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## Vertical distribution of paralytic toxin-producing species, *Protogonyaulax* sp. in Funka Bay, Hokkaido

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UCHIDA, T., KAWAMATA, K. and NISHIHAMA, Y. 1980. Vertical distribution of paralytic toxin-producing species, *Protogonyaulax* sp. in Funka Bay, Hokkaido. Jap. J. Phycol. 28: 133-139.

The vertical distribution of the dinoflagellate *Protogonyaulax* sp., an organism causing paralytic scallop poisoning, was investigated from July to September, 1978, in Funka Bay, Hokkaido. During the period of abrupt thermocline, a relatively high cell density was only found in the middle layer. At the surface, water temperature was near 20°C, while at the bottom it was maximum 10°C. Cell density measurements show that *Protogonyaulax* sp. aggregated at the 8-14°C layer. This is the reason of aggregation in the middle water layer. A similar vertical distribution pattern is also considered to be maintained at night since distribution under dim light at daybreak was almost the same as that recorded during daytime. It is concluded that, unlike most other dinoflagellates studied, the vertical distribution pattern of *Protogonyaulax* sp. is significantly affected by temperature, but not by light. The application of the results obtained is briefly discussed for practical use in scallop cultivation.

*Key Index Words:* *Dinoflagellate*; *Gonyaulax*; *paralytic toxin*; *Protogonyaulax*; *scallop cultivation*; *temperature*; *thermocline*; *vertical distribution*.

*Gonyaulax catenella* and related species are linked with paralytic shellfish poisoning which can cause human illness and sometimes death (BURKE *et al.* 1960, PRAKASH 1963, PRAKASH and TAYLOR 1966, MACLEAN 1977). Since 1976, members of Hokkaido Institute of Public Health have found paralytic toxins in scallops from Funka Bay (Official data). In June 1978, a higher level of toxicity than that measured before was observed. Investigations were made to determine causative phytoplankton species which appeared parallel with the scallop poisoning. A *G. catenella* like species<sup>1)</sup> (*Protogonyaulax* sp.) was revealed to have

close correlation with shellfish toxicity, and this dinoflagellate was found to be present in the middle water layer (NISHIHAMA *et al.* 1979).

NISHIHAMA *et al.* (1979) have cultured this species and assayed the toxicity of laboratory grown individuals. Their findings show that *Protogonyaulax* sp. contains a potent paralytic toxin. Ecological studies on such a toxic dinoflagellate are of immediate practical importance for all those interested in the mariculture of filter-feeding shellfishes. Therefore, we studied the vertical distribution of this species in Funka Bay to correlate its occurrence with

1) This alga was quoted as *Gonyaulax catenella* like species in the previous report (NISHIHAMA *et al.* 1979). The genus *Protogonyaulax* is established by TAYLOR (1978).

temperature.

**Methods**

From July to September 1978, routine seawater samplings were carried out to determine water temperature, salinity and phytoplankters at both Sawara and Toyoura stations (Fig. 1). At each station seawater was collected from different depths with a VAN DORN plastic sampler. Immediately after pouring the seawater sample into a plastic bucket its temperature was measured. From samples brought back to the laboratory, salinity was measured with a salinometer (MC-5, Electronic Switchgear (London) LTD). For identification and counting of *Protogonyaulax* cells, each one

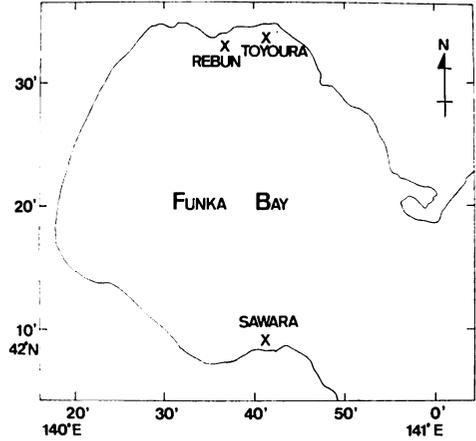


Fig. 1. Location of sampling stations.  
Water depth: Toyoura: 38 m, Sawara: 45 m, Rebun: 45 m.

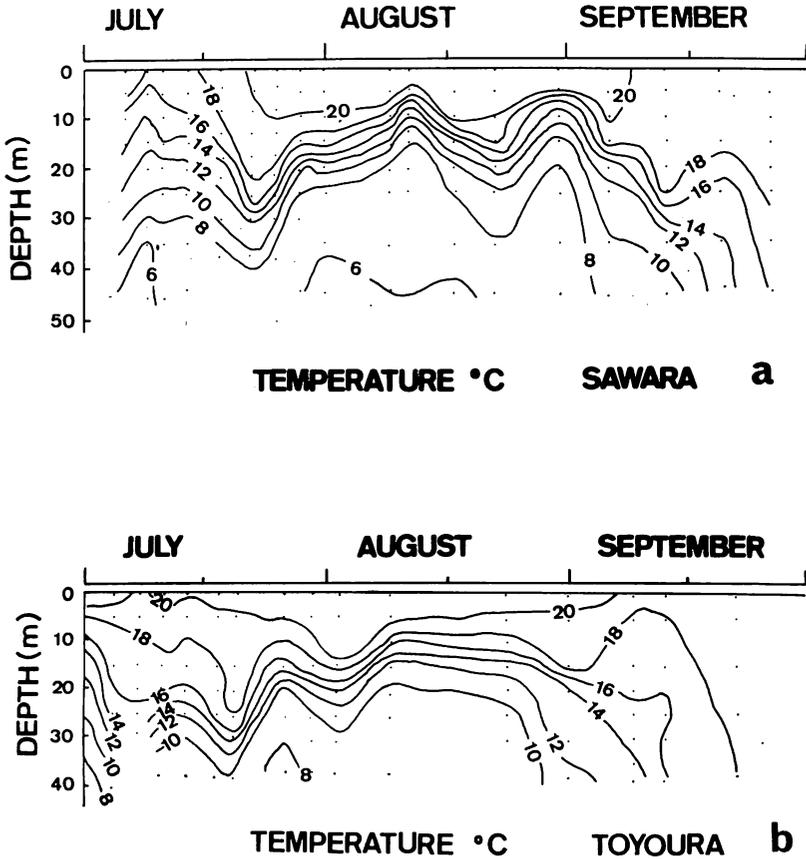


Fig. 2. Vertical seasonal distribution of water temperature at Sawara (a) and Toyoura (b).

liter sample from different depths was fixed with 2% ammonia-neutralized formalin. After concentration to 2 ml by allowing the phytoplankters to settle, the contents of a 0.1 ml sample was counted on a ruled slide.

## Results

Temperature and salinity stratification is clearly observed during the period of investigation as shown in Figs. 2 and 3. These results show vertical stability of water in this season.

At both stations, *Protogonyaulax* cells were relatively abundant during July to August (Fig. 4). From the end of August until September, the number of dinoflagellate cells decreased and remained below 200 cells/l. At the end of September they could hardly be detected as phytoplankters.

During the whole period of investigation, maximum cell density of *Protogonyaulax* sp. was  $24.9 \times 10^3$  cells/l at Sawara, 35 m depth, July, 21; and  $45.9 \times 10^3$  cells/l at Toyoura, 20 m, August, 8. In both cases, the cells aggregated in the middle layer. At Sawara, the layer containing more than 1,000 cells/l was narrow (5–14 m), although its depth varied considerably day by day. At Toyoura, temperature stratification was less pronounced and the layer containing more than 1,000 cells/l was wider (5–20 m) than at Sawara. During this period, a thermocline developed; the maximum temperature of bottom seawater was 10°C, while the surface temperature was approximately 20°C (Fig. 2). At both stations, the vertical distribution of *Protogonyaulax* sp. cells seems to be affected by temperature (Fig. 5). The upper thermal limit of the layer containing more than 1,000 cells/l

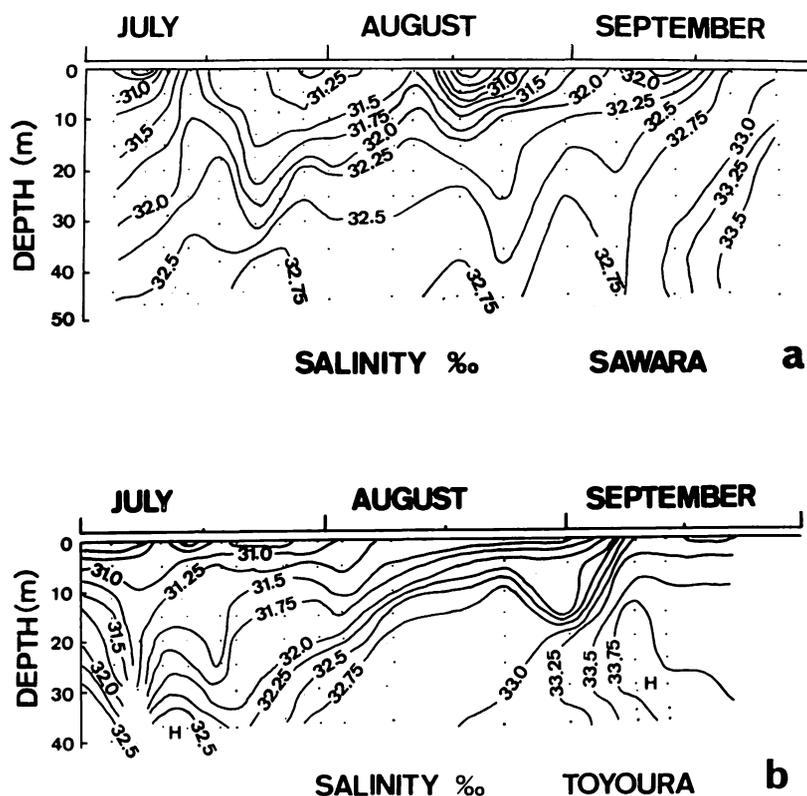


Fig. 3. Vertical seasonal distribution of salinity at Sawara (a) and Toyoura (b).

varied between 10 and 14°C. Furthermore, the maximum density layer of the cells was between the isothermal lines of 10 and 12°C. At Sawara, this relationship between temperature and dinoflagellate density was more clearly expressed (Fig. 5 a).

Since the above mentioned phenomena were based on daytime sampling, the observation on the distribution of *Protogonyaulax* sp. was carried out under dim light (5:00, August, 9) as well as during daylight (15:00, August, 8) at Rebun (Fig. 6). It took about one hour to complete sampling process. The sampling time described shows the time when the sampling had been finished. Since the sunrise was at

4:35, August, 9, 1979, the dim light sampling was conducted during before to after sunrise. As a result, similar distribution pattern was obtained in each case (Fig. 6). A relatively high cell density was recorded at 20 m depth, while only a few cells were observed in other water layers.

Starting from the end of August in Toyoura, high salinity (above 33‰), warm waters gradually intruded (Fig. 3). At Sawara, this intrusion was delayed for about 20 days. As soon as this water mass occupied all the layers, the *Protogonyaulax* sp. cells completely disappeared from the seawater layer.

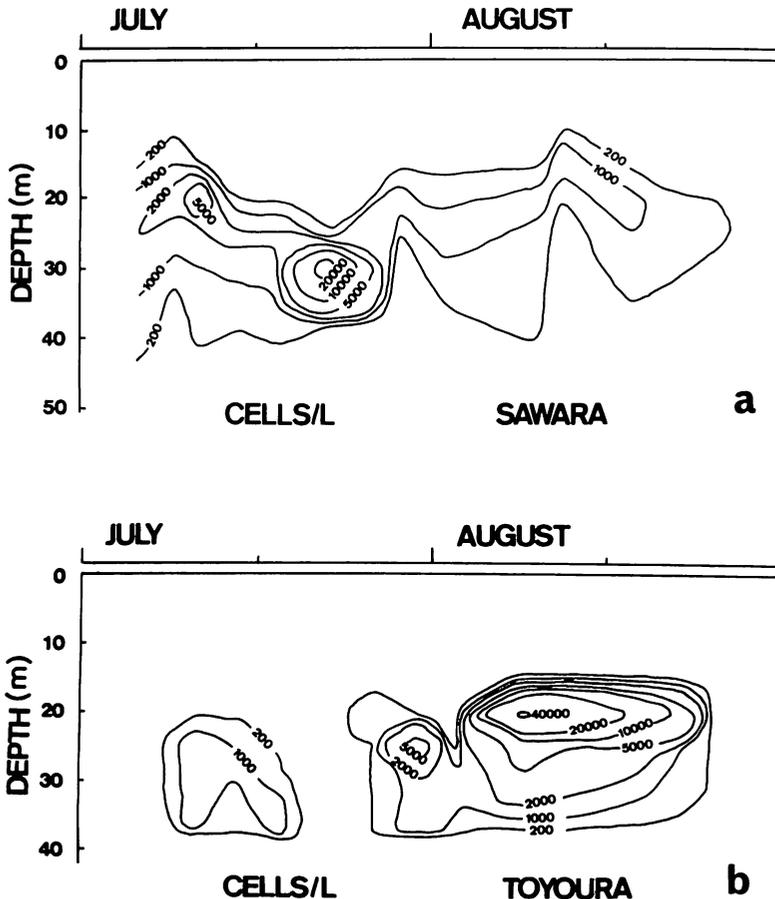


Fig. 4. *Protogonyaulax* sp. vertical seasonal distribution at Sawara (a) and Toyoura (b).

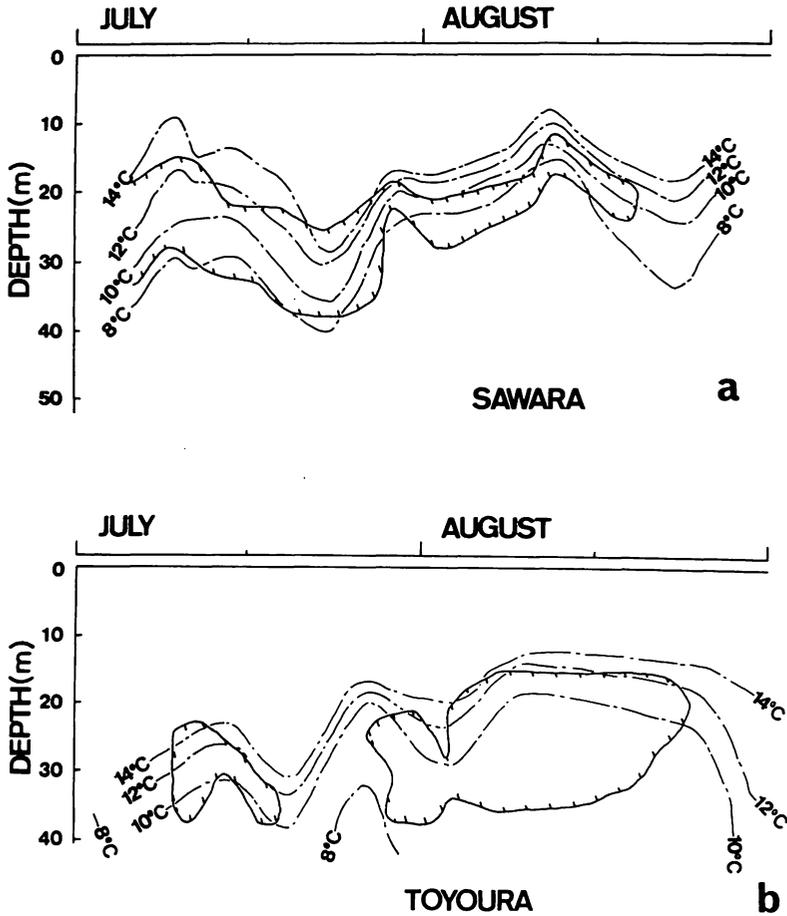


Fig. 5. Relationship between *Protogonyaulax* sp. distribution and water temperature at Sawara (a) and Toyoura (b). Area surrounded by slanted lines contains more than 1,000 cells/l. (Quoted by NISHIHAMA 1980)

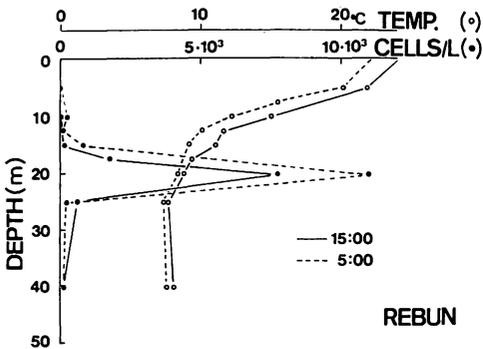


Fig. 6. *Protogonyaulax* sp. vertical distribution under daylight (15:00) and dim light at daybreak (5:00).

### Discussion

Light is thought to be an important factor for dinoflagellates vertical distribution because of the existence of positive or negative phototaxis (HASLE 1950, 1954; NORDLI 1957; WHEELER 1964; EPPLEY *et al.* 1968; SELIGAR *et al.* 1970; TILZER 1973; MACLEAN 1977). The response to light resulted in a diurnal vertical migration of some dinoflagellates in response to light-dark cycles (HASLE 1950, 1954; WHEELER 1964; EPPLEY *et al.* 1968; TILZER 1973). Other than light, some factors are known to influence the migrative behavior of some dinoflagellate species. In *Pyrodinium*

*bahamense*, phototactic behavior is not an obligatory species pattern but a facultative response to nutrient availability (MACLEAN 1977). In addition to phototaxis, endogenous rhythm is a possible factor causing diurnal migration in *Gonyaulax polyedra* and *Cachonina niei* (EPPLEY *et al.* 1968). In Funka Bay, *Protogonyaulax* sp. was shown to respond differently from other related organisms, and no significant diurnal migration was shown. The pattern of vertical distribution was similar under daylight (15:00) and under dim light (5:00). Therefore, the distribution pattern obtained here from midday samplings is most likely maintained also during night.

The vertical distribution of *Protogonyaulax* sp. seems to be determined by temperature: a large number of the cells were found only in the water layer with temperatures of 8–14°C. At Toyoura, water temperature was above 8°C in all water layers examined from July to August, while at Sawara an 8°C isotherm prevailed in the middle layer. This may be the reason why at Toyoura *Protogonyaulax* sp. is distributed in deeper layer as compared to the situation at Sawara. Since this species shows active growth in culture at 20°C (UCHIDA, unpublished), the temperature dependent distribution cannot be explained in the present case merely by a growth phenomenon. These findings can be of importance to investigators involved in scallop cultivation. It is possible to eliminate the accumulation of toxin in cultivated scallops by avoiding the layer containing a dense population of *Protogonyaulax* sp. cells. The layer containing high cell densities can easily be detected by measuring water temperature.

A mid-layer distribution pattern of phytoplankters, as shown in the present study, is not limited to *Protogonyaulax* sp. According to NISHIHAMA (unpublished), in Funka Bay, dinoflagellate *Exuviaella* sp. also aggregates in the middle layer. The density of *Gonyaulax* sp. in Ofunato Bay was also higher in the middle layer (MURANO 1975). However, it is still unclear

whether the vertical distributions of these two species are affected by temperature or other environmental factors. In Funka Bay, *Protogonyaulax* sp. was found in quite low numbers ( $45.9 \times 10^3$  cells/l), even in the highest density layer. The population did not develop a bloom perhaps because the nutrient levels are low during summer (NISHIHAMA *et al.* 1976).

By the end of September, warm water with a high salinity occupied all layers. This is due to the inflow of Tsugaru Warm Current (OHTANI and AKIBA 1970). When the intrusion had completed, *Protogonyaulax* sp. completely disappeared from water sample. This may be partly due to that the low-salinity and cold water mass containing the species was replaced by Tsugaru Warm Current water mass.

#### Acknowledgements

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内田卓志\*・川真田憲治\*\*・西浜雄二\*\*：北海道、噴火湾における麻痺性  
貝毒原因種、*Protogonyaulax* sp. の鉛直分布について

噴火湾産ホタテガイ毒化の原因種である *Protogonyaulax* sp. の鉛直分布を1978年7月から9月迄の3カ月間、噴火湾沿岸の定点において調査した。その結果、本種は水温8~14°Cの中層に局在し、この水温層の上下に伴って出現層が変化した。また細胞密度の最も高い層は常に水温10~12°Cの層に存在した。一方、同一地点で日中および夜明け時の鉛直分布を比較したところ、ほぼ同じパターンが得られ本種は顕著な日周移動をしないものと考えられた。以上の結果および考察から、光依存の鉛直分布を示す他の渦鞭毛藻とは異なり *Protogonyaulax* sp. は水温に依存した鉛直分布を示すと結論される。(\*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, \*\*041-14 茅部郡鹿部村字本別 北海道立栽培漁業総合センター)

## 新刊紹介

七尾善磨： **原色青森県海藻図鑑** 159頁。昭和55年9月1日発行。限定自費出版。頒価3,800円  
(連絡先：青森市大字筒井字桜川361-17, 七尾善磨)

この図鑑は本学会々員、県立青森高校教諭である著者が約20年に亘って採集、観察を続けてきた青森県沿岸産の海藻のうち、比較的大型の緑藻22種、褐藻55種、紅藻127種の合計204種について標本写真191点、生態写真55点、その他4点を纏めたものである。本書は県内小、中、高校の理科教師の参考のために作られたものであり、各種の形態、生育時期、生育場所、産地名が簡明に記されている。巻末には青森県沿岸の海流、海藻の垂直分布、浅虫における年間の生育期、青森県産有用海藻の種類とその利用方法などが認されている。写真は鮮明で形態的な特徴はわかりやすい。種類の同定に関しては尚検討を要するものが2、3見受けられるが全体としてよくまとまった労作である。著者は県内で利用されることを目的としているが、日本北部の他地域においても広く参考になるものであり、又一地方の海藻図鑑が出版されることは少ないので本書が大いに活用されることが期待される。

(小樽商大 山田家正)

**New blade initiation in the perennial red alga  
*Constantinea rosa-marina* (GMELIN) POSTELS  
et RUPRECHT (Cryptonemiales, Dumontiaceae)**

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LINDSTROM, S. 1980. New blade initiation in the perennial red alga *Constantinea rosa-marina* (GMELIN) POSTELS et RUPRECHT (Cryptonemiales, Dumontiaceae). Jap. J. Phycol. 28: 141-150.

New blade initiation is described for the perennial red alga *Constantinea rosa-marina*. New blades are initiated each autumn from a meristematic region in the center of the previous year's blade. During new blade production cells in the meristematic region begin to elongate in a prescribed sequence and attain lengths to 200 times their original size, thereby forming the medullary filaments of the new blade. A predetermined pattern of cell divisions occurs concomitantly to produce the cortical tissue of the new blade.

New blades are produced under short-day conditions in both the field and the laboratory. A laboratory experiment showed that new blade initiation is halted by long-day conditions.

*Key Index Words:* *Constantinea rosa-marina*; *Cryptonemiales*; *development*; *new blade initiation*; *Rhodophyta*.

SETCHELL (1906) distinguished three species of *Constantinea* on the basis of new blade initiation in relation to new stipe production. *Constantinea rosa-marina* (GMELIN) POSTELS et RUPRECHT and *C. simplex* SETCHELL were separated from *C. subulifera* SETCHELL on the basis that the blades of the former two species are peltate (circular, having the stipe attached to the lower surface at the center), "becoming perfoliate only upon the appearance of a new lamina, while in *C. subulifera* the blades are circular and perfoliate from the very beginning." POWELL (1964) and ABBOTT (1968) have described new blade initiation in *C. subulifera* and *C. simplex*, respectively. However, nothing has yet been written on new blade initiation in *C. rosa-marina*, the type species of the genus.

*Constantinea rosa-marina* is a perennial red alga which arises as a multiaxial blade from a cushion-like disc. Early in its development the blade becomes peltate, and the plant maintains this form throughout its life span of up to 18 years or more (the age of the oldest plant collected during this study).

A new blade is produced each fall in the center of the old blade, and it is this terminal blade which produces the reproductive structures. Following reproduction, the outer reproductive part of each blade is shed, and the inner part of the blade gradually erodes so that after a few years an "annual ring" on the stipe is all that remains of a blade.

In order to better understand the relationship between the species of *Constantinea*,

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I undertook a comparative study of the two species occurring in Japan, *C. rosa-marina* and *C. subulifera*. Since POWELL (1964) has already studied *C. subulifera* rather thoroughly in Puget Sound and it is the less common of the two species in Japan, I concentrated on *C. rosa-marina*. In this paper, I describe the initiation of new blades in *C. rosa-marina*. Reproductive structures and reproductive strategy will be described in a subsequent paper.

