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Comparative analysis of photosynthetic properties in ice algae and phytoplankton inhabiting Franklin Bay, the Canadian Arctic, with those in mesophilic diatoms during CASES 03-04

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Abstract: Psychrophilic phytoplankton and ice algae were collected in Franklin Bay, the Canadian Arctic, in late May 2004, and the photosynthetic properties were measured at 4°C using a pulse amplitude modulation fluorometer (Phyto-PAM). Rapid light curve measurements allowed for the assessment of the photosynthetic efficiency (α), maximal electron transport rate ($rETR_{max}$), and minimum saturating irradiance (E_k) in the samples. The values of α in phytoplankton (0.63–0.68) were much larger than those in ice algae (0.10–0.51), and the values of $rETR_{max}$ in phytoplankton (4.6–6.7) were relatively larger than those in ice algae (1.8–4.3). However, E_k showed similar values in both samples and were around 10 μmol photons·m^{-2}·s^{-1}. These values were systematically compared with those obtained from mesophilic marine diatoms (a centric diatom, *Chaetoceros gracilis*, and a pennate diatom, *Phaeodactylum tricornutum*) grown under various irradiances in the laboratory. The highly shade-adapted features of ice algae and phytoplankton were disclosed through this comparative analysis. It was also found that the non-photochemical quenching was much higher in psychrophilic samples than in mesophilic diatoms grown under moderate irradiance. Furthermore, in ice algae and phytoplankton, the decrease in rETR at high irradiances was prominent, showing that they were highly susceptible to photoinhibition. Our comparative analysis using psychrophilic phytoplankton, ice algae and two strains of mesophilic diatoms also revealed that the dependency on the xanthophyll cycle for the protection mechanisms of photosystems were remarkably different between the groups, indicating that the acclimation strategies to growth irradiances were variable between species. Such variable acclimation strategies could be one of the forces that results in a diverse algal flora that enables this region around Franklin Bay to be a productive area, even though the psychrophilic phytoplankton and ice algae are highly shade-adapted.

[$] These authors contributed equally to this article.

key words: ice algae, phytoplankton, photosynthesis, PAM, Franklin Bay

Introduction

In high latitude regions, psychrophilic phytoplankton and ice algae are the major primary producers. They are performing efficient photosynthesis in an environment apparently harsh for photosynthetic organisms (Arrigo *et al*., 1993, 1995; Gosselin *et al*., 1997; Pomeroy, 1997), with low temperatures (Squire, 1990; Suzuki *et al*., 1997), and low incident irradiance (Arrigo *et al*., 1993; Kishino, 1993; Lizotte and Priscu, 1992; SooHoo *et al*., 1987), all of which have close relationships with sea ice. The photosynthetic properties of these organisms show fundamentally shade-adapted features (Arrigo *et al*., 1993; Suzuki *et al*., 1995, 1997). In the ocean, the light environment fluctuates on a wide temporal scale (*e.g.*, annually, or diurnally) (Kirk, 1994). Especially in high latitude areas, the seasonal variation becomes prominent (Sakshaug, 1990). For photosynthetic organisms, especially for shade-adapted phytoplankton, excess incident irradiance is harmful as it destroys photosynthetic systems (Aro *et al*., 1993; Demmig-Adams, 1990). Under such excessive light conditions, the xanthophyll cycle plays an important role to protect these photosystems (Demmig-Adams, 1990). In diatoms, diadinoxanthin and diatoxanthin are the two components of the xanthophyll cycle (diadinoxanthin cycle) (Arsalane *et al*., 1994; Kashino and Kudoh, 2003; Lavaud *et al*., 2002; Olaizola and Yamamoto, 1994). This protection system is important for psychrophilic phytoplankton and ice algae, because excess light is especially harmful at low temperatures (Sonoike, 1998). The light-shade acclimation of these psychrophiles has been reported (*e.g.* Ikeya *et al*., 2000; Lizotte and Sullivan, 1991; McMinn and Hegseth, 2004; Michel *et al*., 1988). For example, it was observed that, in psychrophilic phytoplankton, the shift from low-light to high-light caused a decrease in photosynthetic efficiency (α), and an increase in maximal photosynthetic rate (P_{max}) and the minimum saturating irradiance (E_k) (Ikeya *et al*., 2000). However, studies on the xanthophyll cycle in psychrophiles are limited (*e.g.* Kashino *et al*., 2002; Kudoh *et al*., 2003; Olaizola *et al*., 1992).

The major populations of these psychrophilic phytoplankton and ice algae are diatoms and flagellates (Gosselin *et al*., 1997; Michel *et al*., 1988). The diatoms are important as the primary producer in the world ocean; they are responsible for around 40% of annual carbon fixation in the world oceans (Nelson *et al*., 1995). In view of this importance, many attempts have been made to understand the photosynthetic properties of mesophilic diatoms (Falkowski, 1983; Falkowski *et al*., 1981; Lavaud *et al*., 2002; Olaizola and Yamamoto, 1994; Strzepek and Harrison, 2004). The light environment for these mesophiles and the spatial and temporal changes of such environment are different from those for psychrophiles (Sakshaug, 1990) (psychrophiles experience a long dark period in the winter season and long light period in the summer season). The light-shade acclimation in mesophiles is reported in terms of the change of photosynthetic properties (Falkowski and Owens, 1980; Falkowski, 1983; Falkowski *et al*., 1981; Strzepek and Harrison, 2004). Falkowski *et al.* reported that *Skeletonema costatum* decreased the photosystem I (PS I) content and the maximal rate of photosynthesis with decreasing growth irradiance (Falkowski and Owens, 1980; Falkowski *et al*.,

1981). Strzepek and Harrison (2004) observed the change of the ratio of photosystems by the change of growth irradiance using *Thalassiosira oceanica* and *T. wessflogii*.

Although both psychrophilic and mesophilic diatoms are very important for the ecosystem and the global carbon cycle, the comparative investigation between them seems to be out of focus. The comparison of the photosynthetic properties between both groups could reveal the characteristics which enables psychrophilic algae to perform efficient photosynthesis under extremely low temperatures and low irradiance environments.

