PHOTOPROTECTION OF SEA ICE MICROALGAL COMMUNITIES FROM THE EAST ANTARCTIC PACK ICE[1]

K. Petrou, R. Hill, M. A. Doblin

Plant Functional Biology and Climate Change Cluster and Department of Environmental Sciences, University of Technology, Sydney, PO Box 123, Broadway NSW 2007, Australia.

A.McMinn

University of Tasmania, Centenary Building, 203, Hobart, 7000, Australia

R. Johnson, S. W. Wright

Australian Antarctic Division and Antarctic Climate and Ecosystems Cooperative Research Centre, 203 Channel Highway, Kingston, Tasmania 7050, Australia

P. J. Ralph[2]

Plant Functional Biology and Climate Change Cluster and Department of Environmental Sciences, University of Technology, Sydney, PO Box 123, Broadway NSW 2007, Australia.

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[1]Received __________________ Accepted ______________________

[2]Author for correspondence: e-mail Peter.Ralph@uts.edu.au

ABSTRACT

All photosynthetic organisms endeavour to balance energy supply with demand. For sea ice diatoms, as with all marine photoautotrophs, light is the most important factor for determining growth and carbon fixation rates. Light varies from extremely low to often relatively high irradiances within the sea ice environment, meaning that sea ice algae require moderate physiological plasticity that is necessary for rapid light acclimation and photoprotection. This study investigated photoprotective mechanisms employed by bottom Antarctic sea ice algae in response to relatively high irradiances to understand how they acclimate to the environmental conditions presented during early spring, as the light climate begins to intensify and snow and sea ice thinning commences. The sea ice microalgae displayed high photosynthetic plasticity to increased irradiance, with a rapid decline in photochemical efficiency that was completely reversible when placed under low light. Similarly, the photoprotective xanthophyll pigment diatoxanthin (Dt) was immediately activated, but reversed during recovery under low-light. The xanthophyll inhibitor, dithiothreitol (DTT), and state transition inhibitor, sodium fluoride (NaF) were used in under-ice *in situ* incubations and revealed that non-photochemical quenching via xanthophyll cycle activation was the preferred method for light acclimation and photoprotection by bottom sea ice algae. This study showed that bottom sea ice algae from the east Antarctic possess a high level of plasticity in their light acclimation capabilities and identified the xanthophyll cycle as a critical mechanism in photoprotection and the preferred means by which sea ice diatoms regulate energy flow to PSII.

KEYWORDS

Chlorophyll *a* fluorescence, OJIP transients, photoprotection, sea ice microalgae, xanthophyll cycle.

ABBREVIATIONS

Dd, diadinoxanthin; Dt, diatoxanthin; DTT, dithiothreitol; F_V/F_M, maximum quantum yield of PSII; F_O, minimum fluorescence; F_M, maximum fluorescence; PSI, Photosystem I; PSII, Photosystem II; Q_A, primary plastoquinone acceptor of PSII; Q_B, secondary plastoquinone acceptor of PSII; VAZ, violaxanthin, antheraxanthin and zeaxanthin.

INTRODUCTION

Light regulates growth and photosynthesis in autotrophic organisms. However, the amount of light absorbed often exceeds the capacity for utilization in photochemistry, potentially leading to photoinhibition and eventually damage of the photosynthetic apparatus (Müller et al. 2001). As a result, photosynthetic organisms endeavour to balance energy supply with demand. This is achieved through regulation of the photosynthetic apparatus, where plants and algae attempt to maintain homeostasis between energy conversion through electron transport and energy consumption for carbon fixation (Foyer et al. 1990). Low temperatures often exacerbate photosynthetic sensitivity in plants (Ensminger et al. 2006) which can reduce the capacity for photosystems to cope with high irradiances, leading to increased excitation pressure on photosystem II (PSII) (Hüner et al. 1993, Ivanov et al. 2003). Therefore, energy imbalances between photochemistry and carbon fixation can often occur when plants are exposed to high light or low temperature conditions, resulting in increased PSII excitation pressure and subsequently, photoinhibition (Hüner 1998).

In the case of psychrophilic photoautotrophs (organisms whose optimal growth temperature is below 15°C (Morgan-Kiss et al. 2006)), such as sea ice algae, extremely low temperatures and relatively high light conditions often coincide. This mean that the algae require strategies for coping with low temperatures, as well as a certain level of physiological plasticity for rapid light acclimation and photoprotection. Sea-ice algae occupy a unique habitat characterised by sub-zero temperatures and variable irradiance. In the sea ice environment, light levels can vary from a little as 0.001 (Mock & Gradinger 1999) to more than 10 % of surface irradiance (Palmisano et al. 1987), depending on the ice thickness, snow cover and solar irradiance (Eicken 1992). On an annual cycle, as winter progresses into spring, light intensity and day length increase rapidly (Sakshaug and Slagstad 1991), and as summer approaches, the thinning of ice and melting of snow means that sea ice algae still entrained in the ice matrix are likely to be exposed to relatively high irradiances, while remaining at sub-zero temperatures. These combined conditions may lead to increased excitation pressure on PSII, where the low temperatures reduce the cell's capacity to deal with the increases in light (Hüner et al. 1993, Hüner 1998, Ivanov et al. 2003). Similarly, sea ice algae melted into surface waters in late spring are also exposed to elevated irradiances (McMinn et al. 2003). Therefore, sea ice algae must employ strategies to ensure efficient photosynthesis is maintained, while preventing the formation of reactive oxygen species, and protecting the cell from oxidative damage.

The dominant sea ice algae are pennate diatoms (Thomas and Dieckmann 2002, McMinn et al. 2007). Unlike higher plants and green algae – which use the xanthophyll pigments violaxanthin, antheraxanthin and zeaxanthin (VAZ) – diatoms possess a single-step xanthophyll cycle (Grouneva et al. 2006) utilising diadinoxanthin and its de-epoxidised form diatoxanthin (Dd and Dt, respectively). The de-epoxidised carotenoid pigments effectively dissipate energy from the PSII antenna before it reaches the reaction centre, thus preventing photodamage (Olaizola et al. 1994, Demmig-Adams and Adams 1996). This photoprotective strategy forms a major part of non-photochemical quenching (NPQ), operating over timescales of seconds to minutes. It often requires the formation of a pH gradient (ΔpH) across the thylakoid membrane (Demmig-Adams et al. 1989), and is closely associated with the regulation of the xanthophyll cycle (Krause and Weis 1991).

