European Journal of Phycology

ISSN: 0967-0262 (Print) 1469-4433 (Online) Journal homepage: https://www.tandfonline.com/loi/tejp20

Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta)

Hans Pakker , Chantal Beekman & Anneke Breeman

To cite this article: Hans Pakker , Chantal Beekman & Anneke Breeman (2000) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta), European Journal of Phycology, 35:2, 109-114, DOI: 10.1080/09670260010001735691

To link to this article: https://doi.org/10.1080/09670260010001735691

Published online: 03 Jun 2010.

Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta)

HANS PAKKER, CHANTAL A.C. BEEKMAN AND ANNEKE M. BREEMAN

Department of Marine Biology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

(*Received 11 May 1999; accepted 25 January 2000*)

Repair of DNA damage induced by ultraviolet-B radiation (UVBR) was investigated in the sublittoral red alga *Rhodymenia pseudopalmata* at different temperatures, using immunofluorescent detection of thymine dimers. Photoreactivation of thymine dimers was completed within about 3 h at 6, 12 and 18 °C in the presence of ultraviolet-A radiation (UVAR) and photosynthetically active radiation (PAR) but no repair was found in dark-incubated fragments after 16–17 h. In plants previously exposed to a low UVBR dose (biologically effective dose: $BED_{DNA300} = 1{\cdot}6$ kJ m^{-2}; unweighted irradiance = 0·72 W m^{-2} for 4 h), photoreactivation started within the first hour under UVAR + PAR at 18 °C but repair only started after 1–2 h at 12 and 6 °C. At 6 °C, repair was more efficient after exposure to a high UVBR dose ($BED_{DNA300} = 3{\cdot}9$ kJ m^{-2}; unweighted irradiance = 0·78 W m^{-2} for 5 h) than to a lower dose ($BED_{DNA300} = 1{\cdot}6$ kJ m^{-2}); no such difference was found at 18 °C. It is concluded that *R. pseudopalmata* is able to repair DNA damage induced by high UVBR doses efficiently.

Key words: dark repair, DNA damage, photoreactivation, Rhodophyta, *Rhodymenia pseudopalmata*, temperature, thymine dimer, ultraviolet-B radiation

Introduction

The increase in ultraviolet-B radiation (UVBR, 280–315 nm) caused by ozone depletion has led to increased research on the effects of UVBR on plants (for reviews see Rozema *et al.*, 1997; Franklin & Forster, 1997). Among the primary targets of UVBR is nuclear DNA. DNA damage occurs when UVBR causes covalent bonds between two adjacent pyrimidine dimers. Cyclobutyl pyrimidine dimers (CPDs) are the most abundant type of dimers formed. Of the four possible types of CPDs (TT, TC, CT and CC), thymine dimers are the most frequent (Friedberg *et al.*, 1995). One of the consequences of dimer formation is that DNA polymerase, and thus genome replication, is blocked. This may lead to a delay of cell division (Buma *et al.*, 1996, 2000; Gieskes & Buma, 1997), and ultimately to growth reduction.

Thymine dimers can be restored to their monomers by photoreactivation, a light dependent process which is fast and error-free. Although photoreactivation appears to be a ubiquitous repair mechanism, there are several organisms, including diatoms and higher plants, in which it has not been found (Mitchell & Karentz, 1993). A repair mechanism which is independent of light is nucleotide excision repair or 'dark repair'. Several enzymes are involved in excising the damaged oligonucleotide and in replacing it by a newly synthesized fragment (Mitchell & Karentz, 1993; Karentz, 1994; Britt, 1995). Organisms that display efficient photoenzymatic repair appear to have a reduced capacity for excision repair; conversely, many organisms that lack photoenzymatic repair have a greater capacity to remove CPDs by excision repair (Mitchell & Karentz, 1993). Photoenzymatic and excision repair have been demonstrated in many different organisms, including microalgae (Karentz *et al.*, 1991; Buma *et al.*, 1995), higher plants (Pang & Hays, 1991; Quaite *et al.*, 1994; Taylor *et al.*, 1996) and vertebrates (Hitomi *et al.*, 1997).

