

UVBR-induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic Ocean

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ABSTRACT: UVBR (ultraviolet-B radiation: 280 to 315 nm)-induced DNA damage, measured as cyclobutane pyrimidine dimers (CPDs), was determined in size fractions of natural populations of bacterio- and phytoplankton collected in marine tropical waters. Mean biologically effective UVBR doses in the wind-mixed layer were calculated from DNA dosimeter data. Phytoplankton species composition in these waters was monitored using flow cytometry and pigment analyses. In terms of (divinyl-)chlorophyll *a* concentrations, prochlorophytes and cyanobacteria comprised the largest fraction of the phytoplankton, except in a eutrophic bay at Curaçao, an island located in the southern Caribbean. In terms of cell numbers and amount of DNA, small prochlorophytes and marine bacteria dominated. Small but detectable levels of UVBR-induced DNA damage were found at all locations. In general, more DNA damage was found in the small size fraction (0.2 to 1 µm) than in the larger size fraction (1 to 10 µm). The greatest amount of damage was found in the small size fraction collected in the central Atlantic Ocean (20 CPDs/10⁶ nucleotides), despite the fact that UVBR doses were much higher at other locations. The calculated mean biologically effective UVBR doses in the wind-mixed layer were 2 to 17 times lower as compared with incident UVBR doses. CPD levels determined in cultures of the cyanobacterium *Synechococcus* sp. subjected to UVBR doses similar to those in the wind-mixed layer corresponded with CPD levels measured in the 1 to 10 µm fraction in the field. Our results indicate that UVBR vulnerability is size dependent. Furthermore, the low CPD levels observed in these field communities may be explained by the low mean biologically effective doses received by the cells as a result of wind-induced mixing.

KEY WORDS: Cyclobutane pyrimidine dimers · DNA damage · Marine bacteria · Phytoplankton · *Synechococcus* sp. WH7803 · Ultraviolet-B radiation · UVBR

INTRODUCTION

In tropical oceanic waters UVBR (ultraviolet-B radiation: 280 to 315 nm) penetrates to several tens of meters (Smith & Baker 1979). In a previous study we used a DNA biosensor to measure the penetration of biologically effective (in this case DNA damaging) UVBR in the central Atlantic Ocean and in several

water types around Curaçao, Netherlands Antilles (Boelen et al. 1999). It was shown that biologically effective UVBR may penetrate deep into these waters, giving 1% levels down to 25 m. This low attenuation, together with the high natural UVBR levels in these regions, may have an impact on organisms that are present in these waters. Such stress can result in a decrease in primary (Behrenfeld et al. 1993) or bacterial production (Aas et al. 1996, Jeffrey et al. 1996a,b, Visser et al. 1999), the induction of UV absorbing com-

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pounds (Shibata 1969, Wood 1987, Drollet et al. 1993), or damage to essential macromolecules, e.g. DNA (Jeffrey et al. 1996a,b, Lyons et al. 1998, Wilhelm et al. 1998, Visser et al. 1999).

Structural changes in DNA are considered to be one of the primary consequences of the deleterious effects of UVBR on the cellular level (Karentz et al. 1991, Buma et al. 1995). Cyclobutane pyrimidine dimers (CPDs), especially thymine dimers (T<>T) are the predominant lesions induced by UVBR (Tyrrell 1986). These photoproducts block transcription and replication of the DNA and cause mutations or even cell death (Setlow et al. 1963, Swenson & Setlow 1966).

In the current paper we report on the presence of CPDs in DNA isolated from phytoplankton and bacterioplankton samples in the central Atlantic Ocean and in several water types around Curaçao, the Netherlands Antilles. To relate these results to UVBR doses the cells may have received, mean biologically effective doses were calculated, taking into account wind-induced mixing. Also, a comparison was made with results from laboratory experiments, where a typical marine picophytoplankton from tropical oceanic waters, *Synechococcus* sp. WH7803, was exposed to increasing levels of UVBR.

MATERIALS AND METHODS

Sampling sites. The experiments were carried out in 1996 in several water types around Curaçao and at several stations during the 1996 'Deep Chlorophyll

Table 1. Sampling dates and locations

Code	Location	Latitude (°N)	Longitude (°W)	Date (1996)
st300	DCM cruise	22	38	13 Aug
st400	DCM cruise	34	33	20 Aug
st500	DCM cruise	34	22	26 Aug
AB1	Curaçao, Anna Bay	12	69	14 Nov
PB1	Curaçao, Pest Bay	12	69	7 Nov
OO1	Curaçao, open ocean	12	69	11 Nov

Maximum' (DCM) expedition of RV 'Tydeman' in the central Atlantic Ocean (Fig. 1). Detailed station information is summarized in Table 1. Samples (4 l) of water were collected at several depths using Niskin bottles during the middle of the day and immediately filtered through 10, 1 and 0.2 µm polycarbonate filters (Poretics). The 1 and 0.2 µm filters were frozen in liquid nitrogen and stored at -80°C until DNA isolation.

Samples for flow cytometer analysis (described later) were collected at the central Atlantic stations in the middle of the day. Samples (2 ml) were analyzed immediately or fixed with 0.5% paraformaldehyde (final concentration), frozen in liquid nitrogen and stored at -80°C until further analyses.

