Synergistic effects of UVR and simulated stratification on commensalistic phytoplankton-bacteria relationship in two optically-contrasting oligotrophic Mediterranean lakes

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Abstract

An indirect effect of global warming is a reduction in the depth of the upper mixed layer (UML) causing organisms to be exposed to higher levels of ultraviolet (UVR, 280-400 nm) and photosynthetically active radiation (PAR, 400-700 nm). This can affect primary and bacterial production as well as the commensalistic phytoplankton-bacteria relationship. The combined effects of UVR and reduction in the depth of the UML were assessed on variables related to the metabolism of phytoplankton and bacteria, during in situ experiments performed with natural pico- and nanoplankton communities from two oligotrophic lakes with contrasting UVR-transparency (high-UVR versus low-UVR waters) of southern Spain. The negative UVR effects on epilimnetic primary production (PP) and on heterotrophic bacterial production (HBP), intensified under increased stratification, were higher in the low-UVR than in the high-UVR lake, and stronger on the phytoplanktonic than on the heterotrophic bacterial communities. Under UVR and increased stratification, the commensalistic phytoplankton-bacteria relationship was strengthened in the high-UVR lake where excretion of organic carbon **(**EOC) rates exceeded the bacterial carbon demand (BCD; i.e., %BCD:EOC ratio <100). This did not occur in the low-UVR lake (i.e., %BCD:EOC ratio >100). The greater UVR damage to phytoplankton and bacteria and the weakening of their commensalistic interaction found in the low-UVR lake indicates that these ecosystems would be especially vulnerable to UVR and increased stratification, as stressors related to global climate change. Thus, our findings may have important implications for the carbon cycle in oligotrophic lakes of the Mediterranean region.

Keywords: UVR, Stratification, phytoplankton, bacteria, metabolism

1 Introduction

Rising levels of greenhouse gases (mainly CO_2), attributed to human activities, have led to an increase of 0.56°C in the Earth's surface temperature over the past 150 years (IPCC, 2013). Model predictions indicate greater temperature increases, ranging from 1.5°C to 6.4°C by the end of the century. Major changes in precipitation have accompanied these temperature variations and are expected to become more pronounced (IPCC, 2013). These climate changes affect aquatic ecosystems by increasing water temperature, altering mixing regimes, shortening the thaw time and the duration of ice cover, and/or strengthening water-column stratification (de Senerpont Domis et al., 2013). These alterations in physical conditions have different effects on primary and bacterial production, plankton growth, nutrient supply, and trophic interactions, among other ecological processes (de Senerpont Domis et al., 2013). In addition, variations in stratification patterns are known to strongly affect biogeochemical cycles (van de Waal et al., 2009).

Higher temperatures in the upper layers of freshwater bodies increase density differences between the upper mixed layer (UML) or epilimnion, and deeper waters, augmenting the vertical temperature gradient, and thus the stratification. This process has contrasting effects on nutrient and light availability for organisms' growth. By one hand, stratification reduces the flow of nutrients from deep and nutrient-rich areas into the UML, limiting nutrient availability for growth (Huisman et al., 2006). On the other hand, stratification traps phytoplankton populations in surface layers, increasing the light available for growth, but also exposing them to higher levels of ultraviolet radiation (UVR, 280-400 nm). In this regard, it has been widely reported that greater exposure to UVR exerts an inhibitory effect on autotrophic and heterotrophic organisms (Häder et al., 2011), and that UV-B (280-315 nm) in particular, harms primary and bacterial production (Carrillo et al., 2002), enzymatic activity (Korbee et al., 2012), and cell viability (Helbling et al., 1995), among other effects. However, it has been also reported (Aas et al., 1996; Medina-Sánchez et al., 2002; Gao et al., 2007) that UVR does not produce negative effects and it can even stimulate bacterial production and photosynthetic activity. These opposite effects may be attributable to the high acclimation capacity of organisms in high-UVR ecosystems (Medina-Sánchez et al., 2002; Ruiz-González et al., 2013) or to differences in physical-chemical factors (e.g. temperature or nutrient content) among ecosystems (Harrison and Smith, 2009).

With respect to physical factors, it has been experimentally demonstrated (Helbling et al., 1994) that vertical mixing can alter UVR-induced effects on planktonic organisms by generating a regime of fluctuating irradiance, with high values near the surface and low values at the bottom of the UML. The depth of the UML also influences the mean UVR and PAR irradiance received by organisms and the duration of their residence in the photoactive zone (Neale et al., 2003). Studies on the interactive effects of UVR and vertical mixing on phytoplankton (Helbling et al., 1995; Neale et al., 2003) and bacteria (Bertoni et al., 2011) have shown that these organisms can recover from UVR-induced damage when UVR exposure is subsequently reduced or avoided. The outcome of damage vs. repair depends not only on the amount of damaging UVR received, but also on photo-repair wavelengths (UV-A, PAR) to which organisms are subsequently exposed during the fluctuating radiation regime. Moreover, the effects of different mixing depths, and thus of different mean irradiances, can act synergistically or antagonistically with UVR, depending on the composition, structure, and size of the species as well as on the environmental conditions (Villafañe et al 2007). For instance, Barbieri et al. (2002) found that the impact of UVR in Patagonian coastal waters was negative or positive depending on the fraction of the euphotic zone (Z_{eu}) that was mixed; thus, UVR was used for photosynthesis when vertical mixing reached ~90% of the Z_{eu}, but carbon fixation was reduced by UVR when the UML was shallow (~60% of the Z_{eu}).

Besides increased stratification of the water column, more extreme rainfall events and storms are predicted in many parts of the Earth in the global-change scenario (IPCC, 2013). This would increase the amount of allochthonous dissolved organic matter (DOM) reaching inland and coastal aquatic ecosystems, reducing the penetration of incident UVR (Rose et al., 2009). The UVR filtering characteristics of coloured DOM (CDOM) result in a more effective attenuation of shorter (UV-B) than longer (UV-A, 315-400 nm) wavelengths, as also observed for stratospheric ozone. Concomitantly, the photochemical reactions mediated by UVR lead to (i) the photodegradation of DOM, altering the composition and absorbance of CDOM and; (ii) the photo-oxidation of DOM, producing oxygen free-radicals (Kitidis et al., 2014). These changes would modulate the response of aquatic organisms to UVR (Williamson and Rose, 2010), making more complex to predict the interactive effects of UVR and stratification on the planktonic community.

Recent experiments carried out by our group have demonstrated that fluctuating irradiance increases the harmful UVR effects on primary producers in oligotrophic mountain lakes with high DOM, whereas the opposite effects were detected in those with low DOM content

(Helbling et al., 2013). Several authors have highlighted the importance of the quality of the radiation, which can interact with DOM and either increase or decrease the availability of organic carbon for bacteria (Pérez and Sommaruga, 2007). However, despite the key role of phytoplankton and heterotrophic bacteria production as a link between the microbial and grazing food webs, no studies on the interactive effects of radiation quality and increased stratification on the commensalistic phytoplankton-bacteria relationship have been done in ecosystems with high- and low-CDOM contents.

A growing body of literature supports the strong dependence of planktonic heterotrophic prokaryotes on organic matter released in situ by phytoplankton in the upper layers of aquatic ecosystems (Baines and Pace, 1991; Norrman et al., 1995; Morán et al., 2011). It has also been demonstrated that UVR exposure in the upper layers of the water column can increase the proportion of photosynthate released as exudates (Carrillo et al., 2008; Korbee al., 2012), which would stimulate the growth of UVR-resistant bacteria (Xenopoulos and Schindler, 2003) and give rise to a coupled phytoplankton-bacteria relationship in clear oligotrophic lakes (Carrillo et al., 2002). Coupling between phytoplankton and bacterioplankton has been defined as the capacity of the carbon (C) released by phytoplankton to support the bacterial carbon requirement (Morán et al., 2002) and will therefore differ depending on: (i) the availability of alternative (allochthonous or autochthonous) carbon sources (Gasol et al., 2009), and (ii) the supply of inorganic nutrients (Medina-Sánchez et al., 2010; 2013; López-Sandoval et al., 2011). Although the bacterial dependence on C released by phytoplankton is a well-established paradigm in aquatic microbiology (Cole et al., 1988), it is currently under renewed debate. Thus, Fouilland and Mostajir (2010, 2011) proposed that C dependency of bacteria on phytoplankton is uncertain because C sources other than those from algal origin might support the bacterial growth more significantly. However, Morán et al. (2011) rebutted this idea due to uncertainty found in the application of different conversion factors to raw data and modeled rates in the Fouilland and Mostajir´s calculations.

With this background, the aim of the present study was to improve our understanding about the combined effects of UVR exposure and increased stratification on (i) phytoplanktonic and heterotrophic bacterial production and (ii) the commensalistic relationship between them in lakes with different transparency to UVR. We hypothesised that the interactive effects of UVR and increased stratification will accentuate the harmful UVR effects on primary production (PP) and heterotrophic bacterial production (HBP), thus resulting in a greater C release by phytoplankton, which will strengthen the commensalistic phytoplankton-bacteria

relationship. These effects will be more acute in low-UVR than in high-UVR lakes, where UVR-resistant populations are likely not selected for.

To test our hypothesis, we carried out in situ experiments to assess the combined impact of solar radiation (i.e., quality) and simulated stratification on metabolism of phytoplankton and bacteria, and their commensalistic relationship, in two oligotrophic lakes with contrasting transparency to UVR in the Mediterranean Region.

