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Solar UV radiation modulates daily production and DNA damage of marine bacterioplankton from a productive upwelling zone (36°S), Chile

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Abstract

In upwelling ecosystems, such as the Humboldt Current system (HCS) off Concepción, the effects of solar radiation on bacterioplankton incorporation rates have been related to previous light acclimation and responses to irradiance. In this paper, we study the daily effect of Photosynthetic Active Radiation (PAR, 400–700 nm) and ultraviolet radiation UVR (280–400 nm) on bacterial secondary production (BSP). We also considered the DNA damage–repair response to solar radiation stress by the induction of cyclobutane pyrimidine dimers (CPDs). Experiments were conducted with natural bacterioplankton assemblages (0.2–0.7 μm) collected off Concepción (36°S), during the austral Spring, October–November, 2004. Surface (0.5 m) and subsurface (80 m) bacterioplankton samples were exposed to different solar radiation treatments for 5–20 h. BSP was estimated by ^{14}C-leucine and ^{3}H-thymidine incorporation at several time intervals, whereas CPDs accumulation was assessed using immunoassay techniques. During high irradiance periods, BSP was mainly affected by PAR in both surface and subsurface assemblages and, to a lesser, but significant (Tukey <0.05) extent, by UV-A (320–400 nm) and UV-B (280–320 nm) radiation. Maximum inhibition of BSP in surface waters was 78%; growth rates (μ) and bacterial growth efficiency (BGE) were also low (78% and 66% respectively). Subsurface water assemblages, on the contrary, showed a ~25 fold enhancement of BSP, μ, and BGE. Both types of assemblages had a rapid CPDs accumulation (maximum 60 CPDs Mb^{-1}) during high irradiance periods. Recovery of BSP inhibition and DNA damage in surface bacteria was total after sunset and after the night incubation period, resembling pre-exposure levels. Despite subsurface BSP enhancement during day–night exposure, residual DNA damage was detected at the end of the experiment (20 CPDs Mb^{-1}) suggesting a chronic DNA damage. Our results represent the worst case scenario (i.e., assemblages receiving surface irradiances as may occur in this upwelling zone) and indicate that surface and subsurface bacterial

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assemblages in the HCS are both highly sensitive to solar irradiance. However, they showed different responses, with surface bacteria having more effective photorepair mechanisms, and sustaining higher BSP than subsurface assemblages.

Keywords: Bacterioplankton activity; DNA damage; Humboldt Current System; Photobiology; Photosynthetic active radiation; Ultraviolet radiation; Upwelling

1. Introduction

The energy provided by the Sun modulates marine ecosystems due to the biological transformation of radiant energy into chemical energy. However, normal levels of PAR (400–700 nm), UV-A (320–400 nm) and UV-B (280–320 nm) could damage cellular structure (Chatila et al., 2001 and references therein; Buma et al., 2003) and cause several effects on the metabolism of many organisms (see reviews of Jeffrey et al., 2000; Häder et al., 2003). Solar ultraviolet radiation (UVR) can penetrate deeper in the water column (Jeffrey et al., 1996a,b; Hargreaves, 2003) than previously known (Jerlov, 1950). Thus, any change in the proportion of UV-B reaching the Earth, caused by stratospheric ozone depletion (Frederick et al., 1994; Madronich et al., 1998), will have important consequences for the trophic web (Williamson and Zagarese, 2003).

Most studies of UV effects on marine biota have focused on phytoplankton (Helbling et al., 1992; see reviews of Villafañe et al., 2001, 2003) and also on bacterioplankton (i.e., Sommaruga et al., 1997; Buma et al., 2001a,b; Visser et al., 2002). Previous studies (Helbling et al., 1995; Jeffrey et al., 1996a,b; Herndl et al., 1997) indicate that heterotrophic bacterioplankton cells are more sensitive than phytoplankton to solar UV stress (mainly UV-B; Herndl, 1993; García-Pichel, 1994). Nevertheless, bacteria are generally dominant in terms of numbers and biomass (Ducklow, 1999).

One of the most critical UV effects is the distortion of the DNA helix by the formation of cyclobutane pyrimidine dimers (CPDs) like T<>T, C<>C, and T<>C (Buma et al., 2001a,b; Sinha et al., 2001). When CPDs accumulate, DNA transcription and replication might be blocked and, if the cell cannot repair the damage, then mutagenesis or even cell death may occur (Sinha et al., 2001). Given their turnover rates, bacteria should be able to adapt to UV stress (Joux et al., 1999; Häder et al., 2003) more easily than slow growing organisms. However, their small size precludes the synthesis and accumulation of protecting substances such as UV-absorbing compounds (García-Pichel, 1994). In the absence of protecting pigments, bacterioplankton adapted using repair mechanisms to cope with UV damage (Boelen et al., 2001, 2002; Banaszak, 2003). CPDs photorepair (PR) implies the presence of PAR and UV-A energy (between 350 and 450 nm) to activate the photolyase enzyme (Sancar, 1996a; Buma et al., 2003). Dark reactions as the nucleotide excision repair (NER), imply additional ATP input by the organism (Sancar, 1996b; Herndl et al., 1997). Even though these mechanisms produce an effective repair of the DNA damage, some lesions may persist if the damage/repair ratio is exceeded and the cells cannot cope with the damage. Furthermore, low temperatures and darkness can hamper the enzymatic repair of DNA (Britt, 1996).

Presently, models and experimental approaches are used to elucidate the effects of UVR on bacterioplankton (see e.g. Huot et al., 2000; Arrieta et al., 2000; Chatila et al., 2001). However, the multifactorial nature of the process makes it very difficult to generalize response patterns (Peachey, 2005). Models could simulate mechanisms of net DNA damage and repair considering variables such as dissolved organic matter (liability/composition), spectral irradiance, and vertical mixing of the water column (Huot et al., 2000). Recently, experimental approaches were used (Obernoster et al., 2001) to quantify the effects of UVR on bacterioplankton respiration, biodegradation, and production considering communities inhabiting water parcels from 5000 and 200 m deep brought to the surface.

The high productivity of waters in Central-South Chile (35–36°S), especially during austral summer, is related to wind-forced upwelling, in conjunction with deep upwelling generated by cyclonic eddies and the shoreward advection of Equatorial Subsurface waters (ESSW; highly saline, >34.4; cool, <10.5 °C; and with high nutrient concentration). During upwelling events, the ESSW (usually located below the euphotic zone) raise to depths of 80 m or less (Ahumada et al., 1991; Strub et al., 1998; Leth and Middleton, 2004). The high nutrient concentration of ESSW induces high coastal primary productivity and bacterioplankton secondary production (BSP, Daneri et al., 2000; Troncoso et al., 2003; Cuevas et al., 2004) and helps to sustain more than 50% of the Chilean fish landings (Sobarzo et al., 2001; Cubillos et al., 2002). During windy periods, upwelled water parcels that were not previously exposed

to solar radiation are suddenly exposed to surface or near surface irradiance. Surprisingly, there are few reports for the nearby area of Central-North Chile (Helbling et al., 1993; Montecino and Pizarro, 1995). These studies, together with one report in Central-South Chile within the HCS (Hernandez et al., 2006), highlighted the importance of PAR and UVR inhibiting phytoplankton and bacterioplankton production.

