

Article

Variation in Survival and Growth Following Different Periods of Prolonged Darkness in a Polar Diatom

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Abstract

Phytoplankton are the major primary producers in the Southern Ocean, are important to global nutrient cycles, and are at the base of marine food webs. Polar ecosystems are unique in their extended periods of darkness in the winter, and prolonged darkness has the potential to exert selection that affects diatom population composition if there is differential survival in the dark, the variation in population growth rates in subsequent light periods, or both. We tested whether prolonged darkness has the potential to exert within-species selection on a model polar diatom species by exposing five strains of the polar diatom *Porosira glacialis* to prolonged darkness at two different temperatures in the laboratory. We measured population survival in the dark, growth rate upon re-illumination, and between-strain variability in these traits. We found a pronounced decline in survival and growth rate with time spent in the dark, as well as important intraspecific variation in the tested strains. Declines in survival and growth were exacerbated at a higher temperature. Our results show that the darkness of polar night can exert intraspecific selection in a common Southern Ocean diatom.

Keywords: diatom; polar night; genotype sorting; natural selection; population growth; Southern Ocean

1. Introduction

The Southern Ocean (SO) is an extremely productive habitat fuelled by photosynthetic microbes (phytoplankton). Diatoms are the most abundant group of phytoplankton in the Southern Ocean and make up over 40% of the phytoplankton biomass there [1], where they also play an important role in carbon uptake, biogeochemical cycles, and polar food webs. The SO is disproportionately important in global biogeochemical cycles, given its outsized contribution to biological carbon uptake and underlying nutrient cycling processes [2]. Nutrient export (N, P, and Si) from the Southern Ocean accounts for over 60% of nutrients exported to temperate and tropical latitudes [3,4], and impacts global primary production, ecosystems and fisheries. Since these processes are affected by the composition of diatom communities, understanding how selection shapes both inter- and intraspecific variation in polar diatoms is fundamental to understanding polar ecosystems.

A key driver of primary productivity is light [5], and springtime productivity in polar systems is dependent on the end of the polar night, where phytoplankton populations have just experienced a prolonged period of darkness that can reach up to 4 months depending on ice and snow cover [6,7]. Thus, polar diatoms must both survive prolonged periods of darkness and resume population growth upon re-illumination. Variation in dark survival



Academic Editor: Marcin Dębowski

Received: 28 April 2026

Revised: 18 June 2026

Accepted: 24 June 2026

Published: 10 July 2026

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and subsequent population growth in the light has the potential to drive differences in strain composition within species (and thus the average species trait values) and the species composition of diatom communities [8–10]. These changes in species and community composition can affect polar food webs and biogeochemical cycling if functional traits, such as cell size or lipid content, covary with overwinter survival or spring growth [6]. These shifts in diatom community composition can then affect rates of nutrient uptake and export, as well as the food value of diatoms for grazers [11,12].

Information on how diatoms survive weeks or months of very low or no light is rapidly growing. Diatoms and other polar phytoplankton have specific traits and strategies linked to surviving polar nights. These strategies include reducing metabolism, sexual reproduction and spore formation, and maintaining high photosynthetic efficiency to resume photosynthesis immediately once light becomes available [2,13]. Recent evidence from the polar diatom *Fragilariopsis cylindrus* suggests that diatoms reduce their metabolic rates and rely on lipid stores to survive dark periods [14]. In addition to surviving periods of darkness, a key strategy in diatoms' success at polar latitudes is their ability to resume photosynthesis almost immediately upon re-illumination, even at very low light intensities [10,15]. Maintaining the ability to photosynthesise during extended darkness gives polar diatoms a competitive advantage once light becomes available [9,16]. These and other mechanisms, reviewed in Berge et al. (2015) [17], paint an increasingly detailed picture of how phytoplankton survive through the polar night and grow following it. However, there is a lack of data on variation in dark survival and subsequent photosynthesis-driven population growth, and subsequent effects on diatom community composition [8], limiting our ability to link variation in polar phytoplankton traits, survival, and growth to potential variation in fitness in these taxa.

Studies to date have focused on how single strains survive in the dark. A detailed study by Joli et al. (2024) [14] shows how a wide range of physiological traits change during periods of darkness in a single strain of *Fragilariopsis cylindrus*, but does not address growth following the dark period. Van de Poll et al. (2020) [18] explore the relative survival of two Polar diatom species (*Thalassiosira antarctica*, *Thalassiosira nordenskiöldii*) and two flagellate species (*Rhodomonas* sp., *Micromonas* sp.) over an 8-week darkness period. They found that the diatoms have a higher survival than flagellates, which is consistent with diatoms having a competitive advantage once light becomes available. They also report differences between the two diatom species *T. antarctica* and *T. nordenskiöldii*, but lack the statistical power to explore the cause of this variation in more detail. In addition to variation in dark survival and subsequent population growth within and among species, there is variation in the length of dark periods that populations experience. This is due to differences in latitude and depth, as well as the timing and extent of ice formation and melt [19]. The latter of which is rapidly shifting in polar regions [7,19,20], as warming water and atmospheric temperatures are driving earlier ice melt and later ice formation, with lower overall ice coverage and thickness [21].

In this study, we examine how biological and environmental variation affect diatom dark survival and subsequent growth in the light, using five strains of the common Antarctic diatom *Porosira glacialis*. Single-strain populations were placed in the dark for up to three months at two different temperatures (well below and below but near T_{opt} for these strains). Over three months, they were subsampled five times, and at each sampling time, we measured cell survival and population growth rates upon re-illumination. We found that the proportion of viable cells in each population declines with the length of time spent in the dark. When populations are re-exposed to light, the population growth rate also declines with the length of time spent in the dark. These trends were amplified at the elevated temperature. We found intraspecific variation in survival and growth responses at

both temperatures, indicating that temperature can contribute to genotype sorting within polar diatom species; this intraspecific variation has the potential to drive changes in the composition of species during darkness and subsequent re-illumination. Studies focused on variation in fitness components inform our understanding of how populations evolve from standing variation in response to specific environmental cues, such as changes in the duration of dark periods.

