Effects of the photochemical transformation of dissolved organic matter on bacterial physiology and diversity in a coastal system

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Abstract

The effect of solar radiation on dissolved organic matter (DOM) and the subsequent impact of photo-altered DOM on bacterial activity and community structure were assessed during two experiments in the coastal system of the Ría de Vigo (NW Iberian Peninsula). After exposure of 0.2 μ m filtered seawater for 3.5 days to dark and full sunlight, an inoculum of the bacterial community collected at the same time as the exposed water was added and the mixture was incubated for 4 days in the dark at 15°C. Changes in bacterial production (BP), diversity (assessed by Fluorescence in situ hybridization) and electron transport system (ETS) activity, dissolved organic carbon (DOC) and nitrogen (DON) and DOM humic-like absorption and fluorescence were followed. The exposure to sunlight had no effect on DOM concentrations while an average ($\pm$ SD) decrease in DOM humic fluorescence of 45 ± 10 % was found. The incubations with photo-altered DOM had lower BP (57 ± 11 %), ETS (42 ± 9 %) and bacterial carbon demand (BCD) (42 ± 8 %) compared with the dark incubations, while bacterial growth efficiency (BGE) was unaffected. This suggests that DOM photo-alteration had a negative effect on bacterial metabolism in the study system. The bacterial growth on irradiated DOM resulted in a significant enrichment of the *Gammaproteobacteria* group compared with the dark control, indicating that solar exposure of DOM led to rapid changes in the bacterial community composition of the Ría de Vigo.

Keywords: DOM, Photochemistry, Bacterial activity, CARD-FISH

1. Introduction

Dissolved organic matter (DOM) constitutes the main substrate for heterotrophic bacterial growth and respiration in marine systems (Hedges, 2002). DOM in coastal waters can be differentiated according to its origin as autochthonous or allochthonous. Autochthonous DOM is produced within the system and originates largely from phytoplankton exudation, cell lysis, and grazing (Nagata, 2000), whereas allochthonous DOM primarily originates from terrestrial plant and soil materials (Cauwet, 2002).

There is considerable evidence that coloured DOM (CDOM) can be transformed by UV-B (280–320 nm) and UV-A (320–400 nm) solar radiation into reactive oxygen species (ROS) (Scully et al., 2003), inorganic carbon species (CO_2 and CO) (Bertilson and Tranvik 2000), labile organic and inorganic compounds (Kieber et al., 1989) and further induce the production of biological refractory humic substances (Harvey et al., 1983; Kieber et al., 1997). ROS species may enhance microbial activity, since they can convert biological refractory DOM into labile forms, while they can also lower microbial activity by competing for substrates and causing oxidative stress (Scully et al., 2003; Lesser, 2006). The direct photomineralization of DOC to CO or CO_2 does not provide new bacterial substrate (Bertilson and Tranvik 2000), while the photochemical conversion of biological refractory DOM into new organic and inorganic compounds may stimulate bacterial growth (Vähatalo et al., 2003). Contrarily, sunlight can also induce the biological polymerization and condensation of DOM into refractory materials, representing a source of CDOM (Harvey et al., 1983; Kieber et al., 1997). While some studies suggest that autochthonous DOM gets less and allochthonous DOM more bioavailable after UV exposure, others studies have found the converse (Obernosterer and Herndl 2000; Tranvik and Bertilson, 2001; Sulzberger and Durisch-Kaiser, 2009).

The bacterial community in marine waters is often dominated by a few major bacterial groups: *Alpha*-, *Beta*- and *Gammaproteobacteria*, and the group *Bacteroidetes* (Giovannoni and Rappé, 2000; Giovannoni and Stingl, 2005). Several studies have shown that the DOM composition influences the bacterial community composition (Pérez and Sommaruga, 2006; Teira et al., 2009). However, little is known about the influence of photochemically altered DOM on the bacterial community composition. A recent study by Piccini et al., (2009) in a coastal lagoon suggests that photochemical transformation of DOM stimulates the growth of the *Alpha*- and *Betaproteobacteria* groups. But this finding needs to be investigated in more detail before we can come with general conclusions.

