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

2 Meghana A. Rajanahally[1], Phil J. Lester[1] & Peter Convey[1,2*]

3 [1] School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington
4 6140, New Zealand

5 [2] British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 0ET,
6 United Kingdom

7 [*] corresponding author: meghana.rajanahally@gmail.com (0061 434 965 885)

8

9 **Abstract**

10 Sea ice algae are the primary producers of the ice-covered oceans in both polar regions.
11 Changes in sea ice distribution are potentially altering exposure to photosynthetically active
12 (PAR) and ultra-violet (UV-B) wavelengths of light. Incubations using monospecific cultures
13 of common species from the Ross Sea, Antarctic Peninsula and Arctic Ocean were carried
14 out at ecologically relevant light levels over periods of 7 d to examine their tolerance to
15 conditions likely to be faced during sea ice thinning and melt. Algal responses were assessed
16 using chlorophyll fluorescence techniques and superoxide dismutase activity. Quantum yields
17 of cultures incubated in the dark and at ambient light did not differ. At higher light levels, the
18 Ross Sea and Arctic cultures showed no significant change in photosynthetic health, while
19 those from the Antarctic Peninsula showed a significant decrease. Antarctic cultures showed
20 no detectable changes in superoxide dismutase activity, while the Arctic culture showed
21 dynamic changes, initially increasing, then decreasing to the end of the study. The general
22 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

31 **Introduction**

32 Polar sea ice forms one of the largest ecosystems on Earth. In the Antarctic it covers an area
33 of 19 x 10^6 km^2 at its maximum extent in winter, diminishing to 3 x 10^6 km^2 during the
34 summer (Arrigo, 2014). In the Arctic it oscillates between 15 and 5 x 10^6 km^2. The long-term
35 rate of change is currently greater in the Arctic, where summer ice extent has reduced by 45%
36 in only the past three decades and, in areas of rapid sea ice decrease, the spring retreat now
37 initiates two months earlier and autumn advance one month later as compared to 1979/1980,
38 resulting in the ice-free summer season lengthening by three months (Maksym et al., 2012;
39 Stammerjohn et al., 2012). In the Antarctic overall winter sea ice extent has slightly increased
40 (Turner et al., 2013; http://www.ipcc.ch/report/ar5/wg1/#.UuhKY2SBoYI) but its distribution
41 has changed, with increases in the Ross Sea region and decreases in the Weddell Sea (Arrigo,
42 2014; Turner et al., 2013). In the Antarctic Peninsula and Bellinghausen Sea region, changes
43 in sea ice retreat and advance timing have again led to a three month longer ice-free period
44 (Stammerjohn et al., 2012). In contrast, in the Ross Sea region, sea ice retreat is one month
45 later and advance is one month earlier, leading to a shortening of the summer ice-free season
46 by almost two months. Such changes in sea ice duration and distribution can cause severe
47 ecological disruptions, with potentially negative consequences for the whole ecosystem
48 (Arrigo, 2014; Nicol et al., 2008).

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

56 1998; Mangoni et al., 2009), as well as in the benthic and epiphytic habitats beneath
57 (Majewska et al. 2013). However, this depends on their surviving the melting transition and
58 acclimating very rapidly to the higher photosynthetically active radition (PAR) and ultra-
59 violet-B (UV-B) conditions that they are then exposed to (Mundy et al., 2011), and not being
60 lost through sedimentation (Riebesell et al., 1991).

61 Sea ice can form annually, or survive to become thicker multi-year ice (Arrigo & Thomas,
62 2004) through which light transmission is reduced. Snow cover, and its changing patterns,
63 can further influence the under-ice environment (Arrigo, 2014). An emigration of diatoms
64 from Arctic sea ice has been reported under thickness reducing conditions that could lead to
65 reductions in productivity from the ice algal bloom, thereby impacting overall productivity
66 (Lund-Hansen et al., 2013).

