Biogeosciences, 10, 6391–6401, 2013
www.biogeosciences.net/10/6391/2013/
doi:10.5194/bg-10-6391-2013

Biogeosciences Open Access

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

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Received: 21 March 2013 – Published in Biogeosciences Discuss.: 10 April 2013
Revised: 29 August 2013 – Accepted: 4 September 2013 – Published: 9 October 2013

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 ($\sim$ 390 and $\sim$ 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 (FA) content. Optimal growth rate was observed around 5 °C in a separate experiment. Carbon enrichment only promoted (6 %) growth rate closer to the optimal growth, but not at the control temperature (-1.8 °C). At -1.8 °C and at $\sim$ 960 µatm pCO_2, the total FA content was reduced relative to the $\sim$ 390 µatm treatment, although no difference between pCO_2 treatments was observed at 2.5 °C. A large proportion (97 %) of the total FAs comprised on average of polyunsaturated fatty acids (PUFA) at -1.8 °C. Cellular PUFA content was reduced at $\sim$ 960 relative to $\sim$ 390 µatm pCO_2. Effects of carbon enrichment may be different depending on ocean warming scenario or season, e.g. reduced cellular FA content in response to elevated CO_2 at low temperatures only, reflected as reduced food quality for higher trophic levels. Synergy between warming and acidification 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 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 1970s, and recent models have predicted a sea-ice-free Arctic Ocean during the summer within the next 30 years (Wang and Overland, 2009). In fact, the extent of sea ice in the Arctic reached a record low as reported in September 2012 (Parkinson and Comiso, 2013).

In Antarctica, the climate has been relatively stable from a geological perspective (Petit et al., 1999), enabling biological 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 suggest 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, cellular fatty acid (FA) composition and nutrient stoichiometry can be directly related to the food quality transferred to higher trophic levels and may be negatively affected by ocean acidification (Riebesell et al., 2000; Rossoll et al., 2012; Schoo et al., 2013). However little is known about the combined effects of ocean acidification and warming on microalgal lipid FA composition.

Sea ice plays an important role in carbon biogeochemistry, both in terms of physiochemical and biological processes. Algae, in particular diatoms, inhabiting the bottom part of sea ice and brine channels contribute significantly to the primary production in ice-covered polar areas and provide a substantial carbon source to higher trophic levels. During spring, the early development of bottom ice algae is very important for grazers, as bottom ice algae are abundant and contain high levels of PUFA compared to phytoplankton. The peak in bottom ice algal biomass occurs before the ice edge bloom. Hence, the bottom 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 of sea ice, e.g. salinity, partial pressure of CO_2 (pCO_2), vary substantially during the season. During freezing, pCO_2 is higher 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 intense ice algal growth 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 bottom ice algal community.

Below the optimum temperature, 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 psychrophilic 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 psychrophilic 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 (Wu et al., 2010; Boelen et al., 2011; Torstensson et al., 2012). The combination of increased pCO_2 and alteration of additional environmental factors (e.g. radiation) complicates the response and could explain the species-specific responses (Gao et al., 2012; Li et al., 2012). 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.

Antarctic sea ice algae exhibit a widespread occurrence of carbon concentrating mechanisms (CCMs) to assist carbon fixation in photosynthesis when CO_2 is limited (Tortell et al., 2013). 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 (Giordano et al., 2005). 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 suggest that CA activity in diatoms is costly and is induced at low levels of pCO_2 in order to maintain photosynthetic activity at different pCO_2 (Burkhardt et al., 2001). Hence, the efficiency of CCMs needs to be further addressed, as they 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.

2 Material and methods

2.1 Experimental set-up

The diatom *N. lecointei* was isolated from sea ice from the Amundsen Sea (January 2011) and cultivated in $f/2$ medium with silica (Guillard, 1975) at -1.8 °C. The stock culture was maintained at experimental light and nutrient conditions for one month before the start of the experiment. 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). Experimental treatments were set up using flasks and bubbling system as in 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. At day 0 of the experiment, the stock culture of *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). 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. The constant bubbling created agitation in the culture. Estimated time to shift and settle a new equilibrium of the carbonate system was three days in previous experiments (unpublished data) using the set-up by Torstensson et al. (2012), providing a gradual alteration and possibility to algal acclimation. 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*. At day 0 of the experiment, the stock culture of *N. lecointei* was inoculated (5.2×10^5 cells L^{-1}) in 360 mL batch cultures containing $f/2$ medium with silica prepared from 0.2 µm filtered Antarctic seawater (salinity 33). Water baths equipped with cooling and circulation provided four temperature treatments, ranging from −2.3–8.4 °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.

