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Effects of UV-B Radiation on the Structural and Physiological Diversity of Bacterioneuston and Bacterioplankton

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The effects of UV radiation (UVR) on estuarine bacterioneuston and bacterioplankton were assessed in microcosm experiments. Bacterial abundance and DNA synthesis were more affected in bacterioplankton. Protein synthesis was more inhibited in bacterioneuston. Community analysis indicated that UVR has the potential to select resistant bacteria (e.g., *Gammaproteobacteria*), particularly abundant in bacterioneuston.

Global changes over the next decades are expected to increase the exposure of aquatic organisms to damaging UV wavelengths, particularly UV-B (280 to 320 nm), with far-reaching ecological consequences (5). Therefore, a thorough understanding of the effects of UV radiation (UVR) on the diversity and function of bacterial communities, key players in nutrient cycling in aquatic ecosystems, is necessary.

The effects of UV-B on aquatic organisms depend on the dose of harmful radiation to which they are exposed, which is, in turn, determined by the positioning of the organism in the water column (19). The surface microlayer (SML) represents a unique microbial niche in which the bacterial community (bacterioneuston) is exposed to high doses of solar UVR (2). The higher abundances of microorganisms in the SML than in underlying waters (UW) (e.g., 1) could indicate that bacterioneuston may have adapted to this "extreme environment," making it an interesting model system for the assessment of UV effects on aquatic organisms by testing the hypothesis that bacterioneuston and bacterioplankton respond differently (in terms of abundance, activity, and structural and functional diversity) to UV-B radiation.

Sampling and experimental setup. Samples from the SML and UW were collected in triplicate ($n = 9$) from an estuarine system (Ria de Aveiro, Portugal, latitude 40°38′N, longitude 08°46′N) on three consecutive days in June 2008. Samples were collected around noon, with a clear sky, minimum wind (<2 m s^{-1}), and solar radiation levels ranging from 30 to 35 kJ m^{-2} (climetua.fis.ua.pt/legacy/main/current_monitor/cesamet.htm). Water properties of the original samples are presented as supplemental material. Bacterioneuston was sampled with glass plates (9). Samples from underlying water were taken at a depth of 20 cm. A total of three irradiation experiments (one on each day), with triplicate subsamples, were conducted using unfiltered water samples, since

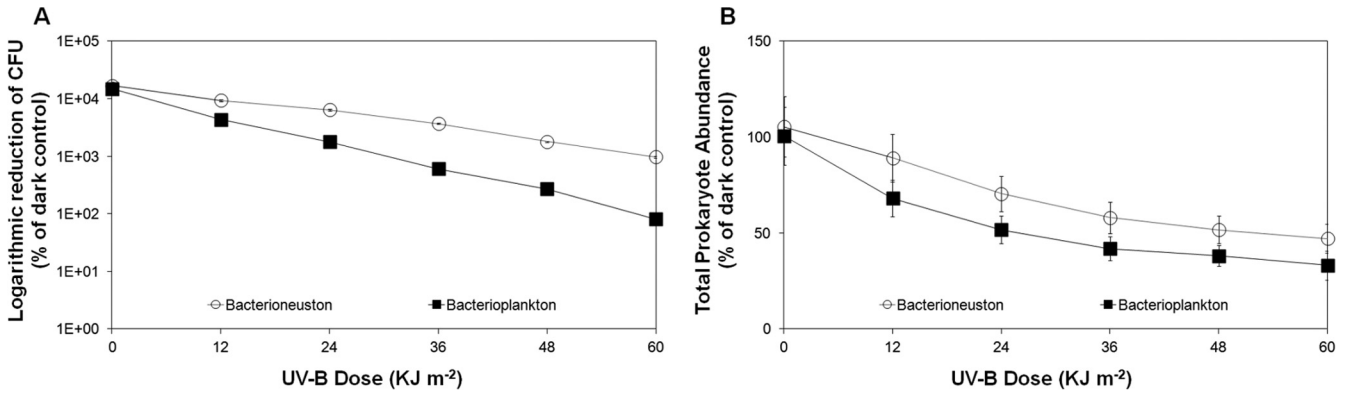

FIG 1 UV-B dose-dependent variation of abundance of culturable bacteria (A) and total prokaryote abundance (B). Results are expressed as percentages of those for the dark controls. Mean values of triplicate determinations in three subsamples from three independent experiments ($n = 27$) were plotted. Error bars represent standard deviations. The absence of error bars indicates that standard deviations are too small to see on the scale used.

