Rapid photoprotection in sea-ice diatoms from the East Antarctic pack ice

Katherina Petrou,[a] Ross Hill,[a,*] Christopher M. Brown,[b,1] Douglas A. Campbell,[b]
Martina A. Doblin,[a] and Peter J. Ralph[a]

[a] Plant Functional Biology and Climate Change Cluster and Department of Environmental Sciences, University of Technology, Sydney, Australia
[b] Department of Biology, Mount Allison University, Sackville, New Brunswick, Canada

Abstract

Photoinhibition and D1 protein re-synthesis were investigated in bottom-dwelling sea-ice microalgal communities from the East Antarctic pack ice during early spring. Bottom-dwelling sea-ice microalgal communities were dominated by diatoms that exhibited rapid photoprotection when exposed to a range of different light levels (10 μmol photons m^{-2} s^{-1}, 50 μmol photons m^{-2} s^{-1}, 100 μmol photons m^{-2} s^{-1}, and 200 μmol photons m^{-2} s^{-1}). Photosynthetic capacity of photosystem II (PSII) dropped significantly over 3 h under 200 μmol photons m^{-2} s^{-1}, but largely recovered when placed in a low-light environment (10 μmol photons m^{-2} s^{-1}) for an additional 3 h. PSII repair rates increased with increasing irradiance, and the D1-protein pool remained steady even under high light (200 μmol photons m^{-2} s^{-1}). Sea-ice diatoms showed a low intrinsic susceptibility to photoinactivation of PSII across all the light treatments, and a strong and irradiance-dependent induction of nonphotochemical quenching, which did not depend upon chloroplast protein synthesis, was also seen. These highly plastic organisms, once thought to be adapted to shade, are in fact well equipped to withstand rapid and relatively large changes in light at low temperatures with minimal long-term effect on their photosynthetic machinery.

Warming is occurring throughout the global ocean (Levitus et al. 2005; Domingues et al. 2008), posing a serious challenge to all marine ecosystems. High-latitude sea-ice ecosystems are among the environments most susceptible to the effects of increasing oceanic and atmospheric temperatures, with observed declines in sea-ice thickness (Giles et al. 2008) and extent (Turner et al. 2005; Serreze et al. 2007) already recorded at both poles. Indeed, the West Antarctic has experienced the greatest warming on the planet (0.56–1.09°C decade^{-1}) over the last 30 yr (Turner et al. 2005, 2006). It is projected that continued warming over both polar regions will lead to further reductions in the duration, thickness, and extent of the annual sea ice (Mayewski et al. 2009), and to changes in snowfall patterns, with precipitation increasing in some areas and declining in others (IPCC 2007). These changes in sea-ice conditions will affect the light climate within the sea ice and hence will affect photosynthesis in bottom-dwelling sea-ice microalgal communities.

The ephemeral sea ice is a defining structural feature of the Antarctic ecosystem (Eicken 1992), and its annual cycle of formation and decay is the major process that drives global thermohaline circulation. Antarctic sea ice provides a niche for a diverse microbial community of bacteria, microalgae, and protists, which in turn provide a vital food source for higher trophic levels during the winter months, when food and sunlight are scarce. Like all photoauto-trophs, sea-ice microalgae must balance light energy entering their photosystems with the energy utilized in carbon fixation (Ensminger et al. 2006). However, unlike most photosynthetic organisms, sea-ice microalgae have to maintain this balance under cold, hypersaline, and often hyperoxic conditions (Thomas and Dieckmann 2002; Morgan-Kiss et al. 2006). In higher plants, low temperatures often result in an increase in relative excitation pressure on photosystem II (PSII), particularly in conjunction with high light (Hüner et al. 1993; Hüner 1998; Ivanov et al. 2003). In response to short-term light stress, nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence is activated. This substantially reduces the effective absorption cross section of PSII to limit over-excitation of the photosystem. Overexcitation of PSII can result in the production of reactive oxygen species in the thylakoid lumen (Müller et al. 2001), causing light-induced damage to the photosynthetic apparatus. Photoinactivation is the primary event that leads to a loss of activity in PSII reaction centers (Nagy et al. 1995; Six et al. 2007; Key et al. 2010). A common site of damage is the D1 protein of PSII (Aro et al. 1993; Tyystjarvi 2008). If the rate of photoinactivation exceeds the rate of protein repair (Aro et al. 1993; Melis et al. 1999), photoinhibition of photosynthesis results because the pool of active PSII centers declines. The extent of photoinhibition is thus dependent on the dynamic balance between the rate of PSII protein photoinactivation and the rate of PSII repair through proteolytic removal of the photoinactivated D1 and resynthesis of a replacement D1 protein. Because photoinactivation is driven by the level of incident light, but the repair is metabolic (which is temperature sensitive), photoinactivation and repair can become imbalanced with decreasing temperature, leading to photoinhibition. In this

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* Corresponding author: ross.hill@uts.edu.au

[1] Present address: Environmental Biophysics and Molecular Ecology Group, Institute of Marine and Coastal Sciences, Rutgers, The State University of New Jersey, New Brunswick, New Jersey

way, the cycle of D1-protein breakdown and resynthesis is important to maintain PSII function under potentially photoinhibiting conditions (Allakhverdiev and Murata 2004).