2. Materials and Methods

2.1. Strain Collection and Cultivation

The diatoms used for this project were isolated from the Southern Ocean during a single cruise in 2017. Each strain was cultured from a single isolated cell, which ensured genetic homogeneity of each strain [22]. *Porosira glacialis* was chosen for this study because it was first found across many sampling stations, and is a relatively common centric diatom in the SO. Second, the culture collection had sufficient isolates of *P. glacialis* to conduct a study of intraspecific variation. Finally, the other characteristics of the *P. glacialis* strains used here are consistent enough that they all have $T_{\max}$ values above 5 °C [22], meaning that two temperature treatments could be used without any isolate being exposed to temperatures above their thermal optimum. The collected strains were continuously cultured since collection at 2 °C under 24 h light, with regular transfers into fresh F/2 media [23]. In preparation for the experimental treatment, 5 strains (Pg590, Pg595, Pg633, Pg654, and Pg716) of the diatom *P. glacialis* were acclimated to temperatures of 1 °C (low) and 4 °C (high), respectively, and 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity for 5 days prior to the beginning of the experimental treatments. Light intensity was monitored throughout (Apogee instruments, North Logan, UT, USA, model MQ-200). All cultures were actively growing and were below carrying capacity at the beginning of the experiment. Cultures had no visible signs of bacterial growth before, during, or after the experiment, but were not completely axenic. See flow cytometry measurements below for further details.

2.2. Experimental Design

This study was designed to measure variation in survival and growth attributable to intraspecific variation and the length of the dark period. This was conducted at two temperatures: below and near the optimal temperature for growth for this species [22]. To accommodate the replication needed, individual culture size was limited, which allowed the growth and monitoring of 60 independent cultures. All populations were grown as single-strain cultures. Experiments were initiated from cultures of approximately equal cell density for dark treatments (3500 cells/mL, ± 1000 cells/mL) and light treatments (350 cells/mL, ± 100 cells/mL). Cultures in the light treatment were started at lower cell densities, as these were in growth phases; they were propagated as semi-continuous cultures throughout the experiment to ensure they never reached carrying capacity (see Supplementary Materials S1, Table S1), with three replicates per strain per treatment. Populations were grown in non-vented culture flasks in 50 mL of F/2 media [23]. Dark treatment flasks were wrapped in three layers of tin foil. Three replicates of each strain-by-treatment combination were maintained in respective 4 °C or 1 °C incubators for the duration of the experiment. All flasks were mixed every 3 days to minimise the chance of sexual reproduction of diatom cells, avoid biofilm formation, and minimise the formation of environmental gradients. For a diagram of the experimental set-up, see Supplementary Materials S2, Figure S1.

Each culture was sampled approximately every 14 days for mortality assays over 2 months, and then at the end of 3 months of experimental treatment. Samples from each culture were re-inoculated in light conditions approximately every 10 days for the duration

of 2 months, and then after 2 months (precise timeline in Supplementary Materials S3, Figure S2). The population growth rates of the re-illuminated samples were measured to assess culture viability. At the end of the 3-month experiment, we opportunistically measured relative cellular neutral lipid content using flow cytometry on the remaining culture volume.

2.3. Mortality Assays

Mortality was assessed by counting live and dead cells from a sample of each culture. Cells were stained with a 1% Evans Blue dye solution made up to a final dye concentration of 0.02% in all sample aliquots, as in Samuels et al. (2019) [24], which was adapted for this species by using a staining time of 1 h, ± 15 min. Samples were concentrated 15-fold by centrifugation in an Eppendorf (Hamburg, Germany) Centrifuge 5424R (8 min, 800 rcf, 2 °C), mounted onto glass slides, and imaged under an Olympus BX43 inverted microscope connected to a Canon EOS800 camera using EOS Utility Software version 2.14. Live cells showed up as pale green or green–gold, while dead cells were stained light grey–blue to dark blue. Between 100 and 200 total cells were counted per sample, and the proportion of dead cells in each sample was calculated from live and dead cell counts. See Supplementary Materials S4 (Figure S3) for counting details and representative images of the stained cells.

2.4. Viability Assays

The population growth rate was measured in the light after maintenance in the dark, or in control cultures maintained in the light. An aliquot of each culture was inoculated into a well on a 24-well plate with fresh F/2 media to a total volume of 1.9 mL. Initial cell densities were measured by fixing cells with Lugol's iodine [25], concentrating 15-fold through centrifugation, and counting cells using Sedgewick Rafter or Neubauer chambers [26].

Post-dark cultures were grown at the temperature treatments they originated from, at $30 (\pm 5) \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ light intensities, with 24:0 days (24 h of light), to reflect the same conditions as the light cultures. Cells were counted on either a Neubauer chamber or a Sedgewick rafter, depending on concentrations, and the population growth rate was calculated as follows:

$$\mu = \ln(N_t/N_0) \times \Delta t^{-1}$$

where N_t is the cell concentration at the time of harvesting, N_0 is the cell concentration at the time of inoculation, and Δt^{-1} is the difference in days between the day of inoculation and the day of harvesting. Finally, μ values were corrected by the expected proportion of live cells at each timepoint, as calculated from the Evan's Blue live/dead proportions.

2.5. Flow Cytometry Measurements, Including Cell Size, Chlorophyll *a*, and Lipid Content

Following 3 months of experimental treatment, 5 mL aliquots of each culture were sampled and stained with BODIPYTM 505/515 (Thermo Fisher, Waltham, MA, USA), which reacts with neutral lipid stores of phytoplankton cells [27]. The fluorescence of each sample was measured using flow cytometry. Relative chlorophyll fluorescence and relative forward scatter (as a proxy for cell size) were measured by flow cytometry at the same time. At least 1000 cells of each sample were measured to estimate these variables.