Culture conditions. A culture of the cyanobacterium *Synechococcus* sp. WH7803 was obtained from the Provasoli-Guillard National Center for Culture of Phytoplankton (CCMP 1334). The cells were grown in artificial sea water supplied with H/2 nutrients (Guillard 1975) in temperature-controlled (22°C) quartz

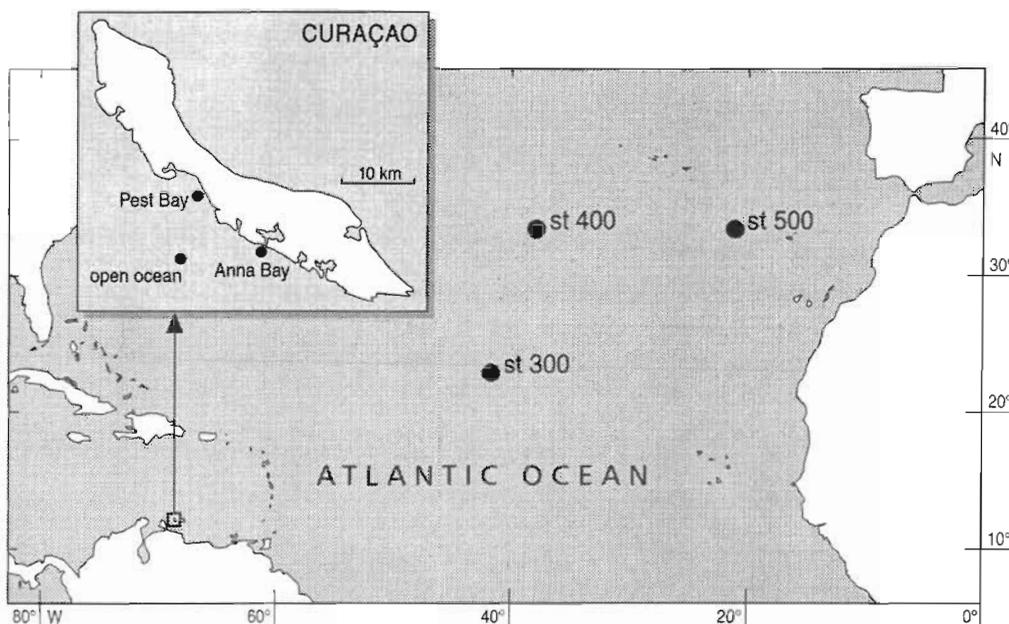


Fig. 1. Sample locations during the 1996 'Deep Chlorophyll Maximum' (DCM) research cruise and at Curaçao (inset)

cuvettes (500 ml). The cultures were kept in suspension by gentle stirring. The cuvettes were illuminated from 1 side with 50 W halogen lamps to provide photosynthetically active radiation (PAR) (14 h light:10 h dark), giving approximately $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 2). After 3 d of acclimation to these PAR levels the cultures were exposed additionally to 2 UVAR fluorescent tubes (Philips 09 N) and 2 UVBR tubes (Philips TL 12) for 6 h d^{-1} in the middle of the light period. UVCR (200 to 280 nm) was removed using 3 mm 305 Schott cut-off filters. The intensity of UVR received by the cultures was varied by changing the distance between the UVR sources and the cuvettes. The amount of UVBR, UVAR (315 to 400 nm) and PAR (400 to 700 nm) was measured using a MACAM SR9910 double monochromator scanning spectroradiometer (Macam Photometrics) equipped with a 4.2 m quartz cable connected to a 4π -collector. Spectra were recorded at 1 nm intervals between 280 and 400 nm and at 5 nm intervals between 400 and 700 nm. Biologically effective doses (BEDs) were calculated from the spectra using the DNA action spectrum of Setlow (1974) normalized at 300 nm (Table 2).

Cells were counted daily using a Coulter counter. Cultures were not axenic, the average amount of bacteria was 12 % of the total biovolume. There was no measurable difference in growth rate (mean of 0.6 d^{-1}) between the cuvettes. Cells were harvested on the 5th day of UVR treatment before (09:30 h), in the middle (13:00 h) and at the end (16:00 h) of the UVR-exposure period. Aliquots (50 ml) of culture were filtered over a Whatman GF/F filter. The filter was immediately frozen in liquid nitrogen and stored at -80°C until further treatment.

DNA isolation and quantification. DNA was extracted from filters using a method modified from Doyle & Doyle (1991). The filters were incubated at 60°C for 30 min with 750 μl preheated CTAB isolation buffer (2% [w/v] CTAB [Sigma], 1.4 M NaCl, 0.2% [v/v] 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl pH=8.0). Then 750 μl CIA (chloroform/isoamylalcohol [24:1]) was added to extract the DNA from cell debris and proteins. The solution was shaken briefly and subsequently centrifuged in an Eppendorf cen-

trifuge at maximum speed ($20\,000 \times g$) for 10 min. The upper (water) phase was transferred to a clean vial and around 0.5 ml (2/3 of total volume) cold isopropanol was added to precipitate the DNA. This mixture was shaken gently, incubated for 1 h at 4°C and centrifuged 30 min at 4°C . The supernatant was removed carefully and 1 ml of 80% ice-cold ethanol was added to the precipitated nucleic acids. The vials were placed at -20°C for 15 min and centrifuged for 30 min ($20\,000 \times g$). The DNA was dried under vacuum and resuspended in $0.1 \times \text{TE}$ buffer (1 mM Tris-HCl pH = 8.0, 0.1 mM EDTA). To remove RNA, the DNA was incubated for 1 h with $75 \mu\text{g ml}^{-1}$ RNase (Boehringer Mannheim) at room temperature.