67 Microalgae are the primary producers found in the complex sea ice ecosystem, with diatoms
68 (Bacillariophyceae) dominating (Arrigo, 2014) and reaching large stocks in the Antarctic due
69 to the nutrient-rich waters of the Southern Ocean. The potential for nutrient limitation in the
70 Antarctic is considered to be low, but local depletion can occur if there are other growth-
71 limiting factors (Harrison & Cota, 1991) such as vertical stratification in the water column
72 and depletion due to the sea ice algal bloom in spring. In both the Antarctic and Arctic factors
73 other than nutrients, such as light and salinity, can also be limiting (Harrison & Cota, 1991).
74 Algal cell concentrations in sea ice can vary by up to six orders of magnitude ($<10^4$ to $>10^9$
75 cells l^{-1}; Arrigo et al., 2010), a range that covers both typical global oceanic values and some
76 of the highest recorded in any aquatic environment. Chlorophyll *a* (Chl *a*) biomass in sea ice
77 varies by region, ice type and season. In the Arctic, volumetric Chl *a* concentrations range
78 from 3 to 800 mg m^{-3}, and they can reach 10,100 mg m^{-3} in the Antarctic (Arrigo et al.,
79 2010). The higher values in the Antarctic are again related to greater nutrient availability, and
80 to lower annual light levels at the highest latitudes in the Arctic.

81 The community composition of the sea ice and associated ecosystems is determined by
82 physical factors including light, salinity, nutrients and temperature (Arrigo & Thomas, 2004;
83 Arrigo et al., 2010; Arrigo, 2014). Specific communities can include 30-170 diatom species.
84 Commonly encountered genera in land-fast ice include *Nitzchia*, *Thalassiosira*,
85 *Fragilariopsis* and *Navicula*. Microlagal biomass varies through the sea ice profile, with the
86 highest levels found in the bottom 20 cm due to the stable light and temperature conditions
87 present in this part of the sea ice (Arrigo & Thomas, 2004). There is also a constant supply of
88 nutrients to this bottom layer through its interface with the underlying seawater (Arrigo,
89 2014). Microalgal blooms in the sea ice are short-lived and are limited by low light and low
90 temperatures (Ratkova et al., 2004). The distribution of algal biomass can also be patchy and
91 show large variability (Rysgaard et al., 2001) due to local conditions such as surface snow
92 cover and strong sub-ice water currents (Lund-Hansen et al., 2013).

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

105 Ultra-violet radiation (UVR) reduces photo-protective capacity in diatoms such as
106 *Phaedactylum tricornutum* (Halac et al., 2009), affecting the xanthophyll cycle and causing a
107 decrease in photosynthetic health when exposed to saturating PAR. However, Halac et al.
108 (2010) also demonstrated how increased temperature could counteract the negative effects of
109 UVR, as well as variation in response due to length of exposure and size of cells, highlighting
110 the importance of studying species-specific responses. High PAR and UVR stress can also
111 lead to detrimental photoreactions (Janknegt et al., 2007), interrupting important metabolic
112 pathways and causing an over-reduction of the electron transport chain (ETC). When this
113 occurs, electrons from the ETC combine with molecular O_2 leading to the formation of
114 reactive oxygen species (ROS). ROS cause damage to photosystem II reaction center
115 proteins, thereby reducing the photosynthetic rate (Van De Poll et al., 2005). In response to
116 this damage, cells produce antioxidant enzymes that actively scavenge ROS intermediates.
117 The first ROS produced is a superoxide anion ($O_2^{\bullet -}$), which can be converted into the highly
118 active hydroxyl radical ($HO^{\bullet}$) through a series of reductions. To avoid this, $O_2^{\bullet -}$is converted
119 into hydrogen peroxide by the enzyme superoxide dismutase (SOD) (Gregory & Fridovich,
120 1973). SOD is key to the cell's antioxidant response (Janknegt et al., 2007). Studies that
121 describe SOD responses in marine polar microalgae are rare due to difficulties such as
122 obtaining sufficient biomass for reliable measurements (Janknegt et al., 2009; Katayama &
123 Taguchi, 2013; Van de Poll et al., 2009).

