Photoacclimation strategy of ice algal community in the seasonal sea ice

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Abstract: Samples containing algal cells were collected from the bottom layers of sea-ice in Saroma-Ko Lagoon, Hokkaido, Japan in early spring 2010–2012. The physical and biogeochemical parameters of brine and sea ice, the structure of the ice algal community, and their photosynthetic characteristics were determined. Light responses of the relative electron transport rate (rETR) of the communities were estimated by quantum yield (ΔF/Fm′) of photosystem II at various light intensities with a pulse amplitude modulation (PAM) fluorometer. Although the compositions of the communities were different between the three years, their maximum quantum yields (Fv/Fm) were well correlated to in situ light attenuation. In contrast, their light acclimation index (Ek), the ratios of maximum rETR to the efficiency of the relative electron transport (α), varied year by year. This suggests that the ice algal communities have effective light-harvesting capabilities and their photoacclimation strategy might be related to the structure of the community.

Key words: chlorophyll a specific light absorption (aφ*), light acclimation index (Ek), maximum photosynthetic capacity (rETRmax), maximum quantum yield (Fv/Fm), photosynthetic efficiency (α)

Introduction

Microalgae are trapped in sea ice when sea ice is formed as suggested by Garrison et al. (1983) and Niimura et al. (2000). They develop as ice algal communities in brine channels and grow to extremely high biomass in the bottom layer by the end of the ice season due to a high nutrient supply from the underlying water (e.g., Horner & Alexander 1972, Smith et al. 1990, Legendre et al. 1991).

Light intensity in the ice algal layer is greatly reduced by absorption and scattering by snow and sea ice and the heterogeneous distribution of brine channels (Gosselin et al. 1986, Perovich et al. 1998, Mundy et al. 2007). Ice algal cells absorb light due to the high cellular contents of chlorophyll a (Chl a) (Mundy et al. 2007, Pogson et al. 2011), requiring the ice algae to photoacclimate (Palmisano et al. 1985, Robinson et al. 1995, Rysgaard et al. 2001). Although the cellular Chl a increases to enhance the cellular absorption of light, the increasing cellular Chl a causes high package effects (Dubinsky et al. 1986), resulting in a low efficiency of cellular light absorption (Qφ) (Fujiki & Taguchi 2002).

To examine the photoacclimation of aquatic photosynthetic micro-organisms, Pulse-Amplitude Modulation (PAM) fluorometry has been effectively employed (Suggett et al. 2010a). The efficiency of photosystem II (PSII) photochemistry, ΦPSII, can be measured by PAM (Suggett et al. 2010b). Potential efficiency of PSII measured with dark-adapted samples, called maximum ΦPSII, has often been used as an index of light adaptation to environmental factors, such as irradiance and nutrients (Franklin et al. 2009). Maximum ΦPSII of ice algae has been reported to vary between 0.022 and 0.65 (Kühl et al. 2001, McMinn & Hesgath 2004). It increases with the duration of the sea ice season, indicating progressive acclimation to the environment (Manes & Gradinger 2009). The ΦPSII can be used to estimate the rates of linear electron transport (ETR) by multiplying the light intensity absorbed by algae. The relationship between rETR and irradiance follows a Rapid Light Curve (RLC, White & Critchley 1999) which can be used to characterize the photosynthetic performance of aquatic photoautotrophs (Kühl et al. 2001). Similar to conventional P versus I curves, RLCs can be di-
vided into two characteristic phases: the light-limited and light-saturated phases. The former phase is referred to as the photosynthetic efficiency ($\alpha$), whereas the latter phase is referred to as the maximum photosynthetic capacity ($rETR_{max}$) (Schreiber 2004). Ice algal communities in the sub-Arctic show relatively higher $\alpha$ up to 0.605 $\mu$ mol electrons ($\mu$mol electrons)$^{-1}$ (McMinn & Hattori 2006), with lower $rETR_{max}$ (e.g., 7.1 $\mu$mol electron m$^{-2}$ sec$^{-1}$, McMinn & Hesgath 2004) than temperate marine diatoms (Cruz & Serodio 2008, McMinn & Lee 2013). The increase in $\alpha$ demonstrates enhanced light utilization efficiency at low irradiance to compensate for the low efficiency of cellular light absorption due to the high cellular Chl content.

