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Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton

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Abstract

The effect of ultraviolet radiation on the bioavailability of dissolved organic matter (DOM) to marine bacterioplankton was investigated in the Mediterranean Sea (Strait of Gibraltar, South and North Aegean Sea) and in the Caribbean Sea off Curaçao. Surface-water samples (collected between 1 and 85 m in depth) exposed to solar radiation did not show a distinct pattern in subsequent bacterial growth. However, samples collected from a pronounced chlorophyll maximum (two stations in the Strait of Gibraltar and four stations in the Aegean Sea) displayed in a 50% lower bacterial activity in the radiation-exposed treatments compared with the dark controls. In contrast, mesopelagic water samples (200–350 m in depth) exposed to surface solar radiation exhibited a two- to fourfold increase in bacterial activity compared with the corresponding dark controls. Addition of the model protein bovine serum albumin (BSA) to mesopelagic-water samples and subsequent exposure to solar radiation resulted in a 50% lower rate of bacterial activity compared with the dark treatments, thus indicating the occurrence of photochemically induced changes to this labile compound. This decrease in bacterial activity in BSA-amended, irradiated water was also detectable in surface waters sampled off Curaçao, whereas BSA amendment to surface water from the Mediterranean Sea did not result in a distinct pattern of bacterial activity.

Our data indicate that exposure of DOM to solar radiation causes a reduced bioavailability of the exposed DOM to bacterioplankton, if the bacterial activity : dissolved organic carbon (DOC) concentration ratio is high (indicative of labile DOM). If the bacterial activity : DOC ratio is low (indicative of more refractory DOM), the bioavailability of the DOM is increased upon exposure to solar radiation.

The processes that regulate the cycling of oceanic dissolved organic matter (DOM), one of the largest organic carbon reservoirs in the Earth's biosphere (Hedges 1988), are still poorly understood. Only the bacterially mediated turnover of the more labile DOM pool, which comprises less than 20% of the total oceanic DOM (Thurman 1985), has been intensively studied. Recently, the direct and indirect effects of solar radiation on the turnover rates of DOM have also received considerable attention. DOM exhibits photoreactive properties and is especially sensitive to the ultraviolet (UV)-B (280–320-nm) and the UV-A (320–400-nm) ranges. It is still unclear, however, to what extent the oceanic DOM pool is altered on a molecular level upon exposure to sunlight, and it is also unclear how this alteration affects the subsequent bioavailability of DOM to bacterioplankton.

There is substantial evidence that exposure to sunlight (especially to UV light) increases the availability of originally refractory DOM to bacterioplankton, which indicates the release of biologically available photoproducts (Lindell et al. 1995; Wetzel et al. 1995; Moran and Zepp 1997; Reitner et al. 1997). In particular, photochemical production of low-molecular-weight organic acids (Kieber et al. 1989; Mopper et al. 1991; Kulovaara et al. 1996), nitrogenous compounds such as ammonium (Bushaw et al. 1996) and free amino acids (Amador et al. 1989), and phosphate (Francko and Heath 1982) has been observed. The photochemical production of all of these molecules will ultimately stimulate bacterioplankton activity. However, other photoproducts formed (via solar radiation) from DOM might inhibit bacterioplankton activity. The action of solar radiation on DOM forms

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carbon gases (CO [Mopper et al. 1991; Valentine and Zepp 1993] and CO_2 [Salonen and Vähätalo 1994; Miller and Zepp 1995; Graneli et al. 1996]) as well as radicals, such as hydrogen peroxide (Cooper et al. 1988; Moffet and Zafiriou 1990; Cooper and Lean 1992; Zika et al. 1993). Therefore, exposure of DOM to solar radiation leads to the formation of growth-promoting and growth-inhibiting substances as well as to loss of dissolved organic carbon (DOC).

The potential impact of surface solar radiation on the pool of labile DOM has, thus far, not been adequately considered. Previous studies show that sunlight can initiate condensation reactions, thus leading to the formation of marine humic substances, thereby incorporating originally labile organic molecules (Harvey et al. 1983; Momzikoff et al. 1983; Kieber et al. 1997). Keil and Kirchman (1994) reported higher abiotic modification rates of the protein RuBPcase if it was exposed to sunlight, compared with exposure in the dark. Naganuma et al. (1996) found decreased availability of UV-exposed peptone to *Escherichia coli*, and Tranvik and Kokalj (1998) detected reduced bioavailability of algal-derived DOC exposed to UV radiation in the presence of dissolved humic substances.

Recently, Benner and Biddanda (1998) found decreased bioavailability of sunlight-exposed DOM from surface waters, whereas enhanced bacterial growth was observed for deep-water DOM exposed to surface solar radiation (as compared with the dark control). These authors suggested that two contrasting photochemically induced alterations take place in solar irradiation–exposed DOM. These differential photochemically induced changes in the DOM pool probably act concomitantly. The resulting net effect of solar radiation on the bioavailability of DOM will ultimately be determined by the prevalence of photoproducts that stimulate or inhibit subsequent bacterial growth.

In this study, we tested the hypothesis that solar radiation has opposing effects on the bioavailability of DOM for bacterioplankton depending on its initial biological reactivity. In order to compare the photoreactivity of DOM of different biological reactivities, presumably ''younger'' DOM was collected from the chlorophyll maximum layer, and ''older'' DOM was collected from the mesopelagic-water layers (>200 m in depth) and exposed to surface solar radiation prior to inoculation of bacterioplankton. Additionally, bovine serum albumin (BSA) was used as a model protein in order to investigate the effects of sunlight on a labile compound in the presence of DOM of different biological reactivities.

