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THE SHORT-TERM EFFECT OF IRRADIANCE ON THE PHOTOSYNTHETIC PROPERTIES OF ANTARCTIC FAST-ICE MICROALGAL COMMUNITIES[1]

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Although sea-ice represents a harsh physicochemical environment with steep gradients in temperature, light, and salinity, diverse microbial communities are present within the ice matrix. We describe here the photosynthetic responses of sea-ice microalgae to varying irradiances. Rapid light curves (RLCs) were generated using pulse amplitude fluorometry and used to derive photosynthetic yield (Φ_{PSII}), photosynthetic efficiency (α), and the irradiance (E_k) at which relative electron transport rate (rETR) saturates. Surface brine algae from near the surface and bottom-ice algae were exposed to a range of irradiances from 7 to 262 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$. In surface brine algae, Φ_{PSII} and α remained constant at all irradiances, and $rETR_{max}$ peaked at 151 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$, indicating these algae are well acclimated to the irradiances to which they are normally exposed. In contrast, Φ_{PSII}, α, and $rETR_{max}$ in bottom-ice algae reduced when exposed to irradiances >26 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$, indicating a high degree of shade acclimation. In addition, the previous light history had no significant effect on the photosynthetic capacity of bottom-ice algae whether cells were gradually exposed to target irradiances over a 12 h period or were exposed immediately (light shocked). These findings indicate that bottom-ice algae are photoinhibited in a dose-dependent manner, while surface brine algae tolerate higher irradiances. Our study shows that sea-ice algae are able to adjust to changes in irradiance rapidly, and this ability to acclimate may facilitate survival and subsequent long-term acclimation to the postmelt light regime of the Southern Ocean.

Key index words: **Antarctic sea ice; E_k; PAM fluorometry; photoinhibition; rapid light curves; $rETR_{max}$; α; Φ_{PSII}**

Abbreviations: **α, light-limited photosynthetic efficiency; Φ_{PSII}, effective quantum yield of PSII; E_k, minimum saturating irradiance; F, fluorescence yield; F'_m, F_m, maximum fluorescence in light and in dark, respectively; PAM, pulse amplitude modulated; $rETR_{max}$, relative maximum electron transport rate photosynthetic efficiency; RLC, rapid light curve**

Diverse microbial communities that are integral to the energy base of the Southern Ocean are present in the brine inclusions and interstices of sea ice (Legendre et al. 1992, Garrison 1991). Although the microalgal component of the sea-ice microbial community can acclimate to the physicochemical gradients that characterize the sea-ice matrix, algal production is strongly influenced by nutrient availability (McMinn et al. 1999, Lizotte and Sullivan 1992), temperature (Arrigo and Sullivan 1992, Ralph et al. 2005), salinity (Arrigo and Sullivan 1992, Ryan et al. 2004, Ralph et al. 2007), and available light (Palmisano et al. 1985).

During sea-ice formation, microbial communities become trapped in brine channels within the ice. In land fast sea ice (sea ice attached to land), the microbial communities that occur close to the ice surface commonly include diatoms and dinoflagellates, where they are frequently exposed to supersaturating irradiances (Robinson et al. 1997). As a result, these surface brine communities have high photosynthetic tolerances to surface irradiance (Ralph et al. 2005). In contrast, bottom-ice algae (predominantly diatoms) are exposed to as little as 0.1% of the surface irradiance (McMinn et al. 2007), and these algae are highly shade-acclimated (Lizotte and Sullivan 1991). In the Ross Sea fast ice, the greatest sea-ice microbial community biomass usually occurs in the bottom few centimeters (McMinn et al. 2007), where the environmental conditions are relatively stable and favorable for growth. However, surface brine algae and bottom-ice communities may still encounter a range of environmental conditions. Light conditions can change rapidly due to daily solar fluctuations or storms that may modify the ice–water interface and redistribute snow cover on the surface. Surface brine algae may be confronted with reduced irradiance and bottom-ice algae with greatly increased irradiance. Is the sea-ice community able to acclimate to these fluctuations, or are they so badly stressed by the sudden change in irradiance that they rapidly die? Their success in surviving within the sea ice and eventually establishing a phytoplankton bloom when the sea ice melts in spring may be determined by their ability to photoacclimate to rapid changes in irradiance.

The ability of the photosynthetic apparatus to acclimate to solar fluctuations in ambient irradiance is crucial for maintaining photosynthesis and

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enabling cell function at both limiting and excessive light levels (Ralph and Gademann 2005). Sea-ice algae may respond to increases or decreases in irradiance within minutes via photoinhibition, or over a longer time period by photoacclimation (Lizotte and Sullivan 1991, Robinson et al. 1997, Han et al. 2000, McMinn et al. 2003, Ryan et al. 2004, Ralph et al. 2005). Photoacclimation requires a change at the physiological and biochemical composition level, whereas photoinhibition occurs mainly at the electron transport chain in PSII (Han et al. 2000). For example, the decrease in chl *a* concentration in sea-ice microalgae after a 3–4 d exposure to high irradiances and subsequent increase after a similar exposure to lower irradiances (Lizotte and Sullivan 1991) are photoacclimation responses. Photoinhibition is an often reversible reduction in photosynthetic capacity that occurs in plants when exposed to high irradiance (Han et al. 2000) and occurs via the closing down of reaction centers of PSII and a decrease in electron transport rate (ETR). Thus, at high midday irradiances, a short-term photoinhibitory response was observed in bottom-ice algae from McMurdo Sound (McMinn et al. 2003), although McMinn and Hattori (2006) found no evidence of photoinhibition in a similar community from the Saroma Ko Lagoon, Hokkaido.

Photoinhibition of PSII under normal circumstances is thought of as a photoprotective mechanism rather than damaging and is directly related to chloroplast-encoded protein synthesis, in particular the D1 protein (Anderson et al. 1997). Under normal photosynthesis, photoinactivation of PSII and repair via D1 synthesis occur in parallel, even in low-light conditions. In supersaturating irradiances, the rate of D1 inactivation exceeds its capability for repair, resulting in an apparent photoinhibition (Lee et al. 2001). The photoinactivation of PSII has been shown to occur in a light dosage manner and is dependent on the number of photons absorbed rather than the rate of photons absorbed (Anderson et al. 1997, Han et al. 2000, Lee et al. 2001).

