2010

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GEOPHYSICAL RESEARCH LETTERS, VOL. 37, L09604, doi:10.1029/2010GL042666, 2010

Subtle biological responses to increased CO_2 concentrations by *Phaeocystis globosa* Scherffel, a harmful algal bloom species

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Received 25 January 2010; revised 19 March 2010; accepted 6 April 2010; published 7 May 2010.

[1] Recent investigations into the role of carbon dioxide on phytoplankton growth and composition have clearly shown differential effects among species and assemblages, suggesting that increases in oceanic CO_2 may play a critical role in structuring lower trophic levels of marine systems in the future. Furthermore, alarming increases in the occurrence of harmful algal blooms (HABs) in coastal waters have been observed, and while not uniform among systems, appear in some manner to be linked to human impacts (eutrophication) on coastal systems. Models of HABs are in their infancy and do not at present include sophisticated biological effects or their environmental controls. Here we show that subtle biological responses occur in the HAB species *Phaeocystis globosa* Scherffel as a result of CO_2 enrichment induced by gentle bubbling. The alga, which has a polymorphic life history involving the formation of both colonies and solitary cells, exhibited altered growth rates of colonial and solitary forms at [CO_2] of 750 ppm, as well as increased colony formation. In addition, substantial modifications of elemental and photosynthetic constituents of the cells (C cell^{-1}, N cell^{-1}, potential quantum yield, chl *a* cell^{-1}) occurred under elevated CO_2 concentrations compared to those found at present CO_2 levels. In contrast, other individual and population variables (e.g., colony diameter, total chlorophyll concentration, carbon/nitrogen ratio) were unaffected by increased CO_2. Our results suggest that predictions of the future impacts of *Phaeocystis* blooms on coastal ecosystems and local biogeochemistry need to carefully examine the subtle biological responses of this alga in addition to community and ecosystem effects. **Citation:** Wang, Y., W. O. Smith Jr., X. Wang, and S. Li (2010), Subtle biological responses to increased CO_2 concentrations by *Phaeocystis globosa* Scherffel, a harmful algal bloom species, *Geophys. Res. Lett.*, *37*, L09604, doi:10.1029/2010GL042666.

1. Introduction

[2] Experimental studies have shown that carbon dioxide concentrations can influence phytoplankton in both direct and indirect manners. For example, CO_2 levels influenced the assemblage composition in experiments conducted in a variety of marine systems [*Riebesell*, 2004; *Tortell et al.*, 2002], and it was proposed that such effects were modulated through the physiological mechanisms of carbon acquisition (carbon concentrating mechanisms (CCMs) [*Raven and Johnston*, 1991; *Riebesell et al.*, 1993]). That is, species with CCMs were stimulated by CO_2 additions, whereas those forms that depend on HCO_3^- were less impacted, since HCO_3^- is the form that a majority (90%) of inorganic carbon in marine waters occurs. In Peruvian coastal waters increasing CO_2 concentrations to 750 ppm resulted in the dominance by diatoms and a decrease in *Phaeocystis* [*Tortell et al.*, 2002], but with no change in total biomass; however, less dramatic effects were observed elsewhere [*Tortell et al.*, 2008; *Feng et al.*, 2010]. *Rost et al.* [2003] suggested that photosynthesis of the harmful algal bloom (HAB) species *Phaeocystis globosa* was close to saturation at present CO_2 levels, and that CO_2 half-saturation constants were independent of ambient CO_2 levels. However, there have been few other studies on the effects of enhanced carbon dioxide concentrations on HABs, as most studies have tried to elucidate the role of nutrient inputs on their formation [*Glibert et al.*, 2005]. While a causative mechanism is lacking from many systems, there is a general agreement that eutrophication is associated with increased numbers, intensity and duration of HABs [*Sunda et al.*, 2006], and as such, they are expected to have increased impacts on coastal systems in the future. It is unknown if the attendant increase in CO_2 that has occurred in coastal systems (and can be expected to occur in the future) has had (or will have) any significant impact on phytoplankton assemblage composition and HAB generation, but the potential impact may be substantial.