2 Methods

2.1 Model ecosystems

The study was performed during September 2011 in two Spanish oligotrophic lakes: La Caldera Lake in Sierra Nevada National Park (37º 03'N; 3º 19'W, 3050 m a.s.l.) (Granada) and La Conceja Lake in Ruidera Natural Park (38º 55' N; 2º 47' W, 850 m a.s.l.) (Ciudad Real). La Caldera is a mixed oligotrophic (total phosphorus [TP] < 0.3 µM and chlorophyll *a* Chl *a* < 5 µg L^{-1}) high-mountain lake above the treeline on a siliceous bedrock in a glacial cirque (Carrillo et al., 2006). This lake has a surface area of 2 ha, a mean depth of 4.3 m, with a maximum depth inter-annually variable from 2 to 14 m. UVR of considerable intensity penetrates deeply in the lake due to the high transparency of the water and low values of dissolved organic carbon (DOC; < 0.08 mM) as reported in Carrillo et al. (2008), and Helbling et al. (2013). Therefore, this lake is called hereafter the "high-UVR" lake. The pelagic community is relatively simple (Carrillo et al., 2006) and it is characterized by the scarcity of ciliates, absence of heterotrophic nanoflagellates and autotrophic picoplankton, and no size overlap exist between phytoplankton and heterotrophic bacteria (Medina-Sánchez et al., 2002). La Conceja is a stratified oligotrophic lake (TP < 0.03 µM and Chl *a* < 5 µg L^{-1}), although it has an elevated nitrate concentration which can exceed 800 µM due to agricultural use of the land. This lake has a surface area of 29 ha and maximum depth of 14 m. The DOC content ranges from 0.15 to 0.25 mM. Therefore, this lake is called hereafter the "low-UVR" lake. The autotrophic community is composed of pico- and nanoplankton (Rojo et al., 2012).

2.2 Experimental setup

To assess the interactive effects of solar radiation quality ("UVR" factor) and stratification conditions ("STRAT" factor) on PP, HBP, TPR (total planktonic respiration, < 45-µm fraction) and BR (bacterial respiration < 1-µm fraction in the high-UVR lake alone), samples were collected from the surface (0-0.5 m) epilimnetic water. An acid-cleaned 6-L horizontal Van Dorn sampler was used to collect the water that was pre-screened through a 45-µm mesh to remove large zooplankton prior to the experiments. Samples for PP were placed in 50-mL quartz flasks and those for HBP, TPR, and BR in 25-mL quartz flasks. In the low-UVR lake, samples for PP, HBP and TPR analyses were also gathered from the hypolimnetic water below the thermocline at 6 m depth, where UV-B did not reach the cells. The idea behind sampling these two communities in the low-UVR lake was to compare the responses of responses of phytoplankton and bacterial communities that had different light histories and degree of acclimation to solar radiation when exposed to similar light quality treatments and irradiance conditions. Since this sharp contrast did not occur in the clear lake, only samples from the 0-0.5 m were used in these experiments.

The experimental design consisted of three (for TPR and BR), four (for PP, HBP) or two (for TPR in the low-UVR lake) "UVR" treatments combined with the two stratification conditions: 1) The UVR treatments (triplicates for each condition) were: (i) PAB: full solar radiation, uncovered quartz flasks; (ii) PA: exclusion of UV-B (280-320 nm), wrapping the flasks with Folex 320 film (Folex, Germany); (iii) P, control: exclusion of UVR (280-400 nm), wrapping the flasks with Ultraphan UV Opak395 film (Digrefa, Germany); (iv) Dark: wrapping the flasks with black tape. The optical properties of the filters used for the radiation treatments have been published elsewhere (Villafañe et al., 2003); the filters were replaced before each experiment and tested using a double-beam spectrophotometer (Perkin-Elmer Lambda 40). 2) The stratification treatments were: (i) Subsurface, samples incubated at 0.5 m depth; (ii) Mixed, samples subjected to vertical mixing from 0 to 5 m depth. To simulate these reductions in the depth of the UML (i.e. from 5 m to near the surface) two round trays containing the samples were exposed in situ to solar radiation. One tray was placed at 0.5 m depth (Subsurface) subjected to irradiance oscillations associated with waves at the surface. This treatment represents the worst-case scenario in terms of solar radiation (i.e., high summer irradiance conditions), in combination with a sharp increase of thermal stratification (i.e., simulating the formation of near-surface thermoclines) during the usually warm Mediterranean summer. Transient thermoclines trapping phytoplankton very close to the surface have previously been detected in aquatic environments (Neale et al., 2003). The second tray was vertically moved between the surface and 5 m depth to simulate the

irradiance changes in the upper 5 m of the water column (mixed). The speed of movement was 1 m every 2 min, achieved by a custom-made mixing simulator, using a frequency-controlled DC motor (Maxon motor, Switzerland) to impose a linear transport rate on the vessels from the surface to the mixing depth and back. The tray was placed on a boat anchored in a deep area of each lake in such a manner as to avoid shadows or any type of interference from the shoreline or boat. All incubations lasted for 3.5 h centered on local noon, and a total of 10 cycles (from the surface to 5 m depth to the surface again) were completed for the mixed condition.

Unfortunately, space restrictions within the trays prevented the performance of all experimental treatments in the low-UVR lake for TPR, which was measured only in samples exposed to PAB and P in the subsurface and mixed treatments. The overlapping between autotrophic and heterotrophic picoplankton precluded the measurements of BR in the low-UVR lake.

2.3 Physical measurements

Incident solar radiation was continuously monitored by means of a BIC radiometer (deck unit, Biospherical Instruments Inc., CA, USA) that has three channels in the UVR region of the spectrum (305, 320, and 380 nm) and one broad-band channel for PAR (400-700 nm). Vertical profiles of solar radiation in the water column were performed at noon using a BIC radiometer (underwater unit) with temperature and depth sensors, in addition to the aforementioned channels. Vertical profiles of temperature and pH in the water column were measured using a multiparameter probe (Turo Water Quality Analysis T-611 Sandy Bay, Tasmania, Australia). These profiles were done daily at noon, and the temperature data were used to estimate the strength and depth of the epilimnion.

2.4 Chemical analyses

Chemical and biological variables were sampled with a 6-liter Van Dorn sampler at the deepest central station at four depths in the high-UVR lake (surface, 5, 8, and 10 m) and six in the low-UVR lake (surface, 2, 4, 6, 8, and 10 m). Water samples were taken to determine the bacterial abundance (BA, 20 mL), phytoplankton species composition and abundance (250 mL), and Chl *a* (1L). Samples were also collected for the chemical determination of total

nitrogen (TN), TP, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), nitrate (NO_3^-), and soluble reactive phosphorus (SRP). The samples for TDN, TDP, NO_3; and SRP analyses were filtered through GF/F Whatman filters (47 mm in diameter) before the determinations. Samples for TP and TDP were persulfate-digested at 120°C for 30 min and determined (as for SRP) using 10-cm quartz cuvettes (following the acid molybdate technique, APHA 1992). TN and TDN samples were also persulfate-digested and measured as NO_3^- by means of the ultraviolet spectrophotometric screening method (APHA, 1992). Blanks and standards were run in all procedures. DOC values were determined by filtering the samples through pre-combusted (2h at 500ºC) glass fiber filters (Whatman GF/F) and acidifying them with HCl. Samples were then measured in a total organic carbon analyzer (TOC V CSH/CSN Shimadzu).

2.5 Analysis biological variables

Chl a concentration: For measurements of the Chl *a* concentration, water samples from different depths in the water column were filtered onto Whatman GF/F filters (25 mm in diameter), which were frozen at -20°C until analyses. For Chl *a* analysis, samples were thawed and placed in centrifuge tubes (15 mL) with 5 mL of acetone (90%) for 24 h in the dark at 4ºC. Next, the samples were centrifuged, and the fluorescence of the supernatant was measured with a fluorometer (LS 55 Perkin Elmer, USA) (APHA, 1992).

Identification and cell counting: Samples for the identification and counting of phytoplankton were placed in 250-mL brown glass bottles and fixed with Lugol's reagent (approx. 1% vol/vol). Sub-samples (100 mL) were settled for 48 h in Utermöhl chambers (Hydro-Bios GmbH), and species were then identified and counted using an inverted microscope (Leitz Fluovert FS, Leica, Wetzlar, Germany). Phytoplankton biovolumes were estimated from measurements of 20–30 cells of each species using image analysis (Inverted microscope Axio Observer A1, Zeiss – High resolution microscopy camera Axiocam HRc, Zeiss). Cell volume was calculated according to Carrillo et al. (1995), and converted to phytoplankton carbon using the conversion factors reported by Rocha and Duncan (1985). Bacterial abundance (BA) was determined by the 4', 6-diamidino-2-phenylindole (DAPI) direct-count method described by Porter and Feig (1980). Water samples were fixed with neutralized formaldehyde (2%), stained with DAPI to a final concentration of 2.5 µg mL^{-1}, and then filtered onto 0.2-µm pore-size polycarbonate black filter. At least 400 cells per sample were

counted by epifluorescence microscopy (Karl Zeiss AX10). Bacterial biomass (BB) was estimated from bacterial biovolume, measured from bacterial images obtained by transmission electron microscopy (TEM) as described by Medina-Sánchez et al. (1999).