Although psychrophilic microalgae have evolved metabolic strategies to photosynthesize and grow at low temperatures (Morgan-Kiss et al. 2006), little is known about how they acclimate to increases in irradiance and what photoprotective mechanisms they employ under such circumstances. Previous studies from both polar regions have found bottom-dwelling sea-ice microalgae to have great capacity for low-light acclimation (Lizotte and Sullivan 1991; Thomas et al. 1992), and they are thus described as shade adapted (Cota 1985; Palmisano et al. 1985). However, a recent investigation into photoprotection with a laboratory culture of the diatom *Fragilariopsis cylindrus*, a dominant bipolar sea-ice microalgal species, showed a high capacity for NPQ under moderately high light exposure (250 μmol photons m^{-2} s^{-1}) (Kropuenske et al. 2009), suggesting that sea-ice microalgae possess high-light photoprotective capabilities. Developing an understanding of the physiological plasticity of sea-ice microalgae is of particular importance when trying to predict their capacity for acclimation to rapid environmental change, specifically the increased irradiances expected with predicted ice thinning.

To date, there have been no studies on photoinhibition of Antarctic bottom-dwelling sea-ice microalgal communities. Therefore, the main purpose of this study was to determine whether these organisms, previously assumed to be shade adapted (Cota 1985; Palmisano et al. 1985; Thomas and Dieckmann 2002), are indeed susceptible to photoinhibition in the early spring, within the context of predictions for thinning sea-ice conditions in a warmer climate. The hypothesis, that bottom-dwelling Antarctic sea-ice microalgae are able to avoid photoinhibition through rapid photoprotective mechanisms, was tested.

Methods

Sample collection and experimental protocol—Sea-ice microalgae were collected in Austral spring (September and October 2007) from four different locations ($n = 4$) in the East Antarctic pack ice (from 64.24°S to 65.35°S and 121.31°E to 128.05°E) during the Sea Ice Physics and Ecosystem eXperiment (SIPEX) voyage aboard the R/V *Aurora Australis*. Measurements were made twice at the first location and then once at each of the following three sites. D1-protein determinations were made on samples collected from only the final three sites ($n = 3$) due to very low biomass at the first location. Ice-core thickness varied from 47 cm to 130 cm, with measured ambient light ranging from around 2.3 μmol photons m^{-2} s^{-1} to 57 μmol photons m^{-2} s^{-1}, depending on ice thickness, snow cover, and incident irradiance. Under-ice photosynthetically active radiation (PAR) was measured using a 2π underwater sensor (LI-189, LICOR) connected to a mechanical arm. Thus measurements were made at a 2-m distance from the core hole in order to avoid direct sunlight intrusion. Measurements were made hourly from 12:00 h until 18:00 h. Bottom-dwelling sea-ice microalgae were collected using
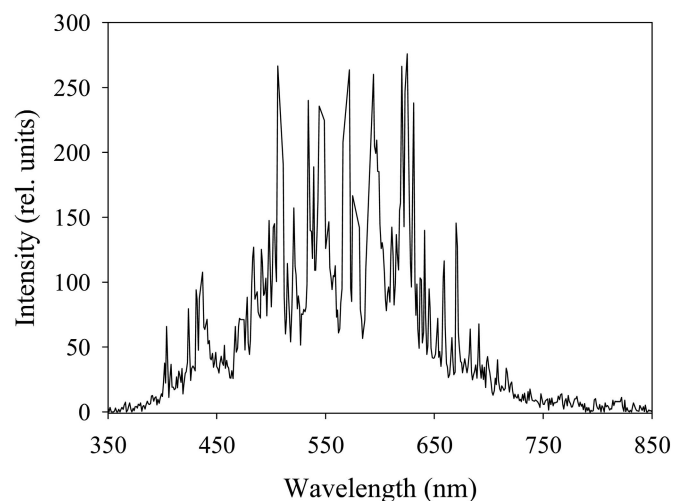

Fig. 1. The relative spectrum of light between the wavelengths of 350–850 nm emitted from the metal halide light source. Spectral output was measured through polyethylene jars and water.

an ice auger (internal diameter: 90 mm), and the bottom 20 mm of the core was then sawed off under black plastic and melted in filtered (0.22 μm) seawater and brine over 24 h at 4°C in the dark. To avoid osmotic stress, salinity was checked every 5 h to ensure that salinity remained within the range of 30 to 35. If salinity dropped lower than 30 during melting, more filtered brine (salinity 75) was added. Brine water was extracted by means of the incomplete coring of holes in the sea ice and by collecting the hypersaline interstitial sea-ice water that filled the cored hole. Before experimental procedures began, a 5-mL aliquot of microalgal sample was preserved in 1% glutaraldehyde for later microscopic cell identification and size determination.