[3] Models incorporating CO_2 impacts on phytoplankton or HAB generation are in their infancy [*Anderson and Ramsdell*, 2005; *Franks*, 2008], largely because of the difficulty of incorporating non-linear effects of biological processes. Such effects can be subtle, but have substantial attendant impacts on food webs and biogeochemical cycles. One example is the HAB genus *Phaeocystis*. *Phaeocystis* occurs in eutrophic coastal waters such as the Baltic and North Seas and South China Sea, as well as in lesser eutrophied regions such as the Greenland Sea and in Norwegian fjords [*Lancelot et al.*, 1998; *Schoemann et al.*, 2005]. It can have substantial ecosystem effects that result from its substantial production and accumulation; the organic matter subsequently is oxidized and contributes to the formation of spatially extensive hypoxic zones [*Peperzak and Poelman*, 2008]. It also has been shown to have haemolytic properties

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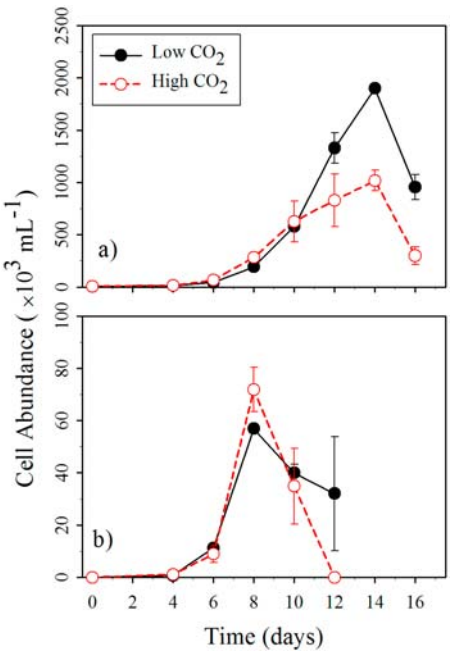

Figure 1. Growth of (a) solitary *Phaeocystis globosa* cells and (b) colonial *P. globosa* cells under ambient (380 ppm) and elevated (750 ppm) CO_2 concentrations. The maximum solitary cell abundance was attained under ambient CO_2 levels, whereas the maximum colonial cell abundance was attained under elevated CO_2. Maximum cell numbers were significantly different ($p < 0.05$; Two-way Repeated Measures ANOVA followed by Tukey's test). Total cell abundance was significantly greater under ambient CO_2 conditions.

[*van Rijssel et al.*, 2007], can disrupt aquaculture [*Qi et al.*, 2004], and upon partial degradation is responsible for the genesis of large organic foams that are blown onto beaches, thus negatively impacting tourism [*Lancelot et al.*, 1987]. The genus has an unusual polymorphic life history, in that it exists both as small (ca. 5 μm) solitary cells and as colonies (usually 2 mm or more in diameter [*Rousseau et al.*, 1994]), which are composed of non-flagellated cells embedded in a ball-shaped mucopolysaccharide matrix [*Schoemann et al.*, 2005]. Each morphotype has a distinct ecological role, with solitary cells ingested by microzooplankton within the microbial food web, but colonies being grazed to a far lesser degree and generally sinking passively to subeuphotic depths [*Reigstad and Wassmann*, 2007]. Both forms contribute large amounts of organic matter to the water column, and thus contribute to the generation of hypoxic zones in numerous locations.

2. Methods

[4] We conducted experiments designed to assess the impact of enhanced CO_2 concentrations on the nuisance HAB species *Phaeocystis globosa*. We hypothesized that enhanced inorganic carbon levels would induce a change in the partitioning of carbon among solitary cells, colonial cells, and the colonial matrix, but not necessarily bulk community properties. Experiments were conducted in a walk-in growth chamber at 20°C using constant irradiance (100 μmol photons m^{-2} s^{-1}) and a 12:12 h photoperiod. The *P. globosa* Scherffel (CCMP 1528) culture was obtained from the Pravasoli-Guillard culture collection at Bigelow Laboratory, Boothbay Harbor, ME. Flagellated, solitary *P. globosa* cells for the experiments were collected by passing the culture twice through a 10 μm sieve under gravity to remove colonies [*Tang*, 2003], and experiments were initiated from an initial cell density of 5×10^3 cells mL^{-1}. After growth our culture contained a mixture of colonies and solitary cells, but a majority of cells were solitary. Nutrient depletion did not occur, and bubbling alone did not induce significant changes in colony or cellular abundance among the three particulate matter pools.

