Vs. 3 17 May 2012

Ms. submitted to Limnology & Oceanography

Annual variability in light modulation of bacterial heterotrophic activity in surface northwestern Mediterranean waters

Clara Ruiz-González,* Martí Galí, Thomas Lefort, Clara Cardelús, Rafel Simó and Josep M. Gasol

Institut de Ciències del Mar-Consejo Superior de Investigaciones Científicas

Barcelona, Spain

*Corresponding author: clara.ruiz.glez@gmail.com

Condensed running head: Seasonal effects of sunlight on bacteria

Acknowledgements

We are grateful to V. Balagué, I. Forn, H. Sarmento, A. Gomes, and all the people participating in the Blanes Bay Microbial Observatory sampling program for sampling assistance and coordination, and for providing chlorophyll *a* concentrations. This work has been supported by the Spanish Ministry of Science and Innovation through projects 'Fuentes de Materia Orgánica, Diversidad microbiana y funcionamiento (respiración y Uso del carbono) del ecosistema marino pelágico costero'-MODIVUS (Ciencias y Tecnologías Marinas-CTM2005-04795/MAR), 'Surface Mixing Modulation of the Exposure to solar Radiation'-SUMMER (Ciencias y Tecnologías Marinas-CTM2008-03309/MAR), and by a Ph.D. studentship to C. R.-G.

Abstract

The effect of photosynthetically available radiation (PAR, 400-700 nm) and ultraviolet radiation (UVR, 280-400 nm) on marine bacterial heterotrophic activity was assessed monthly throughout a seasonal cycle in Blanes Bay (northwestern Mediterranean Sea). Seawater samples amended with ^{3}H-leucine were exposed to solar radiation under three radiation treatments: PAR + UVR (280-700 nm), PAR + UVA (320-700 nm), and PAR only. Parallel reference incubations in the dark and under a fixed artificial light source (PAR only) were also performed. Exposure to high UVR doses caused strong inhibition of ^{3}H-leucine incorporation rates (LIR), whereas natural PAR doses did not cause overall significant effects. Within UVR, UVA radiation accounted for most of the reduction in LIR, and this effect was modulated by the proportionality of the experimental light to the previous light exposure history of the samples. Constant (artificial) PAR-only exposure led to a general but seasonally variable increase in bacterial heterotrophic production compared to the dark controls, with large increases in spring and lower changes during summer. This pattern was likely caused by the stimulation of the bacterial group *Gammaproteobacteria*, which showed higher numbers of cells active in ^{3}H-leucine uptake after light exposure. Again, the previous light history of the samples seemed to partly explain the measured effects. Overall, our results show variable responses of bacterial activities to light manipulations, depending on seasonally changing light conditions and communities, and stress the importance of realistic simulation of light exposure conditions for ecosystem-relevant photobiological studies with microbial plankton.

Introduction

Abundance and activity of marine heterotrophic bacteria are influenced by parameters that fluctuate at different time scales. Temperature, nutrient concentration, dissolved organic matter (DOM) availability, and the composition of the microbial community have been regarded among the main factors controlling DOM consumption by bacteria (White et al. 1991; Cotner et al. 2000; Cottrell and Kirchman 2003). However, the relative importance of solar radiation as a modulator of bacterial production across spatial or seasonal patterns has received little attention. In surface waters, bacteria are exposed to damaging solar ultraviolet radiation (UVR, 280-400 nm) that can cause inhibition of metabolic activities such as synthesis of protein and deoxyribonucleic acid (DNA) (Herndl et al. 1993; Sommaruga et al. 1997), oxygen consumption (Pakulski et al. 1998), or amino acid and adenosine triphosphate (ATP) uptake (Alonso-Sáez et al. 2006). Nevertheless, there is also evidence of a positive effect of UVA (320-400 nm) and photosynthetically available radiation (PAR, 400-700 nm) on bacterial activity due to photoenzymatic repair (Kaiser and Herndl 1997), to the ability of some bacteria to derive energy from light using bacteriochlorophyll *a* or proteorhodopsin (Béjà et al. 2000; Kolber et al. 2000), or due to the reported light-stimulation of cyanobacterial uptake of amino acids and related compounds (Church et al. 2004; Michelou et al. 2007; Mary et al. 2008). In addition, UVR can photolyze some recalcitrant DOM into more readily utilizable forms, making it more available to heterotrophs and thus enhancing their activity or, by contrast, initially labile DOM can be rendered more recalcitrant upon UVR exposure (Benner and Biddanda 1998; Obernosterer et al. 1999). Finally, heterotrophic bacteria might react to increased or decreased release of photosynthate from light-affected phytoplankton. All in all, the

80 aforementioned processes indicate that the interactions between heterotrophic bacteria
81 and light are far from simple.
82 The Mediterranean Sea is characterized by relatively high solar radiation levels
83 owing to a weak cloud cover and a high penetration of solar radiation into the
84 oligotrophic and transparent water column. However, although some UVB (280-320
85 nm) and UVA underwater measurements are available for the Mediterranean
86 (Sommaruga et al. 2005; Llabrés et al. 2010), there is still a remarkable dearth of data
87 on UVR attenuation varying at different scales. Moreover, most of the studies on
88 UVR effects on Mediterranean prokaryotes drive conclusions from occasional
89 experiments conducted mostly during spring or summer (Sommaruga et al. 2005;
90 Alonso-Sáez et al. 2006; Llabrés et al. 2010).
91 A current time series study in the Blanes Bay Microbial Observatory
92 (northwestern Mediterranean), a shallow oligotrophic coastal area, shows a great
93 seasonal variability in underwater PAR and UVR profiles (M. Galí unpubl.). This,
94 joined to the fact that both, seasonal bacterial taxonomic succession (Schauer et al.
95 2003) and differential sensitivities to UVR of different bacterial groups, have been
96 described for this coastal region (Alonso-Sáez et al. 2006), suggests that seasonal
97 variability in the bacterial responses to UVR is likely to occur in the area.
98 We incubated natural bacterioplankton with radiolabeled leucine in UVR-
99 transparent vials throughout a seasonal cycle to characterize the effects of natural
100 radiation levels on bacterial heterotrophic activity as compared to the values measured
101 with an artificial constant light source. The observed responses were further compared
102 with in situ changes in physical (temperature, irradiance, light history, mixing layer
103 depth) or biological (chlorophyll *a,* primary productivity, bacterial abundance, and
104 community composition) parameters in order to search for potential causes of the

variability. Since both, light levels and bacterial taxonomic composition change through the seasonal cycle, the differential responses of bacterial communities to sunlight are essential to understand the role of solar radiation as a modulator of organic matter fluxes in marine ecosystems. The results obtained have potential implications for assessing the suitability of standard dark protocols for a realistic measurement of heterotrophic bacterial activity.

Methods

*Sampling and basic parameters***.** A series of monthly experiments were carried out with waters from a shallow (≈20 m depth) coastal station (the Blanes Bay Microbial Observatory, BBMO, northwestern Mediterranean Sea, http://www.icm.csic.es/bio/projects/icmicrobis/bbmo) between January 2008 and April 2010. Surface waters (0.5 m depth) were sampled at about 9:00 a.m. at 800 m offshore (41º 40'N, 2º 48'E), filtered through a 200 μm mesh and transported within an hour to the lab under dim light. Seawater temperature was measured in situ with a SAIV A-S 204 conductivity–temperature–depth (CTD) probe and underwater PAR and UVR profiles were obtained with a profiling ultraviolet (PUV) 2500 radiometer (Biospherical Instruments). Chlorophyll *a* (Chl *a*) concentration was determined by filtering 150 mL of seawater on GF/F filters (Whatman), extracting the pigment in acetone (90% v:v) in the dark at 4ºC for 24 h, and measuring fluorescence with a Turner Designs fluorometer.

