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Picophytoplankton cell death induced by UV radiation: Evidence for oceanic Atlantic communities

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Abstract

We analyzed the effect of ambient levels of visible and ultraviolet radiation (UVR) on picophytoplankton cell death by exposing natural communities of picophytoplankton (*Prochlorococcus, Synechococcus,* and picoeukaryotic cells) from the Atlantic Ocean to different levels of natural solar radiation, from that received just below the surface to 23% lower levels and dark conditions. Underwater oceanic levels of UVR and visible light can induce significant cell death in picophytoplankton communities. The decay rates of living cells induced by solar radiation was highest for *Prochlorococcus* sp., which showed an average decay rate of -0.24 ± 0.053 h^{-1} (mean $\pm$ SE) in the experiments, whereas *Synechococcus* sp. showed the lowest decay rate of -0.021 ± 0.008 h^{-1} (mean $\pm$ SE) in treatments ranging from the full incident irradiance to 23% of the irradiance incident below the ocean surface. Decay rates decreased significantly upon removal of UVR, demonstrating a major effect of UVR on cell death, although ambient levels of visible light alone still induced cell death in *Prochlorococcus* and picoeukaryotic populations, but not in *Synechococcus* sp. The high cell death of *Prochlorococcus* induced by total solar radiation resulted in short half-life values for this genus, ranging between 1.5 and 13.4 h across treatments. The half-life times for *Synechococcus* sp. and eukaryotic picoplankton cells exposed to UVR were longer, varying from 8.8 to 14.7 h and from 2.1 to 31.7 h, respectively. The UVR doses required to reduce the picophytoplankton populations by 50% (LRD_{50}) differed among the three groups, with considerably lower doses required for *Prochlorococcus* sp. *Prochlorococcus* sp. is highly sensitive to solar radiation, contrasting with the higher tolerance of *Synechococcus* sp. High, but taxon-specific, phytoplankton mortality induced by ambient UVR levels may act as a primary driver of the community structure of autotrophs and affect the dynamics of the microbial food web in clear, oligotrophic, oceanic waters.

Solar ultraviolet radiation (UVR, 280–400 nm) is increasingly recognized to exert a major influence on biological and chemical processes in the aquatic environment, including the growth and productivity of phytoplankton (Holm-Hansen et al. 1993; Neale 2001). Inhibition of photosynthetic rates by UVR has been observed in many regions of the oceans, such as tropical, temperate, and polar areas (Smith et al. 1992; Behrenfeld et al. 1993; Helbling et al. 1993). UVR affects photosynthetic energy-harvesting enzymes and other cellular proteins, as well as photosynthetic pigment contents (Häder et al. 1998). UVR exposure can also induce severe DNA damage in aquatic organisms, including phytoplankton (Buma et al. 2001; Helbling et al. 2001; Boelen et al. 2002). In addition, reactive oxygen substances (ROS), such as O_2^-, H_2O_2, $^1O_2$, and the OH^- radical, formed as a result of UV photolysis of dissolved organic matter, are strong oxidants and cause lipid peroxidation of cell membranes and other cellular damage (Murphy 1983; Tyrrell 1991). Phytoplankton have a variety of cellular systems for ultraviolet photoprotection and to repair the cell damage caused by UVR exposure; however, phytoplankton cells exposed to UVR may die if those systems are not sufficiently efficient. Small phytoplankton cells, which dominate the warm oligotrophic ocean (Agawin et al. 2000), have been predicted to have low levels of photoprotection and to be, therefore, more vulnerable to UVR (Garcia-Pichel 1994).

Water transparency to UVR is highest in the oligotrophic regions of the oceans, where UVR can penetrate several meters into the water column (Smith et al. 1992), being a potential source of damage for planktonic organisms. Picophytoplankton communities, composed by *Synechococcus* sp., *Prochlorococcus* sp., and small eukaryotic phytoplankters, are predicted to be particularly vulnerable to UVR (García-Pichel 1994) and are the dominant primary producers in the oligotrophic areas of the oceans (Waterbury et al. 1986; Partensky et al. 1999). These picophytoplanktonic cells inhabiting clear waters may receive high doses of ultraviolet and solar radiation that could severely damage them. Whether these communities are able to cope with the UVR levels they receive or, in contrast, experience considerable cell death remains, however, untested.

Sizeable phytoplankton lysis rates have been, however, reported in the oligotrophic ocean (Agustí et al. 1998, Agustí et al. 2001) and an important proportion, >40%, of *Prochlorococcus* and *Synechococcus* cells have been found to be dead in the surface of the Central Atlantic Ocean (Agustí 2004). The distribution of dead picocyanobacteria cells through the water column has been linked to underwater light levels (Agustí 2004), suggesting that high light and UVR may induce cell death. Despite these indications, the induction of picophytoplankton cell death by the direct effect of visible and ultraviolet radiation levels in ocean waters, has not yet been tested.

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The goal of this study is to assess the effect of solar and UV radiation on the cell death of picophytoplanktonic communities from the surface of the Atlantic Ocean and to compare the sensitivity to UVR of *Prochlorococcus*, *Synechococcus*, and picoeukaryotic phytoplankton. Cell death induced by natural underwater solar radiation was tested experimentally on natural communities of picophytoplankton from the surface waters of the Atlantic Ocean. Cell death of the different populations was quantified using the cell-digestion assay (Agustí and Sánchez 2002), a membrane-permeability test used to discriminate living and dead cells. The half-life of the different populations and the 50% lethal UVR doses were calculated from the decay of living cells and were used to evaluate the differential sensitivity of natural picophytoplankton populations to UVR.