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

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

134

135 **Methods**

136 Culture methodology

137 Monospecific stock cultures were maintained in Victoria University of Wellington at a PAR
138 level of 50 µmol m^{-2} s^{-1} and 4°C in f/2 medium (Ausaqua Pty Ltd., Australia) for 5-6 months
139 prior to these experiments. The Ross Sea species, *Thalassiosira antarctica*, used in the
140 experiments described below were isolated from sea ice at Cape Evans in the summer of
141 2010/2011. A stock culture of *Chaetoceros socialis* from the Antarctic Peninsula was
142 obtained from Dr. Claire Hughes (University of East Anglia) as part of studies under the
143 Rothera Time Series maintained by the British Antarctic Survey near its Rothera Research
144 Station in Ryder Bay (Marguerite Bay, 67°34'S, 68° 9'W) during the austral summer of
145 2008/2009 (http://www.antarctica.ac.uk//staff-profiles/webspace/mmm/RaTS/RaTS.html). A
146 stock culture of the same nominate species from the Arctic was obtained from Assoc. Prof.
147 Else Hegseth (University of Tromso), originally collected from Billefjorden, Svalbard, in
148 summer 2011.

149 Light treatments

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

154 Ryan et al., 2011). Sub-samples (400 ml) of monospecific cultures (n=3 at each level) were

155 incubated under each of four light levels (L0=0 µmol m^{-2} s^{-1}, L1=1 µmol m^{-2} s^{-1} of PAR,

156 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

157 mW m^{-2} of UV-B) at 4°C for 168 h in a temperature controlled water bath (Haake, Cleveland,

158 OH, USA). These light levels are intended to be representative of those found in the water

159 column below the sea ice (L0), at the bottom of the sea ice (L1), a quarter of the way above

160 the water-ice interface (L2), or at the middle of the sea ice profile (L3). The separate PAR

161 and UV-B levels were combined to reflect that the increase in PAR is synchronous with an

162 increase in UV-B through the sea ice profile.

163 The 400 ml samples were incubated following standard approaches described by Ryan et al.

164 (2012) in 500 ml opaque containers which were placed under 5W LED lights (Greenlights,

165 Taiwan) and a Philips Ultraviolet-B TL 12/40W UV-B tube. Different sections of the water

166 bath were separated from each other with opaque black plastic sheets and the various levels

167 of light and UV-B were obtained with appropriate shade cloth. The UV-B tube was also

168 covered with a polyvinyl chloride (PVC) sheet to filter out harmful UV-C radiation. PAR

169 levels were measured using a SpectroSense 2 meter with an SKR 1850 radiometer (bandwith

170 400 – 700 nm) and UV-B with an SKU 430 broad band UV-B radiometer (bandwidth 280 -

171 315 nm) (Skye Instruments, UK).

172 Samples of 1 ml for cell counts were taken at 0, 48 and 168 h from all replicates in each

173 incubation, preserved using 2% Lugol's Iodine and stored at 4°C. Fifty ml of sample was

174 used for the measurement of MAA content following the protocol described by Ryan et al.

175 (2002) and Rajanahally et al. (2014). However, as no detectable amounts of MAAs were

176 produced, no further consideration of MAAs is included here.

177 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).

202 *Superoxide dismutase (SOD)*

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

213 Sub-sampling, PAM fluorometry, and statistical analyses

214 PAM fluorometry is a widely used tool for studying the photosynthetic health of microalgae
215 (Hancke et al., 2008; Katayama & Taguchi, 2013; McMinn & Hegseth, 2004; Parkhill et al.,
216 2001; Ryan et al., 2009) as it provides a non-invasive method to study smaller cultures. A
217 Water PAM fluorometer (Walz, Effeltrich, Germany) was used to measure effective quantum
218 yield for photosystem II (ϕ_{PSII}). Before commencing observations, the cells were dark
219 acclimated. Thereafter, all PAM measurements were made on cells exposed to light as the
220 cells were subjected to actinic light used to make the PAM measurements. The Water PAM
221 was also used to generate rapid light curves (RLC). An RLC describes the effective quantum
222 yield as a function of irradiance (Ralph & Gademann, 2005). Each sample was treated with a
223 series of eight increasing actinic light treatments (0, 8, 20, 35, 55, 75, 96, 114, 150 µmol m^{-2}
224 s^{-1}), after which a strong saturating pulse was applied and ϕ_{PSII} was recorded. The RLC took
225 90 s to generate. The electron transport rate (ETR) values were calculated by multiplying the
226 ϕ_{PSII} value by the irradiance just applied. As the ϕ_{PSII} value is a ratio and ETR is derived from