The amount of DNA was determined fluorometrically using Picogreen dsDNA quantitation reagent (dilution 1:400, Molecular Probes) on a 1420 Victor multilabel counter (EG&G Wallac, excitation 485 nm, emission 535 nm).

Quantification of cyclobutane pyrimidine dimers.

The amount of CPDs in DNA was determined using an immunodotblot procedure (Boelen et al. 1999). In brief, heat-denatured DNA samples (0.06 to 1000 ng) were blotted onto a nylon membrane. To detect CPDs, the membrane was incubated with the primary antibody H3 (Roza et al. 1988). This was followed by an incubation with the secondary antibody (rabbit anti-mouse) coupled to alkaline phosphatase. Chemiluminescent detection was done using Lumi-Phos Plus (GibcoBRL) in combination with a photographic film. The H3 antibody is raised against cyclobutane thymine dimers but also has a high affinity for 5'TC dimers (Fekete et al. 1998). To allow quantification of the amount of damage in the sample DNA, the samples were compared with a dilution series of standard DNA. The amount of CPDs in the standard DNA was determined before by calibrating it against DNA isolated from irradiated Hela cells, kindly provided by Dr Len Roza. The amount of CPDs in this DNA was determined by Roza et al. (1988) by means of HPLC. The detection limit of the CPD assay is $<1 \text{ CPD}/10^6$ nucleotides.

Calculation of mixing depth and mean doses. To estimate the daily BED that the plankton actually

Table 2. Irradiance conditions during UVR-treatments. Biologically effective UV-dose (BED) weighted with the action spectrum of Setlow (1974), normalized at 300 nm

Cuvette no.	UVBR (W m^{-2})	UVAR (W m^{-2})	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	BED ($\text{J m}^{-2} \text{d}^{-1}$)
1	0	0.33	345	0
2	0.076	1.4	370	300
3	0.15	2.9	370	900
4	0.30	4.6	340	1600
5	0.54	7.8	390	2900

Table 3. Calculated mean daily biologically effective doses (BED_m) at the sampling sites, taking into account wind-induced vertical mixing. Biologically effective dose just below the surface (BED_0) and attenuation coefficient for the induction of DNA damage (K_d) were measured using a DNA dosimeter (Boelen et al. 1999). The depth of the wind-mixed layer (WML) was estimated using the wind speed (W_{10}). *Depth of the calculated wind-mixed layer exceeded the actual depth in the bays: here real depths were used to calculate BED_m

Code	Location	BED_0 ($J\ m^{-2}\ d^{-1}$)	K_d (m^{-1})	W_{10} ($m\ s^{-1}$)	WML (m)	BED_m ($J\ m^{-2}\ d^{-1}$)
st300	DCM cruise	884	0.18	8	20	239
st400	DCM cruise	592	0.20	10	25	118
st500	DCM cruise	456	0.18	3	7.5	250
AB1	Curaçao, Anna Bay	880	1.60	6	10*	55
PB1	Curaçao, Pest Bay	2263	0.26	7	15*	569
OO1	Curaçao, open ocean	2235	0.35	6	15	423

received at the various sampling sites, the depth of the wind-mixed layer was combined with the attenuation coefficient and the dose just under the surface (Table 3).

Subsurface doses and attenuation coefficients were determined using a DNA dosimeter (Boelen et al. 1999). In short, small acid-cleaned quartz tubes were filled with a solution of $10\ \mu g\ ml^{-1}$ calf thymus DNA (Sigma) in TE-buffer (10 mM Tris-HCl pH = 8.0, 1 mM EDTA). The tubes were sealed with parafilm, after which duplicate tubes were incubated at several water depths during a period of the day. After incubation, the tubes were kept in the dark at $4^\circ C$ during transportation. Subsequently, the DNA solution was stored at $-20^\circ C$ until CPD analyses. Calibration of biodosimeter levels was done by use of an underwater, double-monochromator, scanning spectroradiometer (Macam Photometrics), equipped with a 4.2 m quartz cable connected to a 4π -collector. The conversion factor between scalar irradiance and the DNA dosimeter was: 1 CPD per million nucleotides = $3.1\ J\ m^{-2}$ (Setlow weighted, $N = 300$) (Boelen et al. 1999).

Average biologically effective attenuation coefficients (K_d) were determined from linear regressions of natural logarithmic biologically effective irradiation against depth using the log linear part of the curve. The daily doses at 0 m (BED_0) were calculated by extrapolating the curves to the surface. Daily doses were expressed in $J\ m^{-2}\ d^{-1}$ (Setlow weighted, normalized at 300 nm) by using the conversion factor 3.1, as described above.

Depth of the wind-mixed layer (WML, in m) was estimated by calculating half of the Ekman depth D_E (Veth 1991):

$$WML = 0.5D_E \approx 2.5W_{10}$$

where W_{10} = wind speed ($m\ s^{-1}$) at standard height (10 m). The calculated values for WML corresponded with occasionally measured CTD profiles (C. Veth pers. comm., data not shown).

