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PHOTOSYNTHETIC PIGMENT COMPOSITION OF ICE ALGAL AND PHYTOPLANKTON ASSEMBLAGES IN EARLY SPRING IN SAROMA KO LAGOON, HOKKAIDO, JAPAN

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Abstract: Ice algae were collected from sea ice of 35 cm thickness at Saroma Ko lagoon in early February, 1996 The sea ice was sectioned into 5 cm height intervals from the bottom so as to separate ice algae according to vertical distribution. Phytoplankton were also harvested at the same site in mid-April, 1996, a few days after the sea ice disappeared. Photosynthetic pigments of ice algae and phytoplanktonic algae were analyzed by reverse-phase HPLC (high performance liquid chromatography). Chlorophyll *a*, *c*, β-carotene, and several kind of carotenoids, such as fucoxanthin, diadinoxanthin or diatoxanthin, were detected, but chlorophyll *b* was not recognized, suggesting that diatom species were predominant in ice algae and phytoplanktonic algae. The pigment composition of ice algae changed according to their vertical distribution The ratios of chlorophyll *c* and carotenoids to chlorophyll *a* in ice algae harvested from the top of the sea ice were higher than those of ice algae collected from the lower position, in other words, ice algae in the lower position, *i.e.*, where they experienced less light decreased the ratio This suggests that ice algae adopted very peculiar light-shade adaptation, because vascular plant or green algae have the opposite response so that they decrease the ratio of auxiliary pigments to chlorophyll *a* with increasing light intensity Pigment composition of phytoplanktonic algae resembled that of ice algae collected from the uppermost portion of sea ice.

key words: ice algae, photosynthetic pigments, reverse-phase HPLC, diatom, light-shade adaptation

Introduction

Massive ice algal growth is commonly observed in brine pockets or channels formed mainly in the bottom of sea ice (WELCH and BERGMANN, 1989; POULIN, 1990). Accordingly, ice algae have to perform photosynthesis under very severe circumstances such as sub-zero temperature and low light intensity. Light intensity at the bottom of sea ice where ice algae mainly exist is severely lowered, below 1% compared to the light

Abbreviations: Chl; chlorophyll, HPLC; high performance liquid chromatography, LHC, light harvesting complex, PS I and PS II, photosystem I and II, PSU, photosynthetic unit, RT, retention time

intensity on the surface of sea ice (SooHoo *et al.*, 1987; KISHINO, 1993; KUDOH, 1994; KUDOH *et al.*, 1997). Moreover, the incident light intensity fluctuates considerably because of the variable weather, growing sea ice, accumulating snow and so on. However, they carry out active photosynthesis so as to largely support a fertile ecosystem in the high latitude ocean as a primary producer (HORNER, 1990; PRIDDLE 1990).

Why are they able to perform active photosynthesis against adverse circumstances? Only limited investigations have been reported concerning the fundamental mechanism of photosynthesis and its regulation of ice algal photosynthetic apparatus, which works properly regardless of the non-satisfactory environment.

In this work, for the first step to understand the characteristics of ice algal photosynthesis, we investigated how ice algae regulate the light harvesting system, which performs the primary step in executing photosynthesis, in relation to the change of surrounding light intensity. Ice algae were collected at Saroma Ko lagoon and classified by the vertical position they occupied. The light history they experienced should be different so that one from the upper region in the original sea ice received higher light irradiance than another from the bottom region. The pigment compositions were analyzed by HPLC and compared with one another.

Materials and Methods

Sampling procedure

Ice algal samples were collected from sea ice of 35 cm thickness using an Ice Core Drill (inside diam. 7.5 cm) under 5–8 cm snow cover in the eastern basin of Saroma Ko lagoon (44°07′N, 143°57′E) on 7 February, 1996. Light intensities above/under the sea ice at the sampling site were measured by means of PAR (Photosynthetic Active Radiation) spherical and 2π sensors (SPQA and UWQ, LI COR,) at the same time. Cored sea ice was immediately sectioned in every 5 cm height from the bottom so as to be separated into seven sections, and each sliced sample was stored in an opaque container. Those sliced samples were crushed and allowed to melt for several hours under room temperature. 250 m*l* of each melted sample was filtered onto a glass fiber filter (GF/F, Whatman), and those were stored at -85°C until further analysis. Another portion was used for microscopic observation to check the dominant algal species.