2.6 Analysis of biotic functional variables

Primary production and excreted organic carbon: For PP measurements, samples of phytoplankton communities were placed in 50-mL round quartz flasks (three clear and one dark per radiation treatment), inoculated with 0.37 MBq of $NaH^{14}CO_3$ (specific activity: 310.8 MBq $mmol^{-1}$, DHI Water and Environment, Germany), and exposed to solar radiation in situ, as described above. were placed in 20-mL scintillation vials and acidified with 100 µL of 1 N HCl for 24 h (no bubbling) to remove inorganic The total organic carbon (TOC) produced was measured on 4-mL aliquots before filtration. The samples for PP were filtered onto 0.2-µm filters (25 mm diameter, Nuclepore, Whatman), under low vacuum (< 100 mm Hg) to minimize cell breakage. Excretion of organic carbon (EOC) was measured on 4-mL aliquots from the filtrates (< 0.2 µm). Both filters and filtrates ^{14}C before the addition of liquid scintillation cocktail (Ecoscint A) to the vials. The amount of organic carbon produced was obtained by counting disintegrations per minute (dpm), using an autocalibrated scintillation counter (Beckman LS 6000 TA). The total CO_2 in the lake water was calculated from alkalinity and pH measurements (APHA, 1992). In all calculations, dark values were subtracted from the corresponding light values (more details in Carrillo et al., 2002). The %EOC was estimated as:

$$\%EOC = 100x(EOC/TOC). \quad (1)$$

Heterotrophic bacterial production: Samples for HBP measurements were placed in 25-mL quartz flasks and exposed in situ for 3.5 h under the radiation and stratification conditions as described above. Then, the HBP was determined as incorporation of ^3H-thymidine (S.A= 52 Ci $mmol^{-1}$, Amersham Pharmacia) into the bacterial DNA, in darkness. Briefly, ^3H-thymidine was added to independent sets of five (three replicates + two blanks per treatment) sterile microcentrifuge tubes filled with 1.5 mL of the pre-exposed samples to a final (saturating) concentration of 15.2 nM. The vials were then incubated at in situ temperature in darkness for 1 h. After incubation, the incorporation of ^3H-thymidine was stopped by adding trichloroacetic acid (TCA, 6% final concentration). Likewise, blanks were TCA-killed before the radiotracer was added. After the cold TCA extraction, the precipitate was collected by

centrifugation at 14000 rpm for 10 min. The conversion factor 1.5×10^{18} cell mol^{-1} was used to estimate the number of bacteria produced per mol of incorporated ^{3}H-thymidine (Bell, 1993). The factor 20 fgC $cell^{-1}$ was applied to convert bacterial production into C (Lee and Fuhrman, 1987).

Respiration rates: Samples for TPR (<45µm fraction) and BR (<1µm fraction) measurements were placed in 25-mL quartz flasks and exposed in situ for 3.5 h under the radiation and stratification conditions described above. TPR and BR rates were measured in darkness using optode sensor-spots (SP-PSt3-NAU-D5-YOP; PreSens GmbH, Germany) and an optic-fibre oxygen transmitter (Fibox 3; PreSens GmbH, Germany) connected to a computer. Data were recorded using the OxyView 3.51 software (PreSens GmbH). The system was calibrated by a two-point calibration, together with data of atmospheric pressure and temperature, before each experiment, following the manufacturer's recommendations. Measurements were made at the initial time (t_0) and then every hour during 8 h. Every oxygen measurement was done during 30 sec with a frequency of 1 datum per sec; only the last 10 data points of each measurement were used in our analysis to ensure the stability of the data. Oxygen data were then adjusted to a linear model via least-squares regression. Slope of the regressions provided the oxygen consumption rates (µM O_2 h^{-1}) (Warkentin et al., 2007). Oxygen was converted into carbon units using a respiratory quotient of 1 (del Giorgio and Cole, 1998).

The bacterial carbon demand (BCD) is the HBP plus BR. The bacterial growth efficiency (BGE) is the proportion of C entering the bacterial pool that is incorporated into the biomass, and was calculated as $BGE = HBP/BCD$. The absence of size-overlapping between phytoplankton and bacteria in the high-UVR lake (Medina-Sánchez et al., 2002) allowed for a direct measurement of BR. This, however, was not possible in the low-UVR lake, since autotrophic picoplankton and bacteria co-existed in the < 3 µm fraction. Therefore, BCD in this lake was estimated by assuming that BR values lie within two limits: (i) a conservative value of 75% of TPR, which is an average value based on data reported for oligotrophic waters (Lemeé et al., 2002); and (ii) a potential minimum value of 50% of TPR (Robinson, 2008), comparable with direct measurements made in this study on the TPR *v*s. BR in La Caldera lake (Herrera *et al.*, unpubl. data).

2.7 Data calculation and statistical analysis

The effect size of the UVR was quantified as:

$$\text{Effect size of UVB (\%)} = 100 \times [(C_P - C_{PAB})/C_P) - ((C_P - C_{PA})/C_P)] \quad (2)$$

$$\text{Effect size of UVA(\%)} = 100 \times [(C_P - C_{PA})/C_P] \quad (3)$$

where C_P, $C_{PA,}$ and C_{PAB} represent the carbon production by phytoplankton or bacteria in samples under the P, PA and PAB treatments, respectively. We used propagation errors to calculate the variance of the effect-size (as percentage) due to UV-B and UV-A. The change (Δ) in the effect size of UV-B and UV-A, between the subsurface and mixed treatments, was calculated as the difference of the effect size for each radiation band.

The effects of solar radiation quality ("UVR" factor) and stratification ("STRAT" factor) on the response variables were tested using two-way ANOVA. When the interactive effects were significant, a post hoc Bonferroni's test was used to determine significant differences among treatments. The normality (by Kolgomorov-Smirnov's test) and homoscedasticity (by Cochran, Hartley & Bartlett's test) were checked for each data group before ANOVA application. HBP data from the hypolimnetic community in the low-UVR lake were log-transformed to meet ANOVA assumptions. Significance of the effect size of UV-B and UV-A on PP and HBP between subsurface and mixed conditions was evaluated using *t*-test. Regression analyses were done to assess the dependence of the BGE on the EOC rates for the experimental data in each lake. Statistica 7.1 software for Windows was used for the statistical analyses.

3 Results

3.1 Physical, chemical, and biological variables in the water column

The lakes greatly differed in their transparency to UVR, but not to PAR (Table 1). Thus, in the high-UVR lake, the 1% of the surface energy at 305 nm reached the bottom of the lake, whereas in the low-UVR lake most of the UVR energy was attenuated in the upper layers (1% of the surface energy at 305 nm reached only ca. 1 m depth). This differential penetration of solar UVR resulted in two contrasting environments, with organisms being exposed to UV-B throughout the water column in the high-UVR lake but only in the upper 1-2 m of the water column in the low-UVR lake. This was related to the different DOC concentrations in the lakes, reaching values of 0.07 and 0.18 mM in the high-UVR and low-UVR lakes, respectively (Fig. 1a, b). Vertical temperature profiles also differed between the lakes: the temperature was 14°C, ranging only 0.4 ºC between the surface and bottom in the high-UVR lake, whereas a weak thermal stratification between 2-3 m was detected in the low-UVR lake,

where the temperature ranged from 22 to 19.5°C between the surface and bottom layers (Fig. 1a, b).

The concentrations of total dissolved and inorganic forms of N and P were homogeneous in the water column in both lakes; therefore, only mean values are reported in Table 1. TN values were higher in the low-UVR than in the high-UVR lake, by up to one order of magnitude, and NO_3^- constituted most of the TN (90% in the low-UVR and 68% in the high-UVR lake). By contrast, TP values were < 0.16 µM and mostly in organic form in both lakes. The NO_3^-:TP ratio was >100 in the high-UVR lake and > 10,000 in the low-UVR lake, indicating a strong P limitation (Table 1).

Chl *a* concentrations had small variations with depth in both lakes (Fig. 1c, d). However, the vertical distribution of phytoplankton and bacteria differed between the lakes: in the high-UVR lake (Fig.1c) bacterial abundance was rather homogeneous, but phytoplankton abundance was higher at the deepest depth; however, in the low-UVR lake (Fig.1d) the abundances of bacteria and phytoplankton were rather uniform with depth. Mean phytoplankton and bacterial abundance values were greater in the high-UVR than in the low-UVR lake (Table 1). In terms of taxonomic composition, the Chlorophyceae *Monoraphidium* sp. represented >90% and ~80% of the total abundance and biomass, respectively, in the high-UVR lake, whereas the Bacillariophyceae, *Cyclotella ocellata* was the dominant species in the low-UVR lake (>75% abundance and 95% biomass).

3.2 Variations in solar mean irradiance during experiments

The mean irradiance for the three wavelengths within the UVR and PAR region received by the samples under the experimental conditions are shown in Table 2. The mean irradiance at 305nm, 320 nm and 380 nm in the high-UVR lake were 2.8-, 2.5-, and 1.9-folds higher, respectively, in the subsurface than in the mixed conditions. The ratios between subsurface and mixed treatments in the low-UVR lake were 8.7-, 7.1-, and 3.7- for the 305 nm, 320 nm, and 380 nm wavelengths, respectively. The energy ratio at 380 and 305 nm (i.e., UVA_{380}:UVB_{305} ratio) had higher values in the low-UVR lake as compared to the high-UVR lake, reflecting the lower penetration of UV-B in the former.

