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Long-Term Experiment on Physiological Responses to Synergetic Effects of Ocean Acidification and Photoperiod in the Antarctic Sea Ice Algae *Chlamydomonas* sp. ICE-L

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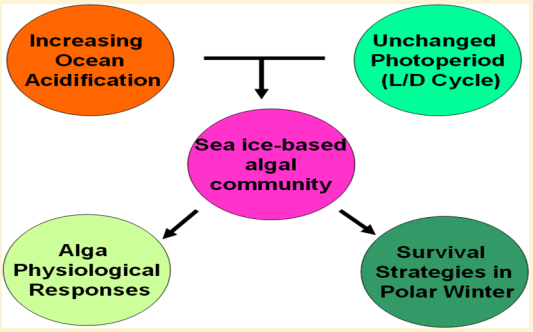
Supporting Information

ABSTRACT: Studies on ocean acidification have mostly been based on short-term experiments of low latitude with few investigations of the long-term influence on sea ice communities. Here, the combined effects of ocean acidification and photoperiod on the physiological response of the Antarctic sea ice microalgae *Chlamydomonas* sp. ICE-L were examined. There was a general increase in growth, PSII photosynthetic parameters, and N and P uptake in continuous light, compared to those exposed to regular dark and light cycles. Elevated pCO_2 showed no consistent effect on growth rate (p = 0.8) and N uptake (p = 0.38) during exponential phrase, depending on the photoperiod but had a positive effect on PSII photosynthetic capacity and P uptake. Continuous dark reduced growth, photosynthesis, and nutrient uptake. Moreover, intracellular lipid, mainly in the form of PUFA, was consumed at 80% and 63% in low and high pCO_2 in darkness. However, long-term culture under high pCO_2 gave a more significant inhibition of growth and F_v/F_m to high light stress. In summary, ocean acidification may have significant effects on *Chlamydomonas* sp. ICE-L survival in polar winter. The current study contributes to an understanding of how a sea ice algae-based community may respond to global climate change at high latitudes.



INTRODUCTION

Ocean acidification, derived from anthropogenic CO_2 emissions, is a major environmental issue of the 21st century.[1−5] According to the Intergovernmental Panel on Climate Change (IPCC), the atmospheric partial pressure of carbon dioxide (pCO_2) has increased from 285 ppm in the preindustrial age to the current level of 384 ppm and will approach ∼1000 ppm by 2100.[6] While an ocean uptake of ∼25% of anthropogenic CO_2 provides an invaluable service by mitigating CO_2-related global warming, unabated CO_2 emissions are likely to cause a 100−150% increase in the hydrogen ion concentration and a 0.3−0.4 drop in the pH of surface waters.[7,8] Ocean acidification, accompanied by substantial warming, is projected to threaten the biodiversity and function of marine ecosystems, from the deep sea to coastal estuaries and from the tropical oceans to polar regions.[9−13]

Recent laboratory and field studies have revealed that organisms with calcareous shells or exoskeletons may be unable to function as oceans acidify over the next 100 years.[14−17] However, some non-calcifying phytoplankton taxa, such as nitrogen-fixing cyanobacteria or diatoms, may benefit from the increasing CO_2 levels in seawater.[18−20] Minor effects of ocean acidification on the growth of several macroalgae were also found.[21] Despite an increasing interest in the understanding of the impacts of elevated CO_2 concentrations on a wide range of marine organisms, research has primarily focused on low latitude regions and on short time scale studies.[22] It is still not possible to make meaningful projections of the impacts on high latitude marine environments, which are particularly vulnerable to ocean acidification due to the high solubility of CO_2 in cold waters.[22,23] Moreover, most results are based on single stressor studies, and a comprehensive understanding of the synergistic effects of the main climate variables on ocean productivity during longer time scales is lacking.[24−26]

Phototrophic organisms in Antarctic oceans usually experience strong seasonal light variation, which is characterized by long periods of continuous light during polar summer or total darkness during the polar winter.[27] The large variation in incident solar radiation requires considerable physiological adaptations for polar organisms to survive.[28,29] These organisms rely on a variety of species-specific strategies to survive the long periods of darkness. The production of cysts or resting cells, adjustment of metabolic rates, or increasing

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metabolic diversity have all been recognized as important overwintering strategies for both heterotrophic and autotrophic organisms.[30,31] Although such acclimation mechanisms have been adapted by polar organisms to survive longtime darkness in winter, additional global climate change co-stressors may challenge them in the future.

McMinn and Martin[32] reported that although Arctic and Antarctic phytoplankton are able to survive temperature increases of up to 6 °C in the dark, elevated temperatures in winter reduces the viability of some polar microalgae.[32] Here we examine whether they can also survive in an increasingly acidified ocean. Shadwick et al.[33] compared the complete annual cycles of the CO_2 system between Arctic and Antarctic seasonal cycles and demonstrated that the Arctic system is more vulnerable to anthropogenic change due to lower alkalinity, enhanced warming, and nutrient limitation. Torstensson et al.[23] also showed that polar oceans are particularly susceptible to ocean acidification and warming and that elevated pCO_2 and temperature caused a synergistic effect on the growth and lipid profiling of the Antarctic sea ice diatom *Nitzschia lecointei*. Nevertheless, to our knowledge, the physiological response of polar organisms to survive winter darkness under ocean acidification is poorly understood.

Chlamydomonas sp. ICE-L is one of the green microalgae that can thrive in extreme environments in the Antarctic.[34] Despite extensive research showing that it can undergo specific ecological and physiological adaptations to harsh habitats, including low temperature, intense solar irradiance, excessive UV radiation, and nutrient depletion, its survival strategy to overwinter is mostly lacking.[35,36] Even less investigation has been undertaken on the impacts of climate change on its adaptation mechanism during winter. Under this scenario, we aim to present a comprehensive overview of multiaspect cellular events associated with prolonged dark treatment under current and ocean acidification conditions in the Antarctic microalgae *Chlamydomonas* sp. ICE-L. Moreover, to test physiological differences of reacclimated microalgae under light, we examined and compared photosynthetic and growth responses to stressor between samples treated in current and elevated CO_2.

EXPERIMENTAL DETAILS

Sampling and Culture Conditions. The Antarctic ice microalgae *Chlamydomonas* sp. ICE-L was isolated from pack ice near the Zhongshan Research Station of Antarctica (69.8°S, 77.8°E) and grown axenically in Provasoli seawater medium.[37] Cultures were incubated with aeration in a plant growth chamber (GXZ, Ruihua, Wuhan, China) at 5 ± 1 °C with a 12 h:12 h light:dark (L:D) photoperiod. During light periods, photosynthetic actinic radiation (PAR), which was provided by a 17 W, cool-white lamp, averaged approximately 40 μmol photons m^{-2} s^{-1}. When exponential growth phase was reached, the cultures were incubated in fresh culture medium that was bubbled with low (pCO_2 = 390 μatm, current CO_2 levels) or high CO_2 (pCO_2 = 1000 μatm, predicted CO_2 levels in 2100). The corresponding pH of the culture medium was 7.68 and 8.02 respectively, which was monitored with a pH meter (Orion ROSS, Thermo Electron Corp., Beverly, MA, USA). Parameters in the carbonate system were calculated using the CO2SYS Package[38] based on the pH, temperature, salinity, and total alkalinity (TA). TA was measured using a 848 Titrino plus automatic titrator (Metrohm, Riverview, FL, USA) on 100 mL of GF/F filtered samples.

