Journal of Experimental Marine Biology and Ecology 406 (2011) 54–62

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Emiliania huxleyi shows identical responses to elevated pCO_2 in TA and DIC manipulations

C.J.M. Hoppe *, G. Langer, B. Rost

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

ARTICLE INFO

Article history:
Received 28 February 2011
Received in revised form 6 June 2011
Accepted 8 June 2011
Available online 2 July 2011

Keywords:
Calcification
CO_2 manipulation
Coccolithophores
Ocean acidification
Photosynthesis

ABSTRACT

With respect to their sensitivity to ocean acidification, calcifiers such as the coccolithophore *Emiliania huxleyi* have received special attention, as the process of calcification seems to be particularly sensitive to changes in the marine carbonate system. For *E. huxleyi*, apparently conflicting results regarding its sensitivity to ocean acidification have been published (Iglesias-Rodriguez et al., 2008a; Riebesell et al., 2000). As possible causes for discrepancies, intra-specific variability and different effects of CO_2 manipulation methods, i.e. the manipulation of total alkalinity (TA) or total dissolved inorganic carbon (DIC), have been discussed. While Langer et al. (2009) demonstrate a high degree of intra-specific variability between strains of *E. huxleyi*, the question whether different CO_2 manipulation methods influence the cellular responses has not been resolved yet. In this study, closed TA as well as open and closed DIC manipulation methods were compared with respect to *E. huxleyi's* CO_2-dependence in growth rate, POC- and PIC-production. The differences in the carbonate chemistry between TA and DIC manipulations were shown not to cause any differences in response patterns, while the latter differed between open and closed DIC manipulation. The two strains investigated showed different sensitivities to acidification of seawater, RCC1256 being more negatively affected in growth rates and PIC production than NZEH.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Since the industrial revolution, anthropogenic activities such as the burning of fossil fuels or changes in land use have increased atmospheric pCO_2 values from about 280 μatm to 385 μatm (Lüthi et al., 2008; Tans, 2009). About one third of the emitted CO_2 has already been taken up by the oceans, leading to increased DIC concentrations in surface waters (Wolf-Gladrow et al., 1999). The subsequent changes in speciation, such as increased CO_2 concentrations $[CO_2]$ and decreased CO_3^{2-} concentrations $[CO_3^{2-}]$, lead to decreasing oceanic pH values (Broecker et al., 1971). This process, commonly referred to as ocean acidification, has diverse effects on marine organisms, communities and ecosystems (e.g. Bijma et al., 1999; Kleypas et al., 1999; Raven et al., 2005; Tortell et al., 2002).

There have been several studies investigating ocean acidification effects on phytoplankton on the single species and community level (for review see Fabry et al., 2008; Rost et al., 2008). CO_2 perturbation experiments are the prime tools to mimic future CO_2 scenarios and to study organism responses. These experiments can be conducted by i) equilibrating with air of a certain pCO_2, ii) the addition of $NaHCO_3$, Na_2CO_3, or iii) the addition of a strong acid or base (Riebesell et al., 2010). In any of these perturbations, the seawater carbonate system will react by increasing or decreasing the relative proportions of the carbonate species or the DIC concentration according to its new equilibrium state. The most common perturbation methods, leading to very similar speciation with regard to pH, $[CO_2]$, $[CO_3^{2-}]$ and Ω_{Ca} (calcite saturation state), are the manipulation of dissolved inorganic carbon (DIC) by aeration with a certain pCO_2 (while keeping total alkalinity (TA) constant) and manipulation of the TA by the addition of HCl or NaOH (while DIC stays constant). DIC manipulations reflect current changes in the marine carbonate chemistry. Even though TA perturbations differ regarding the quantity manipulated, they mimic the carbonate speciation as occurring during ocean acidification quite closely (Schulz et al., 2009).

