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Acclimation of photosynthetic properties in psychrophilic diatom isolates under different light intensities

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Abstract: Acclimation of growth and photosynthetic properties was examined for diatom strains, isolated from Saroma Ko lagoon (44° N, 144° E) in early spring, under different light intensities at 1℃. The relatively high specific growth constants under low irradiances were recognized for both *Chaetoceros* sp. and *Thalassiosira* sp. Further examination of photosynthesis in *Chaetoceros* sp. showed that growth under low irradiance caused an increase in the photosynthetic efficiency for Chl *a*-specific rate of gross O_2 evolution (α^B) and a decrease in the Chl *a*-specific rate of dark O_2 respiration (R_d^B), which was extrapolated from the initial slope of net O_2 evolution rate *vs.* irradiance curve. These changes explain the low light compensation point for net photosynthetic O_2 evolution (I_c) and the high efficiency for growth under low irradiance conditions. Neither Chl *a*-specific amounts of light-harvesting pigments, such as fucoxanthin and Chl *c*, nor cellular content of Chl *a*, but Chl *a*-specific amount of diadinoxanthin was varied by the change in irradiance condition. The increase of α^B under low growth irradiance corresponded with a decrease in the amount of diadinoxanthin relative to Chl *a*. However, the Chl *a*-specific maximum photosynthetic rate (P_m^B) was retained at the same level and photoinhibition could not be observed under illumination up to ~800 μE m^{-2} s^{-1}. The light intensity at which photosynthesis was light-saturated (I_k, $=P_m^B/\alpha^B$) was much higher than the incident growth irradiances (76 and 88 μE m^{-2} s^{-1} under the growth irradiances of 11 and 42 μE m^{-2} s^{-1}, respectively), although the value of I_k for the former was significantly smaller (*t*-test, $P<0.001$) than that for the latter. Thus, the capacity of photochemical reaction around photosystems seems to remain large even under low irradiance. These properties may indicate that the diatom cells could efficiently utilize light-energy under large variation of irradiance even on a short time-scale.

key words: ice algae, acclimation, photosynthesis, respiration, diadinoxanthin

Introduction

In high latitudinal seas, the distribution and production of algae are greatly affected by sea ice (Horner, 1985; Medlin and Priddle, 1990). Due to reflection and attenuation by ice and snow at the surface of the sea, irradiance is greatly reduced to 5-10% of the surface irradiance under the surface snow coverage and further to less than 1% at the

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bottom of the ice sheet (Maykut and Grenfell, 1975; Kishino, 1993; Kudoh *et al.*, 1997). The attenuation of irradiance in ice and snow is much more rapid than in ocean water. Under such low irradiance, massive algal cells, mainly composed of diatoms, are accommodated at the bottom of the sea ice and utilize light-energy efficiently at the canopy of the productive structure in the ocean (Meguro *et al.*, 1966; Horner and Shrader, 1982). Flux of light-energy has been considered as a controlling factor of growth and biomass of ice algae though excess irradiance, physical structure of the ice, nutrient flux and predation may be other possible causes of biomass variation (Smith *et al.*, 1988; Welch and Bergman, 1989).

It has been recognized that ice algal cells, which occur at the bottom of the ice, are capable of photosynthesizing under extremely low light intensities. Very low light compensation points for photosynthesis have been reported for the bottom assemblages of ice algae (*cf.* Horner, 1985). Variation of photosynthetic capabilities has also been recognized for ice algal assemblages which experience temporal and spatial variation of light conditions (*cf.* Cota and Smith, 1991). The investigation of photosynthetic pigment composition of ice algal assemblages has indicated that the ratios of Chl *a* to Chl *c* and carotenoids, such as fucoxanthin, change at different vertical positions in the sea ice (Kashino *et al.*, 1998). Observation of the time variation of pigment composition in the ice algae at the bottom of the ice has also shown that the amount of diatoxanthin relative to diadinoxanthin increases in proportion to the increase of irradiance from sunrise to afternoon (Kashino *et al.*, 1999). Light-energy absorbed by these pigments is transformed to ATP and reductants, such as NADPH, by photochemical reactions in photosystems and/or dissipated by non-photochemical quenching with the aid of, for example, the xanthophyll cycle (Olaizoa *et al.*, 1994; Arsalane *et al.*, 1994; Kashino *et al.*, 1999). Excess light-energy transferred to reaction centers in photosystems causes photoinhibition (*cf.* Krause, 1988). Thus, photosynthetic pigments may be adjusted so as to maintain photosynthesis and growth under ambient attenuated but variable light conditions. Dark respiration has also been considered as a determinant of efficiency for growth under low irradiance; this is especially important in the diel cycle of solar radiation (Kudoh *et al.*, 1997).