Aliquots of melted ice cores (70 mL) were subsampled into 16 clear, standard, polyethylene jars. Half of the jars were treated with 500 μg mL^{-1} of the chloroplast protein synthesis inhibitor lincomycin (Aro et al. 1993; Singleton and Sainsbury 1994), and the remaining half were left untreated. Maximum quantum yield of PSII was measured in all samples prior to the jars being placed into one of four light treatments (10 μmol photons m^{-2} s^{-1}, 50 μmol photons m^{-2} s^{-1}, 100 μmol photons m^{-2} s^{-1}, or 200 μmol photons m^{-2} s^{-1}), with subsequent measurements being made after 0.5 h, 1 h, 2 h, and 3 h. Light was supplied by a metal halide lamp (Osram), and light levels were adjusted using neutral density filters (Lee Filters). The spectral quality during the incubation (*see* Fig. 1 for photon emission spectra) was measured using a spectrophotometer (Ocean Optics). Jars containing melted algal samples were incubated in a custom-built flow-through chamber maintained at -1.8°C. Following the 3-h exposure period, all jars were placed into the lowest light treatment (10 μmol photons m^{-2} s^{-1}) and were left for a further 3 h before being tested for photosynthetic recovery.

Chlorophyll a *fluorescence*—Chlorophyll *a* fluorescence measurements were made using a Water-PAM (Pulse

Amplitude Modulated) Fluorometer (Walz, GmbH). Sub-samples (3 mL) from each jar were transferred at each time point into a quartz cuvette and were dark-adapted for 10 min before measuring maximum quantum yield of PSII ($F_V : F_M$) (measuring light frequency = 6; photomultiplier gain = 10–26 (depending on algal density); output gain = 1; damping = 1; saturating pulse intensity > 3000 μmol photons m^{-2} s^{-1}; and saturating pulse width = 0.6 s). A 10-min dark-adaptation period was chosen as the minimum time required to reach maximum F_M, based on prior measurements of different time intervals. Maximum quantum yield of PSII was calculated as the difference between the minimum fluorescence value (F_O) and the maximum fluorescence value (F_M) (i.e., $F_M - F_O = F_V$) and divided by F_M: ($F_M - F_O$)/F_M (Schreiber 2004).

D1-protein determination—For D1-protein (encoded by the psbA gene) determination, samples were taken initially and after 3 h of light exposure for comparison of protein levels. Samples were filtered onto 47-mm-diameter glass microfiber (GF/F) filters (Whatman), placed in a 2-mL cryovial, and frozen at -80°C for later analysis. Protein quantification was performed using the protein extraction method and immunoblotting techniques described in Six et al. (2007) and Brown et al. (2007, 2008). The anti-psbA antibody for D1 detection and protein quantification standard (www.agrisera.com) were used to immunodetect and quantify D1 levels.

Determination of PSII photoinactivation and repair rates—For parameterization of photoinactivation during the exposure period, we estimated an effective target size for photoinactivation (σ_i with units of nm^2 $PSII^{-1}$) as the exponential decay rate of $F_V : F_M$ plotted vs. the cumulative photon dose (Six et al. 2007) incident on the algal suspensions in the presence of lincomycin. The $F_V : F_M$ value at 0.5 h was used as the initial 100% $F_V : F_M$ value (Six et al. 2007). This allowed better separation of the change in $F_V : F_M$ as a result of the cumulative absorption of photons during the photoinactivation phase from those during the initial phase dominated by induction of NPQ. The $|\sigma_i|$ parameterization allowed estimation of the photoinactivation rate by multiplying $|\sigma_i|$ with incident irradiance (photons m^{-2} s^{-1}). $|\sigma_i|$ was not the physical size of an entity, but rather it expressed, in units of nominal area per PSII, the probability that a photon incident upon the algae community caused the photoinactivation of a PSII center. Repair rates of PSII were estimated using the difference in exponential decay rates of $F_V : F_M$ plotted against time in the absence and presence of lincomycin (Six et al. 2007; Key et al. 2010), starting after an initial 0.5-h period when induction of rapid NPQ processes dominated the responses. The ability to acclimate to increased irradiance depends on the rate of PSII repair (R_{PSII} s^{-1}), which must either equal or exceed photoinactivation rates in order to avoid photoinhibition.

Data analysis—Quantification of D1 immunoblots was performed using a charge-coupled device (CCD) imager (Kodak Carestream 4000MMPro) and associated software (Carestream Molecular Imaging). Changes in $F_V : F_M$ and D1-protein level were analyzed by a temporally dependent, general linear model ANOVA with three fixed factors (i.e., time, light, and lincomycin) in conjunction with a Tukey's post hoc pairwise comparison (α = 0.05). One-way ANOVA was used to detect differences in the R_{PSII} rate among light treatments (α = 0.05). To ensure that assumptions of normality and equal variance for both parametric tests were satisfied, the Kolmogorov-Smirnov test for normality and Levene's test for homogeneity of variance were applied to all analyses. In all cases the assumptions were met. Analyses were performed using Minitab statistical software (version 15.1.0.0 2006).

