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# Metabolomic and physiological analyses of two picochlorophytes from distinct oceanic latitudes under future ocean acidification and warming

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#### ABSTRACT

Phytoplankton are cosmopolitan marine photosynthetic organisms that are vital to biogeochemical cycles and marine ecosystems. The current rise in atmospheric CO<sub>2</sub> and surface ocean temperatures are poised to disrupt the ecological niches of phytoplankton. Picochlorophytes, a broad taxon of small green eukaryotic phytoplankton, have been shown to perform well under future rising oceanic CO<sub>2</sub> and temperature scenarios. This study investigates the acclimation responses of cosmopolitan picochlorophytes from the Chlorella-lineage under high CO<sub>2</sub> (1000 p.p.m.) and a rise of 4 °C (8 °C – polar picochlorophyte; 32 °C, tropical picochlorophyte). In order to determine how the future ocean warming and acidification might affect picochlorophytes, a polar strain of Chlorella and a tropical Parachlorella were selected, and their physiology and GCMS-based metabolomics were investigated. Growth rate and cellular dimensions (diameter, volume, and surface area) of Chlorella significantly increased in all environmental future scenarios compared to Parachlorella. Photosynthetic parameters of the picochlorophytes studied showed acclimation, with high temperature and high CO2 triggering the adaptation of  $F_{y}/F_{m}$ , NPQ<sub>max</sub>, and  $E_{k}$  of *Chlorella* and *Parachlorella*, respectively. High CO<sub>2</sub> induced the most changes in the Chlorella metabolome, altering the levels of metabolites related to amino acids and their derivatives, glutathione production, carbohydrates, and photochemical quenching. Combined high CO2/temperature altered Parachlorella's metabolome, though with a small number of biomarkers detected. This study provided evidence to support the hypothesis that picochlorophytes could thrive in a more acidified and warmer ocean.

#### 1. Introduction

Marine phytoplankton are globally distributed photosynthetic organisms (Dasgupta et al., 2009) with a rich diversity shaped over millions of years through endosymbiotic events (Coelho et al., 2013; Falkowski et al., 2004), lateral gene transfer (Parker et al., 2008), and adaptation to various ecotypes within species (Benner et al., 2013; Langer et al., 2009). As key primary producers (Field et al., 1998), marine phytoplankton fix carbon dioxide photosynthetically and form the base of the oceanic food web (Pierella Karlusich et al., 2020). Marine phytoplankton play an important role in the biological carbon pump and are vital in the global carbon cycle, remediating the amount of carbon in the atmosphere (Alvain et al., 2008). However, human-driven emissions of  $CO_2$  are irreversibly causing acidification and warming of the ocean surface at an unprecedented rate (Intergovernmental Panel on Climate Change, 2023). Ocean acidification and warming have been demonstrated to threaten ecosystem functions of phytoplankton, including a reduction in nutrient transfer to higher trophic levels (J.R. Bermúdez et al., 2016), reduction of species richness (Tatters et al., 2013), and an undermining of the biological pump efficiency (Brussaard et al., 2013).

Picochlorophytes are a functional group of small eukaryotic green picophytoplankton with a maximum size of 5  $\mu$ m (Barber, 2007). They are no less important than larger phytoplankton in terms of carbon export (Richardson and Jackson, 2007) and are often found to be the dominant phytoplankton in some marine environments (Boysen et al., 2021; Zubkov et al., 1998). Picochlorophytes have consistently been

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