3.3 Joint effects of UVR and stratification on phytoplanktonic and bacterial metabolism in the high-UVR lake

The PP values did not show significant differences between subsurface and mixed conditions in the PAB treatment, while samples under the PA and P treatments had significant higher PP values at subsurface than at mixed conditions (Fig. 2a). A significant UVR×STRAT effect was found for PP (Table 3) and according to our hypothesis; the subsurface incubations resulted in higher UV-B (11.5%) and UV-A (18.3%) inhibition as compared to the mixed incubations (Table 4). UVR at subsurface also significantly increased the rates of EOC, with significantly higher values in samples under the PAB and PA treatments (Fig. 2b). Likewise, the %EOC was significantly affected by UV-B, increasing to 22% and 21% in subsurface and in mixed treatments, respectively (Fig. A1 in Appendix A). Like PP, HBP did not differ between PAB-subsurface and PAB-mixed treatments. However, HBP was significantly lower under PA-subsurface than under PA-mixed treatments (Fig. 2c) resulting in a significant UVR×STRAT effect (Table 3). By contrast, only the "UVR" factor significantly affected BR (Fig. 2d, Table 3), with the lowest BR value determined in the PAB-subsurface treatment (Fig. 2d). BGE had higher values in the PAB-subsurface treatment as compared to the other radiation treatments at subsurface conditions; other comparisons between paired treatments did not result in significant differences of BGE (Fig. 2e). There was, nevertheless, a significant UVR×STRAT interaction on BGE (Table 3). No relationship was found between EOC rate and BGE ($R^2 = 0.149$ $p > 0.05$). Finally, to quantify the capacity of EOC released by phytoplankton to support the bacterial C demand (BCD) in each treatment, the BCD:EOC ratio (as a percentage) was calculated (Fig. 2f). Carbon released by phytoplankton resulted in excess to meet BCD (i.e., BCD:EOC values < 100%) only in the PAB-subsurface treatment (Fig. 2f).

3.3. Joint effects of UVR and stratification on phytoplanktonic and bacterial metabolism in the low-UVR lake

UVR exerted negative effects on both epilimnetic (Fig. 3) and hypolimnetic (Fig. 4) communities. For the epilimnetic community, PP was significantly lower in the PAB than in PA and P treatments at subsurface conditions, while UVR did not affect PP at mixed conditions (Fig. 3a). A significant UVR×STRAT effect on PP (Table 3) was found, with the lowest PP values at PAB-subsurface treatment. The highest values of UV-B (37%) and UV-A (25%) inhibition were found at subsurface (Table 4). As for PP, EOC was significantly lower in the PAB than in the PA and P treatments at subsurface, but not significant differences among radiation treatments at mixed conditions were found (Fig. 3b). %EOC did not show

differences due to radiation in none of the stratification treatments (Fig. A2 in Appendix A). HBP showed significant higher values in dark treatments at subsurface than at mixed conditions (Fig 3c) generating a significant interactive effect of UVR×STRAT on HBP (Table 3). Noticeably, a strong inhibition of HBP by UV-B and UV-A in subsurface and in mixed conditions was found (Table 4). By contrast, the estimated BR was not significantly affected by any factor (Table 3; Fig. 3d shown $BR_{50\%}$). UVR was the only factor that significantly reduced BGE values in both mixed and subsurface conditions (Fig. 3e). No relationship between EOC rate and BGE was found (R^2 = 0.055 p > 0.05). The BCD:EOC (%) was < 100% for every experimental condition except for that under PAB-subsurface treatment, where the BCD:EOC (%) reached values from ~ 100% (assuming BR = 50% of TPR) to 145% (assuming BR = 75% of TPR) (Fig. 3f). Thus, in this latter case (PAB- subsurface), EOC was not enough to meet BCD.

For the hypolimnetic community, UVR was the only factor that significantly inhibited PP (Fig. 4a). Samples under the PAB and PA treatments had significantly lower PP values than those under the P in both subsurface and mixed conditions (Fig. 4a). The EOC rates were significantly lower in the PAB and PA treatments than in the P treatment at subsurface (Fig.4b). No significant differences among both stratification treatments were determined when comparing each radiation treatment (Fig. 4b). HBP was significantly inhibited only by UV-B (Fig. 5c), whereas it was stimulated by PA and P, at the subsurface conditions (Fig. 4c). Under mixing, however, UVR did not affect HBP. Therefore, subsurface exposure triggered the inhibition due to UV-B by 45.6 % (Table 4). Only UVR, as a single factor, significantly affected BR (Table 3), with the lowest values under the PAB-mixed treatment (Fig. 4d), whereas only the STRAT factor affected BGE, with the lowest BGE values in the PAB-subsurface treatment (Fig. 4e). The BCD:EOC was < 100% under all conditions (assuming BR = 50% or 75% of TPR), indicating the EOC was always capable of supporting BCD (Fig. 4f).

Summarizing, and taking into account the changes (Δ) in the inhibitory UVR effect (UV-B and UV-A) on PP and HBP with increased stratification (Table 4), our results reveal greater UV-B sensitivity of: (i) epilimnetic phytoplankton and heterotrophic bacteria communities in the low-UVR lake than in the high-UVR lake; (ii) epilimnetic phytoplankton than heterotrophic bacteria in both lakes; and (iii) hypolimnetic heterotrophic bacterial than phytoplankton community in the low-UVR lake. In addition, significant interactive UVR×STRAT effects were observed on the BCD:EOC (%) only in the epilimnetic

communities (Table 3). Thus, partially supporting our hypothesis, the BCD:EOC (%) significantly decreased under PAB-subsurface treatment in the high-UVR lake but increased in the low-UVR lake.

4 Discussion

The main outcome of our work is that the increased stratification of the water column altered the commensalistic phytoplankton-bacteria relationship in oligotrophic lakes. The present study is the first, so far, directly assessing the interactive effects of UVR and stratification changes on phytoplankton, bacteria and their commensalistic relationship in freshwater ecosystems. Furthermore, in our complex experimental approach, we simulated reductions in the depth of the UML due to the stratification of the water column (one of the potential consequences of global warming; Gao et al., 2012; de Senerpont-Domis et al., 2013). Under these conditions, we measured the extracellular carbon release by phytoplankton, and directly determined the BR because these are the key variables implied in the bacterial carbon demand to C-supply ratio. Moreover, since a strong feedback between physical processes (e.g. mixing, stratification) and changes in DOC concentration in small lakes have previously been reported (Read and Rose, 2013), we further achieved an advance in our knowledge by investigating two oligotrophic ecosystems that differed in their UVR penetration in the water column due to their DOC content, as model lakes representing two ends of an optical gradient of transparence to UVR in Mediterranean inland waters. This provides a framework for disentangling the complex processes that underlie biological interactions under changing physical (stratification, UVR) and chemical (DOC) conditions, which can then modify the C flux in aquatic ecosystems.

4.1 Sensitivity of phytoplankton and bacteria to UVR and stratification

Despite the physical and ecological differences between the two lakes, PP and HBP responses to the joint effect of UVR and stratification were quite similar in that the latter augmented the effect size of UVB, mainly on the epilimnetic communities in both ecosystems. This effect reached a higher magnitude in the low-UVR lake (Table 4), which coincided with a greater relative exposure to UV-B (9-fold) and an more accentuated decrease in the UV-A:UV-B ratio (58%) at shallower layer in the low-UVR than in the high-UVR lake. This result agrees with the findings of higher UVR damage on primary producers in low-UVR lakes than in

high-UVR lakes as reported by Helbling et al. (2013), although in their study this response was found only under fluctuating irradiances. The results presented here indicate increased susceptibility to UVR of bacteria and phytoplankton communities relatively less exposed to UV-B during their life cycles (Pakulski et al., 2007; Harrison and Smith, 2011a). Interestingly, the UVR effect on %EOC was only significant in the high-UVR lake; the release of C has been described as a protective mechanism to prevent photosystem damage from reducing power excess under high irradiance of PAR (Wood and Van Valen, 1990) and also of UVR (Carrillo et al., 2002, 2008). The lack of this "escape valve", which helps to prevent over-excitation of PSII, might be the final cause of the higher sensitivity of phytoplankton communities in the low-UVR lakes. In addition, a higher sensitivity to UVR was found for epilimnetic phytoplankton than for bacteria mainly at subsurface condition, suggesting that photosynthetic processes are more sensitive under extreme conditions that mimic the global-warming scenario. This result contrasts to previous reports of greater UVR damage to bacterioplankton than to phytoplankton in oligotrophic waters of the Mediterranean Sea (Bertoni et al., 2011), the northern South China Sea (Yuan et al., 2011), high-mountain lakes (Sommaruga et al., 1997) and boreal lakes (Xenopoulos and Schindler, 2003).

Taken all together, our results show that increased stratification, by trapping the cells in a shallower epilimnion, with increased UVR exposure, triggered or exacerbated the inhibitory effect of UVR on phytoplanktonic and bacterial metabolism measured under mixed conditions. Because this negative effect was greater in high-DOC waters, we propose that the "ideal" photoprotective DOM may become harmful on planktonic communities in a scenario of increased stratification. Our proposal is based on the indirect harmful UV-B effects due to the free radicals (O_2^-, H_2O_2, OH^-) generated by photo-oxidation of the DOC (Banaszak, 2003; Pullin et al., 2004) which can exacerbate the negative UVR effect in low-UVR lakes. In addition, DOC would become bleached and therefore the lake would be more UVR transparent (Reche et al., 2001), thus increasing the negative effect of UVR on organisms. However, cell acclimation to UVR or a shift in the taxonomic composition towards UVR-resistant species could counteract the net negative UVR effect in a long-term scale.

