



## Differential impacts of global change variables on coastal South Atlantic phytoplankton: Role of seasonal variations



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### ABSTRACT

Global change is associated to the increase in temperature (T), nutrient inputs (Nut) and solar radiation in the water column. To address their joint impact on the net community production [NCP], respiration [CR] and PSII performance ( $\Phi_{PSII}$ ) of coastal phytoplankton communities from the South Atlantic Ocean over a seasonal succession, we performed a factorial design. For this, we used a  $2 \times 2 \times 2$  matrix set-up, with and without UVR, ambient and enriched nutrients, and *in situ* T and *in situ* T + 3 °C. The future scenario of global change exerted a dual impact, from an enhancement of NCP and  $\Phi_{PSII}$  during the pre-bloom to an inhibition of both processes towards the bloom period, when the *in situ* T and irradiances were lower and the community was dominated by diatoms. The increased inhibition of NCP and  $\Phi_{PSII}$  during the most productive stage of the annual succession could produce significant alterations of the CO<sub>2</sub>-sink capacity of coastal areas in the future.

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### 1. Introduction

Coastal areas represent a small fraction (~5%) of the total oceanic surface, however, they constitute the most productive ecosystems on Earth (Rousseaux and Gregg, 2014; Uitz et al., 2010). These areas are also considered biogeochemical hot spots because they receive large inputs of nutrients (Nut) and organic carbon from land and open ocean thus supporting high metabolism and primary production (Cloern et al., 2014). Coastal areas also present highly variable environmental conditions e.g., light, temperature (T) among others, making them particularly interesting model systems to evaluate the responses of organisms in a scenario of global change. Global change is a process largely related to human-derived activities e.g., the release of high amounts of CO<sub>2</sub> into the atmosphere due to industrialization (IPCC, 2013). Such atmospheric changes

derive in more acidified and warmer water bodies, receiving higher levels of solar radiation (including ultraviolet radiation [UVR, 280–400 nm]) due to increased stratification of the water column (Williamson et al., 2014). In addition, due to the increasing human pressures through agriculture, livestock, and industry, higher population densities in areas close to the coast, and consequently higher waste removal (Cloern et al., 2016), coastal areas are incurring greater nutrient inputs through rivers, and these inputs expected to intensify during the next few decades (Rabalais et al., 2009).

The effects of variables associated to global change on coastal communities have been largely explored individually in both, laboratory and field studies. The validity of such approaches, however, is being challenged by recent research that reveals interactive effects among environmental variables that affect the responses as compared to the individual effects (Boyd et al., 2015, 2016). Thus, studies assessing multi-variable impacts are more appropriate as they provide more reliable information about future impacts of global change on aquatic ecosystems. For example, solar UVR is an abiotic factor that strongly influences the responses of phytoplankton under global change conditions. Although a huge body of

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literature has shown the negative effects of UVR on several targets (e.g., photosystem II [ $\Phi_{PSII}$ ], proteins, DNA) and processes (e.g., growth, nutrients uptake, photosynthesis, respiration) (Häder et al., 2015) other studies have also found positive effects (e.g., higher photorepair of DNA, enhanced photosynthesis; Gao et al., 2007; Barbieri et al., 2002). Part of these contrasting responses, however, occurs due to the interaction of UVR with other variables. In this sense, nutrient enrichment generally tends to counteract the negative effects of solar UVR exposure (Agustí et al., 2009; Harrison and Smith, 2013; Villafañe et al., 2014) therefore acting antagonistically. Increased temperature frequently acts in an antagonistic manner with UVR either on short- (e.g. Sobrino and Neale, 2007) and mid-term scales (Helbling et al., 2011). This antagonistic effect improves phytoplankton photochemical performance (Helbling et al., 2011; Villafañe et al., 2015; Wong et al., 2015), increasing growth rates (Morán et al., 2010) or even protein synthesis and nitrogen uptake (Toseland et al., 2013), especially when the tested organisms are below their optimal thermal limit.

In spite of the ecological and socio-economic importance that implies the prediction of the effects of multiple global change variables on aquatic ecosystems, up to date scarce experimental studies have considered how the interaction between UVR, Nut and T could impact on primary producers (Cabrerizo et al., 2014; Doyle et al., 2005; Durán et al., 2016). These studies have reported a wide range of effects under a multi-factor scenario, ranging from inhibition of phytoplankton growth (Doyle et al., 2005) to enhancement of photosynthesis and of excretion of organic carbon (Durán et al., 2016). One study carried by our group (Cabrerizo et al., 2014) further highlighted the species-specificity of responses under the joint effect of these variables – UVR, Nut and T. Moreover, most of the studies simulating a scenario of global change, however, have been performed during rather short periods of time, neglecting the natural environmental heterogeneity that can also alter the biological responses of phytoplankton. This is especially important, as aquatic ecosystems experience natural variations in their physical and chemical parameters, together with a temporal succession of species. Thus, and to address this gap of knowledge i.e., the responses of organisms to global change conditions tied to the natural variability of the environment, we designed experiments to quantify how a future scenario of UVR under increased Nut and T could alter the physiology of phytoplankton communities during the pre-bloom to bloom period in coastal South Atlantic Ocean waters. Thus we worked not only with communities that changed along the season but that also had different light and thermal history due to variable irradiances/mixing conditions, and *in situ* T due to the transition from fall to winter. We performed experiments during almost three months, manipulating simultaneously the Nut concentrations, T and radiation quality. Over this period, we measured the net community production [NCP], community respiration [CR] and the effective photochemical quantum yield ( $\Phi_{PSII}$ ) on different phytoplankton communities of Patagonian coastal waters.

