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6   **Annual variability in light modulation of bacterial heterotrophic activity in**

7   **surface northwestern Mediterranean waters**

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18   Condensed running head: Seasonal effects of sunlight on bacteria
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32 Abstract

33   The effect of photosynthetically available radiation (PAR, 400-700 nm) and

34 ultraviolet radiation (UVR, 280-400 nm) on marine bacterial heterotrophic activity

35 was assessed monthly throughout a seasonal cycle in Blanes Bay (northwestern

36 Mediterranean Sea). Seawater samples amended with ^{3}H-leucine were exposed to

37 solar radiation under three radiation treatments: PAR + UVR (280-700 nm), PAR +

38 UVA (320-700 nm), and PAR only. Parallel reference incubations in the dark and

39 under a fixed artificial light source (PAR only) were also performed. Exposure to high

40 UVR doses caused strong inhibition of ^{3}H-leucine incorporation rates (LIR), whereas

41 natural PAR doses did not cause overall significant effects. Within UVR, UVA

42 radiation accounted for most of the reduction in LIR, and this effect was modulated

43 by the proportionality of the experimental light to the previous light exposure history

44 of the samples. Constant (artificial) PAR-only exposure led to a general but

45 seasonally variable increase in bacterial heterotrophic production compared to the

46 dark controls, with large increases in spring and lower changes during summer. This

47 pattern was likely caused by the stimulation of the bacterial group

48 *Gammaproteobacteria*, which showed higher numbers of cells active in ^{3}H-leucine

49 uptake after light exposure. Again, the previous light history of the samples seemed to

50 partly explain the measured effects. Overall, our results show variable responses of

51 bacterial activities to light manipulations, depending on seasonally changing light

52 conditions and communities, and stress the importance of realistic simulation of light

53 exposure conditions for ecosystem-relevant photobiological studies with microbial

54 plankton.

55

Introduction

56   Introduction

57   Abundance and activity of marine heterotrophic bacteria are influenced by

58   parameters that fluctuate at different time scales. Temperature, nutrient concentration,

59   dissolved organic matter (DOM) availability, and the composition of the microbial

60   community have been regarded among the main factors controlling DOM

61   consumption by bacteria (White et al. 1991; Cotner et al. 2000; Cottrell and Kirchman

62   2003). However, the relative importance of solar radiation as a modulator of bacterial

63   production across spatial or seasonal patterns has received little attention. In surface

64   waters, bacteria are exposed to damaging solar ultraviolet radiation (UVR, 280-400

65   nm) that can cause inhibition of metabolic activities such as synthesis of protein and

66   deoxyribonucleic acid (DNA) (Herndl et al. 1993; Sommaruga et al. 1997), oxygen

67   consumption (Pakulski et al. 1998), or amino acid and adenosine triphosphate (ATP)

68   uptake (Alonso-Sáez et al. 2006). Nevertheless, there is also evidence of a positive

69   effect of UVA (320-400 nm) and photosynthetically available radiation (PAR, 400-

70   700 nm) on bacterial activity due to photoenzymatic repair (Kaiser and Herndl 1997),

71   to the ability of some bacteria to derive energy from light using bacteriochlorophyll *a*

72   or proteorhodopsin (Béjà et al. 2000; Kolber et al. 2000), or due to the reported light-

73   stimulation of cyanobacterial uptake of amino acids and related compounds (Church

74   et al. 2004; Michelou et al. 2007; Mary et al. 2008). In addition, UVR can photolyze

75   some recalcitrant DOM into more readily utilizable forms, making it more available

76   to heterotrophs and thus enhancing their activity or, by contrast, initially labile DOM

77   can be rendered more recalcitrant upon UVR exposure (Benner and Biddanda 1998;

78   Obernosterer et al. 1999). Finally, heterotrophic bacteria might react to increased or

79   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

105 variability. Since both, light levels and bacterial taxonomic composition change

106 through the seasonal cycle, the differential responses of bacterial communities to

107 sunlight are essential to understand the role of solar radiation as a modulator of

108 organic matter fluxes in marine ecosystems. The results obtained have potential

109 implications for assessing the suitability of standard dark protocols for a realistic

110 measurement of heterotrophic bacterial activity.

