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RESEARCH ARTICLE

The interacting effect of prolonged darkness and temperature on photophysiological characteristics of three Antarctic phytoplankton species

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Abstract

Photophysiological characteristics of the Southern Ocean phytoplankton species *Phaeocystis antarctica, Geminigera cryophila*, and *Chaetoceros simplex* were assessed during 7 weeks of darkness and subsequent recovery after darkness at 4 and 7°C. Chlorophyll *a* fluorescence and maximum quantum efficiency of PSII decreased during long darkness in a species-specific manner, whereas chlorophyll *a* concentration remained mostly unchanged. *Phaeocystis antarctica* showed the strongest decline in photosynthetic fitness during darkness, which coincided with a reduced capacity to recover after darkness, suggesting a loss of functional photosystem II (PSII) reaction centers. The diatom *C. simplex* at 4°C showed the strongest capacity to resume photosynthesis and active growth during 7 weeks of darkness. In all species, the maintenance of photosynthetic fitness during darkness was clearly temperature dependent as shown by the stronger decline of photosynthetic fitness at 7°C compared to 4°C. Although we lack direct evidence for this, we suggest that temperature-enhanced respiration rates cause stronger depletion of energy reserves that subsequently interferes with the maintenance of photosynthetic fitness during long darkness. Therefore, the current low temperatures in the coastal Southern Ocean may aid the maintenance of photosynthetic fitness during the austral winter. Further experiments should examine to what extent the species-specific differences in dark survival are relevant for future temperature scenarios for the coastal Southern Ocean.

KEYWORDS
algae, Antarctica, *Chaetoceros simplex*, climate change, *Geminigera cryophila*, light, photoacclimation, photosynthesis *Phaeocystis antarctica*

INTRODUCTION

Irradiance within the polar circles is characterized by extreme variability due to seasonal changes in solar angle. This forces the principal primary producers of polar oceans, phytoplankton, to survive periods with the sun below the horizon, that, depending on latitude, can last from days to months. In contrast with the Northern hemisphere, Southern hemisphere phytoplankton does not experience the full extent of the

Abbreviations: chl a_{F0}, chlorophyll *a* fluorescence; chl *a*, chlorophyll *a*; FRRf, fast repetition rate fluorometry; F_v/F_m, maximum quantum efficiency of PSII; NPQ, non photochemical quenching; PSII, photosystem II.

polar night (Ljungström et al., 2021). Seasonal extremes in irradiance for Southern Ocean phytoplankton are most pronounced in coastal regions, as these extremes coincide with extensive seasonal sea ice cover, further reducing exposure to irradiance and expanding the period that phytoplankton experience insufficient irradiance to drive net photosynthesis. The permanently open waters within the Polar Front are further away from the pole and experience reduced seasonal fluctuations. However, phytoplankton in this region are subjected to deep vertical mixing during the austral winter and early spring, which also can result in periods of darkness for phytoplankton estimated to range from days to months (McMinn & Martin, 2013). Therefore, it is relevant to investigate how Antarctic phytoplankton responds to long darkness and if these responses can potentially influence phytoplankton composition. Algae that experience long darkness display a decrease in maximum quantum efficiency of photosystem II (PSII) and decreased light harvesting pigments (Lacour et al., 2019; Morin et al., 2020; van de Poll et al., 2019; Veuger & van Oevelen, 2011). Transcripts encoding for light-harvesting protein complexes were significantly reduced after 1–7 d of darkness in the Antarctic sea ice diatom *Fragilariopsis cylindrus* (Kennedy et al., 2019; Mock et al., 2017), but changes in pigments and PSII photochemistry typically become visible after weeks of darkness. The decline in photosynthetic fitness during darkness and the subsequent ability to recover after long darkness differs among species and possibly among taxonomic groups (van de Poll et al., 2019). By influencing fitness and survival of the phytoplankton species, long darkness can change the over wintering phytoplankton community composition and that of the following spring (van de Poll et al., 2020).

Southern Ocean sea water temperature varies between –1.9 and 6.9°C within the Polar Front (Freeman & Lovenduski, 2016). Although highly variable among species, the average estimated optimum temperature for growth of Antarctic phytoplankton is around 5.2°C (Coello-Camba & Agustí, 2017), suggesting that temperature may constrain productivity in most of the Southern Ocean. A temperature increase from –0.8 to 7°C elevated photosynthetic carbon incorporation 2-fold in Antarctic algal assemblages during short incubations (Neori & Holm-Hansen, 1982). Elevated temperature also increases respiration rates. Respiration of Southern Ocean *Chaetoceros* and *Phaeocystis antarctica* strains increased significantly when grown under 4°C compared to 1°C, respectively (Bozzato et al., 2019). When in darkness, (autotrophic) phytoplankton rely mostly on stored energy reserves, which may be depleted faster at a higher temperatures (McMinn & Martin, 2013; Schaub et al., 2016). The effect of temperature on photosynthetic characteristics of Antarctic sea ice diatoms during darkness were found to be modest, but a temperature increase to 10°C shortened dark survival by nearly a factor 10 (Reeves et al., 2011).