Mean biologically effective doses (BED_m) were calculated assuming constant attenuation as

$$BED_m = \frac{BED_0(1 - e^{(-K_d WML)})}{K_d WML}$$

(Helbling et al. 1994), where BED_0 = biologically effective daily dose just below the surface and K_d = attenuation coefficient for induction of DNA damage.

Pigment composition. In order to estimate phytoplankton composition, 10 l samples of water were filtered over a Whatman GF/F glass-fiber filter. Near Curaçao the samples were collected at the surface. At the cruise stations samples were collected at different depths between 5 and 30 m. The filters were frozen immediately in liquid nitrogen and stored at $-80^\circ C$ until further analyses. Pigments were determined by HPLC as described by Kraay et al. (1992). Divinylchlorophyll *a* concentrations were determined as described by Veldhuis & Kraay (1990). Phytoplankton class abundances were calculated from the pigment data using the CHEMTAX program, as introduced by Mackey et al. (1996).

Flow cytometry. At the stations st300, st400 and st500 in the central Atlantic Ocean (Fig. 1) species composition and relative DNA content per species cluster were determined using a flow cytometer (Coulter XL-MCL or Elite-ESP) in combination with the nucleic acid stain Picogreen, as described by Veldhuis et al. (1997). Four clusters were identified: heterotrophic bacteria, *Prochlorococcus* sp., *Synechococcus* sp. and small eukaryotic algae.

These data were used to calculate the relative contribution, in terms of cell numbers and amount of DNA, of the species groups which were collected on the filters used for DNA isolation. We assumed that all the heterotrophic bacteria and *Prochlorococcus* sp. cells were retained on the $0.2\ \mu m$ filter, whereas *Synechococcus* sp. and eukaryotic algae were retained on the $1\ \mu m$ filter. This gives an indication of which species groups contributed most to the total amount of DNA damage.

The actual contribution is difficult to predict since there may be differences in sensitivity to DNA damage and yield of DNA isolation between the various groups.

RESULTS

Dose-response experiments

A UVBR dose-related induction of CPDs was found in cultures of the marine picophytoplankter *Synechococcus* sp. WH7803 (Fig. 2). In general, CPD levels increased during UVR treatment as well as with increasing UVR intensity. No signal was found in *Synechococcus* cells not exposed to UVR. The highest level of photoproducts (60 to 70 CPDs/ 10^6 nucleotides) was found at the end of the UVR treatment in the culture exposed to the highest BED (2900 $\text{J m}^{-2} \text{d}^{-1}$). Samples taken just prior to the daily UVR treatment showed residual DNA damage. This indicates that at the given PAR levels and UVR doses not all damage was repaired between UVR treatments.

Species composition at field locations

In terms of (divinyl-)chlorophyll *a* concentrations, prochlorophytes (*Prochlorococcus* sp.) and cyanobacteria (*Synechococcus* sp.) comprised the largest fraction of the phytoplankton, except at the eutrophic Anna Bay station off Curaçao (Table 4). At this station chlorophyll *a* concentrations were substantially higher and diatoms, dinoflagellates and cyanobacteria formed the bulk of the phototrophic community.

At the central Atlantic stations, heterotrophic bacteria and *Prochlorococcus* sp. dominated in terms of cell numbers and DNA quantity, as measured by flow cytometry (Table 5). Typically, more than 97% of the total DNA content originated from these small organisms at the central Atlantic stations. When these data were subdivided into 2 size classes, the smallest size

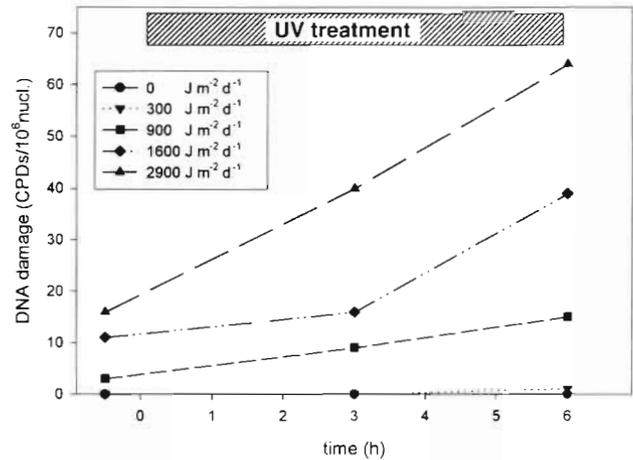


Fig. 2. Dose-response curves for the induction of CPDs in cultures of *Synechococcus* sp. WH7803. Biologically effective doses were calculated using the DNA action spectrum of Setlow (1974), normalized at 300 nm (Table 2). Cells were harvested on the 5th day of UVR treatment before (09:30 h), in the middle (13:00 h) and at the end (16:00 h) of the UVR exposure period

fraction (<1 μm) consisted of 62% heterotrophic bacteria and 38% prochlorophytes, in terms of DNA content. In the fraction >1 μm , cyanobacteria and eukaryotic algae each contributed 50% to the total amount of DNA (Table 6).