The potential effects that UVBR may exert on macroalgal DNA have been largely overlooked, in contrast with the increasing amount of information available for higher plants (Pang & Hays, 1991; Chen *et al.*, 1994; Quaite *et al.*, 1994; Britt, 1995; Taylor *et al.*, 1996; Takeuchi *et al.*, 1996, 1998; Hada *et al.*, 1998; Kang *et al.*, 1998) and microalgae (Karentz *et al.*, 1991; Mitchell & Karentz, 1993; Karentz, 1994; Buma *et al.*, 1995, 1996, 1997, 2000; Gieskes & Buma, 1997). Recently, Pakker *et al.* (2000) showed that the low intertidal to upper sublittoral red alga *Palmaria palmata* is able to photoreactivate UVBR-induced thymine dimers within a few hours. Dark repair proceeded more slowly, but after 19 h approximately 67% of thymine dimers were removed. Photoenzymatic repair was found to be temperature dependent in this species, with higher

Correspondence to A.M. Breeman. e-mail: a.m.breeman@biol.rug.nl

repair efficiencies of thymine dimers at higher temperatures.

In the present study, we investigated the repair of UVBR-induced thymine dimers in the red alga *Rhodymenia pseudopalmata* (Lamouroux) Silva from Brittany (France). This species mainly grows in the sublittoral on rocks and kelp stipes but it may also be found in shady pools and crevices in the lower littoral zone (Irvine, 1983). Repair of thymine dimers was determined after exposure to low and to high UVBR doses, both under photoreactivating light and in darkness, and at three temperatures (6, 12 and 18 °C), simulating local seawater temperatures at different times of year (US Navy, 1981). Due to ozone depletion, high UVBR levels may occur during the main growing season in spring, at relatively low temperatures. In summer, relatively high temperatures may coincide with high UVBR levels due to the high solar angle.

Materials and methods

Plant material

Rhodymenia pseudopalmata was collected in August 1996 from the upper sublittoral at Pointe de Mousterlin (Brittany, France), cleaned, and cultured in the laboratory as a unialgal isolate in aerated 5 litre glass tanks in autoclaved seawater with half-strength Provasoli enrichment (Starr & Zeikus, 1987). The Provasoli enrichment was modified by using Hepes as buffer (McFadden & Melkonian, 1986) instead of Tris, to reduce bacterial growth. Plants were grown at 12 °C under a photoperiod of 16 : 8 h L : D with a photon irradiance of 10–35 μmol photons m^{-2} s^{-1}.

Irradiance measurements

In all experiments, lamp spectra were measured with a MACAM SR9910 double monochromator scanning spectroradiometer (Macam Photometrics, UK) with a cosine corrected sensor. The biologically effective dose of UVBR (BED_{DNA300}) was calculated using the DNA action spectrum of Setlow (1974), normalized to 1 at 300 nm. The use of action spectra is necessary in order to be able to compare data obtained from different UVBR sources (for full details see Rundel, 1983; Caldwell *et al*., 1986; Cullen & Neale, 1997). Although the action spectrum of Setlow (1974) was obtained from various biological systems that have no shielding in the UV range, whereas the multilayered structure and the presence of UVR screening/absorbing substances affect the spectral composition of the light reaching the target DNA in the plant (Sinha *et al*., 1998), the use of a spectral weighting function is preferable to quantification of the dose as unweighted UVBR (Cullen & Neale, 1997). Weighted doses were chosen based on Björn & Murphy's (1985) calculations of the BED_{DNA300} at the collection locality. The highest dose (3·9 kJ m^{-2}) represents the maximum BED_{DNA300} at the sea surface in midsummer under a 20% ozone reduction scenario (Björn & Murphy, 1985). In fact, the actual BED_{DNA300} in the field would be less because of tidal immersion of the plants for all or part of the day. The lower dose (1·6 kJ m^{-2}) was chosen arbitrarily and represents about 40% of the higher dose. The only documented data on UVBR attenuation in the water column at this location (Franklin & Forster, 1997, table 1) give a UVBR penetration depth of 2·4 m (depth at which irradiance ($\lambda = 320$ nm) is reduced to 10% of that immediately below the surface), which fits well with the Jerlov (1976) water types (oceanic type III to coastal type 1) recorded for the area (Lüning, 1990). As attenuation further increases towards shorter wavelengths, even the lower dose used in the experiments ($BED_{DNA300} = 1{\cdot}6$ kJ m^{-2}) probably surpasses the UVBR levels to which plants are often exposed in the field.