The present study was conducted to determine the acclimation of photosynthetic properties of psychrophilic diatoms which experience the development of sea ice and variation of the light-environment at low temperature in the ice. Saroma Ko lagoon is located at the southernmost limit of the seasonal sea-ice area in the Northern Hemisphere (44°N, 144°E) and the surface is mostly covered by sea ice during winter (Taguchi and Takahashi, 1993; Shirasawa, 1993). The ice greatly affects the light environment and the growth of algal cells in the lake (Kishino, 1993; Tada *et al.*, 1993; Kudoh, 1993). Several strains were isolated from the surface water of Saroma Ko lagoon in the last period of ice coverage. Among the isolates, two strains, *Chaetoceros* sp. and *Thalassiosira* sp., which were easy to grow in experimental culture without cloging and adhesion to the culture flask, were used for the culture experiment to examine their growth ability. Further analysis was restricted mainly to the former strain because of the non-uniform distribution in the culture medium and the difficulty of experimental treatment, such as fragility of the latter cells. The two strains may not be necessarily typical ice algae but have planktonic nature (*cf.* Kashino *et al.*, 1998).

It has been reported that small unicellular *Chaetoceros* spp. occurs as the dominant species in, for example, water lenses formed by melting pack ice in the Weddell Sea but may thrive also in pack ice (*cf.* Thomas *et al.*, 1992). Cells were grown under various light intensities which are typically experienced in the sea-ice habitat. The evolution and the consumption of O_2 were measured to determine photosynthetic parameters since they are direct indicates of electron transport activity around photosystems in the steady state as well as of O_2 respiration activity. Then, the observed acclimation of photosynthetic properies were discussed in relation to the variation in photosynthetic pigment composition and respiration activity.

Materials and methods

Two strains of diatoms, *Chaetoceros* sp. B23-p1 and *Thalassiosira* sp. B5-p5, were isolated from surface water on the east coast of Saroma Ko lagoon on 8 April 1998. When the sample was collected, warm weather had caused melting and breaking of the fast ice. The broken-ice floated and covered less than 10% (visual observation) of the surface water. The surface water (-0.4°C) was filled directly into several sterilized tubes of 50 m*l* capacity. The two isolated strains could not proliferate at temperatures higher than 15°C. Cells were grown at 1.0 ± 0.5°C in f/2 medium (Guillard and Ryther, 1962) with gentle reciprocal shaking (60 or 80 strokes min^{-1}). Light was supplied for the culture in conical flasks at 5, 11, 22 and 42 $\mu E\ m^{-2}\ s^{-1}$ by an incandescent lamp with a 16 h : 8 h light and dark cycle. The light period was not exactly the same as the *in situ* condition in April in Saroma Ko lagoon, but was designed to determine a clear relationship between the light intensities of growth irradiance and the photosynthetic pigment composition. Cells were harvested in the late exponential phase after growth for at least three generations.

Photosynthetic O_2 evolution was measured using a Clark-type O_2 electrode (4004, Yellow Spring Instruments) at 1.5 ± 0.1°C under incandescent illumination. The illumination was attenuated with a combination of neutral density filters (Toshiba). The rate of net photosynthetic O_2 evolution was determined from the steadily increasing linear slope of O_2 concentration during 20 min under illumination. The rate of O_2 consumption was determined from the steadily decreasing linear slope of O_2 concentration during 20 min after each illumination (post-illumination respiration). The rate of gross photosynthetic O_2 evolution was derived from the sum of the rate of net photosynthetic O_2 evolution and the rate of post-illumination respiration after corresponding illumination on the assumption that the rate of post-illumination respiration equaled that of respiration in light. The dark respiration rate (R_d) was derived by linear extrapolation of the initial slope of the net photosynthetic O_2 evolution rate *vs.* irradiance curve. The irradiance *vs.* photosynthetic O_2 evolution rate data set was fitted to an exponential function so as to minimize the chi-squared function. Statistical evaluation of each parameter was done following the derivation by Zimmerman *et al.* (1987).

