Impact of low pH/high pCO2 on the physiological response and fatty acid content in diatom Skeletonema pseudocostatum

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pCO2/pH perturbation experiments were carried out under two different pCO2 levels to evaluate effects of CO2-driven ocean acidification on semi-continuous cultures of the marine diatom Skeletonema pseudocostatum CSA48. Under higher pCO2/ lowered pH conditions, our results showed that CO2-driven acidification had no significant impact on growth rate, chlorophyll-a, cellular abundance, gross photosynthesis, dark respiration, particulate organic carbon and particulate organic nitrogen between CO2-treatments, suggesting that S. pseudocostatum is adapted to tolerate changes of ~0.5 units of pH under high pCO2 conditions. However, dissolved organic carbon (DOC) concentration and DOC/POC ratio were significantly higher at high pCO2, indicating that a greater partitioning of organic carbon into the DOC pool was stimulated by high CO2/low pH conditions. Total fatty acids (FAs) were significantly higher under low pCO2 conditions. The composition of FAs changed from low to high pCO2, with an increase in the concentration of saturated and a reduction of monounsaturated FAs. Polyunsaturated FAs did not show significant differences between pCO2 treatments. Our results lead to the conclusion that the balance between negative or null effect on S. pseudocostatum ecophysiology upon low pH/high pCO2 conditions constitute an important factor to be considered in order to evaluate the global effect of rising atmospheric CO2 on primary productivity in coastal ocean. We found a significant decrease in total FAs, however no indications were found for a detrimental effect of ocean acidification on the nutritional quality in terms of essential fatty acids.

Keywords: Diatom, low pH, high CO2, fatty acid, DOC, photosynthesis, elemental composition

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INTRODUCTION

Diatoms dominate the phytoplankton community in coastal ecosystems, contributing to ~20% of global primary production (Nelson et al., 1995). Because of their large size and silica ballast, they contribute a major fraction of the downward flux of particulate organic matter into deep ocean (Alldredge & Jackson, 1995) and therefore constitute major players in carbon sequestration from the atmosphere to the deep ocean (Boyd et al., 2010). Chain-forming centric diatoms are the most successful group of eukaryotic primary producers in productive upwelling coastal ecosystems, supporting higher trophic levels. A prerequisite for their high growth rates is an efficient and regulated acquisition of inorganic carbon (Ci) that compensates for the catalytic inefficiency of the enzyme Ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO) (Burkhardt et al., 2001).

Ocean acidification (OA) is a consequence of increased inorganic carbon content of the ocean surface water due to rising atmospheric CO2. The associated drop in the average surface water pH from ~8.2 to 7.8 represents one of the most rapid OA events on earth over the past 300 Myr (Caldeira & Wickett, 2003). It is a controversial issue whether high CO2/low pH in seawater would significantly promote growth and primary productivity. Responses of diatoms to high pCO2 and decreased pH are likely to be species-specific, with potential winners, neutral and losers (Gao & Campbell, 2014). Diatoms can downregulate the activity of extracellular carbonic anhydrase (Burkhardt et al., 2001), but they differ in their CO2 concentrating mechanisms (CCMs) (Hopkinson et al., 2013). Photosynthetic responses to enhanced CO2 under OA are remarkably diverse, and there is
a large variability both between and within taxonomic groups. Nevertheless, despite the growing body of literature on the topic, clear trends in the photosynthetic responses of phytoplankton to elevated CO₂ have not emerged, and positive effects, if any, are small (Mackey et al., 2015).

In addition to growth and primary production, the elemental composition of phytoplankton may vary under OA. Increasing CO₂ can lead to increased particulate organic carbon (POC) content relative to N and P quotas, i.e., lower C:N ratios have not been observed in response to rising pCO₂ and temperature (Burkhart et al., 1999; Wohlers-Zöllner et al., 2011). Moreover, the current evidence demonstrated that large differences in the elemental composition of marine phytoplankton can arise from nutrient limitation (Geider & LaRoche, 2002), physical factors (Laws & Bannister, 1980; Burkhart et al., 1999) and interspecific variability among algal species with different C:N:P requirements (Geider & LaRoche, 2002).