Forward scatter and relative chlorophyll *a* (red autofluorescence, 488 nm wavelength, filter 695/40 BP) were measured using a BD FACSCANTO II.

Relative neutral lipid content was measured using BODIPYTM 505/515 (Thermo Fisher, Waltham, MA, USA) in the FITC-A channel (488 nm wavelength, filter 530/30 BP). Background (unstained) fluorescence was measured using the undyed sample. BODIPY was added to 200 μL of the sample to a final concentration of $2 \mu\text{g mL}^{-1}$, and then incubated in

the dark for 15 min before measurement with the flow cytometer. Neutral lipid content was determined as the difference in the median per-cell fluorescence between stained and unstained samples. To analyse neutral lipid content and variation, we gated the populations and extracted the Median and SD fluorescence values for BODIPY fluorescence using FlowJo software version 10.10.0 [28]. Gating information is provided in the Supplementary Materials S5.

The role of lipids in cell mortality and viability was analysed using mixed regression models to address four specific hypotheses:

H0. *Lipid content does not explain variation in mortality/growth rate upon re-illumination.*

H1. *Initial lipid content best explains variation in mortality/growth rate.*

H2. *Final lipid content best explains variation in mortality/growth rate.*

H3. *Lipid loss best explains variation in mortality/growth rate.*

We also extracted median relative chlorophyll fluorescence using FlowJo software version 10.10.0 [28]. We then tested the correlation with the variation in mortality using mixed regression models. More information is provided in the Supplementary Materials S6–S8 (Figures S4 and S5). Because changes in relative chlorophyll a do not explain the variation in mortality or growth in this study, they are not discussed further.

The cultures used in this experiment had minimal bacteria, but were not completely axenic. Bacterial populations were monitored by flow cytometry, and did not show any marked difference in abundance over the experiment or between treatments.

2.6. Statistical Analyses

Mortality data was analysed through logistic regressions using the following model:

$$\text{Prop. dead} = \text{intercept} + \beta_1 \times \text{temperature} + \beta_2 \times \text{light} + \beta_3 \times \text{day} + \beta_4 \times \text{temperature} \times \text{light} + \beta_5 \times \text{temperature} \times \text{day} + \beta_6 \times \text{light} \times \text{day} + \beta_7 \times \text{temperature} \times \text{light} \times \text{day} + \beta_8 \times \text{strain} + \text{Error}$$

Growth rate was analysed through a linear regression with the following structure:

$$\text{Growth rate} = \text{intercept} + \beta_1 \times \text{temperature} + \beta_2 \times \text{light} + \beta_3 \times \text{day} + \beta_4 \times \text{temperature} \times \text{light} + \beta_5 \times \text{temperature} \times \text{day} + \beta_6 \times \text{light} \times \text{day} + \beta_7 \times \text{temperature} \times \text{light} \times \text{day} + \beta_8 \times \text{strain} + \text{Error}$$

R and R Studio version 4.3.3 were used for all statistical analyses [29]. Data formatting and manipulation were conducted using the packages dplyr version 1.1.4 [30] and tidyr version 1.3.1 [31]. Statistical analyses were carried out using the packages lme4 version 1.1-36 [32] and lmerTest version 3.2-1 [33]. Graphs were created using ggplot2 version 3.5.1 [34].

3. Results

3.1. Cell Mortality

Because differential survival has the potential to impose selection during periods of darkness, we directly measured variation in mortality over time in our study. Overall, cell mortality was higher in the dark and increased with time; this effect was intensified at the warmer temperature. All strains followed this qualitative trend. Across all strains, there was a stable baseline mortality in the light for the first four timepoints, as expected in semi-continuous cultures that did not sit at the stationary phase. Timepoint 5 showed increased mortality in the light treatment, which is likely an artefact, as mortality is usually steady in standard cultures maintained under standard conditions. Analysing our data

with and without timepoint 5 did not change the trends or the statistical significance of the results that we report.

We found a significant increase in dead cells in the dark over time, including over the first four timepoints where the proportion of dead cells in the light was stable, at both temperatures ($Z = 4.08$, $p < 0.001$). In all strains, cells in the dark were approximately twice as likely to be dead than in the light at any given timepoint and treatment (Odds ratio = 1.97, $Z = 6.56$, $p < 0.001$). This is consistent with wider research that indicates elevated mortality in the dark [10,14,15,35–37]. We found a significant increase in dead cells in the dark over time ($Z = 4.08$, $p < 0.001$) (Figure 1).