Results

Microscopic analyses of the preserved sea-ice microalgal communities revealed a fairly homogeneous community of pennate diatoms consisting almost entirely of members of the genus *Fragilariopsis*, falling into two cell-volume categories: small (1.0 $\pm$ 0.22 $\times$ 10^3 μm^3) and large (1.8 $\pm$ 0.09 $\times$ 10^3 μm^3). Statistical analyses of the $F_V : F_M$ and D1-protein data showed no significant interactions between time, light, and lincomycin. However, individual factors of time, light, and lincomycin were significant ($p < 0.001$, $p < 0.001$, and $p = 0.007$, respectively) for the 100 μmol photons m^{-2} s^{-1} and 200 μmol photons m^{-2} s^{-1} light treatments, as determined by the ANOVA with post-hoc comparison. The sea-ice microalgae exposed to 10 μmol photons m^{-2} s^{-1} or 50 μmol photons m^{-2} s^{-1} showed no significant change in $F_V : F_M$ over the 6-h incubation period, and no statistically significant difference between the lincomycin and non-lincomycin control treatments (Fig. 2a,b) was evident. At 100 μmol photons m^{-2} s^{-1} a significant decline in $F_V : F_M$ ($p < 0.001$) from approximately 0.60 to 0.40 after 3 h was observed; however, no significant difference between the control cells and the lincomycin-treated cells was detected (Fig. 2c). The highest light treatment (200 μmol photons m^{-2} s^{-1}) showed a significant decline in $F_V : F_M$ from 0.60 to 0.35 in the control treatment, and $F_V : F_M$ declined to 0.26 in the presence of lincomycin ($p < 0.001$) after 3 h (Fig. 2d). Here, a significant difference between the control and lincomycin-treated samples ($p = 0.007$) was detected. In the two highest light treatments, most of the observed drop in $F_V : F_M$ occurred within the first 0.5 h, with a subsequent slower phase over the remainder of the treatment. Furthermore, in the high light treatment (200 μmol photons m^{-2} s^{-1}), the subsequent recovery of $F_V : F_M$ in the absence of lincomycin reached 0.50, 84% of the initial value. In contrast, the $F_V : F_M$ in the lincomycin-treated microalgae recovered to 0.37 (only 61% of the initial value) after 3 h of recovery light (10 μmol photons m^{-2} s^{-1}) (Fig. 2d).

