Science of the Total Environment 649 (2019) 224–232

Contents lists available at ScienceDirect

Science of the Total Environment

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

# Comparative effects of seawater acidification on microalgae: Single and multispecies toxicity tests

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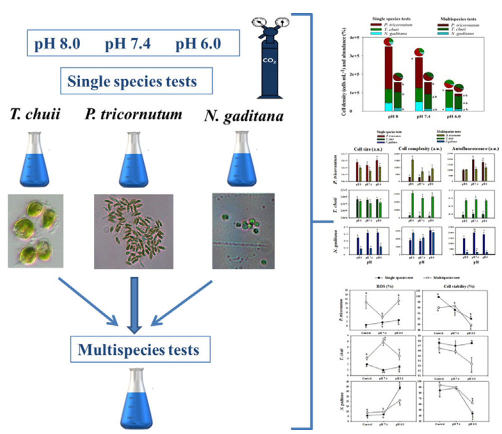
## HIGHLIGHTS

- Different responses were observed depending on the species.
- In this work *N. gaditana* was the most sensitive species to low pH.
- Effects of competence among species were observed in the multispecies control (pH 8.0).
- Effects of competence were eclipsed by the $CO_2$ effects on cultures exposed to pH 6.0.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 29 June 2018
Received in revised form 16 August 2018
Accepted 17 August 2018
Available online 21 August 2018

Editor: D. Barcelo

*Keywords:*
pH
$CO_2$ enrichment
Flow cytometry
Ecotoxicity
Multispecies

## ABSTRACT

In order to gain knowledge about the potential effects of acidification in aquatic ecosystems, global change research based on microalgae as sentinel species has been often developed. However, these studies are limited to single species tests and there is still a research gap about the behaviour of microalgal communities under this environmental stressor. Thus, the aim of this study was to assess the negative effects of $CO_2$ under an ecologically realistic scenario. To achieve this objective, two types of toxicity tests were developed; i) single toxicity tests and ii) multispecies toxicity tests, in order to evaluate the effects on each species as well as the interspecific competition. For this purpose, three microalgae species (*Tetraselmis chuii, Phaeodactylum tricornutum* and *Nannochloropsis gaditana*) were exposed to two selected pH levels (7.4, 6.0) and a control (pH 8.0). The pH values were choosen for testing different scenarios of $CO_2$ enrichment including the exchange atmosphere-ocean (pH 7.4) and natural or anthropogenic sources of $CO_2$ (pH 6.0). The effects on growth, cell viability, oxidative stress, plus inherent cell properties (size, complexity and autofluorescence) were studied using flow cytometry (FCM). Results showed that *T. chuii* was the most resistant species to $CO_2$ enrichment with less abrupt changes in terms of cell density, inherent cell properties, oxidative stress and cell viability. Although *P. tricornutum* was the dominant species in both single and multispecies tests, this species showed the highest decrease in cell density under pH 6.0. Effects of competence were recorded in the multispecies control (pH 8) but this competence

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https://doi.org/10.1016/j.scitotenv.2018.08.225

was eclipsed by the effects of low pH. The knowledge of biological interactions made by different microalgae species is a useful tool to extrapolate research data from laboratory to the field.



## 1. Introduction

Carbon dioxide ($CO_2$) is the most important greenhouse gas (GHG) contributing to climate change present in the atmosphere, being responsible of an approximately 65% of the total increase in "radiative forcing" caused by long-lived GHG, according to the World Meteorology Organization (WMO, 2016). The high concentration of this greenhouse gas is due not only to the strong dependence of global economies on unclean carbon energies (Pires et al., 2011) but also because of the exponential population growth. Greenhouse gases concentration has rapidly increased in the atmosphere from 280 ppm in the preindustrial era (IPCC, 2007) until the current 411.24 ppm (Tans et al., 2018). About 30% of the anthropogenic $CO_2$ is absorbed by the ocean due to the physical and biological interactions between atmosphere and the oceans, leading to the acidification of the marine environments (IPCC, 2014) According to Caldeira and Wickett (2003), ocean pH is predicted to fall 0.3–0.5 units by the year 2100 and 0.5–0.7 units by the year 2300.

The Carbon Capture Storage (CCS) technology has been proposed as a potential solution to mitigate climate change (IPCC, 2005). This technique consists on the $CO_2$ sequestration, transport and storage in geological formations, in the ocean, in mineral carbonates, or for use in industrial processes. In the case of storage in geological formations (such as oil and gas fields, unminable coal beds and deep saline formations) (IPCC, 2005) there is a general scientific agreement on its economic and environmental viability (Reguera et al., 2009) but the potential effects of an accidental $CO_2$ leakage from the storage site in the marine environment are still under study. Apart from these two mentioned sources of $CO_2$ enrichment, there are other natural sources in the marine environment, such as bacterial organic matter degradation and diagenesis (Canfield, 1994), natural $CO_2$ vents (Hall-Spencer et al., 2008; McGinnis et al., 2011) or submarine eruptions such as the one occurring in the Canary Islands (Spain) where pH values between 5.13 and 8.04 have been measured (Santana-Casiano et al., 2012). In order to predict the ecological risk related to $CO_2$ enrichment and hence acidification, many ecotoxicity works have been developed, with a wide range of marine organisms, from primary producers such bacteria (Borrero-Santiago et al., 2016a, 2016b) and microalgae (Bautista-Chamizo et al., 2018) to higher organisms such as amphipods (Basallote et al., 2014; Pasarelli et al., 2017), clams (Rodríguez-Romero et al., 2014), and polychaetes (Pereira et al., 2016).

