View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by College of William & Mary: W&M Publish

W&M ScholarWorks

VIMS Articles

Virginia Institute of Marine Science

2009

Survival and recovery of Phaeocystis antarctica (Prymnesiophyceae) from prolonged darkness and freezing

KW Tang
Virginia Institute of Marine Science

Walker O. Smith Jr.
College of William and Mary

AR Shields
Virginia Institute of Marine Science

DT Elliott
Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/vimsarticles

Part of the Aquaculture and Fisheries Commons

Recommended Citation

Tang, KW; Smith, Walker O. Jr.; Shields, AR; and Elliott, DT, "Survival and recovery of Phaeocystis antarctica (Prymnesiophyceae) from prolonged darkness and freezing" (2009). *VIMS Articles*. 970.
https://scholarworks.wm.edu/vimsarticles/970

This Article is brought to you for free and open access by the Virginia Institute of Marine Science at W&M ScholarWorks. It has been accepted for inclusion in VIMS Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2009) **276**, 81–90
doi:10.1098/rspb.2008.0598
Published online 2 September 2008

Survival and recovery of *Phaeocystis antarctica* (Prymnesiophyceae) from prolonged darkness and freezing

Kam W. Tang*, **Walker O. Smith Jr**, **Amy R. Shields**† **and David T. Elliott**

Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062, USA

The colony-forming haptophyte *Phaeocystis antarctica* is an important primary producer in the Ross Sea, and must survive long periods of darkness and freezing temperature in this extreme environment. We conducted experiments on the responses of *P. antarctica*-dominated phytoplankton assemblages to prolonged periods of darkness and freezing. Chlorophyll and photosynthetic capacity of the alga declined nonlinearly and independently of each other in the dark, and darkness alone would potentially reduce photosynthetic capacity by only 60 per cent over 150 days (approximately the length of the Antarctic winter in the southern Ross Sea). The estimated reduction of colonial mucous carbon is higher than that of colonial cell carbon, suggesting metabolism of the colonial matrix in the dark. The alga quickly resumed growth upon return to light. *Phaeocystis antarctica* also survived freezing, although longer freezing durations lengthened the lag before growth resumption. Particulate dimethylsulfoniopropionate relative to chlorophyll increased upon freezing and decreased upon darkness. Taken together, the abilities of *P. antarctica* to survive freezing and initiate growth quickly after darkness may provide it with the capability to survive in both the ice and the water column, and help explain its repeated dominance in austral spring blooms in the Ross Sea and elsewhere in the Southern Ocean.

Keywords: *Phaeocystis antarctica*; darkness; freezing; recovery; photosynthetic capacity; Southern Ocean

1. INTRODUCTION

The Southern Ocean is one of the harshest environments for marine organisms owing to the extremely low temperatures, variable photoperiods and wide ranges in photon flux densities. Such extremes are greater at more southerly latitudes, and hence the environment in McMurdo Sound (77.9° S) is at the maximum in seasonal forcing in the Antarctic. In McMurdo, air temperatures average −26.1°C in August and −2.9°C in January, and water temperatures are nearly always below −1.5°C. The region experiences complete darkness for five months, and much of the McMurdo Sound is ice covered throughout the year. Austral spring phytoplankton production in the southern Ross Sea is largely regulated by light, and phytoplankton abundance increases over two orders of magnitude in biomass during November–December, and normally declines rapidly in early January to modest chlorophyll levels (less than 1 $\mu g\,l^{-1}$; Smith *et al.* 2000, 2003).

The haptophyte *Phaeocystis antarctica* Karsten (Prymnesiophyceae) has been observed to occur regularly in the southern Ross Sea (e.g. El-Sayed *et al.* 1983; Tremblay & Smith 2007), and well over 60 per cent of the seasonal primary production can be attributed to this species (Smith *et al.* 2006). It generally occurs as colonies, but it has also been observed to occur in solitary form (Mathot *et al.* 2000; Dennett *et al.* 2001). During the colonial phase of its life cycle, *P. antarctica* cells are embedded in a thin mucous skin that forms a hollow, balloon-like colony. The cells reproduce by asexual binary fission, and the colony expands its size to hundreds of micrometres. *Phaeocystis antarctica* has also been found in the ice of the Ross Sea (Garrison & Buck 1987; Garrison *et al.* 2003), leading to the speculation that it is released from the ice upon ice melting to seed the upper water column and provide an inoculum for the phytoplankton bloom that regularly occurs in the austral spring.

Despite the growing oceanographic literature on the southern Ross Sea, a glaring omission in our understanding of the regional ecology is the total absence of data from the austral winter. *Phaeocystis antarctica* in the Ross Sea is subject to prolonged darkness and near-freezing temperatures in the austral winter, and, as a member of the ice biota, may experience temperatures and salinities that may far exceed water column extremes. However, its physiological adaptations to these extreme conditions have never been studied. Survival of long periods of darkness (in some cases for more than 1 year) is not uncommon among phytoplankton (e.g. Bunt & Lee 1972; Smayda & Mitchell-Innes 1974; Antia 1976; Murphy & Cowles 1997). By comparison, cold survival by phytoplankton in nature is less well known. A vast diversity of microorganisms can be found within Antarctic sea ice (Bowman *et al.* 1997; Song & Wilbert 2000; Delille *et al.* 2002; Garrison *et al.* 2005), suggesting that many of them survive dark and cold conditions, and upon ice melting in the spring may return to the water column (Gleitz *et al.* 1996; Stoecker *et al.* 1997). *Phaeocystis antarctica* also plays an

* Author for correspondence (kamtang@vims.edu).
† Present address: National Risk Management Research Laboratory, Office of Research and Development, USEPA, Ada, OK 74820, USA.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2008.0598 or via http://journals.royalsociety.org.

Received 5 May 2008
Accepted 11 August 2008

This journal is © 2008 The Royal Society

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

important role in the region's sulphur cycle, thanks to its prolific production of dimethylsulfoniopropionate (DMSP; DiTullio & Smith 1995). DMSP has been hypothesized to function as an osmolyte and cryoprotectant, especially in polar microalgae (Kirst *et al.* 1991; Karsten *et al.* 1996); hence, the cellular DMSP level of *P. antarctica* may also vary between the austral summer and austral winter.

Successful adaptation to the extreme austral winter conditions in the southern Ross Sea is vital to the growth and survival of *P. antarctica*. To understand these adaptations, ideally, one should study *P. antarctica* in the southern Ross Sea during the austral winter; unfortunately, logistical difficulties have thus far prevented that type of investigation. An alternative, albeit less ideal, approach is to simulate the austral winter conditions in the laboratory and to study the response of *P. antarctica*. We collected natural *P. antarctica*-dominated assemblages from the Ross Sea during the austral summer of 2006–2007 and subjected them to prolonged darkness and freezing. We then examined how *P. antarctica* survived and recovered after being in darkness or freezing conditions. The experiments were far shorter than the austral winter, but it was logistically feasible within the length of our field season. While many investigators used isolates that had been cultivated in the laboratory for many years, we used naturally occurring assemblages from the southern Ross Sea and conducted the experiments at McMurdo Station shortly after collection; hence, we were able to study the phytoplankton close to their natural state and to avoid any unintended population genetic drift prior to the experiments. Similar studies on polar phytoplankton tend to focus on diatoms, whereas our study is the first on the adaptations of *P. antarctica* to prolonged darkness and freezing conditions, and it provides important insights into how this species may survive austral winters in the southern Ross Sea.