A phytoplankton sample was collected in the eastern basin of this lagoon on 17 April, 1996, a few days after the sea ice was melted/broken away, on board the R/V MICHI-SHIO. A vertical haul by a NORPAC net (45 cm opening diam.) equipped with XX13 netting (mesh size, 0.095 mm) from the depth of *ca.* 10 m, nearly double the depth of the Secchi disk reading, was used for the present study. The dominant phytoplankton was microscopically checked, and the remaining portion was collected onto the glass fiber filters (GF/F, Whatman) and stored at -85°C until further analysis.

Pigment analysis

In order to determine the extraction and elution conditions of diatom pigments for this HPLC analysis, an isolated diatom, *Chaetoceros gracilis*, which was maintained in an enriched seawater medium of modified Provasoli ES (MCLACHLAN, 1973) at $50\,\mu E \cdot m^{-2} \cdot s^{-1}$ with a 14L/10D light cycle at 25°C in the logarithmic growth phase,

was used.

Samples of those algae were freeze-dried in the dark and the pigments were extracted by 0.5 m*l* of methanol in combination with sonication for 1 min (Branson 2200) under dim light at room temperature (but see Results and Discussion). They were centrifuged ($8000 \times g$ for 5 min) and 100–180 μl of the resulting supernatants were subjected to HPLC analysis immediately. Reverse-phase HPLC-analysis was carried out according to the method described by VELDHUIS and KRAAY (1990) with some modification. It was performed by Shimadzu LC-10AD with system controller SCL-10A equipped with Prodigy 5 (ODS 3, 100A) column (150 × 4.60 mm) of Phenomenex (Torrance, California), equilibrated with 80% methanol containing 0.02 M ammonium acetate (Solvent A) and eluted with a gradient of 0 to 100% of ethyl acetate/methanol (30/70, v/v, Solvent B) as described below at a flow rate of 0.8 m*l* min^{-1} over a period of 49 min. The ratio of Solvent B increased linearly up to 25% in the first four min (6.25%/min); then gradually increased up to 90% until 37 min (2%/min) and finally 100% at 39 min (5%/min). The ratio of 100% of Solvent B was kept for 10 min so as to elute highly hydrophobic pigments such as pheophytin or β-carotene. The two steeply increasing segments (from 0 to 4 min and from 37 to 39 min) were for time saving since no pigments were recognized in these periods in the present study. Absorption of pigments was detected by Shimadzu photodiodearray detector SPD-M10AV, which was equipped with a W-lamp to provide a measuring beam so as to permit measurement with higher reliability up to the red light region, at wavelengths ranging from 250 nm to 750 nm, and analyzing software Shimadzu CLASS-M10A.

The standard pigments fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene (Water Quality Institute, Denmark) were also subjected to HPLC analysis under the same conditions mentioned above, to identify and quantify the separated algal pigments by comparing retention times and absorption patterns. Chlorophyll concentrations were also estimated by the method of JEFFREY and HUMPHREY (1975).

Results and Discussion

Field study

Sea ice formation in the lagoon started from early January and the lagoon surface was entirely ice-covered by 1 February in this year. Several days before our sampling, seawater infiltrated between snow and the sea ice surface (FUJIYOSHI, personal communication); however, it was frozen by 7 February because cold weather followed.

Ice algal assemblages were visually recognized at the top and bottom parts of collected sea ice at that time. The top part was colored strongly in brown and the bottom few cm was a light golden-yellow color. The algae in the top sea ice seemed to belong to the so-called 'infiltration community' growing *in situ* when seawater invaded the snow-ice interface, and it created a favorable environment for organisms (HORNER *et al.*, 1992). The latter algae were found spreading among sea ice crystals at the ice bottom surface, so were classified as an 'interstitial community'.

Irradiance at the snow surface was *ca.* 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$, reflection was *ca.* 950 $\mu E \cdot m^{-2} \cdot s^{-1}$, and irradiance reaching the sea ice bottom was only 5 $\mu E \cdot m^{-2} \cdot s^{-1}$. Therefore, the infiltration and the interstitial communities seemed to experience *ca.* 5%

and 0.5% of the surface irradiance level, respectively.