In ecotoxicity tests, microalgae are considered as key organisms, due to their position in the food chain as primary producers, but also because of their sensitivity to toxicants, being considered as rapid, cost-effective and ecologically relevant organisms to evaluate emerging pollutants (Arensberg et al., 1995; Prado et al., 2009; Seoane et al., 2017). Evidences about ocean acidification have been found all over the world in marine and coastal areas, and the current research about the effects of $CO_2$ on microalgae is quite wide but it is still limited to single species tests (Wu et al., 2010; Johnson et al., 2013) and most of the literature about $CO_2$ toxicity in microalgae is focused on coccolithophores (Müller et al., 2010; Beaufort et al., 2011; Meyer and Riebesell, 2015; Furukawa et al., 2018). However, to mimic realistic and natural conditions, accuracy is greater when working with multispecies toxicity tests, in order to study community drifts (Franklin et al., 2004; Yu et al., 2007; De Laender et al., 2009; Debenest et al., 2011) since microalgae communities are formed by different species in continuous competition for light and nutrients (Gross, 2003). Also, microalgae are involved in a chemical interaction known as allelopathy, characterized by the production of secondary metabolites to influence the development of other species, bacteria and predators, (Gross, 2003; Legrand et al., 2003; Granéli et al., 2008). The biggest issue when working with multispecies tests is related to the difficulty of distinguishing between species with similar shape and size. In this sense, flow cytometry is currently the most useful technique on this field (Stauber et al., 2002; Franklin et al., 2004; Debenest et al., 2011).

Hence, this work hypothesizes that $CO_2$ enrichment in marine environments may cause negative effects in marine phytoplankton communities. The aim of this study focuses on the determination and comparison of the different effects of $CO_2$ on microalgae using single and multispecies toxicity tests, in order to evaluate also the potential algal-algal interaction under this stressor. In this study, microalgae are used as useful ecological indicators to assess the potential effects of $CO_2$ enrichment on the marine environment. For this purpose, three microalgae species (*Tetraselmis chuii, Phaeodactylum tricornutum* and *Nannochloropsis gaditana*) were exposed to two pH values (7.4, 6.0) and a non-acidified control (pH 8.0) in both single and multispecies cultures. The election of these species has been motivated by their different size and family, but also by their common natural habitat and because of their importance on aquaculture. The pH selection has been based on anthropogenic acidification (for this assessment, pH 7.4 has been selected) and on an extreme value of natural or anthropogenic sources of $CO_2$ enrichment (in this case, pH 6.0 has been selected). To assess the sensitivity of microalgae to $CO_2$ injection, different endpoints such as cell density, size, complexity, autofluorescence, percentage of reactive oxygen species (ROS) and cell viability were studied using flow cytometry techniques. The importance of these sub-lethal endpoints relies on the early warning aspect, before mortality signals appear (Yu et al., 2007). The present work provides a deeper knowledge about the response of these three species to $CO_2$ when exposed by themselves, but also an interesting approach to how the presence of other species influences the microalgae responses to toxicants or external stressors.

## 2. Material and methods

### 2.1. The $CO_2$ Injection System®

The $CO_2$ Injection System® has been designed to mimic seawater acidification (Fig. 1). This system (patent no: ES2618843, University of Cádiz) is located into a non-pressurized chamber which guarantees sterile conditions in order to safely develop toxicity tests with bacteria and microalgae (Borrero-Santiago et al., 2016a). Other physical parameters such as temperature and light are also controllable. With this system, the desired pH is adjusted and controlled using the Aqua Medic AT control hardware and software. The AT control records the data obtained from the pH electrodes at real time. Solenoid valves are automatically opened if the target pH value is deviated $>0.01$ units and $CO_2$ gas is injected through a silicon hose which connects the solenoid valve with the water. This valve is closed when the desired pH is reached. The carbon dioxide is provided by $CO_2$ bottles (Air liquid) (Bautista-Chamizo et al., 2016).

### 2.2. Toxicity tests

Microalgae from the species *Tetraselmis chuii* (Butcher, 1959) (Chlorodendrophyceae), *Phaeodactylum tricornutum* (Bohlin, 1897) (Bacillariophyceae) and *Nanochloropsis gaditana* (Lubián, 1982) (Eustigmatophyceae) were obtained from Servicios Centrales de Investigación en Cultivos Marinos from the University of Cádiz. These