Photosynthetic pigment composition was determined with HPLC as described in Kashino *et al.* (1999). Extraction was done in 90% acetone for over one hour at -20 °C with sonication for one minute before and after the incubation. After centrifuga-

tion, the extracts were subjected to reverse-phase HPLC analysis on a 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) at a flow rate of 0.8 m*l* min^{-1} over a period of 51 min. The ratio of solvent B increased linearly up to 25% in the first four minutes (6.25% min^{-1}), then gradually increased to 100% by 41 min (2.03% min^{-1}). The ratio of 100% of solvent B was kept for 10 min so as to elute highly hydrophobic pigments. Absorption spectra were measured by a Shimadzu photodiodearray detector SPD-M10AV with the analyzing software Shimadzu CLASS-M10A. Pigments of fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene (Water Quality Institute, Denmark) were used as standards. Chlorophyll concentrations were estimated by the method of Jeffrey and Humphrey (1975). The coefficients of variation of estimates with this HPLC system were usually 1.5%, 3.0%, 7.08%, 11.9%, 2.59% and 1.8% for Chl *a*, fucoxanthin, diadinoxanthin, diatoxanthin, Chl *c* and β-carotene, respectively.

For the samples of photosynthesis measurements, the Chl *a* concentration in 90% acetone extracts was determined spectrophotometrically using the absorption coefficient of Jeffrey and Humphrey (1975).

Cell density was determined by counting the number of cells using a hematocytometeric chamber under a light microscope after fixation in glutaraldehyde solution (1%, v/v in seawater) or by turbidity of the culture medium at 750 nm.

Results

Acclimation of growth rate to irradiance

The light intensities 5, 11, 22, 42 μE m^{-2} s^{-1} correspond to *ca.* 0.4–0.8, 0.9–1.8, 1.8–3.6, 3.5–7.0% surface irradiance in Saroma Ko lagoon at diurnal maximum on sunny

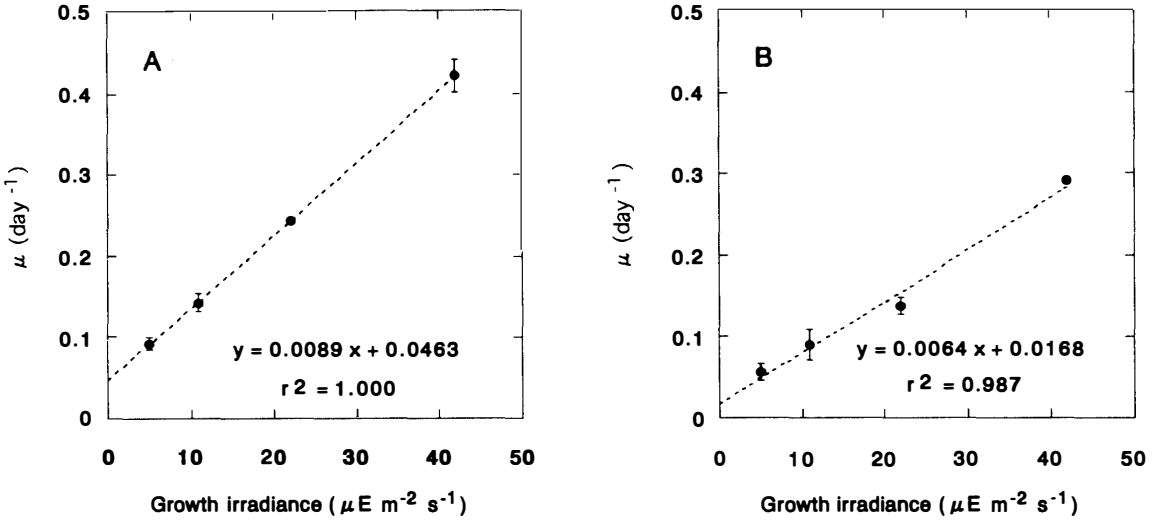

Fig. 1. Growth constant (μ) determined for isolated strains under different growth irradiance. A; *Chaetoceros* sp. B23-p1. B; *Thalassiosira* sp. B5-p5. Broken lines indicate linear regression functions determined for each species. Vertical bars represent standard deviations of the growth constant.

days in early spring (February–March), respectively (*cf.* Kudoh *et al.*, 1997). The growth constant of both strains increased with increasing irradiance, following a linear regression function (Fig. 1). Both the intercept of the ordinate and the slope of the line for *Chaetoceros* sp. B23-p1 were higher than those for *Thalassiosira* sp. B5-p5 (*t*-test, $P<0.001$ for both parameters). This may suggest that the efficiency for light-energy utilization in *Chaetoceros* sp. B23-p1 is higher than in *Thalassiosira* sp. B5-p5. However, the efficiency of *Thalassiosira* sp. B5-p5 might be underestimated due to self-shading, since the latter cells tend to form a soft aggregation at the base of culture-flask under the present culture condition, whereas the former cells disperse in the medium.