In the present study, we assessed the bacterial community response to irradiated DOM in a coastal system dominated by autochthonous inputs. We hypothesize that photochemically altered DOM will have a negative impact on bacterial activity and change the bacterial community composition.

2. Material and methods

2.1. Sampling site

The Ría de Vigo is a large coastal embayment, with a length of 33 km, a surface area of 176 km², and a volume of 3.32 km³. Its hydrographic regime is dictated by wind-driven upwelling and downwelling episodes; northerly winds result in upwelling, which prevails from April to October, with nutrient-rich upwelled water reaching the surface resulting in a high productivity. From November to March, southerly winds predominate, resulting in downwelling forcing warm and nutrient-poor shelf surface water into the ría (Álvarez-Salgado et al., 2003). In the winter, the phytoplankton community consists mainly of small phytoplankton species and diatoms which are followed by a spring diatom bloom. During upwelling episodes, phytoplankton primary production reaches a mean value of 2.5 g Cm⁻² d⁻¹, which decreases to < 0.3 g Cm⁻² d⁻¹ during downwelling

episodes (see review by Álvarez-Salgado et al., 2010). The bacterial abundance varies seasonally between 0.3 and 2.7×10^9 cells l^{-1} and the bacterial production reaches levels between 2.8 and 66.7 $mg\ C\ l^{-1}\ d^{-1}$ (Lekunberri et al., 2010; Teira et al., 2013). The bacterioplankton community is dominated by the groups *Bacteroidetes* and *Roseobacter* (Alonso-Gutiérrez et al., 2009; Teira et al., 2008), although SAR11 can become occasionally abundant in this eutrophic system (Teira et al., 2009).

Two separate experiments were conducted during winter (14 February 2008) and spring (24 April 2008) with water samples collected in the middle segment of the Ría de Vigo ($42^\circ 14.5'N$ and $8^\circ 45.8'W$). Previous studies have shown large differences in DOM concentration and composition between these two periods (Nieto-cid et al., 2005; Lønborg et al., 2010a). Samples were taken at 5 m depth using a $25\ l$ Niskin bottle. Salinity and temperature were measured prior to the sampling with an SBE 9/11 CTD probe. Samples for chlorophyll *a* (Chl. *a*) were collected by filtering seawater (100 - $200\ ml$) through a GF/F filter and analyzed after $90\ \%$ acetone extraction with a Turner Designs 10000R fluorometer (Yentsch and Menzel, 1963).

2.2. Experimental design

Filtration of the collected seawater started within $10\ min$ of collection; one part was filtered through pre-combusted ($450^\circ C$ for $4\ h$) GF/C filters to establish a microbial culture, which was kept in the dark at $15^\circ C$ until used. The second part was gravity filtrated through a dual-stage (0.8 and $0.2\ \mu m$) filter cartridge (Pall-Acropak supor Membrane), which had been pre-washed with Milli-Q water ($>10\ l$).

In the laboratory, the $0.2\ \mu m$ filtered seawater was divided into two experimental treatments; dark (dark) and full sun light treatment (UV). The dark treatments were established by placing the $0.2\ \mu m$ filtered seawater in $500\ ml$ glass bottles covered with aluminium foil and dark plastic bags. The water for the UV treatments was distributed into $270\ ml$ quartz tubes (diameter: $5\ cm$). No

