Patterns in ultraviolet radiation sensitivity of tropical, temperate and Arctic marine macroalgae

van de Poll, Willem

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Chapter 4

Ultraviolet-B-induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes

Willem H. van de Poll, Dieter Hanelt, Kirsten Hoyer, Anita G. J. Buma and Anneke M. Breeman

ABSTRACT

The significance of ultraviolet-B radiation (UVBR: 280-315 nm)-induced DNA damage as a stress factor for Arctic marine macrophytes was examined in the Kongsfjord (Spitsbergen, 78°55.5’N, 11°56.0’E) in summer. UVBR penetration in the water column was monitored as accumulation of cyclobutane-pyrimidine dimers (CPDs) in bare DNA. This showed that UVBR transparency of the fjord was variable, with 1% depths ranging between 4 and 8 m. In addition, induction and repair kinetics of CPDs were studied in several subtidal macrophytes obtained from the Kongsfjord (5-15 m). Surface exposure experiments demonstrated CPD accumulation in Palmaria palmata, Devaleraea ramentacea, Phycodrys rubens, Coccotylus truncatus and Odonthalia dentata. In artificial light, field-collected material of P. palmata, D. ramentacea, P. rubens and Laminaria saccharina showed efficient CPD repair, with only 10% of the artificially induced CPDs remaining after 5 h. No significant differences in repair rate were observed among these species. CPD repair was slower or absent in O. dentata, C. truncatus and Monostroma arcticum, indicating that fast repair mechanisms such as photolyase were not continuously expressed in these species. CPD repair rates were not directly related to the vertical distribution of algae in the water column and to the reported UV sensitivity of the examined species. Dosimeter incubations showed that maximal exposure to DNA damaging wavelengths was low for all examined species. Furthermore, most species collected below the 1% depth for DNA damage displayed efficient CPD repair, suggesting that UVBR-induced CPDs currently impose a minor threat for mature stages of these species growing in the Kongsfjord, Spitsbergen.
INTRODUCTION

Marine macrophytes occurring on rocky substrates are important primary producers in coastal waters. Apart from photosynthetically active radiation (PAR, 400-700 nm), these plants face exposure to the ultraviolet-A (UVAR, 315-400 nm) and ultraviolet-B (UVBR, 280-315 nm) spectral ranges of sunlight. Intensity and duration of UV exposure depend on many factors, *i.e.* the position of plants and attenuation of UV in the water column. Ambient UVBR changes with solar elevation and is influenced by weather and atmospheric conditions. UVBR is also directly related to the stratospheric ozone concentration because ozone selectively filters out the shorter UV-wavelengths. Stratospheric ozone over polar regions is subjected to enhanced seasonal depletion caused by emission of chlorofluorocarbons and other halogenated compounds. Consequently, relative increases in ambient UVBR are most pronounced at high latitude (Herman *et al.* 1996). However, ozone losses over the Arctic are less dramatic than those over Antarctica and enhanced UVBR in spring currently does not exceed maximum summer UVBR intensities in the Arctic (Dahlback 2002).

UVBR has multiple deleterious effects on the cellular level. High-energy UVB quanta are strongly absorbed by nucleic acids and can produce several structural modifications. Cyclobutane-pyrimidine dimers (CPDs) are the most abundant UV-induced DNA lesions (Mitchell and Nairn 1989). These distortions are cytotoxic because they block DNA and RNA polymerases and consequently inhibit genome replication and expression (Jordan *et al.* 1991, Buchholz *et al.* 1995, Taylor *et al.* 1996, Draper and Hays 2000). Plants deploy several elaborate repair pathways to remove damage from their genomes. Photolyase enzymes use light in the 350-450 nm range to reverse recognized CPDs (Pang and Hays 1991, Hada *et al.* 2000). Genome replication in *Arabidopsis* proceeds slower when UVBR exposure is not supplemented with photoreactivating light (Draper and Hays 2000). Moreover, photolyase deficient mutants of *Arabidopsis* and *Synechocystis* are extremely sensitive to UVBR, demonstrating the importance of photolyase for growth and survival in the presence of UVBR (Landry *et al.* 1997, Ng and Pakrasi 2001). A proposed function of photolyase is repair of thymine-rich promoter regions to ensure continuous transcription of essential genes (Sancar 2000). In addition to photolyase, several versatile repair pathways involving multiple enzymatic steps such as nucleotide excision repair and recombination repair have been recognized in plants (Xu *et al.* 1998, Liu *et al.* 2000, Ries *et al.* 2000a). Deficiency of nucleotide excision repair also leads to increased UVBR sensitivity in *Arabidopsis* mutants (Harlow *et al.* 1994).