Photosynthetic characteristics in acclimated cells of *Chaetoceros* sp.

The composition of photosynthetic pigments was determined for cells of *Chaetoceros* sp. B23-p1 grown under various light intensities (Table 1). Neither fucoxanthin, which was the most abundant carotenoid, nor Chl *c*, showed significant change in amount under these conditions. The amount of β-carotene also remained constant with change of irradiance. Cells grown under higher irradiance contained higher amounts of diadinoxanthin. Up to threefold change was observed under the examined irradiance conditions. A slight amount of diatoxanthin was detected only under 42 μE m^{-2} s^{-1}.

The rate of gross photosynthetic O_2 evolution ($P_g(I)$) was further determined to examine the effect of growth irradiance on the light-harvesting properties. The plots of irradiance (I) *vs.* $P_g(I)$ were determined for cells grown under 11 and 42 μE m^{-2} s^{-1} (Fig. 2A and B). Since photoinhibition was not recognized within the examined range of illumination, each plot of $P_g(I)$ could be described as an exponential photosynthesis-irradiance (*P-I*) curve (*cf.* Platt *et al.*, 1980), neglecting the term for photoinhibition ($r^2=0.982$ and 0.951 for cells grown under 11 and 42 μE m^{-2} s^{-1}, respectively):

$$P_g(I)=P_m\ (1-\exp\ (-\alpha I/P_m)), \qquad (1)$$

where α is the initial slope of the *P-I* curve, and P_m is the maximum photosynthetic rate.

Table 1. Photosynthetic pigment composition determined for *Chaetoceros* sp. B23-p1 cells grown under different irradiance conditions*.

Irradiance	5	11	22	42
	(μE m^{-2} s^{-1})			
	(mol [mol Chl *a*]$^{-1}$)			
Chl *c*	0.124	0.204	0.231	0.233
Fucoxanthin	0.504	0.530	0.515	0.546
Diadinoxanthin	0.047	0.080	0.071	0.132
Diatoxanthin	0.000	0.000	0.000	0.009
β-Carotene	0.034	0.035	0.041	0.045

* Data were obtained from independently duplicated experiments in the cases of 11 and 42 μE m^{-2} s^{-1}.