111

112 Methods

113 *Sampling and basic parameters*. A series of monthly experiments were carried

114 out with waters from a shallow (≈20 m depth) coastal station (the Blanes Bay

115 Microbial Observatory, BBMO, northwestern Mediterranean Sea, http://

116 www.icm.csic.es/bio/projects/icmicrobis/bbmo) between January 2008 and April

117 2010. Surface waters (0.5 m depth) were sampled at about 9:00 a.m. at 800 m

118 offshore (41º 40'N, 2º 48'E), filtered through a 200 μm mesh and transported within

119 an hour to the lab under dim light. Seawater temperature was measured in situ with a

120 SAIV A-S 204 conductivity–temperature–depth (CTD) probe and underwater PAR

121 and UVR profiles were obtained with a profiling ultraviolet (PUV) 2500 radiometer

122 (Biospherical Instruments). Chlorophyll *a* (Chl *a*) concentration was determined by

123 filtering 150 mL of seawater on GF/F filters (Whatman), extracting the pigment in

124 acetone (90% v:v) in the dark at 4ºC for 24 h, and measuring fluorescence with a

125 Turner Designs fluorometer.

126 *Experimental setup.* Different experiments were performed during the study

127 period (*see* Fig. 1). From 15 January 2008 to 14 September 2009 incubations for

128 bacterial heterotrophic activity measurements (hereafter leucine incorporation rates,

129 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

155 dark control (bottle wrapped with aluminum foil) were filled with seawater and

156 inoculated with 10 μCi NaH^{14}CO$_3$. The incubation was carried out in a water bath at

157 in situ temperature for 2 hours in a gradient of light irradiance (ca. 10-1500 μmol

158 photons m^{-2} s^{-1}). Circulating water connected to a water bath maintained the

159 temperature. Light was measured with a small size spherical light meter (Illuminova

160 AB). After the incubation, the samples were filtered at low vacuum pressure through

161 cellulose ester filters (Millipore 0.22 μm), and the filters were subsequently exposed

162 overnight to concentrated HCl fumes. Scintillation cocktail (4 mL Optiphase Hisafe 2)

163 was then added to each filter, and the radioactivity was measured in a Beckton-

164 Dickinson LS6000 scintillation counter. Average in situ pPP was estimated from the

165 photosynthesis-irradiance (P-E) curve and the hourly in situ PAR irradiance within

166 the 'actively mixing layer' of the 24 h prior to sampling (*see* below).

167   *Leucine incorporation rates (LIR)*. LIR was monthly estimated using the ^{3}H-

168 leucine incorporation method described by Kirchman et al. (1985). Four 1.2 mL

169 aliquots and two trichloroacetic acid (TCA)-killed controls (5% final concentration)

170 of each sample were incubated with 40 nmol L^{-1} ^{3}H-leucine for 2 h. The incubations

171 were carried out in a water bath at in situ temperature in the dark and under fixed light

172 irradiance (ca. 1500 μmol photons m^{-2} s^{-1}, approximately the surface PAR irradiance

173 of a summer day in this area). The incorporation was stopped by adding cold TCA

174 (5% final concentration) to the vials and samples were kept at -20ºC until processing

175 as described by Smith and Azam (1992). Radioactivity was then counted on a

176 Beckman scintillation counter.

177   From 15 January 2008 to 14 September 2009 LIR was also measured under

178 exposure to natural solar radiation. For that purpose, six UVR-transparent cuvettes (4

179 mL, Plastibrand) of which two were formaldehyde-killed controls (4% final

180 concentration) were amended with ^{3}H-leucine (40 nmol L^{-1} final concentration, 160

181 Ci mmol^{-1}) and incubated for 2 to 3 hours under different radiation conditions: full

182 sunlight spectrum (PAR+UVR), the full spectrum minus UVB (PAR+UVA, covered

183 with the plastic foil Mylar-D, which excludes UVB radiation), PAR only (wrapped

184 with two layers of Ultraphan URUV Farblos which removes all UVR) or darkness

185 (wrapped with aluminium foil inside a black plastic bag to avoid reflection). Cuvettes

186 were incubated at about 5 cm under the surface inside a black tank (200 L) with

187 running seawater to maintain in situ temperature. After incubation, 1.2 mL were

188 transferred from each cuvette to centrifuge tubes, then killed with 120 μL cold TCA

189 (5% final concentration) and processed as described above.

