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Synergism between elevated pCO_2 and temperature on the Antarctic sea ice diatom *Nitzschia lecointei*

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Abstract

Polar oceans are particularly susceptible to ocean acidification and warming. Diatoms play a significant role in sea ice biogeochemistry and provide an important food source to grazers in ice-covered oceans, especially during early spring. However, the ecophysiology of ice living organisms has received little attention in terms of ocean acidification. In this study, the synergism between temperature and partial pressure of CO_2 (pCO_2) was investigated in relationship to the optimal growth temperature of the Antarctic sea ice diatom *Nitzschia lecointei*. Diatoms were kept in cultures at controlled levels of pCO_2 (~ 390 and ~ 960 µatm) and temperature (−1.8 and 2.5 °C) for 14 days. Synergism between temperature and pCO_2 was detected in growth rate and acyl lipid fatty acid content. Carbon enrichment only promoted (3 %) growth rate closer to the optimal growth, but not at the control temperature (−1.8 °C). Optimal growth rate was observed around 5 °C in a separate experiment. Polyunsaturated fatty acids (PUFA) comprised up to 98 % of the total acyl lipid fatty acid pool at −1.8 °C. However, the total content of fatty acids was reduced by 39 % at elevated pCO_2, but only at the control temperature. PUFAs were reduced by 30 % at high pCO_2. Effects of carbon enrichment may be different depending on ocean warming scenario or season, e.g. reduced food quality for higher trophic levels during spring. Synergy between temperature and pCO_2 may be particularly important in polar areas since a narrow thermal window generally limits cold-water organisms.

1 Introduction

High latitude marine environments are particularly vulnerable to ocean acidification due to the high solubility of CO_2 in cold waters (Orr et al., 2005). Therefore, the Southern Ocean has a naturally low carbonate saturation state and is believed to be one of the first oceans to become persistently undersaturated with respect to aragonite (Orr et al., 2005; Steinacher et al., 2009). Along with elevated levels of greenhouse gases in the

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atmosphere, an increase in average sea surface temperature (SST) of 0.74 °C has been recorded from year 1906 to 2005 (IPCC, 2007). Mean SST is predicted to rise 1–4 °C by the year 2100, and the largest impacts are predicted to occur in polar areas (IPCC, 2007). For instance, the summer sea ice extent in the Arctic has declined since the late 1970's, and recent models have predicted a sea ice-free Arctic Ocean during the summer, within the next 30 yr (Wang and Overland, 2009). In fact, the extent of sea ice in the Arctic reached a record low as reported in September 2012 (NSDIC Arctic Sea Ice News and Analysis, 2012, available at http://nsidc.org).

The climate has been relatively stable from a geological perspective in Antarctica, hence enabling adaptation to extreme environmental conditions. However, selection can also result in the loss of important abilities that allow for adaptation to environmental change. Examples of such loss have been reported in ectothermic organisms inhabiting the Southern Ocean, and suggests that Antarctic organisms are particularly sensitive to these types of changes (Somero, 2012). One common adaptation to changes in temperature is alteration of the lipid composition in the cell membrane. According to the hypothesis of "homeoviscous adaptation", the composition of lipids will change when temperature prevent functioning of the membrane beyond the membrane lipids optimal function range (Hazel, 1995). To maintain membrane fluidity at temperatures below that of freezing seawater, sea ice algae increase the proportion of unsaturated fatty acids. For instance, Teoh et al. (2004) reported a significant decrease in polyunsaturated fatty acids (PUFA) at temperatures above 4 °C in an Antarctic strain of *Navicula* sp. In addition, fatty acid (FA) composition and nutrient stoichiometry can be directly related to the food quality transferred to higher trophic levels and is known to be negatively affected by ocean acidification (Rossoll et al., 2012; Schoo et al., 2012). However, little is still known about the effects of ocean acidification on microalgal lipid FA composition.

Sea ice plays an important role in carbon biogeochemistry, both in terms of physiochemical and biological dynamics. Algae inhabiting brine channels of the ice, in particular diatoms, contribute significantly to the primary production in ice covered polar areas,

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providing a substantial carbon source to higher levels. During spring, the early offset from sea ice to blooming ice algae in the water column is very important for grazers, as ice algae are abundant and contain high levels of PUFA compared to phytoplankton. The peak of the sea ice algal bloom occurs before the peak of the pelagic bloom, meaning the sea ice algal community may act as a significant seeding population to the pelagic bloom when dispersed in the marginal ice zone (see Lizotte, 2001). The timing is crucial and a mismatch due to a changing climate could have serious consequences for organisms higher in the food web (Hoegh-Guldberg and Bruno, 2010). Moreover, the physiochemical properties, e.g. salinity, partial pressure of CO_2 (pCO_2), of sea ice vary substantially during the season. During freezing, pCO_2 is increased compared to the surrounding seawater due to concentration of solutes, $CaCO_3$ precipitation and microbial respiration. As the ice becomes warmer and more permeable to liquids, the conditions can be similar to the surrounding seawater and atmosphere (Geilfus et al., 2012). However, during sea ice blooms, the fugacity of carbon dioxide (fCO_2) can be < 100 µatm (Fransson et al., 2011; Geilfus et al., 2012). Hence, the temporal variation of pCO_2 in sea ice is generally higher compared to the surrounding seawater, although pCO_2 in brine can be similar to the atmosphere during the growing season of the ice algal community.

Phytoplankton tend to exhibit elevated growth rates in warmer temperatures within their thermal window (Eppley, 1972). More specifically for polar algae, minor temperature elevations tend to favor pschycrophilic pennate diatom species, with optimum growth temperature usually reported around 4–5 °C (Fiala and Oriol, 1990). In general, optimal photosynthetic activity and growth of pschycrophilic algae often occurs at temperatures above natural conditions (Fiala and Oriol, 1990). In addition, both positive, negative and no responses to increased pCO_2 have been reported in marine diatoms (Yang and Gao, 2012). However, many laboratory studies are performed at, or close to, the optimal growth temperature, rather than at ambient conditions. If temperature and pCO_2 act synergistically, the interpretation of experiments performed at optimal growth could be ambiguous in terms of global change.