Other important proxies for food quality in marine food webs are fatty acid (FA) content and composition. Polynsaturated fatty acids (PUFA) are considered especially important as they represent essential FAs that cannot be synthesized de novo by heterotrophic consumers (Müller-Navarra et al., 2004). Phytoplankton production of PUFAs is highly dependent on the algal physiology and nutrients, and therefore on the environmental conditions (Klein Breteler et al., 2005; Leu et al., 2013). There is a paucity of information on how OA might impact FA contents. Here, we examine the effects of high CO₂/low pH and excess nutrients on growth and physiological rates, elemental composition, carbon partitioning and nutritional quality of Skeletonema pseudocostatum in order to evaluate: (1) how the growth rate, photosynthesis and carbon partitioning of Skeletonema pseudocostatum is affected by a drop of ~0.5 units of pH caused by elevated pCO₂ levels and (2) how physiological rates may affect the fatty acid content under condition of increased CO₂/lowered pH.

**MATERIALS AND METHODS**

**Semi-automatic system for seawater carbonate manipulation**

Experiments were conducted using a semi-automatic mesocosm system for seawater carbonate chemistry manipulation at Calbuco Marine Laboratory in south-central Chile (39°78′S, 73°36′W). CO₂-enriched seawater was produced by bubbling seawater with air-CO₂ mixtures, following the method described by Torres et al. (2013). The system uses mass flow controllers (MFC) to blend atmospheric air with ultra-pure CO₂ (i.e. research grade) to produce different pCO₂ levels (see Table 1). The seawater was continuously bubbled with either ambient or enriched pCO₂-air. The high level of pCO₂, 1123 µatm, corresponded approximately with projected atmospheric levels between years 2100 and 2150 under the RCP 8.5 scenario (Meinshausen et al., 2011). Air/CO₂ mixtures were produced using a bulk technique, where dry air with pure CO₂ were supplied to seawater using an air mass flow controller (MFC) (Aalborg, model GFC; http://www.aalborg.com) and a CO₂ MFC (Aalborg, Model GFC). Dry and filtered air (through a 1 µm particulate filter) was generated by compressing atmospheric air (117 psi) using an oil-free, 4 piston air compressor (Schulz, model MSV12). Pressure in the air and pure CO₂ was maintained at ~10 psi. Air flow in MFC was set manually to 5 l min⁻¹ for treatment and CO₂ flow was set manually to 4.25 ml min⁻¹ to produce the high CO₂ treatment. The CO₂ of blended gas was monitored to allow fine regulation of CO₂ through MFC to reach target pCO₂ in seawater. The pCO₂ monitoring system was based on a CO₂ analyser (Qubit System, model S151), primarily for measuring the CO₂ content in the air-CO₂ mixture.

**Culture conditions**

*Skeletonema pseudocostatum* (CSA 48, non axenic) was isolated from Yaldad Bay, southern Chile (43°1′S–73°7′W) in March 2009 and obtained from the COPAS Sur-Austral strain collection (http://www.ficolab.cl), at the Department of Botany, Concepción University. Cells were grown and acclimated in autoclaved (1 L) and filtered (0.1 µm) natural seawater (salinity: 29.8 PSU) within autoclaved glass bottles (1 L) at the same temperature (14.7 ± 0.6°C), light intensity (195 µmol m⁻² s⁻¹) and at a 16/8 light/dark cycle. Light was measured with a sensor Li-192SA (Li-Cor) and was provided by cold white LED tubes (22 Watts, 6000 K). The time of sampling was kept throughout the acclimatization and experiment. For the acclimatization, seawater was enriched to f/2 medium (Guillard & Ryther, 1962). Cells were maintained in exponential growth phase using a semicontinuous culture. To maintain balanced exponential growth, cultures were diluted with fresh medium every 3–4 days, keeping cell concentrations <4.3 × 10⁶ cell ml⁻¹ during the acclimation. During the first (21–25 November) and second round (26–29 November) of the algal acclimation under low pCO₂ levels, mean pH values ranged between 8.106 and 8.230, respectively (Table 1). In cultures under high pCO₂ conditions, mean pH values ranged between 7.676 and 7.654, respectively. Cultures were acclimated to the respective pH/pCO₂ values for 10 generations.