In Experiment A and B, 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 was performed 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). For this, 3 mL of sample was carefully transferred into a 10.00 mm cuvette and 30 µL *m*-cresol purple was added. The 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 using the 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. Between 35 and 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).

2.3 Photosynthetic activity

The maximum quantum yield of photosystem II (F_v/F_m) was measured by pulse amplitude modulation (PAM) fluorometry, using a Water-PAM (Universal control unit, Water-ED/B, Walz Mess- und Reigeltechnik, Effeltrich, Germany). For each measurement, 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) was calculated using Eq. (1):

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

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, was measured using the radiocarbon technique (Aertebjerg and Bresta, 1984). For this, 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 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):

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

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.

During the ^{14}C measurements, additional incubations of each sample were prepared with addition of the CA-inhibitor acetazolamide (AZ, final concentration 400 µM) (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany). Concentration of inhibitor was chosen after optimization using other diatom species (Morant-Manceau et al., 2007). CA inhibition was determined as the relative difference in primary productivity between untreated and AZ-treated cells.

2.6 Glycerolipid fatty acid composition

For fatty acid analysis, 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. The filters were submerged in boiling isopropanol for 5 min and dried under a stream of N_2. Total lipids were extracted in chloroform:methanol:water (1 : 2 : 0.8, by volume) as described in Andersson et al. (2006). The total lipid extract was subjected to alkaline transesterification (Christie, 1976) and analysed by gas chromatography-mass spectrometry (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. Fatty acid methyl ester standards used were provided from Supelco; synthetic 37 component FAME mix and marine PUFA No.3 from Menhaden oil. 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).

2.7 Inorganic nutrients

For analysis 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).

2.8 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. Student–Newman–Keuls (SNK) post-hoc analysis was performed when necessary. A probability level of < 0.05 was used for statistical significance. SuperANOVA 1.11 and SPSS 19.0 were used for statistical analyses.

3 Results

3.1 Growth and photosynthesis

In Experiment A, an interaction between warming and rising pCO_2 was observed for specific growth rate of *N. lecointei* ($p < 0.05$; two-factor ANOVA; Fig. 1a). Growth rate increased (6 %) significantly in ∼960 µatm compared to the ∼390 µatm pCO_2 at 2.5 °C (Fig. 1a). However, there was no difference in growth rate at −1.8 °C between the pCO_2 treatments. After 14 days of exposure, mean cell densities at −1.8 °C were 3.1×10^7 and 3.0×10^7 cells L^{-1} in the ∼390 and ∼960 µatm pCO_2 treatments, respectively. In the warm treatment mean densities were 1.1×10^8 and 1.5×10^8 cells L^{-1} in the ∼390 and ∼960 µatm pCO_2 treatments, respectively.

In Experiment B, the specific growth rate was significantly different between treatments ($p < 0.001$; two-factor

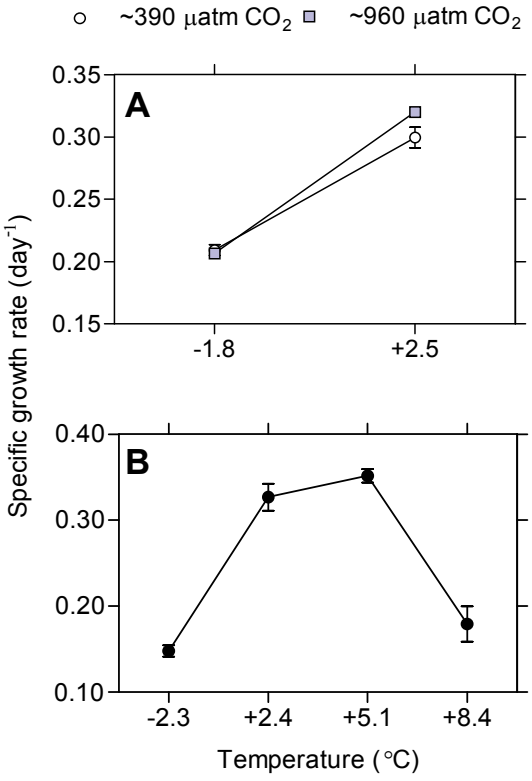

Fig. 1. Specific growth rate (day^{-1}) during 14 days of treatment. **(A)** Interaction of temperature and pCO_2 in Experiment A. ($n = 4$) **(B)** Optimal growth temperature during Experiment B ($n = 5$). Error bars are displaying standard error.