Few studies have utilized chl fluorescence techniques to measure photosynthetic properties of sea-ice bottom algae. Kühl et al. (2001) were the first to measure photosynthesis using a pulse-amplitude-modulated (PAM) (Schreiber et al. 1994) chl fluorometer (diving PAM) in bottom-ice algae in Greenland. Most photosynthetic studies have focused on bottom-ice algae perhaps due to their high biomass (McMinn et al. 1999, 2000, 2003, 2007, Ryan et al. 2004, McMinn and Hattori 2006), with only two studies investigating brine microalgal physiology (Ralph et al. 2005, 2007). Surface brine algae may tolerate surface irradiances up to 350 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$, whereas bottom communities are highly dark acclimated and may be more easily damaged by increases in irradiance.

In the present study, the photosynthetic response of surface brine and bottom-ice algal communities to changes in irradiance was examined using PAM fluorometry. We applied a range of irradiances either gradually or via light shock to algal isolates to determine whether the previous light history modified the photosynthetic response. We measured changes in effective quantum yield (Φ_{PSII}), relative electron transport rate ($rETR_{max}$), photosynthetic efficiency (α), and saturation irradiance (E_k) using the RLC technique (Henley 1993) to (i) determine whether sea-ice algae are able to acclimate to sudden changes in irradiance associated with daily solar fluctuations or storm events and (ii) assess the ability of sea-ice algae to survive these events and contribute to bloom events in the Southern Ocean.

MATERIALS AND METHODS

Physical parameters. Temperature data loggers (MicroDAQ, Contoocook, NH, USA) were embedded at 20 cm intervals through the full 2.5 m thickness of sea ice in the region of Gondwana Station, Terra Nova Bay, Antarctica (164°13 E, 74°38 S). PAR sensors were encased in resin and embedded in the ice at depths of 0.5, 1.5, and 2.3 m, and a further sensor was deployed above the ice to obtain surface irradiances.

Surface brine algae. Surface brine algae were extracted from 2.6 m thick fast ice on 22 November 2006. Three holes were drilled to a depth of 700 mm using a 150 mm diameter ice coring drill (Kovaks, Lebanon, NH, USA, Mark V). To prevent exposure to ambient surface irradiance, the holes were covered with black plastic, and brine from the adjacent ice was allowed to drain into the holes over the following 10 min. Approximately 100 mL of brine was collected from each hole using sterile syringes, transferred to a dark container, and maintained at a temperature of 0°C overnight. Four 20 mL samples were taken from each of the three stock suspensions and allocated to 25 mL glass vials (12 vials total). All vials were placed in a refrigerated water bath and kept in the dark for 2 h at −1.8°C. One vial from each ice core was used as a dark control ($n = 3$), and the other three were allocated to separate light treatments ($n = 3 \times 3 = 9$). The ambient irradiance of the two communities was determined directly by measuring irradiance with HOBO PAR sensors (MicroDAQ) embedded in the sea ice at 50 cm and immediately below the ice, and calculating the % transmission.

Bottom-ice algae. Core samples of sea ice were taken using the ice coring drill on 26 and 29 November, and 4 and 5 December 2006. Cores were withdrawn from the ice shrouded in a black cloth, and the bottom 400 mm was transferred to a black plastic bag to avoid light shock to the algae. Samples were collected from three ice cores on each day. There was a 1–2 cm platelet-ice layer on the underside of the ice, and ice algae were concentrated in a firm consolidated region 2 cm thick above the platelets. The bottom 100 mm of the ice core containing ice algae was melted in three times the volume of filtered (0.7 μm) seawater overnight to avoid salinity shock (Ralph et al. 2007). This method maintained the salinity of the sample at ~31‰, which is slightly less than ambient levels. During the melting process, the algae were kept in dark boxes at −1.8°C. Other extraction methods, such as direct collection by scuba divers, were not practical at this remote field site.

Once melted, the cells were filtered through a 60 μm mesh filter (Madison Filter, Auckland, New Zealand) to remove large chains of cells to obtain a uniform suspension, diluted to a standardized concentration of chl (measured on a Water-PAM fluorometer; Walz Gmbh, Effeltrich, Germany), and homogenized using a battery drill with a plastic stirrer (Ryobi 12W,

Hong Kong, China). Twelve 25 mL sample vials of bottom-ice algae were prepared as described above.

PAM fluorometry. PAM fluorometry measures chl *a* fluorescence, providing a tool to assess the effective quantum yield of photosynthesis of PSII (Φ_{PSII}). Apart from the initial dark incubation, the cells were not dark acclimated before RLCs were performed. A weak measuring light allows fluorescence yield to be monitored without inducing photosynthesis. This minimum fluorescence under light-acclimated conditions is termed F' and measures the proportion of closed PSII reaction centers, and it is proportional to the chl concentration. When cells are exposed to a strong saturating actinic light (3,000 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) for 0.8 s, all PSII reaction centers are rapidly closed, and chl fluorescence increases to maximum (F'_m) before it declines back to an equilibrium level. Effective quantum yield (Φ_{PSII}) is defined as: $(F'_m - F')/F'_m = \Delta F/F'_m$ (Genty et al. 1989). Since this value is a ratio of two fluorescence measures, it is dimensionless.