Methods

Seawater samples were collected during the COCA-II cruise onboard the R/V *BIO Hespérides* along tropical Atlantic waters. This cruise started on 19 May 2003 in Las Palmas de Gran Canaria and finished at the same place on 15 June 2003. During the cruise, four stations were sampled for UV radiation experiments: station 14 (26°N, 18°W), station 32 (26°N, 26°W), station 42 (21°N, 26°W), and station 66 (21°N, 18°W). The stations were located in oligotrophic waters, except station 66, which was located in the northwest African upwelling area of Mauritania. At stations 14 and 32, seawater samples were collected from a depth of 20 m, and at stations 42 and 66, seawater samples were taken at 5 m. In both cases, seawater samples were incubated in duplicate quartz and glass bottles (100 mL) in incubators on deck with sea-surface recirculating water, to maintain in situ temperature. Bottles were acid cleaned but there was no prefiltration of the sample, to remove grazers, as any additional manipulation could possibly affect already compromised cells and, therefore, add artifacts. Quartz bottles allowed all the radiation (UVR + PAR) to pass through while glass bottles only allowed PAR radiation to pass through. Three levels of radiation, corresponding to the 100%, 57%, and 23% of the incident irradiance were tested parallel to dark controls. The 57% and 23% radiation levels were attained by covering the bottles with a neutral screen and 100% radiation treatment was run without screens. The total duration of the experiments was 7–8 h and duplicated samples were taken from each treatment every 2 h, although the last sample time was taken at 2- or 3-h intervals.

Solar radiation received underwater in the incubation tanks was measured using a PUVR 2500 Biospherical Instruments meter, which measures UVR at 7 wavelengths: 305, 313, 320, 345, 380, and 395 nm. The instrument also has a photosynthetically active radiation (PAR) sensor. Natural PAR and UVR were measured in the incubation tanks (sensor was 0.3 m just below surface) every half-hour during the experiments. UVR values obtained at the different wavelengths were integrated, from 300–400 nm, to calculate the whole incident UV radiation during the experiments. Profiles of underwater PAR and UVR were performed in the four stations by using the PUVR 2500 Biospherical Instruments radiometer.

The thickness of the upper mixed layer (UPM) was calculated from the shallowest depth at which water density differed from surface values by more than 0.05 kg m^{-3}.

The variability in the abundance of *Prochlorococcus* and *Synechococcus* cells during the experiments was determined on-board by flow cytometric analysis of duplicated fresh samples with a FACSCALIBUR flow cytometer (Becton Dickinson) according to the characteristic features of fluorescence for each population (Marie et al. 1999). Analysis of fresh samples that yielded sufficient red fluorescence signals from surface *Prochlorococcus* cells allowed their unambiguous detection by flow cytometry.

At the same time, two additional fresh samples were treated with the cell digestion assay (Agustí and Sánchez 2002) to identify the changes in the abundance of living and dead cells in the populations of picophytoplankton. The cell digestion assay involves use of an enzymatic cocktail (DNase and trypsine) to test cell membrane permeability and thus discriminate living from dead cells (Agustí and Sánchez 2002). Dead cells, with compromised membranes, cannot prevent the enzymes from invading their cytoplasm and are accordingly digested by these enzymes, disappearing from the samples. Live cells with intact membranes are not affected by the enzyme cocktail, so they remain in the sample. One hundred microliters DNase (400 μg DNase mL^{-1} HBSS [Hanks' balanced salts]) were added to 0.5-mL duplicate samples in assay tubes and were incubated at 37°C for 15 min. Then, 100 μL trypsin 1% (in HBSS) were added to the same samples and were incubated at 37°C for 30 min. Finally, samples were counted at the flow cytometer, as described by Agustí and Sánchez (2002) and Agustí (2004).

The half-life time ($t_{1/2}$, the time required to decline to one-half of the initial cell density) of each population was calculated from the decay rate in the abundance of living cells when exposed to irradiance:

$$t_{1/2} = \frac{0.693}{k} \quad (1)$$

where k (h^{-1}) is the decay rate, calculated as the slope of the linear regression between the natural logarithm of the living cells abundance and time, in hours.

Lethal radiation doses, LRD_{50}, represent the radiation exposure required for the phytoplankton living cell abundance to decrease to one-half of its original value. This parameter was calculated from a similar equation used to calculate the half-life time, but where k represented the slope of the relationship between the natural logarithm of the living-cell abundance and radiation doses. UVR doses were calculated by integrating ultraviolet radiation between 300 and 400 nm and, for both UVR and PAR, by integrating the cumulative radiation received during the experiments up to the time of sampling.

Results

UVR values showed a consistent pattern of variation along the day with radiation, for all wavelengths measured, increasing during the morning and reaching their maximum value between 12:30 and 14:30 h, decreasing again after

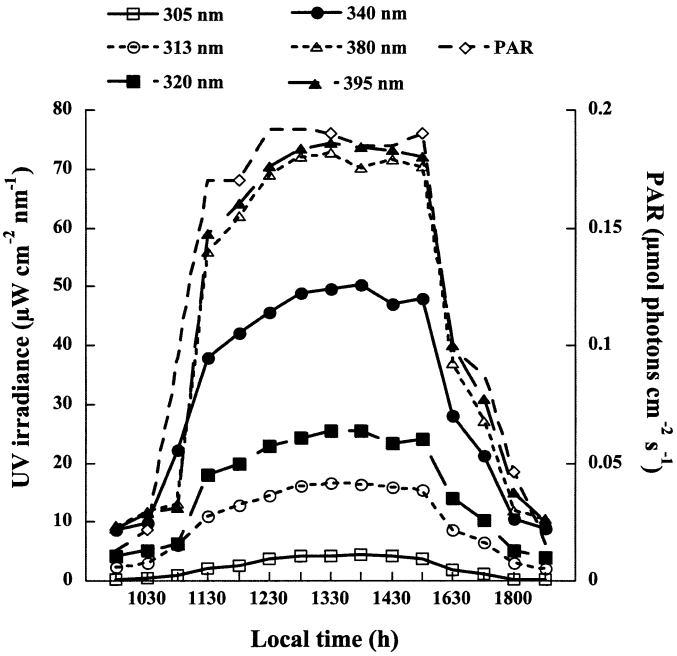

Fig. 1. Daily variation of spectral ultraviolet irradiance (μW cm^{-2} nm^{-1}) and PAR (μmol photons cm^{-2} s^{-1}) received during the experiment at station 14 (26°N, 18°W) on 25 May 2003.

