Aspects of resilience of polar sea ice algae to changes in their environment

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Abstract

Sea ice algae are the primary producers of the ice-covered oceans in both polar regions. Changes in sea ice distribution are potentially altering exposure to photosynthetically active (PAR) and ultra-violet (UV-B) wavelengths of light. Incubations using monospecific cultures of common species from the Ross Sea, Antarctic Peninsula and Arctic Ocean were carried out at ecologically relevant light levels over periods of 7 d to examine their tolerance to conditions likely to be faced during sea ice thinning and melt. Algal responses were assessed using chlorophyll fluorescence techniques and superoxide dismutase activity. Quantum yields of cultures incubated in the dark and at ambient light did not differ. At higher light levels, the Ross Sea and Arctic cultures showed no significant change in photosynthetic health, while those from the Antarctic Peninsula showed a significant decrease. Antarctic cultures showed no detectable changes in superoxide dismutase activity, while the Arctic culture showed dynamic changes, initially increasing, then decreasing to the end of the study. The general lack of significant changes signals the need for further parameters to be assessed during such

experiments. The coupling between the measured parameters appeared to protect photosynthetic health, even though significant effects have been detected in other studies when subjected to PAR or UV-B alone.

Key words: Sea ice algae, *Thalassiosira antarctica*, *Chaetoceros socialis*, photoprotection, stress, ultra-violet B, Ross Sea, Antarctic Peninsula, Arctic Ocean

Introduction

Polar sea ice forms one of the largest ecosystems on Earth. In the Antarctic it covers an area of 19 x 10^6 km^2 at its maximum extent in winter, diminishing to 3 x 10^6 km^2 during the summer (Arrigo, 2014). In the Arctic it oscillates between 15 and 5 x 10^6 km^2. The long-term rate of change is currently greater in the Arctic, where summer ice extent has reduced by 45% in only the past three decades and, in areas of rapid sea ice decrease, the spring retreat now initiates two months earlier and autumn advance one month later as compared to 1979/1980, resulting in the ice-free summer season lengthening by three months (Maksym et al., 2012; Stammerjohn et al., 2012). In the Antarctic overall winter sea ice extent has slightly increased (Turner et al., 2013; http://www.ipcc.ch/report/ar5/wg1/#.UuhKY2SBoYI) but its distribution has changed, with increases in the Ross Sea region and decreases in the Weddell Sea (Arrigo, 2014; Turner et al., 2013). In the Antarctic Peninsula and Bellinghausen Sea region, changes in sea ice retreat and advance timing have again led to a three month longer ice-free period (Stammerjohn et al., 2012). In contrast, in the Ross Sea region, sea ice retreat is one month later and advance is one month earlier, leading to a shortening of the summer ice-free season by almost two months. Such changes in sea ice duration and distribution can cause severe ecological disruptions, with potentially negative consequences for the whole ecosystem (Arrigo, 2014; Nicol et al., 2008).

Sea ice algal communities reach their peak standing biomass in spring, leading to a significant increase in pigment concentration in the ice (Arrigo, 2014). This reduces the amount of light available to the upper water column, affecting algal productivity in the water below the ice (Arrigo et al., 1991; SooHoo et al., 1987). Phytoplankton blooms in the water column are delayed until after the ice algal bloom, whose timing therefore controls that of the subsequent phytoplankton bloom (Arrigo et al., 2012). When the sea ice melts, some of the algae released can provide seed stock for blooms at the ice edge (Cunningham & Leventer,

1998; Mangoni et al., 2009), as well as in the benthic and epiphytic habitats beneath (Majewska et al. 2013). However, this depends on their surviving the melting transition and acclimating very rapidly to the higher photosynthetically active radition (PAR) and ultra-violet-B (UV-B) conditions that they are then exposed to (Mundy et al., 2011), and not being lost through sedimentation (Riebesell et al., 1991).

Sea ice can form annually, or survive to become thicker multi-year ice (Arrigo & Thomas, 2004) through which light transmission is reduced. Snow cover, and its changing patterns, can further influence the under-ice environment (Arrigo, 2014). An emigration of diatoms from Arctic sea ice has been reported under thickness reducing conditions that could lead to reductions in productivity from the ice algal bloom, thereby impacting overall productivity (Lund-Hansen et al., 2013).

