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Photochemical responses of three marine phytoplankton species exposed to ultraviolet radiation and increased temperature: Role of photoprotective mechanisms

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ABSTRACT

We carried out experiments using long-term (5–7 days) exposure of marine phytoplankton species to solar radiation, in order to assess the joint effects of ultraviolet radiation (UVR) and temperature on the photochemical responses and photoprotective mechanisms. In the experiments, carried out at Atlantic coast of Patagonia (43°18.7′S; 65°2.5′W) in spring-summer 2011, we used three species as model organisms: the dinoflagellate *Prorocentrum micans*, the chlorophyte *Dunaliella salina* and the haptophyte *Isochrysis galbana*. They were exposed under: (1) two radiation quality treatments (by using different filters): P (PAR, >400 nm) and PAB (PAR + UV-A + UV-B, >280 nm); (2) two radiation intensities (100% and 50%) and (3) two experimental temperatures: 18 °C and 23 °C during summer and 15 °C and 20 °C in spring experiments, simulating a 5 °C increase under a scenario of climate change. In addition, short-term (4 h) artificial radiation exposure experiments were implemented to study vertical migration of cells pre- and non-acclimated to solar radiation. We observed species-specific responses: *P. micans* displayed a better photochemical performance and a lower inhibition induced by UVR than *D. salina* and *I. galbana*. In accordance, *P. micans* was the only species that showed a synthesis of UV-absorbing compounds (UVACs) during the experiment. On the other hand, non-photochemical quenching (NPQ) was activated in *D. salina* at noon throughout the exposure, while *I. galbana* did not show a regular NPQ pattern. This mechanism was almost absent in *P. micans*. Regarding vertical migration, *I. galbana* showed the most pronounced displacement to deepest layers since the first two hours of exposure in pre- and non-acclimated cells, while only non-acclimated *D. salina* cells moved to depth at the end of the experiment. Finally, temperature partially counteracted solar radiation inhibition in *D. salina* and *I. galbana*, whereas no effect was observed upon *P. micans*. In particular, significant UVR and temperature interactive effects were found in *I. galbana*, the most UVR sensitive species. The joint effects on UVR and temperature, and the species-specific photoprotective responses will affect the trophodynamics and production of aquatic ecosystems in a way that is difficult to predict; however the specificity of the responses suggests that not all phytoplankton would be equally benefited by temperature increases therefore affecting the balance and interaction among species in the water column.

1. Introduction

High intensities of solar radiation, which include ultraviolet (UVR, 280–400 nm) and visible (PAR, 400–700 nm) components may negatively affect some species in the surface layers of the water column. The UVR impact on phytoplankton cells comprises short- and long-term effects such as the decrease of photosynthesis and growth rates, and damage to different cellular targets, such as to the DNA molecule, among others [1,2]. However, some phytoplankton species have evolved photoprotective mechanisms that can be selectively activated, depending on the exposure length and/or species-specific features, thus alleviating the impact of UVR in physiological processes such as photosynthesis. For motile species, one of these short-term mechanisms is the downward migration in the water column [3]. Radiation can trigger movement in flagellated species [4,5]. Avoidance of excessive radiation can result from movement in response to high irradiance [6,7] or also by a circadian response to daily light patterns [8]. In this

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way, flagellate cells maintain an appropriate level of light, and limit the loss of cells in the photic zone, if the water is not too turbulent [9]. However, under pronounced stratification, microorganisms are somewhat physically forced to stay close to the water surface, exposing cells to high radiation.

Another short-term mechanism includes the enzymatic conversion of xanthophylls which helps cells to cope with UVR and high levels of PAR [10,11]. The de-epoxidation of diadinoxanthin, violaxanthin and antheraxanthin reduces any potential damage to PSII by enhancing the energy dissipation of excess light, measured as non-photochemical quenching, NPQ [12]. Over long-term (days) periods of exposure to high radiation levels, more permanent, physiological changes can occur, which are considered part of an acclimation process. One of such long-term mechanisms includes the synthesis of photoprotective compounds such as carotenoids, which act as antioxidants, and UV-absorbing compounds, UVACs – mainly mycosporine like amino acids, MAAs. UVACs exhibit absorption peaks within 310–360 nm [13] and are broadly distributed in tropical, temperate and polar environments, although not all the phytoplankton species synthesize them, e.g., they are less common in chlorophytes [14]. The acclimation ability under UVR is species-specific: Helbling et al. [15] found less photosynthetic inhibition and higher MAAs concentration in centric diatoms as compared to pennate species, whereas Hannach and Sigleo [16] demonstrated that MAAs synthesis was higher in dinoflagellates and haptophytes than in diatoms, chlorophytes and prasinophytes species.