227 this parameter, it is termed relative ETR (rETR). An RLC permits derivation of different
228 parameters that can be used to describe the photosynthetic properties of an algal sample,
229 including rETRmax (the maximum value for rETR), photosynthetic efficiency (α) and
230 saturation irradiance (E_k) (Ryan et al., 2009). To determine these parameters, the rETR data
231 were imported into Microsoft Excel v 10.0 (Microsoft, USA) and the curve was fitted with a
232 "waiting-in-line" function as described by Ritchie (2008). An RLC was generated for each
233 sample at times 0, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h.
234
235 Statistical analyses
236 First, a repeated measures ANOVA was applied to test specifically for changes over time
237 within each treatment. The assumptions of normality and equal variance were satisfied. As
238 the data from successive time points are likely not to be independent the covariance matrix -
239 the matrix of all covariances between time points - was examined. If that matrix was
240 spherical (as confirmed by Mauchly's test of sphericity), then a standard repeated measures
241 ANOVA calculation was carried out. If, however, the sphericity assumption did not hold,
242 then the Greenhouse Geisser adjustment was applied (Greenhouse & Geisser, 1959).
243 Repeated measures ANOVA was used to analyse effective quantum yield and $rETR_{max}$, with
244 'treatment' being the irradiance treatments and the different time points. The significance of
245 differences between pairs of time points was adjusted using Bonferroni's correction. Finally,
246 one-way ANOVA was used to compare the different treatment groups at specific time points.
247 *Post hoc* tests using Bonferroni's correction were used for subsequent pair-wise comparisons,
248 e.g. between different pairs of treatment groups at a time point, or between different time
249 points within a treatment group. All differences referred to are statistically significant at $p <
250 0.05$.
251

252 **Results**

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

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

262 *Quantum Yield (ϕ_{PSII})*

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

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

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

291 *$rETR_{max}$*

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

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

301 2(b)) for all light treatments other than at L0 was a slight increase in $rETR_{max}$ followed by a
302 decrease to reach a level higher than at time 0 h. Algae at L0 did not show any change over
303 time. Those incubated at L2 showed a significant increase in $rETR_{max}$ between 144 and 168
304 h. However, there was no overall significant increase. The general response of *C. socialis*
305 (Arctic Ocean) (Figure 2(c)) for all light treatments other than at L0 was a slight increase in
306 $rETR_{max}$ followed by a decrease to reach a level similar to that at time 0 h. Algae at L0 did
307 not show any change over time. Algae incubated at L2 showed an overall significant increase
308 in $rETR_{max}$.

309 <u>Chlorophyll *a*</u>

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

315 <u>Superoxide dismutase</u>

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

320 Although the general responses appeared to show small and consistent patterns of change
321 over time, the repeated measures ANOVA showed no significant change in SOD activity
322 over 168 h for algae at all light treatments, except for *Chaetoceros socialis* (Antarctic
323 Peninsula) which had a significant increase in the first 48 h for all light treatments followed
324 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).

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

360 When the three species were compared at each light level, there was no significant change in
361 quantum yield for algae incubated in the dark or at ambient low light, while those incubated
362 at higher light exposures showed an overall significant decrease. At both higher light levels,
363 the Arctic culture of *C. socialis* had the highest quantum yield at 168 h, perhaps indicating a
364 better ability to tolerate higher light exposure. During ice melt, algae are obviously exposed
365 to both varying and/or increasing levels of light and other parameters which are more
366 complex than those used in the current study. Van de Poll et al. (2009), studying the Antarctic
367 marine diatom *Chaetoceros brevis*, identified no difference in growth rates of algae exposed
368 to constant or dynamic irradiance regimes in iron-limited or replete conditions. Iron
369 limitation and wind-driven vertical mixing that resulted in a dynamic irradiance regime were
370 frequently co-occurring conditions that affected photosynthetic health of algae. However,
371 their study identified differences in pigment composition, quantum yield and antioxidant
372 capacity between algae exposed to the two irradiance regimes. Cellular pigment
373 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