Microalgae are the primary producers found in the complex sea ice ecosystem, with diatoms (Bacillariophyceae) dominating (Arrigo, 2014) and reaching large stocks in the Antarctic due to the nutrient-rich waters of the Southern Ocean. The potential for nutrient limitation in the Antarctic is considered to be low, but local depletion can occur if there are other growth-limiting factors (Harrison & Cota, 1991) such as vertical stratification in the water column and depletion due to the sea ice algal bloom in spring. In both the Antarctic and Arctic factors other than nutrients, such as light and salinity, can also be limiting (Harrison & Cota, 1991). Algal cell concentrations in sea ice can vary by up to six orders of magnitude ($<10^4$ to $>10^9$ cells l^{-1}; Arrigo et al., 2010), a range that covers both typical global oceanic values and some of the highest recorded in any aquatic environment. Chlorophyll *a* (Chl *a*) biomass in sea ice varies by region, ice type and season. In the Arctic, volumetric Chl *a* concentrations range from 3 to 800 mg m^{-3}, and they can reach 10,100 mg m^{-3} in the Antarctic (Arrigo et al., 2010). The higher values in the Antarctic are again related to greater nutrient availability, and to lower annual light levels at the highest latitudes in the Arctic.

The community composition of the sea ice and associated ecosystems is determined by physical factors including light, salinity, nutrients and temperature (Arrigo & Thomas, 2004; Arrigo et al., 2010; Arrigo, 2014). Specific communities can include 30-170 diatom species. Commonly encountered genera in land-fast ice include *Nitzchia*, *Thalassiosira*, *Fragilariopsis* and *Navicula*. Microlagal biomass varies through the sea ice profile, with the highest levels found in the bottom 20 cm due to the stable light and temperature conditions present in this part of the sea ice (Arrigo & Thomas, 2004). There is also a constant supply of nutrients to this bottom layer through its interface with the underlying seawater (Arrigo, 2014). Microalgal blooms in the sea ice are short-lived and are limited by low light and low temperatures (Ratkova et al., 2004). The distribution of algal biomass can also be patchy and show large variability (Rysgaard et al., 2001) due to local conditions such as surface snow cover and strong sub-ice water currents (Lund-Hansen et al., 2013).

Various studies have examined the effects of particular environmental parameters on monospecific cultures (e.g. Davidson et al., 1994; Hannach & Sigleo, 1998; Leu et al., 2010; Martin et al., 2012; Ryan et al., 2012). However, very few have examined the effect of multiple stressors (Halac et al., 2010; Hancke et al., 2008; Petrou et al., 2011; Salleh & McMinn, 2011) or compared common algal species from different parts of the polar regions (Petrou & Ralph, 2011). Brief periods of exposure to high light levels caused significant changes to the photosynthetic activity and composition (affecting its quality as a dietary item) of a common polar microalga, *Thalassiosira antarctica* var. *borealis* (Leu et al., 2006). Exposure to higher PAR and UVR caused a decline in the quantum yield of photosystem II (PSII) and led to a significant reductions in C:P and N:P ratios. There was also a difference in the effects of PAR and UVR, with the algae affected strongly by increase in PAR but not UVR.

Ultra-violet radiation (UVR) reduces photo-protective capacity in diatoms such as *Phaedactylum tricornutum* (Halac et al., 2009), affecting the xanthophyll cycle and causing a decrease in photosynthetic health when exposed to saturating PAR. However, Halac et al. (2010) also demonstrated how increased temperature could counteract the negative effects of UVR, as well as variation in response due to length of exposure and size of cells, highlighting the importance of studying species-specific responses. High PAR and UVR stress can also lead to detrimental photoreactions (Janknegt et al., 2007), interrupting important metabolic pathways and causing an over-reduction of the electron transport chain (ETC). When this occurs, electrons from the ETC combine with molecular O_2 leading to the formation of reactive oxygen species (ROS). ROS cause damage to photosystem II reaction center proteins, thereby reducing the photosynthetic rate (Van De Poll et al., 2005). In response to this damage, cells produce antioxidant enzymes that actively scavenge ROS intermediates. The first ROS produced is a superoxide anion ($O_2^{\bullet -}$), which can be converted into the highly active hydroxyl radical ($HO^{\bullet}$) through a series of reductions. To avoid this, $O_2^{\bullet -}$ is converted into hydrogen peroxide by the enzyme superoxide dismutase (SOD) (Gregory & Fridovich, 1973). SOD is key to the cell's antioxidant response (Janknegt et al., 2007). Studies that describe SOD responses in marine polar microalgae are rare due to difficulties such as obtaining sufficient biomass for reliable measurements (Janknegt et al., 2009; Katayama & Taguchi, 2013; Van de Poll et al., 2009).

The primary aims of the current study were (1) to examine the photosynthetic (measured using chlorophyll fluorescence techniques) and antioxidative (measured using the RF/NBT assay) responses of sea ice algae in laboratory treatments combining ecologically relevant PAR and UV-B exposures, in order to study the capacity of common species to tolerate increased radiation levels such as might be experienced during ice melt and the thinning of sea ice, and (2) to compare acclimatory abilities across species. The study used monospecific

cultures of common algal species obtained from three regions with different patterns of environmental variation, namely the Ross Sea, the Antarctic Peninsula and the Arctic Ocean. Assessing the capacity of these algae for photo-protection will provide a better foundation for predictions relating to their response in context of climate change.