Sunlight-induced CPD accumulation has been detected in phytoplankton and terrestrial plants from a wide range of latitudes, indicating that CPD induction can exceed repair rates (Ballaré *et al.* 1996, Jeffrey *et al.* 1996a, Rousseaux *et al.* 1999, Boelen *et al.* 2001, Buma *et al.* 2001a, b). CPD accumulation is accompanied by decreased growth rates in several phytoplankton and macrophyte species (Buma *et al.* 2000, Poll *et al.* 2001, 2002a). Furthermore, ambient UVBR exposure can reduce the
growth rates of various macrophytes (Grobe and Murphy 1994, Aguilera et al. 1999, Franklin et al. 1999, Makarov 1999, Altamirano et al. 2000, Kuhlenkamp et al. 2001, Pang et al. 2001). Increasing UVBR mediated by ozone depletion may elevate the DNA damage burden in Arctic macrophytes and consequently depress productivity and alter species composition. Although previous experiments suggested that macrophytes possess DNA repair pathways similar to those in higher plants (Pakker et al. 2000a, b), responses of Arctic macrophytes to DNA damage are unknown. Because Arctic macrophytes are exposed to extremely low UVBR, responses that specifically remove UVBR induced DNA damage such as photolyase may be less developed in these species. This could increase the vulnerability of these plants to enhanced UVBR. Thus, information on repair responses is required to evaluate the ecological relevance of DNA damage as a stress factor for Arctic macrophytes.

Several experiments are presented to assess CPD formation in Arctic macrophytes during summer in Spitsbergen. DNA dosimeters were deployed to examine the in situ transparency of the water column for wavelengths that induce CPDs. Combined with spectroradiometer data, the maximum daily CPD loads were estimated for bare DNA at several depths. In addition, CPD accumulation after exposure to surface solar UVBR and in vivo repair of artificially induced CPDs were examined for macrophytes obtained from the Kongsfjord.

MATERIALS AND METHODS

All experiments were performed in Ny-Ålesund, Spitsbergen (78°55.5’N, 11°56.0’E) between June 12 and July 4, 2001. DNA dosimeters were used to estimate the attenuation of UVBR and the maximum daily CPD load in the water column. Small fragments (2-3 cm) cut from field-collected algae were used in outdoor exposure experiments and DNA repair experiments. The algae were collected in the Kongsfjord by SCUBA diving and kept in tanks with running seawater (6±2°C) under continuous low light of 6-12 µmol m⁻² s⁻¹ for at least 12 h before the experiments. Coccotylus truncatus (P.) Wynne and Heine, Devaleraea ramentacea (L.) Guiry, Palmaria palmata (L.) Kuntze, Odonthalia dentata (L.) Lynbg, Phycodrys rubens (L.) Batters and Monostroma arcticum Wittrock were collected from depths between 8-15 m, whereas Laminaria saccharina (L.) Lamour was collected from 5-6 m.

DNA dosimeters and UVBR attenuation

Attenuation of wavelengths that induce CPDs was monitored in the Kongsfjord between June 13 and July 2, 2001. Quartz tubes containing 1.5 mL DNA solution (~10 µg/mL calf thymus DNA, Sigma, St. Louis, USA) were used as dosimeters (Boelen et al. 1999). Duplicate dosimeters were simultaneously exposed for 2-4 days at 0.15, 0.3, 0.6, 1.2, 2.4 and 4.8 m depth in the fjord. Collected dosimeters were stored at -20°C until CPD detection. Diffuse vertical attenuation coefficients (Kd) for DNA damage were calculated from linear regression of natural log-transformed
vertical CPD profiles versus depth. This provided estimates of UVBR attenuation over the incubation period. In addition, 1% depths were calculated for this time interval.