Material and methods

Study sites—Experiments were performed during cruises in the Strait of Gibraltar (June 1997) and the Aegean Sea (September 1997) (Fig. 1) as well as off of the CARMABI station at Curaçao (Caribbean Sea, November 1996) (Table 1). The temperature–salinity profiles (Fig. 2) characterize the different water masses sampled in the Strait of Gibraltar and the South and North Aegean Sea. In the Strait of Gibraltar (Fig. 2a), Atlantic surface water (ASW) of low salinity (≤36.5) is transported into the Mediterranean basin, whereas the outflowing high salinity (≈38.4) waters represent Mediterranean deep water (MDW). According to the temperature–salinity data, we differentiated between ASW, MDW, and intermediate water (IMW), which represented ASW mixed with MDW (Fig. 2a). At Sta. I and II, a pronounced chlorophyll maximum was detectable within the nutrient-depleted surface layer (0–70 m), whereas near-surface waters at Sta. III to VII were characterized by high concentrations of inorganic nutrients (NO_3: 2.5–7 μM; PO_4: 0.23–0.43 μM), thus indicating that substantial input of nutrient-rich deeper waters was occurring. No distinct chlorophyll maximum layer was detected at these stations. The surface waters of the eastern stations (Sta. V, VI, and VII; Fig. 2a) exhibited a higher salinity (≈37.5) than did the ASW, and these waters were thus regarded to be IMW. Samples considered to be typical mesopelagic water were collected from the MDW, except at Sta. I, where the mesopelagic water exhibited temperature–salinity values characteristic of IMW (Fig. 2a).

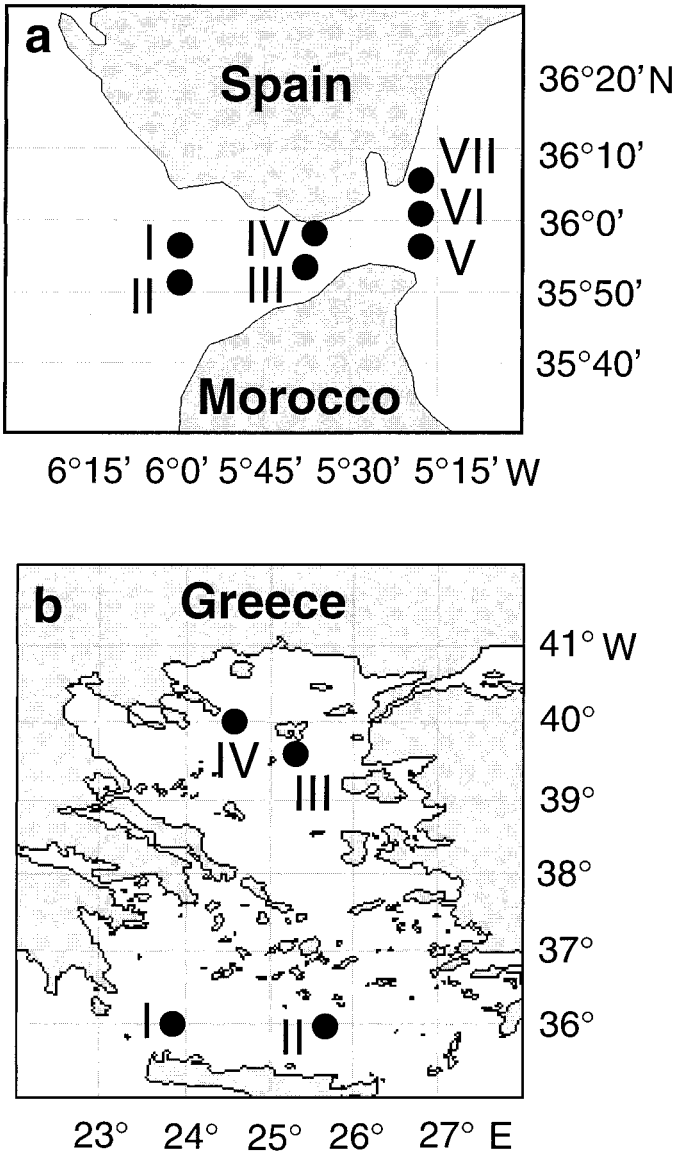

Fig. 1. Maps of the Strait of Gibraltar (a) and the Aegean Sea (b), with sampling sites indicated by dots.

Table 1. Summary of all experiments performed in the Strait of Gibralter, the Aegean Sea, and the Caribbean Sea. Concentration of dissolved organic carbon (DOC, in μM), bacterial abundance in the 0.8–μm filtrate (BA, numbers $\times 10^5$ ml^{-1}) and chlorophyll *a* (chl *a*, in μg L^{-1}) values are given for the corresponding sampling depths. Irradiation is given for one wavelength in the UVB (320 nm) and the UVA (380 nm) range; values are integrated over the 6-h exposure period around noon (Caribbean Sea, $n = 12$). ASW, Atlantic surface water; IMW, intermediate water; MDW, Mediterranean deep water; SW, surface water; MW, mesopelagic water.

Station	Water type	Depth (m)	DOC (μM)	Irradiation ($\times 10^3$ Wm^{-2}nm^{-1}) 320 nm	Irradiation ($\times 10^3$ Wm^{-2}nm^{-1}) 380 nm	BA ($\times 10^5$ ml^{-1})	chl *a* (μg L^{-1})
Strait of Gibraltar							
Surface water							
I	ASW*	50	90	4.84	12.51	6.00	0.60
II	ASW*	50	80	5.24	13.45	1.62	0.38
III	ASW	50	75	5.33	13.66	5.79	0.09
IV	ASW	30	75	5.45	13.77	0.50	0.62
V	IMW	50	82	5.28	13.27	4.27	0.51
VI	IMW	30	116	4.26	10.82	3.33	2.97
VII	IMW	50	78	5.32	13.48	0.95	2.69
Mesopelagic water							
I	IMW	200	55	4.84	12.51	0.71	<0.05
II	MDW	350	78	5.24	13.45	0.38	<0.05
III	MDW	350	92	5.33	13.66	0.22	<0.05
IV	MDW	200	75	5.45	13.77	0.43	<0.05
V	MDW	200	82	5.28	13.27	1.34	0.14
VI	MDW	300	ND†	4.26	10.82	1.94	<0.05
VII	MDW	350	81	5.32	13.48	1.21	<0.05
Aegean Sea							
Surface water							
I	SW*	85	92	3.41	10.44	2.45	0.17
II	SW*	85	66	3.96	10.56	1.95	0.34
III	SW*	15	111	3.23	8.93	3.09	0.42
III	SW	80	71	3.23	8.93	1.38	0.33
IV	SW*	20	117	3.08	8.58	3.12	0.15
Mesopelagic water							
I	MW	300	80	3.41	10.44	0.86	<0.05
II	MW	300	58	3.96	10.56	0.52	<0.05
IV	MW	300	72	3.08	8.58	0.66	<0.05
Caribbean Sea							
	SW	1	ND	2.85 ± 0.6	7.08 ± 1.4	5.16 ± 2.0	ND

* Samples taken from a distinct chlorophyll maximum.
† ND, not determined.