Experimental setup. Different experiments were performed during the study period (*see* Fig. 1). From 15 January 2008 to 14 September 2009 incubations for bacterial heterotrophic activity measurements (hereafter leucine incorporation rates, LIR) were conducted under natural radiation conditions inside a seawater-flushed tank

130 placed outside the laboratory in Barcelona, ca. 100 km south of the sampling site in
131 the same coastline. Irradiance spectrum was manipulated with light filters, and the
132 experimental PAR and UVR doses were monitored using a PUV 2500 radiometer
133 placed underwater next to the samples. Throughout the entire study period (January
134 2008 to April 2010), parallel dark and artificial light (PAR-only; ca. 1500 μmol
135 photons m^{-2} s^{-1}) LIR incubations were additionally carried out inside an indoor
136 incubator at in situ temperature. These experiments were aimed at standardizing the
137 responses of seasonally changing communities to constant irradiance conditions. .
138 Between 17 March 2009 and 13 April 2010, additional parallel dark and artificial
139 PAR incubations were performed for microautoradiography combined with catalyzed
140 reporter deposition-fluorescence in situ hybridization (MAR-CARD-FISH) analyses
141 (*see* below). The objective was the identification of the bacterial taxonomic groups
142 responsible for the patterns found in LIR. The rest of the variables (primary
143 production, bacterial and picophytoplankton abundances, composition of the bacterial
144 assemblages) were monitored throughout the whole period at the sampling site.
145 *Abundance of prokaryotes***.** Aliquots for bacterial abundance were preserved
146 with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), frozen
147 immediately in liquid nitrogen, and stored at -80ºC until quantification with a
148 fluorescent activated cell sorting (FACSCalibur) flow cytometer (Becton Dickinson)
149 of cells stained with SybrGreen I (Molecular Probes, Gasol and del Giorgio [2000]).
150 *Synechoccocus* and *Prochlorococcus* cells were enumerated by flow cytometry and
151 distinguished by their different sizes and pigment properties in unstained samples
152 (Marie et al. 1999).
153 *Primary production.* Particulate primary production (pPP) was determined using
154 the ^{14}C technique (Steeman-Nielsen 1952). Fourteen 70 mL-bottles (Iwaki) and one

dark control (bottle wrapped with aluminum foil) were filled with seawater and inoculated with 10 μCi $NaH^{14}CO_3$. The incubation was carried out in a water bath at in situ temperature for 2 hours in a gradient of light irradiance (ca. 10-1500 μmol photons m^{-2} s^{-1}). Circulating water connected to a water bath maintained the temperature. Light was measured with a small size spherical light meter (Illuminova AB). After the incubation, the samples were filtered at low vacuum pressure through cellulose ester filters (Millipore 0.22 μm), and the filters were subsequently exposed overnight to concentrated HCl fumes. Scintillation cocktail (4 mL Optiphase Hisafe 2) was then added to each filter, and the radioactivity was measured in a Beckton-Dickinson LS6000 scintillation counter. Average in situ pPP was estimated from the photosynthesis-irradiance (P-E) curve and the hourly in situ PAR irradiance within the 'actively mixing layer' of the 24 h prior to sampling (*see* below).

Leucine incorporation rates (LIR). LIR was monthly estimated using the ^{3}H-leucine incorporation method described by Kirchman et al. (1985). Four 1.2 mL aliquots and two trichloroacetic acid (TCA)-killed controls (5% final concentration) of each sample were incubated with 40 nmol L^{-1} ^{3}H-leucine for 2 h. The incubations were carried out in a water bath at in situ temperature in the dark and under fixed light irradiance (ca. 1500 μmol photons m^{-2} s^{-1}, approximately the surface PAR irradiance of a summer day in this area). The incorporation was stopped by adding cold TCA (5% final concentration) to the vials and samples were kept at -20ºC until processing as described by Smith and Azam (1992). Radioactivity was then counted on a Beckman scintillation counter.

From 15 January 2008 to 14 September 2009 LIR was also measured under exposure to natural solar radiation. For that purpose, six UVR-transparent cuvettes (4 mL, Plastibrand) of which two were formaldehyde-killed controls (4% final

concentration) were amended with ^{3}H-leucine (40 nmol L^{-1} final concentration, 160 Ci $mmol^{-1}$) and incubated for 2 to 3 hours under different radiation conditions: full sunlight spectrum (PAR+UVR), the full spectrum minus UVB (PAR+UVA, covered with the plastic foil Mylar-D, which excludes UVB radiation), PAR only (wrapped with two layers of Ultraphan URUV Farblos which removes all UVR) or darkness (wrapped with aluminium foil inside a black plastic bag to avoid reflection). Cuvettes were incubated at about 5 cm under the surface inside a black tank (200 L) with running seawater to maintain in situ temperature. After incubation, 1.2 mL were transferred from each cuvette to centrifuge tubes, then killed with 120 μL cold TCA (5% final concentration) and processed as described above.

Microautoradiography combined with catalyzed reporter deposition-fluorescence in situ hybridization (MAR-CARD-FISH). In the experiments between 17 March 2009 and 13 April 2010, 30 mL samples were amended with trace amounts of ^{3}H-leucine (0.5 nmol L^{-1} final concentration, 160 Ci $mmol^{-1}$) and incubated in parallel in the dark or in the light (PAR-only, ca. 1500 μmol photons m^{-2} s^{-1}) for 2 to 3 hours. After exposure, samples were fixed overnight with paraformaldehyde (PFA, 1% final concentration) at 4ºC in the dark and filtered on 0.2 μm polycarbonate filters (GTTP, Millipore). Sections of the filters were then hybridized following the CARD-FISH protocol (Pernthaler et al. 2002). A few horseradish peroxidase (HRP)-probes were used in order to characterize the composition of in situ bacterial communities: Gam42a that targets most *Gammaproteobacteria* (Manz et al. 1992), NOR5-730 for the NOR5 clade (Eilers et al. 2000*b*), SAR11-441R for the SAR11 cluster (Morris et al. 2002), Ros537 targeting the *Roseobacter* clade (Eilers et al. 2001), CF319a for clades belonging to *Bacteroidetes* (Manz et al. 1996), CYA339 for the cyanobacteria (Nübel et al. 1997), and Eub338-II-III for inclusion of most *Eubacteria* (Daims et al.

1999). The relative abundance of each group was checked by cutting smaller pieces from each filter and staining them with 4,6-diamidino-2-phenylindole (DAPI, 1 μg mL^{-1}). Between 500-800 DAPI-positive-cells were counted manually within a minimum of 10 fields under an Olympus BX61 epifluorescence microscope.

For microautoradiography, we followed the protocol described in Alonso-Sáez and Gasol (2007). On the basis of previous studies (Alonso-Sáez et al. 2006; Ruiz-González et al. 2012) only potentially photostimulable groups (i.e., *Gammaproteobacteria,* NOR5, *Cyanobacteria*, and *Roseobacter*) were subjected to microautoradiographic analysis. The optimal exposure times were determined for each sampling point and ranged from 2 to 19 days. After development, the slides were dried overnight, stained with DAPI and 500 to 700 hybridized cells were counted manually by epifluorescence microscopy within a minimum of 10 fields.

Measurement and calculation of PAR and UVR doses. A radiometer (Biospherical PUV 2500) was used in the field and also placed inside the incubation tanks with the sensor covered by ca. 5 cm of water, and the downwelling cosine irradiance reaching the samples was recorded at a frequency of 5 s^{-1}. The wavelengths measured included 6 bands in the UVR (305, 313, 320, 340, 380 and 395 nm, in units of mW cm^{-2} nm^{-1}) and one integrated band in the visible (PAR, in μmol photons cm^{-2} s^{-1}). The mean spectral irradiance in the 6 UVR bands was converted to mean UVB and UVA irradiance (mW cm^{-2}) by integrating over the spectrum (sum of trapezoids), between 305 – 320 nm and 320 – 395 nm respectively. Finally, the mean UVB, UVA, and PAR irradiance was multiplied by the duration of each experiment in order to obtain the radiation dose (in kJ m^{-2} for UVB and UVA, and mol photons m^{-2} for PAR).