headspace was left in either the glass bottles or the quartz tubes, and all incubators were sealed with ground glass stoppers. The samples were thereafter placed in a recirculation water bath (water depth: 2.5 cm) in the terrace of the laboratory and exposed to 100 % natural sunlight for 3.5 days encompassing the natural light/dark cycle. This exposure time was fixed on basis of the decay rate constants of coloured dissolved organic matter previously obtained by Nieto-Cid et al., (2006) in winter (0.25 d^{-1}) and spring (0.40 d^{-1}) in the Ría de Vigo. At these rates, a colour loss of 60-75% would occur in 3.5 days. Before and after sunlight exposure, subsamples were collected for the analysis of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN: NH_4^+ , NO_2^- , NO_3^-) and phosphate (DIP: HPO_4^{2-}), and DOM optical properties (absorption and induced fluorescence). Within 30 min after sunlight exposure, the water was combined into two different (dark and UV) carboys and the microbial community collected at the time of sample collection (3.5 days before), was added in a ratio of 1 part of microbial culture to 9 parts of exposed water. The water was thereafter transferred into 500 ml glass bottles and incubated in the dark at a constant temperature of 15°C , with four replicate bottles being used for sub-sampling at incubation times 0, 1, 2, and 4 days. Unfiltered water from these bottles was used to follow changes in bacterial abundance (BA), production (BP), electron transport system (ETS) activity and diversity using Catalysed Reporter Deposition-Fluorescence In Situ Hybridisation (CARD-FISH). Samples for the analysis of DIN, DIP, DOC, TDN and CDOM absorption were collected in four replicates at day 0 and 4. DOM fluorescence (FDOM) was measured at incubation day 0, 1, 2, and 4. Samples for the dissolved phase were collected after filtration through $0.2 \mu\text{m}$ filters (Pall Supor membrane Disc) in an acid-cleaned glass filtration system under low N_2 flow pressure. All glassware used was acid washed in 10 % HCl and rinsed with Milli-Q and sample water prior to use.

2.3. Sample analysis

BA was determined on day 0, 1 and 2 by fixing the samples (1-2 h) with formol in the dark, filtered onto 0.2 μm polycarbonate filters, and stored at -20°C until counted. The samples were stained with a DAPI-mix before counted on a Leica DMBL microscope equipped with a 100-W Hg-lamp; more than 800 DAPI-stained cells were counted per sample. Bacterial biomass was calculated from BA, using a carbon conversion factor of $30 \text{ fg C cell}^{-1}$, which is representative for coastal bacterial assemblages (Fukuda et al., 1998).

BP was measured by [^3H] thymidine incorporation (Fuhrman and Azam, 1980) on days 0, 1 2 and 4. Four replicate 9.9-ml samples and 2 trichloroacetic acid killed samples were added an aqueous stock solution of [^3H - methyl] thymidine (40 nmol final concentration). The samples were incubated in the dark at 15°C for 2 h, 10 ml of ice-cold Trichloroacetat (TCA) was thereafter added and samples were filtered onto 0.2 μm polycarbonate filters (presoaked in non-labelled thymidine), washed with 95% ethanol and autoclaved Milli-Q water. The filters were hereafter dried at room temperature (24 h) and mixed with 10 ml of scintillation fluid (Sigma-Flour). The radioactivity incorporated into cells was counted using a Beckman spectral liquid scintillation counter. Thymidine incorporated into bacterial biomass was converted to carbon production using the theoretical conversion factors $2 \times 10^{18} \text{ cells mol}^{-1}$ thymidine (Fuhrman and Azam, 1980) and a carbon conversion factor of $30 \text{ fg C cell}^{-1}$ (Fukuda et al., 1998).

The ETS activity was measured on days 0, 1, 2 and 4 using the reduction of 2-(4-iodophenyl)-3-(4-nitro-phenyl)-5-phenyl tetrazolium chloride (INT) (Martínez-García et al., 2009). The activity was measured using 1 h incubations of 3 replicate samples (10 ml) and 1 formol-killed control. The incubations were terminated by adding formol and filtering onto 0.2 μm polycarbonate filters. The filters were hereafter stored frozen (-20°C) until further processing (Martínez-García et al., 2009). The respiration rates derived from ETS activity (R_{ETS} , in $\mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$) were obtained by multiplying the in vivo ETS activity (in $\mu\text{mol INTF l}^{-1}$) by an empirically derived conversion factor

of 12.8 (Martínez-García et al., 2009). The microbial respiratory quotient (RQ), defined as the ratio of mol CO₂ production per mol O₂ was calculated using the average RQ values (range 1.35-1.53) calculated by Lønborg et al., (2010a) for the same sampling period.