The warming climate strongly modifies polar regions, with ongoing reductions in sea ice extent and rises in temperature expected in the future. Although the Southern Ocean is a key region for atmospheric and oceanic heat exchange (Huguenin et al., 2022), most regions within the Polar Front zone are currently expected to experience limited increases in sea surface temperature (Sallée, 2018), with the exception of the West Antarctic Peninsula. In this research, we tested photophysiological responses during darkness and after recovery from darkness in three Antarctic phytoplankton species from different taxonomic groups: *Phaeocystis antarctica* (Haptophyceae), *Geminigera cryophila* (Cryptophyceae), and *Chaetoceros simplex* (Bacillariophyceae). The specific strains of these species were isolated in coastal locations but have a widespread distribution in the Southern Ocean. Furthermore, the effect of a 3°C difference in temperature on the responses to darkness was investigated by performing the experiment at 4 and 7°C. We expected species-specific responses during darkness, with the diatom showing reduced effects on photosynthetic characteristics when compared to the flagellated species, as this was reported previously (van de Poll et al., 2019). In addition, we hypothesized stronger effects of darkness on photophysiological characteristics at elevated temperature due to assumed higher respirational losses at elevated temperature.

MATERIALS AND METHODS

Strains and cultivation

Phaeocystis antarctica (Haptophyceae, CS 243) and *Chaetoceros simplex* (Bacillariophyceae, CS 624, ANACC, 3–5 μm) were isolated from the coastal waters of Prydz Bay, Antarctic, and obtained from the Australian National Algal Culture Collection (CSIRO, Hobart, Tasmania). *Geminigera cryophila* (Cryptophyceae, CCMP 2564) was isolated from the coastal waters of McMurdo Sound, Antarctica. The algae were maintained in sterilized sea water with a salinity of 35, enriched with F/2 nutrients (Guillard, 1975; Guillard & Ryther, 1962) under irradiance of 15 μmol photons · m^{-2} · s^{-1} with a 16:8 h light:dark cycle in a cold room at 4°C. Cultures of these species were acclimated for 3 weeks prior to the dark incubation experiment to 20 μmol photons · m^{-2} · s^{-1} (Osram Biolux fluorescent lamps, irradiance measured by a Li-cor Li-250A light meter with a cosine sensor) under a 16:8 h light:dark cycle at 4 and 7°C. The temperature

of the 7°C cultures was maintained by a cryostat (Neslab) controlled circulating water bath.

Dark incubation experiment

Three replicates (0.5-L conical flasks) of each species were prepared for the 4 and 7°C experiments by diluting actively growing cultures 10 times in fresh sea water with double strength F/2 medium (to prevent nutrient depletion during the recovery experiment). The chlorophyll *a* concentrations of these cultures were between 45 and 100 μg chlorophyll $a \cdot L^{-1}$ prior to dark incubation. The cultures were allowed to acclimate to the new conditions overnight and were subsequently transferred to darkness. The flasks were wrapped in aluminum foil to minimize light exposure during sampling and put in an aluminum box (4°C). The temperature of the dark incubation at 7°C was maintained in a closed box with circulating water of a cryostat (Neslab). Cultures were sampled for FRRf at *t*-zero (during the light period, before dark incubation) and weekly during seven consecutive weeks of darkness for chlorophyll *a* concentration and for photosynthetic characteristics using FRRf (see below). In addition, weekly samples were obtained for recovery experiments during the seven consecutive weeks of darkness (see below). Sampling of the dark experiment was completed in less than 1 min, under weak light (<1 μmol photons $\cdot m^{-2} \cdot s^{-1}$) to prevent light exposure of the dark incubated cultures.

Recovery experiments

To investigate the ability to recover after long darkness, dark incubated samples (25 mL) were transferred to 50-mL transparent cultivation flasks and exposed to continuous irradiance of 20 μmol photons $\cdot m^{-2} \cdot s^{-1}$ for 5 d at 4 and 7°C. Afterward, a 10-mL sample was obtained to assess chlorophyll *a* concentration (chl *a*, see below), and a 3.5-mL sample was used to determine photosynthetic characteristics (FRRf, see below). The temperature of the recovery experiment at 7°C was maintained by circulating water from a cryostat (Neslab).

Chlorophyll *a* concentration

Changes in chl *a* concentration were assessed during dark incubation and after the recovery experiments. For this purpose, 10-mL samples were filtered on 25-mm GF/F (Whatman), snap frozen in liquid nitrogen, and stored at −80°C. The filters were freeze died for 48 h prior to extraction, and chl *a* was extracted in 3 mL of 90% acetone for 48 h at 4°C in darkness (van Leeuwe et al., 2006). Afterward, the chl *a* concentration was measured using a calibrated Turner designs Trilogy fluorometer.

Fast repetition rate fluorometry (FRRf)

After 30 min dark acclimation on ice, Fast Repetition Rate fluorometry (FRRf) was used to determine chlorophyll *a* fluorescence (chl a_{F0}) and maximum quantum efficiency of PSII (F_v/F_m). A volume of 3.5 mL was pipetted in the ice water cooled cuvette of the FRRf (FastOcean Sensor fitted with an Act2 laboratory system, and Act2Run software, Chelsea Technologies Group). The instrument was used in a single turnover mode with a saturation phase of 100 flashlets on a 2 μs pitch and a relaxation phase of 40 flashlets on a 60 μs pitch. The excitation wavelength of the FRRf's light-emitting diodes was 450 nm. Measurements in darkness were used for this manuscript (i.e., measurements without actinic light).

Chlorophyll *a* fluorescence: Chlorophyll *a* concentration ratio

To investigate the changes in chl *a* fluorescence, the ratio between chl *a* fluorescence relative to the chl *a* concentration was calculated weekly for each culture as the chl *a* fluorescence (chl a_{F0}) measured in 3.5 mL by the FRRf relative to the chl *a* concentration calculated in 3.5-mL culture.