Despite their importance to primary production at high latitudes, there have not been many studies into the strategies used in photoacclimation of sea ice algae (Kudoh et al. 2003), especially with regard to their capacity to acclimate to rapid environmental change. Many studies describe bottom sea ice algae to be among the Earth's most shade-adapted plants (Cota 1985, Palmisano et al. 1985, Thomas and Dieckmann 2002, McMinn et al. 2003, 2007, Lazzara et al. 2007). However, Lizotte and Sullivan (1991a, 1991b, 1992) found a high level of plasticity in sea ice algae under both elevated and lowered light conditions, suggesting high photoacclimation capacities. More recently, Ralph et al. (2005) tested tolerances of surface sea ice algae (with higher *in situ* light environment) to high light and found that even at freezing temperatures, surface communities were able to use NPQ effectively, suggesting a sun-adapted physiology. Similarly, laboratory studies on temperate diatoms have revealed that some diatom species express a very high capacity for NPQ that is highly dependent on xanthophyll cycle activity (Jakob et al. 2001, Lavaud et al. 2002, 2004, Ruban et al. 2004). Therefore, the aim of this study was to investigate photoprotection and photoinhibition by bottom sea ice algae from the east Antarctic pack ice in response to relatively high irradiances and determine the mechanisms employed to photoacclimate to the environmental conditions presented during early spring, as the light climate begins to intensify and snow and sea ice thinning commences.

MATERIALS AND METHODS

Algal sampling and experimental protocol

Investigations into sea ice algal photoprotection were conducted during the RSV *Aurora Australis* Sea Ice Physics and Ecosystem eXperiment (SIPEX) voyage to the east Antarctic sea-ice zone. Sea-ice samples were collected in austral spring (September/October 2007) from four different ice stations (from 64.30 to 65.01°S and 116.80 to 119.15°E). Ice core thickness varied from 570 – 1300 mm with under-ice light climates ranging from approximately 2.3-153 µmol photons · m^{-2} · s^{-1}, depending on ice thickness, snow cover and incident irradiance (dependent on local weather and time of day). Bottom sea-ice algal assemblages were collected using an ice auger (internal diameter 90 mm) and the bottom 20 mm of the core was then sawn off under black plastic and returned to the ship where they were then melted in filtered (0.22 µm) seawater/brine mix over 24 h at 4°C in the dark. To avoid osmotic stress, salinity levels were checked every 5 h to ensure that the samples remained between 30-35 psu. If salinity dropped below 30, more filtered brine (collected from sack holes at each site) was added. This mixture of 0.22 µm filtered brine and seawater was used to minimise dilution of cell density. Before experimental procedures began, a 5 ml aliquot of sea ice was preserved in 1% glutaraldehyde for later microscope cell identification. For on board incubations, aliquots of melted out sea ice algae (100 mL) were sub-sampled in triplicate into clear 120 mL polyethylene jars and placed in different light treatments (10, 50, 100, 200 µmol photons · m^{-2} · s^{-1}). Light was supplied by a metal halide lamp (400W M59/E; Osram GmbH, Munich, Germany) and light levels were obtained using neutral density filters (Lee Filters, Burbank, CA, USA). Samples were incubated in a custom built flow-through chamber (-1.8°C) for a maximum of 8 h. Chlorophyll *a* fluorescence measurements were made for each treatment at 0, 1, 2, 3 and 5 h of incubation, after which all remaining jars were placed into the lowest light treatment (10 µmol photons · m^{-2} · s^{-1}) for an additional three hours to encourage recovery from photoinhibition. The same experiment was repeated on five occasions with algae collected from four different ice stations.

To further investigate the potential mechanisms of photoprotection in sea ice algae, two short (6 h) inhibitor experiments were conducted using a custom designed under-ice incubator. Quadruplicate sea ice algal aliquots of (45 mL) were incubated under the ice for 6 h either in the presence or absence of the xanthophyll inhibitor dithiothreitol (DTT; 100 µM (Olaizola et al. 1994)). Dithiothreitol (DTT) has previously been shown to prevent the de-epoxidation of Dd and consequently NPQ in the diatom *Phaeodactylum tricornutum* (Olaizola et al. 1994, Casper-Lindley and Bjorkman 1998). The second incubation experiment used an inhibitor of state transitions, sodium fluoride (NaF; 0.05 M (Canaani et al. 1984)) and was also incubated for 6 h under the ice. Fluorescence measurements were made every 2 h from 12:00 until 18:00 h and under ice photosynthetically active radiation (PAR) was recorded hourly using a

2π underwater sensor connected to a light meter (LI-189, LiCOR, Lincoln, Nabraska, USA). Due to time required for the study and time restrictions at each ice station, the two under-ice incubations (DTT and NaF) were conducted on different days at different ice stations.

Chlorophyll a *fluorescence:*

Photosystem II (PSII) photochemical efficiency was determined through measurements of maximum quantum yield (F_V/F_M) using a Water-PAM (Pulse Amplitude Modulated) fluorometer (Walz GmbH, Effeltrich, Germany). A 3 mL aliquot of sample from each treatment was transferred to a quartz cuvette and after a 5 min dark-adaptation period, minimum fluorescence (F_O) was recorded. Upon application of a saturating pulse of light (saturating pulse width = 0.6 s; saturating pulse intensity > 3000 µmol photons · m^{-2} · s^{-1}) maximum fluorescence (F_M) was determined. From these two parameters F_V/F_M was calculated according to the equation $(F_M-F_O)/F_M$ (Schreiber 2004).