ANOVA). The growth rate increased when temperature increased from the ambient up to 5.1 °C where the highest growth rate was detected. At further temperature increase, the specific growth rate declined (Fig. 1b). Growth rates were significantly differentiated between all treatments ($p < 0.01$; SNK test). The one factor ANOVA was performed with heterogeneous variances due to inability of transformation, as the F-test is rather robust to heteroscedasticity in balanced designs (Underwood, 1997). At the end of the experiment mean cell density was 4.1×10^6 cells L^{-1} in the −2.3 °C treatment, 5.1×10^7 cells L^{-1} in 2.4 °C, 7.2×10^7 cells L^{-1} in 5.1 °C and 6.5×10^6 cells L^{-1} in 8.4 °C.

In Experiment A, F_v/F_m ($p < 0.001$; two-factor ANOVA; Fig. 2) and primary productivity ($p < 0.001$; two-factor ANOVA; Fig. 3a) were significantly higher (9 and 50 %, respectively) at 2.5 °C compared to −1.8 °C. However, no interactions between treatments or main effects of pCO_2 were observed ($p > 0.05$; two-factor ANOVA). One sample from the ^{14}C-incubation was lost due to technical error. Inhibition of primary productivity was observed in 15 measurable samples where AZ was added, giving a mean inhibition of 12 % (Fig. 3b). However, no significant interaction or main effects were detected ($p > 0.05$; two-factor ANOVA).

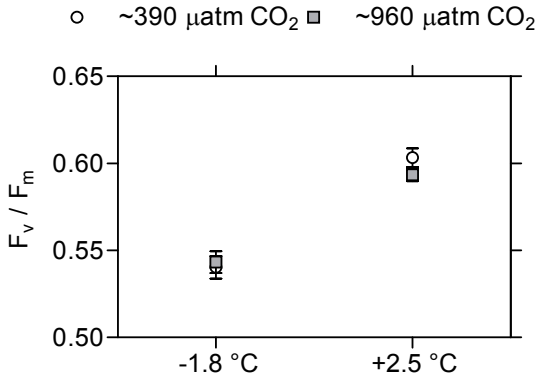

Fig. 2. Maximum quantum yield (F_v/F_m) measurements after 14 days of treatment in Experiment A. Error bars are displaying standard error ($n = 4$).

3.2 Glycerolipid fatty acid composition

There was a significant interaction between treatments for total FA content in Experiment A ($p < 0.05$; two-factor ANOVA; Fig. 4). At −1.8 °C, total FA content was reduced from 193 ± 26 (mean ± standard error) to 121 ± 17 fg FA $cell^{-1}$ in the ∼960 µatm treatment compared to the ∼390 µatm treatment. However, there was no difference between the two pCO_2 treatments at 2.5 °C, 45 ± 5 and 41 ± 3 fg FA $cell^{-1}$ at ∼390 and ∼960 µatm, respectively. No interactions were observed for cellular content of SAFA, MUFA and PUFA ($p > 0.05$; two-factor ANOVA; Fig. 4). However, cellular PUFA content was significantly lower ($p < 0.001$; two-factor ANOVA) in the 2.5 °C treatment (36 ± 2 fg FA $cell^{-1}$) compared to the −1.8 °C treatment (150 ± 17 fg FA $cell^{-1}$). Cellular PUFA content also decreased significantly (from 110 ± 29 to 76 ± 18 fg FA $cell^{-1}$) with increased pCO_2 ($p < 0.05$; two-factor ANOVA). No significant difference in SAFA and MUFA content was detected between treatments ($p > 0.05$; two-factor ANOVA). Statistical analyses of SAFA and MUFA were executed with heterogeneous variances, due to inability of transformation.

The cellular FA content for each fatty acid is presented in Table 1. Among the FAs the omega-3 PUFAs 18 : 3n-3 (α-linolenic acid, ALA), 18 : 4n-3 (stearidonic acid, SDA) and 20 : 5n-3 (eicosapentaenoic acid, EPA) were dominating, representing 92 % of the total FA content at −1.8 °C (Table 1). The most common FA in all treatments was ALA. PUFAs represented on average 97 % of the total FA content at −1.8 °C (Table 1).

