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## Physio-ecological responses of Patagonian coastal marine phytoplankton in a scenario of global change: Role of acidification, nutrients and solar UVR



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#### ABSTRACT

The aim of our study was to experimentally determine the future combined effects of solar UVR (280–400 nm), nutrient enrichment and acidification on a natural phytoplankton community (late phase of the bloom) of the Chubut River estuary (Patagonia, Argentina). We exposed the community to two radiation conditions (i.e., with and without UVR) under a future scenario of global change as compared to present conditions, using a cluster environmental approach. We combined short- (hours) and mid-term (days) incubations under solar radiation of the two clusters (present vs. future) to focus on changes in phytoplankton photosynthesis, growth and biodiversity. Our results indicate that the future conditions of increased nutrient availability and acidification, together with solar radiation, would shape the coastal phytoplankton community of Patagonia. The observed change for future conditions was towards a community dominated by relatively large diatoms, with high growth rates, little or no UVR-inhibition of photosynthesis, and better light-utilization efficiency. Given the speciesspecificity in responses, our results should not be generalized, especially considering that open waters of Patagonia are characterized by recurrent blooms of coccolithophorids that could be severely affected by acidification process. This future global change scenario in coastal waters, however, will produce significant increases in primary production, with phytoplankton communities that may sequester significant carbon amounts and thus they might sustain important secondary production (including fisheries) if their nutritional quality is not compromised.

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#### 1. Introduction

Global change, due to anthropogenically-derived activities, involves rapid alterations in several variables, such as temperature, pH, radiation and nutrient loads (IPCC, 2013). The results of such changes have been translated into warming, acidification and eutrophication of water bodies causing, for example, changes in phenology and biodiversity patterns, effects in the metabolism of aquatic organisms, reduction of water quality and modification of biogeochemical cycles (Wrona et al., 2006; Winder and Sommer, 2012; Häder et al., 2014). All aquatic ecosystems show variable sensitivity to global change and even estuaries, with organisms already adapted to large variability in physical, chemical and biological properties (Wilson, 2008; Lancelot and Muylaert, 2011) are vulnerable due to variations in sea level, riverine inputs, acidification and modifications in climate (Scavia et al., 2002). Therefore, there has

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been an increasing interest in evaluating the effects and impacts of global change in estuaries (Bricker et al., 2008; Bianchi and Allison, 2009; Gillanders et al., 2011), with particular emphasis on phytoplankton, as they are responsible for a high share of primary production (Cloern et al., 2014).

The evaluation of the responses and impacts of global change variables is rather complex, as they usually interact in a synergistic or antagonistic manner, thus enhancing or reducing individual effects (Folt et al., 1999). For example, exposure to ultraviolet radiation (UVR, 280–400 nm) generally causes deleterious effects on phytoplankton metabolism (Villafañe et al., 2003). However, antagonistic effects of UVR and increased temperatures on photosynthesis and growth have been observed (Sobrino and Neale, 2007; Halac et al., 2010), although synergistic effects have been determined as well (Lesser, 1996; Halac et al., 2013). The combined impact of UVR exposure and higher input of nutrients generally improves photosynthetic performance of phytoplankton (Bergmann et al., 2002; Cabrerizo et al., 2014) but in some cases high amounts of phosphorus unmask negative UVR effects (Carrillo et al., 2008). Also acidification in the presence of UVR enhanced phytoplankton growth and production (Domingues et al., 2014) due to

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the downregulation of carbon concentrating mechanisms (Giordano et al., 2005); however, it may also reduce metabolic processes of calcifying organisms (Riebesell, 2004; Gao et al., 2012). Thus, it is obvious that there are no generalized responses, as they depend not only on the species, but also on the type of interaction involved among the factors considered under study.

In this study we aim to experimentally determine the combined effects of UVR exposure, nutrient addition and increased acidification on a natural phytoplankton community of the Chubut River estuary (Patagonia, Argentina). In particular, our study focuses on changes in metabolism and biodiversity of phytoplankton, that in turn may have important consequences for an area that sustains high secondary production (Bogazzi et al., 2005; Skewgar et al., 2007). To achieve this objective, we exposed phytoplankton to solar radiation (with and without UVR) to both, present conditions of nutrients and pH, and a future scenario of global change, in which higher nutrient inputs (due to increased land use and agricultural activities, higher dust depositions, etc.) as well as increased acidification (due to the release of high amounts of CO<sub>2</sub> in the atmosphere) are expected (IPCC, 2013). Even though factorial analysis are generally used to study the combined impacts of different variables (e.g., Feng et al., 2008; Coello-Camba et al., 2014; Sobrino et al., 2014), they are extremely difficult to perform when multiple variables are considered; moreover, they are sometimes, logistically impractical. Therefore, and to carry out the comparison we used a cluster (Boyd et al., 2010; Xu et al., 2014) instead of a factorial approach, in which present conditions of the global change variables considered were grouped and compared with future conditions of the same variables, which were based on the projections made by the Intergovernmental Panel on Climate Change (IPCC, 2013).