In the South and North Aegean Sea (Fig. 2b), the temperature–salinity profiles indicated highly saline water masses (salinity ≈39) below 50 m in depth. A pronounced chlorophyll maximum within the upper 100 m was detectable at all stations (Table 1).

In the Caribbean Sea, nearshore reef water was sampled off Curaçao (Netherlands Antilles, 12°05′N, 69°00′W). Chlorophyll (Chl) *a* concentrations at this study site ranged between 0.2 and 0.4 μg L^{-1} in late fall when we performed the experiments (Gast pers. comm.).

For Mediterranean Sea samples, the term ''surface water'' (SW) refers to water taken from a 15–85-m depth, whereas the term ''chlorophyll maximum layer'' (CM layer) refers to a subset of surface-water samples collected from a distinct phytoplankton fluorescence peak. In the Strait of Gibraltar, the SW that we collected originated either from the ASW or from the IMW (Fig. 2a, Table 1), whereas in the Aegean Sea, a surface chlorophyll maximum (15–20 m in depth) or a deep chlorophyll maximum (85 m in depth) was sampled. In the Caribbean Sea, SW was taken at 1 m in depth. The term ''mesopelagic water'' (MW) refers to water collected from a 200–350-m depth, which represents high-salinity water masses.

Experimental setup—Based on the previous conductivity-temperature-depth (CTD)–hydrocast, two sampling depths were chosen, one in the SW and one in the MW. Water samples were taken between 0700 and 0900 h using rinsed 10-L Niskin bottles mounted on a rosette. As soon as the samples were on deck, they were gently filtered, first through 0.8-μm polycarbonate filters (Nuclepore, 47-mm filter diameter) and then through 0.2-μm filters (Nuclepore, 47-mm filter diameter). The filtrates of the SW and MW were subsequently dispensed into 500-ml glass flasks (which had been prerinsed with 1 N HCl and subsequently rinsed with Milli-Q water). One part of the filtrate was amended with the protein BSA (final concentration, 458 μM C). The BSA-amended and unamended SW and MW were then exposed

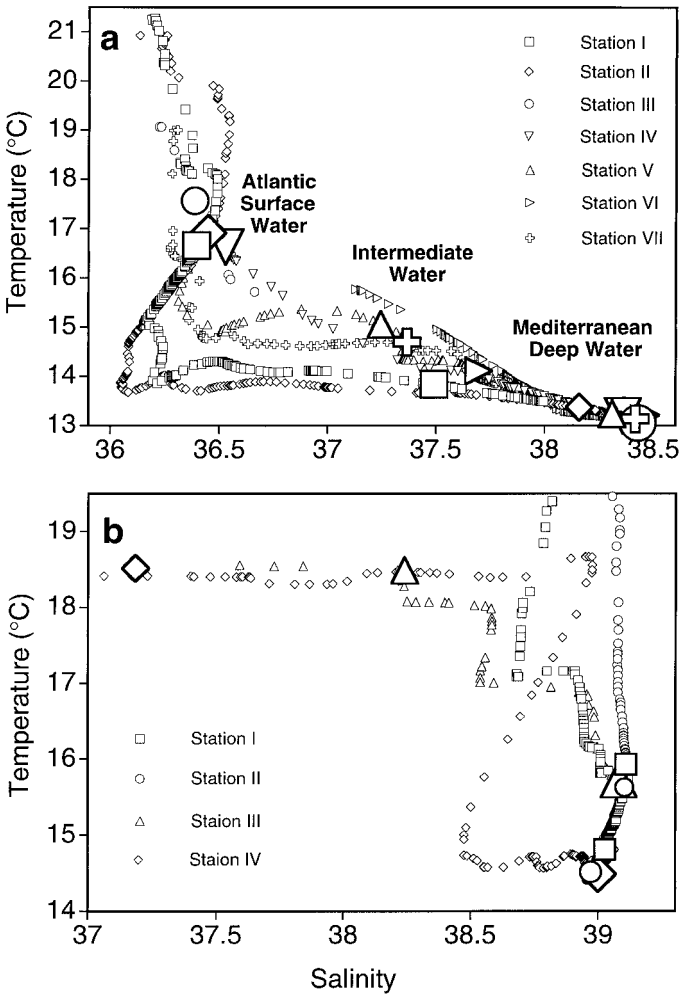

Fig. 2. Temperature–salinity profiles of the sampling sites in the Strait of Gibraltar (a) and the Aegean Sea (b). Oversized symbols indicate characteristics of the water samples used in the experiments. For further explanations, see Materials and methods.

in quartz tubes (250-ml volume) to surface solar radiation on board, beginning at around 1200 h and extending for 6 h. The corresponding dark controls were wrapped in aluminum foil. The incubations were performed in a water bath on the deck of the ship, and temperature was kept constant (SW temperature) using a flow-through system. The quartz tubes were stoppered at both ends with Teflon foil, which had been rinsed with 1 N HCl and rinsed extensively with Milli-Q water before use. After exposure to solar radiation, the different treatments were inoculated with a natural bacterial community (0.8-μm filtrate, 1 : 10 inoculum ratio) that had been collected at the corresponding depth (SW or MW). These dilution cultures were kept in the dark for 16 h before samples for bacterial abundance and bacterial production measurements were taken. A 16-h incubation period—in the dark—was chosen because time-course experiments using a similar experimental approach and water from the Mediterranean Sea had revealed a lag phase of bacterial growth of between 0 and 16 h. No systematic difference in the duration of the lag phase was detectable in the different treatments. Thus, a 16-h incubation period, as chosen in this study, was most likely long enough to allow bacteria to enter the exponential growth phase. Generally we used the same experimental protocol in the Caribbean Sea experiment. Additionally, in the Caribbean Sea, experiments were also performed with BSA-amended artificial seawater in order to determine whether the observed pattern in bacterial production is due to the interaction of the added BSA with the natural DOM. Artificial seawater was prepared according to Parsons et al. (1984).