[5] For each treatment triplicate, 1-L bottles were equilibrated at 380 (ambient) or 750 ppm (predicted to be near the atmospheric CO_2 concentration in 2100 [*Intergovernmental Panel on Climate Change*, 2007]); targeted pCO_2 values in f/2 seawater (salinity 30) medium were achieved by continuous, gentle bubbling (16.7 mL min^{-1}) with filtered air and commercially prepared air-CO_2 mixtures (GTS, Richmond, VA), respectively. pH and DIC concentrations were monitored throughout the experiments, with pH being 8.20 and 7.96 at the two different pCO_2 concentrations. Subsamples were harvested every two days to quantify chlorophyll *a*, particulate organic carbon and nitrogen, cell abundances of solitary and colonial cells, cellular and colony characteristics, and photosynthetic capacity (potential quantum yield) of *P. globosa.* Chlorophyll *a* (Chl *a*) was determined by filtering 10–20 ml samples from each of the triplicate flasks through a GF/F glass fiber filter, extracting in 7 mL 90% acetone, and stored overnight in darkness at −20°C. Fluorescence was measured on Turner Designs-700 fluorometer before and after acidification [*Knap et al.*, 1996]. Samples for microscopic enumeration of solitary cells and colonies of *P. globosa* were preserved with Lugol's solution (1–4%). Solitary cell concentration was determined with 1 mL Sedgwick-Rafter chambers; colony

Table 1. Population, Growth Rates and Cellular Properties as Influenced by CO_2 Concentrations[a]

Variable	$[CO_2]$ = 380 ppm	$[CO_2]$ = 750 ppm
pH[2] *	8.20 ± 0.048	7.96 ± 0.026
Chlorophyll *a*[b] (μg L^{-1})	269 ± 15.3	244 ± 30.8
Particulate Organic Carbon[1] (μg mL^{-1})*	16.9 ± 0.98	24.1 ± 0.60
μ_{Chl}[c] (d^{-1})	0.44 ± 0.04	0.41 ± 0.02
$\mu_{SC\ Abund}$[c] (d^{-1})*	0.51 ± 0.03	0.44 ± 0.03
$\mu_{max\text{-}SC\ Abund}$[d] ($d^{-1}$)	0.74 ± 0.14	0.73 ± 0.10
$\mu_{max\text{-}CC\ Abund}$[d] ($d^{-1}$)*	0.81 ± 0.03	1.06 ± 0.22
Colony diameter[e] (μm)	82.8 ± 22.0	83.11 ± 21.1
Colonial cell density[f] (cells $colony^{-1}$)	43.8 ± 19.6	38.7 ± 20.3
Chl $cell^{-1}$ (pg $cell^{-1}$)*	0.158 ± 0.014	0.258 ± 0.029
POC $cell^{-1}$ (pg $cell^{-1}$)*	14.7 ± 1.32	19.5 ± 5.05
F_v/F_m[1,]*	0.45 ± 0.02	0.58 ± 0.01

[a]Asterisks indicate significant ($p < 0.05$; t-test) difference between the two CO_2 treatments; μ is the growth rate, and μ_{max} the maximum growth rate. Growth rate subscripts indicate from what variable each was calculate; SC and CC are solitary cells and colonial cells, respectively.
[b]Determined from day 14.
[c]Determined from days 4–14.
[d]Determined from days 6–8.
[e]Determined from day 8.
[f]Determined from day 10.

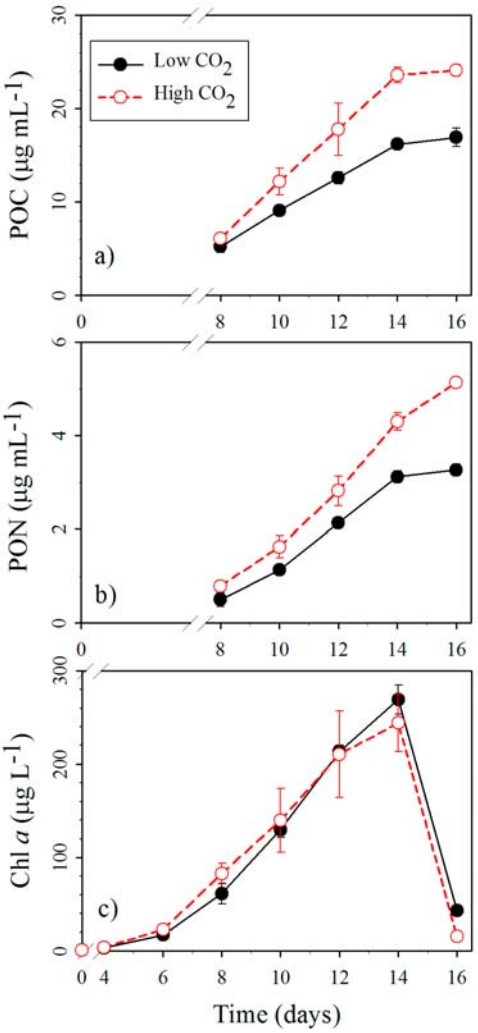

Figure 2. Temporal patterns of (a) particulate organic carbon, (b) particulate organic nitrogen, and (c) total chlorophyll *a* in *Phaeocystis globosa* cultures grown with ambient (380 ppm) and elevated (750 ppm) CO_2 concentrations. Elevated carbon dioxide increased the total POC and PON significantly ($p < 0.05$; Two-way Repeated Measures ANOVA followed by Tukey's test) at 8 days and beyond, but no significant changes were observed in total chlorophyll *a* concentrations.

