Role of nitrogen versus phosphorus availability on the effect of UV radiation on bacterioplankton and their recovery from previous UV stress

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ABSTRACT: The influence of nitrogen (N) versus phosphorus (P) availability on the sensitivity of marine bacterioplankton to solar radiation and on their recovery from UV stress was assessed in laboratory experiments under high versus low substrate concentrations. Bacterioplankton were exposed to artificial solar radiation closely resembling natural radiation levels for 4 h and aliquots were subsequently exposed to different radiation ranges for 3 h. Bacterial activity was significantly reduced after exposure to solar radiation, as compared to the activity measured prior to the exposure, only in P-deplete conditions under both, high, and low substrate conditions. Exposure of the bacterioplankton to different radiation ranges following exposure to the full range of solar radiation revealed that nucleotide excision repair is probably more important than the photoenzymatic DNA repair mechanism. Recovery from previous UV stress was similar under N- and P-deplete bacterial growth at high substrate conditions. Under low substrate conditions, however, the recovery efficiency was significantly lower under P- than under N-deplete conditions. Thus, we conclude that to a large extent, P availability determines the sensitivity of bacterioplankton to UV radiation and the recovery efficiency from previous UV stress in oligotrophic surface waters.

KEY WORDS: UV radiation · Bacterioplankton · Substrate availability · Nitrogen · Phosphorus

INTRODUCTION

Ultraviolet-B (UV-B, 280 to 320 nm) radiation penetrating into the water column influences the carbon and energy flux in the euphotic layer of the oceans and therefore has impact on the oceanic biogeochemical cycles (Moran & Zepp 1997). In oligotrophic oceanic waters, the 10% level of surface solar radiation of the 340 nm wavelength is at about 30 to 40 m depth and that of 380 nm at 60 to 70 m depth (Obernosterer et al. 2001). Thus, about half of the photic zone is influenced by UV radiation (Obernosterer et al. 2001).


One of the direct, primary targets of UV radiation in microorganisms is DNA. The major forms of DNA damage induced by low wavelength solar radiation are cyclobutane dimers and pyrimidine (6-4) pyrimidone photoproducts (Karentz et al. 1991, Buma et al. 1995).
In contrast to eukaryotic microorganisms and cyanobacteria, heterotrophic bacteria do not produce UV-absorbing pigments to shield off high energy UV radiation (Garcia-Pichel & Castenholz 1993). Microorganisms have developed several mechanisms to repair DNA lesions, which are either induced under solar radiation in the presence of UV-A (320 to 400 nm) and photosynthetic active radiation (PAR; 400 to 700 nm), or induced in the dark (Friedberg 1985). These repair mechanisms are common to all bacteria (Arrage et al. 1993). The photoenzymatic repair (PER) is a light-dependent process where the photolyase absorbs photons from the higher UV-A and lower PAR range (360 to 430 nm) to repair the DNA damage (Friedberg 1985, Karentz 1994, Chen et al. 1995). The induction of photolyase, therefore, does not require energy in the form of ATP, whereas the other important DNA repair mechanism, the nucleotide excision repair (NER), requires ATP (Friedberg 1985).

In a study performed in the coastal northern Adriatic Sea, it has been reported that the recovery rate of bacterioplankton from previous UV stress is higher in the presence of UV-A and PAR than in the dark (Kaiser & Herndl 1997). This indicates that PER of DNA damage might be, at least under certain conditions, more important than NER. In a recent paper, Arrieta et al. (2000) showed that bacterial strains exhibit considerable interspecific variability in their sensitivity to UV radiation and that they use efficiently either PER or NER, but not both.

The aim of this study was to determine the influence of the availability of nitrogen (N) and phosphorus (P) on the impact of solar radiation including UV on marine bacterioplankton and on their recovery from previous UV stress. The rationale behind this is that P is a major element of the DNA and therefore essential for cell division while N is primarily used for the synthesis of proteins and therefore, biomass. Thus, the availability of P versus N should have different effects on the response of bacterioplankton to UV stress, and possibly on the recovery from it. As an integrative parameter to assess bacterial activity, we chose leucine incorporation into bacterial macromolecules rather than measuring the dynamics of DNA damage.