Experimental Setup. In the first experiment (Experiment I, EI), conducted to explore the combined effects of photoperiod and partial pressure of CO_2 (pCO_2) on the physiological responses of *Chlamydomonas* sp. ICE-L, six combination treatments were implemented: low pCO_2 and regular light and dark cycle (**LLD**), low pCO_2 and continuous light (**LLL**), low pCO_2 and continuous darkness (**LDD**), high pCO_2 and regular light and dark cycle (**HLD**), high pCO_2 and continuous light (**HLL**), and high pCO_2 and continuous darkness (**HDD**). The experimental treatments were set up with a flask and tubing system similar to Zou and Gao.[39] All treatments were administered in triplicate. Cultures in exponential growth phase were inoculated into 5000 mL Erlenmeyer flasks containing 3000 mL of acidified Provasoli seawater medium. The initial cell concentration used for the experiment was 2.2×10^5 cells mL^{-1}. The low and high pCO_2 levels were manipulated in a CO_2 chamber, which was programmed to supply 390 μatm and 1000 μatm CO_2 by bubbling. The regular light and dark cycle (12 h/12 h) was switched at 9:00 a.m. and 21:00 p.m.; the continuous light was set at 20 μmol photons m^{-2} s^{-1} to maintain the same irradiance as the ambient photoperiod. Flasks in continuous darkness were wrapped in aluminum foil to prevent light penetration. Six controls, prepared acidified Provasoli seawater medium but without algal cultures under each treatment combination, were used to monitor the stability of the pH in the culture systems. Experiment I ran for 28 days. During this period, physiological parameters including growth, PSII photosynthetic activity, and nutrient uptake rate were determined by sampling at 0, 1, 3, 5, 7, 14, 21, and 28 days. In addition, a further 2 cultures (3 replicates) were incubated in darkness under low (LDD) or high pCO_2 (HDD) to examine the lipid profile changes during the experiment; 400 mL cultures were sampled for lipid and fatty acid analysis at 0, 7, 14, and 28th day.

A second experiment (Experiment II, EII) was set up to investigate the photosynthetic responses of *Chlamydomonas* sp. ICE-L to stressor after reacclimation to light. HDD and LDD cultures in EI were supplemented with fresh culture medium and reacclimated in regular light and dark cycle under low or high pCO_2 conditions. Cells treated in LDD and reacclimated in low pCO_2 or high pCO_2 were marked as LDDL or LDDH, respectively. Cells treated in HDD were reacclimated only in high pCO_2 and marked as HDDH. All treatments were cultured semicontinuously for 28 days until they reached exponential phase at a cell concentration of 8.6×10^5, 8.9×10^5, and 9.1×10^5 cells mL^{-1}, respectively. A 100 mL sample of each treatment was exposed to high light of 400 μmol photons m^{-2} s^{-1} for 12 h. PSII photosynthetic performance and cell numbers were monitored during the treatments.

Growth Measurements. A 0.5 mL sample in EI was collected at each sampling point and preserved in Lugol's solution to estimate microalgal growth by directly counting cells using a hemocytometer under an optical microscope (Nikon, Tokyo, Japan). In EII, cells were first dyed with trypan blue (Solarbio, Cat. No. C0040, Lot. No. 20110505) and then counted microscopically using hemocytometer to check cell viability according to Gao et al.[40]

Chlorophyll Fluorescence Measurements. Variable in vivo chlorophyll fluorescence was measured using the pulse amplitude modulated (PAM) technique and a Dual-PAM-100 (Walz, Effeltrich, Germany) instrument. The minimum (F_0) and maximum fluorescence (F_m) was measured after a dark acclimation period of 15 min. The F_m yield of an illuminated

sample was denoted as F_m' and F_t was the real-time fluorescence yield. The PSII maximum quantum yield was calculated as F_v/F_m, where F_v is the variable fluorescence emission ($F_m - F_0$).[41] The relative electron transport rate (rETR) was calculated as rETR = Y(II) × PAR × 0.5, where PAR is the photosynthetic actinic radiation and 0.5 assumes that half of the absorbed light is distributed to PSII.[41] The rapid fluorescence light-response curve (LRC) was measured by exposing the samples to 10-step increasing irradiance (8 up to 827 μmol photons m^{-2} s^{-1}) at intervals of 30 each seconds. From LRC, light utilization efficiency (α) was obtained.

Inorganic Nutrients. The concentration of dissolved inorganic nutrients NO_2^{-1}, NO_3^{-1}, NH_4^+, and PO_4^{3-} was analyzed photometrically using an AutoAnalyzer (BRAN and LUEBBE AA3, Germaby). Nutrient uptake rates were calculated as follows: NUR = $(C_0 - C_t)V/N/t$, where NUR is the nutrient uptake rate (μmol $cell^{-1}$ h^{-1}); C_0 and C_t are the nutrient concentrations (μmol L^{-1}) at the beginning and the end of the experiment, respectively; V is the volume of water (L); N is the average cell numbers (cells), and t is the time interval (h). The nutrient removal efficiency (NRE, %) was calculated as follows: NRE = 100 − (100 × C_t/C_0).

Total Lipid and Fatty Acid Analysis. The total lipid contents of *Chlamydomonas* sp. ICE-L at sampling time under different pCO_2 were analyzed by the gravimetric method,[42] and intracellular lipid droplets were strained and detected with lipophilic fluorescent dye BODIPY 505/515 (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene; Invitrogen Molecular Probes, Carlsbad, CA) according to Xu et al.[43] For the fatty acid analysis, it was performed as described by An et al.[34]

Statistical Analysis. The significance of the variance between treatments was analyzed using two-way ANOVA or repeated measures ANOVA by the software SPSS 17.0 (SPSS, Chicago, IL, USA). Post hoc tests were examined using Tukey's test for two-way ANOVA and Dunnett's test for multivariate ANOVA. The assumptions of homogeneity of variance and normality were assessed by scatter plots of residuals and normal curves of residuals, respectively. All tests were run using the software SPSS 17.0. The significance level was set at 0.05 for all tests unless otherwise stated.

RESULTS

Variation of Carbonate System in Experiment I. The variation of parameters of the seawater−carbonate system including pH, DIC, HCO_3^-, CO_3^{2-}, CO_2, and pCO_2 under different treatments was monitored and is presented in Supplementary Figure S1. The initial corresponding pH under pCO_2 of 390 and 1000 ppm was 8.02 ± 0.08 and 7.68 ± 0.06. Due to the density of the cultures, the carbonate chemistry of culture was difficult to maintain. During the culture period, there was a variation of the carbonate system to a certain degree. pH varied from initial value 8.02 ± 0.08 to 8.18 ± 0.15, 8.74 ± 0.01, and 8.28 ± 0.01 under treatment of LLD, LLL, and LDD. Under high pCO_2 treatment, it changed from 7.68 ± 0.06 to 8.10 ± 0.02, 8.11 ± 0.01, and 8.16 ± 0.03 under treatment of HLD, HLL, and HDD. However, although these variations occurred, a clear difference of pH (df = 18, F = 35.370, $p < 0.001$) between low and high CO_2 cultures was maintained throughout the experiment. Therefore, the results are reliable and have guiding significance.

