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Effects of salinity changes on coastal Antarctic phytoplankton physiology and assemblage composition

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ABSTRACT

A natural marine phytoplankton assemblage from a coastal environment of Antarctica was experimentally exposed to low salinity sea water (30 vs 34 in the control) during 8 days in order to study their physiological and community responses to hypoosmotic stress conditions. Hypoosmotic conditions favour water influx into the cells, which results in increased turgor pressure and increased oxidative stress. This stress is linked to a number of other cellular toxic processes, including damages to proteins, enzyme inactivation and DNA breakage. Inhibition of the instantaneous growth rate started after 48 h exposure to low salinity, but at the end of experiment, growth was significantly higher in the low than in the normal (control) salinity treatment. Hypoosmotic conditions prevented phytoplankton biomass accumulation, as evidenced by reduced Chlorophyll-a concentrations as compared to the control treatment. However, in terms of cell numbers and species composition, we observed a gradual replacement of big centric by small pennate diatoms, which became dominant by the end of the experiment. In addition, the content of reactive oxygen species (ROS) and 2-thiobarbituric acid-reactive substances (TBARS), which are indicative of oxidative stress, were studied. In the low salinity treatments, ROS concentrations were significantly higher than control values on days 4 and 6, decreasing thereafter to nearly initial values. TBARS content increased during the first 48 h and then decreased until around day 0 values. This coincided with significant increased values of the antioxidants α-tocopherol and β-carotene in low salinity treatments over the control. These results suggest the existence of protection mechanisms against lipid peroxidation, and lead to the conclusion that the response to stress is species-specific, so that at the community level a change in the relative abundance of phytoplankton taxa appears as a response to hypoosmotic conditions. This could have important consequences for the trophic food web dynamics in areas influenced by high fresh water inputs.

1. Introduction

Phytoplankton are currently responsible for about 50% of global planetary primary production (Falkowski and Raven, 2007) and their change contributes as a substantial sink for CO_2 in marine ecosystems. Size structure is an important characteristic of phytoplankton communities, and relates to the magnitude of carbon fixed and exported into the deep sea (Armstrong et al., 2002; Schloss et al., 2007), as well as to the transfer of carbon to higher trophic levels (Waite et al., 1997). At the cellular level, phytoplankton size relates to physiological features such as growth, photosynthesis, respiration rates, light absorption (Finkel, 2001), as well as nutrient uptake and requirements (Hein et al., 1995).

Mean air temperatures along the Western Antarctic Peninsula have increased significantly (1–2 °C) over the past 50 years (Steig et al., 2009). This has long-term consequences on sea ice and ice shelf dynamics (Smith and Stammerjohn, 2001), as well as on glacial melting (Dierssen et al., 2002). Melting ice masses contribute to the globally rising sea level (Vaughan, 2006), promoting water column stratification, especially in shallow coastal environments, where freshwater is likely impacting on the structure and function of coastal food webs (Dierssen et al., 2002). In particular, Moline et al. (2004) have demonstrated an association between decreased salinities attributed to warmer environmental conditions and the dominance of cryptophytes rather than diatoms during austral summer. However, under low field surface salinity conditions in the Western Antarctic Peninsula Piquet et al. (2011) found changes to diatoms dominating the assemblages as well as in bacteria. Much of our current understanding on the effects of low salinity on marine phytoplankton is based on cultured algae under

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laboratory conditions (Fujii et al., 1999; Thessen et al., 2005; Petrou et al., 2011) and on limited observations from field studies (Vernet et al., 2011; Knox, 2007). To our knowledge, no experimental results are available on the response of Antarctic natural marine phytoplankton assemblages to changes in salinity. The variation in phytoplankton composition along salinity gradients has generally been ascribed to the fact that most phytoplankton species are stenohaline (i.e., having very narrow salinity tolerance range) and suffer osmotic stress upon exposure to salinity changes (Bisson and Kirst, 1995; Lionard et al., 2005). Any drastic change in salinity that is strong enough, could change local phytoplankton assemblages and establish a new stable community (Chakraborty et al., 2011). Low salinity stress in algae as well as in other plants severely disturbs the cellular homeostasis brought about by differences between the internal and exogenous concentration of inorganic ions (predominantly Na^+ and Cl^-), causing water influx, i.e., an increase in cell volume and ion efflux (Guillard, 1962). Thus, salt stress has effects on a variety of metabolic pathways, ranging from photosynthesis (Allakhverdiev et al., 2002), membrane lipid biosynthesis (Sakamoto and Murata, 2002; Singh et al., 2002), to an increase in cells' respiratory activity to maintain osmotic balance (Qasim et al., 1972). Some of the effects are mediated by the liberation of reactive oxygen species (ROS), causing additional oxidative stress (Mittler, 2002). Due to the inherent instability and reactivity of most ROS and their very low steady-state levels, their analysis is a much more difficult task than the determination of concentration of antioxidants and activities of antioxidant enzymes (Jakubowski and Bartosz, 2000). Oxidative stress has been linked to a number of cellular toxic processes, including damages to proteins (Prasad et al., 1995), membrane lipid peroxidation, enzyme inactivation and DNA breakage (Halliwell and Gutteridge, 2007). The membrane-permeable non-fluorescent 2-7-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation has been used for detecting several ROS in biological media (McDowell et al., 2013). DCFH-DA was initially thought to be useful as a specific indicator for hydrogen peroxide. However, it was already demonstrated that DCFH is oxidized by other ROS, including superoxide anion radical, hydroxyl radical, peroxyl, alkoxyl, hydroperoxyl and peroxynitrite which are products of normal metabolism (Halliwell and Gutteridge, 2007). The increased DCFH-DA oxidation will be referred to as an index of oxidative stress. It has been shown that ROS are responsible for the degradation of chlorophyll a and the decrease in the activity of photosystem II (PSII) in phytoplankton photosynthetic antenna (Saison et al., 2010), and cause inhibition of diatom growth (Hernando et al., 2011). However, it was also described that ROS might act as secondary messengers in the activation of stress-response signal transduction pathways and defence mechanisms (Mittler, 2002).