The aim of this paper is to evaluate the daily impact of solar radiation (PAR and UVR) on (i) bacterioplankton production and (ii) the induction of DNA damage in two natural bacterioplankton assemblages inhabiting surface and subsurface waters off Central-South Chile (36°S). In addition we determined photoreactivation (PR) and nucleotide excision repair (NER) of the already induced DNA damage of both bacterial assemblages.

2. Materials and methods

2.1. Sampling

Seawater (100 l) was collected from a coastal station in Coliumo Bay and a shelf station located 18 nautical miles offshore (Fig. 1). Shelf water was obtained on board the R/V Kay Kay, using an acid (1 N HCl) washed Go-Flo bottle (30 l). The water was transported in the dark, in acid-cleaned polycarbonate containers, and kept at *in situ* temperatures during transport until beginning of the experiments. The sampling (coastal and offshore) stations were selected based on previous oceanographic information (Ahumada and Chuecas, 1979; Arcos et al., 1995; Strub et al., 1998; Figueroa and Moffat, 2000). These studies described the distribution of the hydrological properties of the area, indicating that both sampling zones are under the influences of typical upwelling water masses (ESSW). This type of water is bringed to the surface by southerly winds action mainly during spring–summer periods and disappears from the surface during winter (Faundez et al., 2001).

Four experiments (different dates) were carried out at the Dichato Marine Station (Universidad de Concepción, Chile) during October–November 2004. Two experiments were conducted with typical ESSW collected below the photic layer at the Concepción shelf (80 m depth). The other two were done with surface water collected in Coliumo Bay (0.5 m depth) representing the already upwelled waters. The experiments were designed to determine the impact of solar radiation (PAR, UV-A, and UV-B) on bacterial secondary production (BSP) and DNA, and to establish the photorepair/nucleotide excision repair (PR/NER) mechanisms in the bacterioplankton assemblages. Bacterial BSP was measured by radiolabeled leucine and thymidine incorporation and DNA damage was quantified by CPDs induction. The bacterioplankton assemblages used in the experiments (0.7–0.2 μm) from the two depths differed thus in their light history.

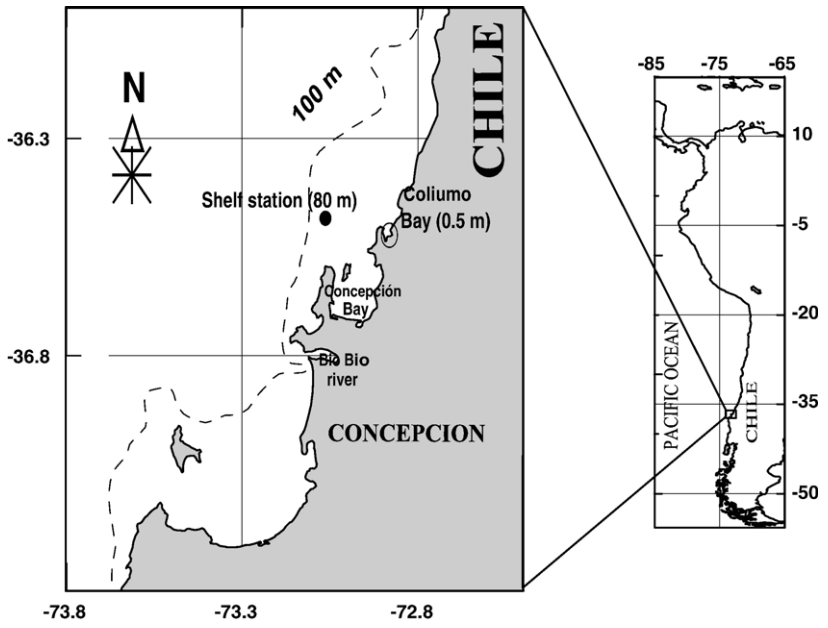

Fig. 1. Map of the study zone in Central-South Chile upwelling zone; sampling sites and depths are marked as Shelf Station (80 m) and Coliumo Bay (0.5 m).

2.2. Experimental settings

During the same day of water collection, bacterioplankton cells were separated by filtration onto Whatman GF/F filters (47 mm, 0.7 μm). Subsequently, 60 l of the filtered water was distributed among plexiglass UV-transparent containers (Rhom and Haas), previously cleaned with HCl 10% v/v. All the containers were exposed to incident solar radiation, submersed in an outdoor water bath where temperature was controlled by continuously pumping surface Coliumo Bay water. The temperature during the incubations was 18±3 °C for both surface and subsurface samples. The temperature used resembles that encountered by water from 80 m when it reaches the surface due to upwelling. The temperature variations for each sampling time were considered also for bacterial respiration calculations (see Section 2.4) according to del Giorgio et al. (1997).

Various radiation arrangements were implemented to determine the impact of different wavebands on BSP and DNA throughout the day. The incubations started during early morning and samples were obtained close to local noon, sunset and the morning of the following day. These experiments were designed to assess the impact of solar radiation during the morning and afternoon; also, the PR capacity of different wavebands was determined during the afternoon as well as NER. Basically, a combination of three radiation treatments was used in our experimental setup: A) PAB treatment — unfiltered samples receiving full solar radiation (280–700 nm); B) PA treatment — samples covered with PR Montagefolie filter (No. 10155099, Folex; 50% transmission at 320 nm) so they were exposed to irradiances from 320 to 700 nm; and C) P treatment — samples covered with Ultraphan UV Opak filter (Digefra film, Munich, Germany; 50% transmission at 395 nm) so they received irradiances from 400 to 700 nm. The spectral characteristics of these filters are published elsewhere (Villafañe et al., 2003). A total of ten containers were exposed to solar radiation and the combinations or radiation treatments were as follows: 1) two containers exposed under the PAB treatment that were collected at noon (to obtain morning impact); 2) two containers that were under the PAB treatment during the morning and then covered with a filter so they were under a PA treatment during the afternoon (morning impact followed by afternoon recovery under PA); 3) two containers that were under the PAB treatment during the morning and then covered with a filter so they were under a P treatment during the afternoon (morning impact followed by afternoon recovery under P); 4) two containers that were under the P treatment during the morning and then the filter was removed so they were under a PAB treatment during the afternoon (afternoon impact by UVR); and finally two containers exposed under a PAB treatment for the whole experimental period that were collected after ca 20 h (following morning).

At each sampling time (start, noon, sunset and early next morning), the water collected from each container (5 l) was used for several analyses (as outlined below): 50 ml of sample was used for BSP determinations; 50 ml for bacterial cell counts; 100 ml for nutrients determination; and the rest was used to determine CPDs.