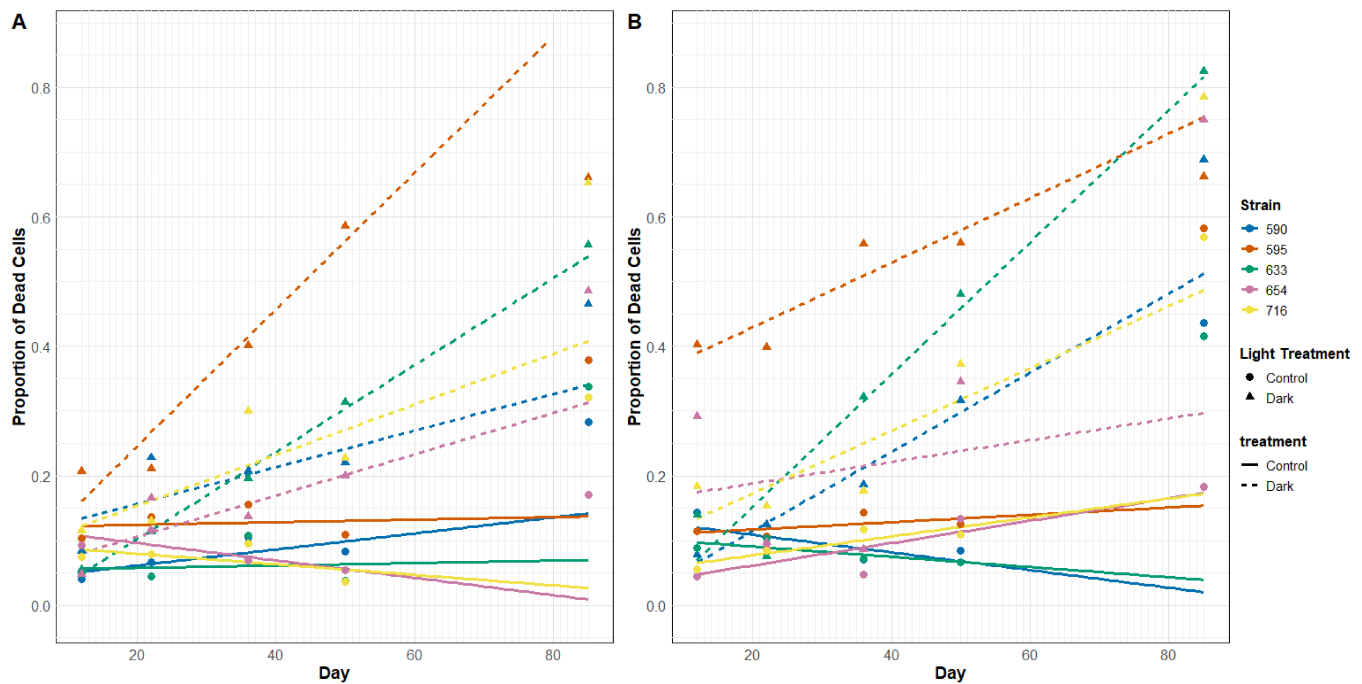


Figure 1. Proportion of dead cells over time of both light and temperature treatments. (A) Shows the proportion of dead cells in the control light cultures (round points and solid lines) and dark cultures (triangles and dashed lines) in the 1 °C treatment. (B) Shows the proportion of dead cells in the control light cultures and dark cultures in the 4 °C treatment. The colours of each line correspond to the 5 different strains of *Porosira glacialis*. Because a stable baseline proportion of dead cells in the light was observed for 50 days, we fit a stable baseline for control light treatments. Fits including timepoint 5 can be found in Supplementary Materials S9 (Figure S6).

There was significant variation in mortality between strains. When fitted as independently varying terms in our model, each strain had a p value < 0.05 . Strains Pg590 and Pg654 had consistently lower mortality, while strains Pg595 and Pg716 had the highest overall mortality. Strain Pg633 had the highest increase in mortality over time in the dark, in particular at 4 °C.

The variation in relative chlorophyll a content did not explain the variation in dark survival or subsequent growth. See the Supplementary Materials S6, for statistical details.

3.2. Population Growth Rate upon Re-Illumination

We measured the population growth rate upon re-illumination after different times spent in the dark. Overall, the population growth decreased after being maintained in the dark relative to cultures maintained in the light at the same temperature by an average of 0.0001329μ per day over all strains (Figure 2), ($t = -5.158$, $p < 0.001$, $DF = 288$). Populations maintained at the higher temperature (4 °C) grew faster than cells maintained at 1 °C ($t = 5.714$, $p < 0.001$, $DF = 288$). However, the growth rate decreased more rapidly over time

in populations maintained at 4 °C than at 1 °C ($t = -3.009$, $p = 0.003$, $DF = 288$) in both dark and light treatments (Figure 2). The decrease in the growth rate in the light treatment between timepoints 4 and 5 is likely an artefact, as previously mentioned, and excluding this data point does not alter the overall trends of our data.