2. MATERIAL AND METHODS

(a) *Collection of Ross Sea* P. antarctica*-dominated assemblages*

More than 100 l of surface water was collected from the Ross Sea during a dense phytoplankton bloom dominated by *P. antarctica*. The water was maintained under low irradiance in an environmental room on the *RVIB N. B. Palmer* after collection, and transported to McMurdo Station within 5 days. Upon arrival in McMurdo, the water was maintained at -1°C under continuous light at approximately 95 µmol photons $m^{-2}\,s^{-1}$. Prior to the experiments, the Ross Sea water was diluted with 0.2 µm filtered McMurdo Sound surface water (collected from a flowing seawater system) to the desired initial chlorophyll concentrations (approx. 3 $\mu g\,l^{-1}$ for light–dark (LD) experiments; approx. 14 $\mu g\,l^{-1}$ for freeze–thaw (FT) experiments). Experimental water was amended with f/10 nutrients (Gillard & Ryther 1962) and distributed into polycarbonate carboys (see below).

(b) *LD experiment*

Nutrient-amended Ross Sea water was distributed into six 20 l clear carboys (pre-washed with 10% HCl and rinsed with Milli-Q water). Three carboys were maintained under continuous cool-white fluorescent irradiance at 95 µmol photons $m^{-2}\,s^{-1}$ (measured at the surface of the carboys) for the light treatment, whereas the other three carboys were wrapped under multiple layers of black plastic for the dark treatment. All carboys were incubated at -1°C in aquaria with running seawater that provided gentle mixing to the carboy during the incubation. Every 5–10 days, samples were collected from each carboy for size-fractionated chlorophyll (total and greater than 20 µm), *P. antarctica* solitary cell abundance, *P. antarctica* colony abundance and size, particulate DMSP ($DMSP_p$), particulate organic carbon (POC), particulate organic nitrogen (PON), photosynthetic capacity and dissolved nutrients. On day 30, the remaining seawater in the dark-treatment carboys was split into six carboys; three of which remained in the dark, whereas the other three were returned to the light to follow their recovery. The recovery carboys were sampled in a similar manner. The entire LD experiment lasted 60 days.

(c) *High-frequency dark sampling*

We conducted a separate experiment to generate a more detailed temporal trend of photosynthetic capacity in dark incubation. The natural assemblage was placed in an acid-washed carboy and immediately placed in darkness at -1°C as described above, after which we sampled at high frequency for photosynthetic capacity and total chlorophyll.

(d) *FT experiment*

Ross Sea water was placed into six carboys, which were then placed in a Percival growth chamber under dim illumination (approx. 34 µmol photons $m^{-2}\,s^{-1}$). The initial water temperature was approximately -1°C, and was lowered by approximately 2°C every 2 hours until -11°C was reached, at which time the carboy content appeared as solid ice (visual observation). Three of the carboys were kept frozen at -11°C for 15 days (FT_{15}); the other three remained frozen for 30 days (FT_{30}). At the end of the freezing period, the carboys were removed from the freezer and allowed to gradually thaw at room temperature. Before complete thawing, the carboys were placed in a flow-through aquarium at -1°C to complete the thawing and maintain that temperature for subsequent sampling. The aquarium was illuminated by continuous cool-white fluorescent light at an irradiance of 95 µmol photons $m^{-2}\,s^{-1}$. Samples were drawn before freezing, immediately after thawing and every 7 days thereafter for size-fractionated chlorophyll, *P. antarctica* solitary cell and colony abundances, colony size, $DMSP_p$, POC, PON, photosynthetic capacity and dissolved nutrients.

3. RESULTS

(a) *LD experiment: light treatment*

Microscopic observations confirmed that the assemblage was almost entirely *P. antarctica* at the start of the experiment (i.e. no other phytoplankton species was found in significant numbers). Solitary cell abundance in the light treatment increased exponentially during the first 25 days to 1.8×10^{4} cells ml^{-1}, after which it declined to approximately 1.1×10^{4} cells ml^{-1} until day 40 (figure 1). Colonial abundance increased slightly in the first 5 days, but remained relatively constant thereafter (approx. 210 colonies ml^{-1}; figure 1). The greater than 20 µm fraction chlorophyll concentration (largely composed of colonies) increased from less than 2 $\mu g\,l^{-1}$ on day 0 to 210 $\mu g\,l^{-1}$ on day 25 (approx. 80% of the total chlorophyll; figure 2). During this time, the geometric mean of colony diameter increased steadily from 93 to

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

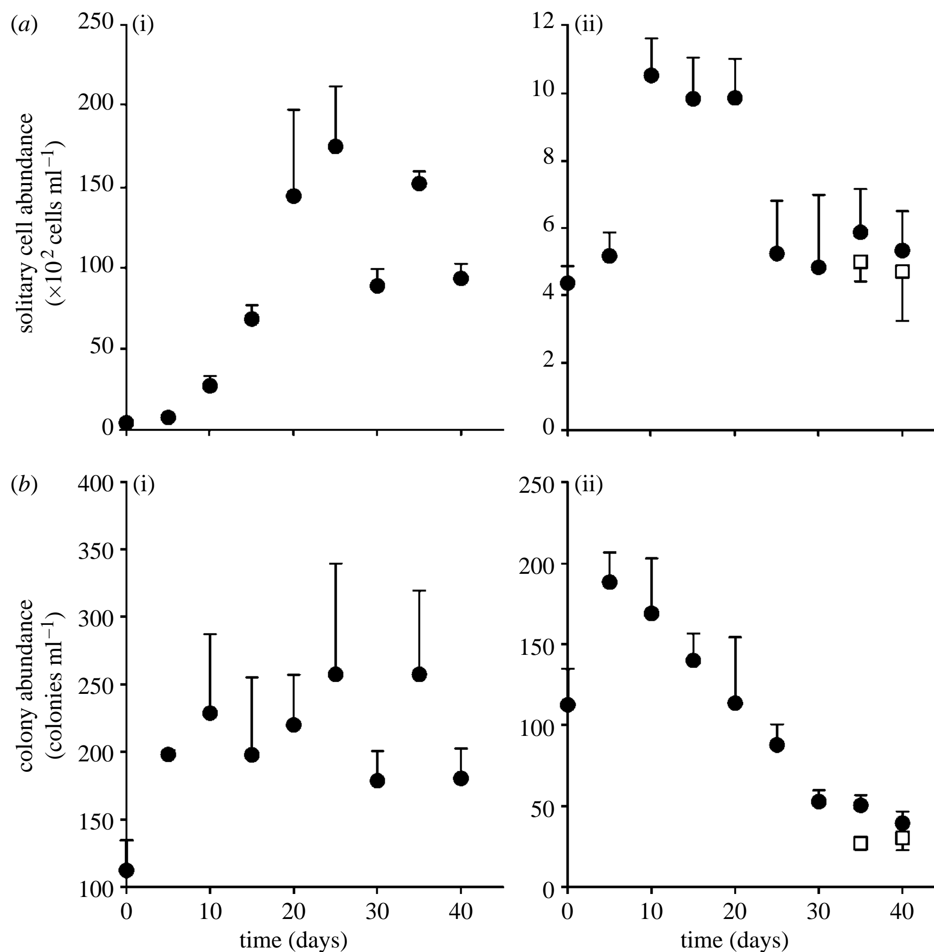

Figure 1. *Phaeocystis antarctica* (*a*) solitary cell and (*b*) colony abundances in the (i) light and (ii) dark treatment as indicated by filled circles (mean + s.d.; $n = 3$). Recovery treatment is indicated by open squares (mean − s.d.; $n = 3$).