Microscopic observation revealed that diatom species dominated in both ice algal and phytoplankton communities. *Detonula confervacea* (centric diatoms) and ribbon-shaped colony forming *Navicula* and *Nitzschia* spp. (pennate diatoms) were the dominant ice algal species (*cf.* KAWANOBE-KIKUCHI and KUDOH, 1995). Centric diatoms dominated in the top part; however, pennate species dominated in the lower parts. In the phytoplankton samples, *Chaetoceros* spp., *Thalassiosira* spp. (centric diatom) and the former ice algal pennates contributed the major part of the algal biomass.

Determination of HPLC analysis conditions

Photosynthetic pigments of ice algae and phytoplankton were analyzed by means of HPLC. Before analysis, the best way to extract pigments was determined by preliminary examination of cultured marine diatoms, *Chaetoceros gracilis*, because this process would seriously affect the qualitative and quantitative results of the following HPLC analysis. The cultured sample (50 μl of *ca.* 120 μgChl·ml^{-1}) was freeze-dried because the water might prevent the full extraction of the pigments (but see the latter discussion). Various extraction solvents and procedures were adopted depending upon the types of algae so as to extract lipid-soluble pigments efficiently without denaturation (summarized by ROWAN, 1989). Many extraction solvents used for the previous pigment analysis mentioned in ROWAN (1989), except extremely volatile solvents such as ether, were checked; we finally adopted methanol extraction in combination with sonication under dim light at room temperature. Methanol gave the highest extraction efficiency for all extracted pigments in these samples and quite good reproducibility.

Figure 1 shows a 3-D view of the typical elution pattern of pigments extracted

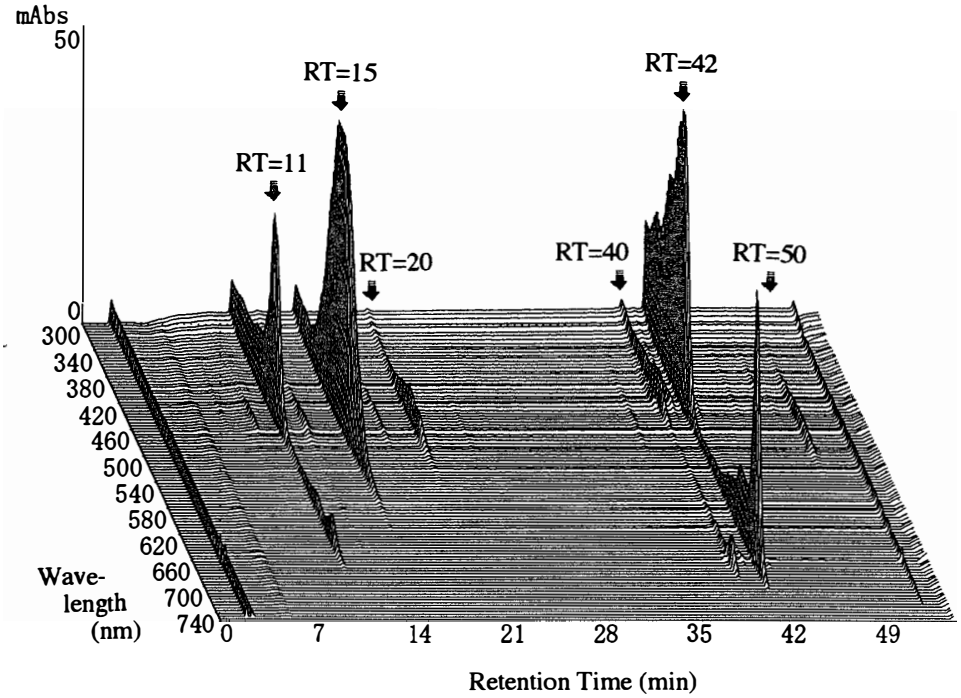

Fig. 1. A typical profile of HPLC analysis represented in 3-D view.
Pigments extracted from cultured *Chaetoceros gracilis* were tested. See text for detail.