concentration, colony size, and cells per colony were measured in 24-well multi-plates using Nikon inverted microscope with a calibrated micro-ruler. All samples were enumerated within two days of collection and preservation to prevent cellular and colony disintegration. POC/PON concentrations were determined by filtering 10–20 mL subsamples through pre-combusted (4 h, 450°C) GF/F filters, rinsing with ca. 5 mL 0.01N HCl to remove particulate inorganic carbon adsorbed to the filter, and drying in combusted glass vials at 60°C. All filters were pyrolized using a Carlo-Erba Model EA 1108 elemental analyzer. Maximum potential quantum yield (F_v/F_m) of PSII was measured on dark adapted samples for 30 min (see auxiliary material).[1]

[1]Auxiliary materials are available in the HTML. doi:10.1029/2010GL042666.

Solitary cell and colony growth rates were calculated using an exponential growth equation.

3. Results and Discussion

[6] We found a marked effect of CO_2 on the carbon partitioning of *Phaeocystis globosa*; that is, increased CO_2 concentrations resulted in a change in cell numbers as well as the numbers of colonies (Figure 1). Specifically, at elevated CO_2 concentrations maximal solitary cell biomass decreased by 46%, whereas the numbers of colonies increased by over 26%. Maximum growth rates of colonies were also significantly increased by elevated CO_2 levels (Table 1), with the growth rate of colonies increasing over 30% (from 0.81 to 1.06 d^{-1}; $p < 0.05$; t-test); μ_{max} of solitary cells (measured over the same interval as colonial cell

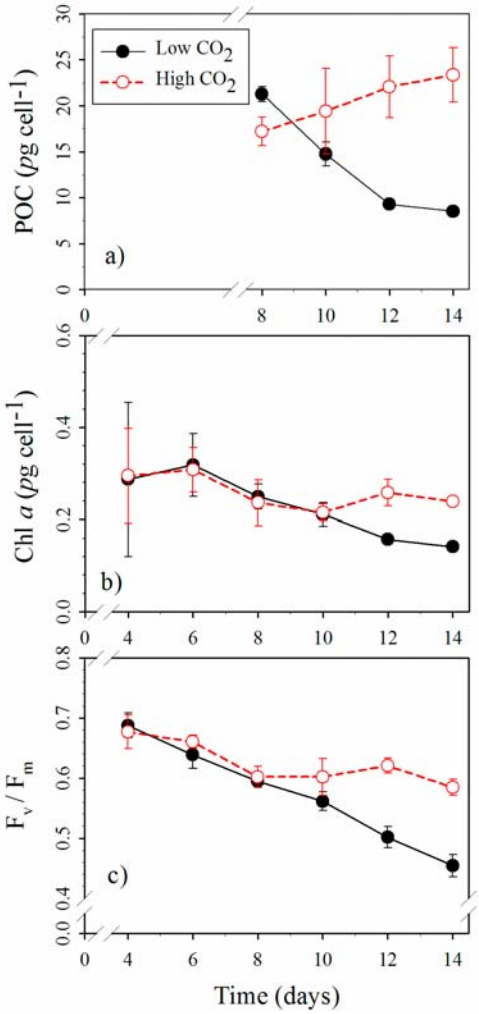

Figure 3. Temporal patterns of (a) particulate organic carbon per cell, (b) chlorophyll *a* per cell, and (c) F_v/F_m (an index of photosystem quantum yield) in *Phaeocystis globosa* cultures grown with ambient (380 ppm) and elevated (750 ppm) CO_2 concentrations. Elevated carbon dioxide increased the C $cell^{-1}$, chl *a* $cell^{-1}$ and F_v/F_m significantly ($p < 0.05$; Two-way Repeated Measures ANOVA followed by Tukey's test) after 8–10 days.