Data analysis

Photophysiology was assessed from changes in F_v/F_m, chl a_{F0}, and chl *a* concentration during darkness and after subsequent recovery experiments. Differences between measurements obtained during dark incubation and after the corresponding recovery experiments ($n = 3$) at 4 and 7°C were tested for significance using a *t*-test, or Mann–Whitney Rank Sum test when data did not have a normal distribution. The effect of temperature and species on changes in chl *a* concentration, chl a_{F0}, F_v/F_m, and the chl a_{F0}:chl *a* concentration ratio of dark incubated cultures were tested for significance by calculating decline rates over the linear part of the data of individual cultures at 4 and 7°C. Decline rates ($n = 3$) were compared using a *t*-test, or Mann–Whitney Rank Sum test when data did not have a normal distribution. Differences were considered significant at $p < 0.05$.

RESULTS

Chlorophyll *a* concentration during darkness and after recovery

The chl *a* concentrations in the cultures of all species did not show significant increasing nor decreasing trends during the 7 weeks of darkness at 4 and 7°C (Figure 1). Differences between dark incubated chl *a* and chl *a* after subsequent recovery experiments were species specific and dependent on the time in darkness and on temperature. Chlorophyll *a* of *Phaeocystis antarctica* at 4°C was significantly higher after recovery experiments in the cultures that had been incubated for up 3 weeks in darkness (chl *a* after recovery was on average 141% ± 53% of dark chl *a*), whereas chl *a* after recovery experiments was significantly lower when compared with dark chl *a* for cultures that were incubated longer

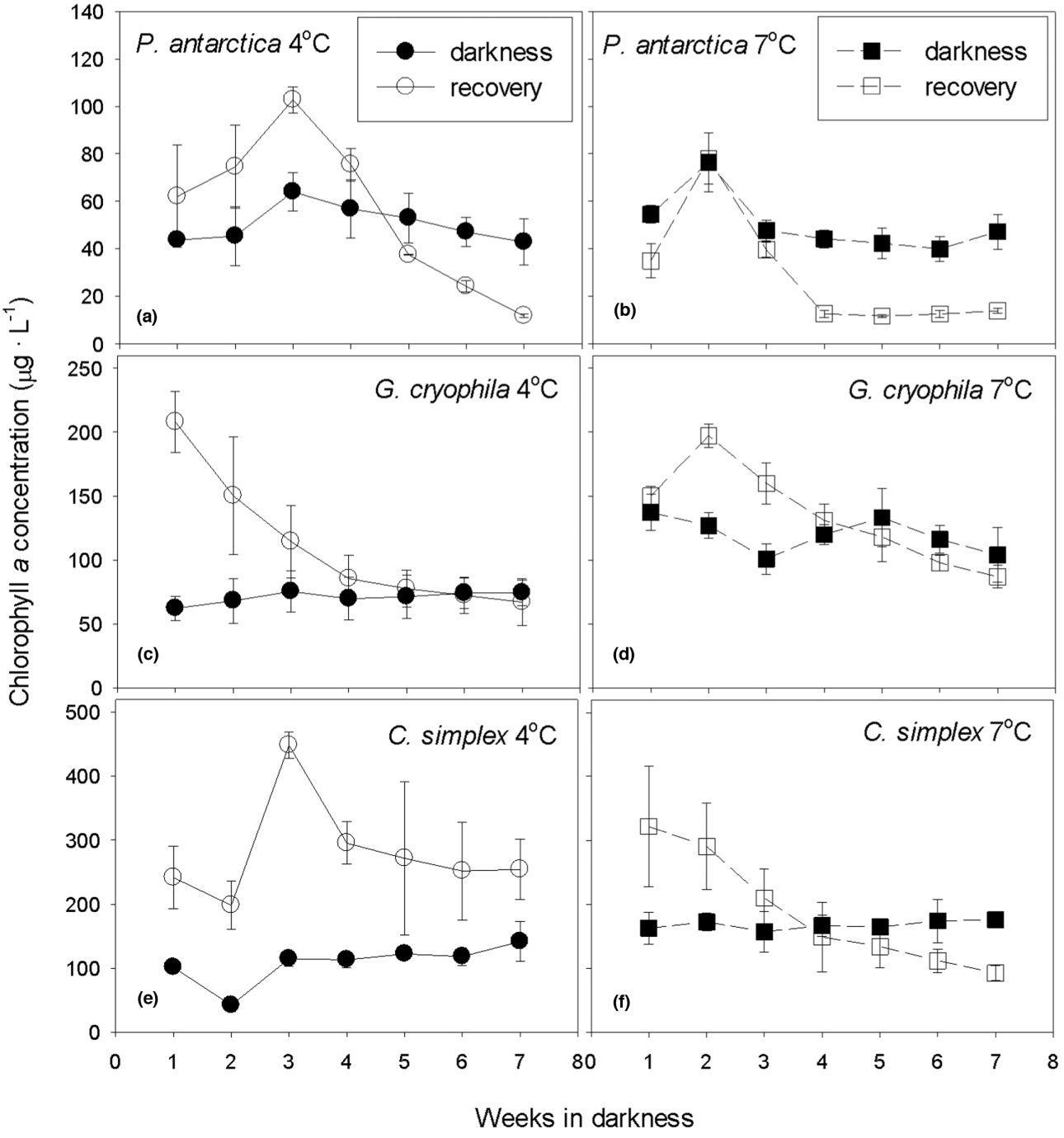

FIGURE 1 Chlorophyll *a* concentrations during the 7 weeks of darkness and after 5 days of subsequent recovery in irradiance of *Phaeocystis antarctica* (a, b), *Geminigera cryophila* (c, d), and *Chaetoceros simplex* (e, f) cultivated, incubated at 4 and 7°C. Mean and standard deviations are shown for measurements on three replicate cultures.