The 'light' history of microbial communities, i.e., their previous UVR and PAR exposure at the sampling site, was calculated as a function of spectral irradiance at the water subsurface, vertical mixing depth, and underwater attenuation of solar radiation (Vallina and Simó 2007). For this purpose, two distinct exposure regimes were considered: 'seasonal exposure' and 'maximum daily exposure'. Seasonal exposure was calculated by combining the mean irradiance of the three days prior to sampling with the seasonal mixed layer depth (MLD), whereas maximum daily exposure was calculated as the combination of 'actively mixing layer' depth (mLD) with average irradiance at noon ± 2 hours of the previous day. Total solar irradiance (with hourly resolution) was obtained from a meteorological station located 5 km southwest from the BBMO sampling station (Malgrat de Mar, Catalan Meteorological Service, SCM). MLD and mLD were calculated from temperature profiles obtained from CTD casts, binned at 1 m intervals. MLD was defined as the depth where a jump in temperature larger than 0.15 ºC was encountered relative to 1 m depth, while mLD was defined as the depth showing a 0.03 ºC departure from the 1 m reference. These criteria were optimized for our particular dataset, and yielded mLD or MLD estimates that were consistent with the vertical profiles of other variables (M. Galí unpubl.). Diffuse attenuation coefficients of downwelling radiation ($K_{d,\lambda}$) were calculated as the slope of the linear regression between the natural logarithm of spectral cosine irradiance ($E_{d,\lambda,,z}$) and depth (z). $K_{d,320}$ and $K_{d,380}$ were chosen as representative of UVB and UVA attenuation respectively, while PAR (and its corresponding $K_{d,PAR}$) was originally measured in one integrated band.

Statistical analyses. The Shapiro-Wilk *W*-test for normality of data and the Levene's test for homogeneity of variance were applied prior to analysis, and either one-way analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis Test

were used to analyze statistically significant ($p < 0.05$) differences in the measured variables, and post hoc analyses (Tukey's Honestly Significant Difference test) for comparison among different light treatments or seasonal averages. Correlations between variables were calculated using the Pearson's correlation coefficient. These statistical analyses were performed using the JMP software (SAS Institute).

Results

Background information. The seasonal variation of the basic parameters in the study area is shown in Fig. 1 for 2008-2010. The summer period was characterized by high surface temperatures (20-25ºC), a strongly stratified water column, and low Chl *a* concentrations (< 0.4 μg L^{-1}) while the opposite trend was observed in the well mixed winter waters showing the lowest temperatures (~ 12ºC) and largest Chl *a* peaks (up to 2 μg L^{-1}; Fig. 1A, B). In situ particulate primary production (pPP) also varied seasonally, reaching higher values in late winter (> 1.5 mg C m^{-3} h^{-1} in 2008) and variable peaks during spring and summer (Fig. 1C). No winter pPP peak showed up in 2009, although it is possible that we missed it by missing the March sampling.

LIR values showed large variability among sampling dates (range 2-135 pmol leucine L^{-1} h^{-1}, Fig. 1C). Maximum values tended to be observed after Chl *a* or pPP peaks, higher values were generally found in summer and spring compared to autumn and winter, as reflected by a positive correlation between LIR and temperature (Pearson's $r = 0.65$, $n = 36$, $p < 0.0001$). Bacterial abundances ranged three-fold from 0.4 to 1.2 x 10^6 cells mL^{-1} and tended to be higher during the summer. *Synechococcus* and *Prochlorococcus* abundances also varied seasonally (Fig. 2A), with greater numbers in summer and autumn, respectively.

278 The bacterial community (composition assessed by CARD-FISH) was always
279 dominated by the SAR11 group (average of 35% of cell counts) followed by similar
280 proportions of *Bacteroidetes* (15%) and *Gammaproteobacteria* (13%). The latter two
281 groups showed a strong seasonality, increasing their numbers during winter and
282 peaking in spring in both years, closely following the Chl *a* peaks (up to 25% or 30%
283 of cell counts for *Bacteroidetes* and *Gammaproteobacteria*, respectively). Conversely,
284 the SAR11 group did not show any obvious seasonal pattern, with numbers ranging
285 from 20% to 60% of total DAPI counts. *Roseobacter* and the gammaproteobacterial
286 group NOR5 presented much lower numbers (average 5% and 2% of total DAPI
287 counts, respectively) with abundance peaks generally coinciding with maximal Chl *a*
288 concentrations.

289 *Seasonal responses of bacteria to natural solar radiation.* Exposure of samples
290 to natural sunlight radiation caused a general but variable inhibition of LIR compared
291 to the dark control (Fig. 3). The lowest LIR were observed under full sunlight (UVR
292 inclusive) exposure (up to 60% inhibition, average 20%). PAR alone caused a
293 significant decrease of LIR compared to the dark control in only five out of the twenty
294 five experiments (range 20% - 32% decrease) and, exceptionally, significant increases
295 (range 20% - 60%) were observed (Tukey's test, $p < 0.05$). The degree of inhibition
296 due to UVR, as compared to PAR, was significantly correlated with the UVR doses
297 measured during the incubations (Table 2, Pearson's $r = 0.51$, $p < 0.02$, $n = 24$).
298 However, this effect seemed to be mainly driven by UVA-induced inhibition ($r =$
299 0.53, $p < 0.01$, $n = 24$), as no significant correlation was observed between LIR
300 measured under PAR or UVB radiation and their respective doses (Table 2).

301 The sensitivity of LIR to UVR was not correlated with any other measured
302 parameter including water transparency, salinity, Chl *a*, dissolved organic carbon

303 (DOC) or nutrient concentration, primary productivity, depth of the mixed layer, nor
304 bacterial community composition or the abundance of the different bacterial groups as
305 described by CARD-FISH (*n* values from 8 to 26, *p* values from 0.062 to 0.989,
306 details not shown). Only sea surface temperature was positively correlated with UVR-
307 driven inhibition ($r = 0.40$, $p < 0.05$, $n = 25$), yet probably this simply reflects the
308 obvious relationship between warmer temperatures and greater UVR levels.

309 Seasonally averaged changes in LIR measured under natural light conditions are
310 summarized in Table 1 as percentages of the dark control. No differences were found
311 among seasons for LIR measured under PAR radiation. Conversely, a tendency for
312 lower activities was found in spring and summer samples exposed to both UVA and
313 UVR, yet not significantly different at the level of $p < 0.05$. When annually averaged,
314 LIR measured under PAR+UVA and full sunlight were 15% and 20% lower,
315 respectively, than LIR measured in the dark (Tukey's test $p < 0.05$). LIR measured
316 under natural PAR did not significantly differ from that in the dark (Table 1).

317 In order to find out which regions of the spectrum were responsible for these
318 effects, the relative contribution of UVA and UVB to the total LIR inhibition (Fig.
319 4A) was calculated as follows:

320 $$\text{Inhibition due to UVX} = (LIR_{PAR}\text{-}LIR_{UVX})\cdot 100/LIR_{PAR} \quad (1)$$

321 where LIR_{PAR} represents the ^{3}H-leucine incorporation rates under PAR-only
322 incubation treatment, and LIR_{UVX} means the LIR measured under each UVR
323 treatment. Inhibition due to UVB was calculated as the difference between the relative
324 inhibition due to UVA and UVR. We found that the contribution of each type of UVR
325 varied throughout the year although, in most cases, UVA was responsible for most of
326 the observed inhibition (Fig. 4A), and no seasonality was apparent in the contributions

of each UVR fraction. The inhibition due to each fraction did not correlate to the measured UVA:UVB ratio of irradiances during incubations (Fig. 4B) but to the ratio estimated at the sampling site (calculated from the in situ UV irradiance measured during the 4 hours of maximum insolation of the previous day, *see* Methods); thus, inhibition by experimental UVB was found to increase with the in situ UVA:UVB ratios ($r = 0.60$, $p < 0.003$, $n = 25$, Fig. 4C). In other words, the lower the UVB doses relative to UVA received by the plankton community prior to sampling, the higher the inhibition caused by UVB during the incubations. This was partially explained by the difference between the in situ UVA:UVB ratio and the one experienced by samples during our incubations. Experimental overexposure to UVB relative to the conditions microbial plankton was acclimated to would cause a larger deleterious effect of UVB.