After acclimatization, cells from respective pH/pCO₂ treatments were inoculated in autoclaved polycarbonate carboys filled with 20 L of autoclaved seawater (29 November) at the same temperature and light intensity, and carboys were positioned randomly in the experimental system. Carboys were closed with rubber stoppers pierced with glass capillaries for inlet and outlet of air/CO₂ mixture. Four carboys were used for low pCO₂ and four for high pCO₂ treatments, while two control carboys without cells were followed for monitoring abiotic changes in carbonate system parameters. Under these culture conditions, cells were grown for ~6 generations. Samples for carbonate system parameters were taken on 1 and 3 December (Table 1). The harvesting of samples was carried out on 3 December. Cell concentrations at the time of sampling were ~22 × 10⁶ cell ml⁻¹ at low pCO₂ and ~14.5 × 10⁶ cell ml⁻¹ at high pCO₂. During the experimental period, the photosynthetic activity and cell density increased, leading to an increase in pH (0.11 unit under low CO₂ and 0.018 unit under high CO₂). The concentration of DIC and
Table 1.  

<table>
<thead>
<tr>
<th>Date</th>
<th>Period</th>
<th>Treatment</th>
<th>pH (NBS)</th>
<th>DIC (mmol kg⁻¹)</th>
<th>pCO₂ (μatm)</th>
<th>TA (μmol kg⁻¹)</th>
<th>HCO₃⁻ (mmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21–25.11.2013</td>
<td>1st round</td>
<td>L</td>
<td>6.16 ± 0.07</td>
<td>2.14 ± 0.53</td>
<td>7.67 ± 0.30</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>26–29.11.2013</td>
<td>2nd round</td>
<td>H</td>
<td>6.18 ± 0.08</td>
<td>2.16 ± 0.56</td>
<td>7.64 ± 0.33</td>
<td>0.03 ± 0.01</td>
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</tr>
</tbody>
</table>

First round of acclimation was carried out between 21 and 25 November and second round between 26 and 29 November. Experiment started on 29 November and ended on 3 December.

**Chemical analysis**

Samples for nutrient analysis (NO₃⁻, NO₂⁻, PO₄³⁻ and Si (OH)₄) were taken every day during the acclimation and experimental periods (Table 2). Samples were filtered (GF/F) and frozen (−20°C) until analysis following Strickland & Parsons (1968). Daily pH samples were collected in 50 ml syringes and immediately transferred to a 25 ml thermostatted cell at 25.0 ± 0.1°C for standardization, and measurements were conducted with a pH electrode with a glass combined double Ag/AgCl junction (Metrohm model 6.025(8.600) calibrated with standard National Bureau of Standards (NBS) calibration buffer Metrohm® 4 (Code 6.2307.200), 7 (Code 6.2307.210) and 9 (Code 6.2307.220). The estimated analysis error for this analysis was estimated as <0.01 pH. For dissolved inorganic carbon (DIC) and DOC determination, separate 30 ml subsamples were collected with a sterile syringe and filtered through a Swinex containing a GF/F filter that had been precombusted for 4−5 h at 450°C directly into 40 ml glass 200 Series I-CHEM® vials. For DIC analyses, the septa of vials were exchanged for butyl rubber septa to prevent diffusion of CO₂ (DOE, 1994). Samples for DIC analysis were preserved with 50 µl of a saturated solution of mercuric chloride (DOE, 1994). Immediately after opening the sample bottle, a digital syringe withdrew a small amount of sample (0.5 ml), acidified it with 10% phosphoric acid and subsequently measured the evolved CO₂ with a LICOR 6262 non-dispersive infrared gas analyser. Certified seawater reference materials from A. Dickson were used to ensure the quality of DIC determination by preparing a calibration curve covering the range of DIC from 200−2000 µeq l⁻¹ (Dickson et al., 2003), with a resulting precision averaging ≈ 0.1% (range 0.05−0.5%). Temperature and salinity data were used to calculate the other carbonate system parameters (e.g. pCO₂, HCO₃⁻). Analyses were performed using CO₂SYS software for MS Excel (Pierrot et al., 2006) set with Mehrbach solubility constants (Mehrbach et al., 1973) refitted by Dickson & Millero (1987). The KHSO₄ equilibrium constant determined by Dickson (1990) was used for all calculations.

For POC and particulate organic nitrogen (PON) analysis, a subsample (1 l) was filtered through combusted (4−5 h at 450°C) GF/F filters to concentrate particles. Filters were dried at ~60°C for 24 h and held in a desiccator until analysed. DOC samples were bubbled with CO₂−free nitrogen for 7 min to ensure complete removal of DIC. DOC and POC measurements were conducted by the G.G. Stable Isotope Hatch, Laboratories at the University of Ottawa, Canada, with an analytical precision of 2%. All DOC and POC samples were run on an total inorganic carbon-total organic carbon (TIC-TOC) analyser (OI Analytical Analyzer, Model 1030). Data were normalized using internal standards.