**Maximum daily CPD load**
The UV model of Björn and Murphy (1985) calculates a maximum daily dose of 467 J m$^{-2}$ weighted with the action spectrum for DNA damage (normalized to 1 at 300 nm, [Setlow 1974]) for a clear sky in mid summer in Spitsbergen. CPD concentrations for this maximum dose were estimated by correlating UVBR doses derived from spectroradiometer measurements and CPD concentrations in dosimeters incubated just below the water surface (see below). Because shielding of quartz tubes is minimal and repair mechanisms are absent in DNA solution, we assume that this is the maximum CPD concentration that can accumulate just below the water surface in Spitsbergen over a 24 h period. Vertical CPD profiles for maximum and minimum attenuation were used to calculate the penetration of the maximum CPD load under these conditions. These potential CPD profiles were compared with the collection depth of the algae and their highest position in the water column as reported by Vögele (unpublished).

**UVBR measurements**
Ambient UVBR was measured with a 32 channel spectroradiometer of the Alfred Wegener institute, equipped with a cosine diffuser (Groß et al. 2001, Hanelt et al. 2001). This device is installed on the roof of the NDSC building (Network for the Detection of Stratospheric Changes, Koldewey Station) in Ny-Ålesund and continuously monitors incident UVBR. Biologically effective radiation was calculated with the action spectrum for DNA damage over 5 min averages. The spectroradiometer was located approximately 200 m from the site where algae and dosimeters were exposed. Spectral energy distribution for the repair experiments was measured with a MACAM SR9910 double monochromator scanning spectroradiometer equipped with a cosine collector (Macam Photometrics, Livingston, UK).

**Exposure experiments just below the water surface**
On 20 June 2001, series of 3 fragments of *P. palmata*, *D. ramentacea*, *P. rubens*, *C. truncatus* and *O. dentata* were exposed to sunlight to investigate CPD accumulation. The fragments were spread out under UV transparent Plexiglas (farblos 2458, Röhm, Munich, Germany) with and without 395 nm cut-off foil (Digefra, Munich, Germany) in running sea water (6±2°C). The algae were incubated directly below the water surface for ~4 h around noon. In parallel, 2 DNA dosimeters were exposed under UV transparent Plexiglas. After exposure, algal fragments were preserved on activated silica gel in darkness. Exposure experiments with *P. palmata*, *P. rubens* and dosimeters were repeated several times in June and the beginning of July.
**CPD repair experiments in artificial light**

Repair of artificially induced CPDs was examined in *P. palmata, D. ramentacea, P. rubens, C. truncatus, O. dentata, M. arcticum* and *L. saccharina*. For each species, series of 6 fragments were covered by WG 305 (3 mm, Schott, Germany) and UG5 filters (Schott, Mainz, Germany) in 0.5 cm sea water (7±2°C) and exposed for 45 min to 3 TL 20W/12 lamps (Philips, Eindhoven, Netherlands) for CPD induction. This resulted in 2.3 W m\(^{-2}\) UVBR and a dose of 2814 J m\(^{-2}\) weighted with the DNA damage action spectrum (Fig. 1, Table 1). After replacing the filters by a WG 335 nm filter (3 mm, Schott), fragments were exposed to photoreactivating light (Fig.1, Table 1). Two RUVAR (Philips) and 3 Biolux L18W/72 lamps (Osram, Germany) provided photoreactivating light of 9.23 W m\(^{-2}\) UVAR and 13.8 W m\(^{-2}\) PAR. Kinetics of CPD removal were monitored by collecting series of 6 fragments after 0, 10, 20, 40, 75, 150 and 300 min of photoreactivating light exposure. Collected fragments were preserved in activated silica gel in darkness. In addition, CPD repair in *P. palmata* was studied under reduced sunlight to compare repair under artificial and natural light. In the latter treatment, fragments were exposed outdoors in a bath with running sea water (7±2°C) under 3 layers of neutral density screen and a WG 335 nm filter (Fig. 1). Fragments of *P. palmata* were also kept in darkness for up to 7 h after CPD induction to estimate the contribution of repair pathways that act independent of light.

**Figure 1.** Spectral energy distribution during CPD induction (1), CPD repair (2) and CPD repair in reduced sunlight (3). Note that irradiance in (3) was not constant.