An RLC was recorded on each sample using the PAM system. Each cuvette was treated with a series of eight increasing actinic light treatments. After each 20 s period of actinic light, a strong saturating pulse was applied and Φ_{PSII} was recorded. The ETR values were calculated by multiplying the Φ_{PSII} by the irradiance just applied. Since Φ_{PSII} is dimensionless, the ETR value is properly termed relative ETR (rETR). No correction for the proportion of light shared by PSI (often assumed to be 0.5) was applied to the rETR, as the relative proportion of PSI and PSII is not known in this system. Similarly, no absorption coefficient (often assumed to be 0.84) was applied as this is unknown also. All RLCs can be described by the parameters $rETR_{max}$, maximum relative electron transport rate (μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}); E_k, the irradiance at the onset of saturation of photosynthesis (μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}); and α, light-limited photosynthetic efficiency; and the initial slope of the RLC, which gives an indication of the efficiency of light utilization. To determine these photosynthetic parameters, rETR data were imported into Sigmaplot v. 8.0 (Systat Software Inc., Chicago, IL, USA), and the curve was fitted with a Marquardt-Levenberg regression algorithm (Platt et al. 1980):

$$P = P_s(1 - e^{-(\alpha E_d/P_s)})e^{-(\beta E_d/P_s)} \quad (1)$$

where P is photosynthesis (here rETR), P_s is the maximal potential rETR in the absence of a photoinhibitory response, α is the initial slope of the RLC before the onset of saturation, and E_d is the downwelling irradiance (400–700 nm). The β signifies the slope of the curve after the onset of photoinhibition. To find the $rETR_{max}$, the following equation was utilized:

$$rETR_{max} = P_s(\alpha/\alpha + \beta)(\beta/\alpha + \beta)^{\beta/\alpha} \quad (2)$$

E_k can be described as follows:

$$E_k = rETR_{max}/\alpha \quad (3)$$

Water bath/PAR illumination source. In the water bath, a box made of black acrylic plastic was separated into 24 individual chambers, each containing 6 × 5 mm holes to allow throughput of coolant (20% clear ethylene glycol in water). Each chamber had an individual clear acrylic lid, to which neutral density filters (Wellington Photographic Supplies, Wellington, New Zealand) could be fitted to modify the light environment within each individual chamber. The lids were interchangeable from chamber to chamber, and this allowed a random allocation of each of six different light levels (0, 7, 26, 75, 151, and 262 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}). A light bank of 35–50 W quartz halogen lamps (Philips, New Zealand) was suspended over the water bath, and fans were employed to cool the lamp housings. The lamps were arranged to provide a uniform field of light over the water bath incubator.

Experiment 1. Gradually increasing light exposure (ramping): Here we test the response of algae to gradually increasing exposure to the six light levels listed above. All the brine and bottom-ice algae (except the dark controls) were exposed to incremental steps of irradiance for 2 h each. The brine algae were collected on 26 November 2006, and the experiment was run on 27 November. The dates for the bottom-ice algae run were 29/30 November 2006. Vials were placed in the appropriate chamber sequentially 4 min apart to allow time for individual RLCs to be performed on each sample 2 h later. At the end of each light incubation period, the vials were agitated gently, and then a 0.5 mL subsample was removed from each vial and diluted with 2.5 mL of 0.7 μm filtered seawater. The original sample was immediately returned to the incubator and placed in a new, randomly allocated position where it received the next highest irradiance. Each subsample was placed in a quartz cuvette and inserted into the Water PAM system, and an RLC was generated (see below). The PAM cuvette holder was kept in a cool box along with ice packs to ensure that the subsamples were not temperature shocked prior to taking measurements. When the RLC was completed, the diluted suspension was discarded. At the end of the full 12 h treatment, the dark control samples were shifted directly to the highest irradiance for a further 2 h.

Experiment 2. Gradually increasing light exposure and maintenance at each step (ramp and hold): This experiment is very similar to Experiment 1 but tests whether the bottom-ice algal response to each irradiance changes with time (experiment dates 4/5 December). Eighteen vials (6 × 3 replicates) were exposed to the six consecutive light levels as described above for Experiment 1, but at each transfer of vials from one irradiance to the next, three vials were held back and maintained at that prior irradiance for the rest of the treatment period.

Experiment 3. Sudden irradiance increase (light shock), then maintenance at each step: This experiment tests whether prior exposure to lower light levels as achieved in the previous experiments has any influence on photosynthesis (experiment dates 5/6 December). At the end of the dark acclimation, three vials were transferred directly to each of the six irradiances described above and held there for a further 4 h.

Statistical analyses. Differences in brine and bottom-ice algae with respect to change in irradiance treatments for effective quantum yield (Φ_{PSII}), $rETR_{max}$, α, and E_k were measured using the Pearson's correlation coefficient with the test statistic (r) at 0.05. Normality of data was assessed using the Anderson-Darling test. Mean values were compared using a one-way analysis of variance (ANOVA, test statistic F) in the form of a generalized linear model (Quinn and Keough 2002) with subsequent Tukey's pair-wise comparisons. When normality was not achieved after transformation, mean values were compared using a Kruskal–Wallis (K–W) test (test statistic H) with subsequent Dunn's procedure for pair-wise comparisons. Individual differences between brine and bottom algae at a particular irradiance were compared using a t-test (test statistic t).

RESULTS

The samples were collected from a region of snow-free sea ice during early summer when air temperatures fluctuated between +8 and −12°C. PAR levels varied between 100 and 1,200 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} from midnight to midday. Ice temperatures were −7°C at 0.7 m depth (surface brine algae collection) and −1.8°C at the ice–water interface (bottom-ice algae collection). The midday light levels measured in the ice during collections

were ~80 μmol photons · m^{-2} · s^{-1} at 0.5 m depth (surface brine algae) and <1 μmol photons · m^{-2} · s^{-1} at 2.3 m depth, just above the bottom-ice algal layer.