### MATERIALS AND METHODS

To evaluate the response of marine bacterioplankton to exposure to solar radiation under N- versus P-deplete conditions and the impact of N versus P availability on the recovery from previous UV stress, 4 laboratory experiments were performed with freshly collected bacterioplankton communities incubated at high and low substrate concentrations and exposed to artificial solar radiation. The 4 experiments with high and low substrate concentrations were performed between 22 to 26 January and 4 to 9 February 1999, respectively.

**Experimental setup.** Generally, dilution cultures with bacterioplankton communities inoculated in artificial seawater amended with nutrients were used in the experiments. Seawater was collected in acid-rinsed carboys at high tide from the Netherlands Institute for Sea Research’s (NIOZ) pier located at the southern entrance to the Dutch Wadden Sea (Marsdiep, coastal North Sea). This water was filtered through 0.8 μm polycarbonate filters (Millipore) to remove most of the non-bacterial particles. From the 0.8 μm filtrate, 200 ml were inoculated into 1.8 l of artificial seawater amended with nutrients were used in the experiments. Seawater was collected in acid-rinsed carboys at high tide from the Netherlands Institute for Sea Research’s (NIOZ) pier located at the southern entrance to the Dutch Wadden Sea (Marsdiep, coastal North Sea). This water was filtered through 0.8 μm polycarbonate filters (Millipore) to remove most of the non-bacterial particles. From the 0.8 μm filtrate, 200 ml were inoculated into 1.8 l of artificial seawater amended with glucose, NH₄⁺, PO₄³⁻ and a mixture of amino acids at different concentrations to simulate high- and low-substrate conditions (Table 1). Natural bacterioplankton assemblages were inoculated within 0.5 to 1.5 h after sampling. The dilution cultures were held at 17 to 18°C in the dark for 24 h. Subsequently, the dilution cultures were split into aliquots of 1 l and enriched with either PO₄³⁻ or NH₄⁺ to create an imbalance in N- or P-availability (as compared to the elemental composition of bacteria [C:N:P = 45:9:1, Goldman et al. 1987]) under high- and low-substrate conditions (Table 1). After 12 h, 200 ml of the N- and P-limited cultures were filled into combusted quartz tubes and exposed to artificial solar radiation for 4 h (Fig. 1). Immediately thereafter, the cultures were transferred to various radiation ranges for 3 h to evaluate the potential of the bacteria to recover from the previous UV stress under different nutrient conditions (Fig. 1). Immediately before and after the exposure to the full range of artificial solar radiation, bacterial

### Table 1. Summary of nutrient conditions during the initial phase of the dilution cultures in artificial seawater lasting for 24 h and after N- or P-enrichment for the subsequent exposure to artificial solar radiation. Nutrient concentrations are given in µM, the concentration of added amino acid (AA) mixture in µM N. Glu-C: glucose-C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial nutrient regime (in µM)</th>
<th>Added nutrients (in µM)</th>
<th>Number of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glu-C  NH₄⁺  PO₄³⁻  AA</td>
<td>NH₄⁺ or  PO₄³⁻</td>
<td></td>
</tr>
<tr>
<td>High-nutrient regime</td>
<td>100  10  1  0.4</td>
<td>40  4</td>
<td>4</td>
</tr>
<tr>
<td>Low-nutrient regime</td>
<td>20  2  0.2  0.4</td>
<td>8  0.8</td>
<td>4</td>
</tr>
</tbody>
</table>
activity was measured via leucine incorporation as described below.

To evaluate the role of different wavelength ranges on the recovery efficiency, 3 different radiation regimens were established following the exposure to the full spectrum of solar radiation: UV-A + PAR excluding UV-B by wrapping the quartz tubes in Mylar-D foil (Dupont); PAR alone by wrapping tubes in vinyl chloride foil (CI Kasei); and a dark control (tube wrapped in aluminum foil). After 3 h of exposure to the different radiation regimens, bacterial activity was re-assessed.

