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# Phytoplankton interactions can alter species response to present and future $CO_2$ concentrations

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ABSTRACT: Ocean acidification is a direct consequence of carbon dioxide ($CO_2$) dissolution in seawater and has the potential to impact marine phytoplankton. Although community composition and species interactions may be affected, few studies have taken the latter into account. Here, we assessed how species interactions and competition shape physiological responses by testing monospecific and mixed cultures of (1) the haptophyte *Phaeocystis globosa* and the chain-forming diatoms *Chaetoceros* sp. and *Asterionellopsis glacialis* under present $CO_2$ levels, and (2) *Chaetoceros* sp. and *P. globosa* under increasing $CO_2$. The interactions established between the 3 phytoplankton cultures were species- and abundance-dependent. The 2 diatoms did not interact; however, in the presence of *P. globosa* the growth rates of *A. glacialis* decreased and those of *Chaetoceros* sp. increased (depending on a *Chaetoceros* sp. abundance threshold). Conversely, when *Chaetoceros* sp. was reasonably abundant, *P. globosa* was also positively affected (alternating between an abundance/biomass-dependent commensalistic and/or mutualistic interaction). Under enhanced $CO_2$ concentrations, the responses of *Chaetoceros* sp. and *P. globosa* mixed cultures were altered, mainly due to *Chaetoceros* sp. showing a physiological optimum at higher $CO_2$ concentrations than *P. globosa*. While *P. globosa* was hindered by increased $CO_2$, *Chaetoceros* sp. registered augmentation of growth rates, chain length and cellular elemental quotas up to ~750 µatm. Our work emphasizes the role of species interactions when addressing effects of enhanced $CO_2$ on marine phytoplankton. Species-specific response trends to increasing $CO_2$ concentrations revealed significant alterations to species interaction and biomass build-up, which may consequently affect future phytoplankton communities' composition and dynamics.

KEY WORDS: Species interaction · Phytoplankton · $CO_2$ · Biomass ratios · Growth rates · Cellular quotas · Chain length



## INTRODUCTION

Since the Industrial Revolution, anthropogenic-related gas emissions have increased carbon dioxide ($CO_2$) atmospheric concentrations, from ~280 to ~400 ppm. In a continued 'business as usual' scenario, atmospheric concentrations are expected to reach up to ~1000 ppm by the year 2100 (IPCC 2014). The consequent increase in $H^+$ ions will concomitantly lead to a drop of ~0.35 units in mean ocean pH by the year 2100, a process referred to as ocean acidification (e.g. Feely et al. 2004). Ocean acidification

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and carbonation can affect various marine organisms, altering their elemental composition, calcium carbonate ($CaCO_3$) production, growth and photosynthesis (e.g. Riebesell et al. 2000, Riebesell 2004, Feng et al. 2009, Lohbeck et al. 2012).

Phytoplankton assemblages are the general base of marine food webs, and changes in their abundance and/or community composition produce bottom-up effects through higher trophic levels (Menge et al. 1997, Ware & Thomson 2005, Vargas et al. 2006). These autotrophic organisms coexist and interact inside complex communities, competing for resources such as nutrients (N or P) and light (Terry et al. 1983, Leonardos & Geider 2004, Matthiessen et al. 2012). Optimum $CO_2$ concentrations for physiological processes (e.g. photosynthesis, growth rates) differ among phytoplankton species (Riebesell 2004), which suggests potential changes in community structure and succession stemming from increased $CO_2$ availability (Tortell et al. 2002). Despite this, few studies have focused on the potential interaction effects between phytoplankton species under variable $CO_2$ concentrations (Trimborn et al. 2013).

*Chaetoceros* sp., *Phaeocystis globosa* and *Asterionellopsis glacialis* are cosmopolitan species, often present in phytoplankton communities in the Northeast Atlantic (Rahmel et al. 1995, Seuront & Vincent 2008). Both chain-forming diatoms (i.e. *Chaetoceros* sp. and *A. glacialis*) typically precede *P. globosa*-dominated blooms in the NE Atlantic (Rousseau et al. 2002). However, only *Chaetoceros* sp. has been found embedded within *P. globosa* colonies (Rousseau et al. 1994, Seuront et al. 2006). Rousseau et al. (1994) reasoned that the lateral chaetae present in *Chaetoceros* sp. could be used as substrate on which *P. globosa* colonies could grow. Conversely, *P. globosa* can hinder the growth of other algal species via allelopathy (Liu et al. 2010), which enables this species to frequently dominate phytoplankton communities (Lancelot & Mathot 1987, Schoemann et al. 2005). Other *Phaeocystis* species have been seen to decrease phytoplankton growth rates (Hansen & Eilertsen 2007) and affect the survival of more complex taxa, e.g. shellfish (Peperzak & Poelman 2008) and cod larvae (Aanesen et al. 1998).

$CO_2$ concentrations predicted for the year 2100 (~1000 ppm) may provoke distinct effects, depending on the phytoplankton species. Increased $CO_2$ can promote growth rates in diatoms, e.g. *Chaetoceros* sp., by increasing carbon availability (Beardall & Raven 2004, Tortell et al. 2008, Barcelos e Ramos et al. 2014). On the other hand, diatom and *P. globosa* growth rates may not show significant increases (Rost et al. 2003), potentially due to their high affinity for inorganic carbon, leading to carbon-saturated phytoplankton cells near present-day $CO_2$ concentrations (Riebesell 2004).

The effects of increasing $CO_2$ concentrations on phytoplankton interactions have not been well studied. Eggers et al. (2014) found that the initial community composition, due to the presence of different species and/or different ratios, can exert a stronger effect on biomass than increased $CO_2$ alone. Moreover, at the species level and under increased $CO_2$, growth rates of *Chaetoceros debilis* were altered by co-occurring *Pseudo-nitzchia subcurvata* (in relative higher biomass ratios) (Trimborn et al. 2013). Thus, it is essential to improve our understanding of how species interaction will affect phytoplankton community composition responses in the future. Within this context, in this study we first aimed to clarify the role of phytoplankton species interactions under current-day $CO_2$ levels. Successively, we performed a second experiment testing how such interactions shape species' responses to increasing $CO_2$ levels. To this intent, we used 2 species known to interact in nature (*P. globosa* and *Chaetoceros* sp.) and a third species (*A. glacialis*) which has not been reported to co-occur with *P. globosa* blooms nor interact with *Chaetoceros* sp.