Despite that between 6 and 11% of the global primary productivity occurs in the South Atlantic Basin (Rousseaux and Gregg, 2014; Uitz et al., 2010) and although Patagonian waters constitute one of the most important fishery areas of the Atlantic Ocean Basin (De Carli et al., 2012; Góngora et al., 2012), they continue to be a relatively unexplored area. The area has continuous inputs of nutrients from the river into the sea due to agricultural and urban activities (Helbling et al., 2010), and a clear bloom (dominated by diatoms, mainly *Odontella aurita*) during winter time and pre- and post-bloom periods (dominated by pico-nanoplankton cells, mainly flagellates) have also been reported for this site (Villafañe et al., 2004, 2013).

With this background in mind, our working hypothesis was that

a future global change scenario will reduce the NCP and  $\Phi_{PSII}$  performance, and will enhance the CR in the pre-bloom as compared to the bloom communities, as increased T will displace such communities above of the optimal growing temperatures experienced inside the annual thermal limits (17–9 °C). Thus, through our simulations of future global change conditions we tested the impacts of a multi-variable scenario on the communities varying during the seasonal succession.

## 2. Material and methods

### 2.1. Study site and sampling

Water samples were collected at the seawater side of the Chubut River estuary, in Patagonian coastal waters (Chubut Province, South Atlantic Ocean, Argentina) (Fig. 1). The experiments were done during the period April 5 to June 14, 2013, with field samples collected every week (10 experiments in total). Surface seawater (salinities > 31) samples (ca. 20 L) were collected in the afternoon-evening of the day previous to the experimentation at Egi station (43° 20.5' S, 65° 02.0' W) (Fig. 1) during high tide. The samples were pre-screened through a 180  $\mu$ m Nitex mesh to eliminate mesozooplankton, and put into an acid-cleaned (1 N HCl) opaque container and immediately transported to the Estación de Fito-biología Playa Unión (EFPU, 10–15 min away from the sampling site) where experiments were performed as described below. Once at the laboratory, samples were pre-acclimated to the *in situ* T registered during the sampling moment or either to the *in situ* T + 3 °C overnight before being used in experimentation.

### 2.2. Experimental set up

The UVR  $\times$  Nut  $\times$  T effects on NCP, CR and  $\Phi_{PSII}$  were assessed using a factorial design set up with a 2  $\times$  2  $\times$  2 matrix. All experimental units were run in triplicate. The original seawater sample was divided in two sub-samples that were put into two opaque containers. In one of them, the nutrients were kept under ambient conditions (i.e., without modification, as at the time of collection) whereas the other was enriched in macronutrients by 45  $\mu$ M for nitrate + nitrite, 1.8  $\mu$ M for phosphate, and 5.5  $\mu$ M for silicate over their respective ambient concentration, simulating larger inputs by the Chubut river. Samples from these two Nut conditions were placed in 50 mL quartz round vessels, 24 for oxygen and 24 for  $\Phi_{PSII}$  measurements, and exposed to: a) two radiation treatments, PAB (UVR + PAR, >280 nm), uncovered vessels, and P (PAR, >400 nm) vessels covered with UV Opak 395 filter (Difegra); and, b) two T treatments (*in situ* and *in situ* + 3 °C). The increase in 3 °C represents predicted values by the end of century for South Atlantic surface waters by IPCC (2013, scenario RCP 8.5).

All vessels containing the samples were put in a rotating system, to ensure homogeneous exposures, inside an illuminated environmental chamber (Sanyo MLR-350, Japan). The chamber kept the desired temperature *in situ* or *in situ* + 3 °C constant for each experimental condition. Due to logistical limitations inside the environmental test chamber, it was first set to the *in situ* water temperature and the following day to the increased temperature. To avoid alterations in the acclimation conditions and in the physiological state of the communities, we took new samples for the *in situ* + 3 °C experiments. We found no significant differences between samples taken during two consecutive days for each experiment (data not shown). Radiation levels were provided by 10 Philips daylight fluorescent tubes for PAR and 5 Q-Pannel UVA-340 tubes for UVR. The samples were exposed to constant irradiances of 164.1, 42.8 and 0.7 W m<sup>-2</sup> for PAR, UV-A and UV-B, respectively. The spectral output of the lamps was checked using a

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