![Spectral energy distribution](image)

**Table 1.** Irradiance conditions (W m\(^{-2}\)) during the CPD induction and repair experiments.

<table>
<thead>
<tr>
<th>condition</th>
<th>wavelength</th>
<th>CPD induction</th>
<th>CPD repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVBR</td>
<td>280-315 nm</td>
<td>2.31</td>
<td>0.00</td>
</tr>
<tr>
<td>UVAR</td>
<td>315-400 nm</td>
<td>4.20</td>
<td>9.23</td>
</tr>
<tr>
<td>PAR</td>
<td>400-700 nm</td>
<td>0.04</td>
<td>13.8</td>
</tr>
</tbody>
</table>

*In vivo* CPD repair rates were obtained from linear regression on natural log-transformed CPD data *versus* time, assuming that repair processes result in
exponential removal of CPDs over time (Malloy et al. 1997). The slope of the regression line represents the repair rate, whereas the intercept equals the initial CPD concentration after CPD induction. The analysis of variance (ANOVA) procedure for regression lines was followed to test for differences in slopes and intercepts among species.

**Extraction of DNA**

DNA of replicates was extracted separately. Silica-dried fragments of ~0.03 g were ground to powder under liquid nitrogen and suspended in 0.9 mL extraction buffer (2% cetyl-trimethylammonium bromide [CTAB], 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl pH 8, 0.2% polyvinyl-polypyrrolidone [PVPP], 0.01% sodium dodecyl sulfate [SDS], 0.2% â-mercaptoethanol). After 45 min of extraction at room temperature, samples were washed twice with 0.6 mL chloroform-isoamyl alcohol (24:1, vol/vol) followed by centrifugation for 10 min (20000 g). DNA was precipitated with a half volume of isopropanol for 20 min at −20°C and pelleted by 20 min of centrifugation at 4°C. The pellet was washed with 70% cold ethanol, vacuum dried and dissolved in 0.3 mL TE buffer (pH 8). After 30 min of RNase (0.02 µg) treatment at 37°C, samples were stored at −20°C. DNA concentrations were quantified fluorometrically (PicoGreen, Molecular probes, Eugene, OR, USA) using a 1420 Victor multilabel counter (Wallac, Inc. Gaithersburg, MD, USA).

**Detection of CPDs**

Portions of 100 (exposure experiments) or 50 ng (repair experiments, dosimeters) DNA were heat denatured for 10 min at 90°C, followed by 30 min on ice, and vacuum-blotted on nitrocellulose membrane (Portran BA79, 0.1 µm pore size, Schleicher and Schuell, Dassel, Germany) with a Minifold 1 SRC96D dotblot apparatus (Schleicher and Schuell). Calibration series of UV-irradiated DNA with equal concentrations (100 or 50 ng) were included. The UV-irradiated DNA was calibrated against UV-irradiated HeLa DNA with a known CPD concentration that was kindly provided by A. Vink, TNO, Zeist, Netherlands. After washing with 200 1 phosphate-buffered saline (PBS), DNA was fixed to the membrane at 80°C for 2 h. Nonspecific binding sites were blocked with 5% skimmed milk powder in PBST (PBS and 0.1% (vol/vol) Tween 20, Sigma) for 30 min at room temperature. CPDs (T-T and 5'T-C dimers, [Roza et al. 1988]) were recognized by the H3 antibody (Affitech, Oslo, Norway) during overnight incubation at 4°C (1:3000 diluted in PBST with 0.5% skimmed milk powder [wt/vol]). Repeated washing with PBST was followed by incubation with HRP-conjugated rabbit anti-mouse (Dako, Glostrup, Denmark) for 2 h at room temperature (1:15000 diluted in PBST with 0.5% skimmed milk powder [wt/vol]). This complex was detected by exposing the sealed membrane to photosensitive films after incubation with ECL western blotting detection reagent (Amersham, Buckinghamshire, UK). Grey scale values of scanned films were determined in ImageQuant (Molecular Dynamics, Sunnyvale, USA) and CPD concentrations were calculated from the linear part of the film.
RESULTS

**UVBR attenuation in the Kongsfjord**
The vertical CPD profiles obtained by the dosimeters allowed accurate calculation of $K_d$ values (Fig. 2). Low $K_d$ values were detected at the start of the monitored period, resulting in a calculated 1% depth for DNA damage of ~8 m. $K_d$ values increased towards the end of the period because of influx of turbid meltwater and sediments, which decreased calculated 1% depths for DNA damage to ~4 m (Fig. 2, Table 2).

**Figure 2.** Attenuation of biologically effective UVBR in the Kongsfjord as determined at several depths with DNA dosimeters for two intervals in June. CPD concentrations per million nucleotides (Mb) were natural log-transformed. Two replicate dosimeters were incubated at each depth.