Derived from RLC data, the light saturation index ($E_k=rETR_{max}/\alpha$) is defined to indicate irradiance where the saturation of $rETR$ becomes dominant (Schreiber 2004). Due to the higher $\alpha$ and lower $rETR_{max}$, the $E_k$ of the ice algal community becomes lower than for pelagic phytoplankton (McMinn et al. 2005). $E_k$ can also be employed to characterize the response to the ambient light climate at different latitudes on a global scale (McMinn & Hattori 2006) and seasonal aclimation on a regional scale (Rysgaard et al. 2001). In the seasonal sea ice formed at one of the southern limits of sea ice, such as Saroma-Ko Lagoon, $E_k$ ranges from 15 to 173 $\mu$mol photons m$^{-2}$ sec$^{-1}$ (McMinn & Hattori 2006, Obata & Taguchi 2009). In Saroma-Ko Lagoon, seasonal sea ice is formed every year in February and March, and the duration of ice algal blooms is less than three months, resulting in thin ice (approximately 50 cm) with high levels of transmitted light (Taguchi & Takahashi 1993). Because the ice algal community is expected to reach its seasonal maximum biomass in thin ice at the end of the ice season, a higher $E_k$ should be expected due to the higher irradiance at the ice algal layer in Saroma-Ko Lagoon compared to the Arctic (Kühl et al. 2001, Rysgaard et al. 2001, Obata & Taguchi 2009).

Based on conventional photosynthesis versus irradiance curves, phytoplankton show two types of photoacclimation strategies: $E_k$-dependent variation and $E_k$-independent variation (Behrenfeld et al. 2004). A similar approach could be applied to the $rETR$ versus $E$ curve in the present study on an ice algal community. The $E_k$-dependent variation is caused by independent alterations in $rETR_{max}$ and $\alpha$. The $E_k$-independent variation is caused by parallel changes in both parameters with no change in $E_k$. In the Arctic Ocean, ice algae show higher values of $rETR_{max}$ and $\alpha$ at the beginning of the sea ice season. Decreases in $rETR_{max}$ are seen during the later parts of the sea ice season (Manes & Gradinger 2009, Hawes et al. 2012). This suggests $E_k$-independent variation in Arctic ice algae.

We hypothesized variable photoacclimation of the ice algal community (along a depth gradient) with the development of sea ice. The hypothesis was proposed because $F_v/F_c$ decreased with increasing light attenuation, whereas the $E_k$-strategy between ice seasons could change according to community structure.

### Materials and Methods

#### Sampling

Sampling was conducted at the end of the ice period each year, at the site off Sakae-Ura, east of Saroma-Ko Lagoon on the northern coast of Hokkaido (44°07'N, 143°57'E) (Fig. 1). The snow depth and ice core thickness were measured with a rule. Water samples were collected from within 0.5-m below the ice-water surface at each site to obtain filtered sea water (FSW) after filtration using glass fiber filters (GF/F, Whatman, UK) with a nominal pore size of 0.7 $\mu$m. Two ice cores, within a 1 m$^2$ radius of each other, were collected from the four corners of the site by using a CRREL (Cold Region Research and Engineering Laboratory) core sampler (Rand & Mellor 1985). Ice cores were immediately placed in a dark box to avoid exposure to ambient irradiance and then transported to a laboratory. The bottom 9 cm of each core was sectioned, in the dark, into three 3 cm long sections. The bottom, middle, and upper third, respectively, were combined for cores from the same station and were melted in a bottle with FSW to avoid osmotic changes.

#### Environmental factors

The photosynthetically available radiation ($PAR_0$) values in the air and at the undersurface of the sea ice ($PAR_z$), with and without snow coverage, were measured simultaneously using a PAR air sensor (Li-Cor, LI-190SA, USA) placed on the surface of the snow and a PAR underwater sensor (Li-Cor, LI-193 A, USA) placed on the undersurface of the sea ice, respectively. Next, a block (30 cm$\times$30 cm width, 36 cm high) for the $PAR_z$ measurements was cut out of the ice and the bottom 3 cm was sliced off before the block was replaced in the original position and another $PAR_z$ measurement was made at the undersurface of the ice. This was repeated twice with two more 3 cm thick slices being removed and $PAR_z$ measurements taken. The remaining 27 cm thick ice block then had the lower 9 cm removed before the block was again deployed and a further $PAR_z$ measurement was taken. This was repeated twice with one further slice and two further $PAR_z$ measurements.
being made. The attenuation coefficient ($K_a$) for each 3-cm algae-containing layer and each 9-cm non-algae containing layer was calculated by formula (1):

$$K_a = \left(-\frac{\ln(PAR_{Z_i}/PAR_{Z_i+1})}{Z_i}\right)$$

(1)