3.3 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 2. A_T increased on average by 5.2 and 7.7 % during the 14 days of experiment in the −1.8 and 2.5 °C

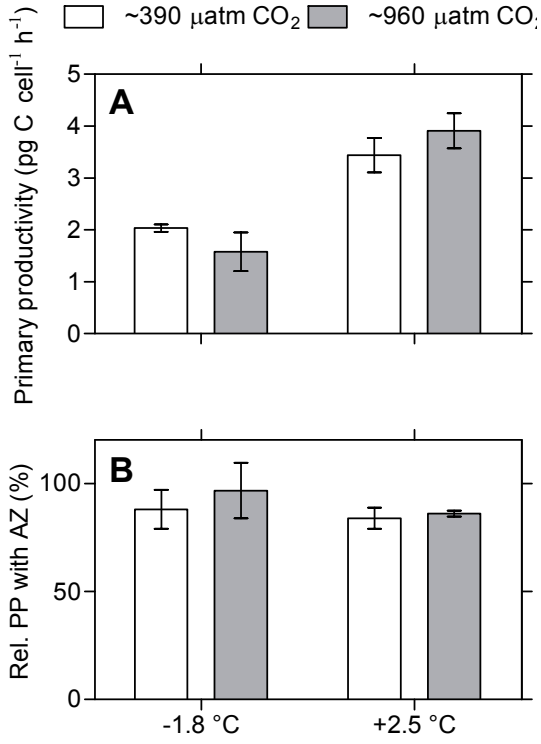

Fig. 3. (A) Primary productivity (pg C h^{-1} $cell^{-1}$) measurements after 14 days of treatment in Experiment A. **(B)** Relative primary productivity (PP) rates after addition of carbonic anhydrase inhibitor acetazolamide (AZ). Carbon fixation is expressed in % in relation to samples incubated without inhibitor. Incubations were performed after 14 days of treatment in Experiment A. Error bars are displaying standard error ($n = 4$).

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 and 2.5 °C temperature treatments, respectively.

Inorganic nutrients were measured to rule out nutrient limitation. Results showed satisfying levels (Table 3), with steady values of F_v/F_m throughout the experiments, suggesting inorganic nutrients were not limiting growth.

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 the 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).

Our 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 either over- or underestimate the effect of carbon enrichment due to a temperature dependency of the response. 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). Furthermore, acclimation and the ability to benefit from elevated carbon dioxide levels could be an alternative explanation to the interaction observed for the growth rate in this study. Since growth rate was higher in the warmer treatment, the potential acclimation rate to high CO_2 was also higher. Although we believe that the difference in accumulated generations in this experiment are too few to confound the results, long-term studies are crucial for understanding acclimation and adaptation to climate change.

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 lower total FA content and PUFA concentration in *Thalassiosira pseudonana* grown at high pCO_2, 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 and 76 % (∼ 390 and ∼ 960 µatm, respectively) at 2.5 °C compared to −1.8 °C 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 the cellular lipid content of phytoplankton from the Antarctic Ocean. Smith and Morris (1980) observed that up to 80 % of assimilated carbon was incorporated in lipids at temperatures below 0 °C. Above 0 °C the incorporation into lipids was less than 20 %. In addition, Smith and Morris (1980) suggested that low light stress may enhance the effect of low temperature on stored lipids. Increased lipid content due to environmental stressors, such as nutrient deficiency, low light and low temperature may be a characteristic response for polar phytoplankton populations (Smith and Morris, 1980). Deficiency of nitrogen elevate cellular lipid content in diatoms in order to accumulate FAs when conditions are unfavorable (Parrish