190 *Microautoradiography combined with catalyzed reporter deposition-*

191 *fluorescence in situ hybridization (MAR-CARD-FISH).* In the experiments between 17

192 March 2009 and 13 April 2010, 30 mL samples were amended with trace amounts of

193 ^{3}H-leucine (0.5 nmol L^{-1} final concentration, 160 Ci mmol^{-1}) and incubated in parallel

194 in the dark or in the light (PAR-only, ca. 1500 μmol photons m^{-2} s^{-1}) for 2 to 3 hours.

195 After exposure, samples were fixed overnight with paraformaldehyde (PFA, 1% final

196 concentration) at 4ºC in the dark and filtered on 0.2 μm polycarbonate filters (GTTP,

197 Millipore). Sections of the filters were then hybridized following the CARD-FISH

198 protocol (Pernthaler et al. 2002). A few horseradish peroxidase (HRP)-probes were

199 used in order to characterize the composition of in situ bacterial communities:

200 Gam42a that targets most *Gammaproteobacteria* (Manz et al. 1992), NOR5-730 for

201 the NOR5 clade (Eilers et al. 2000*b*), SAR11-441R for the SAR11 cluster (Morris et

202 al. 2002), Ros537 targeting the *Roseobacter* clade (Eilers et al. 2001), CF319a for

203 clades belonging to *Bacteroidetes* (Manz et al. 1996), CYA339 for the cyanobacteria

204 (Nübel et al. 1997), and Eub338-II-III for inclusion of most *Eubacteria* (Daims et al.

205 1999). The relative abundance of each group was checked by cutting smaller pieces

206 from each filter and staining them with 4,6-diamidino-2-phenylindole (DAPI, 1 μg

207 mL^{-1}). Between 500-800 DAPI-positive-cells were counted manually within a

208 minimum of 10 fields under an Olympus BX61 epifluorescence microscope.

209   For microautoradiography, we followed the protocol described in Alonso-Sáez

210 and Gasol (2007). On the basis of previous studies (Alonso-Sáez et al. 2006; Ruiz-

211 González et al. 2012) only potentially photostimulable groups (i.e.,

212 *Gammaproteobacteria,* NOR5, *Cyanobacteria*, and *Roseobacter*) were subjected to

213 microautoradiographic analysis. The optimal exposure times were determined for

214 each sampling point and ranged from 2 to 19 days. After development, the slides were

215 dried overnight, stained with DAPI and 500 to 700 hybridized cells were counted

216 manually by epifluorescence microscopy within a minimum of 10 fields.

217   *Measurement and calculation of PAR and UVR doses.* A radiometer

218 (Biospherical PUV 2500) was used in the field and also placed inside the incubation

219 tanks with the sensor covered by ca. 5 cm of water, and the downwelling cosine

220 irradiance reaching the samples was recorded at a frequency of 5 s^{-1}. The wavelengths

221 measured included 6 bands in the UVR (305, 313, 320, 340, 380 and 395 nm, in units

222 of mW cm^{-2} nm^{-1}) and one integrated band in the visible (PAR, in μmol photons cm^{-2}

223 s^{-1}). The mean spectral irradiance in the 6 UVR bands was converted to mean UVB

224 and UVA irradiance (mW cm^{-2}) by integrating over the spectrum (sum of trapezoids),

225 between 305 – 320 nm and 320 – 395 nm respectively. Finally, the mean UVB, UVA,

226 and PAR irradiance was multiplied by the duration of each experiment in order to