Fast induction curves (FICs) were measured at T0 and after 5 h of light exposure, using a double-modulation fluorometer (Photon System Instruments, FL-3500, Brno, Czech Republic) with a 3 s multiple turnover flash at >3000 µmol photons · m^{-2} · s^{-1} light intensity. Fluorescence measurements were recorded every 10 µs for the first 2 ms, every 1 ms until 1 s, then every 500 ms up to 3 s. Prior to all measurements, sub-samples were dark-adapted for 5 min. All FICs were normalised to F_O, where all values were divided by the O step (at 50 µs) and J values (at 700 µs) were compared for statistical differences between light exposure times for each treatment. Data were then normalised to the J step, where data were divided by the J value (700 µs) to evaluate differences in the amplitude of the P step (260 ms) between treatments, as described in Hill et al (2004).

Chlorophyll a *and photoprotective pigments*

Samples (80 - 100 mL) for photoprotective pigments and Chl *a* were collected from the initial population, at the maximum level of photoinhibition, and after recovery in low light (0, 5, and 8 h, respectively). Pigment samples were also taken from the *in situ* incubations in the presence and absence of DTT. Samples were collected for HPLC pigment analysis after 0, 2, and 6 h of incubation. No HPLC samples were obtained from the NaF incubation, as particles of the inhibitor blocked the filter before sufficient cells could be collected on the membrane. Pigment samples were filtered under low vacuum (≤ 20 mm Hg) onto GF/F filters (13 mm Whatman, Göttingen, Germany) in low light (< 10 µmol photons · m^{-2} · s^{-1}) and filters were immediately frozen in liquid nitrogen for subsequent analysis. The pigment extraction method of Mock and Hoch (2005) was used, with modifications described in Wright et al. (2010).

Molar pigment ratios of Chl *c*, fucoxanthin, Dd + Dt, β, β-carotene and the VAZ pool were calculated against Chl *a*. Photoprotective pigment ratios were determined by dividing the total photoprotective pigment (Pp) pool (diatoxanthin, diadinoxanthin, lutein, β, β-carotene, antheraxanthin) by the total pigment pool (photoprotective + photosynthetic; Pp + Ps), which included chlorophylls *a, b,* c_1, c_2, c_3, β,ε-carotene, 19'-butfucoxanthin, fucoxanthin, 19'-hexfucoxanthin and prasinoxanthin. The de-epoxidation ratio (a measure of diadinoxanthin conversion to the photoprotective diatoxanthin) was calculated as the total diatoxanthin (Dt) pool divided by the total diatoxanthin + diadinoxanthin pool (Dt + Dd).

Data analyses

Two-factor analysis of variance (ANOVA) was used to identify changes in HPLC pigments and F_V/F_M between light treatments over time (initial, 5 h and recovery) and Tukey's post hoc test was used to locate the significant differences (α = 0.05). For detecting differences between J and P values, a one-way ANOVA was used (α = 0.05). To determine that all assumptions of normality and equal variance for all parametric tests were satisfied, the Kolmogorov-Smirnov test for normality and Levene's test for homogeneity of variance was applied to all analyses *a priori*. Assumptions were met in all instances with the exception of one. The de-epoxidation ratio (light * time) data were transformed (square root) to ensure equal variances before being analysed by two-factor ANOVA. All analyses were performed using Minitab statistical software (version 15.1.0.0 2006, Minitab Inc, State College, Pennsylvania, USA).

RESULTS

Sea ice microalgal community

Microscopic identification of preserved samples revealed the community of bottom sea ice algae was dominated by the pennate diatoms *Fragilariopsis curta, Fragilariopsis kerguelensis,* and *Pseudonitzschia* sp. and the centric diatom *Chaetoceros dicheata*. This was reflected in the community pigment composition, which contained high concentrations of Chl *a* and *c*, fucoxanthin (23% of total pigment content; Table 1), diadinoxanthin and β, β-carotene (Table 1), characteristic of diatoms. There was also a relatively high proportion (9%) of 19'-hexfucoxanthin (data not shown), which was confirmed by microscopy to be only a small proportion (< 7%) of haptophytes. The molar ratios of pigments showed that the Dd+Dt pool was tenfold greater than the VAZ pool (Table 1), supporting the diatom dominance by the strong presence of photoprotective carotenoids (Dd and Dt) utilised by this algal group. Compared with polar diatom cultures and other field samples, the pigment ratios (Table 1) were well within reported ranges for Chl *c*:chl *a,* fuc:Chl *a* and Dd+Dt:Chl *a* (Sakshaug and Slagstad 1991, Olaizola and Yamamoto 1994, Robinson 1997). However, β, β-car:Chl *a* was below previously reported values and the VAZ pool:Chl *a* was close to values found in temperate diatoms (Dimier et al. 2007).

Chlorophyll a *fluorescence*

There was a significant interaction ($P < 0.001$) between time and light in F_V/F_M. Sea ice algae incubated at the higher light levels of 100 and 200 µmol photons · $m^{-2} \cdot s^{-1}$ showed a dramatic decline in F_V/F_M values for both treatments reaching a minimum of 0.43 in the 100 µmol photons · $m^{-2} \cdot s^{-1}$ and 0.36 in the 200 µmol photons · $m^{-2} \cdot s^{-1}$ treatment after 5 h exposure (Fig. 1). Sea ice algae showed a full recovery to initial F_V/F_M values ($P < 0.001$) in the two highest light treatments (Fig. 1). Sea ice algae incubated at 10 and 50 µmol photons · $m^{-2} \cdot s^{-1}$ showed no change in maximum quantum yield of PSII (F_V/F_M) over the 5 h incubation or subsequent 3 h recovery period.

The amplitude of the FICs declined with increased irradiance after the 5 h exposure (Fig. 2), however when compared statistically for differences in the amplitude of the J step (at 700 µs) no significant differences were detected. In contrast, fast induction curves that were normalised to the J step (Fig. 3) showed a significant decline in the amplitude of the P step (260 ms) with increased irradiance (50, 100 and 200 µmol photons · $m^{-2} \cdot s^{-1}$ treatments) after 5 h of light exposure (P = 0.045, 0.022, 0.004, respectively; Fig. 3b-d).