2.3. Bacterial secondary production (BSP) and growth rates (μ)

Bacterial secondary production (BSP) was estimated by the incorporation of radiolabeled precursors of proteins such as ^{14}C-leucine (Leu) (Simon and Azam, 1989) and of DNA such as [methyl-^{3}H]-thymidine (TdR) (Fuhrman and Azam, 1982, modified by Wicks and Robarts, 1987). At the beginning of the experimentation and after the exposure periods 50 ml of sample was separated, 2 replicates and 2 blanks for TdR and Leu determinations. The samples were dispensed into ambar glass flasks, and inoculated with either TdR (87.1–90 Ci mmol^{-1} s.a., Sigma) or Leu (300–330 mCi mmol^{-1} s.a., Sigma) to saturation concentrations of 7 nM and 50 nM, respectively. The flasks were incubated for 2 h in darkness at constant temperature (18±3 °C), resembling the surface Coliumo Bay temperature. Flask incubations were stopped by the addition of cold trichloroacetic acid (TCA, 50% w/v). The samples were filtered (<200 mm Hg) onto 0.2 μm cellulose ester filters (GSWP Millipore) and the filters washed three times with TCA 5% (for both radioactive tracers), then treated with 3 ml of phenol–chloroform solution (50% w/w) and ethanol (80% v/v) for TdR samples (Riemann and Bell, 1990). The filters were immediately transferred to vials and treated with 1 ml of ethyl acetate and 10 ml Ecolite (+) (ICN). Both Leu and TdR uptake were estimated from dpm, using a Packard (Model 1600TR) liquid scintillation counter; the counting efficiency was calculated from the non-quenched standard of ^{3}H-toluene. BSP from Leu incorporation was calculated using a ratio of cellular carbon to protein of 0.86 and a fraction of Leu in protein of 0.073 (Simon and Azam, 1989).

The cell production rates obtained from moles of TdR incorporated (see Fuhrman and Azam, 1982) were transformed to BSP assuming a widely used conversion factor of 2×10^{18} (Lee and Fuhrman, 1987; Sherr et al., 1997, 2003) which is comparable with previous calculations made for the zone (Troncoso et al., 2003; Cuevas

et al., 2004; Hernandez et al., 2006). In addition, the cell specific incorporation of Leu and TdR (expressed as fg C cell d^{-1}) was calculated as the quotient of either Leu or TdR incorporation to total bacterial number (cell abundance) determined for each water sample. Finally, bacterioplankton growth rates ($\mu = d^{-1}$) were calculated as the ratio of bacterial secondary production (BSP as μg C l^{-1} d^{-1}) over the estimated biomass (B, see Section 2.5).

2.4. Bacterioplankton growth efficiency (BGE)

By definition, BGE is the quantity of biomass synthesized per unit of substrate assimilated (del Giorgio and Cole, 2000) and it can be estimated as:

$$BGE = BSP/(BSP + BR) \quad (1)$$

where BGE is bacterioplankton growth efficiency, BSP is bacterial production, and BR is bacterial respiration.

Bacterioplankton respiration (expressed as μg C l^{-1} d^{-1}) was estimated using the del Giorgio et al. (1997) equation:

$$\log BR = -3.67 + 0.75 \log BA + 0.059 \times T \quad (2)$$

where BA is bacterioplankton abundance (cells ml^{-1}) and T is the incubation temperature (°C).

2.5. Bacterial abundance and biomass

Bacterial abundance (BA) was estimated from samples preserved with formaldehyde (2% final concentration) and stored in darkness at 4 °C. Duplicates (3 ml) were stained with DAPI (4′,6-diamidino-2-phenylindole) and filtered on dark 0.2 μm polycarbonate filters. Ten to twenty random fields (minimum 400 cells) were counted, using a Zeiss-Axioskop epifluorescence microscope (1000×) equipped with quartz optics with UV excitation. The bacterioplankton biomass was calculated using the BA measured at each sampling time and the conversion factor of 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1987; Sherr et al., 1997, 2003).

2.6. Nutrient measurements

Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations were determined following the methodology described by Strickland and Parsons (1972), whereas silicate (Si_2O_3) was determined as in UNESCO (1983). Each 100 ml sample collected for nutrient determination was frozen in plastic flasks for later manual determination with the Agilent UV–Visible spectrophotometer (Model 8453).

2.7. CPDs analyses

The bacterioplankton from 5 l samples separated for CPDs analyses, was concentrated on 0.2 μm polycarbonate filters (GTBP Millipore filters 47 mm) and immediately frozen in liquid nitrogen (−180 °C) until further analysis at the PROIMI Research Center (Tucumán, Argentina). DNA was extracted from the filters using the procedure described by Buma et al. (2001a,b). RNA was removed from the extracts by incubating the samples for 1 h with 75 μg ml^{-1} RNAase (Boerhringer Mannheim) at room temperature. The DNA concentration of the extracts was determined using a fluorometer, and CPDs were determined employing a primary antibody (H3, Affitech, Oslo, Norway) directed mainly to thymine dimers (Boelen et al., 1999). In short, 100 ng of heat denatured DNA samples was blotted onto nitrocellulose membranes (Scheleicher and Shuell, Protran 0.1 μm). To immobilize the DNA, the membranes were baked at 80 °C with the secondary antibody (HRP rabbit–anti-mouse, Dako PO260) for 2 h at room temperature. CPDs were detected using ECL detection reagents (RPN2106 Amersham) in combination with photosensitive films (Kodak-X-AR-5). Finally, these films were scanned and dimers were quantified using Image Quant software (version 4.2 Molecular Dynamics). Each blot contained two dilution series of standard DNA with known amounts of CPDs (Boelen et al., 1999).

2.8. Radiation measurements

Incident solar radiation was monitored every minute during the experimental period (October and November 2004), with a filter radiometer (GUV-511C Biospherical Instruments). The radiometer has a broad band channel for PAR (400–700 nm) and four narrow band channels for UVR (380, 340, 320, and 305 nm). Integrated irradiance values (W m^{-2}) for UV-A (320–400 nm) and UV-B (280–320 nm) were obtained using the Orce and Helbling (1997) model and total ozone column concentrations were obtained from satellite data (http://jwocky.gsfc.nasa.gov).

2.9. Statistical analysis

Two-factor ANOVA analysis was conducted in order to determine significant effects of solar exposure on bacterioplankton metabolic activity and DNA damage for each depth obtained from the incorporation of Leu and TdR. The variables BSP, BGE, abundance, μ and CPDs amount from each experimental treatment (PAB,

PAB-PA, PAB-P, P-PAB) was compared along the day. Factor I was 'experiment' (two levels) and time was factor II (three levels). We also compared the influence of the different treatments at the end of the day on BSP, BGE, abundance, μ and CPDs amount using a two-way ANOVA, with the experiments as factor I and the treatments (P-PAB, PAB-P, PAB-PA) as factor II. Finally, the bacterioplankton PR/NER repair was analyzed with a two-way ANOVA, using the experiments and treatments (PAB at noon, PAB-P, PAB-PA at sunset) as categorical factors. We used log transformed BSP, BGE, and μ rates and the inverse of bacterioplankton abundance, in order to reduce deviations from the homogeneity of variance and normality (Sokal and Rohlf, 1981). All data were tested for homoscedasticity with Cochran's *C*-test (Sokal and Rohlf, 1981). This analysis was done with Statistica 6.0 software. Pairwise multiple comparisons of differences between treatment means were conducted using Tukey's test ($\alpha = 0.05$).