227 obtain the radiation dose (in kJ m^{-2} for UVB and UVA, and mol photons m^{-2} for

228 PAR).

229   The 'light' history of microbial communities, i.e., their previous UVR and PAR

230   exposure at the sampling site, was calculated as a function of spectral irradiance at the

231   water subsurface, vertical mixing depth, and underwater attenuation of solar radiation

232   (Vallina and Simó 2007). For this purpose, two distinct exposure regimes were

233   considered: 'seasonal exposure' and 'maximum daily exposure'. Seasonal exposure

234   was calculated by combining the mean irradiance of the three days prior to sampling

235   with the seasonal mixed layer depth (MLD), whereas maximum daily exposure was

236   calculated as the combination of 'actively mixing layer' depth (mLD) with average

237   irradiance at noon ± 2 hours of the previous day. Total solar irradiance (with hourly

238   resolution) was obtained from a meteorological station located 5 km southwest from

239   the BBMO sampling station (Malgrat de Mar, Catalan Meteorological Service, SCM).

240   MLD and mLD were calculated from temperature profiles obtained from CTD casts,

241   binned at 1 m intervals. MLD was defined as the depth where a jump in temperature

242   larger than 0.15 ºC was encountered relative to 1 m depth, while mLD was defined as

243   the depth showing a 0.03 ºC departure from the 1 m reference. These criteria were

244   optimized for our particular dataset, and yielded mLD or MLD estimates that were

245   consistent with the vertical profiles of other variables (M. Galí unpubl.). Diffuse

246   attenuation coefficients of downwelling radiation ($K_{d,\lambda}$) were calculated as the slope

247   of the linear regression between the natural logarithm of spectral cosine irradiance

248   ($E_{d,\lambda,z}$) and depth (z). $K_{d,320}$ and $K_{d,380}$ were chosen as representative of UVB and

249   UVA attenuation respectively, while PAR (and its corresponding $K_{d,PAR}$) was

250   originally measured in one integrated band.

251   *Statistical analyses*. The Shapiro-Wilk *W*-test for normality of data and the

252   Levene's test for homogeneity of variance were applied prior to analysis, and either

253   one-way analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis Test

254 were used to analyze statistically significant ($p < 0.05$) differences in the measured

255 variables, and post hoc analyses (Tukey's Honestly Significant Difference test) for

256 comparison among different light treatments or seasonal averages. Correlations

257 between variables were calculated using the Pearson's correlation coefficient. These

258 statistical analyses were performed using the JMP software (SAS Institute).

259

260 Results

261   *Background information.* The seasonal variation of the basic parameters in the

262 study area is shown in Fig. 1 for 2008-2010. The summer period was characterized by

263 high surface temperatures (20-25ºC), a strongly stratified water column, and low Chl

264 *a* concentrations (< 0.4 μg L^{-1}) while the opposite trend was observed in the well

265 mixed winter waters showing the lowest temperatures (~ 12ºC) and largest Chl *a*

266 peaks (up to 2 μg L^{-1}; Fig. 1A, B). In situ particulate primary production (pPP) also

267 varied seasonally, reaching higher values in late winter (> 1.5 mg C m^{-3} h^{-1} in 2008)

268 and variable peaks during spring and summer (Fig. 1C). No winter pPP peak showed

269 up in 2009, although it is possible that we missed it by missing the March sampling.

270   LIR values showed large variability among sampling dates (range 2-135 pmol

271 leucine L^{-1} h^{-1}, Fig. 1C). Maximum values tended to be observed after Chl *a* or pPP

272 peaks, higher values were generally found in summer and spring compared to autumn

273 and winter, as reflected by a positive correlation between LIR and temperature

274 (Pearson's $r = 0.65$, $n = 36$, $p < 0.0001$). Bacterial abundances ranged three-fold from

275 0.4 to 1.2 x 10^6 cells mL^{-1} and tended to be higher during the summer. *Synechococcus*

276 and *Prochlorococcus* abundances also varied seasonally (Fig. 2A), with greater