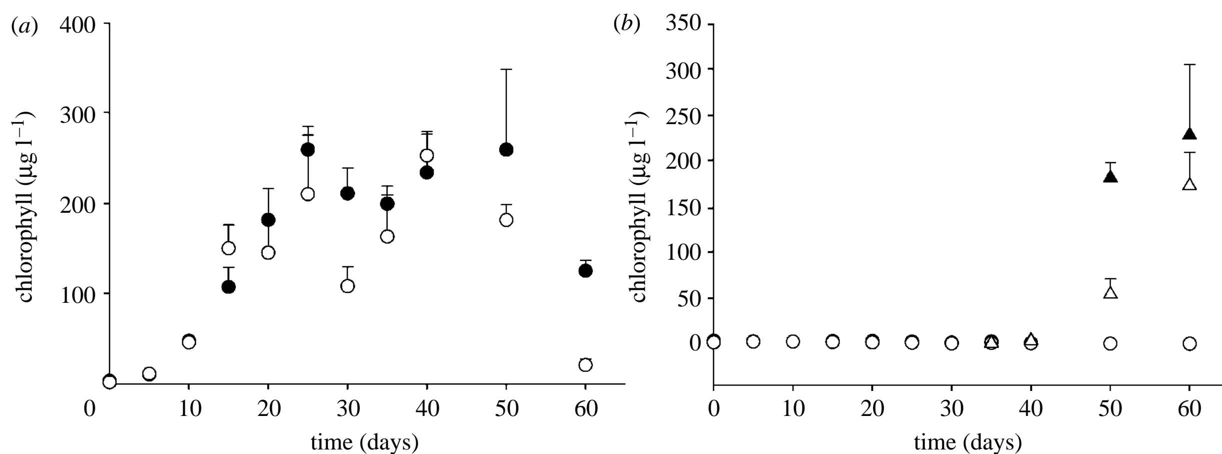

Figure 2. *Phaeocystis antarctica* size-fractioned chlorophyll concentrations in the (*a*) light and (*b*) dark treatment as indicated by circles (mean + s.d.; $n = 3$). Recovery treatment is indicated by triangles (mean + s.d.; $n = 3$). Filled circles, total; open circles, greater than 20 μm; filled triangles, total recovery; open triangles, greater than 20 μm recovery.

157 μm, suggesting that the increase in greater than 20 μm chlorophyll was due to an increase in the number of cells per colony (see figure in the electronic supplementary material). After day 30, the colonies decreased in size slightly, but the final (day 40) mean colony diameter remained larger than the initial value (t-test, $p < 0.001$). The greater than 20 μm chlorophyll concentration also declined sharply from 182 to 21 $\mu g\,l^{-1}$ (to approx. 17% of the total chlorophyll) between days 50 and 60, indicating a decay or disruption of the colonies. Total chlorophyll increased from an initial concentration of 3.4 $\mu g\,l^{-1}$ to a maximum of 260 $\mu g\,l^{-1}$ by day 25 (figure 2), which yields a growth rate of 0.17 d^{-1} when fit to an exponential growth equation.

There was a steady reduction of nitrate in the light treatment from 150 to 54 μM over the first 40 days (see table in the electronic supplementary material). Phosphate also decreased from 8.7 to 0.6 μM in the first 20 days and remained steady or increased slightly afterwards. Ammonium steadily increased from below detection limit on days 0 to 16 μM during the experiment, indicating significant excretion and remineralization in the

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

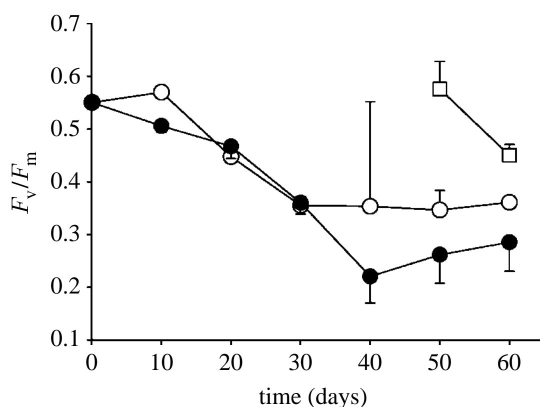

Figure 3. Maximum quantum yield (F_v/F_m) in the light treatment (open circles; mean+s.d.; $n=3$), dark treatment (filled circles; mean−s.d.; $n=3$) and recovery treatment (open squares; mean+s.d.; $n=3$).

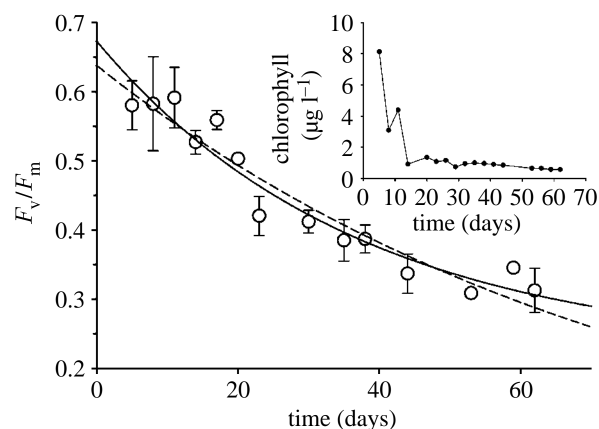

Figure 4. High-frequency measurement of F_v/F_m in dark incubation, with chlorophyll shown in the inset. The dashed line represents an exponential decay function fitted to the data ($Y=0.638\ e^{-0.013X}$ ($r^2=0.922$; $p<0.0001$)). The solid line represents an exponential decay function with a threshold fitted to the data ($Y=0.223+0.450\ e^{-0.027X}$ ($r^2=0.934$; $p<0.0001$)).

water. The POC and PON concentrations of the light treatments followed the general trend of total chlorophyll, and increased steadily from days 0 to 25, after which they fluctuated around rather constant concentrations (see table in the electronic supplementary material). POC did not decrease as dramatically as chlorophyll between days 50 and 60, suggesting that the reduction in chlorophyll was largely a chromatic acclimation. POC normalized to chlorophyll (μg μg^{-1}) varied between 23 and 60 for the entire experiment.

$DMSP_p$ concentration was measured every 10 days. Similar to total chlorophyll and POC/N, $DMSP_p$ increased during the first 20 days, after which it remained relatively constant (see table in the electronic supplementary material). Between days 30 and 40, there was a large variation between replicates, whose cause remains obscure. During the period of high phytoplankton biomass (days 20–50), $DMSP_p$ concentration exceeded 230 nmol l^{-1}. $DMSP_p$ normalized to chlorophyll was 19 nmol μg^{-1} on day 0, but decreased to approximately 0.7–2.3 nmol μg^{-1} for the rest of the experiment. Photosynthetic potential measured as the maximum quantum yield (F_v/F_m; see the electronic supplementary material) in the light treatment was 0.55–0.57 in the first 20 days of the experiments, then decreased to 0.36 by day 30 and remained near that level (figure 3).

(b) *LD experiment: dark treatment and recovery*

In the dark treatment, solitary cell abundance increased from 4.0×10^2 on day 0 to 1×10^3 cells ml^{-1} on day 10, but decreased to approximately 5.0×10^2 cells ml^{-1} between days 20 and 25, and remained relatively constant thereafter (figure 1). Colony abundance increased from 112 to 180 colonies ml^{-1} in the first 5 days, and then steadily decreased (figure 1). The last measured colonial abundance was less than half of the initial value. Not only did the colonial abundance decrease, but the colony size also steadily decreased after day 15, when the geometric mean colony diameter decreased from greater than 100 μm to less than 50 μm (see figure in the electronic supplementary material). The final mean colony diameter was significantly less than the initial colony diameter (*t*-test, $p<0.001$), indicating an absolute loss in colonial materials. Chlorophyll concentrations decreased to near zero during the dark incubation (figure 2). All nutrient concentrations remained nearly constant in the dark treatment except for ammonium, which increased to approximately 7 μM by day 40 (see table in the electronic supplementary material).

