Effects of ultraviolet radiation on growth, cell death and the standing stock of Antarctic phytoplankton

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ABSTRACT: We performed a series of experiments with Antarctic natural phytoplankton communities exposed to natural levels of solar radiation in order to quantify the effect of ambient ultraviolet radiation (UVR) on phytoplankton growth, cell death and their balance. Treatments in which UVR was excluded showed a high increase in biomass, dominated by diatoms, with chl a (chlorophyll a) reaching values as high as 22 µg l⁻¹, 9 times larger than initial values. In contrast, chl a values remained low at the end of the experiments under treatments with full solar radiation. Phytoplankton growth rates were also inhibited by UVR, increasing up to 5 times in UVR-excluded treatments. The percentage of dead cells within Antarctic phytoplankton communities decreased in treatments with UVR blocked. The Antarctic phytoplankton populations studied appeared to be strongly controlled by UV at surface irradiances with biomasses inhibited by up to 80–90%. This suggests that increased UVR levels over Antarctica may reduce phytoplankton growth rates and cause cell death, thus reducing the phytoplankton stock. These effects may have important consequences for the food web in Antarctic waters.

KEYWORDS: UVR · Phytoplankton · Antarctica · Growth · Cell death · Standing stock

INTRODUCTION

Stratospheric ozone levels, essential to protect organisms from excess ultraviolet radiation, significantly declined due to emissions of anthropogenic chlorinated compounds until the mid-1990s (Staehelin et al. 2001), with the largest rates of depletion near the poles, particularly over Antarctica during the austral spring (Solomon 1999). The loss of ozone over Antarctica has resulted in an important increase in ultraviolet B radiation (UVBR) levels with negative impacts on Antarctic marine ecosystems (Smith et al. 1992, Prézelin et al. 1994, Buma et al. 2001). In spite of the efforts made to diminish the loss of stratospheric ozone (i.e. reduction of CFCs and other adverse emissions since the Montreal Protocol), pre-1980 levels of ozone have not been recovered as yet, and the future trajectory toward this recovery involves considerable uncertainties (Weatherhead & Andersen 2006). Thus, significantly enhanced UVBR reaches Antarctic surface waters, particularly during ozone hole episodes, and will likely continue to do so for several decades (Weatherhead & Andersen 2006).

The increased UVBR over Antarctic waters has been reported to suppress oceanic productivity by 6 to 12% (Smith et al. 1992). Evidence of stress induced by UVR in Antarctic photosynthetic plankton has been reported on the basis of various indicators, such as the inhibition of photosynthesis (Smith et al. 1992, Helbling et al. 1994, Prézelin et al. 1994), the formation of cyclobutane pyrimidine dimmers in DNA (CPDs, Prézelin et al. 1998, Buma et al. 2001) and the synthe-
sis of mycosporine-like amino acids (MAA’s, Carreto et al. 1990, Karentz et al. 1991, Villafañe et al. 1995). The growth and survival of phytoplankton cells may be also strongly affected by enhanced UVBR levels in Antarctica (Davidson et al. 1994), affecting the cell density and biomass of Antarctic populations. Although sufficient irradiance is essential for phytoplankton to grow, mesocosm experiments conducted with natural coastal Antarctic plankton (Agustí et al. 2009) showed that Antarctic phytoplankton communities are either light limited or light stressed when exposed to high irradiances, suggesting the role of UVR as a potential inhibitor of phytoplankton growth and biomass (Agustí et al. 2009). There have been only few studies examining the impact of increased UVBR levels on the growth of Antarctic phytoplankton populations in situ, with most studies performed with isolated Antarctic phytoplankton growing in cultures and exposed to artificial light (Davidson et al. 1994, Davidson & Belbin 2002, Nunez et al. 2006). Moreover, the effect of UVR on phytoplankton cell mortality has not yet been tested. As a consequence, the effect of in situ UVR on the balance between growth and death within natural phytoplankton populations has not been examined.

The main goal of this study was to investigate the effect of UVR on the demographic balance between population growth and cell death within natural Antarctic phytoplankton populations. In addition, we examined differences in the responses observed among phytoplankton groups in an effort to assess how UVR could affect the dominance of different groups, thereby shaping phytoplankton community structure in Antarctic waters.