Light measurements—A profiling UV radiometer (Biospherical PUV-510) was used to measure the surface solar radiation at four distinct wavelengths in the UV-B (305-nm, 320-nm) and UV-A (340-nm, 380-nm) regions; it was also used to measure the integrated irradiance of the photosynthetic active radiation (PAR, 400–700 nm).

Bacterial abundance—Bacterial abundance was determined by acridine orange staining and epifluorescence microscopy on black polycarbonate filters (Nuclepore, 0.2-μm pore size, 25-mm diameter) (Hobbie et al. 1977).

Bacterial production—Bacterial production was measured by [^{3}H]thymidine (specific activity: 83 Ci mmol^{-1}, Amersham; final concentration, 20 nM) or [^{3}H]leucine (specific activity: 120 Ci mmol^{-1}; final concentration, 10 nM) incorporation. Samples were incubated in triplicate with two blanks in the dark; blanks were fixed with concentrated formaldehyde (final concentration, 4%, v/v) 10 min before the tracer was added. After incubating for 60 min, the samples were filtered onto 0.2-μm cellulose nitrate filters (Millipore HA, 25-mm–diameter filter) and rinsed twice with 10 ml ice-cold 5% trichloroacetic acid (Sigma Chemical) for 5 min. The filters were dissolved in 1 ml ethylacetate, and after 10 min, 8 ml of scintillation cocktail (Insta-Gold, Canberra Packard) was added. The radioactivity incorporated into bacterial cells was converted to the actual amount of substrate taken up. External isotope dilution was taken into account for leucine incorporation by measuring the leucine concentration of the collected water by *o*-phthaldialdehyde-derivatization and high-performance liquid chromatography (Lindroth and Mopper 1979).

Dissolved organic carbon—Samples for DOC were sealed in precombusted glass ampules and immediately frozen at −20°C. DOC concentrations were determined by the high-temperature combustion method using a Shimadzu TOC-5000 analyzer (Benner and Strom 1993). Standards were prepared with potassium hydrogen phthalate (Kanto Chemical). Both water blank (i.e., Milli-Q water redistilled with 10 mM potassium peroxide sulfate and 20 mM *o*-phosphoric acid; both obtained from Sigma Chemical) and instrument blank were assessed before and after sample analysis. Blanks typically ranged between 3.3 and 4.2 μM (water) and between 1.7 and 2.5 μM (instrument), respectively. The overall analytical precision was always better than 3%.

DOM fluorescence measurements—Water samples for fluorescence measurements were taken before and after ex-

posure to solar radiation. Ten-milliliter subsamples were sealed in precombusted glass ampules and immediately frozen at −20°C. Fluorescence was measured at an excitation of 350 nm and an emission wavelength of 450 nm using a 1-cm quartz cuvette. The fluorometer (Hitachi F 2000) was standardized with a quinine sulfate (QS) solution (1 QS unit = 1 ppb in 0.05 M H_2SO_4).

Statistical analysis—The Wilcoxon test for matched pairs was used to test treatment effects on variables. Statistics were performed with SYSTAT 5.2 (Wilkinson 1990).

Results

Radiation regime during exposure of the different treatments—Radiation was measured at 5-min intervals. Integrated values over the 6-h exposure period indicated that the dose received by the samples at a particular study site varied up to 1.7-fold (Table 1). Higher irradiance was detectable during the cruise in the Strait of Gibraltar in June compared with the Aegean Sea cruise in September. Irradiation values at Curaçao (November) were found to be lower than those found in the Mediterranean Sea. Integrated values of the UV-B wavelengths varied at 305 nm between 4 and 6 × 10^2 W m^{-2} nm^{-1}, and in the UV-A region, values ranged between 5 and 9 × 10^3 W m^{-2} nm^{-1} at 340 nm. The integrated dose of PAR varied between 20 and 36 E m^{-2}.

Bacterial abundance—Bacterial abundance in the 0.8-μm filtrate (subsequently used as inocula for the dilution cultures) of the stations in the Strait of Gibraltar and the Aegean Sea ranged from 0.5 to 6 × 10^5 ml^{-1} in the SW, and in the MW, values ranged from 0.2 to 1.9 × 10^5 ml^{-1} (Table 1). Off Curaçao, bacterial numbers in the 0.8-μm filtrate from 1-m depth ranged between 2.8 and 9 × 10^5 ml^{-1} (Table 1). In the dilution cultures, the 0.8-μm filtrate was diluted to a 1 : 10 ratio with 0.2-μm filtered seawater; after 16 h, bacterial numbers generally increased two- to threefold.

Bacterial response to solar-irradiated DOM in the Caribbean Sea—No significant differences in bacterial activity between unamended irradiated and dark treatments were found. BSA amendment resulted in a 30% lower bacterial activity in the irradiated treatment compared with the bacterial activity in the dark control (Wilcoxon test, $P = 0.012$; $n = 12$) (Table 2). Bacterial activity in the BSA-amended, irradiated treatment was similar to that in the unamended dark treatment. Using artificial seawater amended with the same concentration of BSA and exposed to surface solar radiation, no significant differences in bacterial activity were detected compared with the dark controls (Table 2).

