CO$_2$ sensitivity of Southern Ocean phytoplankton

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[1] The Southern Ocean exerts a strong impact on marine biogeochemical cycles and global air-sea CO$_2$ fluxes. Over the coming century, large increases in surface ocean CO$_2$ levels, combined with increased upper water column temperatures and stratification, are expected to diminish Southern Ocean CO$_2$ uptake. These effects could be significantly modulated by concomitant CO$_2$-dependent changes in the region's biological carbon pump. Here we show that CO$_2$ concentrations affect the physiology, growth and species composition of phytoplankton assemblages in the Ross Sea, Antarctica. Field results from in situ sampling and ship-board incubation experiments demonstrate that inorganic carbon uptake, steady-state productivity and diatom species composition are sensitive to CO$_2$ concentrations ranging from 100 to 800 ppm. Elevated CO$_2$ led to a measurable increase in phytoplankton productivity, promoting the growth of larger chain-forming diatoms. Our results suggest that CO$_2$ concentrations can influence biological carbon cycling in the Southern Ocean, thereby creating potential climate feedbacks. **Citation:** Tortell, P. D., C. D. Payne, Y. Li, S. Trimborn, B. Rost, W. O. Smith, C. Riesselman, R. B. Dunbar, P. Sedwick, and G. R. DiTullio (2008), CO$_2$ sensitivity of Southern Ocean phytoplankton, *Geophys. Res. Lett.*, *35*, L04605, doi:10.1029/2007GL032583.

1. Introduction

[2] The Southern Ocean regulates atmospheric CO$_2$ concentrations over glacial-interglacial cycles [*Sigman and Boyle*, 2000] and contributes disproportionately to the oceanic sequestration of anthropogenic CO$_2$ [*Caldeira and Duffy*, 2000]. The availability of iron and light are believed to exert primary controls on Southern Ocean productivity and biological carbon uptake [*Boyd*, 2002]. In contrast, CO$_2$ has not been considered as a potentially important factor affecting phytoplankton growth and community composition in this region. Recent field studies have demonstrated

CO$_2$ effects on phytoplankton in several oceanic regimes [*Hein and SandJensen*, 1997; *Riebesell et al.*, 2000; *Tortell et al.*, 2002], yet the extent to which the results apply to high latitude regions is unknown. The near freezing temperatures of Antarctic seawater significantly increase CO$_2$ solubility, yielding equilibrium CO$_2$ concentrations more than two-fold higher than those of tropical waters. Southern Ocean carbon isotope data have been interpreted as evidence of CO$_2$-dependent photosynthesis [*Rau et al.*, 1989], and laboratory experiments suggest that CO$_2$ diffusion can limit the growth rates of large Antarctic diatoms [*Riebesell et al.*, 1993]. Recent work has demonstrated, however, that at least some Southern Ocean phytoplankton possess cellular carbon concentrating mechanisms and can utilize the abundant HCO$_3^-$ ion as an inorganic carbon (C$_i$) source [*Cassar et al.*, 2004]. The extent to which CO$_2$ concentrations can regulate C uptake, growth and species composition of Antarctic phytoplankton assemblages has thus far not been examined. To address these questions, we examined the CO$_2$-sensitivity of phytoplankton populations in the Ross Sea, one of the most productive regions in the Southern Ocean [*Arrigo et al.*, 1998].

2. Methods

[3] Sampling and experiments were conducted in the Ross Sea polynya during the Austral summer (December 2005–January 2006), and Austral spring (November–December 2006). Surface water samples (5 m) were collected at 35 stations to examine the physiological mechanisms of inorganic C utilization by in situ phytoplankton assemblages. Phytoplankton were concentrated by gravity filtration onto 2.0 μm pore size filters, and the fraction of cellular HCO$_3^-$ and CO$_2$ uptake was measured in concentrated phytoplankton samples using isotope disequilibrium experiments at a pH of 8.5 and temperature of 2.0°C [*Martin and Tortell*, 2006]. For a subset of samples (11 stations), chlorophyll *a*-normalized maximum C uptake rates (V$_{max}$) were determined by measuring 10 min ^{14}C uptake over a range of external C concentrations, and fitting data to a Michaelis-Menten hyperbolic equation. For these experiments, phytoplankton were concentrated in a C-free buffer (pH 8.0 seawater buffer sparged with CO$_2$-free air), and ^{14}C uptake rates measured across a range of test DIC concentrations. The maximum uptake rates (V$_{max}$) derived from this analysis provide an estimate of the total physiological capacity of phytoplankton for inorganic C uptake under substrate saturating conditions, and are not necessarily related to steady-state C fixation rates.

