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FULL LENGTH ARTICLE

Impact of elevated CO_2 concentrations on the growth and ultrastructure of non-calcifying marine diatom (*Chaetoceros gracilis* F.Schütt)

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Abstract The impacts of different CO_2 concentrations on the growth, physiology and ultrastructure of noncalcifying microalga *Chaetoceros gracilis* F.Schütt (Diatom) were studied. We incubated *Ch. gracilis* under different CO_2 concentrations, preindustrial and current ambient atmospheric concentrations (285 and 385 µatm, respectively) or predicted year-2100 CO_2 levels (550, 750 and 1050 µatm) in continuous culture conditions. The growth of *Ch. gracilis* measured as cell number was decreased by increasing the pCO_2 concentration from nowadays concentration (385 µatm) to 1050 µatm. The lowest percentage changes of oxidizable organic matter, nitrite, nitrate, phosphate and silicate were recorded at a higher pCO_2 (1050 µatm), and this is in consistence with the lowest recorded cell number indicating unsuitable conditions for the growth of *Ch. gracilis*. The minimum cell numbers obtained at higher levels of CO_2 clearly demonstrate that, low improvement occurred when the carbon level was raised. This was confirmed by a highly negative correlation between cell number and carbon dioxide partial pressure ($r = -0.742$, $p \leqslant 0.05$). On the other hand, highest growth rate at pCO_2 = 385 µatm was also confirmed by the maximum uptake of nutrient salts (NO_3 = 68.96 µmol.l^{-1}, PO_4 = 29.75 µmol.l^{-1}, Si_2O_3 = 36.99 µmol.l^{-1}). Total protein, carbohydrate and lipid composition showed significant differences ($p \leqslant 0.05$) at different carbon dioxide concentrations during the exponential growth phase (day 8). Transmission Electron Microscopy of *Ch. gracilis* showed enlargement of the cell, chloroplast damage, disorganization and disintegration of thylakoid membranes; cell lysis occurs at a higher CO_2 concentration (1050 µatm). It is concluded from this regression equation and from the results that the growth of *Ch. gracilis* is expected to decrease by increasing pCO_2 and increasing ocean acidification.

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Introduction

Rising carbon dioxide (CO_2) concentrations in the atmosphere due to human activities are causing the ocean to become more acidic. The pH of the upper ocean has decreased from a preindustrial value of 8.2 to approximately 8.1 today (Royal Society, 2005). It is estimated that the pH of ocean surface waters will be 7.9 by the end of this century and 7.4 by the end of the millennium (Caldeira and Wickett, 2003).

In parallel to the rising CO_2, ocean temperature is increasing, which may increase stratification and decrease the upper-mixed layer depth, thus exposing phytoplankton cells to higher mean light intensities (Boyd et al., 2010). These changes affect marine plankton in various ways, positively as for cyanobacteria, or in most cases, negatively as for coccolithophores. However there is a lack in the understanding of the effect of this increase in carbon for some important organisms such as diatoms, an important primary producer in the ocean.

Diatoms are abundant unicellular algae in aquatic habitats. They have been known to be the bulk of the food that sustains the marine food chain and important fisheries. They can also produce enormous amounts of biomass and are thought to be responsible for about 20% of global carbon fixation. Recent assessments suggest that diatom-mediated export production can influence climate change through the uptake and sequestration of atmospheric CO_2 (Granum et al., 2005). A significant fraction of the organic carbon generated by diatoms remains in the upper ocean and supports production by higher trophic levels and bacteria.

Carbon fixed by microalgae is incorporated into carbohydrates and lipids, so that energy, chemicals or food can be produced from algal biomass (Lee et al., 2001; Olaizola, 2003). It is documented by Riebesell et al. (1993), Hein and Sand-Jensen (1997) that raising CO_2 could lead to enhanced phytoplankton growth and photosynthetic carbon fixation. Taraldsvik and Myklestad (2000) reported that a high pH leads to increased growth, CO_2 uptake and amino acid content in *Skeletonema costatum*. Li and Douglas (2013) studied the interactive effects of pCO_2 and light on the coastal marine diatom *Thalassiosira pseudonana* CCMP 1335 growing under ambient and expected end-of-the-century pCO_2 (750 µatm).