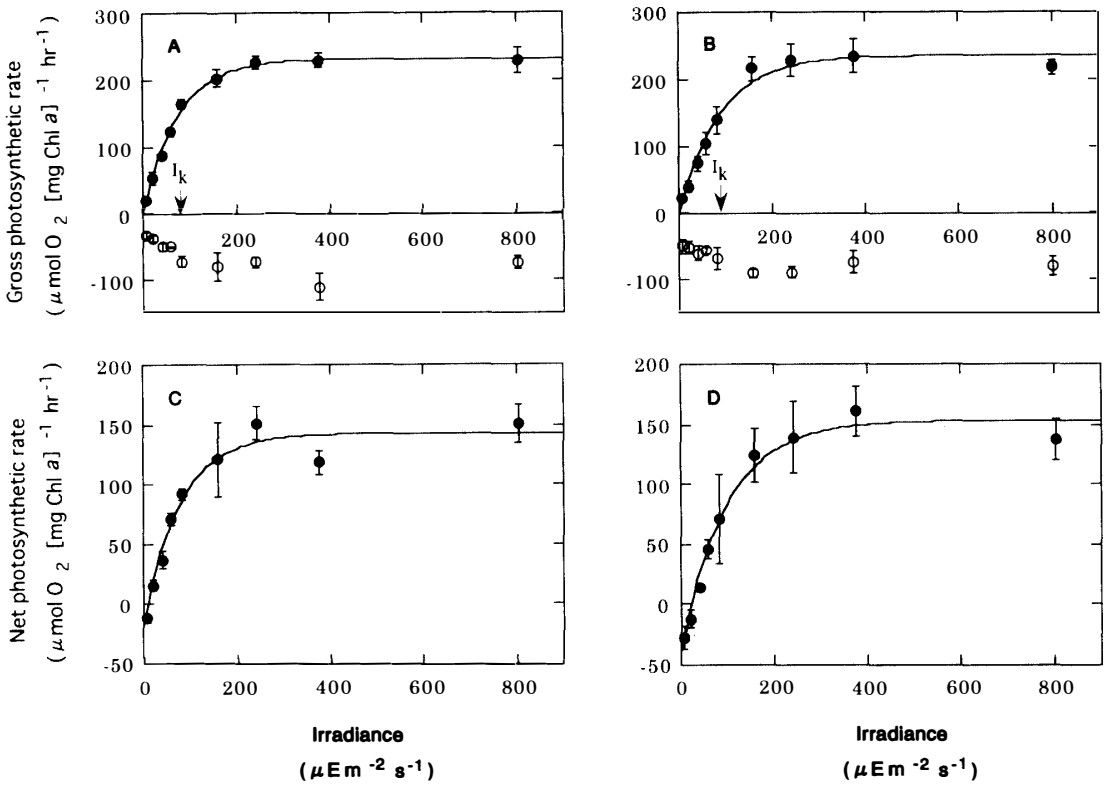

Fig. 2. The rates of gross (A, B) and net (C, D) photosynthetic O_2 evolution determined for *Chaetoceros* sp. B23-p1 cells grown under different growth irradiances (closed circles). The rates of O_2 consumption after respective illumination are also shown as negative values (A, B, open circles). Cells were grown under 11 (A, C) and 42 μE m^{-2} s^{-1} (B, D). Vertical bars represent standard deviations of the rates of photosynthetic O_2 evolution and consumption. Arrows indicate the values of I_k.

The initial slope of the *P-I* curve for Chl *a*-specific rate of gross photosynthesis (α^B) in cells grown under 11 μE m^{-2} s^{-1} was 1.2 times greater than that in cells grown under 42 μE m^{-2} s^{-1} (*t*-test, $P<0.001$) (Table 2). The Chl *a*-specific maximum rate of photosynthesis (P_m^B) changed insignificantly under the two irradiance conditions. The light intensity at which photosynthesis was light-saturated ($I_k, = P_m^B/\alpha^B$), was 76 and 88 μE m^{-2} s^{-1} in cells grown under 11 and 42 μE m^{-2} s^{-1}, respectively. The value of I_k for the former was also significantly smaller than that for the latter (*t*-test, $P<0.001$). These results may suggest that the photosynthetic efficiency of *Chaetoceros* sp. B23-p1 is adjusted to ambient irradiance and the pigment composition varies so as to support efficient light-harvesting under low irradiance.

The rates of dark O_2 consumption (post-illumination respiration) after each illumination for the measurement of $P_g(I)$ increased exponentially with increasing light intensity (Fig. 2A and B). Under weak illumination, such as 8 μE m^{-2} s^{-1}, the rate of post-illumination respiration in the cells grown under 11 μE m^{-2} s^{-1} was lower than that in the cells grown under 42 μE m^{-2} s^{-1} (*t*-test, $P<0.05$), but increased to the maximum and equaled those in the latter cells (*f*-test, $P>0.05$) at light intensities higher than I_k for $P_g(I)$ (76 and 88 μE m^{-2} s^{-1} under the growth irradiances of 11 and 42 μE m^{-2} s^{-1},

Table 2. Parameters of gross photosynthesis-irradiance response determined for *Chaetoceros* sp. B23-p1 cells grown under different irradiance conditions.

		Growth irradiance (μE m^{-2} s^{-1})	
		11	42
α^B	(μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}) (μE m^{-2} s^{-1})$^{-1}$	3.04 (±0.64)	2.66 (±0.84)
$P_m{}^B$	(μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1})	231 (±3)	234 (±5)
I_k	(μE m^{-2} s^{-1})	76 (±16)	88 (±27)
r^2		0.982	0.951
Chl *a*	(10^{-13} g cell^{-1})	3.03 (±0.09)	2.97 (±0.07)

Numbers in parentheses indicate 95% confidence intervals.

respectively).