Combined Effects of Photoperiods and pCO_2 on the Cell Growth in Experiment I. Cell concentration in each carbonate system was monitored throughout the incubation period of 28 days (Figure 1a). Repeated measures ANOVA

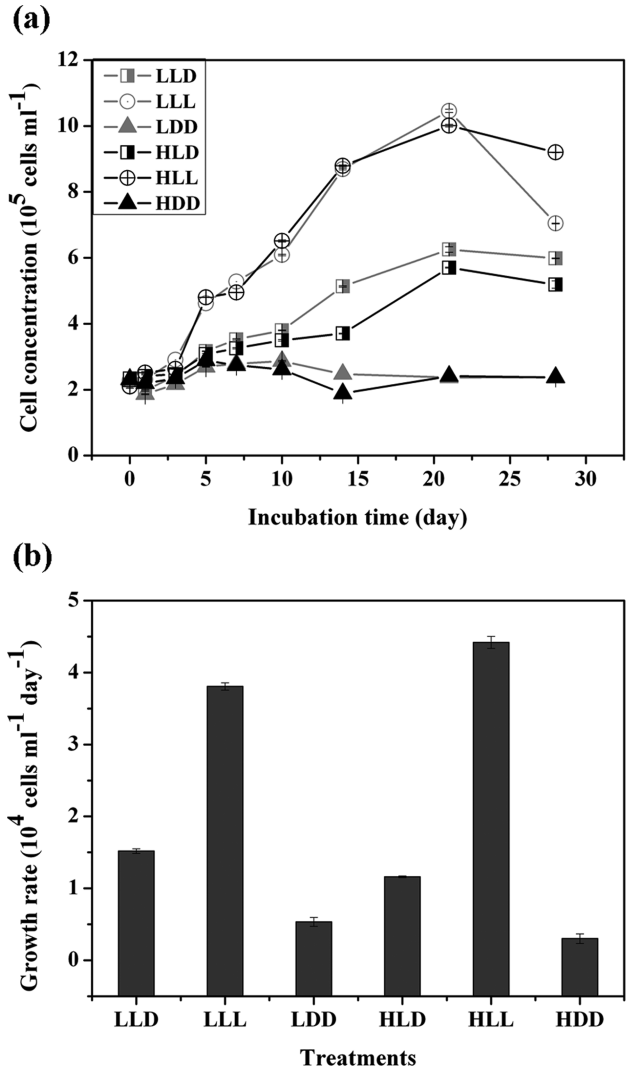

Figure 1. Cell growth of *Chlamydomonas* sp. ICE-L in different photoperiods and pCO_2 during an incubation period of 28 days. (a) Variation of cell concentration during the period. (b) Growth rate of the first 10 days. Treatment of low pCO_2 and regular light and dark cycle marked as LLD, low pCO_2 and continuous light marked as LLL, low pCO_2 and continuous darkness marked as LDD, high pCO_2 and regular light and dark cycle marked as HLD, high pCO_2 and continuous light marked as HLL, and high pCO_2 and continuous darkness marked as HDD. Vertical lines represent standard deviations of triplicate incubations.

indicated there was significant interactive effect of photoperiod and pCO_2 on cell concentration over time (df = 16, F = 540.756, $p < 0.001$). Cells grew exponentially in all treatments during the first 10 days, and the growth rate is presented in Figure 1b. Statistic analysis indicated that the continuous light in low pCO_2 (LLL) increased the growth rate by 151%, whereas the continuous dark in low pCO_2 (LDD) decreased it by 65%, compared to the control (LLD) ($p < 0.001$). By contrast, there was no consistent effect of elevated pCO_2 (1000 ppm) on the growth rate during the first 10 days (p = 0.80), depending on the photoperiod. Although the growth rate of HLL was 1.16 times that of LLL, that in HLD and HDD was only 76% and 56% of LLD and LDD, respectively. By the end

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of experiment, the cell concentration presented a variation trend under different treatments that was similar to the growth rate in exponential phrase. The treatment HLL resulted in higher values of cell concentration than LLL ($p < 0.001$), whereas the HLD and HDD reduced it ($p < 0.001$).

Combined Effects of Photoperiods and pCO_2 on the PSII Photosynthetic Activity in Experiment I. PSII photosynthetic characteristics, including maximum quantum yield (F_v/F_m), relative electron transport rate (rETR) and light utilization efficiency (α), were determined under different photoperiods and pCO_2 conditions (Figure 2). Both photoperiod and pCO_2 had a significant influence on PSII photosynthetic performance ($p < 0.05$), but the magnitude was different among the two factors. During the exponential growth phrase of the first 7 days, the average F_v/F_m, rETR, and α were 0.45, 7.4, and 0.18 in LLD, respectively. Compared to the control, the continuous light (LLL) increased the average F_v/F_m, rETR, and α by 10%, 6%, and 9%, respectively, but the continuous dark (LDD) decreased them by 24%, 21%, and 2%, respectively. On the other hand, the elevated pCO_2 generally increased the levels of these parameters. By the end of experiment at 28 days, F_v/F_m ($p < 0.001$) and rETR ($p < 0.001$) in HLD, HLL, and HDD were significant higher than those in LLD, LLL, and LDD, but the value of α was not significant between the low and high pCO_2 ($p = 0.69$). Moreover, high pCO_2 magnified the positive effects of continuous light and alleviated the negative effects of continuous dark. Under high pCO_2 of 1000 ppm and continuous light (HLL), the average F_v/F_m, rETR, and α during the first 7 days increased by 13%, 18%, and 27%, compared to LLD, whereas under high pCO_2 of 1000 ppm and continuous dark (HDD), they decreased by 19%, 21%, and 0.004%.

Combined Effects of Photoperiods and pCO_2 on Nutrient Uptake Rate in Experiment I. The change in the nutrient concentration of the media and nutrient uptake rates under different photoperiods and pCO_2 are shown in Figure 3. The most significant nutrient decline occurred in nitrogen in continuous light and standard light-dark cycle regardless of the pCO_2 ($p < 0.01$) (Figure 3a). It took only 5 days to remove 92% and 93% of N in LLL and HLL, whereas it took 14 days and 21 days to remove 87% and 96% of N in LLD and HLD. However, not surprisingly, in darkness the depletion of N after 28 days was less than 24% and 29% in LDD and HDD, respectively. The presence of light made a significant difference to the average N uptake rate during the first 5 days (Figure 3b) (df = 2, $F = 228.45$, $p = 0.001$), but differences under low and high pCO_2 were not significant (df = 1, $F = 0.85$, $p = 0.38$).

By contrast, the concentration of P in the culture media decreased significantly in all treatments over the course of the incubation period (df = 14, $F = 137.85$, $p = 0.001$) (Figure 3c). Within the first 5 days, 98%, 98%, 99%, and 99% of P was removed from the cultures under LLD, LLL, HLD, and HLL, respectively. There was also a quick uptake of P in the dark during the first 7 days, but thereafter uptake stopped and in LDD even reversed. Statistical analysis demonstrated that both light (df = 2, $F = 645.17$, $p < 0.001$) and high pCO_2 (df = 1, $F = 131.03$, $p < 0.001$) contributed to P uptake significantly (Figure 3d).