Bacterial response to irradiated DOM originating from the SW layer—The SW collected in the Strait of Gibraltar varied considerably in its physical characteristics. At two stations (I and II) only was a pronounced chlorophyll maximum (at 50 m in depth) detectable within the nutrient-depleted surface layer (0–70 m). At these two stations, bacterial activity in the unamended dark treatments was found to be higher in SW compared with activity in the corresponding MW, whereas MW at Sta. III to VII supported higher bacterial activity compared with the corresponding SW. DOC concentrations were found to be similar for SW and MW at Sta. III to VII (Table 1); however, concentrations of inorganic nutrients (e.g., ΣN_{inorg}, PO_4) were consistently higher in MW compared with SW (by up to a fourfold value). Chl *a* concentrations in the SW layers ranged between 0.09 and 2.9 μg L^{-1}, and concentrations of DOC varied between 75 and 116 μM C (Dafner pers. comm.) (Table 1). When we pooled all the bioassay experiments performed with SW from the Strait of Gibraltar, no significant differences were found between the solar irradiation–exposed treatments and the dark controls in either the unamended or the BSA-amended treatments. Only at Sta. III to VII was significantly higher bacterial activity found in the unamended irradiated treatments, compared with the dark controls, for both leucine and thymidine (Wilcoxon test, $P = 0.022$; $n = 5$).

Table 2. Means ± SE of [^{3}H]leucine incorporation rates of bacteria in dilution cultures with water sampled at 1-m depth in the Caribbean Sea (off Curaçao) or in artificial seawater amended with BSA. BSA, bovine serum albumin. The Wilcoxon test for matched pairs was used to test treatment effects on variables; $n = 12$ for natural seawater, $n = 6$ for artificial seawater, NS – not significantly different.

	[^{3}H]leucine incorporation (pmol L^{-1} h^{-1})	
Seawater Treatment	unamended	+BSA
Natural		
Irradiated	604 ± 98	628 ± 60
	NS	$P = 0.012$
Dark	596 ± 67	927 ± 115
Artificial		
Irradiated		260 ± 62
		NS
Dark		279 ± 42

The stations in the South and North Aegean Sea were characterized by distinct chlorophyll maxima within the upper 100 m of the water column, with Chl *a* concentrations ranging from 0.15 to 0.42 μg L^{-1} (Table 1). At all stations in the Aegean Sea, unamended dark treatments supported higher bacterial activity in SW as compared with MW. Water from these CM layers that had been exposed to surface solar radiation for 6 h showed a significantly decreased bacterial activity (by 40%) for both thymidine and leucine, as compared with the dark control (Wilcoxon test, $P = 0.022$; $n = 5$). BSA-amended SW exhibited no significant difference between the irradiated and the dark treatments.

By pooling the data from the pronounced CM layers of the Strait of Gibraltar and the Aegean Sea, bacterial activity in the irradiated, unamended treatments was significantly lower (by ≈50%) than it was in the corresponding dark controls for both thymidine and leucine incorporation (Wilcoxon test, $P = 0.014$; $n = 6$)(Fig. 3a; results are only shown for thymidine incorporation since leucine incorporation exhibited essentially the same pattern). BSA amendment generally stimulated bacterial activity in waters of the CM layer; how-

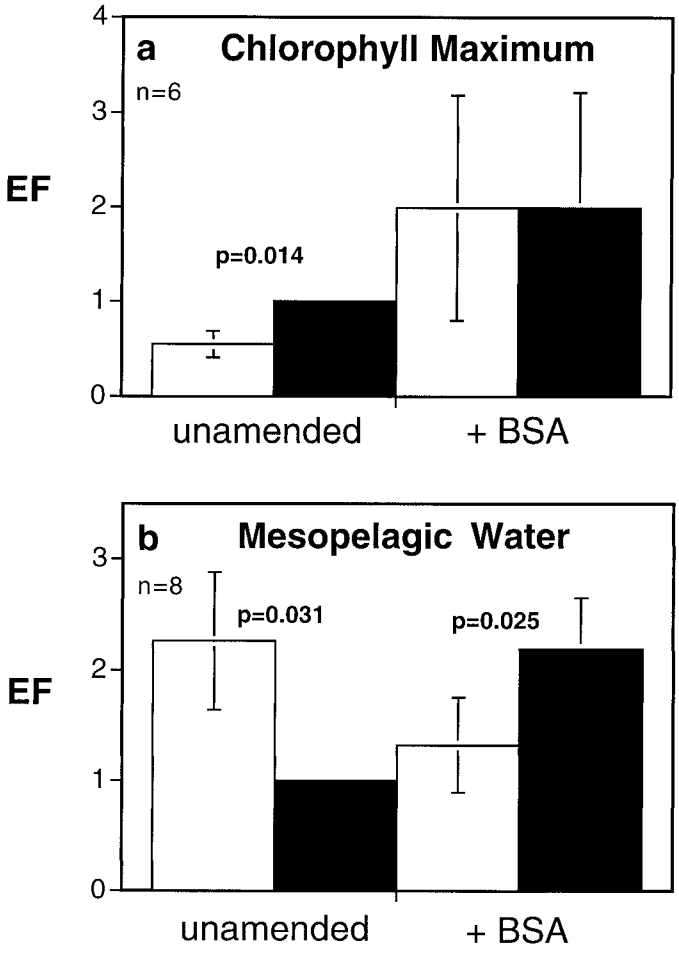

Fig. 3. Mean bacterial activity (as measured by [^{3}H]thymidine incorporation) in irradiated and dark treatments expressed as enhancement factor (bacterial activity in irradiated DOM/bacterial activity in the unamended dark control; EF) of the chlorophyll maximum (a) and the mesopelagic waters (b). Data from the Strait of Gibraltar and the Aegean Sea are pooled. BSA, bovine serum albumin; mean ± SE; significant differences between irradiated and dark treatments of unamended and BSA-amended waters, respectively, are indicated.

ever, exposure to solar radiation had no effect on bacterial activity in the BSA-amended treatments (Fig. 3a).

Bacterial response to irradiated DOM originating from MW— In the Strait of Gibraltar, MW exposed to surface solar radiation and subsequently inoculated with bacterioplankton exhibited higher thymidine incorporation rates compared with the dark controls in three out of five experiments. Leucine incorporation was, on average, four times higher in the irradiated, unamended treatments compared with the dark controls (Wilcoxon test, $P = 0.034$; $n = 5$). BSA amendment of MW led to a significant decrease in the bacterial activity in irradiated treatments compared with the dark controls for both thymidine (by 50%; Wilcoxon test, $P = 0.022$; $n = 6$) and leucine (by 80%; Wilcoxon test, $P = 0.022$; $n = 5$).