17:00 h (Fig. 1). PAR values followed the same variation as observed for UVR during the day (Fig. 1). There were no significant differences between the solar radiation reaching the incubation tank during the different experiments conducted. However, the extinction coefficients of the different stations sampled varied highly, indicating that the UV and visible-light levels experienced by the picocyanobacterial populations at the time of sampling varied between the stations. The highest extinction coefficients for PAR and UVR were observed at station 66, the most eutrophic station, while the lowest coefficients were found at station 32, the most oligotrophic one (Table 1). For the 100% treatments, the depth receiving a comparable irradiance was 0.3 m (the equivalent depth of the sensor in the tank) for the less clear stations (Table 1) and slightly deeper at the clearest waters (Table 1). For the 57% treatments, the PAR levels experienced by phytoplankton in the experiments were equivalent to those reaching 14.4 and 3.4 m for the clearest and more turbid stations, respectively, and varied from 1.1 to 14.1 m for UVR, depending on the wavelength (Table 1). Deepest equivalent depths, varying from 3 to 37.7, depending on light spectra, were calculated for the 23% treatments. In addition, the deepest mixed layer, with 27 m, was located at station 42 (Table 1).

Picophytoplanktonic cells dominated phytoplankton biomass at the most oligotrophic stations (32 and 42) sampled, although their contribution to total biomass was less important at stations located close to the coastal and upwelling areas (stations 14 and 66), where larger cells dominated the total biomass and some picophytoplanktonic taxa, such as *Prochlorococcus* sp., became very scarce.

Synechococcus sp. and *Prochlorococcus* sp. total cell abundance tended to decrease in the experiments, depending on the shading treatment to which they were exposed. Living *Prochlorococcus* sp. cell abundance decreased remarkably following exposure to total radiation (UVR + PAR), indicating a rapid cell death of *Prochlorococcus* sp. induced by total solar radiation (Fig. 2). The mortality of *Prochlorococcus* sp. was very fast in most of the experiments exposed to total radiation, with living-cell abundance typically falling below detection limits after short exposures of 2 or 4 h to 100%, 57%, and 23% total irradiance (UVR + PAR) treatments (Fig. 2). *Synechococcus* sp. cell abundance exposed to total radiation (UVR + PAR) also tended to decrease during the experiments, with a parallel decrease in the abundance of living cells, indicating cell death induced by UVR to be important (Fig. 2). In general, the decay of living cells was higher under the highest irradiance treatments of 100% and 57% solar radiation (Fig. 2).

Prochlorococcus sp. showed high cell mortality in most of the experiments (Fig. 3). At the end of the experiments from stations 14 and 32, the entire *Prochlorococcus* sp. population was dead after exposure to total solar radiation exposure (UVR + PAR). *Prochlococcus* sp. cell mortality following exposure to high PAR (under 100% and 57% PAR treatments) was also very high, with the entire cell population dead by the end of the experiments (Fig. 3). At the experiment from station 42, *Prochlorococcus* sp. did not show the strong mortality observed in the previous experiments, with no significant ($p > 0.05$) changes in the proportion of living cells with respect to the initial values when exposed to UVR + PAR treatments; however, when UVR was removed and PAR was reduced, the proportion of living cells increased significantly ($p < 0.06$ for 57% and 23% PAR treatments; Fig. 3), suggesting the initial population to be stressed already at the onset of the experiments. At station 66, located at the northwest African upwelling, the low abundance of *Prochlorococcus* sp. precluded the analysis of solar-radiation effects on *Prochlorococcus* populations.

Table 1. Values for stations 14, 32, 42, and 66 sampled on the depth, *Z* (m), of the upper mixed layer (UML), extinction coefficient, K_d (m^{-1}), for PAR and some wavelengths in the UVA (380 nm) and UVB (320 nm) bands, and the calculated equivalent depths, for each station, at which the different experimental irradiance treatments (100%, 57%, and 23%) could be attained in the natural conditions.

	Station			
	14	32	42	66
Kd (PAR)	0.048	0.039	0.043	0.166
Kd (380 nm)	0.06	0.0398	0.047	0.255
Kd (320 nm)	0.166	0.108	0.131	0.484
100% *Z* (m) PAR	0.3	1.8	1.2	0.3
100% *Z* (m) 380	0.3	1.8	1.2	0.3
100% *Z* (m) 320	0.3	1.5	1	0.3
57% *Z* (m) PAR	11.6	14.4	13.2	3.4
57% *Z* (m) 380	9.3	14.1	12.3	2.2
57% *Z* (m) 320	3.4	5.2	4.4	1.1
23% *Z* (m) PAR	30.3	37.7	33.2	8.8
23% *Z* (m) 380	24.3	36.9	30.9	5.7
23% *Z* (m) 320	8.8	13.7	11.1	3
Z (m) UML	13	10	27	17

