催され，午前中に提案説明された総会提案案件の審議が行われた。

第2日（4月16日）の午前。前日提案された案件の審議・採決が順次行われた。

まず，「副会長世話担当研究連絡委員会の運営について（申合せ）の一部改正」が採択された。これは，「副会長世話担当研究連絡委員会運営協議会」という名称を「複合領域研究連絡委員会運営協議会」に改めるとともに，運営協議会のより円滑な運営を図るために，必要な措置を講じたものである。

次いで，「学術国際貢献特別委員会の設置について（申合せ）」が採択された。これは，学術の分野における我が国の国際貢献の在り方について検討するための特別委員会を設置したものである。

なお，審議・採決の終了後，さきに会長談話として発表した「旧ソ連邦の科学者に対する緊急の支援措置について（平成4年2月25日）」に関連して，旧ソ連邦の科学者の実情調査のために，当会議からロシアに派遣された第6常置委員会幹事の宅間会員から，その調査結果について報告が行われた。

第2日の午後。各部会が開催され，各部における懸案事項について審議が行われた。

第3日（4月17日）午前には，各常置委員会が，午後には，各特別委員会がそれぞれ開催された。

学術国際貢献特別委員会の設置

本会議は，昨年10月に開催した第113回総会における内閣官房長官からの学術の分野における我が国の国際貢献の在り方についての検討依頼を踏まえ，今回の第114回総会において学術国際貢献特別委員会を設置した。

AASSREC執行委員会の開催

去る3月23日から26日にかけて4日間，ＡＡＳＳＲＥＣ（Association of Asian Social Science Research Councils）執行委員会が日本学術会議の会議室で開催された。外国代表団は前ＡＡＳＳＲＥＣ会長で現副会長のＲ・トリニダード教授（フィリピン社会科学協議会），同じく副会長代行のＪ・Ｊ・スモリッツ教授（オーストラリア社会科学アカデミー），ＡＡＳＳＲＥＣ事務局長のＤ・Ｎ・ダナガーレ教授（インド社会科学研究協議会），同じく事務幹事のＶ・Ｋ・メータ博士（同上）のＡＡＳＳＲＥＣ側4理事と，タイ国バンコック駐在のＵＮＥＳＣＯ人間社会科学地域アドヴァイザーのＹ・アタール博士の5名。

日本側は，現ＡＡＳＳＲＥＣ会長の川田侃日本学術会議副会長のほか，来年9月に川崎市のＫＳＰ（神奈川サイエンス・パーク）で日本学術会議が共催して開く予定の「ＡＡＳＳＲＥＣ第10回日本総会」の組織運営委員会委員長山田辰雄教授（慶応義塾大学，アジア政経学会理事長），同事務局長・平野健一郎教授（東京大学，アジア政経学会前理事長），及び日本学術会議ＡＡＳＳＲＥＣ専門委員会幹事浦田賢治会員（第2部）の3名がオブザーヴァーの資格で参加，連日，時間を措しむかのように，ＡＡＳＳＲＥＣの運営や来たるべき第10回総会の打合せなどについて，熱心な討議が続けられた。

また討議の合間を縫うようにして，外国代表団は近藤次郎日本学術会議会長表敬訪問，日本学術会議運営審議会における挨拶などのほか，川崎市にも赴き市長表敬訪問，ＫＳＰ視察などを精力的に行った。日本学術会議も，近藤会長主宰のレセプションを催し，関係諸国の東京駐在大使館スタッフなどを招いて，アジア・太平洋地域における学術交流と発展のための意見交換の場を設け，友好的な雰囲気のなかで談論が風発，至るところで談笑の花が開いた。

ＡＡＳＳＲＥＣはアジア・太平洋地域の社会科学領域における国際学術上部組織で，いわゆるアンブレラ・オーガニゼイションである。1973年にインドのシムラで「社会科学の教育・研究に関するアジア会議」が開かれた際に設立が合意され，それ以来ＵＮＥＳＣＯの協力のもとに発展を遂げてきた。ＡＡＳＳＲＥＣは加盟各国それぞれの文化的伝統を尊重しつつ，社会科学の研究，教育，知識の普及などを促進することを通して，この地域における社会科学の発達を図ることを目的に，加盟諸国の社会科学協議会，またはこれに類する団体（1国1会員）により構成されている。

加盟国はオーストラリア，インド，中国，ニュージーランド，フィリピンなど，1991年8月現在，15ヵ国であるが，国（くに）会員のほかに，準会員の制度もあり，将来この地域の各国の学協会や研究所等が準会員としてＡＡＳＳＲＥＣの活動に参加する道も開かれている。出版活動としては，隔年に開催される総会における諸報告やシンポジウムなどの出版のほか，定期刊行物「aassrec panorama」が年2回出されている。

ＡＡＳＳＲＥＣには最高決定機関である総会のほかに，会長，副会長（2名制），事務局長の4名で構成される理事会が置かれているが，これにさらにＵＮＥＳＣＯの地域アドヴァイザーが加わって開かれる執行委員会に事実上の運営権限があるようにみえる。今回，日本学術会議で開かれた会議はＡＡＳＳＲＥＣとしては極めて重要な会議であったといえる。ＡＡＳＳＲＥＣはＵＮＥＳＣＯによって承認された「非政府機関（ＮＧＯ）」の地位をもち，絶えずＵＮＥＳＣＯと緊密な関係を保っているが，同じくＵＮＥＳＣＯによって承認されたＮＧＯの地位をもつＩＦＳＳＯ（国際社会科学団体連盟）とも相互協力関係にある。