In the Aegean Sea, unamended MW exposed to surface solar radiation exhibited a significantly higher [^{3}H]thymidine incorporation (by 30%) compared with the dark controls (Wilcoxon test, $P = 0.05$; $n = 3$), whereas the addition of BSA to MW led to a significant decrease in thymidine incorporation (by 20%) in the irradiated treatments compared with the dark controls (Wilcoxon test, $P = 0.05$; $n = 3$).

By pooling the results obtained from the experiments with MW from the Strait of Gibraltar and the Aegean Sea, the following pattern emerged: irradiated unamended MW supported bacterial activity at twofold (for thymidine) and fourfold (for leucine, data not shown) higher rates than it did in the dark controls (Wilcoxon test, $P = 0.031$ and $P = 0.035$, respectively; $n = 8$) (Fig. 3b). In BSA-amended dark treatments, however, bacterial incorporation rates were twice as high as they were in the BSA-amended irradiated treatments for both thymidine and leucine incorporation (Wilcoxon test, $P = 0.025$ and $P = 0.05$, respectively; $n = 8$) (Fig. 3b).

Concentration of DOC and DOM fluorescence—Concentrations of DOC in SW of the Strait of Gibraltar and the Aegean Sea ranged between 66 and 117 μM C, and Mediterranean MW exhibited DOC concentrations ranging from 55 to 92 μM C. DOC-normalized fluorescence was significantly higher in MW compared with samples collected in the CM (Wilcoxon test, $P = 0.014$; $n = 6$; Fig. 4a). Exposure of water from pronounced CM layers to surface solar radiation did not significantly change the fluorescence yield of the DOM, whereas irradiation of MW decreased DOC-normalized fluorescence by ≈30%, as compared with the dark control (Wilcoxon test, $P = 0.004$; $n = 8$). It is interesting to note that the DOM fluorescence of MW decreased upon irradiation to values found in situ in the CM layer. In the Strait of Gibraltar, SW from Sta. III through VII exhibited a decrease in DOM fluorescence upon irradiation, which indicated that mixing of deep water with SW was occurring, as was also evident from the temperature–salinity plot (Fig. 2a) and concentrations of inorganic nutrients. BSA addition did not change the fluorescence pattern in either waters from the CM or in the MW. However, higher DOM fluorescence was observed in MW amended with BSA (Fig. 4b).

Discussion

UV radiation can penetrate the water column to considerable depth, particularly in oligotrophic environments (Fleischmann 1989; Smith 1989). In the subtropical Atlantic, the 10% radiation level of the 320-nm wavelength is at about 25 m in depth, whereas the 10% radiation level of 340-nm and 380-nm wavelengths is at 35 and 60 m in depth, respectively (Obernosterer unpubl. data). Moreover, it has been found that photooxidation of humic-rich DOM (measured as photochemical production of inorganic carbon or photochemical oxygen consumption) is mediated in roughly equal portions by the UV-B, UV-A, and the PAR range (Graneli et al. 1996; Reitner et al. 1997). In the subtropical Atlantic Ocean, photochemical oxygen consumption of SW DOM has been found to be about 90% attributable to UV-A radiation (Obernosterer unpubl. data). The exposure of DOM to solar radiation might result in a continuous bleaching of the chromophore-rich portion, leading to an overall loss in ab-

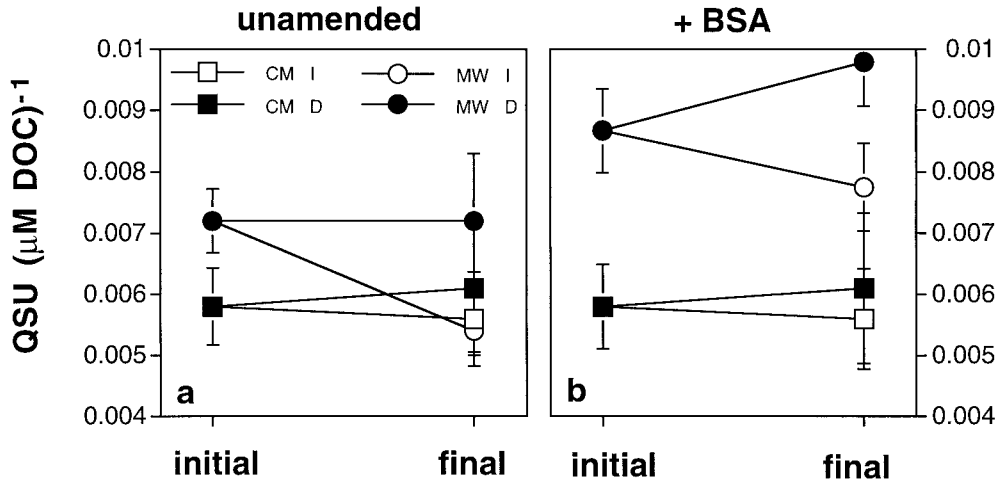

Fig. 4. Fluorescence of DOM sampled in the chlorophyll maximum (CM, $n = 6$) and in the mesopelagic waters (MW, $n = 8$) and exposed unamended (a) or BSA-amended (b) samples (exposure to surface solar radiation for 6 h). Data from the Strait of Gibraltar and the Aegean Sea are pooled. Fluorescence is given in DOC-normalized quinine sulfate units (QSUs). In BSA-amended treatments, the fluorescence was normalized to the original DOC concentration before the addition of BSA. I, irradiated treatments (open symbols); D, dark treatments (full symbols); BSA, bovine serum albumin; mean ± SE.

sorption and fluorescence (Vodacek 1992; Lindell et al. 1995; Morris and Hargreaves 1997). Stability of the water column would enhance this effect for near-surface DOM, whereas upwelling would counteract this effect by transporting chromophore-rich deep water DOM into the surface layers. In this study, DOM fluorescence was found to be significantly higher in waters from the mesopelagic zone compared with waters from the CM.