Experiment 1. Light-ramping treatment: Both the surface brine algae and bottom-ice algae showed a photosynthetic recovery during the initial 2 h of dark incubation. Φ_{PSII} for the surface brine algae recovered to 0.29 from 0.22 (Fig. 1a) and in bottom-ice algae to 0.29 from 0.23 (Fig. 1b). Surface brine algae involved in the light-ramping experiment showed a significantly higher Φ_{PSII} from the brine ice algae maintained in the dark (K–W, $H_7 = 37.53$, $P < 0.001$, Fig. 1a). In contrast, bottom-ice algae involved in light ramping had a significantly lower Φ_{PSII} than those incubated in the dark (K–W, $H_7 = 60.17$, $P < 0.001$, Fig. 1b). Although, Φ_{PSII} increased with increased exposure to light in the surface brine algae (Pearson's, $r_{21} = 0.585$, $P < 0.001$), Φ_{PSII} reached a plateau at 0.33 ± 0.01. The opposite was seen in bottom-ice algae where Φ_{PSII} decreased with exposure to irradiances >26 μmol photons · m^{-2} · s^{-1} (Dunn's pair-wise comparisons, $P < 0.01$ for all comparisons). The Φ_{PSII} of both brine and bottom-ice algae in the dark incubations remained constant over time, with bottom-ice algae having a slightly higher Φ_{PSII} than that of the surface brine algae (surface brine algae, 0.29 ± 0.11; bottom-ice algae, 0.32 ± 0.08). When suddenly exposed to the maximum irradiance of 262 μmol photons · m^{-2} · s^{-1} after 12 h of darkness, the dark-incubated surface brine algae showed no significant change in Φ_{PSII} (*t*-test, $t_5 = 0.30$, $P = 0.773$), nor was Φ_{PSII} significantly different from that for surface brine algae exposed to the incremental series of irradiances (*t*-test, $t_6 = 1.15$, $P = 0.294$). In contrast, when suddenly exposed to an irradiance of 262 μmol photons · m^{-2} · s^{-1} after 12 h of darkness, bottom-ice algae showed a significant drop in Φ_{PSII} to 0.07 ± 0.01 (*t*-test, $t_2 = 18.56$, $P < 0.001$). This new level was not significantly different from that in algae exposed gradually to increasing irradiance over 12–14 h (*t*-test, $t_4 = 0.26$, $P = 0.806$).

Photosynthetic efficiency (α) showed a similar trend to $\Phi_{PSII.}$ The light-exposed surface brine algae had an increase in α with increased irradiance (Pearson's, $r_{64} = 0.588$, $P < 0.0011$, Fig. 1c), and bottom-ice algae showed a decrease in α with increasing irradiance (Pearson's, $r_{67} = 0.637$, $P < 0.001$, Fig. 1d). The cells held in the dark maintained a constant α over time in both the brine (Pearson's, $r_{16} = 0.319$, $P = 0.138$, Fig. 1c) and bottom-ice algae (Pearson's, $r_{16} = 0.359$, $P = 0.143$ Fig. 1d). When these algal cells were suddenly exposed to an irradiance of 262 μE, α values were not dissimilar to those of algae exposed to steadily increasing irradiance (surface brine algae *t*-test, $t_6 = 0.64$, $P = 0.547$; bottom-ice algae *t*-test, $t_1 = 1.06$, $P = 0.329$, Fig. 1, c and d).

$rETR_{max}$ was higher for the surface brine algae with values ranging from 24.0 to 68.3 μmol photons · m^{-2} · s^{-1}. In comparison, the $rETR_{max}$ of bottom-ice algae did not increase above 11.3 ± 2.3 μmol photons · m^{-2} · s^{-1}. Following the same trends, surface brine algae had a significant increase in $rETR_{max}$ when exposed to increasing irradiance (Pearson's, $r_{64} = 0.642$, $P = 0.001$), and bottom-ice algae had a significant decrease in activity (Pearson's, $r_{22} = -0.472$, $P = 0.020$). Brine and bottom-ice algae in the dark incubations maintained a constant $rETR_{max}$; however, surface brine algae maintained a higher $rETR_{max}$ (28.68 ± 2.64, Fig. 1e) than did the bottom-ice algae (7.17 ± 0.62, Fig. 1f). Brine and bottom-ice algae again showed contrasting changes in $rETR_{max}$ when suddenly exposed to high irradiance. The $rETR_{max}$ of surface brine algae significantly increased when moved from darkness to high light (ANOVA, $F_{7,15} = 3.41$, $P = 0.022$), whereas the bottom-ice algae showed a significant decrease in $rETR_{max}$ (*t*-test, $t_2 = 4.91$, $P = 0.039$). In both cases, when the dark-treated cells were moved into high light, $rETR_{max}$ values were not significantly different from the $rETR_{max}$ values recorded from the light-ramping algae.

Maximum saturating irradiance (E_k) was higher in surface brine algae in comparison to bottom-ice algae. When exposed to the light-ramping experiment, surface brine algae had maximum E_k of 219 ± 17.2 μmol photons · m^{-2} · s^{-1} at exposure to 262 μE in comparison to the maximum E_k in bottom-ice algae of 58.03 ± 7.7 μmol photons · m^{-2} · s^{-1} at 151 μmol photons · m^{-2} · s^{-1}. Surface brine algae incubated in increasing light regimes had a significantly higher E_k in comparison to algae in the dark incubations (ANOVA, $F_{1,\ 7} = 11.47$, $P < 0.001$, Fig. 1g) and significantly increased as the incubation irradiance increased (Pearson's, $r_{64} = 0.684$, $P < 0.001$). E_k also significantly increased in bottom-ice algae as the incubation irradiance steadily increased ($r_{22} = 0.660$, $P < 0.001$, respectively). Algae incubated in darkness maintained a constant E_k (surface brine algae, 94.05 ± 7.07 μE, Fig. 1g), (bottom-ice algae, 23.54 ± 4.67 μmol photons · m^{-2} · s^{-1}, Fig. 1h). There was a significant increase in E_k when dark-treated surface brine algae were suddenly exposed to high light (*t*-test, $t_2 = 4.76$, $P = 0.041$). This trend was similar for bottom-ice algae; however, the increase in E_k was not significant.