Methods

Culture methodology

Monospecific stock cultures were maintained in Victoria University of Wellington at a PAR level of 50 μmol m^{-2} s^{-1} and 4°C in f/2 medium (Ausaqua Pty Ltd., Australia) for 5-6 months prior to these experiments. The Ross Sea species, *Thalassiosira antarctica*, used in the experiments described below were isolated from sea ice at Cape Evans in the summer of 2010/2011. A stock culture of *Chaetoceros socialis* from the Antarctic Peninsula was obtained from Dr. Claire Hughes (University of East Anglia) as part of studies under the Rothera Time Series maintained by the British Antarctic Survey near its Rothera Research Station in Ryder Bay (Marguerite Bay, 67°34'S, 68° 9'W) during the austral summer of 2008/2009 (http://www.antarctica.ac.uk//staff-profiles/webspace/mmm/RaTS/RaTS.html). A stock culture of the same nominate species from the Arctic was obtained from Assoc. Prof. Else Hegseth (University of Tromso), originally collected from Billefjorden, Svalbard, in summer 2011.

Light treatments

The light treatments were based on measurements taken in the field in the Ross Sea through the sea ice profile (MAR pers. obs.; see also Rajanahally et al., 2014) and are representative of the PAR and UV- B levels that sea ice algae would be exposed to from the middle to the bottom of the sea ice profile (Eicken, 1992; Petrou & Ralph, 2011; Rajanahally et al., 2014;

Ryan et al., 2011). Sub-samples (400 ml) of monospecific cultures (n=3 at each level) were incubated under each of four light levels (L0=0 μmol m^{-2} s^{-1}, L1=1 μmol m^{-2} s^{-1} of PAR, L2=45 μmol m^{-2} s^{-1} of PAR and 50 mW m^{-2} of UV-B, L3=100 μmol m^{-2} s^{-1} of PAR and 50 mW m^{-2} of UV-B) at 4°C for 168 h in a temperature controlled water bath (Haake, Cleveland, OH, USA). These light levels are intended to be representative of those found in the water column below the sea ice (L0), at the bottom of the sea ice (L1), a quarter of the way above the water-ice interface (L2), or at the middle of the sea ice profile (L3). The separate PAR and UV-B levels were combined to reflect that the increase in PAR is synchronous with an increase in UV-B through the sea ice profile.

The 400 ml samples were incubated following standard approaches described by Ryan et al. (2012) in 500 ml opaque containers which were placed under 5W LED lights (Greenlights, Taiwan) and a Philips Ultraviolet-B TL 12/40W UV-B tube. Different sections of the water bath were separated from each other with opaque black plastic sheets and the various levels of light and UV-B were obtained with appropriate shade cloth. The UV-B tube was also covered with a polyvinyl chloride (PVC) sheet to filter out harmful UV-C radiation. PAR levels were measured using a SpectroSense 2 meter with an SKR 1850 radiometer (bandwith 400 – 700 nm) and UV-B with an SKU 430 broad band UV-B radiometer (bandwidth 280 - 315 nm) (Skye Instruments, UK).

Samples of 1 ml for cell counts were taken at 0, 48 and 168 h from all replicates in each incubation, preserved using 2% Lugol's Iodine and stored at 4°C. Fifty ml of sample was used for the measurement of MAA content following the protocol described by Ryan et al. (2002) and Rajanahally et al. (2014). However, as no detectable amounts of MAAs were produced, no further consideration of MAAs is included here.

Chlorophyll *a* content

178 Twenty-five ml of sample were used for the measurement of chlorophyll *a* content. This was
179 filtered onto GF/F filters which were then placed in containers with 5 ml 100% methanol and
180 extracted in the dark at 4°C for 24 h. The extract was then measured on a digital fluorometer
181 (Turner 10AU, Australia) following the acidification method of Evans et al. (1987).

182 Superoxide dismutase activity

183 Twenty to 80 ml of the sample was stored in a container at -20˚C for measurement of
184 superoxide dismutase (SOD) activity following Janknegt et al. (2007). The variation in
185 volumes collected for determination of SOD activity was due to varying growth rates
186 between species. As SOD activity had not been previously determined for these cultures, it
187 was not possible to identify the minimum volume required *a priori*. Therefore, a minimum of
188 20 ml and, in some cases, a larger volume was collected as a contingency to ensure the level
189 of SOD activity was assayable. These samples were centrifuged (2000 x g, 5 min, 4°C) in 1
190 ml Eppendorf tubes and the pellets were stored at -80°C to preserve enzyme activity.