Effects pCO_2 on Lipid Profile in Darkness. The cellular lipid content declined significantly in complete darkness (df = 3, $F = 76.50$, $p = 0.001$), from an initial value of 40.2% of algal dry weight to 7.9% in the low pCO_2 treatment and to 14.9% in high pCO_2 treatment (Figure 4). There were also significant

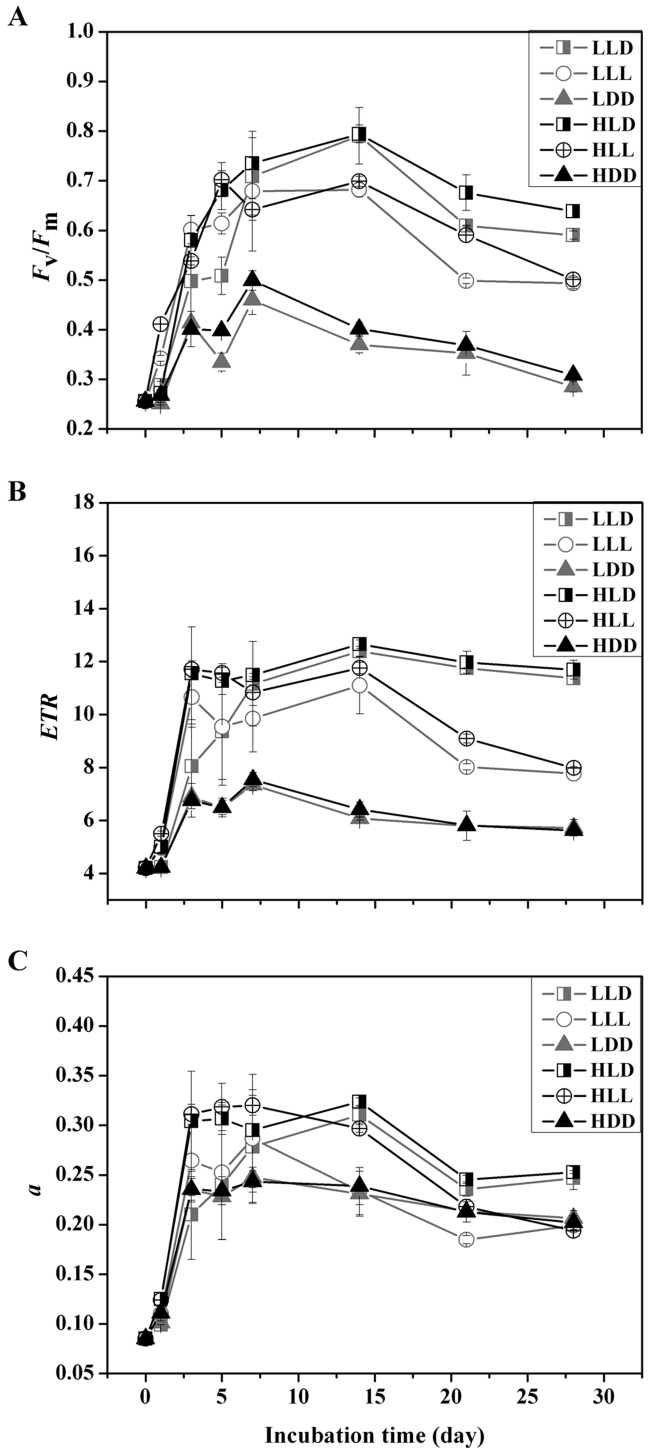

Figure 2. PSII photophysiological responses of *Chlamydomonas* sp. ICE-L to different photoperiods and pCO_2. (a) Maximum quantum yield (F_v/F_m). (b) Relative electron transport rate (rETR). (c) Light utilization efficiency (α). Treatment of low pCO_2 and regular light and dark cycle marked as LLD, low pCO_2 and continuous light marked as LLL, low pCO_2 and continuous darkness marked as LDD, high pCO_2 and regular light and dark cycle marked as HLD, high pCO_2 and continuous light marked as HLL, and high pCO_2 and continuous darkness marked as HDD. Vertical lines represent standard deviations of triplicate incubations.

differences between the low and high pCO_2 treatments at the end of the incubation period (df = 1, $F = 6.62$, $p = 0.02$). This could also be seen in the abundance of intracellular lipid

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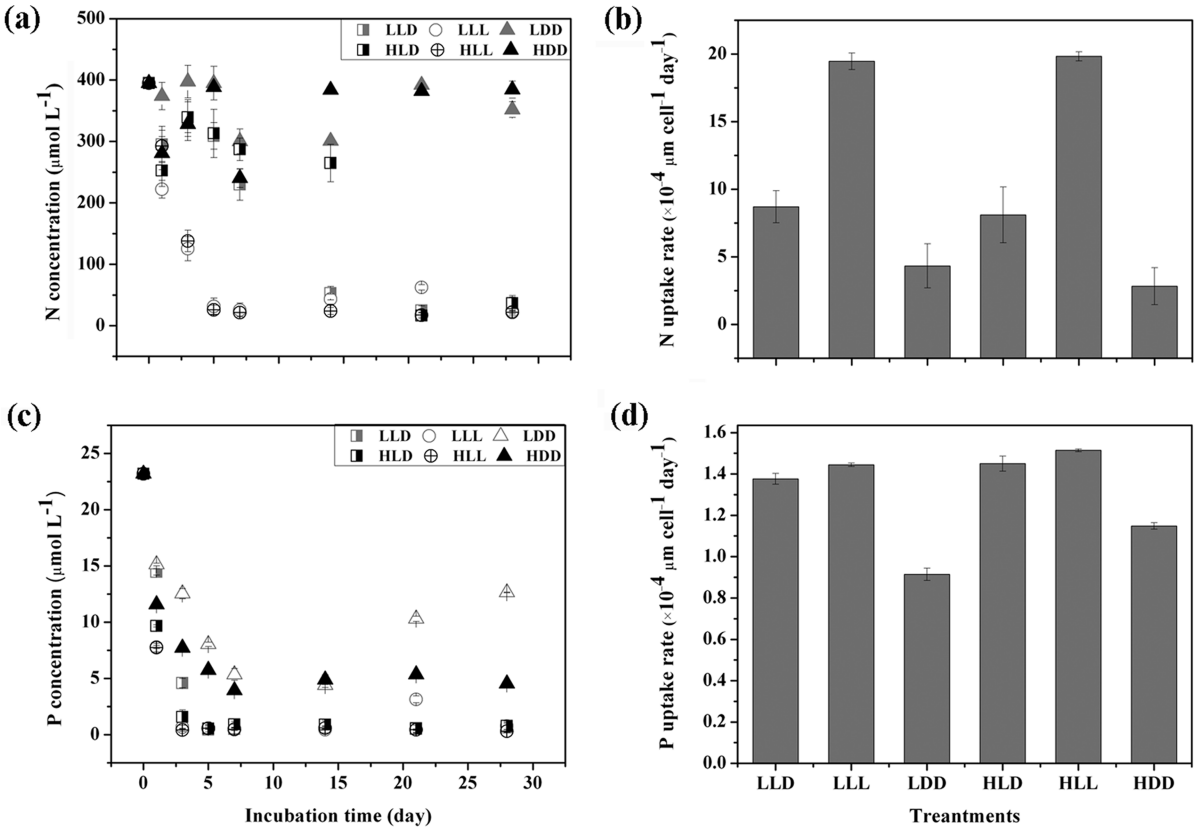

Figure 3. Nutrient analysis during incubation of *Chlamydomonas* sp. ICE-L in cultures grown in different photoperiods and pCO_2. (a) Variation of N concentration over the time. (b) Average N uptake rate during the first 5 days. (c) Variation of P concentration over the time. (d) Average P uptake rate during the first 3 days. Treatment of low pCO_2 and regular light and dark cycle marked as LLD, low pCO_2 and continuous light marked as LLL, low pCO_2 and continuous darkness marked as LDD, high pCO_2 and regular light and dark cycle marked as HLD, high pCO_2 and continuous light marked as HLL, and high pCO_2 and continuous darkness marked as HDD. Vertical lines represent standard deviations of triplicate incubations.