418 **Acknowledgements**

419 We acknowledge support from VUW grant 100241, and FRST Grant VICX0706. MAF
420 permits 2009037596 and 2010040450 were used to transport cultures and samples from
421 Antarctica to New Zealand. We thank Antarctica New Zealand for logistic support in the
422 field. Dr. Claire Hughes and Assoc. Prof. Else Hegspeth are thanked for the provision of

423 cultures originally obtained from the Antarctic Peninsula and Arctic Ocean. Two anonymous
424 reviewers are thanked for constructive comments. PC is supported by NERC core funding to
425 the BAS Biodiversity, Evolution and Adaptation programme. This paper also contributes to
426 the SCAR AnT-ERA international science programme.

427

428 **References**

429 Arrigo, K. R., C. W. Sullivan & J. N. Kremer, 1991. A bio-optical model of Antarctic sea ice.
430 Journal of Geophysical Research: Oceans 96: 10581-10592.
431 Arrigo, K. R. & D. N. Thomas, 2004. Large scale importance of sea ice biology in the
432 Southern Ocean. Antarctic Science 16: 471-486.
433 Arrigo, K. R., T. Mock & M. P. Lizotte, 2010. Primary Producers and Sea Ice. In Thomas, D.
434 N. & G. S. Dieckmann (Eds.), Sea Ice (Second edition), Blackwell Publishing Ltd,
435 Oxford: 283-325.
436 Arrigo, K. R., D. K. Perovich, R. S. Pickart, Z. W. Brown, G. L. van Dijken, K. E. Lowry et
437 al., 2012. Massive phytoplankton blooms under Arctic sea ice. Science 336: 1408-
438 1408.
439 Arrigo, K. R. 2014. Sea ice ecosystems. Annual Review of Marine Science 6: 439-467.
440 Beauchamp, C. & I. Fridovich, 1971. Superoxide dismutase: Improved assays and an assay
441 applicable to acrylamide gels. Analytical Biochemistry 44: 276-287.
442 Beyer, W. F. & I. Fridovich, 1987. Assaying for superoxide dismutase activity: Some large
443 consequences for minor changes in conditions. Analytical Biochemistry 161: 559-
444 566.

445 Cunningham, W.L. & A. Leventer, 1998. Diatom assemblages in surface sediments of the
446 Ross Sea: relationship to present oceanographic conditions. Antarctic Science 10:
447 134–146.
448 Davidson, A. T., D. Bramich, H. J., Marchant & A. McMinn, 1994. Effects of UV-B
449 irradiation on growth and survival of Antarctic marine diatoms. Marine Biology 119:
450 507-515.
451 Eicken, H. 1992. The role of sea ice in structuring Antarctic ecosystems. Polar Biology 12: 3-
452 13.
453 Evans, C. A., J. E. O'Reilly & J. P. Thomas, 1987. A handbook for the measurement of
454 chlorophyll *a* and primary production. Texas A&M University, College Station.
455 Folt, C. L., C. Y. Chen, M. V. Moore & J. Burnaford, 1999. Synergism and antagonism
456 among multiple stressors. Limnology and Oceanography 44: 864-877.
457 Fryer, M. J., J. R. Andrews, K. Oxborough, D. A. Blowers & R. Baker, 1998. Relationship
458 between CO_2 assimilation, photosynthetic electron transport, and active O_2
459 metabolism in leaves of maize in the field during periods of low temperature. Plant
460 Physiology 116: 571-580.
461 Gao, K., J. Xu, G. Gao, Y. Li, D. A. Hutchins, B. Huang, L. Wang, Y. Zheng, P. Jin, X. Cai,
462 D-P. Hader, W. Li, K. Xu, N. Liu & U. Riebesell, 2012. Rising CO_2 and increased
463 light exposure synergistically reduce marine primary productivity. Nature Climate
464 Change 2: 519-523.
465 Garibotti, I. A., M. Vernet & M. E. Ferrario, 2005. Annually recurrent phytoplanktonic
466 assemblages during summer in the seasonal ice zone west of the Antarctic Peninsula
467 (Southern Ocean). Deep Sea Research Part I: Oceanographic Research Papers 52:
468 1823-1841.