**MATERIALS AND METHODS**

**Experimental design.** Five UVR experiments were carried out in Antarctic waters. Three experiments (Expts 1, 2 and 3) were carried out in the Spanish Antarctic Base Juan Carlos I (Livingston Island, South Shetlands Islands) during the ICEPOS-1 expedition in January–February 2004 (Table 1). Two more experiments (Expts 4 and 5) were conducted on board the RV ‘Hespérides’ during the ICEPOS-2 cruise in January–February 2005 (Table 1).

The experiments involved incubating surface seawater sampled at different locations in 2 l bottles submerged in 2000 l incubators, fed with running surface seawater to maintain temperature conditions, and exposed to either natural solar radiation or radiation filtered to exclude UVR. In 2004, the incubators were placed on the shore of South Bay (Livingston Island) with a pumping system to circulate surface water from the bay. In experiments performed during 2005, the incubators were placed on the deck of the vessel, in an area free of shade and with a circulating surface water (3 m depth) system. Duplicate 2 l quartz bottles, that allow all the radiation to pass through (UVR+PAR, photosynthetically active radiation), were used for the total solar radiation treatment and polycarbonate bottles (Nalgene product, Thermo Fisher Scientific), which filtered most UVR radiation, with 0.3 and 35% transmission at 379 and 399 nm respectively, were used for the treatment excluding UVR. Bottles were acid-cleaned and seawater samples were pre-filtered through a 150 µm net to exclude larger grazers in the experiments performed at the Antarctic Base (Expts 1 to 3).

**Table 1. Experimental parameters.** Nutrients (PO₄, SiO₄ and NO₂/NO₃ [µmol l⁻¹]), temperature and salinity were measured at the water sampling location; –: not measured.
Surface water (0.5 m) was sampled using plastic carboys from an inflatable boat, for Expts 2 and 3, and using Niskin bottles (5 m) for Expts 1, 4 and 5. For Expt 1, surface seawater from Foster Port (Deception Island, South Shetland Islands) was used, and we sampled surface seawater at South Bay (Livingston Island) for Expts 2 and 3 (Table 1). Surface seawater for Expts 4 and 5 was sampled around the Antarctic Peninsula at 64° 44’ S, 65° 42’ W and 64° 03’ S, 55° 50’ W (Table 1).

At the time of the ICEPOS-1 experiments, solar radiation was automatically recorded by a meteorological station located in the Spanish Antarctic Base Juan Carlos I, at Livingston Island. The station was provided with a Kipp & Zonen CM11 pyranometer (Delft) for the measurement of global solar radiation with sensitivity in the 305 to 2800 nm, and a Kipp & Zonen CUV3 (Delft) for UVB+UVA (300 to 400 nm) measurements. All the sensors of the meteorological station integrated the radiation every half an hour from samplings taken every second. During experiments performed in ICEPOS-2, solar and UVR were automatically measured by a Weatherlink Vantage Pro. Davis meteorological station (Scientific Sales, Lawrenceville) located on board RV ‘Hespérides’. PAR was measured with the Solar radiation 6450 Davis sensor (from 400 to 1100 nm) every 5 min. In addition, integrated UVR (290 to 390 nm) values in all the wavelengths were obtained every 5 min with the UV 6490 sensor. UV data were integrated every 30 min to be comparable with the 2004 data from the meteorological station placed on Livingston Island.

UV and global radiation data were also transformed to daily doses (kJ m⁻² d⁻¹) and the daily average radiation was calculated from the values obtained every day during the experiments.

**Experimental sampling and analytical procedures.**

Every 2 d, at 10:00 h local time, duplicate samples were taken from each treatment to determine chl a and phytoplankton cell abundance. Variable water volumes (25 to 50 ml, depending on phytoplankton biomass) were filtered through Whatman GF/F filters for fluorometric analysis of chl a concentration (Parsons et al. 1984). Phytoplankton abundance was determined using an epifluorescent microscope. Samples for microscopic examination were preserved in glacial acetic acid (1% final concentration), filtered onto 0.6 µm Nuclepore filters and kept frozen (−80°C) until examination. Cells were counted at magnifications of 200, 400 and 1000× under a Zeiss epifluorescence microscope. The phytoplanktonic cells counted were differentiated into major taxonomic groups and grouped by cell size.