where $Z_i$ is the distance of $i$th ice core from the bottom of the whole ice core. The difference in PAR between the top of the algal layer and the undersurface of the sea ice was estimated to be due to the amounts of PAR absorbed (and scattered) by a combination of sea ice and sea algae. The temperatures in the ice cores at the sites were determined using a thermometer (Testo, Testo110, Japan) by drilling a hole (Takagi, DDR-140KD, Japan) with a diameter of 4.0 mm to the core center at a distance of 1.5 cm from the ice-water interface immediately after the core was recovered in the field. The salinity levels of the mixture of the melted core section with filtered sea water ($S_m$) and of the filtered sea water ($S_i$) alone were calculated based on conductivity in the laboratory (Horiba, ES-51, Japan). The volumes of the samples for the analysis of salinity, nutrient concentrations, species enumeration, and pigment concentrations were corrected in accordance with the method provided by Harrison et al. (1990) to calculate the in situ concentrations in sea ice from formula (2):

$$C_a = [(C_m \times V_m) - (C_i \times V_i)]/V_a$$

(2)

where $C_a$ is the concentration in sea ice, $C_m$ is the concentration in the mixture of sea ice and FSW, $C_i$ is the concentration in FSW, $V_a$ is the volume of sea ice, $V_m$ is the volume of the mixture of sea water and FSW, and $V_i$ is the volume of FSW. The brine volume fraction in the sea ice was calculated from temperature and salinity measurements following the equations provided by Cox & Weeks (1983) and Eicken (2003).

Subsamples for the analysis of nutrients were filtered through a membrane filter with a 0.45 µm pore size (Millex-GC, Millipore, USA), and the filtrates were kept frozen at −60°C in a deep freezer (MDF-C8V1, Sanyo Electronics, Tokyo, Japan) for further analysis. Nutrient concentrations, including nitrate+nitrite, nitrite, silicate, and phosphate in the melted samples, were determined using an Auto Analyzer (BL-TEC, SWAAT, Japan) with a C18 reserved-phase Ultrasphere 3 mm column, using a solvent gradient based on the modified method described by Wright et al. (1997). The peaks were quantified for Chl $a$, DD, and DT using standards obtained from the Danish Hydraulic Institute (Denmark).

**Light absorption analysis**

Light absorption analyses were conducted in 2011 and 2012. Subsamples for light absorption analysis were filtered onto glass fiber filters (GF/F, Whatman UK), frozen using liquid nitrogen, and stored at −60°C. The absorption spectra of the pigments [$a_p(\lambda)$] were measured between 400 and 700 nm using a spectrophotometer equipped with an integrating sphere (UV-2450, Shimazu, Japan) based on the quantitative filter technique (Mitchell 1990). The pigments on the filter were then extracted in 100% methanol for one hour using the procedure of Kishino et al. (1985) to measure $a_d(\lambda)$. Filters moistened with FSW were used as the reference material. The absorption coefficients of the total particles [$a_p(\lambda)$] and detritus [$a_d(\lambda)$] were calculated by integrating the absorption spectra in the range from 400 to 700 nm (Cleveland & Weidemann 1993). The light absorption coefficient [$a_{ph}(\lambda)$] of the ice algal community was calculated by formula (3):

$$a_{ph} (\lambda) = a_p (\lambda) - a_d (\lambda)$$

(3)

The Chlorophyll $a$ (Chl $a$) specific absorption coefficient ($a_p^\ast$) was calculated by formula (4):

$$a_p^\ast = a_{ph} (\lambda)/\text{Chl } a^\prime$$

(4)

where Chl $a^\prime$ is the concentration of Chl $a$ in mg m$^{-3}$. The light absorption efficiency ($Q_p^\ast$) at 675 nm was calculated by formula (4) (Bricaud et al. 1995, Johnsen et al. 1994):

$$Q_p^\ast(675)=a_{ph}^\ast(675)/0.027$$

(5)

where $a_{ph}$ (675) is the absorption by phytoplankton at 675 nm, and 0.027 is the specific absorption coefficient at 675 nm for diatoms (Morel & Bricaud 1981). Contribution of $a_{ph}$ to $K_a$ was calculated by formula (6):

$$K_{ph} = a_{ph} \cdot K_d^{-1}$$

(6)
Fluorescence analysis

Active fluorescence of the melted ice samples that were diluted with FSW was measured using a fluorometer (Water-PAM, Walz, Germany) with red actinic light (660 nm) within eight hours from the time of collection (Garrido et al. 2013). Subsamples of 4 ml were placed in a 15-mm-diameter quartz cuvette in darkness at 0°C for 30 minutes. A measuring light was applied to detect the minimum fluorescence ($F_0$) in the dark-adapted state. When $F_0$ became stable, a saturating pulse of 2,500 µmol photons m$^{-2}$ sec$^{-1}$ for 1 sec was supplied to detect the maximum fluorescence ($F_m$). The steady-state ($F$) and maximum fluorescence ($F'_m$) under actinic light were measured using a measuring light and saturation pulse, respectively, after exposure to nine different levels of the actinic light, ranging from 0 to approximately 900 µmol photons m$^{-2}$ sec$^{-1}$, with the emitting diodes having a maximum intensity at 655 nm. The ratio of $F_0$ to $F_m$ of PSII (maximum quantum yield) and the ratio of $ΔF/F'_m$ of PSII (quantum yield) were calculated from formula (7) (Butler & Kitajima 1975) and formula (8) (Genty et al. 1989), respectively:

$$\frac{F_0}{F_m} = \frac{(F_m - F_0)}{F_m} \quad (7)$$

$$\frac{ΔF}{F'_m} = \frac{(F'_m - F)}{F'} \quad (8)$$

The $rETR$ was calculated from formula (9) (Schreiber et al. 1994):

$$rETR = \frac{PFD(ΔF/F'_m)0.5Qa}{660} \quad (9)$$

where PFD is the photon flux density at the nine different levels of actinic light, which ranged from 0 to approximately 900 µmol photons m$^{-2}$ sec$^{-1}$, and 0.5 is the ratio of the absorbed light going to PSII (Gilbert et al. 2000). The $rETR$ versus irradiance curve was obtained by fitting the curve to formula (8) (Webb et al. 1974), with no indication of photoinhibition:

$$rETR = rETR_{max}(1 - \exp(-\alpha PFD/rETR_{max})) \quad (10)$$

where $rETR_{max}$ is the maximum $rETR$, and $α$ is the efficiency of the relative electron transport, which is the initial slope of the curve. The light saturation coefficient ($E_k$) was calculated from formula (11):

$$E_k = rETR_{max}/α \quad (11)$$

Statistical analysis

The mean and one standard deviation of variables were calculated and reported for the bottom, middle, and top sections of the ice core at 1.5 cm, 4.5 cm, and 7.5 cm on the figures in this study. Significant differences between treatments were analyzed using the Student's t-test for unpaired comparisons and using a paired t-test and Wilcoxon signed-rank test for paired comparisons, using the SigmaPlot software program (System Software, version 11.0, San Jose, USA).

Results and Discussion

Environmental conditions

Sampling was conducted when the ice algal biomass was expected to have reached the seasonal maximum (Obata & Taguchi 2009). These dates corresponded to near the end of the ice season in Saroma-Ko Lagoon. The mean air temperature from the beginning of the freezing of the

Table 1. Freezing period (days from complete ice cover to sampling), mean (±SD) air temperature during the freezing period and salinity in the bottom algal layer.

| Year | Freezing period (days) | Air temperature (°C) | Sea ice temperature (°C) | Sea ice salinity |
|------|------------------------|-----------------------|--------------------------|-----------------|
| 2010 | 30                     | -5.62±5.0             | -1.5±0.00                | 11.7±2.9        |
| 2011 | 42                     | -5.94±4.0             | -1.5±0.00                | 13.7±0.7        |
| 2012 | 32                     | -8.44±4.1             | -1.7±0.03                | 12.5±1.8        |

Table 2. Light conditions, snow depth, and ice thickness in Saroma-Ko Lagoon in 2010, 2011, and 2012. PAR and percentage in parenthesis indicate the incident on the snow surface, top of algal layer, and the transmitted through ice cover. * indicates that the extinction coefficients in the ice core above the algal layer were assumed to be approximately 6 m$^{-1}$.

| Year | Incident on snow surface (µmol photons m$^{-2}$ s$^{-1}$) | Top of algal layer (µmol photons m$^{-2}$ s$^{-1}$) | Transmitted through ice (µmol photons m$^{-2}$ s$^{-1}$) | Snow (cm) | Sea ice (cm) |
|------|--------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------|----------|-------------|
| 2010 | 368±36 (100%)                                         | 18.3±0.40* (5.0%)                              | 9.11±0.2 (2.5%)                                         | 11.9±0.25| 43.7±2.8    |
| 2011 | 1289±31 (100%)                                        | 26.2±2.4 (2.0%)                                 | 12.5±1.2 (0.97%)                                       | 11.1±1.1 | 51.6±1.1    |
| 2012 | 330±1.7 (100%)                                        | 6.03±0.03 (1.8%)                                | 2.23±0.09 (0.68%)                                      | 15.6±1.3 | 40.9±1.1    |
whole lagoon surface to the sampling day was lowest in 2012, although the freezing period of sea ice until the day of sampling was approximately 10 days shorter in 2010 and 2012 than in 2011 (Table 1). The snow coverage of 10 to 20 cm and ice thickness of 40 to 50 cm observed in the three years of this study (Table 2) were similar to values reported earlier in Saroma-Ko Lagoon (Robineau et al. 1997, Shirasawa et al. 2005). The temperatures of the sea water and sea ice were lowest at −1.7°C in 2012 (Fig. 2A). The salinity generally decreased from 14 in the bottom algal layer to 10 in the top algal layer within the bottom 9 cm of the ice core (Fig. 2B). The highest salinity in the bottom algal layer was consistent with previous observations from the same area (Shirasawa et al. 2005). The highest (>39%) and lowest brine volume fractions (<34%) were observed in the bottom and top algal layers in all three years, respectively (Fig. 2C). The values were much higher than in 2006 and 2008 (Nomura et al. 2010). The smaller brine volume fractions in 2012 indicate that the lowest temperature might maintain the configuration of the brine channels due to less melting (Eicken 2003).