[4] In addition to our in situ sampling, we also conducted CO$_2$ manipulation experiments with phytoplankton collected at 3 different Ross Sea locations. For these incubations, we

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used a semi-continuous batch-culture technique [*Tortell et al.*, 2002], where sample bottles were periodically diluted with 0.2 μm filtered seawater to prolong exponential phytoplankton growth. For all experiments, triplicate incubations bottles were bubbled with commercially prepared air standards containing 100, 380, or 800 ppm CO$_2$, and amended with 1 nM Fe (as FeCl$_3$) to promote phytoplankton growth. No additional macronutrients were added to the incubation bottles. Of the three experiments, two were conducted in a deck-board flowing seawater tank with temperatures close to in situ values (0 $\pm$ 1 °C), and light intensity reduced to $\sim$ 30% of surface irradiance levels with two layers of neutral density screening. Due to very cold air temperatures at the beginning of the spring cruise (November 2006), one incubation experiment was run in a temperature controlled growth chamber (0 °C), with a constant blue light irradiance of 165 μmol quanta m^{-2} s^{-1}. Incubation experiments lasted between 10 and 18 days depending upon logistical constraints.

[5] For all incubation experiments, steady-state phytoplankton growth rates (d^{-1}) were determined from linear regressions of the natural logarithm of chlorophyll a concentrations against time. Chlorophyll a concentrations were measured following standard JGOFS procedures (http://usjgofs.whoi.edu/protocols_rpt_19.html). At the end of each incubation experiment, samples were removed for the determination of net primary productivity using 24 h ^{14}C incubations following standard JGOFS protocols. Additional isotope disequilibrium experiments were also conducted (as described above) on the final day of incubations to examine CO$_2$ effects on total inorganic carbon uptake rates and the relative utilization of HCO$_3^-$. Phytoplankton taxonomic composition was analyzed by microscopic examination of gluteraldehyde-preserved samples collected periodically during the incubations, using transmitted light microscopy to identify individual species and epifluoresence microscopy for quantitative cell counts.

3. Results and Discussion

[6] Spring phytoplankton assemblages in the Ross Sea polynya were dominated by monospecific blooms of the prymnesiophyte alga *Phaeocystis antarctica*, while summer assemblages contained a more diverse mixture of *P. antarctica* and various diatom species. Carbon uptake experiments conducted with both spring and summer assemblages demonstrated that all phytoplankton had a high capacity for direct HCO$_3^-$ transport. On average, HCO$_3^-$ transport accounted for 83 ($\pm$7) % of total C uptake by phytoplankton, and there were no statistically significant differences between diatom and *Phaeocystis* dominated assemblages. Our results thus suggest the widespread occurrence of cellular carbon concentrating mechanisms in Ross Sea phytoplankton.