The proportion of dead cells in the phytoplanktonic communities was analyzed in Expts 2, 4 and 5 at the beginning and end of the experiments. A cell membrane permeability test (cell digestion assay, Agustí & Sánchez 2002) was used to identify and count living and dying cells within the communities. The cell digestion assay is based on the brief exposure of the cells to the enzymes trypsin and DNase I, which enter the cytoplasm of cells with damaged plasma membranes (i.e. necrotic or advanced apoptotic cells) resulting in the entire digestion of the cells, while having little or no effect on the viability, morphology or function of live cells (Agustí & Sánchez 2002, Darzynkiewicz et al. 1994). The digestion of dead cells is based on the fragmentation and hydrolysis of DNA by DNase I, and peptide hydrolysis by trypsin, which penetrates the damaged cells. The digested dead cells are undetectable by optical observation and lose any fluorescence signals, and are, therefore, effectively removed from the population (Agustí & Sánchez 2002, Darzynkiewicz et al. 1994). Live cells with intact membranes are not affected by the enzyme cocktail, so they remain in the sample to be counted after the assay.

Stock solutions of DNase I (400 µg ml⁻¹ in HBSS) and trypsin (1% in HBSS) were prepared in HBSS medium (Sigma) and kept frozen at −65°C until use. DNase I stock solution (5 to 10 ml) was added to duplicated experimental samples (25 to 50 ml) and incubated for 15 min at 25°C, as recommended for polar phytoplankton species (Llabrés & Agustí 2008). Then, 5 to 10 ml of trypsin solution were added, followed by incubation for an additional 30 min at 25°C. At the end of the incubation, samples were filtered onto 0.6 µm Nuclepore filters, washed several times with 0.2 µm filtered seawater (to eliminate the enzymes) (Agustí & Sánchez 2002) and fixed with glutaraldehyde (1% final concentration). Filters were kept frozen (−80°C for 3 mo) prior to counting under an epifluorescence microscopy.

Parallel to samples run with the cell digestion assay for the quantification of living cells, duplicate samples to quantify the abundance of the total population (i.e. living and dying cells) were sampled and filtered as described above, preserved with glutaraldehyde (1% final concentration), and kept frozen (−80°C for 3 mo) until examination under epifluorescent microscopy. To quantify the total population and the living/dead cells, both samples were counted at magnifications of 200, 400 and 1000× under a Zeiss epifluorescence microscope, classified into major taxonomic groups and grouped by cell size, as described above for regular counting.

Cells remaining after the enzymatic treatment, i.e. those having intact membranes, were considered to represent living or viable cells, whereas dead ones, with compromised membranes, were digested out by the enzymatic cocktail and were undetectable by the epifluorescence microscope. The fraction of dead phytoplankton cells in the sample was calculated by subtracting the abundance of living cells (after the en-
zyme treatment) from the counts of the untreated sample, and dividing the concentration of dead cells by the cell concentration in the untreated sample, which represent the total (dead plus living) cell concentration.

Net population growth rates were calculated for diatoms and flagellates from the cell abundances obtained at the beginning and end of the experiments as it was the best approach to express differences between treatments. Inhibition percentages of diatom populations due to UVR were calculated as the difference between growth rates obtained in the PAR and UVR+PAR treatments. Also inhibition percentages were calculated for the whole phytoplankton community as the differences between PAR and UVR+PAR treatments in terms of chl a biomass obtained at the end of the experiments. Statistical differences between treatments were determined by Student’s t-test.