Seasonal responses of bacteria to constant PAR exposure. An artificial light source (PAR only) was further used for comparison of the response of bacteria to the same light conditions across seasons. In general, LIR measured in the light was stimulated with respect to the dark control (range 20% to 150% increase), although also inhibition (range 20% to 65% decrease) or no effect at all were occasionally observed (Fig. 5). Again, such variability in the responses was not correlated with other measured parameters such as water transparency, salinity, Chl *a*, DOC and nutrient concentrations, primary productivity or the composition of the bacterial community (*n* values from 17 to 36, *p* values from 0.057 to 0.983, details not shown). However, significant relationships were found between the increase in the light-measured LIR relative to dark and the water temperature (Pearson's $r = -0.41$, $p < 0.02$, $n = 35$), the MLD ($r = 0.41$, $p < 0.02$, $n = 36$), and the maximum daily PAR irradiance (mean irradiance at noon ± 2h of the previous day within the mLD, *see* Methods, $r = -0.33$, $p < 0.05$, $n = 37$). These observations indicate that the light-LIR

352 was generally higher than the dark-LIR when lower irradiances occurred in situ, and
353 conversely, inhibition or no effect were found when the natural irradiances
354 approached the artificial level (i.e., in summer). This explains the positive relationship
355 with MLD: higher light-driven increases in LIR occurred when deeper mixed layers
356 were found, i.e., in response to experimental overexposure relative to in situ
357 underwater irradiance conditions. In terms of seasons, the average photostimulation
358 was maximal in spring (50% increase compared to dark LIR) whereas photoinhibition
359 generally occurred in summer when experimental light levels approached those in the
360 sea (Table 1).

361 *Role of community structure in bulk bacterial responses to sunlight.* To further
362 investigate the reasons underlying such variability in the bacterial responses exposed
363 to a fixed light source, several MAR-CARD-FISH incubations were performed to
364 describe the response of potentially photostimulable bacterial groups. Probes for
365 *Gammaproteobacteria*, *Roseobacter*, NOR5 and *Cyanobacteria* were selected for that
366 purpose (Fig. 6), and hybridizations were carried out in the samples where the
367 differences between dark and light LIR had been significant (either positive or
368 negative, Fig. 5). Given the low abundances of the NOR5 group it was not possible to
369 quantify their number of active cells with accuracy and they were not considered as
370 potential drivers of the observed light-stimulation patterns.

371 Variable numbers of *Gammaproteobacteria* active in the uptake of ^{3}H-leucine
372 were found all through the year (range 40%-90% of active cells), showing higher
373 percentages in summer and spring compared to autumn samples (Fig. 6A). This group
374 showed a repeated pattern of significant stimulation due to light, except in August
375 2009 when they were slightly inhibited with respect to the dark control. Notably, this
376 observation was coincident with the observed decrease in bulk light-LIR (*see* Fig 5).

A good positive correlation was found between the light-driven increases in the number of active *Gammaproteobacteria* and the increase in bulk LIR caused by light ($r = 0.73$, $p < 0.001$, $n = 17$, Fig. 7A). Moreover, significant correlations were found between the number of active *Gammaproteobacteria* and bulk LIR ($r = 0.59$, $p < 0.02$, $n = 17$) and between active *Gammaproteobacteria* and active *Eubacteria* ($r = 0.66$, $p < 0.003$, $n = 17$) measured in the dark (Fig. 7B, C). These correlations were better in the light ($r = 0.67$, $p < 0.004$, $n = 17$ and $r = 0.82$, $p < 0.0001$, $n = 17$, respectively, Figs. 7B, C). Interestingly, *Gammaproteobacteria* abundances seemed to be explained in part by the underwater ambient PAR levels ($r = 0.57$, $p < 0.0005$, $n = 29$), showing greater abundances in more illuminated waters, while no correlation was apparent with temperature, Chl *a* or primary production data. As with bulk LIR, furthermore, the magnitude of light-driven increases in the number of active *Gammaproteobacteria* was negatively correlated with the maximum daily PAR irradiance ($r = -0.49$, $p < 0.05$, $n = 17$).

No other bacterial group seemed to explain the observed bulk light-driven differences due to lightin bulk LIR. Very high percentages of *Roseobacter* were active in ^{3}H-leucine uptake throughout the year (> 95% of labeled cells) but no significant differences were commonly found between dark and light treatments (only in 3 out of 9 sampling dates). The fraction of active *Cyanobacteria* rarely exceeded 10%, and although some differences were detected between treatments (up to two fold increases), their low activity and different stimulation patterns excluded them as candidates responsible for the light enhancement of bulk LIR.

401 Discussion

402 Very few seasonal studies have considered the responses of marine microbial
403 communities to temporally variable natural radiation levels, UVR being often omitted
404 as a significant driver of microbial activities. Given the high transparency to UVR of
405 most oceanic waters, and the reported distinct UVR sensitivity of different bacterial
406 groups (Alonso-Sáez et al. 2006), seasonal variations in light intensity and penetration
407 into the water column might differentially affect the year-round use of DOM by
408 bacteria, thus modulating the effects of other environmental variables that are more
409 easily and frequently measured.

410 *Seasonal responses of bacteria to natural sunlight conditions.* Our approach
411 consisted of short term incubations under natural sunlight with the radioisotope tracer
412 already added, which allows a more realistic estimation of in situ incorporation rates
413 since irradiation and uptake processes are not separated in time. Following Vaughan
414 et al. (2010), any possible UVR effect on the added leucine tracer was discarded since
415 no significant reduction in LIR was found in samples amended with previously
416 exposed ^{3}H-leucine to both natural or artificial UVR (data not shown). Unfortunately,
417 light driven changes in the bioavailability of endogenous DOM could not be
418 discriminated from direct UVR effects on bacteria. On the other hand, short term
419 incubations were thought to prevent or minimize other indirect interactions derived
420 from e.g. effects on viruses or grazers. In any case, we have to bear in mind that the
421 observed bacterial responses are the final balance among all the synergistic and
422 antagonistic effects that are taking place at the same time inside the experimental
423 cuvettes.

With this approach, and as reported by others (Aas et al. 1996; Sommaruga et al. 1997), we found a significant UVR-driven decrease in LIR with respect to the values measured in dark incubations. In general, we found low or no inhibition when irradiance values were low (winter and autumn) and greater inhibition (up to 60%) under high irradiances (spring and summer). This suggests that, under certain circumstances, standard measurements in the dark may severely overestimate bacterial heterotrophic activity. This would be particularly dramatic in highly illuminated waters. Annually averaged, the LIR measured under full sunlight conditions was 20% lower than that measured in the dark.

In contrast, exposure to natural PAR only did not generally affect the measured LIR regardless of the irradiance, except for a few cases where a slight decrease (mainly in spring and summer) or an occasional increase were observed. Other studies have also observed different degrees of inhibition (Aas et al. 1996; Sommaruga et al. 1997; Morán et al. 2001) or stimulation (Aas et al. 1996; Church et al. 2004; Pakulski et al. 2007) when measuring LIR under in situ PAR irradiance, effects that have been attributed to photodynamic processes (Harrison 1967). Morán et al. (2001), though, suggested that PAR-mediated decrease in samples from the same area of our study was due to enhanced bacterial activity in the dark rather than inhibition by light, yet the exact mechanisms were not identified.

An interesting aspect of the UVR effects refers to the relative contribution of UVA and UVB to the total LIR inhibition. In our experiments the contribution of UVA was generally higher than that of UVB (Fig. 4), as also seen by other authors (Sommaruga et al. 1997; Visser et al. 1999). This might be attributed to the fact that even though UVB is more energetic than UVA, the amount of UVA energy that reaches the sea surface is much larger than that in the UVB region. On some

449 occasions, however, higher inhibition by UVB was observed. In those cases, the
450 increase in the relative inhibition by UVB in experiments was associated with deeper
451 in situ mixing layers ($r = 0.61$, $p < 0.002$, $n = 25$, data not shown). Vertical mixing
452 controls the residence time of marine bacteria in surface waters and it has been shown
453 to be an important factor regulating the exposure to and the effects of UVR (Huot et
454 al. 2000; Bertoni et al. 2011). Since the attenuation of UVB in the water column is
455 much stronger than that of UVA or PAR, bacterioplankton transported within a
456 deeper mixing layer (e.g., in winter) will be exposed to higher UVA:UVB ratios than
457 cells confined in a strongly stratified and shallow layer (e.g., in summer), where they
458 will be continuously exposed to deleterious UVB doses and will have fewer chances
459 for UVA- or PAR-driven photorepair of DNA damage (Friedberg et al. 1995). A
460 recent study of Bertoni et al. (2011) shows for the first time that mixing reduces LIR
461 inhibition and that large differences can be found between fixed and vertically moving
462 incubations. Thus, it seems that many of our static incubations led to an overexposure
463 to UVB of organisms naturally inhabiting a well-mixed water column and thus
464 acclimated to lower UVB doses, resulting in an enhanced UVB inhibition compared
465 to UVA. This was supported by the fact that higher inhibition due to UVB was found
466 with increasing in situ UVA:UVB ratios on the previous day (Fig. 4C), that is, with
467 increasing experimental overexposure to UVB proportions relative to conditions in
468 the sea. UVB has often been regarded as the main contributor to bacterial damage
469 (Herndl et al. 1993) but, in view of our results and those recently reported by Bertoni
470 et al. (2011), it is possible that, depending on the environmental characteristics of the
471 samples, the use of artificial UVR lamps or long surface incubations that neglect
472 natural mixing effects and cause overexposure to UVB, result in unrealistic inhibitory
473 observations. All of the aforementioned points out to the relevance of taking into

account the often overlooked light-exposure history of samples (which is in turn dependant on the mixing regime), and further highlights the difficulty of mimicking natural underwater light conditions.