### Materials and Methods

In Japan, *C. rosa-marina* and *C. subulifera* occur along the eastern coast of

Hokkaido from Cape Nosappu (43°23'N lat., 145°49'E long.) to Cape Erimo (41°33'N lat., 143°8'E long.). In the present study, *C. rosa-marina* was found subtidally at depths of 4–10 m, and *C. subulifera* from the low intertidal zone to 2 m subtidally, except for one male plant that was collected at a depth of 5 m at Cape Nosappu. According to Prof. T. MASAKI (pers. comm.), *C. rosa-marina* can be collected in the extreme low intertidal zone of Daikokujima, an offshore island in the cold current region of eastern Hokkaido. Specimens observed during the course of this study came from the following collections:

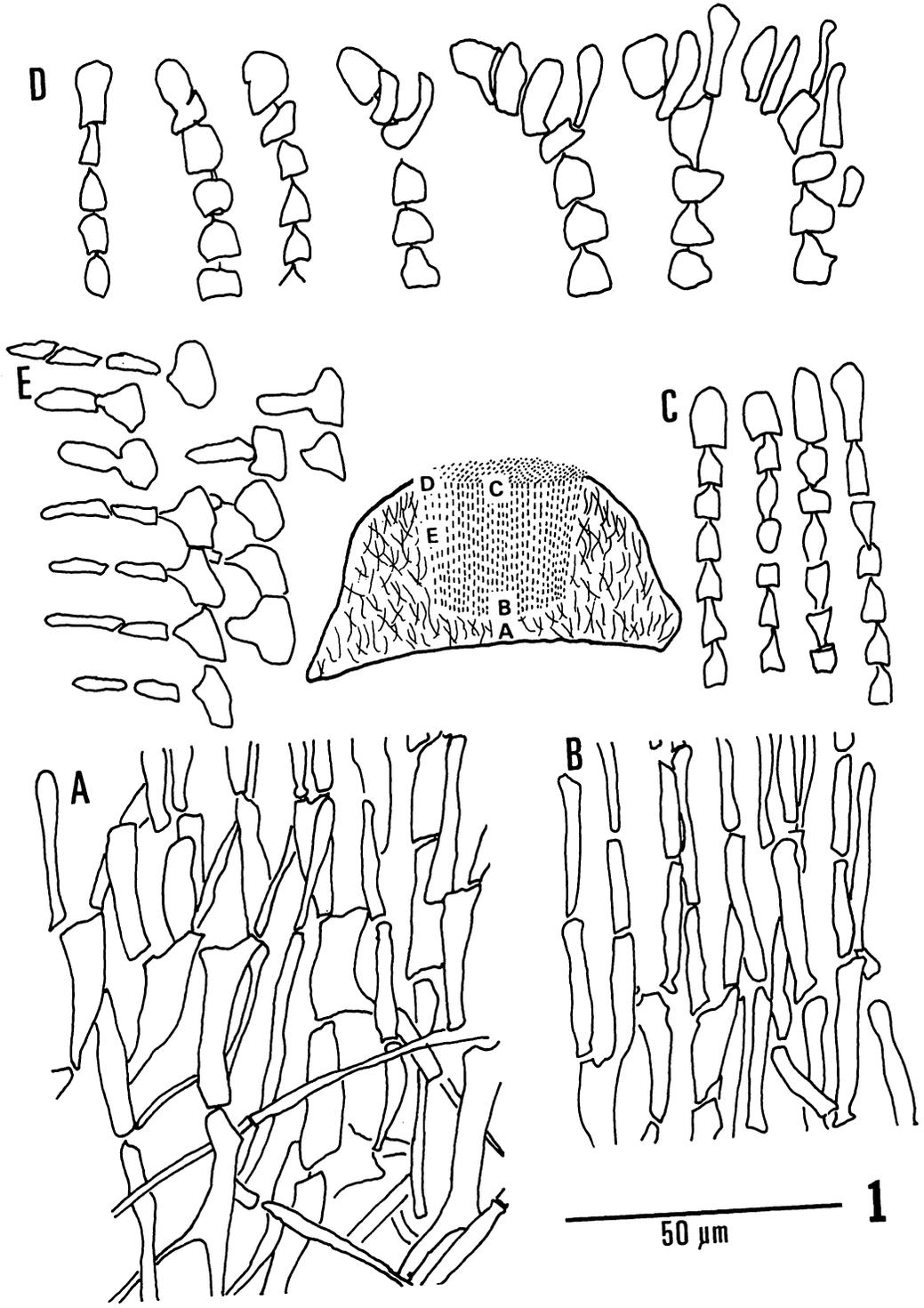
Date	Location	Depth	Collector
May 26, 1978	Kushiro	4–10 m	N. TAZAWA
June 6, 1978	Cape Nasappu	0–5 m	Author
Aug. 5, 1978	” ”	Cast ashore	M. KUROI
Sept. 5, 1978	” ”	” ”	Author
Oct. 17, 1978	” ”	” ”	”
Nov. 29, 1978	” ”	” ”	”
Jan. 26, 1979	” ”	” ”	”

Plants were preserved in 10% formalin in seawater. Sections of the meristematic region were made using a freezing microtome, and glycerine-mounted sections were stained with Cotton blue for microscopic examination.

Plants for the photoperiod experiment on new blade initiation in *C. rosa-marina* were collected September 5 at Cape Nosappu. The upper blade and stipe were removed from the rest of the plant. The upper blades were then trimmed so that a rim less than 1 cm wide remained around the stipe. Plants were put into individual

200 ml glass storage jars with PROVASOLI'S ES medium (PROVASOLI 1968) and maintained in 10°C culture rooms. Eight plants were placed under a 16:8 photoregime, and eight under an 8:16 photoregime. Several plants became moribund during the first month and were terminated. Medium was changed every five days for the first ten weeks and approximately every ten days to three weeks thereafter. Epiphyte growth, especially for plants under long-day conditions, necessitated periodic cleaning of the plants.

Fig. 1. Cross section of mound in center of *Constantinea rosa-marina* blade showing: A. construction of mound below the meristematic zone showing similarity to the blade and stipe below it; B. construction of mound showing transition to meristematic cylinder; C. construction of apical region of meristematic cylinder before actual new blade initiation; D. sequence from left to right of an apical cell switching from transverse to oblique division with the immediately subapical cells subsequently cutting off cells to fill in the space created by deflection of the original apical cell; E. lateral branching of cells of the meristematic cylinder with several branch apices already beginning to divide obliquely.



## Results

### *Morphological observations of*

#### *C. rosa-marina*

New blade initiation in *C. rosa-marina* can be divided into two phases: preparatory and actual. The preparatory phase begins during the summer months when a small mound of tissue appears in the center of terminal blades of *C. rosa-marina* immediately above the stipe. This mound produces a transition from the branched filamentous construction of the blade and stipe below it (Fig. 1A) to the unbranched filamentous construction of a solid cylinder of meristematic tissue at the top of the mound (Fig. 1C). As the meristematic cylinder increases in depth, the apical cells cut off shorter and shorter intercalary cells so that the earlier formed cells at the base of the meristematic cylinder average about  $18\ \mu\text{m}$  in length (Fig. 1B) compared to  $6.5\ \mu\text{m}$  for cells just below the apical cells (Fig. 1C) just prior to actual new blade initiation.

Actual blade initiation involves three processes: (1) branching of the apical cells of the meristematic cylinder to form the cortex of the upper surface of the blade, (2) elongation of the intercalary cells of the meristematic cylinder to form the medulla of the blade and stipe, and (3) branching of the intercalary cells of the meristematic cylinder to form (a) the cortex of the lower surface of the blade and stipe, (b) the secondary cortical filaments of the blade which cross from cortex to cortex, and (c) the secondary medullary filaments of the stipe. These processes are initiated in a prescribed sequence but, once initiated, occur simultaneously.

The first sign of actual blade initiation occurs when the meristematic cylinder is about 75–90 cells deep. At this time, the apical cells of the meristematic cylinder begin to branch (Fig. 1D). This occurs in each apical cell by the formation of an oblique rather than a transverse cell wall. The apical cell is cut off toward the outside of the cylinder, and the immediately

subapical cell begins to elongate at its distal end to fill the space left by the oblique division of the apical cell. Eventually, the distal protrusion of this subapical cell is cut off to form another apical cell, which begins to divide obliquely as the initial apical cell also continues to do. Other subapical cells (to 3–4 cells below the cell in which an oblique division first occurred) may also cut off new apical cells in a similar manner, the net effect being the creation of a layer of cortical tissue which is being pushed out from the center of the new blade by production of new apical (cortical) cells.

As the blade begins to grow in diameter, the intercalary cells of the meristematic cylinder elongate in a prescribed pattern to keep pace with the proliferation of apical cells, and stipe elongation occurs concomitantly (Fig. 2).

As the processes described above continue, the cortex of the lower surface of the new blade is initiated by cells near the outer edge of the meristematic cylinder producing lateral branches at right angles (Fig. 1E). These lateral branches continue to elongate by transverse division, but once they reach the outer surface of the new blade their apical cells begin to divide obliquely in the same manner as the apical cells of the upper surface of the blade (i. e., with the apical cell always cut off toward the margin of the new blade). The formation of these lateral branches keeps pace with production of new cortical cells on the upper surface of the new blade. In addition to forming the cortex of the lower surface of the new blade, lateral branches developing from the outer edge of the meristematic cylinder near its base form the cortex of the stipe, and lateral branches arising from cells within the meristematic cylinder become the filaments which cross from cortex to cortex. Cells of these filaments may form secondary pit connections with other cortical cells. In the center of the meristematic cylinder, these lateral branches tend to grow downward and form the secondary medullary filaments

of the stipe.

By the end of May, when blade and stipe growth appeared to have ceased, the medullary cells of the stipe averaged about 150–500  $\mu\text{m}$  in length (depending on their position and the length of the stipe), whereas most of the medullary cells in a typical blade (radius=70 mm) ranged from 1100–1500  $\mu\text{m}$  in length, with some cells

over 2 mm in length. From October to May cells near the base of the meristematic cylinder therefore elongated only about 10 times, from about 18  $\mu\text{m}$  to 160–230  $\mu\text{m}$ , but those originally near the top of the meristematic cylinder elongated about 200 times, from about 6.5  $\mu\text{m}$  to 1100–1500  $\mu\text{m}$ . Cell width increased by only 4–8 times to about 20–30  $\mu\text{m}$  during the same period.

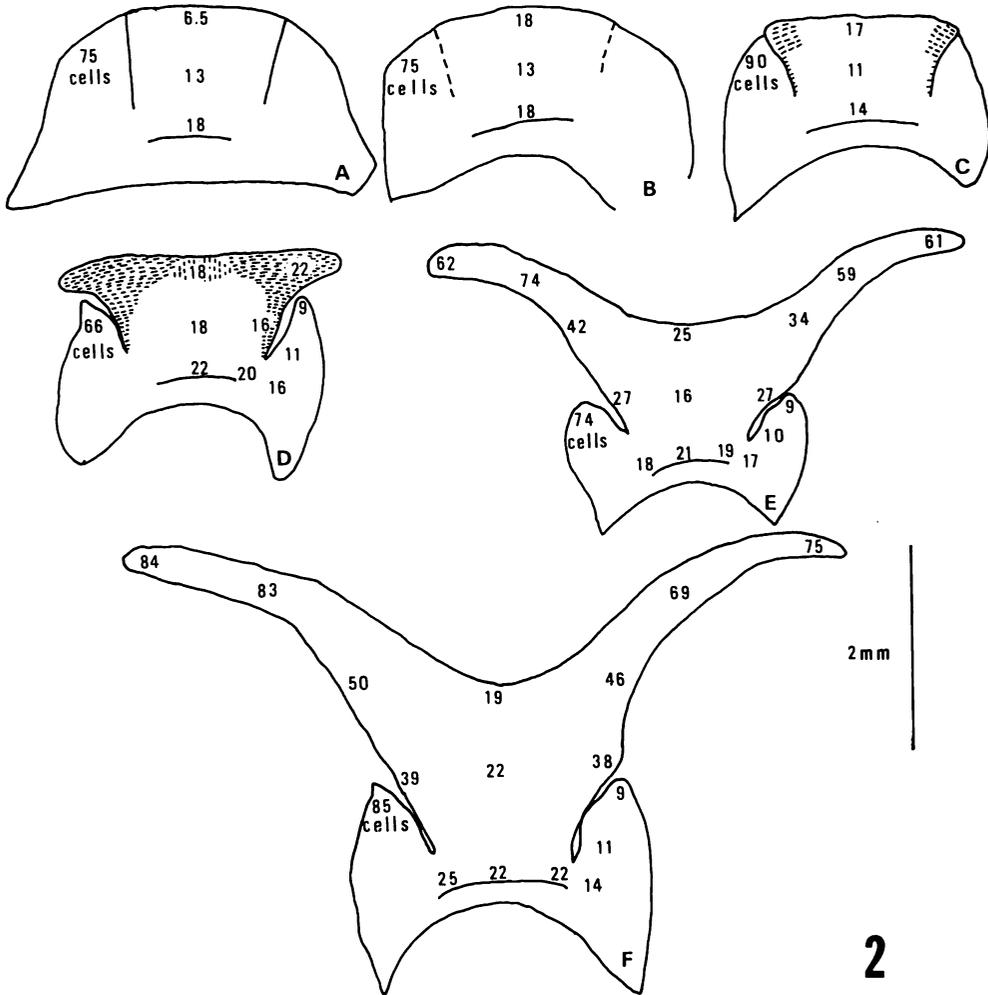


Fig. 2. A series of cross sections of the mound in the center of the *C. rosa-marina* blade showing new blade production and the concomitant elongation of cells in various parts of the meristematic cylinder. Broken lines in B–D indicate areas where lateral branches are beginning to form. Numbers indicate average lengths in  $\mu\text{m}$  of 10 cells in that part of the meristematic cylinder. The depth of the meristematic cylinder at the time apical cell branching was initiated is indicated on the left of each mound. Numbers in the lower right corner of D–F show cell lengths of peripheral cells of the meristematic cylinder which did not take part in new blade production.

The oblique cell divisions responsible for forming the upper surface of a *Constantinea* blade occur about 14–16 times as indicated by the number of cell rows exhibiting that kind of branching in the center of the new blade just above the stipe. Since the cortex of *Constantinea* is only 6–8 cells thick, the first 8–10 rows of cortical cells cut off by oblique divisions must have elongated together with the intercalary cells of the meristematic cylinder to help form the medullary filaments of the blade.

#### *Morphological observations of C. subulifera*

Compared to *C. rosa-marina* the new blades of *C. subulifera* fall apart much more readily when preserved and sectioned. It was therefore not possible to obtain a similar sequence of early blade development stages in *C. subulifera* as in *C. rosa-marina*. However, some comparisons can be made:

The new blade is initiated at the top of the stipe which protrudes from the previous year's blade. A meristematic zone develops at the top of the stipe similar to that in *C. rosa-marina* except that it occurs in a slight depression (probably formed by the cells comprising the meristematic zone elongating less than the surrounding stipe cells). Also, the meristematic zone was

only about 50 cells deep in the specimens examined. Development of the new blade appears to proceed as in *C. rosa-marina* except that fewer cells at the base of the meristematic zone contribute to growth in stipe length, most contributing directly to new blade production. Because of the delicate nature of mature *C. subulifera* blades, it was not possible to determine the final lengths of the medullary filaments derived from the cells of the meristematic zone as was done in *C. rosa-marina*.

#### *Field observations*

In mid October, I observed a wide variety of stages in new blade initiation in *C. rosa-marina*. Some specimens appeared to show no further development than plants collected in early September. However, some specimens showed evidence of very young blades with diameters up to 3.2 mm, hardly as wide as the stipe itself! By late November, all specimens had new blades. In contrast, new blades of *C. subulifera* first appeared in late September or early October. The following table shows average new blade diameter, old stipe length, and new stipe length for terminal blades of specimens of *C. rosa-marina* and *C. subulifera* collected in the fall and early winter.

	Sept. 5	Oct. 17	Nov. 29	Jan. 26
<i>C. rosa-marina</i>			n=72	n=56
new blade diameter <sup>1)</sup>	0	(3.2 max)	9.9±4.5	31.5±11.9
old stipe length <sup>2)</sup>	~1.5	~1.5	~1.5	~1.5
new stipe length	0	0	0.5	3.8±1.1
<i>C. subulifera</i>	n=20	n=13	n=10	n=35
new blade diameter	0	10.6±5.0	30.8±9.2	56.3±16.6
old stipe length	6.2±1.5	7.8±1.7	NM <sup>3)</sup>	6.8±2.1
new stipe length	0	0	NM	2.5±1.4

1) All measurements in mm.

2) Although the mound of tissue cannot technically be called a stipe, it contributes to overall stipe length since the new blade arises out of the upper surface of the mound.

3) NM=not measured.

*Photoperiod experiment*

Under laboratory conditions, one short-day plant terminated October 12 showed early signs of new blade initiation (i. e., branching of the apical cells and production of laterals along the upper outer edge of the meristematic cylinder), but the first macroscopic evidence of blade initiation in

short-day plants did not appear until late October or early November.

When it was evident that all short-day plants were producing new blades but no long-day plants were, the cultures were rearranged so that all long-day plants were moved to short-day conditions, and some of the short-day plants were moved to long-day conditions. Long-day plants moved to short-day conditions showed macroscopic

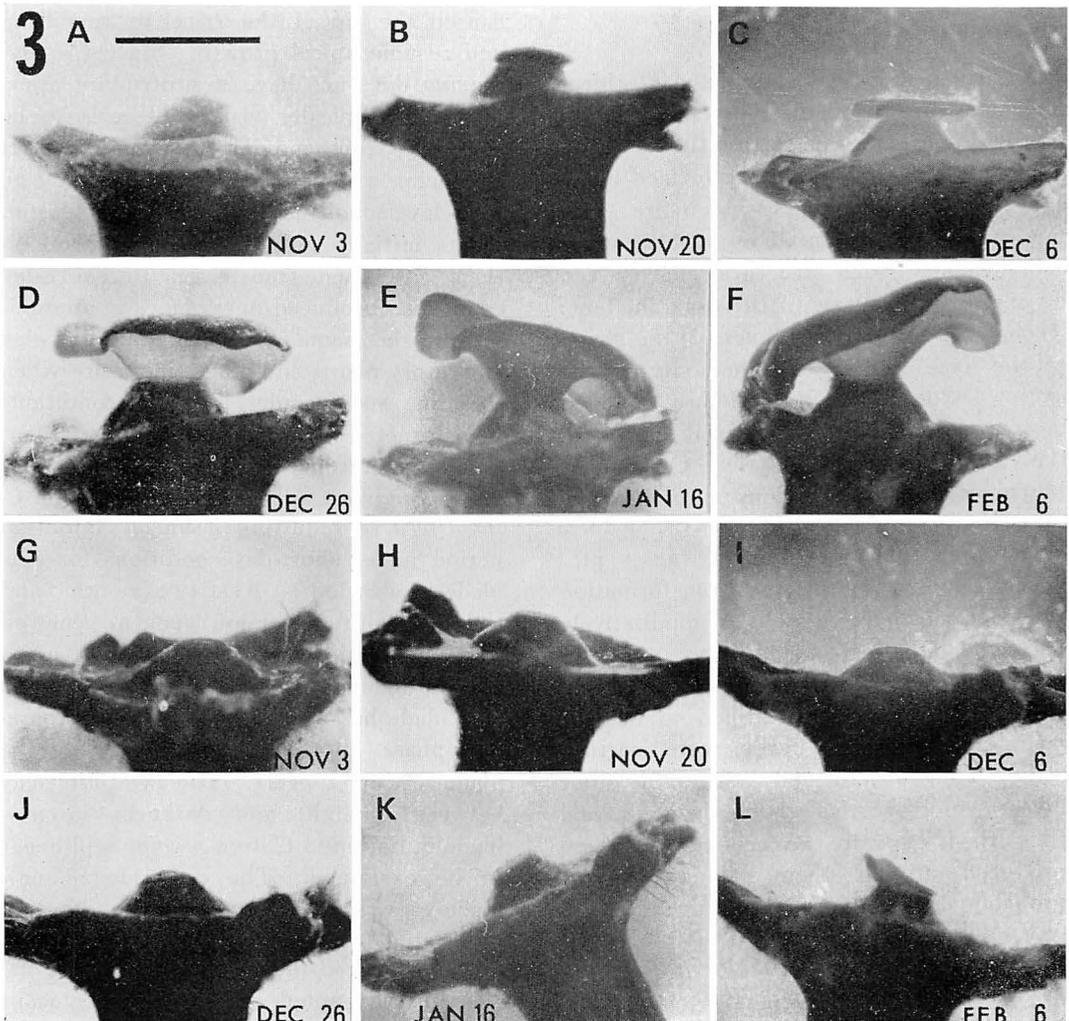


Fig. 3. A-F. Blade center cultured under short-day (8:16) conditions from Sept. 6-Dec. 6 and under long-day (16:8) conditions from Dec. 6-Feb. 6. G-L. Blade center cultured under long-day conditions from Sept. 6-Dec. 6 and under short-day conditions from Dec. 6-Feb. 6. Photographs taken after 8 weeks (Nov. 3), 11 weeks (Nov. 20), 13 weeks (Dec. 6), 16 weeks (Dec. 26), 19 weeks (Jan. 16), and 22 weeks (Feb. 6). Scale = 5 mm.

evidence of new blade initiation after another two months. This period of time corresponded to how long it had taken plants in nature and the plants originally cultured under short-day conditions to initiate new blades after the collection date (Fig. 3). Growth of blades initiated under short-day conditions slowed and eventually appeared to halt when moved to long-day conditions (Fig. 3).

### Discussion

As DIXON (1971) has stated, the Florideophyceae are particularly well suited to morphological analysis of growth and development because all thalli are formed by an aggregation of filaments, which are, with few exceptions, produced by strictly apical cell division. Such structural analysis can be expressed in terms of three parameters: (1) the disposition of the axes, (2) the shape of the axes, and (3) the longevity of the axes. DIXON (1971) himself has confined most of his analyses to uniaxial species in the Nemalionales and the Ceramiales. Morphological observations of multiaxial taxa (NORRIS and KIM 1972; CODOMIER 1972) have shown that medullary tissue is produced sequentially by the transformation of older cortical tissue. A qualitatively different situation appears to exist in *Constantinea rosa-marina* and *C. subulifera*. Here, cells which are destined to become medullary filaments never were cortical cells. They are formed before the outer investment of cortical tissue develops, and they then elongate as cortical cells are produced around them. This situation probably arose because of the peltate and perennial nature of the genus.

BOWEN (1971) states that *Maripelta rotata* (DAWSON) DAWSON, a peltate perennial red alga from southern California and Mexico, undergoes repeated cycles of blade abscission and new blade formation, which she states is unique in the Rhodophyta but nevertheless shows some similarity to vesicle formation in the closely-related genus *Botryocladia*. NEUSHUL *et al.* (1967) have

cultured *Sciadophycus stellatus* DAWSON, another peltate red alga from southern California and Mexico, but no work has been done on new blade initiation in this species.

POWELL (1964) found a critical daylength of about 11–14 hours for between 21 and 28 days was required to completely trigger new blade initiation in *C. subulifera*. Longer daylengths, interruption of the dark period, or a shorter initiation period all caused the tip of the stipe to round up and resume apical growth. Since *C. rosa-marina* does not have a protruding stipe, no macroscopically visible effect is to be expected from moving a plant not yet producing a new blade from short-day to long-day conditions. In my experiment, plants initially put under long-day conditions and then transferred to short-day conditions produced new blades at approximately the same time as did short-day laboratory plants and plants in nature when the time spent under long-day conditions is subtracted from the total time spent under laboratory conditions. Therefore, *C. rosa-marina* appears to differ from *C. subulifera* in requiring a longer initiation period under short-day conditions for new blade production (at least two months) and lacks a definite reversion (such as renewed stipe elongation in *C. subulifera*) when placed under long-day conditions after a new blade has been initiated (the "preparatory phase" in this paper) but before the blade actually appears. The exact interplay between daylight and darkness in new blade initiation in *C. rosa-marina* still needs to be examined. The cessation of new blade growth in *C. subulifera* in the spring was noted by NEUSHUL and POWELL (1964) in field-cultured plants.

As already noted, some differences occur in new blade initiation in *C. rosa-marina* and *C. subulifera*. POWELL (1964) briefly dealt with new blade initiation in *C. subulifera* at the microscopic level. He refers to a flattening of the center of the stipe with concomitant thickening of the "cortical zone" at the tip of the stipe to 50–60

cells. This description and his accompanying figures agree with observations I made on new blade initiation in Japanese *C. subulifera*.

New blade initiation in *C. simplex* also shows some differences from *C. rosa-marina*. According to ABBOTT (1968), *C. simplex* shows a slight depression in the mature blade which marks the meristematic region rather than its occurrence in a mound rising out of the mature blade as in *C. rosa-marina*. In *C. simplex*, ABBOTT distinguished two types of cells in the meristematic zone, central and peripheral, which are responsible for stipe elongation and new blade production, respectively. However, such a clear-cut distinction of cell types is not evident in *C. rosa-marina* or *C. subulifera* where central and peripheral cells both contribute to new stipe production (when it occurs in conjunction with new blade initiation in *C. subulifera*), and also both contribute to the medulla of the new blade.

Some of the morphological differences cited by SETCHELL (1906) for distinguishing the three species of *Constantinea* have been found to not hold up when specimens from various seasons and habitats are examined. Whereas SETCHELL stated that the blades of *C. subulifera* are perfoliate "from the very beginning," POWELL (1964) and the present author (unpublished observations) have found that the stipe does not begin to protrude through a new blade until March to May, leaving a period of five to seven months when the terminal blade is peltate, as in *C. rosa-marina* and *C. simplex*. The development of a small mound in the center of terminal *C. rosa-marina* blades during the summer, while perhaps not making the blade perfoliate, contrasts SETCHELL's statement that a new blade originates in a kind of depression in the center of the old blade. SETCHELL further stated that the internode between the old and the new blade in *C. rosa-marina* "elongates soon after the new blade is fairly well formed," but in the present study stipe elongation was found to occur

simultaneously with new blade production. Furthermore, in many low intertidal specimens of *C. rosa-marina* from Southeast Alaska, I have found that the internodes fail to elongate, an important characteristic cited by SETCHELL for distinguishing *C. simplex* from *C. rosa-marina*. However, my observations should be considered as a clarification of SETCHELL's because the same three species he dealt with then remain valid today, and the best vegetative characteristics for distinguishing them, while not as clear-cut as SETCHELL originally indicated, remain those he stated in 1906, namely, the protruding stipe of *C. subulifera* and the long versus the short internodes of *C. rosa-marina* and *C. simplex*, respectively.