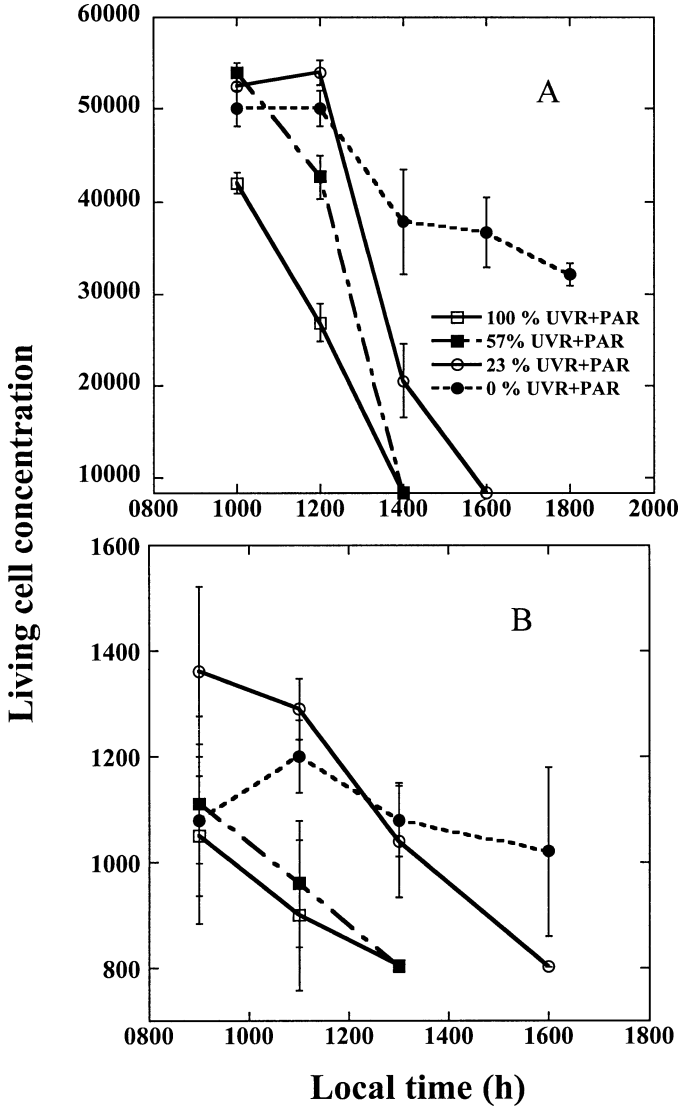

Fig. 2. The decline observed in the abundance of living cells exposed to different levels of solar radiation during the experiments. (A) *Prochlorococcus* sp. (experiment from station 14), and (B) *Synechococcus* sp. (experiment from station 32).

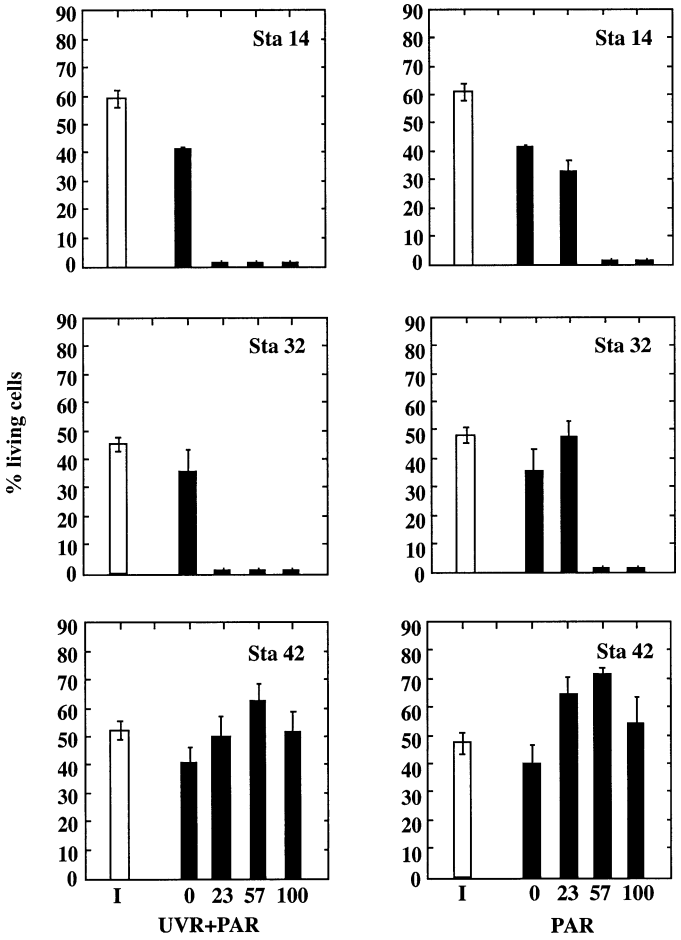

Fig. 3. The percentage of living *Prochlorococcus* sp. cells observed at the beginning of the experiments (I) and by the end of the experiments (after 6–8 h) under dark controls (0), 100%, 57%, and 23% of total solar radiation (UVR + PAR) treatments and under 100%, 57%, and 23% PAR treatments.

The cell mortality of *Synechococcus* sp. induced by solar radiation (Fig. 4) was consistently lower than that observed for *Prochlorococcus* sp. *Synechococcus* sp. showed significant cell mortality under total radiation at the experiment from station 32, where the entire *Synechococcus* sp. population was dead by the end of the experiment under the various UVR + PAR treatments used (Fig. 4). In some of the experimental treatments, *Synechococcus* sp. cell death was not induced (Fig. 4), but when UVR was removed (PAR treatments) or the intensity of UVR + PAR was reduced, the proportion of *Synechococcus* sp. living cells increased toward the end of the experiments (Fig. 4), indicating that UVR was already stressing *Synechococcus* populations in situ.

The highest cell mortality of the small eukaryotic cells was observed at the experiments conducted at station 14, where their populations were totally dead by the end of the experiment, and at station 42, where populations were totally dead under high irradiance treatments (Fig. 5). The abundance of small phytoeukaryotic cells was, however, very low ($<$500 cells mL^{-1}) at station 32, precluding the analysis of solar radiation effects on eukaryotic cells in this experiment. At the treatments with reduced irradiance or under PAR alone, there were no significant differences between the proportion of living cells at the end of the experiment when compared with the dark controls ($p < 0.05$; Fig. 5). Picoeukaryote cell viability tended to decrease in the experiment of station 66 when solar radiation was reduced to 23% of the incident irradiance (Fig. 5).