469 Greenhouse, S.W. & S. Geisser, 1959. On methods in the analysis of profile data.
470 Psychometrika 24: 95-112.
471 Gregory, E. M., & I. Fridovich, 1973. Induction of superoxide dismutase by molecular
472 oxygen. Journal of Bacteriology 114: 543-548.
473 Halac, S., E. Garcia-Mendoza & A. T. Banaszak, 2009. Ultraviolet radiation reduces the
474 photoprotective capacity of the marine diatom *Phaeodactylum tricornutum*
475 (Bacillariophyceae, Heterokontophyta). Photochemistry and Photobiology 85: 807-
476 815.
477 Halac, S. R., V. E. Villafañe & E. W. Helbling, 2010. Temperature benefits the
478 photosynthetic performance of the diatoms *Chaetoceros gracilis* and *Thalassiosira*
479 *weissflogii* when exposed to UVR. Journal of Photochemistry and Photobiology B:
480 Biology 101: 196-205.
481 Hancke, K., T. B. Hancke, L. M. Olsen, G. Johnsen & R. N. Glud, 2008. Temperature effects
482 on microalgal photosynthesis-light responses measured by O_2 production, pulse-
483 amplitude-modulated fluorescence, and ^{14}C assimilation. Journal of Phycology 44:
484 501-514.
485 Hannach, G. & A. C. Sigleo, 1998. Photoinduction of UV-absorbing compounds in six
486 species of marine phytoplankton. Marine Ecology Progress Series 174: 207-222.
487 Harrison, W. G. & G. F. Cota, 1991. Primary production in polar waters: relation to nutrient
488 availability. Polar Research 10: 87-104.
489 Janknegt, P. J., J. W. Rijstenbil, W. H. van de Poll, T. S. Gechev & A. G. J. Buma, 2007. A
490 comparison of quantitative and qualitative superoxide dismutase assays for
491 application to low temperature microalgae. Journal of Photochemistry and
492 Photobiology B: Biology 87: 218-226.

493 Janknegt, P. J., C. M. de Graaff, W. H. van de Poll, R. J. W. Visser, J. W. Rijstenbil & A. G.
494 J. Buma, 2009. Short-term antioxidative responses of 15 microalgae exposed to
495 excessive irradiance including ultraviolet radiation. European Journal of Phycology
496 44: 525-539.
497 Katayama, T. & S. Taguchi, 2013. Photoprotective responses of an ice algal community in
498 Saroma-Ko Lagoon, Hokkaido, Japan. Polar Biology 36: 1431-1439.
499 Leu, E., S.-Å. Wängberg, A. Wulff, S. Falk-Petersen, J. Børre Ørbæk & D. O. Hessen, 2006.
500 Effects of changes in ambient PAR and UV radiation on the nutritional quality of an
501 Arctic diatom (*Thalassiosira antarctica* var. *borealis*). Journal of Experimental
502 Marine Biology and Ecology 337: 65-81.
503 Leu, E., J. Wiktor, J. E. Søreide, J. Berge & S. Falk-Petersen, 2010. Increased irradiance
504 reduces food quality of sea ice algae. Marine Ecology Progress Series 411: 49-60.
505 Lund-Hansen, L. C., I. Hawes, B. K. Sorrell & M. H. Nielsen, 2013. Removal of snow cover
506 inhibits spring growth of Arctic ice algae through physiological and behavioral
507 effects. Polar Biology 37: 471-481.
508 Majewska, R., M.C. Gambi, C.M. Totti & M. De Stefano, 2013. Epiphytic diatom
509 communities of Terra Nova Bay (Ross Sea, Antarctica): structural analysis and
510 relations to algal host. Antarctic Science 25: 501–513.
511 Maksym, T., S. E. Stammerjohn, S. Ackley & R. Massom, 2012. Antarctic sea ice - A polar
512 opposite? Oceanography 25: 140-151.
513 Mangoni, O., M. Saggiomo, M., Modigh, G. Catalano, A. Zingone & V. Saggiomo, 2009.
514 The role of platelet ice microalgae in seeding phytoplankton blooms in Terra Nova
515 Bay (Ross Sea, Antarctica): a mesocosm experiment. Polar Biology 32: 311-323.
516 Martin, A., A. McMinn, M. Heath, E. N. Hegseth & K. G. Ryan, 2012. The physiological
517 response to increased temperature in over-wintering sea ice algae and phytoplankton