<table>
<thead>
<tr>
<th>Date (2001)</th>
<th>$K_d$ (m$^{-1}$)</th>
<th>1% depth (m)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-16 June</td>
<td>0.58</td>
<td>7.94</td>
<td>0.96</td>
</tr>
<tr>
<td>16-18 June</td>
<td>0.66</td>
<td>6.98</td>
<td>0.96</td>
</tr>
<tr>
<td>18-20 June</td>
<td>0.74</td>
<td>6.22</td>
<td>0.96</td>
</tr>
<tr>
<td>20-24 June</td>
<td>0.68</td>
<td>6.77</td>
<td>0.93</td>
</tr>
<tr>
<td>26-28 June</td>
<td>1.28</td>
<td>3.60</td>
<td>0.98</td>
</tr>
<tr>
<td>29 June-2 July</td>
<td>1.24</td>
<td>3.71</td>
<td>0.97</td>
</tr>
<tr>
<td>2-7 July</td>
<td>1.17</td>
<td>3.94</td>
<td>0.97</td>
</tr>
</tbody>
</table>

**Maximum daily CPD load**
CPD concentrations in DNA dosimeters correlated with biologically effective UVBR doses (BED) calculated from spectroradiometer measurements ($R^2$: 0.9, Fig. 3). From this relationship, we calculated that in one day a maximum of 53 CPDs per million nucleotides (Mb) could accumulate in dosimeters incubated just below the surface in midsummer in Spitsbergen. Using minimum and maximum $K_d$ values, we calculated that the algae received no UVBR during time of collection, except for *L. saccharina*
Reported values on the highest position in the algal zonation of the investigated species were used to assess the potential CPD load (Fig. 4).

**Figure 3.** Correlation between CPD concentrations in DNA dosimeters and the BED calculated with Setlow’s DNA damage action spectrum based on spectroradiometer measurements. Dosimeters were exposed just below the water surface, whereas spectroradiometer measurements were performed in air. Dotted lines represent 95% confidence limits.

**Figure 4.** Attenuation of the maximum daily CPD load in DNA dosimeters in the water column in Spitsbergen, calculated for minimum (line) and maximum (dashed line) attenuation. The upper vertical distribution boundary of several species is indicated with horizontal dotted lines.

**Exposure experiments just below the water surface**

Exposure to sunlight resulted in significant CPD induction in *P. rubens*, *C. truncatus*, *O. dentata*, *P. palmata* and *D. ramentacea* compared with PAR-exposed fragments (single factor ANOVAs, *p*<0.05, Fig. 5a). Repeated exposure also showed significant CPD accumulation in *P. rubens*, except on day 204 (Fig. 5b). Accumulated CPDs in this species closely followed the dosimeter results. CPD induction in *P. palmata* was significant on day 201 and 215. CPD concentrations in the dosimeters were always higher than those in the UV-exposed algal fragments (Fig. 5b).
Figure 5. Sunlight induced CPD concentrations per million nucleotides (Mb) in *P. rubens*, *C. truncatus*, *O. dentata*, *P. palmata* and *D. ramentacea* (A). Algal fragments were exposed on 20 June for ~4 h to PAR+UV (filled bars) or PAR just below the surface around noon. *P. rubens* (triangles), *P. palmata* (diamonds) and DNA dosimeters (circles) were exposed just below the surface on several occasions in June and July (B). Closed symbols: PAR+UV, open symbols: PAR. Means and standard deviations of two dosimeters and six algal fragments are shown, except on 20 June, when three fragments were used. Connecting lines are shown to visualize patterns.

**CPD repair experiments in artificial light**

Prior to the experiments, background levels of 0-2 CPDs/Mb were detected in the collected material (Fig. 6). Linear regression explained up to 86% of the variability in natural log-transformed CPD data versus time (Table 3). For *P. palmata*, significant repair was observed under photoreactivating light, whereas no repair was observed in darkness (Fig. 6a). The repair rate in reduced sunlight was not significantly different from that in artificial light (*p*=0.28). Similarly, significant light dependent repair was observed in *D. ramentacea*, *P. rubens*, and *L. saccharina* (Fig. 6b). Repair rates were not significantly different from those of *P. palmata* (*p*=0.27), whereas initial CPD concentrations were different for each species (*p*<0.001). On average, 10% of the initial CPD concentrations remained after 5 h of repair in *P. palmata*, *D. ramentacea*, *P. rubens*, and *L. saccharina*.