The amount of respiration affects the balance of the budget of energy utilization under a given light regime. Thus, the net rate of photosynthetic O_2 evolution ($P_n(I)$) was examined for the cells of *Chaetoceros* sp. B23-p1 grown under 11 and 42 μE m^{-2} s^{-1} (Fig. 2C and D). The plots can also be described as *P-I* curves following the previous exponential function with an additional term of Chl *a*-specific rate of dark respiration ($R_d{}^B$) ($r^2=0.913$ and 0.921 for cells grown under 11 and 42 μE m^{-2} s^{-1}, respectively):

$$P_n(I)=P_m'\ (1-\exp\ (-\alpha' I/P_m'))-R_d, \qquad (2)$$

where α' and P_m' are the same parameters as those in eq. (1) but specifically determined for $P_n(I)$. Due to the enhancement of the rate of post-illumination respiration (Fig. 2 A and B), the initial slope of the *P-I* curve for Chl *a*-specific rate of net photosynthesis ($\alpha^{B'}$) was lower than the α^B value (Table 3). The difference in $\alpha^{B'}$ was negligible between the two irradiance conditions (*t*-test, $P>0.05$). The maximum Chl *a*-specific

Table 3. Parameters* of net photosynthesis-irradiance response determined for *Chaetoceros* sp. B23-p1 cells grown under different irradiance conditions.

		Growth irradiance (μE m^{-2} s^{-1})	
		11	42
$\alpha^{B'}$	(μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}) (μE m^{-2} s^{-1})$^{-1}$	2.05 (±0.81)	1.95 (±0.83)
$P_m{}^{B'}$	(μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1})	155 (±4)	192 (±5)
$R_d{}^B$	(μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1})	17 (±6)	39 (±16)
I_c	(μE m^{-2} s^{-1})	9 (±3)	22 (±9)
r^2		0.913	0.921

Numbers in parentheses indicate 95% confidence intervals.

* Parameters were estimated for rates of net photosynthetic oxygen evolution by non-linear regression to the exponential function $P_n\ (I)^B=P_m{}^{B'}\ (1-\exp\ (-\alpha^{B'} I/P_m{}^{B'}))-R_d{}^B$. $\alpha^{B'}$ and $P_m{}^{B'}$ are initial slope of the *P-I* curve and the Chl *a*-specific rate of maximum photosynthesis, respectively. $R_d{}^B$ (Chl *a*-specific rate of dark respiration) was determined by linear extrapolation of the initial slope of the *P-I* curve.

rate of net photosynthesis (P_m^B') in cells grown under 11 μE m^{-2} s^{-1} was lower than that in cells grown under 42 μE m^{-2} s^{-1} (*t*-test, $P<0.001$). It is notable that R_d^B in the former cells was less than half of that in the latter cells. The light compensation point (I_c) for the former cells decreased to around 40% of that for the latter cells (Table 3).

The R_d^B value in cells grown under 42 μE m^{-2} s^{-1} amounted to 17% of P_m^B and 45% of $P_g(I)$ at the incident growth irradiance of 42 μE m^{-2} s^{-1} (87 μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}), respectively. In cells grown under the growth irradiance of 11 μE m^{-2} s^{-1}, the R_d^B value was 7% of P_m^B but equaled 55% of $P_g(I)$ at 11 μE m^{-2} s^{-1} (31 μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}).

The trend of photosynthetic parameters and relative amount of photosynthetic pigments under the two light conditions was the same when expressed as Chl *a*-basis and cell-basis (data not shown), since significant change in cellular content of Chl *a* could not be observed between the two growth irradiance conditions (*t*-test, $P>0.05$) (Table 2).

Discussion

The composition of major pigments identified for *Chaetoceros* sp. B23-p1 was typical for diatoms (Table 1). The light intensities tested in this study were similar to those observed in the ice sheet in the eastern basin of Saroma Ko lagoon where samples were collected for examination of the photosynthetic pigment composition of natural ice algae assemblages (Fig. 4 in Kashino *et al.*, 1998). Chl *c* and fucoxanthin were the major pigments in both samples. In the natural ice algal samples, the relative amounts of Chl *c* and fucoxanthin to Chl *a* under low light condition were lower than those in the upper layer of the ice. However, the amounts of Chl *c* and fucoxanthin in *Chaetoceros* sp. B23-p1 cells remained almost identical under 5 μE m^{-2} s^{-1}. They were higher in that range than those observed for samples collected from sea ice. The apparent difference in the amounts of Chl *c* and fucoxanthin might be caused by the presence of other algae whose pigment composition was different from that of the strain tested in the present study. Alternatively, it might come from the difference of other conditions, which control variation in the pigment composition, such as light-regime, nutrient flux, salinity and temperature.