The bacterial carbon demand (BCD) was calculated as the sum of BP and ETS activity:

$$BCD = BP + ETS \quad (1)$$

The bacterial growth efficiency (BGE) was calculated as BP divided by the sum of BP and ETS activity:

$$BGE = BP / (BP + ETS) \quad (2)$$

Changes in the bacterial community composition were monitored using CARD-FISH at day 0, 1 and 2. Initially, 15 ml of sample was fixed with 0.2 µm filtered formaldehyde (1-2 % final conc.) and, subsequently, the samples were stored at 4°C in the dark for 12-18 h. Thereafter, each sample was filtered through a 0.2 µm polycarbonate filter (Millipore, GTTP, 25 mm filter diameter) supported by a cellulose nitrate filter (Millipore, 0.45 µm), washed twice with Milli-Q water, dried and stored in a vial at -20°C until further treatment. The abundance of different bacterial populations was determined using a mix of oligonucleotide probes specific for the domain *Eubacteria* (EUB338, EUB338II, EUB338III) (Daims et al., 1999), the *Beta*- (BET42a) (Manz et al. 1992) and *Gammaproteobacteria* (GAM42a) (Manz et al., 1992) subclasses and the class *Flavobacteria* of phylum *Bacteroidetes* (CF319a) (Manz et al., 1996). Additionally, the relative abundance of the SAR11, *Roseobacter*, and SAR86 clusters was also analysed using the specific probes SAR11-441R (Morris et al., 2002), Ros537 (Eilers et al., 2001), and SAR86/1245 (Zubkov et al., 2001). The Eub antisense probe Non338 probe was used as negative control. Filters for CARD-FISH were embedded in low-gelling-point agarose and incubated with lysozyme (Pernthaler et al., 2002; Teira et al., 2004). Filters were cut in sections and hybridized at 35°C with horseradish peroxidase (HRP)-labeled oligonucleotide probes for 2-4 h. Tyramide-Alexa488 was used for signal

amplification (30-40 min) (Teira et al., 2004). We used 55 % of formamide for all probes excepting for SAR11-441R (45 % formamide). Cells were counter-stained with a 4',6-diamidino-2-phenylindole (DAPI)-mix (5.5 parts of Citifluor [Citifluor, Ltd.], 1 part of Vectashield [Vector Laboratories, Inc.] and 0.5 parts of PBS with DAPI (final concentration $1 \mu\text{g ml}^{-1}$). The slides were examined under a Leica DMBL microscope equipped with a 100-W Hg-lamp and appropriate filter sets for DAPI and Alexa488. For each microscope field, 2 different categories were enumerated: 1) total DAPI-stained cells and 2) cells stained with the specific probe. Negative control counts (hybridization with HRP-Non338) averaged 0.5 % of DAPI-stained cells. The counting error, expressed as the percentage of standard error between replicates, was, on average, < 5 % for DAPI counts and < 10 % for CARD-FISH counts. The counting error was relatively higher for the less abundant groups (*Betaproteobacteria* and SAR86).

Inorganic nutrients (NH_4^+ , NO_2^- , NO_3^- and HPO_4^{2-}) were determined by standard segmented flow analysis (SFA) as described in Hansen and Koroleff, (1999). DOC and TDN were measured using a Shimadzu TOC analyser (Pt-catalyst) connected to an Antek-TN measuring unit. Three to five replicate injections of 150 μl were performed per sample. Concentrations were determined by subtracting a Milli-Q blank and dividing by the slope of a daily standard curve of potassium hydrogen phthalate and glycine. Using the deep ocean reference (Sargasso Sea deep water, 2600 m) we obtained a concentration of $46.0 \pm 2.0 \mu\text{mol C l}^{-1}$ (average $\pm$ SD) for DOC and $22.0 \pm 2.0 \mu\text{mol N l}^{-1}$ for TDN. DON concentrations were calculated as the difference between TDN and DIN (DON = TDN – DIN). The DOM consumed over the 4 days incubation was here defined as the bio-available pool (BDOM), and the remaining as the bio-resistant pool (RDOM).

The CDOM absorption was measured in four replicates on a Beckman Coulter DU 800 spectrophotometer using Milli-Q water as a blank. Before analysis samples were warmed to room

temperature. The absorption was measured at a wavelength of 340 nm using a 10 cm quartz cuvette.