than 4 weeks in darkness. *Phaeocystis antarctica* incubated for up to 7 weeks in darkness at 7°C showed mostly significantly decreased chl *a* after the recovery experiments when compared with corresponding samples from darkness. Chlorophyll *a* of *Geminigera cryophila* at 4 and 7°C showed significant increases during recovery experiments in cultures that were incubated for up to 3 weeks in darkness (chl *a* after recovery was on average 237% ± 89% and 157% ± 7% of dark chl *a* at 4 and 7°C, respectively), whereas chl *a* showed no significant increase after experiments with cultures that were incubated longer than 3 weeks in darkness. Recovery experiments with *Chaetoceros simplex* at 4°C incubated for up to 7 weeks into darkness showed significantly increased chl *a* when compared to dark incubated samples (chl *a* after recovery was on average 288% ± 89% of dark chl *a*). Chlorophyll *a* of *C. simplex* at 7°C was significantly increased after recovery experiments with cultures that were incubated for up to 2 weeks in darkness (chl *a* after recovery was 182% ± 33% of dark chl *a*), whereas chl *a* decreased compared to dark chl *a* after recovery experiments with cultures that were incubated longer than 5 weeks into darkness.

Chl *a* fluorescence during darkness and after recovery

Chlorophyll *a* fluorescence (chl a_{F0}) decreased during darkness in all species. Linear chl *a* decline rates calculated over the trajectory from t-zero to minimum values (3–7 weeks of darkness) were species specific and dependent on temperature. Chlorophyll a_{F0} of *Phaeocystis antarctica* showed a linear decline during the 7 weeks of darkness at 4°C and during 3 weeks of darkness at 7°C (Figure 2). Chlorophyll a_{F0} of *Geminigera cryophila* and *Chaetoceros simplex* showed linear decline rates during the 7 weeks of darkness at both temperatures. The chl a_{F0} decline rates of *P. antarctica* were significantly higher when compared to the other species ($p < 0.004$). Chlorophyll a_{F0} decline rates were significantly higher at 7°C than at 4°C for all species ($p < 0.001$). The chl a_{F0} decline rates of *G. cryophila* and *C. simplex* were on average 3.4 times higher at 7°C than at 4°C, whereas *P. antarctica* the decline rate was 2.4 times higher at 7°C.

Chlorophyll a_{F0} of *Phaeocystis antarctica* at 4°C after the recovery experiments was significantly increased compared to the chl a_{F0} when dark incubated after experiments with cultures that had been incubated for up to 5 weeks in darkness ($p = 0.005$), whereas at 7°C, chl a_{F0} was significantly higher compared to the chl a_{F0} when dark incubated after experiments with cultures incubated for up to 2 weeks into darkness ($p < 0.001$; Figure 2). *Geminigera cryophila* at 4 and 7°C showed significant increased chl a_{F0} after recovery experiments with cultures incubated for up to 7 weeks into darkness. *Chaetoceros simplex* at 4°C showed the most pronounced increase in chl a_{F0} after recovery experiments with cultures incubated for up to 7 weeks into darkness as compared with the other species (Figure 2). At 7°C, chl a_{F0} of *C. simplex* was not significantly increased after recovery experiments with cultures that had been incubated for longer than 2 weeks into darkness, whereas chl *a* after the recovery experiments was significantly lower compared to chl *a* of dark incubated in experiments with algae that were in excess of 5 weeks into darkness.

Maximum quantum efficiency of PSII (F_v/F_m) during darkness and after recovery

Maximum quantum efficiency of PSII (F_v/F_m) of species growing at 4 and 7°C were similar at the start of the experiment (*t*-zero, Figure 3). The decline of F_v/F_m during long darkness was species specific and dependent on temperature (Figure 3). Linear F_v/F_m decline rates were highest in *Phaeocystis antarctica*, (weeks 1–7 at 4°C and weeks 1–5 at 7°C). F_v/F_m decline rates of this species were 1.5-fold higher at 7°C compared to 4°C ($p < 0.001$). F_v/F_m of *Geminigera cryophila* and *Chaetoceros simplex* at 4°C remained stable during darkness (at 80% ± 4% of the *t*-zero values). F_v/F_m showed a stronger decline at 7°C compared to 4°C in these species, reaching 67% ± 1% and 42% ± 4% of *t*-zero after 7 weeks of darkness, respectively (Figure 3, Table 1).

F_v/F_m of *Phaeocystis antarctica* at 4°C was significantly increased after recovery experiments with cultures incubated for up to 6 weeks in darkness, whereas at 7°C, F_v/F_m after the recovery experiments was not significantly different from the F_v/F_m of dark incubated cultures that had been incubated longer than 2 weeks in darkness. F_v/F_m of *Gemingera cryophila* at 4°C was significantly increased compared to that of dark incubated samples after recovery experiments with cultures that had been incubated for up to 6 weeks in darkness, whereas at 7°C this was significant for cultures incubated for up to 3 weeks in darkness. F_v/F_m of *Chaetoceros simplex* at 4°C showed full recovery (compared to *t*-zero) during recovery experiments with cultures incubated up to 7 weeks in darkness (Figure 3), whereas at 7°C F_v/F_m showed no significant increase after experiments with cultures that had been incubated longer than 3 weeks in darkness.