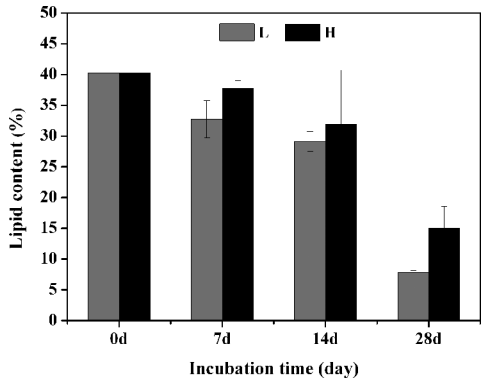

Figure 4. Variation of lipid content (%) of *Chlamydomonas* sp. ICE-L grown in darkness under low and high pCO_2. Treatment in low pCO_2 marked as L, and treatment in high pCO_2 marked as H. Vertical lines represent standard deviations of triplicate incubations.

droplets, using BODIPY 505/515 (Supplementary Figure S2). Moreover, the fatty acid (FA) profiles in darkness under different pCO_2 are shown in Supplementary Table S1. Analysis of FA composition shows that the most of the decline in total lipids resulted from a decrease in PUFA, which declined from an initial value of 65% to 44% and 49% in low and high pCO_2 treatments, respectively.

High Light Effects on *Chlamydomonas* sp. ICE-L in Experiment II. In the high light experiment, the cell concentration did not change over the incubation period of 12 h (p = 0.74) (Figure 5a). However, significant photoinhibition was induced on F_v/F_m with incubation course (p < 0.001) (Figure 5b). The high pCO_2 treatment was more sensitive to high light stress (p = 0.01) than the low pCO_2 treatment. The value of F_v/F_m in LDDL, LDDH, and HDDH was reduced by 59%, 64%, and 71% by the end of experiment, respectively.

DISCUSSION

The Southern Ocean plays a critical role in global ocean circulation as well as global cycling of carbon and nitrogen, taking up 20%−30% of anthropogenic CO_2.[44] Interannual variability in Southern Ocean productivity is most closely related to variability in sea ice.[45] Sea ice provides an extensive habitat for many organisms in polar environments, which routinely experience extremes in irradiance from several months of continuous light in summer to prolonged darkness in winter. Polar alga have adapted to survive these extreme light conditions,[29,32] but ongoing ocean acidification is an additional stress that is further challenging algal survival. Therefore, we examined the combined effects of photoperiods and pCO_2 on the physiological performance of an Antarctic sea ice microalgae, *Chlamydomonas* sp. ICE-L. Particular attention was paid to the analysis of dark survival mechanisms under high pCO_2. The long-term effects of ocean acidification on *Chlamydomonas* sp. ICE-L were further tested by analyzing its photosynthetic responses to high light stress.

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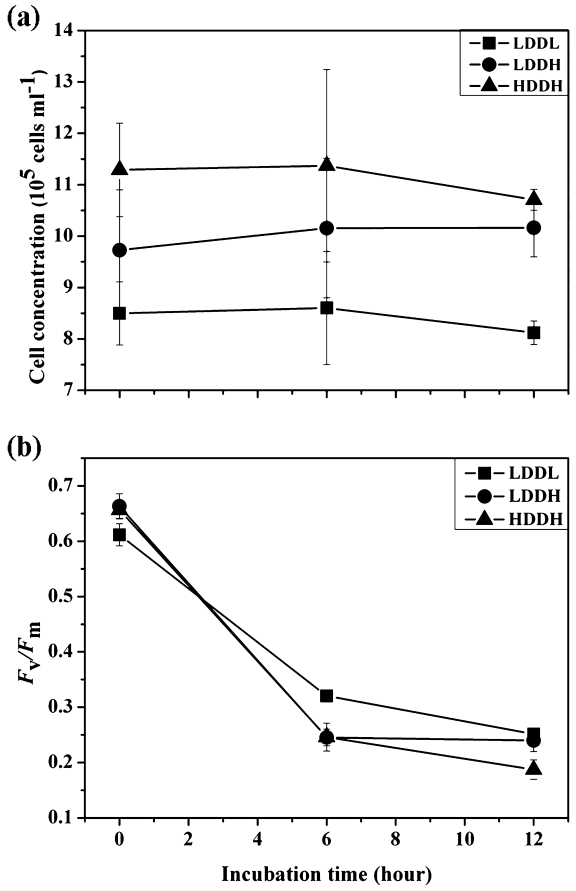

Figure 5. Growth (a) and photosynthetic responses (b) of *Chlamydomonas* sp. to high light stress of 400 μmol photons m^{-2} s^{-1}. LDDL and LDDH represent cells treated in LDD and HDD in EI and recovered under low and high pCO_2, respectively. HDDH represent cells treated in HDD in EI and recovered under high pCO_2. Vertical lines represent standard deviations of triplicate incubations.

Physiological Responses to Different Photoperiods. Most phototrophic organisms regularly experience natural light and dark cycle and have adapted their physiology and behavior to these periodic oscillations in external signals.[46,47] Photosynthesis cannot occur without light, and many of the processes involved in photosynthesis are regulated by redox status or other mechanisms sensitive to light and dark.[48] Productivity in polar oceans is characterized by large-scale spatial and temporal variability, due to the annual expansion and contraction of sea ice and seasonal alternation in incident solar radiation.[29,30] Our results in EI are consistent with the conclusion above and show that photoperiod has a significant influence of on physiological performance of the sea ice microalga *Chlamydomonas* sp. ICE-L. Compared to the regular light and dark cycle, continuous light increases algal growth (Figure 1), PSII photosynthetic performance (Figure 2), and nutrient uptake (Figure 3). Conversely, dark treatment led to negative effects on each parameter mentioned above.

Combined Effects of Ocean Acidification upon Different Photoperiod. Although global climate change is occurring in the polar regions, seasonal changes in daylight hours are naturally unaffected.[29,32] Therefore, in the future polar microalgae will have to survive both at higher temperature and lower pH.[1,49] A mounting body of studies has emphasized the need to consider the effect of the combination of stressors that accompany ocean acidification such as temperature, light, UV, nutrients, and even species ecotype on the physiological responses of phototrophic organisms.[50−53] These studies have shown that there may be positive, negative, or neutral interactions of elevated pCO_2 with other factors, and results may depend on biotic factors such as taxonomic groups, life history, nutrient status, or source population and abiotic factors such as the order magnitude of CO_2 or duration of experiment.[54−56] However, currently there is little information on the combined effects of pCO_2 and photoperiod on the sea ice community. In our study, pCO_2 and photoperiod showed combined effects on the physiological performance of the Antarctic sea ice microalgae *Chlamydomonas* sp. ICE-L. The high pCO_2 magnified the positive effects of continuous light and alleviated the negative effects of continuous dark on PSII photosynthetic parameters (Figure.2). By contrast, there was no consistent effect of elevated pCO_2 (1000 ppm) on growth rate (p = 0.8) or N uptake (p = 0.38) during exponential phrase, depending on the photoperiod. A C-concentrating mechanism (CCM) was previously reported in the model green alga *Chlamydomonas reinhardtii* to uptake inorganic carbon (HCO_3^- and CO_2) for photosynthesis, which was efficient under low CO_2.[57] Passive diffusion of CO_2 was also adapted in some algae. The elevated pCO_2 accompanied by increasing dissolved inorganic carbon (DIC) would reduce the energy costs of photosynthesis. The positive effect of high pCO_2 on photosynthesis of *Chlamydomonas* sp. ICE-L may benefit from increasing inorganic carbon source.