The repair rate was significantly lower in *O. dentata* than in the species mentioned above, with 38% of the initial CPD concentration persisting after 5 h (*p*<0.0001). No significant repair was observed in *C. truncatus* and *M. arcticum* (Fig. 6c, Table 3).
Table 3. CPD induction and repair characteristics in artificial light for seven macrophyte species that were collected from the Kongsfjord (Spitsbergen). Second column: Estimated CPD concentrations after artificial CPD induction with 95% confidence limits. Third column: calculated CPD repair rates from linear regression of natural log-transformed CPD concentrations versus time. $R^2$ values show how well the data are explained by this model.

<table>
<thead>
<tr>
<th>Species (Class)</th>
<th>CPDs/Mb</th>
<th>Repair rate (h$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccotylus truncatus (Rhodophyceae)</td>
<td>56 (49-60)</td>
<td>0.59·10$^{-2}$</td>
<td>0.12·10$^{-2}$</td>
</tr>
<tr>
<td>Devaleraea ramentacea (Rhodophyceae)</td>
<td>24 (20-27)</td>
<td>0.45</td>
<td>0.86</td>
</tr>
<tr>
<td>Odonthalia dentata (Rhodophyceae)</td>
<td>134 (110-160)</td>
<td>0.22</td>
<td>0.48</td>
</tr>
<tr>
<td>Palmaria palmata (Rhodophyceae) lab:</td>
<td>69 (56-81)</td>
<td>0.51</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>field:</td>
<td>50 (41-60)</td>
<td>0.43</td>
</tr>
<tr>
<td>Phycodrys rubens (Rhodophyceae)</td>
<td>72 (55-90)</td>
<td>0.38</td>
<td>0.57</td>
</tr>
<tr>
<td>Laminaria saccharina (Phaeophyceae)</td>
<td>88 (74-110)</td>
<td>0.49</td>
<td>0.73</td>
</tr>
<tr>
<td>Monostroma arcticum (Chlorophyceae)</td>
<td>61 (49-74)</td>
<td>0.64·10$^{-1}$</td>
<td>0.75·10$^{-1}$</td>
</tr>
</tbody>
</table>

Figure 6. *In vivo* repair of artificially induced CPDs in *P. palmata* (A), *D. ramentacea*, *L. saccharina*, *P. rubens* (B), and *C. truncatus*, *M. arcticum*, *O. dentata* (C). Repair in *P. palmata* (A) was studied under artificial light (□), reduced sunlight (○) and in darkness (●), whereas artificial light was used for the other species. Means and standard deviations are shown for six replicates.
DISCUSSION

Because of the high latitude the sun reaches a maximum elevation of only 35º in Spitsbergen, resulting in low daily UVBR doses in summer. Although the polar day is characterized by continuous light, long periods of sunshine are rare on Spitsbergen. Furthermore, vertical CPD profiles of the dosimeters showed that short wavelengths are strongly attenuated in the Kongsfjord compared with tropical and Antarctic coastal waters (Jeffrey et al. 1996b, Boelen et al. 1999, 2000, Buma et al. 2001a). Attenuation in the Kongsfjord is highly variable, and periods with clear water in late spring and early summer typically last only a few days before influx of turbid meltwater decreases water transparency. Consequently, macrophytes encounter much lower UVBR doses than at the surface. Although $K_d$ values obtained with the dosimeters agree with those from previous spectroradiometer measurements in the Kongsfjord, it should be noted that DNA dosimeters provided averages for 2-4 day periods, whereas water transparency can change on shorter time scales (Bischof et al. 1998b, Wiencke et al. 2000, Hanelt et al. 2001).

Accumulated CPDs in the DNA dosimeters correlated with biologically effective UVBR doses derived from spectroradiometer measurements, as was previously found in surveys in the southern Caribbean and Antarctica (Boelen et al. 1999, George et al. 2002). We estimated that approximately 53 CPDs/Mb can accumulate in bare DNA just below the surface in a 24 h interval at the highest solar elevation in midsummer at Spitsbergen. As expected, the maximum CPD load in Spitsbergen is much lower than those found in the Caribbean and Antarctica, where several hundred CPDs can accumulate in one daily cycle (Regan et al. 1992, Jeffrey et al. 1996a, b, Boelen et al. 1999, 2000, Buma et al. 2001a, George et al. 2002).