191 *Cell lysis*

192 For enzyme measurements, cell pellets were thawed on ice, centrifuged (2000 x g, 5 min,
193 4°C) and resuspended in 1 ml cold lysis buffer mixture of 300 µl potassium phosphate buffer
194 (50 mM, pH 7.8) containing DTPA (0.2 mM), riboflavin (1.3 µM), L-methionine (10 mM),
195 NBT (57 µM), and Triton X-100 (0.025% (v/v)). The suspension was then centrifuged (2000
196 x g, 5 min, 4°C) again before the cells were resuspended in a final volume of 500 µl of lysis
197 buffer and sonicated (Ultrasonic liquid processor W-380, Heat Systems-Ultrasonics, Inc.,
198 New York) on ice for three 15 s pulses with a time interval of 10 s between each pulse. All
199 lysates were centrifuged (16000 x g, 5 min, 4°C) and supernatants aliquoted and frozen at -
200 80°C until further analysis. Total aqueous soluble protein content was determined using the
201 improved Bradford assay with BSA as standard (Zor & Selinger, 1996).

Superoxide dismutase (SOD)

SOD assays were performed using the riboflavin/nitroblue tetrazolium (RF/NBT) assay in a microtiter plate format (Beauchamp & Fridovich, 1971; Fryer et al., 1998). Samples of 20 µl of lysate or SOD standard (0.5-500 U ml^{-1}) were added into a final reaction mixture of 300 µl potassium phosphate buffer (50 mM, pH 7.8) containing EDTA (0.1 mM), riboflavin (1.3 µM), L-methionine (10 mM), NBT (57 µM), and Triton X-100 (0.025% (v/v)). Absorbance was read at 560 nm both immediately and after 10 min incubation under a homogenous light field (130 µmol m^{-2} s^{-1}) at 25°C. Standards and samples were measured using the same reaction mixture and a sigmoidal 5-parameter semi logarithmic standard curve (24 standard levels) used to determine SOD activity of samples. One unit of SOD activity was defined as the amount of enzyme that inhibited the NBT reduction by 50% (Beyer & Fridovich, 1987).

Sub-sampling, PAM fluorometry, and statistical analyses

PAM fluorometry is a widely used tool for studying the photosynthetic health of microalgae (Hancke et al., 2008; Katayama & Taguchi, 2013; McMinn & Hegseth, 2004; Parkhill et al., 2001; Ryan et al., 2009) as it provides a non-invasive method to study smaller cultures. A Water PAM fluorometer (Walz, Effeltrich, Germany) was used to measure effective quantum yield for photosystem II (ϕ_{PSII}). Before commencing observations, the cells were dark acclimated. Thereafter, all PAM measurements were made on cells exposed to light as the cells were subjected to actinic light used to make the PAM measurements. The Water PAM was also used to generate rapid light curves (RLC). An RLC describes the effective quantum yield as a function of irradiance (Ralph & Gademann, 2005). Each sample was treated with a series of eight increasing actinic light treatments (0, 8, 20, 35, 55, 75, 96, 114, 150 µmol m^{-2} s^{-1}), after which a strong saturating pulse was applied and ϕ_{PSII} was recorded. The RLC took 90 s to generate. The electron transport rate (ETR) values were calculated by multiplying the ϕ_{PSII} value by the irradiance just applied. As the ϕ_{PSII} value is a ratio and ETR is derived from

this parameter, it is termed relative ETR (rETR). An RLC permits derivation of different parameters that can be used to describe the photosynthetic properties of an algal sample, including rETRmax (the maximum value for rETR), photosynthetic efficiency (α) and saturation irradiance (E_k) (Ryan et al., 2009). To determine these parameters, the rETR data were imported into Microsoft Excel v 10.0 (Microsoft, USA) and the curve was fitted with a "waiting-in-line" function as described by Ritchie (2008). An RLC was generated for each sample at times 0, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h.

Statistical analyses

First, a repeated measures ANOVA was applied to test specifically for changes over time within each treatment. The assumptions of normality and equal variance were satisfied. As the data from successive time points are likely not to be independent the covariance matrix - the matrix of all covariances between time points - was examined. If that matrix was spherical (as confirmed by Mauchly's test of sphericity), then a standard repeated measures ANOVA calculation was carried out. If, however, the sphericity assumption did not hold, then the Greenhouse Geisser adjustment was applied (Greenhouse & Geisser, 1959). Repeated measures ANOVA was used to analyse effective quantum yield and $rETR_{max}$, with 'treatment' being the irradiance treatments and the different time points. The significance of differences between pairs of time points was adjusted using Bonferroni's correction. Finally, one-way ANOVA was used to compare the different treatment groups at specific time points. *Post hoc* tests using Bonferroni's correction were used for subsequent pair-wise comparisons, e.g. between different pairs of treatment groups at a time point, or between different time points within a treatment group. All differences referred to are statistically significant at $p < 0.05$.