Considering OA effects on nutrient uptake, Riebesell et al.[58] reported that although biological carbon consumption of a natural plankton community was enhanced in a high CO_2 ocean, nutrient uptake remained the same. In the present study, rising CO_2 resulted in no significant effects on N uptake (Figure 3b), but P uptake was significantly increased by 5.3%, 4.8%, and 25% under treatments of HLD, HLL, and HDD, compared with LLD, LLL, and LDD, respectively (Figure 3d). Additionally, previous study indicated anthropogenic eutrophication is causing large CO_2 inputs into coastal waters and could induce acifification (Sunda and Cai[59]). Russell et al.[60] found that nutrient levels had an amplification effects on ecosystem upon ocean acidification. Van Den Berg et al.[61] studied the effects of increasing ammonium concentrations in combination with different pH levels on five heathland plant species. It was found that a decline of acid-sensitive species in heathlands was attributed to ammonium toxicity effects in combination with a low pH. During the period of batch culture in Experiment I, nutrient declined significantly (Figure 3) and nutrient limitation possibly occurred. To determine the possible interaction of nutrient limitation with pCO_2, continuous/semicontinuous culture methods would be studied further.

Survival Mechanism to Winter Darkness under Acidification. Deep ocean mixing can expose cells to darkness on time scales of hours to days. Species-specific strategies have been adopted by algal cells to survive darkness. Nymark et al.[62] investigated both the response to prolonged darkness and the reacclimation to moderate intensity white irradiance in the diatom *Phaeodactylum tricornutum*. It was found that dark treatment led to a decrease in nuclear transcriptional activity, total cell pigment pool, and photosynthetic efficiency, accompanied by distinct intracellular changes. Light is also critical to the pattern of reproduction in some micoralgal species. Continuous light leads to asexual reproduction and production of antospores, while darkness can induce zoosporo-

genesis or gemetogenesis.[63] A recent study of the model green alga *Chlamydomonas* (Chlorophyta) found that it could grow heterotrophically in the dark on medium supplemented with acetate, and there is some genetic variation in the survival process. A comparative trial further indicated that outbred sexual populations displayed the most effective evolutionary strategy in darkness.[64] Furthermore, McMinn and Martin[32] reviewed dark survival mechanisms of algae in a warming world and indicated that exposure to elevated temperatures in the dark potentially reduces their viability. Using energy storage products such as lipids and starch have often been detected in polar phytoplankton.[32,65,66] Results in EI show that the cellular lipid content declined significantly during culture in darkness (Figure 4). It seems that the intracellular lipid may be consumed to survive the darkness. Moreover, the high pCO_2 treatment showed less decline of lipid than that of low pCO_2 treatment. In dark, autotrophic and heterotrophic microorganisms were found to incorporate CO_2 through different metabolic pathways and amplify various bacterial carboxylases involved in fatty acids biosynthesis, anaplerotic pathways, and leucine catabolism.[67] The bubbled CO_2 treatment increased the supply of inorganic carbon source from CO_2 and HCO_3^-, which may alleviate the consumption of cellular lipid during dark. However, whether the lower consumption of lipid in high pCO_2 may be of benefit for the sea ice microalga *Chlamydomonas* sp. for surviving the total darkness in winter should studied further.

Long-Term Acidification Displayed Effects Opposite to Those of Short-Term Experiment. Most previous ocean acidification studies have been performed in the laboratory or field conditions and based on short-term experimental periods. This may have created a bias for species and clones that are best adapted to modern CO_2 levels and raises questions regarding the general validity of the observed responses.[9] There is a need to carry out experiments on long-time scales. Low-Décarie et al.[56] conducted a long-term experiment on seven freshwater phytoplankton species under elevated pCO_2 for over 750 generations and found neutral effects of acidification on carbon utilization in all species tested. Long-term culture of the diatom *Thalassiosira pseudonana* of 100 generations over 3 months in low pH 7.8 also showed little effects.[54] The coccolithophore *Gephyrocapsa oceanica* also displayed acclimation to ocean acidification after a long-term experiment lasting for 670 generations at 1000 ppm pCO_2. On the basis of these results, we set up Experiment II where the algae recovered after acidifying treatments were imposed to high light stress. Surprisingly, although high pCO_2-treated algae displayed higher PSII performance during the batch culture in EI, the alga that had been treated in high pCO_2 and also recovered in pCO_2 (HDDH) was the most vulnerable to stress treatment (Figure 5). Therefore, we concluded that it is unauthentic to define the effects of ocean acidification using short-term experiment only. Aside from time scale, mediate and higher CO_2 concentration should be included to assess ocean acidification in the future study.

■ ASSOCIATED CONTENT

Supporting Information

Table S1 showing fatty acid profile (% of total) in the dark under different pCO_2; Figure S1 showing parameters of the seawater−carbonate system in batch cultures under in different photoperiods and pCO_2; Figure S2 showing variation of intracellular lipid droplet during incubation in darkness for 28 days. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Steig, E. J.; Schneider, D. P.; Rutherford, S. D.; Mann, M. E.; Comiso, J. C.; Shindell, D. T. Warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. *Nature* **2009**, *457* (7228), 459−462.

(2) Harley, C. D. G. Climate change, keystone predation, and biodiversity loss. *Science* **2011**, *334* (6059), 1124−1127.

(3) Gao, K. S.; Xu, J. T.; Gao, G.; Li, Y.; Hutchins, D. A.; Huang, B. Q.; Wang, L.; Zheng, Y.; Jin, P.; Cai, X. N.; Häder, D.; Li, W.; Xu, K.; Liu, N.; Riebesell, U. Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. *Nat. Clim. Change* **2012**, *2* (7), 519−523.

(4) Kroeker, K. J.; Kordas, R. L.; Crim, R.; Hendriks, I. E.; Ramajo, L.; Singh, G. S.; Duarte, C. M.; Gattuso, J. P. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biol.* **2013**, *19* (6), 1884−1896.

(5) Steinacher, M.; Joos, F.; Stocker, T. F. Allowable carbon emissions lowered by multiple climate targets. *Nature* **2013**, *499* (7457), 197−201.

(6) Twine, T. E.; Bryant, J. J.; Richter, K. T.; Bernacchi, C. J.; McConnaughay, K. D.; Morris, S. J.; Leakey, A. D. B. Impacts of elevated CO_2 concentration on the productivity and surface energy budget of the soybean and maize agroecosystem in the Midwest US. *Global Change Biol.* **2013**, *19* (9), 2838−2852.

(7) Hall-Spencer, J. M.; Rodolfo-Metalpa, R.; Martin, S.; Ransome, E.; Fine, M.; Turner, S. M.; Rowley, S. J.; Tedesco, D.; Buia, M. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **2008**, *454* (7200), 96−99.

(8) Shi, D.; Xu, Y.; Hopkinson, B. M.; Morel, F. M. M. Effect of ocean acidification on iron availability to marine phytoplankton. *Science* **2010**, *327* (5966), 676−679.

(9) Langer, G.; Geisen, M.; Baumann, K. H.; Kläs, J.; Riebesell, U.; Thoms, S.; Young, J. R. Species-specific responses of calcifying algae to changing seawater carbonate chemistry. *Geochem. Geophy. Geos.* **2006**, *7* (9), 1 DOI: 10.1029/2005GC001227.

(10) Przeslawski, R.; Ahyong, S.; Byrne, M.; Woerheide, G.; Hutchings, P. Beyond corals and fish: the effects of climate change on non-coral benthic invertebrates of tropical reefs. *Global Change Biol.* **2008**, *14* (12), 2773−2795.