As expected, UVR was the main factor that affected the non-acclimated hypolimnetic community, since PP and HBP underwent negative UV-B and UV-A effects in both subsurface and mixed conditions (Table 4). These responses reflect the higher sensitivity of the hypolimnetic than the epilimnetic community to UVR, because only the hypolimnetic community was negatively affected by UVR under mixed conditions. These results agree with

previous reports of higher photosynthetic impairment under UVR exposure of phytoplankton from deep chlorophyll maxima (Harrison and Smith, 2011b) or from the bottom of the mixed layer (Xenopoulos and Schindler, 2003).

Nevertheless, HBP of the hypolimnetic community was stimulated by UV-A and PAR when exposed to shallower conditions. These results suggest that the hypolimnetic bacteria possessed photorepair mechanisms, *via* UV-A and PAR-promoted photolyase activity (DNA repair), which may be activated after 4 h of UVR and PAR exposure (Jeffrey et al. 1996; Bertoni et al. 2012). This photorepair mechanism has a low energy cost and may be an important adaptive mechanism to attenuate the gross negative effect of UVR when a non-UVR-acclimated bacterioplankton community is exposed to high PAR and UV-A intensity and harmful UV-B levels in ecosystems with low nutrient availability (Medina-Sánchez et al., 2002). Notwithstanding, and in agreement with our hypothesis, photorepair mechanisms were insufficient to completely counteract UVR-induced damage, this being concordant with a sharp decrease in the UVA/UVB ratio (58%) in the upper layers (subsurface conditions). Moreover, the increased HBP found after exposure of samples to higher PAR intensity in the upper layers is consistent with the previously reported stimulatory effect of PAR on HBP (Morán et al., 2001, Medina-Sánchez et al., 2002, Pakulski et al., 2007). Besides, a potential presence of aerobic anoxygenic phototrophic bacteria (Bertoni et al. 2011, Mašín et al., 2012, Ferrara et al., 2011) should not be ruled out to account for the increased HBP under high PAR in the low-UVR lake.

4.2 UVR and increased stratification effect on the commensalistic phytoplankton-bacteria relationship

As noted above, UVR and stratification exerted an interactive effect on PP and HBP in the epilimnetic layer in both lakes. However, this interactive effect was only exerted on EOC in the low-UVR lake, where the EOC rates values were 3-fold higher (except under PAB-subsurface treatment) than in the high-UVR lake. The carbon released by phytoplankton is composed mainly of low-molecular-weight compounds that are readily assimilable by bacteria (Amon et al., 2001). This source of carbon is preferred by bacteria, even in lakes with considerable input of terrestrial carbon to subsidize their growth (Kritzberg et al., 2005, 2006), because the non-readily assimilable organic matter, mostly composed of high

molecular-weight (HMW) compounds, must be hydrolyzed by bacterial ectoenzymes before the assimilation.

Quantification of the dependence of heterotrophic bacteria on organic substrate released by phytoplankton requires an accurate assessment of the BCD (Morán et al., 2002). Our study offers a quite precise estimate of the BCD, because both HBP and BR were directly measured in the high-UVR lake, due to absence of size overlap between auto- and heterotrophic organisms. In the low-UVR lake, where segregation between both biological fractions was not feasible, BR was estimated from direct measurements of TPR and the reported percentages of the latter variable accounted for BR (i.e. 50 and 75%; Lemeé et al., 2002; Robinson, 2008). This procedure brought about a min-max range where the actual BR should safely fall. In addition, its reliability is supported in that our estimated mean BGE and BR values fell within the range reported for oligotrophic ecosystems (Biddanda et al., 2001; Amado et al., 2013).

In the high-UVR lake, BGE was increased under full-sunlight and subsurface conditions, reflecting greater changes in bacterial respiration than in production. The reduction in BR and, as a consequence, the increase in bacterial growth efficiency could be interpreted as a tolerance-related mechanism under full-sunlight exposure in accordance with the non-inhibitory effect of UV-B on HBP found under shallower conditions. By contrast, in the low-UVR lake, BGE values were lower under full sunlight and subsurface (stratified) conditions. The lack of the inhibitory effect of full sunlight (PAB *vs.* P) on TPR (and hence BR) concomitantly with a strong inhibitory effect of UV-B on HBP determined a reduction in bacterial growth efficiency according to the high sensitivity of the bacterial community. The differences in the bacterial responses between the lakes could be the outcome of specific bacterial composition inhabiting each lake. These results agree with previous laboratory findings of a negative UV-B effect on BGE or BR in some bacterial strains isolated from alpine lakes, but a positive effect on others, suggesting a strain-specific response (Hörtnagl et al., 2010). Nevertheless, changes in BGE are frequently observed when bacterial growth is limited by substrate availability (del Giorgio and Cole, 1998; López-Urrutia and Morán, 2007). Although our experiments were not specifically designed to test the role of organic substrates on BGE, we did not find a significant direct relationship between EOC rate and BGE in each lake. Thus, our data support the view that BGE can be altered by direct solar UVR impact.

Regarding the commensalistic phytoplankton-bacteria relationship, it was noticeable that in the high-UVR ecosystem, EOC rates increased with full sunlight under subsurface conditions,

reaching values that exceeded the C demand of a bacterial community which seemed to have undergone an inactivation or dormancy under PAB, reflected by lower respiration. This slowing of the bacterial metabolism, concomitant with an increase in the availability C released by phytoplankton, was the mechanism that determined the "coupling" phytoplankton-bacteria relationship. However, the fate of the C released by phytoplankton could be a transitory accumulation in lake water until its consumption by enhanced bacterial metabolic processes (growth and respiration) after an improvement in the light conditions, or could be definitively incorporated into the dissolved-C pool of the lake water.

In the low-UVR ecosystem, particularly to the epilimnetic community, the strong inhibitory effect of UV-B at subsurface on PP (i.e. decreasing C incorporation) was also reflected in a lesser C release by phytoplankton under these conditions. These decreased EOC rates did imply a change in their capability to meet the BCD, which ranged from barely sufficiency (if a 50% loss of TPR is assumed) to non-sufficiency (if a 75% loss of TPR is assumed). Therefore, the estimated min-max interval for each experimental condition shows an unexpected trend to a weakening of the bacterial dependence on phytoplankton C under full-sunlight and subsurface condition in the low-UVR lake, which may be induced by global warming. These results partially support our hypothesis because the interaction between UVR and stratification strengthened the commensalistic phytoplankton-bacteria relationship (decreasing %BCD:EOC ratio to <100) in the high-UVR lake, but weakened (increasing %BCD:EOC ratio to >100) this relationship in the low-UVR lake (Figs. 2f and 3f). Moreover, they underline the capability of UVR in altering the efficiency of phytoplankton C excretion to support bacterial demands in optically contrasting ecosystems. Since the interaction of UVR and simulated stratification on this crucial biotic interaction in high-UVR and low-UVR lakes has not been previously examined, more data is needed in order to generalize these responses by microbial organisms, not only on short-term (as considered in this study) but also on long-term basis.

To summarize our findings, we propose a conceptual functioning model that embraces both contrasting model ecosystems (Fig. 5). According to the global-warming scenario, it is expected that: (i) the vertical stratification of aquatic ecosystems will intensify (de Senerpont Domis et al., 2013); (ii) the depth of the mixed layer will be altered as a consequence of micro-stratification in shallow lakes (van de Waal et al 2009); and (iii) microbial communities and DOC will be confined within a highly irradiated layer. Based on our results, the synergistic effect of UVR and increased stratification on the microbiota might strengthen the

C flux through the microbial loop in the high-UVR lake (or increasing the DOC pool in the lake) but might weaken it in the low-UVR lake. Therefore, our results showing a greater UVR damage in the low-UVR lake imply that these types of ecosystem might be especially vulnerable to these factors related to global change.

Author Contributions

Conceived and designed the experiments: PC, WH, VV. Performed the experiments: PC, JMMS, CD GH WH VV. Analyzed the data: PC, JMMS, WH. Contributed reagents/materials/analysis tools: PC. Wrote the paper: PC, JMMS, WH. VV

Acknowledgements

This study was supported by Ministerio Español de Medio Ambiente, Rural y Marino (PN2009/067) and Ciencia e Innovación (CGL2011-23681), Junta de Andalucía (Excelencia CVI-02598 and P09-RNM-5376), Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET (PIP No. 112-201001-00228) and Fundación Playa Unión; GH and CD were supported by the Spanish Government - Formación de Profesorado Universitario Grant. The authors are indebted to the staff of Sierra Nevada National Park and Lagunas de Ruidera Natural Park for permission to work, to E. Jiménez-Coll for the bacterial production analysis, and to D. Nesbitt for writing assistance in English. We also thank two anonymous reviewers for helping us to improve the quality of this manuscript. This is the contribution Nº 151 of Estación de Fotobiología Playa Unión.

References

American Public Health Association (APHA): Standard methods for the examination of water and wastewater, 18th edn. American Public Health Association, Washington, DC1992.

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

Amado, A.M., Meirelles-Pereira, F., Vidal, L.O., Sarmento, H., Suhett, L., Farjalla, V.F, Cotner, J.B., and Roland, F.: Tropical freshwater ecosystems have lower bacterial growth efficiency than temperate ones. Front. Microbiol., 4, 1-8, 2013.

Amon, R.M.W., Fitznar, H-P., and Bennerm, R.: Linkages among the bioreactivity, chemical composition and diagenetic state of marine dissolved organic matter. Limnol. Oceanogr., 46: 287–297, 2001.

Baines, S. B., and Pace, M. L.: The production of dissolved organic-matter by phytoplankton and its importance to bacteria- patterns across marine and fresh-water systems. Limnol. Oceanogr., 36, 1078-1090, 1991.