The absorption coefficient, $a_{\text{CDOM}}(340)$, was calculated as:

$$a_{\text{CDOM}}(340) = \frac{2.303 \cdot A(340)}{L} \quad (3)$$

Where $A(340)$ is the absorbance measured at 340 nm minus the average residual absorbance from 600 to 700 nm (m^{-1}). The factor 2.303 converts from base 10 to base e logarithms and the denominator L is the cell path-length in meters.

The CDOM fluorescence was measured in four replicates on a Perkin Elmer LS 55 luminescence spectrophotometer equipped with a xenon discharge lamp, equivalent to 20 kW for 8 μs duration. Measurements were performed at a constant temperature of 20°C in a 1 cm quartz fluorescence cell. Milli-Q water was used as a reference and Excitation/emission (Ex/Em) measurements were performed at Ex/Em = 340 nm/440 nm (termed $F(340/440)$) characteristic of humic-like materials (Coble, 1996; Lønborg et al., 2010b). $F(340/440)$ were expressed in ppb equivalents of quinine sulphate (QSU), by calibrating the LS 55 Perkin Elmer at Ex/Em: 350 nm/450 nm against a quinine sulphate dihydrate (QS) standard dissolved in 0.05 mol l^{-1} sulphuric acid.

In this paper unpaired t-tests as described in Sokal and Rohlf (1995) were used to test the significance (95% confidence level) of the differences observed in bacterial abundance, activity, and relative abundance of different phylogenetic groups between the Dark and UV treatments, with all statistical analysis conducted in Statistica 6.0.

3. Results

3.1. Environmental chemical and optical conditions

Contrasting hydrographic conditions occurred during the two study periods: salinities were 35.4 and 25.0, temperatures 13.4 and 15.5 °C and Chl a concentrations 1.13 and 8.42 mg m^{-3} during

the winter and spring samplings, respectively. Both DIP and DIN concentrations were higher during winter, while DOC, DON, $a_{\text{CDOM}}(340)$ and $F(340/440)$ were higher during spring (Table 1). The $F(340/440)$ to DOC ratio indicated a larger allochthonous influence and lower DOM quality during spring (Table 1).

Incident irradiance during the UV treatments was taken from the meteorological observatory of the Cies Islands, ~ 10 km from the base laboratory, showing that over the 3.5 days the UV samples were exposed to 268 kJ (winter) and 890 kJ (spring) of total solar radiation. These solar intensities are comparable to average values found for these periods in the NW Iberian Peninsula.

3.2. Photochemical induced changes in chemical and optical parameters

The 3.5 days dark and UV-light exposure did not have any significant impact on nutrient or DOM concentrations (Table 1). In the dark treatments, significant differences were not found in $a_{\text{CDOM}}(340)$ and $F(340/440)$ compared with the field data, while strong changes occurred in the UV treatments (Table 1). The UV-light degradation led to a decrease of $a_{\text{CDOM}}(340)$ of $0.06 \pm 0.02 \text{ m}^{-1}$ (about 20% of the initial absorption) in winter and $0.36 \pm 0.06 \text{ m}^{-1}$ in spring (about 50% of the initial absorption). For the case of $F(340/440)$, the decrease was $0.6 \pm 0.2 \text{ QSU}$ (about 40% of the initial fluorescence) in winter and $1.6 \pm 0.2 \text{ QSU}$ (about 50% of the initial fluorescence) in spring. DOM absorption and fluorescence were significantly different in the dark and UV treatments (t-test, $p < 0.003$, $n = 8$). The $F(340/440)$ to DOC ratio in the dark treatment was not different from the field measurements, while decreases were found in the UV-light exposed samples (Table 1).

3.3. Effects of photochemical alterations on the bacterial community

After the UV exposure, water samples were inoculated with the original bacterial community of the exposed water. The bio-available DOC (BDOC) measured over the 4 days incubation period was $4 \pm 2 \text{ (UV)}$ and $12 \pm 3 \text{ } \mu\text{mol C l}^{-1}$ (Dark) in winter and $13 \pm 4 \text{ (UV)}$ and $11 \pm 2 \text{ } \mu\text{mol C l}^{-1}$

(Dark) in spring (Table 1). Bio-available DON (BDON) varied only slightly (between 1.4 ± 0.7 and $1.7 \pm 1.0 \mu\text{mol N l}^{-1}$), and there was no difference between the dark and UV treatments (Table 1). During the 4 days incubation, $a_{\text{CDOM}}(340)$ and $F(340/440)$ remained constant in both the dark and UV treatments (data not shown).