## MATERIALS AND METHODS

### Experimental setup

Monospecific cultures of *Chaetoceros* sp. (most likely *Chaetoceros affinis*, CCMMG_2, October 2011), *Phaeocystis globosa* (CCMMG_8, October 2011) and *Asterionellopsis glacialis* (CCMMG_1, October 2011) isolated in the NE Atlantic were grown for at least 14 generations in 2 consecutive dilute batch cultures (12 d in total) in 0.2 µm filtered and UV sterilized seawater (salinity: 35.5 ± 0.5). Inside a closed and controlled environment chamber, average light intensity was 180 ± 10 µmol $m^{-2}$ $s^{-1}$, with a 14 h light:10 h dark cycle; temperature was maintained at 20°C. On a daily basis, all culture bottles were shifted randomly in the chamber and rotated vertically (20 times gently) to avoid sedimentation, aggregation and self-shading. Cultures were enriched with 5.9 ± 0.3 µmol $l^{-1}$ of phosphate, 66.3 ± 1.4 µmol $l^{-1}$ of nitrate and 63.0 ± 1.8 µmol $l^{-1}$ of silicate (increase of total alkalinity upon the addition of $Na_2SiO_3$ was compensated by HCl addition), and following f/8 (modified f/2) medium concentrations for

trace metals and vitamins (Guillard & Ryther 1962, Barcelos e Ramos et al. 2014).

After 12 d of acclimation to the applicable $CO_2$ levels, 2 sets of experiments were conducted. First (Expt I), in order to quantify the effects of interactions among the 3 species, cells were grown under ambient $CO_2$ levels of ~400 µatm and $pH_T$ of ~8.03 (reported on total scale) for 5 d (see Table A1 in the Appendix). A total of 21 bottles (~1 l) were used, comprising triplicates for each of the following conditions: monospecific cultures (100% of total biomass of *A. glacialis* [A], *P. globosa* [P] and *Chaetoceros* sp. [C]); *A. glacialis* + *P. globosa* (initial 60% + 40% [AP]); *A. glacialis* + *Chaetoceros* sp. (initial 80% + 20% [AC]); *P. globosa* + *Chaetoceros* sp. (initial 45% + 55% [PC]); and *P. globosa* + *Chaetoceros* sp. (initial 85% + 15% [Pc]), summarized in Table 1 (including biovolume proportions). To distinguish the last 2 treatments, lower case nomenclature for *Chaetoceros* sp. was used to represent its relatively lower biomass ratio. Initial relative biomass ratios between species were chosen to reflect different stages of diatoms to *P. globosa* blooms (Seuront & Vincent 2008); i.e. in the case of mixed cultures of *P. globosa* and *Chaetoceros* sp., 2 biomass ratios were chosen to determine potential differences in species response arising from distinct initial relative contributions. Species initial abundances were estimated from previous cultures, taking into account a 3% dissolved inorganic carbon (DIC) drawdown threshold and intended biomass ratios. After the experiments were terminated, biomass ratios were calculated more precisely through species- and $CO_2$-specific particulate organic carbon (POC) quotas, applied to each species' initial cell abundance. Final cell densities in the first experiment reached a maximum of 7000 cells $ml^{-1}$ for *A. glacialis* (except AP: 2500 cell $ml^{-1}$), 10000 cells $ml^{-1}$ for *P. globosa* and 2000 cells $ml^{-1}$ for *Chaetoceros* sp. (except PC: 4000 cell $ml^{-1}$).

In Expt II, the interaction between *Chaetoceros* sp. and *P. globosa* (cell number proportion: 1% + 99%; biovolume proportion: 6% + 94%; biomass proportions: 15% + 85%, to simulate a *P. globosa* bloom; see Tables 1 & 4) was tested for 4 d, over increasing $pCO_2$ conditions: ~400, 900, 1100, 1500 and 2400 µatm (initial $pCO_2$), corresponding to initial $pH_T$ values of ~8.06, 7.75, 7.67, 7.54 and 7.36 respectively, in a total of 15 bottles (initial, final and mean carbonate chemistry are provided in Table 2). Final cell densities in Expt II varied similarly in mixed and monospecific cultures, but reached not more than 6000 cells $ml^{-1}$ for *Chaetoceros* sp. and 40000 cells $ml^{-1}$ for *P. globosa*, thus minimizing changes in seawater carbonate chemistry during incubation (DIC drawdown < 3%). Sampling started at the onset of the light phase.

Table 1. Initial biomass and biovolume proportions for treatments in Expts I and II containing *A. glacialis* (A), *P. globosa* (P) and *Chaetoceros* sp. (C) and tested under varying $CO_2$ levels. Cell biovolumes were assumed to be: *Asterionellopsis glacialis*, 500 $µm^3$; *Phaeocystis globosa*, 87 $µm^3$; *Chaetoceros affinis*, 589 $µm^3$ (Naz et al. 2013, Vogt et al. 2013)

| Treatment | By biomass | | | By biovolume | | | $pCO_2$ (µatm) |
|---|---|---|---|---|---|---|---|
| | *A. glacialis* | *P. globosa* | *Chaetoceros* sp. | *A. glacialis* | *P. globosa* | *Chaetoceros* sp. | |
| **Expt I** | | | | | | | |
| A | 100% | – | – | 100% | – | – | 390 |
| P | – | 100% | – | – | 100% | – | 390 |
| C | – | – | 100% | – | – | 100% | 390 |
| AP | 60% | 40% | – | 79% | 21% | – | 390 |
| AC | 80% | – | 20% | 93% | – | 7% | 390 |
| PC | – | 45% | 55% | – | 60% | 40% | 390 |
| Pc | – | 85% | 15% | – | 89% | 11% | 390 |
| **Expt II** | | | | | | | |
| P | – | 100% | – | – | 100% | – | 390, 890, 1100, 1530, 2430 |
| C | – | – | 100% | – | – | 100% | |
| Mix | – | 85% | 15% | – | 94% | 6% | |

## Carbonate chemistry

Carbonate chemistry was manipulated using $NaHCO_3$ and HCl in a closed system, increasing DIC and maintaining total alkalinity (TA) constant according to Schulz et al. (2009). TA was assessed by potentiometric titration following Dickson et al. (2003), using a MetrohmTitrino Plus 848 equipped with a 869 Compact Sample Changer. Measurements were corrected using certified reference material provided by Dickson. $pH_T$ was measured using a glass electrode cell (WTW 340i pH meter) and corrected with TRIS seawater buffer (provided by A. Dickson) according to Dickson et al. (2007). Seawater carbonate chemistry was calculated from measured temperature, salinity, $pH_T$ and TA using CO2Sys (Pierrot et al. 2006) based on Lewis & Wallace (1998), with the equilibrium constants determined by Mehrbach et al. (1973), as refitted by Dickson & Millero (1987).