Banaszak, A.T.: Photoprotective physiological and biochemical responses of aquatic organisms. in: UV effects in aquatic organisms and ecosystems, edited by: Helbling, E. W., and Zagarese, H. E., Royal Society of Chemistry, Cambridge, UK, pp. 329–356, 2003.

Barbieri, E.S., Villafañe, V.E., and Helbling, E.W.: Experimental assessment of UVR effects on temperate marine phytoplankton when exposed to variable radiation regimes. Limnol. Oceanogr., 47,1648–1655, 2002.

Bell, R.T.: Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine, in: Handbook of methods in aquatic microbial ecology, edited by Kemp B.F., Sherr E.B., and Cole J.J., Lewis Publishers, Boca Raton, FL, 495–503, 1993.

Bertoni, R., Jeffrey, W. H., Pujo-Pay, M., Oriol, L., Conan, P., and Joux, F.: Influence of water mixing on the inhibitory effect of UV radiation on primary and bacterial production in Mediterranean coastal water. Aquat. Sci., 73, 377-387, 2011.

Biddanda, B.A., Ogdahl, M., and Cotner, J.B.: Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnol. Oceanogr., 46, 730–739, 2001.

Carrillo, P., Reche, I., Sánchez-Castillo, P., and Cruz-Pizarro, L.: Direct and indirect effects of grazing on the phytoplankton seasonal succession in an oligotrophic lake. J. Plankton Res., 17, 1363–1379, 1995.

Carrillo, P., Medina-Sánchez, J.M., Villar-Argaiz, M., Delgado-Molina. J.A., and Bullejos, F.J.: Complex interactions in microbial food webs: Stoichiometric and functional approaches. Limnética, 25, 189-204, 2006.

Carrillo, P., Delgado-Molina, J. A., Medina-Sánchez, J. M., Bullejos, F. J., and Villar-Argaiz, M.: Phosphorus inputs unmask negative effects of ultraviolet radiation on algae in a high-mountain lake, Global Change Biol., 14, 423-439, 2008.

Carrillo, P., Medina- Sánchez, J. M., and Villar-Argaiz, M.: The interaction of phytoplankton and bacteria in a high-mountain lake: Importance of the spectral composition of solar radiation, Limnol. Oceanogr., 47, 1294-1306, 2002.

Cole, J. J., Findlay, S., and Pace, M. L.: Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser., 43, 1–10, 1988.

de Senerpont Domis, L.N., Elser, J. J., Gsell, A. S., Huszar, V. L. M., Ibelings, B. W., Jeppesen, E., Kosten, S., Mooij, W. M., Roland, F., Sommer, U., Van Donk, E., Winder, M., and Lurling, M.: Plankton dynamics under different climatic conditions in space and time. Freshwater Biol., 58, 463-482, 2013.

del Giorgio, P. A., and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu. Rev. Ecol. Syst., 29, 503-541, 1998.

Ferrara, I., Gasol, J.M,, Sebastián, M., Hojerová, E., and Koblížek, M.: Comparison of growth rates of aerobic anoxygenic phototrophic bacteria and other bacterioplankton groups in coastal Mediterranean waters. Appl. Environ. Microb., 77, 7451-7458, 2011.

Gao, K., Wu, Y., Li, G., Wu, H., Villafañe, V.E., and Helbling, E.W.: Solar UV radiation drives CO_2 fixation in marine phytoplankton: a double-edged sword. Plant Physiol., 44, 54–59, 2007.

Fouilland, E. and Mostajir, B.: Revisited phytoplanktonic carbon dependency of heterotrophic bacteria in freshwaters, transitional, coastal and oceanic waters. FEMS Microbiol. Ecol., 73, 419–429, 2010.

Fouilland, E. and Mostajir, B.: Complementary support for the new ecological concept of 'bacterial independence on contemporary phytoplankton production' in oceanic waters. FEMS Microbiol. Ecol., 78, 206–209, 2011.

Gao K, Helbling, E.W., Häder, D.P., Hutchins, D.A.: Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming Mar. Ecol. Prog. Ser., 470, 67–189, 2012.

Gasol, J. M., Vázquez-Domínguez, E., Vaqué, D., Agustí, S., and Duarte, C.M.: Bacterial activity and diffusive nutrient supply in the oligotrophic Central Atlantic Ocean. Aquat. Microb. Ecol., 56, 1–12, 2009.

Genty, B. E., Briantais, J.M., and Baker, N.R.: Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and non-photorespiratory conditions. Plant Physiol. Biochem., 28, 1–10,1989.

Häder, D.P., Helbling, E.W., Williamson, C.E., and Worrest, R.C.: Effects of UV radiation on aquatic ecosystems and interactions with climate change. Photochem. Photobiol. Sci., 10, 242–260, 2011.

Harrison J.W, and Smith, R.E.H.: Effects of ultraviolet radiation on the productivity and composition of freshwater phytoplankton communities. Photochem. Photobiol. Sci., 8, 1218–1232, 2009.

Harrison J.W. and Smith, R.E.H.: The spectral sensitivity of phytoplankton communities to ultraviolet radiation-induced photoinhibition differs among clear and humic temperate lakes Limnol. Oceanogr., 56, 2115–2126, 2011a.

Harrison J. W. and Smith, R.E.H.: Deep chlorophyll maxima and UVR acclimation by epilimnetic phytoplankton. Freshwater Biol., 56, 980–992, 2011b.

Helbling, E.W., Villafañe, V. E., and Holm-Hansen, O.: Effects of ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing, in: Ultraviolet Radiation in Antarctica: Measurements and Biological Effects, edited by Weiler, S. and Penhale, P., American Geophysical Union, Washington, DC, USA, Antarctic Research Series, Vol. 62, 207–227, 1994.

Helbling, E.W., Marguet, E.R., Villafañe, V.E., and Holm-Hansen, O.: Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation. Mar. Ecol. Prog. Ser., 126, 293-298, 1995.

Helbling, E. W., Carrillo, P., Medina-Sanchéz, J. M., Duran, C., Herrera, G., Villar-Argaiz, M., and Villafañe, V. E.: Interactive effects of vertical mixing, nutrients and ultraviolet radiation: in situ photosynthetic responses of phytoplankton from high-mountain lakes in Southern Europe, Biogeosciences, 10, 1037-1050, 2013.

Kitidis, V., Tilstone, G.H., Serret, P., Smyth, T. J. Torres, R., and Robinson, C.: Oxygen photolysis in the Mauritanian upwelling: Implications for net community production. Limnol. Oceanogr., 59, 299–310, 2014.

Hörtnagl, P., Pérez, M.T., and Sommaruga, R.: Contrasting effects of ultraviolet radiation on the growth efficiency of freshwater bacteria. Aquat. Ecol., 45, 125-136, 2010.

Huisman, J., Pham, Thi, N., Karl, D.M., and Sommeijer, B.: Reduced mixing generates oscillations and chaos in the oceanic deep chlorophyll maximum. Nature, 439, 322–325, 2006.

IPCC: Climate Change 2013, Summary for Policymakers, edited by: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xla, Y., Bex, V., and Mldgley,P., Cambridge University Press, Cambridge, UK and New York, NY, USA, 2013.

Jeffrey, W. H., Pledger, R. J., Aas, P., Hager, S., Coffin, R. B., VonHaven, R., and Mitchell, D. L.: Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation, Mar. Ecol.-Prog. Ser., 137, 283-291, 1996.

Korbee, N., Carrillo, P., Mata, M. T., Rosillo, S., Medina-Sanchéz, J. M., and Figueroa, F. L.: Effects of ultraviolet radiation and nutrients on the structure-function of phytoplankton in a high-mountain lake, Photochem. Photobiol. Sci., 11, 1087-1098, 2012.

Kritzberg, E.S., Cole, J.J., Pace, M.M, and Granéli, W.: Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs? Aquat. Microb. Ecol., 38, 103–111, 2005.

Kritzberg, E.S., Cole, J.J., Pace, M.M, and Granéli, W.: Bacterial growth on allochthonous carbon in humic and nutrient enriched lakes: results from whole-lake ^{13}C addition experiments. Ecosystems, 9, 489–499, 2006.

Lee, S., and Fuhrman, J. A.: Relationships between biovolume and biomass of naturally derived marine bacterioplankton, Appl. Environ. Microb., 53, 1298-1303, 1987.

Lemeé, R., Rochelle-Newall, E., Van Wambeke, F., Pizay, M. D., Rinaldi, P., and Gattuso, J. P.: Seasonal variation of bacterial production, respiration and growth efficiency in the open NW Mediterranean Sea, Aquat. Microb. Ecol. , 29, 227-237, 2002.

López-Sandoval, D.C., Fernández, A., and Marañon, E.: Dissolved and particulate primary production along a longitudinal gradient in the Mediterranean Sea. Biogeosciences, 8, 815–825, 2011.

López-Urrutia, A., and Morán, X.A.G.: Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling. Ecology, 88, 817–822, 2007.

Mašín, M., Čuperová, Z., Hojerová, E., Salka, I., Grossart, H.P., and Koblížek, M.: Distribution of aerobic anoxygenic phototrophic bacteria in glacial lakes of northern Europe. Aquat. Microb. Ecol., 66, 77-86. 2012.

Medina-Sánchez, J. M., Villar-Argaiz, M., and Carrillo, P.: Modulation of the bacterial response to spectral solar radiation by algae and limiting nutrients, Freshwater Biol., 47, 2191-2204, 2002.