Recent advancements in fluorescence measurements (pulse amplitude modulation method, PAM), enabled us to assess the photosynthetic properties in phytoplankton in a different way from the ^{14}C method (Franklin and Badger, 2001; Hartig *et al*., 1998; Wolfstein and Hartig, 1998), and recently, the PAM method is being applied to assess the photosynthesis in psychrophilic phytoplankton and ice algae (Kashino *et al*., 2002; Kudoh *et al*., 2003; McMinn and Hegseth, 2004). This method can assess the photosynthetic status in a very short time (min to tens min) using natural water samples with concentrations as low as 0.1 μgChl/*l*. These features of this simple method are especially useful for the assessment of the photosynthetic performance in psychrophilic phytoplankton and ice algae field *in situ*. Accordingly, to put the fluorescence method to practical use, it is very important to accumulate data not only from phytoplankton and ice algae in wide variety environments, but also from algae cultivated under various conditions.

In this study, we determined the parameters of photosynthetic activities in psychrophilic phytoplankton and ice algae inhabiting the Arctic Ocean, including the effect of the xanthophyll cycle activity. Furthermore, we determined the photosynthetic parameters in mesophilic marine diatoms, and systematically compared them with those of psychrophilic phytoplankton and ice algae to elucidate their photosynthetic properties which achieve the high primary production in the Arctic Ocean. This work was performed as a part of Canadian Arctic Shelf Exchange Study project (CASES).

Materials and methods

Sampling of ice algae and phytoplankton

Ice algae were collected from the bottom of the sea ice at Takatuk Station (126° 10.8′, 70° 16.2′) in Franklin Bay, the Canadian Arctic (Fig. 1), on 23, 26 and 30 May 2004 around noon. Around this time, the sun began to remain above the horizon, and the sea ice began to decrease depending on the increasing irradiances and temperature in this area. During this sampling period, the weather was calm and fine, and the irradiance was *ca*. 1000 μmol photons$\cdot m^{-2}\cdot s^{-1}$ on the ice around noon. An ice corer (inside diameter, *ca*. 90 mm) was used to cut the sea ice, which had a thickness of *ca*. 200 cm with 20 cm snow cover. The bottom part of the collected sea ice showed a brown color. The colored part of 4 cm thickness was immediately sliced horizontally using an ice saw. The sliced sea ice was maintained at around 0°C to keep it frozen, and to avoid low-salinity stress on ice algae. The sea ice was kept in a thermos box during transportation to the laboratory on board, which took less than 1 h. The suspension of ice algae that seeped from the brine pockets was used in the following experiments. To

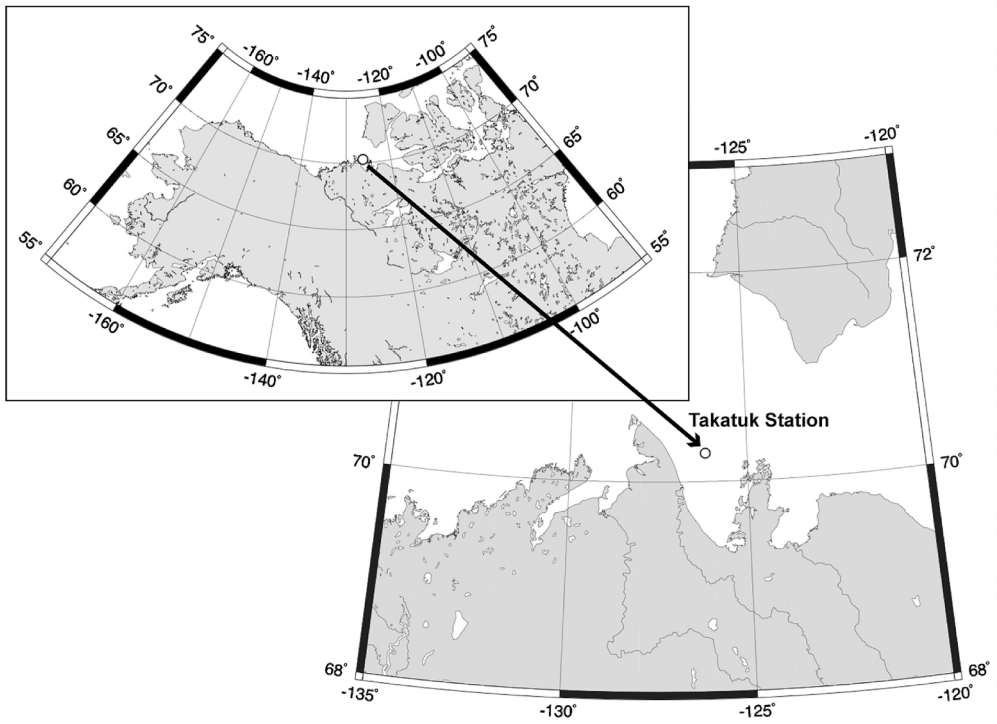

Fig. 1. Location map of the field station.

collect a phytoplankton sample, seawater was pumped up from just beneath the sea ice using the borehole that was drilled by the ice corer to collect ice algae. The collected seawater was immediately transferred into plastic bottles that were covered by black clothes and kept at around 0°C in the dark in a thermos box. The seawater samples containing phytoplankton were directly used without concentration in the following measurements. During these sampling procedures and transportation to the laboratory on board, the samples of ice algae and phytoplankton were kept in the dark to avoid exposure to any direct sunlight. The fluorescence measurements were performed immediately in the laboratory, which was within 60 min.

Culture of mesophilic marine diatoms

A centric diatom, *Chaetoceros gracilis* Schütt (UTEX LB 2658), and a pennate diatom, *Phaeodactylum tricornutum* Böhlin (UTEX 642) were grown photoautotrophically in sterile F/2 medium (Guillard and Ryther, 1962), with air-bubbling under continuous light at 25°C for 1 to 3 days. The light was supplied from one side and the irradiance was adjusted to 3, 20, 100, 350 μmol photons$\cdot m^{-2}\cdot s^{-1}$ by changing the distances from the light source (150 W halogen lamp, Trad HL-150, Sankyo, Osaka, Japan). Cells in optically thin stages were used for the experiments.

Measurement of fluorescence

A pulse amplitude-modulation chlorophyll fluorometer, Phyto-PAM, (Heinz Walz GmbH, Effeltrich, Germany) with control and analysis software, Phyto-Win, was used for measurements of photosynthetic fluorescence of ice algae, phytoplankton and cultivated mesophilic diatoms. The equipment was placed in a cold room to keep the algal sample at *ca*. 4°C during the measurements for ice algae and phytoplankton. The Chl concentrations of measured samples were between 15 and 360 $\mu g/l$ for ice algae, and between 0.18 and 0.28 $\mu g/l$ for phytoplankton as assessed by Phyto-PAM. To measure fluorescence in cultivated mesophilic diatoms, the temperature was kept at 25°C. The Chl concentrations were between 30 and 700 $\mu g/l$. The difference of Chl concentration in these ranges did not affect the results of fluorescence measurement, which was assessed by cultivated *C. gracilis* in various Chl concentrations (data not shown).