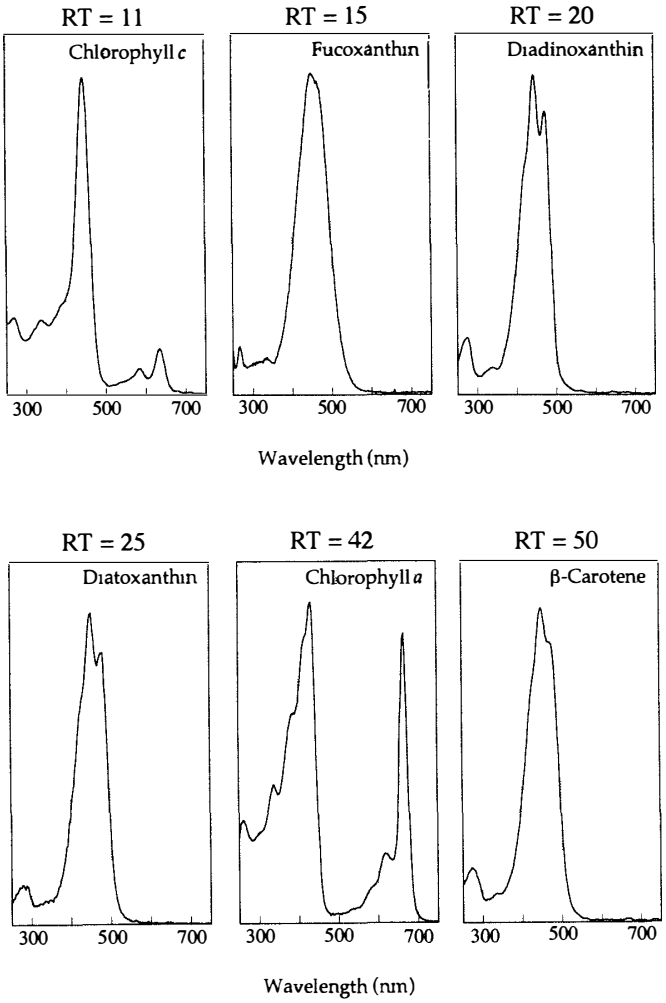

Fig. 2. Absorption pattern of each pigment separated from cultured *Ch gracilis* in Fig. 1.

from the cultured diatoms. Six large and several minor pigments were separated. According to their absorption patterns and retention times, those pigments could be identified. Pigments eluted around 11 min (retention time, RT = 11 min), 15 min, 20 min, 25 min, 42 min and 50 min were chlorophyll (Chl) *c*, fucoxanthin, diadinoxanthin, diatoxanthin, Chl *a* and *β*-carotene, respectively (Fig. 2). Signals found at RT = 39–41 min showed the same absorption spectrum as Chl *a* (RT = 42 min), but a slightly hydrophilic state. The other minor signals retained at 9, 12, 18 min were thought to be degraded Chls since they have large absorption both around the blue and red regions, but those pigment quantities were negligible in the cultured sample. If there were Chl derivatives such as chlorophyllide *a* (phytol-less Chl *a*, which is produced by chlorophyllase), pheophorbide *a* (magnesium- and phytol-lacking Chl *a*) and pheophytin *c* and *a* (magnesium-less Chl *c* and *a*), those should be separated at nearly the same position as Chl *c* (because chlorophyllide *a* has a similar molecular structure to Chl *c*), between Chl *c* and Chl *a*, and later positions of the original pigments in the present elution condition, respectively.

It was clear that every pigment showed significant absorption around 431 nm (Fig.

1), so, signals at 431 nm were routinely monitored to detect the pigments.

Analysis of ice algal and phytoplankton pigments

Ice algal pigments showed nearly the same quality composition as measured in the cultured diatoms. Green algal light-harvesting pigments of Chl *b* and lutein, or alloxanthin and peridinin of other xanthophyll pigment group of the Cryptophyceae and Dinoflagellate, were not detected, confirming the result of microscopic observation that diatom species were predominant. Signals from degraded Chl *a* and Chl *c* were also negligible, as found in the cultured diatoms.

The chromatograms in Fig. 3 indicate that there were significant peaks at 11, 15, 20, 42 and 50 min in all samples, which were Chl *c* (RT=11), fucoxanthin (RT=15), diadinoxanthin (RT=20), Chl *a* (RT=42) and *β*-carotene (RT=50), respectively. Diatoxanthin (RT=25) was detected only in the bottom 0–5 cm sea ice sample. Peaks at RT=8–9 and 39–41 were Chl *c*- and *a*-like pigments (showed the same absorption patterns) which were neither chlorophyllide *a* nor pheophorbides. Those were also detected in the phytoplankton sample, and tended to be found much more in the sample from the upper portion of the sea ice, *i.e.*, where they experienced higher light intensity.