Results

Effect of different light levels on the photosynthetic performance of *Thalassiosira antarctica* (Ross Sea), *Chaetoceros socialis* (Antarctic Peninsula) and *C. socialis* (Arctic)

Initially a two way repeated measures ANOVA was conducted for all parameters with time as the 'within' factor and treatment and species as 'between' factors (Table 1). This demonstrated that both time alone and all the interaction combinations (excepting the three-way time-treatment-species interaction on E_k) had a highly significant influence on each parameter. Since the significant 3-way interactions make it impossible to interpret the treatment effects, repeated measures analyses were carried out for each species separately to test for the effect of all treatments and time.

Quantum Yield (ϕ_{PSII})

A repeated measures ANOVA was carried out for each species separately to test for effect of all treatments and time (Table 2). Although the general response, except for *Chaetoceros socialis* (Antarctic Peninsula) at L2, appeared to show consistent patterns over time, the repeated measures ANOVA for algae incubated at all light treatments was not significant for ϕ_{PSII} over 168 h.

All the algal cultures showed no significant difference in quantum yield between those incubated in the dark (L0) or at ambient low light (L1) levels. For both the Antarctic species, quantum yield was lowest for algae incubated at L2 and highest for cultures incubated in the dark or at ambient light. For both species, quantum yield decreased significantly over the first two hours. However, quantum yield for *T. antarctica* at L2 did not show a significant change over 168 h while *C. socialis* showed an overall decrease. In the Arctic *C. socialis*, there was no overall change in quantum yield over 168 h. However, at the end of the incubation, the quantum yield for the algae at ambient low light level was the highest and that of those in the

dark and at the highest light exposure were the lowest. The general response for *Thalassiosira antarctica* (Ross Sea) (Figure 1(a)) at L2 and L3 was an initial decrease followed by a recovery in ϕ_{PSII}, then maintenance of a stable value over the rest of the incubation. Algae at L0 and L1 showed very little change over 168 h. The general response for *C. socialis* (Antarctic Peninsula) (Figure 1(b)) at L0 and L1 was a slight increase followed by ϕ_{PSII} remaining stable over 168 h. For algae incubated at L2 and L3, there was a sharp decrease over the first 2 h, followed by a recovery in ϕ_{PSII} between 2 and 4 h. However, algae at the higher light treatments of L2 and L3 then showed a gradual decrease in ϕ_{PSII} to reach levels similar to those at 2 h. Although the repeated measures ANOVA showed no significant change in ϕ_{PSII} over 168 h for this species at L0, L1 and L3, those at L2 showed a significant decrease over the full 168 h period. *C. socialis* (Arctic Ocean) (Figure 1(c)) showed a gradual decrease in ϕ_{PSII} for algae incubated at L0 and a gradual increase for algae incubated at L1. Algae incubated at L2 showed an initial decrease in ϕ_{PSII} followed by a recovery to reach the same levels as at the start of the incubation. Algae incubated at L3 also showed an initial decrease, but this was followed by a recovery to reach a ϕ_{PSII} lower than that at time 0 h.

$rETR_{max}$

A repeated measures ANOVA was carried out for each species separately to test for effect of all treatments and time (Table 3). Although the general response showed changes over time, the repeated measures ANOVA showed no significant change in $rETR_{max}$ over 168 h for algae at all treatments, except for both *Chaetoceros* cultures at L2.

The general response of *Thalassiosira antarctica* (Ross Sea) (Figure 2(a)) at L2 and L3 was an initial increase followed by $rETR_{max}$ varying little throughout the incubation and not showing any change from time 0 h. Algae at L0 showed a slight decrease in $rETR_{max}$ before it stabilized for the rest of the incubation. Algae incubated at L1 showed an increase in $rETR_{max}$ over the 168 h. The general response of *Chaetoceros socialis* (Antarctic Peninsula) (Figure

2(b)) for all light treatments other than at L0 was a slight increase in $rETR_{max}$ followed by a decrease to reach a level higher than at time 0 h. Algae at L0 did not show any change over time. Those incubated at L2 showed a significant increase in $rETR_{max}$ between 144 and 168 h. However, there was no overall significant increase. The general response of *C. socialis* (Arctic Ocean) (Figure 2(c)) for all light treatments other than at L0 was a slight increase in $rETR_{max}$ followed by a decrease to reach a level similar to that at time 0 h. Algae at L0 did not show any change over time. Algae incubated at L2 showed an overall significant increase in $rETR_{max}$.