With regard to climate change and its effects on the world's oceans, calcifying organisms are of major importance. Coccolithophores are considered to account for a significant fraction of the pelagic biogenic carbonate precipitation (Baumann et al., 2004; Milliman, 1993) and are mainly responsible for creating and maintaining the oceans vertical gradient in total alkalinity (Wolf-Gladrow et al., 1999). This group of marine calcifying phytoplankton has received special attention within the framework of ocean acidification research as they were shown to exhibit distinct sensitivity to elevated pCO_2 values (Fabry et al., 2008; Rost et al., 2008). Riebesell et al. (2000) reported a reduction in calcification in the most prominent coccolithophore *Emiliania huxleyi* under future CO_2 scenarios. Since then, several studies have confirmed the sensitivity of this species to acidification (Delille et al., 2005; Feng

* Corresponding author. Tel.: +49 471 4831 1630; fax: +49 471 4831 1425.
E-mail address: Clara.Hoppe@awi.de (C.J.M. Hoppe).

0022-0981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2011.06.008

et al., 2008; Langer et al., 2009; Sciandra et al., 2003). These findings have recently been challenged by Iglesias-Rodriguez et al. (2008a), who observed enhanced calcification under elevated pCO_2 in *E. huxleyi*. The authors attributed these striking differences to the application of different manipulation methods. As the TA manipulation used in Riebesell et al. (2000) do not mimic future scenarios as closely as DIC manipulations, Iglesias-Rodriguez et al. (2008a, 2008b) claimed that their results represent more realistic responses of *E. huxleyi* to ocean acidification.

As two different strains were used in these studies (PLYB92/11 in Riebesell et al., 2000; NZEH in Iglesias-Rodriguez et al., 2008a), intraspecific variability between *E. huxleyi* strains may have also caused differences in the response patterns. Intra-specific variability has been shown to lead to different responses for four strains of *E. huxleyi* (Langer et al., 2009). The strains used in Langer et al. (2009), however, neither included the one used by Riebesell et al. (2000) nor the one used by Iglesias-Rodriguez et al. (2008a). Therefore, the findings by Langer et al. (2009) are suggestive but not unambiguous with regard to the discrepancy between Riebesell et al. (2000) and Iglesias-Rodriguez et al. (2008a). Shi et al. (2009) compared responses of one *E. huxleyi* strain (NZEH) in growth, POC and PIC quota and production from closed TA and open DIC manipulations. Even though the cells were responding differently in the two manipulations, it remains unclear whether this was due to differences in the carbonate chemistry or mechanical effects of gas bubbling, occurring in the open DIC manipulation only.

As the reasons for differences between Riebesell et al. (2000) and Iglesias-Rodriguez et al. (2008a) are still unresolved, the aim of this study was to compare the effects of different CO_2 manipulation methods. To this end, the responses of two strains of *E. huxleyi* to changing carbonate chemistry were investigated in three different manipulation approaches. First, ecophysiological responses to TA (as applied by Riebesell et al., 2000) and DIC manipulations (as applied by Iglesias-Rodriguez et al., 2000a) were compared. Additionally, to further investigate the differences between TA and open DIC manipulations as found by Shi et al. (2009), the effect of mechanical perturbation was examined by comparing closed pre-equilibrated with open continuously aerated DIC manipulated incubations.

2. Material and methods

2.1. Cultures and media preparation

Monoclonal cultures of two strains of the coccolithophore *E. huxleyi* (NZEH / PLY M219, isolated near New Zealand, supplied by the Plymouth Culture Collection, http://www.mba.ac.uk/culturecollection.php; RCC1256, isolated near Iceland, supplied by the Roscoff Culture Collection, http://www.sb-roscoff.fr/Phyto/RCC) were grown in 0.1 μm sterile-filtered North Sea seawater. The salinity was 32.38 (Guildline Autosal 8400B salinometer, Ontario, Canada).

The seawater was enriched with vitamins and trace metals according to f/2 media (Guillard and Ryther, 1962; except for iron which was added in a concentration of 1.94 μmol L^{-1} $FeCl_3$). Seawater was also enriched with nitrate (NO_3^-) and phosphate (HPO_4^{2-}) to yield concentrations of 100 and 6 μmol L^{-1}, respectively. Nutrient concentrations were measured colorimetrically using a continuous flow analyzer (Evolution III, Alliance Instruments, Salzburg, Austria).

Dilute-batch cultures were grown in 2 L borosilicate bottles at 15 ± 0.2 °C. Daylight lamps (Lumilux De Luxe T8, Osram, München, Germany) provided light intensities of 170 ± 15 μmol photons m^{-2} s^{-1} as measured with a Li-Cor datalogger (Li-Cor, Lincoln, USA) equipped with a 4π-sensor (Walz, Effeltrich, Germany). A light: dark cycle of 16:8 h was applied and all samples were taken between 6 and 10 hours after the beginning of the light phase.