The decay rates of living cells induced by solar radiation varied greatly across the different populations analyzed. *Prochlorococcus* sp. showed the highest rate of -0.24 ± 0.053 h^{-1} under total solar radiation exposure (mean $\pm$ SE; Fig. 6), whereas *Synechococcus* sp. showed the lowest decay rate, an order of magnitude below that for *Prochlorococcus* sp., under total solar radiation exposure (-0.021 ± 0.008 h^{-1}, mean $\pm$ SE; Fig. 6). Living-cell decay rates were sig-

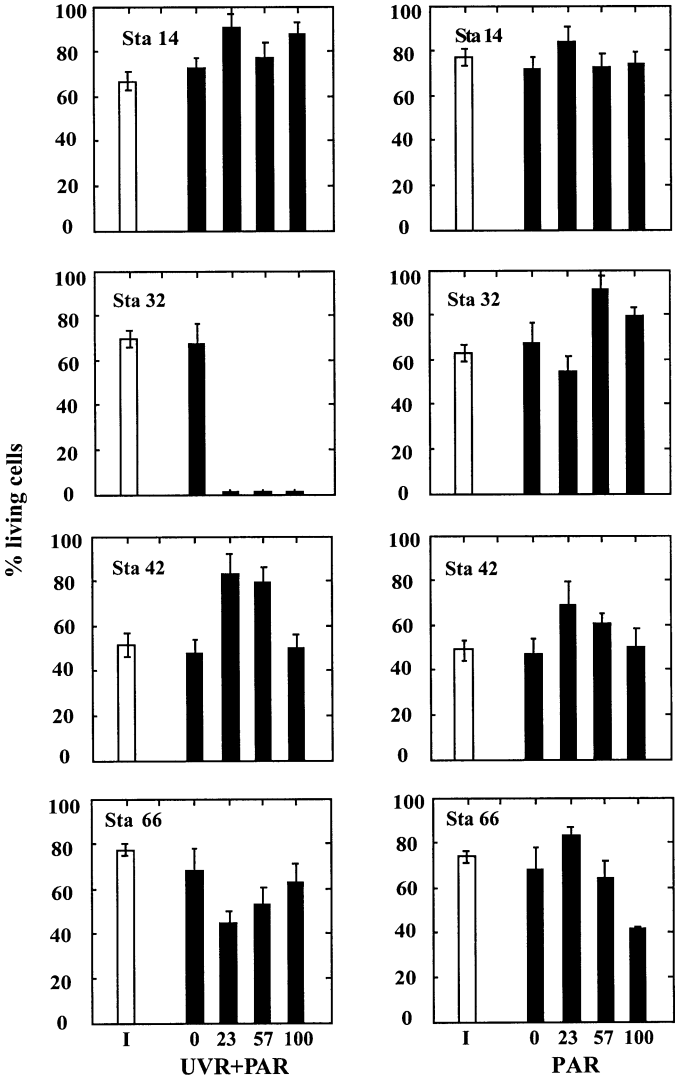

Fig. 4. The percentage of living *Synechococcus* sp. cells observed at the beginning of the experiments (I) and by the end of the experiments (after 6–8 h) under dark controls (0), 100%, 57%, and 23% of total solar radiation (UVR + PAR) treatments and under 100%, 57%, and 23% PAR treatments.

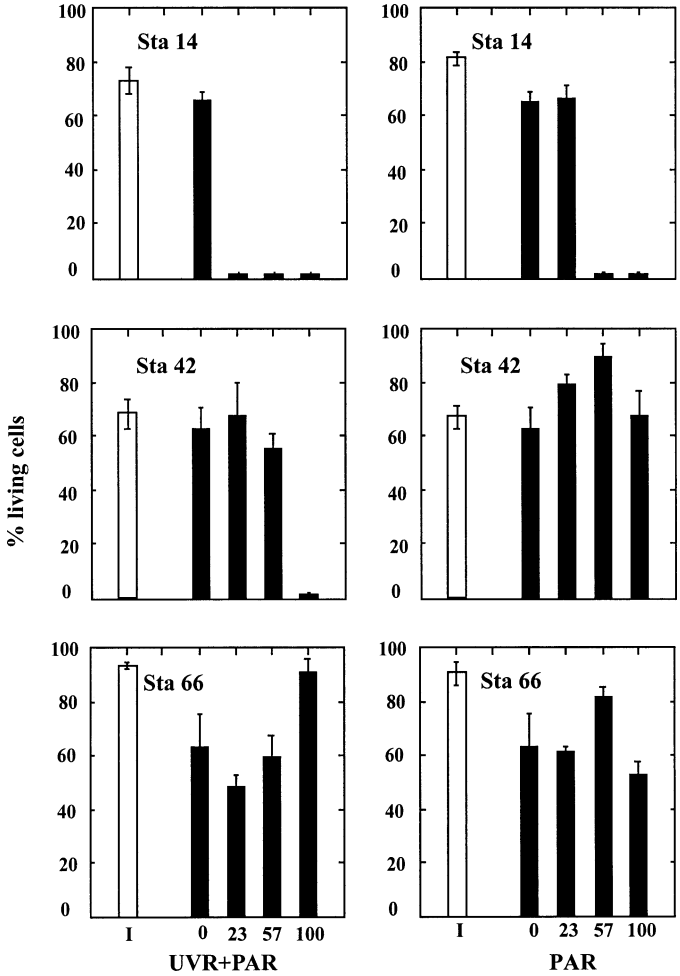

Fig. 5. Similar percentages as described in Figs. 3 and 4 but for populations of small eukaryotic cells found in the picophytoplankton communities.

nificantly lower ($p > 0.05$) when cells were exposed only to PAR and were almost undetectable for *Synechococcus* sp. under PAR exposure alone (Fig. 6). The high decay rates observed for *Prochlorococcus* sp. implied very short half-life times for this taxa, ranging between 1.5 and 13.4 h when exposed to total radiation (Table 2). The shortest half-life values corresponded to the 100% and 57% UVR + PAR treatments, while exposure to 23% of the incident UVR + PAR resulted in extended half-life times (Table 2). The half-life times for *Synechococcus* sp. could be only calculated from the experiment made at station 32 because there was no detectable decay of *Synechococcus* sp. living cells in the experiments conducted at the other stations. The half-life for *Synechococcus* sp. varied between 8.8 and 14.7 h, much longer than that obtained for *Prochlorococcus* sp. For picoeukaryotic phytoplankton, the half-life times obtained under total radiation exposure varied between 2.1 and 31.7 h (Table 2). The half-life values for *Prochlorococcus* sp., *Synechococcus* sp., and eukaryotes exposed to PAR were longer than those obtained for the same communities exposed to total solar radiation that included UVR (Table 2). Half-life values for *Prochlorococcus* sp. exposed to PAR were also short and varied between 1.6 and 8.8 h, with no detectable cell mortality induced by PAR in most of the experiments (Table 2).