Light curves were obtained by running a rapid light curve protocol in Phyto-Win software. Here, the photosynthetic rate was expressed as the relative Electron Transfer Rate (rETR) (McMinn and Hegseth, 2004), and given by the equation:

$$rETR = [Fm' - F]/Fm' \times PAR = \Delta F/Fm' \times PAR = Y \times PAR,$$

where F and Fm′ are the transient and maximum fluorescence levels at a given time, and PAR is photosynthetically available radiation. The data were exported into KaleidaGraph (ver 3.6 for Macintosh, Synergy Software, Reading, PA), and were fitted to the following equation (Eilers and Peeters, 1988; Zonneveld, 1998) using a Levenberg-Marquardt regression algorithm.

$$rETR = E/(aE^2 + bE + c),$$

where E is irradiance, and a, b and c are regression coefficients to fit to the curve. The photosynthetic parameters were calculated as:

$$\alpha = 1/c,$$
$$rETR_{max} = [b + 2(ac)^{0.5}]^{-1},$$
$$E_k = rETR_{max}/\alpha,$$

where $rETR_{max}$ represents the maximum rETR.

To determine the non-photochemical quenching (NPQ) using Phyto-PAM, a train of saturating flashes with 20 s intervals was supplied during actinic light illumination for 300 s and subsequent darkness for 300 s, and fluorescence was recorded for 600 s. The intensities of actinic light were 90, 180 and 350 μmol photons$\cdot m^{-2} \cdot s^{-1}$. The value of NPQ was calculated by the following equation:

$$NPQ = (Fm - Fm')/Fm'.$$

Pigment analysis

Photosynthetic pigments were extracted using *N,N*-dimethylformamide (Furuya *et al*., 1998), and were analyzed with high performance liquid chromatography according to Kashino and Kudoh (2003).

Results

Photosynthetic properties of phytoplankton and ice algae

Figures 2A to C show the light curves measured for phytoplankton collected from the beneath of sea ice on different dates in late May 2004. The initial slopes are steep in all samples, implying shade-adapted characteristics. Over 30 μmol photons$\cdot m^{-2}\cdot s^{-1}$, the rETR decreased gradually suggesting that photoinhibition had occurred. Similar characteristics were observed in ice algae (Figs. 2D to F), which were collected at the

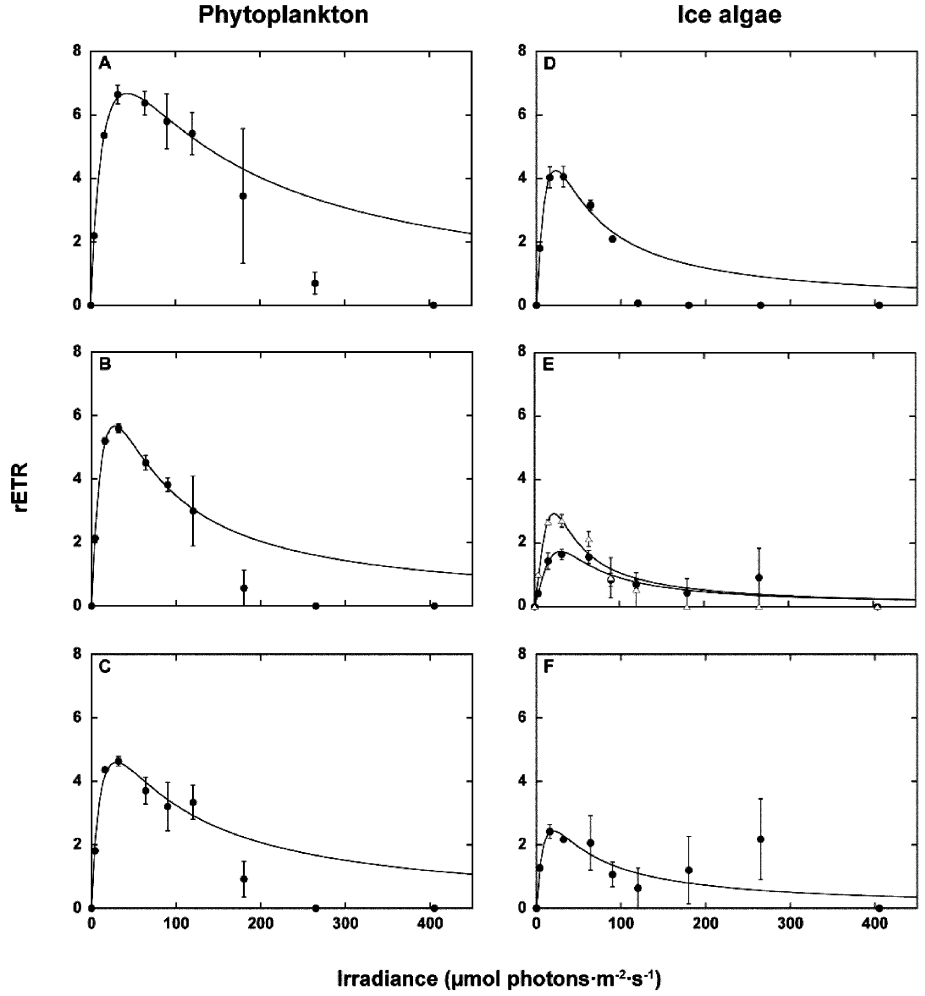

Fig. 2. Light curves measured for phytoplankton and ice algae. A to C, phytoplankton; D to F, ice algae. A and D, collected on 23 May 2004, B and E, collected on 26 May 2004; C and F, collected on 30 May 2004. Two ice algal samples were measured on 26 May 2004 (E). Using the aliquots, light curves were measured 3 or 4 times for each sample (2 times for one ice algal sample (closed symbol) on 26 May 2004). Error bars represent the standard errors. Note: in some data points, error bars are smaller than the symbols.

Table 1. Photosynthetic parameters obtained from Fig. 2.