The UV pre-treatment had a negative impact on the BP compared with the dark treatment during both experiments (Table 1; Figure 1a, b). The BP peaked in both periods at day 0 or 1, decreasing thereafter reaching average values of 0.13 ± 0.09 and $0.02 \pm 0.01 \mu\text{mol C l}^{-1} \text{ d}^{-1}$ after 4 days in the dark and UV treatments, respectively (Table 1; Figure 1a, b). The impact of UV pre-exposure on the BP was evaluated by comparing the average BP measured at each sampling day in the two treatments, showing a statistical significant difference (t-test, $p < 0.05$, $n = 8$), suggesting a negative impact of DOM photoalteration on BP.

The initial BA was 7.9 (winter) and 7.1 (spring) $\times 10^5$ bacteria ml^{-1} , increasing to maximum abundances of 21.1 (winter) and 11.2 (spring) $\times 10^5$ bacteria ml^{-1} in the dark and 19 (winter) and 14.0 (spring) $\times 10^5$ bacteria ml^{-1} in the UV treatment at day 1 (Figure 1c, d). This corresponded to an average bacteria biomass growth (BG) of $2.2 \pm 1.6 \mu\text{mol C l}^{-1}$ in the dark and $1.6 \pm 0.1 \mu\text{mol C l}^{-1}$ in the UV treatment. There was no statistical significant difference in BA between the two pre-treatments (t-test, $p > 0.05$, $n = 6$). Microscopic examination revealed that no heterotrophic nanoflagellates were present in both types of incubations (data not shown).

The initial ETS activity was higher in the dark treatments (Table 1). It increased after day 0 reaching values between 4.4 ± 0.2 and $6.2 \pm 0.2 \mu\text{mol C l}^{-1} \text{ d}^{-1}$ in the dark and 2.4 ± 0.1 to $4.5 \pm 0.1 \mu\text{mol C l}^{-1} \text{ d}^{-1}$ in UV treatments after 1 to 2 days. In the winter experiment, the ETS activity declined after day 2, reaching final average values of 1.8 ± 0.1 (dark) and $0.2 \pm 0.1 \mu\text{mol C l}^{-1} \text{ d}^{-1}$ (UV). In the spring experiment, the ETS activity increased after day 2 in the dark treatment reaching final respiration rates of $5.7 \pm 0.6 \mu\text{mol C l}^{-1} \text{ d}^{-1}$, while it decreased in the UV treatments

reaching rates of $3.8 \pm 0.1 \mu\text{mol C l}^{-1} \text{d}^{-1}$ (Table 1: Figure 1 e, f). The impact of the UV pre-treatment on ETS activity was evaluated by comparing the average ETS measurements of 3 replicate samples for the two treatments and combining both sampling periods (winter and spring), which showed a statistical significant difference, suggesting that the DOM photoalteration had a negative impact on ETS activity (t-test, $p < 0.05$, $n = 8$).

The initial BCD was higher in the dark treatments during both experiments, with average $\pm$ SD values of $3.46 \pm 2.06 \mu\text{mol C l}^{-1} \text{d}^{-1}$ in the dark compared to $0.66 \pm 0.24 \mu\text{mol C l}^{-1} \text{d}^{-1}$ in the UV treatments (Table 1). The BCD peaked in both experiments at incubation day 1, decreasing thereafter to reach average values of 3.88 ± 2.06 (dark) and $2.04 \pm 1.80 \mu\text{mol C l}^{-1} \text{d}^{-1}$ (UV) after 4 days (Table 1). The integrated BCD (BCD_{int}) was calculated by integrating the BCD estimates over the incubation period, resulting in average values of 12.72 ± 11.73 (dark) and $11.34 \pm 5.50 \mu\text{mol C l}^{-1}$ (UV) (Table 1). A t-test comparing the average BCD showed a significant negative impact of the DOM photoalteration (t-test, $p < 0.05$, $n = 8$).