Seasonal responses of bacteria under invariable PAR conditions. In addition to the intensity and spectral characteristics of sunlight, the response of bacteria to solar radiation has been shown to depend on, or interact with, many other environmental or biological factors, such as temperature (Bullock and Jeffrey 2010), nutrient status (Pausz and Herndl 2002), or the specific sensitivities of different bacteria (Alonso-Sáez et al. 2006). Hence, in order to search for other potential causes of seasonality in the bacterial responses, we excluded the effect of experimental light variability by incubating a parallel set of samples under a fixed artificial PAR source (equivalent to the annual mean surface irradiance in this area). Unlike natural PAR exposure, this indoor approach led to a general stimulation of activity relative to the dark control. Unexpectedly, though, such an effect was more pronounced in spring than in summer, when no changes or even inhibition occurred. This seasonality in the light effects of different samples receiving exactly the same irradiance discarded an exclusive dependence of bacterial responses on light intensity and suggested that causes other than irradiance influenced the observed variability. During our annual cycle neither pPP, DOC, Chl *a* or nutrient concentration, nor the composition of the bacterial communities (in terms of group abundances) seemed to influence the light-driven responses of LIR to natural sunlight. In a recent study conducted also in the BBMO we showed that responses to natural solar radiation at the community level are influenced by taxon-specific sensitivities to sunlight and their relative contribution to total activity (Ruiz-González et al. 2012). Hence, we wanted to check whether the

observed seasonality in the bacterial responses to a fixed irradiance was also influenced by the activity of some particular groups.

Role of bacterial community composition in bulk responses to sunlight. Single-cell activity analyses were performed in order to search for bacterial groups responsible for the observed light driven changes in bulk activity. Several studies that also reported similar PAR-driven stimulation of bulk LIR attributed it to light-enhanced amino acid uptake by cyanobacteria (Church et al. 2004; Michelou et al. 2007; Mary et al. 2008). Our MAR-CARD-FISH data (Fig. 6C), conversely, discarded this group as the main driver of the observed responses due to the low numbers of active cells and the lack of significant light-enhancement in these numbers concomitant with LIR increases. However, bacterial groups other than cyanobacteria have also been shown to augment their activity under the light (Alonso-Sáez et al. 2006; Mary et al. 2008; Straza and Kirchman 2011), in what has been attributed to the photoheterotrophic capabilities of some phylotypes containing light-harvesting proteorhodopsins or bacteriochlorophyll *a* (Béjà et al. 2000; Kolber et al. 2000). With the aim to check whether photoheterotrophic bacteria were responsible for the observed increases in LIR, *Gammaproteobacteria*, *Roseobacter* and the NOR5 group were probed on the basis of previously published data indicating that these taxa occasionally presented light-enhanced activity in this area (Alonso-Sáez et al. 2006; Ruiz-González et al. 2012). SAR11 and *Bacteroidetes* were not considered due to the reported negative sensitivity to light of the former and the lack of responses to sunlight and low contribution to total active cells of the latter (Alonso-Sáez et al. 2006; Ruiz-González et al. 2012).

While the great majority of *Roseobacter* cells were active in both light and dark treatments throughout the year, increases in the number of active cells due to light

were often negligible, so it is unlikely that they accounted for the observed light-stimulated LIR. Conversely, the numbers of active *Gammaproteobacteria* cells significantly increased in the light during most of the study period, showing a stimulation pattern very similar to that of bulk LIR. Even the observed LIR decrease in August was mirrored by a decrease in active *Gammaproteobacteria*, thus pointing to a major role of this group in the community response to light. This role, remarkable because *Gammaproteobacteria* were not the most abundant prokaryotes, was further supported by the good positive correlations observed between the number of active cells within this group, the number of active *Eubacteria* cells, and LIR. Some members of the *Gammaproteobacteria* maintain large numbers of ribosomes during extended periods of non-growth, which allow them to rapidly initiate growth upon changing environmental conditions (Eilers et al. 2000*a*). Thus, they might also take quick advantage of changes in light conditions and respond faster than other groups.

Interestingly, the *Gammaproteobacteria* showed preference for highly illuminated environments as seen by the good correlation between the seasonal irradiance and cell abundances, not seen with other variables. This is in accordance with their apparent ability to benefit from light and it is further supported by the relatively high resistance to UVR reported for these bacteria in the study area (Alonso-Sáez et al. 2006; Ruiz-González et al. 2012).

Within *Gammaproteobacteria*, the NOR5 clade was analyzed in detail because one sequenced member of the group had shown capability for aerobic anoxygenic photosynthesis (Fuchs et al. 2007). Unfortunately, their very low abundances throughout most of the year prevented an accurate quantification of active cells and discarded them as major drivers of increases in LIR. However, in a parallel study in

548 the BBMO we found that their ^{3}H-leucine uptake was occasionally stimulated by
549 natural PAR (Ruiz-González et al. 2012). On 26 May 2009, when they comprised up
550 to 90% of all *Gammaproteobacteria*, they might indeed have driven the light response
551 of the whole group.

552 *Influence of the previous light exposure history on bacterial responses to light.*
553 In agreement with the observations with samples exposed to natural radiation
554 conditions, also in the artificial light experiments the previous light exposure history
555 seemed to partially explain the observed light effects on LIR. Interestingly, the lower
556 the maximal daily irradiance bacteria had been exposed to in the sea, the larger the
557 increase caused by our artificial light source. In contrast, smaller or even negative
558 effects of light were observed when the natural maximal irradiance was more similar
559 to the experimental irradiance, i.e., in summer. This same pattern was also apparent in
560 the numbers of active *Gammaproteobacteria,* supporting their role in driving the
561 responses to light at the community level despite their low abundances. In other
562 words, the bigger the difference between the natural and the experimental light
563 conditions, the greater the effects we should expect. This finding is similar to that of
564 Straza and Kirchman (2011) who found that the magnitude of the light-driven effects
565 on bacterial activity was lower with greater light exposure prior to sampling, albeit the
566 reasons behind this observation have not yet been elucidated.

567 Among the possible explanations, we may consider a seasonally variable
568 photoheterotrophic response of the *Gammaproteobacteria* or other taxa containing
569 proteorhodopsins (PR) or bacteriochlorophyll *a* (BChl *a*). It has been hypothesized
570 that photoheterotrophy would be more advantageous in nutrient poor conditions
571 (Kolber et al. 2000); thereby, highly illuminated oligotrophic waters such as those of
572 Blanes Bay, where strong year-round phosphorous limitation of LIR is known to

573 occur (Pinhassi et al. 2006), might select for phylotypes with photoheterotrophic
574 capabilities. A similar strategy was described by Gómez-Consarnau et al. (2007) for a
575 cultured PR-containing *Bacteroidetes* isolated from the BBMO, which indeed showed
576 higher photostimulation of growth when growing on low DOM concentrations;
577 however, to date no field measurement has consistently supported this hypothesis. In
578 Blanes Bay it makes sense that lower PR-photoheterotrophy occurs in summer, when
579 DOC accumulates (C. Romera-Castillo unpubl.), presumably due to nutrient
580 limitation of microbial activities. Instead, photoheterotrophy might be higher in
581 spring, when DOC levels are at their minimum but there already is enough light. If
582 this was the case in Blanes Bay, and PR-containing bacteria were more active or
583 abundant in spring than in summer, greater light-driven increases would be expected
584 in spring upon exposure to high light conditions.