Sample	Date (DD/MM/YY)	α	$rETR_{max}$	E_k	Y
Phytoplankton	23/05/04	0.681	6.68	9.81	0.642
	26/05/04	0.631	5.67	8.99	0.644
	30/05/04	0.637	4.60	7.22	0.554
Ice algae	23/05/04	0.505	4.25	8.42	0.522
	26/05/04	0.101	1.75	17.3	0.278
	26/05/04	0.203	2.95	14.6	0.113
	30/05/04	0.418	2.44	5.83	0.372

α, photosynthetic efficiency (initial slope of light-curve).
$rETR_{max}$, maximum electron transfer rate.
E_k, minimum saturating irradiance.
Y, quantum yield of PS II ($\Delta F/Fm'$).

same location and the same dates. However, the apparent maximum value of rETR ($rETR_{max}$) seemed to be lower than that of phytoplankton. Photosynthetic parameters derived from light curves in Fig. 2 are summarized in Table 1. The photosynthetic efficiency expressed as α was between 0.63 and 0.68 in phytoplankton samples, whereas the values in ice algae were much lower; between 0.10 and 0.51. Phytoplankton which lived beneath the sea ice appears much more dark-adapted than ice algae which inhabited the bottom of the sea ice. This is also supported by the values of the photoadaptive index (minimum saturating irradiance, E_k); E_k was smaller in phytoplankton (7.2–9.8) than in ice algae (5.8–17.3). However, the $rETR_{max}$, which corresponds to the maximum photosynthetic rate, showed otherwise; the values in ice algae were much smaller than in phytoplankton. Table 1 also shows the values of quantum yield of PS II (Y; $\Delta F/Fm'$). They were between 0.55 and 0.64 in phytoplankton, and between 0.11 and 0.52 in ice algae, and are comparable to those reported for phytoplankton and ice algae in previous studies (McMinn and Hegseth, 2004; McMinn *et al*., 2005).

Non-photochemical quenching (NPQ) in phytoplankton and ice algae

When phytoplankton was illuminated, NPQ progressively increased during illumination, and promptly decreased to almost the initial level after turning off the light (Figs. 3A and B). This indicates that the xanthophyll cycle was functioning to protect photosystems under high irradiances. The level of NPQ was almost the same, irrespective of the intensity of actinic light in the phytoplankton collected on 23 May 2004 (Fig. 3A). However, in the phytoplankton collected on 30 May 2004, the actinic light of 90 μmol photons$\cdot m^{-2}\cdot s^{-1}$ yielded the highest NPQ, while the actinic light of 240 μmol photons$\cdot m^{-2}\cdot s^{-1}$ yielded the lowest NPQ during the illumination (Fig. 3B). Similarly, the NPQ increased in ice algae after the onset of actinic light. The maximum amplitude of NPQ which was observed during the measurement was 2.5 after illumination of 300 s under the irradiance of 350 μmol photons$\cdot m^{-2}\cdot s^{-1}$ in ice algae collected on 23 May 2004 (Fig. 3C), which was comparable to the value observed in Fig. 3B. The NPQ activity was somewhat smaller in the ice algae collected on 26 and 30 May 2004

(Figs. 3D and E), and amplitudes were comparable to those for phytoplankton collected on 23 May 2004 (Fig. 3A). However, unlike phytoplankton, the NPQ did not decrease after turning off the actinic light in ice algae. This indicates that, in ice algae, the photoinhibition is the major origin in the observed NPQ.

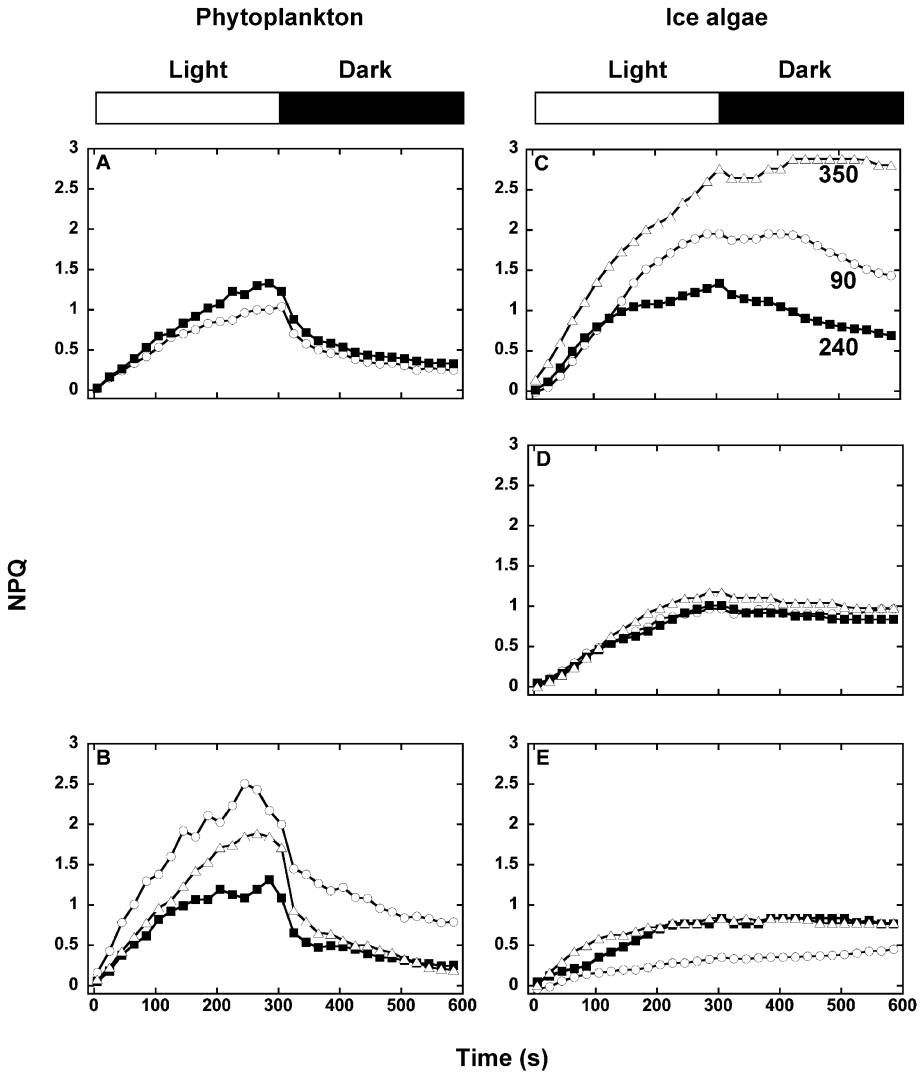

Fig. 3. Kinetics of NPQ measured in phytoplankton and ice algae. During the illumination of actinic light for 300 s and the subsequent darkness for 300 s, the NPQ were measured every 20 s. The intensities of actinic light were 90 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (open circle), 240 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (closed square), and 350 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (open triangle). Panels A and C, phytoplankton and ice algae measured on 23 May 2004; panel D, ice algae measured on 26 May 2004; panels B and E, phytoplankton and ice algae measured on 30 May 2004.