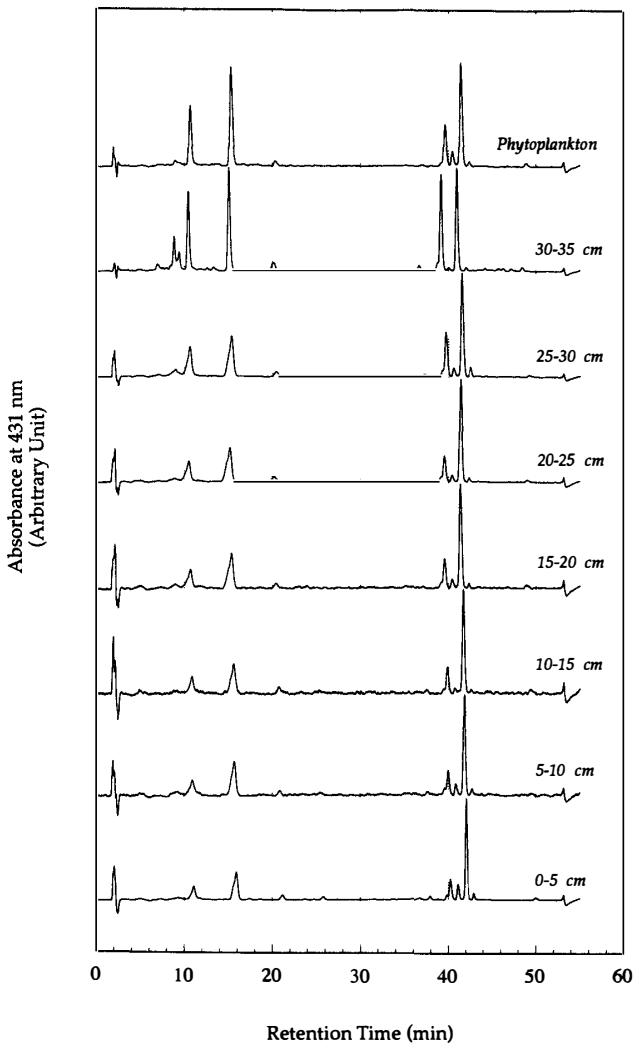

Fig. 3. Chromatogram profiles of ice algal and phytoplanktonic pigments. Chromatograms of ice algae are arrayed according to the vertical position in the sea ice. 0–5 cm represents the chromatogram of pigments extracted from ice algae collected from the bottom of the sea ice, and 30–35 cm is from uppermost level. The chromatograms were adjusted so that the height of Chl *a* would become the same in each chromatogram.