Nitrate and silicate concentrations were usually higher than 1 µM, except in the middle and top algal layers in 2012 (Fig. 2D) and all three algal layers in 2012 (Fig. 2E), respectively. Phosphate concentrations were usually higher than 0.1 µM, except for the top algal layer in 2011 and all three algal layers in 2012 (Fig. 2F). The nutrient concentrations in the bottom algal layer were usually equal to or higher than the other two algal layers combined because the brine was replaced by sea water flowing into the ice when the brines had flowed out of the sea ice due to gravity with increasing temperature (Eicken 2003). The nutrient concentrations in the water column were usually higher than in the algal layers, except for nitrate in the bottom algal layer in 2012. The higher nitrate concentrations in the bottom algal layer could have been maintained by minimal gravity drainage at the lowest temperature (Fig. 2A). Only nitrate concentrations (NO₃) were related to the brine volume (%), as NO₃=0.24%–5.2 (%=0.79, p<0.05). The significance of the relationship should be treated with caution because nitrate is usually promptly utilized by the ice algal community (Smith et al. 1997) where the Chl a concentration was enhanced. However the Chl a concentration did not correlate with the brine volume in this study.
Biomass and species

The Chl $a$ concentration and total cell volume showed similar temporal and spatial variations (Figs. 3A & 3B). Size of xanthophyll cycle pigment (DD+DT/Chl $a$) peaks and activity levels of xanthophyll cycle pigments (DT/ DD+DT) indicated maxima in the top algal layer and minima in the bottom algal layer, except in 2011 when there was little difference along the vertical gradient (Figs. 4A & 4B). The decrease in DD+DT/Chl $a$ and DT/DD+DT with depth of the algal layers suggests photoacclimation of the ice algal community to low irradiance (Demers et al. 1991, Sigleo et al. 2000). The mean cell volumes of the total ice algal community were $>8 \times 10^3 \mu m^3$ cell$^{-1}$ in 2010, whereas mean cell volumes were $<5 \times 10^3 \mu m^3$ cell$^{-1}$ in 2011 and 2012. Based on the cell volumes the largest species were pennate diatoms, such as *Pinnularia quadratarea* var. *constricta* and *Entomoneis* sp., cell volumes of which were approximately $13 \times 10^3 \mu m^3$ cell$^{-1}$ (Table 3). In 2010 and 2011, the most abundant species were larger centric diatoms such as *Odontella aurita*, *Detonulla confervacea*, and *Thalassiosira* spp., with volumes greater than $3.2 \times 10^3 \mu m^3$ cell$^{-1}$ and smaller pennate diatoms such as *Achnanthes* sp. and *Navicula transitans* var. *delicatula*, with volumes less than $4.1 \times 10^3 \mu m^3$ cell$^{-1}$.

Table 3. Mean cell volume and abundance of most common 8 ice algal species observed in Saroma-Ko Lagoon in 2010, 2011, and 2012.