Photosynthetic properties in mesophilic diatoms

Because major populations in ice algae and phytoplankton were diatoms (Dr. Michel Poulin, pers. commun.), we measured the photosynthetic properties of mesophilic marine diatoms to address the characteristics of photosynthetic systems in psychrophilic phytoplankton and ice algae. To clarify the acclimation properties to the growth irradiance, the cells were grown under a wide range of irradiances (3, 20, 100 and 350 μmol photons$\cdot m^{-2}\cdot s^{-1}$). Figure 4 shows the quantum yield of PS II ($\Delta F/Fm'$) in *C. gracilis* and *P. tricornutum*. To include the physiological status in the growth conditions, fluorescence was measured without dark-adaptation. A similar strategy was adopted for ice algae and phytoplankton by McMinn *et al.* (2005). The quantum yield of PS II in *P. tricornutum* cells was around 0.65 and did not change much, irrespective of the growth irradiances. The value in *C. gracilis* decreased slightly with an increase in the growth irradiance (0.68 in cells grown under 3 μmol photons$\cdot m^{-2}\cdot s^{-1}$, and 0.47 in cells grown under 350 μmol photons$\cdot m^{-2}\cdot s^{-1}$).

Figure 5 shows the typical light-curves observed in two diatom cells grown under various irradiances. In the centric diatom, *C. gracilis*, $rETR_{max}$ seems to increase when the cells were grown under higher irradiances (Figs. 5A–D). In all *C. gracilis* cells, photoinhibitory effects were observed under high irradiances. However, the level of photoinhibition was much lower than those observed in ice algae and phytoplankton (Fig. 2). Similar characteristics to *C. gracilis* were observed in a pennate diatom, *P. tricornutum* (Figs. 5E–H). The photoinhibitory effect was also observed under high irradiance. From the rapid light curve experiments with cells grown under different irradiances (Fig. 5), photosynthetic parameters were obtained (Table 2). In both strains, the value of α decreased gradually, but slightly, when the growth irradiance

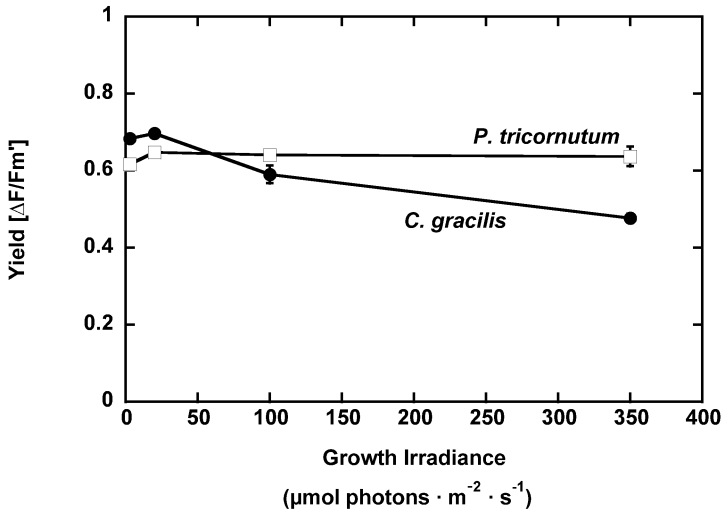

Fig. 4. Quantum yield of PS II ($\Delta F/Fm'$) in *Chaetoceros gracilis* (closed circle) and *Phaeodactylum tricornutum* (open square) grown under wide range of irradiances. Cells were not dark-adapted. Each data point includes 2 to 5 independent experiments. Error bars represent standard errors. Note: in some data points, error bars are smaller than the symbols.