Changes in D1-protein levels followed the patterns observed in the chlorophyll fluorescence data (Fig. 2a–d). Under high light (200 μmol photons m^{-2} s^{-1}) the lincomycin-treated cells showed a decline in D1-protein content from 0 h to 3 h that was at the edge of significance ($p = 0.052$; paired, one-tailed *t*-test, $n = 3$). At 3 h, the D1 content was significantly lower in the lincomycin-treated
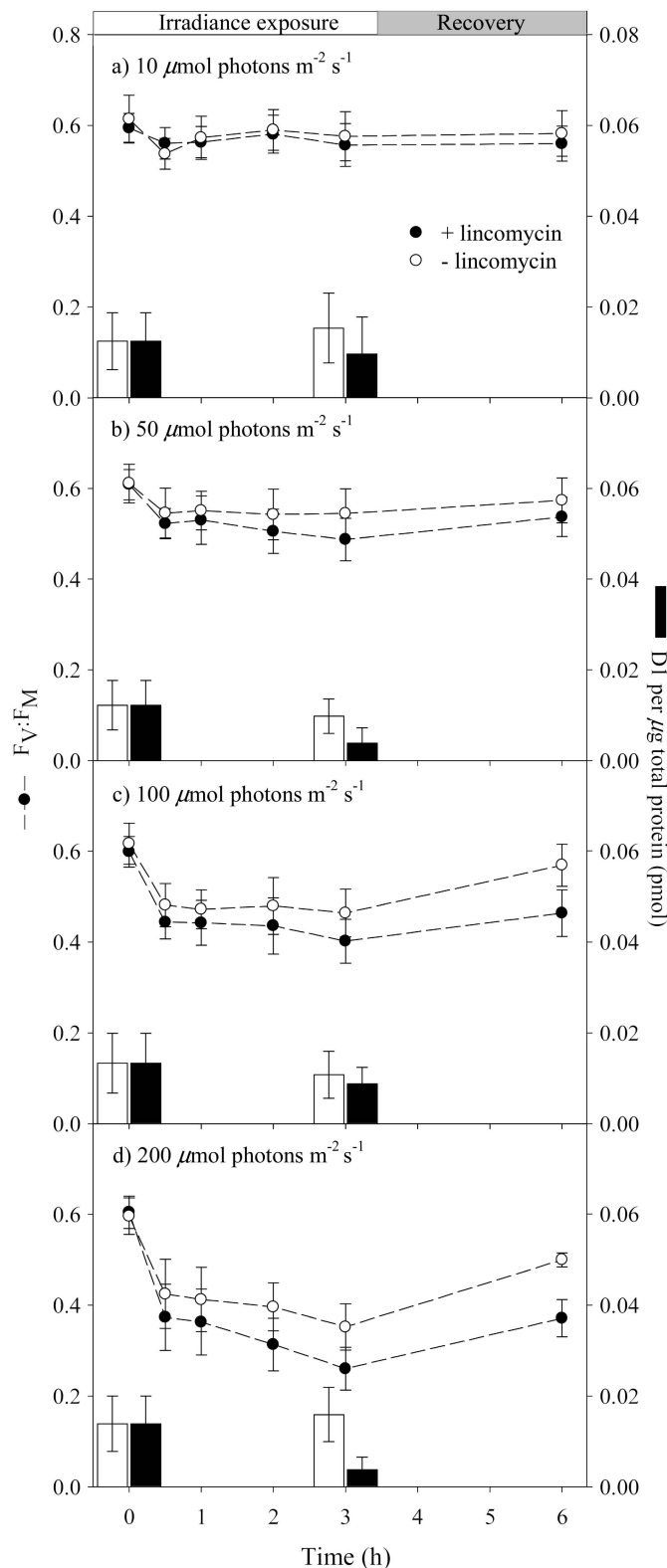
Fig. 2. Maximum quantum yield of PSII ($F_V : F_M$) of sea-ice microalgal communities in the presence (closed circles) and absence (open circles) of lincomycin exposed to (a) 10 μmol photons m^{-2} s^{-1}, (b) 50 μmol photons m^{-2} s^{-1}, (c) 100 μmol photons m^{-2} s^{-1}, and (d) 200 μmol photons m^{-2} s^{-1}. Samples were exposed to light conditions for 3 h followed by 3 h of recovery light (10 μmol photons m^{-2} s^{-1}). Data represent means ($n = 5 \pm$ SEM). Vertical bars represent pmol of D1 μg^{-1} of total protein in sea-ice microalgal communities in the presence (black bars) and absence (white bars) of lincomycin exposed to 10 μmol photons m^{-2} s^{-1}, 50 μmol photons m^{-2} s^{-1}, 100 μmol photons m^{-2} s^{-1}, and 200 μmol photons m^{-2} s^{-1} (a–d, respectively) at 0 h and 3 h of exposure. Data represent means ($n = 3 \pm$ SEM).

cells than in the control cells ($p = 0.018$; paired, one-tailed t-test, $n = 3$). This demonstrates that the control cells synthesize D1 in sufficient amounts to counter the loss from the D1 pool that was observed in the lincomycin-treated cells. In the other light and inhibitor treatments the changes in D1 protein were not large enough to reach statistical significance.

The effective target size for photoinactivation of PSII, $|\sigma_i|$, was comparable when estimated for each light level separately (Allakhverdiev and Murata 2004). Therefore, the data from the four light treatments were pooled for an overall estimate of $|\sigma_i|$ prevailing across the range of treatment lights (Fig. 3). The functional repair rate of PSII (R_{PSII}) was estimated as the difference in the exponential decay rates for $F_V : F_M$ in the absence and presence of lincomycin (Fig. 4), which blocks D1 repair. R_{PSII} was not determined in the light level of the control; it was not detectable. However, when comparing the other light levels, there was a significant increase from 8.55×10^{-6} (50 μmol photons m^{-2} s^{-1}) to 2.27×10^{-5} PSII s^{-1} ($p = 0.006$) when measured under the highest irradiance (Table 1). The repair rates achieved (R_{PSII}) were faster than the estimated rate of photoinactivation $|\sigma_i| \times$ irradiance (E) under the three determined light treatments (Table 1).