**Radiation conditions.** Artificial solar radiation was provided by 3 different types of light sources. Two HQI-T Powerstar (Osram) lamps provided PAR, 2 TL 100W/10R fluorescent light tubes (Philips) were used to provide UV-A, and 3 UVA-340 fluorescent light tubes (Q-Panel) provided UV-A and UV-B. The solar simulator was adjusted to 30 to 60% of the local maximum radiation intensity in late spring measured on a cloudless day (Table 2). To maintain constant water-temperature conditions during the exposure to the different regimens of artificial solar radiation (17 to 18°C), the treatments were kept in a flow-through water bath connected to a temperature control unit (LAUDA RCS/RC-6).

**Bacterial production.** Bacterial production was determined via the incorporation of L-[14C] leucine (specific activity [SA] 55.0 mCi mmol⁻¹, Amersham) into bacterial macromolecules (Simon & Azam 1989). Duplicate 5 ml samples and 1 formaldehyde-fixed blank (2% final concentration) were spiked with 20 nM leucine (final concentration) and incubated in the dark at 17°C for 20 to 30 min. Thereafter, the samples were fixed by adding 0.5 ml of concentrated formalin. Samples were filtered onto 0.2 μm cellulose-nitrate filters (Millipore) and the filters rinsed twice with 5 ml of 5% ice-cold trichloroacetic acid. The filters were then placed in scintillation vials and a scintillation cocktail of 1 ml ethylacetate and 8 ml liquid (Ultima Gold, Packard) was added. After 12 h, the radioactivity was determined with a LKB-Wallac 1211 RackBeta liquid scintillation counter. Bacterial production was calculated using the conversion factor of 3090.84 g bacterial C produced per mol leucine incorporated (Simon & Azam 1989).

**Statistical analyses.** The results from the 4 experiments were pooled for statistical analyses. Unless stated otherwise, statistical analyses were performed using the Wilcoxon matched-pair test and the non-parametric Friedman test (SPSS Systat 7.0).
RESULTS

Bacterioplankton response to full solar-radiation exposure

The response of bacterial communities held at high versus low substrate regimes and exposed to the full range of artificial solar radiation is shown in Fig. 2. The initial bacterial production (i.e. before exposure to artificial solar radiation) in the dilution cultures with high substrate levels was $19.6 \pm 11.9 \mu g C l^{-1} h^{-1}$ (n = 4) for P-deplete and $11.6 \pm 6.1 \mu g C l^{-1} h^{-1}$ (n = 4) for N-deplete cultures. Exposure to artificial solar radiation at high substrate levels for 4 h resulted in a decline in bacterial activity in the P-deplete cultures by $46 \pm 24\%$ (n = 4; Fig. 2a). Bacterial activity in the N-deplete dilution cultures continued to increase (by $42 \pm 21\%$; n = 4) as compared to the initial bacterial activity prior to exposure (Fig. 2a).

Bacterial cultures held at low substrate levels exhibited an initial production (prior to exposure to artificial solar radiation) of $3.7 \pm 0.4 \mu g C l^{-1} h^{-1}$ (n = 4) under P-deplete and $3.3 \pm 1.2 \mu g C l^{-1} h^{-1}$ (n = 4) under N-deplete conditions. After the 4 h exposure to the full range of artificial solar radiation, bacterial activity declined by $46 \pm 16\%$ (n = 4) under P-deplete conditions, whereas bacterial production under N-deplete conditions increased slightly to $105 \pm 54\%$ (n = 4; Fig. 2b). As for the exposure to the high-substrate levels, also at low-substrate levels bacterial production was significantly reduced under P- as compared to N-deplete conditions (Wilcoxon, p = 0.068; for each n = 4).

Recovery of the bacterioplankton community from previous UV stress

At high substrate levels, bacterioplankton previously exposed to the full range of solar radiation for 4 h, and subsequently exposed to different ranges of solar radiation for an additional 3 h exhibited significantly
higher recovery efficiency under dark conditions in both N- and P-deplete cultures (Friedman, p = 0.046 for both treatments; n = 4) than under PAR + UV-A and PAR conditions (Fig. 2a). There was no significant difference between the recovery under PAR + UV-A and PAR radiation in either the P- or the N-deplete cultures.