Initial BGE was on average $19 \pm 10 \%$ in the dark and $10 \pm 8 \%$ in the UV treatments (Table 1). The BGE generally declined after day 1, reaching average values of 3 ± 1 (dark) and $1 \pm 1 \%$ (UV) after 4 days incubation (Table 1; Figure 1g, h). The BGE was higher during winter, in agreement with the higher BG during this period. The integrated BGE (BGE_{int}) showed average values of 6 ± 2 (dark) and $5 \pm 2 \%$ (Table 1). There was no significant difference in BGE between the two pre-treatments (t-test, $p > 0.05$, $n = 8$).

The percentage of DAPI-counts detected by the mix of Eub338 probes represented on average $94 \pm 10 \%$ of the cells (Table 2). In the winter experiment, the initial bacterial community was dominated by *Bacteroidetes* (48 %), with only minor contributions of SAR11 (13 %) and *Roseobacter* (6 %) (Table 2). The contribution of *Roseobacter* increased in both treatments reaching 32 and 14 % in the UV-light and dark treatments at day 2. *Gammaproteobacteria* also increased in

both pre-treatments but the contribution was 2.3-fold larger in the UV-light than in the dark treatment, while *Bacteroidetes* decreased from 48 to 13 (dark) and 23% (UV) at day 2 (Table 2).

During spring, the bacterial community was initially dominated by *Bacteroidetes* (37 %) and *Gammaproteobacteria* (32 %), but *Roseobacter* (18 %) also contributed (Table 2). *Bacteroidetes* increased rapidly in the dark incubation reaching 54 % after 1 day of incubation, while it decreased in the UV treatment to 21 % (Table 2). The contribution of *Gammaproteobacteria* increased in the UV-light pre-treatment (48% at day 2) but decreased in the dark incubation (15%), while the *Roseobacter* was relatively more abundant in dark incubations. Both *Betaproteobacteria* and SAR86 showed very low relative abundances throughout the experiments (0–5 % of DAPI counts) (Table 2). The abundance of *Gammaproteobacteria* was positively impacted by the DOM photoalteration (t-test, $p < 0.05$), while none of the other groups showed statistically significant difference between the dark and UV treatments.

4. Discussion

Solar radiation has previously been shown to affect DOM bioavailability, nutrient dynamics and bacterial community composition (e.g. Abboudi et al., 2008; Obernosterer and Benner, 2004; Obernosterer et al., 1999; Tranvik and Bertilsson, 2001). Our study demonstrates a negative impact of photo-altered DOM on the bacterial activity of the Ria de Vigo, while the abundance of *Gammaproteobacteria* was positively impacted.

4.1. Photochemical induced changes on chemical and optical parameters

The effect of UV light on the DOM pool is determined by its origin and previous sunlight exposure (Obernosterer and Benner, 2004). Our UV treatments showed no detectable DOC (detection limit $\sim 2 \mu\text{mol C l}^{-1}$) photodegradation to inorganic carbon, while slight changes in DOC bioavailability were found (Table 1). In the UV treatments, a decrease in CDOM absorption and

F(340/440) to DOC ratio was found, suggesting changes in the DOM composition and quality (Table 1). The CDOM absorption at 340 nm decreased 20-50 %, which is comparable with other studies (< 50 %; Blough and Del Vecchio, 2002; Nieto-Cid et al., 2006).

4.2. *Effect of photochemical changes on the bacterial community during incubation studies*