RESULTS

The experimental conditions differed greatly among the ICEPOS-1 and ICEPOS-2 experimental periods. The incident UVR was much greater during ICEPOS-1 (Expts 1 to 3) than that received during the ICEPOS-2 cruise (Expts 4 and 5, Fig. 1). UVR was high throughout most of the ICEPOS-1 period, with maximum instantaneous UVR around 70 kJ m$^{-2}$ (Fig. 1), compared to maximum values of 40 kJ m$^{-2}$ and values regularly 30 kJ m$^{-2}$ during ICEPOS-2 (Fig. 1). Accordingly, daily UVR doses were, on average, 2 to 3 times higher in experiments carried out during ICEPOS-1 (Expts 1, 2 and 3, Table 1) than in those conducted during ICEPOS-2 (Expts 4 and 5, Table 1).

The time series of phytoplankton abundance, as chl a concentration, during the experiments clearly showed a suppression in response to UVR exposure (Fig. 2). These responses were strongest for Expts 2 and 3, where clear response of phytoplankton abundance to UVR exclusion was observed. Chl a reached high values of 15 and 23 µg l$^{-1}$ after 6 and 8 d, respectively, representing an increase of 5 and 9 times, respectively, over the treatments receiving total solar radiation. Phytoplankton abundance was clearly suppressed when exposed to UVR+PAR irradiance (Fig. 2), showing only a small increase with values staying below 5 µg l$^{-1}$ (Fig. 2). The increase in phytoplankton abundance when UVR was excluded was much more moderate in experiments performed during the ICEPOS-2 cruise (Expts 4 and 5, Fig. 2), consistent with the lower UVR during these experimental periods, with a maximum chl a concentration of 8 µg l$^{-1}$ achieved in Expt 4 (Fig. 2); this represents an increase of 1.7 times over the treatments receiving total solar radiation. Smaller changes were observed during Expt 5 (Fig. 2).

Phytoplankton communities were dominated by diatoms, represented by the genera *Thalassiosira*, *Eucampia*, *Chaetoceros*, *Fragilaria* and *Pseudo-nitzschia*. Other phytoplankton groups, mainly flagellated populations, were also present and were mostly dominated by *Cryptophyceae* and other groups, such as *Phaeocystis* (free form). In all experiments, the responses observed in chl a concentration were consistent with those in phytoplankton cell concentration. Diatoms, which tended to dominate the biomass of phytoplankton communities in all experiments, increased their cell density when UVR was excluded between 9 to 21 times in Expts 1, 2, 3 and 4, with respect to the total radiation controls. However, in Expt 5 the cell...
density increased only 1.3 times when UVR was excluded relative to the total radiation control.

Phytoplankton net growth rates, calculated from changes in cell abundance, were strongly affected by UVR as indicated by the results of the different experiments in which the exclusion of UVR resulted in higher growth rates with respect to controls (Fig. 3). Differences between replicates were very small, which infers strong consistency to the results obtained. Growth rates of diatoms were inhibited by UVR in all the experiments (Fig. 3) showing high values of 0.8 and 0.5 d\(^{-1}\) in some of the experiments when UVR was removed (Fig. 3). In addition, in Expt 1 the growth rate reached by diatoms following UVR removal was moderate, but negative net growth rates were obtained in the total radiation control indicating strong UVR inhibition (Fig. 3). Flagellate net growth rates were negative in Expts 1, 4 and 5. However in Expts 2 and 3, flagellate growth rates were positive and increased when UVR was excluded (Fig. 3).

The percentages of dead cells within the populations of diatoms and flagellates were calculated at the end of Expts 2, 4 and 5 (Fig. 4). In general, the percentages of dead cells were higher in the control for both diatoms
and flagellates (Fig. 4). The percentage of dead cells was significantly lower for both diatoms and flagellates when UVR was removed in Expts 2 and 5 (Student's t-test, p < 0.05). However, when excluding UVR in experiment 4 (Fig. 4), the percentage of dead flagellate cells was not significantly different between treatments (Student's t-test, p > 0.05, Fig. 4). These results suggested a general induction of cell death by UVR in diatoms, reflected in a larger pool of dead cells of 13.5, 22 and 5.2% for diatoms (for Expts 2, 5 and 4, respec-
Differences in the average daily UVR doses received during the experiments explained the differences in the response of phytoplankton biomass to UVR across experiments, as indicated by the positive and significant relationship between the percentage of inhibition and the UVR dose ($R^2 = 0.99$, 2nd order polynomial equation, Fig. 5A). Accordingly, the extent of inhibition of phytoplankton biomass increased with increasing UVR to represent more than 80% biomass reduction when mean daily doses exceeded 450 kJ m$^{-2}$ d$^{-1}$ (Fig. 5A). A similar pattern, with a positive increase in the percent inhibition with increasing mean daily UVR dose was observed for diatom abundance ($R^2 = 0.61$, Fig. 5B).