Chlorophyll *a*

Repeated measures ANOVA indicated a significant effect of time ($p<0.005$) and the interaction between time and species ($p<0.005$), but no significant interaction between time and light. However, Levene's test for equality of variances was significant at each time point, indicating that the data were highly variable. The chlorophyll data were, thus, too variable to permit conclusions to be drawn about the responses of the algae.

Superoxide dismutase

Two way repeated measures ANOVA conducted with time as the 'within' factor and treatment and species as 'between' factors identified significant 3-way interactions. Repeated measures analyses were, therefore, carried out for each species separately to test for the effect of all treatments and time (Table 4).

Although the general responses appeared to show small and consistent patterns of change over time, the repeated measures ANOVA showed no significant change in SOD activity over 168 h for algae at all light treatments, except for *Chaetoceros socialis* (Antarctic Peninsula) which had a significant increase in the first 48 h for all light treatments followed by a significant decrease by 168 h, resulting in no overall change over 168 h.

325 The general response of *Thalassiosira antarctica* (Ross Sea) (Figure 3(a)) in the dark and at
326 low light levels was an increase in SOD activity. At the two higher light levels, SOD activity
327 increased over the first 48 h, followed by a decrease by the end of the incubation at 168h. The
328 general response of *Chaetoceros socialis* (Antarctic Peninsula) (Figure 3(b)) at all light levels
329 was an increase in SOD activity over the first 48 h, followed by a decrease to reach a similar
330 level at 168 h to that at the start of the incubation. That of *C. socialis* (Arctic Ocean) (Figure
331 3(c)) for all light treatments was a large decrease in SOD activity over the first 48 h, followed
332 by a slight recovery by 168 h.

333

334 **Discussion**

335 Each of the species studied here is a common member of ice algal communities from each of
336 three regions with different patterns of environmental variation, namely the Ross Sea, the
337 Antarctic Peninsula and the Arctic Ocean. In the western Antarctic Peninsula (WAP) region,
338 diatoms respond positively to increase in irradiance, with the effect being modulated by cell
339 volume (Vernet et al., 2008). Although there has been a decrease in summer sea ice in this
340 region leading to a decrease in phytoplankton blooms containing large, chain-forming
341 diatoms (Garibotti et al., 2005), flagellate blooms are still able to maintain daily productivity
342 (Vernet et al., 2008). These species-specific tolerances emphasise the variability that could be
343 observed in environmentally similar regions with varying community composition.

344 In terms of the response of quantum yield to exposure to different light levels, differences
345 between the same species from different regions could indicate specialization according to
346 the niche they occupy. Species-specific sensitivities to environmental change have been
347 observed elsewhere, suggesting that there is a link between photosynthetic capacity and
348 ecological niche occupancy (Petrou & Ralph, 2011).

The combinations of PAR and UV-B exposures used, which mimic those that might reasonably be expected in the natural environment, mean that it is difficult to differentiate why a lack of change in quantum yield was observed at the higher light exposures. These exposures combine two parameters that are complementary to each other in the natural ecosystem, giving a better indication of how algae may tolerate increased radiation levels during summer and/or due to thinner sea ice conditions. The failure to find detectable levels of MAA production may indicate that MAAs are not amongst the mechanisms used by these algal species for photo-protection, unlike Rajanahally et al.'s (2014) clear demonstration of MAA production in experimental exposures of mixed multi-species algal communities soon after collection in the field in the same region of the Ross Sea used to source one of the cultures used here.

When the three species were compared at each light level, there was no significant change in quantum yield for algae incubated in the dark or at ambient low light, while those incubated at higher light exposures showed an overall significant decrease. At both higher light levels, the Arctic culture of *C. socialis* had the highest quantum yield at 168 h, perhaps indicating a better ability to tolerate higher light exposure. During ice melt, algae are obviously exposed to both varying and/or increasing levels of light and other parameters which are more complex than those used in the current study. Van de Poll et al. (2009), studying the Antarctic marine diatom *Chaetoceros brevis*, identified no difference in growth rates of algae exposed to constant or dynamic irradiance regimes in iron-limited or replete conditions. Iron limitation and wind-driven vertical mixing that resulted in a dynamic irradiance regime were frequently co-occurring conditions that affected photosynthetic health of algae. However, their study identified differences in pigment composition, quantum yield and antioxidant capacity between algae exposed to the two irradiance regimes. Cellular pigment concentrations increased three-fold under dynamic as compared to constant irradiance, under

374 iron-replete conditions. In iron-limited conditions smaller differences were detected in
375 cellular pigment concentrations between the two regimes, suggesting reduced acclimation
376 potential.