3. Results

3.1. Radiation measurements

Spring ozone concentrations at Concepción decreased from the beginning of October towards the end of November, 2004, values ranged between 329 and 239 DU (Fig. 2a; Total Ozone Mapping Spectrometer — TOMS; http://jwocky.gsfc.nasa.gov). Daily doses of PAR, UV-A, and UV-B are shown in Fig. 2b–d. Surface

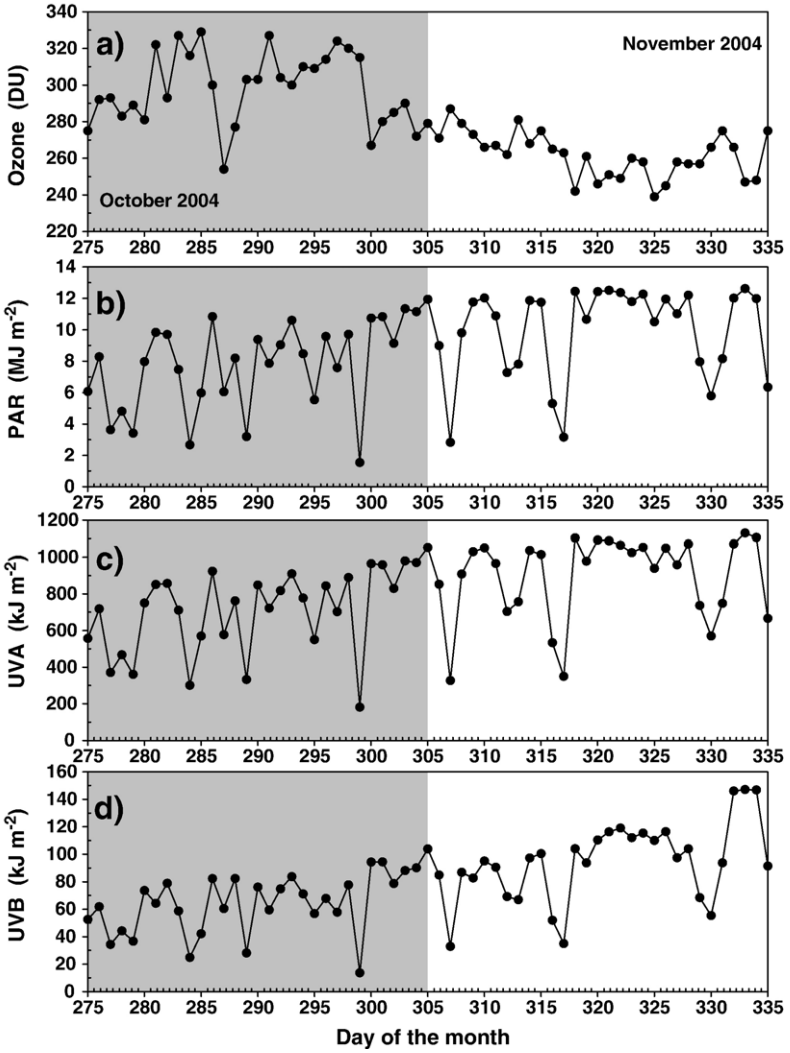

Fig. 2. Atmospheric radiation conditions in Coliumo Bay during October and November 2004. (a) Ozone column concentrations obtained from TOMS images (Dobson units). (b) Daily PAR dose in MJ m^{-2}. (c) Daily UV-A dose in kJ m^{-2}. (d) UV-B dose in kJ m^{-2}. All measured with a GUV-511C radiometer located at the Dichato Marine Biology Station in Coliumo Bay.

radiation reached a maximum on November 28 with PAR values of 12.6 MJ m^{-2} followed by UV-A and UV-B values of 1131 and 147 kJ m^{-2}, respectively. During these months the observed variability was mainly due to cloud cover, which also occurred during the experimental days (i.e., October 6 and 29, 2004). During these dates, the maximum irradiances reached 395.8 W m^{-2} (PAR), 35.5 W m^{-2} (UV-A), and 4.9 W m^{-2} (UV-B) (Fig. 3a–b). Similar values were observed on November 23 and 26, 2004, when maximum irradiances were 451 W m^{-2} (PAR), 39 W m^{-2} (UV-A), and 6.3 W m^{-2} (UV-B) (Fig. 3c–d). During the experiments, some differences were detected in the measured radiation due to cloud cover. For example, scatter clouds were present during the afternoon on October 6 (Fig. 3a), while heavy cloud conditions were observed during the whole morning of November 26 (Fig. 3d).

3.2. Day–night effects on bacterioplankton secondary production and growth

Surface bacterial assemblages (0.5 m) had a BSP (based on Leu incorporation) that varied between 0.56 and 5.49 fg C $cell^{-1}$ d^{-1} (Fig. 4a). There was a significant inhibition of BSP at local noon (Tukey < 0.05), that decreased to 78% of the BSP value at time zero (t_0). The rate of inhibition was less during the afternoon, and no significant differences were observed between noon and