Table 2. Initial, final and mean carbonate chemistry parameters in Expt II for *Chaetoceros* sp. (C), *Phaeocystis globosa* (P) and mixed (Mix) cultures under varying $CO_2$ levels. Dissolved inorganic carbon (DIC) and $pCO_2$ were calculated from $pH_T$ and total alkalinity (TA), nutrients, salinity (35 PSU) and temperature (20°C) using CO2Sys (Lewis & Wallace 1998)

| $CO_2$ level | $pH_T$ | TA (µmol kg$^{-1}$) | DIC (µmol kg$^{-1}$) | $pCO_2$ (µatm) |
|---|---|---|---|---|
| **Initial** | | | | |
| 1 $CO_2$ | 8.061 | 2435.3 | 2099.2 | 389.0 |
| 2 $CO_2$ | 7.753 | 2410.2 | 2242.8 | 891.2 |
| 3 $CO_2$ | 7.673 | 2435.7 | 2301.9 | 1108.4 |
| 4 $CO_2$ | 7.541 | 2411.6 | 2330.3 | 1533.2 |
| 5 $CO_2$ | 7.357 | 2424.1 | 2408.1 | 2427.3 |
| **Final** | | | | |
| C 1$CO_2$ | 8.135 | 2431.4 | 2045.9 | 312.8 |
| C 2$CO_2$ | 7.943 | 2402.2 | 2156.0 | 534.8 |
| C 3$CO_2$ | 7.872 | 2438.1 | 2211.4 | 657.5 |
| C 4$CO_2$ | 7.683 | 2408.7 | 2280.5 | 1066.1 |
| C 5$CO_2$ | 7.426 | 2409.9 | 2374.2 | 2031.4 |
| P 1$CO_2$ | 8.175 | 2433.4 | 2019.8 | 278.5 |
| P 2$CO_2$ | 7.894 | 2404.9 | 2170.3 | 610.4 |
| P 3$CO_2$ | 7.792 | 2431.9 | 2245.2 | 811.2 |
| P 4$CO_2$ | 7.719 | 2411.1 | 2271.0 | 972.5 |
| P 5$CO_2$ | 7.438 | 2419.2 | 2377.3 | 1985.7 |
| Mix 1$CO_2$ | 8.252 | 2433.4 | 1985.7 | 223.1 |
| Mix 2$CO_2$ | 7.991 | 2411.2 | 2135.3 | 470.7 |
| Mix 3$CO_2$ | 7.879 | 2436.1 | 2217.0 | 644.1 |
| Mix 4$CO_2$ | 7.796 | 2414.7 | 2245.1 | 795.8 |
| Mix 5$CO_2$ | 7.530 | 2417.8 | 2352.6 | 1570.6 |
| **Mean** | | | | |
| C 1$CO_2$ | 8.098 | 2433.372 | 2072.6 | 350.9 |
| C 2$CO_2$ | 7.848 | 2406.202 | 2199.4 | 713.0 |
| C 3$CO_2$ | 7.773 | 2436.899 | 2256.7 | 882.9 |
| C 4$CO_2$ | 7.612 | 2410.147 | 2305.4 | 1299.7 |
| C 5$CO_2$ | 7.392 | 2416.972 | 2391.2 | 22294 |
| P 1$CO_2$ | 8.118 | 2434.386 | 2059.5 | 333.8 |
| P 2$CO_2$ | 7.824 | 2407.571 | 2206.5 | 750.8 |
| P 3$CO_2$ | 7.733 | 2433.806 | 2273.6 | 959.8 |
| P 4$CO_2$ | 7.630 | 2411.364 | 2300.6 | 1252.9 |
| P 5$CO_2$ | 7.397 | 2421.653 | 2392.7 | 2206.5 |
| Mix 1$CO_2$ | 8.156 | 2434.391 | 2042.5 | 306.0 |
| Mix 2$CO_2$ | 7.872 | 2410.714 | 2189.1 | 681.0 |
| Mix 3$CO_2$ | 7.776 | 2435.885 | 2259.5 | 876.2 |
| Mix 4$CO_2$ | 7.669 | 2413.138 | 2287.7 | 1164.5 |
| Mix 5$CO_2$ | 7.443 | 2420.943 | 2380.4 | 1999.0 |

### Cell numbers and growth rates

Cell abundances were determined by inverted light-microscopy (Nikon Eclipse TS100, 200× magnification) from samples fixed with Lugol (2% of total volume). From a given sample volume (~2 ml), cell numbers were counted and corresponding abundance (cells ml$^{-1}$) estimated. On average, 1000 *P. globosa*, 400 *Chaetoceros* sp. and 400 *A. glacialis* cells were counted per sample. We projected relatively fast timings for culture renewal and low initial cell numbers to simultaneously allow for correct $CO_2$ acclimation and prevent colonies from reaching large cell numbers, due to cell count accuracy implications (see Table 4 for final cell abundances). Given that colony formation is fueled by nutrient depletion (Rousseau et al. 1990), we registered low occurrence of *P. globosa* colonies which were composed of <10 cells (continuous nutrient rich environment). Thus, distinction between isolated and colony forms was deemed redundant and cells were counted directly. Concurrently, the number of cells per chain was recorded for each diatom species. Cellular growth rates (µ) were calculated as:

$$\mu = [\ln(N_f) - \ln(N_i)] / \Delta t$$

where $N_f$ and $N_i$ refer to the final and initial cell abundances and $\Delta t$ to the duration of the incubation period in days.