377 In the current study, the Arctic cultures had the highest SOD activity and the two Antarctic
378 cultures showed equal activity at the start of all incubations. At all light exposures, the SOD
379 activity did not show a difference between treatments at 168 h. This lack of variation in SOD
380 production at the different light treatments was unexpected (cf. Janknegt et al., 2009; Van de
381 Poll et al., 2009), with algae incubated at the higher light levels predicted to express greater
382 activity. It is possible that even the higher irradiance exposures used in this study were not
383 high enough to cause stress in these algal cultures. Other studies that have observed oxidative
384 stress have often used irradiances exceeding 1000 μmol m^{-2} s^{-1} (Van de Poll & Buma, 2009).

385 Janknegt et al. (2009), conversely, found that SOD activity in some microalgae decreased
386 with exposure to high irradiance. An increase in the de-epoxidation of the xanthophyll
387 pigments led to more efficient recovery of PS II and reduced the production of hydrogen
388 peroxide, in turn reducing the need for production of SOD. The variation in SOD activity was
389 correlated with the cell size of the species examined, with those that had a higher surface area
390 to volume ratio showing an increase in SOD activity whereas those with lower ratio showed a
391 decrease. In the species examined in the current study, *C. socialis* cells are known to be
392 smaller than those of *T. antarctica*. SOD activity in *C. socialis* from the Arctic (but not the
393 Antarctic Peninsula) was higher than that of *T. antarctica.* The difference in SOD activity
394 between cultures of *C. socialis* obtained from the Antarctic Peninsula and the Arctic could be
395 related to the degree of change in the two regions, although to confirm this would require
396 detailed measuring and experimental studies of cultures obtained from multiple locations.

397 This study took place over a one week manipulation period in order to examine any effect of
398 length of incubation on acclimatory abilities. For all three species, there were very few

399 significant changes in photosynthetic parameters between 2 and 7 d, which strongly supports
400 the utility of shorter incubation periods in experimental studies of this type. In the natural
401 environment, the time taken for sea ice to melt can vary considerably and is influenced by
402 various factors such as snow cover and initial thickness (Thomas & Dieckmann, 2003).

403 In this study, the combination of exposure to PAR and UV-B led to a lack of differentiation
404 in algal photosynthetic health, contrasting with the significant effects on photosynthetic
405 parameters and MAA production that was previously observed in monospecific Ross Sea
406 cultures when subjected to increases in PAR or UV-B separately (Rajanahally, 2014). During
407 and after sea ice melt, algae face increases in PAR, UV-B and temperature and decreases in
408 salinity, and the consequences of these effects cannot be estimated simply in an additive
409 fashion (Folt et al., 1999). These responses will be further complicated by responses to
410 changing CO_2 concentrations and ocean acidification processes (e.g. Gao et al., 2012, Rost et
411 al., 2006). It is therefore essential for multivariate studies to be conducted that combine as
412 many of these factors as possible in order to give insight into how algae tolerate the entirety
413 of the processes of ice melt and release into the pelagic environment. Studies including
414 evaluation of the photo-protective responses of these algae, such as that described here, will
415 help assess their ability to survive current and predicted magnitudes of climate change and,
416 hence, assess risks to the productivity of these ice-covered regions of the global oceans.

417

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Table 1. Summary of results of statistical analyses of changes in quantum yield of photosystem II, $rETR_{max}$, Alpha(α) and E_k for *Thalassiosira antarctica* (Ross Sea), *Chaetoceros socialis* (Antarctic Peninsula) and C. *socialis* (Arctic) over 168 h experimental treatments at different combinations of PAR and UV-B levels (L0=0 μmol m^{-2} s^{-1}; L1=1 μmol m^{-2} s^{-1}; L2=45 μmol m^{-2} s^{-1} and 50 mWm^{-2}; L3=100 μmol m^{-2} s^{-1} and 100 mWm^{-2}) at 4°C, analysed using repeated measures ANOVA. Detailed analyses are presented in Tables 2 and 3.

Source of variation	Time	Time x Treatments	Time x Species	Time x Treatments x Species
ϕ_{PSII}	<0.001	<0.001	<0.001	<0.001
$rETR_{max}$	<0.001	<0.001	<0.001	<0.001
Alpha(α)	<0.001	<0.001	<0.001	<0.001
E_k	0.002	<0.001	0.001	0.064

Table 2. Changes in quantum yield of photosystem II over 168 h experimental treatments at different combinations of PAR and UV-B levels (L0=0 μmol m^{-2} s^{-1}; L1=1 μmol m^{-2} s^{-1}; L2=45 μmol m^{-2} s^{-1} and 50 mWm^{-2}; L3=100 μmol m^{-2} s^{-1} and 100 mWm^{-2}) at 4°C, analysed using repeated measures ANOVA. Significant changes identified by *post hoc* pairwise analyses are also listed.