In order to keep cultures in exponential growth phase and to prevent significant changes in carbonate chemistry as well as attrition of nutrients in the media, cultures were diluted regularly (cell densities never exceeded 72,000 cells mL^{-1}). Cultures were kept at experimental temperatures, light intensities and cell densities for at least two weeks, followed by another week being pre-acclimated to experimental pCO_2 levels (5–7 generations).

2.2. CO_2 perturbation experiments

Different CO_2 manipulation methods (closed TA, closed and open DIC manipulation) were applied to test ecophysiological responses to different CO_2 concentrations. In the alkalinity manipulations, carbonate chemistry was adjusted by addition of calculated amounts of HCl or NaOH (1 N Titrisol, Merck, Darmstadt, Germany) to seawater for which DIC concentrations were known. The manipulated media were stored in 2 L borosilicate bottles, which were sealed immediately with Teflon-lined screw caps without head space to avoid CO_2 exchange with the atmosphere.

DIC manipulations and incubations were conducted in 2 L borosilicate bottles equipped with glass frits for aeration. The media were sparged continuously with humidified, 0.2 μm-filtered air of different partial pressures of CO_2 (180, 380, 750 and 1000 μatm). Gas flow rates were 130 ± 10 mL min^{-1}. Gas mixtures were generated using a custom-made gas flow controller. CO_2-free air (<1 ppmv CO_2; Dominick Hunter, Willich, Germany) was mixed with pure CO_2 (Air Liquide Deutschland, Düsseldorf, Germany) by a mass flow controller based system (CGM 2000 MCZ Umwelttechnik, Bad Nauheim, Germany). The CO_2 concentration was regularly controlled with a non-dispersive infrared analyzer system (LI6252, LI-COR Biosciences, Bad Homburg, Germany) calibrated with CO_2-free air and purchased gas mixtures of 150 ± 10 and 1000 ± 20 ppmv CO_2 (Air Liquide Deutschland, Düsseldorf, Germany). Experiments were started after 48 h of aeration in order to ensure equilibration. Bottles of the closed DIC treatments were sealed without head space with Teflon-lined screw caps. A roller table was used to keep the cells in suspension. Bottles of the open DIC treatments (only applied to strain NZEH) were sparged continuously with the respective gases over the duration of the experiment. Sedimentation of cells was minimised by aeration and shaking of bottles twice a day.

2.3. Determination of carbonate chemistry

Samples for TA measurements were 0.6 μm-filtered and stored in 150 mL borosilicate bottles at 3 °C. TA was determined by duplicate potentiometric titrations (Brewer et al., 1986) using a TitroLine alpha plus autosampler (Schott Instruments, Mainz, Germany), and calculation from linear Gran plots (Gran, 1952). Certified Reference Materials (CRMs, Batch No. 54) supplied by A. Dickson (Scripps Institution of Oceanography, USA) were used to correct the measurements. The average reproducibility was ± 5 μmol kg^{-1} (n = 10).

DIC samples were filtered through 0.2 μm cellulose-acetate syringe-filters and stored head-space free in 5 mL gas-tight borosilicate bottles at 3 °C. DIC was measured colorimetrically in triplicates with a QuaAAtro autoanalyzer (Seal Analytical, Mequon, USA) with an average reproducibility of ± 5 μmol kg^{-1} (n = 20). CRMs (Batch No. 54) were used to correct the measurements. Shifts in DIC concentrations due to CO_2 exchange were prevented by opening the storage vials less than one minute prior to each measurement.

Seawater pH was determined potentiometrically on the NBS scale using a glass electrode/reference electrode cell (Schott Instruments, Mainz, Germany), which included a temperature sensor and was two-point calibrated with NBS buffers prior to every set of measurements. Average repeatability was found to be ± 0.02 pH units (n = 30).

Calculations of the carbonate system were based on measurements of DIC, pH, temperature, salinity und nutrient concentrations. They were performed with the programme CO_2sys (Pierrot et al., 2006). The dissociation constants of carbonic acid of Mehrbach et al.

Download English Version:

https://daneshyari.com/en/article/4396277

Download Persian Version:

https://daneshyari.com/article/4396277

Daneshyari.com