Experimental set-up

Apical fragments of 1–2 cm were used for the experiments. They were incubated in large aerated 2 litre Duralex glass beakers (Schott, Mainz, Germany) containing 1·6 litres of seawater, which were placed in a cryostat-controlled water bath ($\pm 0{\cdot}2$ °C). Square plastic frames, which held the appropriate cut-off filter to remove undesired wavelengths, were placed on top of the beakers. Fluorescent lamps were placed immediately above the cut-off filters at an appropriate distance. First, thymine dimers were induced by incubating plant fragments in an aerated 2 litre glass beaker under TL12 UVBR lamps (Philips, The Netherlands), either with an Ultraphan UBT 500 μm or an UBT 140 μm cut-off filter (Digefra, Munich, Germany). After the UVB lamps were switched off, repair of thymine dimers was monitored in the light and in darkness. One half of the fragments were placed under ultraviolet-A radiation (UVAR; provided by QPanel 340 lamps, Cleveland, OH, USA) plus photosynthetically active radiation (PAR; provided by Lumilux Deluxe Biolux lamps, Osram, Germany) in order to monitor the time course of repair in the light. A 320 nm filter (130 μm, Folex, Dreieich, Germany) was used to block the UVBR emitted by the QPanel lamp. The other half of the fragments were transferred immediately after UVBR treatment to another aerated beaker, which was kept in darkness by wrapping it with aluminium foil, and placed in the same water bath. Duplicate samples, each consisting of two to six fragments, were taken at different times during the experiment (see below). Samples were dabbed dry, put in silica gel and stored immediately at 4 °C in the dark prior to DNA extraction (see below).

Repair of thymine dimers

Repair of UVBR-induced thymine dimers was studied at 6, 12 and 18 °C. Fragments were acclimated to the experimental temperature for 1 week. Plants grew well during acclimation at 12 and 18 °C but little growth took

place at 6 °C. In the first experiment, plants were exposed to a relatively low dose of UVBR (BED_{DNA300} = 1·6 kJ m^{-2}; unweighted irradiance = 0·72 W m^{-2} for 4 h). The UBT 500 μm cut-off filter was used to block low-wavelength UVBR. Irradiation conditions during the 17 h repair period were: UVAR = 2·0 W m^{-2}, and PAR = 30 μmol photons m^{-2} s^{-1}. Samples were taken before UVBR exposure (control), directly after UVBR exposure, and during the light repair period at t = 1, 2, 3 and 17 h. Dark-incubated fragments were sampled after 17 h only.

When we found efficient repair of thymine dimers after exposure to a low UVBR dose, the experiment was repeated at 6 and 18 °C using a much higher UVBR dose. Fragments were irradiated for 5 h with UVBR. The UBT 140 μm cut-off filter was used in order to increase the BED_{DNA300} to 3·9 kJ m^{-2}, while keeping the unweighted irradiance approximately the same as in the previous experiment (0·78 W m^{-2}). The irradiance conditions during the light repair period (16 h) were the same as in the previous experiment. Samples were taken before UVBR exposure (control), directly after UVBR exposure, and during the light repair period at t = 0, 0·5, 1, 1·5, 2·25, 3 and 16 h. Dark-incubated fragments were sampled after 16 h only.

DNA extraction

Silica gel-dried samples (20–30 mg) were homogenized in liquid nitrogen in a sterile 2 ml Eppendorf tube using a small plastic pestle. Subsequently, DNA was extracted using the CTAB (Hexadecyltrimethyl-ammonium-bromide) procedure (modified after Doyle & Doyle, 1987): 0·6 ml 2% CTAB extraction buffer (1·4 M NaCl; 20 mM EDTA; 100 mM Tris HCl pH 8·0) with 2% (w/v) polyvinylpyrrolidone (PVP) and 0·2% (v/v) 2-mercaptoethanol was added and samples placed on a turntable for 2 h at room temperature; 2/3 volume chloroform–isoamyl alcohol (CIA; 24: 1 v/v) was added, mixed and centrifuged (14 000 rpm, 10 min). The supernatant containing the DNA was removed and the previous step repeated. DNA was precipitated with 2/3 volume cold (−20 °C) isopropanol followed by centrifugation (Eppendorf, 14 000 rpm, 30 min). The pellet was washed twice with 70% cold (−20 °C) ethanol, vacuum-dried and dissolved in 0·3 ml Tris/EDTA buffer (TE). After RNase treatment (30 min at 37 °C), DNA was quantified fluorometrically with the PicoGreen dsDNA quantitation assay (Molecular Probes). Samples were either processed immediately or stored at −20 °C.