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S・リンドストローム：多年生紅藻オキツバラ *Constantinea*  
*rosa-marina* の新葉形成

多年生紅藻オキツバラ (*Constantinea rosa-marina*) の新葉の形成を記述した。新葉は前年の葉の中央部に  
ある形成部位から秋に開始される。新葉の形成中、形成部位の細胞は予定の様式で伸長してもとの大きさの200  
倍の長さ達し、新葉の髄部となる。同時に既定の様式で細胞分裂が起って新葉の皮層部が形成される。

新葉は天然でも実験室でも短日条件下で形成される。実験室の長日条件下で新葉の形成は停止する。(060 札幌市北区北10条西8丁目 北海道大学理学部植物学教室, 現住所: Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109, U. S. A.)

## Observations on the girdle of the genus *Amphora* (Diatoms)

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GOTOH, T. 1980. Observations on the girdle of the genus *Amphora* (Diatoms). Jap. J. Phycol. 28: 151-155.

Although the girdle with numerous divisions of *Amphora* has been termed intercalary bands, it is a problem whether it has a set of numerous same single bands or a set of different elements. SEM investigation has been done in order to answer this question. As a result it became clear that the girdle is composed of numerous same single bands, therefore we should term it correctly connecting bands (pleurae). Further, concerning to the connection between a valve mantle and a connecting band, it became evident that the margin of the valve mantle overlaps the one side of the connecting band.

*Key Index Words:* *Amphora*, *Bacillariophyceae*, *connecting band*, *diatom*, *girdle structure*, *intercalary band*.

During the last several years, the taxonomical criteria of diatoms have become evident by using electron microscope. However, there are few reports on the structure of the girdle of the genus *Amphora*, which has been adopted as one of the taxonomical criteria observed by using light microscope. According to the past descriptions, the girdle of some of the genus *Amphora*, for instance *Amphora coffeaeformis* (AGARDH) KÜTZING which has numerous divisions, were termed only intercalary bands (cf. KARSTEN 1899, p. 104; HUSTEDT 1930, p. 345; PATRICK & REIMER 1975, p. 78 as *A. coffeiformis*). If we follow in MÜLLER's definition concerning to the girdle elements (MÜLLER 1886, 1895; cf. VON STOSCH 1975), an intercalary band is not exist without a connecting band by reason that the intercalary band is an element which is inserted between a valve and a connecting band. On the girdle with numerous divisions of the genus *Amphora*, it is a problem whether it has a set of numerous same single bands or a set of different elements. If the girdle is a set

of numerous same single bands, we should term it correctly connecting bands. The purpose of this study is to clarify this problem by the observation using scanning electron microscope.

### Materials and Methods

A species of the genus *Amphora* having numerous girdle elements was obtained from the film-like diatom assemblages which developed in one of the culture tanks of the rotifer *Brachionus plicatilis*. The diatom materials were collected from there and then cleaned by heating in concentrated sulphuric acid, and adequate amounts of KNO<sub>3</sub> were added, then they were washed by distilled water until the supernatant became neutral. Preparations mounted in Pleurax were examined by using a Nikon Apophot microscope. For the scanning electron microscope examination, the material was cleaned as above and dehydrated in ethanol or iso-amyl acetate, and a drop of the suspension was dried by natural drying method or by critical point

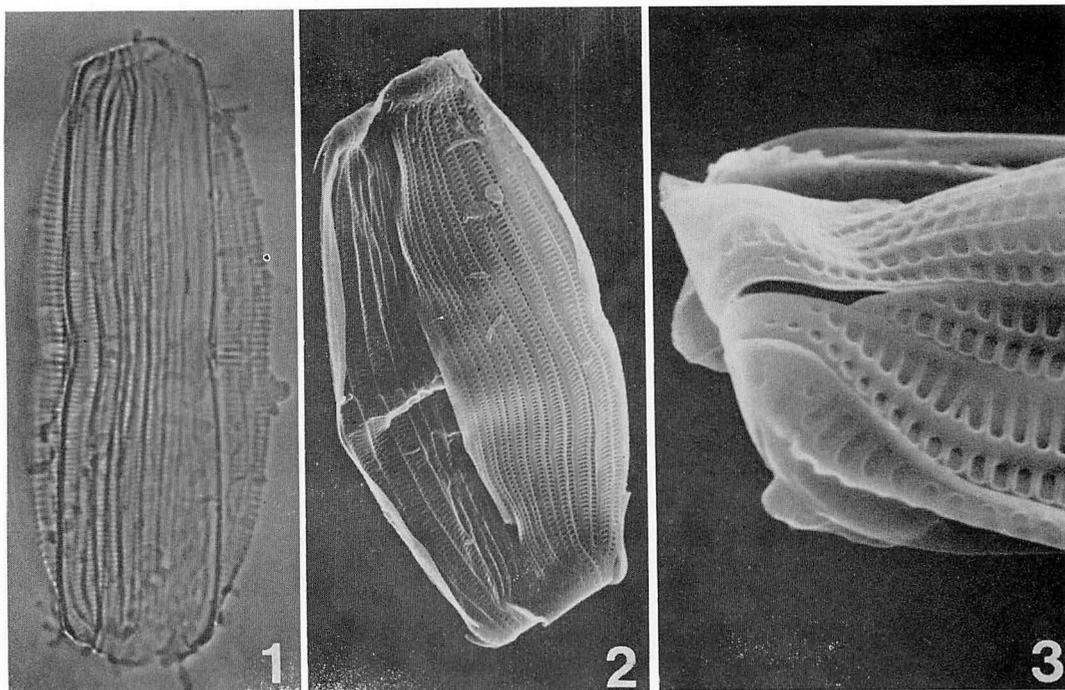
drying method on stubs, and coated with gold (roughly 200 Å in thickness) by using a GIKO IB 3 ion coater. These were examined by using a HITACHI S-450 unit in the Institute of Life Science, Kinki University, at an accelerating voltage of 20 kV, and photographed on Kodak Tri-X pan film.

### Observations

This taxon is nearly akin to *Amphora*

*castellata* GIFFEN and *A. turgida* var. *africana* CHOLNOKY. But it differs from those taxa in several characters. In this report, therefore, the author deals with it as *Amphora* sp. The valve structure and taxonomical considerations of this diatom will be reported in another place.

*Light microscope observations:* In this specimen, several linear series of short dashes are observed at the ventral and the dorsal part of the frustule (Fig. 1).

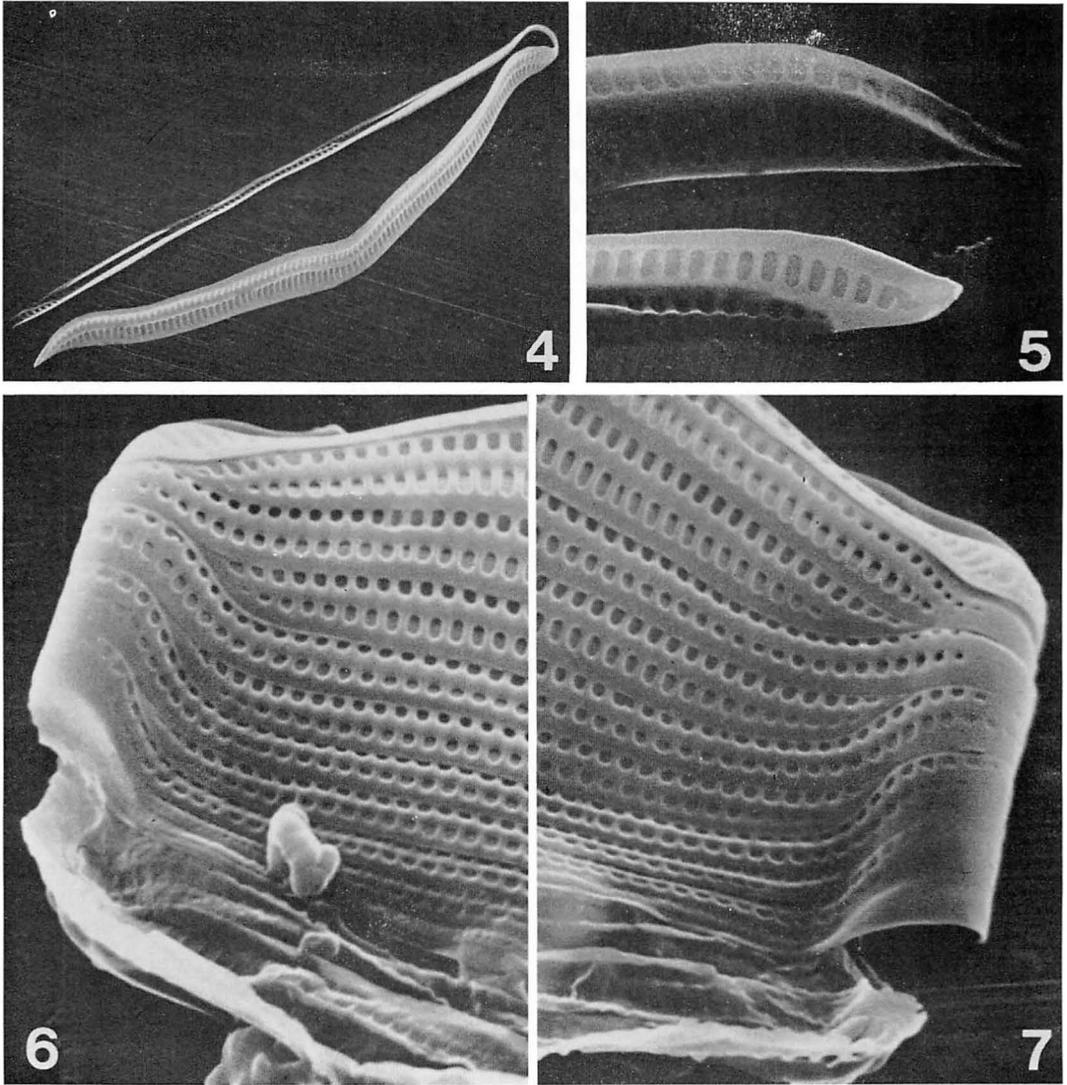


Figs. 1-3. *Amphora* sp.

1. The ventral part of the cell with several linear series of short dashes. LM.  $\times 2000$ . 2. The dorsal part of the cell. Right: epitheca; left: hypotheca, with newly formed and weakly silicified hypocingulum. SEM.  $\times 2300$ . 3. The incomplete dorsal apex of the epitheca showing a separate part and the connection between the valve mantle and the first connecting band. The valve mantle overlaps the first connecting band. SEM.  $\times 10000$ .

*Scanning electron microscope observations:* The dorsal part of the girdle is divided by several longitudinal lines (Fig. 2). By observation of a separate part (Fig. 3) and of a single bobby-pin like open band (Fig. 4), it is understood that the cingula are composed of several open bands (7-9 per one cingulum). Each open band has the point-

ed endings (Fig. 5) and two longitudinal rows of poroid areola, at the ventral and the dorsal part (Fig. 2). And the breadth of the open band in the dorsal part is somewhat broader than in the ventral part (Fig. 4). Externally, faintly raised axial ribs are laid at the portion of the both edges, respectively, and more silicified axial

Figs. 4-7. *Amphora* sp.

4. Single element of the cingulum, bobby-pin like open band. Upper: ventral side; lower: dorsal side. SEM.  $\times 3000$ . 5. Showing the pointed endings and the rices (or the vela) of a band. Upper: interior of the dorsal side; lower: exterior of the ventral side. SEM.  $\times 10000$ . 6 and 7. The two apices of the dorsal part of the cell. Showing the position of the opening of open bands changed alternately, and the connections among the valve mantle and the first connecting band and each connecting band. SEM.  $\times 9100$ .

ribs are raised in the center or in somewhat eccentric advalvar portion, and from which the transverse ribs are developed alternately to the advalver and abvalvar direction (Figs. 3, 4, 6, 7). Transverse ribs decrease their height gradually away from the axial rib (Figs. 3, 4). Internally, each

ribs is slightly raised (Fig. 5). The rounded oblong to square portion, surrounded by the ribs is a rica (or a velum: not be observed in detail) (Figs. 3, 5, 6, 7). The rica (or the velum) is about  $0.2\text{--}0.9\ \mu\text{m}$  long and  $0.2\ \mu\text{m}$  wide in the dorsal part. These poroid areolae agree with the ornamenta-

tion of the valve. Connections of each open bands are as follows; the margin of the valve mantle overlaps the one side of the first band and the other side of the first band overlaps the margin of the second band, and in the same way each open bands is connected in the abvalvar direction (Figs. 2, 3, 6, 7). The position of the opening of the open bands changes in the pervalvar direction, alternately (Figs. 6, 7).

### Discussion

The structure of the cingulum in this taxon seems to be a set of the single elements, although it is recognized that the breadth of the band and the size of the *rica* (or the *velum*) increase gradually from the opening of the cingulum to the advalvar direction, but is not divided clearly by their gradual changes. According to the terminology of the diatom girdle amended by VON STOSCH (1975), in this case, the first bands connected with the valve mantles are termed *valvocopulae*, and the other several bands are termed *connecting bands* (*pleurae*). However, in the terminology proposed by ANONYMOUS (1975) and ROSS *et al.* (1979), a term *valvocopula* is restricted to use for the special case. In this taxon, there is no reason to separate the first band by term from the others because of the each bands has no significant difference in structure. SCHOEMAN & ARCHIBALD (1978) showed the structure of the girdle of *Amphora veneta* var. *capitata* HAWORTH. This species has numerous bands, and is similar in the structure and the ornamentations to this taxon. KARSTEN (1899) gave many illustrations of *Amphora*, and showed that some of them had the girdle consisted of the numerous single elements. In these observations it is evident that the girdle of this taxon, probably of another species of *Amphora* having numerous girdle elements too, has been expressed as the *intercalary bands*, however we should term it correctly *connecting bands*.

Three types of connections between the valve mantle and the cingulum were shown

by ROUND (1972 a, 1972 b) from a point of view of the relationship between the morphology of cell and the reduction of cell size during cell division. He showed that in the centric diatom *Stephanodiscus* the cingulum overlaps the margin of the mantle (ROUND 1971). However, VON STOSCH (1975) indicated the three possible interpretation on ROUND's SEM micrographs, and questioned whether the cingulum overlaps the margin of the valve mantle or not in *Stephanodiscus*. In the materials of the genus *Amphora*, the structure of the connection between the valve mantle and the first connecting band is same as the connecting band's juncture, i.e. the margin of the valve mantle overlaps the one side of the first connecting band. This type is similar to one of the three illustrations figured by ROUND (1972 a, fig. 1 C) and strictly speaking it agrees with the description and the illustration given by VON STOSCH (1975, fig. 12 c), but the structure so-called 'slit' edge is not observed in this taxon.

The alternate change of position of the opening of the open bands is observed not only in this taxon but also in *connecting bands* of *Chaetoceros septentrionale* OESTRUP (DUKE, LEWIN & REIMANN 1973). The structural pattern of the girdle elements is common between the pennate diatom *Amphora* and the centric diatom *Chaetoceros*. This is very interesting from a taxonomical point of view.

As one of the taxonomical criteria, the measurement of the number of the *connecting bands* (until now, they were termed *intercalary bands*) under light microscope has been done by some authors. In this case, it is out of the question whether the measuring of the numbers of the bands for unit is appropriate to the taxonomical criterion or not. It is necessary to pay attention to the measurement because of its complexity. As the *epicingulum* and the *hypocingulum* overlap one another, it is a fact that the measurement is the sum of two numbers in the case which the overlapping can not be distinguished. Also

it must be designated clearly whether the numbers were measured by the numbers of the bands or by the numbers of rows of the areolae.

### Acknowledgements

I should like to express my gratitude to Prof. Dr. K. NEGORO, Department of Fisheries, Faculty of Agriculture, Kinki University, for his suggestions and continuing guidance, and to Mr. T. YOSHIDA for his supply of the diatom materials. I also wish to thank Mr. H. KOMIYA and Mr. Y. HORIUCHI, Institute of Life Science, Kinki Univ., for their advice and helps with SEM.

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### 後藤敏一：珪藻 *Amphora* 属の殻帯の観察

*Amphora* 属の中には殻帯 (girdle) が多くの縦線をもつものがある。従来の報告では上記のような殻帯は中間帯環 (intercalary band) と表記されているのみで接続帯環 (connecting band) の存在は明らかでない。上記のような構造をもつ *Amphora* sp. の殻帯の SEM による観察で次の3点が明らかになった。1) 殻帯は同一構造の開放帯環 (open band) の連なり [半殻帯 (cingulum) あたり7~9本] である。故に従来、中間帯環と表記されてきたのは誤りで、正しくは接続帯環と表記されるべきである。2) それぞれの開放帯環はその開口部を貫殻軸方向に交互に変換している。3) 殻套 (valve mantle) とそれに接続する接続帯環の結合部は前者が後者の端部を覆っている。接続帯環相互の結合も同様で殻 (valve) に近い方の端部はその前の接続帯環に覆われ、半殻帯の開口に近い端部は次の接続帯環の端部を覆っている。(術語訳は主に小林弘博士によるものである。)(577 大阪府東大阪市小若江3-4-1, 近畿大学教養部)

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**新 刊 紹 介**  
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BOLD, H. C., ALEXOPOULOS, C. J. and DELEVORYAS, T.: **Morphology of Plants and Fungi.**  
 4th edition. x+819 pp. Harper & Row, New York. 1980.

著名な藻類学者の著書で植物学の教科書として1957年に発行された“Morphology of Plants”は1967年、1973年に改訂されてそれぞれ第2版、第3版が発行された。本書はその第4版となっているけれども、標題は“Morphology of Plants and Fungi”と変更され、著者も菌類学者の ALEXOPOULOS と古植物学者の DELEVORYAS を加えて3人の共著となって、内容も第3版の668ページから約150ページも増加している。

BOLDに代表されるアメリカ学派の傾向は、第3版においても植物を門 Division のレベルで細分化する方向であり、これはドイツの ENGLER 系のもつと対照的であった。今回の第4版でもその傾向ははっきりしており最近の WHITTAKER らの5 kingdom system の方向を全面的にとり入れて、本書の標題を‘Plants and Fungi’としたのであろう。原核生物の取扱い、用語は MARGULIS らのもつと少し異なるところもあるが、本書では動物(原生動物を含む)を除く残りの生物群の大別として次の様な system を採用している。

Superkingdom I. Prokaryonta

Kingdom A. Monera (細菌 Bacteria と藍藻 Cyanochloronta の2門を含む)

Superkingdom II. Eukaryonta

Kingdom A. Phyta (Plantae: 緑藻 Chlorophycophyta など25の Division からなる)

Kingdom B. Myceteae (Fungi: 粘菌 Gymnomycota, 鞭毛菌 Mastigomycota, 無鞭毛菌 Amastigomycota の3 Division に分けられる)

各植物群の記述の形式は以前の版と大体同様で、印象的な図や写真が多数あり、これも新しい、より適切なものと取換られているものもある。共著者の専門分野を反映してか、コケ・シダの部分と、菌類の部分のページ数の増加が目立っている。その割には藻類の部分は改訂が少ないように思われる。

標題は「形態学」となっているとはいえ、分類学の教科書として、現在出版されているものの中でも、最もよいものの1つであることは疑いない。(邦価 約7,700円)

(北大理 吉田忠生)

## The asexual reproduction of Japanese *Pandorina morum* BORY (Chlorophyta, Volvocales)

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NOZAKI, H. 1980. The asexual reproduction of Japanese *Pandorina morum* BORY (Chlorophyta, Volvocales). Jap. J. Phycol. 28: 157-158.

The asexual reproduction in Japanese strains of *Pandorina morum* BORY was observed in detail under controlled laboratory conditions. As a result, my observation was different from the results reported by COLEMAN (1959) with regard to the parental gelatinous matrix.

*Key Index Words:* Asexual reproduction; Chlorophyta; gelatinous matrix; *Pandorina morum*; Volvocales.

The sexual process of Japanese strains of *Pandorina morum* BORY was described in detail in the previous paper (NOZAKI & KAZAKI 1979). Though we did not describe, we observed the asexual reproduction in these strains and discussed it to be similar to the results reported by COLEMAN (1959). Later, however, as I observed it more carefully, I have obtained the different results from hers with regard to the parental gelatinous matrix. In the present paper I described the asexual reproduction of Japanese strains of *P. morum* in detail.

The strains used in the present study as well as the methods of culture and observation are the same as in the previous study (NOZAKI & KAZAKI 1979).

In the asexual reproduction, each cell of the colony performs daughter colony formation equally. Previous to the cell division, the constitutive cells, the size of which has attained to about 15  $\mu\text{m}$  in surface diameter, become separated from one another to be spherical in shape in the swollen gelatinous matrix. Following the progress of the cell division, the gelatinous matrix becomes more swollen to reveal its internal structure (Fig. 1), which forms a keystone-shaped space for each parental cell; i.e.

16 spaces in case of a 16-celled parental colony. At last the gelatinous matrix attains to about 130  $\mu\text{m}$  in length as a whole in case of a 16-celled parental colony.

Each parental cell is embedded in this keystone-shaped space and conducts the colony formation. Namely, it performs usually 4 longitudinal divisions successively to form a 16-celled plakea, and a spherical colony, in which each cell has a cup-shaped chloroplast with a single basal pyrenoid, is formed as a result of inversion. After the

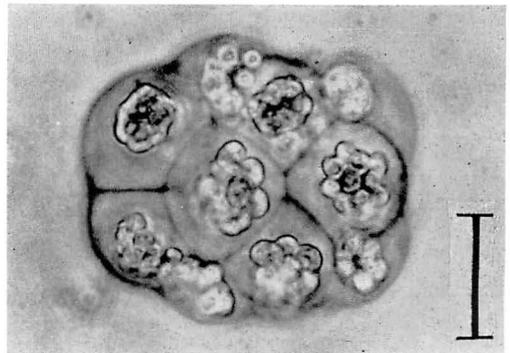


Fig. 1. Asexual reproduction of *Pandorina morum* showing plakeas, daughter colonies and internal structure of parental gelatinous matrix. Scale 30  $\mu\text{m}$ .

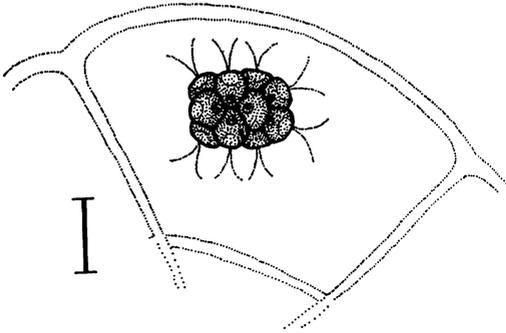


Fig. 2. A daughter colony of *Pandorina morum* before secretion of new gelatinous matrix in each keystone-shaped space of parental gelatinous matrix. Note a pyrenoid and two flagella of equal length in each individual cell, and a parental stigma in one cell of daughter colony. Scale 10  $\mu\text{m}$ .

inversion, each individual cell begins to project two flagella of equal length (Fig. 2). Up to this time, the stigma of the parental cell has remained to one of the cells of the plakea or daughter colony. When the projection of the flagella has nearly completed, a new gelatinous matrix is secreted and a new stigma appears from each individual cell. As a result, the daughter colony, which has the same form as the parental colony except in size, is formed in the keystone-shaped space of the parental gelatinous matrix and then swims away from the matrix. One side of a square plakea with 16 cells is about 20  $\mu\text{m}$  long. The 16-celled daughter colony, just after its formation, is measured 16–18  $\mu\text{m}$  in length.

Although COLEMAN (1959) did not report

the keystone-shaped space in which each daughter colony is formed, this space is clearly recognized in my observation in the same strains used by her (In 50-3, In 50-11, In-BI II-9 and Cal-68-8). This disagreement of the two observations may be caused by the difference of the methods of observation; in the present study, the materials were stained with methylene blue and observed with a phase contrast microscope. It is considered that this keystone-shaped space in the asexual reproduction is resulted from the direct swelling of the gelatinous matrix which surrounds the keystone-shaped cell tightly in the vegetative phase. Such a structure of the gelatinous matrix of *Pandorina morum* was recently reported by FULTON (1978) using a electron microscope. Furthermore, it is noteworthy that in 19th century, PRINGSHEIM (1870) had already reported such spaces, in which daughter colonies were formed, with his natural collection of *P. morum*.

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#### 野崎久義：本邦産 *Pandorina morum* BORY (緑藻・オオヒゲマワリ目) の無性生殖について

前報で筆者ら(野崎・加崎 1979)は本邦産の *Pandorina morum* BORY の有性生殖について報告したが、無性生殖は報告しなかった。今回、筆者は前報と同じ株の *P. morum* の無性生殖の過程を培養条件下で詳細に観察した。その過程は基本的には COLEMAN (1959) の結果と同一であったが、親のゼラチン様膜に関しては異なる結果を得た。(223 神奈川県横浜市港北区日吉四丁目一番二号 慶応義塾高等学校)

## Toxicities of trace metals on *Chlorella vulgaris* isolated from palm oil mill sludge

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SIVALINGAM, P. M. 1980. Toxicities of trace metals on *Chlorella vulgaris* isolated from palm oil mill sludge. Jap. J. Phycol. 28: 159-164.

Studies on the influences of ten trace metals, viz. Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn, at different concentrations, i. e., 0.5, 1, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200 and 300 ppms, on the proliferation of *Chlorella vulgaris* isolated from scum of palm oil mill sludge indicated that at most instances the productivity maxima occur after a culture period of 15 days as compared to the control which is 10 days. A definite pattern of inhibition by concentration was only observable for the trace metals of Cd, Cr and Cu while the other elements reflected independent effects.