Experiment 2. Ramp-and-hold treatment: In this experiment, bottom-ice algae were exposed to progressively higher irradiances exactly as in Experiment 1, but this time, three replicates were maintained at each prior light increment (Fig. 2). As before, Φ_{PSII} decreased in a dose-dependent manner (Tukey's pair-wise comparisons, $P < 0.001$, Fig. 2a), where the curve exactly matched the Φ_{PSII} curve shown in Figure 1b. The bottom-ice algal cells maintained at each irradiance had a stable Φ_{PSII} at

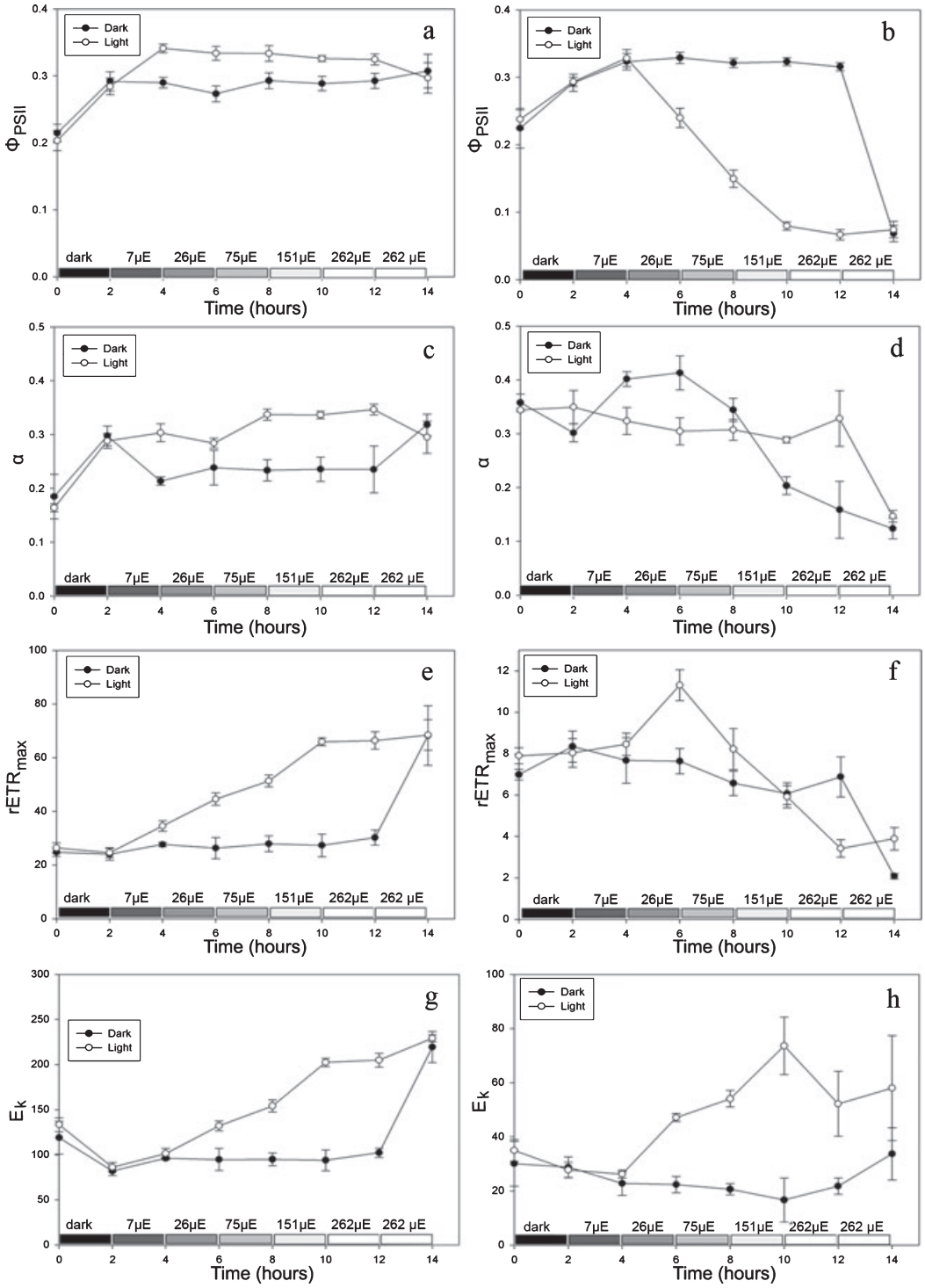

FIG. 1. Changes in measures of photosynthetic activity in surface brine algae (left column) and bottom-ice algae (right column) when exposed to increasing irradiance. After 12 h, the samples from the dark incubation were transferred to the highest irradiance treatment for an additional 2 h. (a and b) Effective photosynthetic yield (Φ_{PSII}). (c and d) Photosynthetic efficiency (α). (e and f) Relative electron transport rate ($rETR_{max}$). (g and h) Minimum saturating irradiance (E_k). Units: μmol photons · m^{-2} · s^{-1}. Data are means ± 1 SE.

each particular light level for the duration of the measurements (Tukey's pair-wise comparisons, $P = ns$ for all comparisons). The $rETR_{max}$ and α showed a similar pattern, where there was a significant decrease in activity >26 μmol photons · m^{-2} · s^{-1} treatment (Tukey's pair-wise comparisons, $P < 0.001$, Fig. 2b) followed by maintenance of activity at each level (Tukey's pair-wise comparisons,