During the recovery (when some of the dark-adapted assemblage was returned to light on day 30), there was only a small increase in the chlorophyll concentration in the first 10 days (figure 2). However, after day 50 (i.e. 20 days after return to light), the chlorophyll concentration increased rapidly to approximately 230 μg l^{-1}; the greater than 20 μm fraction also increased to approximately 170 μg l^{-1} (74% of the total). The calculated growth rate during the 30-day recovery period is 0.17 d^{-1}. The POC and PON concentrations follow the trend of chlorophyll for both the dark and the recovery treatments (see table in the electronic supplementary material). Chlorophyll in the dark treatment decreased more rapidly than POC, resulting in a C : Chl (μg μg^{-1}) ratio exceeding 240 after day 30. Conversely, the phytoplankton assemblage synthesized chlorophyll more rapidly than POC once returned to light, as evidenced by the drop in the C : Chl ratio from above 240 to 36.

$DMSP_p$ decreased by 76 per cent within the first 10 days of dark incubation (see table in the electronic supplementary material), compared with a 30 per cent reduction in chlorophyll and POC, but remained at 9–16 nmol l^{-1} after day 10. In the recovery treatment (days 30–60), $DMSP_p$ remained low and was not significantly different from the dark treatment (two-way RM ANOVA; $p=0.202$, 0.001 and 0.192 for treatment, time and interaction, respectively), in stark contrast with the chlorophyll and POC concentrations, both of which showed rapid increases during the recovery.

Photosynthetic potential (F_v/F_m) in the dark treatment was significantly lower than that in the light treatment over time (two-way RM ANOVA; $p=0.045$, <0.001 and 0.006 for treatment, time and interaction, respectively). In the recovery treatment, F_v/F_m was measured on days 50 and 60 (i.e. 20 and 30 days after return to light), when it reached 0.45–0.58 (figure 3), comparable with the initial values in the light treatment.

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

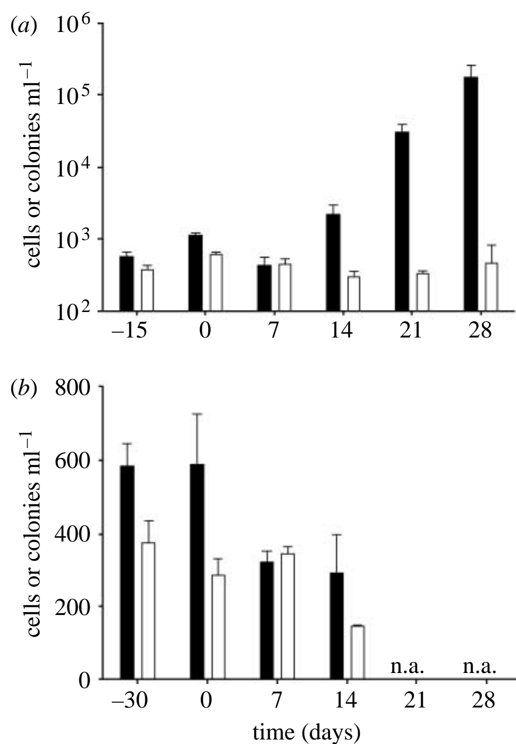

Figure 5. *Phaeocystis antarctica* solitary cell (filled bars) and colony (open bars) abundances (mean+s.d.; $n=3$) in (*a*) 15 day and (*b*) 30 day freezing treatments. Freezing began on days −15 and −30, and recovery started on day 0. n.a.; data not available.

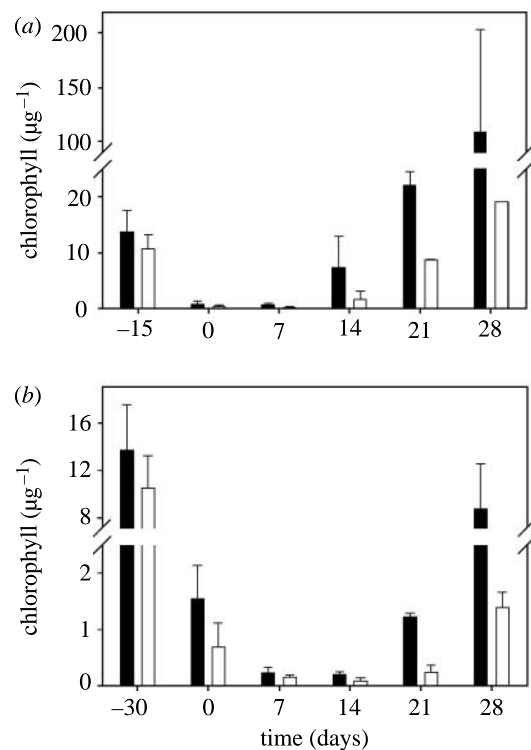

Figure 6. *Phaeocystis antarctica* size-fractioned chlorophyll concentrations (mean+s.d.; $n=3$) in (*a*) 15 day and (*b*) 30 day freezing treatments. Freezing began on days −15 and −30, and recovery started on day 0. Filled bars, total; open bars, greater than 20 μm.

(c) *High-frequency dark sampling*

A 62-day incubation in darkness resulted in a gradual reduction in the photosynthetic capacity (figure 4). F_v/F_m was near maximal at the start of the experiment, and declined, but at a reduced rate relative to chlorophyll concentrations, over time. The temporal change in F_v/F_m can be described by an exponential decay function with a threshold (figure 4), suggesting that over longer periods of darkness at least some residual photosynthetic capacity is maintained. If the equation is extrapolated to 150 days of darkness, the residual F_v/F_m would equal 0.23, or approximately 40 per cent of the initial F_v/F_m.

(d) *Freeze–thaw experiment: FT$_{15}$ treatment*

In the FT_{15} treatment, the solitary cell abundance remained close to the pre-freezing levels in the first 7 days of recovery, after which it increased rapidly to 1.8×10^5 cells ml^{-1} (figure 5). Colony abundance, on the other hand, remained relatively constant throughout the entire experiment at approximately 420 colonies ml^{-1} (figure 5). Chlorophyll concentrations decreased from a pre-freezing level of 14 to 1 μg l^{-1} after 15 days of freezing (a 93% decrease), while the greater than 20 μm fraction dropped from 11 to 0.4 μg l^{-1} (a 96% decrease; figure 6). Total and greater than 20 μm chlorophyll concentrations increased to 108 and 19 μg l^{-1}, respectively, after 28 days of recovery. The FT_{15} growth rates derived from cell count and chlorophyll data were 0.18 and 0.17 d^{-1}. Colony diameter decreased significantly during freezing (*t*-test; $p=0.017$), from a geometric mean of 117 to 85.8 μm (figure 7). Microscopic observations showed that although colonies were visible on day 0 of the recovery, most of them appeared ghost-like; that is, the colonial matrix was

