



Effects of CO₂ enrichment on two microalgae species: A toxicity approach using consecutive generations

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H I G H L I G H T S

- The paper addresses the potential impacts of CO₂ enrichment in the marine environment.
- Two different marine microalgae species were used through four consecutive generations.
- *T. chunii* showed a slight adaptation through generations, in terms of metabolic activity.
- *P. tricorutum* was the most sensitive one with almost total growth inhibition in the fourth generation.
- The results give valuable data about the transgenerational effects of CO₂ enrichment on microalgae.

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As a result of the increasing pressure provoked by anthropogenic activities, the world climate is changing and oceans health is in danger. One of the most important factors affecting the marine environment is the well-known process called ocean acidification. Also, there are other natural or anthropogenic processes that produce an enrichment of CO₂ in the marine environment (CO₂ leakages from Carbon Capture and Storage technologies (CCS), organic matter diagenesis, volcanic vents, etc). Most of the studies related to acidification of the marine environment by enrichment of CO₂ have been focused on short-term experiments. To evaluate the effects related to CO₂ enrichment, laboratory-scale experiments were performed using the marine microalgae *Tetraselmis chunii* and *Phaeodactylum tricorutum*. Three different pH values (two treatments - pH 7.4 and 6.0 - and a control - pH 8.0) were tested on the selected species across four consecutive generations. Seawater was collected and exposed to different scenarios of CO₂ enrichment by means of CO₂ injection. The results showed different effects depending on the species and the generation used. Effects on *T. chunii* were shown on cell density, chlorophyll-*a* and metabolic activity, however, a slight adaptation across generations was found in this last parameter. *P. tricorutum* was more sensitive to acidification conditions through generations, with practically total growth inhibition in the fourth one. The conclusions obtained in this work are useful to address the potential ecological risk related to acidification by enrichment of CO₂ on the marine ecosystem by using consecutive generations of microalgae.

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1. Introduction

Oceans health is under high and still increasing pressure from the current climate change provoked by anthropogenic activities (Wernberg et al., 2011). Anthropogenic carbon emissions are mainly originated by the burning of fossil fuel and worsened because of deforestation (Bijma et al., 2013). CO₂ values have increased from 280 ppm in the preindustrial era (IPCC, 2007) until

the current 411.25 ppm measured at Mauna Loa (NOAA, May 2018).

While renewable energy sources seem to be the key to mitigating climate change, the Carbon Capture and Storage (CCS) technology has been suggested as the best temporarily option to reduce carbon emissions (EPA, 2014). But this technique is associated with a great concern about the risk that a potential CO₂ leakage could cause in the marine environment (Ardelan et al., 2009). Therefore, the CCS technology is considered another potential source of acidification by CO₂ enrichment (Khosrovyan et al., 2014; Pasarelli et al., 2017; De Orte et al., 2018).

The immediate effects of climate change on oceans are warming (IPCC, 2007) and acidification, since oceans act as a sink of CO₂ (Sabine et al., 2004). There is a continuous flux of CO₂ across the interface atmosphere–ocean, which causes the alteration of the seawater chemistry, with the consequent phenomena known as ocean acidification (OA) (Raven, 2005). Previous studies related to this issue point out that although some marine species will be tolerant to these changes, the whole ecosystem balance could be damaged (Turley and Gatusso, 2012).

There are also many natural sources of CO₂ enrichment in the marine environment, such as the organic matter degradation and diagenesis in the sediment (Canfield, 1994), the natural CO₂ vents (Hall-Spencer et al., 2008; McGinnis et al., 2011) or the submarine eruption in the Canary Islands (Spain) where pH values between 5.13 and 8.04 have been measured (Santana-Casiano et al., 2012).

As it has been suggested in multiple research works, marine acidification will cause a wide range of responses from individuals but also from ecosystems as a whole (Riebesell et al., 2000; Zondervan, 2007). Many contradictory trends have been reported about those effects of acidification on marine ecosystems; including positive, neutral or negative responses, depending on the physiological processes involved. The magnitude of acidification effects on microalgae has been proved to be a species-specific issue (Hinga, 2002). In the case of microbial communities, the processes affected by acidification include primary productivity, trace gases emission, nitrogen fixation and organic matter degradation, among others (Das and Mangwani, 2015). Changes on the ecosystem are related to the potential modification of its composition, structure, energy flow and resources (Blackford, 2010) with still unknown consequences to the environment.