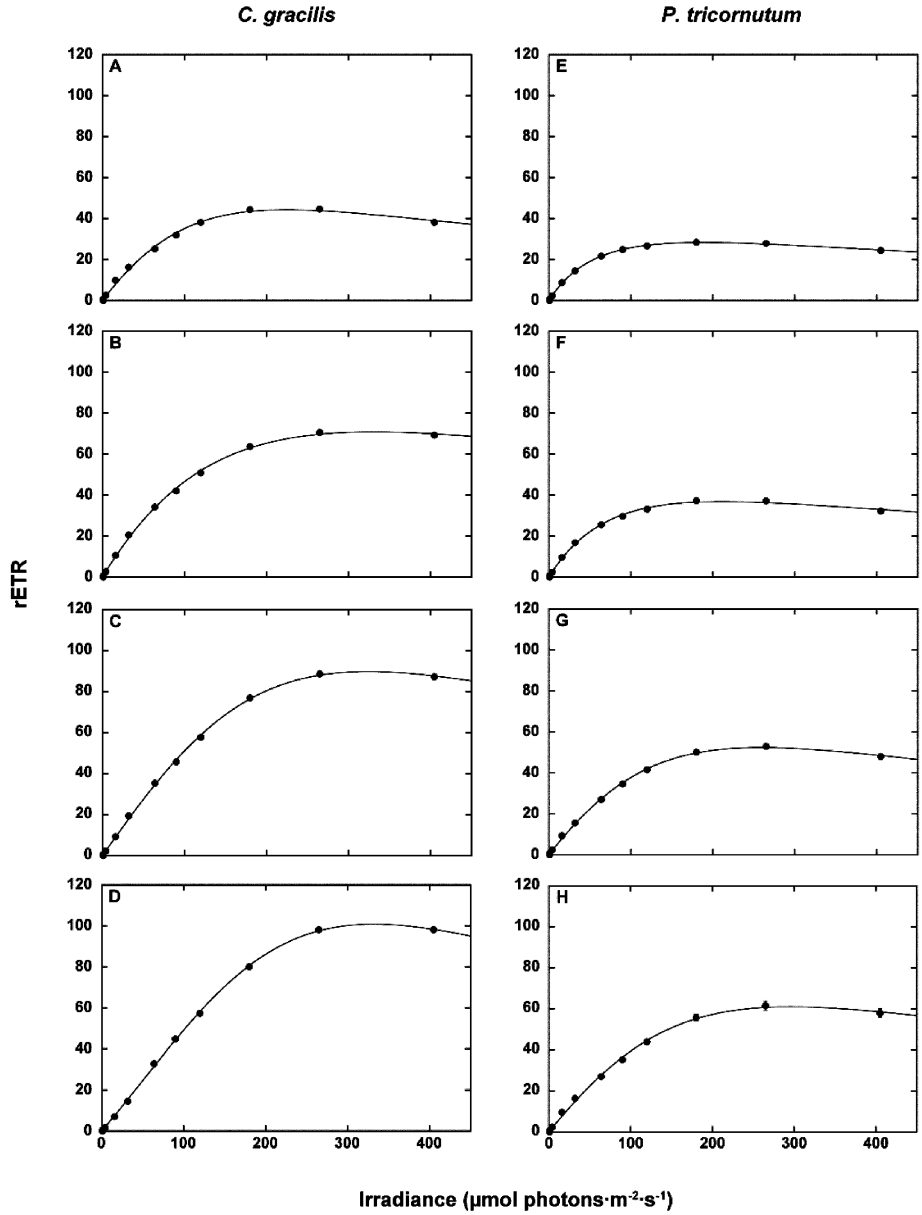

Fig. 5. Typical light curves observed for *Chaetoceros gracilis* (A to D) and *Phaeodactylum tricornutum* (E to H). Diatom cells were grown under 3 (A and E), 20 (B and F), 100 (C and G), and 350 (D and H) μmol photons$\cdot m^{-2}\cdot s^{-1}$. Using the aliquots, light curves were measured 3 times in each culture. Error bars represent the standard errors. Note: in some data points, error bars are smaller than the symbols.

Table 2. Changes in photosynthetic parameters due to the change of growth irradiance in mesophilic diatoms, *Chaetoceros gracilis* and *Phaeodactylum tricornutum*. Values obtained from light curves measured in diatom cells grown as shown in Fig. 5.

Strain	PAR at growth	α	$rETR_{max}$	E_k
C. gracilis	3	0.494	44.3	89.7
		(0.0274)	(3.55)	(6.14)
	20	0.629	72.4	116
		(0.0465)	(4.82)	(16.7)
	100	0.533	88.5	166
		(0.0593)	(9.21)	(6.52)
	350	0.433	90.9	210
		(0.0426)	(10.4)	(5.59)
P. tricornutum	3	0.664	23.6	36.0
		(0.0747)	(3.19)	(6.84)
	20	0.570	42.0	74.4
		(0.0581)	(4.89)	(12.9)
	100	0.482	56.6	118
		(0.0433)	(5.92)	(12.8)
	350	0.466	64.3	142.1
		(0.0465)	(21.0)	(35.6)

PAR; μmol photons$\cdot m^{-2}\cdot s^{-1}$.
Numbers in parentheses show standard deviations ($n=3$ or 4).

increased. The $rETR_{max}$ increased prominently with increasing growth irradiance in both strains. The value of E_k also increased with increasing growth irradiance. These properties against the change of ambient irradiances agree well with the well-known light-shade acclimation of higher plants (Evans *et al*., 1988).

These changes could be closely related to the change of accessory pigment ratios or antenna size. Therefore, pigment composition was analyzed (Fig. 6). Unexpectedly, the ratio of fucoxanthin, which is the major accessory pigment in the light-harvesting complex (fucoxanthin-chlorophyll protein complex), to Chl *a*, and the ratio of Chl *c* to *a* did not show marked changes for a wide range of growth irradiance in both mesophilic strains (Fig. 6). However, the sum of the amount of diadinoxanthin and diatoxanthin increased significantly with increasing growth irradiance, especially in *P. tricornutum*. These two pigments are the components of xanthophyll cycle in diatoms which is responsible for the NPQ.

Non-photochemical quenching in mesophilic diatoms

Figure 7 shows the typical kinetics of NPQ under different actinic illumination in diatom cells grown under various irradiances. In *C. gracilis* cells grown under 3 μmol photons$\cdot m^{-2}\cdot s^{-1}$ (Fig. 7A), the NPQ increased promptly and reached a maximum level within 70 s after onset of actinic light in all actinic light conditions. After turning off the actinic light, it decreased promptly to the initial level (data not shown). The maximum level of the NPQ was dependent on the intensity of the actinic light; a higher actinic light induced a higher NPQ. However, the intensity of actinic light did not

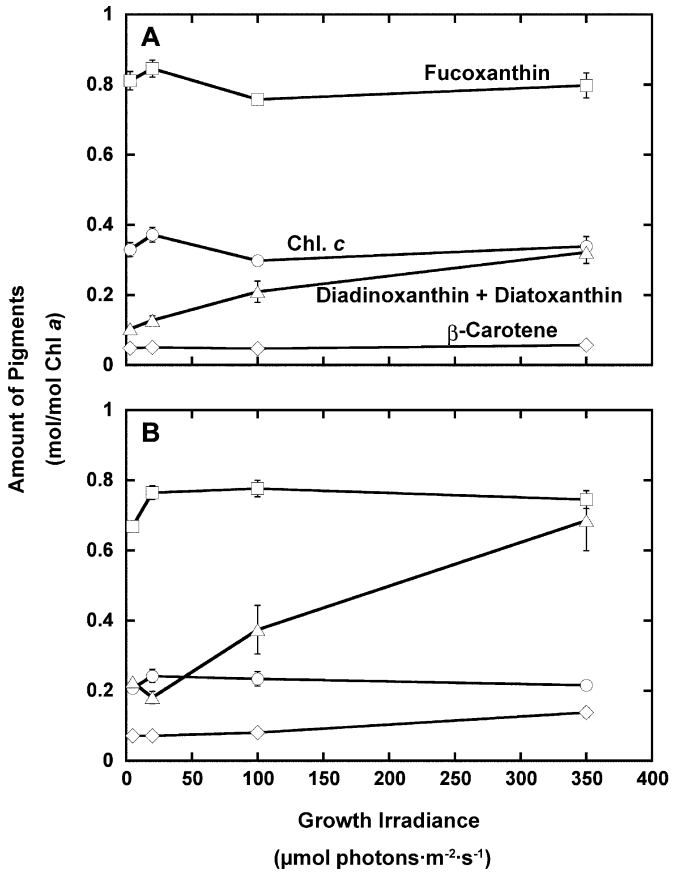

Fig. 6. Changes of pigment composition due to the growth irradiance. The cells were grown as in Fig. 5. A: *C. gracilis*; B: *P. tricornutum.* The amounts are expressed in the molar ratio against Chl *a.* Each data point includes 3 to 5 independent experiments. Open circle, Chl *c*; open square, fucoxanthin; open triangle, diadinoxanthin plus diatoxanthin; open diamond, β-carotene. Error bars are standard errors. Note: in some data points, the error bars are smaller than the symbols.

affect the period so much that was required to reach the maximum level for NPQ. The same properties were observed in all cells, including *P. tricornutum* cells, which were grown under different irradiances (Figs. 7A to H). It is noteworthy that the quantum yield of PS II was kept at the same level throughout the illumination (data not shown).