277 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   Inhibition due to UVX = $(LIR_{PAR}-LIR_{UVX})\cdot100/LIR_{PAR}$ (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

327 of each UVR fraction. The inhibition due to each fraction did not correlate to the

328 measured UVA:UVB ratio of irradiances during incubations (Fig. 4B) but to the ratio

329 estimated at the sampling site (calculated from the in situ UV irradiance measured

330 during the 4 hours of maximum insolation of the previous day, *see* Methods); thus,

331 inhibition by experimental UVB was found to increase with the in situ UVA:UVB

332 ratios ($r = 0.60$, $p < 0.003$, $n = 25$, Fig. 4C). In other words, the lower the UVB doses

333 relative to UVA received by the plankton community prior to sampling, the higher the

334 inhibition caused by UVB during the incubations. This was partially explained by the

335 difference between the in situ UVA:UVB ratio and the one experienced by samples

336 during our incubations. Experimental overexposure to UVB relative to the conditions

337 microbial plankton was acclimated to would cause a larger deleterious effect of UVB.

338   *Seasonal responses of bacteria to constant PAR exposure.* An artificial light

339 source (PAR only) was further used for comparison of the response of bacteria to the

340 same light conditions across seasons. In general, LIR measured in the light was

341 stimulated with respect to the dark control (range 20% to 150% increase), although

342 also inhibition (range 20% to 65% decrease) or no effect at all were occasionally

343 observed (Fig. 5). Again, such variability in the responses was not correlated with

344 other measured parameters such as water transparency, salinity, Chl *a*, DOC and

345 nutrient concentrations, primary productivity or the composition of the bacterial

346 community (*n* values from 17 to 36, *p* values from 0.057 to 0.983, details not shown).

347 However, significant relationships were found between the increase in the light-

348 measured LIR relative to dark and the water temperature (Pearson's $r = -0.41$, $p <$

349 0.02, $n = 35$), the MLD ($r = 0.41$, $p < 0.02$, $n = 36$), and the maximum daily PAR

350 irradiance (mean irradiance at noon ± 2h of the previous day within the mLD, *see*

351 Methods, $r = -0.33$, $p < 0.05$, $n = 37$). These observations indicate that the light-LIR

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

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

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

377 A good positive correlation was found between the light-driven increases in the

378 number of active *Gammaproteobacteria* and the increase in bulk LIR caused by light

379 ($r = 0.73$, $p < 0.001$, $n = 17$, Fig. 7A). Moreover, significant correlations were found

380 between the number of active *Gammaproteobacteria* and bulk LIR ($r = 0.59$, $p <$

381 0.02, $n = 17$) and between active *Gammaproteobacteria* and active *Eubacteria* ($r =$

382 0.66, $p < 0.003$, $n = 17$) measured in the dark (Fig. 7B, C). These correlations were

383 better in the light ($r = 0.67$, $p < 0.004$, $n = 17$ and $r = 0.82$, $p < 0.0001$, $n = 17$,

384 respectively, Figs. 7B, C). Interestingly, *Gammaproteobacteria* abundances seemed to

385 be explained in part by the underwater ambient PAR levels ($r = 0.57$, $p < 0.0005$, $n =$

386 29), showing greater abundances in more illuminated waters, while no correlation was

387 apparent with temperature, Chl *a* or primary production data. As with bulk LIR,

388 furthermore, the magnitude of light-driven increases in the number of active

389 *Gammaproteobacteria* was negatively correlated with the maximum daily PAR

390 irradiance ($r = -0.49$, $p < 0.05$, $n = 17$).

391   No other bacterial group seemed to explain the observed bulk light-driven

392 differences due to lightin bulk LIR. Very high percentages of *Roseobacter* were active

393 in ^{3}H-leucine uptake throughout the year (> 95% of labeled cells) but no significant

394 differences were commonly found between dark and light treatments (only in 3 out of

395 9 sampling dates). The fraction of active *Cyanobacteria* rarely exceeded 10%, and

396 although some differences were detected between treatments (up to two fold

397 increases), their low activity and different stimulation patterns excluded them as

398 candidates responsible for the light enhancement of bulk LIR.