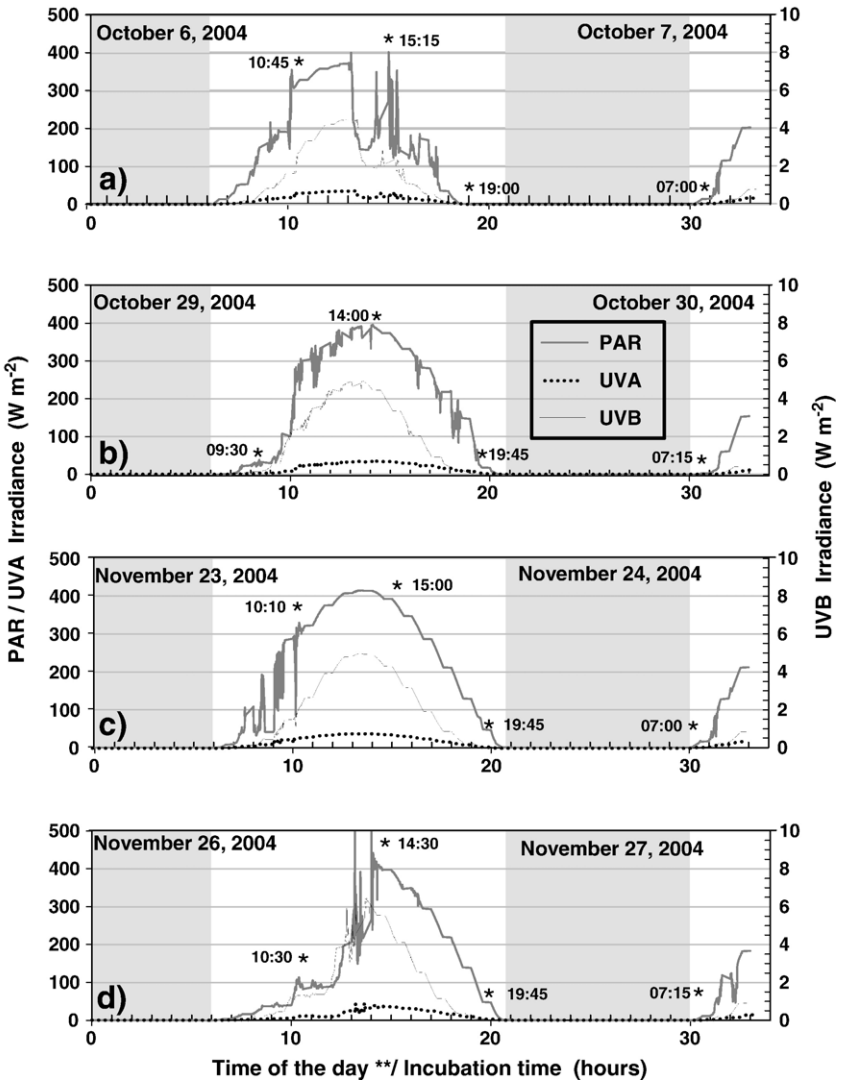

Fig. 3. Irradiance conditions during the experimental days, indicating sampling hours. PAR, UV-A, and UV-B irradiances are expressed as W m^{-2}. (a) October 6, 2004; experiment 1 with 80 m waters. (b) October 29, 2004; experiment 1 with 0.5 m waters. (c) November 23, 2004; experiment 2 with 0.5 m waters. (d) November 26, 2004; experiment 2 with 80 m waters.

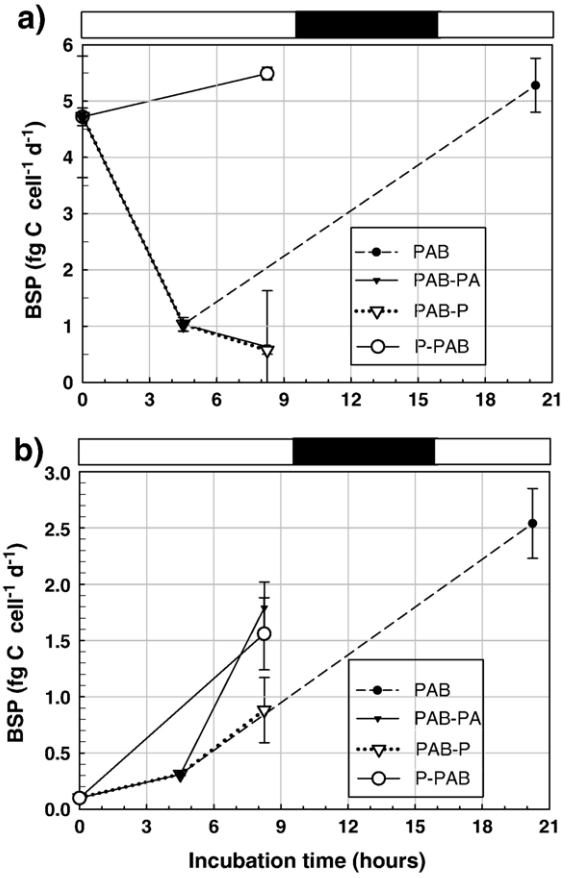

Fig. 4. Bacterioplankton secondary production (BSP) normalized per cell abundance as fg C cell^{-1} d^{-1} for surface (a) and subsurface waters (b) estimated from Leu incorporation. PAB: BSP after full sunlight and dark exposure. PAB-PA: BSP after morning sunlight (PAB) and afternoon PAR plus UV-A exposure. PAB-P: BSP after morning sunlight (PAB) and afternoon PAR exposure. P-PAB: BSP after morning PAR and afternoon sunlight (PAB) incubation. Vertical lines indicate the standard deviation of the samples (2 replicates and 2 experiments). Surface water experiments were conducted on October 29 and November 23, 2004, and subsurface water experiments on October 6 and November 26, 2004. Horizontal bars above the plot indicate light (open bars) and dark (filled bar) incubation periods.

afternoon BSP (Tukey>0.05), when filters were placed on top of the containers and the UV-B stress was eliminated (Fig. 4a). The afternoon BSP values in the PAB-PA and PAB-P treatments were 12% and 13% of the initial value, respectively. Additionally, surface assemblages recovered during the night period, with a significant 12% increase in BSP (Tukey<0.05) as compared to the t_0 values. BSP inhibition was also compared in the different exposure treatments. When surface samples were exposed only to PAR irradiances during the morning and to full solar radiation during the afternoon (P-PAB), BSP increased significantly (Tukey<0.05) by 16% of the initial value (Fig. 4a) suggesting some degree of acclimation to the new conditions of irradiance.

On the other hand, subsurface prokaryote assemblages (80 m samples), showed a differential enhancement response in terms of diurnal BSP (Fig. 4b). These subsurface assemblages had, however, lower BSP values (0.1–2.54 fg C cell^{-1} d^{-1}) than the surface bacterioplankton. There was a slow but significant increase (2.1 times of t_0; Tukey<0.05) of BSP in the samples exposed to full solar radiation (PAB treatment) at noon. A BSP enhancement was also observed at

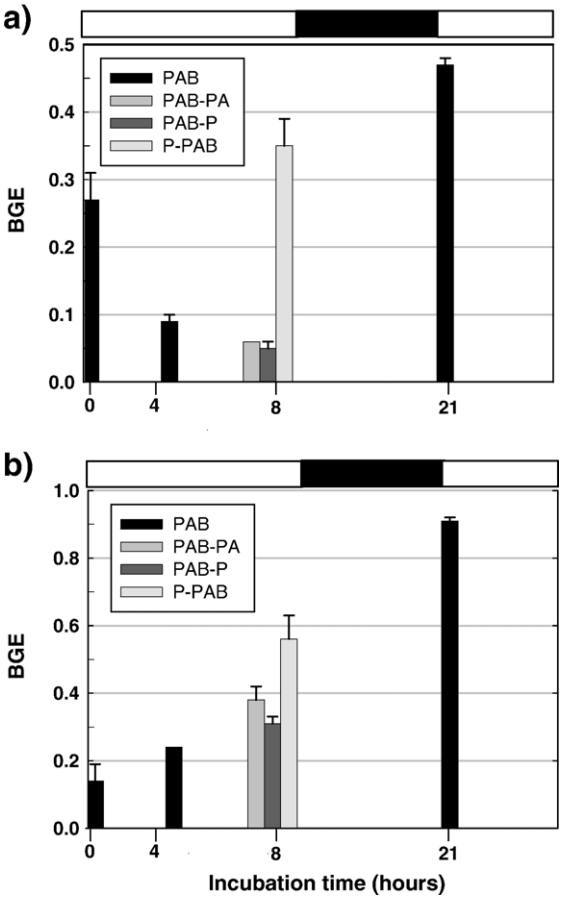

Fig. 5. Bacterioplankton growth efficiency (BGE) for surface (a) and subsurface waters (b) estimated from Leu incorporation as a function of the incubation time. Open bars show pre-exposure levels. PAB: BGE during the morning exposure. PAB-PA: BGE obtained after full morning sunlight and afternoon PAR plus UV-A radiation exposure. PAB-P: BGE after morning sunlight (PAB) and afternoon PAR radiation exposure. P-PAB: BGE after PAR incubation during the morning and full sunlight (PAB) during the afternoon. Vertical lines indicate the standard deviation of the samples (2 replicates and 2 experiments). Surface water experiments were conducted on October 29 and November 23, 2004, and subsurface water experiments on October 6 and November 26, 2004. Horizontal bars above the plot indicate light (open bars) and dark (filled bar) incubation periods.

sunset with maximal values in the PAB-PA treatment, and minimal in the PAB-P treatment. Despite the conspicuous effects of PAR plus UV-A on BSP at sunset, a substantial increase in BSP (Tukey<0.001) was also found during the dark period reaching a value in the PAB treatment that was approximately 24 times higher than that at t_0.