UVR levels at field locations

The BEDs just underneath the surface at the sample locations varied between 456 and 2263 $\text{J m}^{-2} \text{d}^{-1}$ (Table 3). The central Atlantic waters (st300 to st500) appeared to be most transparent for UVBR, with biologically effective attenuation coefficients between 0.18 and 0.2 m^{-1} . Waters off Curaçao were also shown to be UVBR-transparent, except the eutrophic Anna Bay (Table 3).

The calculated wind-mixed layer varied between 7.5 and 20 m (Table 3). In those cases where the calculated

Table 4. Estimated contributions of various phytoplankton classes at the sample sites. The contribution is expressed in nanograms of (divinyl-)chlorophyll *a* per liter as determined by interpretation of pigment HPLC data using the CHEMTAX program. Values shown for the Tydemans stations (st300, st400 and st500) are averaged values of samples collected between 5 and 30 m

Stn	Prochlorophytes	Cyanobacteria	Dinoflagellates	Diatoms	Prymnesiophytes	Chrysophytes	Chlorophytes	Cryptophytes
st300	13	22	0	0	11	3	0	0
st400	13	21	0	0	11	3	0	0
st500	13	23	0	0	12	4	1	0
AB1	0	2622	1789	901	65	253	309	62
PB1	51	22	0	0	7	3	18	5
OO1	78	94	15	17	28	13	61	14

Table 5. Relative contribution, in cell numbers and amount of DNA, of 4 species clusters as analyzed by flow cytometry at the Tydeman stations. Shown are averaged values of 4 or 5 samples collected between 0 and 40 m

Stn	Heterotrophic bacteria		<i>Prochlorococcus</i> sp.		<i>Synechococcus</i> sp.		Eukaryotic algae	
	% cells	% DNA	% cells	% DNA	% cells	% DNA	% cells	% DNA
st300	81.5 ± 1.1	63.7 ± 1.6	18.3 ± 1.1	35.7 ± 1.6	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.4 ± 0.08
st400	77.1 ± 2.2	57.0 ± 3.0	22.2 ± 2.3	40.9 ± 3.2	0.7 ± 0.19	1.4 ± 0.42	0.1 ± 0.02	0.8 ± 0.11
st500	80.3 ± 0.5	61.4 ± 0.7	18.9 ± 0.7	36.1 ± 1.3	0.6 ± 0.40	1.4 ± 0.89	0.2 ± 0.01	1.2 ± 0.06

Table 6. Relative contribution, in cell numbers and amount of DNA, of the species groups from Table 5 subdivided into 2 size classes. Assumed is that all the small heterotrophic bacteria and *Prochlorococcus* sp. were present on the 0.2 µm filter and the *Synechococcus* sp. and eukaryotic algae on the 1 µm filter

Stn	0.2 µm filter				1 µm filter			
	Heterotrophic bacteria		<i>Prochlorococcus</i> sp.		<i>Synechococcus</i> sp.		Eukaryotic algae	
	% cells	% DNA	% cells	% DNA	% cells	% DNA	% cells	% DNA
st300	81.7 ± 1.1	64.1 ± 1.6	18.3 ± 1.1	35.9 ± 1.6	62.3 ± 4.4	35.3 ± 4.2	37.7 ± 4.4	64.7 ± 4.2
st400	77.7 ± 2.3	58.2 ± 3.2	22.3 ± 2.3	41.8 ± 3.2	83.6 ± 7.4	63.7 ± 11	16.4 ± 7.4	36.3 ± 11
st500	81.0 ± 0.7	63.0 ± 1.0	19.0 ± 0.7	37.0 ± 1.0	73.2 ± 14	49.6 ± 17	26.8 ± 14	50.4 ± 17

depth of the wind-mixed layer exceeded the actual depth of the sample locations (Anna Bay and Pest Bay) the real depth was used.

At the central Atlantic stations the mean BED in the wind-mixed layer varied between 118 and 250 J m⁻² d⁻¹. The highest BED_m was found at Stn 500 due to the relatively shallow wind-mixed layer. The mean BEDs as calculated for the stations off Curaçao were much higher due to the high daily doses at the surface, except in the eutrophic Anna Bay, where the high attenuation coefficient (1.6 m⁻¹) caused a very low BED_m (Table 3).

DNA damage in field samples

We detected thymine dimers in DNA in all field samples collected in the central Atlantic Ocean and off Curaçao. Values varied from 20 CPDs/10⁶ nucleotides in the 0.2 to 1 µm fraction collected in the central Atlantic Ocean to almost 0 in some samples collected in Anna Bay and Pest Bay off Curaçao (Fig. 3).

No clear relationship was found between sample depth and amount of CPDs, except for Stn 500, where there seemed to be a trend with depth. This station was characterized by strong stratification of the water column with a wind-mixed layer of only 7.5 m. At the other stations the calculated wind-mixed layer exceeded the sample depth.

Occasionally samples were taken in the morning and evening. No significant trend was seen during the day (data not shown).

There was a significant difference (2-way ANOVA without replication, p < 0.001) between the amount of CPDs detected in the 2 size fractions. We detected more photoproducts in DNA isolated from the 0.2 µm filters (Fig. 3A) than in DNA isolated from the 1 µm filters (Fig. 3B).