Chlorophyll a *and photoprotective pigments*

The ratio of photoprotective pigments to total pigments (photoprotective + photosynthetic) showed a significant increase ($P = 0.018$) over time between 0 h and recovery, but no difference was detected between light treatments (Fig. 4a). Similarly, the pool of diadinoxanthin (Dd) increased significantly from 0 h to recovery ($P = 0.003$), again with no differences between light treatments (Fig. 4b). In contrast, the diatoxanthin (Dt) pool increased significantly over time and between treatments ($P < 0.001$), with a peak in Dt concentration after 5 h exposure to high light (100 and 200 µmol photons · $m^{-2} \cdot s^{-1}$), followed by a decline after 3 h under recovery light (Fig. 4c). The increase in the de-epoxidation ratio correlated positively with increased irradiance, where after 5 h exposure there was a significant increase in the de-epoxidation ratio in the two upper light treatments ($P < 0.001$). This ratio declined significantly towards initial values following 3 h under recovery light (Fig. 4d), however, recovery values were significantly higher than initial values in the highest two light treatments (Fig. 4d).

Under ice incubations

The two sites used for the *in situ* experiments had different under ice light environments because of differences in ice thickness, snow cover and incident irradiance. The DTT incubations were conducted under 40-120 mm of snow cover and 570 mm of ice, while the NaF incubations were performed under 70-80 mm of surface snow with ice that was 430 mm thick. Noon surface irradiance was similar at both sites (840 and 800 µmol photons · $m^{-2} \cdot s^{-1}$, respectively), however, the weather changed rapidly during the DTT incubation, diminishing light levels considerably (Fig 5a). Under-ice PAR during the DTT incubation reached a maximum of 50 µmol photons · $m^{-2} \cdot s^{-1}$ at midday and declined to 2.3 µmol photons · $m^{-2} \cdot s^{-1}$ by 18:00 h (Fig 5a). In contrast, the midday PAR values for the NaF incubation were much higher, reaching a maximum of 153 µmol photons · $m^{-2} \cdot s^{-1}$ by 13:00 h before declining to 30 µmol photons · $m^{-2} \cdot s^{-1}$ by 18:00 h (Fig 5b). The under ice incubations showed a significant decline in F_V/F_M from 0.56 to 0.49 in the absence and 0.56 to 0.32 in the presence of the xanthophyll cycle inhibitor DTT ($P < 0.001$) within the first two hours (Fig. 5a). Furthermore, the decline in F_V/F_M in the presence of DTT was significantly greater ($P = 0.002$) than that measured in the absence of DTT. The de-epoxidation ratio remained constant in the presence of the xanthophyll inhibitor DTT, while there was a mid-afternoon increase in control samples (Fig. 5c). However, after 6 h the ratio was similar in both, when under ice

PAR reached less than 10 µmol photons · m^{-2} · s^{-1} (Fig. 5a). In the NaF incubations, there was a concomitant significant decline in F_V/F_M within the first two hours in the absence (P = 0.019) and presence (P = 0.002) of the state transition inhibitor (Fig. 5b). The de-epoxidation ratio increased with exposure to higher irradiances and was still high by 18:00 h in the absence of NaF (Fig. 5d). Unfortunately, no data were available for the de-epoxidation ratio in the presence of NaF.

DISCUSSION

Short-term photoacclimation and recovery in bottom sea ice microalgae have been measured here for the first time on Antarctic communities. This study has identified the photosynthetic mechanisms utilised to regulate energy flow to PSII and photoprotection in Antarctic bottom sea ice algae during the early Austral Spring. It is also the first study to apply the xanthophyll inhibitor DTT on natural Antarctic populations under *in situ* conditions and link the observed responses with antenna pigment changes. It has been clearly demonstrated that Antarctic bottom sea ice algal communities (dominated by pennate diatoms) displayed a high level of resilience to increases in irradiance, where the significant decline in PSII efficiency under high light (20% of full sunlight), was rapidly reversed under reduced irradiance. This ability to recover quickly would suggest that the sea ice algal photosystems were not damaged by the irradiances applied. Such resilience to changes in irradiance has been observed in earlier studies. Lizotte and Sullivan (1991b) measured rates of long-term photo-adaptation in bottom ice algae comparable to those reported for temperate algae when exposed to variable changes in light conditions over 5 days. Similarly, Ralph et al (2005) showed surface sea ice algae were able to acclimate efficiently to irradiances of up to 350 µmol photons · m^{-2} · s^{-1}. These studies (including the present study) are in contrast to previous studies that concluded sea ice algae are obligate shade-adapted species. However, *in situ* environmental conditions from which the algae were collected must be considered. For example, Ralph et al (2005) tested surface sea ice communities, whose natural light environment would be much higher than that of algae that live on the bottom of the ice. Similarly, the east Antarctic has the shortest ice season and therefore least snow accumulation within the entire frozen ocean (Arrigo et al. 1998), so the ice is generally thinner and consequently the light environment higher than other locations around the Antarctic, while at the same time, given the latitude, the algae do not experience a polar night.

The primary mechanism being utilised for photoprotection during the decline in F_V/F_M upon illumination was heat dissipation via xanthophyll cycle activation, as evidenced by increases in the photoprotective pigment de-epoxidation ratio and the *de novo* synthesis of Dt. The absence of any further decline in photosynthetic efficiency following the first hour of light exposure, suggests that the activation of the xanthophyll pigments was likely sufficient to dissipate all excess light energy reaching the antenna and avoid long-term irreversible photodamage. Massive NPQ capacities have been previously observed in temperate diatoms (Ruban et al. 2004), and a strong correlation between NPQ and Dt pigment concentration has been measured (Olaizola and Yamamoto 1994, Jakob et al. 2001, Lavaud et al. 2002). The pigment data in this study are consistent with these previous findings, in that de-epoxidation of Dd to Dt increased with an increase in irradiance, with the increase in the de-epoxidation ratio directly related to the increase in the total Dt pool. This ratio reverted after the 3 h low-light recovery period, confirming that it was a temporary photoprotective measure. Indeed, Antarctic sea ice algae have been shown to maintain sufficiently high level of D1 protein re-synthesis under relatively high irradiances (Petrou et al. 2010). Based on the data presented here, it is impossible however, to rule out other potential NPQ mechanisms being utilised by the sea ice algae under high light. There have been studies that suggest that xanthophyll activity and NPQ in diatoms might be independent (Eisenstadt et al. 2008), with two quenching sites having been identified and only one of those located on the PSII antenna (Miloslavina et al. 2009).