| Species            | Cell volume ($\mu m^3$ cell$^{-1}$) | Algal layer | Maximum Abundance ($\mu m^3$ m$^{-3}$) |
|--------------------|-------------------------------------|-------------|-----------------------------------------|
| *Odontella aurita* | 4,400                               | Top         | 20                                      | 6.1 1.1 |
|                    |                                     | Middle      | 40                                      | 4.6 0.61 |
|                    |                                     | Bottom      | 17                                      | 0.61 0.01 |
| *Detonulla confervacea* | 3,200                             | Top         | 1.4                                     | 29 15 |
|                    |                                     | Middle      | 4.3                                     | 35 40 |
|                    |                                     | Bottom      | 6.5                                     | 40 48 |
| *Thalassiosira* spp. | 11,000                              | Top         | 2.2                                     | 6.8 3.7 |
|                    |                                     | Middle      | 3.5                                     | 4.9 7.3 |
|                    |                                     | Bottom      | 3.4                                     | 6.3 7.8 |
| *Pinnularia quadratarea* var. *constricta* | 13,000                               | Top         | 0.20                                    | 0.85 11 |
|                    |                                     | Middle      | 0.34                                    | 2.7 18 |
|                    |                                     | Bottom      | 0.20                                    | 11 160 |
| *Navicula transitans* var. *delicatula* | 4,100                                | Top         | 0.22                                    | 4.2 31 |
|                    |                                     | Middle      | 0.060                                   | 6.8 17 |
|                    |                                     | Bottom      | 0.20                                    | 9.8 76 |
| *Achnanthes* sp.    | 720                                 | Top         | 0.02                                    | 0.25 7.4 |
|                    |                                     | Middle      | 0.03                                    | 0.88 19 |
|                    |                                     | Bottom      | 0.02                                    | 3.0 5.3 |
| *Nitzschia frigida* | 1,900                               | Top         | 0.042                                   | 0.10 2.2 |
|                    |                                     | Middle      | 0.12                                    | 0.55 3.9 |
|                    |                                     | Bottom      | 0.020                                   | 0.42 15 |
| *Entomoneis* sp.    | 13,000                              | Top         | 0.082                                   | 1.1 6.5 |
|                    |                                     | Middle      | 0.55                                    | 0.73 4.4 |
|                    |                                     | Bottom      | 1.2                                     | 1.7 0.28 |
| Total              |                                     | Top         | 25.1                                    | 51.1 79.8 |
|                    |                                     | Middle      | 51.1                                    | 67.9 114 |
|                    |                                     | Bottom      | 29.7                                    | 87.4 322 |

The most common 8 species had a similar relative abundance in the three algal layers in 2010 and 2011 (Fig. 5).
When the brine volume indicated similar values, such as in 2010 and 2011 (Fig. 2C), the algal biomass and species structure were similar in the three algal layers (Fig. 3A, Fig. 5). In shallow water, such as Saroma-Ko Lagoon, similar species would be expected to appear every ice season because ice algal cells can be recruited from resting spores as suggested by Palmisano & Sullivan (1982). Centric diatoms dominate the phytoplankton community at the beginning of the period of sea ice formation in Saroma-Ko Lagoon (Niimura et al. 2000). Pennate diatoms comprised 76.8±7.3% of the total number of phytoplankton cells toward the end of the ice season in the present study in Saroma-Ko Lagoon. Pennate diatoms were dominated by *Pinnularia quadratarea* var. *constricta*, *Navicula transitans* var. *delicatula*, and *Nitzschia* spp. (Fig. 5). In 2010 and 2011, the high dominance of centric diatoms suggests that the species composition might have remained relatively similar in the three algal layers, because the Chl a biomass remained low in all three algal layers.

**Light attenuation and absorption**

The light attenuation coefficients in the 16 cm snow layer in 2012 were 0.63±0.25 m⁻¹ (Fig. 6A). The attenuation coefficients in the top 27 cm of ice without apparent Chl a were approximately 6 m⁻¹, whereas the attenuation coefficients in the bottom 9 cm core with Chl a ranged from 6.5 m⁻¹ in the top algal layer to 33 m⁻¹ in the bottom algal layer. When the attenuation by Chl a was estimated from the Chl a concentrations (Fig. 3A) and the chlorophyll a specific absorption (Fig. 3C), their mean contribution to the total attenuation coefficient $K_{ph}$ was similar in each of the three algal layers, at 31±7%. The attenuation coefficients in the bottom algal layer were enhanced most by the increase in the ice algal biomass. Although the relative light penetrations of 2.5% in 2010 and 0.97% in 2011 at the undersurface of the sea ice were larger than the value of 0.68% in 2012 (Table 2), the percent PAR transmission at the undersurface of the sea ice remained within the range from 3.3 to 0.72% (Kishino 1993, Obata & Taguchi 2009).

The lowest light penetration in 2012 may have been due to
the thickest snow cover (Table 2) and the coldest air temperature \((p < 0.05, \text{Table 1})\).

The Chl \(a\) specific absorption coefficient in the bottom algal layer was smallest in 2010 and 2012, whereas a uniform distribution between the algal layers was observed in 2011 (Fig. 3C). The highest \(a_{ph}^a\) in 2010 was associated with the lowest Chl \(a\) per cell volume (Fig. 5B) as predicted from phytoplankton studies (e.g., Bricaud et al. 1995). The absorption efficiency \((Q^a)\) at 675 nm in the three algal layers in 2010 and 2011 was 0.81±0.12 and 0.47±0.04, respectively and lowest 0.37±0.06 in 2012 (Fig. 3D). The lowest \(Q^a\) (0.30±0.02) was observed in the bottom algal layer in 2012 (Fig. 3D). The lowest \(a_{ph}^a\) and \(Q^a\) associated with the highest Chl \(a\) biomass, particularly in 2012, were caused by a higher accumulation of Chl \(a\) in the cells due to lower irradiance causing the package effect (Dubinsky et al. 1986). The package effect might be related with the change in species composition (Fig. 5), although this remains a topic for further research.