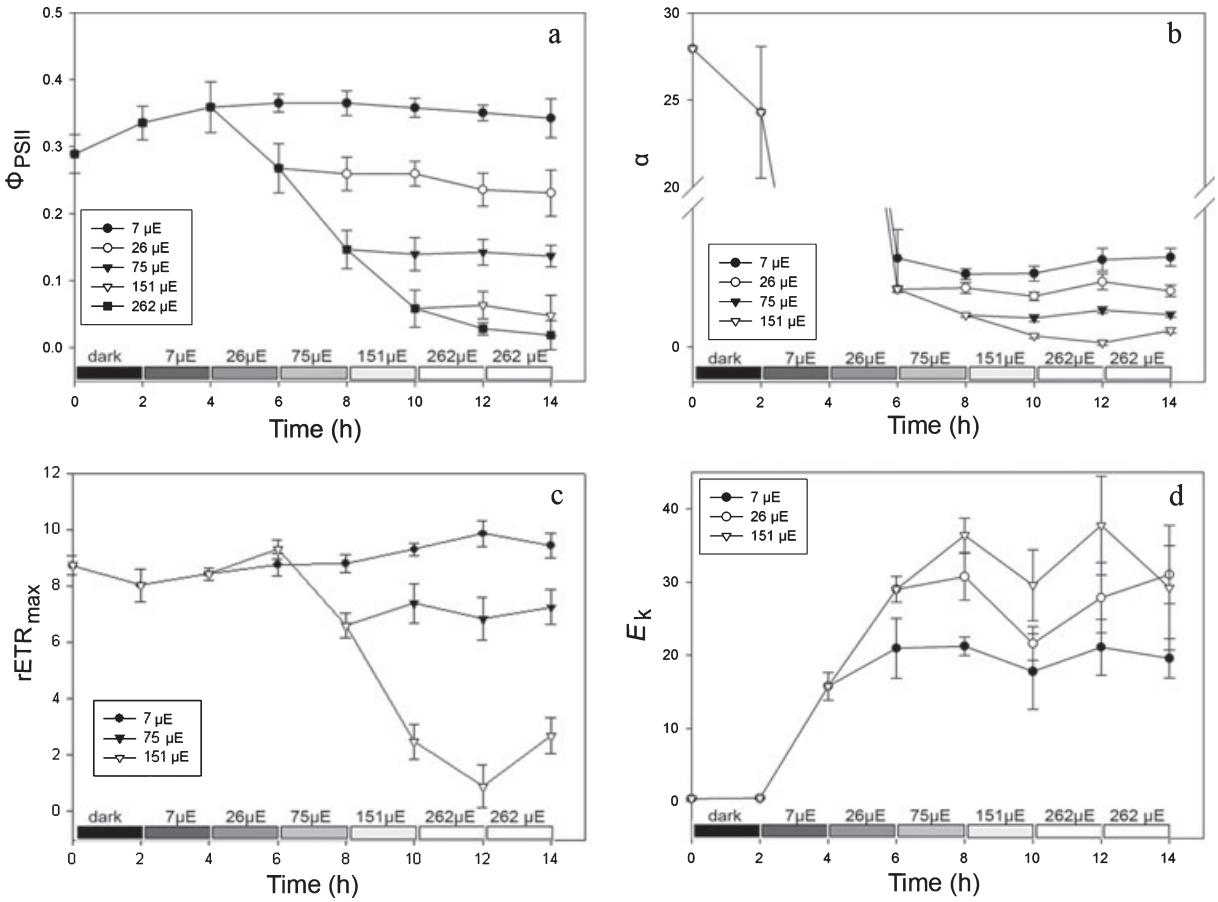

FIG. 2. Changes in photosynthetic activity of bottom-ice algae when exposed to increments of increased irradiance (µmol photons $\cdot m^{-2} \cdot s^{-1}$, µE). (a) Effective photosynthetic yield (Φ_{PSII}). (b) Photosynthetic efficiency (α). (c) Relative maximum electron transport rate ($rETR_{max}$). (d) Minimum saturating irradiance (E_k). At each irradiance level, three bottom-ice algae samples were maintained at that constant irradiance for the remainder of the experiment. Data are means ± 1 SE.

$P = ns$). When bottom-ice algae were exposed to an irradiance of 7 µmol photons $\cdot m^{-2} \cdot s^{-1}$, $rETR_{max}$ was maintained at 8.9 (Fig. 2c), similar to the dark control cells in the previous experiment (Fig. 1f). Algae left at 26 and 262 µmol photons $\cdot m^{-2} \cdot s^{-1}$ had $rETR_{max}$ values that were not significantly different from those of algae maintained at 75 and 151, respectively, and are therefore not shown. E_k showed a similar trend to the light-ramping experiment (Fig. 1h) where activity increased with increasing irradiance; however, these trends were not significant (Tukey's pair-wise comparisons, $P = ns$, Fig. 2d).

Experiment 3. Light-shock treatment: After 2 h of incubation in the dark, bottom-ice algal cells were light shocked to the same irradiances used in the previous two experiments. Φ_{PSII} rapidly declined to levels similar to those shown in Figures 1b and 2a at the same irradiances (Tukey's pair-wise comparisons, $P < 0.001$ for all comparisons, Fig. 3). Maintenance of Φ_{PSII} at the same level occurred in the subsequent 3 h culture (Tukey's pair-wise comparisons, $P = ns$). There were no trends detected in the other photosynthetic parameters.

DISCUSSION

Brine and bottom-ice algae are two photosynthetically and taxonomically distinct communities in land fast sea ice (McMinn et al. 2003, Ralph et al. 2005). In these experiments, Φ_{PSII} in both dark-incubated brine and bottom-ice algae was lower, at ~0.3, than we have observed in algae from other locations in the Ross Sea (see McMinn et al. 2003, Ralph et al. 2005). In other studies in eastern Antarctica, Ryan et al. (2004) found a Φ_{PSII} of 0.34 in bottom-ice algae, which is comparable to the results of the present study. Bottom-ice algae were prescreened for large-chain diatoms to gain a uniform suspension of cells and allow the different treatments to be comparable. This prescreening might perhaps explain the reduced $\Phi_{PSII.}$ However, Φ_{PSII} was also low in the surface brine algae, which did not undergo prescreening. Considerable effort was spent trying to improve isolation and incubation conditions in the present study. For example, the bottom-ice algae were carefully melted out from the ice overnight (in the dark) to minimize light shock and in excess filtered seawater to minimize salinity