The difference between the initial and the final Dd and Dt pigment concentrations can be attributed to *de novo* synthesis. Unexpectedly, there was an increase in Dd concentration under high light – as normally Dd concentration would decline when it de-epoxidises to form Dt – demonstrating the occurrence of *de novo* synthesis of Dd during the experiment. Some of this synthesised Dd was likely converted to Dt, as shown by the significant increase in Dt concentration after 8 h, but it is impossible to rule out that some of the Dt may have come from direct conversion from violaxanthin (Lohr and Wilhelm 2001). The presence of de-epoxidation in the initial samples can be attributed to the dark period during melting out. It has been shown that prolonged dark periods (between 16 and 70 h) lead to an increase in Dt concentration in diatoms (Jakob et al. 1999, 2001). The de-epoxidation ratios correlate well with other studies; ratios of 0.22 were obtained from cultures of *Fragilariopsis cylindrus* grown at 5 µmol photons m^{-2} s^{-1} (Kropuenske et al. 2009) compared with 0.20 at 10 µmol photons · m^{-2} · s^{-1} in this study. In cells grown at 65 and 125 µmol photons · m^{-2} · s^{-1} the ratio increased to 0.51 and 0.73 respectively (Kropuenske et al. 2009), while values of 0.22, 0.32 and 0.39 were obtained for the 50, 100 and 200 µmol photons · m^{-2} · s^{-1} light treatments, respectively. The discrepancy between the two studies may be due to differences in photoacclimation times to the new light intensities (5 h compared to weeks) and the specific organisms studied. The previous study looked at a single diatom species, whereas our lower ratio under higher light was a mixed community response, where the heterogeneity in the present community would likely yield variability in pigment ratios.

The FICs in this study revealed a rapid exponential rise in O-J (ie: rapid photochemical reduction of Q_A) for the sea ice diatoms. This would suggest a lack of PSII enriched grana in the thylakoid (Antal et al. 2009) resulting in a diminished energetic connectivity between

PSII units, and a large PSII antenna size (or absorption cross sectional area of PSII antenna) suggesting that cells possess a very high capacity for photoacclimation under moderate ambient light (Antal et al. 2009). These physiological characteristics would be advantageous to sea ice algae as they would optimise light capture under the ice, yet provide moderate resilience to relatively large changes in incoming irradiance.

The significant drop in the JIP component of the FIC transients at all three higher light levels (50, 100, and 200 µmol photons · m^{-2} · s^{-1}), suggests the maximum fluorescence (F_M) of sea ice algae to be most impacted by high irradiances. The JIP rise, or *thermal* phase of the transient, is indicative of the reduction of Q_B and plastoquinone (PQ) (Lazar 1999, 2006, Strasser 2004). This light-induced reduction of the PQ pool (evident by the decline in the P step) means that sea ice algae under high light have a reduced capacity for electron transport even when they are able to protect their photosystems from damage via rapid xanthophyll cycling. Changes in the P step of the curve have also been attributed to incomplete closure of PSII reaction centres due to changes in the distribution of excitation energy between the two photosystems (Franck et al. 2002). However, since there is no evidence of state transitions in this study or ever having been confirmed in diatoms at all (Owens 1986), changes in P as a result of state transition quenching in these sea ice algal communities are unlikely. However, the possibility of contributions from photosystem I (PSI) in the form of cyclic electron transport cannot be ruled out (Eisenstadt et al. 2008). The presence of a decline in the signal following the P step has been attributed to relaxation of the ΔpH (Antal et al. 2009). Here, the decline after P was most strongly evident in the highest light treatment, suggesting ΔpH-dependent NPQ activation was greatest at 200 µmol photons · m^{-2} · s^{-1}.

The under-ice incubations revealed that bottom sea ice algae were not photoinhibited by the *in situ* environmental conditions. Thus, the substantial decline in F_V/F_M observed in the DTT incubation suggests that the level of xanthophyll activity in combination with other potential photoprotective mechanisms was sufficient in providing protection, because in the absence of the xanthophyll inhibitor the decline in F_V/F_M was significantly less. By comparing the responses from the two *in situ* inhibitor incubations (DTT and NaF), we were able to demonstrate the preferential use of xanthophyll cycling over state transition quenching as a means of photosystem regulation and photoprotection. De-epoxidation ratios support the use of xanthophyll cycling as a preferred means of photoprotection by bottom sea ice diatoms. The similarity of the ratios after the 6 h incubation was likely due to relaxation of the xanthophyll pigment Dt into Dd in the control sea ice algae in response to non-inhibiting irradiances. While this study did not exclude the possibility that sea ice algae can use state transition quenching to prevent over-excitation of PSII, it showed that under the observed conditions, the algae only utilised energy-dependent quenching in the light climate they were exposed to. Despite the greater PAR during the NaF *in situ* experiment, there was no difference in the changes in F_V/F_M with or without NaF. In contrast, the light levels experienced during the +DTT incubation, while lower, still lead to a significant difference between the control and DTT inhibited algae. This confirms the role of Dt in photoprotection, where the presence of epoxide-free forms of Dd (i.e. Dt), mediates the excess energy dissipation from the pigment bed, providing protection from photoinhibitory damage (Demmig-Adams and Adams 1996). This leads to the conclusion that xanthophyll activity plays an essential role in regulating energy flow to PSII, providing critical protection against photodamage under increased light climates.