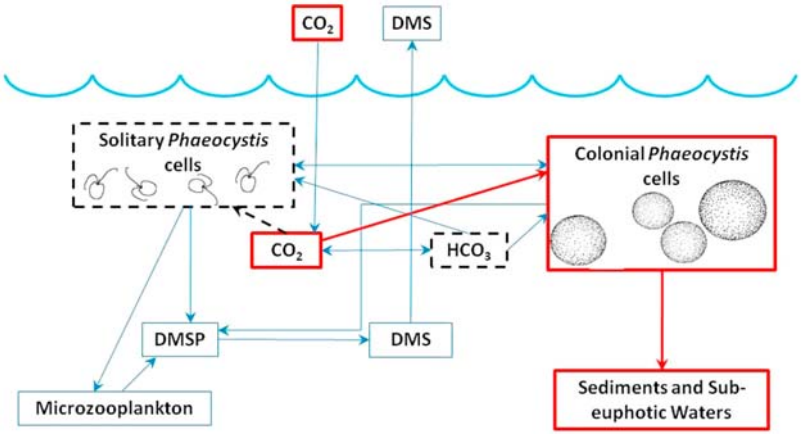

Figure 4. Conceptual diagram depicting the relationships among changes in *Phaeocystis* morphotype standing stocks and growth rates and carbon and sulfur pools. Solid, red heavy boxes and lines represent increasing pool sizes and rates of exchange; dashed black boxes and lines represent decreasing pool sizes and exchange rates. Solid, blue thin boxes and lines represent fluxes that may change, but are not addressed in this study. Process rates per cell are assumed to remain constant. Increased CO_2 concentrations result in increased colonial biomass and growth, which results in increased flux of organic matter to depth; conversely, decreased solitary cell growth will influence microzooplankton grazing in an unknown manner. Sulfur dynamics also will be impacted, but the impacts are poorly constrained.

μ_{max}) showed no change. However, the growth rate of solitary cells was significantly decreased during exponential growth (from day 4–14) by 16% (from 0.51 to 0.44 d^{-1}; $p < 0.05$; t-test). No change was observed in colony diameter, which averaged 82 μm on day 8 (Table 1), increasing from 38 μm at the initiation of exponential growth (increases in colonial diameter are normally observed and are a function of the age of the colony, suggesting that the absolute amount of mucilage was unaffected by increased CO_2). Furthermore, no significant change was observed in cell abundance per colony or total chlorophyll *a* (Figure 2 and Table 1). Elevated CO_2 concentrations also produced increased population particulate organic carbon (POC) and nitrogen (PON) concentrations (Figure 2), and as a result generated significantly increased cellular ratios of C and N (Figure 3). No significant change was noted in the POC/PON ratio. Thus, increased levels of CO_2 result in not only altered population indices, but induced modifications of cellular indices as well; conversely, population properties do not uniformly reflect these important changes. Perhaps most importantly, carbon partitioning (that is, the formation of colonies and the production of the extracellular matrix relative to solitary cell production) was significantly increased by elevated CO_2.

[7] While many of these changes were noted during exponential growth, changes in the photosynthetic efficiency or potential quantum yield (F_v/F_m) were observed only at maximum biomass and during stationary phase (Figure 3c). As growth rates decreased (likely due to limitation by irradiance in the cultures), potential quantum yield in ambient CO_2 treatments also decreased dramatically (from 0.67 to 0.45), whereas they remained relatively high (from 0.67 to 0.58) under elevated CO_2 concentrations. This may have led to the increased chlorophyll per cell values we observed during stationary phase, and suggests that under elevated carbon dioxide cells may be physiologically acclimated to respond to improved environmental conditions (increased light or nutrient levels) that might occur within or after a bloom.

4. Implications

[8] Our results suggest that while substantial population-level responses (e.g., biomass, growth rates) to elevated carbon dioxide can occur, additional and far more subtle biological responses also occur (changes in the abundance of colonies), and that it is these responses that may have considerably more significant ecological and biogeochemical impacts (Figure 4). Enhanced colony production would be expected to increase the flux of carbon to depth via passive sinking, and in shallow regions, contribute to the formation of hypoxic zones and markedly influence local biogeochemical cycles. Additionally, given that *Phaeocystis* species are essential components of the sulfur cycle via their active release of DMSP and DMS [*Matrai et al.*, 1995], it is likely that the enhanced formation of colonies will influence sulfur and carbon dynamics [*Simó*, 2001]. Simultaneously, less organic matter would be processed by the food web, which potentially might alter local and regional trophic processes. Predicted future increases in CO_2 concentrations can be expected to exert unpredictable influences on plankton communities, and these changes most likely will have substantial consequences to coastal waters that are at present difficult to predict. Such biological responses need to be taken into account when attempting to evaluate the oceanic response to future increases in CO_2 levels.

[9] **Acknowledgments.** This work was supported by NSF grant to WOS (OCE 0726258) and by the Visiting Scholar Program of PRC. We thank K. Tang and J. Dreyer for technical assistance.

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