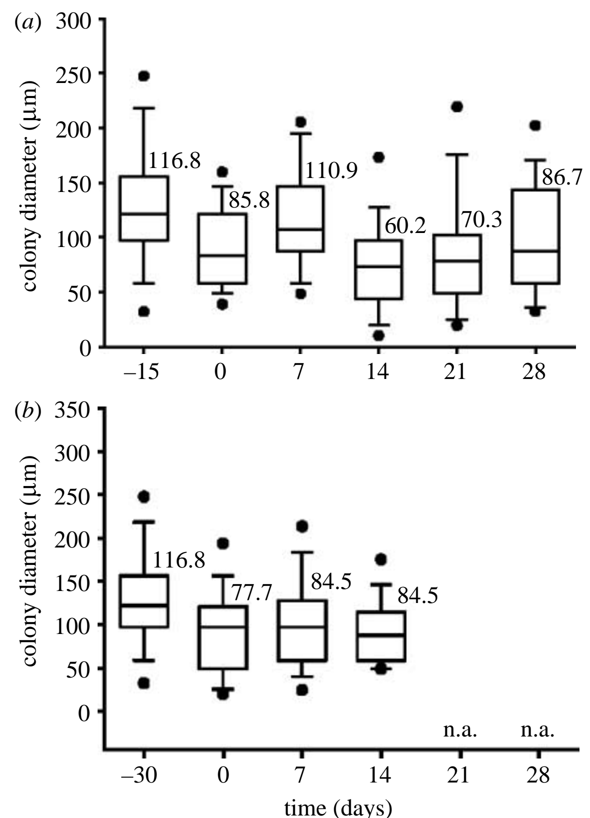

Figure 7. *Phaeocystis antarctica* colony diameter in (*a*) 15 day and (*b*) 30 day freezing treatments. Colony diameters are presented in box plots showing 5, 10, 25, 50, 75, 90 and 95 percentiles. The number next to each box represents the geometric mean of the pooled data of three replicates. Freezing began on days −15 and −30, and recovery started on day 0. n.a., data not available.

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

Table 1. POC/N (μmol l^{-1}) and $DMSP_p$ (nmol l^{-1}) concentrations in the FT_{15} and FT_{30} experiments. (Standard deviations are given in parentheses.)

	15 days of freezing			30 days of freezing		
days of recovery	POC	PON	$DMSP_p$	POC	PON	$DMSP_p$
pre-freezing	89.5 (4.9)	10.9 (0.5)	60.0 (5.2)	89.5 (4.9)	10.9 (0.5)	60.0 (5.2)
0	69.0 (6.4)	9.4 (0.7)	43.9 (35.3)	63.6 (4.1)	8.4 (0.2)	
7	96.0 (2.7)	14.5 (0.4)		68.5 (2.8)	9.3 (1.1)	
14	109 (14.8)	19.1 (3.3)	1870 (3130)	90.3 (11.7)	14.7 (3.0)	2010 (258)
21	224 (12.7)	39.3 (2.4)		100 (5.3)	17.5 (0.9)	
28	554 (140)	92.3 (23.4)	69.2 (28.0)	97.5 (11.6)	17.2 (2.5)	2640 (1560)

intact, but without healthy, embedded cells. At the end of the 28 day recovery period, the mean colony diameter remained smaller than the initial value.

Unlike chlorophyll, POC and PON decreased by only 23 and 14 per cent, respectively, after 15 days of freezing, indicating that the cellular carbon and nitrogen were less labile during freezing (table 1). During the recovery, POC rebounded and exceeded the pre-freezing level after 7 days. Indeed, by day 28, POC increased to 554 μmol l^{-1}, which approaches the POC level after 30 days of continuous growth in the light (760 μmol l^{-1}; see table in the electronic supplementary material). $DMSP_p$ was measured for the initial and days 0, 14 and 28 of the recovery period. Similar to POC, $DMSP_p$ decreased by only 27 per cent, from 60 to 44 nmol l^{-1} during freezing (table 1). There was considerable variation in $DMSP_p$ during the recovery period, especially on day 14. Nonetheless, $DMSP_p$ rebounded and increased to greater than 700 nmol l^{-1} by the end of the experiment, which is higher than the $DMSP_p$ level attained under continuous growth (286 nmol l^{-1}; see table in the electronic supplementary material). F_v/F_m decreased from 0.60 (pre-freezing) to 0.28 during freezing, but quickly recovered to the pre-freezing level within 14 days of recovery, after which it declined slightly but remained high, at 0.47 towards the end of the experiment (figure 8).

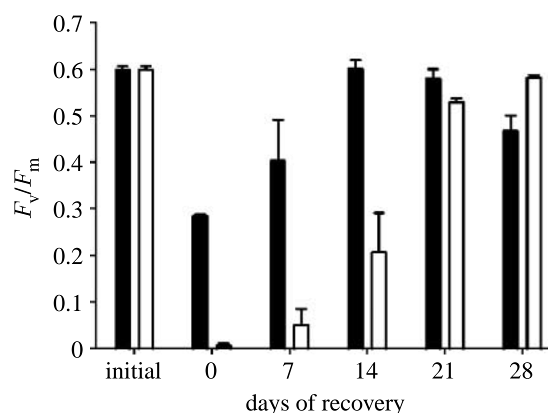

Figure 8. Maximum quantum yield (F_v/F_m) in 15 day (filled bars) and 30 day (open bars) freezing treatments (mean + s.d.; $n=2$ or 3).

(e) *Freeze–thaw experiment: FT_{30} treatment*

In the FT_{30} treatment, solitary cell and colony abundances after thawing were similar to the pre-freezing values (figure 5). However, unlike the FT_{15} treatment, there was no indication of an increase in solitary cell and colony abundances until 14 days after thawing. Size-fractioned chlorophyll decreased by an order of magnitude after freezing (figure 6). During the 28 day recovery period, total chlorophyll increased from 1.6 to 8.8 μg l^{-1}, whereas the greater than 20 μm chlorophyll increased from 0.7 to 1.4 μg l^{-1} (16% of the total). The calculated recovery growth rates were 0.02 to 0.06 d^{-1}, much lower than that in the FT_{15} treatment. The mean colony diameter significantly decreased by 33 per cent after 30 days of freezing (t-test; $p=0.003$), and remained low throughout the recovery (figure 7).

POC and PON decreased by 24–29% after freezing (table 1). More importantly, the recovery of POC and PON was much slower than in the FT_{15} treatment, and the POC concentration did not return to the pre-freezing level until day 14; further increments were also small. Unfortunately, we lost the $DMSP_p$ sample for day 0 recovery; thus, only data for days 14 and 28 are shown (table 1). Similar to the FT_{15} treatment, $DMSP_p$ showed a considerable amount of variation, although it clearly increased and reached greater than 2600 nmol l^{-1} by day 28. Because the corresponding increase in chlorophyll was relatively modest, $DMSP_p$ normalized to chlorophyll reached 10^4 nmol μg^{-1} on day 14 and 300 nmol μg^{-1} on day 28. Photosynthetic potential (F_v/F_m) was reduced to 0.005 after the freezing, and then it recovered but very slowly, and did not return to the pre-freezing level until day 28 (figure 8).

4. DISCUSSION

It is physiologically remarkable that *P. antarctica* assemblages not only survived but also resumed active growth and photosynthesis after long periods of darkness and freezing conditions. In the southern Ross Sea, among the most southern oceanic bodies of water on Earth, *P. antarctica* often dominates the spring blooms (Smith & Asper 2001), although its life history during the austral winter remains unknown. McMurdo Sound experiences up to five months of complete darkness, and it remains largely frozen for most of the year. Rich diversity and abundance of microbial organisms are frequently found within sea ice around the continent (e.g. Garrison *et al.* 1986; Archer *et al.* 1996; Delille *et al.* 2002), and in view of this, it is perhaps not surprising that our results show the ability of *P. antarctica* to withstand environmental extremes. Sea ice organisms not only remain viable, but at least a fraction of them remain metabolically active within the ice (Kottmeier & Sullivan 1990; Grossmann 1994). The presence of *P. antarctica* in sea ice in this region has also been observed (Garrison & Buck 1987;

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

Lizotte 2001; Garrison *et al.* 2003), but its physiological condition is unknown. Although the austral winter in McMurdo Sound lasts much longer than we simulated in this study, our results do suggest that *P. antarctica* could physiologically survive the extreme seasonal conditions and live within sea ice until the austral spring, when the residual might be released upon ice melting and the populations reseed the water column.