At low substrate levels, bacterioplankton generally showed a higher recovery efficiency under N- than under P-deplete conditions in all 3 radiation treatments: UV-A + PAR, PAR, dark (Friedman, p = 0.046; n = 4; Fig. 2b). As for the high substrate levels, bacterioplankton held in the dark following exposure to the full range of solar radiation exhibited a higher recovery than either the UV-A + PAR or the PAR treatment. Fig. 3 shows the ratio between the bacterial production measured at the end of the recovery period (for the 3 different radiation regimens) and the production after exposure to the full range of solar radiation. While there was no significant difference at the high substrate level between P- and N-deplete conditions (Fig. 3a), at low substrate levels significantly higher recovery efficiencies were obtained for N- than for P-deplete bacterioplankton (Friedman, p = 0.046; Fig. 3b).

**DISCUSSION**

To ensure comparable conditions over the course of the experiments, we used artificial solar radiation and artificial seawater amended with substrates at specific concentrations.

Artificial solar radiation was provided by a lamp configuration which closely resembles natural solar radiation up to a wavelength of about 700 nm. The intensity of the radiation was chosen to be 30 to 60% (depending on the wavelength considered) of the maximum radiation, measured on 2 cloudless days in late spring, and the exposure time was limited to 4 h. Thus, the radiation dose provided in these experiments was close to natural conditions in the near-surface layers of the North Sea (Table 2).

Artificial seawater amended with glucose and major inorganic nutrients was used to ensure that the same amount of nutrients was potentially available in all the experiments. Since the chemical composition of natural DOM is rather complex and its characterization on a molecular level is not possible at present, it also remains unknown at which rate all the different DOM compounds are incorporated into bacterioplankton cells. Moreover, the composition of the DOM changes considerably, even over short-time scales (Burney et al. 1982, Herndl & Malacic 1987, Suttle et al. 1991). Despite the lack of detailed knowledge on the chemical composition of marine DOM, complex chemical alterations of the DOM pool take place upon its exposure to solar radiation, particularly to UV radiation. On the one hand, it has been shown that a suite of compounds produced from parent DOM is readily bioavailable, making the DOM pool generally more accessible for bacterioplankton utilization (Moran & Zepp 1997, Tranvik & Kokalj 1998, Obernosterer et al. 1999). However, on the other hand, originally labile DOM might be photochemically altered to become more refractory (Benner & Biddanda 1998, Obernosterer et al. 1999, 2001, Pausz & Herndl 1999). It is impossible to account for all these complex photochemical alterations of natural DOM which might have contrasting effects on bacterioplankton. Thus, we used artificial seawater amended with nutrients which do not absorb solar radiation efficiently. We therefore assume that in the experimental approach used in this study, photochemical processes on the substrate pool are not biasing our results.

The substrate concentrations used in this study were meant to allow investigation of the bacterioplankton response under both high and low concentrations of bioavailable substrates where either N or P availability is controlling bacterial growth. The N:P ratios of the added nutrients were chosen on the basis of the elemental composition of bacterioplankton, with a ratio of C:N:P = 45:9:1 (Goldman et al. 1987).

In previous studies on the impact of solar radiation on bacterioplankton, it has been found that exposure to surface-level solar radiation leads to significantly reduced bacterial activity (Herndl et al. 1993, Jeffrey et al. 1996, Joux et al. 1999, Arrieta et al. 2000). In our study, a significant reduction in bacterioplankton activity upon exposure to lower levels of solar radiation than used in the previous studies was only detectable under P-deplete conditions (Fig. 2). Thus, upon direct exposure, bacterioplankton are more sensitive to solar radiation under P- than under N-deplete conditions.