In this study, we tested the hypothesis that the capacity for short-term photosynthetic and pigment variations (activated as a photoprotective response to high light) in bottom sea ice algae are dependent on the ecological characteristics of the community (ie: low-light adaptation and low temperature photoinhibition). This study dispelled the idea that all bottom sea ice algae are obligate shade-adapted species; indeed even at the onset of spring (when cells were collected) they were able to acclimate rapidly and effectively without any lasting damage to their photosynthetic machinery. This plasticity is due to a number of physiological strategies including, enhanced photoacclimation capacity by possessing a large PSII antenna size, rapid and sustainable xanthophyll cycling for photoprotection, as well as efficient *de novo* synthesis of carotenoid pigments to increase the NPQ potential and help sustain photoprotection over longer periods of time. The ecological importance of such findings is that as spring approaches, light period and light intensity rapidly increase (Sakshaug and Slagstad 1991) and as the sea ice melts, cells are released into the water column experiencing even greater irradiances. The resilience and photoacclimative capabilities of the sea ice algae will mean minimal photoinhibition under increased light conditions and therefore minimal impact on productivity in the sea ice zone.

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Table 1: Percent cellular contribution of fucoxanthin to total pigment concentration and molar pigment ratios of Chl *c*, fucoxanthin (Fuco), diadinoxanthin + diatoxanthin (DdDt), β, β-carotene (β-car) and violaxanthin + antheraxanthin + zeaxanthin (VAZ) to Chl *a* for bottom ice algae from the East Antarctic pack ice from September to October 2007. Values represent the mean (n = 9 ± SD).

	Fuco %	*Chl c:Chl a*	*Fuco:Chl a*	*DdDt:Chl a*	β-*car:Chl a*	*VAZ:Chl a*
Mean	23.27 ± 0.652	0.287 ± 0.020	0.634 ± 0.020	0.113 ± 0.015	0.016 ± 0.001	0.011 ± 0.006

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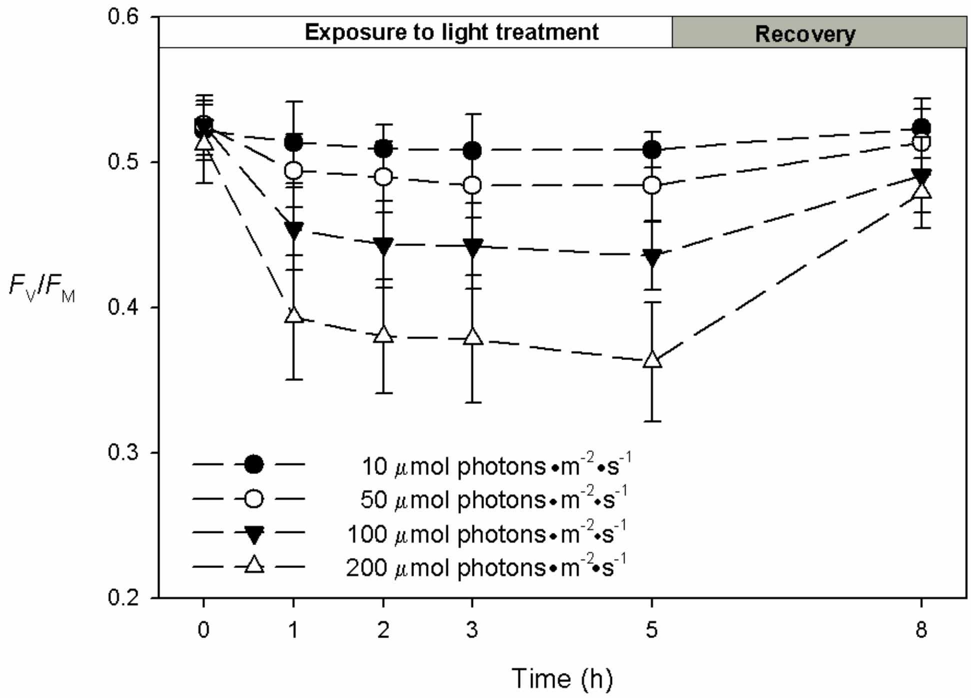

Figure 1: Maximum quantum yield of PSII (F_V/F_M) of bottom sea ice algal communities exposed to 10, 50, 100 and 200 µmol photons · m^{-2} · s^{-1}. Samples were exposed to light conditions for 5 h followed by 3 h recovery period (10 µmol photons · m^{-2} · s^{-1}). Data represent means (n = 5 ± SD).