In the light treatment of the LD experiment, the estimated growth rate (0.17 d^{-1}) was lower than the growth rates reported by other investigators (0.41 d^{-1} by Smith *et al.* 1999; 0.21–0.27 d^{-1} calculated from fig. 2 of Sedwick *et al.* 2007). This was unlikely due to nutrient limitations in our incubation because the nitrate concentration was much higher than the typical value for the region (17.7–23.1 μM; Smith *et al.* 2006). Rather, light was likely to be limiting in our experiments when compared with *in situ* conditions (Smith *et al.* 2000). $DMSP_p$ normalized to chlorophyll was comparable with the values observed in *P. antarctica*-dominated waters in the southern Ross Sea (average 5.4 nmol μg^{-1}; calculated from DiTullio & Smith 1995). F_v/F_m in the light treatment was also at the high end of the range observed in the Ross Sea (Peloquin & Smith 2007), indicating that the phytoplankton was photosynthetically very active in the experiment and was not under micronutrient (i.e. iron) stress or limitation.

In the dark treatment, both colony abundance and colony size decreased over time. Applying the algorithms of Mathot *et al.* (2000), we estimated the colonial cell carbon and colonial mucous carbon concentrations, which decreased by 89 and 95 per cent, respectively, over the first 40 days. The higher reduction rate of mucous carbon suggests that *P. antarctica* is able to metabolize the colonial matrix as an energy reserve in darkness, similar to the observations by Lancelot & Mathot (1985) for *P. pouchetii*. The significant decline in F_v/F_m in the dark treatment indicates a parallel deterioration of the photosynthetic apparatus of the cells induced by darkness. This probably results from an absolute requirement of autotrophs for energy to maintain protein and structural integrity, proton gradients across membranes, and active enzymes and electron transport systems. However, while F_v/F_m declined quite rapidly in the first 40 days of dark incubation, it stabilized afterwards, and the final F_v/F_m (0.29) on day 60 indicates that the assemblage was still photosynthetically viable. A similar temporal trend was also observed in the high-frequency dark sampling. The gradual decrease in F_v/F_m with time in the dark incubation can be described by a simple exponential decay function with a threshold ($r^2=0.934$), although an exponential decay without a threshold also gives a significant regression ($r^2=0.922$). Although there is no *a priori* reason to select one function over the other, a function with a threshold implies the maintenance of some background F_v/F_m; indeed, the residual quantum yield after 150 days would equal 0.23, or approximately 40 per cent of the initial F_v/F_m. An F_v/F_m value of 0.23 is close to the minimum of *in situ* F_v/F_m observed in the Ross Sea in February (Peloquin & Smith 2007). Regardless of the representation of the decay of photosynthetic capacity, the results suggest that the *P. antarctica* assemblage would retain some minimal photosynthetic potential even after being in total darkness for the entire winter.

More remarkably, in the LD experiment, F_v/F_m quickly recovered upon return to light, and reached a level comparable with the initial value within 20 days. This fast recovery of photosynthetic capacity was in agreement with the recovery of chlorophyll (both total and greater than 20 μm) and POC/N, but not $DMSP_p$, suggesting that photosynthetic activity was more tightly coupled to chlorophyll and biomass syntheses than to DMSP production during the recovery, and that both solitary and colonial cells recovered at a similar rate. The estimated growth rate during the recovery was the same as that in the light treatment, which also confirms the ability of *P. antarctica* to quickly recover from prolonged darkness. Similar ability has been demonstrated by many other phytoplankton species (e.g. Furusato *et al.* 2004; Popels *et al.* 2007), especially polar species that naturally experience long periods of darkness (e.g. Palmisano & Sullivan 1983; Peters & Thomas 1996). By maintaining the ability to resume photosynthesis upon exposure to light, the cells have the ability to initiate growth in austral spring, even within the deep mixed layers that are commonly found in the Ross Sea. Other studies have clearly shown that *P. antarctica* is the first species to grow and accumulate in the austral spring in the Ross Sea, and that it can harvest low irradiance levels to do so (Arrigo *et al.* 1999; Moisan & Mitchell 1999; Van Hilst & Smith 2002); maintenance of a positive photosynthetic capacity after long periods of darkness could contribute to its ability to grow in the austral spring.

In both of the FT experiments, solitary cells recovered more rapidly than colonies in terms of abundance and chlorophyll. There was also a distinct difference between the FT_{15} and FT_{30} treatments. In the FT_{15} treatment, solitary cell abundance, total chlorophyll and POC/N increased and exceeded the pre-freezing levels after 28 days of recovery, whereas, in the FT_{30} treatment, all of these parameters recovered only modestly, with the exception of $DMSP_p$. In fact, the estimated recovery growth rate based on cell abundance and chlorophyll in the FT_{15} treatment was 0.17–0.18 d^{-1}, which is the same as the rate of growth under continuous light. By contrast, the recovery growth rate in the FT_{30} treatment was only 0.04 d^{-1} (average). Similarly, the quantum yield of *P. antarctica* decreased by 50 per cent after being frozen for 15 days, whereas an additional 15 days of freezing resulted in 98 per cent further reduction in F_v/F_m, lower than even the estimated F_v/F_m in total darkness for 150 days. Thus, it appears that freezing is physiologically more damaging than darkness to the photosynthetic apparatus, such as by disrupting membrane structures and enzymatic functions. The recovery of F_v/F_m was twice as fast when the phytoplankton was frozen for the shorter duration, indicating a longer lag in recovery with increasing duration of freezing. Such an increased lag might also occur in nature, where the freezing and extreme cold conditions can last longer.

However, it should be emphasized that our freezing conditions were different from those encountered in nature. For example, although under *in situ* conditions exchange is restricted, some exchange (either via tidal flushing or diffusion) does occur between the ice, brine and seawater. Furthermore, brine channels grow and migrate during the austral winter, and organisms can migrate to maintain themselves in this hypersaline (but liquid) environment. Our freezing treatment had no

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

exchanges, and also had much less brine channel (and hypersaline liquid) formation. Ice temperatures *in situ* during winter are also far colder than those we used (−11°C), and, at the surface, temperatures of ice can easily approach those of the air (approx. −60°C). By contrast, brine temperatures are much less extreme, but still could be lower than those we tested. Thus, our experimental conditions do not necessarily mimic winter *in situ* conditions, and the biological responses may not be completely analogous. Furthermore, the length of freezing we used (up to 30 days) was also quite different, as annual ice in the southern Ross Sea can exist for 150 days or more. While our results strongly suggest that *P. antarctica* can survive freezing, *in situ* observations remain necessary to confirm both the extremes in the environment as well as the biotic response.