#### 2. Materials and methods

#### 2.1. Study area and sampling

The Chubut River estuary is characterized by a wide range of physical, chemical, and biological variables due to the interaction between the river and the sea (Helbling et al., 2010); particularly, historical nutrient concentrations are in the range of 0.20–21  $\mu\text{M}$ , 0.19–6.4  $\mu\text{M}$ , and 1.7–236  $\mu\text{M}$  for nitrate + nitrite, phosphate and silicate, respectively (Helbling et al., 2010). The study area has been the focus of several investigations, and the annual succession of phytoplankton has been previously described (Villafañe et al., 2004), with three periods clearly distinguished: pre-bloom, bloom and post-bloom, in which the winter bloom is dominated by microplankton diatom species, whereas during

the rest of the year nanoplanktonic flagellates are the dominant group. Also, natural phytoplankton communities from the Chubut River estuary have been studied in a context of global change i.e., with investigations aiming to determine the effects of solar UVR on photosynthesis (Villafañe et al., 2004, 2008), as well as in combination with other factors such as vertical mixing (Barbieri et al., 2002) and increased temperature (Villafañe et al., 2013; Helbling et al., 2011).

Seawater used in the experiments was collected during high tide on the 14th of September, 2014 at the mouth of the Chubut River estuary (Egi station, 43° 18.8′S, 65° 02.0′W — Fig. 1) with an acid-washed (1 N HCl) bucket. The water was pre-screened through a 200  $\mu m$  Nitex mesh to eliminate large zooplankton, placed in 25-L opaque acid-washed containers, and transported to the Estación de Fotobiología Playa Unión (EFPU, 10 min away from the sampling site) where experiments were performed as described below.

#### 2.2. Experimental set-up

The responses of phytoplankton collected during the late phase of the bloom were tested by comparing two conditions, using environmental clusters with all factors (Morris, 1991; Quinn and Keough, 2002; Boyd et al., 2010): a) Present, in which nutrient concentration as well as pH remained as in the ambient conditions at the moment of sampling (NO<sub>3</sub>: 5.6  $\mu$ M, PO<sub>4</sub><sup>3</sup>: 3.6  $\mu$ M and SiO<sub>3</sub><sup>2</sup>: 1.7  $\mu$ M, pH 8.2) and, b) Future, in which nutrients were increased to reach concentrations of  $NO_3^-$ : 58  $\mu$ M,  $PO_4^{3-}$ : 20  $\mu$ M and  $SiO_3^{2-}$ : 23  $\mu$ M, and additions of  $CO_3^{2-}$  (as  $Na_2CO_3$ ),  $HCO_3^{-}$  (as  $NaHCO_3$ ) and HCI (0.01 N) were used to increase the pCO<sub>2</sub> and dissolved inorganic carbon (DIC), to the levels expected for the year 2100, reaching a pH value of 7.6 (Gattuso et al., 2010). The experimental set-up for testing these two conditions was as follows: On the day of collection (during the evening) the seawater was dispensed into 12 UVR-transparent containers (10-L capacity; LDPE Cubitainers, Nalgene, Fig. 2), with 6 of them set with the present and the other 6 with the future conditions. Two radiation treatments were implemented, with half of the containers from each condition receiving the full solar spectrum (PAB treatment, >280 nm; uncovered containers) and the other half receiving only PAR (Photosynthetically Active Radiation, >400 nm; containers covered with Ultraphan 395 nm filter, Fig. 2); these two radiation treatments thus represented different pre-acclimation conditions.

The containers were put in a water bath with running water for in situ temperature control (9  $\pm$  1 °C, controlled every hour using handheld digital thermometers) and exposed to solar radiation for five days. The containers were routinely shaken manually so that

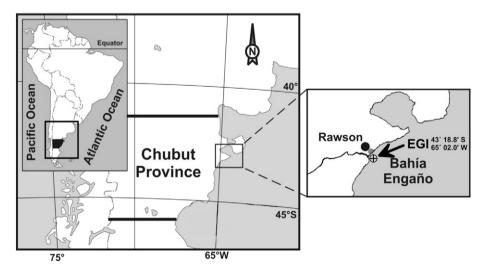


Fig. 1. Map of the study area indicating the sampling site at Egi station at the mouth of the Chubut River estuary (43° 18.8'S, 65° 02.0'W), Chubut Province, Argentina.

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