The variation observed in cell death and half-life values was largely dependent on the different UVR and PAR doses received at the different treatments in the experiments. The exposure to solar radiation resulted in the decay of the populations of picocyanobacteria and eukaryotes, with the abundance of living cells decreasing as the doses of UVR increased (Fig. 7). The ultraviolet lethal radiation doses needed to reduce the populations to the half, LRD_{50}, for *Prochlorococcus* exposed to total irradiance varied between 141 and 659 kJ m^{-2} (Table 3) averaging 320 $\pm$ 169 kJ m^{-2}. The ultraviolet LRD_{50} for *Synechococcus* sp. showed a maximum value of 539 kJ m^{-2}, with ultraviolet LRD_{50} for picoeukaryotic phytoplankton varying between 201 and 1,280 kJ m^{-2} (Table 3). Under PAR exposure, when UVR was removed, PAR LRD_{50} for *Prochlorococcus* sp. showed also lower val-

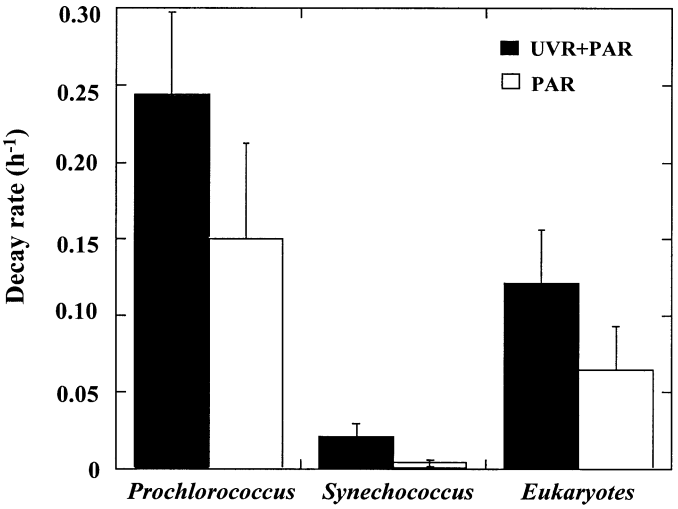

Fig. 6. Cell decay rates (h^{-1}) for living cells of Atlantic picophytoplanktonic communities induced by total solar radiation (UVR + PAR) and PAR after removing UVR. Columns represent the average cell decay rates obtained at the different treatments from all the experiments. Bars represent the standard errors of the averaged rates.

ues than those detected for *Synechococcus* sp. and picoeukaryotic cells (Table 3).

Discussion

The results obtained demonstrated that picophytoplankton cells from the surface of the Atlantic Ocean may be severely affected by exposure to ambient levels of ultraviolet and solar radiation, causing abrupt cell mortality. The sensitivity to solar radiation and ultraviolet bands differed, however, between the different picophytoplankton groups that cohabitate in the ocean, with *Prochlorococcus* sp. showing the highest sensitivity to UVR and PAR and *Synechococcus* sp. the lowest. The damage induced by UVR in natural phytoplankton communities has been previously documented by demonstrating the inhibition of photosynthesis (e.g., Cullen et al. 1992; Buma et al. 2001) or the detection of cyclobutane pyrimidine dimers (CPDs) formed as a result of UVR-induced DNA damage (Boelen et al. 2002). Although it is expected that UVR and high solar radiation should induce phytoplankton cell mortality, the direct consequences of UVR on natural phytoplankton cell death had never been quantified before (Castenholz and Garcia-Pichel 2000). Our results demonstrate that indeed UVR damage causes considerable cell death in picophytoplankton communities from the surface of the Atlantic Ocean. The cell death of picophytoplankton described here should be the consequence of the severe injury that UVR exerts on different cell constituents. UVR can induce great protein and DNA damage in phytoplanktonic cells (Buma et al. 2001; Helbling et al. 2001; Boelen et al. 2002). In addition, reactive oxygen substances, formed as a result of UV photolysis of dissolved organic matter, are strong oxidants and cause lipid peroxidation of cell membranes and other cellular damage (Murphy 1983; Tyrrell 1991). In photosynthetic organisms, UVR affects photosynthetic energy-harvesting enzymes and other cellular proteins, as well as photosynthetic pigment contents (Häder et al. 1998). Photo-protection, achieved through a variety of mechanisms and sunscreen substances, and cell repair mechanisms should help to avoid the strong damage that UVR could induce in cells (Banaszak 2003). The picophytoplankton communities analyzed here experienced high cell death in some of the experiments, indicating that the levels of photoprotection and repair systems were insufficient to overcome the cell damage induced by solar radiation and, in consequence, picophytoplanktonic cells died. The high sensitivity to solar radiation of picophytoplankton observed here could be explained by the small size of their cells (Garcia-Pichel 1994). The minimum load of sunscreen substances for effective cell protection is predicted to be physically unattainable for photosynthetic cells smaller than 1-μm radius (Garcia-Pichel 1994). *Prochlorococcus* sp. (0.3-μm cell radius) should be below the threshold size needed to accommodate sunscreen substances in its tiny cells, the smallest within oxygenic photosynthetic organisms, consistent with

Table 2. Half-life time vlaues for *Prochlorococcus*, *Synechococcus*, and picoeukaryotes at the different UVR + PAR and PAR levels established in the experiments. Ndect = no detected mortality; (—) = not determined.