585 On the other hand, preliminary data from the BBMO indicate that BChl *a*-
586 containing bacteria are abundant in spring and summer and found at very low
587 numbers in winter (I. Ferrera, unpubl.). Should light supplement any energy for
588 growth, the experimental overexposure experienced by spring microbes might have
589 induced a photoheterotrophic response stronger than that of summer BChl *a*-
590 containing bacteria, already exposed to high light conditions in situ. In any case, no
591 direct evidence is so far available of photoenhanced leucine uptake by these kinds of
592 mixotrophic organisms, so, we cannot unequivocally determine whether the observed
593 light increases in activity were mainly the result of bacterial photoheterotrophy.

594 A rapid response of bacteria to photosynthate leaks from phytoplankton upon
595 light exposure could provide a plausible alternative explanation.
596 *Gammaproteobacteria* abundances seemed to follow the peaks in Chl *a*, as if they
597 were rapidly responding to short-term variations in PP. It is thus likely that

598 overexposure of algae with respect to their previous in situ light conditions would
599 have resulted in an enhanced release of DOM, thus stimulating the activity of
600 *Gammaproteobacteria*. However, the estimated excess of pPP due to the difference
601 between the artificial and the in situ irradiances did not exhibit the same variability of
602 the LIR and the active *Gammaproteobacteria* cells. Unfortunately, we did not
603 measure the rates of photosynthetically extracellular release (PER), which has
604 sometimes been shown to increase due to abrupt changes in irradiance (Mague et al.
605 1980) and in this area may vary throughout the year independently from particulate
606 PP (Alonso-Sáez et al. 2008). Thus, a potential role of light-induced PER in light LIR
607 increase could not be ruled out.

608 Differences in DOM quality might also explain the variations in the bacterial
609 responses. In the northwestern Mediterranean, Tedetti et al. (2009) reported a
610 significant PAR-exposure enhancement of DOM bioavailability and bacterial activity
611 in spring, whereas light exposure of DOM in summer caused inhibition of LIR,
612 mainly due to UVA. We cannot test if this was the case in our study because we had
613 no data of DOM phototransformations and bioavailability; nonetheless, the facts that
614 the largest light-driven increases in LIR were observed in spring and that UVA was
615 the main inhibitor of bacterial activity support this argumentation.

616 Finally, the heterotrophic uptake of DOM reported for many algae (Amblard
617 1991) further complicates the picture. Significant numbers of the diatom *Chaetoceros*
618 spp. labeled for ^{3}H-leucine were found in March 2009 microautoradiography filters
619 (details not shown), yet no differences were visually apparent between the numbers of
620 active diatoms in the light and dark bottles. Similarly, radiolabeled *Pseudonitzschia*
621 spp. cells were often found throughout the study period, but again no obvious
622 differences were observed between the two treatments. Using a

623 microautoradiographic approach like ours, Paerl (1991) unveiled that large
624 phytoplankton (mainly diatoms) from different oceanic regions occasionally showed
625 active incorporation of organic substrates, mainly during bloom events, which seemed
626 to be the case in March 2009. Since diatoms dominate Blanes Bay phytoplankton
627 assemblages in spring (Gutiérrez-Rodríguez et al. 2011), it is also possible that they
628 contributed partially to the measured uptake of ^{3}H-leucine. Further research is needed
629 to determine the ecological relevance of this generally overlooked role of
630 phytoplankton in DOM fluxes.

631 In summary, compared to the dark incubation values, the observed light effects
632 on bacterial activity ranged from a 60% UVR-driven decrease up to an increase of
633 150% due to exposure to high-intensity artificial PAR. This variability, though, was
634 certainly not only a function of irradiance levels. It seemed to be strongly dependent
635 on how much our incubation conditions differed from the natural irradiance levels
636 previously experienced by the organisms. This underlines the importance of knowing
637 the previous light exposure history in order to accurately mimic in situ light
638 conditions, or at least to avoid misinterpretation of the results. Similarly, the bacterial
639 community structure seemed to play an important role in the observed responses,
640 since particular taxa appeared to drive some of the patterns.

641 Given the importance of bacteria for carbon and energy fluxes and nutrient
642 cycling in the pelagic ocean, the observed effects of light on bacteria may have
643 ecosystem implications such as seasonal shifts in the dominant pathways of DOM use
644 by different bacteria, accumulation of labile DOM in the surface ocean due to
645 inhibition of microbial consumption or even potential changes in community structure
646 through selection of UVR-resistant bacterial groups.

Our results may also have important methodological implications. Measurements of bacterial heterotrophic production are generally performed in the dark, which has the advantages of avoiding algal stimulation, circumventing the problem of reproducing ambient light levels, and allowing comparison among different studies. Dark measurements, however, do not capture the here described effects of sunlight on bacterial activity and may hence result in severe over- or underestimation. Incubations under realistic light conditions are recommended, particularly if the role of bacteria is to be integrated into quantitative models of carbon cycling for description on prediction purposes. How to reproduce realistic light conditions experimentally for routine measurements remains quite a challenge.

658 References

659 Aas, P., M. M. Lyons, R. Pledger, D. L. Mitchell, and W. H. Jeffrey. 1996.
660 Inhibition of bacterial activities by solar radiation in nearshore waters and the Gulf of
661 Mexico. Aquat. Microb. Ecol. **11:** 229-238.

662 Alonso-Sáez, L., and J. M. Gasol. 2007. Seasonal variations in the contributions
663 of different bacterial groups to the uptake of low-molecular-weight compounds in
664 northwestern Mediterranean coastal waters. Appl. Environ. Microbiol. **73:** 3528-3535.

665 Alonso-Sáez, L., J. M. Gasol, T. Lefort, J. Hofer, and R. Sommaruga. 2006.
666 Effect of natural sunlight on bacterial activity and differential sensitivity of natural
667 bacterioplankton groups in northwestern Mediterranean coastal waters. Appl.
668 Environ. Microbiol. **72:** 5806-5813.

669 Alonso-Sáez, L., E. Vázquez-Domínguez, C. Cardelús, J. Pinhassi, M. M. Sala,
670 I. Lekunberri, V. Balagué, M. Vila-Costa, F. Unrein, R. Massana, R. Simó and J. M.
671 Gasol. 2008. Factors controlling the year-round variability in carbon flux through
672 bacteria in a coastal marine system. Ecosystems **11:** 397-409.

673 Amblard, C. 1991. Carbon heterotrophic activity of microalgae and
674 cyanobacteria: ecological significance. Ann. Biol.-Paris **30:** 6-107.

675 Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F.
676 Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar.
677 Ecol.-Progr. Ser. **10:** 257-263.

678 Béjà, O., L. Aravind, E. V. Koonin, M. T. Suzuki, A. Hadd, L. P. Nguyen, S.
679 Jovanovich, C. M. Gates, R. A. Feldman, J. L. Spudich, E. N. Spudich, and E. F.
680 DeLong. 2000. Bacterial rhodopsin: Evidence for a new type of phototrophy in the
681 sea. Science **289:** 1902-1906.

682 Benner, R., and B. Biddanda. 1998. Photochemical transformations of surface
683 and deep marine dissolved organic matter: Effects on bacterial growth. Limnol.
684 Oceanogr. **43:** 1373-1378.
685 Bertoni, R., W. H. Jeffrey, M. Pujo-Pay, L. Oriol, P. Conan, and F. Joux. 2011.
686 Influence of water mixing on the inhibitory effect of UV radiation on primary and
687 bacterial production in Mediterranean coastal water. Aquat. Sci. **73**: 377-387.
688 Bullock, A. K., and W. H. Jeffrey. 2010. Temperature and solar radiation
689 interactions on ^{3}H-leucine incorporation by bacterioplankton in a subtropical estuary.
690 Photochem. Photobiol. **86**: 593-599.
691 Church, M. J., H. W. Ducklow, and D. A. Karl. 2004. Light dependence of
692 [^{3}H]leucine incorporation in the oligotrophic North Pacific ocean. Appl. Environ.
693 Microbiol. **70:** 4079-4087.
694 Cotner, J. B., R. H. Sada, H. Bootsma, T. Johengen, J. F. Cavaletto, and W. S.
695 Gardner. 2000. Nutrient limitation of heterotrophic bacteria in Florida Bay. Estuaries
696 **23:** 611-620.
697 Cottrell, M. T., and D. L. Kirchman. 2003. Contribution of major bacterial
698 groups to bacterial biomass production (thymidine and leucine incorporation) in the
699 Delaware estuary. Limnol. Oceanogr. **48:** 168-178.
700 Daims, H., A. Bruhl, R. Amann, K. H. Schleifer, and M. Wagner. 1999. The
701 domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*:
702 Development and evaluation of a more comprehensive probe set. Syst. Appl.
703 Microbiol. **22:** 434-444.
704 Eilers, H., J. Pernthaler, and R. Amann. 2000*a*. Succession of pelagic marine
705 bacteria during enrichment: a close look at cultivation-induced shifts. Appl. Environ.
706 Microbiol. **66:** 4634-4640.