Microalgae play an essential role on ecosystems (Cairns et al., 1992). Being the base of the marine trophic chain, higher trophic levels depend on this group (McCormick and Cairns, 1994) which means that any change in these organisms could provoke severe consequences in the marine ecosystem. Ocean acidification research has been focused on studies with photoautotrophic organisms such as phytoplankton, since photosynthesis is a key process in elemental cycles, giving energy to higher trophic levels by the organic matter production, using CO₂ and inorganic nutrients (Riebesell et al., 2010). Some attributes of microalgae including short life cycle, high sensibility to environmental stressors and rapid growth, give them the role of a productive indicator on climate change (McCormick and Cairns, 1994). Also it is remarkable to mention that microorganisms' assays are not subject to ethical restrictions, different from investigation with higher organisms such as fish or invertebrates (Debelius et al., 2009).

The majority of research in this topic is focused on short-term studies (Kroeker et al., 2013). For this reason, the knowledge on multi-generational effects of acidification in marine ecosystems is limited (Reusch, 2014; Sunday et al., 2014). This lack of knowledge is limiting the comprehension of how marine ecosystems will deal with acidification (Rodríguez-Romero et al., 2015). Therefore, there is a risk to over or under estimate the species responses to this phenomenon.

The aim of this study is to evaluate the effects of acidification by

CO₂ enrichment in two marine microalgae species across consecutive generations, using the species *Tetraselmis chuii* and *Phaeodactylum tricorutum*. Acidification scenarios were conducted in laboratory with a CO₂ injection system. Bubbling CO₂ into the water is a very efficient technique to manipulate carbonate chemistry and it is one of the five best practices recommended by Riebesell et al. (2010). The election of the pH values was based on two potential phenomena. On the one hand, the process known as anthropogenic ocean acidification caused by the increase of CO₂ concentration in the atmosphere derived from human activities (pH 7.4). This pH has been established according to the predictions of a decrease in the pH of 0.5 units for 2100 (Caldeira and Wickett, 2003). On the other hand, the pH 6.0 scenario based on a potential CO₂ leakage from sub-seabed carbon capture and storage (CCS) emplacement (Pasarelli et al., 2017; De Orte et al., 2018). Also, another scenario was tested as a control (pH 8.0). The species chosen for this work are equally relevant for the ecosystems and research: *Tetraselmis chuii*, which has been deeply studied for its adequacy on biodiesel production and as a CO₂ sink, and *Phaeodactylum tricorutum*, a widely used species on toxicity tests because of its sensitiveness. Both of them are important species in aquaculture as a nutrient for multiple species. This work pretends to raise awareness about the potential risks of the pH decrease in seawater to the marine ecosystem as a whole, working under the hypothesis that a scenario of acidification by CO₂ enrichment may represent a danger for the development of microalgae not only immediately but also across generations.

2. Material and methods

2.1. The CO₂ Injection System

The CO₂ Injection System[®] (P201200753, Cádiz) has been developed for the simulation of the CO₂ enrichment process in laboratory, through the use of toxicity bioassays (Fig. 1). With this system, organisms are exposed to different pH values in order to assess the potential adverse effects of acidification by enrichment of CO₂ on marine ecosystem. This experiment was developed according to the methodology described in Bautista-Chamizo et al. (2016).

2.2. Seawater analysis

Seawater aliquots (50 mL of filtered sample) were analysed with an automatic alkalinity titrator (Metler Toledo, T50). Carbonate system speciation was calculated using the measured values of pH and total alkalinity (TA), with the CO₂SYSS software (Pierrot et al., 2006) with constant dissociation from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and KSO₄ according to Dickson (1990).

2.3. Toxicity tests

The microalgae species (*Tetraselmis chuii* and *Phaeodactylum tricorutum*) were obtained from “Servicios Centrales de Investigación en Cultivos Marinos” at the University of Cádiz. Experiments were performed in triplicate using Erlenmeyer flasks previously sterilized through HNO₃ (10%) washes and autoclaved. Temperature was kept at 22 °C (±2) and illumination was provided by eight fluorescent lights (36 W) in continuous. Each flask was filled with 200 mL of filtered (Millipore 0.22 μm) seawater (pH 8.0 ± 0.1, salinity 34) enriched with f/2 medium (Sunda and Guillard, 1976). Exponentially-growing populations of microalgae were exposed to three acidification scenarios defined by different pH values (8.0 as a control, 7.4 and 6.0). The initial cell density was

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