### Biomass and elemental ratios

Samples for cellular particulate organic nitrate (PON), phosphorus (POP) and POC were filtered gently (around 200 mbar) onto GF/F filters that had been pre-combusted (6 h, 450°C). Samples were stored at −20°C until analyzed. POC and PON were determined following Sharp (1974) using an elemental analyzer (Thermo Flash ES) coupled to an isotope ratio mass spectrometer (Thermo Delta V PLUS) via a Thermo-Conflo V. POP was determined colorimetrically using a spectrophotometer (Cary 60), following Hansen & Koroleff (1999). At the end of the experiment, cellular quotas were calculated by dividing final organic particulate matter (P, C and N) concentrations by the coincident cell abundances. Similar to cell counts, no distinction was made between *P. globosa* colony-forming and isolated cells due to the low number and size (<10 cells) of *P. globosa* colonies and residual abundance of colony mucus (Mathot et al. 2000).

### Data analysis

Statistical tests were done using R v.3.2.2 (R Development Core Team 2016). Differences in cellular growth rates between conditions were investigated by resorting to 1-way analysis of variance (ANOVA) for each species (*A. glacialis*, *P. globosa* and *Chaetoceros* sp.), using different species treatments as fixed factors. Before analysis, homogeneity of variances was evaluated with Cochran's test (Winer et al. 1991). Significant differences among treatments were de-

Table 3. ANOVA and post hoc Tukey HSD tests performed comparing species-specific growth rates in monospecific and mixed culture treatments. df: degrees of freedom; mean sq: mean squares. Significant p-values are highlighted in **bold**. See Table 1 for treatment designations

| *Asterionellopsis glacialis* | | | | |
|---|---|---|---|---|
| ANOVA | df | Mean sq | *F* | p |
| Treatments | 2 | 0.0316 | 56.8 | **0.0001** |
| Residuals | 6 | 0.0006 | | |
| Tukey HSD | | | | p |
| A & AP | | | | **0.0002** |
| A & AC | | | | 0.5297 |
| AP & AC | | | | **0.0003** |

| *Phaeocystis globosa* | | | | |
|---|---|---|---|---|
| ANOVA | df | Mean sq | *F* | p |
| Treatments | 3 | 0.0237 | 21.32 | **0.0004** |
| Residuals | 8 | 0.0011 | | |
| Tukey HSD | | | | p |
| P & PC | | | | **0.0062** |
| P & Pc | | | | 0.9441 |
| P & AP | | | | 0.0623 |
| PC & Pc | | | | **0.0032** |
| PC & AP | | | | **0.0002** |
| AP & Pc | | | | 0.1349 |

| *Chaetoceros* sp. | | | | |
|---|---|---|---|---|
| ANOVA | df | Mean sq | *F* | p |
| Treatments | 3 | 0.0046 | 8.21 | **0.0080** |
| Residuals | 8 | 0.0006 | | |
| Tukey HSD | | | | p |
| C & PC | | | | **0.0239** |
| C & AC | | | | 0.7721 |
| C & Pc | | | | **0.0067** |
| AC & PC | | | | 0.8372 |
| AC & Pc | | | | 0.3400 |
| PC & Pc | | | | **0.0468** |

termined using the post hoc Tukey's honestly significant difference test (Tukey's HSD) ($\alpha = 0.05$). Differences between *Chaetoceros* sp. growth rates in monospecific and mixed treatments under increasing $CO_2$ levels were also assessed by 1-way ANOVA.

In addition, in order to more thoroughly understand how $CO_2$ shapes the response of phytoplankton cells, we used a regression analysis approach, as described in Riebesell et al. (2010, Chap. 4). Thus, species growth rates in response to changing carbonate chemistry speciation were fitted following Megard et al. (1984). This fit was used to describe the stimulating/inhibiting effects of light on growth rates and is applied here in a similar fashion, balancing a coupled (TA constant at varying DIC) carbonate system with stimulating effects due to increasing substrate ($CO_2$ and $HCO_3^-$) and inhibiting effects due to increasing $H^+$ concentrations. Data fitting was performed using the 'nlinfit' function in MATLAB (The Mathworks).

## RESULTS

Growth rates of the species tested were, in some cases, significantly affected by the presence of other species (Table 3, Fig. 1). Growth rates of *Chaetoceros* sp. (Fig. 1A) increased and *A. glacialis* (Fig. 1C) decreased in the presence of *P. globosa* (Fig. 1B,C). However, due to similar growth rates, *A. glacialis* and *P. globosa* biomass ratios remained fairly unaltered during the experiment (Fig. 2A), while the higher growth rates of *Chaetoceros* sp. led to significantly increased relative biomass at the end of the experiment (Fig. 2B–D). Moreover, the positive effects of *P. globosa* on *Chaetoceros* sp. growth rates were only detected when the abundance of the latter was above a minimum threshold, since under the 85:15 (Pc) ratio (similar to Expt I), final *Chaetoceros* sp. abundance was less than half of the 45:55 (PC) ratio (just below 2000 compared to 5000 cells $ml^{-1}$ respectively, Table 4). *P. globosa* was also positively affected by *Chaetoceros* sp., but only under initial similar relative biomass (45:55; PC treatment).

Starting from ambient ~400 µatm, increasing $CO_2$ levels enhanced *Chaetoceros* sp. growth rates at up to ~750 µatm, whereas *P. globosa* showed a continuous downward trend after ~500 µatm (Fig. 3). Further increases in $CO_2$ levels led to decreased growth rates for both species. Adding to the positive $CO_2$ effects, *Chaetoceros* sp. (final abundance ~6000 cells $ml^{-1}$; Table 4) growth rates also benefited significantly from the presence of *P. globosa* (ANOVA, $F_{1,8} = 46.9$, $p < 0.001$), whereas *P. globosa* was, once again, not influenced by the ~15% relative biomass of *Chaetoceros* sp.

