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Will temperature and salinity changes exacerbate the effects of seawater acidification on the marine microalga *Phaeodactylum tricornutum*?

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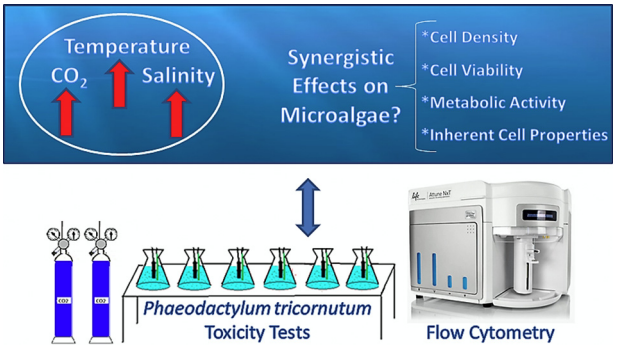
HIGHLIGHTS

- Combined effects of pH, temperature and salinity were studied on a marine diatom.
- A novel CO_2 injection system was used for performing microalgae toxicity test.
- Synergistic effects were found on cell viability, cell size and autofluorescence.
- Results are useful to address the potential impact of climate change.

GRAPHICAL ABSTRACT



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ABSTRACT

To evaluate the effects related to the combination of potential future changes in pH, temperature and salinity on microalgae, a laboratory experiment was performed using the marine diatom *Phaeodactylum tricornutum*. Populations of this species were exposed during 48 h to a three-factor experimental design ($3 \times 2 \times 2$) with two artificial pH values (6, 7.4), two levels of temperature (23 °C, 28 °C), two levels of salinity (34 psu, 40 psu) and a control (pH 8, Temp 23 °C, Sal 34 psu). The effects on growth, cell viability, metabolic activity, and inherent cell properties (size, complexity and autofluorescence) of *P. tricornutum* were studied using flow cytometry. The results showed adverse effects on cultures exposed to pH 6 and high temperature and salinity, being the inherent cell properties the most sensitive response. Also, linked effects of these parameters resulted on cell viability and cell size decrease and an increase of cell autofluorescence. The conclusions obtained from this work are useful to address the potential effects of climate change (in terms of changes on pH, salinity and temperature) in microalgae.

1. Introduction

Climate change will influence oceans and coasts in different ways (Philippart, 2007). The rising of temperature is one of the most evident

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consequences of this process, and it is the reason why glaciers and the Greenland ice cover are melting (Teng et al., 2006). The effects will be of a great magnitude: sea level rise, floodings (Frei et al., 2006), heat waves, hurricanes, droughts and other extreme events (IPCC, 2007). These global environmental changes are happening at an exceptional speed with no precedents in the geological past (IPCC, 2014). Moreover, climate change will have other negative impacts apart from extreme weather events; it will also cause changes in terrestrial and marine ecosystems (Root et al., 2003; Svenning et al., 2009).

Oceans, in their role of carbon dioxide (CO_2) sinks, are acting as a buffer of climate change (Egilsdottir et al., 2009). However, as a result of this CO_2 absorption, the ocean chemistry is being altered, with a decrease of the seawater pH (Raven, 2005). Ocean pH has fallen about 0.1 units since pre-industrial times (Orr et al., 2005) and it is predicted to decrease about 0.3–0.5 units by the year 2100 and 0.5–0.7 units by the year 2300 (Caldeira and Wickett, 2003). In order to mitigate climate change by reducing the CO_2 already available in the atmosphere, the Carbon Capture and Storage technology is getting importance as a short-term solution (IPCC, 2014). By injecting CO_2 in the sub-seabed storage sites, this technology has the capacity to capture 40 million tons of CO_2 worldwide (Global CCS Institute, 2016). Yet, this technology has some associated risks, such as a potential CO_2 leakage, which could lead to an extreme acidification of the surrounding area of the escape, reaching pH values of 6.0 or even lower (Santana-Casiano et al., 2012). Seawater acidification has a wide and complex variety of responses within marine organisms and adverse effects are not necessarily observed, since sensitivities to acidification are species, population and even life-stage specific (Ries et al., 2009; Wang et al., 2015).

Global average temperature has increased by 0.7 °C during this last century (Hansen et al., 2006) and according to IPCC (2007) it is predicted to keep rising between 1.8 °C and 4 °C by the end of this century. Salinity is expected to change as a result of this global warming phenomenon, being reduced or increased depending on the region latitude.

This environmental problem has been addressed by many authors in studies dealing with the effects of seawater acidification on marine higher organisms (Basallote et al., 2012; Rodríguez-Romero et al., 2016), microalgae (Iglesias-Rodriguez et al., 2008; Müller et al., 2010; Johnson et al., 2013; Li et al., 2017) and bacteria (Borrero-Santiago et al., 2016a, 2016b; Díaz-García et al., 2016). However, current studies rarely take into account the potential synergistic effects of environmental factors under climate change scenarios. Only a few research studies are focused on the effects of CO_2 enrichment when combined with changes on temperature or salinity (Matozzo et al., 2012; Ko et al., 2014; Pereira et al., 2016), but there is still a lack of research about the potential effects of these three combined environmental factors on microalgae.