Bacterioplankton BA (data not shown) and BGE (Fig. 5) resembled the observed BSP trend. Since μ (growth) rates showed the same trend as BSP and BGE during the experimental period, we only reported the mean μ values (Table 1). The average of the estimated BGE for surface waters ranged from 0.05 to 0.47 (Fig. 5a), whereas the average μ ranges varied from 0.3 to 2.7 d^{-1} (see Leu, Table 1). Both BGE and μ decreased significantly (Tukey<0.05), during the morning (PAB treatment) and sunset (PAB-P and PAB-PA treatments) and were significantly lower (Tukey<0.05), than the values in the P-PAB treatment (Fig. 5a, Table 1). Additionally, a significant increase (Tukey<0.05), in both bacterioplankton BGE and μ was detected after a dark period as compared to the t_0 value. Assemblages from 80 m had slightly higher BGE (0.14–0.91, Fig. 5b) and μ (1.5–12.7 d^{-1}, Table 1) than surface assemblages. BGE and μ increased significantly (Tukey<0.05), after the night incubation periods (Fig. 5b, Table 1).

The BSP estimated from the daily TdR incorporation, measured in parallel for surface and subsurface bacterioplankton samples, had different trends (Fig. 6). The BSP for surface assemblages in the PAB treatment decreased from 1.61 fg C $cell^{-1}$ d^{-1} at t_0, to 0.26 fg C $cell^{-1}$ d^{-1} at local noon (Fig. 6a). The observed inhibition of 84% at local noon decreased with time and BSP increased in samples where UV-B was screened off during the afternoon (PAB-PA and PAB-P treatments). Nevertheless, BSP was still inhibited after the dark period, and had a value of 56% of the initial BSP value (Fig. 6a). In subsurface assemblages (80 m), BSP oscillated between 0.17 and 7.69 fg C $cell^{-1}$ d^{-1} (Fig. 6b). Contrary to the surface water trend, BSP was enhanced approximately 44 times with respect to the very low t_0 value. At sunset, the maximum BSP was observed under PAB-PA treatment (25 times over initial BSP values) followed by P-PAB, both differed significantly (Tukey<0.01) from the low PAB-P (Fig. 6b).

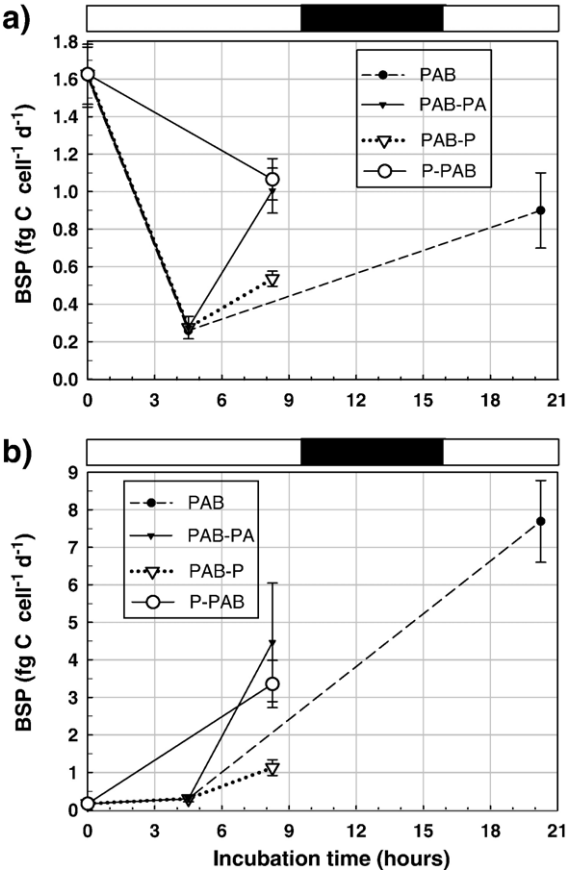

Fig. 6. Bacterioplankton secondary production (BSP) normalized per cell abundance as fg C $cell^{-1}$ d^{-1} for surface (a) and subsurface waters (b) estimated from TdR incorporation. PAB: BSP after sunlight and dark exposure. PAB-PA: BSP after morning sunlight (PAB) and afternoon PAR plus UV-A exposure. PAB-P: BSP after morning sunlight (PAB) and afternoon PAR exposure. P-PAB: BSP after morning PAR and afternoon sunlight (PAB) incubation. Vertical lines indicate the standard deviation of the samples (2 replicates and 2 experiments). Surface water experiments were conducted on October 29 and November 23, 2004, and subsurface water experiments on October 6 and November 26, 2004. Horizontal bars above the plot indicate light (open bars) and dark (filled bar) incubation periods.

Table 1
Mean bacterioplankton growth rates (μ) in d^{-1} calculated from Leu and TdR incorporation for the four experiments carried out in the Concepción upwelling zone during October and November 2004 (explanation in the text)

Sampling depth (m)	t_0, X±Sd	PAB, X±Sd	PAB-P, X±Sd	PAB-PA, X±Sd	P-PAB, X±Sd	Night, X±Sd
Leu (0.5 m)	2.3±0.54	0.5±0.1	0.3±0.01	0.3±0.04	2.7±0.5	2.6±0.1
TdR (0.5 m)	0.8±0.08	0.1±0.03	0.3±0.02	0.5±0.06	0.5±0.05	0.4±0.1
Leu (80 m)	0.5±0.1	1.5±0.1	4.4±1	8.9±1	7.8±2	12.7±1.5
TdR (80 m)	0.1±0.05	0.1±0.04	0.6±0.1	2.2±0.79	1.7±0.31	3.8±0.54

The treatments were averaged at each sampling time.

BGE estimates from surface assemblages were low (0.03–0.13, Fig. 7a), while mean μ values varied from 0.1 to 0.8 d^{-1} (Table 1). After a complete day–night cycle, significant increases were noticed in BGE and μ for the PAB treatment as compared to local noon values (Fig. 7a, Tukey<0.05). Subsurface assemblages had higher BGE values (0.03–0.51, Fig. 7b) and μ (0.1–3.8 d^{-1}, Table 1) than the surface ones. These rates increased during the day and the highest BGE and μ were detected after a night incubation period.