**Maximum quantum yield and relative electron transport rate**

The largest \(F_v/F_m\) was usually observed in the bottom algal layer in all three years (Fig. 7A). Over all maximum \(F_v/F_m\) (0.69±0.01) was observed in 2012. These results might suggest that the ice algal community, particularly in the bottom algal layer, acclimated well to low-light conditions by accumulating cellular Chl \(a\) (Fig. 3A) in the large volume cells (Fig. 3B) of pennate species (Fig. 5). It resulted in the lowest values of \(a_{ph}^a\) and \(Q^a\) (Figs. 3C & 3D). \(\Phi_{PIL\text{max}}\) and \(\alpha\) estimated from the rETR and \(E\) curve in each algal layer exhibited 2 fold variations between years (Table 4).

The negative relationship at Saroma-Ko Lagoon between \(\Phi_{PIL\text{max}}\) and % irradiance \((p < 0.01, \text{Fig. 8})\) suggests an acclimation to lower irradiance, although phytoplankton is not generally thought to be sensitive to growth irradiance (Kolber et al. 1988, Parkhill et al. 2001) and
Pennate diatoms, such as *F.* m  from 0.36 to 0.42 (McMinn et al. 2008). The highest *E*₂ values were observed in thicker ice (Meiners et al. 2009). Although a significant difference in *E*₂ was observed among the three years, a similar response of photoacclimation in the ice algal community was recognized in this study. The *α* and *r*ETRₘₚₓ increased with depth in 2010 and the maximum *α* and *r*ETRₘₚₓ were 0.095±0.016 µmol electrons (µmol photons)⁻¹ and 21.9±7.4 µmol electrons m⁻² sec⁻¹ in the bottom algal layer, respectively (Figs. 7B & 7D). The *α* increased with depth to 0.093±0.010 µmol electrons (µmol photons)⁻¹, whereas, *r*ETRₘₚₓ stayed at similar levels at approximately 17.5±0.7 µmol electrons m⁻² sec⁻¹ in 2011. The *r*ETRₘₚₓ and *α* decreased with depth in 2012 and the minimum *α* and *r*ETRₘₚₓ were 0.068±0.0012 µmol electrons (µmol photons)⁻¹ and 5.33±0.50 µmol electrons m⁻² sec⁻¹ in the bottom algal layer, respectively, because high biomass and low light absorption were observed (Figs. 3A & 3B). A significant relationship between *r*ETRₘₚₓ and *α* was observed in 2010 (Y=288X−6.8, r²=0.754, p<0.01) and 2012 (Y=33.9X+3.7, r²=0.507, p<0.01), whereas no significant relationship was observed in 2011 (Fig. 9). The significant, positive trends between *α* and *r*ETRₘₚₓ in 2010 and 2012 could be interpreted as *E*ₚ-independent variation (Behrenfeld et al. 2004) although the number of data was limited to 12 for each ice season. In 2011, there seemed to be a positive trend but it was not statistically significant, although *E*ₚ (220±50 µmol photons m⁻² sec⁻¹) was similar to in 2010 (Fig. 7C). The algal community in 2012 enhanced its photoacclimation ability to a low, narrow range of irradiance (1.8%, Table 2) by lowering *E*ₚ, whereas the ice algal communities in 2010 and 2011 exhibited different photoacclimation abilities.

### Table 4. *r*ETR versus Irradiance curves at the top, middle, and bottom algal layer in 2010, 2011, and 2012. All curves were significant at *r*²>0.999 at *p*<0.001.

| Algal layer | Year | *r*ETR | Equation |
|-------------|------|--------|----------|
| Top         | 2010 | 9.87   | 1−e (−0.072 PAR/9.87) |
|             | 2011 | 18.0   | 1−e (−0.145 PAR/18.0) |
|             | 2012 | 7.29   | 1−e (−0.219 PAR/7.29) |
| Middle      | 2010 | 11.9   | 1−e (−0.084 PAR/11.9) |
|             | 2011 | 17.8   | 1−e (−0.172 PAR/17.8) |
|             | 2012 | 9.04   | 1−e (−0.232 PAR/9.04) |
| Bottom      | 2010 | 20.8   | 1−e (−0.136 PAR/20.8) |
|             | 2011 | 16.2   | 1−e (−0.200 PAR/16.2) |
|             | 2012 | 5.34   | 1−e (−0.224 PAR/5.34) |

**Fig. 8.** *F*ₜ/*F*ₚₑ as a function of irradiance in the algal layer in 2010 (solid circle), 2011 (open circle), and 2012 (solid triangle). Vertical bars indicate the standard deviations from the mean.