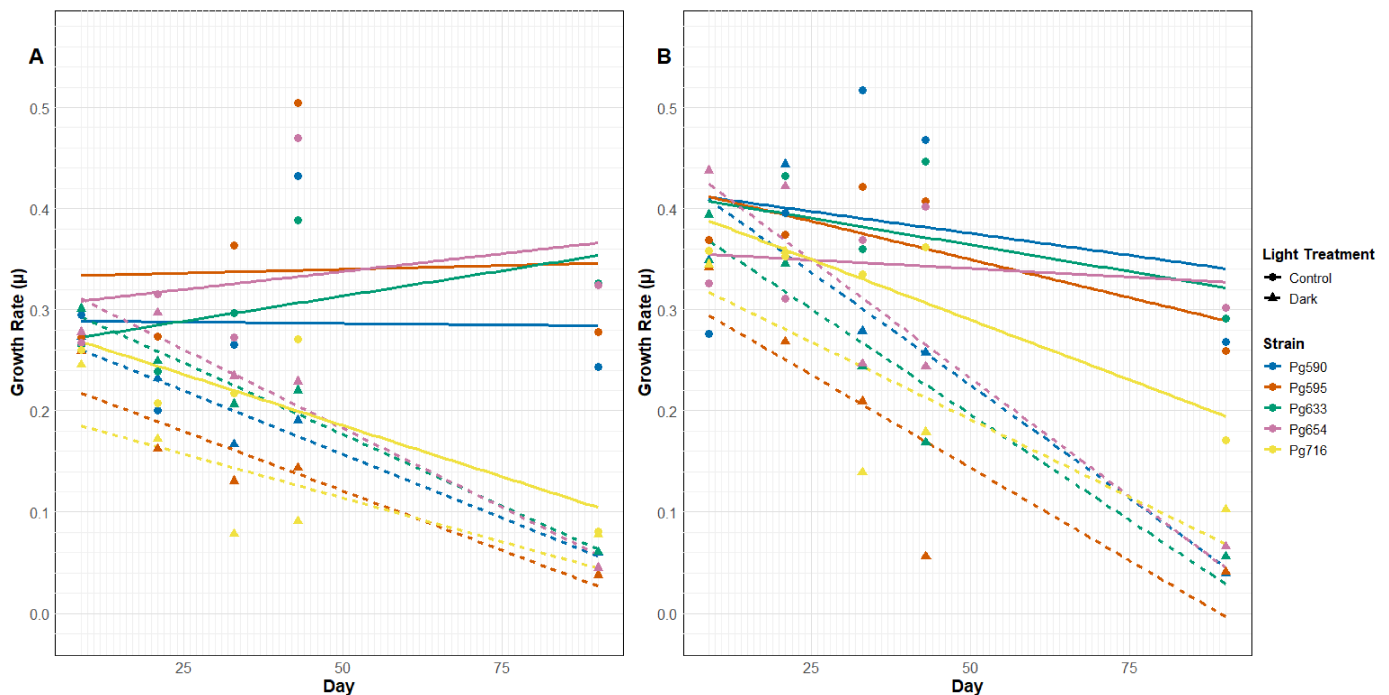


Figure 2. Growth rates upon re-illumination and re-inoculation of all temperature and light treatment combinations. (A) Shows the subsequent growth rates from the control light cultures (round points and solid lines) and dark cultures (triangles and dashed lines) in the 1 °C treatment. (B) Shows the subsequent growth rates from the control light cultures and dark cultures in the 4 °C treatment. The colours of each line correspond to the 5 different strains of *Porosira glacialis*. Solid lines represent control treatment, dashed lines represent dark-treatment.

The population growth rate upon re-illumination varied between strains. This effect was driven by a single strain, Pg 716 ($t = -4.989$, $p < 0.001$). And over both light treatments, strain explained little of the variance in the growth rate (~5% overall, and ~7% in the dark treatment), with most of the variance in growth upon re-illumination being due to the light treatments themselves (~23%).

Considering both our results for cell mortality and growth rate upon re-illumination, we found that both survival and growth rate upon re-illumination decreased significantly over time spent in the dark. Strain variation attributable to differences between strains was more important in mortality responses than in growth rate responses.

3.3. Lipid Content

Polar diatoms are unusually lipid-rich, which is put forward as a partial explanation of their ability to survive long periods of dark or low light [14,38,39]. To test whether variation in neutral lipid content explained survival at this taxonomic level, we measured neutral lipid content in our populations at the end of 3 months in the dark at both temperatures. Though being maintained in the dark depletes intracellular neutral lipid stores ($F = 440.3932$, $p < 0.001$, $DF = 56$) (see Supplementary Materials S10, Figure S7), as expected, lipid stores correlated poorly with both mortality and growth rate in this experiment for populations maintained in the dark. However, variation in lipid content in the dark in this experiment

was very low, suggesting that variation between, rather than within, species is more likely to underlie lipid-related mortality effects in polar diatoms.

For all results below, only populations maintained in the dark, and mortality and population growth upon re-illumination at the final timepoint, are considered, so that the below discusses how the absolute amount or concentration of neutral lipids explains variation in dark survival and growth only.

We considered the proportion of dead cells as a result of the median lipid concentration, strain, and temperature, including an interaction between lipid concentration and strain. This model best captured the variation in the data, with an R^2 of 54%.

Even when including median lipid concentration per cell in our analysis, temperature was the most important predictor of mortality ($t = 4.470$, $p < 0.01$), with an increase in mortality of 5.9% per degree and of 17.7% between the 1 °C and 4 °C treatments on average. We also found that strains differed in their baseline mortality within particular treatments when median lipid concentration was included as a predictor (Pg595: $t = -2.350$, $p = 0.029724$, Pg654: $t = -1.751$, $p = 0.096154$, and Pg716: $t = -2.004$, $p = 0.059531$). Finally, our model also suggests some variability in the mortality response to decreases in lipid concentration depending on strain.

Interestingly, we found that lipid concentration, calculated as the average lipid content per median cell spherical volume, may affect mortality at the final timepoint. Given the relatively low power of this study, we cannot discount that lipid concentration may have an effect on cell mortality, with our results suggesting that higher lipid concentration leads to a slightly lower proportion of dead cells (mortality) (effect size = -32.325 , $t = -1.986$, $p = 0.061682$). Given the statistical effect at even a very small study size, this association requires more study.

We also examined whether lipid content explained the variation in growth rate upon re-illumination. The variation in absolute lipid content or lipid concentration in cells maintained in the dark did not explain the variation in growth rates upon re-illumination at the end of the experiment. A model fitting a three-way interaction of cell size (using forward scatter as an approximation), strain, and temperature best explained the variation in the data (adjusted $R^2 = 79\%$) (see the Supplementary Materials S5; Growth rate Hypothesis Models). Here, size is an important predictor of growth rate upon re-illumination ($t = -3.454$, $p = 0.00618$) (Figure 3), with smaller cells having faster growth rates (estimate = 0.000003597 increase in growth rate per decrease in unit size). Temperature is another important predictor of growth rate upon re-illumination, with a 1 °C increase in temperature associated with a 0.2532 decrease in growth rate upon re-illumination ($t = -2.789$, $p = 0.01916$, $DF = 1$).

This statistical model detects variation between strains. Here, strain Pg716 has a baseline growth rate significantly lower than other strains (using strain Pg590 as the reference strain), with a baseline growth rate of 0.435μ being lower than strain Pg590 ($t = -2.622$, $p = 0.02552$, $DF = 4$). This model further indicates that strains Pg654 and Pg716 respond differently to an increase in temperature than other strains. The overall trend is for the growth rate to decrease with time spent at the higher temperature in the dark. These two strains showed less of a decrease in growth rate over time spent in the dark at the elevated temperature (strain Pg654: $t = 3.643$, $p = 0.00453$; strain Pg716: $t = 2.613$, $p = 0.02589$).