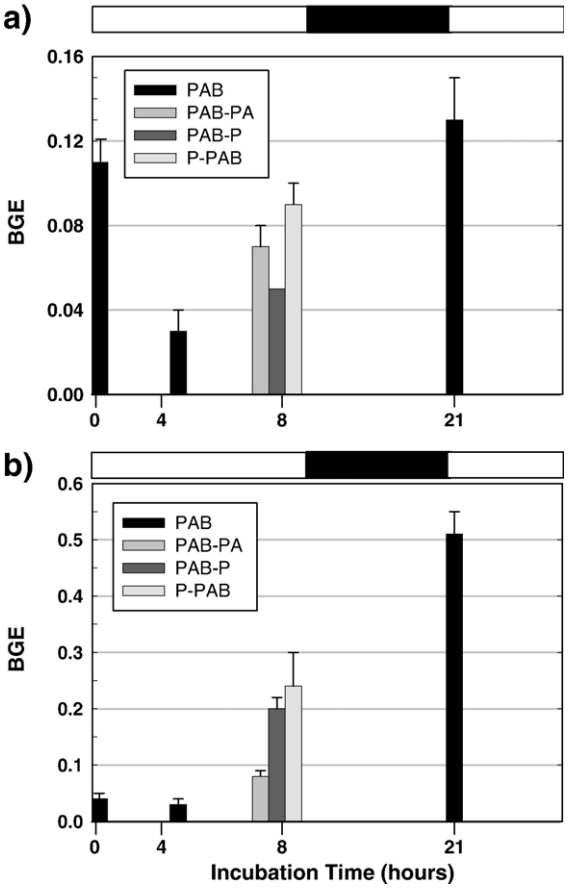

Fig. 7. Bacterioplankton growth efficiency (BGE) for surface (a) and subsurface waters (b) estimated from TdR incorporation as a function of the incubation time. Open bars show pre-exposure levels. PAB: BGE during morning exposure. PAB-PA: BGE obtained after morning sunlight and afternoon PAR plus UV-A radiation exposure. PAB-P: BGE after morning sunlight (PAB) and afternoon PAR radiation exposure. P-PAB: BGE after PAR incubation in the morning and full sunlight (PAB) in the afternoon. Vertical lines indicate the standard deviation of the samples (2 replicates and 2 experiments). Surface water experiments were conducted on October 29 and November 23, 2004, and subsurface water experiments on October 6 and November 26, 2004. Horizontal bars above the plot indicate light (open bars) and dark (filled bar) incubation periods.

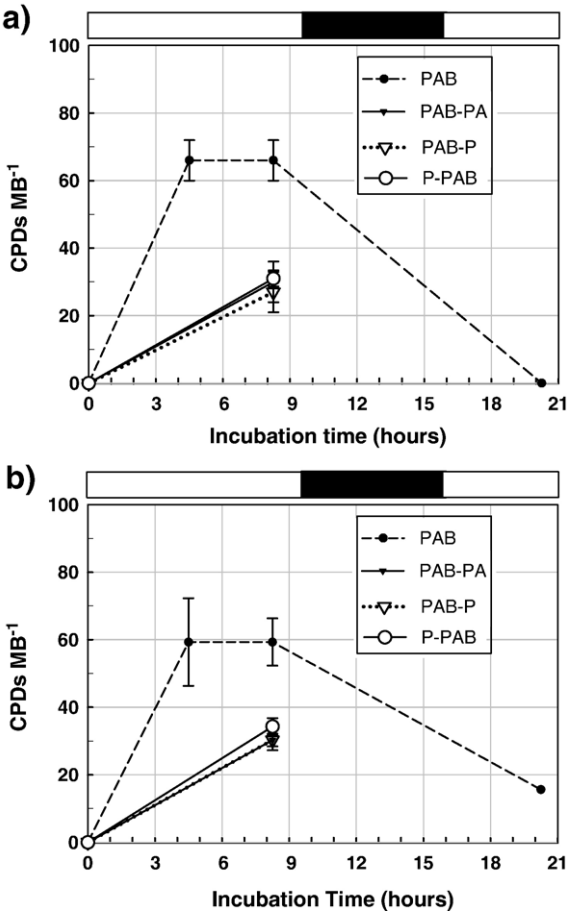

Fig. 8. Bacterioplankton CPDs accumulation for surface (a) and subsurface waters (b) as a function of the incubation time. PAB: CPDs accumulation after sunlight and night exposure. PAB-PA: CPDs accumulation after morning sunlight and afternoon PAR plus UV-A radiation exposure. PAB-P: CPDs accumulation after morning sunlight (PAB) and afternoon PAR radiation exposure. P-PAB: CPDs accumulation after PAR incubation in the morning and full sunlight (PAB) in the afternoon. Vertical lines indicate the standard deviation of the samples (2 replicates and 2 experiments). Surface water experiments were conducted on October 29 and November 23, 2004, and subsurface water experiments on October 6 and November 26, 2004. Horizontal bars above the plot indicate light (open bars) and dark (filled bar) incubation periods.

3.3. Bacterioplankton CPDs accumulation

DNA damage was evaluated via CPDs formation and was plotted as a function of the exposure time throughout the day for each bacterial assemblage (Fig. 8). Both assemblages had a similar induction of CPDs throughout the day, with relatively high values at noon reaching approximately 60 CPDs MB^{-1}. In addition, both assemblages photorepaired the UV-B-induced damage in samples in which UV-B has been screened

off by filters (PAB-P, and PAB-PA treatments) during the afternoon, having a significant decrease (Tukey < 0.05), in the CPDs MB^{-1}. The observed photorepair was more efficient in subsurface samples (Tukey < 0.05), with a 51% repair (Fig. 8b) as compared to the surface assemblages with a 41% repair capacity (Fig. 8a). There were also some differences in the dark repair capacity of the two assemblages, while surface samples had a complete removal of CPDs during the night (Fig. 8a), subsurface assemblages still had almost 16 CPD MB^{-1}, suggesting an incomplete (50%) dark repair mechanism (Fig. 8b). It is interesting to note that both assemblages had zero CPDs during early morning (previous exposure time, t_0).