DISCUSSION

Clearly, our method of CPD detection is a sensitive tool to detect low amounts of DNA damage in natural plankton samples. No or negligible signals were found in non-UVBR-exposed cultures of *Synechococcus* cells and in some field samples, excluding aspecific binding of antibody to isolated DNA.

The results demonstrate that bacterio- and phytoplankton undergo UVBR stress in the field via CPD induction (Fig. 3). Even though UVAR may induce CPDs as well, this occurs at a very slow rate, being several orders of magnitude lower than those induced as a result of UVBR exposure (Giacomoni 1995). Moreover, CPD induction by UVAR could not be detected in living phytoplankton cells (Buma et al. 1997). Generally, CPD numbers were low compared to the damage found in the culture experiments. Although the culture experiments demonstrated the vulnerability of a single species whereas in the field more resistant or sensitive species/clones may prevail, the amount of CPDs measured in field samples from the same size fraction as *Synechococcus* cells

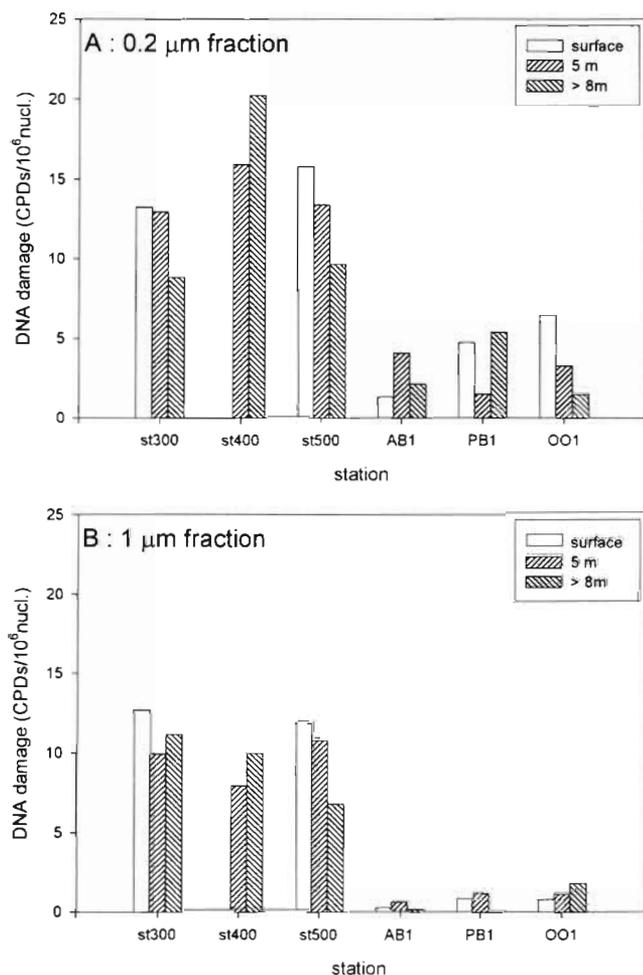


Fig. 3. DNA damage (CPDs) measured in 2 different size fractions of field samples collected between 13:00 and 16:00 h at several depths. Samples were collected between 0 and 2 m depth (surface), at 5 m depth (5 m) and at 15 m depth, except for AB1 (Anna Bay), where samples were taken at 8 m depth (>8 m). At Stn 400 no surface samples were collected (0.2 µm fraction: fraction between 0.2 and 1 µm; 1 µm fraction: fraction between 1 and 10 µm). For information on sampling stations, codes and dates see Table 1

(1 to 10 µm, Fig. 3b) corresponded to CPD levels induced in *Synechococcus* cultures exposed to the lowest UVBR doses (up to $900 \text{ J m}^{-2} \text{ d}^{-1}$, Fig. 2). This is not surprising because the calculated mean BEDs in the mixed layer are much lower as compared to incident UVBR doses: the maximal BED_0 was $2263 \text{ J m}^{-2} \text{ d}^{-1}$, whereas the maximal BED_m was only $569 \text{ J m}^{-2} \text{ d}^{-1}$. This emphasizes the importance of including mixing phenomena in UVBR effect studies: even though incident UVBR doses at these latitudes may be high, the extent of mixing strongly reduces the UVBR level experienced by plankton cells collected from surface waters. On the other hand, cells mixed from deeper waters, for instance close to the 1%

UVBR depth, to shallower depths, will experience elevated UVBR levels. In this way, damage is distributed throughout the whole mixed layer.

Comparable low amounts of DNA damage were measured by Visser et al. (1999) in field samples collected off Curaçao and in Antarctic zooplankton collected by Malloy et al. (1997). Jeffrey et al. (1996b) measured DNA damage in bacterio- and phytoplankton in the Gulf of Mexico combined with DNA dosimeter measurements. While Jeffrey et al. (1996b) measured comparable BEDs, the number of CPDs they found in bacterioplankton was much higher (up to 400 CPDs/Mbp DNA $\approx 200 \text{ CPDs}/10^6$ nucleotides) than the levels found in our study. Besides differences in sampling procedures, DNA isolation and DNA damage quantification methods, seasonal variations, differences in species composition and mixing depth could explain the discrepancies in the results.

Since the depth of the wind-mixed layer exceeded the sample depth at most stations, depth-dependent trends in photodamage were not expected, and were not observed. Only at Stn 500, a decreasing trend with depth was found (Fig. 3). Here, the calculated wind-mixed layer was only 7.5 m.