		Half-life (hours)					
		Prochlorococcus sp.		*Synechococcus* sp.		Picoeukaryotes	
Station	% radiation	UVR + PAR	PAR	UVR + PAR	PAR	UVR + PAR	PAR
14	100	1.7	1.6	Ndect	32.8	2.1	3.15
	57	1.5	1.5	Ndect	39.8	2.8	—
	23	2.1	3.2	Ndect	Ndect	4.2	4.6
32	100	2.6	4.3	10.5	Ndect	—	—
	57	2.9	8.8	14.7	Ndect	—	—
	23	8.1	Ndect	8.8	Ndect	—	—
42	100	6.2	Ndect	14.7	Ndect	6.8	65.8
	57	—	Ndect	Ndect	Ndect	28.3	Ndect
	23	13.4	Ndect	Ndect	Ndect	31.7	Ndect
66	100	—	—	Ndect	Ndect	9.7	17.2
	57	—	—	Ndect	Ndect	11.7	30.7
	23	—	—	Ndect	Ndect	9.7	9.1

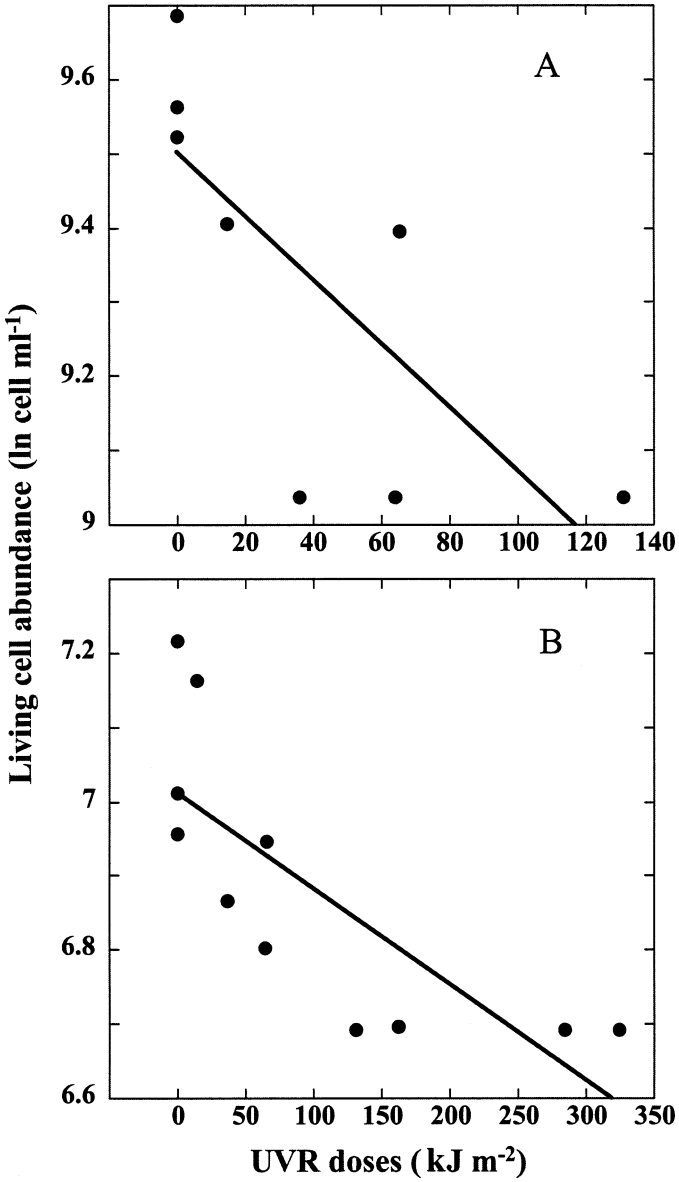

Fig. 7. The relationship between living cell abundance (ln) of *Prochlorococcus* sp. (A) and *Synechococcus* sp. (B) and UV integrated doses received at the different sampling events during the experiment from station 32. The solid lines represent the fitted linear regression equations $R^2 = 0.563$ and $R^2 = 0.603$ for *Prochlorococcus* sp. (A) and *Synechococcus* sp. (B), respectively.

its highest sensitivity to UVR-induced death, as demonstrated here (Garcia-Pichel 1994). Picocyanobacteria lack the UVR screens scytonemin or MAAs (micosposine-like aminoacids) known to protect larger cyanobacteria or phytoplanktonic groups (Castenholz and Garcia-Pichel 2000). *Synechococcus* sp. showed, however, the highest resistance to solar radiation, indicating that the populations examined from these genera have better photo-protection or repair systems than *Prochlorococcus* and picoeukaryotes. *Synechococcus*, as other cyanobacteria, can prevent inhibition of photosystem-II by the capacity to rapidly exchange D1 proteins (Campbell et al. 1998). Within 15 min of moderate ultraviolet B (UVB) exposure, *Synechococcus* was able to change the expression of a family of three genes encoding photosystem II D1 proteins (Campbell et al. 1998). Differences observed in the sensitivity to UVR between *Synechococcus* sp., *Prochlorococcus* sp., and picoeukaryotes could be also the consequence of different DNA repair efficiencies, which appear to be species specific in phytoplankton and depends on the initial level of damage (Small and Greimann 1977; Karentz et al. 1991). Indeed, cellular properties, such as morphology, DNA base content and sequence, placement of organelles, antioxidants and repair capabilities, could also play an important role in explaining differences in UV tolerance across taxa (Karentz et al. 1991; Laurion and Vincent 1998). Recent studies on genomics of picocyanobacteria have identified DNA repair capacities in *Prochlorococcus* and *Synechococcus (*e.g., Dufresne et al. 2005), although they found high variability in the repair capacity among different *Prochlorococcus* strains, some strains of which are lacking important genes for DNA reparation (Hess at al. 2001; Dufresne et al. 2005). There are indeed recent studies describing differences among cyanobacteria groups in the presence of specific genes coding for proteins with a role in the adaptation to high visible light (e.g., Bhaya et al. 2002; Rocap et al. 2003). Previous studies with some cultured strains of picocyanobacteria reported different growth responses to visible light, showing that *Synechococcus* sp. is better adapted to growth under high visible light than *Prochlorococcus* (Kana and Glibert 1987; Moore et al. 1995), although these studies did not consider ultraviolet radiation effects.