518 in McMurdo Sound, Antarctica and Tromso Sound, Norway. Journal of Experimental
519 Marine Biology and Ecology 428: 57-66.
520 McMinn, A. & E. N. Hegseth, 2004. Quantum yield and photosynthetic parameters of marine
521 microalgae from the southern Arctic Ocean, Svalbard. Journal of the Marine
522 Biological Association of the United Kingdom 84: 865-871.
523 Mundy, C., M. Gosselin, J. Ehn, C. Belzile, M. Poulin, E. Alou et al. 2011. Characteristics of
524 two distinct high-light acclimated algal communities during advanced stages of sea
525 ice melt. Polar Biology 34: 1869-1886.
526 Nicol, S., J. Clarke, S. J. Romaine, S. Kawaguchi, G. Williams & G. W. Hosie, 2008. Krill
527 (*Euphausia superba*) abundance and Adélie penguin (*Pygoscelis adeliae*) breeding
528 performance in the waters off the Béchervaise Island colony, East Antarctica in 2
529 years with contrasting ecological conditions. Deep Sea Research Part II: Topical
530 Studies in Oceanography 55: 540-557.
531 Parkhill, J.-P., G. Maillet & J. J. Cullen, 2001. Fluorescence-based maximal quantum yield
532 for PSII as a diagnostic of nutrient stress. Journal of Phycology 37: 517-529.
533 Petrou, K., S. A. Kranz, M. A. Doblin & P. J. Ralph, 2011. Photophysiological responses of
534 *Fragilariopsis cylindrus* (Bacillariophyceae) to nitrogn depletion at two temperatures.
535 Journal of Phycology 48: 127-136.
536 Petrou, K. & P. Ralph, 2011. Photosynthesis and net primary productivity in three Antarctic
537 diatoms: possible significance for their distribution in the Antarctic marine ecosystem.
538 Marine Ecology Progress Series 437: 27-40.
539 van de Poll, W. H., M. A. van Leeuwe, J. Roggeveld & A. G. J. Buma, 2005. Nutrient
540 limitation and high irradiance acclimation reduce PAR and UV-induced viability loss
541 in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). Journal of Phycology
542 41: 840-850.