Nevertheless, species occurring in the higher subtidal such as *D. ramentacea* are challenged by CPD induction in summer. In contrast, the deep water species *P. rubens*, *C. truncatus* and *O. dentata* receive low maximum CPD loads even under minimum attenuation, showing that they are continuously protected from UVBR exposure by the water column. Exposure to sunlight just below the surface resulted in significant CPD accumulation in all examined macrophytes. Shielding of DNA and repair processes presumably cause the differences between CPD concentrations in algae and bare DNA used in the dosimeters. Because low light-adapted subtidal plants were exposed, all spectral ranges were far more intense than in their natural habitat. Exposure to PAR and especially PAR and UVR resulted in severe bleaching in *P. rubens*, *C. truncatus* and *O. dentata*, whereas this was less pronounced in *P. palmata* and *D. ramentacea*, demonstrating a higher tolerance for excessive light in the latter species. Furthermore, the lower CPD levels in these species suggest a higher degree of repair. However, morphological characteristics and the presence of UV-absorbing compounds may also account for these differences (Hoyer et al. 2001, Poll et al. 2002a). Morphology-related variation in CPD induction was previously observed in Antarctic diatoms (Karentz et al. 1991a). CPD accumulation in PAR-exposed
fragments of *P. rubens* cannot be readily explained because PAR wavelengths are not involved in CPD formation.

A simple exponential model provided reasonable fits for CPD repair rates in *D. ramentacea, L. saccharina, P. palmata* and *P. rubens*. However, *in vivo* repair is undoubtedly a more complex process that is influenced by factors such as chromatin structure (Suter *et al.* 1997). Repair rates appeared faster at the beginning, indicating involvement of slow and fast components in the repair process. Due to the low resolution of the data set we could not discriminate between these components.

CPD repair in *P. palmata* was exclusively detected in samples that were exposed to photoreactivating light, suggesting an important role of photolyase in removing the majority of CPDs. Slow repair in darkness was previously observed in *Rhodymenia pseudopalmata, P. palmata* and in many higher plant species (Pang and Hays 1991, Quaite *et al.* 1994, Sutherland *et al.* 1996, Pakker *et al.* 2000a, b). However, exact contributions of repair pathways remain uncertain because dark repair pathways such as recombination repair are also enhanced by light exposure (Ries *et al.* 2000a). In yeast and *Escherichia coli*, nontranscribed strands are preferentially repaired by photolyase whereas transcribed strands are repaired by nucleotide excision repair, showing that both repair pathways co-occur (Livingstone-Zatchej *et al.* 1997). Deficiencies both in photolyase and nucleotide excision repair increase UVBR sensitivity in *Arabidopsis* (Britt *et al.* 1993, Harlow *et al.* 1994, Landry *et al.* 1997).

CPD repair in artificial light was similar to that in reduced sunlight despite obvious differences in spectral composition. Apparently, light-dependent reversal of CPDs is saturated at relatively low photon levels, as was also observed for higher plants (Pang and Hays 1991, Taylor *et al.* 1996). Significant CPD repair was found within 1 h after CPD induction. This suggests that photolyase activity was present before CPD induction because *the novo* synthesis of these enzymes takes 2-8 h in higher plants (Buchholz *et al.* 1995).