[7] Over the range of ambient *P*CO$_2$ encountered during our surveys (120–410 ppm), we found a strong inverse relationship between maximum (i.e. substrate-saturated) C uptake rates (V$_{max}$) and surface water *P*CO$_2$ (r^2 = 0.68, p = 0.002), indicating that phytoplankton upregulate inorganic carbon transport systems in response to decreased surface water CO$_2$ concentrations. The apparent CO$_2$-dependent regulation of V$_{max}$ cannot simply be explained by the co-variation between *P*CO$_2$ and Fe or light availability across our survey stations. At the stations we sampled for V$_{max}$ determinations, mixed layer depths (calculated as the minimum depth where density exceeded surface values by 0.02 kg m^{-3}) averaged 85 $\pm$ 15 m and were not correlated to surface water *P*CO$_2$ (r^2 = 0.05, p = 0.52, n = 11). Similarly, surface water dissolved Fe concentrations (mean 0.07 $\pm$ 0.02 nmol L^{-1}) were not correlated to *P*CO$_2$ (r^2 = 0.01, p = 0.38, n = 45). Low Fe concentrations were likely limiting to phytoplankton across much of our survey area as indicated by low photosynthetic efficiency (F$_v$/F$_m$ values of 0.05 to 0.35), and ship-board bioassay experiments. Overall, our survey results indicate that CO$_2$ availability modulates phytoplankton C uptake under conditions of significant vertical mixing and Fe stress in the Ross Sea.

[8] Our incubation experiments provided further compelling evidence for a specific CO$_2$ effect on C uptake by Ross Sea phytoplankton. For three independent experiments, short-term maximum C uptake capacity increased significantly under low CO$_2$ conditions, by a factor of $\sim$2-fold relative to the highest CO$_2$ treatment (Figure 1). Since all incubation bottles experienced a similar light, Fe and macronutrient regime, we can attribute the observed treatment effects specifically to the experimental CO$_2$ manipulations. Given the resource costs associated with carbon concentrating mechanisms [*Raven and Johnston*, 1991], this CO$_2$-dependent regulation of cellular C transport has important implications for the growth and net productivity of Ross Sea phytoplankton. To address this, we measured ^{14}C-based net primary productivity (24 h) and chlorophyll a-based growth rates in sub-samples from our CO$_2$ incubation experiments. For the *Phaeocystis*-dominated springtime phytoplankton assemblages, there was a statistically significant increase in ^{14}C fixation between 100 and 380 ppm CO$_2$ (t-test, p < 0.05), but no further effects observed at 800 ppm CO$_2$ (Figure 2, top). Steady-state growth rates for these assemblages showed a parallel increase from 100 to 380 ppm CO$_2$, although this increase was not statistically significant due to variability among replicates. For the diatom-dominated summer phytoplankton assemblages, net ^{14}C fixation increased monotonically with increasing *P*CO$_2$ (Figure 2, bottom), and a regression of net ^{14}C productivity vs. *P*CO$_2$ was statistically significant at the 0.05 level. The response of chlorophyll a-specific diatom growth rates was generally consistent with that observed in ^{14}C, although only the increase in growth rates between 380 and 800 ppm CO$_2$ was statistically significant (p < 0.05. We interpret the CO$_2$-dependent increase in net C fixation and growth rates as a result of lowered energetic costs of C assimilation under high CO$_2$ conditions where cells downregulate inorganic carbon transport (Figure 1).