Received 27 July 2011 **Accepted** 7 January 2012
Published ahead of print 13 January 2012
Address correspondence to Angela Cunha, acunha@ua.pt.
Supplemental material for this article may be found at http://aem.asm.org/.

doi:10.1128/AEM.06344-11

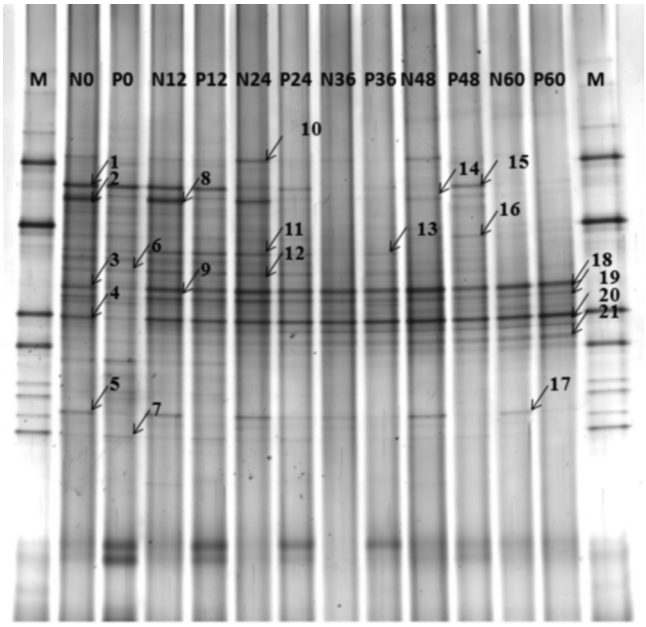

FIG 2 Representative denaturing gradient gel of bacterioneuston and bacterioplankton exposed to different UV-B doses (0, 12, 24, 36, 48, or 60 kJ m^{-2}), in which sequenced bands are indicated by arrows. M, marker; N, bacterioneuston; P, bacterioplankton.

preliminary experiments did not show a significant effect of grazers on the photobiological responses, in terms of abundance, activity, or diversity of the bacterial communities (data not shown). For the experiments, subsamples were transferred to uncovered petri dishes, forming a 1.5-mm layer. Irradiation was conducted with UV-B lamps (Philips UV-B TL 100 W/01; maximum emission peak at 311 nm; preburned for 1 h to ensure stability of light emission) for 4 h at room temperature (25°C ± 0.5) with magnetic stirring. The cumulative UV-B dose (60 kJ m^{-2}; determined using a DM 300 spectroradiometer; Bentham Instruments, Reading, United Kingdom) was equivalent to ambient surface UVR levels at 40 to 44°N latitude on clear summer solstice days (16). Aliquots were collected at predetermined UV-B doses (0, 12, 24, 36, 48, and 60 kJ m^{-2}) for analysis. Dark controls were included in all experiments.

Effects of UV-B on abundance and community composition. Bacterial abundance, determined from colony counts and epifluorescence microscopy (12), was less reduced (one-way analysis of variance [ANOVA]; $P < 0.05$) in bacterioneuston than in bacterioplankton (Fig. 1A and B), indicating an enhanced UV tolerance of bacterioneuston, as was previously observed in experiments with bacterial isolates (15). Denaturing gradient gel electrophoresis (DGGE) profiling of 16S rDNA sequences (14) showed a reduction in structural diversity (17; assessed from the Shannon diversity index) of 14% and 25% in bacterioneuston and bacterioplankton, respectively (Fig. 2), thus demonstrating the relevance of UV-B radiation as a driver of the structure of bacterial communities. Sequencing of selected, cloned DGGE bands (see the supplemental material) revealed the predominance of ribotypes affil-