It was found that the toxicities of the trace metals examined on the proliferation of *Chlorella vulgaris* fall into the following category: Cu>Cr>Cd=Zn>Co>Fe=Pb>Ni>Hg>Mn.

*Key Index Words:* *Chlorella*; *palm oil mill sludge*; *toxicity*; *trace metals*.

The production of palm oil is of prime national interest from the viewpoint of economics as a foreign exchange earner in the Malaysian context. Concomitantly, it is also the major pollutant of our aquatic environment culminating in at least 42 rivers been grossly polluted while 16 others fairly. Numerous attempts have been made to eliminate this organic pollution problem by local scientists (COLLIER and CHICK 1977, DALZELL 1977, MUTHURAJAH and DEVENDRAN 1975, RAJAGOPALAN and SIVALINGAM 1975, SEOW 1977, WEBB *et al.* 1975). However, these approaches are yet to meet the legislation promulgated by the Ministry of Environment, Science and Technology, Malaysia (MAHESWARAN 1978).

In this connection, SIVALINGAM *et al.* (1979) and SIVALINGAM (1980) have demonstrated that through the propagation of *Chlorella vulgaris* isolated from the scum of palm oil mill sludge the B. O. D. load could be lowered from 1,080 ppm to 40 ppm. It is envisaged that with the availability

of this new information and other presently investigated technology the overcoming of this agro-based industrial pollution problem is just round the corner.

Knowledge on this strain of *Chlorella vulgaris* which has the capability to sustain under such noxious conditions is still lacking. Hence, the author has attempted to verify toxicity effects of various trace metals on its proliferation at different concentration levels with time. The results of this investigation are presented here.

### Materials and Methods

Isolated *Chlorella vulgaris* (Fig. 1) cells from palm oil mill sludge (SIVALINGAM *et al.* 1979, SIVALINGAM 1980) were used in all the experiments that are to follow. Experiments on the effects of the various trace metals were performed in the most suitable dilution by 4 times of fermented palm oil mill sludge. The various trace metals used in the experiments were salts

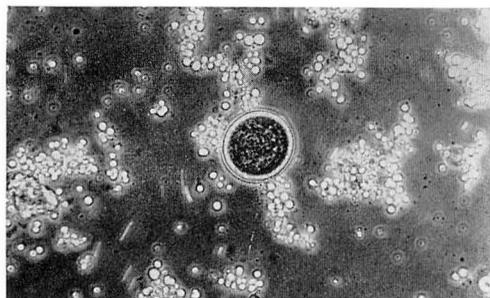


Fig. 1. Isolated *Chlorella vulgaris* cells from the scum of palm oil mill sludge (200  $\times$  magnification).

of the following:  $\text{CdCl}_2 \cdot \frac{1}{2} \text{H}_2\text{O}$ ,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuCl}$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{FeCl}_3$ ,  $\text{HgCl}_2$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \cdot \text{H}_2\text{O}$ ,  $\text{Pb}(\text{NO}_3)_2$  and  $\text{ZnCl}_2$ , at concentrations of 0.5, 1, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200 and 300 ppms, respectively. As blank the culture medium did not contain any of the trace metals. Each amendment of the trace metals concentration were performed in triplicates and their mean propagation of inoculated *Chlorella vulgaris* cells were followed periodically every five days for a period of 25 days. The number of cells for each inoculation was  $3.8 \times 10^3/\text{ml}$  of the culture medium. All the above-

Table 1. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of cadmium with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/ml with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.913	3.911
0.5	3.8	<u>5.897</u>	5.16	<u>10.647</u>	4.586	2.62
1.0	3.8	2.621	3.302	<u>9.828</u>	5.897	3.276
1.5	3.8	<u>5.897</u>	2.889	<u>9.009</u>	5.241	3.931
2.5	3.8	<u>3.931</u>	3.715	<u>4.504</u>	3.276	2.62
5	3.8	3.275	4.128	<u>5.733</u>	3.276	2.62
10	3.8	2.621	4.128	<u>6.961</u>	4.586	1.966
15	3.8	<u>3.931</u>	3.096	<u>8.190</u>	3.276	3.276
25	3.8	3.275	3.715	<u>7.371</u>	3.276	2.62
50	3.8	<u>6.552</u>	3.302	<u>6.142</u>	3.276	3.931
100	3.8	1.310	4.128	<u>6.142</u>	3.276	1.31
200	3.8	3.275	3.096	<u>4.504</u>	3.276	2.62
300	3.8	2.621	4.128	<u>6.142</u>	3.276	1.966

— : productivity maximum

mentioned culture experiments were performed in a "Nikko Tron" at 17,000 lux actinic light intensity with a day-light period of 12 hours and chamber temperature of 28°C.

## Results

Tables 1–10 demonstrate the various toxicity effects of the trace metals examined. It is evident that almost all the concentrations of the trace metals have a maximum productivity period on the 15th day of culture as compared to the reference experiment of 0 trace metal treatment which is only 10 days. These productivity peaks are greater than the control in the following order: Cr, 1.26, 1.167 and 1.07 times for the 0.5, 1.0 and 1.5 ppm concentrations, Co; 1.118, 1.118, 1.216, 1.07 and 1.677 times for the 1.5, 5, 10, 25 and 200 ppm concentrations, Cr; 1.26 and 1.67 times for the 0.5 and 1.0 ppm concentrations, Fe; 1.216, 1.216, 1.507, 1.751, 1.217, 1.216 and 1.119 for the 0.5, 2.5, 10, 15, 50, 100 and 200 ppm concentrations, Hg; 1.459, 1.41, 1.459, 1.459, 1.07, 1.119, 1.119, 1.119 and

Table 2. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of cobalt with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/ml with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	1.310	4.953	<u>8.190</u>	4.586	2.62
1.0	3.8	0.655	4.54	<u>7.371</u>	5.897	1.966
1.5	3.8	0.655	5.366	<u>9.418</u>	1.310	2.62
2.5	3.8	0.655	4.334	<u>6.125</u>	3.276	1.31
5	3.8	0.012	5.16	<u>9.418</u>	4.581	1.966
10	3.8	0.655	3.096	<u>10.237</u>	5.897	1.966
15	3.8	1.310	5.779	<u>7.371</u>	3.936	2.62
25	3.8	0.015	5.16	<u>9.009</u>	<u>6.552</u>	3.276
50	3.8	0.013	4.128	<u>7.780</u>	6.552	2.62
100	3.8	0.011	3.302	<u>6.142</u>	6.552	2.62
200	3.8	0.045	5.779	<u>9.828</u>	3.276	1.966
300	3.8	0.032	3.096	<u>2.866</u>	2.621	1.31

— : productivity maximum

Table 3. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of chromium with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	5.241	<u>5.159</u>	<u>10.237</u>	9.172	3.276
1.0	3.8	3.931	6.191	<u>9.828</u>	7.862	3.931
1.5	3.8	3.275	5.572	<u>7.780</u>	7.207	3.931
2.5	3.8	3.275	5.159	<u>7.780</u>	6.552	3.931
5	3.8	3.275	2.064	4.095	<u>6.552</u>	2.62
10	3.8	2.621	1.445	2.457	<u>2.621</u>	1.31
15	3.8	2.621	1.0319	<u>2.860</u>	2.621	1.31
25	3.8	1.966	1.445	1.638	<u>3.276</u>	1.31
50	3.8	1.966	1.0319	2.457	<u>2.621</u>	1.31
100	3.8	2.097	1.238	1.228	<u>2.621</u>	0.655
200	3.8	2.293	1.857	2.047	<u>2.621</u>	1.21
300	3.8	2.293	1.657	1.638	<u>1.966</u>	1.31

— : productivity maximum

Table 4. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of copper with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	3.931	5.572	<u>15.560</u>	6.552	3.931
1.0	3.8	1.966	4.747	<u>6.901</u>	5.241	3.931
1.5	3.8	0.378	4.128	<u>7.371</u>	5.897	3.276
2.5	3.8	0.021	2.064	4.095	<u>5.241</u>	1.21
5	3.8	0.022	2.064	<u>4.095</u>	3.936	1.966
10	3.8	0.025	1.238	<u>3.685</u>	3.936	1.966
15	3.8	0.023	1.0319	<u>4.095</u>	3.276	1.966
25	3.8	0.021	1.0319	<u>5.323</u>	1.966	2.62
50	3.8	0.027	1.0319	<u>4.914</u>	1.966	2.62
100	3.8	0.024	1.0319	<u>5.323</u>	1.966	0.655
200	3.8	0.020	1.238	<u>3.685</u>	3.276	0.655
300	3.8	0.019	1.651	2.647	<u>5.897</u>	0.542

— : productivity maximum

1.119 times for 0.5, 1.0, 1.5, 2.5, 5.0, 10, 50, 100 and 300 ppm concentrations, Mn ; 1.702, 1.605, 1.605, 1.799, 1.508, 1.264, 1.653, 1.167, 1.167, 1.216, 1.362 and 1.459 times for the

Table 5. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of iron with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation time (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	2.621	6.191	<u>10.237</u>	3.276	3.931
1.0	3.8	2.621	5.159	<u>7.780</u>	3.276	3.931
1.5	3.8	<u>0.655</u>	6.191	<u>8.190</u>	5.241	2.62
2.5	3.8	<u>0.655</u>	5.366	<u>10.237</u>	3.931	2.62
5	3.8	1.310	4.411	<u>8.190</u>	5.897	3.276
10	3.8	0.561	6.312	<u>12.694</u>	4.586	3.276
15	3.8	3.275	7.842	<u>14.741</u>	7.862	5.897
25	3.8	2.621	7.223	<u>7.371</u>	5.897	5.241
50	3.8	3.931	7.223	<u>10.237</u>	7.862	5.897
100	3.8	3.276	6.604	<u>10.237</u>	9.828	6.552
200	3.8	2.621	5.572	<u>9.418</u>	6.552	6.552
300	3.8	3.256	6.191	<u>8.190</u>	5.897	5.897

— : productivity maximum

Table 6. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of mercury with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	2.621	5.159	<u>12.286</u>	7.207	3.276
1.0	3.8	3.931	4.128	<u>11.875</u>	4.586	3.931
1.5	3.8	<u>5.241</u>	3.508	<u>12.286</u>	6.552	3.931
2.5	3.8	3.931	4.953	<u>12.286</u>	5.897	3.931
5	3.8	0.655	2.063	<u>9.009</u>	4.581	2.62
10	3.8	0.655	3.090	<u>9.418</u>	3.276	3.276
15	3.8	0.321	2.063	<u>6.142</u>	2.621	1.966
25	3.8	0.378	2.063	<u>8.190</u>	1.966	1.21
50	3.8	0.378	1.0318	<u>9.418</u>	3.276	2.62
100	3.8	0.983	1.0318	<u>9.418</u>	2.621	1.966
200	3.8	0.421	0.826	<u>8.190</u>	3.936	1.966
300	3.8	2.621	2.063	<u>9.148</u>	3.276	1.21

— : productivity maximum

0.5, 1.0, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200 and 300 ppm concentrations, Ni ; 1.07, 1.264, 1.654, 1.09, 1.216, 1.313, 1.313 and 1.012 for the 0.5, 1.0, 2.5, 5, 10, 15, 25 and 100

Table 7. Proliferation of *Cholrella vulgaris* on exposure to different concentrations of manganese with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	5.241	<u>7.223</u>	<u>14.33</u>	7.207	3.931
1.0	3.8	3.931	6.398	<u>13.512</u>	5.241	3.276
1.5	3.8	2.621	6.191	<u>13.512</u>	5.897	3.276
2.5	3.8	2.621	6.811	<u>15.151</u>	5.897	3.276
5	3.8	3.276	<u>7.223</u>	<u>12.694</u>	5.897	3.276
10	3.8	2.621	<u>7.223</u>	<u>10.647</u>	6.552	4.586
15	3.8	2.621	<u>7.223</u>	<u>13.922</u>	6.552	6.531
25	3.8	1.966	6.191	<u>9.828</u>	6.552	<u>10.483</u>
50	3.8	2.621	6.191	<u>9.828</u>	5.241	1.966
100	3.8	2.621	<u>7.223</u>	<u>10.237</u>	9.172	6.522
200	3.8	1.310	6.604	<u>11.467</u>	7.862	1.966
300	3.8	0.655	6.604	<u>12.285</u>	7.207	2.62

—: productivity maximum

Table 8. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of nickel with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	6.552	5.159	<u>9.009</u>	3.936	3.276
1.0	3.8	<u>6.552</u>	4.540	<u>10.647</u>	3.276	2.62
1.5	3.8	<u>6.552</u>	4.540	<u>8.190</u>	4.586	2.62
2.5	3.8	<u>4.586</u>	4.953	<u>13.922</u>	4.586	3.931
5	3.8	<u>6.552</u>	5.779	<u>9.009</u>	3.276	3.276
10	3.8	3.275	4.747	<u>10.237</u>	3.936	2.62
15	3.8	3.275	4.747	<u>11.056</u>	4.581	3.276
25	3.8	3.275	4.128	<u>11.056</u>	3.276	2.62
50	3.8	1.310	2.683	<u>4.504</u>	3.276	2.62
100	3.8	<u>3.275</u>	2.477	3.685	<u>8.517</u>	1.21
200	3.8	1.310	2.064	<u>3.276</u>	1.966	1.21
300	3.8	<u>2.621</u>	1.857	<u>2.866</u>	2.621	1.966

—: productivity maximum

(20-day culture period) ppm concentrations, Pb; 1.679, 1.678, 1.679, 1.07, 1.07, 1.679, 1.945 and 2.189 times for the 0.5, 1, 1.5, 2.5, 15, 100, 200 and 300 ppm concentra-

Table 9. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of lead with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	3.275	6.191	<u>14.133</u>	3.276	3.261
1.0	3.8	3.275	5.159	<u>14.131</u>	3.276	3.258
1.5	3.8	3.275	5.159	<u>14.132</u>	5.241	4.013
2.5	3.8	3.275	4.128	<u>9.009</u>	3.931	2.911
5	3.8	2.621	4.128	<u>6.961</u>	5.897	3.623
10	3.8	1.966	3.715	<u>7.371</u>	4.586	2.431
15	3.8	1.310	5.159	<u>9.009</u>	7.862	5.821
25	3.8	1.310	4.128	<u>7.371</u>	5.897	3.897
50	3.8	1.966	7.842	<u>9.148</u>	7.862	3.213
100	3.8	0.655	5.159	<u>14.133</u>	9.828	6.714
200	3.8	0.655	5.366	<u>16.379</u>	6.552	2.381
300	3.8	2.621	4.334	<u>18.427</u>	5.897	3.438

—: productivity maximum

Table 10. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of zinc with time

Conc. (ppm)	Cell No. ( $\times 10^3$ )/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	<u>8.42</u>	7.37	3.931	3.911
0.5	3.8	3.276	6.604	<u>10.237</u>	5.897	5.241
1.0	3.8	3.276	5.779	6.961	<u>8.517</u>	2.62
1.5	3.8	3.931	6.191	<u>12.285</u>	6.552	3.276
2.5	3.8	2.621	3.715	<u>8.190</u>	7.207	1.966
5	3.8	1.966	2.064	<u>12.694</u>	3.276	1.31
10	3.8	1.310	2.064	2.047	<u>5.241</u>	1.21
15	3.8	1.310	2.477	<u>4.095</u>	3.276	0.655
25	3.8	1.310	1.857	<u>18.427</u>	3.276	0.655
50	3.8	0.655	1.445	<u>4.504</u>	3.276	1.21
100	3.8	0.655	2.064	<u>5.733</u>	3.936	0.655
200	3.8	3.276	2.064	<u>4.095</u>	3.276	0.655
300	3.8	2.621	2.064	2.866	<u>3.276</u>	0.665

—: productivity maximum

tions and Zn; 1.216, 1.012, 1.459, 1.508 and 2.188 times for the 0.5, 1 (20-day culture period), 1.5, 5 and 25 ppm concentrations.

Hence it is evident that at certain con-

centrations of the different trace metals they act as a stimulant instead of a toxicant as compared to the contents though the maximum productivity is delayed by 5 days.

### Discussion

This study obviously indicates that the toxicities of the various trace metals are such that the peak of maximum productivity is shifted by 5 days as a delayed phenomenon. Also, it is noticeable that most of the trace metals indicate some sort of an inhibitory action at the early stages of culturing since normally there is a drop in cell numbers as compared to the initial inoculation.

The increase in cell numbers at the 15-day cultures of the trace metal experiments as compared to the maximum of the controls at 10-day cultures is of great interest. This could be attribute to the fact that the metals could be acting as a "kink" in its life cycle. The thought of probably excreted products by *Chlorella vulgaris* capable in complexing with metals and rendering the metals less toxic as suggested by OVERNELL (1976) should also be considered. Further, this algal species could also be less sensitive to toxic metals as compared to *Chlorella pyrenoidosa* as reported by WONG *et al.* (1979), SCHROLL (1978) and for other algae by SPARLING (1968).

Finally, the author is of the opinion that this strain of *Chlorella vulgaris* isolated from the scums of palm oil mill sludge appears to be fairly tolerant to high concentrations of trace metals contaminations and that studies should be performed to verify whether the organism bioaccumulates them or there in reality exists a mechanism of complexing by excreted extracellular substances to curb the toxicity effects of the trace metals.

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P. M. シバリンガム：ヤシ油工場汚泥から分離された *Chlorella vulgaris* に及ぼす微量金属の影響

ヤシ油工場汚泥表面から分離された *Chlorella vulgaris* の生長に及ぼす10種類の微量金属の影響を研究した。使用した金属はカドミウム、コバルト、クロム、銅、鉄、水銀、マンガン、ニッケル、鉛、亜鉛で、それぞれ 0.5, 1, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200, 300 ppm の濃度であった。

*Chlorella vulgaris* の生長に及ぼす微量金属の毒性は次のような順序となる：Cu>Cr>Cd=Zn>Co>Fe=Pb>Ni>Hg>Mn. (School of Biological Sciences, Universiti Sains Malaysia, Minden, Pulau Pinang, Malaysia)

## The occurrence of *Zonaria stipitata* on the southern coasts of Taiwan

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*Zonaria stipitata* was erected by TANAKA and NOZAWA in 1962 based on the specimens collected at Tanegashima, a small island situated at the southwestern part of Japan. This brown alga is a tropical, subtropical to warm temperate, deep water species found in the southwestern islands of Japan, Ryukyu Islands (TANAKA and NOZAWA 1962), South Vietnam (TANAKA and NOZAWA 1962, HO 1969) and Guam (TSUDA 1972).

During three independent diving surveys on the southern part of Taiwan by Dr. YING-MING CHEN of the Department of Geology, National Taiwan University, at Siaoliuchiu Island on September 9, 1972 and Mr. C. S. CHEN and Miss J. E. LEWIS of the Institute of Zoology, Academia Sinica at Wanlitung, Pingtung Hsien on February 19, 1977 and Lanyeu Island on December 12, 1979 respectively, many specimens of this brown alga were collected at depths of 5 to 20 meters and sent to the writers. Subsequent examinations of the thalli showed that they possess some characters which had not been mentioned in the original descriptions or elsewhere of this alga. The plants at hand reach 5 cm high, growing from a perennial felted rhizoidal base which is up to 1 cm across and 0.5 cm in thickness. From the base arise several cylindrical, slender stalks up to 1.5 cm high. Stalks are usually simple but may occasionally give rise to form one to a few laterals. They are smooth at first, later usually becoming covered with many brown hairs. The stalk is composed of comparatively thick-walled cells which are subspherical to polygonal in the cortical

region and cylindrical in the median portion.

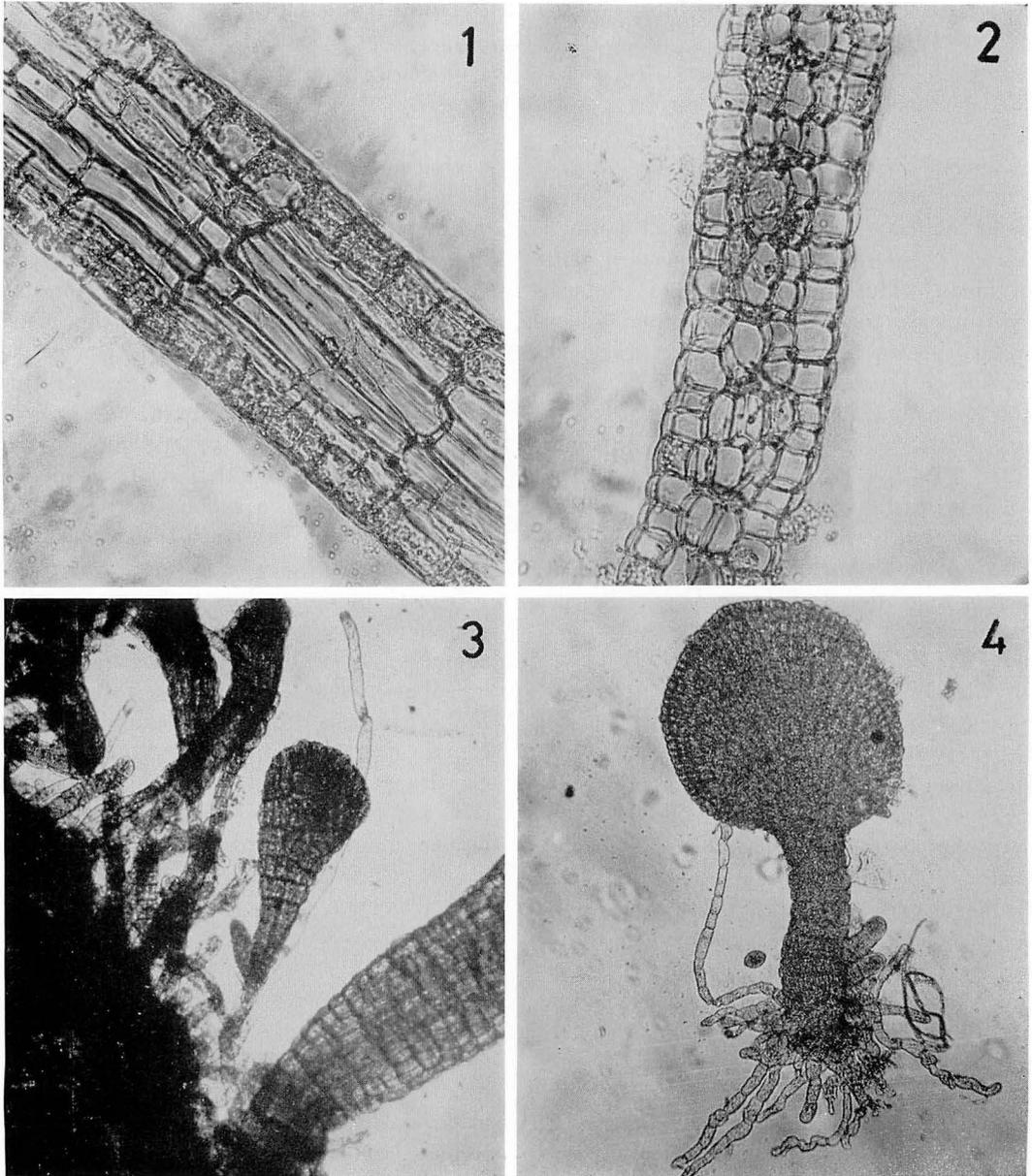
From the top of each stalk arises a membranous blade. The blade is fan-shaped at first, later becoming lobed or lacinate by splitting. Both surfaces of the blade are usually smooth; neither concentric bands of hairs nor veins are present. But in some mature blades, there are rows of brown hairs on the abaxial side of the blade extending from the basal portion to the upper portion in radial directions, and these look like veins on blade when they grow thicker.

Growth of the thallus is by means of a peripheral series of initials. The blade is composed of a layer of epiderms and a medulla of two or more layers of cells. Epidermal cells are cubic to cylindrical and contain many subspherical chromatophores. On the margin of the blade the medullary cells are large and elongated (Fig. 1), but become shorter toward the central portion of the blade (Fig. 2). Therefore, at the central portion of the blade the medullary cells are cuboidal in shape. In a transverse section of a frond it will often be seen that in width, several epidermal cells correspond to a single medullary cell on the margin of the blade (Fig. 1). But progressing toward the median portion of the blade, only one or two epidermal cells correspond to each medullary cell in width (Fig. 2). The margins of young blades are smooth, but as they grow older, some of the marginal initial cells divide periclinally to form filaments (Fig. 3). These filaments are simple, uniseriate, and with chromatophores, but in rare cases they may branch

sparingly.

Of particular significance was the formation of gemmae (Fig. 3) along the margin of the thallus. The gemmae originate either from the apical or cells in the me-

dian portion of the filaments which are produced from the edge of the blade, especially from those on the older or damaged portions. The first division of the gemmae initial is transverse. Gemmae are fan-



Figs. 1-4. *Zonaria stipitata* TANAKA et NOZAWA.

1. A transverse section through the marginal part of the thallus  $\times 300$ .
2. A transverse section through the median part of the thallus  $\times 200$ .
3. A portion of the thallus, showing filaments and various stages of gemmae growing out from the margin of the thallus  $\times 60$ .
4. A mature gemmae separated from a thallus  $\times 30$ .

shaped with long, uniseriate or multiseriate stalks (Fig. 3). It seems that further development of blade, stalk and basal portion may cause detachment of the mature gemmae from the mother plant (Fig. 4). KUMAGAE (1977) reported that the same kind of gemmae formation was observed in two other species of Dictyotaceae, *Zonaria diesingiana* J. AGARDH and *Pachydictyon coriaceum* (HOLMES) OKAMURA and under cultural experiment the gemmae grew into adult thalli.