Detection of thymine dimers

The procedure for the immunoassay for the quantification of thymine dimers was slightly modified after Vink *et al.* (1994) and Boelen *et al.* (1999). Heat-denatured samples containing 0·2 mg DNA in 100 or 200 μl phosphate-buffered saline (PBS) were transferred to a nylon filter (Nytran, pore size 0·45 μm, Schleicher & Schuell, Dassel, Germany) using a Minifold I SRC96D slotblot apparatus (Schleicher & Schuell). A dilution series (0–150 ng) of standard damaged calf thymus DNA was added as a reference. The filter was then dried for 2 h at 80 °C to immobilize the DNA. To block non-specific sites, the filter was incubated for 30 min in PBS-T (PBS + 0·1% (v/v) Tween 20, Sigma) with 5% (w/v) skimmed milk powder. Subsequently, the filter was washed in PBS-T (3 ×) and incubated with the primary antibody, the thymine-dimer-specific H3 (Roza *et al.*, 1988). It has been shown (Fekete *et al.*, 1998) that this antibody has a high affinity for TC as well as for TT dimers but, as the latter predominate (Mitchell & Karentz, 1993), we will refer to them both as 'thymine dimers' for the sake of brevity. After washing (3 ×), the filter was incubated with the secondary antibody (rabbit anti-mouse conjugated with alkaline phosphatase, Dako, 1: 5000 in 0·5% skimmed milk-powder PBS-T). After washing (4 ×), the filter was transferred to a buffer containing a detection reagent (Lumi-Phos Plus, Gibco BRL), which emits light during breakdown of the alkaline phosphatase. The filter was sealed in a transparent folder (Photogene development folder, Gibco BRL) and exposed to a photosensitive sheet (Kodak X-AR-5) for 1–30 min, depending on the amount of damage in the samples. The calf thymus dilution series was used to adjust exposure time of the film in order to ensure linearity of the signal over the relevant range. After the sheet had been developed and fixed, it was scanned with a UMAX PS-2400X scanner and the grey-scale values were quantified with the software program Image QuaNT (Molecular Dynamics). Because the BED_{DNA300} is linearly correlated with both accumulated DNA damage (Britt *et al.*, 1993) and grey-scale values up to approximately 200 units (Boelen *et al.*, 1999), grey-scale values were used as a relative measure for the amount of DNA damage. Time-series for repair rates, together with control samples taken before and directly after UVBR exposure, were always processed on the same blot, to allow direct comparison.

Results

After a low UVBR dose (BED_{DNA300}: 1·6 kJ m^{-2}), repair of thymine dimers under subsequent irradiation with UVAR + PAR was found under all conditions (Fig. 1). Sixty to one hundred per cent of thymine dimers were repaired within 3 h, and complete repair was observed after 16–17 h. At 18 °C repair started within the first hour of exposure to UVAR + PAR but at 12 and 6 °C repair started only after 1–2 h. When the UVBR dose was raised during the second experiment (BED_{DNA300}: 3·9 kJ m^{-2}), complete repair under UVAR + PAR was again found at all temperatures. In fact, repair rates at 6 °C were higher after plants had been exposed to the high than to the lower UVBR dose (Fig. 1*A*). Virtually no repair was observed in the samples incubated in the dark for up to 16–17 h at any temperature (Fig. 1).