Determination of fatty acids (FAs) was conducted from water samples onto filtered through MFS GF/F filters to concentrate particles. Saturated (SAFA) and unsaturated fatty acids (MUFA: monounsaturated and PUFA: polyunsaturated) were measured on separate filters dried at 50°C for 24 h and held in a desiccator until analysed. The fatty acid concentrations were measured after extraction and methylation.
Inorganic nutrient concentrations (± SE, N = 4) and nutrient ratios (± SE, N = 4) during (A) acclimation and (B) experimental period and under two different CO2 concentrations; Low pCO2 (L); High pCO2 (H).

<table>
<thead>
<tr>
<th>Date</th>
<th>Period</th>
<th>Treatment</th>
<th>pCO2</th>
<th>NO3⁻ [µM]</th>
<th>PO4³⁻ [µM]</th>
<th>NO2⁻ [µM]</th>
<th>Si(OH)₄ [µM]</th>
<th>Np</th>
<th>Si:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>21–25.11.2013</td>
<td>1st round</td>
<td>L</td>
<td>315 ± 37</td>
<td>12 ± 0.8</td>
<td>1.6 ± 0.5</td>
<td>88 ± 29</td>
<td>26 ± 2.5</td>
<td>0.28 ± 0.09</td>
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<tr>
<td>26–29.11.2013</td>
<td>2nd round</td>
<td>L</td>
<td>244 ± 13</td>
<td>10 ± 2.3</td>
<td>2.2 ± 1.4</td>
<td>97 ± 53</td>
<td>25 ± 7</td>
<td>0.39 ± 0.2</td>
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</tr>
<tr>
<td>21–25.11.2013</td>
<td>1st round</td>
<td>H</td>
<td>345 ± 25</td>
<td>12 ± 1.2</td>
<td>1.5 ± 0.8</td>
<td>99 ± 32</td>
<td>29 ± 3.5</td>
<td>0.28 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>26–29.11.2013</td>
<td>2nd round</td>
<td>H</td>
<td>268 ± 47</td>
<td>12 ± 3.5</td>
<td>1.7 ± 0.8</td>
<td>105 ± 30</td>
<td>22 ± 3.7</td>
<td>0.38 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>1.12.2013</td>
<td>E</td>
<td>L</td>
<td>284 ± 10</td>
<td>11.09 ± 0.5</td>
<td>1.01 ± 0.5</td>
<td>84.1 ± 6</td>
<td>25.5 ± 0.7</td>
<td>0.31 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td></td>
<td>280 ± 19</td>
<td>11.79 ± 0.2</td>
<td>0.9 ± 0.7</td>
<td>85.73 ± 3</td>
<td>23.8 ± 1.3</td>
<td>0.33 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>3.12.2013</td>
<td>E</td>
<td>L</td>
<td>196 ± 25</td>
<td>10.5 ± 8.0</td>
<td>3.7 ± 2.2</td>
<td>48.4 ± 12</td>
<td>16.5 ± 3.7</td>
<td>0.27 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td></td>
<td>197 ± 45</td>
<td>11.43 ± 0.8</td>
<td>0.85 ± 0.7</td>
<td>65.24 ± 12</td>
<td>17.4 ± 4.7</td>
<td>0.33 ± 0.1</td>
<td></td>
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</tbody>
</table>

First round of acclimation was carried out between 21 and 25 November and second round between 26 and 29 November. Experiment started in 29 November and ended on 3 December.

(Kattner & Fricke, 1986) with a gas chromatograph Perkin Elmer Sigma 300 equipped with a programmable temperature vaporizer-injector, a fused Omegawax 53 capillary column, and a flame ionization detector.