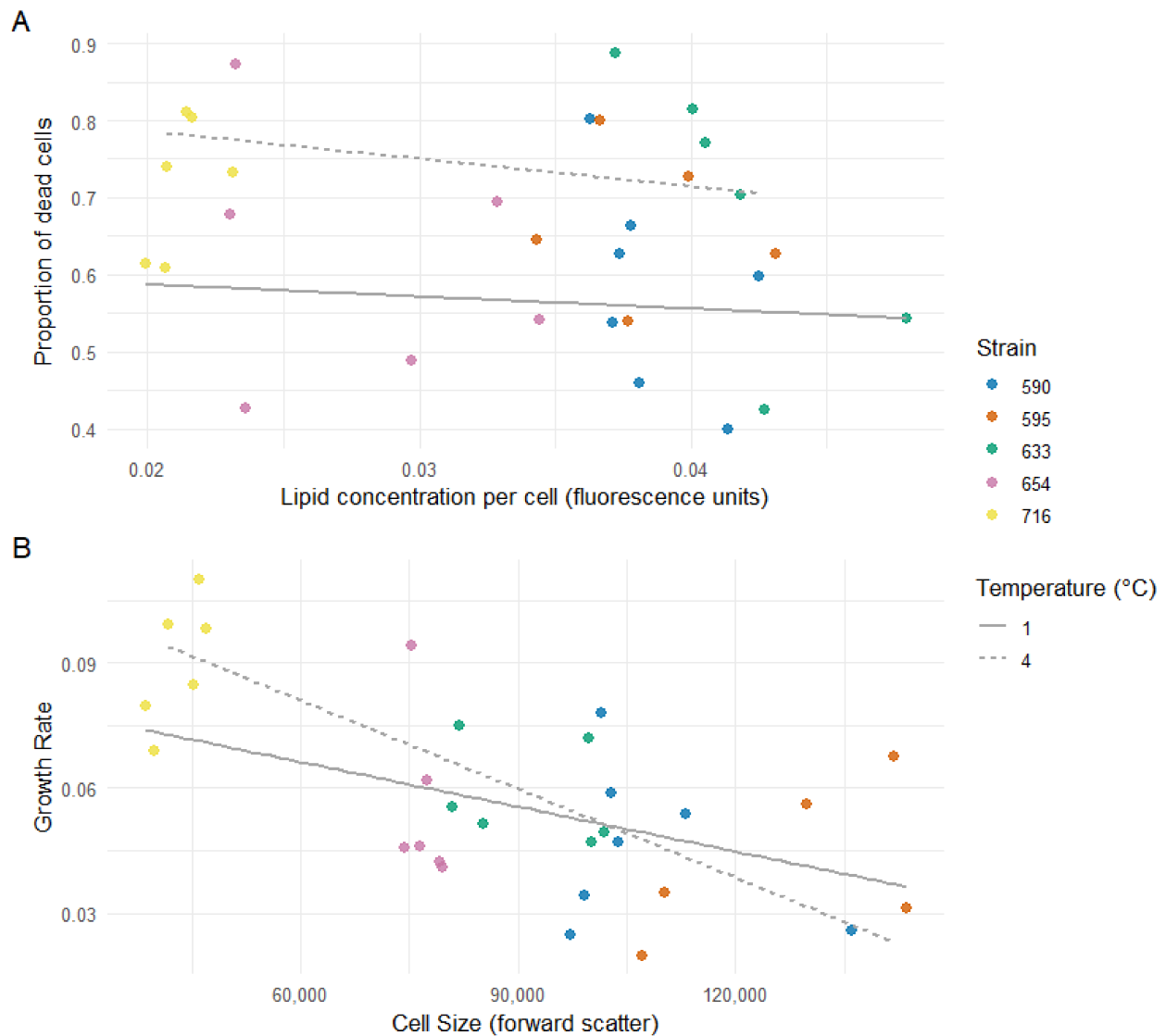


Figure 3. (A) Mortality against lipid concentration in *Porosira glacialis* cells. The solid line denotes cultures from the 1 °C temperature treatment. The dotted line denotes cultures from the 4 °C temperature treatment. All data concerns only cultures maintained in the dark. (B) The growth rate as a function of median cell size (forward scatter) of *Porosira glacialis* strains. The solid line denotes cultures from the 1 °C temperature treatment. The dotted line denotes cultures from the 4 °C temperature treatment. All data concerns only cultures maintained in the dark.

4. Discussion

In this study, we investigated intraspecific variation in mortality and growth rate upon re-illumination of five strains of *P. glacialis* subjected to different durations of darkness at two temperatures. We found that mortality increased with time spent in the dark, while the population growth rate upon re-illumination decreased. We found significant intraspecific variation in both mortality in the dark and growth rate upon re-illumination, suggesting that both dark survival and growth after dark have the potential to shape diatom populations through the action of natural selection.

The variation in mortality is consistent with the variation in one or more of the processes described in Joli et al. (2024) [14], where the polar diatom *Fragilariopsis cylindrus* survives polar nights by entering a state of hypometabolism, with respiration, consumption of cellular lipid stores, and autophagy providing the small amounts of energy required by the cells in this state. In contrast with previous studies, which found minimal mortality

during periods of darkness in polar diatoms in laboratory experiments in different species and under different culture conditions than those used in our study [10,15], we found relatively high mortality in the dark. The differences between our results and others point towards the need to better understand, first, intraspecific variation in responses and, second, how specific culture conditions other than darkness affect cell mortality in the dark. Here, we found higher mortality overall at a higher temperature, but other environmental factors, such as cell density, culture volume, and type of media used, may also play a role. In addition, since these cultures were not entirely axenic, bacterial activity differing between temperature treatments may also affect our results.

All populations grew when reintroduced into the light after a period of darkness, in line with previous studies [10,18,40,41], but their population growth rates decreased with time spent in the dark. In previous experiments, viability has generally been assessed using photosynthetic efficiency, and many of these studies report high photosynthetic efficiency following prolonged darkness [10,14,15,40,41]. The logic of linking photosynthetic efficiency with viability is the presumed ability to resume growth if the photosynthetic machinery is working, but it does not make precise predictions about how rapidly a population should grow after reintroduction into the light. In our study, we focused on the population growth rate upon re-illumination to understand how a key fitness component (population growth rate) varies with time spent in the dark. We did not find evidence that variation in chlorophyll *a* content explained variation in the survival or growth of strains exposed to darkness. This may indicate that while a decrease in chlorophyll is associated with dark survival in general, variation in this trait is low, at least within species.