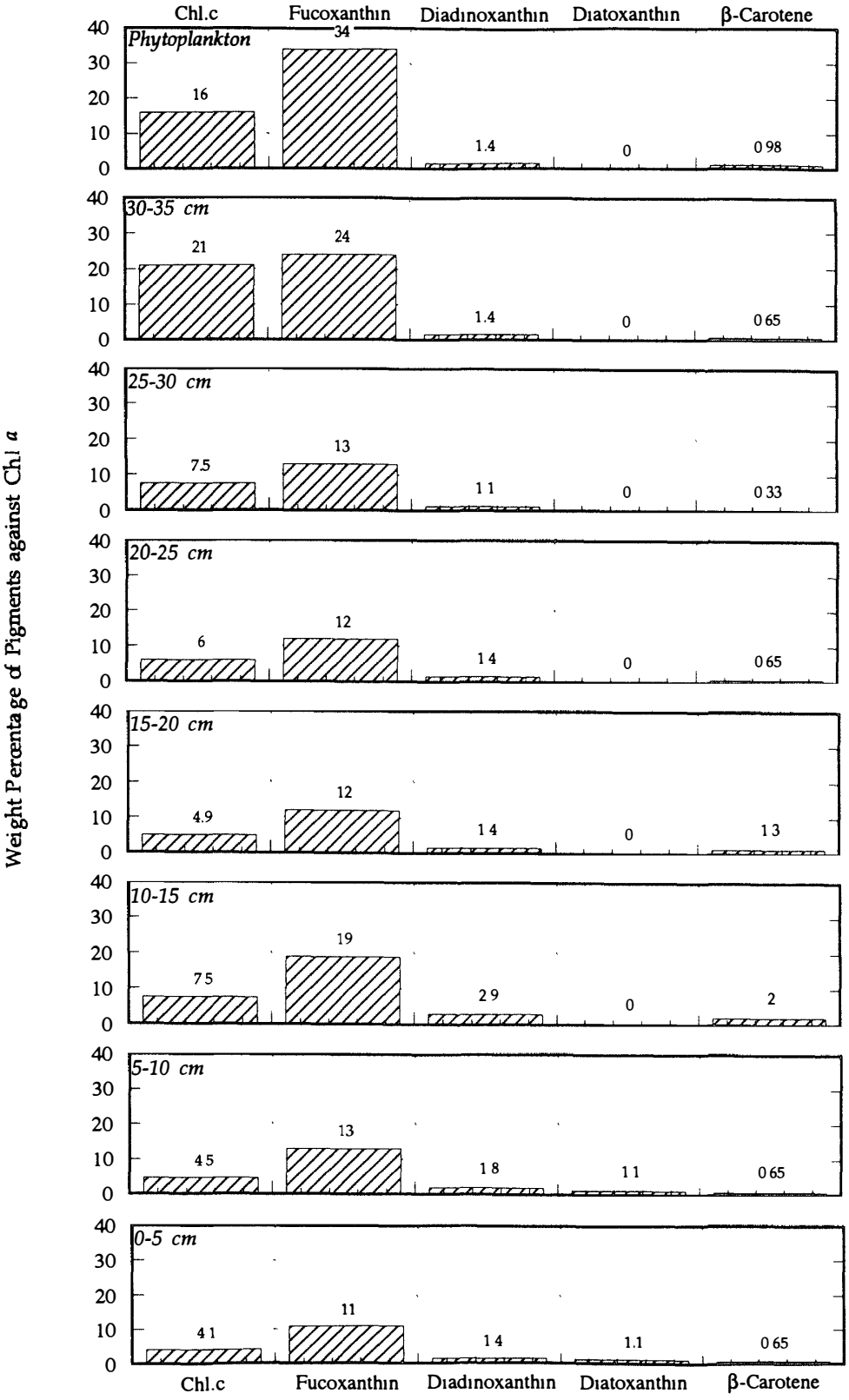

Fig. 4. Pigment compositions of phytoplankton and ice algae according to vertical position. The compositions are expressed in relative weight ratios *vs.* Chl *a*.

Although the origins and the roles of those pigments were unknown, the following quantitative analysis assumed those Chl *c*-like and Chl *a*-like pigments to belong to Chl *c* and Chl *a*, respectively.

Ratios of each pigment to Chl *a* in the ice algal samples showed clear differences between the 30–35 cm sample and the lower ones (Fig. 4). The ratios of Chl *c* and fucoxanthin tended to become higher in the top position. The ratios of those pigments were more than three times larger than those for the sample from the bottom of the sea ice. Diatoxanthin was found in the lower two samples, but the amount was small relative to that of Chl *a*. The ratios of diadinoxanthin and *β*-carotene seemed rather stable at all levels.

Chromatogram of phytoplankton samples, which were collected a few days after sea ice disappearance, showed similar patterns to the ice algal samples from the top of the sea ice (Fig. 3, uppermost). Ratios of Chl *c* and fucoxanthin to Chl *a* were rather large (Fig. 4, uppermost).

Light histories of each sample in the present study should show large differences. Generally, light intensity in the sea ice bottom sample is the lowest, because snow on the sea ice reflects 70–95% of direct sunlight (KUDOH, unpublished data), and most of what remains is absorbed by the snow and sea ice, so that only *ca.* 1% or less of the direct sunlight (photosynthetically active radiation) penetrates to the bottom part of the sea ice (KISHINO, 1993; KUDOH *et al.*, 1997). The light history at the top of the sea ice, therefore, is thought to account for 3–30% of the direct sunlight. Light intensities measured at the sampling site in this study supported this hypothesis. The vertical haul of the water column in April is assumed to have collected phytoplankton that had been exposed to 10–100% light levels because it was taken from the 10% light level depth to the surface.