Diatoms present high activities of antioxidant enzymes, such as catalase, glutathione peroxidase and glutathione reductase and of non-enzymatic antioxidants such as the lipophilic alpha-tocopherol (αT), and beta-carotene (βC) allowing the cells to cope with potentially damaging conditions (Rijstenbil, 2001; Hernando et al., 2011).

Within this context, the main goal of the present study was to experimentally characterize the responses of a natural phytoplankton assemblage from a coastal Antarctic environment that is increasingly subject to changes in salinity by seasonal and long-term influx of freshwater from glacier melting (Rückamp et al., 2011). To reach this goal, we studied the phytoplankton assemblage's biomass, composition, growth and physiology (photosynthesis, respiration and oxidative stress). We hypothesize that low salinity stress induces significant changes in these parameters.

2. Materials and methods

2.1. Experimental set-up

The experiments were performed at Potter Cove (King George Island/25 de mayo, South Shetland Islands, Antarctica, 62°14′S, 58°38′W) in the Carlini (former Jubany) station facility from February 25th (Day 0) to March 5th 2011 (Day 8) using a natural plankton assemblage (Fig. 1). Before the experiment (on February 24th), six 100 l polyethylene tanks (microcosms) were thoroughly cleaned with diluted HCl and rinsed with distilled water. Seawater was collected at ~5 m depth in the outer Potter Cove (Fig. 1). The water was filtered through a 300 μm Nitex net placed inside a water distributor to avoid the passage of mesozooplankton and larger organisms. No nutrients were added to the water. Each microcosm was covered with plastic sheets between 23 h and 7 h of the following day and during storms in order to avoid contamination by particles, snow or fresh water from rain. Temperature and salinity were measured every 8 h using a Horiba U-10 conductimeter (Kyoto, Japan). Temperature was kept around 1 ± 0.7 °C, during the whole experiment, similar to the average in situ water temperature at the moment of sampling. To attain this, the microcosms were placed within a larger water tank connected to a stainless steel sea-water pump (Lowara, Italy), pumping coastal water from 4 to 6 m depth (varying with the tides) and ~25 m from the coastline. The water in each microcosm was mixed manually in order to maintain homogeneity in the water column and preventing cells from settling. The effectiveness of such procedure was previously confirmed (Hernando, 2011). The experimental design included two different salinity treatments (three replicates per treatment): a control with natural ambient salinity (34; Normal Salinity Treatment, NST), and low salinity conditions (30; Low Salinity Treatment, LST). The low value was chosen according to the average measurements, taken before the experiment, from the upper 5 m of Potter Cove in an area close to the Fourcade glacier during high fresh water run-off. In order to lower the salinity in the microcosms, 10 l of distilled water was added to each LST replicate. The same volume of GF/F filtered sea water was added to each replicate in NST, in order to keep the same dilution factor than for LST.

200 ml of water samples from each microcosm was taken at the beginning of the experiment and at days 1, 2, 4, 6 and 8 at 9 a.m. and filtered upon GF/F filters as follows: determination of chlorophyll-a (Chl a) content, DCFH-DA (Molecular Probes Inc, Eugene, OR, USA) oxidation rate, 2-thiobarbituric acid reactive substances (TBARS) content, αT and βC content assays. In addition, 150 ml were taken for cell counts. All these samples, except those taken for assessing DCFH-DA oxidation rate, which were analyzed few minutes after sampling, were stored during two months at −80 °C until analyses.

2.2. Chl a analyses and cell counts

Chl a filtrates were extracted in 7 ml absolute methanol (Holm-Hansen and Riemann, 1978). Fluorescence readings of the extracts (24 h later) were used for the calculation of Chl a concentration, after correction for phaeopigments (Holm-Hansen et al., 1965) and calibration with standard Chl a with a Shimadzu RF-1501 spectrofluorometer. Subsamples for the identification and enumeration of phytoplankton were fixed with an acidic lugol solution and kept it in cold, dark conditions until their analysis. In addition, qualitative phytoplankton samples were taken at the beginning and at the end of the experiment, concentrating the cells using a 20 μm mesh net. For quantitative estimations, cells were enumerated with a phase contrast Leica DMIL LED inverted microscope according to the procedures described by Utermöhl (1958). Subsamples were settled in a composite sedimentation chamber. At least 100 cells of the dominant taxa were counted in one or more strips of the chamber or random fields at 250 or 400×, depending on their concentration and size. The whole chamber bottom was also scanned at 100× to count large and sparse species. Cell biovolumes were calculated by approximation to the nearest geometric shapes proposed by Hillebrand et al. (1999). Cell carbon content was estimated with two different carbon-to-volume ratios, one for diatoms and one for all the other algae groups (Menden-Deuer and Lessard, 2000). Qualitative samples were examined using phase-contrast microscopy under a Leica DM 2500 microscope. For diatom frustules observation in order