The proportion of *Chaetoceros* sp. chains with ≥3 cells in length increased with increasing $CO_2$ from ambient levels, peaking at ~1300 µatm and slightly decreasing at the highest level (~2000 µatm; Fig. 4). Overall, longer chains were also observed in the mixed cultures. Cellular contents (C, N and P) of *Chaetoceros* sp. in the monospecific cultures increased (Fig. 5A–C) with $CO_2$ levels, stabilizing beyond ~1000 µatm, whereas *P. globosa* remained unchanged, from 400 µatm across increasing $CO_2$. *Chaetoceros* sp. consistently presented higher concentrations of these elements, due to its inherently larger cell size (up to 32 µm, compared to 9 µm for *P. globosa*) (Furnas 1978, Rousseau et al. 1994) and biovolume (average 589 $\mu m^3$, compared to 87 $\mu m^3$ for *P. globosa*) (Naz et al. 2013, Vogt et al. 2013). Mixed

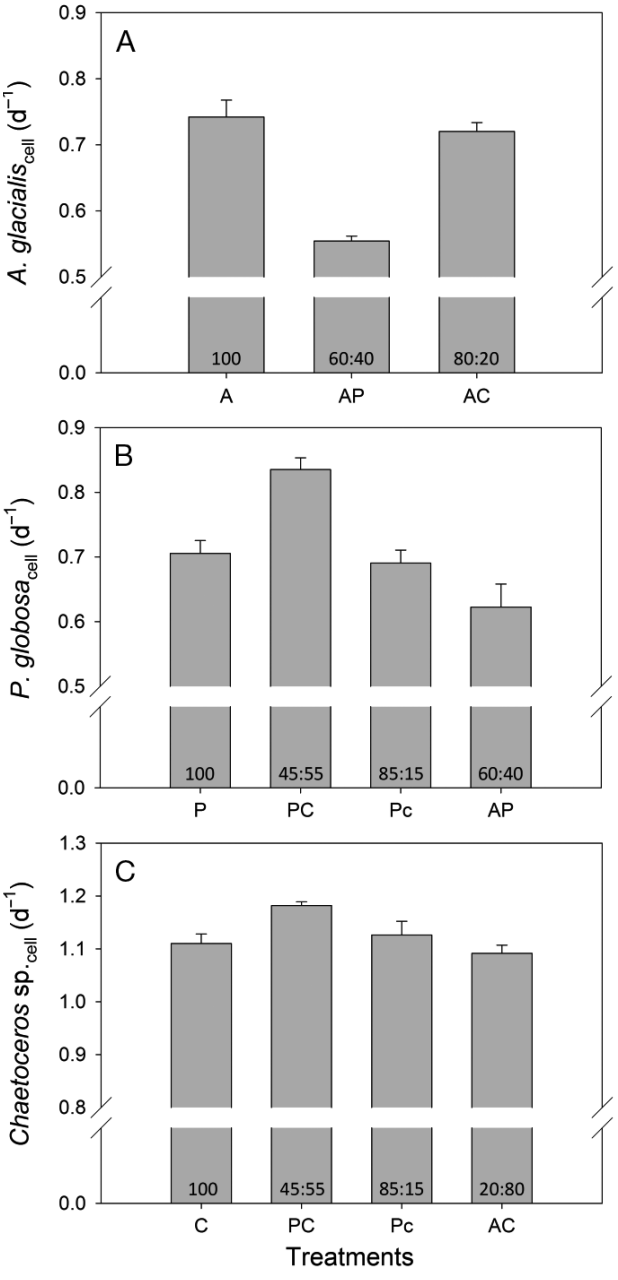


Fig. 1. Mean (±SD) growth rate of (A) *Asterionellopsis glacialis*, (B) *Phaeocystis globosa* and (C) *Chaetoceros* sp. in the various initial particulate organic carbon (POC) biomass ratio treatments. A: *A. glacialis*; C: *Chaetoceros* sp.; AP: *A. glacialis* (60%) + *P. globosa* (40%); AC: *A. glacialis* (80%) + *Chaetoceros* sp. (20%); PC: *P. globosa* (45%) + *Chaetoceros* sp. (55%); Pc: *P. globosa* (85%) + *Chaetoceros* sp. (15%)

cultures showed a slight increase of cellular element quotas in relation to monospecific cultures of *P. globosa* with increasing $CO_2$ levels. Finally, as $CO_2$ affected cellular element quotas similarly, there were no changes in POM ratios with increasing $CO_2$ (data not shown).

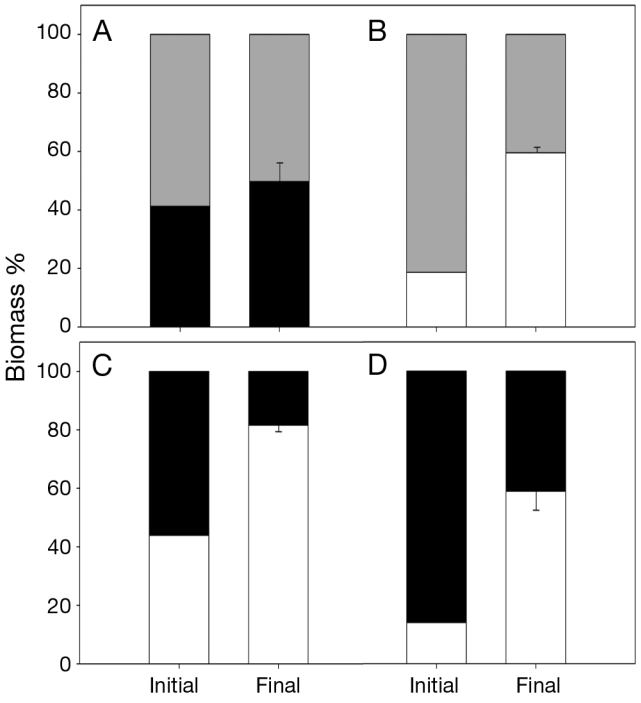


Fig. 2. Initial and final mean (+SD) biomass ratios calculated from cell abundance and species-specific particulate organic carbon quotas for (A) AP: *Asterionellopsis glacialis* (60%) + *Phaeocystis globosa* (40%); (B) AC: *A. glacialis* (80%) + *Chaetoceros* sp. (20%); (C) PC: *P. globosa* (45%) + *Chaetoceros* sp. (55%); and (D) Pc: *P. globosa* (85%) + *Chaetoceros* sp. (15%). Black, grey and white depict *P. globosa*, *A. glacialis* and *Chaetoceros* sp. biomass percentage, respectively