Medina-Sánchez, J. M., Villar-Argaiz. M., Sánchez-Castillo, P., Cruz-Pizarro, L., and Carrillo P.: Structure changes in a planktonic food web: biotic and abiotic controls. J. Limnol., 58: 213–222, 1999.

Medina-Sánchez, J. M., Carrillo, P., Delgado-Molina, J. A., Bullejos, F. J., and Villar-Argaiz, M.: Patterns of resource limitation of bacteria along a trophic gradient in Mediterranean inland waters, FEMS Microbiol. Ecol., 74, 554-565, 2010.

Medina-Sánchez, J.M., Delgado-Molina, J.A., Bratbak, G., Bullejos, F.J., Villar-Argaiz, M., Carrillo, P.: Maximum in the middle: Nonlinear response of microbial plankton to ultraviolet radiation and phosphorus. PLoS One 8: e60223, doi:10.1371 /journal.pone.0060223, 2013

Morán, X.A.G., and Alonso-Sáez, L.: Independence of bacteria on phytoplankton? Unsufficient support to Fouilland & Mostajir's (2010) suggested new concept. FEMS Microbiol. Ecol., 78, 203–205, 2011.

Morán, X.A.G., Massana, R., and Gasol, J.M.: Light conditions affect the measurement of oceanic bacterial production via leucine uptake. Appl. Environ. Microb., 67, 3795-3801, 2001.

Morán, X.A.G., Estrada, M., Gasol, J. M., and Pedrós-Alio, C.: Dissolved primary production and the strength of phytoplankton bacterioplankton coupling in contrasting marine regions, Microbial Ecol., 44, 217-223, 2002.

Neale, P. J., Helbling, E. W., and Zagarese, H. E.: Modulation of UVR exposure and effects by vertical mixing and advection, in: UV effects in aquatic organisms and ecosystems, edited by: Helbling, E. W., and Zagarese, H. E., Royal Society of Chemistry, Cambridge, UK, 108-134, 2003.

Norrman, B., Zweifel, U.L., Opkinson, C.S. Jr. and Fry, B.: Production and utilization of dissolved organic carbon during an experimental diatom bloom. Limnol. Oceanogr., 40, 898–907, 1995.

Pakulski, J.D., Baldwin, A., Dean, A., Durkin, S., Karentz, D., Kelley, C.A., Scott, K., Spero, H.J., Wilhelm, S., and Jeffrey, W.H..: Responses of heterotrophic bacteria to latitudinal variation in solar irradiance. Aquat. Microb. Ecol., 47, 153–162, 2007.

Pérez, M.T., and Sommaruga, R.: Interactive effects of solar radiation and dissolved organic matter on bacterial activity and community structure. Environ. Microbiol., 9**,** 2200–2210, 2007.

Porter, K.G., and Feig, Y.S.: The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr., 25, 943-948, 1980.

Pullin, M. J., Bertilsson, S., Goldstone, J.V., and Voelker, B.M.: Effects of sunlight and hydroxyl radical on dissolved organic matter: Bacterial growth efficiency and production of carboxylic acids and other substrates. Limnol. Oceanogr., 49, 2011–2022, 2004.

Read, J. S., and Rose, K. C.: Physical responses of small temperate lakes to variation in dissolved organic carbon concentrations, Limnol. Oceanogr., 58, 921-931, 2013.

Reche, I., Pulido-Villena, E., Conde-Porcuna, J. M., and Carrillo, P.: Photoreactivity of dissolved organic matter from high mountain lakes of Sierra Nevada (Spain). Arct. Antarc. and Alpine Res., 33, 426-434, 2001

Robinson, C.: Hetrotrophic bacterial respiration, in: Microbial ecology of the oceans. 2nd ed, edited by Kirchman D., Wiley, New York, 299–334, 2008.

Rocha, O. and Duncan, A.: The relationship between cell carbon and cell volume in freshwater algal species used in zooplankton studies, J. Plankton Res., 7, 279–294, 1985.

Rojo, C., Herrera, G., Rodrigo, M.A., Lorente, M.J., and Carrillo, P.: Mixotrophic phytoplankton is enhanced by UV radiation in a low altitude, P-limited Mediterranean lake. Hydrobiologia, 698**,** 97-110, 2012.

Rose, K. C., Williamson, C. E., Saros, J. E., Sommaruga, R., and Fischer, J. M.: Differences in UV transparency and thermal structure between alpine and subalpine lakes: implications for organisms, Photochem. Photobiol. Sci., 8, 1244-1256, 2009.

Ruiz-González, C., Simo, R., Sommaruga, R., and Gasol, J.M.: Away from darkness: a review on the effects of solar radiation on heterotrophic bacterioplankton activity, Front. Microbiol., 4, 131-131, 2013.

Sommaruga, R., Sattler, B., Oberleiter,A., Wille, A., Sommaruga-Wögrath, S., Psenner, R., Felip, M., Camarero, L., Pina, S., Gironés, R., and Catalán, J.: An in situ enclosure experiment to test the solar UV-B impact on microplankton in a high altitude mountain lake: II. Effects on the microbial food web. J. Plankton Res., 21, 859-876, 1999.

van de Waal, D.B., Verschoor, A., Verspagen, J.M.H., Van Donk, E., and Huisman, J.: Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Front. Ecol. Environ. Sci., 8, 145–152, 2009.

Warkentin, M., Freese, H. M., Karsten, U., and Schumann, R.: New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor spots, Appl. Environ. Microb., 73, 6722-6729, 2007.

Villafañe, V.E., Sundbäck, K., Figueroa, F.L., Helbling, E.W.: Photosynthesis in the aquatic environment as affected by UVR. in: UV effects in aquatic organisms and ecosystems, edited by: Helbling, E. W., and Zagarese, H. E., Royal Society of Chemistry, 357-397, 2003

Villafañe, V.E., Gao, K., Li, P., Li, G., and Helbling, E.W.: Vertical mixing within the epilimnion modulates UVR-induced photoinhibition in tropical freshwater phytoplankton from southern China, Freshwater Biol., 52, 1260-1270, 2007.

Williamson, C. E., and Rose, K. C.: When UV meets freshwater, Science, 329, 637-639, 2010.

Wood, A.M., and Van Valen L.M.: Paradox lost? On the release of energy-rich compounds by phytoplankton. Mar. Microb. Food Webs, 4, 103–116. 1990.

Xenopoulos, M.A., and Schindler, D.W.: Differential responses to UVR by bacterioplankton and phytoplankton from the surface and the base of the mixed layer. Freshwater Biol.,48, 108–122, 2003

Yuan, X., Yin, K., Harrison, P. J., and Zhang, J.: Phytoplankton are more tolerant to UV than bacteria and viruses in the northern South China Sea. Aquat. Microb. Ecol., 65, 117–128, 2011.

Tables

Table 1. Mean values of the main physical, chemical and biological variables measured in the water column in Lake La Caldera (high-UVR lake) and in Lake La Conceja (low-UVR lake). Values of vertical attenuation coefficients (kd, m^{-1}) in the UVR (305, 320, 380 nm) and photosynthetically active radiation (PAR, 400-700 nm) regions are shown. Values are mean (±SD) of concentrations for four (La Caldera lake) o six (La Conceja lake) depths of: inorganic, total and dissolved nitrogen (N) and phosphorus (P), Chlorophyll *a*, and phytoplankton and bacterial abundances. TN: Total Nitrogen; TDN: Total Dissolved Nitrogen; NO^-_3: Nitrate; TP: Total Phosphorus; TDP: Total Dissolved Phosphorus; SRP: Soluble Reactive Phosphorus; Chl *a*: Chlorophyll *a* concentration; PA: Phytoplankton Abundance; PB: Phytoplankton Biomass; BA: Bacterial Abundance. BB: Bacterial Biomass

Variable	high-UVR lake	low-UVR lake
kd_{305}	0.61	4.84
kd_{320}	0.52	2.53
kd_{380}	0.34	0.93
kd_{PAR}	0.25	0.28
TN (µM)	21.50 ± 1.54	787.1 ± 10.7
TDN (µM)	20.71 ± 1.46	786.4 ± 12.9
NO_3^-(µM)	14.28 ± 1.02	702.1 ± 6.7
TP (µM)	0.10 ± 0.003	0.06 ± 0.012
TDP (µM)	0.051 ± 0.002	0.038 ± 0.012
SRP (µM)	0.02 ± 0.001	0.018 ± 0.012
Chl *a* (µg L^{-1})	2.02 ± 0.42	2.66 ± 0.46
PA (cell mL^{-1}) x 10^3	7.03 ± 1.65	4.03 ± 0.72
PB (µgC L^{-1})	15.10 ± 4.31	95 ± 5.72
BA (cell mL^{-1}) x 10^6	1.94 ± 0.17	1.28 ± 0.21
BB (µgC L^{-1})	8.66 ± 1.32	0.98 ± 0.03

Table 2: Mean irradiances in subsurface and mixed layers during the incubations for 305 nm, 320 nm and 380 nm within the UVR wavelengths (μW cm^{-2} nm^{-1}) and for PAR (μmol photons m^{-2} s^{-1}). The ratio of the mean irradiances of 380 and 305 nm is also presented.