**Biological measurements**

Concentrations of *S. pseudocostatum* cells were determined from samples preserved with acid Lugol’s. Cell counting was performed using a Neubauer hemocytometer and optical microscope (OLYMPUS CX31). Specific growth rates were calculated using an exponential curve fitted for each replicate of the treatments. The slope of the exponential curve was considered as the growth rate for each pCO2 treatment. Cell size was measured using an epifluorescence microscope (OLYMPUS IX51), choosing at random 40 individual cells for each replicate of each pCO2 treatment. For biovolume calculation we used a cylinder geometric model according to Sun & Liu (2003). For determination of total chl-a, samples were filtered onto GF/F filters and stored at −20°C. Chl-a was extracted in acetone 95% and measured with a fluorometer (Trilogy Model 7200–040, Turner Designs, Sunnyvale, CA, USA) before and after acidification (Lorenzen, 1966). Chl-a, POC, PON and DOC concentrations were normalized per cell (pg cell⁻¹), assuming that changes in Chl-a, N and C from other sources (e.g. lysis) were not significant in our cultures. Gross photosynthesis (GP) and dark respiration (DR) rates were estimated from changes observed in dissolved oxygen concentrations after incubating in vitro in light and dark bottles (Strickland, 1966). Water from three 20 l carboys was transferred to 125 ml borosilicate (i.e. gravimetrically calibrated) using a silicone tube; three time-zero bottles, three light bottles and three dark bottles for replicate were used. The light and dark bottles were incubated at the same temperature and light regime as the 20 l polycarbonate carboy cultures for 6 h; the dissolved oxygen from time-zero bottles was measured at the beginning of the experiment. Dissolved oxygen was measured using a fibre optical oxygen transmitter (Optical Oxygen meter FiBOX, PreSens®). The average of coefficient of variation for replicates was 0.8%. Net photosynthesis (NP) was calculated as the difference in the dissolved oxygen concentration between ‘light’ incubated samples and ‘time zero’ samples. Dark respiration (DR) was calculated as the difference between ‘dark’ incubated samples and ‘time zero’ samples. Dark respiration rates are expressed as a negative O2 flux. Gross photosynthesis (GP) was calculated as the difference between NP and DR (Gaarder & Gran, 1927). GP and DR per cell were expressed in fmol cell⁻¹ h⁻¹.

**Statistical analysis**

In order to evaluate algal responses to experimental conditions, Student’s t-test was used for each chemical and biological parameter. The Shapiro–Wilk statistic (Shapiro & Wilk, 1965) was used to check the data for normality distribution and a Levene test checked the homoscedasticity.

**RESULTS**

**Carbonate system**

During the experimental period (day 3.12.2013), the carbonate system parameters under simulated CO2-driven ocean acidification showed significant differences in the pCO2 concentration (t = −11.52, df = 6, P < 0.0001) and pH values (t = 26.05, df = 6, P < 0.0001) between both CO2 treatments (Table 1). Significant differences were also found in HCO3⁻ (t = −2.88, df = 6, P = 0.028) and CO2 (t = −11.44, df = 6, P < 0.0001). There were no significant differences in DIC concentrations (t = −2.41, df = 6, P = 0.05) between CO2 treatments, although it was close to the minimal accepted probability. As expected, no significant differences were found in the total alkalinity (TA) (t = −1.22, df = 6, P = 0.26).

**Biological parameters**

The impact of high pCO2 on *S. pseudocostatum* physiology was assessed by comparing 12 parameters between low and high pCO2 treatments (Figure 1). Although cell volume was higher at low pCO2 level (442 ± 103 µm³) compared with high pCO2 level (361 ± 42 µm³), there were no significant differences between pCO2 treatments (t = 1.38, df = 6, P > 0.05). There were also no significant differences in growth rates (t = 0.53, df = 6, P > 0.05), cell-normalized Chl-a (t = 2.28, df = 6, P > 0.05), cellular abundance (t = 2.16, df = 4, P > 0.05), gross photosynthesis and respiration rates (t = 2.08, df = 4, P > 0.05; t = −0.74, df = 1.1, P > 0.05, respectively), POC (t = −0.08, df = 5, P > 0.05), and
PON ($t = -0.75$, df = 5, $P > 0.05$) C:N ratio ($t = 1.57$, df = 5, $P > 0.05$), R:P ratio ($t = -1.72$, df = 1.1, $P > 0.05$). In contrast, DOC/POC ratio and DOC per cell significantly increased at high $p\text{CO}_2$ levels ($t = -3.91$, df = 2.4, $P < 0.05$; $t = -2.51$, df = 6, $P < 0.05$, respectively). In percentage terms, DOC/POC and DOC per cell increased by 40.4 and 48.4% at high $p\text{CO}_2$, respectively.