[9] The magnitude of the CO$_2$-dependent growth and productivity effects we observed for Ross Sea phytoplankton ($\sim$10–20%) is consistent with results reported in other oceanic regions [*Hein and SandJensen*, 1997], yet significantly smaller than that observed in Fe bioassay experiments [*Coale et al.*, 2003]. Our measurements may, however, considerably underestimate the true response of net C fixation to increasing CO$_2$ levels as they do not account for the production of dissolved organic carbon (DOC). Previous studies have shown that DOC production
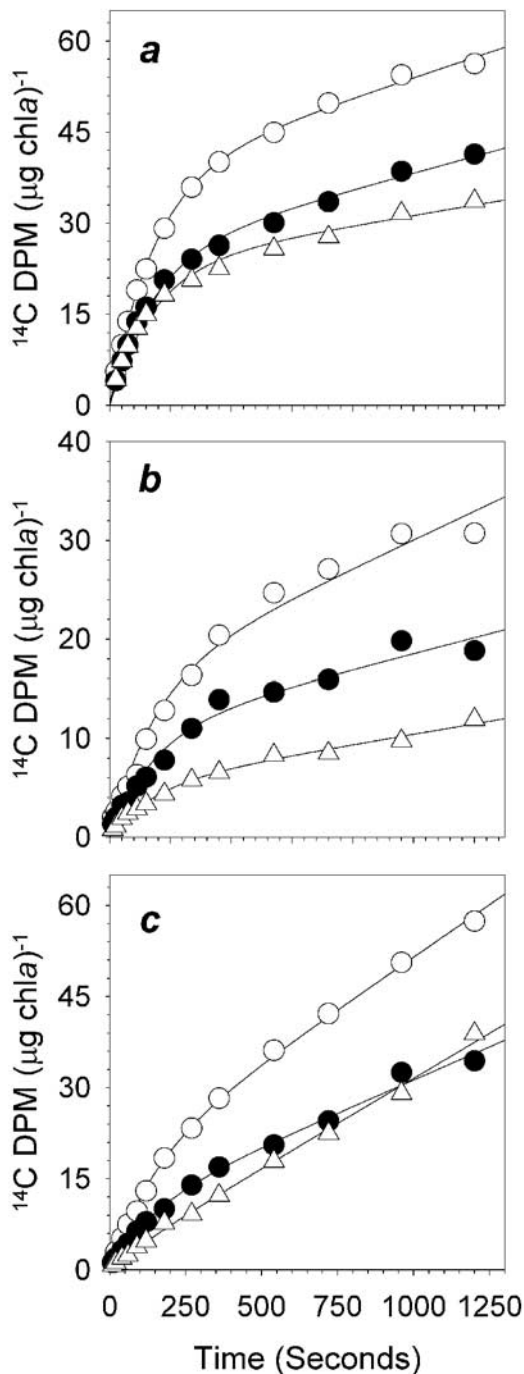
can be as high as 20% of net C fixation in the Ross Sea [*Hansell and Carlson*, 1998], and recent work suggests that elevated CO$_2$ can increase dissolved organic carbon release by phytoplankton [*Engel et al.*, 2004]. This effect, if present in the Ross Sea assemblages, would act to increase the magnitude of CO$_2$-stimulated carbon fixation.