The differences in growth irradiances caused the differences of maximum amplitude of NPQ. The maximum amplitude of NPQ induced by actinic light of 350 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} was smaller in cells grown under 20 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (Fig. 7B) than the cells grown under 3 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (Fig. 7A), and further decreased monotonically in cells grown under higher irradiances (Figs. 7C and D). The NPQ was smallest in cells grown under 350 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (Fig. 7D). In *P. tricornutum* cells, the situation was different (Figs. 7E to H). The largest NPQ was also

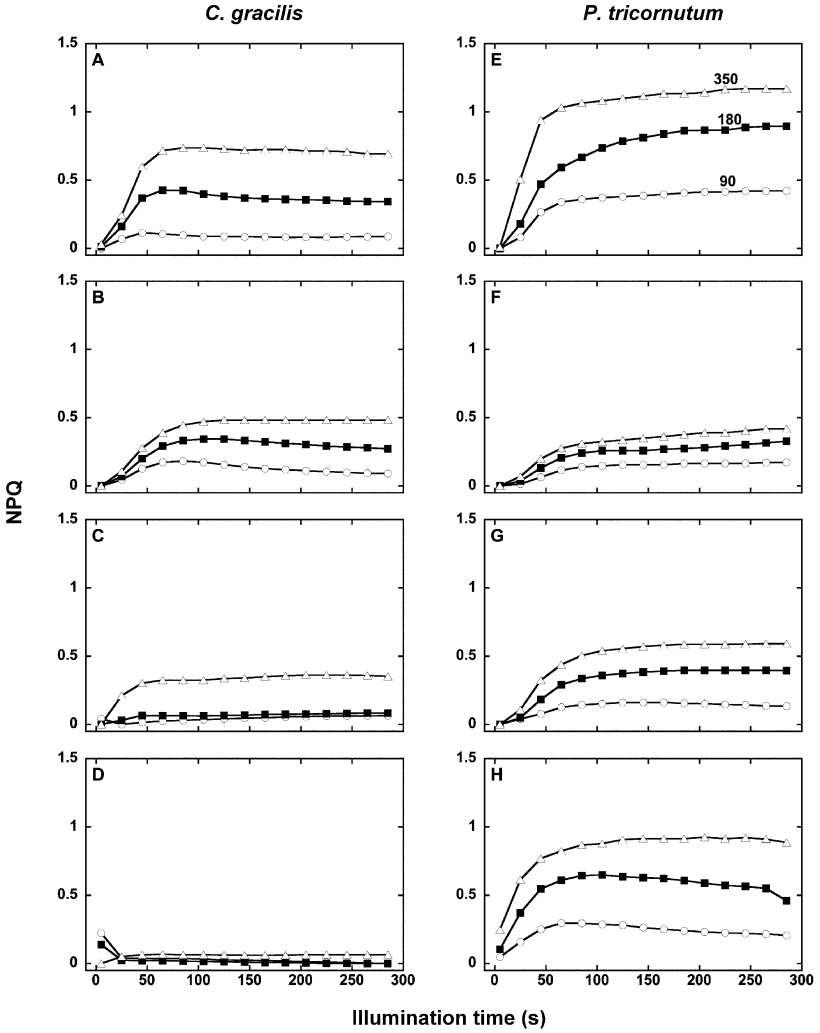

Fig. 7. Kinetics of NPQ measured in *Chaetoceros gracilis* (A to D) and *Phaeodactylum tricornutum* (E to H) grown under wide range of irradiances. Cells were not dark-adapted. During the illumination of actinic light for 300 s, the NPQ were measured in every 20 s. The intensities of actinic light were 90 (open circle), 180 (closed square), and 350 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} (open triangle). Diatom cells were grown under 3 (A and E), 20 (B and F), 100 (C and G), and 350 (D and H) μmol photons$\cdot$m$^{-2}\cdot$s^{-1}.

observed in cells of the lowest growth irradiance in *P. tricornutum* (Fig. 7E). However, if the growth irradiance was larger than 20 μmol photons$\cdot$m$^{-2}\cdot$s^{-1}, the maximum amplitude of NPQ increased steadily in higher growth irradiances (Figs. 7F to H). We sometimes experienced unusual transient NPQ in *P. tricornutum* grown under the highest irradiance; highest NPQ at the onset of actinic light and a following gradual decrease of NPQ (data not shown). Nonetheless, the level of NPQ in such cells was

higher than that in cells grown under 100 μmol photons$\cdot m^{-2}\cdot s^{-1}$.

Discussion

Changes in photosynthetic properties due to the acclimation of two mesophilic diatom stains (centric and pennate diatoms) to growth irradiances were systematically investigated using a Phyto-PAM. Photosynthetic properties of the ice algae and phytoplankton inhabiting Franklin Bay, the Canadian Arctic, were also measured and compared with those of mesophilic diatoms.

The quantum yield of PS II in mesophilic *P. tricornutum* cells was around 0.65 in all cells grown under various irradiances, and that in mesophilic *C. gracilis* changed slightly from 0.68 in the lowest growth irradiance cells to 0.47 in the highest growth irradiance cells (Fig. 4). The smaller apparent quantum yield in diatom cells could be related to the large accessory pigment pool (Fig. 6). The values of quantum yields of the PS II in phytoplankton were smaller than the values from these mesophilic strains, and the values in ice algae were much smaller (Table 1). Furthermore, the values varied significantly among ice algal samples. Similar small values were reported for natural psychrophilic phytoplankton and ice algae (summarized in McMinn and Hegseth, 2004). It is not yet known whether the difference between the values of mesophilic diatoms and psychrophilic phytoplankton (and ice algae) was caused by the difference in growth and measurement temperatures. However, it is possible that the psychrophilic phytoplankton in the early summer season configure their photosynthetic machinery to achieve lower quantum yields to maintain an effective photosynthetic reaction under the low ambient temperature and irradiance. The mechanisms of such variation in the quantum yield will be resolved using cultivated psychrophilic phytoplankton, and we are now preparing for this investigation.