## DISCUSSION

### Species interactions

The presence of allelochemicals produced by *Phaeocystis globosa* generally has deleterious effects on the physiological fitness of other phytoplankton organisms, such as decreased growth and photosynthetic carbon fixation rates (Huang et al. 1999, van Rijssel et al. 2007, Liu et al. 2010). Indeed, *Asterionellopsis glacialis* growth rates decreased in the presence of *P. globosa*, while the latter was not significantly affected. Such allelopathic effects are likely to be maintained or enhanced under increasing $CO_2$ concentrations due to potential increased allelochemical toxicity (Wang et al. 2010, Tatters et al. 2013). Consequently, a $CO_2$-linked shift in the nature of this specific species interaction is unlikely. Therefore, the effects of the interaction between *P. globosa* and *A. glacialis* on the response of the latter to increasing $CO_2$ were not tested here. Given the known allelo-

Table 4. Initial and final abundance (cells $ml^{-1}$; mean ± SD for Expt I) of *Asterionellopsis glacialis* (A), *Phaeocystis globosa* (P and Mix), and *Chaetoceros* sp. (C for similar relative biomass ratio; c for lower relative biomass ratio and Mix) at the end of both experiments

| Treatment | *A. glacialis* (cells $ml^{-1}$) | | *P. globosa* (cells $ml^{-1}$) | | *Chaetoceros* sp. (cells $ml^{-1}$) | |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final |
| **Expt I** | | | | | | |
| A | 160 | 6604 ± 804 | – | – | – | – |
| P | – | – | 250 | 7895 ± 269 | | |
| C | – | – | – | – | 10 | 2049 ± 39 |
| AP | 160 | 2559 ± 96 | 250 | 5726 ± 947 | – | – |
| AC | 160 | 5877 ± 387 | – | – | 10 | 2356 ± 175 |
| PC | – | – | 125 | 8179 ± 765 | 12 | 4430 ± 149 |
| Pc | – | – | 300 | 9531 ± 926 | 6 | 1690 ± 207 |
| **Expt II** | | | | | | |
| P $1CO_2$ | – | – | 1000 | 39 535 | – | – |
| P $2CO_2$ | – | – | 769 | 38 429 | – | – |
| P $3CO_2$ | – | – | 628 | 25 444 | – | – |
| P $4CO_2$ | – | – | 814 | 28 768 | – | – |
| P $5CO_2$ | – | – | 1179 | 13 875 | – | – |
| C $1CO_2$ | – | – | – | – | 11 | 3239 |
| C $2CO_2$ | – | – | – | – | 11 | 5059 |
| C $3CO_2$ | – | – | – | – | 5 | 5128 |
| C $4CO_2$ | – | – | – | – | 6 | 2470 |
| C $5CO_2$ | – | – | – | – | 8 | 1875 |
| Mix $1CO_2$ | – | – | 1000 | 39 513 | 11 | 5534 |
| Mix $2CO_2$ | – | – | 769 | 33 513 | 11 | 7162 |
| Mix $3CO_2$ | – | – | 628 | 19 456 | 5 | 7041 |
| Mix $4CO_2$ | – | – | 814 | 28 275 | 6 | 3341 |
| Mix $5CO_2$ | – | – | 1179 | 13 201 | 8 | 3111 |

pathic potential of the *Phaeocystis* genus (Hansen & Eilertsen 2007), the apparent higher susceptibility of *A. glacialis* to *P. globosa* allelochemicals (bacteriocidal and volatile sulphur compounds) is likely the reason for the lack of *A. glacialis* found embedded on *P. globosa* colonies in nature.

However, the positive effects of species interaction between *Chaetoceros* sp. and *P. globosa* were unexpected and therefore further investigated. Even though the *Chaetoceros* species was not identified (most likely *Chaetoceros affinis*), evidence from previous studies has shown that responses to species interaction and elevated $pCO_2$ do not vary significantly within this genus (Rousseau et al. 1994, Beardall & Raven 2004, Seuront & Vincent 2008, Gaebler-Schwarz et al. 2010, Trimborn et al. 2013). Under increasing $CO_2$ concentrations, *Chaetoceros* sp. maintained higher growth rates in the mixed cultures. This response was observed in Expts I and II, but was only significant above a cell abundance threshold. At low *Chaetoceros* sp. abundances, the proba-

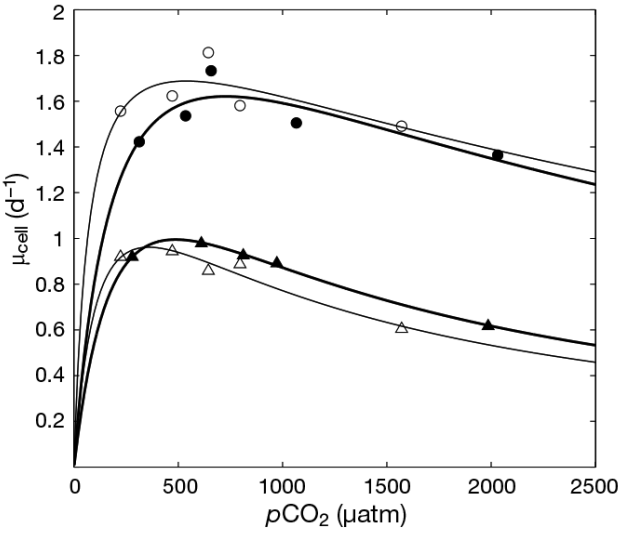


Fig. 3. Growth rates (μ) of *Chaetoceros* sp. and *Phaeocystis globosa* in monospecific (filled) and mixed (open) cultures, with increasing $pCO_2$ levels. Circles: *Chaetoceros* sp.; triangles: *P. globosa*. Solid lines (thick: monospecific cultures; thin: mixed cultures) represent non-linear data fitting (see details in 'Materials and methods') of *Chaetoceros* sp. and *P. globosa* growth rates across $CO_2$ concentrations