[10] Comparison of the CO$_2$ responses of spring and summer Ross Sea phytoplankton assemblages suggests some differences between diatom and *Phaeocystis*-dominated communities. Whereas all phytoplankton assemblages showed evidence of decreased net C fixation under low *P*CO$_2$ conditions, only the diatom-dominated summer assemblages showed a clear increase in both growth rates and net C fixation under the highest *P*CO$_2$ treatment (Figure 2, bottom). Beyond these broad taxonomic differences, microscopic examination of samples from the incubation experiments also revealed significant CO$_2$-dependent
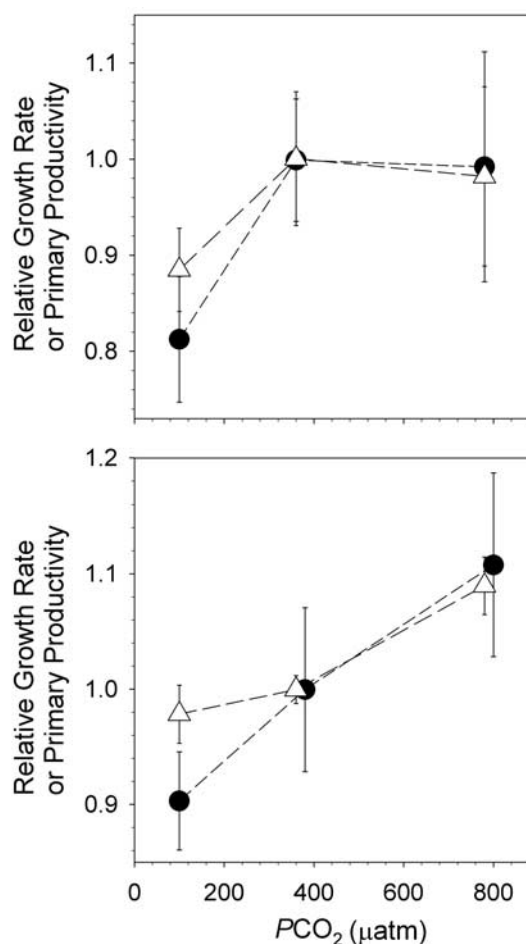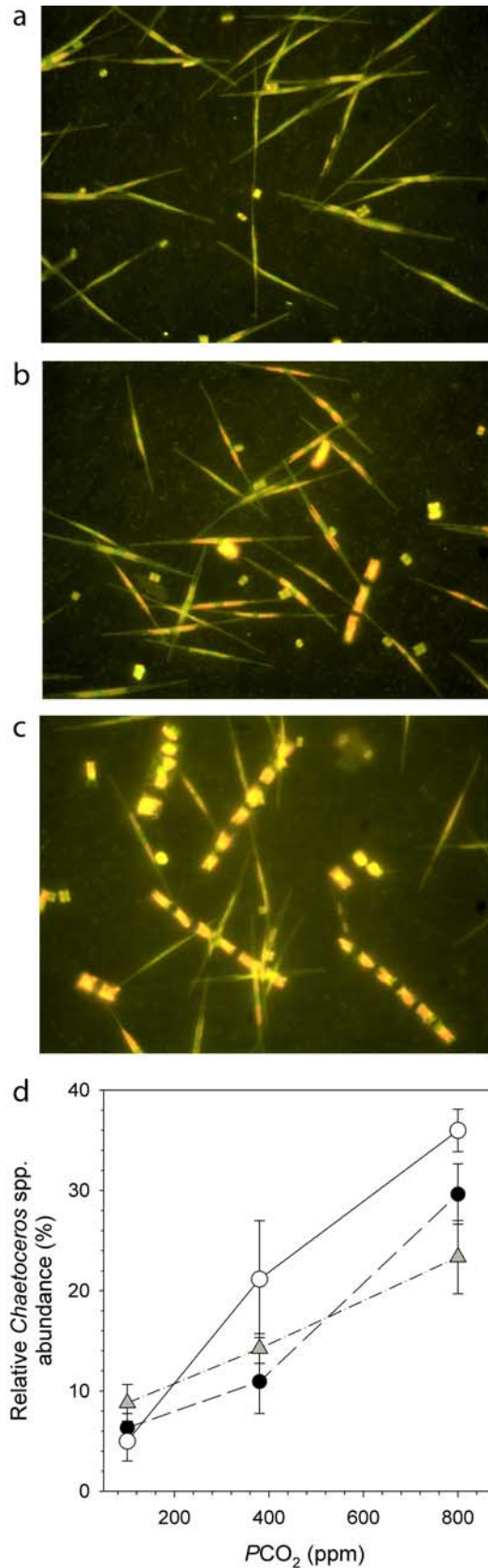

Figure 1. Effects of *P*CO$_2$ manipulations on carbon uptake by Ross Sea phytoplankton assemblages. (a) Results of an experiment conducted in January 2006. (b, c) The results of two experiments conducted in November–December, 2006. Open circles, 100 ppm CO$_2$; closed circles, 380 ppm CO$_2$; triangles, 800 ppm CO$_2$. The amount of curvature in the time-course reflects the fraction of HCO$_3^-$ utilization, while the final slope is proportional to total C uptake rates. The relative fraction of HCO$_3^-$ utilization is 0.87, 0.9, and 0.95 for the data in Figures 1a, 1b, and 1c, respectively, with no statistically significant differences among the CO$_2$ treatments.