No reproductive organs were observed in any of our specimens. Therefore, from the specimens at hand and the reports of TANAKA and NOZAWA (1962), HO (1969) and TSUDA (1972), it seems that the formation of reproductive organs rarely, if ever, occurs. Gemmae formation appears to be common and may be the only method of propagation in nature.

### Acknowledgement

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### 江永棉・周宏農：台湾産のエツキシマオオギについて

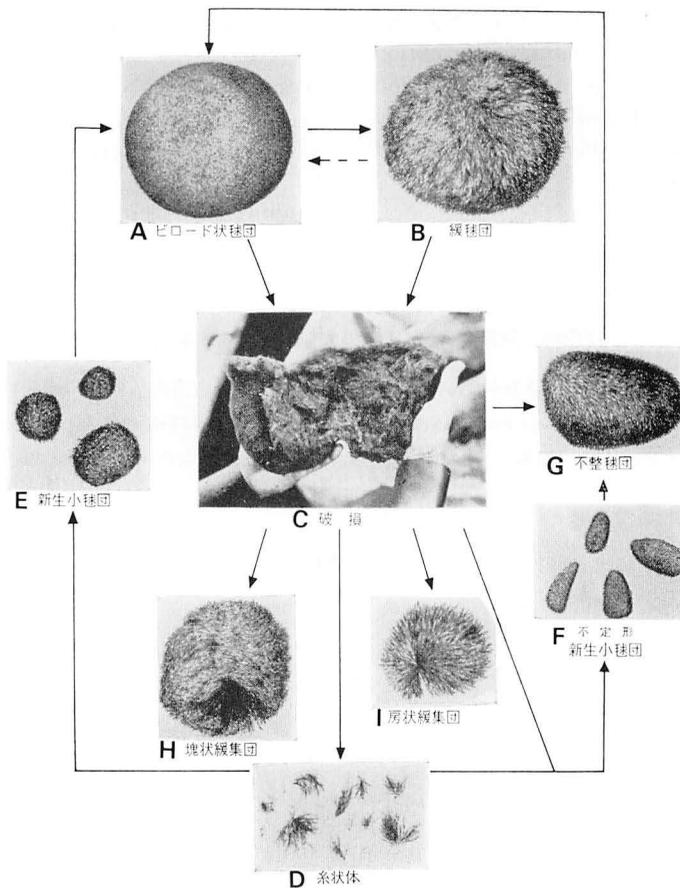
エツキシマオオギ *Zonaria stipitata* TANAKA et NOZAWA が台湾南部の地点から採集された。この標本には生殖細胞の形成はみられなかった。葉体の縁辺から生ずる一列細胞糸に無性芽が生じているのが観察された。天然ではこの無性芽によって繁殖していると考えられる。(中華民國台北市 國立台湾大学海洋研究所)

黒木宗尚: 阿寒湖のマリモの英文紹介 Munenao KUROGI: Lake Ball "Marimo" in Lake Akan

In Lake Akan in eastern Hokkaido grow a great number of beautiful lake balls called "Marimo". The balls are 3-20 cm or more in diameter and have a velvety green surface (Fig. A). They stand unrivaled in the world in figure and size, and are protected as a Special Natural Monument of Japan. The balls are free floating

above the sandy or clayey bottom 1-3 m deep in the northern part of the lake. Visitors can see the balls exhibited at the aquarium on Islet Churui situated near the northern shore of the lake.

The balls are aggregates composed of a number of branched uniseriate filaments of cylindrical cells of the green alga,



Formation of lake ball, Marimo, in Lake Akan.

A. typical beautiful lake ball showing a velvety surface, B. lake ball showing a bushy surface at the calm bottom, C. broken old large ball, D. separate short filaments, E. small aggregates newly formed of irregularly entangled short filaments, F. small fragments of broken old ball entangled with short filaments, G. irregularly shaped aggregate with a velvety surface, H. irregularly shaped loose aggregate, I. separate tufted filament.

*Cladophora sauteri* (NEES) KÜTZING. The branched filaments radiate from the center of the ball and the branches and rhizoids stick to each other to form a stable ball. Large balls have a central cavity caused by the death of filaments in the center. In some balls one or two not-so-clear concentric rings, probably indicating the interruption of growth, are visible in cross section.

The formation of the lake balls at Lake Akan occurs in two ways. Separate short filaments (Fig. D) are irregularly entangled with each other by the wind generated wave action near the shore to form small aggregates (Fig. E). Over many years, the small aggregates grow into stable balls with a regularly radial construction. The balls also form from broken fragments of old large balls with large central cavities (Fig. C). The small fragments, which are entangled with short filaments (Fig. F), and the large fragments are at first irregularly shaped, and then develop into compressed

ovate or elliptical aggregates with a velvety surface (Fig. G). They finally become spherical after being rolled along the sandy bottom for a long time.

On the muddy bottom of the lake 3–10 m deep unattached separate short filaments (Fig. D), tufted filaments (Fig. I) and loose irregular aggregates (Fig. H) accumulate abundantly as an extensive stratum 10–30 cm thick. Filaments attached to rock or other substrates are not found in this lake.

Reproduction of this alga in the lake seems to be carried out vegetatively by fragmentation. The occurrence of reproductive cells, zoospores and gametes, is unknown in the field materials.

This is a summary of a part of the paper by KUROGI, M., YAMADA, I. and YOSHIDA, T. (1976), Distribution, shape and actual amount of the lake ball, Marimo, in Lake Akan (in Japanese). (Department of Botany, Faculty of Science, Hokkaido University, Sapporo 060)

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**新 刊 紹 介**  
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**藻類名詞及名称** 159 pp. 科学出版社, 北京, 1979, 0.80 元.

中国においても藻類学の研究が最近更に活潑化して来ており, 1979 年には中国藻類学会も創立された(藻類 28: 27 参照)。この様なときに英語の術語と学名を中国語と対照した小冊子が発行されたのは時宜を得ていることだろう。この学術用語集というべきものは 1964 年に朱浩然が編集した同じ名前の本を改訂したもので, 多数の研究者の協力の下に 2800 の学術用語と, 4300 の学名が集録され, 中国語と対比されている。

術語に関しては日本語と共通のものも多く, 字体の違いを考慮すれば比較的容易に理解できる。ラテン語の学名に対する中国名に関しては, 古来からあるものの他, 最近つけられたものも相当ある様で, 1962 年に発行された曾呈奎らの編集した「中国經濟海藻志」(科学出版社, 北京) と比較するとよくわかる。藻類の名前に関してそのいくつかを以下に挙げてみる。中国においては簡字体の採用が進行しているので, 日本の活字で表記できないものもかなりある。よく似ていても字体の違うものがあり, それらは無視して日本の字で示す。

<i>Acanthopeltis japonica</i>	日本刺盾藻	ユイキリ
<i>Acetabularia calyculus</i>	傘藻	ホソエガサ
<i>Achnanthes brevipes</i>	短柄曲壳(殼)藻	
<i>Agarum cribrosum</i>	孔叶(葉)藻	アナメ
<i>Anabaena cylindrica</i>	柱孢魚腥藻	
<i>Bangia fuscopurpurea</i>	紅毛藻	ウシケノリ
<i>Batrachospermum moniliforme</i>	串珠藻	カワモズク
<i>Ceramium tenerimum</i>	柔質仙菜	ケイギス
<i>Ceratium hirundinella</i>	角甲藻	エンビツノモ
<i>Chara braunii</i>	布氏輪藻	シャジクモ
<i>Chlorella vulgaris</i>	小球藻	
<i>Chondrus ocellatus</i>	角叉菜	ツノマタ
<i>Cladophora glomerata</i>	団集剛毛藻	カモジシオグサ
<i>Closterium acerosum</i>	鋭新月藻	
<i>Codium fragile</i>	刺松藻	ミル
<i>Ectocarpus confervoides</i>	水云	ケナシシオミドロ
<i>Euglena caudata</i>	尾裸藻	
<i>Fucus evanescens</i>	枯墨角藻	ヒバマタ
<i>Gelidium amansii</i>	石花菜	マクサ
<i>Gigartina pacifica</i>	太平洋杉藻	イボノリ
<i>Glaucocystis nostochinearum</i>	灰胞藻	
<i>Gonyaulax spinifera</i>	具刺膝沟藻	
<i>Gracilaria verrucosa</i>	江蕨	オゴノリ
<i>Halimeda opuntia</i>	仙掌藻	サボテングサ
<i>Laminaria longissima</i>	長海带	ナガコンブ
<i>Laurencia obtusa</i>	鈍形凹頂藻	マギレソゾ
<i>Mallomonas helvetia</i>	黄魚鱗藻	
<i>Merismopedia minima</i>	細小平裂藻	
<i>Micrasterias apiculata</i>	尖刺微星鼓藻	
<i>Monostroma angicava</i>	袋礁膜	エゾヒトエグサ
<i>Navicula maculata</i>	斑点舟形藻	
<i>Oedogonium autumnale</i>	秋熟鞘藻	

(北大理 吉田忠生)

## 防波堤直立面の植生から見た各種海藻の好適な生育場所

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SAITO, Y. 1980. Suitability of habitats for certain algae in respect to their vegetations as they appear on vertical substrata. Jap. J. Phycol. 28: 171-176.

The previously reported suitability of habitats for some algae is re-examined on the basis of further data collected from thirty-five vertical transects on the breakwaters of five fisherman's wharfs near Hakodate, Hokkaido. As a result the tendency for *Ptilota pectinata* (GUNNERUS) KJELLMAN to be a shade alga is reconfirmed. *Corallina pilulifera* POSTELS et RUPRECHT, formerly reported as a sun form is now considered to be a species abundant on the outer side of the breakwaters instead. *Myelophycus intestinale* SAUNDERS, *Nemalion vermiculare* SURINGAR, *Lomentaria hakodatensis* YENDO and *Laurencia nipponica* YAMADA, all have tendencies similar to those of *Corallina pilulifera*. *Analipus japonicus* (HARVEY) WYNNE (reported previously as *Heterochordaria abietina* (RUPRECHT) SETCHELL et GARDNER), can no longer be considered a sun form in light of the additional data from the present study. *Phyllospadix iwatensis* MAKINO and *Rhodomela laris* (TURNER) AGARDH are reported here as species which cannot grow on vertical substrata.

*Key Index Words:* benthic marine algae; ecology; relative cover; suitable direction; vertical substratum.

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筆者は白尻で海藻植生の調査を実施した際、ほぼ北方に面した防波堤の直立面では *Ptilota pectinata* (GUNNERUS) KJELLMAN クシベニヒバが著しく多かったのに、なだらかに傾斜した自然岩礁上には、ほとんど見出されなかった (SAITO and ATOBE 1970) ことなどに興味を抱き、その後、周辺の漁港防波堤側面の植生の観察に重点を置き始めた。まず、豊浦の漁港防波堤の5測区で調査を行ない、白尻漁港の防波堤から得た結果も対比して、直立面の海藻植生の種の組成は基質の面する方向に影響されることや、前述のクシベニヒバは陰性種であって、*Analipus japonicus* (HARVEY) WYNNE マツモや *Corallina pilulifera* POSTELS et RUPRECHT ビリヒバは陽性種と推定されたことについても述べた (SAITO et al. 1971)。その後、年次的に延長された防波堤の新旧各段階の基質上の植生の比較から遷移を論じて極相の査定法を考案し (SAITO et al. 1976)、また、それまでに集めた防波

堤側面の観察結果を使用して、前述の極相査定法を吟味した (齋藤ほか 1977)。このように、より多くの漁港で観察を進め、海藻の生態学的特性や、植生の遷移に関する知見もたくわえたいものと考えていたが、近年、防波堤の周辺には波浪を防除するためコンクリートのテトラポッドを投入することが多くなり、結果的に直立面の植生の発達も思わしくなく、観察に困難の伴うことも多くなったので、1973年夏の調査以来、観察を実施していない。しかしながら、現在手もとにある5つの漁港の35測区での観察結果を用いて、前報 (SAITO et al. 1971) のクシベニヒバやマツモ、ビリヒバその他の好適生育面について吟味することもできるので、前報の訂正や補足も兼ねて報告し、参考に供したいものと思う。

ここに、資料を集める野外調査で助力いただいた谷口和也、跡部進、長縄三郎、佐々木久雄、宮坂広司、渡辺敬一の諸君に感謝の意を表わす。

## 方 法

資料は前回(斎藤ほか 1977), 植生の極相査定法を吟味するのに用いたもので, Fig. 1 の各地で集めた。また, 観察の年月日は, 防波堤の築造後の年数や観察者名とともに Table 1 に示した。このようにして集めた各種海藻の各測区における出現状態から, 明らかに防波堤の内側には生育できないと思われる4種を選び出すことができた。また, 各測区での被度の大小を比較すると, どのような環境によく適応できる種であるかを推測できるものもあった。そのような種の多寡は, それぞれ別の観察者によることが多く, 被度の値には個人差もあると思われるので, 各測区における全海藻種の被度の合計を100とし, 個々の種の示した被度の値を相対値に変え, 直立面の向く方向や, 内側向きか, 外側向きかの別とともに図示してみた。なお, この地域のなだらかに傾斜した自然岩礁上で普通に産しながら, 直立面で見出されなかった種についても最後にふれた。

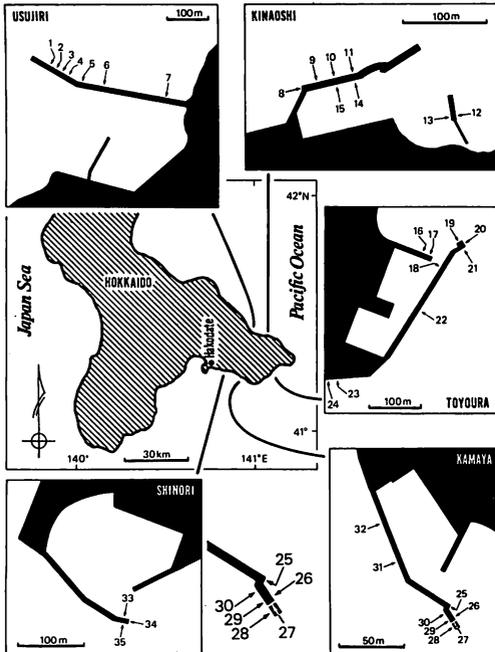


Fig. 1. A map showing the location and topography of the study sites and Transects 1-35.

## 結果と考察

### 1. 外向きの側面に多い種

*Myelophycus intestinale* SAUNDERS キタイワヒ

ゲは防波堤外側の8測区(第1, 8~11, 16, 17及び第29)にのみ出現し, 内側測区には全く見られない。

*Nemalion verniculare* SURINGAR ウミゾウメンは防波堤外側の10測区(第1~4, 6, 7, 28及び第30~32)にのみ出現し, 内側測区には全く見られない。

*Lomentaria hakodatensis* YENDO コスジフシツナギは防波堤外側の13測区(第2, 4~6, 9~11, 16, 17, 22, 24, 30及び第31)と防波堤頂端面の1測区(第27)に出現し, 内側測区には全く見られない。

*Laurencia nipponica* YAMADA ウラソソは防波堤外側の6測区(第12, 23, 24, 28, 31及び第32)と防波堤頂端面の2測区(第20及び第27)に出現し, 内側測区には全く見られない。

*Corallina pilulifera* ピリヒバは前報(SAITO *et al.* 1971)で, 臼尻の第7測区と豊浦の第18~22測区における観察結果から, 陽性種と推定された。その後, 他の多くの測区で得た値とともに分布状態を図示すると Fig. 2 のようになる。これによっても, 本種が南向きの側面で大きい相対被度を示す傾向を読みとることができよう。しかしながら, Fig. 2 からはそれにも増して明確な, 防波堤内側部分での低い相対被度値を読みとることもできる。内側部分にありながら, 例外的に大きい値を示す第25測区は, 調査の2年前

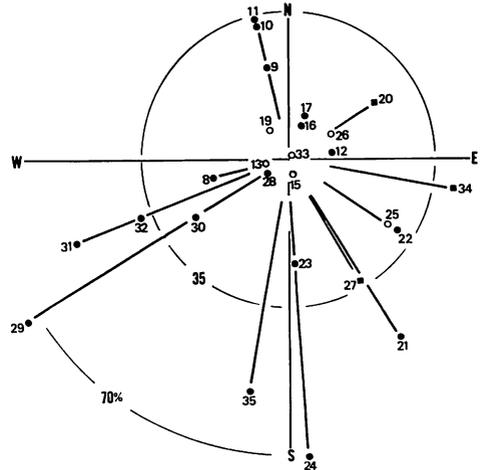


Fig. 2. Relation between relative cover (distance from the origin) and the substratum direction for *Corallina pilulifera* POSTELS *et* RUPRECHT. A black spot denotes the wave-exposed outer side transect. A black square indicates the transect was on the end of the breakwater. A white spot indicates a wave-protected inner side transect.

Table 1. Observed date, age of substratum and observers for each transect

Transect	Observed date	Substratum was built	Approximate age of substratum in years	Observers
1	31 July 1973	July 1971	2	MIYASAKA, WATANABE & SAITO
2	31 July 1973	June 1971	2	MIYASAKA, WATANABE & SAITO
3	31 July 1973	July 1970	3	MIYASAKA, WATANABE & SAITO
4	31 July 1973	July 1969	4	MIYASAKA, WATANABE & SAITO
5	30 July 1973	Before 1951	22+	MIYASAKA, WATANABE & SAITO
6	30 July 1973	Before 1951	22+	MIYASAKA, WATANABE & SAITO
7	11 & 12 July 1968	Before 1951	17+	TANIGUCHI, ATOBE & SAITO
8	22 June 1971	Before 1962	9+	NAGANAWA & SAITO
9	22 June 1971	Before 1962	9+	NAGANAWA & SAITO
10	23 June 1971	Before 1962	9+	NAGANAWA & SAITO
11	23 June 1971	Before 1962	9+	NAGANAWA & SAITO
12	24 June 1971	1962-1967	4-9	NAGANAWA & SAITO
13	24 June 1971	1962-1967	4-9	NAGANAWA & SAITO
14	23 June 1971	Before 1962	9+	NAGANAWA & SAITO
15	23 June 1971	Before 1962	9+	NAGANAWA & SAITO
16	18 June 1973	Before 1951	22+	MIYASAKA, WATANABE & SAITO
17	18 June 1973	Before 1951	22+	MIYASAKA, WATANABE & SAITO
18	30 June 1969	1951-1954	15-18	TANIGUCHI, ATOBE, NAGANAWA & SAITO
19	29 June 1969	1962	7	TANIGUCHI, ATOBE, NAGANAWA & SAITO
20	29 June 1969	1962	7	TANIGUCHI, ATOBE, NAGANAWA & SAITO
21	29 June 1969	1962	7	TANIGUCHI, ATOBE, NAGANAWA & SAITO
22	28 June 1969	Before 1951	18+	NAGANAWA & SAITO
23	17 June 1973	1955-1959	14-18	MIYASAKA, WATANABE & SAITO
24	17 June 1973	1955-1959	14-18	MIYASAKA, WATANABE & SAITO
25	4 June 1970	1949-1955	15-21	TANIGUCHI, NAGANAWA & SAITO
26	4 June 1970	1958*	12	TANIGUCHI, NAGANAWA & SAITO
27	4 June 1970	1958*	12	TANIGUCHI, NAGANAWA & SAITO
28	5 August 1971	September 1970	1	NAGANAWA, SASAKI & SAITO
29	4 June 1970	1958*	12	TANIGUCHI, NAGANAWA & SAITO
30	5 August 1971	October 1968	3	NAGANAWA, SASAKI & SAITO
31	13 July 1971	1949-1955	16-22	NAGANAWA, SASAKI & SAITO
32	13 July 1971	1949-1955	16-22	NAGANAWA, SASAKI & SAITO
33	21 August 1970	1956	14	NAGANAWA & SAITO
34	21 August 1970	1956	14	NAGANAWA & SAITO
35	21 August 1970	1956	14	NAGANAWA & SAITO

\* The substratum for Transects 26, 27 and 29 was first built as a base of a lighthouse, which was isolated before the building of the substratum for Transect 30.

まで防波堤の頂端部分であったし、第26測区は防波堤とは離れて孤立した灯台の基礎であった。調査の2年前に第30測区の位置する部分が築造され、それ以後に第25, 26測区の部分は内側の性格を備えるに到った (Fig. 1, Table 1)。環境の変化があっても、植生はさほど急速に変化しない、ということについては前述した (斎藤ほか 1977) が、これらの測区では該種が高い被度で残存していたものであろう。また、外側に面しておりながら、内湾的性格も有すると思われる第16, 17測区でピリヒバの相対被度は小さく、内側の第14, 18測区では全く出現しない (Fig. 2)。これらの事実から、前報 (斎藤ほか 1977) でもふれたように、本種は防波堤の外側に多産し、内側で激減する種とみるべきものであろう。なお、白尻の各測区は外側に面しているのに、ピリヒバは全く出現しない。その理由について今回明言するのはむづかしいが、該地がかなり噴火湾に近いので、すでにその内湾的性格が現われている、という可能性も考えられるのではなからうか。

以上の種が外側測区に多く、内側測区で全く生じないか、あるいは激減する理由の知見は、目下のところ乏しい。しかしながら、考えられる理由の一つとして波浪の存否をあげ得ようし、他の一つとしては汚染物質も含めた海水成分の問題があろう。そしてウミゾウメンは、自然岩礁上にあっても外洋向きの波浪の当る部分に多産することを見るならば、波浪の存在がその生育に重要な意味を有するもののように思われる。また、ウラボソは汚染された水の中での生育が困難な種と筆者は考えている。すなわち、筆者らの学部からほど近い函館湾の奥の部分に防波堤があって、以前から好適な海藻の採集場所として利用されてきた。しかしながら、その防波堤で筆者や知人はソゾ属の種を採集したことはない。波浪の弱いこのような環境でソゾ属は生育できないものか、と考えたこともあったが、汚染の程度さえ軽ければ、生育できるものようである。すなわち、南オーストラリアの Kangaroo Island にある American River Inlet という静穏な入江ではソゾ属も多く、筆者も何種か採集した経験をもつし、ウラボソに近縁な *Laurencia tasmanica* HOOKER et HARVEY の産地としては SAITO and WOMERSLEY (1974) に記録もあるので参照せられたい。

## 2. 内側向き側面に多い種

*Ulva pertusa* KJELLMAN アナアオサは、前報 (斎藤ほか 1977) でもふれたように、各測区の値でみると前述のピリヒバとは相対被度の相関係数が  $-0.634$  と

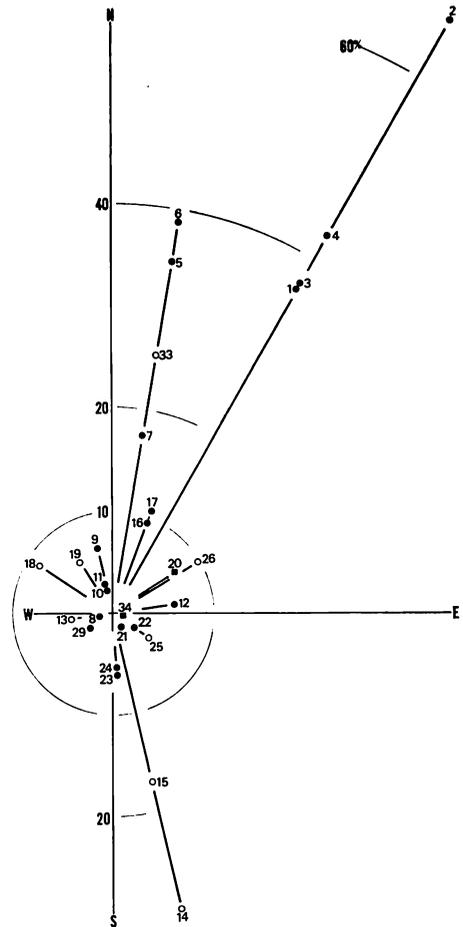


Fig. 3. Relation between relative cover and the substratum direction for *Ulva pertusa* KJELLMAN. See the caption of Fig. 2 for further explanation.