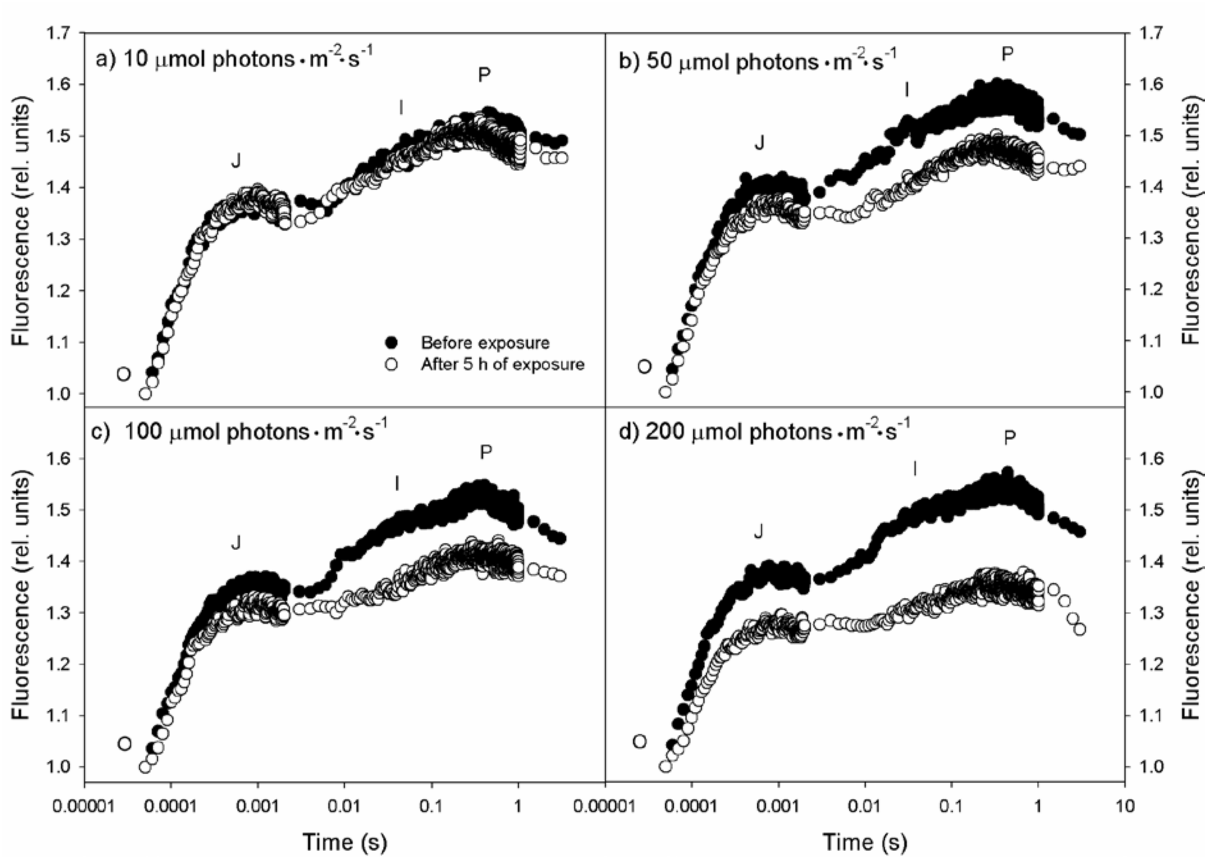

Figure 2: OJIP transients (normalised to the O step) of bottom sea ice algal communities exposed to 10, 50, 100 and 200 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} (a-d respectively), initially and after 5 h light exposure. Data represent means of each treatment (n = 5).

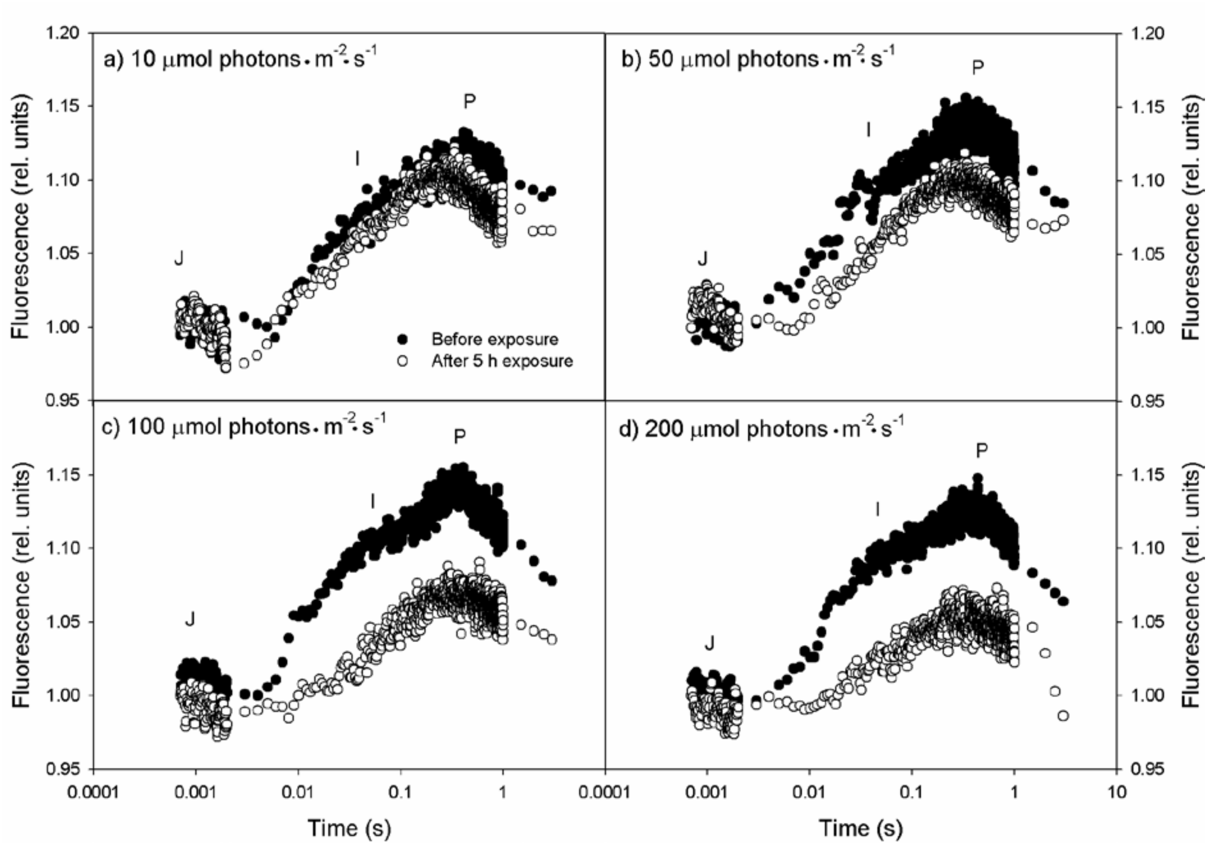

Figure 3: OJIP transients (normalised to the J step) of bottom sea ice algal communities exposed to 10, 50, 100 and 200 µmol photons · m^{-2} · s^{-1} (a-d respectively), at 0 h and after 5 h of light exposure. Data represent means (n = 5).