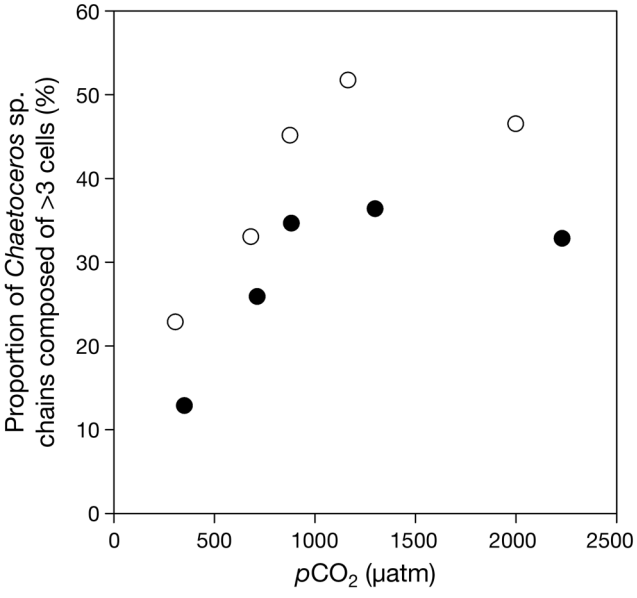


Fig. 4. Proportion of *Chaetoceros* sp. chains composed of more than 3 cells under increasing $CO_2$ levels. White (open): mixed cultures; black (filled): monospecific cultures

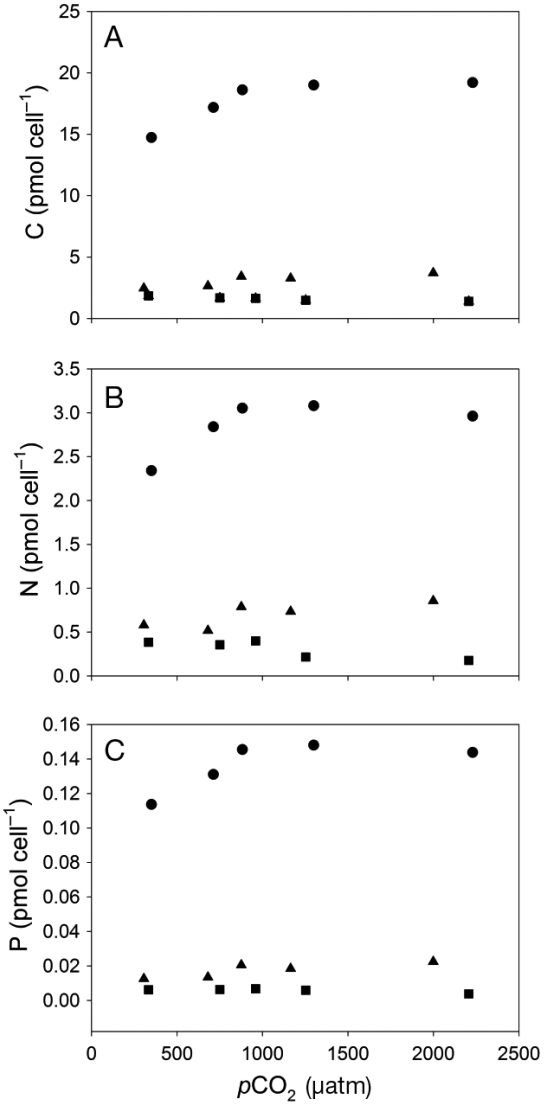


Fig. 5. Cellular element quotas of monospecific *Chaetoceros* sp. (circles), *Phaeocystis globosa* (squares) and mixed cultures of *Chaetoceros* sp. + *P. globosa* (triangles), under increasing $CO_2$ (µatm) concentrations: (A) carbon, (B) nitrogen and (C) phosphorus. Cell quotas were determined based on final particulate organic matter (C, N and P) concentration and total cell abundance in the culture

bility of *P. globosa* cells encountering *Chaetoceros* sp. cells is diminished, which dampens the otherwise observed effects prompted by species interaction. In parallel, *P. globosa* also benefited from the presence of *Chaetoceros* sp., but only when relative biomasses were similar at the beginning of the experiment. *P. globosa* may stop profiting noticeably when *Chaetoceros* sp. is below a certain abundance threshold, as the number of *Chaetoceros* sp. cells present may not be sufficient to prompt measurable interaction effects at a *P. globosa* population level. Another possible explanation is that allelochemical producers can be negatively affected by their own exudates when they are maintained in the same medium (Hansen & Eilertsen 2007), which would account for lower growth rates registered when high *P. globosa* cell abundance was present.

Beneficial interactions have been previously reported for phytoplankton species. In particular, the growth rates of the diatom *Phaeodactylum tricornutum* increase in the presence of several macro- and microalgae exudates (Vasconcelos & Leal 2008). Moreover, the presence of metal-complexing properties in extracellular organic material exuded by *Alexandrium tamarense* is directly related to increased growth rates and abundance of *Chaetoceros* sp. (Weissbach et al. 2010). Regarding the interaction between the species used in our study, *Phaeocystis* has been reported to supposedly use *Chaetoceros* sp. as a substratum on which to grow colonies (Rousseau et al. 1994, Seuront et al. 2006, Seuront & Vincent 2008, Gaebler-Schwarz et al. 2010). *Phaeocystis* sp. colonies are embedded in transparent mucus matrixes consisting of extracellular carbohydrate polymers such as polysaccharides and glucan, which, beyond serving as matrix structural components, are used as energy storage and carbon supply to maintain metabolism and protein synthesis in cells in the dark (Alderkamp et al. 2006). Carbohydrates in the form of polysaccharides and glucan are also used by *Chaetoceros* sp. cells for similar purposes (Myklestad & Haug 1972). Moreover, work with *Chaetoceros* sp. has shown that polysaccharides in the seawater form highly bioavailable organic associations with iron (a highly limiting nutrient in some parts of the ocean), potentially increasing its uptake (Hassler et al. 2011). Thus, our findings may be explained through the fact that co-occurrence with *Phaeocystis* potentially benefits *Chaetoceros* sp. by facilitating iron uptake and providing a chemotrophic substrate (Seuront et al. 2006).

### Species response to varying $CO_2$ concentrations

Previous studies on the effects of increased $CO_2$ concentrations on phytoplankton physiology reported higher growth rates for *Chaetoceros* sp. (up to ~800 µatm) in monospecific cultures (Beardall & Raven 2004) and when mixed with *Pseudo-nitzchia subcurvata* (Trimborn et al. 2013). The higher availability of inorganic carbon is thought to benefit species which are able to down-regulate their carbon concentrating mechanisms, reallocating that energy to other processes such as storage and growth in gen-

eral (Riebesell et al. 1993, Raven 2003, Beardall & Raven 2004). In our study, growth rates of *Chaetoceros* sp. benefited from increased $CO_2$ levels up to ~750 µatm, while growth rates of *P. globosa* displayed a downward trend for $CO_2$ higher than ~500 µatm. Being already close to $CO_2$ saturation under present-day $CO_2$ concentrations, our growth rate data supported the previous finding that *P. globosa* has a high affinity for inorganic carbon (Rost et al. 2003) as its growth rates plateaued at a lower $pCO_2$ than *Chaetoceros* sp.