4. Discussion

Natural bacterioplankton assemblages in a wide range of ecosystems are sensitive to PAR and UVR solar levels (i.e. Jeffrey et al., 2000; Obernoster and Herndl, 2002; Buma et al., 2003; Alonso et al., 2006). The effects of solar radiation described for marine bacteria had been mainly deleterious on surface waters (Jeffrey et al., 1996b), as well as in bacterioplankton from several meters depth in the water column (i.e. in tropical regions, Buma et al., 2003). In our study, maximum solar irradiance induced a BSP inhibition in bacterioplankton from surface waters, whereas an enhancement effect, under the same solar stimulus, was observed in subsurface assemblages. The inhibition of surface BSP had been detected in other productive areas such as the Gulf of Mexico (Jeffrey et al., 1996a,b) and the Gulf of Aqaba (Boelen et al., 2002; Buma et al., 2003). In highly productive waters the Concepción upwelling zone solar impact on bacterioplankton was as previously suggested by McManus and Peterson (1988) where an inhibition of surface BSP was associated especially with clear skies and active winds during the upwelling periods. Wind, on the other hand, could induce vertical mixing in the water column (Huot et al., 2000; Jeffrey et al., 2000), and thus the cells would be transported to deeper layers where repair processes dominate. However, as occurred in our case, the 80 m bacterioplankton assemblages reacted oppositely to the surface assemblages by keeping or enhancing their initial activity (i.e., TdR and Leu incorporation, BGE, and μ). This extreme scenario, of incubating subsurface the water samples as if they were transported upward and received full solar radiation, usually occurs off Concepción (36°S) during upwelling periods when the ESSW rise to the surface by the action of winds (Strub et al., 1998; Sobarzo et al., 2001). Even so, the bacterioplankton response to solar radiation is more complex because it could vary not only with depth but also with the season. For instance, during summer subsurface assemblages (80 m) at Concepción had daily BSP inhibition (mainly due to PAR ~ 50%, Hernandez et al., 2006), as compared to the enhancement response during spring (present study, same depth).

The variability of bacterioplankton responses to solar radiation off Concepción (regarding time or depth) could be explained by the temporal predominance of different prokaryote groups in the water column (see e.g. Joux et al., 1999; Arrieta et al., 2000; Winter et al., 2001) as well as by the previous light history (degree of sensitivity) of the bacterial strains (Helbling et al., 1995; Boelen et al., 2000, 2001, 2002). Within the HCS, some reports showed nitrificant prokaryotes inhabiting the base of the oxygen minimum zone (1% photic depth) off northern Chile (21–23°N, Castro-Gonzalez et al., 2005; Molina, 2005). It was observed, during two years, that archaea (mainly Crenarquea), usually dominates waters off Concepción below 40 m with abundances up to 50%; while at surface waters eubacteria were more common ~ 84% (Levipan, 2006). In spite of these findings the role of prokaryote diversity and the effect of solar radiation on their ecological function (i.e. BSP, BGE, μ rates and BA) remain unknown in this upwelling ecosystem.

The BGE and μ rates differed in both bacterioplankton assemblages following the BSP trend; this result may support the idea of a degree of sensitivity to PAR and UV of the bacterioplankton assemblages (Pakulski et al., 1998). Indeed, fast growing bacteria with good nutritional status might be less sensitive to solar radiation than slow growing or starving bacteria despite environmental changes (del Giorgio and Cole, 2000; Jeffrey et al., 2000). During 2003 and 2004 high BA values during summer (January), decreasing to low values in winter (July), for surface assemblages in the Concepción area (Levipan, 2006). This BA variation might be related to the irradiance changes due to cloud cover, and probably contribute to explain the inhibition effect on subsurface bacterial assemblages (as BSP inhibition) during summer (Hernandez et al., 2006), and, the BSP enhancement during spring (present study). Changes in BA were significant (Tukey < 0.05) within a day for solar exposure treatments of surface waters and the initial surface waters values (data not shown), and also dark repair values with solar treatments. The BSP and BA findings in a daily scale during spring and in summer could indicate a seasonal trend for the total prokaryote component in the water column, where bacterioplankton response to solar radiation is limited by their photobiological history and species-specific diversity associated to the upwelled waters. Nevertheless the differences on the bacterioplankton metabolic response will not be necessarily reflected in

Table 2
Average nutrients concentrations in the water samples at each depth used in the experiments

Water depth (m)	Date	μM Si (X±Sd)	μM NO_2 (X±Sd)	μM NO_3 (X±Sd)
80 m	6-Oct-04	28±0.12	0.2±0.02	19±0.17
0.5 m	28-Oct-04	17±0.15	0.5±0.02	11±0.19
0.5 m	23-Nov-04	29±0.80	1.8±0.00	13±0.55
80 m	26-Nov-04	31±0.31	2.5±0.04	20±0.08

the DNA damage–repair mechanism as what occurred in our case.

The CPDs accumulation was very similar for surface and subsurface bacterial assemblages. Both depths samples had zero CPDs during early morning when the experiment started, although the reasons were different: Surface assemblages had no CPDs as their repair mechanisms (both PR and NER) were effective during a daily cycle to remove any UV-B-induced CPDs. However subsurface assemblages had no CPDs as these cells have no previous exposure to UVR at 80 m. After solar exposure, the repair mechanisms PR (mostly due to UV-A) and NER were observed, accounting for 50% each in surface bacterial assemblages but ~50% (PR) and 25% (NER) in subsurface assemblages. Therefore, PR and NER systems were present but not equally effective in repairing DNA (Joux et al., 1999; Buma et al., 2003). The 80 m assemblages would start a new day with chronic DNA damage, so it would be more difficult to overcome the solar stress in addition to the energetic cost of the NER (e.g. Arrieta et al., 2000; Jeffrey et al., 2000; Buma et al., 2001a; Visser et al., 2002).

Our results showed differences regarding the BSP inhibition and CPDs accumulation throughout the day. These processes were uncoupled or could vary inversely as compared to other studies in productive zones where the BSP from subsurface samples diminished and the CPDs increased together with the solar exposure (i.e. Jeffrey et al., 1996a). This impact of UVR on subsurface assemblages BSP and the DNA damage might have important implications in upwelling events that occur during periods of high irradiances (i.e. summer), if changes in bacteria assemblages cannot cope with UVR levels at that time. Future research in the Concepción upwelling zone should include changes in substrate liability induced by solar exposure (Obernoster et al., 1999, 2001). DOM photo-dissociation coupled with the high concentration of nutrients present in subsurface waters might have enhanced the BSP and compensated the DNA damage from daytime incubations, considering besides that the BSP increases with the age of the upwelled water (Herbland, 1978; Gocke et al., 1983). Furthermore, the nutrient levels measured during the experiments (Table 2) and the high bacterial activity after the night period (for both bacterial assemblages) support the idea that bacterioplankton did not experience additional stress due to a lack of nutrients.

In conclusion, the differences in sensitivity between bacterioplankton assemblages are more obvious in terms of metabolism (Leu and TdR incorporation, BGE, or μ rates), than on DNA damage (CPDs accumulation). Evidently, there are some mechanisms that allow the strains to survive high doses of UVR that remain to be described. Therefore, future studies should also point out, as in the case of Concepción, i) the description of the species-size relation (i.e. within archaea and eubacteria) with the damage induction (Joux et al., 1999; Wilhelm et al., 2003), ii) biogeochemical and physical factors that trigger the defense against solar radiation (i.e. DOM liability, temperature, mixing), iii) definition of several other repair or protective ways to deal with UV-B and UV-A indirect stress (Agogué et al., 2005) and finally iv) description of the wide range of differences along latitudinal and temporal scales (i.e. seasonal) thus developing a comparison of UV-B vulnerability.

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