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Combined effects of temperature and salinity on fatty acid content and lipid damage in Antarctic phytoplankton

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ABSTRACT

We investigated the effects of ocean warming and glacial melting on phytoplankton assemblage composition and physiology in coastal Antarctica by exposing assemblages to a 4 °C increase in seawater temperature (T) and a 4 psu decrease in salinity (S) with respect to ambient values in a 6-day microcosm experiment. Seawater samples from Potter Cove in King George Island (Antarctica) were placed in outdoor microcosms and exposed to four treatments: ambient S-ambient T (S0T0, control), low S-ambient T (S-T0), ambient S-high T (S0T+), and low S-high T (S-T+). The relative abundance of unsaturated fatty acids (UFAs) 20:5ω3, 18:4ω3 and 16:1ω7 in relation to saturated FAs (14:0 and 16:0) significantly increased in all treatments at 24 h, compared to the control. At the same time, we detected a significant increase in the production of Thiobarbituric Acid Reactive Substances (TBARS), used as a proxy for lipid damage, in the S-T0 and the S0T+ treatments. In contrast, in S-T+, concentrations of TBARS remained significantly lower than in the control throughout the experiment. Although phytoplankton species composition did not change during the experiment, an increase in the relative abundance of diatoms (> 20 µm) was found in all treatments compared to the control at 24 h, with no further changes during the rest of the experiment. Furthermore, the relative abundance of small diatoms (10–20 µm) increased only in S0T+, and small prasinophytes decreased at S-T+ at the end of the incubation period.

Our results show a stable unsaturated to saturated FA ratio under the synergistic effects of high temperature and lower salinity, which may help protect phytoplankton cells from lipid damage. When phytoplankton assemblages were exposed to high temperature or low salinity, separately, the proportion of unsaturated FAs increased after 48 h. This increase in FAs resulted in greater lipid damage, which could be potentially avoided, as shown by previous studies, by antioxidant responses or changes in osmoregulatory proteins and FA synthesis by the activation or inactivation of desaturase enzymes. Variations in FA content due to changing environmental conditions can alter the quality of phytoplankton as a food source with potentially critical implications for the marine food web.

1. Introduction

The marine waters around the Western Antarctic Peninsula (WAP) have experienced the fastest rate of warming as well as the greatest sea ice loss on the planet (Vaughan et al., 2003). In view of the expected minimum global temperature rise of 1–4 °C over the next century (Turner et al., 2016), future trends in coastal Antarctic waters may include further widespread warming, sea ice retreat and runoff from glacial melting. Changes in seasonal sea ice dynamics and increased water column stratification from freshwater inputs will largely affect mixing regimes, nutrient supply, and light availability for Antarctic phytoplankton (Massom and Stammerjohn, 2010). Moreover, increasing winter convective storms (or mesocyclonic activity) in the atmosphere can result in intensified advection of moist warm air and

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more rain, which can lead to further melting of the ice cap (Falk and Sala, 2015) and increased inputs of glacial freshwater into coastal marine waters.

Meltwater input has already been shown to have an effect on Antarctic phytoplankton physiology and biomass. Hernando et al. (2015) showed that a few phytoplankton species from Potter Cove (King George Island or 25 de Mayo Island, Antarctica) exposed to hypotonic stress were able to grow at their natural rates while others showed decreased photosynthetic efficiency and inhibition of cell division. Although the composition of phytoplankton assemblage remained unchanged, low salinities affected the evenness of the phytoplankton assemblage and changed the relative contribution of diatoms (Hernando et al., 2015). Changes in composition and size of primary producers, especially diatoms, can ultimately affect higher trophic levels and thus the overall functioning of marine ecosystems. It is therefore essential to understand how the combined effects of warming and freshening of surface waters is affecting plankton composition and the size-structure of phytoplankton assemblages.

The energy flow in marine ecosystems can be quantified by the presence of trophic markers, such as fatty acids (FAs), lipid building blocks that are transferred from primary producers to consumers in the food web. In particular, FAs are important for the flow of energy between primary producers and primary consumers since polyunsaturated FAs (PUFAs, with two or more double bonds in the carbon chain) are almost exclusively synthesized by autotrophic organisms. However, a few PUFAs can be produced by animals also through elongation and desaturation of short-chain FAs (De Troch et al., 2012). PUFAs serve as precursors for important animal hormones and are also essential in animal's diets (Danielsdottir et al., 2007). In aquaculture studies, PUFAs were essential for maintaining high growth and reproduction rates and for the survival of marine organisms (Müller-Navarra et al., 2000). Moreover, FAs produced by microalgae are critical for the proper functioning of their own membranes, and provide the matrix for a wide variety of metabolites. As such, they play a crucial role in algal responses to environmental variability (Piepho et al., 2012). Under a sudden change in environmental conditions, de novo synthesis of unsaturated FAs cannot occur rapidly, but the desaturation of FAs may be adjusted by transferring specific acyl groups to other polar lipids and allowing rapid adaptive membrane reorganization (Makewicz et al., 1997; Khozin-Goldberg and Cohen, 2006). The properties of membranes are intimately related to the fluidity of the constituent lipids. In particular, salinity changes can induce elongation and desaturation of FAs chains to allow osmoregulation in microalgae (Azachi et al., 2002). Temperature stress can also induce changes in FAs in cell membranes. The most common regulatory strategy is a change in the lipid composition by increasing or decreasing the degree of FA unsaturation (homeoviscous adaptation, Hazel, 1995). Therefore, microalgae can survive in diverse and extreme conditions because of their ability to modify the type and quantity of their cellular lipids (Sato et al., 2000).