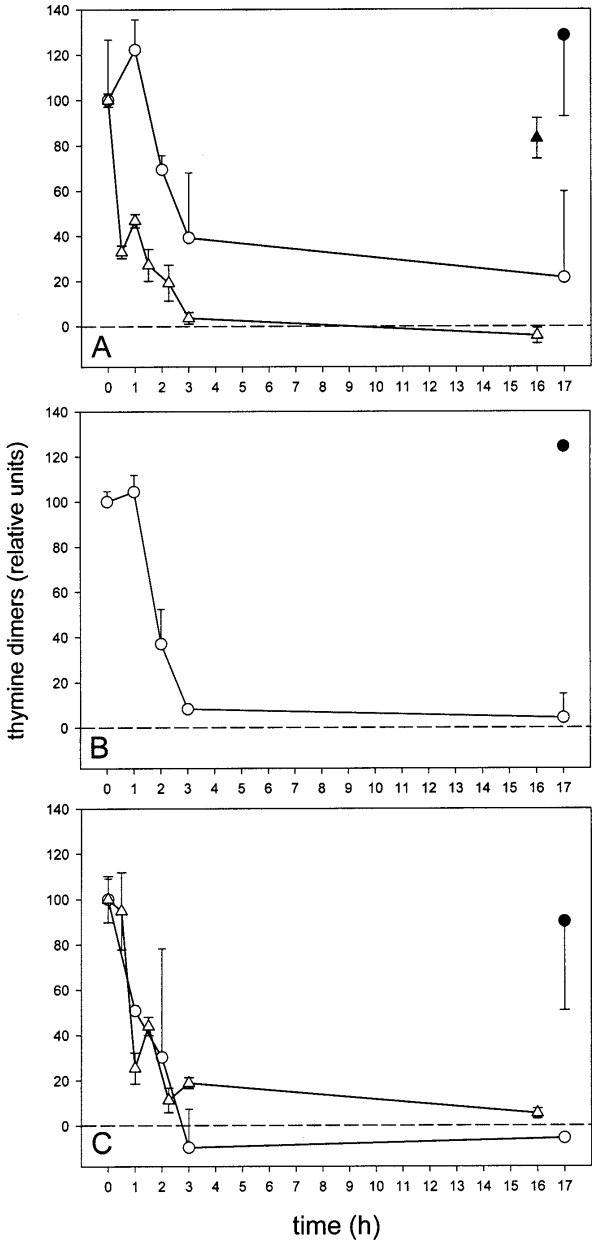

Fig. 1. Time course of the removal of thymine dimers (expressed as percentage of the damage induced) after a low dose (circles: BED_{DNA300} = 1·6 kJ m^{-2}; unweighted irradiance = 0·72 W m^{-2} for 4 h) and after a high dose (triangles: BED_{DNA300} = 3·9 kJ m^{-2}; unweighted irradiance = 0·78 W m^{-2} for 5 h) of UVBR at 6 °C (*A*), 12 °C (*B*) and 18 °C (*C*). Open symbols: under UVAR + PAR (UVAR = 2·0 W m^{-2}; PAR = 30 μmol photons m^{-2} s^{-1}); filled symbols, in darkness. Averages are given of two independent DNA extractions with standard deviations. Broken lines indicate average values of controls, taken before UVBR exposure.

Discussion

Fast photoreactivation of thymine dimers has been shown to occur in some higher plants (Taylor *et al.*, 1996; Takeuchi *et al.*, 1996), and also in the lower intertidal to upper sublittoral red alga *Palmaria palmata* (Pakker *et al.*, 2000). In these cases, complete photoreactivation took place within 2–4 h following UVBR exposure. *Rhodymenia pseudopalmata* also showed efficient photoreactivation at all temperatures tested. No nucleotide excision repair (dark repair) was detected for a period of 16–17 h after exposure to UVBR in *R. pseudopalmata*. In contrast, about 67% of thymine dimers were repaired after 19 h of darkness in *P. palmata*. Differences in the rates of photoenzymatic and dark repair have been found even among closely related species of Antarctic diatoms (Karentz *et al.*, 1991). *R. pseudopalmata* evidently belongs to the group of organisms that display efficient photoenzymatic repair but have a reduced capacity for excision repair (Mitchell & Karentz, 1993). Other examples include several species of Antarctic diatoms (Karentz *et al.*, 1991), higher plants (Pang & Hays, 1991; Quaite *et al.*, 1994; Taylor *et al.*, 1996) and archaebacteria (Eker *et al.*, 1991; McCready, 1996).