399

400

Discussion

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

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

424   With this approach, and as reported by others (Aas et al. 1996; Sommaruga et

425   al. 1997), we found a significant UVR-driven decrease in LIR with respect to the

426   values measured in dark incubations. In general, we found low or no inhibition when

427   irradiance values were low (winter and autumn) and greater inhibition (up to 60%)

428   under high irradiances (spring and summer). This suggests that, under certain

429   circumstances, standard measurements in the dark may severely overestimate

430   bacterial heterotrophic activity. This would be particularly dramatic in highly

431   illuminated waters. Annually averaged, the LIR measured under full sunlight

432   conditions was 20% lower than that measured in the dark.

433   In contrast, exposure to natural PAR only did not generally affect the measured

434   LIR regardless of the irradiance, except for a few cases where a slight decrease

435   (mainly in spring and summer) or an occasional increase were observed. Other studies

436   have also observed different degrees of inhibition (Aas et al. 1996; Sommaruga et al.

437   1997; Morán et al. 2001) or stimulation (Aas et al. 1996; Church et al. 2004; Pakulski

438   et al. 2007) when measuring LIR under in situ PAR irradiance, effects that have been

439   attributed to photodynamic processes (Harrison 1967). Morán et al. (2001), though,

440   suggested that PAR-mediated decrease in samples from the same area of our study

441   was due to enhanced bacterial activity in the dark rather than inhibition by light, yet

442   the exact mechanisms were not identified.

443   An interesting aspect of the UVR effects refers to the relative contribution of

444   UVA and UVB to the total LIR inhibition. In our experiments the contribution of

445   UVA was generally higher than that of UVB (Fig. 4), as also seen by other authors

446   (Sommaruga et al. 1997; Visser et al. 1999). This might be attributed to the fact that

447   even though UVB is more energetic than UVA, the amount of UVA energy that

448   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

474   account the often overlooked light-exposure history of samples (which is in turn

475   dependant on the mixing regime), and further highlights the difficulty of mimicking

476   natural underwater light conditions.

477   *Seasonal responses of bacteria under invariable PAR conditions.* In addition to

478   the intensity and spectral characteristics of sunlight, the response of bacteria to solar

479   radiation has been shown to depend on, or interact with, many other environmental or

480   biological factors, such as temperature (Bullock and Jeffrey 2010), nutrient status

481   (Pausz and Herndl 2002), or the specific sensitivities of different bacteria (Alonso-

482   Sáez et al. 2006). Hence, in order to search for other potential causes of seasonality in

483   the bacterial responses, we excluded the effect of experimental light variability by

484   incubating a parallel set of samples under a fixed artificial PAR source (equivalent to

485   the annual mean surface irradiance in this area). Unlike natural PAR exposure, this

486   indoor approach led to a general stimulation of activity relative to the dark control.

487   Unexpectedly, though, such an effect was more pronounced in spring than in summer,

488   when no changes or even inhibition occurred. This seasonality in the light effects of

489   different samples receiving exactly the same irradiance discarded an exclusive

490   dependence of bacterial responses on light intensity and suggested that causes other

491   than irradiance influenced the observed variability. During our annual cycle neither

492   pPP, DOC, Chl *a* or nutrient concentration, nor the composition of the bacterial

493   communities (in terms of group abundances) seemed to influence the light-driven

494   responses of LIR to natural sunlight. In a recent study conducted also in the BBMO

495   we showed that responses to natural solar radiation at the community level are

496   influenced by taxon-specific sensitivities to sunlight and their relative contribution to

497   total activity (Ruiz-González et al. 2012). Hence, we wanted to check whether the

498   observed seasonality in the bacterial responses to a fixed irradiance was also

499   influenced by the activity of some particular groups.