Eilers, H., J. Pernthaler, F. O. Glockner, and R. Amann. 2000*b*. Culturability and in situ abundance of pelagic bacteria from the North Sea. Appl. Environ. Microbiol. **66:** 3044-3051.

Eilers, H., J. Pernthaler, J. Peplies, F. O. Glockner, G. Gerdts, and R. Amann. 2001. Isolation of novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. Appl. Environ. Microbiol. **67:** 5134-5142.

Friedberg, E. C., G. C. Walker, and W. Siede. 1995. DNA repair and mutagenesis. ASM Press.

Fuchs, B. M., S. Spring, H. Teeling, C. Quast, J. Wulf, M. Schattenhofer, S. Yan, S. Ferriera, J. Johnson, F. O. Glöckner, and R. Amann. 2007. Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. P. Natl. Acad. Sci. USA **104:** 2891-2896.

Gasol, J. M., and P. A. del Giorgio. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci. Mar. **64:** 197-224.

Gómez-Consarnau, L., J. M. González, M. Coll-Lladó, P. Gourdon, T. Pascher, R. Neutze, C. Pedrós-Alió, and J. Pinhassi. 2007. Light stimulates growth of proteorhodopsin-containing marine *Flavobacteria*. Nature **445:** 210-213.

Gutiérrez-Rodríguez, A., M. Latasa, R. Scharek, R. Massana, G. Vila, and J. M. Gasol. 2011. Growth and grazing rate dynamics of major phytoplankton groups in an oligotrophic coastal site. Estuar. Coast. Shelf S **95**: 77-87.

Harrison, A. P. 1967. Harmful effects of light, with some comparisons with other adverse physical agents. Annu. Rev. Microbiol. **21**:143–156.

730 Herndl, G. J., G. Müller-Niklas, and J. Frick. 1993. Major role of ultraviolet B
731 in controlling bacterioplankton growth in the surface layer of the ocean. Nature **361:**
732 717-719.
733 Huot, Y., W. H. Jeffrey, R. F. Davis, and J. J. Cullen. 2000. Damage to DNA in
734 bacterioplankton: A model of damage by ultraviolet radiation and its repair as
735 influenced by vertical mixing. Photochem. Photobiol. **72:** 62-74.
736 Kaiser, E., and G. J. Herndl. 1997. Rapid recovery of marine bacterioplankton
737 activity after inhibition by UV radiation in coastal waters. Appl. Environ. Microbiol.
738 **63:** 4026-4031.
739 Kirchman, D., E. Knees, and R. Hodson. 1985. Leucine incorporation and its
740 potential as a measure of protein-synthesis by bacteria in natural aquatic systems.
741 Appl. Environ. Microbiol. **49:** 599-607.
742 Kolber, Z. S., C. L. Van Dover, R. A. Niederman, and P. G. Falkowski. 2000.
743 Bacterial photosynthesis in surface waters of the open ocean. Nature **407:** 177-179.
744 Llabrés, M., S. Agustí, P. Alonso-Laita, and G. J. Herndl. 2010. *Synechococcus*
745 and *Prochlorococcus* cell death induced by UV radiation and the penetration of lethal
746 UVR in the Mediterranean Sea. Mar. Ecol.-Progr. Ser. **399:** 27-37.
747 Mague, T. H., E. Friberg, D. J. Hughes, and I. Morris. 1980. Extracellular
748 release of carbon by marine phytoplankton: A physiological approach. Limnol.
749 Oceanogr. **25:** 262-279.
750 Manz, W., R. Amann, W. Ludwig, M. Vancanneyt, and K. H. Schleifer. 1992.
751 Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria*:
752 problems and solutions. Syst. Appl. Microbiol. **15:** 593-600.
753 Manz, W., R. Amann, W. Ludwig, M. Vancanneyt, and H. Schleifer. 1996.
754 Application of a suite of 16S rRNA-specific oligonucleotide probes designed to

755 investigate bacteria of the phylum *Cytophaga–Flavobacter–Bacteroides* in the natural
756 environment. Microbiology **142**: 1097-1106.
757 Marie, D., C. Brussard, F. Partensky, and D. Vaulot. 1999 Flow cytometric
758 analysis of phytoplankton, bacteria and viruses, p. 11.11.1-11.11.15. *In* J. P.
759 Robinson, Z. Darzynkiewicz, P. N. Dean, A. Orfao, P. S. Rabinovitch and C. C.
760 Stewart [eds.], Current protocols in cytometry. John Wiley and Sons.
761 Mary, I., G. A. Tarran, P. E. Warwick, M. J. Terry, D. J. Scanlan, P. H. Burkill,
762 and M. V. Zubkov. 2008. Light enhanced amino acid uptake by dominant
763 bacterioplankton groups in surface waters of the Atlantic Ocean. FEMS Microbiol.
764 Ecol. **63:** 36-45.
765 Michelou, V. K., M. T. Cottrell, and D. L. Kirchman. 2007. Light-stimulated
766 bacterial production and amino acid assimilation by cyanobacteria and other microbes
767 in the North Atlantic Ocean. Appl. Environ. Microbiol. **73:** 5539-5546.
768 Morán, X. A. G., R. Massana, and J. M. Gasol. 2001. Light conditions affect the
769 measurement of oceanic bacterial production via leucine uptake. Appl. Environ.
770 Microbiol. **67:** 3795-3801.
771 Morris, R. M., M. S. Rappé, S. A. Connon, K. L.Vergin, W. A. Siebold, C. A.
772 Carlson, S. J. Giovannoni. 2002. SAR11 clade dominates ocean surface
773 bacterioplankton communities. Nature **420**: 806-810.
774 Nübel, U., F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S
775 rRNA genes from cyanobacteria. Appl. Environ. Microbiol. **63:** 3327-3332.
776 Obernosterer, I., B. Reitner, and G. J. Herndl. 1999. Contrasting effects of solar
777 radiation on dissolved organic matter and its bioavailability to marine
778 bacterioplankton. Limnol. Oceanogr. **44:** 1645-1654.

779 Paerl, H. W. 1991. Ecophysiological and trophic implications of light-
780 stimulated amino acid utilization in marine picoplankton. Appl. Environ. Microbiol.
781 **57:** 473-479.
782 Pakulski, J. D., P. Aas, W. Jeffrey, M. Lyons, L. Von Waasenbergen, D.
783 Mitchell, and R. Coffin. 1998. Influence of light on bacterioplankton production and
784 respiration in a subtropical coral reef. Aquat. Microb. Ecol. **14**: 137-148.
785 Pakulski, J. D., A. Baldwin, A. L. Dean, S. Durkin, D. Karentz, C. A. Kelley, K.
786 Scott, H. J. Spero, S. W. Wilhelm, R. Amin, and W. H. Jeffrey. 2007. Responses of
787 heterotrophic bacteria to solar irradiance in the eastern Pacific Ocean. Aquat. Microb.
788 Ecol. **47**: 153-162.
789 Pausz, C., and G. J. Herndl. 2002. Role of nitrogen versus phosphorus
790 availability on the effect of UV radiation on bacterioplankton and their recovery from
791 previous UV stress. Aquat. Microb. Ecol. **29:** 89-95.
792 Pernthaler, A., J. Pernthaler, and R. Amann. 2002. Fluorescence in situ
793 hybridization and catalyzed reporter deposition for the identification of marine
794 bacteria. Appl. Environ. Microbiol. **68:** 3094-3101.
795 Pinhassi, J., L. Gómez-Consarnau, L. Alonso-Sáez, M. M. Sala, M. Vidal, C.
796 Pedrós-Alió, and J. M. Gasol. 2006. Seasonal changes in bacterioplankton nutrient
797 limitation and their effects on bacterial community composition in the NW
798 Mediterranean Sea. Aquat. Microb. Ecol. **44**: 241-252.
799 Ruiz-González, C., T. Lefort, M. Galí, M. M. Sala, R. Sommaruga, R. Simó,
800 and J. M. Gasol. 2012. Seasonal patterns in the sunlight sensitivity of
801 bacterioplankton from Mediterranean surface coastal waters. FEMS Microb. Ecol. **79**:
802 661-674.