(11) Roleda, M. Y.; Morris, J. N.; McGraw, C. M.; Hurd, C. L. Ocean acidification and seaweed reproduction: increased CO_2 ameliorates the

negative effect of lowered pH on meiospore germination in the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae). *Global Change Biol.* **2012**, *18* (3), 854−864.
(12) Shaw, E. C.; McNeil, B. I.; Tilbrook, B.; Matear, R.; Bates, M. L. (2013) Anthropogenic changes to seawater buffer capacity combined with natural reef metabolism induce extreme future coral reef CO_2 conditions. *Global Change Biol.* **2013**, *19* (5), 1632−1641.
(13) Sorte, C. J. B.; Ibáñez, I.; Blumenthal, D. M.; Molinari, N. A.; Miller, L. P.; Grosholz, E. D.; Diez, J. M.; D'Antonio, C. M.; Olden, J. D.; Jones, S. J.; Dukes, J. S. Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. *Ecol. Lett.* **2013**, *16* (2), 261−270.
(14) Hoegh-Guldberg, O.; Mumby, P. J.; Hooten, A. J.; Steneck, R. S.; Greenfield, P.; Gomez, E.; Harvell, C. D.; Sale, P. F.; Edwards, A. J.; Caldeira, K.; Knowlton, N.; Eakin, C. M.; Iglesias-Prieto, R.; Muthiga, N.; Bradbury, R. H.; Dubi, A.; Hatziolos, M. E. Coral reefs under rapid climate change and ocean acidification. *Science* **2007**, *318* (5877), 1737−1742.
(15) Crawley, A.; Kline, D. I.; Dunn, S.; Anthony, K. E. N.; Dove, S. The effect of ocean acidification on symbiont photorespiration and productivity in *Acropora Formosa*. *Global Change Biol.* **2010**, *16* (2), 851−863.
(16) Anthony, K. R. N.; Maynard, J. A.; Diaz-Pulido, G.; Mumby, P. J.; Marshalls, P. A.; Cao, L.; Hoegh-Guldberg, O. Ocean acidification and warming will lower reef resilience. *Global Change Biol.* **2011**, *17* (5), 1798−1808.
(17) Jin, P.; Gao, K.; Beardall, J. Evolutionary responses of a coccolithophorid *Gephyrocapsa oceanica* to ocean acidification. *Evolution* **2013**, *67* (7), 1869−1878.
(18) Hood, M.; Broadgate, W.; Urban, E.; Gaffney, O. In *Ocean Acidification, A Summary for Policymakers from the Second Symposium on the Ocean in a High-CO_2 World*, 2009. Available at http://coralreef.noaa.gov/education/oa/resources/ocacidpolicymakers.pdf.
(19) Riebesell, U.; Tortell, P. D. *Effects of Ocean Acidification on Pelagic Organisms and Ecosystems*; Gattuso, J. P., Hanson, L., Eds.; Oxford University Press: Oxford, 2011; pp 99−121.
(20) Xu, D.; Gao, Z. Q.; Li, F.; Fan, X.; Zhang, X. W.; Ye, N. H.; Mou, S. L.; Liang, C. W.; Li, D. M. Detection and quantitation of lipid in the microalga *Tetraselmis subcordiformis* (Wille) Butcher with BODIPY 505/515 staining. *Bioresour. Technol.* **2012**, *127*, 386−390.
(21) Israel, A.; Hophy, M. Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biol.* **2002**, *8* (9), 831−840.
(22) Orr, J. C.; Fabry, V. J.; Aumont, O.; Bopp, L.; Doney, S. C.; Feely, R. A.; Gnanadesikan, A.; Gruber, N.; Ishida, A.; Joos, F.; Key, R. M.; Lindsay, K.; Maier-Reimer, E.; Matear, R.; Monfray, P.; Mouchet, A.; Najjar, R. G.; Plattner, G. K.; Rodgers, K. B.; Sabine, C. L.; Sarmiento, J. L.; Schlitzer, R.; Slater, R. D.; Totterdell, I. J.; Weirig, M. F.; Yamanaka, Y.; Yool, A. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature.* **2005**, *437* (7059), 681−686.
(23) Torstensson, A.; Hedblom, M.; Andersson, J.; Andersson, M. X.; Wulff, A. Synergism between elevated pCO_2 and temperature on the Antarctic sea ice diatom *Nitzschia lecointei*. *Biogeosci. Discuss.* **2013**, *10*, 6637−6669.
(24) Poloczanska, E. S.; Babcock, R. C.; Butler, A.; Hobday, A.; Hoegh-Guldberg, O.; Kunz, T. J.; Matear, R.; Milton, D. A.; Okey, T. A.; Richardson, A. J. Climate change and Australian marine life. *Oceanog. Mar. Biol. Annu. Rev.* **2007**, *45*, 407−478.
(25) Fabry, V. J.; Seibel, B. A.; Feely, R. A.; Orr, J. C. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* **2008**, *65* (3), 414−432.
(26) Byrne, M.; Ho, M.; Selvakumaraswamy, P.; Nguyen, H. D.; Dworjanyn, S. A.; Davis, A. R. Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proc. R. Soc. B* **2009**, *276* (1663), 1883−1888.
(27) Kennedy, F.; McMinn, A.; Martin, A. Effect of temperature on the photosynthetic efficiency and morphotype of *Phaeocystis antarctica*. *J. Exp. Mar. Biol. Ecol.* **2012**, *429*, 7−14.
(28) Reeves, S.; McMinn, A.; Martin, A. The effect of prolonged darkness on the growth, recovery and survival of Antarctic sea ice diatoms. *Polar Biol.* **2011**, *34* (7), 1019−1032.
(29) Hu, H. Adaptation of Antarctic freshwater green algae to extreme environments. In *Polyextremophiles*; Springer: New York, 2013; pp 425−436.
(30) Martin, A.; McMinn, A.; Heath, M.; Hegseth, E. N.; Ryan, K. G. The physiological response to increased temperature in over-wintering sea ice algae and phytoplankton in McMurdo Sound, Antarctica and Tromsø Sound, Norway. *J. Exp. Mar. Biol. Ecol.* **2012**, *428*, 57−66.
(31) Vick, T. J.; Priscu, J. C. Bacterioplankton productivity in lakes of the Taylor Valley, Antarctica, during the polar night transition. *Aquat. Microb. Ecol.* **2012**, *68*, 77−90.
(32) McMinn, A.; Martin, A. Dark survival in a warming world. *Proc. R. Soc. B* **2013**, *280* (1755), 20122909.
(33) Shadwick, E.; Trull, T.; Thomas, H.; Gibson, J. Vulnerability of polar oceans to anthropogenic acidification: Comparison of Arctic and Antarctic seasonal cycles. *Sci. Rep.* **2013**, DOI: 10.1038/srep02339.
(34) An, M. L.; Mou, S. L.; Zhang, X. W.; Ye, N. H.; Zheng, Z.; Cao, S. N.; Xu, D.; Fan, X.; Wang, Y. T.; Miao, J. L. Temperature regulates fatty acid desaturases at a transcriptional level and modulates the fatty acid profile in the Antarctic microalga *Chlamydomonas* sp. ICE-L. *Bioresour. Technol.* **2013**, *134*, 151−157.
(35) Kan, G. F.; Zheng, Z.; Jiang, Y. H.; Miao, J. L.; Zhang, B. T.; Li, G. Y. Salt resistance of Antarctic ice microalga *Chlamydomonas* sp. *J. Fish. Sci. China* **2006**, *13*, 73−78.
(36) Mou, S. L.; Zhang, X. W.; Ye, N. H.; Miao, J. L.; Cao, S. N.; Xu, D.; Fan, X.; An, M. L. Analysis of ΔpH and the xanthophyll cycle in NPQ of the Antarctic sea ice alga *Chlamydomonas* sp. ICE-L. *Extremophiles* **2013**, *17* (3), 477−484.
(37) Provasoli, L. In *Media and prospects for the cultivation of marine algae*, Cultures and Collections of Algae. Proceedings of the US-Japan Conference, Hakone, September 1966; Japanese Society for Plant Physiology, 1968; pp 63−75.
(38) Pelletier, G.; Lewis, E.; Wallace, D. *CO2SYS. XLS: A calculator for the CO2 system in seawater for Microsoft Excel/VBA*; Washington State Department of Ecology/Brookhaven National Laboratory: Olympia, WA/Upton, NY, USA, 2007.
(39) Zou, D.; Gao, K. Effects of elevated CO_2 on the red seaweed *Gracilaria lemaneiformis* (Gigartinales, Rhodophyta) grown at different irradiance levels. *Phycologia* **2009**, *48* (6), 510−517.
(40) Gao, Z. Q.; Li, D. M.; Meng, C. X.; Xu, D.; Zhang, X. W.; Ye, N. H. Survival and proliferation characteristics of the microalga *Chlamydomonas* sp. ICE-L after hypergravitational stress pretreatment. *Icarus* **2013**, *226* (1), 971−979.
(41) Hiriart-Baer, V. P.; Arciszewski, T. J.; Malkin, S. Y.; Guildford, S. J.; Hecky, R. E. Use of pulse-amplitude-modulated fluorescence to assess the physiological status of Cladophora sp. along a water quality gradient. *J. Phycol.* **2008**, *44* (6), 1604−1613.
(42) Ben-Amotz, A.; Tornabene, T. G. Chemical profile of selected species of microalgae with emphasis on lipids. *J. Phycol.* **1985**, *21* (1), 72−81.
(43) Xu, J.; Gao, K. Future CO_2-induced ocean acidification mediates the physiological performance of a green tide alga. *Plant Physiol.* **2012**, *160* (4), 1762−1769.
(44) Landrum, L.; Holland, M. M.; Schneider, D. P.; Hunke, E. Antarctic sea ice climatology, variability, and late twentieth-century change in CCSM4. *J. Clim.* **2012**, *25* (14), 4817−4838.
(45) Arrigo, K. R.; van Dijken, G. L.; Bushinsky, S. Primary production in the Southern Ocean, 1997−2006. *J. Geophys. Res.: Oceans* **2008**, DOI: 10.1029/2007JC004551.
(46) Wijnen, H.; Young, M. W. Interplay of circadian clocks and metabolic rhythms. *Annu. Rev. Genet.* **2006**, *40*, 409−448.
(47) Pruneda-Paz, J. L.; Kay, S. A. An expanding universe of circadian networks in higher plants. *Trends Plant Sci.* **2010**, *15* (5), 259−265.