平成4年(1992年)度共同主催国際会議

日本学術会議では，我が国において開催される学術関係国際会議のうち毎年おおむね6件について，学・協会と共同主催している。

本年もまた，6件の国際会議を共同主催することとしており，その概要は，次のとおりである。

◆第5回世界臨床薬理学会議（7月26日～31日）

この会議は，臨床薬理学に関する研究を発展させるため討論を行い，最新の研究情報を交換することを目的として横浜市（横浜国際平和会議場）において開催される。

参加予定人数は3,000人（国外1,500人，国内1,500人），参加予定国数は49か国。

◆第14回国際平和研究学会総会（7月27日～31日）

この会議は，平和学に関する研究を発展させるため討論を行い，最新の研究情報を交換することを目的として京都市（国立京都国際会館及び立命館大学）において開催される。

参加予定人数は450人（国外250人，国内200人），参加予定国数は45か国。

◆第8回国際バイオレオロジー会議（8月3日～8日）

この会議は，バイオレオロジー学に関する研究を発展させるため討論を行い，最新の研究情報を交換することを目的として横浜市（横浜国際平和会議場）において開催される。

参加予定人数は500人（国外150人，国内350人），参加予定国数は26か国。

◆国際地質科学連合評議会及び第29回万国地質学会議（8月24日～9月3日）

国際地質科学連合評議会は，同連合の最高決定機関であり，運営事項を協議，決定することを目的とするものである。また，万国地質学会議は，地質学に関する研究を発展させるため討論を行い，最新の研究情報を交換することを目的として京都市（国立京都国際会館）において開催される。

参加予定人数は5,300人（国外3,200人，国内2,100人），参加予定国数は94か国。

◆第9回国際光合成会議（8月30日～9月5日）

この会議は，光合成に関する研究を発展させるため討論を行い，最新の研究情報を交換することを目的として名古屋市（名古屋国際会議場）において開催される。

参加予定人数は1,000人（国外500人，国内500人），参加予定国数は41か国。

◆第11回国際光生物学会議（9月7日～12日）

この会議は，光生物学に関する研究を発展させるため討論を行い，最新の研究情報を交換することを目的として京都市（国立京都国際会館）において開催される。

参加予定人数は1,000人（国外600人，国内400人），参加予定国数は52か国。

御意見・お問い合わせ等がありましたら，下記までお寄せください。

〒106 東京都港区六本木7-22-34

日本学術会議広報委員会　電話03(3403)6291

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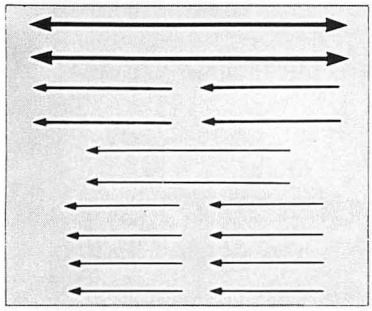

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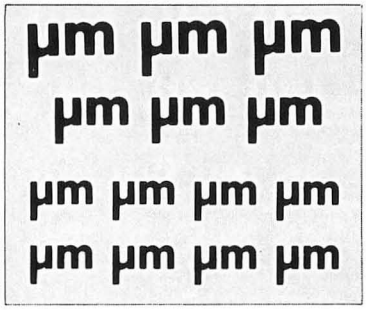

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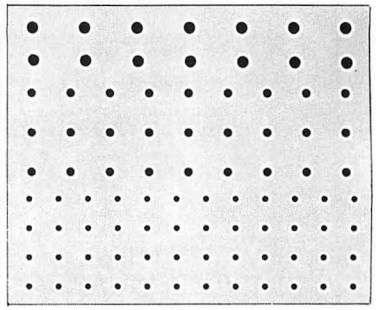

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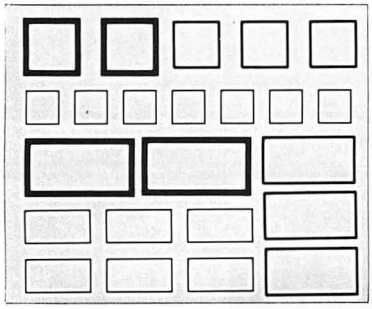

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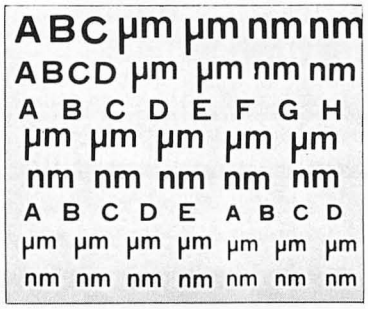

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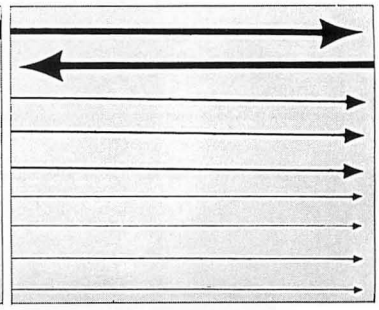

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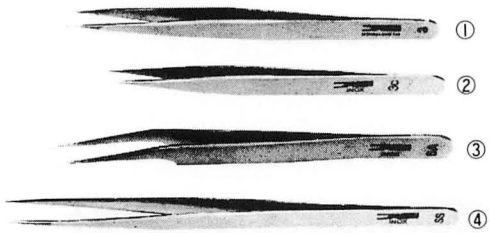

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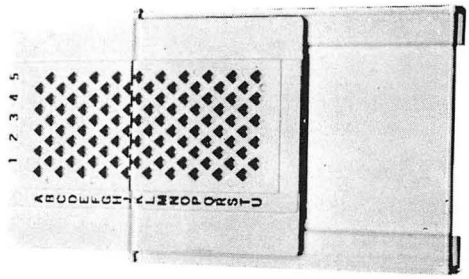

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