Discussion

Antarctic sea-ice diatoms from the East Antarctic show rapid photoprotection when exposed to high-light conditions, responding with an irradiance-dependent initial decline in the maximum quantum yield of PSII ($F_V : F_M$) within 0.5 h following the increase in illumination. We hypothesize that this initial, rapid, irradiance-dependent drop in $F_V : F_M$ represents an induction of NPQ to dissipate excess excitation energy (Kropuenske et al. 2009). Subsequent to the initial 0.5 h, the cells showed a further, slower decline in $F_V : F_M$, which was negligible in low light but was detectable in higher light. When cells were returned to low light, they showed some recovery within 3 h, demonstrating their photosynthetic resilience to changes in light environment. The recovery observed in control samples can be attributed to both PSII repair and relaxation of NPQ processes, whereas the recovery in the lincomycin-treated cells is solely due to the decline in NPQ because PSII repair was blocked. It is clear from this study that some of these highly plastic organisms, once thought to be shade adapted (Cota 1985; Palmisano et al. 1985; Thomas and Dieckmann 2002), are in fact well equipped to deal with rapid and relatively large changes in light level (10 μmol photons m^{-2} s^{-1} to 200 μmol photons m^{-2} s^{-1}), even at low temperatures, with minimal lasting effects to their photosynthetic machinery.
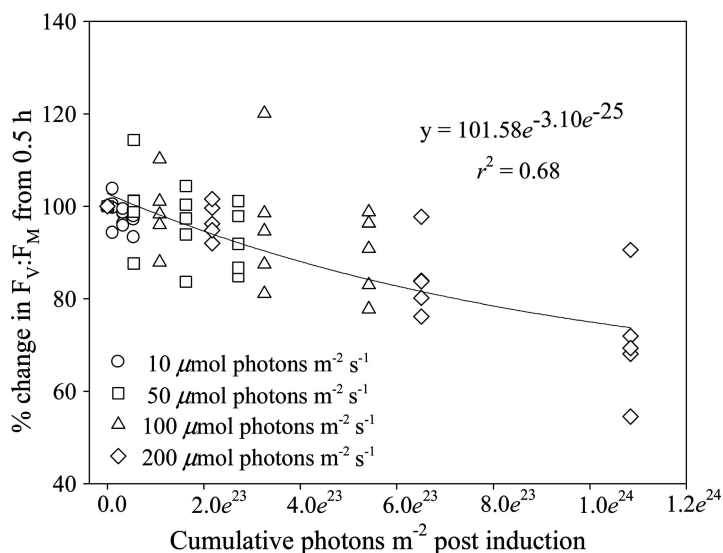
Fig. 3. Percentage change in $F_V:F_M$ of sea-ice microalgae in the presence of lincomycin expressed as a function of cumulative photons postinduction for the light treatments of 10 μmol photons m^{-2} s^{-1}, 50 μmol photons m^{-2} s^{-1}, 100 μmol photons m^{-2} s^{-1}, and 200 μmol photons m^{-2} s^{-1}. Data have been fitted with an exponential decay function. Dots represent individual replicates.

During the 3-h exposure to the highest irradiance (200 μmol photons m^{-2} s^{-1}), the sea-ice diatoms showed an upregulation of their PSII repair rate at the highest irradiance (Table 1), which allowed them to maintain their pools of D1 protein (Fig. 2). The sea-ice diatom PSII repair rates were slower compared with diatoms of similar volume measured at temperate temperatures (Key et al. 2010). This is to be expected because lower temperatures slow down metabolic processes (Morgan-Kiss et al. 2006). Nevertheless, the sea-ice diatoms did induce measurable PSII repair, which kept pace with photoinactivation rates even at the highest irradiance tested, 200 μmol photons m^{-2} s^{-1}, which was many times higher than the measured light in their under-ice environment (2.3 μmol photons m^{-2} s^{-1} to 57 μmol photons m^{-2} s^{-1}). Beyond modulation of PSII repair, two important factors contribute to the strong capacity of the sea-ice diatom community to withstand an increase in light. The sea-ice diatoms, like temperate diatoms (Key et al. 2010), show a low intrinsic susceptibility to PSII photoinactivation, which means that even modest PSII repair rates can keep pace with the limited photoinactivation across the range of light levels measured.

In parallel, the sea-ice diatoms show a significant, irradiance-dependent capacity to rapidly induce a reversible down-regulation of PSII within 0.5 h of a shift to increased light. This downregulation occurred in both the presence and absence of lincomycin, and was reversible to equivalent extents in the presence and absence of lincomycin, within 3 h after a return to low light. This mechanism thus does not depend upon contemporaneous chloroplastic protein synthesis, and shows a kinetic pattern that is likely associated with a NPQ mechanism. NPQ can enable cells to balance the total amount of absorbed energy with their capacity for utilization in metabolic activity, and thus serves to limit photo-oxidative damage (Ruban et al. 2004). Kropuenske et al. (2009) also found that in the sea-ice diatom *Fragilariopsis cylindrus*, the addition of lincomycin had no effect on patterns of NPQ induction and caused only a 5% decline in $F_V:F_M$ compared with a control treatment (Kropuenske et al. 2009). Similarly, minimal functional sensitivity to lincomycin was observed in the large diatom *Coscinodiscus wailesii* (Key et al. 2010). In all of these cases, NPQ and a high intrinsic resistance to photoinactivation allowed the diatoms to endure exposure to high light, even with modest PSII repair rates.

This photosynthetic pattern of diatoms contrasts with an Antarctic psychrophilic chlorophyte, *Chlamydomonas raudensis* (Pocock et al. 2007) isolated from Lake Bonney in the Taylor Valley, Antarctica, which depends heavily upon a rapid PSII repair cycle. In *C. raudensis*, PSII repair functions maximally at low temperatures (8°C), generating unusually rapid recovery (given the low temperatures) from

Table 1. Photophysiological properties of sea-ice diatom communities under four light levels. $|\sigma_i|$, effective target size of PSII photoinactivation; R_{PSII}, repair rate or PSII ($n = 3$, values in parentheses $\pm$ SEM). n.d. = not determined.