803 Schauer, M., V. Balagué, C. Pedrós-Alió, and R. Massana. 2003. Seasonal
804 changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic
805 system. Aquat. Microb. Ecol. **31:** 163-174.
806 Smith, D., and F. Azam. 1992. A simple, economical method for measuring
807 bacteria protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food
808 Webs **6:** 107-114.
809 Sommaruga, R., J. S. Hofer, L. Alonso-Sáez, and J. A. Gasol. 2005. Differential
810 sunlight sensitivity of picophytoplankton from surface Mediterranean coastal waters.
811 Appl. Environ. Microbiol. **71:** 2154-2157.
812 Sommaruga, R., I. Obernosterer, G. J. Herndl, and R. Psenner. 1997. Inhibitory
813 effect of solar radiation on thymidine and leucine incorporation by freshwater and
814 marine bacterioplankton. Appl. Environ. Microbiol. **63:** 4178-4184.
815 Steeman-Nielsen, E. 1952. The use of radioactive carbon for measuring organic
816 production in the sea. J. Cons. Int. Explor. Mer. **18**: 117-140
817 Straza, T. R. A., and D. L. Kirchman. 2011. Single-cell response of bacterial
818 groups to light and other environmental factors in the Delaware Bay, USA. Aquat.
819 Microb. Ecol. **62:** 267-277.
820 Tedetti, M., F. Joux, B. Charriere, K. Mopper, and R. Sempéré. 2009.
821 Contrasting effects of solar radiation and nitrates on the bioavailability of dissolved
822 organic matter to marine bacteria. J. Photoch. Photobio.-B **201:** 243-247.
823 Vallina, S. M., and R. Simó. 2007. Strong relationship between DMS and the
824 solar radiation dose over the global surface ocean. Science **315:** 506-508.
825 Vaughan, P., A. Bullock, F. Joux, and W. H. Jeffrey. 2010. The effects of solar
826 radiation on the stability of ^{3}H-thymidine and ^{3}H-leucine during bacterioplankton
827 production measurements. Limnol. Oceanogr.: Meth. **8:** 562-566.

828 Visser, P. M., E. Snelder, A. J. Kop, P. Boelen, A. G. J. Buma, and F. C. Van
829 Duyl. 1999. Effects of UV radiation on DNA photodamage and production in
830 bacterioplankton in the coastal Caribbean Sea. Aquat. Microb. Ecol. **20**: 49-58.
831 White, P. A., J. Kalff, J. B. Rasmussen, and J. M. Gasol. 1991. The effect of
832 temperature and algal biomass on bacterial production and specific growth-rate in
833 fresh-water and marine habitats. Microb. Ecol. **21:** 99-118.

Tables

Table 1. Seasonally and annually averaged LIR measured under different light conditions (PAR, PAR+UVA, and PAR+UVR) presented as percentages of the dark controls. Last column: Seasonally averaged LIR measured under artificial PAR light (art. light) as percentage of the dark control. Values are average ± standard errors of sampling dates (*n* ranges from 4 to 25). Different superscript letters indicate significant differences among seasonal averages measured under different light conditions (Tukey's test, $p < 0.05$).

	In situ light conditions			Dark vs. art. light
	Average PAR LIR (% of dark control)	Average UVA LIR (% of dark control)	Average UVR LIR (% of dark control)	Average light LIR (% of dark control)
Winter	93 ± 3^{a}	86 ± 4^{a}	90 ± 5^{a}	119 ± 9^{a}
Spring	105 ± 10^{a}	86 ± 11^{a}	75 ± 15^{a}	151 ± 13^{a}
Summer	97 ± 6^{a}	77 ± 8^{a}	76 ± 7^{a}	96 ± 10^{b}
Autumn	100 ± 0^{a}	100 ± 0^{a}	88 ± 7^{a}	114 ± 16^{ab}
Annual average	99 ± 4	85 ± 4	81 ± 5	120 ± 6

Table 2. Linear correlation analysis between inhibition of bacterial heterotrophic activity due to exposure to the different fractions of the spectrum and the PAR and UVR doses received by samples during the experiments. Statistically significant results ($p < 0.05$) are marked in bold.

Inhibition vs. dose	*r*	*p*-value	*n*
% of dark value			
PAR inhibition vs. PAR dose	-0.188	0.379	24
% of PAR value			
UVA inhibition vs. UVA dose	-0.532	**0.007**	24
UVR inhibition vs. UVR dose	-0.514	**0.010**	24
% of UVA value			
UVB inhibition vs. UVB dose	-0.035	0.872	24

Figure legends

Fig. 1. (A) Temporal dynamics of water column temperature and the depths of the mixing (mL) and mixed (ML) layers (*see* Methods for calculations). (B) Surface water temperature (Temp), chlorophyll *a* (Chl *a*) and 'seasonal' PAR irradiance (i.e., mean irradiance within the ML of the 3 days prior to sampling, *see* text). (C) In situ particulate primary production (pPP) and leucine (Leu) incorporation rates (LIR) over the period January 2008-April 2010 in Blanes Bay.

Fig. 2. (A) Seasonal variability in the abundance (abund.) of heterotrophic bacteria (Bac), and the two cyanobacterial genus (Cya) *Synechococcus* (Syn) and *Prochlorococcus* (Proc). Percentages of bacterial groups detected by CARD-FISH with HRP probes specific for: (B) *Bacteroidetes* (Bcdt), SAR11, and *Roseobacter* (Ros); (C) *Gammaproteobacteria* (Gam) and NOR5.

Fig. 3. Bacterial heterotrophic production measured under PAR (black bars), PAR+UVA (grey bars), and PAR+UVR (white bars) expressed as percentage of the dark control.

Fig. 4. (A) Relative contribution of UVA (grey bars) and UVB (black bars) to total inhibition (inhib.) of bacterial production with respect to the PAR treatment. Variation of the relative contribution of UVB to total UVR-inhibition with respect to (B) the ratio UVA:UVB experienced by samples during incubations or (C) to the in situ UVA:UVB ratio (calculated from the in situ UV irradiance measured during the 4 hours of maximum radiation of the previous day, *see* Methods). 'ns' indicates dates were there were no significant differences between PAR and UVA or UVR treatments (Tukey's test, $p < 0.05$).

Fig. 5. Seasonal variability in bacterial heterotrophic production measured in the dark and under a fixed light source (ca. 1500 μmol photons m^{-2} s^{-1}). Asterisks indicate significant differences between dark and light incubations (Tukey's test, $p < 0.05$).

Fig. 6. Percentage of positively hybridized cells with probes for (A) *Gammaproteobacteria*, (B) *Roseobacter*, and (C) *Cyanobacteria* taking up ^{3}H-leucine (average ± standard deviation) as measured by MAR-CARD-FISH after exposure to a fixed light (ca. 1500 μmol photons m^{-2} s^{-1}, open bars) or kept in the dark (black bars). Asterisks indicate significant differences between light and dark treatments (Tukey's test, $p < 0.05$). Only samples where a clear light-driven response in LIR was apparent were analyzed.

Fig. 7. (A) Comparison between the variation in LIR measured in the light (scaled to the dark control value) and the light-driven increase in active *Gammaproteobacteria* (Gam, expressed as % of the dark numbers). Relationships between the percentages of active *Gammaproteobacteria* and (B) bulk LIR or (C) percentages of active *Eubacteria* (Eub) incubated in the dark (black dots, solid line) or under an artificial light source (open dots, dotted line).

834