The high $p\text{CO}_2$ treatment exhibited significant differences in FA concentration and composition. Total FA concentration was significantly decreased ($t = 5.69$, df = 6, $P < 0.05$) under high $p\text{CO}_2$ ($0.208 \pm 0.04 \text{ mg l}^{-1}$) compared with low $p\text{CO}_2$ ($0.07 \pm 0.01 \text{ mg l}^{-1}$) (Figure 2A). The relative amount of SAFAs was significantly higher ($t = -4.53$, df = 6, $P < 0.05$) and the amount of MUFAs lower ($t = 6.1$, df = 6, $P < 0.05$) at high $p\text{CO}_2$ compared with low $p\text{CO}_2$ treatment. In contrast, polyunsaturated fatty acids (PUFA) did not show significant differences between low $p\text{CO}_2$ ($t = 2.2$, df = 6, $P > 0.05$) (Figure 2B). This is exemplified by some essential fatty acids such as docosahexaenoic acid (DHA, 22:6) and arachidonic acid (ARA, 20:4), which showed similar concentrations under low ($0.0008 \text{ mg l}^{-1}$ and 0.001 $\mu\text{g l}^{-1}$, respectively) and high ($0.0002 \text{ mg l}^{-1}$ and 0.002 $\mu\text{g l}^{-1}$, respectively) $p\text{CO}_2$ treatments.

**DISCUSSION**

In the coastal domain, surface waters are commonly exposed to levels in partial $p\text{CO}_2$ higher than expected at equilibrium with the atmosphere (Hofmann et al., 2011; Yu et al., 2011), which is mostly associated with biological processes such as daily time cycles of photosynthesis and respiration (Shamberger et al., 2011) and oceanographic processes such as riverine discharges and coastal upwelling events (Cao et al., 2011). In consequence, diatoms inhabiting coastal areas may be capable of tolerating larger ranges of pH and $p\text{CO}_2$. However, this high variability also may mean that planktonic organisms inhabiting coastal regions are already operating at the limits of their physiological tolerances. Thus, future OA may drive the physiology of these marine organisms up to the edge in their tolerance range.

The present study showed that high $p\text{CO}_2$ had no significant impact on cell volume, growth rate, abundance, chl-a, C/N ratio, and photosynthesis rates in the diatom

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**Fig. 1.** Physiological parameters: growth rate, gross photosynthesis (GP), dark respiration (DR), cellular concentration of Chl-a, particulate organic carbon (POC) and particulate organic nitrogen (PON), respiration losses in % (R:P ratio), C:N ratio, carbon partitioning (DOC/POC ratio) and DOC concentration measured during semi-continuous cultures of *S. pseudocostatum* grown at exponential growth phase under two different $p\text{CO}_2$ concentrations; Low $p\text{CO}_2$: 212 ± 30 $\mu\text{atm}$; High $p\text{CO}_2$: 1050 ± 40 $\mu\text{atm}$. Error bars indicate standard errors.

**Fig. 2.** Fatty acid concentration and composition of *Skeletonema pseudocostatum* cultured at different $p\text{CO}_2$ treatments. (A) Total fatty acid and (B) percentage of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids relative to total fatty acids during the exponential growth phase cultured at low $p\text{CO}_2$: 212 $\mu\text{atm}$ (N = 3) and high $p\text{CO}_2$: 1050 $\mu\text{atm}$ (N = 3) treatments. Error bars indicate standard errors.
S. pseudocostatum (Figure 1). Although some studies have indicated that elevated pCO$_2$ is expected to have a stimulative effect on growth rates (Kim et al., 2006; King et al., 2011; Low-Décarie et al., 2011) and primary productivities (Riebesell et al., 2007), many other studies have shown that elevated pCO$_2$ concentration did not affect the growth rates in diatom monocultures, including S. costatum (Chen & Gao, 2003, 2004), Thalassiosira pseudonana (Crawford et al., 2011; King et al., 2015), and Chaetoceros brevis (Boelen et al., 2011), or in diatom-dominated natural phytoplankton assemblages (Tortell et al., 2000). Furthermore, Berge et al. (2010) have also shown that low pH/high pCO$_2$ conditions do not affect the growth rate and production rates of eight species of phytoplankton representing diatoms, dinoflagellates, cryptophytes and haptophytes. Berge et al. (2010) also showed that 49 strains of a total 33 species of phytoplankton exhibited similar growth rates at pH 7.8 compared with more alkaline levels of pH (8.1–8.2), suggesting that marine phytoplankton are adapted to tolerate the modelled average pH drop due to ocean acidification by the year 2100. Our findings are consistent with these findings, suggesting that S. pseudocostatum tolerates well changes of 0.5 units of pH due to manipulated pCO$_2$ levels.