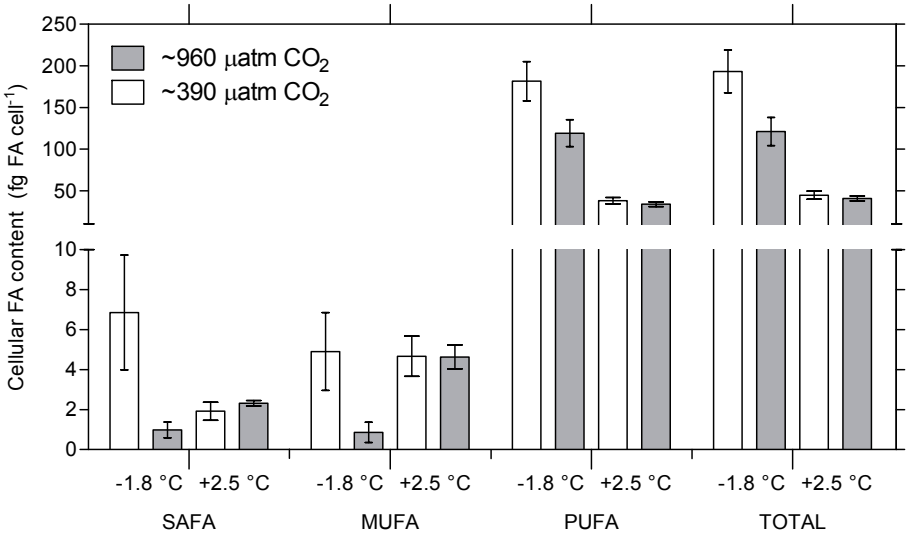

Fig. 4. Cellular fatty acid content (fg FA cell^{-1}) in *N. lecointei* after 14 days of treatment in Experiment A. SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids. Error bars represent standard error ($n = 4$).

Table 1. Cellular content of acyl lipid fatty acid in *N. lecointei* after 14 days of treatment in Experiment A. Values within brackets represents standard error ($n = 4$).

Fatty acid	Content (fg FA cell^{-1})			
	−1.8 °C		2.5 °C	
	~ 390 µatm	~ 960 µatm	~ 390 µatm	~ 960 µatm
16 : 0	2.5 (0.9)	0.6 (0.3)	1.9 (0.4)	2.0 (0.1)
16 : 1n-7	2.7 (1.0)	0.7 (0.5)	4.3 (0.9)	4.3 (0.4)
16 : 4	5.4 (1.0)	2.8 (1.5)	2.1 (0.3)	1.6 (0.4)
18 : 0	4.3 (2.0)	0.4 (0.1)	0.1 (0.03)	0.3 (0.1)
18 : 1n-9	2.3 (1.2)	0.2 (0.1)	0.4 (0.2)	0.3 (0.2)
18 : 2n-6	4.2 (2.7)	0.0 (0.0)	0.04 (0.03)	0.1 (0.1)
18 : 3n-3	101.6 (9.7)	72.5 (26.6)	25.3 (5.0)	19.4 (2.5)
18 : 4n-3	49.9 (24.2)	29.1 (17.4)	5.5 (2.7)	6.7 (1.0)
20 : 5n-3	20.3 (4.9)	14.7 (5.0)	5.3 (0.6)	5.9 (0.4)
Total	193.1 (25.8)	121.0 (16.8)	44.7 (4.89)	40.7 (2.8)

and Wangersky, 1987). However, no signs of nutrient deficiency were detected in this study. These findings suggest a major reduction of *N. lecointei* FA content in response to climate change.

Bottom ice algae 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 high PUFA concentrations in all treatments. FA composition in cultures and natural algal communities in polar oceans have shown PUFA proportions of approximately 20–60 % of the total FA content (Fahl and Kattner, 1993; Falk-Petersen et al., 1998; Teoh et al., 2004; Leu et al., 2006). However, PUFA in *N. lectonei* represented 94–98 % of the FAs at −1.8 °C. Significantly lower PUFA proportions were measured at 2.5 °C; 81.5 % at ~ 390 µatm and 84.5 % at ~ 960 µatm pCO_2, reflecting the importance of PUFA as a cold adaption and illustrating the ecological role of *N. lectonei*, as it is considered a high-quality food source for grazers.

The level of carbon dioxide also appears to alter FA content under certain temperature conditions in *N. lecointei*. Rossoll et al. (2012) showed a similar 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. However, no alteration of SAFA and MUFA content was detected in *N. lecointei*, and the response of pCO_2 appeared temperature dependent. Carbon level only affected total FA content

Table 2. Measured and calculated mean values of pH_T, A_T, $[HCO_3^-]$, $[CO_3^{2-}]$, $[CO_2]$ and pCO_2 with CO2SYS at initial measurements (before aeration of the medium) and after 14 days of treatment in Experiment A. Values within brackets represents standard error ($n = 4$).