**DISCUSSION**

Our results clearly demonstrate that the high UVR levels over Antarctic waters strongly inhibit phytoplankton growth rates and enhance cell death, thereby greatly suppressing phytoplankton biomass. Even relatively modest UVR (200 kJ m$^{-2}$ d$^{-1}$) resulted in growth and biomass suppression, resulting in the widespread observation of impacts of UVR in phytoplankton biomass and growth across experiments, despite the 3-fold variability in average daily UVR doses among experimental periods. UVR undoubtedly inhibited phytoplankton biomass with chl $a$ values remaining low (below 4.5 µg l$^{-1}$) at the end of the experiments under treatments receiving full solar radiation. In contrast, UVR exclusion resulted in high chl $a$ values (as high as 22 µg l$^{-1}$) by the end of the experiments, revealing the strong effect of UVR in suppressing phytoplankton biomass in Antarctic waters. Similarly, when UVR was excluded, growth rates increased up to 5 times. Diatom growth rates reached rather high values when UVR was excluded (up to 0.8 d$^{-1}$), comparable to rates reported for experimentally-induced blooms (Agustí & Duarte 2000), despite the low temperature of Antarctic waters. This suggests that the low growth rates of Antarctic phytoplankton in situ (Mura & Agustí 1996) may result from suboptimal conditions, such as UVR inhibition. In fact, results of mesocosm experiments (Agustí et al. 2009) performed with coastal Antarctic plankton and manipulating the light conditions demonstrated that there is a narrow window of irradiance in which Antarctic phytoplankton growth is adequate, and that phytoplankton communities are either light limited or stressed by high irradiance at irradiances outside this range. Those experimental results (Agustí et al. 2009) suggest that UVR plays an important role in the inhibition of phytoplankton development in Antarctic waters, exerting a control upon phytoplankton biomass.

UVR has been reported to suppress growth rates of Antarctic phytoplankton in cultures (Karentz et al. 1991). The few available experiments carried out with natural phytoplankton populations grown under UVR ambient conditions also demonstrate growth inhibition (Davidson & Belbin 2002, Nunez et al. 2006).
Here we show that ambient UVR not only suppresses Antarctic phytoplankton growth but also induces important cell death. In most of the UVR experiments, diatoms and flagellates experienced cell death, with percentages of dead cells decreasing when UVR was filtered out. In fact, cell death caused by UVR has also been described in phytoplankton from other marine environments, including tropical and temperate areas (Llabrés & Agustí 2006, Agustí & Llabrés 2007), but the role of UVR in causing cell death in natural phytoplankton from Antarctic waters has not been examined before. Comparable parameters, such as cell survival, were analysed before in culture studies with diatoms isolated from Antarctica (Karentz et al. 1991, Davidson et al. 1994) and in natural communities, but these were restricted to the analysis of the decay in cell abundance (Davidson & Belbin 2002). Our estimates of cell death induced by UVR were, however, based on direct counts of living and dead cells allowing the detection of dead cells in growing phytoplankton populations.

Diatoms dominated the phytoplankton community in the experiments reported here. The data available on flagellates do not allow us to reach a clear conclusion as to their sensitivity to UVR relative to diatoms because in 3 out of 5 experiments flagellates did not respond, even when UVR was filtered out. However, in the experiments in which a flagellate response was observed (Expts 2 and 3) diatoms presented significantly higher percentages of inhibition to UVR than flagellates did (48 and 28%, respectively, p < 0.05, in Expt 2; 76 and 40%, respectively, p < 0.05, in Expt 3).