	Control	Low	Medium	High
Light levels (μmol photons m^{-2} s^{-1})	10	50	100	200
$|\sigma_i|$ (nm^2 PSII^{-1})	n.d.	3.1×10^{-7} (7.3×10^{-5})	3.1×10^{-7} (7.3×10^{-5})	3.1×10^{-7} (7.3×10^{-5})
R_{PSII} (PSII s^{-1})	n.d.	8.55×10^{-6} (2.14×10^{-6})	8.57×10^{-6} (3.57×10^{-6})	2.27×10^{-5}* (4.58×10^{-6})
Photoinactivation (P) vs. repair (R)	n.d.	R≥P	R≥P	R≥P

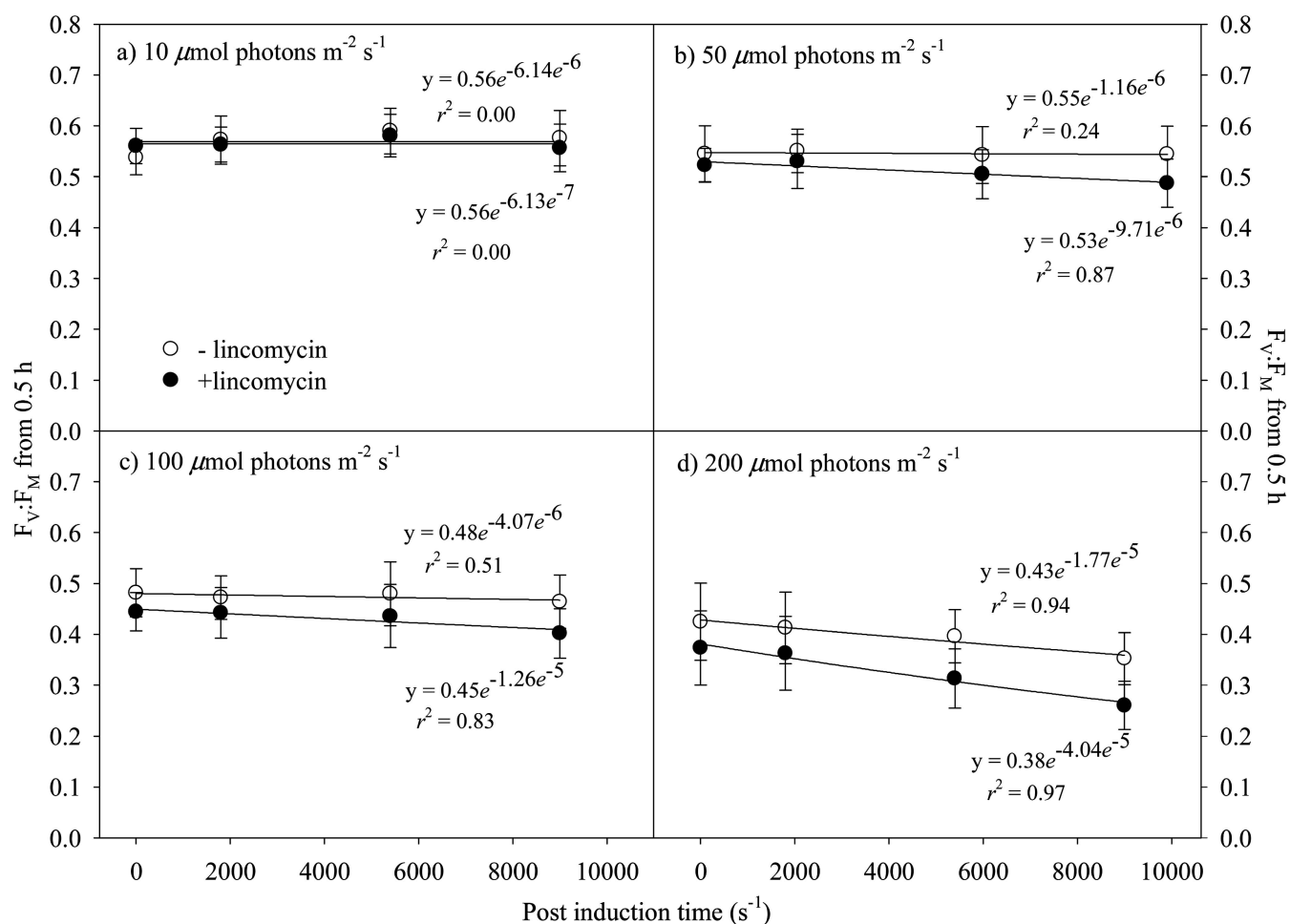* Significantly different from all other light treatments at $p < 0.01$.