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The carbon-fixating enzyme RUBISCO can only utilize dissolved CO_2 as an inorganic carbon source. Since cellular uptake of dissolved inorganic carbon (DIC) creates a gradient in the water surrounding the cell, diatoms may suffer from CO_2-limitation at the cell surface during a bloom (e.g. Riebesell et al., 1993). Almost all marine algal groups have evolved carbon concentrating mechanisms (CCMs) to assist carbon fixation in photosynthesis when CO_2 is limited (see Reinfelder, 2011). It has also been suggested that CCMs are an evolutionary response to the change of atmospheric gases over geological time, where the ratio of O_2 and CO_2 has increased (Hopkinson et al., 2011). Carbonic anhydrase (CA) is a major CCM, catalysing the reversible dehydration of HCO_3^- into CO_2 externally at the surface of algal cells (CA_{ext}), or internally in the cell at the site of carbon fixation (CA_{int}) (Morant-Manceau et al., 2007; Hopkinson et al., 2011). Studies suggests that CA activity in diatoms is induced at low levels of pCO_2 in order to maintain photosynthetic activity at different pCO_2 (Burkhardt et al., 2001). Hence, as CCMs are considered costly, their efficiency will play an important role in determining the success of different photoautotrophs in a high-CO_2 world.

To understand the effects of climate change, combined effects of different stressors need to be addressed. Since temperature and CO_2 concentration play an important role in microalgal physiology, there is great potential for synergism between these two environmental stressors. The goal of this investigation was to evaluate potential synergism between elevated pCO_2 and temperature on the ecophysiology of a sea ice diatom and relate the response to its optimal growth temperature. This was performed during two laboratory perturbation experiments. In Experiment A, the physiology of the Antarctic sea ice diatom *Nitzschia lecointei* van Heurck 1909 was examined after being exposed to a worst-case scenario of elevated SST and increased pCO_2 by year 2100. In Experiment B, we addressed the optimal growth temperature of *N. lecointei* in order to relate the response of CO_2 enrichment to its temperature optimum.

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2 Material and methods

2.1 Experimental setup

The diatom *N. lecointei* was isolated from sea ice in the Amundsen sea (January 2011) and cultivated in f/2 medium with silica (Guillard, 1975) at −1.8 °C until the experiments started. In this study (Experiment A), we simulated a worst-case scenario according to the predicted models of SST and pCO_2 by the year 2100 (IPCC, 2007). During the experiment, *N. lecointei* was inoculated (1.6×10^6 cells L^{-1}) in 220 mL batch cultures containing f/2 medium with silica, prepared from 0.2 µm filtered Antarctic seawater (salinity 33). The experimental treatments were set up using flasks and tubing system similar to Torstensson et al. (2012). The temperature treatments (−1.8 ± 0.2 °C and 2.5±0.2 °C) were maintained using two identical cooling and circulating water baths (Hetofrig, Birkerød, Denmark) containing a mix of MilliQ water and transparent ethylene glycol. Quadruplicated cultures were constantly bubbled (∼ 6 mL min^{-1} $flask^{-1}$) with synthetic air prepared with two different pCO_2 levels (390 and 960 µatm, Air Liquide, Malmö, Sweden) within each temperature treatment. In addition, four separate replicates were used for initial measurements. One control (f/2 medium without diatoms) per treatment combination was used to monitor the carbonate system in absence of *N. lecointei* (i.e. alkalinity perturbation and carbon uptake).

A second experiment (Experiment B) was set up to investigate the optimal growth temperature of *N. lecointei*. *N. lecointei* was inoculated in batch cultures to an initial concentration of 5.2×10^5 cells L^{-1} in 360 mL f/2 medium with silica, prepared from 0.2 µm filtered Antarctic seawater (salinity 33). Water baths equipped with cooling and circulation provided five temperature treatments, ranging from −2.3 °C to 8.4 °C (−2.3 ± 0.3 °C, 2.4 ± 0.7 °C, 5.1 ± 0.1 °C and 8.4 ± 0.1 °C), where five borosilicate culture flasks were deployed in each bath. The water baths were filled with a mix of MilliQ water and colourless ethylene glycol. To prevent carbon limitation and create agitation, the flasks were constantly bubbled with air.

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For both experiments, the irradiance was provided from Osram Lumilux Cool Daylight L36W/865 tubes, with a photosynthetic active radiation (PAR) irradiance at 25 µmol photons m^{-2} s^{-1} (light : dark cycle 23 : 1), corresponding to an irradiance within sea ice at high latitudes. The light intensity was not limiting according to the light saturation point (E_k) as measured with rapid light curves using Water-PAM (see below). Temperatures in the water baths were monitored using temperature loggers (HOBO Pendant, Onset Computer Corporation, Pocasset, USA). Sampling occurred on day 0 and day 14 in both experiments.

2.2 Carbonate system

pH was measured at the total scale (pH_T) using the spectrophotometric method with *m*-cresol purple indicator (Clayton and Byrne, 1993). 3 mL of sample was carefully transferred into a 10.00 mm cuvette and 30 µL *m*-cresol purple was added. Temperature of the sample was immediately measured using a thermistor (A Precision, Ama-Digit ad 15th, Amarell GmbH & Co. KG, Kreuzwertheim, Germany). The pH_T was determined after calculations of Clayton and Byrne (1993).

Total alkalinity (A_T) was measured potentiometrically using an automatic titration system (Metrohm 888 Titrando, Metrohm Aquatrode Plus Pt1000). Samples were stored in darkness at 3 °C until analysis within 24 h. 35–40 g of sample was weighed and titrated with 0.05 M HCl and the Gran equivalence point was determined according to Haraldsson et al. (1997). For corrections of A_T, a certified reference material (CRM) from Scripps Institution of Oceanography, CA, USA, was titrated in triplicates prior to titration of samples. The variation within triplicates was 0.5 % of the average A_T.

The carbonate system (pCO_2, $[HCO_3^-]$, $[CO_3^{2-}]$ and $[CO_2]$) was described using the chemical speciation program CO2SYS (Pierrot et al., 2006) with measured values of pH_T, A_T, salinity and in situ temperature. The dissociation constants for carbonic acid, K_1 and K_2 used in calculations were determined by Mehrbach et al. (1973) and refitted by Dickson and Millero (1987). The dissociation constant for SO_4^- was determined by Dickson (1990).