*Φ*ₚₛᵢₐₘₓ is used as an indicator of nutrient stress in phytoplankton (Cleveland & Perry 1987, Geider et al. 1993). The highest *Φ*ₚₛᵢₐₘₓ in the bottom algal layers at the end of ice algal blooms in the Arctic (Manes & Gradinger 2009) also suggests acclimation to lower irradiance.

The highest *Φ*ₚₛᵢₐₘₓ in 2012 (Fig. 8) is also associated with the predominance of pennate diatoms (Fig. 5). Pennate diatoms exhibit higher *Φ*ₚₛᵢₐₘₓ than centric diatoms (McMinn et al. 2008). Pennate diatoms, such as *Navicula vanhoeffenii*, *Nitzschia frigida*, and *Navicula* spp. exhibited high values of *F*ₜ/*F*ₚₑ from 0.46 to 0.63, whereas centric diatoms, such as *Detonula conferaeva*, *Odontella aurita*, and *Thalassiosira nordenskioeldii*, exhibited lower values of *F*ₜ/*F*ₚₑ from 0.36 to 0.42 (McMinn et al. 2008).

**Photoacclimation of ice algal community and the relationship between *α* and *r*ETRₘₚₓ**

Little vertical difference in *E*ₚ was observed in each year (Fig. 7C) because of no statistical difference between algal layers (*p*>0.05). The lowest *E*ₚ (82±3 µmol photons m⁻² sec⁻¹) was observed in 2012, whereas the other two years showed significantly higher *E*ₚ, with 199±41 in 2010 and 223±47 µmol photons m⁻² sec⁻¹ in 2011. The relatively high *E*ₚ values in the present study might be due to the thickness of the ice, because relatively lower *E*ₚ values were observed in thicker ice (Meiners et al. 2009). Although a significant difference in *E*ₚ was observed among the three years, a similar response of photoacclimation in the ice algal community was recognized in this study. The *α* and *r*ETRₘₚₓ increased with depth in 2010 and the maximum *α* and *r*ETRₘₚₓ were 0.095±0.016 µmol electrons (µmol photons)⁻¹ and 21.9±7.4 µmol electrons m⁻² sec⁻¹ in the bottom algal layer, respectively (Figs. 7B & 7D). The *α* increased with depth to 0.093±0.010 µmol electrons (µmol photons)⁻¹, whereas, *r*ETRₘₚₓ stayed at similar levels at approximately 17.5±0.7 µmol electrons m⁻² sec⁻¹ in 2011. The *r*ETRₘₚₓ and *α* decreased with depth in 2012 and the minimum *α* and *r*ETRₘₚₓ were 0.068±0.0012 µmol electrons (µmol photons)⁻¹ and 5.33±0.50 µmol electrons m⁻² sec⁻¹ in the bottom algal layer, respectively, because high biomass and low light absorption were observed (Figs. 3A & 3B). A significant relationship between *r*ETRₘₚₓ and *α* was observed in 2010 (Y=288X−6.8, r²=0.754, p<0.01) and 2012 (Y=33.9X+3.7, r²=0.507, p<0.01), whereas no significant relationship was observed in 2011 (Fig. 9). The significant, positive trends between *α* and *r*ETRₘₚₓ in 2010 and 2012 could be interpreted as *E*ₚ-independent variation (Behrenfeld et al. 2004) although the number of data was limited to 12 for each ice season. In 2011, there seemed to be a positive trend but it was not statistically significant, although *E*ₚ (220±50 µmol photons m⁻² sec⁻¹) was similar to in 2010 (Fig. 7C). The algal community in 2012 enhanced its photoacclimation ability to a low, narrow range of irradiance (1.8%, Table 2) by lowering *E*ₚ, whereas the ice algal communities in 2010 and 2011 exhibited different photoacclimation abilities.
climation strategies, such as higher $E_i$ (Fig. 7C), with low cellular Chl a contents (Fig. 3A).

The photoacclimation of the ice algal community to lower irradiance along the depth gradient was accelerated by increasing cellular Chl a concentrations, decreasing DD+DT/Chl a (Fig. 4A) and DT/DD+DT (Fig. 4B), and the domination of pennate diatoms in the total diatom assemblages. Although $F_v/F_m$ were highly correlated with light attenuation (Fig. 8), the $E_i$ strategy of photoacclimation of the ice algal community with the development of sea ice was similar within a single ice season but varied among the different ice seasons due to the involvement of different species in the sea ice. Because the $E_i$-independent strategy of photoacclimation is not yet well understood, as suggested by Behrenfeld et al. (2004), further investigation on photosynthetic mechanisms is needed for various groups of ice algae.

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