Source of variation	df	MS	*F*	p	*post hoc* comparisons
***Thalassiosira antarctica* (Ross Sea)**					
Within subjects					
Time	2.340	0.072	54.188	<0.001	No change over 168 h
Time x Treatments	7.021	0.027	20.116	<0.001	(see text for details)
Error	18.723	0.001			
Between subjects					
Treatments	3	0.479	91.685	<0.001	(L0=L1) > L3 > L2
Error	8	0.005			
***Chaetoceros socialis* (Antarctic Peninsula)**					
Within subjects					
Time	4.325	0.034	41.242	<0.001	Decrease over 168 h
Time x Treatments	12.974	0.029	34.796	<0.001	(see text for details)
Error	34.597	0.001			
Between subjects					
Treatments	3	0.687	143.726	<0.001	(L0=L1) >L3 >L2
Error	8	0.003			
***C. socialis* (Arctic Ocean)**					
Within subjects					
Time	3.249	0.074	67.017	<0.001	No change over 168 h
Time x Treatments	9.746	0.029	26.218	<0.001	(see text for details)
Error	25.991	0.001			
Between subjects					
Treatments	3	0.329	78.456	<0.001	L1 > (L0=L3) > L2
Error	8	0.004			

Table 3. Changes in $rETR_{max}$ of photosystem II over 168 h experimental treatments at different combinations of PAR and UV-B levels (L0=0 μmol m^{-2} s^{-1}; L1=1 μmol m^{-2} s^{-1}; L2=45 μmol m^{-2} s^{-1} and 50 mWm^{-2}; L3=100 μmol m^{-2} s^{-1} and 100 mWm^{-2}) at 4°C, analysed using repeated measures ANOVA. Significant changes identified by *post hoc* pairwise analyses are also listed.

Source of variation	df	MS	*F*	p	*post hoc* comparisons
***Thalassiosira antarctica* (Ross Sea)**					
Within subjects					
Time	1.755	707.828	43.814	<0.001	No change over 168 h
Time x Treatments	5.266	229.137	14.184	<0.001	(see text for details)
Error	14.043	16.155			
Between subjects					
Treatments	3	703.075	149.478	<0.001	L1 > L2 >L3 >L0
Error	8	4.704			
***Chaetoceros socialis* (Antarctic Peninsula)**					
Within subjects					
Time	2.824	157.000	38.798	<0.001	No change over 168 h
Time x Treatments	8.471	82.856	20.475	<0.001	(see text for details)
Error	22.590	4.047			
Between subjects					
Treatments	3	489.269	119.725	<0.001	L1 > L2 > L3 > L0
Error	8	4.087			
***C. socialis* (Arctic Ocean)**					
Within subjects					
Time	2.929	620.532	283.378	<0.001	Increase over 168 h
Time x Treatments	8.786	78.510	35.853	<0.001	(see text for details)
Error	23.429	2.190			
Between subjects					
Treatments	3	670.216	82.660	<0.001	L2 > (L1 = L3) > L0
Error	8	8.108			

Table 4. Changes in SOD activity over 168 h experimental treatments at different combinations of PAR and UV-B levels (L0=0 μmol m^{-2} s^{-1}; L1=1 μmol m^{-2} s^{-1}; L2=45 μmol m^{-2} s^{-1} and 50 mWm^{-2}; L3=100 μmol m^{-2} s^{-1} and 100 mWm^{-2}) at 4°C, analysed using repeated measures ANOVA. Significant changes identified by *post hoc* pairwise analyses are also listed.

Source of variation	df	MS	*F*	p	*post hoc* comparisons
Thalassiosira antarctica **(Ross Sea)**					
Within subjects					
Time	2	55299.731	3.817	0.044	No change over 168 h
Time x Treatments	6	23650.055	1.632	0.202	(see text for details)
Error	16	14488.0404			
Between subjects					
Treatments	3	35853.695	2.271	0.157	L0 = L1 = L2 = L3
Error	8	15790.600			
Chaetoceros socialis **(Antarctic Peninsula)**					
Within subjects					
Time	2	326604.643	23.878	<0.001	No change over 168 h
Time x Treatments	6	18888.579	1.381	0.281	(see text for details)
Error	16	13677.826			
Between subjects					
Treatments	3	11406.406	0.588	0.640	L0 = L1 = L2 = L3
Error	8	19382.839			
C. socialis **(Arctic Ocean)**					
Within subjects					
Time	1.255	1307937.01	47.731	<0.001	Decrease over 168 h
Time x Treatments	3.766	16582.805	0.605	0.659	(see text for details)
Error	10.042	27402.151			
Between subjects					
Treatments	3	12677.400	0.910	0.478	L0 = L1 = L2 = L3
Error	8	13924.998			