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2.3 Photosynthetic activity

The maximum quantum yield of photosystem II (F_v/F_m) was measured using a Water-PAM (Universal control unit, WATER-ED/B, Walz Mess- und Reigeltechnik, Effeltrich, Germany). 4 mL of well-mixed sample was dark-adapted at experimental temperature for 15 min. After dark adaptation, minimum fluorescence (F_0) was determined by applying a low level of light and the maximum fluorescence (F_m) by exposing the sample to a short saturation pulse of measuring light (> 3000 µmol photons m^{-2} s^{-1} for 0.8 s). Variable fluorescence ($F_v = F_m - F_0$) and maximum quantum yield (F_v/F_m) were determined for all samples.

2.4 Growth rate

Cell count samples were preserved in 2.5 % (final concentration) glutaraldehyde and kept dark at 7 °C until analyses. Counting was performed using a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). For flow estimation, 1.0 µm ultrasonicated FluoSpheres (Invitrogen, Eugene, OR, USA) were used as an internal standard. The FluoSphere concentration was calibrated against TruCount absolute counting beads (BD Biosciences, San Jose, CA, USA) in triplicated runs. Samples were analysed until a minimum of 300 cells were counted. Specific growth rate (µ day^{-1}) was calculated using Eq. (**??**):

$$\mu = \frac{\ln D_x - \ln D_0}{t_x - t_0} \quad (1)$$

Where D_x is the cell concentration at day x, D_0 is the cell concentration at day 0, t_x is the time in days at day x and t_0 is the time in days at day 0.

2.5 Primary productivity

Total primary productivity, i.e. without separating particulate and dissolved carbon productivity (PP_{POC} and PP_{DOC}), was measured using the radiocarbon technique

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(Aertebjerg and Bresta, 1984). 10 mL of sample was incubated for 1 h with 3 µCi $H^{14}CO_3^-$ (PerkinElmer, Inc., Waltham, MA, USA) at experimental irradiance and temperature in 20 mL glass scintillation vials. One blank from each treatment was wrapped in tin foil for incubation in darkness. After the incubation, 200 µL of formaldehyde was added to all samples to stop the carbon uptake. Two drops of 1 M HCl were added and the samples were bubbled with N_2 gas for 1 h to remove the remaining DIC. 10 mL of Insta-Gel Plus (PerklinElmer, Inc., Waltham, MA, USA) scintillation cocktail was added to samples and vials were shaken rigorously. Primary productivity was measured by counting ionizing radiation from the decay of ^{14}C, in a liquid scintillation counter (Packard Tri-Carb 2900TR Liquid Scintillation Analyzer). Carbon uptake was corrected for dark uptake and estimated using Eq. (2):

$$^{12}C = \frac{^{14}C_S \cdot {}^{12}C_A \cdot 1.06}{^{14}C_A \cdot t} \quad (2)$$

Where ^{12}C is the carbon uptake rate ($mg\,C\,L^{-1}\,h^{-1}$), $^{14}C_S$ is the radioactive activity (DPM) in the sample, $^{12}C_A$ is the available DIC ($mg\,L^{-1}$), 1.06 is a constant adjusting the fixation rate due to the discrimination of ^{14}C during carbon fixation, $^{14}C_A$ is the added activity of ^{14}C to the sample (DPM) and t is the incubation time (h). Carbon uptake was normalized to cell concentrations.

2.6 Carbonic anhydrase activity

During the ^{14}C incubation for primary productivity (see above), additional incubations of each sample were prepared with the addition of 400 µM (final concentration) acetazolamide (AZ) (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany). Concentration of CA inhibitor was chosen after optimization using other diatom species (Morant-Manceau et al., 2007).

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2.7 Glycerolipid fatty acid composition

40 mL of sample was filtered onto pre-combusted (400 °C, 4 h) GF/F filters. Filters were immediately flash-frozen in liquid nitrogen and transferred to −80 °C until extraction. A total lipid extract was obtained from the filters after submergence in boiling 2-propanol as described in Andersson et al. (2006). The total lipid extract was subjected to alkaline transesterification (Christie, 1976) and analyzed by GC-MS using an Agilent 7820 GC coupled to an Agilent 5975 mass selective detector. Di-nonadecanoyl phosphatidylcholine was used as internal standard. The FA methyl esters were separated on a 30 m DB-23 capillary column (J&W Scientific, Folsom, CA, USA) using helium as a carrier gas at a constant flow of 30 cm s^{-1}. The oven was held at 150 °C for 3 min and after that increased by 1 °C min^{-1} to a final temperature of 200 °C. The injector and the detector interface were kept at 250 °C. The methyl esters were identified by retention time and mass spectra compared to authentic lipid standards. Hereafter, the total amount of FA methyl esters is referred to as total FA and grouped by affiliation to: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

2.8 Inorganic nutrients

10 mL of sample was 0.2 µm filtered and stored at −20 °C until analysis of dissolved inorganic nutrients, NO_3^-, NO_2^-, PO_4 and Si. Samples were analysed at the Sven Lovén Center for Marine Research, Kristineberg, Sweden, using colorimetric methods according to Grasshoff et al. (1999).

2.9 Statistics

Statistical analysis of data was performed using one and two factor ANOVAs. To test the data for homogeneity of variances, Levene's test was used and Student–Newman–Keuls (SNK) post-hoc analysis was performed when necessary.

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3 Results

3.1 Growth and photosynthesis

In Experiment A, an interaction between temperature and pCO_2 was observed for specific growth rate of *N. lecointei* (Fig. 1a). Growth rate increased significantly at 960 µatm compared to the 390 µatm pCO_2 at 2.5 °C. However, there was no difference in growth rate at −1.8 °C. In addition, F_v/F_m (Fig. 2), primary productivity (Fig. 3) and specific growth rate increased significantly with temperature. One sample from the ^{14}C-incubation was lost due to technical error. Statistical analyses results from Experiment A are displayed in Table 1.

3.2 Glycerolipid fatty acid composition

There was a significant interaction between treatments for total FA content in Experiment A (Fig. 4). At −1.8 °C, total FA content was reduced by 39 % in the 960 µatm treatment. However, there was no difference between the two pCO_2 treatments at 2.5 °C. FA contents decreased with 65–76 % at 2.5 °C compared to −1.8 °C. The relative composition of FAs in the treatments is presented in Table 2. Fatty acid composition grouped into SAFA, MUFA and PUFA is shown in Fig. 4. PUFA content per cell was significantly lower in the 2.5 °C treatment compared to the −1.8 °C. Cellular PUFA content also decreased significantly with increasing pCO_2. No difference in SAFA and MUFA content was detected between treatments. Statistical analysis for SAFA and PUFA was executed with heterogeneous variances, due to inability of transformation (Underwood, 1997).