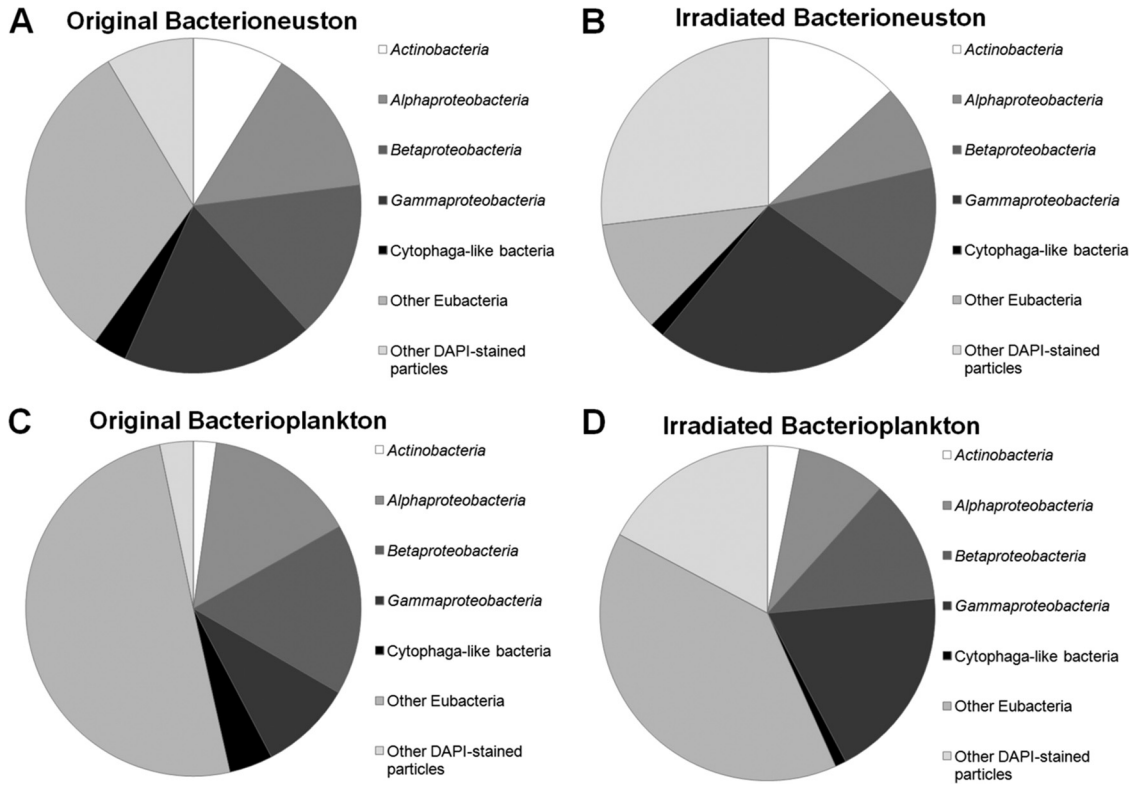

FIG 3 Mean relative abundances (expressed as percentages of total DAPI counts) of specific bacterial groups detected by FISH in original and irradiated bacterioneuston and bacterioplankton communities. Mean values ($n = 27$) were plotted.