Chlorophyll *a* fluorescence:Chlorophyll *a* concentration ratio

Because chl a_{F0} declined during darkness whereas chl *a* concentrations of corresponding cultures remained stable, the ratio of chl a_{F0} relative to chl *a* concentration

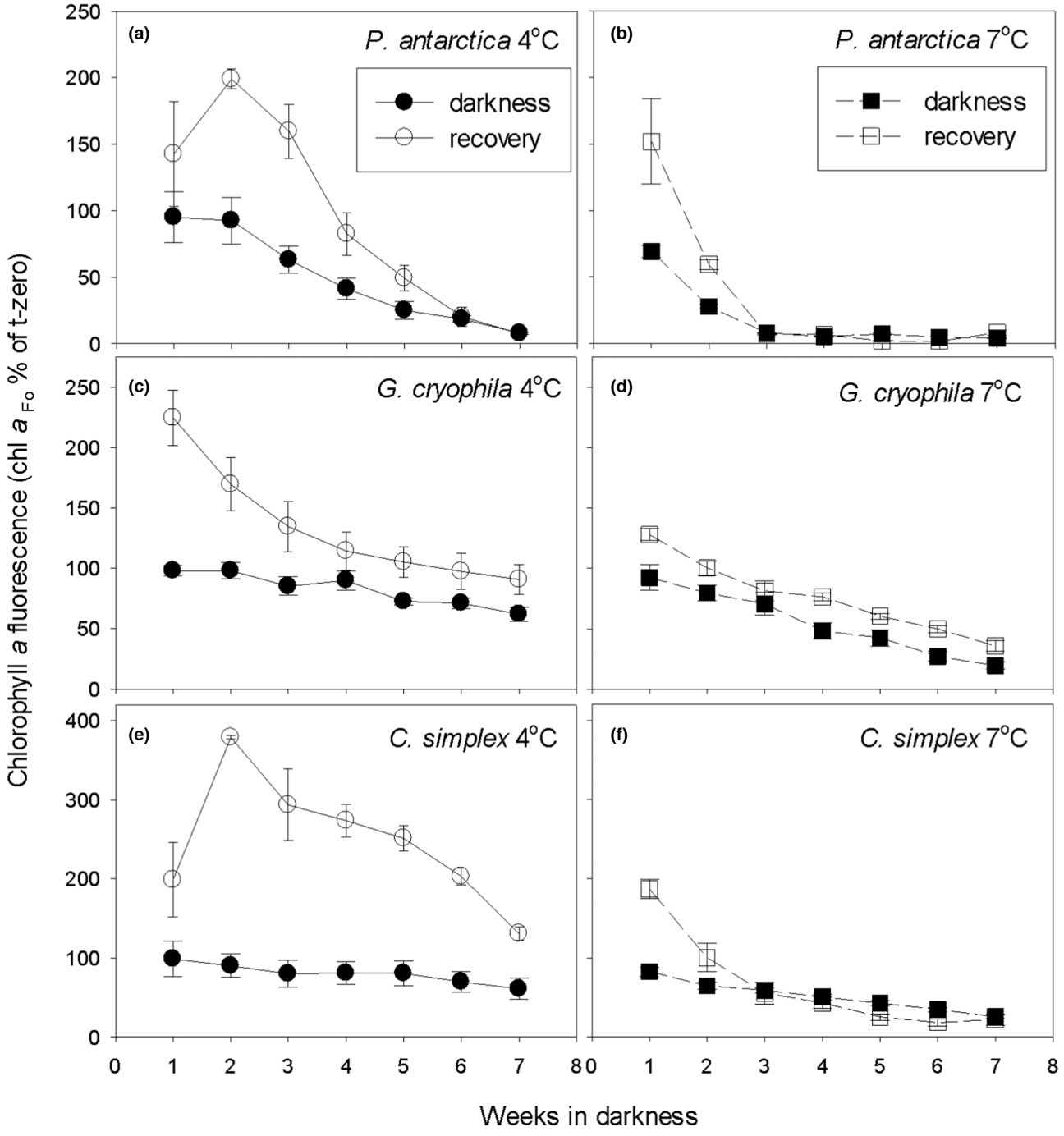

FIGURE 2 Chlorophyll *a* fluorescence (chl a_{Fo}) during the 7 weeks of darkness and after 5 days of subsequent recovery in irradiance of *Phaeocystis antarctica* (a, b), *Geminigera cryophila* (c, d), and *Chaetoceros simplex* (e, f) cultivated and incubated at 4 and 7°C. Chl *a* fluorescence was normalized to *t*-zero. Mean and standard deviations are shown for measurements on three replicate cultures.

(chl a_{F0}:chl *a* ratio) declined in all species (Figure 4). The decline of this ratio in *Phaeocystis antarctica* was significantly higher at 7°C (weeks 1–3) as compared to 4°C (weeks 1–7; Table 1). The decline rates of this ratio were not significantly different at 4 and 7°C in *Geminigera cryophila* and *Chaetoceros simplex*.

The chl a_{F0}:chl *a* ratio increased during the recovery experiments (not shown). Differences in this ratio between darkness and after recovery were most pronounced after 7 weeks of darkness. In *Phaeocystis antarctica* the chl a_{F0}:chl *a* ratio increased by a factor of 3.2 and 1.6 at 4 and 7°C, respectively, after 7 weeks of darkness (results not shown). This was mainly due to a decreased chl *a* concentration after recovery (Figure 1). The effect of temperature was significant in *P. antarctica*. In *Geminigera cryophila* the chl a_{F0}:chl *a* ratio increased on average 2-fold (no temperature effect), whereas in *C. simplex* the ratio increased 2.9- and