The pattern of variation in the amount of diadinoxanthin against the incident irradiance was also different between the two samples. In the natural samples, the amount of diadinoxanthin did not change in the vertical profile (Kashino *et al.*, 1998). However, the amount of diadinoxanthin in *Chaetoceros* sp. B23-p1 increased with increasing growth irradiance. The relative amount of diadinoxanthin against Chl *a* in cells grown under 42 μE m^{-2} s^{-1} was three times as high as that under 5 μE m^{-2} s^{-1}. It was also notable that the amount of diadinoxanthin in *Chaetoceros* sp. B23-p1 is higher than that that in the natural ice algal samples observed by Kashino *et al.* (1998). Diatoxanthin and β-carotene were minor components in both culture and natural samples. Further investigation is required to understand the environmental control of the pigment composition in natural ice algal assemblages.

Diadinoxanthin and diatoxanthin have been known to be involved in the xanthophyll cycle in Chromophytes and play a photoprotective role against relatively short-

term increase in light intensity (Arsalane *et al.*, 1994). Rapid de-epoxidation of diadinoxanthin to diatoxanthin occurs under excessive irradiance. The amount of diatoxanthin is related to the rate of thermal de-excitation in photosystem II, the reacting site of O_2 evolution (Olaizoa *et al.*, 1994). When the efficiency of the reaction in photosystem II was examined by the gross photosynthetic O_2 evolution at low irradiances, the value of α^B under 42 μE m^{-2} s^{-1} was around 87% of that under 11 μE m^{-2} s^{-1} (Table 2). The amount of diadinoxanthin in the former cells was around 1.7 times of that in the latter cells. The present result showed that the increase in the amount of diadinoxanthin possibly reduced the efficiency of photosynthetic O_2 evolution (α^B) at low irradiances. However, significant change in the rate of O_2 evolution of P_m^B was not observed during the 20 min intervals in the cells grown under the two irradiance conditions (Table 2).

Kashino *et al.* (1999) found that ice algae collected from the bottom part of the ice of Saroma Ko lagoon had high capacity of rapid de-epoxidation of diadinoxanthin upon illumination at 100 μE m^{-2} s^{-1}. Further prolonged illumination at higher irradiances might depress photosynthesis, since the de-epoxidation into diatoxanthin slowed down after 20 min of illumination at 100 μE m^{-2} s^{-1} (Kashino *et al.*, 1999). However, prolonged illumination increased the amount of diadinoxanthin (Kashino *et al.*, 1999) as observed in our culture experiments. These results may indicate that the size of the pool of diadinoxanthin may be adjusted by the light environment and determined the photochemical efficiency at low light intensities as well as protective ability upon relatively short-term (from minutes to hours) change of irradiance.

The two strains of diatoms commonly showed a linear relationship between the intensity of growth irradiances and the growth constant (Fig. 1). If loss of energy by respiration is considered, the growth would collapse at some light intensity and the regression lines for the growth constant *vs.* irradiance would be extrapolated to the abscissa. However, the extrapolated lines did not cross the abscissa but the ordinate, because of relatively high values of growth constant under the low irradiances. This may suggest that the efficiency of light-energy utilization increases at low irradiances in both strains. The ability to grow under low irradiance could be realized in the response of net photosynthesis (Table 3). If no acclimation occurred, the photosynthetic performance determined for cells grown under 42 μE m^{-2} s^{-1} shows that they would not grow well under 11 μE m^{-2} s^{-1}, since I_c was as high as 22 μE m^{-2} s^{-1}. However, the I_c value decreased to around 40% when the growth irradiance decreased to 11 μE m^{-2} s^{-1}. Although the initial slope of the *P-I* curve for net photosynthesis ($\alpha^{B'}$) was not significantly different between the two irradiance conditions (*t*-test, $P>0.05$), the decrease of diadinoxanthin in the latter cells might result in reduction of I_c, because of significantly higher α^B (*t*-test, $P<0.001$). The change in the photosynthetic pigment composition was not solely responsible for the observed change in I_c; the decrease in dark respiration rate might also contribute to the decrease in I_c.

Reduction of respiration has been recognized to be possibly important in temperature acclimation of psychrophilies (Tilzer and Dubinski, 1987; Thomas *et al.*, 1992; Smith *et al.*, 1994). The rates of respiration or loss of assimilated carbon in psychrophilic diatoms have been shown to be low in this context (Bunt *et al.*, 1966; Thomas *et al.*, 1992; Smith *et al.*, 1994). Low dark respiration rate also means low energy loss

under irradiance fluctuating below I_c and is one possible way to decrease I_c (Kudoh *et al.*, 1997). The present results show that the number of R_d^B in cells grown under 42μE m^{-2} s^{-1} was halved by decrease of growth irradiance to 11μE m^{-2} s^{-1} (Table 3). Such acclimation makes the diatom cells possible to elevate net photosynthesis and can also explain the high growth efficiency under low and diurnally oscillating irradiance.