We measured a significant difference between the number of CPDs detected in the 2 size fractions. In DNA isolated from the 0.2 to 1 µm fraction, consisting mainly of heterotrophic bacteria and prochlorophytes, more photoproducts were detected compared to the 1 to 10 µm fraction (mainly *Synechococcus* sp. and eukaryotic algae). Similar differences were found by Jeffrey et al. (1996b) in samples collected in the Gulf of Mexico. This effect can be explained by differences in cell size. Karentz et al. (1991) found a positive trend between surface area:volume ratio and photoproduct induction in cultures of Antarctic phytoplankton. Additionally, Garcia-Pichel (1994) calculated that picoplankton, due to their small cell size, can not efficiently use UVR-absorbing compounds, such as mycosporine-like amino acids. Therefore, smaller organisms would have less ability to protect themselves from UVR damage.

Generally, the amount of DNA damage was higher in the central Atlantic Ocean than at the stations off Curaçao (Fig. 3). These differences cannot be explained by the amount of UVBR the plankton received at the sample locations. Despite the fact that the penetration of UVBR into the water column was lower at Curaçao, the mean biologically effective doses were higher, except at the eutrophic Anna Bay (Table 3). Differences in photorepair rate may have played a role as well. Although speculative, photorepair rates may not have been optimal at all stations. Since photorepair depends on levels of UVR and PAR (Friedberg et al. 1995), the final amount of residual CPDs as measured

in the samples will depend on the UVAR/UVBR and PAR/UVBR ratios experienced by the cells. At Curaçao, UVBR levels were higher than at the central Atlantic stations. It is highly likely that incident UVAR and PAR were also higher at Curaçao. Finally, UVAR:PAR:UVBR ratios could be higher as well, due to differences in water characteristics such as yellow substance concentration (Boelen et al. 1999). Thereby residual DNA damage could be lower at these locations.

Another possibility for the lower amount of DNA damage found at the stations near Curaçao is a difference in species composition. The ratio of cyanobacteria to eukaryotic algae, in terms of chlorophyll *a* concentrations, at the Atlantic stations was higher than at the stations off Curaçao (Table 4). This implies that at the stations near Curaçao relatively more of the total amount of DNA in the 1 to 10 μm fraction originated from larger eukaryotic phototrophic organisms. Since large cells are generally less sensitive to UVBR, less DNA damage may have occurred at these stations. At the central Atlantic stations most of the cellular DNA in the surface layer originated from small marine heterotrophic bacteria and *Prochlorococcus* sp. (Table 5). These results correspond to those of Paul et al. (1985) that most (70 to 99%) of the particulate DNA in offshore samples is in the 0.2 to 1 μm fraction. No flow cytometer samples were collected at the stations off Curaçao, but bacterial numbers at the Atlantic stations (results not shown) were comparable with bacterial numbers found in 1994 and 1995 near Curaçao by Gast et al. (1998). Since the amount of (divinyl-)chlorophyll *a* contributed by prochlorophytes was higher at the OO1 and PB1 stations than in the central Atlantic Ocean (Table 4), more prochlorophytes relative to heterotrophic bacteria were collected on the 0.2 μm filters. This relatively higher amount of prochlorophytes could be responsible for the lower amount of DNA damage found at these stations.

In conclusion, our results demonstrate that the amount of CPDs in a population is difficult to predict from incident irradiances. Also, given the differences found between the 2 size fractions, it is of major importance to distinguish between various species groups. Likely mixing phenomena play an important role in the occurrence of UVBR stress in marine organisms. Neale et al. (1998) calculated interactive effects of ozone depletion and vertical mixing on UVR-mediated inhibition of photosynthesis. They showed that UVBR stress may either be diminished or enhanced, depending on the mixing depth. Our next objective is to study the effect of wind-induced, vertical mixing on the rate of DNA damage formation. This will be done by excluding mixing by *in situ* incubation experiments at several depths.