In contrast to dark recovery, $DMSP_p$ increased rapidly in both the FT_{15} and FT_{30} treatments during recovery. We can only speculate on the reasons for this response. $DMSP_p$ is thought to function as a cryoprotectant, among other things, in polar microalgae (Kirst *et al.* 1991; Karsten *et al.* 1996). If this is its function, then the ability to produce and maintain DMSP would be particularly important for survival in an ice-covered environment such as the Ross Sea. High ambient $DMSP_p$ concentrations have been found associated with high *P. antarctica* biomass in the Ross Sea (DiTullio & Smith 1995; DiTullio *et al.* 2003). In the FT_{15} treatment, $DMSP_p$ decreased only modestly during the freezing process, and DMSP–carbon expressed as the percentage of total particulate carbon remained almost constant over the 15 days of freezing (0.32–0.33%, mol mol^{-1}). Thus, it appears that *P. antarctica* maintained a rather constant internal pool of DMSP during freezing. During the recovery, $DMSP_p$ production appeared to resume much faster than that of chlorophyll, particularly after 30 days of freezing, and subsequently very high $DMSP_p$ concentrations were observed. Recovery from darkness, however, shows the opposite trend: chlorophyll increased much more rapidly than $DMSP_p$. Because chlorophyll is required for harvesting light, and DMSP apparently is needed for cryoprotection, it is logical that the *P. antarctica* assemblages responded to darkness by increasing chlorophyll production during recovery, and responded to freezing by increasing DMSP during recovery. In our experiments *P. antarctica* was exposed to a constant level of stress (i.e. darkness or freezing), whereas, in the natural environment, the phytoplankton may experience fluctuations between short periods of darkness and freezing, e.g. during repeated formation and break-up of ice and transition between the austral autumn and winter. To study how *P. antarctica* responds to these fluctuations, one would have to subject *P. antarctica* to, instead of continuous darkness and freezing, cycles of light–dark and freeze–thaw conditions.

Our results, taken in total, suggest that *P. antarctica* has a number of adaptations to the harsh extremes it encounters in the southern Ross Sea. It can survive freezing and long periods of darkness, and also begin growth from both of these conditions relatively rapidly upon return to normal conditions. Indeed, our results suggest that these adaptations may make it well suited to survive the Antarctic winter in ice, and to initiate growth early in the following spring. The use of ice as a survival mechanism may not be unique, but clearly positions *P. antarctica* to grow at the outset of spring. Indeed, embedment in ice may allow *P. antarctica* and other species to maintain their populations at the surface and avoid vertical dilution due to deep winter mixing (mixed layers near the Ross Ice Shelf can be above 600 m by the end of winter; http://usjgofs.whoi.edu/jg/dir/jgofs/), and upon spring and release into the water column, can quickly resume growth.

If sea ice is an essential habitat that maintains *P. antarctica* populations and facilitates their growth during the following spring, then the effects of climate change might alter this ability to form large blooms in the Ross Sea. The Ross Sea sector (the region between 145° W and 165° E) over the past three decades has increased in ice concentration significantly (Kwok & Comiso 2002). However, this increase is not uniform over the entire region, and certain regions on the continental shelf in the Ross Sea have actually decreased slightly (and other regions on the shelf have increased slightly). If such trends in ice cover continue, changes in the habitat and distribution of *P. antarctica* can be expected, which will ultimately alter the success the species has as a planktonic form. It is impossible at this time to constrain the environmental changes that will occur in the coming years, but it can be expected that further increases in air temperatures will lead to a changed Ross Sea: earlier disappearance of ice; more rapid release of ice biota into the water; and a disruption of the coupling between ice and water. Despite our limited ability to predict the exact changes in the ecological interactions between biota and environment, such changes are likely to alter the regional ecosystem and energy transfers significantly, and generate an altered yet unknown Ross Sea in the decades to come.

We thank the support staff of Raytheon Polar Services and McMurdo Station, and all the participants of the ESCAP (Ecology of Solitary and Colonial Antarctic *Phaeocystis*) project. Funding was provided by National Science Foundation OPP grant ANT-0440478. A.R.S., an employee of the US Environmental Protection Agency (EPA), participated in this research prior to her employment with EPA. It was conducted independent of her EPA employment and has not been subjected to the Agency's peer and administrative review. Therefore, the conclusions and opinions drawn are solely those of the authors and are not necessarily the views of the Agency. This is VIMS contribution 2956.

REFERENCES

Antia, N. J. 1976 Effects of temperature on the darkness survival of marine microplanktonic algae. *Microb. Ecol.* **3**, 41–54. (doi:10.1007/BF02011452)

Archer, S. D., Leakey, R. J. G., Burkill, P. H., Sleigh, M. A. & Appleby, C. J. 1996 Microbial ecology of sea ice at a coastal Antarctic site: community composition, biomass and temporal change. *Mar. Ecol. Prog. Ser.* **135**, 179–195. (doi:10.3354/meps135179)

Arrigo, K. R., Robinson, D. H., Worthen, D. L., Dunbar, R. B., DiTullio, G. R., VanWoert, M. & Lizotte, M. P. 1999 Phytoplankton community structure and the drawdown of nutrients and CO_2 in the Southern Ocean. *Science* **283**, 365. (doi:10.1126/science.283.5400.365)

Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. 1997 Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl. Environ. Microbiol.* **63**, 3068–3078.

Bunt, J. S. & Lee, C. C. 1972 Data on the composition and dark survival of four sea-ice microalgae. *Limnol. Oceanogr.* **17**, 458–461.

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

Delille, D., Fiala, M., Kuparinen, J., Kuosa, H. & Plessis, C. 2002 Seasonal changes in microbial biomass in the first-year ice of the Terre Adélie area (Antarctica). *Aquat. Microb. Ecol.* **28**, 257–265. (doi:10.3354/ame028257)

Dennett, M. R., Mathot, S., Caron, D. A., Smith Jr, W. O. & Lonsdale, D. J. 2001 Abundance and distribution of phototrophic and heterotrophic nano- and microplankton in the southern Ross Sea. *Deep Sea Res. II* **48**, 4019–4037. (doi:10.1016/S0967-0645(01)00079-0)

DiTullio, G. R. & Smith Jr, W. O. 1995 Relationship between dimethylsulfide and phytoplankton pigment concentrations in the Ross Sea, Antarctica. *Deep Sea Res. I* **42**, 873–892. (doi:10.1016/0967-0637(95)00051-7)

DiTullio, G. R., Jones, D. R. & Geesey, M. 2003 Dimethylsulfide dynamics in the Ross Sea during austral summer. In *Biogeochemistry of the Ross Sea* (eds G. R. DiTullio & R. B. Dunbar). AGU Antarctic Reseach Series, pp. 279–294. Washington, DC: American Geophysical Union.

El-Sayed, S. Z., Biggs, D. C. & Holm-Hansen, O. 1983 Phytoplankton standing crop, primary productivity, and near-surface nitrogenous nutrient fields in the Ross Sea, Antarctica. *Deep Sea Res. A. Oceanogr. Res. Papers* **30**, 871–886. (doi:10.1016/0198-0149(83)90005-5)

Furusato, E., Asaeda, T. & Manatunge, J. 2004 Tolerance for prolonged darkness of three phytoplankton species, *Microcystis aeruginosa* (Cyanophyceae), *Scenedesmus quadricauda* (Chlorophyceae), and *Melosira ambigua* (Bacillariophyceae). *Hydrobiologia* **527**, 153–162. (doi:10.1023/B:HYDR.0000043198.08168.d3)

Garrison, D. L. & Buck, K. R. 1987 Algal assemblages in Antarctic pack ice and in ice-edge plankton. *J. Phycol.* **23**, 564–572.

Garrison, D. L., Sullivan, C. W. & Ackley, S. F. 1986 Sea ice microbial communities in Antarctica. *Bioscience* **36**, 243–250. (doi:10.2307/1310214)

Garrison, D. L., Jeffries, M. O., Gibson, A., Coale, S. L., Neenan, D., Fritsen, C., Okolodkov, Y. B. & Gowing, M. M. 2003 Development of sea ice microbial communities during autumn ice formation in the Ross Sea. *Mar. Ecol. Prog. Ser.* **259**, 1–15. (doi:10.3354/meps259001)

Garrison, D. L., Gibson, A., Coale, S. L., Gowing, M. M., Okolodkov, Y. B., Fritsen, C. H. & Jeffries, M. O. 2005 Sea-ice microbial communities in the Ross Sea: autumn and summer biota. *Mar. Ecol. Prog. Ser.* **300**, 39–52. (doi:10.3354/meps300039)

Gillard, R. R. L. & Ryther, J. H. 1962 Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.* **8**, 229–239.