This entire suit of individual and biochemical responses is in turn affected by temperature. For example, the dinoflagellates *Lingulodinium polyedrum* and *Ceratium furca* showed differences in swimming speeds at different temperatures: Between 22 and 26 °C, both species migrated at a rate of 0.7–1.0 m h^{-1}, while temperatures below *ca*. 20 °C caused a marked decrease in swimming speed [17]. Moreover, *Gyrodinium dorsum* cells showed significant higher velocity at 32 °C than at 11 °C [18]. The synthesis of protective compounds is also affected by temperature, as it regulates the enzymatic cell machinery. In this regard, Halac et al. [19] demonstrated that the diatoms *Chaetoceros gracilis* and *Thalassiosira weissflogii*, at 23 °C and during short-term exposures to solar radiation showed lower photoinhibition as compared to samples exposed at 18 °C, mainly due to heat dissipation processes (NPQ) mediated by xantophyll pigments, which was more efficient at high temperatures.

So far, long-term studies about the interactive effects of UVR and temperature on phytoplankton photosynthesis and the associated photoprotective mechanisms are rather scarce. Sobrino and Neale [20] demonstrated that photosynthesis in phytoplankton exposed to UVR is highly dependent on temperature. Higher temperatures decreased the sensitivity to UVR due to the temperature dependence of repair mechanisms. However, Lionard et al. [21] did not find any significant effect of temperature or UV-B (280–315 nm) or their interaction, neither on photosynthetic performance nor in diadinoxanthin-based xanthophyll cycle pool size, likely associated to the presence of diatoms, the dominant algal group in the studied communities. This variability in the interactive effects of UVR and temperature on phytoplankton may be partly explained by other factors, such as the optimal temperature range for UVR sensitivity and the capacity of acclimation in each species under different temperatures.

In a context of climate change, it is essential to know the extent of these combined effects of different variables as well as the mechanisms that phytoplankton cells use to cope with their potential impact. Thus the aim of this study was to evaluate the long-term (days) photochemical responses to UVR of three phytoplankton species characteristic of Patagonian waters in terms of (a) effective photochemical efficiency, and (b) three key photoprotective mechanisms – dissipation of excess energy, synthesis of photoprotective compounds, i.e., carotenoids/UVACs, and vertical migration – that might help to mitigate the negative effects of UVR on the photosynthetic process. As temperature increase could interact antagonistically with UVR levels to reduce its negative effects, we also asked whether a potential increase in temperature, such as it may occur in a context of climate change [22], would influence the studied responses. Moreover, we also evaluated the effects of an attenuated irradiance condition such as occurring when cells are in deeper layers in the water column e.g. when the water column was mixed by wind. Because of the utmost importance of Patagonia within a photobiology context, i.e., the region normally receives high levels of UVR and it is periodically under the influence of ozone depletion events [23], this kind of studies are essential to assess the potential responses of local phytoplankton species under a scenario of climate change.

2. Materials and methods

2.1. Culture collection/study site

Prorocentrum micans Ehrenberg (Dinophyceae), *Dunaliella salina* (Dunal) Teodoresco (Chlorophyceae) and *Isochrysis galbana* Parke (Prymnesiophyceae) from the Microalgae Culture Collection at Estación de Fotobiología Playa Unión (EFPU, Argentina) were grown in 1 l Erlenmeyer flasks in f/2 medium [24] with a photoperiod 12L:12D in a chamber (Sanyo model ML 350). Cells were pre-acclimated during two weeks prior to experimentation at the local oceanic mean surface temperature corresponding to the experimental season: 18 °C, experiments with *P. micans* during the period 9–15 February 2011, or 15 °C, experiments with *D. salina* and *I. galbana* during the periods 12–16 and 21–25 November 2011, respectively. During this pre-acclimation period, the cultures received constant PAR – 235 μmol photons m^{-2} s^{-1}, equivalent to the saturation light value (I_k) for coastal Patagonian areas [25]. Nevertheless, in the water column, cells would experience irradiances above and below that level, depending not only on the position of the cells in the water column but also on other factors, such as the depth of the upper mixed layer and the attenuation coefficient in the water column, among others. Light was provided by cool white fluorescent lamps (Philips daylight) and photon flux densities were measured with a spherical micro quantum sensor (Walz GmbH, model US-SQS/WB). Cells were harvested during the exponential growth phase and used in the experiments as described below. Experiments to determine the long-term responses of these three phytoplankton species to solar radiation were performed at EFPU located in the Patagonian coast of Argentina (43°18.7′S; 65°2.5′W).

2.2. Experimentation/sampling protocol

Two types of experiments were carried out to evaluate: (a) Long-term effects on photosynthesis and photoprotective compounds when cells were exposed to solar radiation conditions, and (b) Short-term effects on vertical migration of both, pre-acclimated cells to solar radiation and no-acclimated cells, when exposed to an artificial radiation source.

2.2.1. Solar radiation exposure experiments

Prior to experimentation cells in exponential growth were diluted (6:1) with f/2 medium [24]; this reduced the cell concentration and avoided self-shading effects. Samples (duplicates for each treatment) were incubated under solar radiation during 7 (*P. micans*) or 5 days (*D. salina* and *I. galbana*) in quartz tubes (300 ml) under the following treatments: 1) Radiation quality