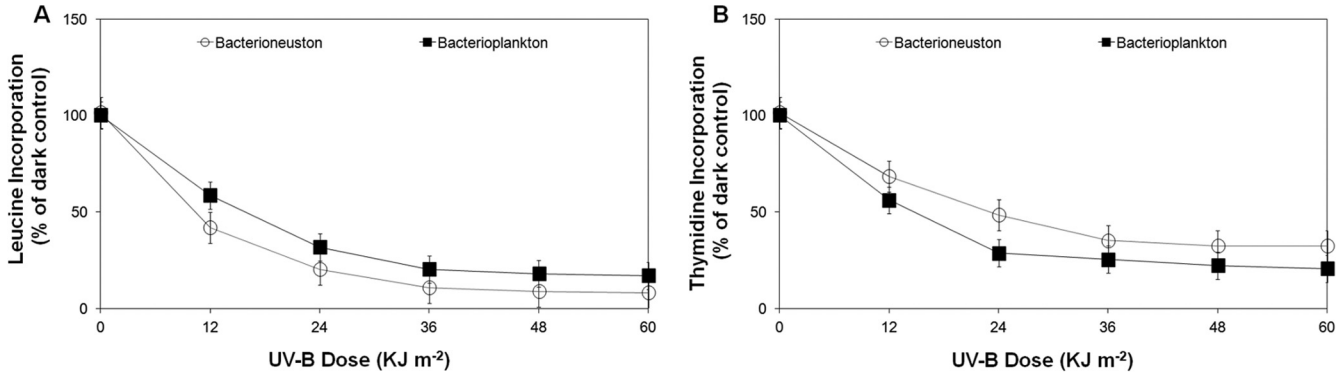

FIG 4 UV-B dose-dependent variation of leucine incorporation (protein synthesis) (A) and thymidine incorporation (DNA synthesis) (B). Results are expressed as percentages of those for the dark controls. Mean values ($n = 9$) were plotted. Error bars represent standard deviations. The absence of error bars indicates that standard deviations are too small to see on the scale used.

iated with *Bacteroidetes-Chlorobi* (band 1), *Firmicutes* (band 2), and *Gammaproteobacteria* (bands 3 and 4) in the original bacterioneuston and bacterioplankton samples. After irradiation, *Gammaproteobacteria*-affiliated ribotypes dominated both communities. Two strong bands (identified in Fig. 2 as bands 3 = 9 = 18 and 4 = 20) affiliated with *Gammaproteobacteria* persisted throughout the irradiation period in bacterioneuston and bacterioplankton, suggesting the selection of resistant strains by UVR in both communities. These bands were already prominent in the original bacterioneuston sample and occurred with equal predominance in bacterioneuston and bacterioplankton after irradiation. *Gammaproteobacteria* have already been reported as resistant to UVR (3).

The effects of UV exposure on community composition were also studied by fluorescence *in situ* hybridization (FISH) using Cy3-labeled oligonucleotide probes for the domain *Bacteria* (4, 6) and classes *Alphaproteobacteria* (8), *Betaproteobacteria* (10), *Gammaproteobacteria* (10), *Cytophaga*-like bacteria (10), and *Actinobacteria* (10). The appropriate controls were included. FISH results confirmed the dominance of *Gammaproteobacteria* in