More than 90 % of the PUFA comprised of the omega-3 fatty acids 18 : 3n-3 (α-linolenic acid, ALA), 18 : 4n-3 (stearidonic acid, SDA) and 20 : 5n-3 (eicosapentaenoic acid, EPA). The most common FA was 18 : 3n-3, which accounted for roughly half of the total FA content in all treatments. 18 : 4n-3 accounted for about 20 % of the lipid content and 20 : 5n-3 for about 10 % (Table 2).

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3.3 Carbonic anhydrase activity

A reduction in carbonic anhydrase (CA) activity was observed in 15 measurable samples where AZ was added, giving a mean inhibition of 12 % (Fig. 5). However, no significant difference between treatments was detected on CA activity.

3.4 Optimal growth temperature

The specific growth rate increased significantly in Experiment B when temperature increased from the ambient, while the highest growth rate was detected in the 5.1 °C treatment. At further temperature increase the specific growth rate declined (Fig. 1b). Growth rates were significantly different (Table 1), and all treatments were significantly separated according to the SNK test. The one factor ANOVA was performed with heterogeneous variances due to inability of transformation (Underwood, 1997).

3.5 Experimental conditions

Measured values of pH_T and A_T, with additional calculated parameters from CO2SYS for $[HCO_3^-]$, $[CO_3^{2-}]$, $[CO_2]$ and pCO_2 after 14 days in Experiment A, are displayed in Table 3. A_T increased on average by 5.2 % and 7.7 % during the 14 days of experiment in the −1.8 °C and 2.5 °C temperature treatments, respectively. When comparing samples and blanks (0.2 µm filtered seawater) at day 14, the algae contributed with 0.8 % and 2.6 % increase of A_T in the −1.8 °C and 2.5 °C temperature treatments, respectively.

Inorganic nutrients were measured to rule out nutrient limitation. Results showed satisfying levels (Table 4), with steady values of F_v/F_m (Fig. 2) throughout the experiment suggesting inorganic nutrients were not limiting growth.

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4 Discussion

In this study, we report synergistic effects between temperature and pCO_2 on growth and FA content of *N. lecointei*, suggesting complexity in physiological responses to climate change. In the control temperature, growth rate was unaffected by pCO_2. However, growth rate in the warmer treatment was promoted by elevated pCO_2. Since increased pCO_2 in combination with warmer temperature stimulated growth, this interaction suggests a physiological constraint in the lower temperature, making *N. lecointei* unable to utilize the extra carbon available to potentially increase growth rate. With a warmer temperature this constraint seemed to diminish and growth was no longer limited by temperature. The synergy between temperature and pCO_2 may be particularly important in polar areas, where many physiological processes are limited by a narrow thermal window (e.g. Pörtner, 2002). Furthermore, these findings emphasize the importance of selecting a realistic temperature when designing perturbation experiments, which may be especially important in polar organisms where temperature regulates many physiological processes. Choosing a temperature close to the optimum growth temperature, rather than at ambient conditions, might paradoxically both over- and underestimate the effect of carbon enrichment. Earlier studies suggest that elevated temperature within the temperature range of the species increases growth rate and photosynthesis in phytoplankton and ice algal species (Eppley, 1972; Montagnes and Franklin, 2001; Torstensson et al., 2012). However, not all observations are consistent with the latter studies. Mock and Hoch (2005) did not detect a difference in maximum quantum yield after a 4 month long steady-state culturing of the polar diatom *Fragilariopsis cylindrus* in −1 °C and 7 °C. On the contrary, a decreasing trend in the maximum quantum yield with increasing temperature was shown by Morris and Kromkamp (2003). However, the latter study was carried out with the benthic diatom *Cylindrotheca closterium* acclimated and grown at 20 °C, but treated with temperatures ranging from 5 °C to 35 °C. Acclimation and ability to benefit from elevated carbon dioxide levels could also explain the interaction observed for the growth rate in this study.

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Since growth was faster in the warmer treatment, the number of generations exceeded the numbers in the colder treatment. There is a possibility that more generations of *N. lecointei* acclimated to high pCO_2 in the high temperature treatment, hence the promoted growth rate. Thus, long-term studies may be crucial for understanding acclimation and adaptation to climate change.

Photosynthesis is just one process contributing to growth, meaning other temperature-sensitive processes may also regulate growth rates, e.g. nitrogen uptake and assimilation (Kuebler et al., 1991). If these processes are affected by ocean acidification, the responses may be temperature dependent. Since pCO_2 did not affect the primary productivity, F_v/F_m or CA activity of *N. lecointei*, it seems plausible that a process other than photosynthesis was affected by the treatments in order to alter the growth of *N. lecointei*. However, primary productivity was measured as total primary productivity ($PP_{POC} + PP_{DOC}$) in this study. Therefore, a treatment-dependent shift in the $PP_{POC} : PP_{DOC}$ ratio could mask a possible shift in PP_{POC}. Engel et al. (2013) performed a study on natural phytoplankton communities in the Arctic fjord Kongsfjorden, Svalbard, and during ^{14}C incubations of primary producers they observed an increase in PP_{DOC} after enrichment of pCO_2. Hence, overconsumption of carbon may increase the production and exudation of DOC and should be considered when interpreting our results. Another theory suggests that photosynthesis and growth have differences in acclimation potential when temperatures increase, e.g. photosynthetic activity has a higher potential to be positively affected by elevated temperature than growth (Longhi et al., 2003). The primary productivity and growth of *N. lecointei* increased with temperature in this study. Similar results have been reported in earlier studies, where carbon fixation increased in phytoplankton populations from the Bering Sea treated with elevated temperatures (Hare et al., 2007).