The variation in growth rates upon illumination measured here is sufficient to drive genotype sorting [42]—natural selection acting on pre-existing variation in fitness—during a short diatom bloom. The effect of growth rate variation in the light driven by time previously spent in the dark on genotype composition is illustrated in Figure 4. The narrow bars in panels A and B show the projected composition of a 14-day bloom when dark-driven changes to growth are taken into account, whereas C and D show the composition for a 14-day bloom from the same starting point when dark-driven changes to growth rate are not taken into account. This variation in growth upon re-illumination affects projections of the strain composition of the species. For example, after 85 days in the dark at 1 °C, strain Pg595 consistently showed the highest mortality, while strains Pg654 and Pg590 had just over half as much mortality by the end of the 3-month dark period as Pg595. By the end of the dark period, the proportion of Pg595 in the community is lower than at the beginning of the dark period (Figure 4A,C). If growth in the light is projected without taking a period of darkness into account, the proportion of Pg595 further decreases due to relatively low growth (Figure 4C). In contrast, if the effect of dark exposure on subsequent growth in the light is taken into account, Pg595 makes up a larger proportion than expected of the post-dark bloom due to relatively rapid growth after darkness (Figure 4A). Differences in how strains of the same species of polar diatom survive prolonged periods of darkness (Figure 2) suggest that the polar night can act as a selective event that shapes polar diatom communities [12,38,43], and that growth rates after periods of darkness differ from growth rates without dark exposure. Both variation in mortality and in subsequent growth in the light measured here have the potential to drive genotype sorting in a common polar diatom species; the combined effects on survival and viability indicate the potential for large darkness-driven differences in diatom community composition between autumn and spring in the Southern Ocean.

The differences in cell survival and population growth rate following a period of darkness are exacerbated by the effect of temperature in this study. At the elevated temperature treatment (4 °C), the average mortality was both higher and the growth rate on

re-illumination was lower than in the low temperature treatment (1 °C). Elevated temperature tends to increase metabolic activity [22,44], and it is plausible that higher mortality at 4 °C is driven by cells having higher metabolic activity at warmer temperatures than at cooler ones, which in turn affects their dark survival [14,39]. The rank order of strains' growth rates also changed between the 1 °C and 4 °C treatments, indicating that temperature not only exacerbates the effects of the polar night, but it may independently change community composition since strains differ in their tolerance to warming, as illustrated in Figure 4. This suggests that diatom community composition is not only shaped by a strain's ability to survive and resume growth following the polar night, but also by the temperatures these seasonal phenomena are experienced at.