(48) Farré, E. M.; Weise, S. E. The interactions between the circadian clock and primary metabolism. *Curr. Opin. Plant Biol.* **2012**, *15* (3), 293−300.
(49) Monaghan, A. J.; Bromwich, D. H.; Chapman, W.; Comiso, J. C. Recent variability and trends of Antarctic near-surface temperature. *J. Geophys. Res.: Atmos.* **2008**, DOI: 10.1029/2007JD009094.
(50) Stecher, A. Combined effects of ocean acidification and tidal emergence on the performance and gene expression in the intertidal brown seaweed Fucus serratus. Master's thesis, Universität Bremen, 2011.
(51) Koch, M.; Bowes, G.; Ross, C.; Zhang, X. H. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biol.* **2013**, *19* (1), 103−132.
(52) Martin, S.; Cohu, S.; Vignot, C.; Zimmerman, G.; Gattuso, J. P. One-year experiment on the physiological response of the Mediterranean crustose coralline alga, *Lithophyllum cabiochae*, to elevated pCO_2 and temperature. *Ecol. Evol.* **2013**, *3* (3), 676−693.
(53) Kroeker, K. J.; Kordas, R. L.; Crim, R.; Hendriks, I. E.; Ramajo, L.; Singh, G. S.; Duarte, C. M.; Gattuso, J. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biol.* **2013**, *19* (6), 1884−1896.
(54) Crawfurd, K. J.; Raven, J. A.; Wheeler, G. L.; Baxter, E. J.; Joint, I. The response of *Thalassiosira pseudonana* to long-term exposure to increased CO_2 and decreased pH. *PloS One* **2011**, *6* (10), e26695.
(55) Kelly, M. W.; Padilla-Gamiño, J. L.; Hofmann, G. E. Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Global Change Biol.* **2013**, *19* (8), 2536−2546.
(56) Low-Décarie, E.; Jewell, M. D.; Fussmann, G. F.; Bell, G. Long-term culture at elevated atmospheric CO_2 fails to evoke specific adaptation in seven freshwater phytoplankton species. *Proc. R. Soc. B* **2013**, *280* (1754), 20122598.
(57) Yamano, T.; Tsujikawa, T.; Hatano, K.; Ozawa, S. I.; Takahashi, Y.; Fukuzawa, H. Light and low-CO_2-dependent LCIB−LCIC complex localization in the chloroplast supports the carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. *Plant Cell Physiol.* **2010**, *51* (9), 1453−1468.
(58) Riebesell, U.; Schulz, K. G.; Bellerby, R. G. J.; Botros, M.; Fritsche, P.; Meyerhöfer, M.; Neill, C.; Nondal, G.; Oschiles, A.; Wohlers, J.; Zöllner, E. Enhanced biological carbon consumption in a high CO_2 ocean. *Nature.* **2007**, *450* (7169), 545−548.
(59) Sunda, W. G.; Cai, W. J. Eutrophication induced CO_2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric PCO_2. *Environ. Sci. Technol.* **2012**, *46* (19), 10651−10659.
(60) Russell, B. D.; Thompson, J. A. I.; Falkenberg, L. J.; Connell, S. D. Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats. *Global Change Biol.* **2009**, *15* (9), 2153−2162.
(61) Van Den Berg, L. J.; Dorland, E.; Vergeer, P.; Hart, M. A.; Bobbink, R.; Roelofs, J. G. Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. *New Phytol.* **2005**, *166* (2), 551−564.
(62) Nymark, M.; Valle, K. C.; Hancke, K.; Winge, P.; Andresen, K.; Johnsen, G.; Bones, A. M.; Brembu, T. Molecular and photosynthetic responses to prolonged darkness and subsequent acclimation to re-illumination in the diatom *Phaeodactylum tricornutum*. *PloS One* **2013**, *8*, e58722.
(63) Přibyl, P. Light is a crucial signal for zoosporogenesis and gametogenesis in some green microalgae. *Eur. J. Phycol.* **2013**, *48*, 106−115.
(64) Bell, G. Evolutionary rescue of a green alga kept in the dark. *Biol. Lett.* **2013**, *9* (1), 20120823.
(65) Fryxell, G. A. Marine phytoplankton at the Weddell Sea ice edge: seasonal changes at the specific level. *Polar Biol.* **1989**, *10* (1), 1−18.
(66) McKnight, D. M.; Howes, B. L.; Taylor, C. D.; Goehringer, D. D. Phytoplankton dynamics in a stably stratified Antarctic lake during winter darkness. *J. Phycol.* **2000**, *36* (5), 852−861.
(67) Alonso-Sáez, L.; Galand, P. E.; Casamayor, E. O.; Pedrós-Alió, C.; Bertilsson, S. High bicarbonate assimilation in the dark by Arctic bacteria. *ISME J.* **2005**, *4* (12), 1581−1590.