The ratios of Chl *c* and fucoxanthin to Chl *a* tended to become higher in phytoplankton and upper growing ice algae which might have experienced higher light intensity. Also, the relative Chl *c*/fucoxanthin ratio also showed remarkable variability. The former response indicates that algae (diatoms) which lived in higher light condition increased light harvesting accessory pigments. This seems quite peculiar, and opposite to the response of green vascular plants, green algae and cyanobacteria (MELIS, 1990), which tend to increase such light-harvesting accessory pigments when they experience shady environments.

Earlier work on diatom responses of photosynthetic apparatus indicated that the photosynthetic unit (PSU) size (Chl/PS I) was found to vary inversely with growth irradiance (PERRY *et al.*, 1981), and PS II/PS I stoichiometry also inversely varied (FALKOWSKI *et al.*, 1981). In addition, the Chl *a*-Chl *c*-Fucoxanthin light harvesting complex associated with PS II (SUGAHARA *et al.*, 1971; OWENS and WOLD, 1986) (Light-Harvesting-Complex: LHC, hereafter) seems to vary its size depending upon growth light conditions (SMITH and MELIS, 1988). The latter result, that diatoms grown under different light showed a variable ratio of Chl *c*/fucoxanthin, may suggest flexible control among LHC components. This in turn suggests that diatoms have great flexibility in adjusting their photosynthetic apparatus to the surrounding light environment by changing the numbers of PSU per cell, relative PS II/PS I ratio and PS II associated size of LHC.

If the ice algae changed their PSU size and PS II/PS I ratio as indicated by previous papers (PERRY *et al.*, 1981; FALKOWSKI *et al.*, 1981; SMITH and MELIS, 1988), such that diatoms grown in low light increase both PSU size and PS II/PS I ratio, then the data presented here suggest that diatoms grown in low light have Chl *c* and fucoxanthin-poor LHC, while those grown in bright light have Chl *c*- and fucoxanthin-rich LHC. Diatoms grown in bright light have relatively Chl *c*- and fucoxanthin-rich LHC systems, suggesting that diatoms change it to balance energy distribution between two photosystems. This is because the whole reaction of PS II is slower than that of PS I. Under light-rich condition, to make sufficient electron offer to PS I, it is necessary to speed up the PS II which can be accelerated by energy supply from LHC even if the diatom reduces PSU size and PS II/PS I ratio. Or increased fucoxanthin might simply play a role to protect photosystems from extremely high light intensity. Indeed, the xanthophyll cycle, which dissipates excessive excitation energy, may really exist because non-photochemical quenching was observed in the fluorescence induction kinetics with repetitive application of a saturating light pulse (data not shown, but see DEMMIG-ADAMS, 1990; OLAIZOLA *et al.*, 1994). Further experimental and repetitive sample analyses are necessary to confirm and justify these hypotheses.

Differences of relative pigment compositions observed in this study do not necessary emerge from the response of single algae to the surrounding light environment. Species favored in shade, having much Chl *a* relative to Chl *c* and fucoxanthin, might dominate at the sea ice bottom, or, species having Chl *c*- and fucoxanthin-rich LHC might grow well in the top sea ice and in the water column after disappearance of the sea ice. Species compositions among present samples were not the same, as mentioned previously.

Accordingly, it has become possible to analyze the pigment compositions of ice algae and plankton through this work. One analytical problem remains unsolved; Chl *a* and *c* seem to be modified, especially in algae which grow in higher light intensity. It is highly probable that the algae in higher light might have these different forms of Chls, or they might have uncertain materials which could make Chls denature during the process of pigment extraction adopted in the present study. The recent trend in pigment extraction is not to use methanol, which may cause denaturation of pigments (MANTOURA and LLEWELLYN, 1983). In the present study their recommended solvents were also checked, but extraction efficiency and repeatability were not so good as with methanol. Cross checking using both methanol and other solvents will tell us whether the present result was caused by an artifact. However, it is true that natural diatoms can change there LHC components such that those grown in bright light have Chl *c* and carotenoid-rich LHC, and those grown in lower light have Chl *c* and carotenoids-poor LHC.

For the comparative study of natural algal light-shade adaptation or acclimation, the ice algal community is an excellent natural sample because diatom species comprise almost the total algal biomass and potentially experience quite large light variations among melt water ponds, the snow-sea ice interface, brine pockets, interstitial water, ice bottom, and ice-water interface (summarized by HORNER, 1985).

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