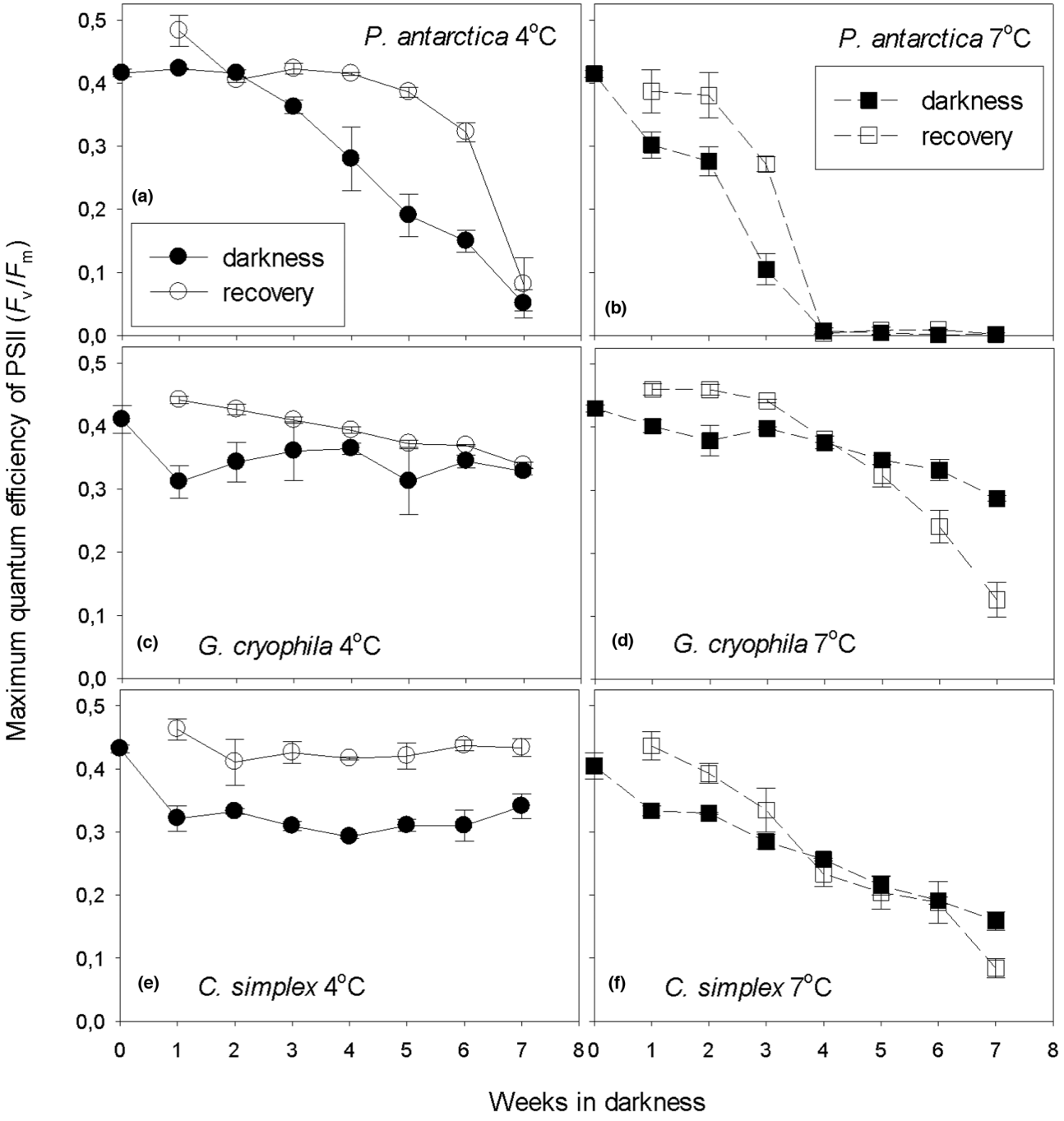

FIGURE 3 Maximum quantum efficiency of PSII (F_v/F_m) during the 7 weeks of darkness and after 5 d of subsequent recovery in irradiance of *Phaeocystis antarctica* (a, b), *Geminigera cryophila* (c, d), and *C. simplex* (e, f) cultivated and incubated at 4 and 7°C. Mean and standard deviations are shown for measurements on three replicate cultures.

2.1-fold at 4 and 7°C, respectively, after recovery. The differences in chl a_{F0}:chl *a* ratios during darkness and after recovery at 7°C in *Chaetoceros simplex* were driven by a decreased chl *a* concentration after recovery at 7°C.

DISCUSSION

We investigated the photophysiology of Antarctic phytoplankton species during long darkness, the potential of photophysiological characteristics to recover from long darkness, and how these responses interacted with temperature. The ability to maintain functional photosystems during darkness is essential for a fast resumption of photosynthesis when returned to irradiance, and species-specific variability in maintaining photosynthetic fitness during darkness can subsequently influence phytoplankton composition. The Antarctic species did not show pronounced changes in chl *a* concentration during darkness, whereas chlorophyll *a* fluorescence of PSII declined (chl a_{F0}). This

Decline rates (darkness)	Temperature	*P. antarctica*	*G. cryophila*	*C. simplex*
chl a_{F0} (week^{-1})	4°C	−58.0 (12.3)	−9.1 (3.3)	−23.4 (1.6)
	7°C	−141.7 (9.5)	−30.9 (3.4)	−79.8 (5.2)
chl a_{F0}:chl *a* (week^{-1})	4°C	−0.49 (0.12)	−0.06 (0.004)	−0.12 (0.03)
	7°C	−0.74 (0.06)	−0.06 (0.007)	−0.15 (0.01)
F_v/F_m (week^{-1})	4°C	−0.065 (0.004)	ns	ns
	7°C	−0.101 (0.007)	−0.017 (0.002)	−0.03 (0.002)

Note: Decline rates were calculated over weeks 1–7 (*C. simplex* and *G. cryophila*) and over weeks 1–7 and 1–3 for *Phaeocystis antarctica*, at 4 and 7°C, respectively.