543 van de Poll, W. H. & A. G. J. Buma, 2009. Does ultraviolet radiation affect the xanthophyll
544 cycle in marine phytoplankton? Photochemical and Photobiological Sciences 8: 1295-
545 1301.
546 van de Poll, W. H., P. J. Janknegt, M. A. van Leeuwe, R. J. W. Visser & A. G. J. Buma,
547 2009. Excessive irradiance and antioxidant responses of an Antarctic marine diatom
548 exposed to iron limitation and to dynamic irradiance. Journal of Photochemistry and
549 Photobiology B: Biology 94: 32-37.
550 Rajanahally, M.A. 2014. Antarctic microalgae: physiological acclimation to environmental
551 change. MSc Thesis, Victoria University of Wellington, Wellington.
552 Rajanahally, M.A., D. Sim, K. G. Ryan & P. Convey, 2014. Can bottom ice algae tolerate
553 irradiance and temperature changes? Journal of Experimental Marine Biology and
554 Ecology 461: 516-527
555 Ralph, P.J. & R. Gademann, 2005. Rapid light curves: A powerful tool to assess
556 photosynthetic activity. Aquatic Botany 82, 222-237.
557 Ratkova, T. N., A. F. Sazhin & K. N. Kosobokova, 2004. Unicellular inhabitants of the White
558 Sea underice pelagic zone during the early spring period. Oceanology 44: 240-246.
559 Riebesell, U., I. Schloss & V. Smetacek, 1991. Aggregation of algae released from melting
560 sea ice: implications for seeding and sedimentation. Polar Biology 11: 239-248.
561 Ritchie, R., 2008. Fitting light saturation curves measured using modulated fluorometry.
562 Photosynthesis Research 96: 201-215.
563 Rost, B., U. Riebesell & D. Sultemeyer, 2006. Carbon acquisition of marine phytoplankton:
564 Effect of photoperiod length. Limnology and Oceanography 51: 12-20.
565 Ryan, K. G., A. McMinn, K. A. Mitchell & L. Trenerry, 2002. Mycosporine-like amino acids
566 in Antarctic sea ice algae, and their response to UVB radiation. Zeitschrift fur
567 Naturforschung 57: 471-477.

568 Ryan, K. G., R. O. M. Cowie, E. Liggins, D. McNaughtan, A. Martin & S. K. Davy, 2009.
569 The short-term effect of irradiance on the photosynthetic properties of antarctic fast-
570 ice microalgal communities. Journal of Phycology 45: 1290-1298.
571 Ryan, K. G., M. L. Tay, A. Martin, A., McMinn & S. K. Davy, 2011. Chlorophyll
572 fluorescence imaging analysis of the responses of Antarctic bottom-ice algae to light
573 and salinity during melting. Journal of Experimental Marine Biology and Ecology
574 399: 156-161.
575 Ryan, K. G., A. McMinn, E. N. Hegseth & S. K. Davy, 2012. The effects of ultraviolet-B
576 radiation on Antarctic sea-ice algae. Journal of Phycology 48: 74-84.
577 Rysgaard, S., M. Kuhl, R. N. Glud & J. W. Hansen, 2001. Biomass, production and
578 horizontal patchiness of sea ice algae in a high-Arctic fjord (Young Sound, NE
579 Greenland). Marine Ecology Progress Series 223: 15-26.
580 Salleh, S. & A. McMinn, 2011. Photosynthetic response and recovery of Antarctic marine
581 benthic microalgae exposed to elevated irradiances and temperatures. Polar Biology
582 34: 855-869.
583 SooHoo, J. B., A. C. Palmisano, S. T. Kottmeier, M. P. Lizotte, S. L. SooHoo & C. W.
584 Sullivan, 1987. Spectral light absorption and quantum yield of photosynthesis in sea
585 ice microalgae and a bloom of *Phaeocystis pouchetii* from McMurdo Sound,
586 Antarctica. Marine Ecology Progress Series 39: 175-189.
587 Stammerjohn, S., R. Massom, D. Rind & D. Martinson, 2012. Regions of rapid sea ice
588 change: An inter-hemispheric seasonal comparison. Geophysical Research Letters 39.
589 Thomas, D. N. & G. S. Dieckmann, 2003. Sea ice: An introduction to its physics, chemistry,
590 biology and geology, Wiley-Blackwell, Oxford.
591 Turner, J., N. E. Barrand, T. J. Bracegirdle, P. Convey, D.A. Hodgson, M. Jarvis et al. 2013.
592 Antarctic climate change and the environment: an update. Polar Record 50: 237-259.

593 Vernet, M., D. Martinson, R. Iannuzzi, S. Stammerjohn, W. Kozlowski, K. Sines et al. 2008.
594 Primary production within the sea-ice zone west of the Antarctic Peninsula: Sea ice,
595 summer mixed layer, and irradiance. Deep Sea Research Part II: Topical Studies in
596 Oceanography 55: 2068-2085.
597 Zor, T. & Z. Selinger, 1996. Linearization of the Bradford protein assay increases its
598 sensitivity: Theoretical and experimental studies. Analytical Biochemistry 236: 302-
599 308.

600

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			