In the water column, solar radiation is attenuated depending on its wavelengths, leading to a characteristic composition of light depending on depth (Jerlov 1976, Kirk 1994). Mixing of the upper layers of the water column is frequently limited due to diurnal stratification of these layers caused by solar heating (Obernosterer et al. 2001). If mixing of the upper layers of the water column takes place, because of either strong wind-induced mixing or surface cooling of the uppermost layers, bacterioplankton are mixed into deeper layers where UV-B is already largely attenuated while UV-A and PAR are still present. Under such conditions, the PER mechanism can be effective as long as there is sufficient short-wavelength PAR present. To evaluate the importance of the different radiation regimens, at different substrate concentrations, on the recovery efficiency of bacterioplankton from previous UV stress,
we shielded off different parts of the solar radiation spectrum from the quartz tubes in which the bacterioplankton were incubated. At high substrate levels, no significant difference in the recovery efficiency of bacterioplankton between N- and P-deplete conditions was detectable (Fig. 3a). At low substrate levels, however, P-deplete bacteria recovered from previous UV stress at significantly lower rates than N-deplete bacteria (Fig. 3b). Differences in the radiation regimens were generally less important for the recovery efficiency than the nature of the growth limiting element at low substrate levels (Fig. 3b). This indicates that light-independent repair mechanisms, among them most likely NER, were more important in this study than PER. In a previous study performed with bacterioplankton of the northeastern Adriatic Sea, Kaiser & Herndl (1997) found that bacterial recovery from previous UV stress is higher under UV-A + PAR exposure than under dark conditions. Hence, it was concluded from these experiments that PER is more important than NER in repairing DNA damage caused by UV radiation (Kaiser & Herndl 1997). However, as has been shown recently on bacterial strains isolated from different parts of the world’s oceans, there are remarkable strain-specific differences in the efficiency of PER and NER to repair DNA damage (Arrieta et al. 2000). In the present study, we expected PER to dominate under P-deplete conditions since it does not require additional P, whereas in N-deplete cultures the less error-prone NER was expected to prevail. Such a pattern in DNA repair, however, could only have been obtained in our experimental setting if bacterial species were capable of switching between the different modes of DNA repair. As shown by Arrieta et al. (2000), bacterial strains use efficiently either PER or NER, but not both. Thus, bacterial strains have limited capabilities to switch modes, resulting in a rather uniform pattern of bacterial recovery from UV stress independent of the radiation regimens in our experiments.

Species-specific differences in the sensitivity to and the recovery from UV stress might lead to shifts in the community composition in surface waters. This has been addressed in a previous study where bacterioplankton, collected from the same sampling site as used in this study, were exposed to different radiation regimens, and the composition of the bacterioplankton community was subsequently documented by denaturing-gradient gel electrophoresis (Winter et al. 2001). Only subtle changes in the bacterioplankton community were found upon exposure to solar radiation as compared to communities held in the dark (Winter et al. 2001). Even the most UV-sensitive bacterial species in these exposure experiments was present in the surface waters of the North Sea throughout the summer, indicating efficient repair of UV-induced DNA damage under natural conditions.

Regardless of the repair mechanism used by bacterioplankton species, we have shown that the sensitivity of bacterioplankton to UV radiation and the recovery from previous UV stress depends to a large extent on the availability of P. In the surface waters of the global ocean, however, P is frequently limiting bacterioplankton activity (Rivkin & Anderson 1997, Kuipers et al. 2000). Thus, under these conditions, bacterioplankton appear to be more susceptible to UV than under N-deplete conditions. Our results indicate, therefore, that the availability of P for bacterioplankton growth determines the direct impact of UV radiation on bacterioplankton in the oceanic near-surface layers.

Acknowledgements. Financial support was provided by a TMR grant (project # ENV4-CT97-5077) and the COMET project of the ELOISE cluster (project # EVK1-1999-00175) of the Commission of the European Union. This is Royal NIOZ contribution # 3705. This work is in partial fulfillment of the requirements for a PhD degree from the University of Vienna, Austria, by C.P.

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Editorial responsibility: John Dolan, Villefranche-sur-Mer, France
Submitted: April 5, 2002; Accepted: June 3, 2002
Proofs received from author(s): July 23, 2002