Diatoms are ubiquitous marine photosynthetic eukaryotes responsible for approximately 20% of global primary productivity, playing a central role in the biogeochemical cycling of important nutrients (Rosenwasser et al., 2014). *Phaedactylum tricornutum* (Bacillariophyceae) (Bohlin, 1897), which has been found in great abundance in coastal and oceanic waters, was selected for the study as it is considered a model organism in ecotoxicological studies (OECD, 1984; USEPA, 1985; ISO, 1987) and also because of its importance in aquaculture as nutrient for higher organisms.

This work hypothesizes that the combination of pH, temperature and salinity changes could cause unwanted effects in marine phytoplankton. Therefore, the objective of this work is to provide an integrated knowledge of the microalgae response to future and potential global warming, seawater acidification and increased salinity. Such understanding will be of great importance in order to manage and fight climate change effects on marine ecosystems. For this purpose, three pH values (6, 7.4, and a non-acidified control pH 8), two temperature values (23 °C, 28 °C) and two salinity values (34 psu, 40 psu) were selected to investigate the effects on key physiological parameters of the microalgae *P. tricornutum*.

2. Material and methods

2.1. Sampling and algae culture conditions

Seawater was collected from Río San Pedro, in the Bay of Cádiz (Atlantic coast of South-West Spain) as this location has been proved to be a valid reference station in many previous ecotoxicology works (Riba et al., 2004; Basallote et al., 2014; Borrero-Santiago et al., 2017). The natural properties of this seawater were: pH of 8, temperature 23 °C and salinity of 34 psu (standard deviation ± 0.1). *P. tricornutum* was obtained from Servicios Centrales de Investigación en Cultivos Marinos from the University of Cádiz. Stock cultures were cultivated using this seawater (pH 8, Temp 23 °C, Sal 34 psu) under a 24 h light regime.

2.2. CO_2 injection system

In order to reproduce an event of seawater acidification or a potential CO_2 leakage, a CO_2 injection was used. This system (patent no: ES2618843) described by Borrero-Santiago et al. (2016ab), is inside of a non-pressurized chamber, and it has been designed to guarantee the sterile conditions during the development of the toxicity test. It also permits the control of other parameters such as temperature and light. Briefly, the semi-automatic system consists on the injection of CO_2 through silicone hoses regulated by an AT control software (Aqua Medic 8.0), which acts according to the data received from the pH meters. The precision of the pH values has a minimal variety of ±0.1. The experimental set-up was developed according to Bautista-Chamizo et al. (2016).

2.3. Toxicity tests

Toxicity tests were performed in triplicate and using sterilized Erlenmeyer flasks, previously washed with HNO_3 (10%) and autoclaved. Continuous illumination was provided by six fluorescent lights (36 W). Each flask was filled with 150 mL of filtered (Millipore 0.22 μm) seawater to avoid bacteria and other microalgae, enriched with f/2 medium (Sunda and Guillard, 1976) and silicate. Flasks were shaken at 4-h intervals to avoid cell precipitation and flasks position was changed with respect to the light source at 8-h intervals to ensure that all of them received the same level of illumination.

Exponentially-growing (72 h) populations of microalgae were exposed to a three factor experimental design ($3 \times 2 \times 2$) with two artificial pH values (6,7.4), adjusted by CO_2 injection, and a control (non-acidified natural seawater, pH 8), two levels of temperature (23 °C, 28 °C) and two levels of salinity (34 psu, 40 psu) (standard deviation 0.1). The selected pH values are related to two different scenarios; a predicted future value (pH 7.4) and the scenario of a potential CO_2 leakage (pH 6). The experimental stressors represent the average surface water temperature and salinity of the study area (23 °C, 34 psu) and the potential future scenarios of temperature (28 °C) and salinity (40 psu). All the possible combinations were tested, with a result of twelve treatments, which are detailed as follows: single stressor treatments (testing changes on pH, temperature (Temp) and salinity (Sal)), dual combination of stressors treatments (testing changes on: pH × Temp, pH × Sal, Sal×Temp) and a triple combination of stressors treatments (pH × Temp×Sal) being the combination: pH 8, Temp 23 °C and Sal 34 psu our control.

The initial cell density was established at 3×10^4 cells mL^{-1}. The experiments were run for 48 h. After this short time period an entire cell cycle is able to be completed (Seoane et al., 2017) and microalgae are able to experience metabolic adjustment in response to stressors (Esperanza et al., 2015). All the parameters were measured at the end of the experiment with a flow cytometer. Toxicity tests were developed according to international standards (OECD, 1984).

Desirable pH values were maintained by bubbling CO_2 into the water. High salinity was achieved by evaporation, and adjusted by