Diatoms have the capacity for simultaneous transport of CO$_2$ and HCO$_3^-$ during photosynthesis and increase their affinities of both transport systems in response to diminishing supply of carbon substrate (Burkhardt et al., 2001). However, the proportion at which CO$_2$ and HCO$_3^-$ are taken up and the extent to which Ci uptake is affected by changes in CO$_2$ supply vary among phytoplankton species (Nimer et al., 1998; Elzenga et al., 2000; Burkhardt et al., 2001; Rost et al., 2003). For example, Phaeodactylum tricornutum takes up CO$_3^-$ preferentially over HCO$_3^-$ from seawater whereas Thalassiosira weissflogii takes up HCO$_3^-$ preferentially to CO$_2$ under depletion of CO$_2$; thus for Phaeodactylum tricornutum one would expect a pronounced response in photosynthetic C fixation under enhanced CO$_2$. However, P. tricornutum showed increased photosynthetic electron transport rates, but no change or very modest increases in growth (5–13%; Wu et al., 2010; Li et al., 2014) or carbon fixation (Burkhardt et al., 2001) under high pCO$_2$. In our experiment (3 December), significant differences in HCO$_3^-$ concentrations were observed between pCO$_2$ treatments (Table 1), suggesting that both free CO$_2$ and HCO$_3^-$ were probably an important inorganic carbon source for Skeletonema pseudocostatum cells.

Rising pCO$_2$ might affect primary producers in terms of saving energy required for active inorganic carbon acquisition, whereas low pH could potentially increase metabolic demand to maintain cellular homeostasis relative to the increased acidity (Gao et al., 2012). Nevertheless, our results did not show significant differences in the respiration rates between low and high pCO$_2$ levels (Figure 1), which suggest no significant changes in the balance between production and consumption (see R:P ratio) between low and high pCO$_2$ levels.

Furthermore, our results also showed that there were no significant differences in the elemental composition (C:N ratio) between the pCO$_2$ treatments (Figure 1), indicating that increasing CO$_2$ would not increase the POC:PON ratio. In contrast, DOC per cell and the DOC/POC ratio were significantly higher at high pCO$_2$, suggesting that extracellular carbon release relative to particulate carbon production increased under elevated pCO$_2$. The extracellular release of photosynthesis products is especially common during nutrient-depleted growth conditions (Kim et al., 2011; Borchard & Engel, 2012), since phytoplankton exude DOC to the environment to reduce the energy costs associated with storing surplus compounds (Wood & Van Valen, 1990). However, it has also been reported to occur independent of nutrient availability (Hessen et al., 2004; Hessen & Anderson, 2008) and under continuous CO$_2$ enrichment (Song et al., 2013). In our experiment cells were under nutrient-replete conditions (Table 2) and continuous CO$_2$ enrichment, so the DOC increase under high pCO$_2$ conditions seems not to be nutrient dependent. The higher DOC release triggered by elevated CO$_2$ is consistent with other studies carried out in natural phytoplankton assemblages (Riebesell et al., 2007; Kim et al., 2011; Engel et al., 2013) and monocultures (Engel et al., 2004; Borchard & Engel, 2012). Most experimental studies have suggested that greater assimilation of carbon into organic matter at high CO$_2$ levels may increase the extracellular organic matter release from phytoplankton. However, our study showed that gross photosynthesis, POC concentration and C:N ratio were not significantly different between pCO$_2$ treatments, which rules out this process. Enrichment of CO$_2$ and increased acidity have also been found to stimulate photosynthesis in diatoms T. pseudonana and P. tricornutum (Gao et al., 2012), a process by which oxygen is consumed and CO$_2$ released under light conditions, as well as a process by which glycolate is lost to the outside medium as an excreted product. It has been suggested photosynthesis is important for maintaining electron flow to prevent photoinhibition under stress conditions (i.e. high CO$_2$ levels) (Heber et al., 1996), as well as under drought stress (Wingler et al., 1999). In addition, the formation of photosynthetic metabolites, such as glycine, serine and glycolate has also been measured under salt stress in C$_3$ plants (Downton, 1977; Di Martino et al., 1999). Therefore, photosynthesis may play a protective role under stress conditions and consequently contribute partially to the release of DOC triggered under high pCO$_2$ levels. We also recognize that our experiment was not axenic and consequently the increase of DOC in the bottles could also be produced by lysis and transformation of POC to DOC by bacteria or chemical hydrolysis (Carlson, 2002). The greater partitioning of organic carbon into the DOC pool under high CO$_2$/low pH conditions may have implications for long-term C storage in aquatic ecosystems, as DOC components can be both important precursors in the creation of large particle aggregates and also in the formation of recalcitrant DOC (Engel 2002) via heterotrophic metabolism in the upper layers of aquatic ecosystems (Jiao et al., 2010). Important questions arise regarding the increase of DOC production under acidification condition scenarios, e.g. (i) How the increase of DOC will affect the C cycling through bacteria; (ii) the formation of transparent exopolymer particles and consequently, the export of organic matter to the deep ocean (Passow, 2002), and (iii) nutrient competition between bacteria and phytoplankton.