Fig. 4.   Maximum quantum yield of PSII ($F_V : F_M$) of sea–ice algae in the presence (closed circles) and absence (open circles) of lincomycin expressed as a function of time postinduction for the light treatments of (a) 10 μmol photons m^{-2} s^{-1}, (b) 50 μmol photons m^{-2} s^{-1}, (c) 100 μmol photons m^{-2} s^{-1}, and (d) 200 μmol photons m^{-2} s^{-1}. Data have been fitted with an exponential decay function. Differences in exponential decay rates of lincomycin and control samples were tested: decay rates were significantly different at 10 μmol photons m^{-2} s^{-1} and 50 μmol photons m^{-2} s^{-1} (p = 0.024 and 0.012, respectively), but no differences were found between the control and lincomycin decay rates at 100 μmol photons m^{-2} s^{-1} and 200 μmol photons m^{-2} s^{-1}. Data represent means (n = 5, SEM).

photoinhibition after high light exposure (Pocock et al. 2007). However, the irradiance used by Pocock et al. (2007) was three times greater (600 μmol photons m^{-2} s^{-1}) than the irradiance applied in this study. Furthermore, diatoms and chlorophytes such as *C. raudensis* may rely upon a different mechanism in response to changes in light (Six et al. 2009). The combination of limited PSII photoinactivation and a reasonably effective D1 protein resynthesis in these sea-ice diatoms explains the remarkable capacity for these supposedly shade-adapted psychrophiles to withstand higher light levels with limited long-term photodamage.

Cell size could also be an important factor in responses to increasing light because cell volume has been correlated with D1 repair rates across a range of temperate diatoms (Key et al. 2010; Six et al. 2009). In a recent study, small diatoms (1.19 $\times$ 10^1 μm^3) showed faster repair rates and greater photoinactivation (up to 45%) under high light exposure compared with larger diatoms (2.13 $\times$ 10^7 μm^3), whereas PSII repair rates were four-fold slower (Key et al. 2010). The larger cells allocate more of their total cellular

protein pool to D1 and have a smaller effective absorption cross-sectional area of PSII (Key et al. 2010). Thus, they are less susceptible to photoinhibition and can better respond to short-term high light exposure. The sea-ice diatoms from this study were intermediate in cell size (1–1.8 $\times$ 10^3 μm^3) compared with those studied by Key et al. (2010), and they had PSII repair rates close to those of the larger-sized cells. Interestingly, while the D1-protein content of sea-ice diatoms was typical of that measured in other diatom species (0.01–0.03 pmol D1 μg^{-1} total protein) (Key et al. 2010), the susceptibility to photoinactivation, $|\sigma_i|$, in the sea-ice diatoms was smaller than would be expected based on their cell volume; this is rather comparable to the largest centric diatoms. However, further investigation is needed to confirm any correlation between cell size and photoinactivation in pennate diatoms. It is possible that these sea-ice diatoms have a relatively small antenna size, which would limit light damage (Melis et al. 1999). This would support the observation that with increased light, the preferred strategy adopted by these sea-

ice diatoms is rapid photoprotection through nonradiative heat dissipation via xanthophyll cycling (K. Petrou unpubl.). This strategy was also found in *F. cylindrus*, which showed almost complete reliance on xanthophyll activity for photoprotection when exposed to high light (Kropuenske et al. 2009). Similarly, many temperate diatoms have displayed a high level of xanthophyll-dependent NPQ as an immediate photoprotective response (Lavaud et al. 2002, 2004; Ruban et al. 2004). If this remains true for all sea-ice diatoms, by maximizing xanthophyll activity they are able to limit D1-protein inactivation as well as subsequent damage to their photosystems from excess light energy.

This is the first study to measure D1 protein and PSII repair rates in natural populations of Antarctic bottom-dwelling sea-ice microalgae exposed to elevated irradiance. While it contradicts the idea that sea-ice algae are obligate shade-adapted photoautotrophs, the discrepancy is likely due to differences in the environmental conditions from which the algae were collected. For example, this study was conducted in the East Antarctic, which has the shortest ice season and the area with least snow accumulation within the Southern Ocean (Arrigo et al. 1998). Consequently, the ice is thinner, and the irradiance is higher than in many other locations around the continent. Any such differences in environmental condition influence the photosynthetic condition of the sea-ice algae. The photosynthetic plasticity to high light exhibited by these natural populations of Antarctic sea-ice diatom communities demonstrates their inherent ability to effectively protect their photosystems from damage even at subzero temperatures. Phytoplankton are often regarded as sentinels of climate change because their rapid population-turnover rates mean populations will respond rapidly to altered environmental conditions. This observed capacity for acclimation to rapid environmental change—specifically increased irradiances—combined with short life cycles and therefore considerable adaptive potential, suggests that the diatom communities observed in this study show potential to adjust to a world with thinner annual sea ice and greater light penetration. However, synergistic effects of increasing irradiance with elevated ocean temperature and ultraviolet irradiation and ocean acidification remain unknown, and these interactions need to be tested in order to fully comprehend their potential to affect sea-ice algae communities.

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