なり、両種の多寡には負の相関が認められた。結局、アナアオサは前記したピリヒバとは逆に、防波堤の内側測区での多産が示唆される。その分布状態は Fig. 3 の示す通りであるが、ここで白尻の7測区のを除外してみれば、内側に面する測区で比較的高い相対被度を示すことが知られる。なお、アナアオサを全く欠く測区は第27, 28, 30, 31, 32及び第35測区であって、いずれも防波堤の外側か頂端方向に設けられた測区である。以上のことから、アナアオサは防波堤の内側部分に多く、外側では少ない種とみることができよう。前記したように、白尻の各測区が噴火湾に近いので、外側の測区でも内湾的性格を示すものとすれば、そこにアナアオサの多産する理由の説明も容易とならう。

## 3. 北向き側面に多い種

*Ptilota pectinata* クシベニヒバは、白尻の第7測区と豊浦の第18~22測区での観察結果から、北向き側面に多かったのが陰性種とみなされた (SAITO *et al.* 1971)。同種はこの地域で汐首岬以西にも分布はみられるものの、防波堤側面で顕著な群落を形成することはないので、白尻、木直及び豊浦の各漁港防波堤の第1~24測区での生育状況を比較してみた (Fig. 4)。それによると、北方に向いた各測区内の該種はかなり大きい値の相対被度を示し、その傾向は基質が防波堤の外側にあっても内側に面していても、あるいは防波堤の頂端部にあっても変わらないように見受けられる。また、西向きから約10度ほど南の第8, 13測区でも高い相対被度値で出現した (Fig. 4) が、そのうち木直の第8測区は、その南を斜めに走る防波堤で被われて (Fig. 1)、日中は日かげになるので、このような値が得られたものであろう。より南向きになった第21, 23測区での該種の相対被度値は著しく低く (Fig. 4)、また、同様に南を向いた第14, 15, 22及び第24測区で該種は全く出現しない。以上の事実から、クシベニヒバは陰性種であろう、とした推定は妥当であったものといえよう。

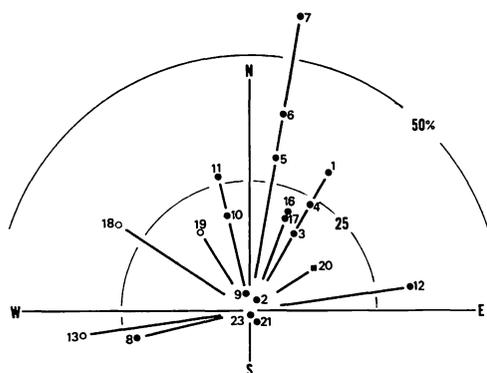


Fig. 4. Relation between relative cover and the substratum direction for *Ptilota pectinata* (GUNNERUS) KJELLMAN. Because of the distribution of the species, the relation is cited for Transects 1-24. See the caption of Fig. 2 for further explanation.

4. 好適生育面の不明瞭になった *Analypus japonicus* マツモ

各測区におけるマツモの出現状況を Fig. 5 に示したが、第1, 3, 7, 13~15及び第28測区で該種は出現しなかった。そのうち、第1, 3及び第28測区は築

造後1~2年という新しい防波堤上にある (Table 1) ので、多年生のマツモはまだ、群落を形成するに到らなかったものであろう。残る測区中、第13~15測区は、いずれも防波堤の内側に面するし、同じく内側に面する他の多くの測区のマツモの相対被度も、さして大きい値を示しているとはいえない (Fig. 5)。大きい値の第26測区は、前述したように、かつて灯台の基礎として防波堤とは別に築造され、植生調査時の2年前に防波堤と連結されて、その一部になった所にある (Fig. 1, Table 1)。その結果、まだ高い値の被度を示すマツモが残存していたものであろう。とすれば、該種は防波堤の外側部分に多産するものではあるまいか、との推定もできようが、Fig. 1によって、多少の内湾的性格があると思われる第16, 17測区での相対被度が著しく大きい (Fig. 5) ことを見るなら、その推定にも多少の無理があるといわなければならない。前報 (SAITO *et al.* 1971) では、今回の第7及び第18~22測区での観察結果から、マツモを陽性種かと考えたのであったが、Fig. 5によって明示されるように、その性質もここで否定されるべきものと考えられる。

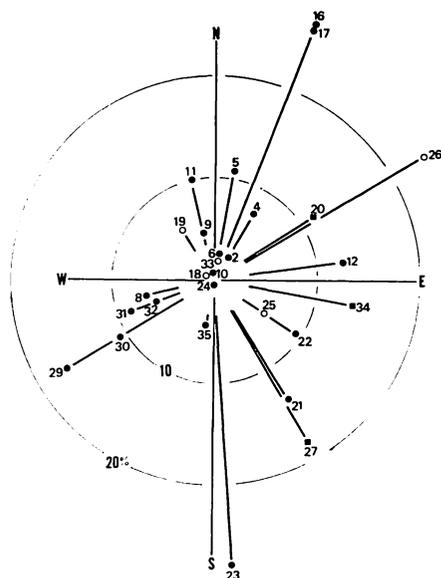


Fig. 5. Relation between relative cover and the substratum direction for *Analypus japonicus* (HARVEY) WYNNE. See the caption of Fig. 2 for further explanation.

## 5. 直立面に生育しない種

海産顕花植物の *Phyllospadix iwatensis* MAKINO スガモと紅藻の *Rhodomela larix* (TURNER) AG-

ARDH フジマツモは、ともにこの地域の岩礁斜面ならば潮間帯域にも多産する種であって、白尻の岩礁斜面での生育については SAITO and ATOBE (1970) によっても明らかである。その他の各漁港付近の岩礁斜面でも普通に生育することは、未発表ながら、筆者らの調査で知られている。しかしながら、今回の 35 測区には全く出現せず、岩礁斜面上とは著しい対照を示す。したがって、これら両種は直立面での生育ができない種と考えるべきものであろう。ここでスガモの生育場所についてみるならば、岩礁の凹入部や漸深部付近で、腐植質や砂泥の堆積した所に発達した根をおろすのが普通である。とすれば、そのような基質のない直立面での生育は、該種の大型な種子の付着が困難なことに相俟って、むつかしいことというべきであろう。

後者のフジマツモも、岩礁斜面の潮間帯部分では凹入部に多いようではあるが、直立面に生育できない理由を示唆できるほどの知見は持たない。また、*Rhodomela subfusca* (WOODWARD) AGARDH イトフジマツモ直立面の 35 測区で見出すことはできなかったが、岩礁斜面でフジマツモほど多産するものではないので、特記するのをさけた。

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## エゾヤハズの四分孢子発生機構の解析 VI

### 四分孢子発生におよぼすサイトカラシンBの影響

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OHMORI, T. and UEKI, Y. 1980. An analysis of tetraspore development in *Dictyopteris divaricata* VI. Effects of cytochalasin B on tetraspore development. Jap. J. Phycol. 28: 177-181.

Tetraspores of *Dictyopteris divaricata* were cultured in seawater solutions of cytochalasin B at various concentrations. Cytochalasin B at 100 mg/l or more inhibited rhizoid formation. In this concentration of cytochalasin B, tetraspores developed into multicellular spherical germlings lacking rhizoids. After the spores developed into 2-4 celled germlings, rhizoidal protuberances began to appear. These protuberances were greater in number as compared with the controls.

Under continuous unilateral illumination, tetraspores were cultured in seawater solution of cytochalasin B at 100 mg/l. The first segmentation wall of the germlings was formed at random and had no relation to the direction of the light. Rhizoids arising afterwards were formed away from the light source. In cytochalasin B seawater solution, therefore, a definite relation was not seen between the direction of the first segmentation wall and that of the rhizoid.

*Key Index Words:* cytochalasin B; *Dictyopteris divaricata*; morphogenesis; Phaeophyta; photopolarization; rhizoid formation; tetraspore development.

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サイトカラシンBは  $C_{29}H_{37}NO_5$  の化学式をもつ一種の抗生物質で、細胞内のマイクロフィラメントの存在様式に影響をおよぼすと考えられている物質である。サイトカラシンBは、原形質流動を可逆的に抑制し、動物細胞では細胞質の分裂を阻害するなど数多くの効果が知られている。海藻に対するサイトカラシンBの効果は、ヒバマタ目に属する *Fucus* (QUATRANO 1973), および *Pelvetia* (NELSON and JAFFE 1973, 安部 1978) の受精卵について調べられているに過ぎない。本研究では、アミジグサ目に属するエゾヤハズ *Dictyopteris divaricata* の四分孢子をサイトカラシンBを含む海水で培養したところ、いくらかの効果がみられたので、その結果を報告する。

### 材料と方法

本研究では、1978年6月4日と翌1979年6月11日に、岡山県玉野市渋川で採集したエゾヤハズの四分孢子体を用いて実験を行なった。採集後、藻体を一晚暗所に放置し、翌日濾過海水を満した大型シャーレに浸して四分孢子を放出させた。2時間以内に放出された四分孢子を遠沈して集め、実験に用いた。

まず、四分孢子の発生に影響するサイトカラシンBの有効濃度を決定するため、10, 50, 100, 120, 150, 200 mg/lのサイトカラシンBを含む海水溶液を作り、小型シャーレ(径6 cm)を用いて培養した。光は自然光で、培養温度は17.5°Cであった。サイトカラシンBは水に不溶なので、その10 mgを0.5 mlのdimethyl sulfoxide (DMSO)に溶解し、これに純水9.5 mlを加

えて貯蔵液とした。この貯蔵液を濾過海水で必要な濃度に稀釈して培養液とした。

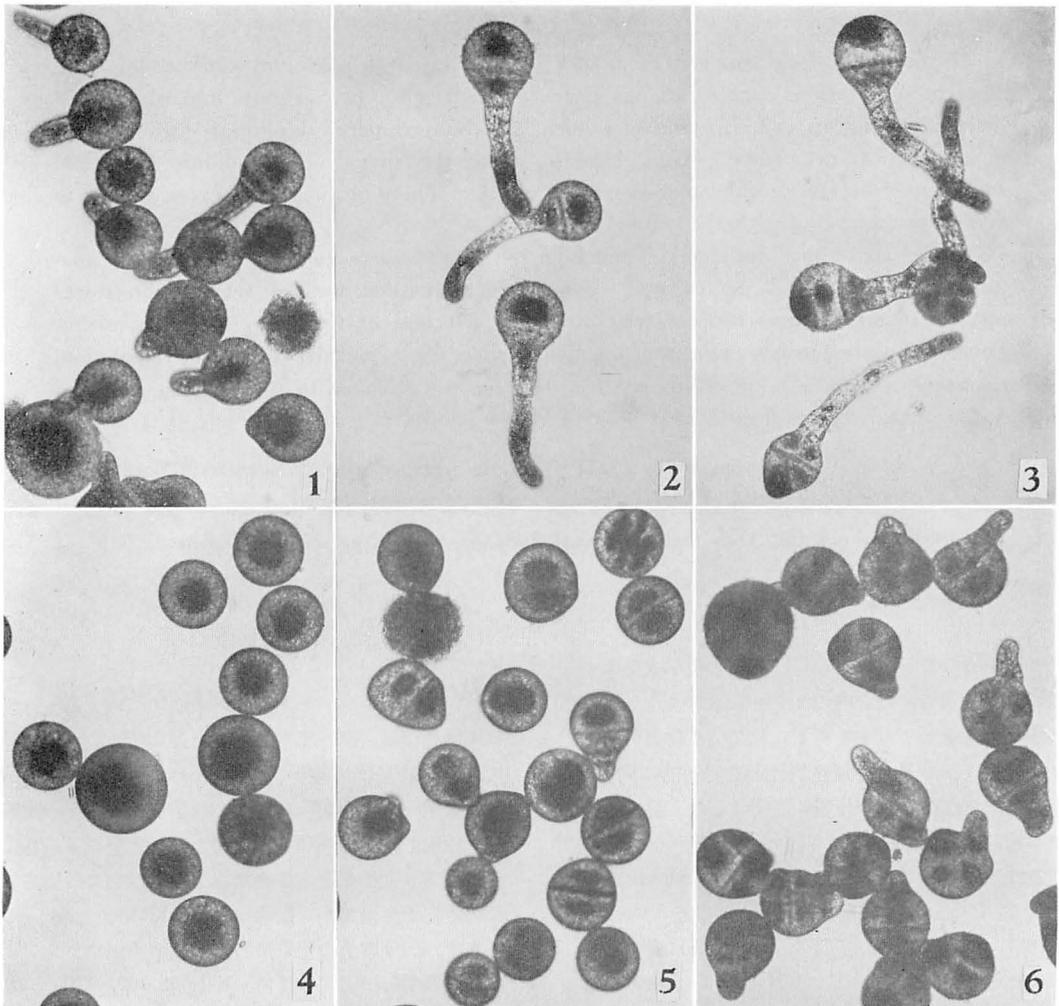
さらに、光による仮根の定位 (photopolarization) にサイトカラシンBがどのような影響を与えるのかを調べるため、100 mg/l サイトカラシンBを含む海水溶液中で培養した四分胞子に光を一側から6~72時間照射し、照射後は暗培養に切り替えて、培養5日後に仮根の伸出方向を観察した。一方照射の実験に用いたシャーレは、一方を開けて黒紙で包み、他の方向からは光が入らないようにした。光源には15 Wの白色蛍光灯を用いて1000 luxの光を与え、室温(23.0±

0.5°C)で実験を行なった。

## 結 果

### 1. 四分胞子の発芽におよぼすサイトカラシンBの影響

エゾヤハズの四分胞子を濾過海水で培養すると、培養14~19時間後に胞子の一端にふくらみを生じ仮根突起を形成する (Fig. 1)。培養22~36時間後に、仮根突起が伸長する方向とは直交して、第一分割壁が胞子の中央よりやや仮根よりの位置に形成される (Fig. 2)。培養2日後の胞子の発芽率は93.9%、培養4日後の発



Figs. 1-6. Stages in the development of tetraspores in *Dictyopterus divaricata*. ( $\times 100$ )

1. 24 hr in seawater. 2. 48 hr in seawater. 3. 72 hr in seawater. 4. 24 hr in cytochalasin B at 100 mg/l. 5. 48 hr in cytochalasin B at 100 mg/l. 6. 72 hr in cytochalasin B at 100 mg/l.

芽率は98.6%であった。dimethyl sulfoxide(DMSO)が胞子発芽およびその後の発生におよぼす影響を調べるために、150 mg/l サイトカラシン B 溶液中に含まれている DMSO と同濃度の DMSO 海水溶液中で四分胞子を培養した。その結果、培養2日後の発芽率は67.0%と低かったが、おくらせて発芽するものが多く、培養4日後の発芽率は87.4%と正常のものに近い値を示すようになった。

サイトカラシン B 溶液中で培養した場合は、その濃度が50 mg/l 以下の場合には濾過海水中での発芽率と変わらず、その影響は見られなかった。100 mg/l の濃度では、培養24時間を経過しても、仮根突起を生じたものは全く見られず (Fig. 4)、培養2日後の発芽率は50.4%と低かった。しかし培養3日後には94.1%と正常に近い発芽率に回復した。120 mg/l 以上の濃度では、濃度が高くなるにしたがって発芽率は低くなり、150 mg/l の濃度では培養2日後の発芽率は49.6%、4日後の発芽率は69.1%であった。

#### 2. 発生におよぼすサイトカラシン B の影響

海水中で四分胞子が発芽する時には、最初に仮根突起を形成し、その後胞子の核が分裂して、2核の間に第一分割壁が形成される。100 mg/l サイトカラシン B 海水溶液で培養すると、四分胞子は培養36時間ぐらい経過した頃に、仮根突起を生じることなく胞子の核は分裂して、胞子の中央部に第一分割壁が形成される (Fig. 5)。続いて第一分割壁に直交して第二分割壁

が入り、胞子は4細胞となる。胞子が2~4細胞に分裂した後 (培養36~60時間後) に、初めて仮根突起が生じてくる (Fig. 6)。培養2日後には、このような仮根のない多細胞の発芽体が79.1%も生じた (Table 1)。サイトカラシン B の濃度が高くなるにしたがって、異常な発芽体の数が増加すると共に、仮根突起の形成もおくらせてくる。培養4日後では、100 mg/l の濃度で仮根をもたない発芽体は26.7%と培養2日後に比べ著しく減少した。200 mg/l の濃度では、培養4日後になっても78.7%のものがまだ仮根突起を形成していなかった (Table 1)。胞子が2~4細胞に分割された後に仮根突起を生じる場合、第一分割壁が走る方向と仮根の伸出部位との間には決まった関係は見られない。第一分割壁に直交した位置に仮根突起を生じる場合もある。この場合、仮根の生長は著しく阻害されていた。サイトカラシン B の濃度が50 mg/l 以下の場合には、正常に発生しその影響はみられない。

濾過海水中で培養すると、ほとんどの四分胞子は1本の仮根を形成し、2本の仮根を生じたものは1.3%に過ぎない。サイトカラシン B 海水溶液で培養した場合には、仮根の数が増加する傾向がみられる。100 mg/l の濃度では、10.5%のものが2本の仮根を形成し、0.5%のものが3本の仮根を形成していた (Table 2, Fig. 7)。2本の仮根を生じる場合、それぞれの仮根が反対の方向に伸出することもあれば、同一方向に

Table 1. Percentage of multicellular germlings without rhizoid in cultures with various concentrations of cytochalasin B

	Seawater	DMSO	Cytochalasin B concentration mg/l					
			10	50	100	120	150	200
2 Days	17.3	8.3	7.7	7.5	79.1	77.0	86.7	88.9
3 Days	9.8	9.9	4.4	6.4	32.5	65.8	77.6	86.2
4 Days	0.9	7.2	3.5	5.1	26.7	42.1	63.9	78.7
7 Days	0.9	4.7	3.8	1.8	13.4	16.0	29.8	36.3

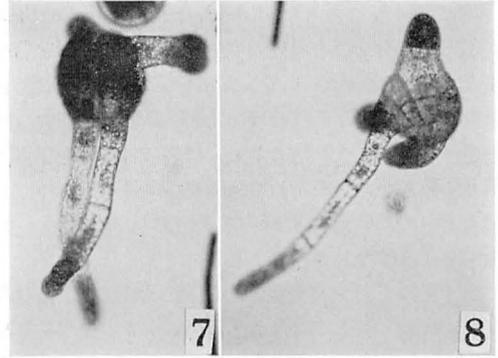
Table 2. Percentage of germlings with several rhizoids in cultures with various concentrations of cytochalasin B for 7 days

Number of rhizoids	Seawater	Cytochalasin B concentration mg/l					
		10	50	100	120	150	200
1	98.7	96.9	94.9	89.0	88.6	88.0	83.5
2	1.3	4.6	4.6	10.5	9.5	9.1	16.5
3	0	0.5	0.5	0.5	1.8	2.9	0

2本の仮根を生じる場合もあった。さらに、サイトカラシンBは直立苗の数も増加させる。濾過海水で7日間培養したものでは、1本の直立苗をもつものが93.0%、2本のが2.2%であった。100 mg/l サイトカラシンBでは、1本の直立苗をもつものが79.0%と減少し、2本もつものが8.9%、3本のが0.8%と増加した (Table 3)。

### 3. Photopolarization におよぼす影響

100 mg/l サイトカラシンB 海水溶液で四分胞子を培養し、これに一方から白色蛍光灯で1000 luxの光を照射した。その結果、胞子は仮根突起を生ずることなく第一分割壁を形成するが、その第一分割壁が走る方向は、入ってくる光の方向とは関係がなく、全く任意の方向に形成された (Table 4)。分割壁形成後、おくれで仮根突起は生じてくる。この仮根突起の伸出方向は光によって影響され、大多数の発芽体が入



Figs. 7-8. Germlings of *Dictyopteris divaricata* in cultures with cytochalasin B at 100 mg/l for 7 days. (×100)

7. A germling with three rhizoids and an erect shoot. 8. A germling with a rhizoid, an erect shoot and two protuberances.

Table 3. Percentage of germlings with several erect shoots in cultures with various concentrations of cytochalasin B for 7 days

Number of erect shoots	Seawater	Cytochalasin B concentration mg/l					
		10	50	100	120	150	200
0	4.8	5.3	3.3	11.3	6.6	22.5	29.7
1	93.0	88.9	85.8	79.0	84.7	66.9	60.1
2	2.2	4.9	10.2	8.9	6.6	8.5	8.0
3	0	0.8	0.8	0.8	2.1	1.4	2.2

Table 4. The direction of the first segmentation wall of apolar germlings to the light in cultures with cytochalasin B at 100 mg/l for 3 days

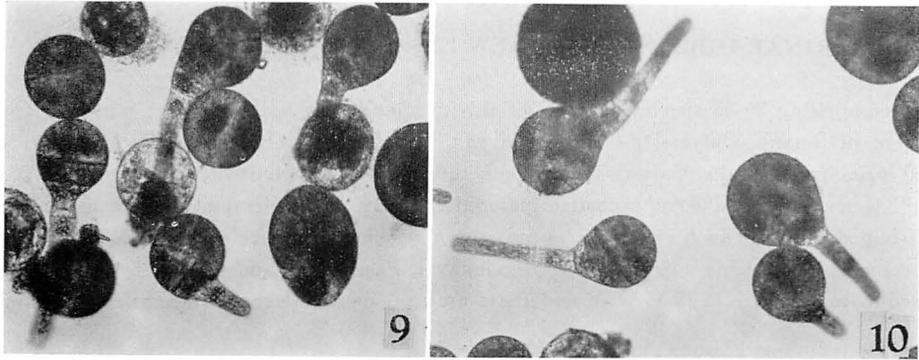
	Direction of the wall to light		Total
	Parallel	Vertical	
Number	63	52	115
%	54.8	45.2	100

てくる光の方向とは反対の側に仮根突起を形成した (Table 5, Fig. 9)。培養開始後から12時間連続一方照射したものでは53.5%、18時間照射したものでは75.5%、24時間照射したものでは85.1%の発芽体が反光源側に仮根を形成した (Table 5)。濾過海水中で培養した場合は、培養開始後9~12時間の間の一方照射が仮根定位に対して最も有効であった (大森・植木1979)。今回の実験では、光が仮根の伸出方向を決定する時間帯が海水中の場合よりもおくられている。これは

サイトカラシンBの影響により、胞子発生の経過が全体としておくられていることに起因していると思われる。第一分割壁は任意の方向に形成されるが、仮根突起が反光源側に形成されるので、第一分割壁に平行に仮根を伸出する発芽体もあれば、分割壁に直交する位置に仮根突起を生じる発芽体もあって、その発芽体の形態はさまざまとなるのである。

### 考 察

エゾヤハズ四分胞子をサイトカラシンB海水溶液で培養すると、その濃度が50 mg/l以下の場合には影響が見られなかった。100 mg/l以上の濃度では、その濃度が高くなるにしたがって発芽率は低下し、発生におくれが生じた。高濃度のサイトカラシンBは細胞分裂には影響を与えないが、仮根の形成を著しく阻害する。100 mg/l サイトカラシンB海水溶液中では四分胞子は仮根突起を生じることなく細胞分裂を行なって多細胞体となる。胞子が2~4細胞に分割された後に、ようやく仮根突起が生じてくる。生じる仮根の数



Figs. 9-10. Germlings of *Dictyopteris divaricata* in cultures with cytochalasin B at 100 mg/l for 96 hr. ( $\times 100$ )

9. Germlings under the continuous unilateral illumination. The light from the upper part of the figure. 10. Germlings in the dark.

Table 5. Percentage of orientation of the rhizoidal outgrowth under the unilateral illumination in cultures with cytochalasin B at 100 mg/l. The light source was in the south

Illumination time	South	North	East	West
0-6 hr L	6.7	60.0	20.0	13.3
0-12 hr L	4.7	53.5	25.6	16.3
0-18 hr L	4.1	75.5	10.2	10.2
0-24 hr L	0	85.1	8.5	6.4
0-36 hr L	0	78.6	16.7	4.8
0-48 hr L	0	84.8	9.1	6.1
0-60 hr L	0	78.6	14.3	7.1
0-72 hr L	0	77.4	12.9	9.7

は1本とは限らないで、増加する傾向がみられる。これは、サイトカラシンBの存在のもとでは、孢子が多細胞になった後に仮根形成が始まるので、分割されたそれぞれの細胞が仮根突起を形成しようとするためと考えられる。

QUATRANO (1973) は *Fucus distichus* の受精卵を 10 mg/l サイトカラシンB で培養すると、エゾヤハズの発芽体でみられたのと同じような多細胞体になり、仮根をもたない胚が生ずることを報告している。NELSON and JAFFE (1973) は *Pelvetia fastigiata* の受精卵で、1~5 mg/l のサイトカラシンB が仮根突起の形成を抑制することを示し、これは仮根形成部位の生長が妨げられるためであると考えている。

QUATRANO (1973) は *Fucus distichus* の受精卵で、50~100 mg/l サイトカラシンB が光による極性

軸の固定をおくらすことを述べている。NELSON and JAFFE (1973) は *Pelvetia fastigiata* の受精卵で 30~100 mg/l のサイトカラシンB が、安部 (1978) はエゾイシゲ *Pelvetia wrightii* の受精卵で 40 mg/l のサイトカラシンB が photopolarization を抑制することを報じている。エゾヤハズでは、100 mg/l という高濃度のサイトカラシンB 海水溶液でも photopolarization を抑制することではなく、分割壁形成後に、入ってくる光の方向とは反対側に仮根を形成した。

エゾヤハズの四分胞子を濾過海水で培養すると、反光源側に仮根を伸出する。その後、仮根の伸出方向とは直交して第一分割壁が形成される。すなわち、第一分割壁の形成方向は仮根の伸出方向によって決定されている。しかし、サイトカラシンB の溶液中では第一分割壁の形成方向は at random であって、秩序はなく、その後に出してくる仮根の形成部位は光に影響されて、第一分割壁が影響を与えている様子はみられなかった。

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**ALGAE MARINAE HIBERNICAE—A NEW EXSICCATA OF IRISH MARINE ALGAE**

Sets, comprising 20-25 specimens each, of this new exsiccata will be issued annually from the Department of Botany, University College, Galway, Republic of Ireland (GALW). Material will be collected principally in the Galway region both intertidally and subtidally. Fifty sets of 20 specimens will be available in 1980 as exchange material to interested institutions and private individuals.

It is hoped, through such exchange, to build up a working reference collection. Currently about 3,000 specimens of benthic marine algae (Rhodophyta, Phaeophyta and Chlorophyta) are housed in the phycological herbarium in GALW and these are available on loan to recognised institutions.

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## 紅藻アリュウシャンノコギリヒバについて<sup>1)</sup>

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MASUDA, M. and YAMADA, I. 1980. On the identity of the so-called *Odonthalia aleutica* (Rhodophyta, Rhodomelaceae) in Japan. Jap. J. Phycol. 28: 183-189.