Chrismadha and Borowitzka (1994) reported that the protein content was increased with carbon dioxide additions in the diatom *Phaeodactylum tricornutum*. Chu et al. (1996) observed an opposite effect in the diatom *Nitzschia inconspicua* with increases in lipids and carbohydrates at protein expenses when the culture was enriched with 5% (v/v) of carbon dioxide. Araújo and Garcia (2005) studied the changes in protein, carbohydrates and lipids in the diatom *Chaetoceros wighamii* under different CO_2 conditions. Changes in productivity and the way in which diatoms allocate carbon into carbohydrates may affect the ecosystem function and the efficiency of the biological carbon pump in a low pH ocean (Thornton, 2009).

Few studies were done on the effect of CO_2 changes on the ultrastructure of non-calcifying algae. Jian-Rong and Kunshan (2002) investigated the ultrastructure response of fresh water green algae *C. reinhardtii* and *Scenedesmus obliques* to elevated CO_2 concentrations.

The potential for marine organisms to adapt to increasing CO_2 and broader implications for ocean ecosystems is not well known; both are high priorities for future research. So the objective of this research was to determine how seawater acidification affects the growth, protein, carbohydrate and total lipids in the non-calcified diatom species *Chaetoceros gracilis*. Also, little has been documented with regard to the effect of CO_2 changes on the ultrastructure of noncalcifying algae.

Materials and methods

Culture media

Unialgal *Ch. gracilis* F.Schütt stock cultures were kindly provided from the Invertebrate Lab., National Institute of Oceanography and Fisheries, Alexandria, Egypt. Stock culture was incubated at 24 ± 1 °C, pH 7.5 under a cool white fluorescent light with continuous illumination (3000 Lux). The algal species were cultured in a sterile f/2-enriched seawater medium (Guillard, 1975).

Experimental design

Cultures were incubated in triplicate 1 L Erlenmeyer flasks containing autoclaved f/2-enriched seawater medium with initial cell count (5×10^4 cell.ml^{-1}) using the stock culture growth conditions. Triplicate bottles were equilibrated at five different pCO_2 concentrations (pCO_2 ~280 µatm – pH = 8.22; 385 µatm – pH = 8.11; 550 µatm – pH = 8.02; 750 µatm – pH = 7.85 and 1050 µatm, pH = 7.74) using acid–base addition (±0.02 pH units), total alkalinity was kept constant at 2700 µatm, and the cultures incubated at 28 °C.

Chemical analysis of culture media

Sea water salinity was measured using a Beckman Induction Salinometer (model RS-7C). Dissolved oxygen was measured using a highly accurate modified Winkler method (Grasshoff et al., 1983).

The initial and final concentrations of nitrite, nitrate, phosphate and silicate were determined using a Seal AA3 Autoanalyzer (Seal Analytical, Fareham, U.K.) with appropriate standards in the bracket of the expected concentrations. Aliquots of 50 ml of culture were filtered through 0.22 mM filter for the determination of nitrite, nitrate, phosphate and silicate concentrations. 35 ml of culture was used for measuring the ammonia content according to Grasshoff et al. (1983).

PCO_2 adjustment in the bottles was achieved by acid base addition according to Riebesell et al. (2010) and volume of HCl or NaOH to be added was calculated using SEACARB then followed by carbonate bicarbonate addition to get the required alkalinity. PCO_2 adjustment was done at the beginning of the experiment only, PCO_2 and carbon dioxide system parameters were identified by measuring pH and total alkalinity (A_T) using reference material batch 19 provided from Dickson laboratory, then pCO_2 is calculated from CO_2SYS. The head space was about 100 ml, the bottle is 1 liter and the culture was 900 ml. Oxidizable organic matter was determined by the permanganate oxidation method (FAO, 1975).

Culture aliquots were sampled for the initial pH measured with a Metrohm (827 pH), pH electrode calibrated with a TRIS buffer on total (T) scale buffers following Dickson et al. (2007). Total alkalinity (A_T) was measured following