	Initial	Treatment			
		−1.8 °C		2.5 °C	
		∼ 390 µatm	∼ 960 µatm	∼ 390 µatm	∼ 960 µatm
pH_T	8.386 (0.017)	8.040 (0.004)	7.816 (0.018)	8.059 (0.019)	7.828 (0.024)
A_T (µmol kg^{-1})	2332 (7)	2448 (3)	2459 (13)	2506 (9)	2519 (5)
$[HCO_3^-]$ (µmol kg^{-1})	1840 (21)	2231 (4)	2323 (11)	2234 (11)	2349 (7)
$[CO_3^{2-}]$ (µmol kg^{-1})	199 (6)	87 (1)	54 (2)	111 (4)	69 (4)
$[CO_2]$ (µmol kg^{-1})	9 (0.5)	28 (0.3)	49 (2)	24 (1)	43 (2)
pCO_2 (µatm)	162 (8)	408 (4)	713 (29)	416 (20)	746 (42)

Table 3. Inorganic nutrient concentration after 14 days in Experiment A and B.

Experiment	Treatment		Concentration (µmol 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

of *N. lecointei* at low temperature, while an increased pCO_2 lead 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, LA), was also found. Zhang et al. (2011) showed a relationship between decreasing temperature and increasing activity of an omega-3 FA desaturase in a psychrophilic strain of *Chlamydomonas* sp., suggesting a correlation between desaturase activity and the shift from 18:2 PUFA to 18:3 PUFA. Mayzaud et al. (2013) demonstrated that increasing temperatures decreased the amount of omega-3 PUFA in natural blooming phytoplankton in an Arctic fjord. Loss of PUFA results in reduced food quality for organisms grazing on primary producers, and may have serious ecological consequences. 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 from *T. pseudonana* grown at high 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 performed in the Arctic show changed foraging patterns in Little auks (*Alle alle*) due to a lack of energy-rich prey near breeding sites, resulting in decreased reproductive success (Steen et al., 2007). In addition to reduced food quality, an alteration in FA content at subzero temperatures could also affect the ability of *N. lecointei* to tolerate low temperature stress due to reduced membrane fluidity. 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 the growth of *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 microalgae. 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 bottom ice algae like *N. lecointei* will be negatively affected by further temperature increase (data not shown). The primary productivity and F_v/F_m of *N. lecointei* increased with elevated temperature in Experiment A. Kennedy et al. (2012) observed highest F_v/F_m in the warmest part of sea 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. 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.

Energy savings from down-regulation of CCMs are suggested to increase carbon fixation in phytoplankton, when sufficient levels of carbon are achieved without the use of energy-consuming CCMs (Raven, 1991; Hopkinson et al., 2011). However, the physiological responses to ocean acidification in diatoms tend to be very species-specific (Wu et al., 2010; Boelen et al., 2011; Torstensson et al., 2012). The growth rate of *T. pseudonana* was unaffected by elevated pCO_2, even though CCMs were down-regulated and photosynthesis up-regulated (Yang and Gao, 2012). This observation was explained by an increased rate of mitochondrial respiration. In our study, primary productivity was reduced in 15 measurable samples that were treated with the CA inhibitor AZ. The inhibition was of the 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 depends on the concentration of the inhibitor (Morant-Manceau et al., 2007), 12 % might be an underestimation.

Bottom ice algae play an important role as a food source for grazers in the early season and as seeding populations to the pelagic 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 bottom ice algae more than the microalgae themselves, since the microalgae nevertheless end up in the water column during the melting season. The upper and middle sections of the sea ice would in a warming scenario become less cold in general during the season, resulting in more porous sea ice with less saline brine. However, during formation of sea ice levels of dissolved carbon dioxide increase, as the water freezes and the solutes become more concentrated within the non-frozen brine channel matrix. Nonetheless, 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). However, the pCO_2 treatments in this study are still considered relevant to conditions in the ice when the bottom ice algae are starting to grow. Nevertheless, it will be important to consider the natural variability of pCO_2, 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 must be considered when assessing physiological effects of ocean acidification on photoautotrophs. Hence, the growth effects of 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 this must be considered when assessing results from ocean acidification perturbation experiments.

Acknowledgements. This study was financed by the Swedish Research Council (VR Project No. 2007-8365) and Lindhes Advokatbyrå AB foundation. We would like to thank A. Al-Handal for species identification.

Edited by: U. Riebesell

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