The photosynthetic parameters in mesophilic diatoms obtained from light-curves changed depending on the growth irradiances (Table 2). The changing pattern of these parameters resembles that in the well-known sun-shade acclimation in higher plant leaves (Evans *et al*., 1988). The index of photosynthetic efficiency (α) decreased gradually, and the maximal photosynthetic rate ($rETR_{max}$) and the minimum saturating irradiance (E_k) increased remarkably with increasing growth irradiance in *P. tricornutum* (Table 2). Similar characteristics were also observed in *C. gracilis*, although the values of these parameters are different between the two strains (Table 2). This result shows that the photosynthetic parameters obtained by the PAM method could be used to assess the physiological status of sun and shade acclimation in diatoms. In addition, it can be emphasized that the basic acclimation manner against the change of growth irradiance may be similar for most photosynthetic organisms. In this context, it is noteworthy that the antenna size seems to be the same in a wide range of growth irradiances judging from the ratio of accessory pigments to Chl *a*. This is contrasting from the result reported for a marine diatom, *S. costatum*, in which the antenna size increased under low growth irradiance (Falkowski and Owens, 1980). Further comparative experiments are necessary to explain this discrepancy.

The α value in phytoplankton resembled that in *P. tricornutum* grown under lower irradiances (Tables 1 and 2). However, the values of $rETR_{max}$ and E_k in phytoplankton

(Table 1) were much smaller than those in *P. tricornutum* grown under the lowest irradiance (Table 2). These properties indicate that the phytoplankton had shade-acclimated features. The α values in ice algae were much smaller than those in phytoplankton (Table 1). Because the ice algae inhabited the bottom part of sea ice, the light availability should have been higher than the phytoplankton living beneath the sea ice. It is reasonable that the ice algae possess a sun-acclimated feature contrary to phytoplankton in this case. However, when the values of $rETR_{max}$ and E_k were compared with those of phytoplankton, the feature becomes complicated; the values of $rETR_{max}$ in ice algae were smaller than those in phytoplankton, and the values of E_k in ice algae were comparable to those in phytoplankton. These results imply that there were other factors to influence the photosynthetic performance in ice algae and phytoplankton. Since the photosynthetic properties were different, even between the two mesophilic strains as was shown in this work, the species (or genus) composition should also be carefully considered in future research.

In mesophilic diatoms, NPQ increased when higher actinic light was supplied (Fig. 7), which is reasonable since the xanthophyll cycle actively functions under high irradiance to dissipate excess light energy to protect photosystems (Demmig-Adams, 1990). This is consistent with our former study on natural phytoplankton inhabiting Baffin Bay (Kashino *et al*., 2002). In *P. tricornutum*, the level of NPQ was higher in cells grown under higher growth irradiances when the same amplitude of actinic light was illuminated, except the cells of the lowest growth irradiance (Fig. 7). The sum of the ratio of diadinoxanthin and diatoxanthin to Chl *a* increased from 0.22 (lowest growth irradiance) to 0.68 (highest growth irradiance) (Fig. 6B). This increase of xanthophyll cycle pigments should have resulted in higher NPQ in the *P. tricornutum* cells of higher growth irradiances. These results indicate that the *P. tricornutum* cells acclimate to high irradiance environments to increase the amount of xanthophyll cycle pigments to protect their photosystems. The NPQ was highest in *P. tricornutum* cells grown under the lowest growth irradiance in all actinic light tested. The reason is not clear at this stage, but it could be explained as follows:

The xanthophyll cycle is the only mechanism to protect photosystems from excess irradiance in cells acclimated to an environment with very low irradiance, and cells growing under moderate irradiances may configure the total photosynthetic systems allowing it to respond to higher irradiances in addition to the xanthophyll cycle. A second possibility is that the actinic light was only excessive to cells of the lowest growth irradiances.

In *C. gracilis*, the level of NPQ decreased steadily with increasing growth irradiances (Figs. 7A to D), which was quite different from *P. tricornutum* (Figs. 7E to H). It seems that *C. gracilis* cells grown under higher irradiances did not need the help of the xanthophyll cycle, since the actinic light was not excessive compared to the growth irradiances, while the *C. gracilis* cells grown under lower irradiances needed the help of the xanthophyll cycle to maintain their efficient photosynthetic systems under the relatively higher actinic light. The difference of the occurrence of NPQ between *C. gracilis* and *P. tricornutum* (Fig. 7), implies that the acclimation strategies to growth irradiances are quite different between species, which can also be presumed for psychrophilic phytoplankton and ice algae (Fig. 3). Our preliminary analysis on the molecular

composition of cells grown under various light conditions suggested that the configuration of photosystems was different between these strains, which will be reported elsewhere.

The increase of NPQ upon the onset of actinic light was also observed in ice algae and phytoplankton (Fig. 3). Generally, the level of NPQ is significantly higher under all actinic light (Fig. 3) compared with mesophilic diatoms (Fig. 7), with one exception (Fig. 3E). This could be closely related to the result that mesophilic diatom cells show the highest NPQ level when they were grown under the lowest irradiance tested, showing that ice algae and phytoplankton had acclimatized to the shade environment. The rate of increase in the NPQ and the level of NPQ were not so different irrespective of the intensity of actinic light, which was quite different from the results obtained in mesophilic diatoms. This could also be explained by the shade-adapted feature of these organisms; even the lowest actinic light supplied in this work ($90\,\mu$mol photons$\cdot m^{-2}\cdot s^{-1}$) was excessively high to induce the maximum activity of xanthophyll cycle. However, it is noteworthy that the rates of increase in NPQ were slower than those observed in mesophilic diatoms. This reflects that ice algae and phytoplankton are highly susceptible to photoinhibition (Fig. 2) and NPQ includes a contribution from photoinhibition. It is surprising that, in phytoplankton, the NPQ level promptly decreased under the subsequent darkness (Figs. 2A and B).

Through our research, it was revealed that the photosynthetic properties of ice algae and phytoplankton inhabiting Franklin Bay in early summer (late May) are typical to photosynthetic organisms highly acclimated to the shade environment. In spite of the shade-adapted features, the variable acclimation strategies between species could be one of the forces to make a diverse algal flora which enables the Arctic Ocean to be a productive area.

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