The efficiency of photorepair of thymine dimers is surprisingly high in *Rhodymenia pseudopalmata* in view of its shaded, predominantly sublittoral habitat. Repair rates were as high as those found in *Palmaria palmata*, which is also common on rocks in the lower intertidal zone. In both species, thymine dimers were repaired within a few hours after exposure to a high UVBR dose (BED_{DNA300} of 3·2–3·8 kJ m^{-2} in *P. palmata*; maximum BED_{DNA300} of 3·9 kJ m^{-2} in *R. pseudopalmata*), representing the maximum daily dose at the sea surface in midsummer under 20% ozone reduction (Björn & Murphy, 1985). In fact, photoreactivation efficiencies in the field may be even higher than those found in the experiments. First, the UVAR and PAR levels used in the experiments (2·0 W m^{-2} and 30 μmol photons m^{-2} s^{-1}, respectively) were lower than those occurring in the field on a sunny day, and repair efficiencies have been found to increase with increasing PAR levels (Takeuchi *et al.*, 1996; Taylor *et al.*, 1996; Gieskes & Buma, 1997; Kang *et al.*, 1998). Secondly, plants exposed to natural sunlight may be less sensitive than those grown in the laboratory because they have more UV-absorbing cell components (Mitchell & Karentz, 1993; Sinha *et al.*, 1998) or because of photolyase induction or activation. For example, Takayanagi *et al.* (1994) found that alfalfa seedlings grown under natural sunlight sustained less damage and had higher repair rates of dimers than plants grown under fluorescent light.

Effects of temperature on photoreactivation have been documented for higher plants (Pang & Hays, 1991; Takeuchi *et al.*, 1996), Archaea (Eker *et al.*, 1991; Grogan, 1997) and for the red alga *Palmaria palmata* (Pakker *et al.*, 2000). Effects of temperature were also found in *Rhodymenia pseudopalmata*, although they were not very strong. The main effect was a slower start of photoreactivation at 6 and 12 °C than at 18 °C after exposure to a low UVBR dose. The fast onset of repair in plants grown at 18 °C indicates that high levels of photolyases were already present at the start of the repair period. Plants grown at lower temperatures may have lower constitutive levels of photolyase or cofactor concentrations (Mitchell & Karentz, 1993), and may be unable to start repair until after induction of photolyase activity, which may take at least 1 h (e.g. Pang & Hays, 1991; Chen *et al.*, 1994; Buma *et al.*, 1996).

At 6 °C, repair of thymine dimers started faster in *Rhodymenia pseudopalmata* after exposure to a high than to a low dose of UVBR. Quaite *et al*. (1994) demonstrated that the rate of photoreactivation and excision repair increases with high initial levels of damage. Similarly, Karentz *et al*. (1991) found a correlation of increased repair with increased damage among Antarctic species of diatoms. However, in *R. pseudopalmata*, no dose-dependent effects on repair rates were observed at 18 °C. Varying rates of repair may be the result of complex interactions depending on the initial amount of damage and the temperature characteristics of the repair systems, which are at present poorly understood and should be investigated further.

Acknowledgements

The authors want to thank Professor M.D. Guiry for his help with identification of *Rhodymenia pseudopalmata*, and Dr Anita Buma and Mr Peter Boelen for their advice and support. This research was carried out within the project 'Effects of ultraviolet radiation on marine macroalgae and seagrasses', which was financed by the European Commission (Environment and Climate Programme, ENV4-CT96–0188; DG XII).

References

Björn, L. O. & Murphy, M. (1985). Computer calculation of solar ultraviolet radiation at ground level. *Physiol. Veg.*, **23**: 555–561.

Boelen, P., Obernosterer, I., Vink, A.A. & Buma, A.G.J. (1999). Attenuation of biologically effective UV radiation in tropical Atlantic waters measured with a biochemical DNA dosimeter. *Photochem. Photobiol.*, **69**: 34–40.

Britt, A.B. (1995). DNA damage and repair in plants. *Annu. Rev. Plant Mol. Biol.*, **47**: 75–100.

Britt, A.B., Chen, J.-J., Wycoff, D. & Mitchell, D. (1993). A UV-sensitive mutant of *Arabidopsis* defective in the repair of pyrimidine-pyrimidinone (6–4) dimers. *Science*, **261**: 1571–1574.

Buma, A.G.J., van Hannen, E.J., Rosa, L., Veldhuis, M.J.W. & Gieskes, W.W.C. (1995). Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *J. Phycol.* **31**: 314–321.

Buma, A.G.J., van Hannen, E.J., Veldhuis, M.J.W. & Gieskes, W.W.C. (1996). UV-B induces DNA damage and DNA synthesis delay in the marine diatom *Cyclotella* sp. Proceedings of the Second Algal Photobiology Workshop, Malaga, October 1994. *Sci. Mar.*, **60** (Suppl. 1): 101–105.