Oxidative stress by free oxygen radicals (ROS) have been linked to changes in FA content and composition. The capacity of algae for environmental adaptation can also be determined by their responses to oxidative stress. ROS are a variety of molecules (chemical species with one unpaired electron) physiologically generated from the metabolism of molecular oxygen (González et al., 2015). Oxidative stress has been linked to a number of toxic cellular processes, including damage to proteins (Prasad, 1995), membrane lipid peroxidation, enzyme inactivation and DNA breakage (Halliwell and Gutteridge, 2007). A cell experiences oxidative damage when the production rate of ROS is higher than the rate of antioxidant activity that protects it (Halliwell, 2006).

The lipid peroxides produced by ROS can damage FAs, resulting in the formation of Thiobarbituric Acid Reactive Substances (TBARS), including malondialdehyde (MDA) (González et al., 2015). An increase in TBARS is therefore expected as ROS production increases. A decrease in PUFA content has been observed to coincide with increased levels of MDA under osmotic stress (Singh et al., 2002). These responses, which are temporarily associated with an increase in electrolyte leakage, suggest that osmotic stress induces cellular membrane damage via lipid peroxidation (Aziz and Larher, 1998). Cellular membranes, made up of large amounts of PUFAs, are highly susceptible to attack by ROS and consequently experience changes in membrane fluidity, permeability, and cellular metabolic functions (Bandyopadhyay et al., 1999; Schuhmann et al., 2011).

The objective of this study was to determine the effects of ocean warming and glacial melting on the composition of phytoplankton assemblages and phytoplankton physiology in coastal Antarctica. We exposed coastal seawater samples to a 4 °C increase in temperature (T) and a 4 psu decrease in salinity (S) with respect to ambient values in a 6-day microcosm experiment. We evaluated both the combined and separate effect of T and S on phytoplankton responses. Our ultimate goal was to test the hypothesis that an increase in seawater T and a decrease in S result in the production of ROS in phytoplankton assemblage, which in turn affect the phytoplankton FA composition.

2. Materials and methods

2.1. Sampling and experimental design

Microcosm experiments were conducted at the Carlini Station located on the shores of Potter Cove in King George Island, South Shetland Archipelago, Antarctica (62°14′S, 58°38′ W) between January 16th and 22th of 2014. The experimental set-up consisted of twelve 100-L plastic tanks (or microcosms) that were pre-cleaned with ~10% HCl and thoroughly rinsed with distilled water. Seawater was collected just outside the entrance to Potter Cove (Fig. 1) from 5 m depth with Niskin bottles, and was pre-filtered through a 300 μm Nitex screen to exclude mesozooplankton. The mesh pore size was selected according to a preliminary analysis of the composition of the zooplankton assemblage and to avoid removing long chains of diatoms. All treatments and controls were incubated for 6 days exposed to natural sunlight and without the addition of nutrients.

The experimental design consisted of four combinations of salinity and temperature (treatments), with three replicates per treatment: (1) ambient salinity (34 psu) and ambient temperature (1–2 °C) (S0T0), (2) low salinity (30 psu) and ambient temperature (S-T0), (3) ambient salinity and high temperature (5–6 °C) (S0T+), and (4) low salinity and high temperature (S-T+). For the low salinity treatments, the target salinity of 30 psu was obtained by adding 10 L of distilled water to the S- microcosms. Similarly, 10 L of seawater (filtered through a 0.7 μm

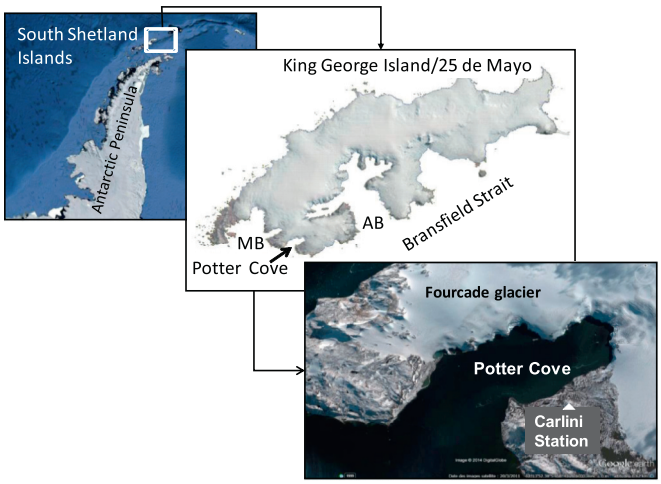

Fig. 1. Location of Potter Cove (62°14′S, 58°38′W) in King George (or 25 de Mayo) Island on the South Shetland Archipelago of the Antarctic Peninsula. AB: Admiralty Bay; MB: Maxwell Bay.