The red alga which has been called *Odonthalia aleutica* (MERTENS ex C. AGARDH) J. AGARDH by Japanese phycologists was compared with *Odonthalia setacea* (RUPRECHT) PERESTENKO, *O. annae* PERESTENKO and genuine *O. aleutica*. The Japanese alga is distinguished from *O. setacea* by the following features. (1) The thallus color is almost black in drying in the Japanese alga and dark red in *O. setacea*. (2) The midribs are absent in the Japanese alga but they are evident in *O. setacea*. (3) The Japanese alga has semiglobose cystocarps, but *O. setacea* has urceolate cystocarps with elevated neck.

The alga in question agrees with *O. annae* in every respect and they are conspecific. The original description of *O. annae* is emended to include the reproductive features. The type specimen of *O. annae* collected from Iturup Island, Kuriles is tetrasporangial. The tetrasporangial stichidia are borne on the distal portion of lateral branches. They are arranged first in an alternate-distichous manner, later shifting to a spiral arrangement and appear in tufts. They are 950-2600  $\mu\text{m}$  in length and 200-230  $\mu\text{m}$  in diameter. Two tetrasporangia are formed in each of 6 to 23 successive segments of the stichidia. The tetrasporangia are 115-145  $\mu\text{m}$   $\times$  100-125  $\mu\text{m}$ . The cystocarpic specimen collected from Bering Island, Commander Islands has semiglobose cystocarps which measure 875-1225  $\mu\text{m}$  in length and 1000-1300  $\mu\text{m}$  in diameter.

The Japanese alga differs from genuine *O. aleutica* in two features. (1) The main stems of the Japanese alga are compressed, but those of *O. aleutica* are terete. (2) The cystocarps are semiglobose in the Japanese alga, whereas they are ovoid in *O. aleutica*. However, the status of genuine *O. aleutica* is still uncertain whether it is an independent species or synonymous with *O. floccosa* (ESPER) FALKENBERG.

*Key Index Words:* *Odonthalia annae*, *Odonthalia aleutica*, *Odonthalia setacea*, *Rhodomelaceae*, *Rhodophyta*, *taxonomy*.

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紅藻アリュウシャンノコギリヒバ *Odonthalia aleutica* (MERTENS ex C. AGARDH) J. AGARDH は最初 *Rhodomela aleutica* の名前で C. AGARDH (1822) によって発表され、後に J. AGARDH (1841) によってノコギリヒバ属 *Odonthalia* へ移されたも

のである。C. AGARDH の観察した標本はアリュウシャン列島の Unalaska Island で採集され、CHAMISSO によって C. AGARDH に送られたもので、*Fucus aleuticus* MERTENS の未発表名が付けられていた。C. AGARDH は原記載の発表以前に本種を図

1) 本研究は文部省科学研究費補助金 (課題番号 374218) による研究の一部である。

示しているが、それがフサノコギリヒバ *O. floccosa* (ESPER) FALKENBERG に類似しているために、本種は前者と混同されてきた。KYLIN (1925) によれば SETCHELL and GARDNER (1903) と COLLINS (1913) の報告はフサノコギリヒバのそれである。

ソ連邦で最近まで *O. aleutica* と呼ばれてきた種に対して、PERESTENKO (1977) は *O. setacea* (RUPRECHT) PERESTENKO の新組合せを提唱している。この種は *Atomaria setacea* RUPRECHT (1850) にもとづくもので、J. AGARDH (1863) 以来 *O. aleutica* の異名として扱われてきた。RUPRECHT (1850) と PERESTENKO (1977) はこの種が C. AGARDH (1822) の記載した *O. aleutica* とは全く異なった種であるとしている。

わが国で *O. aleutica* は最初 OKAMURA (1932) によって北海道東岸から報告され、アリウシャンノコギリヒバの和名が与えられた。しかしながら、わが国でアリウシャンノコギリヒバと呼ばれてきた種は真の *O. aleutica* 及び *O. setacea* ともいくつかの点で異なる。本論文はわが国でアリウシャンノコギリヒバと呼ばれてきた種を、真の *O. aleutica*, *O. setacea* 及び *O. annae* と比較して、その正体を明らかにすることを目的とする。

## 材 料

下記の標本を観察に使用した。

アリウシャンノコギリヒバ *Odonthalia aleutica* sensu OKAMURA, non J. AGARDH: (1) 霧多布, 北海道, 1915年8月(未成熟体, 岡村金太郎採); (2) 厚岸小島, 北海道, 1915年(未成熟体, 岡村金太郎採); (3) Robben Island, Sakhalin, 1930年7月(四分胞子体及び嚢果を付けた個体, 時田肇採); (4) 羅臼, 北海道, 1968年5月(四分胞子体及び嚢果を付けた個体, 増田道夫採)。以上のうち(1)~(3)の腊葉標本は岡村博士の同定によるもので(OKAMURA 1932), 北海道大学理学部標本庫(SAP)に保管されている。(3)の四分胞子体を Fig. 7 に示した。

*Odonthalia annae*: (1) 基準標本, Kassetka Bay, Iturup Island (エトロフ島), 1967年7月(四分胞子体, L. PERESTENKO 採); (2) Bering Island, Commander Islands, 1972年6月(嚢果を付けた個体及び未成熟体, L. PERESTENKO 採)。(1)はLeningradのコマロフ植物研究所から借用し,(2)は北海道大学理学部へ寄贈されたものである。

*Odonthalia setacea*: (1) Mednyi Island, Com-

mander Islands, 1972年7月(L. PERESTENKO 採); (2) Bering Island, Commander Islands, 1972年7月(T. ZAKHODNOVA 採)。両者とも嚢果を付けた個体でPERESTENKO博士の同定による腊葉標本で、コマロフ植物研究所より寄贈されたものである。前者を Fig. 1 に示した。

## 結果と考察

### *Odonthalia setacea* との比較

*O. setacea* については RUPRECHT (1850) 及び PERESTENKO (1977) の記載があるが、その特徴的な形質について図示されていないので、今回観察した標本で重要な2つの形質について図を与えておく。観察した *O. setacea* の標本は共に成熟した嚢果を付けた個体である(Fig. 1)。腊葉標本の色は暗赤色で、嚢果を通常の小枝に多数形成している。藻体の構造で顕著な特徴として、主軸及び主軸下部の側枝によく発達した中肋が認められる(Figs. 2, 3)。中肋は本属の他の種、ノコギリヒバ *O. dentata* や *O. ochotensis* などと同様に主軸及び側枝の中央部の皮層細胞が顕著に発達した結果、形成される。嚢果の形は隆起した頸部をもつ壺形で、大きさは1075~1300  $\mu\text{m}$  の高さ、823~1125  $\mu\text{m}$  の直径である(Figs. 4, 5)。嚢果には距(calcar)がないものが大部分であるが(Fig. 4)、極く少数のものに僅かに発達した距がみられる(Fig. 5)。これらの特徴については RUPRECHT (1850) 及び PERESTENKO (1977) の記載と一致する。わが国でアリウシャンノコギリヒバと呼ばれてきた種は以下の3つの形質で明瞭に *O. setacea* と区別される。(1) 前者の藻体の色は腊葉標本で殆んど黒色(生時は暗褐色)であるのに対し、*O. setacea* のそれは暗赤色である。(2) 前者には中肋が存在しないが、後者では顕著に発達した中肋がみられる。(3) 嚢果の形が前者は半球形であるが、後者は隆起した頸部をもつ壺形である。

### *Odonthalia annae* との比較

PERESTENKO (1973) が与えた *O. annae* の原記載には生殖器官についての記述がないが、今回借用した基準標本(Fig. 6)には四分胞子嚢の形成がみられた。四分胞子嚢は通常の側枝の先端付近に房状に生じた特別な小枝(四分胞子嚢枝, tetrasporangial stichidia)に形成されている(Fig. 8)。四分胞子嚢枝の長さは950~2600  $\mu\text{m}$ , 直径200~230  $\mu\text{m}$  で基部がやや細くなっている。四分胞子嚢の大きさは115~145  $\mu\text{m}$  × 100~125  $\mu\text{m}$  で1節に2個ずつ配列している。四分胞子嚢枝は短いもので連続した6~10節に四分胞子嚢

を形成し、長いものでは15~23節連続して形成している。連続した成熟節の全てに四分孢子囊が存在することは少なく、四分孢子が放出されたものでは2個の蓋細胞のみがみられる。アリュウシャンノコギリヒバの四分孢子囊枝は房状に集合して生じることは少ないが、大きさ、四分孢子囊を形成する節の数では両者はよく一致する (Fig. 9)。

囊果を付けた *O. annae* の個体は Bering Island で採集された標本のなかにみられた。この個体はかなり

老成した状態で完全な形をした囊果の数は少ないが、その形は半球形で、大きさは  $875\sim 1225\ \mu\text{m} \times 1000\sim 1300\ \mu\text{m}$  である (Figs. 10, 11)。短い距をもつもの (Fig. 10) と全く欠くもの (Fig. 11) がみられる。OKAMURA (1932) は距を欠くとしているが、彼の図 (pl. 286, figs. 8, 9) は明らかに距をもつ囊果があることを示している。Figs. 12, 13 にアリュウシャンノコギリヒバの囊果を示したが、形と大きさが *O. annae* のそれとよく一致する。ノコギリヒバ属において囊果

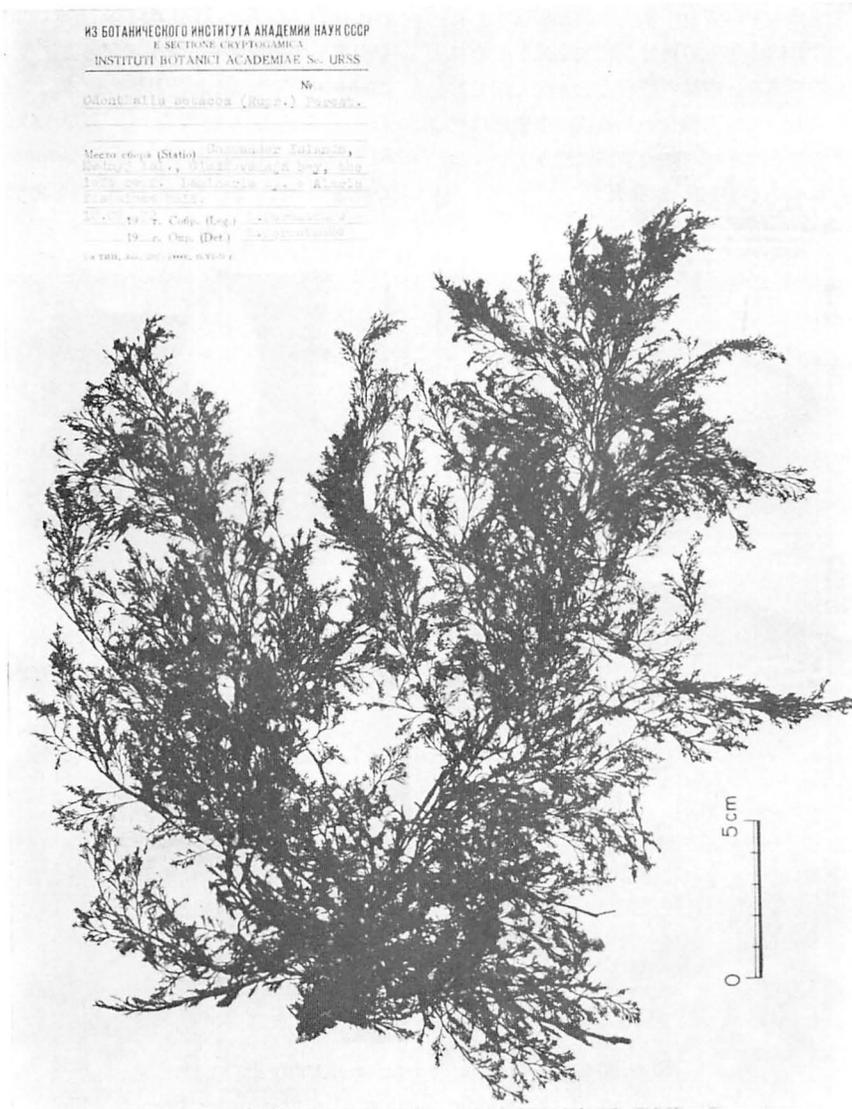
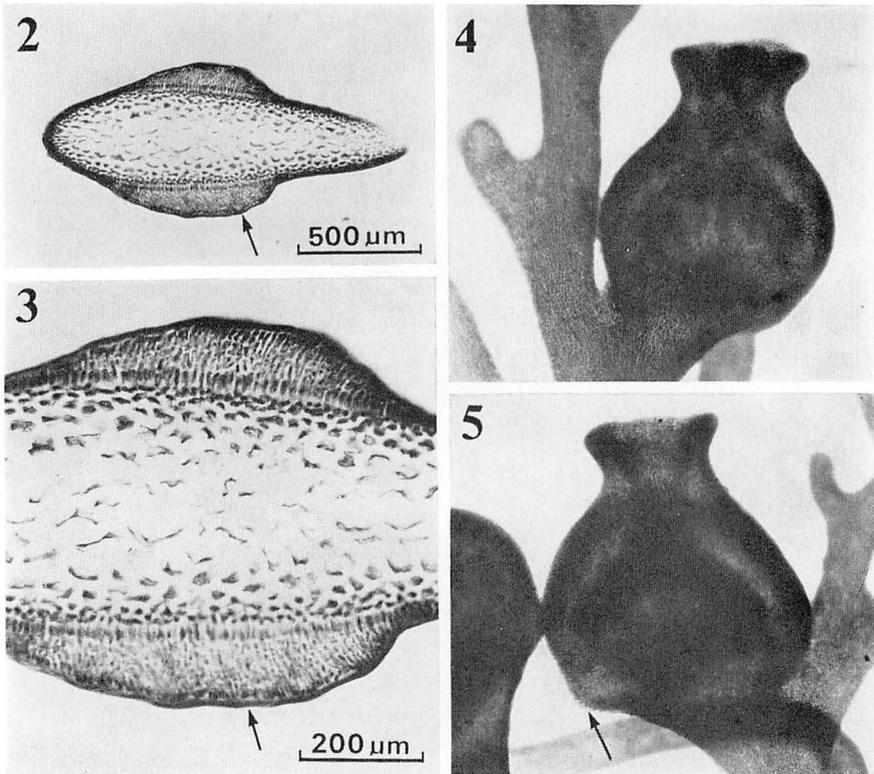


Fig. 1. Cystocarpic specimen of *Odonthalia setacea* (RUPR.) PEREST. collected from Mednyi Island, Commander Islands on July 10, 1972 by L. PERESTENKO.

の形と大きさが種を分ける分類学的形質として重要であることが、本属の11種について比較検討した増田(1979)によって指摘されている。両者はこの点において同一種ないし極めて近縁な種であることを示している。生殖器官の特徴の他に、藻体の色が生体では暗褐色、腊葉標本では黒色に近くなること、中肋を欠くこと、藻体の幅が狭いこと (Figs. 6, 7) などから、両者は同一種であると思われる。OKAMURA (1932) は「体は大部分中肋なくあるいは中肋全く不判明なれども、下部においていずれか一方の側に、もしくは両側に皮層の増厚することにより漸次中肋を形成す」としているが、これは多年生の部分が肥大生長を始めたことを示すものである。中肋の形成方法と全く同じであるが、本種においては主軸ないし側枝の中央部に規則的に発達することはない。この事実は培養実験においても確かめられている (増田, 未発表)。

*Odonthalia aleutica* との比較

次にアリュウシャンノコギリヒバと真の *O. aleutica* との比較であるが、その前に C. AGARDH (1820, 1822) と J. AGARDH (1863) の *O. aleutica* がそれぞれ異質のものを指している可能性に注目する必要がある。両者の記載で最も顕著な差異は中肋の有無と囊果の形である。C. AGARDH の記載には中肋について触れられていないし、また図にも示されていない。J. AGARDH は藻体の下部において皮層が発達することによって中肋が形成されることを明確に示している。囊果の形は C. AGARDH の記載では卵形であるのに対し、J. AGARDH のそれは RUPRECHT (1850) を引用して壺形であるとしている。このことから、AGARDH 父子はそれぞれ別の標本にもとづいて記載したと考えざるを得ない。J. AGARDH の記載は RUPRECHT (1850) の *Atomaria setacea* のそれと多くの点で一致する。RUPRECHT (1850) は MERTENS の *Fucus aleuticus* とそれにもとづいたはずの



Figs. 2-5. *Odonthalia setacea* (RUPR.) PEREST.

2, 3. Cross section of the middle portion of a first order branch which issues from the lower portion of the main stem, showing well-developed midribs (arrows). 4, 5. Cystocarps (an arrow indicates a very short calcar in Fig. 5). All photomicrographs from the specimen shown in Fig. 1. Scale in 2 applies also to 4 and 5.

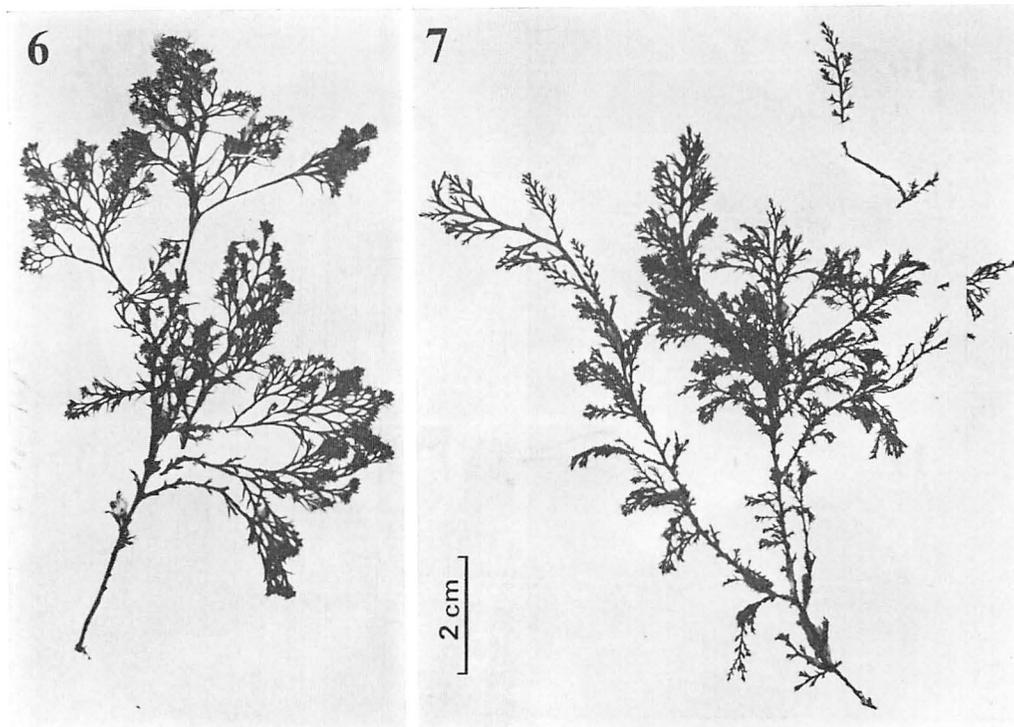


Fig. 6. Type specimen of *Odonthalia annae* PEREST. collected from Iturup Island, Kuriles on July 19, 1967 by L. PERESTENKO.

Fig. 7. Tetrasporangial specimen of *Odonthalia aleutica* sensu OKAMURA, non J. AG. collected from Robben Island, Sakhalin in July, 1930 by J. TOKIDA (Herb. OKAMURA in SAP). Scale in 7 applies also to 6.

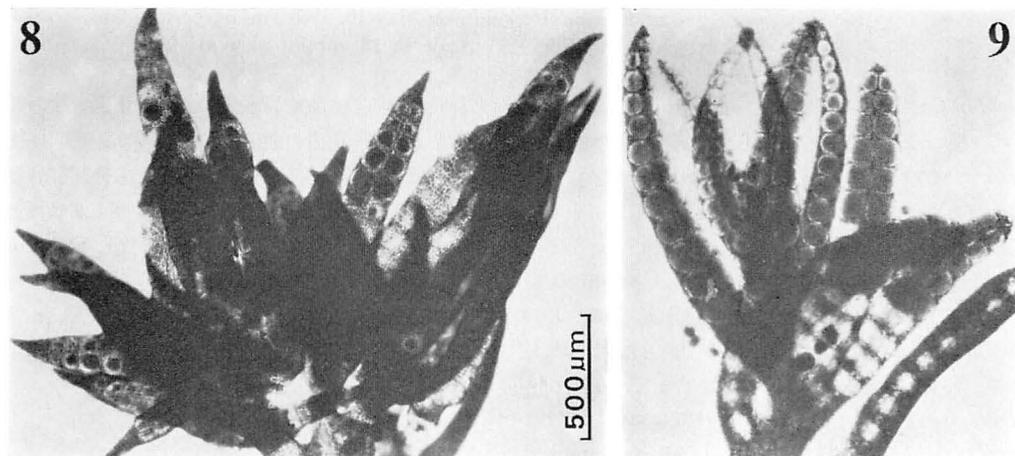
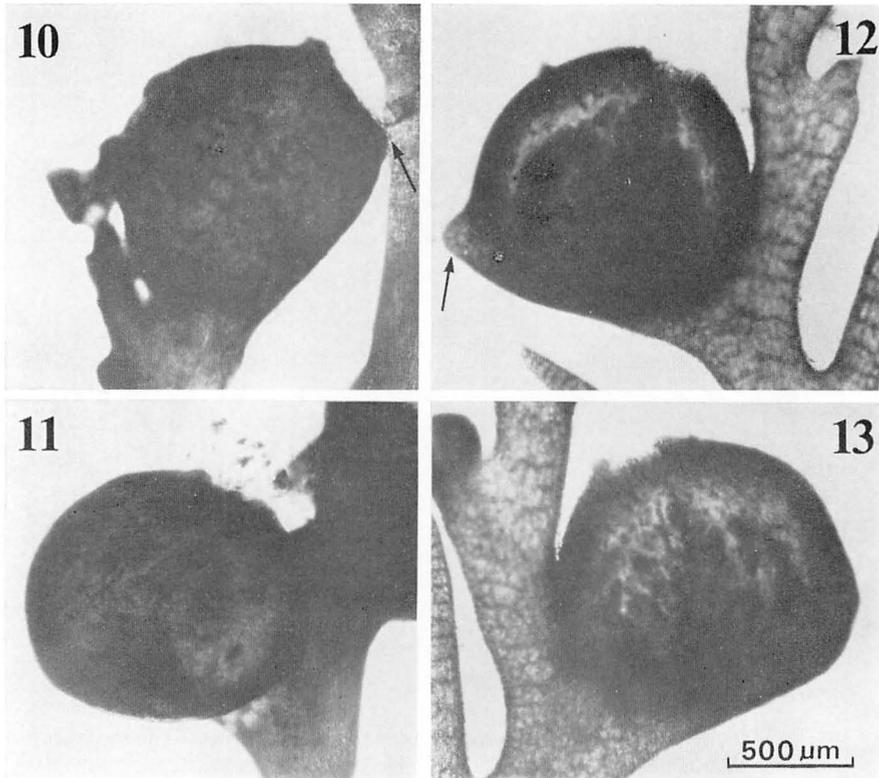


Fig. 8. Tetrasporangial stichidia of *Odonthalia annae* PEREST. (photomicrograph of the type specimen).

Fig. 9. Tetrasporangial stichidia of *Odonthalia aleutica* sensu OKAMURA, non J. AG. (photomicrograph of a specimen collected at Rausu, Hokkaido on May 16, 1968). Scale in 8 applies also to 9.



Figs. 10, 11. Cystocarps of *Odonthalia annae* PEREST. collected from Bering Island, Commander Islands on June 30, 1972 by L. PERESTENKO (an arrow indicates a very short calcar in Fig. 10).

Figs. 12, 13. Cystocarps of *Odonthalia aleutica* sensu OKAMURA, non J. AG. collected at Rausu, Hokkaido on May 16, 1968 (an arrow indicates a short calcar in Fig. 12). Scale in 13 applies also to 10-12.