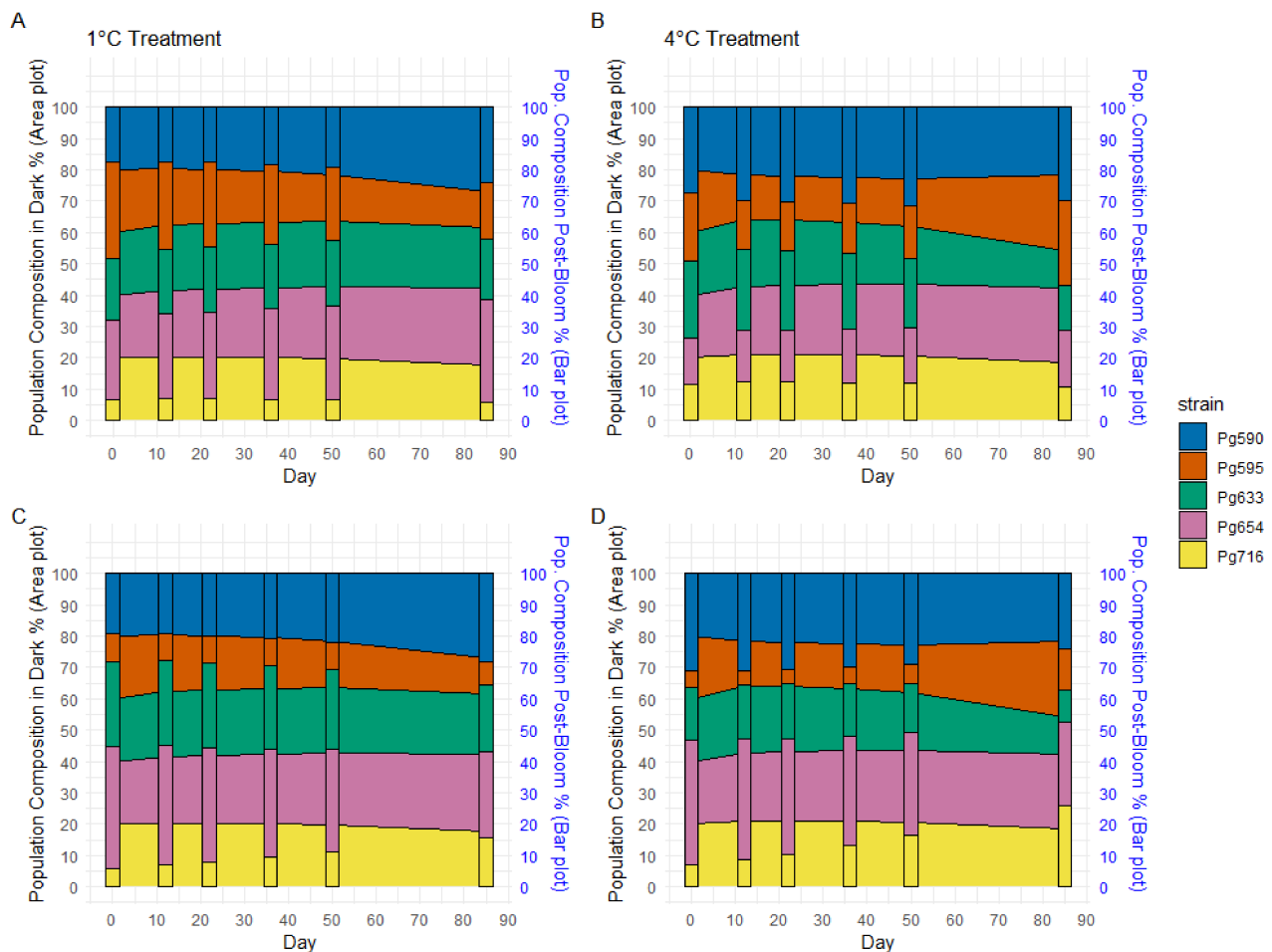


Figure 4. Projected population composition over time spent in the dark (main graph) and following a 14-day bloom (narrow bars), starting after the time indicated in the dark and the corresponding population composition. Panels (A,C) show the 1 °C treatment, panels (B,D) show the 4 °C treatment. The area plot shows changes in population composition by strain over time spent in the dark in all panels, where changes in population composition are due to differential mortality in the dark. The bar chart (narrow bars) corresponds to the population composition following a 14-day bloom after reintroduction to the light on the day indicated. This is calculated from the projected growth rates using the average strain growth rate after maintenance in the dark for the indicated duration in panels (A,B) and the projected growth rates using the overall average strain growth rates when maintained in the light in panels (C,D).

Cell survival over prolonged dark periods may be mediated by their ability to enter a state of hypometabolism and the consumption of cellular stores [14,38,39,44]. Based on this, we conducted a post hoc experiment that measured lipid content after ~3 months

of darkness in the *P. glacialis* populations. Consistent with this, diatoms from the dark treatment in our experiment depleted their cellular lipid stores compared to diatoms in the light treatment [44] (see Supplementary Materials S10, Figure S7). However, we found little evidence that variation in lipid content explains the variation in strains' ability to survive prolonged darkness or subsequent growth. Instead, temperature emerged as the best predictor of mortality in the lipid and mortality model, indicating that it is the strain's differential ability to tolerate elevated temperatures that drives variation in mortality in this experiment.

Relative neutral lipid content correlated poorly with cell growth after re-illumination, and intraspecific differences in this effect are explained mainly by cell size ($t = -3.454$, $p = 0.00618$; 40% of variance in the model) and temperature interaction with strains (explains 16% of variance in the model). There was a weak correlation between relative lipid concentration and mortality ($t = -1.986$, $p = 0.061682$). However, this measurement was made at a single timepoint and within a single species, and variation in lipid content was low. This is a suggestive result given the low power of this experiment to detect it. Further explorations of the role of lipid content in mortality should be conducted using a greater range of lipid content, and by studying both neutral and polar lipids.

To the best of our knowledge, this is one of the few studies that estimated mortality through direct counts of live and dead cells rather than proxy measurements, such as cell concentrations or chlorophyll fluorescence [10,18]. To the best of our knowledge, only the study in [36] directly assesses cell integrity to investigate survival, and similarly found large variation in this between strains. This approach allows us to avoid potential confounding variables, such as a final cell division in the dark (See Supplementary Materials S11, Figure S8), varying rates of chlorophyll degradation between strains and treatments [36], or the preservation of intact but dead cells in cold systems. The overall trends based on this approach agree with wider research, which supports decreasing survival over long periods of darkness [10,14,15,36]; however, attrition here was higher and more variable than previously reported.

One key methodological consideration is that our experiment used complete darkness. Recent evidence suggests that even minimal levels of light are sufficient to maintain baseline activity of photosystems [41] and that very faint light signals can be detected under water, even during the polar night [45]. Using complete darkness to interrogate responses to polar nights has proven to be a useful starting point, and comparing responses in complete darkness and under prolonged periods of extremely low light would contextualise results from our and similar experimental set-ups. While laboratory experiments are not realistic and do not draw their utility from realism in terms of their representation of environments outside of the laboratory, understanding the environmental simplifications used is vital to making causal links between environmental cues and organismal responses. We suggest testing whether complete darkness is a suitable laboratory simplification for the very low light levels of polar winters in future work. Similarly, our experiment used light conditions with no darkness; this establishes an extreme and replicable starting point for understanding, which can be expanded to include day length and gradual illumination effects. There is a necessary trade-off between experiments that use a large number of environmental conditions and those that use many replicated strains. Both types of studies are needed to establish organismal responses to environmental variation and biological variation in those responses.

Our results demonstrate substantial intraspecific variation in both dark survival and population growth rate upon re-illumination in *Porosira glacialis*. This highlights the potential of the polar night to be a selective event between and within species of diatoms. The interaction between dark duration and temperature further amplifies intraspecific vari-

ability in our study, indicating that warming has the potential to modify not only overall mortality and viability, but also the frequency and relative performance of strains emerging from winter. Together, our findings emphasise the importance of considering intraspecific variability and demographic responses, rather than single strains, when predicting how polar diatom populations and microalgal communities might respond to seasonal darkness and ongoing ocean warming.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/phycolgy6030073/s1>.

Author Contributions: P.M. and S.C. designed the research. P.M. collected the data. P.M. and S.C. analysed the data. P.M. and S.C. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by a British Phycological Society Grant, 2023_BPS_0234, to S.C. and the University of Edinburgh MSc programme in Ecology, Evolution and Biodiversity.

Institutional Review Board Statement: Not applicable; this study did not involve humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data, code, and generated datasets is available at datashare.ed.ac.uk under the same title as the manuscript.

Acknowledgments: We thank Shravan Raghu, Simin Gao, and Katharin Balbirnie Cumming for laboratory assistance and training.

Conflicts of Interest: The authors declare no competing interests.

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