The *in situ* specific growth constant of ice algae has been assumed to be 0.06–0.26 d^{-1} in the growing season in Saroma Ko lagoon and elsewhere in higher latitudes (Cota *et al.*, 1987; Welch and Bergman, 1989; Cota and Sullivan, 1990; Kudoh *et al.*, 1997). The irradiance condition may changes more rapidly than the generation time of ice algae, due to the development, melting and erosion of the ice, diel variation in solar angle and temporal change of snow coverage. It has been shown that the acclimation of pigment content in a microalga occurred after at least one generation time (Post *et al.*, 1985). The adjustment of respiration may allow a rapid change in the efficiency of light utilization without large change in cellular constituents, such as the light-harvesting pigment-protein complex.

Weger *et al.* (1989) have demonstrated in the study for a mesophile diatom that the rate of respiration in light increases to around twice as high as the dark respiration rate. The light-enhanced respiration has possibly been ascribed to increased substrate supply from photosynthesis (Weger *et al.*, 1989). It has also been suggested that the O_2 consumption rate measured immediately after illumination was enhanced by the illumination (referred to as "enhanced post-illumination respiration") (Falkowski *et al.*, 1985). The post-illumination respiration determined in the present study also increased with increasing illumination up to around I_k for $P_g(I)$ (Fig. 2A and B). This might affect the estimation of the dark respiration rate (R_d^B), although extrapolation to the dark condition possibly eliminates such an enhancement of respiration by pre-illumination. The derived dark respiration rates also apparently related to the $P_g(I)$ at the incident growth irradiance (around 50% of $P_g(I)$).

The *P-I* curves of gross photosynthetic O_2 evolution in *Chaetoceros* sp. B23-p1 show that I_k was much higher than the incident growth irradiances (76 and 88μE m^{-2} s^{-1} under the growth irradiances of 11 and 42μE m^{-2} s^{-1}, respectively) (Fig. 2 and Table 2). Photoinhibition could not be observed up to around 800μE m^{-2} s^{-1} under the examined conditions. The P_m^B values obtained for the two irradiance conditions are rather high in the range of values collected for various ice algal assemblages, if converted on the assumption of a photosynthetic quotient of 1 : 1 (2.8 *vs.* 0.05–3.9 mg C (mg Chl *a*)$^{-1}$ h^{-1}) (Cota and Smith, 1991; Thomas *et al.*, 1992). Thomas *et al.* (1992) examined the effect of irradiance as well as temperature on the *P-I* curves for carbon fixation in psychrophilic *Chaetoceros* sp. isolated from the Weddell Sea. Their I_k value of 24–31μE m^{-2} s^{-1} estimated for cells grown under 5μE m^{-2} s^{-1} was much lower than 76μE m^{-2} s^{-1} obtained under 11μE m^{-2} s^{-1} in the present study (Table 2), although their I_k value of 64–74μE m^{-2} s^{-1} for cells grown under 55μE m^{-2} s^{-1} was similar to that under 42μE m^{-2} s^{-1} in our case. The difference in the response of the *P-I* curves may reflect the difference in the physiological characteristics of the two psychrophilic *Chaetoceros* and/or deviation between the photosynthetic response of carbon fixation and that of O_2 evolution under higher irradiance conditions, especially in low-light cells.

The present results possibly indicate that the activity of photosystems as long as O_2

evolution is not impaired under wide a range of irradiance under either level of growth irradiance. The values of P_m^B are 2.7 times and even 7.5 times as high as the gross photosynthetic rates under light intensities of the incident growth irradiances of 42 and 11 μE m^{-2} s^{-1}, respectively (234 *vs.* 87, and 231 *vs.* 31 μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}, *cf.* Fig. 2 and Table 2). Thus, the capacity of photochemical reaction around photosystems seems to remain large even under the incident irradiance such as in the ice layer. Increase of diadinoxanthin would raise the potential to protect photosystems against higher irradiance due to rapid energy dissipation in the xanthophyll cycle. Conversely, decrease of diadinoxanthin seems to increase the photochemical efficiency at low light. Adjustment of dark respiration rate in response to incident irradiances also makes the diatom cells possible to elevate net photosynthesis and would explain the high efficiency of growth under low irradiance. These properties may explain the functioning of photosystems and efficient light utilization under large variation of irradiance even on a short time scale.

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