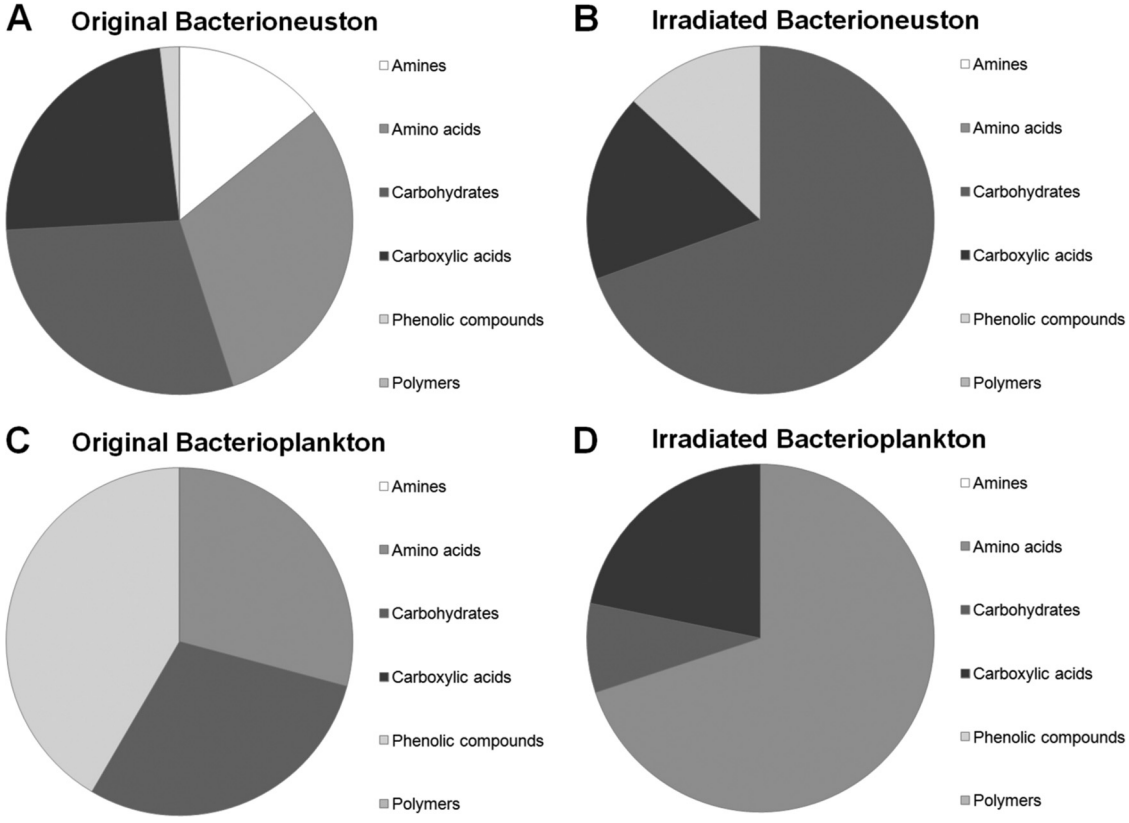

FIG 5 Mean relative consumption of different substrate categories present in Biolog EcoPlates before and after exposure of bacterioneuston and bacterioplankton communities to a total dose of 60 kJ m^{-2} of UV-B radiation. Mean values ($n = 9$) were plotted.

bacterioneuston and an increase of up to 10% (determined by one-way ANOVA; $P < 0.05$) in the relative abundance of *Gammaproteobacteria* after irradiation of bacterioneuston and bacterioplankton (Fig. 3). In general, the different bacterial groups quantified by FISH followed a similar trend of variation in bacterioneuston and bacterioplankton (see the supplemental material), with the exception of *Actinobacteria*, which increased in abundance by 32.2% in bacterioneuston during irradiation, remaining unaffected in bacterioplankton. *Actinobacteria* have been proposed to be genetically adapted to high UV levels (20). The results suggest that bacterioneuston may contain a pool of UV-resistant bacteria that are selected for upon UV exposure.

Effects of UV-B on activity. The rates of leucine and thymidine incorporation were used as proxies for protein and DNA synthesis, respectively (11, 18). Leucine incorporation was more affected in bacterioneuston (Fig. 4A) by UV-B, while in bacterioplankton thymidine incorporation was more inhibited (Fig. 4B) (one-way ANOVA; $P < 0.05$). Reducing protein synthesis upon UV-B exposure could be a metabolic strategy to enhance survival, as actively growing bacteria are more susceptible to stress (7), and this could underlie the lower impact of UV on bacterioneuston abundance.

The effects of UVR on the physiological profiles of bacterioneuston and bacterioplankton assessed with Biolog EcoPlates (13) included a shift in the spectrum of carbon sources used as well as substantial differences in the metabolic profiles of bacterioneuston and bacterioplankton before and after UV exposure (Fig. 5) In bacterioneuston, UV exposure resulted in a 31.2%, 14.4% and a 6.3% decrease in the use of amino acids, amines, and carboxylic acids and an increase in the consumption of carbohydrates and phenolic compounds of 42.3% and 11.6%, respectively. In bacterioplankton, irradiation caused a decrease in the utilization of carbohydrates and phenolic compounds of 21.7% and 42.9%, respectively, and an increase in the consumption of amino acids and carboxylic acids of 42.2% and 22.5%, respectively.

In conclusion, the present work demonstrates that UV radiation has clear effects on the structure and function of estuarine bacterial communities and that the SML environment may select for a bacterial community metabolically adapted to a high level of UV exposure.

ACKNOWLEDGMENTS

This work was supported by CESAM (Centre for Environmental and Marine Studies, University of Aveiro) and the Portuguese Foundation for Science and Technology (FCT) in the form of a Ph.D. grant to A.L.S. (SFRH/BD/40160/2007) and a Postdoctoral grant to I.H. (SFRH/BPD/63487/2009).

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