Abbreviation: ns, no significant linear trend.

TABLE 1 Linear decline rates of chlorophyll *a* fluorescence (chl a_{F0}), the ratio between chl a_{F0} and chl *a* (chl a_{F0}:chl *a*), and the maximum quantum efficiency of PSII (F_v/F_m), during darkness in *Phaeocystis antarctica*, *Geminigera cryophila*, and *Chaetoceros simplex*.

coincided with decreasing maximum quantum efficiency of PSII (F_v/F_m). Loss of chl a_{F0} and F_v/F_m can be a consequence of non-photochemical quenching (NPQ; Falkowski & Kolber, 1995). Previously, activation of xanthophyll cycle pigments (de-epoxidation of xanthophyll cycle) during long darkness had been observed in diatoms (Jakob et al., 1999) and Arctic diatoms (Lacour et al., 2019; van de Poll et al., 2019), suggesting that this may have a function in maintaining photosynthetic fitness during darkness. However, the role of de-epoxidation of xanthophylls during long darkness remains unresolved. *Phaeocystis antarctica* has similar xanthophyll cycle pigments as *Chaetoceros simplex* but showed highly reduced photosynthetic fitness during long darkness when compared to the latter species. *Chaetoceros simplex* at 4°C fully restored F_v/F_m after recovery, and the chl *a* concentration increased strongly after recovery, indicating active growth when returned to light. In cultures of *P. antarctica*, *Geminigera cryophila*, and *C. simplex* maintained at 7°C, reduced chl a_{F0} and F_v/F_m corresponded to lower photosynthetic fitness, as indicated by a subsequent reduced capacity to recover during 5 d of re-illumination. Furthermore, cultures of *P. antarctica* (4 and 7°C), *G. cryophila* (7°C), and *C. simplex* at (7°C) showed further losses of chl *a* concentration after the recovery experiments. This indicates that other, less reversible processes have a role in the reduced chl a_{F0}, and F_v/F_m of these cultures (van de Poll et al., 2011). Here, the decrease of chl a_{F0} and F_v/F_m may imply a progressive decline of functional PSII reaction centers during long darkness, with antennae chl *a* possibly degrading upon reillumination due to reactive oxygen species formation during the recovery experiment.

Overall, the decline in photosynthetic fitness during darkness of the Antarctic species showed similarities to those of Arctic diatoms and flagellated phytoplankton species (van de Poll et al., 2019). The latter study documented a loss of chl a_{F0}, and F_v/F_m accompanied by loss of chl *a* and other light harvesting pigments. In contrast, no loss of chl *a* was observed in the Antarctic species during 7 weeks of darkness. Previously, Reeves et al. (2011) also reported no changes in chl *a* in three Antarctic diatom species during prolonged darkness. However, Tang et al. (2008) reported a decline in chlorophyll *a* concentrations in dark incubated algal assemblages dominated by *Phaeocystis antarctica*. Therefore, responses to long darkness appear to be variable between species and strains.

The tested Antarctic species are common representatives of major taxonomic groups of the Southern Ocean, with highly diverging photophysiology and growth strategies. Interestingly, responses to long darkness showed similarity to proposed survival mechanisms of the evergreen Scots Pine (of high-latitude coniferous forests) during the low temperatures experienced in early spring (Bag et al., 2020). The responses of the tree species (reduced chl *a*, F_v/F_m, increased NPQ) coincided with a rearrangement of thylakoid membrane structure. Therefore, structural changes in the light harvesting antennae complexes of phytoplankton species during long darkness cannot be ruled out. Such changes may facilitate the ability to maintain some photosynthetic potential at the expense of maximizing short-term photosynthetic gains when returned to irradiance.

Responses to darkness of the Antarctic phytoplankton species were species specific, in line with responses of Arctic phytoplankton (van de Poll et al., 2019). *Phaeocystis antarctica* showed the most pronounced reduction of chl a_{F0} and F_v/F_m during darkness and impaired recovery after darkness compared with *Chaetoceros simplex* and *Geminigera cryophila*. A comparable study with Arctic phytoplankton species showed that diatoms can maintain photosynthetic fitness during darkness, whereas this declined in the flagellated species that were tested (van de Poll et al., 2019). The Antarctic diatom *C. simplex* at 4°C maintained a strong photosynthetic fitness throughout 7 weeks of darkness in contrast to *G. cryophila* and *P. antarctica*. Therefore, *C. simplex* can rapidly resume growth after long periods of darkness, which is consistent with Kvernvik et al. (2018) who observed fast resumption of photosynthesis after the Arctic polar night in centric diatom dominated field samples. The Antarctic cryptophyte *G. cryophila* at 4°C showed a declining capacity to resume photosynthesis during 7 weeks of darkness. However, the decrease in chl a_{F0}