Further synergism was detected in the FA concentration of *N. lecointei*. The cellular FA content in −1.8 °C was higher at ambient pCO_2 compared to the elevated pCO_2 treatment. Rossoll et al. (2012) also reported higher total FA content and PUFA concentration in *Thalassiosira pseudonana* grown at 380 µatm compared to 750 µatm,

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which suggests that diatoms reduce their FA content in response to ocean acidification. This could have major consequences on the food quality transferred to higher trophic levels (Rossoll et al., 2012). However, we only observed a pCO_2-related decline in FA concentration at −1.8 °C, suggesting that temperature in combination with pCO_2 plays an important role in FA stoichiometry. The total FA contents were reduced by 65–76 % in the higher temperature compared to the colder temperature. Hence, the effect of elevated temperature on *N. lecointei* FA content is also of great importance. Temperature has previously been well documented to impact on the cellular lipid content of phytoplankton from the Southern Ocean. Smith and Morris (1980) observed that up to 80 % of assimilated carbon during ^{14}C incubations was incorporated in lipids when incubated in temperatures below 0 °C. At incubations above 0 °C the incorporation into lipids was less than 20 %. In addition, Smith and Morris (1980) suggested low light stress to enhance the effect of stored lipids as response to low temperature. Increased lipid content due to environmental stressors, such as nutrient deficiency, low light and low temperature, is suggested to be a characteristic response for polar phytoplankton populations (Smith and Morris, 1980). Deficiency of nitrogen is well known to elevate cellular lipid content in diatoms in order to accumulate FAs when conditions are unfavorable (Parrish and Wangersky, 1987). Hence, inorganic nutrient levels are of importance to cellular FA content, i.e. the storage of FAs and lipids is built up when access is reduced. However, no signs of nutrient deficiency were detected in this study, e.g. inorganic nutrient concentrations and F_v/F_m. These findings suggest a major reduction of *N. lecointei* FA content in response to climate change, highlighting the synergy between environmental changes.

Sea ice algae are known to contain high amounts of FA and are an important food source for grazers in polar areas, especially prior to the pelagic spring bloom. Therefore, a loss of sea ice in polar areas may seriously impact food webs due to grazer essential FA being altered, e.g. PUFA concentration in the food may be reduced. The FA composition analysis revealed noticeable high PUFA concentrations in all treatments. PUFA content is generally high when algae are growing at optimal culture conditions.

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Studies of FA composition in cultures and natural algal communities sampled from water, sea ice, platelet ice, interstitial water and under ice water in polar oceans have shown PUFA concentrations of approximately 20–60 % (Fahl and Kattner, 1993; Falk-Petersen et al., 1998; Teoh et al., 2004; Leu et al., 2006). However, PUFA in *N. lectonei* represented 94.1–98.5 % of the lipids in the control temperature, reflecting the importance of PUFA as a cold adaption. Significantly lower PUFA concentrations were measured in the warmer treatment; 81.5 % at ambient carbon level and 84.5 % at elevated pCO_2. These results are consistent with previous knowledge about FAs and adaption to a warmer environment (Teoh et al., 2004). Furthermore, the level of carbon dioxide also appears to alter FA content under certain temperature conditions in *N. lecointei*. In a previous study, Rossoll et al. (2012) showed a decrease in PUFA concentration, at the expense of increasing SAFA, as a result of elevated carbon levels. The authors also observed a decline in total FA content at high pCO_2. The results from the latter and present studies are somewhat consistent, i.e. cellular FA and PUFA content decreases due to elevated carbon levels. However, no alteration of SAFA and MUFA content was detected in *N. lecointei*, and the response of pCO_2 appeared temperature dependent. Thus, carbon level only affects total FA content of *N. lecointei* at low temperature, while an increased pCO_2 leads to deterioration of FA content. The PUFA present in the samples were mainly omega-3 PUFA, however in three out of four treatments a small fraction (< 2 %) of non-omega-3 PUFA, 18 : 2n-6 (linoleic acid), was found as well. Decreasing PUFA content, mainly omega-3 PUFAs, is consistent with earlier studies. Zhang et al. (2011) showed a relationship between decreasing temperature and increasing activity of an omega-3 FA desaturase, suggesting a correlation between desaturase activity and the decrease of 18 : 2 PUFA and the increase of 18 : 3 PUFA. The study was performed on a strain of the green alga *Chlamydomonas* sp. isolated from Antarctic sea ice. In addition, Mayzaud et al. (2013) also demonstrated that increasing temperatures decreased the amount of omega-3 PUFA in natural blooming phytoplankton in the Arctic fjord Kongsfjorden, Spitsbergen, Svalbard. Alteration of cellular FA content may not only affect the primary producers. A loss of PUFA results in

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altered food quality for organisms grazing on primary producers. Rossoll et al. (2012) showed a major decrease in somatic growth and egg production in grazing copepods (*Acartia tonsa*), as a response to deteriorated food quality, i.e. reduced total FA and PUFA : SAFA, from *T. pseudonana* grown at increased pCO_2. This type of event may have significant effects throughout the food web. It is suggested that poor food quality in copepods and amphipods is negatively correlated with breeding performance in marine bird species in polar areas. Studies from the Arctic show changed foraging patterns, with Little Auks (*Alle alle*) taking longer trips, due to a lack of energy-rich prey near breeding sites, resulting in decreased reproductive (Steen et al., 2007). In addition, an alteration in the FA content at subzero temperatures could also affect the ability of *N. lecointei* to tolerate low temperature stress, e.g. during freezing. Hence, an alteration in FA composition in the foundation of the food web may have severe consequences at higher trophic levels. However, few ocean acidification studies have so far focused on trophic transfer in the ecosystem.

This study demonstrates that *N. lecointei* is clearly favored by higher temperatures compared to ambient levels. According to Experiment B, optimal growth temperature for *N. lecointei* was around 5 °C. It has previously been suggested that maximal growth rate occurs at temperatures above in situ levels (Fiala and Oriol, 1990; Longhi et al., 2003), which indicates that a narrow thermal window generally limits Antarctic algae. This suggests that carbon enrichment might not be beneficial at ambient conditions since temperature primarily regulates growth. However, ambient temperatures may become closer to the peak of the thermal window during an ocean warming event and promote the effect of carbon enrichment. Temperature is essential to enzyme activity and metabolic processes where the speed of reactions increases with increasing temperature. Results from this study indicate that *N. lecointei* is more efficient in utilizing available resources at higher temperatures (5.1 °C), which is within reasonable limits of what could be expected considering reaction speed. At 8.4 °C, specific growth rate was still 17 % higher than at −1.8 °C. However, psychrophilic sea ice algae like *N. lecointei* will be negatively affected by further temperature increase (data not shown).