Figure 2. Effects of *P*CO$_2$ manipulations on phytoplankton growth rates and net primary productivity. (top) Data from an incubation conducted during the spring growth season (November–December, 2006). (bottom) Data from the summer (January 2006). Closed circles, net primary productivity; triangles, steady-state growth rates. Error bars represent standard errors of mean values (n = 3). ^{14}C uptake was measured for 24 hours on the final day of incubation experiments, while phytoplankton growth rates (rate of chlorophyll *a* increase with time) were measured continually throughout the experiments.

shifts in diatom species composition. In the summer experiments, where diatoms dominated the phytoplankton community over much of the Ross Sea, the relative abundance of the small pennate diatom *Pseudo-nitzschia subcurvata* decreased dramatically in the high CO_2 treatment, being replaced primarily by the larger chain-forming centric diatom *Chaetoceros* spp. (subgenus *Hyalochaetae*) (Figures 3a, 3b, and 3c). A similar CO_2-dependent increase in *Chaetoceros* abundance was also observed in the spring experiments despite the overwhelming dominance (> 90%) of *Phaeocystis* in the phytoplankton community (Figure 3d). This CO_2-dependent species shift may be explained by the effects of PCO_2 on the expression of inorganic carbon uptake by the phytoplankton assemblages. Larger chain-forming *Chaetoceros* species may be at a competitive disadvantage for C uptake under low CO_2 conditions which induce an upregulation of cellular C transport (Figure 1), and favor small cells such as *Pseudo-nitzschia* with high surface area to volume ratios.

4. Implications

[11] The CO_2-dependent regulation of phytoplankton physiology, productivity and species assemblage composition has important biogeochemical implications. While iron supply exerts proximate control on primary productivity over large parts of the Southern Ocean [*Boyd et al.*, 2000], other variables such as light and silicic acid concentrations interact with iron to determine the relative growth rates and ultimate species composition of phytoplankton assemblages over the annual cycle [*Boyd*, 2002]. Our results show that CO_2 can also have a significant effect in controlling phytoplankton processes in the Southern Ocean. In regions subject to natural iron fertilization (through upwelling of deep waters [*Coale et al.*, 2005], aeolian input [*Cassar et al.*, 2007], island effects [*Blain et al.*, 2007], or melting sea ice [*Sedwick and DiTullio*, 1997]), increased CO_2 levels may promote an observable increase in phytoplankton productivity, specifically stimulating diatom-dominated assemblages and promoting a shift towards larger chain-forming species (Figure 3)]. Such diatom species, and in particular the chain forming *Chaetoceros* spp., are prolific bloom formers with a very high capacity for organic carbon export to the sediments [*Stickley et al.*, 2005]. Potential CO_2-dependent productivity increases and algal species shifts could thus act to increase the efficiency of the biological pump, enhancing Southern Ocean CO_2 uptake and contributing to a negative feedback on increased atmospheric CO_2. This feedback could be further amplified by predicted increases in surface water stratification [*Sarmiento et al.*, 1998] and Fe supply resulting from the

Figure 3. Effects of CO_2 manipulations on the species composition of Ross Sea phytoplankton assemblages. Epifluoresence microscope photographs of summer phytoplankton assemblages cultured with (a) 100 ppm CO_2, (b) 380 ppm CO_2, or (c) 800 ppm CO_2. (d) The relative abundance of *Chaetoceros* in all three incubations (including spring and summer). Percent abundances are relative to total diatom counts.

melting of sea ice and glaciers [*Sedwick and DiTullio*, 1997] over the coming century.

[12] Our results also bear relevance to the interpretation of paleoceanographic records of Southern Ocean biogeochemistry. To the extent that we can extrapolate our bottle incubation experiments to the open ocean, we suggest that the low CO$_2$ concentrations of the glacial Southern Ocean may have restricted the growth of larger, chain-forming diatoms despite higher iron inputs, instead favoring smaller, more weakly silicified taxa that are seldom preserved in the sediments. This effect could lead to the apparent decrease in total opal fluxes that has been reported for some glacial Antarctic sediments [*Mortlock et al.*, 1991; *De La Rocha et al.*, 1998]. Many authors have suggested that changes in Southern Ocean phytoplankton productivity could explain part of the observed glacial atmospheric CO$_2$ drawdown [*Sigman and Boyle*, 2000]. Our work is the first, however, to provide direct evidence that CO$_2$ concentrations can, in turn, affect phytoplankton physiology and community structure in this region. Future field and modeling studies should consider CO$_2$ variability when attempting to understand the potential response of Southern Ocean phytoplankton to past and future global change.

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