The DOM pool in the SW is spatially and temporally closely coupled to phytoplankton production and is therefore at least partially directly or indirectly fueled with freshly released DOM. Because of the rapid remineralization of this freshly produced DOM by bacterioplankton, turnover times are in the range of minutes to weeks (Lancelot and Billen 1985; Fuhrman 1987). Little is known about the photoreactivity of biologically labile compounds. Most simple organic substances detectable in seawater do not efficiently absorb radiation in the solar wavelength spectrum that reaches the Earth's surface (Zafiriou 1977). Only a few compounds, such as carbonyl compounds, organic iodocompounds, riboflavin, tryptophane, thiamine, and disulfides are known for their chromophoric structure (Zafiriou 1977). Nonabsorbing compounds of the DOM might be involved in secondary reactions promoted by photosensitizers (Zafiriou et al. 1984; Tsao and Eto 1994; Brugger et al. 1998). Tranvik and Kokalj (1998) presented evidence on decreased availability of UV-exposed freshly produced algal DOM in the presence of dissolved humic substances, pointing to direct physicochemical reactions between these compound classes.

In this study, water taken from the CM and exposed to surface solar radiation decreased subsequent bacterial activity (by 50%) compared with the treatment that was maintained in the dark (Fig. 3a). Recently, Benner and Biddanda (1998) found reduced bioavailability of irradiated water collected in the euphotic zone of the Gulf of Mexico. In their study, bacterial activity (as measured by [^{3}H]leucine incorporation) was measured immediately after the exposure of DOM to surface solar radiation for 5–9 h. This led to a 75% reduction in bacterial activity in the irradiated treatment compared with the dark control. In our study, the bioavailability of the DOM was assessed after 16 h, yielding a 50% reduction in bacterial activity in the irradiation-exposed treatment compared with the dark control. Thus, the observed effects of UV exposure on the bioavailability of DOM are sustained for at least 16 h.

UV-mediated transformation of several classes of labile compounds has been described, for example, for triglycerides and fatty acids (Kieber et al. 1997) and proteins (Keil and Kirchman 1994). These transformations might lead to humification processes with an immobilization of nitrogen species, such as ammonia, amines, or amino acids (Kieber et al. 1997), or phosphate. Thus, some components of the labile fraction of the DOM pool might become less labile upon UV radiation and might further immobilize potentially available nitrogen and phosphorus sources. However, the exact chemical reactions involved in the UV-mediated transformation of the organic and inorganic nutrient pool are largely unresolved (Kieber et al. 1997; Miller and Moran 1997; Moran and Zepp 1997).

While the DOM pool consists of labile and refractory components throughout the water column, more refractory compounds prevail in deeper water, such as in the mesopelagic layer (Benner et al. 1992). Although this presumably ''older'' DOM is more resistant to biological degradation, it is at least partially readily able to be photochemically degraded (Kieber et al. 1989; Mopper et al. 1991; Bushaw et al. 1996). This is also indicated by the decrease in DOM fluorescence of irradiated MW (Fig. 4a). In our study, mean bacterial activity increased by a factor of two for thymidine and increased fourfold for leucine incorporation when MW

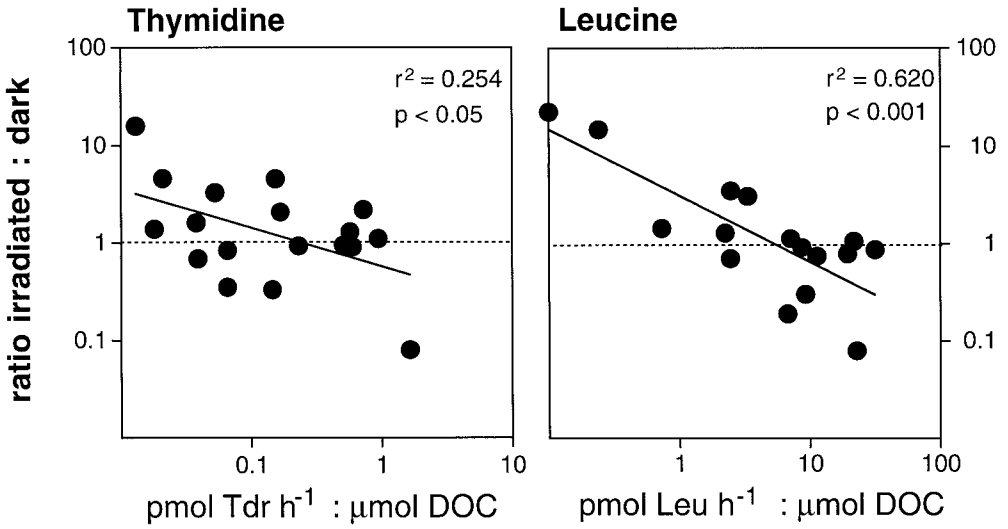

Fig. 5. Relation between bacterial activity, measured as [^{3}H]thymidine (Tdr) and [^{3}H]leucine (Leu) incorporation, in the dark treatment normalized to DOC and the ratio of bacterial activity measured in the irradiated treatment to bacterial activity measured in the dark treatment (irradiated : dark ratio). A bacterial activity ratio (irradiated : dark treatment) of <1 indicates lower bacterial activity in the treatment exposed to solar radiation compared with the bacterial activity in the dark treatment.

was exposed to solar radiation. This enhancement in bacterial activity indicates photoinduced cleavage of biologically labile substrates, as was shown previously (*see* Moran and Zepp [1997] and citations therein).