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The primary productivity and F_v/F_m of *N. lecointei* increased with elevated temperature in Experiment A. Kennedy et al. (2012) described the relationship between temperature and photosynthesis within sea ice. This was performed by measuring F_v/F_m in the haptophyte *Phaeocystis antarctica* distributed at different depths in the ice, while excluding the factor of salinity. The highest level was observed in the warmest part of the ice, around −2 °C, close to the ice/water interface, while the lowest level was measured in the coldest part, around −6 °C, at the top of the ice (Kennedy et al., 2012). In addition, Ralph et al. (2005) studied brine channel communities dominated by diatoms and showed similar results, i.e. the photosynthetic performance was higher at −1.8 °C than at −10 °C. Hence, the pattern with warmer temperature and higher photosynthetic performance, which is also shown for *N. lecointei* in this study, continues in temperatures below −1.8 °C.

The possible down-regulation of CCMs due to elevated pCO_2 has previously been discussed elsewhere. Raven (1991) suggested that phytoplankton using CCMs would favour from elevated pCO_2, since costs for carbon fixation seemed to be lower with elevated growth and photosynthesis as a result. Contrary to Raven (1991), Hopkinson et al. (2011) suggested energy savings from down-regulation of CCMs would increase carbon fixation, when sufficient levels of carbon are achieved without the use of energy-consuming CCMs. However, the responses tend to be very taxa specific. It has been reported that the photosynthetic performance of the Antarctic diatom *Chaetoceros brevis* remains unaffected at both a reduction by half and a doubling of present day pCO_2 (Boelen et al., 2011). Yang and Gao (2012) reported that the growth rate of *T. pseudonana* was unaffected, even though CCMs were down-regulated and photosynthesis up-regulated when treated with pCO_2 of 1000 µatm. The authors explained this observation by an increased rate of mitochondrial respiration and loss of carbon, corresponding to the enhanced carbon fixation. However, down-regulation of CCMs has more recently been suggested to occur at high pCO_2 (Wu et al., 2008; Hopkinson et al., 2011). In this study, a mean reduction of CA activity was observed in 15 measurable samples inhibited with AZ during the ^{14}C incubation. The inhibition was of the

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same magnitude in all treatments, suggesting that *N. lecointei* has the same requirement of carbon regardless of treatment level, i.e. *N. lecointei* may already be saturated in carbon at the ambient pCO_2. Nevertheless, this shows that CA as a CCM is present in *N. lecointei* and that it provides for at least 12 % of the carbon assimilation, which is a substantial part of the total carbon uptake. However, since inhibition of CA is species specific and depending on concentration of inhibitor (Morant-Manceau et al., 2007), 12 % might be an underestimation.

Sea ice algae play an important role as a food source for grazers in the early season and as seedling populations to the spring bloom. An elevated SST would change the distribution of sea ice and could result in an ice free summer, as is predicted for the Arctic within the next 30 yr (Wang and Overland, 2009). This might affect organisms dependent on sea ice algae more than the first year ice inhabiting algae itself, since the algae nevertheless end up in the water column during the melting season. However, the sea ice would also become warmer in general during the season, resulting in more porous sea ice with less saline brine. In addition, levels of dissolved carbon dioxide within sea ice during formation would theoretically increase, similar to the increasing salinity of the brine in the non-frozen matrix within the ice. However during spring and summer, measured levels of fCO_2 were below ambient atmospheric level (Fransson et al., 2011), indicating that increased levels of dissolved carbon dioxide in brine are not as elevated as they may be during formation. This suggests that the temporal variation of pCO_2 in brine is quite high compared to the surrounding seawater (Geilfus et al., 2012). Hence, the pCO_2 treatments in this study are still considered relevant to condition in the ice when the sea ice algae are starting to bloom. However, natural variability of pCO_2 will be important to consider, especially when modelling the results from ocean acidification experiments.

We hereby suggest that pCO_2 and temperature can act synergistically to promote growth rate and affect the FA composition of sea ice diatoms. We also emphasize that the temperature optimum is important to consider when assessing physiological effects of ocean acidification on photoautotrophs. Hence, the physiological effects of

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pCO_2 may be more pronounced closer to the optimal temperature of the species, although this temperature is generally higher than ambient conditions. However, the gap between optimal and ambient SST will probably be reduced during a future ocean warming event and may be important to consider when assessing results from ocean acidification perturbation experiments.

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Table 1. Results from statistical analyses after 14 days in Experiment A and B, using one and two factor ANOVA. Analysed parameters are maximum quantum yield (F_v/F_m), specific growth rate (Growth), primary productivity (PP), relative primary productivity with inhibitor Acetazolamide (AZ), total fatty acids (FA), saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Experiment	Parameter		df	*F* value	*P* value
A	F_v/F_m	Temp	1	104.0	< 0.001
		pCO_2	1	0.4	0.555
		Temp·pCO_2	1	1.3	0.273
		Error	12		
	Growth (μday^{-1})	Temp	1	397.5	< 0.001
		pCO_2	1	3.0	0.108
		Temp·pCO_2	1	5.1	0.044
		Error	12		
	PP ($pgCh^{-1}cell^{-1}$)	Temp	1	38.8	< 0.001
		pCO_2	1	0.4	0.562
		Temp·pCO_2	1	2.6	0.132
		Error	11		
	Relative PP with AZ (%)	Temp	1	0.9	0.374
		pCO_2	1	0.4	0.537
		Temp·pCO_2	1	0.2	0.668
		Error	11		
	Total FA ($nmol cell^{-1}$)	Temp	1	53.9	< 0.001
		pCO_2	1	6.9	0.022
		Temp·pCO_2	1	5.3	0.041
		Error	12		
	SAFA ($nmol cell^{-1}$)	Temp	1	1.4	0.267
		pCO_2	1	3.5	0.086
		Temp·pCO_2	1	4.6	0.053
		Error	12		
	MUFA ($nmol cell^{-1}$)	Temp	1	2.8	0.122
		pCO_2	1	3.0	0.109
		Temp·pCO_2	1	3.0	0.113
		Error	12		
	PUFA ($nmol cell^{-1}$)	Temp	1	62.7	< 0.001
		pCO_2	1	5.4	0.039
		Temp·pCO_2	1	4.1	0.067
		Error	12		
B	Growth (μday^{-1})	Temp	3	420.8	< 0.001
		Error	16		

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Table 2. Ratio of fatty acids in *N. lecointei* expressed in % after 14 days of treatment in Experiment A. Values within brackets represents standard error (n = 4).