The effects of lowered pH and increased pCO$_2$ was also evaluated on nutritional quality of S. pseudocostatum. Total FAs were significantly different between pCO$_2$-treatments, being 63.26% higher under low pCO$_2$ compared with high pCO$_2$ treatment (Figure 2A). These results agree with other studies that showed a significant decline in total FAs of the...
centric diatom *T. pseudonana* under elevated CO$_2$ (750 µatm) compared with present-day CO$_2$ (380 µatm) (Rossell *et al.*, 2012). These authors found that the relative amount of SAFAs was significantly higher at high CO$_2$ and a ~20% decline in the relative amount of PUFAs. Our findings showed that the relative amount of SAFAs was significantly higher (44 to 63%) and MUFAs significantly lower (44 to 10%) at high pCO$_2$ compared with the low pCO$_2$ treatment. In contrast, lowered pH and elevated CO$_2$ did not affect the contribution of PUFAs to total fatty acids significantly (Figure 2B). The important increase of saturated and decrease of monounsaturated FA contents and total FAs under acidification may affect the transfer of lipids to higher trophic levels. However, the nutritional quality in terms of essential FAs remains unchanged. Most lipids consist mainly of hydrocarbon chains with varying numbers of double bonds. SAFAs have hydrocarbon chains with single bonds while polysaturated FAs contain more than one double bond and include many compounds essential for higher trophic levels, such as for copepod egg production, hatchling and maturity (Jonasdottir *et al.*, 2005; Klein Breter et al., 2005). Our findings are consistent with other studies showing no detrimental effects of high pCO$_2$ on the nutritional quality in terms of essential fatty acids (Leu *et al.*, 2013). Many other responses can be expected in the total FAs and components depending on phytoplankton functional group and species. For example, declining PUFA content at elevated pCO$_2$ was reported for the Antarctic prasinosphyte *Pyramimonas gelidicola* (Wynn-Edwards *et al.*, 2014), the sea-ice diatom *N. lecioatei* (Torstensson *et al.*, 2013) and the diatom *Cylindrotheca fusiformis* (Bermúdez *et al.*, 2015). No detectable differences attributable to pCO$_2$ treatment in the fatty acids component has been observed for the centric diatoms *T. pseudonana* and *T. weissflogii*, the green algae *Dunalieila salina*, the euryhaline microalgae *Chlorella autotrophica* (King *et al.*, 2015) and the dinoflagellate *Gymnodinium* sp. (Wynn-Edwards *et al.*, 2014). In contrast, high CO$_2$ increased the accumulation of total lipids and polysaturated fatty acids in the chloroplasty *Scenedesmus obliquus* and *Chlorella pyrenoidosa* (Tang *et al.*, 2011). The cellular processes involved in FA synthesis under changing pH and pCO$_2$ levels are not fully understood. Because pH might act as a regulation signal for the formation of cell membranes by controlling the production of its synthesizing enzymes (Young *et al.*, 2016), it has been proposed that a higher saturation degree at high CO$_2$ levels (increase of SAFAs) may be a mechanism to control the internal cell-pH because a membrane of short chain FA is less fluid and permeable to CO$_2$ (Rossell *et al.*, 2012).

Our findings suggest that growth, gross photosynthesis and C:N ratio were not necessarily connected to CO$_2$-driven changes in composition and content of FAs in *S. pseudocostatum*. In agreement, other studies showed that CO$_2$-driven changes in the growth rate of the centric diatom *Thalassiosira weissflogii* were not reflected by significant changes in the elemental composition and fatty acid composition, which indicate bidirectional responses to changes in CO$_2$ (King *et al.*, 2015). Since ocean acidification has the potential to alter phytoplankton biochemistry, our results highlight the importance for understanding the cellular processes involved in FA synthesis under rising CO$_2$ decreasing pH which will finally determine the carbon transfer efficiency to higher trophic levels in a changing ocean.

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