Buma, A.G.J., Engelen, A.H. & Gieskes, W.W.C. (1997). Wavelength dependent induction of thymine dimers and growth rate reduction of the marine diatom *Cyclotella* sp. exposed to ultraviolet radiation. *Mar. Ecol. Progr. Ser.*, **153**: 91–97.

Buma, A.G.J., van Oijen, T., van de Poll, W., Veldhuis, M.J.W. & Gieskes, W.W.C. (2000). On the high sensitivity of the marine prymnesiophyte *Emiliania huxleyi* to ultraviolet-B radiation. *J. Phycol.*, in press.

Caldwell, M.M., Camp, L.B., Warner, C.W. & Flint, S.D. (1986). Action spectra and their key role in assessing biological consequences of solar UV-B radiation change. In *Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life* (Worrest, R.C. & Caldwell, M.M., editors), 87–111. Nato ASI series, vol. G8. Springer, Berlin.

Chen, J.-J., Mitchell, D.L. & Britt, A.B. (1994). A light-dependent pathway for the elimination of UV-induced pyrimidine (6–4) pyrimidinone photoproducts in *Arabidopsis*. *Plant Cell*, **6**: 1311–1317.

Cullen, J.J. & Neale, P.J. (1997). Biological weighting functions for describing the effects of ultraviolet radiation on aquatic systems. In *The Effects of Ozone Depletion on Aquatic Ecosystems* (Häder, D.-P., editor), 97–118. R.G. Landes, Austin.

Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, **19**: 11–15.

Eker, A.P.M., Formenoy, L. & de Wit, L.E.A. (1991). Photoreactivation in the extreme halophilic archaebacterium *Halobacterium cutirubrum*. *Photochem. Photobiol.*, **53**: 643–652.

Fekete, A., Vink, A.A., Gaspar, S., Berces, A., Modos, K., Ronto, G.Y. & Roza, L. (1998). Assessment of the effects of various UV sources on inactivation and photoproduct induction in phage T7 dosimeter. *Photochem. Photobiol.*, **66**: 527–531.

Franklin, L.A. & Forster, R.M. (1997). The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur. J. Phycol.*, **32**: 207–232.

Friedberg, E.C., Walker, G.C. & Siede, W. (1995). *DNA repair and Mutagenesis*. ASM Press, Washington DC.

Gieskes, W.W.C. & Buma, A.G.J. (1997). UV damage to plant life in a photobiologically dynamic environment: the case of marine phytoplankton. *Plant Ecol.*, **128**: 16–25.

Grogan, D.W. (1997). Photoreactivation in an archaeon from geothermal environments. *Microbiology*, **143**: 1071–1076.

Hada, H., Hashimoto, T., Nikaido, O. & Shin, M. (1998). UVB-induced DNA damage and its photorepair in nuclei and chloroplasts of *Spinacia oleracea* L. *Photochem. Photobiol.*, **68**: 319–322.

Hitomi, K., Kim, S.-T., Iwai, S., Harima, N., Otoshi, E., Ikenaga, M. & Todo, T. (1997). Binding and catalytic properties of *Xenopus* (6–4) photolyase. *J. Biol. Chem.*, **272**: 32591–32598.

Irvine, L.M. (1983). *Seaweeds of the British Isles*, vol. 1, *Rhodophyta*, part. 2A, *Cryptonemiales* (*sensu stricto*), *Palmariales*, *Rhodymeniales*. British Museum (Natural History), London.

Jerlov, N.G. (1976). *Marine Optics*. Elsevier Oceanographic Series 14. Elsevier, Amsterdam.

Kang, H.-S., Hidema, J. & Kumagai, T. (1998). Effects of light environment during culture on UV-induced cyclobutyl pyrimidine dimers and their photorepair in rice (*Oryza sativa* L.). *Photochem. Photobiol.*, **68**: 71–77.

Karentz, D. (1994). Ultraviolet tolerance mechanisms in Antarctic marine organisms. In *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects* (Weiler, C.S. & Penhale, P.A., editors), 93–100. Antarctic Research series 62. American Geophysical Union, Washington, DC.

Karentz, D., Cleaver, J.E. & Mitchell, D.L. (1991). Cell survival characteristics and molecular responses of Antarctic phytoplankton to UV radiation. *J. Phycol.*, **27**: 326–341.