Our results are not consistent with previous studies showing diatoms apparently less vulnerable to UVBR as compared with other phytoplanktonic organisms (Karentz et al. 1991, Bischof et al. 1998, Buma et al. 2001) and attributing UVBR resistance to their large size (Karentz et al. 1991) and presence of photoprotective compounds (MAA) (Buma et al. 2001). In contrast, diatoms showed very little response in MAA induction under ultraviolet-B (280 to 320 nm) wavelengths (Riegger & Robinson 1997) compared to colonies of the flagellate Phaeocystis Antarctica, in which a maximal induction of MAA was reported (Riegger & Robinson 1997) for the same wavelengths. Other features affecting the sensitivity of diatoms to UVR could be the optical qualities, similar to quartz, of the siliceous cell walls (Karentz et al. 1991). Moreover, differences in the competition for nutrients between diatoms and flagellates, or possible effect of small grazers on flagellates, may also influence the responses observed between both phytoplankton groups.

UVR was known to suppress phytoplankton growth, but our results show that it also induces cell death, thereby suggesting that the overall impact on the net balance of the phytoplankton populations must be greater than was considered previously. Indeed, previous studies have revealed higher chl a and biomass values in treatments without UVR (Hernando et al. 2002, Hernando & Ferreyra 2005).

Environmental conditions were optimized in our incubation experiments, since mixing of the water column was eliminated, resulting in higher PAR levels. Shallow upper mixed layers and stability within the water column are conditions that allow cells to have enough light to saturate photosynthesis and to maintain turnover rates of repair mechanisms at PSII and to induce sufficient MAA synthesis (Bracher & Wiemcke 2000). The reduced mixing in the experiments should result in greater UVR doses than those received by Antarctic phytoplankton growing under strong mixing conditions of the water column. However, ice melting and increased air temperature in summer contribute to stabilization of the water column and reduce mixing as described for South Bay, where a pycnocline at about 4 m depth was reported (Agustí & Duarte 2000). Hence, the percent inhibition of phytoplankton biomass by ambient UVR reported in this study (approx. 80 to 90%) should be close to that of phytoplankton biomass in this Antarctic coastal location when UVR doses were high. Previous studies have calculated that primary production in Antarctic waters is UVR-inhibited by 6 to 12% (Smith et al. 1992) in the water column, but our results indicate that inhibition of phytoplankton biomass and losses due to cell death at the surface should be added to the reported negative effects of UVR on Antarctic phytoplankton. Indeed, solar UVBR during the spring ozone depletion has been reported to reduce biomass production of other primary producers such as Antarctic vascular plants, with an inhibition of more than 22% (Xiong & Day 2001).

The strong positive relationship between the percent inhibition of phytoplankton biomass and ambient UVR doses derived here provides further evidence of the importance of UVR effects on natural phytoplankton communities. UVR doses in Antarctica have increased about 30 to 50% due to stratospheric ozone depletion relative to pre-disturbance levels (Weatherhead & Andersen 2006, Agustí 2007). The steep UVR dose-biomass response relationship derived here suggests that an increase in UVR doses of 30 to 50% may result in an additional inhibition of phytoplankton biomass of up to 40% relative to pre-disturbance UVR doses. Because phytoplankton is the basis of the entire food web of Antarctica, a major suppression of phytoplankton biomass and growth must have clear impacts on the entire food web. The possible effect of phytoplankton suppression by UVR on the Antarctic food web has not, however, been assessed. The inferences drawn from the results presented here that Antarctic phytoplankton must have been strongly suppressed since the
onset of stratospheric ozone depletion in the early 1980s are consistent with reports of a major concurrent decline in krill (Atkinson et al. 2004), the main consumer of phytoplankton. This observation suggests that impacts of phytoplankton suppression due to enhanced UVR on the Antarctic food web should be explored.

CONCLUSIONS

The results of the present study indicate that phytoplankton populations from the Antarctic Peninsula show growth inhibition and enhanced cell death under ambient UVR doses. The decreasing growth and the increasing cell death influence the net balance of the phytoplankton populations that appear to be strongly controlled by UVR, with phytoplankton biomasses suppressed by up to 80–90% in the presence of ambient UVR doses. These results show that springtime ozone depletion over Antarctica results in elevated levels of UVBR reaching surface waters, which may reduce phytoplankton stocks with consequences for the associated Antarctic food web.

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