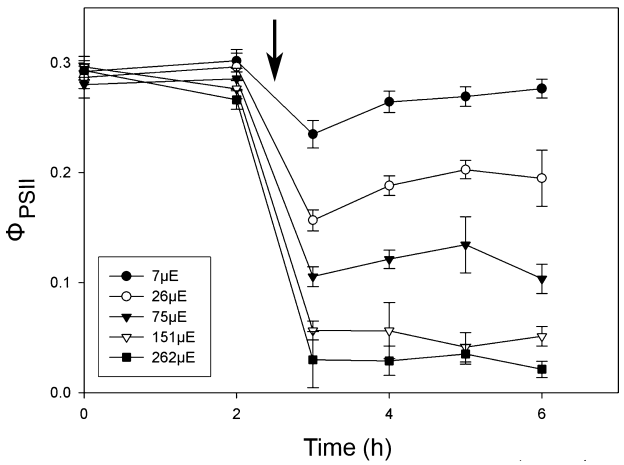

FIG. 3. Changes in effective photosynthetic yield (Φ_{PSII}) of bottom-ice algae. Algae were incubated in the dark for 2 h, then light shocked with a particular irradiance (μmol photons · m^{-2} · s^{-1}, μE) level (arrow). Data are means ± 1 SE.

shock (final salinity ~31‰ compared to ambient seawater salinity of 35‰). Ralph et al. (2007) have noted that low salinities during melting of 22‰ reduce Φ_{PSII}, but when maintained close to ambient salinities, physiological damage is minimized. We believe that the Φ_{PSII} of 0.3 reflects the state of health of the algae at this location at this time. It should be noted that Φ_{PSII} in marine phytoplankton is usually lower than that for land plants and is often lower than that found in seagrasses (Ralph and Gademann 2005).

The bottom-ice community in this study was dominated by *Berkelya adeliensis*, whereas the other studies were dominated by *Nitzschia stellata* (Ralph et al. 2005) or *Fragillariopsis curta* (McMinn et al. 2003), which could perhaps explain these discrepancies in Φ_{PSII}. The surface brine community was composed predominantly of an unidentified dinoflagellate (possibly *Polarella* sp.).

The bottom-ice algae in this study were collected from 2.6 m thick annual ice, and the under-ice ambient irradiance was <1 μmol photons · m^{-2} · s^{-1}. Melted samples of these algae were exposed to gradually increasing irradiances and showed a typical response for highly shade-acclimated photosynthetic organisms (Fig. 1b). The Φ_{PSII} initially rose, but then photoinhibition was initiated at irradiances >7 μmol photons · m^{-2} · s^{-1}. As the exposure to light was increased gradually beyond 7 μmol photons · m^{-2} · s^{-1} over a 12 h period, Φ_{PSII} declined in a dose-dependent manner. At irradiances >151 μmol photons · m^{-2} · s^{-1}, Φ_{PSII} in bottom-ice algae was as low as 0.01. When other bottom-ice algae samples, which had been held in the dark for the same period, were suddenly exposed to high irradiance, they showed a rapid photoinhibitory response (Fig. 1b). The Φ_{PSII} dropped to the same value as for the samples of bottom-ice algae that had been gradually exposed to light at this level. In addition, α decreased to match the values seen in light-treated cells, while $rETR_{max}$ did not change through the course of the experiment at any treatment. This finding indicates that previous light history had no influence on the short-term response of bottom-ice algae to increased numbers of light photons.

The surface brine algae in this study were collected ~0.7 m from the surface. At this depth in the ice, the irradiance at midday on a clear day reached ~80 μmol photons · m^{-2} · s^{-1}, and these algae are therefore acclimated to much higher light intensities than the algae at the bottom of the ice. In our incubation experiments, the Φ_{PSII} increased initially with exposure to light and maintained this high level above that of dark-treated cells for the duration of the experiment. There was no significant decrease in photosynthetic capacity even at the highest irradiance of 262 μmol photons · m^{-2} · s^{-1}, suggesting no photoinhibition or damage to the photosynthetic apparatus. These values are comparable to those of Ralph et al. (2005) where surface brine algae from a Cape Hallett also demonstrated extensive photosynthetic activity over a range of irradiances. They observed surface brine algae to have phototolerance up to 370 μmol photons · m^{-2} · s^{-1}, with no evidence of photoinhibition. As for the bottom-ice algae, the previous light history of surface brine cells had no effect on the photosynthetic capacity. On exposure to 262 μmol photons · m^{-2} · s^{-1}, the dark-control and the light-exposed cells had the same photosynthetic activity, regardless of their previous light history. In addition, α, $rETR_{max}$, and E_k all increased in the brine dark-control cells to an activity level similar to that of the light-exposed cells.

Shade-acclimated plants have higher α, indicating that they can utilize lower irradiances more effectively (Dubinsky et al. 1986, Kirst and Wiencke 1995). When comparing dark-incubated brine and bottom-ice algae in the light-ramping experiment, α was higher in the bottom-ice algae (0.32 ± 0.02) than surface brine algae (0.23 ± 0.03). When observing algal cells incubated in increasing irradiances, α reached a maximum of 0.41 in bottom-ice algae when exposed to 26 μmol photons · m^{-2} · s^{-1}, which declined as irradiance increased >75 μmol photons · m^{-2} · s^{-1}, indicating the effectiveness of low-light utilization in bottom-ice algal communities. In the high-light-acclimated surface brine algae, α increased with irradiance and reached a maximum of 0.35 at 151 μmol photons · m^{-2} · s^{-1}, again showing algal communities are well acclimatized to their ambient light environment.

Surface brine algae had an $rETR_{max}$ of between 21 and 68 μmol photons · m^{-2} · s^{-1}. High $rETR_{max}$ values are typical of algae exposed to high irradiance, but our values were higher than those found by Ralph et al. (2005), where $rETR_{max}$ was ~18 μmol photons · m^{-2} · s^{-1}. In contrast, bottom-ice algae in our study only reached an $rETR_{max}$ of

11 μmol photons · m^{-2} · s^{-1}, which is consistent with that found in shade-acclimated algae in other studies (McMinn et al. 1999). McMinn et al. (2003) found in situ $rETR_{max}$ in bottom-ice algae to be at a maximum value of 11 μmol photons · m^{-2} · s^{-1} at midday at in situ irradiances of <5 μmol photons · m^{-2} · s^{-1}. Maximum $rETR_{max}$ occurred at irradiances of 151 and 26 μmol photons · m^{-2} · s^{-1} in brine and bottom algae, respectively, further confirming their photosynthetic acclimatization to their environment (Cota 1985).