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LITERATURE CITED

- Aas P, Lyons MM, Pledger R, Mitchell DL, Jeffrey WH (1996) Inhibition of bacterial activities by solar radiation in nearshore waters and the Gulf of Mexico. *Aquat Microb Ecol* 11:229–238
- Behrenfeld M, Hardy J, Gucinski H, Hanneman A, Lee HI, Wones A (1993) Effects of ultraviolet-B radiation on primary production along latitudinal transects in the South Pacific Ocean. *Mar Environ Res* 35:349–363
- Boelen P, Obernosterer I, Vink AA, Buma AGJ (1999) Attenuation of biologically effective UV radiation in tropical Atlantic waters, as measured with a biochemical DNA dosimeter. *Photochem Photobiol* 69:34–40
- Buma AGJ, van Hannen EJ, Roza L, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *J Phycol* 31:314–321
- Buma AGJ, Engelen AH, Gieskes WWC (1997) Wavelength-dependent induction of thymine dimers and growth rate reduction in the marine diatom *Cyclotella* sp. exposed to ultraviolet radiation. *Mar Ecol Prog Ser* 153:91–97
- Doyle JJ, Doyle JL (1991) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15
- Drollet JH, Glaziou P, Martin PMV (1993) A study of mucus from the solitary coral *Fungia fungites* (Scleractinia: Fungiidae) in relation to photobiological UV adaptation. *Mar Biol* 115:263–266
- Fekete A, Vink AA, Gaspar S, Berces A, Modos K, Ronto G, Roza L (1998) Assessment of the effects of various UV sources on inactivation and photoproduct induction in phage T7 dosimeter. *Photochem Photobiol* 68:527–531
- Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM Press, Washington, DC
- Garcia-Pichel F (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnol Oceanogr* 39:1704–1717
- Gast GJ, Wiegman S, Wieringa E, van Duyl FC, Bak RPM (1998) Bacteria in coral reef water types: removal of cells, stimulation of growth and mineralization. *Mar Ecol Prog Ser* 167:37–45
- Giacomini PU (1995) Open questions in photobiology: II. Induction of nicks by UV-A. *J Photochem Photobiol B Biol* 29:83–85
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York, p 29–60
- Helbling EW, Villafane Y, Holm-Hansen O (1994) Effects of ultraviolet radiation on Antarctic marine photosynthesis with particular attention to the influence of mixing. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. *Antarctic Res Ser* 62, American Geophysical Union, Washington DC, p 207–229
- Jeffrey WH, Aas P, Lyons MM, Coffin RB, Pledger RJ, Mitchell DL (1996a) Ambient solar radiation-induced photo-

- damage in marine bacterioplankton. *Photochem Photobiol* 64:419–427
- Jeffrey WH, Pledger RJ, Aas P, Hager S, Coffin RB, von Haven R, Mitchell DL (1996b) Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. *Mar Ecol Prog Ser* 137: 283–291
- Karentz D, Cleaver JE, Mitchell DL (1991) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *J Phycol* 27: 326–341
- Kraay GW, Zapata M, Veldhuis MJW (1992) Separation of chlorophylls c1, c2 and c3 of marine phytoplankton by reversed-phase-c18-high-performance liquid chromatography. *J Phycol* 28:708–712
- Lyons MM, Aas P, Pakulski JD, van Waasbergen L, Miller RV, Mitchell DL, Jeffrey WH (1998) DNA damage induced by ultraviolet radiation in coral-reef microbial communities. *Mar Biol* 130:537–543
- Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. *Mar Ecol Prog Ser* 144: 265–283
- Malloy KD, Holman MA, Mitchell D, Detrich HW (1997) Solar UVB-induced DNA damage and photoenzymatic DNA repair in Antarctic zooplankton. *Proc Natl Acad Sci USA* 94:1258–1263
- Neale PJ, Davis RF, Cullen JJ (1998) Interactive effects of ozone depletion and vertical mixing on photosynthesis of Antarctic phytoplankton. *Nature* 392:585–589
- Paul JH, Jeffrey WH, DeFlaun M (1985) Particulate DNA in subtropical oceanic and estuarine planktonic environments. *Mar Biol* 90:95–101
- Roza L, van der Wulp KJM, McFarlane SJ, Lohman PHM, Baan RA (1988) Detection of cyclobutane thymine dimers in DNA of human cells with monoclonal antibodies raised against a thymine dimer-containing tetranucleotide. *Photochem Photobiol* 48:627–633
- Setlow RB, Swenson PA, Carrier WL (1963) Thymine dimers and inhibition of DNA synthesis by ultraviolet radiation of cells. *Science* 142:1464–1465
- Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc Nat Acad Sci USA* 71:3363–3366
- Shibata K (1969) Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant Cell Physiol* 10:325–335
- Smith RC, Baker KS (1979) Penetration of UV-B and biologically effective dose-rates in natural waters. *Photochem Photobiol* 29:311–323
- Swenson PA, Setlow RB (1966) Effects of ultraviolet radiation on macromolecular synthesis in *Escherichia coli*. *J Mol Biol* 15:201–219
- Tyrrell RM (1986) Repair of genetic damage induced by UV-B (290–320 nm) radiation. In: Worrest RC, Caldwell MM (eds) Stratospheric ozone reduction, solar ultraviolet radiation and plant life. Springer-Verlag, Berlin, p 139–149
- Veldhuis MJW, Kraay GW (1990) Vertical distribution and pigment composition of a picoplanktonic prochlorophyte in the subtropical North Atlantic: a combined study of HPLC-analysis of pigments and flow cytometry. *Mar Ecol Prog Ser* 68:121–127
- Veldhuis MJW, Cucci TL, Sieracki ME (1997) Cellular DNA content of marine phytoplankton using two new fluorochromes: Taxonomic and ecological implications. *J Phycol* 33:527–541
- Veth C (1991) The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia-Weddell Sea. *J Mar Syst* 2:451–464
- Visser PM, Snelder E, Kop AJ, Boelen P, Buma AGJ, Van Duyl FC (1999) Effects of UV radiation on DNA photodamage and production in bacterioplankton in the coastal Caribbean Sea. *Aquat Microb Ecol* 20:49–58
- Wilhelm SW, Weinbauer MG, Suttle CA, Pledger RJ, Mitchell DL (1998) Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. *Aquat Microb Ecol* 14:215–222
- Wood WF (1987) Effect of solar ultra-violet radiation on the kelp *Ecklonia radiata*. *Mar Biol* 96:143–150

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