Differences in CPD repair rates were observed among species although they were collected from depths where little UV penetrated. Efficient repair was found in *D. ramentacea, L. saccharina* and *P. palmata*, which also occur in shallower habitats. The repair rates are slower than those reported from similar experiments with Antarctic zooplankton that occurs near the surface (Malloy *et al.* 1997). Obviously, Arctic plants growing below 5 m have much lower requirements for CPD repair. Surprisingly, the repair rate of *P. rubens* was not different from those observed in *P. palmata, L. saccharina* and *D. ramentacea*. Together with *C. truncatus* and *O. dentata*, *P. rubens* predominantly occurs at greater depths and is highly sensitive to UV light and excessive PAR (Aguilera *et al.* 1999, Karsten *et al.* 2001, Poll *et al.* 2001, 2002a). Although the repair experiments show that CPD repair occurs in *P. rubens* under favorable light conditions, exposure to sunlight resulted in strong accumulation of CPDs, suggesting minimal repair in this species. Presumably, high light-induced oxidative stress interfered with the CPD repair response in this experiment. The extreme sensitivity of *P. rubens, C. truncatus* and *O. dentata* to high
PAR probably prevents these species from growing in UV exposed habitats. The lack of repair in *O. dentata*, *M. arcticum* and *C. truncatus* suggest that photolyase enzymes were absent in these algae. Similar diversity in DNA repair responses was observed among Antarctic diatoms and marine bacteria (Karentz *et al.* 1991a, Joux *et al.* 1999). However, it is unlikely that these macrophyte species are unable to express photolyase because light-dependent CPD repair was previously observed in cultivated Arctic *C. truncatus* (W. H. van de Poll, unpublished). Thus, low photolyase activity may result from adaptation to low light. In higher plants, *in vitro* photolyase activity of dark- grown plants can increase two- to five-fold after exposure to light (Langer and Wellmann 1990, Buchholz *et al.* 1995, Ahmad *et al.* 1997, Hada *et al.* 1999, Takahashi *et al.* 2002, Waterworth *et al.* 2002). Consequently, the CPD repair rates may not represent the maximum repair rates for these plants because all species were acclimated to low light. Furthermore, nutrient status and condition of the collected plants is unknown. Nitrate in the Kongsfjord is typically depleted early in summer (Aguilera *et al.* 2002a). A shortage of this nutrient may enhance UV sensitivity in algae (Litchman *et al.* 2002). Although regulation of DNA repair is unknown, other protective responses such as the production of UV-absorbing compounds are regulated by UVAR and blue light in macrophytes (Karsten *et al.* 1998). Because blue light and UVAR penetrate deeper in the water column than UVBR, similar regulation of DNA repair responses appears likely. In addition, species that frequently endure UVBR exposure may have increased constitutive expression levels of repair enzymes. Juvenile stages of kelps are more sensitive to UVBR than mature plants because UV penetrates deeper into thin thalli (Dring *et al.* 1996a, Huovinen *et al.* 2000, Wiencke *et al.* 2000). Actively growing plants may have a higher requirement for DNA repair because of the inhibitory effect of unrepaired CPDs on cell division. In higher plants, photolyase expression is developmentally regulated (Pang and Hays 1991, Hidema and Kumagai 1998, Hada *et al.* 2001).

**CONCLUSIONS**

Despite low ambient UVBR in summer, exposure just below the surface causes CPD accumulation in all examined species. However, risks of CPD induction are generally low in the Kongsfjord because of low transparency of the waterbody for UVBR. Furthermore, few species inhabit the intertidal and upper subtidal because of mechanical stress from sea ice. Although differences in repair responses were observed, macrophytes appear well prepared for UV exposure since efficient repair of CPDs was shown for several species that were collected below the maximum 1% depth for DNA damaging wavelengths. It is difficult to predict if increased UVBR will elevate CPD levels in Arctic macrophytes because regulation of repair and the threshold above which repair mechanisms are saturated are unknown. Furthermore, physical factors such as temperature, light and nutrient availability could impose constraints on DNA repair responses. For instance, DNA repair in *P. palmata*
proceeds slower at low temperatures (Pakker et al. 2000b). Especially repair pathways that involve expression of many genes such as nucleotide excision repair could be inhibited by these factors.

It has been suggested that the Arctic marine flora is vulnerable to increased UVBR because of genetic adaptation to low ambient UVBR. But the current experiments provided no evidence to support this. Moreover, Arctic and temperate isolates of cold temperate macrophytes were equally sensitive to artificial UV treatments at 6 and 12°C (Poll et al. 2002a). Similarly, the endemic Arctic species *D. ramentacea* adapts efficiently to UV exposure (Karsten et al. 1999, Bischof et al. 2002a). Other studies also showed that photosynthesis of Arctic macrophytes has the potential to acclimate to UVBR (Bischof et al. 1999, Karsten et al. 2001). During glacial episodes, Arctic species were forced to retreat to lower latitudes with higher UVBR levels. This may partly account for their comparatively high UV tolerance. In conclusion, we find that CPD induction is low in the Arctic compared with tropical and temperate regions, whereas the CPD repair capacity of the mature stages of examined Arctic macrophytes appears sufficient to prevent CPDs accumulation in their natural habitat. Consequently, UVBR-induced CPD formation is expected to be a minor stress factor for the examined Arctic macrophytes.

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