Fatty acid	Concentration (%)			
	−1.8 °C		2.5 °C	
	390 µatm	960 µatm	390 µatm	960 µatm
16 : 0	1.5 (0.5)	0.5 (0.3)	4.4 (0.9)	5.4 (0.4)
16 : 1n-7	1.4 (0.4)	0.5 (0.3)	10.2 (1.6)	11.6 (1.2)
16 : 4	3.1 (0.6)	2.2 (1.1)	5.4 (1.2)	4.3 (1.1)
18 : 0	2.1 (0.9)	0.3 (0.1)	0.2 (0.1)	0.8 (0.4)
18 : 1n-9	1.1 (0.6)	0.2 (0.1)	0.8 (0.3)	0.8 (0.5)
18 : 2n-6	2.0 (1.2)	0.0 (0.0)	0.1 (0.1)	0.3 (0.3)
18 : 3n-3	54.8 (7.0)	54.7 (18.3)	54.6 (6.1)	47.1 (3.1)
18 : 4n-3	24.6 (9.9)	31.2 (20.8)	13.8 (8.2)	16.5 (2.1)
20 : 5n-3	9.6 (2.2)	10.4 (2.6)	10.8 (0.4)	13.2 (0.8)

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Table 3. Measured and calculated in situ mean values of pH_T, A_T, $[HCO_3^-]$, $[CO_3^{2-}]$, $[CO_2]$ and pCO_2. Values in brackets represents standard error (n = 4).

	Treatment			
	−1.8 °C		2.5 °C	
Parameter	390 µatm	960 µatm	390 µatm	960 µatm
pH_T	8.040 (0.004)	7.816 (0.018)	8.059 (0.019)	7.828 (0.024)
A_T (µmol kg^{-1})	2448 (3)	2459 (13)	2506 (9)	2519 (5)
$[HCO_3^-]$ (µmol kg^{-1})	2231 (4)	2323 (11)	2234 (11)	2349 (7)
$[CO_3^{2-}]$ (µmol kg^{-1})	87 (1)	54 (2)	111 (4)	69 (4)
$[CO_2]$ (µmol kg^{-1})	28 (0.3)	49 (2)	24 (1)	43
pCO_2 (µatm)	408 (4)	713 (29)	416 (20)	746 (42)

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Table 4. Inorganic nutrient concentration after 14 days in Experiment A and B.

Experiment	Treatment		Concentration ($\mu mol\,L^{-1}$)		
	Temperature (°C)	pCO_2 (μatm)	NO_3^-/NO_2^-	PO_4	SiO_2
A	−1.8	390	254	4.3	87
	−1.8	960	357	8.5	103
	2.5	390	408	7.4	108
	2.5	960	341	6.6	79
B	−2.3	N/A	231	5.1	89
	2.4	N/A	358	6.8	96
	5.1	N/A	410	8.4	118
	8.4	N/A	444	7.7	132

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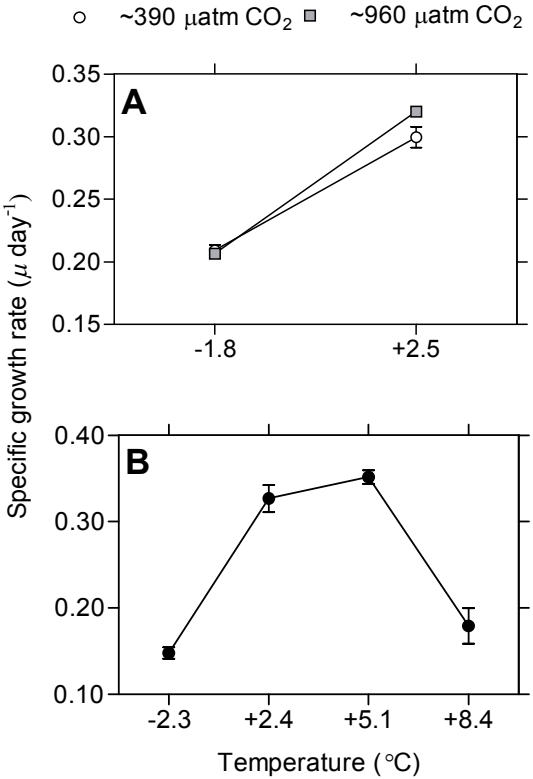

Fig. 1. Specific growth rate (µday-1) during 14 days of treatment. **(A)** Interaction in Experiment A. ($n = 4$) **(B)** Optimal growth temperature during Experiment B ($n = 5$). Error bars are displaying standard error.

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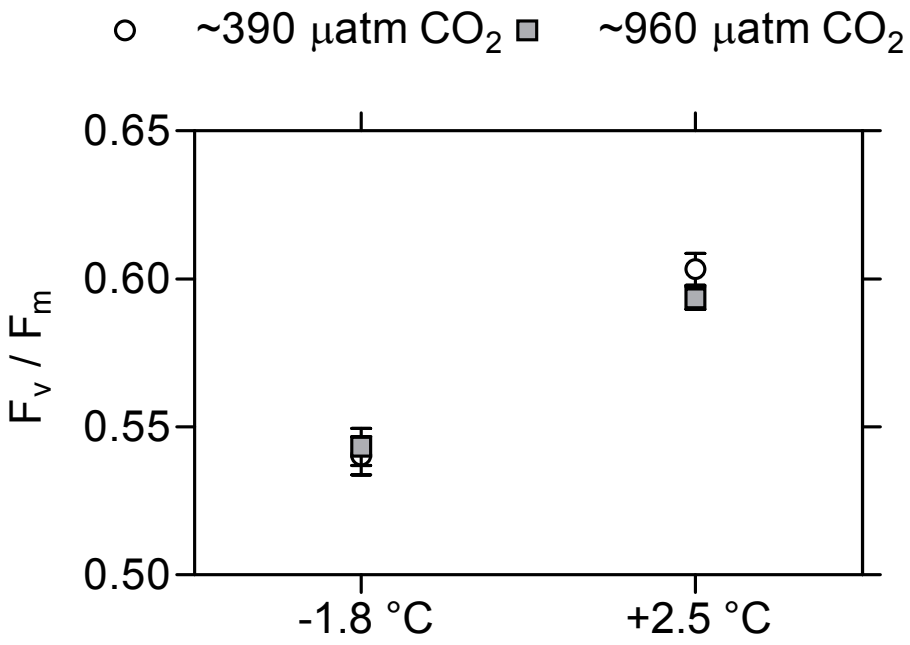

Fig. 2. Maximum quantum yield (F_v/F_m) measurements after 14 days of treatment in Experiment A. Error bars are displaying standard error (n = 4).