Irradiance at the onset of saturation (E_k) is often utilized to quantify shade acclimation (Kirst and Wiencke 1995, Ralph and Gademann 2005). E_k was much higher in surface brine algae with values of 80 to 219 μmol photons · m^{-2} · s^{-1} in comparison to bottom algae (24 to 58 μmol photons · m^{-2} · s^{-1}), illustrating their respective light environments. Similar values were recorded in low-light- and high-light-acclimated seagrass leaves (Ralph and Gademann 2005). McMinn et al. (2007) found E_k of similar values for bottom-ice algae from 21 to 69 μmol photons · m^{-2} · s^{-1}. In this study, E_k increased with light intensity in both the brine and the bottom-ice algae. A similar trend was also observed in bottom-ice algae in situ by McMinn et al. (2003) where E_k varied between 2.1 and 18 μmol photons · m^{-2} · s^{-1} with increasing ambient irradiance.

The response of brine and bottom algae dark-treated cells to a sudden change in light observed above suggests that the previous light history of the cells did not affect the short-term response to new light levels. We tested this light history response with two further experiments. First, bottom-ice algae were exposed to increased irradiance, but some samples were left behind and maintained at the same irradiance (ramp and hold). Bottom-ice algae showed increased photoinhibition as irradiance level increased with a response curve that is remarkably similar to that in the previous experiment. In addition, the Φ_{PSII} of cells ''left behind'' was maintained at a constant steady-state value for that irradiance. In the light-shock experiment, bottom-ice algae were maintained in the dark and then directly shifted to the five target irradiances. The same steady-state values of Φ_{PSII} as in the previous two experiments were recorded at each irradiance. These observations from all three experiments strongly support the conclusion of Anderson et al. (1997) who demonstrated in pea plants that the rate of decline of PSII function is related to the number of photons absorbed rather than the rate of photon delivery and concluded that loss of PSII happens in a light dosage-dependent manner. In their experiments, the number of functional PSII centers in pea leaves declined with increasing photon exposure, where there was always reciprocity between the irradiance and the time of exposure. The law of reciprocity states that an equal amount of photoinactivation is induced by an equal dose of photon exposure and holds in the presence of a D1 inhibitor (Anderson et al. 1997, Han et al. 2000, Lee et al. 2001). However, when D1 protein is present, the decline of active PSIIs stabilizes, and there is no further net loss of PSIIs with photon exposure (Lee et al. 2001). D1 degradation and turnover are highly regulated in high-light-acclimated plants, which, like surface brine algae, have a higher photosynthetic tolerance to high irradiance than their shade-acclimated counterparts. Bottom-ice algae, like shade-acclimated plants (Aro et al. 1993, Anderson et al. 1997), will perhaps have a reduced capacity for D1 regulation and show a lowered ability to repair PSII and will perhaps adhere more to the reciprocity laws. When both photoinactivation and repair of PSII occur, a steady state is reached (Tyystjärvi et al. 1992). Bottom-ice algae in the lower irradiance treatment showed a higher steady-state fraction of functional PSIIs than those exposed to higher irradiance. The maintenance of photosynthetic parameters in bottom-ice algae at a near steady state in response to the ramp-and-hold experiment suggested a balance between photoinactivation of PSIIs and the rate of D1 protein turnover.

There was no significant difference between the 151 and 262 μmol photons · m^{-2} · s^{-1} light treatments in the bottom-ice algal photosynthetic parameters. Although these parameters decreased with these high-irradiance treatments, bottom-ice algae still produced some photosynthetic activity. A residual amount of functional PSIIs must be able to survive at high irradiances and perhaps are photoprotected in some way. Photoinactivated PSII complexes have been found to accumulate in stacked granal domains and photoprotect the function of neighbors (Aro et al. 1993, Lee et al. 2001). As well as photoprotection from the photoinactivated PSII complexes, shade-acclimated algal cells have additional mechanisms that protect them from short-term photon exposure. Xanthophyll cycling and cyclic electron flow around the PSII complex (Robinson et al. 1997) and chloroplast intracellular movement for self-shading (Lizotte and Sullivan 1991) are photoprotective responses seen by algae in response to high irradiance.

Sea-ice algae species composition is a subset of that occurring in the water column (Lizotte and Sullivan 1991), and it is likely that these species are part of the phytoplankton seasonally and become incorporated into the sea ice during freezing (Rozanska et al. 2008). Some microalgal species have been collected in both sea ice and the water column, which can be explained by cyclic entrapment in sea ice and subsequent melting of the sea ice in spring (Garrison et al. 1983). Vertical mixing of phytoplankton in the water column also causes changes in irradiance exposure (Falkowski 1984), and therefore, sea-ice algae must have a broad scope for light acclimation. Surface brine algae appear to have photosynthetic

tolerance and regulation of PSII repair over a wider range of irradiances. In bottom-ice algae, photoinhibition occurs in a dose-dependent manner, and although turnover rate of the D1 protein may be slower than that in the surface brine algae, photosynthesis is still maintained. A residual number of active PSIIs survive at high irradiances, which must be extremely important for the survival of the algae. The present study did not investigate long-term photosynthetic responses to increased irradiance; however, it has been suggested that acclimation does occur over days to weeks (Prezelin and Matlick 1980, Palmisano et al. 1985). Brine and bottom algae are able to survive initial rapid changes in irradiance, such as those that may occur during storm events or with daily solar fluctuations. Brine algae may contribute to sea-ice production even when irradiance is low and snow and ice cover may be thick. When exposed to less rapid changes in irradiance due to melting at the ice, or annual light fluctuations, it is likely that the short-term photosynthetic responses of sea-ice algae will help them survive until they can photoacclimate to their new environment.

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THE SHORT-TERM EFFECT OF IRRADIANCE ON THE PHOTOSYNTHETIC PROPERTIES OF ANTARCTIC FAST-ICE MICROALGAL COMMUN

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