For all three picophytoplanktonic groups, cell death was lowest at the experiments from station 42 and 66. Both stations were located in the area under the influence of the northwest African upwelling. This suggests that higher nutrient availability may exert a positive effect in the protec-

Table 3. LRD_{50} for UVR (KJ m^{-2}) and PAR (mol photons m^{-2}) treatments required for 50% reduction in the populations of *Synechococcus* sp., *Prochlorococcus* sp., and picoeukaryotes. Ndect = no detected mortality, (—) = not determined.

	Prochlorococcus sp.		*Synechococcus* sp.		Eukaryotes	
Station	UVR	PAR	UVR	PAR	UVR	PAR
14	141	6.6	Ndect	257	201	17.3
32	162	17.6	539	Ndect	—	—
42	659	Ndect	Ndect	Ndect	785	Ndect
66	—	—	Ndect	Ndect	1280	65.8
Mean±SE	320±169	12.1±5.5	>539	>257	755±311	41.5±24

tion–repair capacities of cells or, alternatively, that the specific composition of the communities, with its associated capacity of photoprotection–repair, differed with respect to the communities inhabiting more oligotrophic waters. In any case, although no cell death was observed in some experiments at these stations, the stress induced by UVR and high PAR was detected by the observation of an increase in the percentage of living cells of *Prochlorococcus* sp., *Synechococcus* sp., and eukaryotes after removing UVR or when solar radiation was reduced to the 57% or 23% levels, suggesting that the picophytoplanktonic populations were UVR stressed in the ambient waters. Variability between experiments could also be the result of the different light history of the cells in the different stations as a result of different light penetration and mixing conditions in the water column.

The degree of lethality in the experiments was dependent on the doses to which the communities were exposed. The use of radiation gradients in the experiments allows us to calculate the 50% lethal UVR doses for *Prochlorococcus* sp., *Synechococcus* sp., and picoeukaryotes. The ultraviolet lethal radiation doses required to kill 50% of the picophytoplankton populations (LRD_{50}) differed among the three picophytoplanktonic groups, with the doses for *Prochlorococcus* sp. considerably lower than those required to decimate the populations of the other two groups. In some of the experiments, the ultraviolet LRD_{50} could not be calculated for *Synechococcus* sp. due to its higher resistance and longer experiments (e.g., 2 or 3 d) would have been required to quantify LRD_{50} for *Synechococcus* sp. The ultraviolet radiation dose of 162 kJ m^{-2} needed to reduce the *Prochlorococcus* sp. population to half, found at the experiment of station 32, is equivalent to the daily dose of UVR penetrating at a depth of 63 m at this station (Agustí unpubl. data). This calculation, which does not reproduce the changes in UVR relative to PAR with depth, indicates that the layer over which phytoplankton is exposed to lethal UVR doses can be considerable in the clear ocean, deeper than expected from calculations based on UVR-induced DNA damage alone (Smith and Baker 1979; Boelen et al. 2002). Further analysis should consider how the changing ratio of UVR to PAR with depth may affect the resistance of the cells to UVR. Existing indices of underwater UVR penetration for DNA damage are based on the UVR sensitivity of organisms other than phytoplankton (e.g., anchovy eggs for Setlow's DNA action spectra; Smith and Baker 1979) and should have sensitivity to UVR that strongly differs from that observed here for picophytoplankton because UVR sensitivity appears to be highest for small cells (García-Pichel 1994).

The cell decay rates induced by solar radiation in *Prochlorococcus* sp. were indeed remarkably fast, resulting in very short, <2 h, cell half-lives, indicating that *Prochlorococcus* sp. cells should experience strong cell mortality, induced by solar radiation, at the surface waters of the oligotrophic ocean. This result is in agreement with reports of a high proportion of dead *Prochlorococcus* cells in surface subtropical Atlantic Ocean waters (Agustí 2004). A high proportion of dead *Prochlorococcus* sp. cells have been indeed observed down to considerable depths (100 m) in the oligotrophic South Atlantic Subtropical Gyre (Agustí 2004). The differential sensitivity between the picophytoplanktonic groups to solar radiation observed here is also in agreement with the contrasting patterns in the prevalence of death *Prochlorococcus* and *Synechococcus* cells with increasing relative irradiance in the Atlantic Ocean (Agustí 2004), which suggested *Prochlorococcus* sp. to be more vulnerable to high irradiance. Our results also showed small eukaryotic cells to be highly sensitivity to UVR and solar radiation. This sensitivity was, although not as high as that observed for *Prochlorococcus*, quite significant, indicating that the UVR and PAR levels present at the surface layers of the oligotrophic ocean should be also an important stressor for small phytoeukaryotic cells, potentially affecting their abundance, distribution, and population dynamics.

The high picophytoplankton decay rates induced by solar radiation, together with the high proportion of dead cells reported for picocyanobacteria at the Atlantic Ocean (Agustí 2004), point to high phytoplankton cell mortality in these waters, in agreement with the high phytoplankton lysis rates reported for the oligotrophic subtropical north Atlantic (Agustí et al. 2001). The report of elevated picophytoplankton cell loss through UVR damage helps improve our understanding of the dynamics of oceanic production, by explaining the fate of excess growth that cannot be accounted for by grazing losses in natural systems (Christaki et al. 1999). High, but taxon-specific, phytoplankton mortality by UVR may act as a primary driver of the community structure of autotrophs and affect, through the release of substances after cell death, the dynamics of the microbial food web in clear oceanic waters.

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