Luening, K. (1990). *Seaweeds: Their Environment, Biogeography and Ecophysiology*. Wiley, New York.

McFadden, G.I. & Melkonian, M. (1986). Use of Hepes buffer for microalgal culture media and fixation for electron microscopy. *Phycologia*, **25**: 551–557.

McCready, S. (1996). The repair of ultraviolet light-induced DNA damage in the halophilic archaebacteria, *Halobacterium cutirubrum*, *Halobacterium halobium* and *Haloferax volcanii*. *Mutat. Res.*, **364**: 25–32.

Mitchell. D.L. & Karentz, D. (1993). The induction and repair of DNA photodamage in the environment. In *Environmental UV Photobiology* (Young, A.R., Björn, L.O., Moan, J. & Nultsch, W., editors), 345–377. Plenum Press, New York.

Pakker, H., Martins, R.S.T., Boelen, P., Buma, A.G.J., Nikaido, O. & Breeman, A.M. (2000). Effects of temperature on the photoreactivation of Ultraviolet-B induced DNA damage in *Palmaria palmata* (Rhodophyta). *J. Phycol.*, in press.

Pang, Q. & Hays, J.B. (1991). UV-B-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. *Plant Physiol.* (*Bethesda*), **95**: 536–543.

Quaite, F.E., Takayanagi, S., Ruffini, J. Sutherland, J.C. & Sutherland, B.M. (1994). DNA damage levels determine cyclobutyl pyrimidine dimer repair mechanisms. *Plant Cell*, **6**: 1635–1641.

Roza, L., van der Wulp, K.J.M., McFarlane, S.J., Lohman, P.H. & Baan, R.A. (1988). Detection of cyclobutane thymine dimers in DNA of human cells with monoclonal antibodies raised against thymine dimer-containing tetranucleotide. *Photochem. Photobiol.*, **48**: 627–633.

Rozema, J., van de Staaij, J., Björn, L.O. & Caldwell, M. (1997). UV-B as an environmental factor in plant life: stress and regulation. *Trends Ecol. Evol.*, **12**: 22–28.

Rundel, R.D. (1983). Action spectra and estimation of biologically effective UV radiation. *Physiol. Plant.*, **58**: 360–366.

Setlow, R.B. (1974). The wavelength of sunlight effective in producing skin

cancer: a theoretical analysis. *Proc. Natl. Acad. Sci. USA*, **71**: 3363–3366.

Sinha, R.P., Klisch, M., Groeniger, A. & Häder, D.P. (1998). Ultraviolet screening/absorbing substances in cyanobacteria, phytoplankton and macroalgae. *J. Photochem. Photobiol. B*, **47**: 83–94.

Starr, R.C. & Zeikus, J.A. (1987). UTEX: the Culture Collection of Algae at the University of Texas at Austin. *J. Phycol.*, **23** (Suppl.): 1–47.

Takayanagi, S., Trunk, J.G., Sutherland, J.C. & Sutherland, B.M. (1994). Alfalfa seedlings grown outdoors are more resistant to UV-induced damage than plants grown in a UV-free environmental chamber. *Photochem. Photobiol.*, **60**: 363–367.

Takeuchi, Y., Murakami, M., Nakajima, N., Kondo, N. & Nikaido, O. (1996). Induction and repair of damage to DNA in cucumber cotyledons irradiated with UV-B. *Plant Cell Physiol.*, **37**: 181–187.

Takeuchi, Y., Murakami, M., Nakajima, N., Kondo, N. & Nikaido, O. (1998). The photorepair and photoisomerization of DNA lesions in etiolated cucumber cotyledons after irradiation by UV-B depends on wavelength. *Plant Cell Physiol.*, **39**: 745–750.

Taylor, R.M., Nikaido, O., Jordan, B.R., Rosamond, J., Bray, C.M. & Tobin, A.K. (1996). Ultraviolet-B induced DNA lesions and their removal in wheat (*Triticum aestivum* L.) leaves. *Plant, Cell Environ.*, **19**: 171–181.

US NAVY (1981). *Marine Climatic Atlas of the World*, vol. 9, *World-Wide Means and Standard Deviations*. US Government Printing Office, Washington, DC.

Vink, A.A., Bergen Henegouwen, J.B., Nikaido, O., Baan, R.B. & Roza, L. (1994). Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. *J. Photochem. Photobiol. B*, **24**: 25–31.