500   *Role of bacterial community composition in bulk responses to sunlight.* Single-

501   cell activity analyses were performed in order to search for bacterial groups

502   responsible for the observed light driven changes in bulk activity. Several studies that

503   also reported similar PAR-driven stimulation of bulk LIR attributed it to light-

504   enhanced amino acid uptake by cyanobacteria (Church et al. 2004; Michelou et al.

505   2007; Mary et al. 2008). Our MAR-CARD-FISH data (Fig. 6C), conversely,

506   discarded this group as the main driver of the observed responses due to the low

507   numbers of active cells and the lack of significant light-enhancement in these numbers

508   concomitant with LIR increases. However, bacterial groups other than cyanobacteria

509   have also been shown to augment their activity under the light (Alonso-Sáez et al.

510   2006; Mary et al. 2008; Straza and Kirchman 2011), in what has been attributed to the

511   photoheterotrophic capabilities of some phylotypes containing light-harvesting

512   proteorhodopsins or bacteriochlorophyll *a* (Béjà et al. 2000; Kolber et al. 2000). With

513   the aim to check whether photoheterotrophic bacteria were responsible for the

514   observed increases in LIR, *Gammaproteobacteria*, *Roseobacter* and the NOR5 group

515   were probed on the basis of previously published data indicating that these taxa

516   occasionally presented light-enhanced activity in this area (Alonso-Sáez et al. 2006;

517   Ruiz-González et al. 2012). SAR11 and *Bacteroidetes* were not considered due to the

518   reported negative sensitivity to light of the former and the lack of responses to

519   sunlight and low contribution to total active cells of the latter (Alonso-Sáez et al.

520   2006; Ruiz-González et al. 2012).

521   While the great majority of *Roseobacter* cells were active in both light and dark

522   treatments throughout the year, increases in the number of active cells due to light

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523 were often negligible, so it is unlikely that they accounted for the observed light-

524 stimulated LIR. Conversely, the numbers of active *Gammaproteobacteria* cells

525 significantly increased in the light during most of the study period, showing a

526 stimulation pattern very similar to that of bulk LIR. Even the observed LIR decrease

527 in August was mirrored by a decrease in active *Gammaproteobacteria*, thus pointing

528 to a major role of this group in the community response to light. This role,

529 remarkable because *Gammaproteobacteria* were not the most abundant prokaryotes,

530 was further supported by the good positive correlations observed between the number

531 of active cells within this group, the number of active *Eubacteria* cells, and LIR.

532 Some members of the *Gammaproteobacteria* maintain large numbers of ribosomes

533 during extended periods of non-growth, which allow them to rapidly initiate growth

534 upon changing environmental conditions (Eilers et al. 2000*a*). Thus, they might also

535 take quick advantage of changes in light conditions and respond faster than other

536 groups.

537   Interestingly, the *Gammaproteobacteria* showed preference for highly

538 illuminated environments as seen by the good correlation between the seasonal

539 irradiance and cell abundances, not seen with other variables. This is in accordance

540 with their apparent ability to benefit from light and it is further supported by the

541 relatively high resistance to UVR reported for these bacteria in the study area

542 (Alonso-Sáez et al. 2006; Ruiz-González et al. 2012).

543   Within *Gammaproteobacteria*, the NOR5 clade was analyzed in detail because

544 one sequenced member of the group had shown capability for aerobic anoxygenic

545 photosynthesis (Fuchs et al. 2007). Unfortunately, their very low abundances

546 throughout most of the year prevented an accurate quantification of active cells and

547 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

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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.

647   Our results may also have important methodological implications.

648   Measurements of bacterial heterotrophic production are generally performed in the

649   dark, which has the advantages of avoiding algal stimulation, circumventing the

650   problem of reproducing ambient light levels, and allowing comparison among

651   different studies. Dark measurements, however, do not capture the here described

652   effects of sunlight on bacterial activity and may hence result in severe over- or

653   underestimation. Incubations under realistic light conditions are recommended,

654   particularly if the role of bacteria is to be integrated into quantitative models of carbon

655   cycling for description on prediction purposes. How to reproduce realistic light

656   conditions experimentally for routine measurements remains quite a challenge.

657

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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).

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