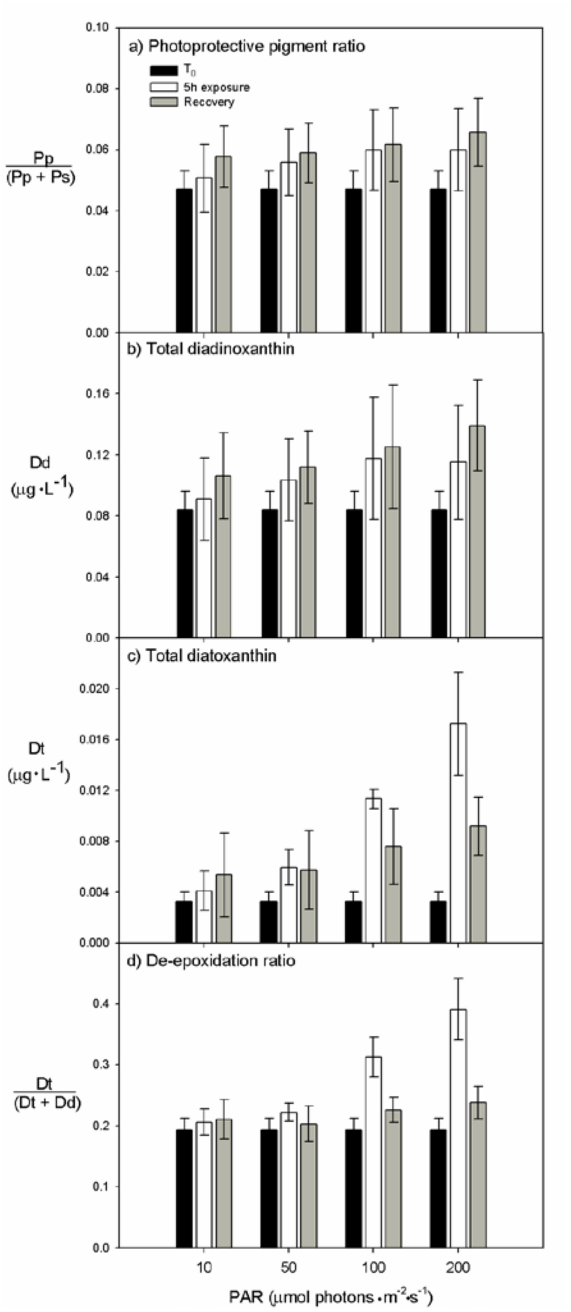

Figure 4: Photoprotective pigment ratios (a), total Dd and Dt pools normalised to chl *a* (b & c, respectively) and square root transformed de-epoxidation ratios (d) for bottom sea ice algal communities exposed to 10, 50, 100 and 200 μmol photons · m^{-2} · s^{-1}. Samples were exposed to light conditions for 5 h followed by 3 h of recovery light (10 μmol photons · m^{-2} · s^{-1}). Data represent means (*n* = 5 ± SD). (Pp, Photoprotective pigment pool; Ps, Photosynthetic pigment pool; Dd, diadinoxanthin pool; Dt, diatoxanthin pool).

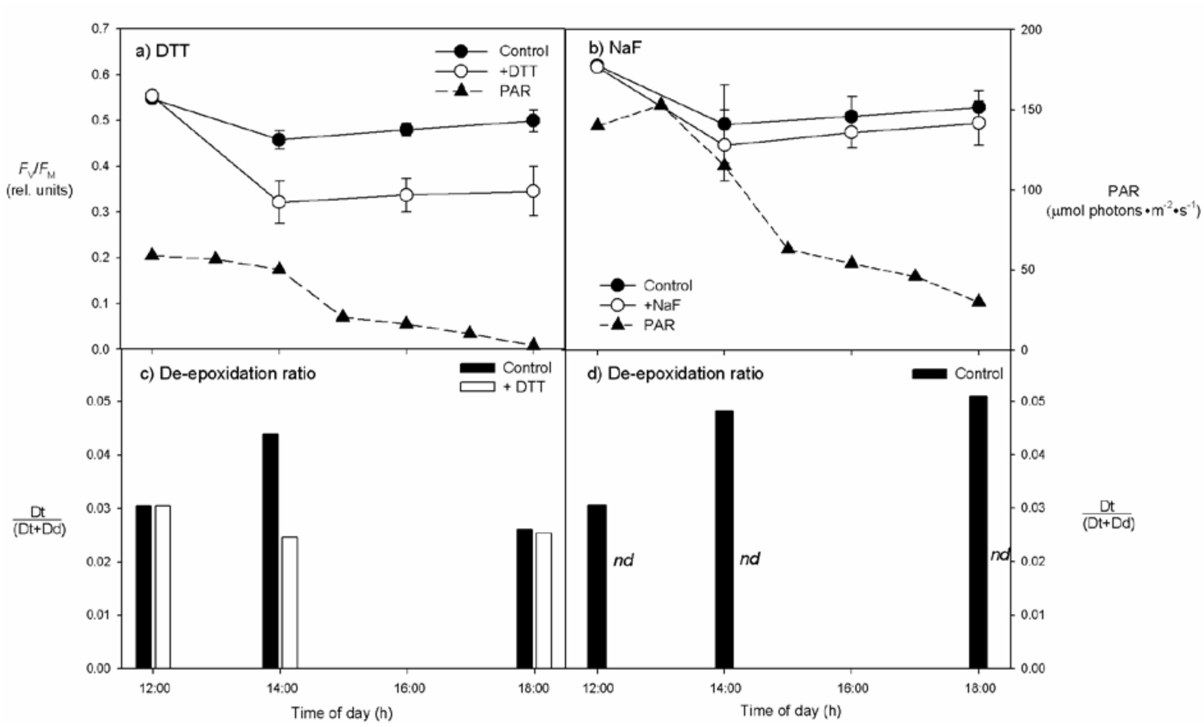

Figure 5: Maximum quantum yield of PSII (F_V/F_M), under-ice PAR, and the de-epoxidation ratios for bottom sea ice algal communities exposed to *in situ* under ice light climate over 6 h in the presence and absense of the xanthophyll inhibitor DTT (a & c) and the state transition inhibitor NaF (b & d). F_V/F_M data represent the mean (n = 4 ± SD). PAR and pigment data are single measurements; *nd*, no data.