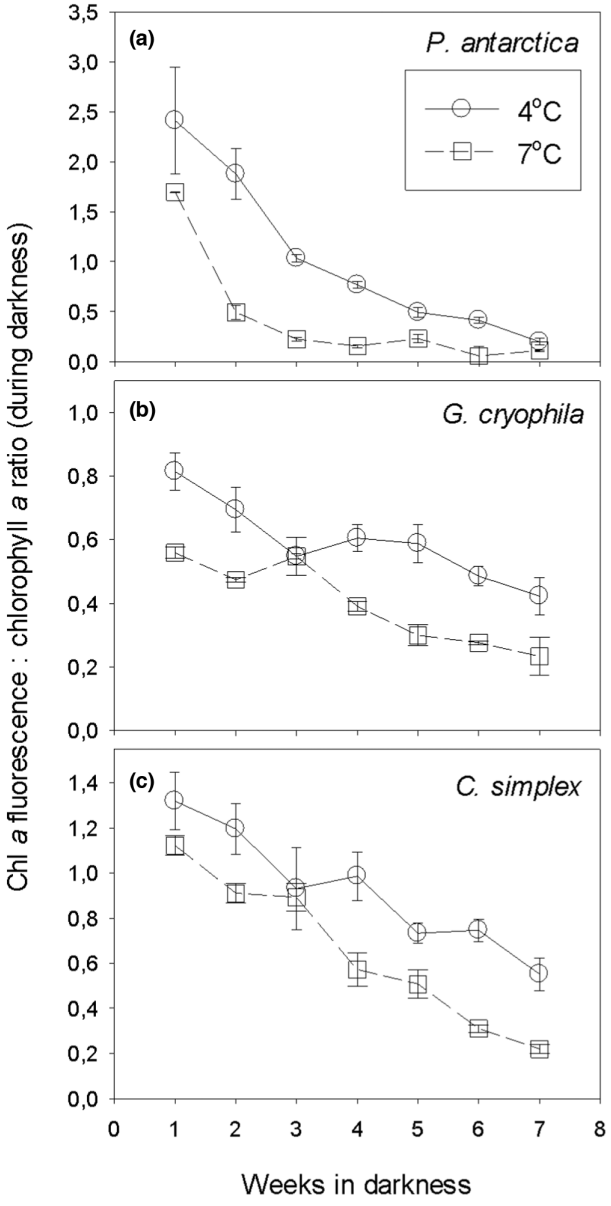

FIGURE 4 Chlorophyll *a* fluorescence: chlorophyll *a* concentration ratio during the 7 weeks of darkness in *Phaeocystis antarctica* (a, b), *Geminigera cryophila* (c, d), and *Chaetoceros simplex* (e, f) cultivated and incubated at 4 and 7°C. Mean and standard deviations are shown for measurements on three replicate cultures.

and F_v/F_m of this species were less severe when compared to Arctic *Rhodomonas* sp. cultivated at the same temperature.

Changes in photophysiology of *Phaeocystis antarctica*, *Geminigera cryophila*, and *Chaetoceros simplex* during 7 weeks of darkness were influenced by the temperature at which the algae were grown and incubated in darkness. Photosynthetic characteristics and recovery capacity declined more strongly at 7 than at 4°C during darkness. Although we lack direct evidence for this, we interpret the temperature effect as evidence for an important role of respiration rates in dark survival. Long darkness may affect the maintenance of photosystems and the photosynthetic machinery, as resources may be allocated to other crucial processes. For example, darkness caused upregulation of protein pathways associated with cellular respiration and downregulation of expression of light harvesting proteins in *Fragilariopsis cylindrus* (Kennedy et al., 2019). Metabolic rates, including respiration rates, have been shown to increase at elevated temperatures (Bozzato et al., 2019). Consequently, stored products become more rapidly depleted at higher temperature during darkness (McMinn & Martin, 2013; Schaub et al., 2016). This also implies that the low temperature experienced during the austral winter of the coastal Southern Ocean aids dark survival of autotrophic phytoplankton by slowing down metabolic activity. In support of this, much longer survival (around 20 weeks) of *P. antarctica* was observed at 0°C compared with survival at our experimental temperatures (3 and 6 weeks at 7 and 4°C, respectively; Tang et al., 2008). Linear extrapolation of our results points to 15 weeks of dark survival at 0°C for this species, in line with the above results. Algal respiration rates in darkness are known to vary by two orders of magnitude (Geider & Osborne, 1989). A study of six Antarctic benthic diatoms showed 5-fold variability in respiration rates between species as well as temperature dependence of respiration rates (Prelle et al., 2022). Therefore, differences in respiration rates and the capacity to store energy reserves may to a large extent explain how long species can maintain photosynthetic fitness in darkness. We acknowledge that other processes also can contribute to the ability to survive for long periods without irradiance, such as resting spore formation and mixotrophy. The latter may be particularly important in flagellated species but was not investigated in this study.

Numerous studies have aimed to explain how irradiance and other factors influences the composition of Southern Ocean phytoplankton (e.g., Alderkamp et al., 2012; Arrigo et al., 2010; Camoying et al., 2022; Trimborn et al., 2019). In addition to their findings, fitness after long darkness may be a factor that influences early spring phytoplankton composition due to species-specific responses to long darkness. Our experiments at 4 and 7°C do not provide information on the influence of long darkness on photosynthetic fitness under the current coastal Southern Ocean temperature regime, which is around the freezing point in the regions that are most impacted by seasonal variability in sunlight. However, these experiments increase our understanding of species-specific responses of algal photophysiology during long darkness. Our results show that *Phaeocystis antarctica* has reduced photosynthetic fitness after long darkness when compared to the other species and that loss of photosynthetic fitness during darkness is temperature dependent. Further experiments should

examine to what extent species-specific differences in dark survival are relevant for future temperature scenarios for the coastal Southern Ocean.

AUTHOR CONTRIBUTIONS

Willem van de Poll: Conceptualization (lead); formal analysis (lead); investigation (equal); methodology (lead); project administration (supporting); resources (lead); software (equal); supervision (lead); validation (equal); visualization (equal); writing – original draft (lead); writing – review and editing (lead). **Talia Abi Nassif:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (lead); resources (supporting); software (equal); supervision (supporting); validation (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting).

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