*Chaetoceros* sp. benefited from the presence of *P. globosa* at increased $CO_2$ levels. Indeed, in the second experiment the growth rates of *Chaetoceros* sp. were always higher in the mixed cultures, while maintaining the trend of monospecific cultures. However, *P. globosa* was not positively affected by *Chaetoceros* sp. over increasing $CO_2$, likely due to the high *P. globosa*:*Chaetoceros* sp. ratio (85:15), i.e. high relative biomass of *P. globosa*, and/or high allelochemical concentration in the medium.

*Chaetoceros* sp. chain length increased up to ~1300 µatm, suggesting that this species' chain length might increase at $CO_2$ concentrations predicted for the year 2100. Given that chain length was also enhanced by the presence of *P. globosa*, it appears that increasing $CO_2$ (up to a threshold) additively affects both growth rates and chain length. Over ~1000 µatm, a simultaneous decrease of growth rate and increase in chain length has been shown for other species (Beardall et al. 2009, Barcelos e Ramos et al. 2014). Furthermore, chain-forming diatoms, such as *A. glacialis*, are suggested to have the capacity to create microenvironments, increasing pH within the chain (Barcelos e Ramos et al. 2014). The ability to ameliorate unfavorable pH levels would become particularly relevant at high $CO_2$ levels, as it would reduce the effects of pH-induced physiological stress in the cells. The increase in chain length with increasing $CO_2$ reported by Barcelos e Ramos et al. (2014) and found in *Chaetoceros* sp. here may indicate that this species also possesses this characteristic. In *Chaetoceros* sp., cellular element quotas increased up to ~750 $CO_2$ µatm with increasing growth rates and then remained relatively constant at higher $CO_2$, despite the decline in growth rates at these levels.

## CONCLUSIONS

Our findings provide a step forward towards understanding how phytoplankton communities can be distinctly impacted, depending on species and relative biomass composition, by increased $CO_2$ levels. Moreover, our study highlights the fact that specific species interactions may modulate $CO_2$ effects, and consequently shape community response. Indeed, we confirmed the hypothesis of Rousseau et al. (1994) that *P. globosa* and *Chaetoceros* sp. possess a specific species interaction that differs from most *P. globosa* interactions previously reported. As an example, *A. glacialis* growth rates were hindered, most likely due to the presence of *P. globosa* allelochemicals. Thus, we reason that allelopathic susceptibility might explain why *A. glacialis* is not found embedded in *P. globosa* colonies in nature. Previous reports theorized that *P. globosa* could use *Chaetoceros* sp. as physical substrate for the growth of their colonies, defining a commensalistic interaction where *P. globosa* benefited and *Chaetoceros* sp. was unaffected (Rousseau et al. 1994, Seuront et al. 2006, Seuront & Vincent 2008). Although this may be true under specific conditions, we showed that the interaction dynamics may vary with relative biomass and abundance. Here, we found that *Chaetoceros* sp. may also benefit from this interaction above a *P. globosa* biomass proportion/abundance threshold, likely due to facilitated iron uptake and higher nutrient availability triggered by the presence of *P. globosa* exudates (commensalism/mutualism, depending on relative biomass ratios). This explains the close interaction in nature between the 2 species and indicates that *Chaetoceros* sp. is tolerant to *P. globosa* allelochemicals.

In general, increasing $CO_2$ resulted in increased growth rates, chain length and cellular element quotas in *Chaetoceros* sp. (up to ~900 µatm). Conversely, *P. globosa* has a $CO_2$ optimum close to the present-day level, and showed negative effects to $CO_2$ levels exceeding ~500 µatm. Species interactions altered *Chaetoceros* sp. physiological response to increased $CO_2$ (additively). Thus, in the expected 'business as usual' $CO_2$ emission scenario by the year 2100, *Chaetoceros* sp. could benefit from enhanced $CO_2$ concentrations, whereas *P. globosa* biomass and growth rates may decrease. Theoretically, however, this effect may be counterbalanced by the consequent increase in *Chaetoceros* sp. relative biomass (if *P. globosa* relative biomass is matched), potentially shifting the nature of species interaction from commensalistic to mutualistic. Given the important role of *P. globosa* for the sulphur and carbon cycles, future modifications to biogeochemical cycles are to be expected. However, simultaneously, the formation of a *P. globosa*-dominated harmful algal bloom (HAB) is likely to be reduced.

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## Appendix.

Table A1. Initial, final and mean carbonate chemistry parameters in Expt I for cultures under varying $CO_2$ levels. Dissolved inorganic carbon (DIC) and $pCO_2$ were calculated from $pH_T$ and total alkalinity (TA), nutrients, salinity (36 PSU) and temperature (20°C) using CO2Sys (Lewis & Wallace 1998)

| | Treatment | $pH_T$ | TA (µmol $kg^{-1}$) | DIC) (µmol $kg^{-1}$) | $pCO_2$ (µatm) |
|---|---|---|---|---|---|
| Initial | All | 8.034 | 2406 | 2134 | 418 |
| Final | A | 8.039 ± 0.01 | 2403 ± 2 | 2131 ± 4 | 417 ± 8 |
| | P | 8.043 ± 0.01 | 2402 ± 1 | 2126 ± 0 | 407 ± 3 |
| | C | 8.053 ± 0.01 | 2403 ± 1 | 2120 ± 6 | 395 ± 11 |
| | AP | 8.054 ± 0.04 | 2405 ± 2 | 2122 ± 26 | 397 ± 14 |
| | AC | 8.044 ± 0.01 | 2404 ± 4 | 2126.5 ± 11 | 406 ± 16 |
| | PC | 8.058 ± 0.01 | 2405 ± 2 | 2118.9 ± 7 | 391 ± 14 |
| | Pc | 8.056 ± 0.03 | 2405 ± 2 | 2120 ± 19 | 394 ± 23 |

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