*Rhodomela aleutica* C. AG.=*Odonthalia aleutica* が同一種ではないことを指摘して、真の *Fucus aleuticus* MERTENS に新しく *Atomaria setacea* と名付けたのであるが、それが正しいことを示していると判断できる。AGARDH 父子の記載のくい違いがどのような理由で生じたのか不明であるが、CHAMISSO によって C. AGARDH に送られた標本が MERTENS の *Fucus aleuticus* とは別のものであった可能性がある (RUPRECHT 1850)。したがって、真の *O. aleutica* の定義は C. AGARDH (1822) に依らなければならない。本種の基準標本は Lund の Botanical Museum に保管されていないとのことであるので (Dr. O. ALMBORN 私信)、現在のところ、C. AGARDH の原記載と図以外に資料は見当たらない。これらとアリウジャンノコギリヒバを比較すると以下の2点で差異が認められる。(1) *O. aleutica* の主軸は円

筒状であるのに対し、後者のそれは下部を除いて扁平である。(2) 前者の囊果の形は卵形であるが、後者のそれは半球形である。両方の形質とも分類学的形質として重要であり、アリウジャンノコギリヒバと真の *O. aleutica* が異なった種であることを示している。*O. aleutica* は RUPRECHT (1850) が指摘しているように、フサノコギリヒバ *O. floccosa* に近いと思われるが、基準標本が見当たらないので正確な決定はできない。

#### 結 論

以上述べたように OKAMURA (1932) 以来、わが国の研究者によってアリウジャンノコギリヒバ *Odonthalia aleutica* と呼ばれてきた種は真の *O. aleutica*、*O. setacea* とも異なり、*O. annae* と同一種と考えられる。和名については OKAMURA (1932) の命名が明

らかに学名にもとづいたもので、*O. annae* の和名としては不適當とも考えられるが、*O. aleutica* が独立した種として認められるまではそのまま使用したい。

本稿の御校閲を戴いた北海道大学理学部黒木宗尚教授、貴重な標本をお送り戴いたコマロフ植物研究所の L. P. PERESTENKO 博士に厚く御礼申し上げます。

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## 日本藻類学会々則

- 第 1 条 本会は日本藻類学会と称する。
- 第 2 条 本会は藻学の進歩普及を図り、併せて会員相互の連絡並に親睦を図ることを目的とする。
- 第 3 条 本会は前条の目的を達するために次の事業を行なう。
1. 総会の開催 (年 1 回)
  2. 藻類に関する研究会、講習会、採集会等の開催
  3. 定期刊行物の発刊
  4. その他前条の目的を達するために必要な事業
- 第 4 条 本会の事務所は会長が適当と認める場所におく。
- 第 5 条 本会の事業年度は 1 月 1 日に始まり、同年 12 月 31 日に終る。
- 第 6 条 会員は次の 4 種とする。
1. 普通会员 (藻類に関心を持ち、本会の趣旨に賛同する個人で、役員会の承認するもの)。
  2. 団体会員 (本会の趣旨に賛同する団体で、役員会の承認するもの)。
  3. 名誉会員 (藻学の発達に貢献があり、本会の趣旨に賛同する個人で、役員会の推薦するもの)。
  4. 賛助会員 (本会の趣旨に賛同し、賛助会員会費を納入する個人又は団体で、役員会の推薦するもの)。
- 第 7 条 本会に入会するには、住所、氏名 (団体名)、職業を記入した入会申込書を会長に差出すものとする。
- 第 8 条 普通会员は毎年会費 4,000 円 (学生は 2,500 円) を前納するものとする。但し、名誉会員 (次条に定める名誉会長を含む) は会費を要しない。外国会員の会費は 5,000 円とする。団体会員の会費は 5,000 円とする。賛助会員の会費は 1 口 15,000 円とする。
- 第 9 条 本会には次の役員を置く。
- 会長 1 名 幹事 若干名 評議員 若干名 会計監事 2 名
- 役員任期は 2 カ年とし重任することが出来る。但し、会長と評議員は引続き 3 期選出されることは出来ない。役員選出の規定は別に定める (付則第 1 条～第 4 条)。本会に名誉会長を置くことが出来る。
- 第 10 条 会長は会を代表し、会務の全体を統べる。幹事は会長の意を受けて日常の会務を行う。会計監事は前年度の決算財産の状況などを監査する。
- 第 11 条 評議員は評議員会を構成し、会の要務に関し会長の諮問にあずかる。評議員会は会長が招集し、また文書をもって、これに代えることが出来る。
- 第 12 条
1. 本会は定期刊行物「藻類」を年 4 回刊行し、会員に無料で頒布する。
  2. 「藻類」の編集・刊行のために編集委員会を置く。
  3. 編集委員会の構成・運営などについては別に定める内規による。
- (付 則)
- 第 1 条 会長は国内在住の全会員の投票により、会員の互選で定める (その際評議員会は参考のため若干名の候補者を推薦することが出来る)。幹事は会長が会員中よりこれを指名委嘱する。会計監事は評議員会の協議により会員中から選び総会において承認を受ける。
- 第 2 条 評議員選出は次の二方法による。
1. 各地区別に会員中より選出される。その定員は各地区 1 名とし、会員数が 50 名を越える地区では 50 名までごとに 1 名を加える。
  2. 総会において会長が会員中より若干名を推薦する。但し、その数は全評議員の 1/3 を越えることは出来ない。
- 地区割は次の 7 地区とする。北海道地区。東北地区。関東地区 (新潟、長野、山梨を含む)。中部地区 (三重を含む)。近畿地区。中国・四国地区。九州地区 (沖縄を含む)。
- 第 3 条 会長、幹事及び会計監事は評議員を兼任することは出来ない。
- 第 4 条 会長および地区選出の評議員に欠員を生じた場合は、前任者の残余期間次点者をもって充当する。
- 第 5 条 会員がバックナンバーを求めるときは各号 1,000 円とし、非会員の予約購読料は各号 1,500 円とする。
- 第 6 条 本会則は昭和 55 年 1 月 1 日より改正施行する。

## 紅藻サエダの生長、成熟におよぼす温度と照度の影響

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NOTOYA, M. and SAITO, Y. 1980. Life history, growth and environmental condition for reproduction in *Microcladia elegans* OKAMURA (Ceramiales, Rhodophyta). Jap. J. Phycol. 28: 191-195.

The life cycle of *Microcladia elegans* OKAMURA was completed in culture and revealed to be the "Polysiphonia-type". Growth and reproduction were examined under several conditions of temperature and light intensity using unialgal cultures. Optimum conditions appear to be near 15°C to 20°C and 500 to 8000 lux. Under the above conditions, the life history was completed in 3-4 months under a 12:12 photoperiod in modified GRUND medium.

*Key Index Words:* Ceramiales; culture; growth; life cycle; light; *Microcladia elegans*; reproduction; Rhodophyta.

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*Microcladia elegans* OKAMURA サエダは函館の立待岬で3月から9月頃、打ち揚げとしてよく見られ、成熟体は4月下旬から5月上旬に多い。そこで四分胞子体から胞子を得、生活史を完結させるとともに種々の温度、照度条件下で培養し、生長と成熟におよぼす影響について調べることもできたのでここに報告する。

### 材料と方法

採集は函館の立待岬で1979年1月から11月まで30数回にわたって実施したが、培養に用いたのは1979年5月23日に得た四分胞子体である。四分胞子の採集と培養方法は前報(能登谷 1979)と同様に行ない、単藻培養とした。初めに生活史の完結のため温度10°C, 15°C, 20°Cの3段階とし、照度は1000 luxから4000 luxの間で1日12時間照明の条件下で培養してみた。その後、ここで得た四分胞子と果胞子を用いて温度と照度が発芽体の生長、成熟におよぼす影響を調べるため、温度を5°C, 10°C, 15°C, 20°C, 25°C, 30°Cの6段階とし、500 lux, 1000 lux, 2000 lux, 4000 lux, 8000 luxの5段階の照度を組み合わせ、計30条件を設定した。各条件の発芽体は7日目ごとに最もよく生長した体について高さを測定し、成熟についても調べた。藻体の成熟に関しては、雄は体表面に精子器が形

成された時、雌は受精毛の発出が確認された時、更に囊果の形成が見られた時、また四分胞子体では藻体中に四分胞子囊が形成された時をそれぞれの成熟時期として記録した。培養液はGRUND 改変培地(MCLA-CHLAN 1973)を用い、測定時に全量を換水した。

### 結果と考察

#### 1. 生活史

培養温度は前記のように10°C, 15°C, 20°Cの3段階で行なったが、最も速く生活史を完結したのは20°Cであったので、以下に20°Cでの発生経過について記す。

野外で採集した体から得た四分胞子の直径は43~59 μm, 平均51.7 μmで、球形の深紅色を呈していた(Fig. 1-A)。胞子は数時間後に基質のスライドグラスに附着し、約24時間後には発芽を開始して仮根と直立部の伸長が見られ、他の多くのイギス目植物と同様に直立型(猪野 1947)の発生を示した(Fig. 1-B)。そして発芽後7日目には高さ0.4 mm前後の体に生長し、葉状を呈した(Fig. 1-C)。30日目には高さ3~4 mmに生長し、体のやや先端寄りの表層細胞が細分して雄性器官の成熟が確認された(Fig. 1-D)。精子器は帯状に形成され、その部分はわずかに盛り上り、色彩もうすれる(Fig. 1-D, E)。この時期に他の発芽体

には体の先端付近から受精毛を発出するものが見られ雌性体の成熟が認められた (Fig. 1-F)。発芽後 35 日目には、ほとんどの発芽体は成熟し、雌雄の両配偶体の比率は約 1:1 であった。発芽後 60 日目には、成熟した囊果の形成が観察された (Fig. 1-G)。これら囊果を形成した体は新しい培養液を満した容器に移して培

養を続けたところ、翌日に果胞子の放出が見られた (Fig. 1-H)。果胞子は深紅色の球形で、四分胞子の直径よりわずかに大きく、直径 48~57  $\mu\text{m}$  で平均は 53.6  $\mu\text{m}$  となったので、本種でも果胞子は四分胞子より大きいという傾向が明らかになった。果胞子の発生過程は四分胞子の場合とはほぼ同様に進み (Fig. 1-I, J)、発

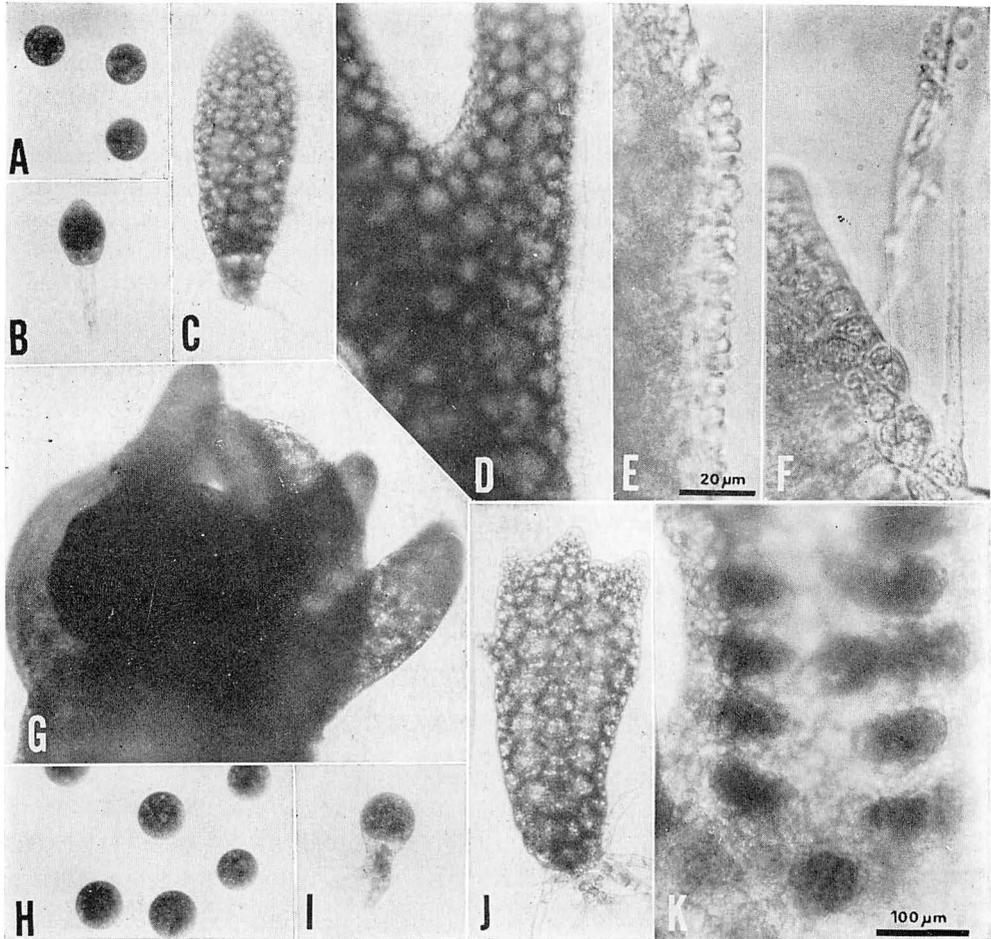


Fig. 1. Life history of *Microcladia elegans* OKAMURA in culture.

A-G. Successive stages of male and female gametophyte formation from tetraspore germlings. A. Liberated tetraspores from a natural specimen. B. One-day-old germling with rhizoid. C. Seven-day-old germling showing shape of blade. D. Thirteen-day-old male gametophyte in mature stage. E. Close up of a part of male gametophyte showing spermatangia at the blade edge. F. A part of female gametophyte showing trichogyne. G. Sixty-day-old carposporophyte showing mature gonimoblast.

H-K. Successive stages of tetrasporophyte formation from carpospore germlings. H. Carpospores liberated from a cultured specimen. I. One-day-old germling. J. Seven-day-old germling showing shape of blade. K. Thirtyfive-day-old germling showing mature tetrasporangia. Use scale in K for A-D and G-K. Use scale in E for E and F.

芽後 35 日目には高さが 4~5 mm に達し、発芽体の先端寄りに四分孢子囊の成熟が観察された (Fig. 1-K)。四分孢子囊は初めは白く透明な部位が 2 列に形成され、下方から次第に暗紅色に変わり、孢子囊の成熟が認められ、やがて成熟した孢子囊から孢子が放出される。その孢子は野外で採集した体から得たものと形状、色彩ともに差異は認められなかった。

以上の結果から、サエダはこれまで報告されている多くのイグス目の種 (UMEZAKI 1977) と同様に、完全なイトグサ型の生活史を示すことが明らかになった。

## 2. 生長と成熟に及ぼす温度と照度の影響

上記の培養によって得られた四分孢子と果孢子を用いて、温度や照度が生長、成熟に及ぼす影響を調べた。

培養温度は 5°C から 30°C の範囲で行なった。果孢子と四分孢子とも 30°C ではどの照度条件でも 2~4 日間で枯死した。低温の 5°C では枯死することなく、

低照度条件におけるほど速く生長するが、500 lux から 8000 lux までの照度下の発芽体は直立体の長さが 70 日間の培養でも 90~180  $\mu\text{m}$  に達するのみで、他の温度条件のものに比べて生長は極端に遅い。果孢子と四分孢子それぞれの発芽体の 10°C から 25°C における生長と成熟は Fig. 2 と Fig. 3 に示した通りである。これで明らかなように、両性孢子の生長過程はともに似た傾向を示した。すなわち、10°C、15°C、20°C では発芽後 5~7 週目までは各照度条件とも生長に大きな差は見られないが、培養期間が長くなるにつれて低照度で高い生長率が見られ、25°C では 3~4 週目頃から各照度条件による差が大きくなる。発芽体の形態は一般に高温では体の幅が狭く、低温では広い。また、高照度におけるほど枝分れの多い体となり、低照度で分枝の少ない棒状の体となる傾向が明らかであった。更に体色は、高照度におけるほど黄色が強くなり、温度と照度による形態、体色の変化が顕著であった。

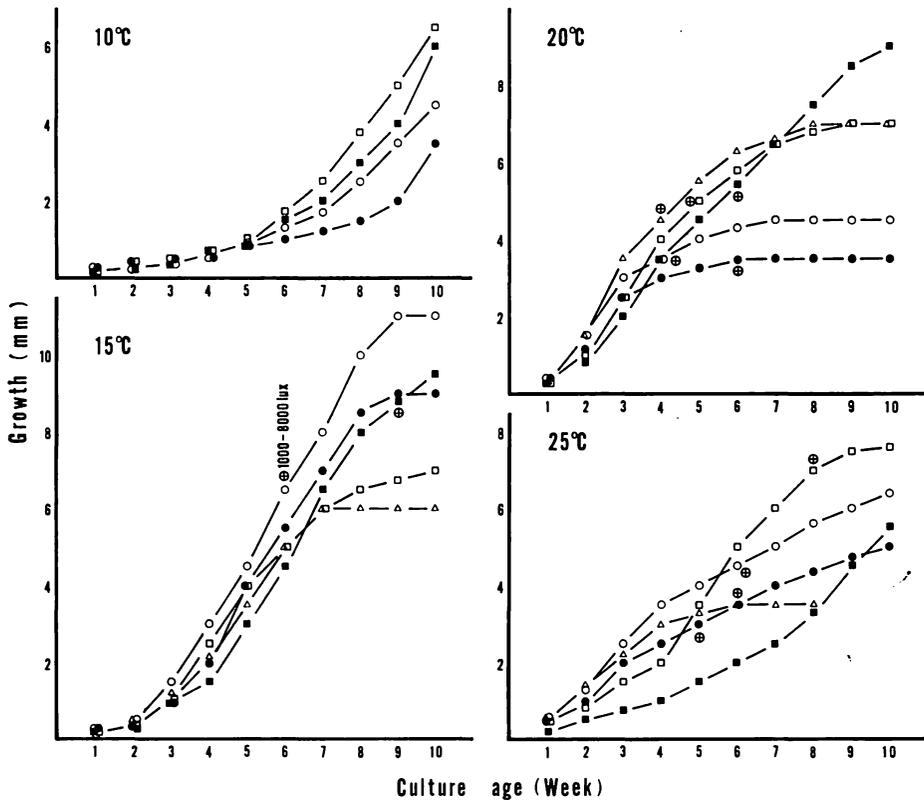


Fig. 2. Growth of carpospore gemlings of *Microcladia elegans* OKAMURA under various temperatures and light intensities. Closed rectangle, 500 lux; open rectangle, 1000 lux; closed circle, 2000 lux; open circle, 4000 lux; open triangle, 8000 lux.  $\oplus$  indicates the presence of mature tetrasporangia.

果孢子発芽体 (Fig. 2) の成熟時期は 20°C の 4000 lux, 8000 lux のやや高照度条件下で最も早く, 孢子発芽後 4 週目に成熟が認められた。次いで 5 週目に 25°C の 2000 lux と 20°C の 1000 lux の条件下のものが成熟し, 6 週目になると 15°C の 1000~8000 lux, 20°C の 2000 lux と 500 lux, 25°C の 4000 lux, 8000 lux のものが成熟した。更に 10 週目までには 15°C から 25°C までのほとんどの条件下で成熟が見られた。一方, 10°C における発芽体では 8000 lux の高照度条件のものは 2 週目までに枯死するが, 他の条件では生育が見られた。しかし, 発芽後 10 週目に至っても四分孢子囊の形成は観察できなかった。そこで 10°C の条件下のものはその後も培養を続けたところ, 2 カ月

後には各照度とも四分孢子囊の形成が見られ, 結局 4 カ月半の培養期間で 10°C から 25°C に至るすべての温度, 照度条件において成熟し, 孢子の放出が見られたことになる。

四分孢子発芽体 (Fig. 3) の成熟については, 孢子放出後 4 週目に 20°C の 1000~8000 lux と 25°C の 1000~8000 lux で雌雄両性株が成熟し, 5 週目には 15°C の 1000~4000 lux, 20°C の 500 lux でも成熟に至ったので, 15°C の 500 lux, 25°C の 500 lux を除くすべての条件で精子器の形成と受精毛の発出が認められたことになる。囊果の形成は照度条件にかかわらず, 20°C では 8~9 週目に, 15°C では 9 週目に観察されたが, 25°C では 10 週目に至っても囊果を形成した

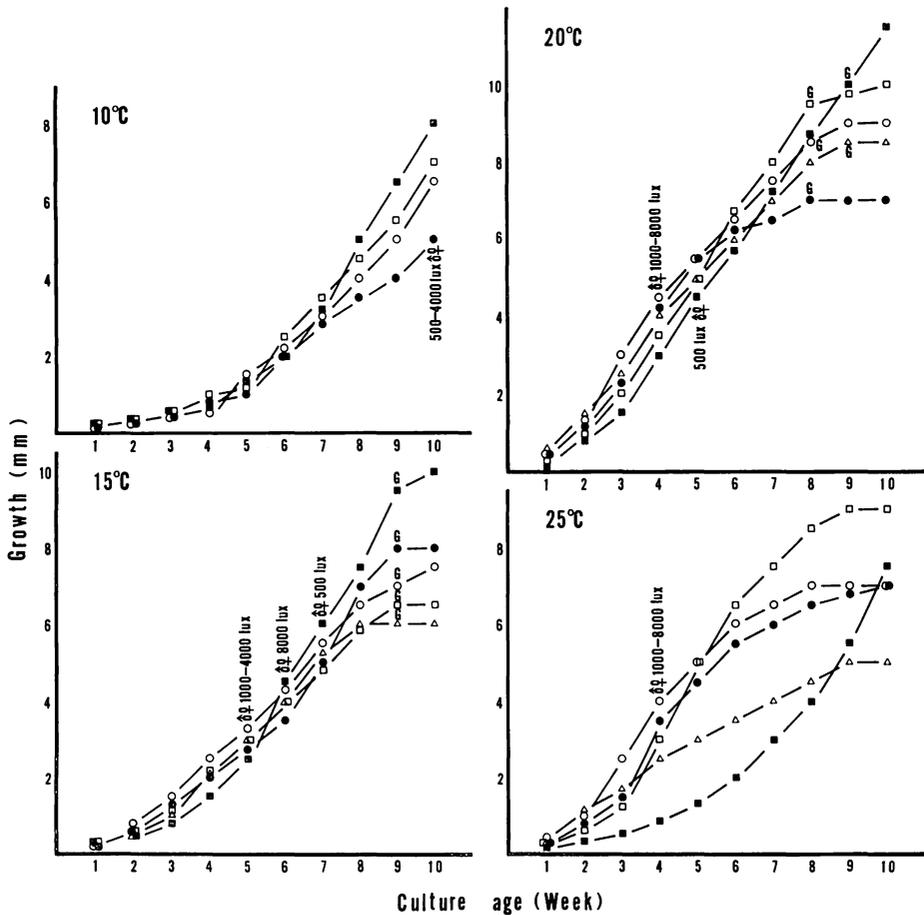


Fig. 3. Growth of tetraspore germlings of *Microcladia elegans* OKAMURA under various temperatures and light intensities. Closed rectangle, 500 lux; open rectangle, 1000 lux; closed circle, 2000 lux; open circle, 4000 lux; open triangle, 8000 lux. ♂ and ♀ indicate the presence of mature spermatangia or trichogynes. G indicates gonimoblasts presence.

体は得られなかった。そこで更に2カ月間培養を継続したが、生殖器官を形成することはなかった。10°Cでの発芽体では8000 luxを除く500~4000 luxの条件下で10週目に及んで雌雄の生殖器官の成熟が認められた。その後2カ月間培養を継続した結果、1000 luxで1個の嚢果が認められただけであった。

以上のように、サエダは培養温度25°C以下で生育することはできるが、15°Cから20°Cが生長、成熟の速い条件と考えられる。中でも最も短期間に生活史を完結させた条件は20°C、4000 luxで、その期間は3カ月であった。逆に成熟の遅い条件は5°Cで、成熟は確かめられなかった。10°C、1000 luxで9カ月間の培養期間を必要とし、条件による生活史完結のための期間に著しい差異が見られた。また、25°Cの条件では、四分孢子嚢と雌雄生殖器官の成熟を認めることはできたが、嚢果の成熟は見られないので、25°Cは生育できる温度条件の上の限界に近いものと考えられる。

これら種々の条件下で得られる藻体の形態、色彩を

本種の採集された立待岬における天然の藻体と比較すると、本培養条件の10~15°C、1000~2000 luxで生長した体とよく類似することから、当地での生育環境はこれに近いものと推測される。

本研究に際し、材料の採集その他で御助力をいただいた赤城敏正氏に謝意を表します。

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学 会 録 事

新 入 会

住 所 變 更



## 投 稿 案 内

**I. 編集の方針** 本誌には藻学と応用藻学に関する会員の未発表の論文・速報・総説、その他雑報(分布資料・ニュース・新刊紹介・抄録など)を掲載します。論文・速報はデータや考察の独創性の有無に重点を置いた編集委員会の審査を経た後に受理されます。原稿の取捨、掲載順序、体裁などは編集委員会および編集幹事で決めます。論文と速報は和文または英文とし、その他は和文を原則とします。論文と総説は刷上り6頁、速報は2頁、雑報は原則として2頁以内を無料とします。頁の超過は制限しませんが、頁の超過分(1頁7,000円)、折込み、色刷りなどの費用は著者負担となります。和文原稿では5枚が、英文原稿では2枚が刷上り1頁となる見当です。

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

例: *Batrachospermum ectocarpum* Sirod., Discussion, sec, min, hr, nm,  $\mu$ m, mm, cm, m,  $\mu$ l, ml, l,  $\mu$ g, mg, g, N, M, ppm, lux, g (gravity), 25°C など

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(単行本) ①, ② 共通 広瀬弘幸<sup>1)</sup> 1959.<sup>2)</sup> 藻類学総説.<sup>3)</sup> 内田老鶴圃, 東京.<sup>4)</sup>

(単行本中の1章) ① DREBES, G.<sup>1)</sup> 1977.<sup>2)</sup> Sexuality,<sup>3)</sup> p. 250-283.<sup>4)</sup> ② In D. WERNER (ed.),<sup>4)</sup> The biology of diatoms.<sup>3)</sup> Blackwell Sci. Pub., London.<sup>4)</sup>

(叢書中の分冊) ① HUSTEDT, F.<sup>1)</sup> 1930.<sup>2)</sup> Bacillariophyta.<sup>3)</sup> ② In A. PASCHER (ed.),<sup>4)</sup> Süßwasser-Flora Mitteleuropas. ed. 2. vol. 10.<sup>3)</sup> Gustav Fischer, Jena.<sup>4)</sup>

(雑誌の中の1論文) ① 森 通保<sup>1)</sup> 1970.<sup>2)</sup> *Batrachospermum ectocarpum* SIROD. の分類学的研究.<sup>3)</sup> ② 藻類 8<sup>3)</sup>: 1-8.<sup>4)</sup>

① KOBAYASI, H. and ANDO, K.<sup>1)</sup> 1978.<sup>2)</sup> New species and new combinations in the genus *Stauroneis*.<sup>3)</sup> ② Jap. J. Phycol. 26<sup>3)</sup>: 13-18.<sup>4)</sup>

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