Gleitz, M., Grossmann, S., Scharekm, R. & Smetacek, V. 1996 Ecology of diatom and bacterial assemblages in water associated with melting summer sea ice in the Weddell Sea, Antarctica. *Antarct. Sci.* **8**, 135–146. (doi:10.1017/S095410209600020X)

Grossmann, S. 1994 Bacterial activity in sea ice and open water of the Weddell Sea, Antarctica: a microautoradiographic study. *Microb. Ecol.* **28**, 1–18. (doi:10.1007/BF00170244)

Karsten, U., Kück, K., Vogt, C. & Kirst, G. O. 1996 Dimethylsulfoniopropionate production in phototrophic organisms and its physiological function as a cryoprotectant. In *Biological and environmental chemistry of DMSP and related sulfonium compounds* (eds R. P. Kiene, P. T. Visscher, M. D. Keller & G. O. Kirst), pp. 143–153. New York, NY: Plenum Press.

Kirst, G. O., Thiel, C., Wolff, H., Nothnagel, J., Wanzek, M. & Ulmke, R. 1991 Dimethylsulfoniopropionate (DMSP) in ice-algae and its possible biological role. *Mar. Chem.* **35**, 381–388.

Kottmeier, S. T. & Sullivan, C. W. 1990 Bacterial biomass and production in pack ice of Antarctic marginal ice edge zones. *Deep Sea Res. A. Oceanogr. Res. Papers* **37**, 1311–1330. (doi:10.1016/0198-0149(90)90045-W)

Kwok, R. & Comiso, J. C. 2002 Spatial patterns of variability in Antarctic surface temperature: connections to the Southern Hemisphere Annular Mode and the Southern Oscillation. *Geophys. Res. Lett.* **29**, 1705. (doi:10.1029/2002GL015415)

Lancelot, C. & Mathot, S. 1985 Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short-and long-term incubations with 14 C-bicarbonate. *Mar. Biol.* **86**, 219–226. (doi:10.1007/BF00397507)

Lizotte, M. P. 2001 The contributions of sea ice algae to Antarctic marine primary production. *Am. Zool.* **41**, 57–73. (doi:10.1668/0003-1569(2001)041[0057:TCOSIA]2.0.CO;2)

Mathot, S., Smith Jr, W. O., Carlson, C. A., Garrison, D. L., Gowing, M. M. & Vickers, C. L. 2000 Carbon partitioning within *Phaeocystis antarctica* (Prymnesiophyceae) colonies in the Ross Sea, Antarctica. *J. Phycol.* **36**, 1049–1056. (doi:10.1046/j.1529-8817.2000.99078.x)

Moisan, T. A. & Mitchell, B. G. 1999 Photophysiological acclimation of *Phaeocystis antarctica* Karsten under light limitation. *Limnol. Oceanogr.* **44**, 247–258.

Murphy, A. M. & Cowles, T. J. 1997 Effects of darkness on multi-excitation *in vivo* fluorescence and survival in a marine diatom. *Limnol. Oceanogr.* **42**, 1444–1453.

Palmisano, A. C. & Sullivan, C. W. 1983 Physiology of sea ice diatoms. II. Dark survival of three polar diatoms. *Can. J. Microbiol.* **29**, 157–160.

Peloquin, J. A. & Smith Jr, W. O. 2007 Phytoplankton blooms in the Ross Sea, Antarctica: interannual variability in magnitude, temporal patterns, and composition. *J. Geophys. Res.* **112**, C08013. (doi:10.1029/2006JC003816)

Peters, E. & Thomas, D. N. 1996 Prolonged darkness and diatom mortality: I. Marine Antarctic species. *J. Exp. Mar. Biol. Ecol.* **207**, 25–41. (doi:10.1016/S0022-0981(96)02520-8)

Popels, L. C., MacIntyre, H. L., Warner, M. E., Zhang, Y. & Hutchins, D. A. 2007 Physiological responses during dark survival and recovery in *Aureococcus anophagefferens* (Pelagophyceae). *J. Phycol.* **43**, 32–42. (doi:10.1111/j.1529-8817.2006.00303.x)

Sedwick, P. N., Garcia, N. S., Riseman, S. F., Marsay, C. M. & DiTullio, G. R. 2007 Evidence for high iron requirements of colonial *Phaeocystis antarctica* at low irradiance. *Biogeochemistry* **83**, 83–97. (doi:10.1007/s10533-007-9081-7)

Smayda, T. J. & Mitchell-Innes, B. 1974 Dark survival of autotrophic, planktonic marine diatoms. *Mar. Biol.* **25**, 195–202. (doi:10.1007/BF00394965)

Smith, W. O. & Asper, V. L. 2001 The influence of phytoplankton assemblage composition on biogeochemical characteristics and cycles in the southern Ross Sea, Antarctica. *Deep Sea Res. I* **48**, 137–161. (doi:10.1016/S0967-0637(00)00045-5)

Smith, W. O., Nelson, D. M. & Mathot, S. 1999 Phytoplankton growth rates in the Ross Sea, Antarctica, determined by independent methods: temporal variations. *J. Plankton Res.* **21**, 1519–1536. (doi:10.1093/plankt/21.8.1519)

Smith Jr, W. O., Marra, J., Hiscock, M. R. & Barber, R. T. 2000 The seasonal cycle of phytoplankton biomass and primary productivity in the Ross Sea, Antarctica. *Deep Sea Res. II* **47**, 3119–3140. (doi:10.1016/S0967-0645(00)00061-8)

Smith Jr, W. O., Dinniman, M. S., Klinck, J. M. & Hofmann, E. 2003 Biogeochemical climatologies in the Ross Sea, Antarctica: seasonal patterns of nutrients and biomass. *Deep Sea Res. II* **50**, 3083–3101. (doi:10.1016/j.dsr2.2003.07.010)

Downloaded from http://rspb.royalsocietypublishing.org/ on November 5, 2018

Smith Jr, W. O., Shields, A. R., Peloquin, J. A., Catalanao, G., Tozzi, S., Dinniman, M. S. & Asper, V. A. 2006 Interannual variations in nutrients, net community production, and biogeochemical cycles in the Ross Sea. *Deep Sea Res. II* **53**, 815–833. (doi:10.1016/j.dsr2.2006.02.014)

Song, W. & Wilbert, N. 2000 Ciliates from Antarctic sea ice. *Polar Biol.* **23**, 212–222. (doi:10.1007/s003000050029)

Stoecker, D. K., Gustafson, D. E., Merrell, J. R., Black, M. M. D. & Baier, C. T. 1997 Excystment and growth of chrysophytes and dinoflagellates at low temperatures and high salinities in Antarctic sea-ice. *J. Phycol.* **33**, 585–595. (doi:10.1111/j.0022-3646.1997.00585.x)

Tremblay, J. E. & Smith Jr, W. O. 2007 Primary production and nutrient dynamics in polynyas. In *Polynyas: windows to the world* (eds W. O. Smith & D. G. Barber), pp. 239–270. Amsterdam, The Netherlands: Elsevier.

Van Hilst, C. M. & Smith Jr, W. O. 2002 Photosynthesis/irradiance relationships in the Ross Sea, Antarctica, and their control by phytoplankton assemblage composition and environmental factors. *Mar. Ecol. Prog. Ser.* **226**, 1–12. (doi:10.3354/meps226001)