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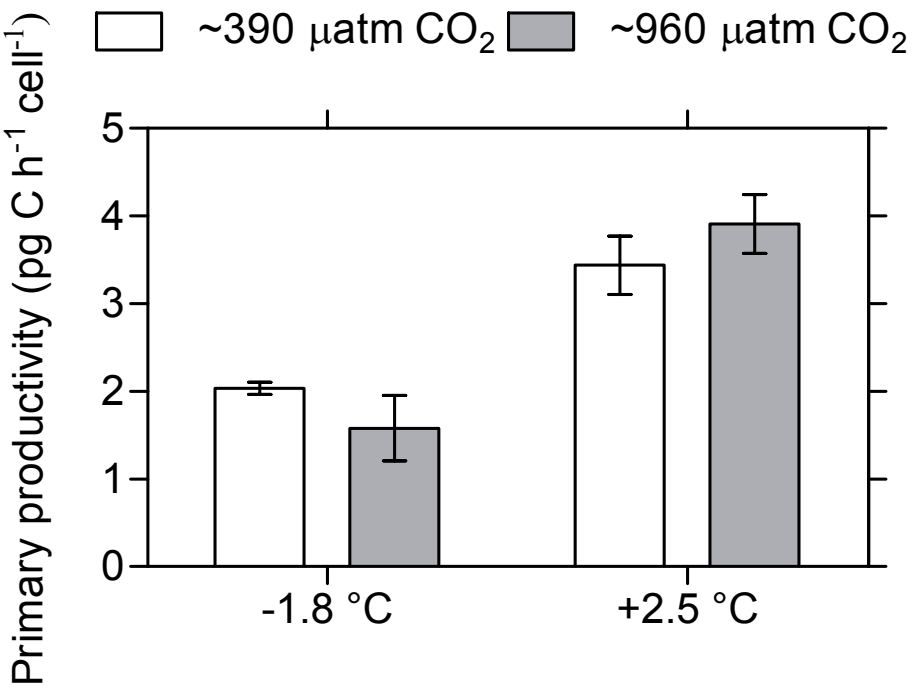

Fig. 3. Primary productivity (pg C h^{-1} $cell^{-1}$) measurements after 14 days of treatment in Experiment A. Error bars are displaying standard error (n = 4).

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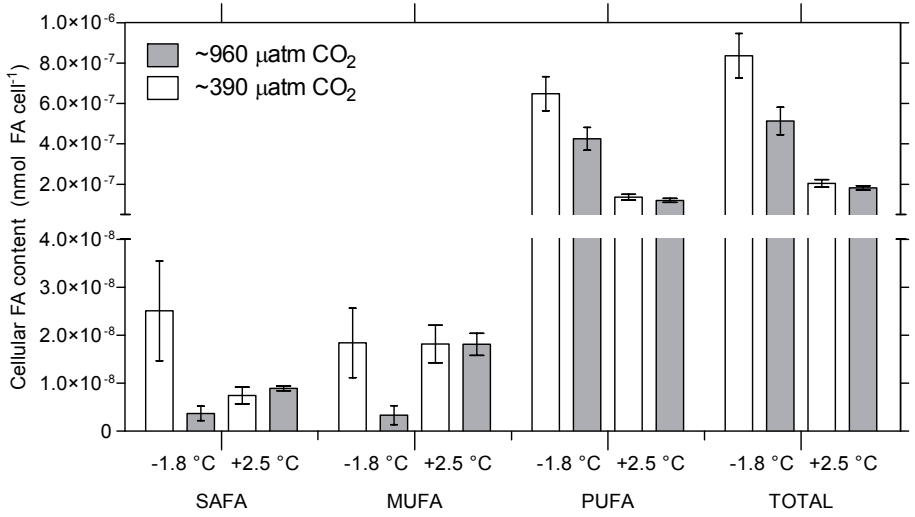

Fig. 4. Cellular fatty acid content (nmol FA cell^{-1}) in *N. lecointei* after 14 days of treatment in Experiment A. SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids. Error bars represent standard error (n = 4).

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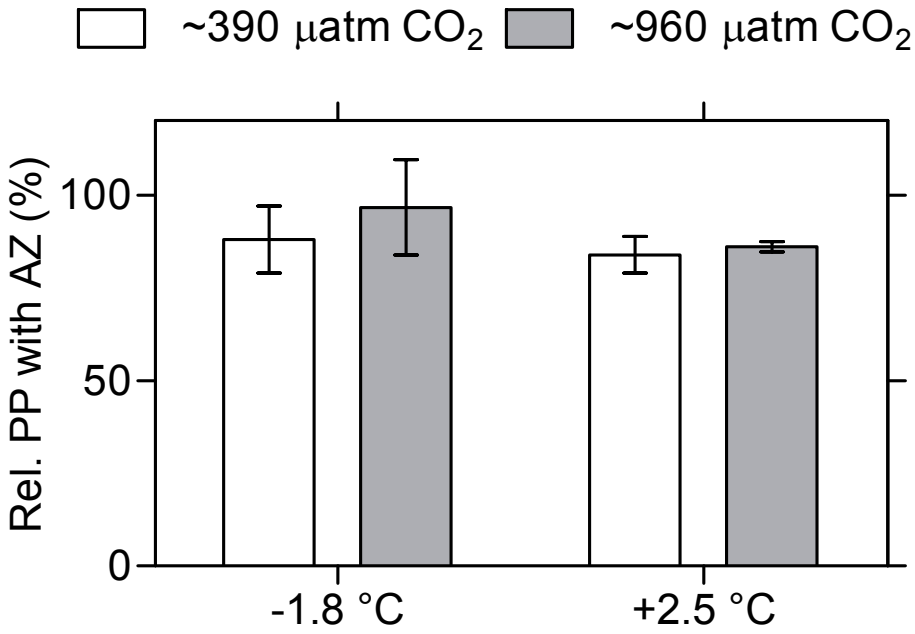

Fig. 5. Relative primary productivity (PP) rates after addition of carbonic anhydrase inhibitor acetazolamide (AZ). Carbon fixation is expressed in % in relation to samples incubated without inhibitor. Incubations were performed after 14 days of treatment in Experiment A. Error bars represent standard error (n = 4).

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