Based on the pooled data from the two sampling areas in the Mediterranean Sea (Fig. 3), it is evident that the response of the bacterioplankton community to DOM that had been exposed to solar radiation is related to the initial bioavailability of the DOM. As an integrative measure of the bioavailability of the nutrient pool, we used the ratio between bacterial activity (thymidine and leucine incorporation) and the DOC concentration (Fig. 5). A high bacterial activity : DOC concentration ratio indicates rapid utilization of the DOC and is therefore considered to indicate a greater amount of labile DOC. On the contrary, a lower ratio indicates the more refractory nature of the DOC pool. Slow uptake of DOC might also indicate that other elements are limiting bacterial growth. If we relate the bacterial activity : DOC concentration ratio to the response of the bacterioplankton to the DOM pool that had been exposed to solar radiation, compared with the dark treatment, clear trends become apparent (Fig. 5). With increasing bacterial activity : DOC concentration ratios, the bacterial activity in the solar radiation–exposed treatment : dark treatment ratios declined (Fig. 5). Thus, with increasing initial bioavailability of the DOC, exposure of the DOC pool to solar radiation led to decreasing postexposure availability of the DOC pool, as indicated by bacterial incorporation ratios in the irradiated versus the dark treatment (<1). If the initial bioavailability of the DOC pool is low (indicated by a low bacterial activity to DOC concentration ratio), exposure of the DOC pool to solar radiation leads to stimulated bacterial activity.

Photoreactivity of BSA-amended DOM—Only in some cases did addition of BSA to water from the CM layers increase bacterial activity in the dark treatments. This might be partially explained by the fact that bacterial activity was limited by elements other than C or N. Exposure of BSA-amended DOM from the CM to surface solar radiation did not affect subsequent bacterioplankton activity (Fig. 3a). In contrast, in the BSA-amended, irradiated MW, a decrease in bacterial activity (compared with that seen in the dark control) was found. Thus, while exposure to solar radiation of unamended MW enhanced subsequent bacterial activity, as compared with the dark control, the opposite effect was observed with BSA-amended, irradiated water, which supported lower bacterial activity than did the corresponding dark control (Fig. 3b). Off Curaçao, where water was sampled at 1 m in depth, decreased bioavailability of irradiation-exposed, BSA-amended DOM was observed as well (Table 2). BSA itself does not efficiently absorb light in the $\geq$300-nm region; thus, our model protein is probably interacting with photoreactive DOM species. This is also indicated by experiments conducted with BSA-amended artificial seawater, in which no differences in bacterial activity between irradiated and dark treatments were observed (Table 2). DOM fluorescence of MW that was either amended with BSA or left unamended decreased upon exposure to solar radiation, which indicated the presence of photochemically induced changes in the DOM pool. In a recent study, Tranvik and Kokalj (1998) found that the inhibition of bacterial mineralization of UV-irradiated, algal-derived DOC was more pronounced in the presence of dissolved humic matter. Thus, the availability of photoreactive DOM, such as in this study's MW, might be a possible explanation for the effective transformation of labile, nonchromophoric compounds (i.e., BSA) into more refractory material.

Exposure of BSA-amended samples could also lead to photooxidation of BSA–carbon into dissolved inorganic car-

bon, as shown for humic- and phytoplankton-derived DOM (Graneli et al. 1996; Tranvik and Kokalj 1998). However, rates of DOM photooxidation are too low to account for the difference in bacterioplankton activity that was observed in this study (Miller and Moran 1997; Pausz and Herndl 1999).

We suggest that two irradiation-induced processes lead to contrasting effects on the bioavailability of DOM to bacterioplankton. Generally, photochemical modification of DOM can lead to both a stimulation as well as an inhibition of subsequent bacterial activity. We assume that these processes occur concurrently, resulting in a net positive or net negative effect on bacterial activity depending on the original bioavailability and, thus, depending indirectly on the origin and age of the parent DOM. While a net positive (stimulating) effect on bacterioplankton activity (as indicated by ratios of bacterial activity in the irradiated and the dark treatments; >1) seems to prevail in DOM from deeper water layers, a net negative effect (ratios of irradiated to dark treatments, <1) seems to be dominant in waters containing a higher fraction of labile compounds, such as in the CM layers.

Based on our results on the differential effects of radiation on the bioavailability of DOM to bacterioplankton and depending on the initial biological reactivity of the DOM, the following scenario emerges: The supply of fresh, labile DOM in SW is closely coupled to phytoplankton production that is derived either directly from algal exudation or indirectly from zooplankton grazing on phytoplankton. This fresh, labile DOM combined with the bacterioplankton is exposed to a certain radiation regime, depending on the depth of the DOM within the upper 100 m of the water column. This exposure to solar radiation significantly affects DOM and microorganisms if they are confined in the upper water layers because of the establishment of a diurnal thermocline (Milot-Roy and Vincent 1994; Doney et al. 1995). Bacterioplankton have been shown to be inhibited by UV radiation (Herndl et al. 1993; Aas et al. 1996). This confinement of DOM in the high–solar radiation environment of the upper water layers in combination with inhibited bacterial activity might lead to an accumulation of photochemically altered DOM that is susceptible to further abiotic transformations. As indicated by our results, the originally labile fraction of the DOM pool might be rendered more refractory upon irradiation. On the other hand, mixing events will increase the proportion of more refractory DOM in SW that is potentially available for photolysis upon exposure to radiation. These contrasting processes take place on a relatively short time scale. Mixing of the upper water column in the late afternoon because of surface cooling (Doney et al. 1995) will transport the photoproducts to deeper layers. Bacterioplankton have been found to recover quickly from UV stress (Kaiser and Herndl 1997); thus, the activity of bacterioplankton mixed into deeper layers will strongly depend on the bioavailability of the recently produced photoproducts from the parent DOM. According to our results, the bioavailability after exposure to solar radiation will be related to the original DOM bioavailability. Although freshly released, labile DOM might be photochemically rendered more refractory upon exposure to radiation, more refractory DOM might become susceptible to bacterioplankton uptake after photoinduced alteration.

The input of MW into surface layers is a frequently observed phenomenon that is not restricted to Mediterranean waters. Moreover, the oceanic conveyor belt leads to large-scale upwelling of deep waters in certain areas of the world's oceans (McDonald and Wunsch 1996). According to our results, these regions should exhibit enhanced bacterial activity in the SW because of photolysis of refractory, deep-water DOM. In stratified SW, however, solar radiation can lead to a transformation of labile compounds to more refractory material and, consequently, to reduced bacterioplankton activity. This differential response of bacterioplankton to the photochemically altered DOM pool obviously depends on the molecular structure of the DOM, a fact that complicates predictions and modeling approaches related to the impact that increasing UV radiation has on the overall carbon turnover of the ocean.

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