Wavelength		305 nm	320 nm	380 nm	PAR	UV-A_{380}:UV-B_{305}
high-UVR lake	Subsurface	3.90	23.40	60.10	1480	15.41
	Mixed	1.40	9.50	31.50	900	22.50
low-UVR lake	Subsurface	1.44	12.90	47.90	1428	33.26
	Mixed	0.16	1.80	12.80	824	80.00

Table 3. Results of the two-way ANOVA of the interactive effect of "UVR" (PAB, PA, P, Dark) and "stratification (subsurface and mixed) factors on carbon incorporation of phytoplankton (PP, in µgC L^{-1} h^{-1}), and Excreted Organic Carbon (EOC, in µgC L^{-1} h^{-1}), Heterotrophic Bacterial Production (HBP, in µgC L^{-1} h^{-1}), Bacterial Respiration (BR, in µgC L^{-1} h^{-1}) was directly measured in the high-UVR lake or it was calculated as 50% of Total Planktonic Respiration (TPR) in the low-UVR lake; Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD):Excreted Organic Carbon (EOC; as a percentage). Numbers in bold indicate, $p < 0.05$. df1, df2, and df3, df4, are the degrees of freedom.

		PP				EOC		%EOC		HBP				BR		BGE		BCD:EOC (%)	
		df_1	df_2	$F_{df1,df2}$	*p*	$F_{df1,df2}$	*p*	$F_{df1,df2}$	*p*	df_3	df_4	$F_{df3,df4}$	*p*	df_1	df_2	$F_{df1,c}$	*p*	$F_{df1,df2}$	*p*	$F_{df1,df2}$	*p*
high-UVR lake																					
Epilimnetic	STRAT	1	12	42.29	**<0.001**	44.00	**<0.001**	0.02	0.896	1	16	6.41	**0.022**	1	12	1.07	.321	0.26	0.619	6.15	**0.029**
	UVR	2	12	124.12	**<0.001**	6.33	**0.013**	27.25	**<0.001**	3	16	8.65	**0.001**	2	12	12.38	**0.001**	7.22	**0.009**	35.47	**<0.001**
	UVR x STRAT	2	12	20.90	**<0.001**	0.11	0.895	0.80	0.473	3	16	5.46	**0.009**	2	12	3.71	0.056	4.80	**0.029**	14.59	**0.001**
low-UVR lake																					
Epilimnetic	STRAT	1	12	0.61	0.450	2.46	0.143	0.24	0.634	1	16	7.37	**0.015**	1	8	5.28	**0.05**	1.45	0.263	18.76	**0.002**
	UVR	2	12	6.78	**0.011**	9.78	**0.003**	0.01	0.986	3	16	27.96	**<0.001**	1	8	0.14	0.72	46.13	**<0.001**	14.42	**0.005**
	UVR x STRAT	2	12	16.71	**<0.001**	16.51	**<0.001**	0.21	0.816	3	16	6.38	**0.005**	2	8	0.63	0.45	0.06	0.810	44.86	**<0.001**
	STRAT	2	12	0.33	0.574	4.33	0.060	0.02	0.899	1	16	32.98	**<0.001**	1	8	0.29	0.604	6.01	**0.040**	4.65	0.063
Hypolimnetic	UVR	2	12	41.58	**<0.001**	52.75	**<0.001**	2.51	0.123	3	16	12.05	**<0.001**	1	8	8.39	**0.020**	0.15	0.711	0.81	0.394
	UVR x STRAT	2	12	0.39	0.688	3.21	0.076	0.63	0.547	3	16	7.98	**0.002**	2	8	0.90	0.372	5.24	0.061	1.99	0.196

Table 4. Effect size of UV-B and UV-A on primary production (PP, in µgC L^{-1} h^{-1}); and heterotrophic bacterial production (HBP, in µgC L^{-1} h^{-1}) in the experimental conditions. The change (Δ) in effect size of UV-B and UV-A was calculated as difference effect size of UV-B and UV-A between subsurface and mixed treatments. Numbers in bold indicate $p < 0.05$

		PP				HBP			
		%UV-B	Δ%UV-B	%UVA	Δ%UV-A	%UV-B	Δ%UV-B	%UV-A	Δ%UV-A
high-UVR lake									
Epilimnetic	Subsurface	37.3 ± 2.4	**11.55**	25.6 ± 7.6	**18.32**	2.7 ± 18.3	-20.3	51.9 ± 26.7	**110.2**
	Mixed	25.7 ± 5.0		7.3 ± 7.1		23.0 ± 1.5		-58.3 ± 0.2	
low-UVR lake									
Epilimnetic	Subsurface	33.7 ± 4.2	**40.00**	17.4 ± 13.9	27.41	42.9 ± 6.2	-4.2	30.0 ± 8.7	1.2
	Mixed	-6.3 ± 10.9		-10.0 ± 23.5		47.1 ± 2.0		28.2 ± 6.7	
Hypolimnetic	Subsurface	27.2 ± 22.5	0.09	20.8 ± 28.9	-5.98	52.1 ± 5.8	**45.6**	12.0 ± 24.4	-11.5
	Mixed	27.1 ± 5.6		26.8 ± 12.8		6.5 ± 12.2		23.6 ± 2.6	

Figure captions

Fig. 1: Water column characteristics of the high-UVR lake (a, c), and low-UVR lake (b, d). Depth profiles of temperature (°C), and dissolved organic carbon (DOC in µM) (a, b); phytoplanktonic and bacterial abundances (cell mL^{-1}), and chlorophyll *a* concentration (µg L^{-1}) (c, d). Each symbol represents the mean of triplicate samples while the horizontal error lines are the standard deviation.

Fig. 2: Metabolic variables of epilimnetic community under different radiation treatments (PAB, PA, PAR, Dark) and different stratification conditions (subsurface versus mixed) in the high-UVR lake. (a) Primary Production (PP, in µgC L^{-1} h^{-1}); (b) Excretion of Organic Carbon rates (EOC, in µgC L^{-1} h^{-1}); (c) Heterotrophic Bacterial Production (HBP, in µgC L^{-1} h^{-1}); (d) Bacterial Respiration (BR, in µgC L^{-1} h^{-1}); (e) Bacterial Growth Efficiency (BGE); (f) Bacterial Carbon Demand :Excretion of Organic Carbon ratio (BCD:EOC) as a percentage. The horizontal line in (f) represents a balanced commensalistic phytoplankton-bacteria relationship. The vertical error lines on top of the bars are the standard deviation whereas the letters indicate differences among treatments.

Fig. 3: Metabolic variables of epilimnetic community under different radiation treatments (PAB, PA, PAR, Dark) and different stratification conditions (subsurface versus mixed) in the low-UVR lake. (a) Primary Production (PP, in µgC L^{-1} h^{-1}); (b) Excretion of organic carbon rates (EOC, in µgC L^{-1} h^{-1}); (c) Heterotrophic Bacterial Production (HBP, in µgC L^{-1} h^{-1}), (d) Bacterial Respiration ($BR_{50\%}$ in µgC L^{-1} h^{-1}) calculated as 50% of Total Planktonic Respiration (TPR); (e) Bacterial Growth Efficiency (BGE); (f) Bacterial Carbon Demand: Excretion of Organic Carbon ratio (BCD:EOC) as a percentage. The broken-lines indicate the min-max range of BCD:EOC ratio, with BCD calculated assuming BR as either 50% or 75% of TPR. The horizontal line in (f) represents a balanced commensalistic phytoplankton-bacteria relationship. The vertical error lines on top of the bars are the standard deviation whereas the letters indicate differences among treatments.

Fig. 4: Metabolic variables of hypolimnetic community under different radiation treatments (PAB, PA, PAR, Dark) and different stratification conditions (subsurface versus mixed) in the low-UVR lake. (a) Primary Production (PP, in µg C L^{-1} h^{-1}); (b) Excretion of organic carbon rates (EOC, in µg C L^{-1} h^{-1}); (c) Heterotrophic Bacterial

Production (HBP, in µg C L^{-1} h^{-1}), (d) Bacterial Respiration ($BR_{50\%}$ in µg C L^{-1} h^{-1}) calculated as 50% of Total Planktonic Respiration (TPR); (e) Bacterial Growth Efficiency (BGE); (f) Bacterial Carbon Demand: Excretion of Organic Carbon ratio (BCD:EOC) as percentage, calculated assuming BR as either 50% or 75% of TPR. The horizontal line in (f) represents a balanced commensalistic phytoplankton-bacteria relationship. The vertical error lines on top of the bars are the standard deviation whereas the letters indicate differences among treatments.

Fig. 5: Epilimnetic phytoplankton-bacteria relationship under PAB-subsurface and PAB-mixed conditions in high-UVR lake (a, c) and low-UVR lake (b, d). The sizes of the boxes are proportional to the magnitude of the rates (in µgC L^{-1} h^{-1}). The absolute numbers are values of excretion of organic carbon (EOC) rates and Bacterial Carbon Demand (BCD), and the percentage numbers are values of %BCD:EOC ratio. The thicknesses of the arrows indicate the relative magnitude of a particular carbon flux. The broken-lines arrows indicate that EOC is not enough to satisfy BCD. Thick black lines represent the %BCD:EOC ratio, indicating either coupled (solid lines) or uncoupled (broken lines) phytoplankton-bacteria relationship. PP: Primary Production; HBP: Heterotrophic Bacterial Production; BGE:Bacterial Growth Efficiency. Numbers are rates of C flux (in µgC L^{-1} h^{-1}).

Appendix A

Fig. A. Percentage of excretion of organic carbon rates (%EOC) under different radiation (PAB, PA, PAR) and stratification conditions (subsurface versus mixed) in (1) epilimnetic community in the high-UVR lake, (2) epilimnetic community in the low-UVR lake, (3) hypolimnetic community in the low-UVR lake. The vertical error lines on top of the bars are the standard deviation whereas the letters indicate differences among treatments.