Labile organic and inorganic compounds released from DOM by UV-light have been shown to stimulate bacterial production and respiration (e.g. Moran and Zepp, 1997). The decrease in humic-like fluorescence during the UV pre-treatments suggests that coloured, high molecular weight compounds were transformed into colourless low molecular weight forms or inorganic carbon; the final outcome for the bacterial production and ETS activity was negative. Decreases in bacterial activity after DOM exposure to sunlight has been reported before in other systems (e.g. Tranvik and Kokalj, 1998; Obernosterer et al., 1999) and has been explained by (1) DOC photo-oxidation to CO or CO₂ (Bertilson and Tranvik 2000); (2) production of ROS radicals (Scully et al., 2003); and/or (3) autochthonous DOM getting less bioavailable after UV exposure due to polymerization and condensation reactions (Harvey et al., 1983; Obernosterer et al., 1999). As we found no difference in DOM concentration between dark and UV treatments, photo-oxidation to CO or CO₂ is not a likely cause for the negative effect. ROS species can lower bacterial growth by competing for substrate and causing oxidative stress (Scully et al., 2003; Petasne and Zika, 1997; Lesser, 2006). In this study we did not measure ROS production, so we cannot exclude this as a possible reason for the decrease in bacterial activity. It has repeatedly been shown that photochemical transformations of allochthonous DOM enhance the bacterial production by converting UV-absorbing complex molecules into labile compounds (e.g. Moran and Zepp, 1997). In contrast, when autochthonous DOM is exposed to UV light it becomes less accessible to bacterial degradation (Tranvik and Bertilson, 2001). The net effect of UV radiation on DOM bioavailability depends therefore on the contribution of allochthonous and autochthonous organic matter.

Bioavailable DOM in the Ría de Vigo has previously been shown to be primarily of autochthonous marine phytoplankton origin (Lønborg et al., 2010a). As slightly higher BDOC values were found in the dark treatments during winter when the contribution of allochthonous DOM was lowest (high salinity and FDOM/DOC ratio), the most likely reason for the negative effect was that the originally labile DOM pool became more bio-resistant upon irradiation (Obornosterer et al., 1999). This suggests that solar-driven DOM transformation decreases the bacterial transfer of energy and nutrients to the microbial food web in the Ría de Vigo. Though, it should be kept in mind when extrapolating our findings that we only conducted experiments during the winter and spring periods.

The initial BGE was on average 19 ± 10 % in the dark and 10 ± 8 % in the UV treatments, which is comparable to values previously reported for the Ría de Vigo (range 7–55%; Lønborg et al. 2011) and the average value found for marine systems (~20%; del Giorgio and Cole 1998). During the 4 days incubation period we found a steep decrease in the BGE, suggesting that as the labile DOM got consumed more carbon was used for energy production than for biomass production (del Giorgio and Cole 1998), leading to low BGE values (< 4 %) at end of the incubations. The studies that have addressed how photo-altered DOM affects BGE have reported both positive and negative responses (e.g. Moran et al., 2000; Pullin et al., 2004). In our study, no significant effect of the UV exposure on BGE was found. This suggests that bacteria in the dark and UV incubations used the same energy per unit biomass produced and that BP and BR responded in the same way to photo-altered DOM. In this study, BGE was calculated using respiratory ETS activity. Most previous studies measuring ETS have measured potential in vitro activity (after ETS enzymes isolation and addition of a superabundance of electrons donors), showing large variations in the relation between respiration and ETS activity (R/ETS ratio) (Martínez-García et al., 2009). In this study we used the in vivo ETS activity (living cells are incubated and natural levels of electron donors are present) which has a rather constant R/ETS ratio (Martínez-García et al., 2009). This

suggests that the use of ETS activity as a measure of respiration cannot explain the lack of response in BGE. The differences in the response of BGE to photo-altered DOM between studies may therefore be explained by varying nutrient availability and DOM composition between study areas.

As the microbial cultures added to the laboratory incubations had been collected together with the exposed samples, they had been stored 3.5 days before use. This could have impacted the composition of the added bacterial community, but as the contribution of the different groups to the total bacterial abundance was approximately the same as that found by Teira et al. (2009), for the same sampling period, the storage seems to have had a minor effect. Alteration of DOM by solar radiation has previously been suggested to cause rapid changes in the bacterial community composition in marine systems, but few studies have identified which specific bacterial groups are influenced (Abboudi et al., 2008; Piccini et al., 2009). In this study, we based our bacterial community analysis on FISH probes which does not allow any detailed phylogenetic identification of the bacteria, but provides valuable information on the proportions and actual abundance of different phylogenetic groups. An enrichment of *Gammaproteobacteria* has repeatedly been observed during incubations and in response to changes in nutrient and organic matter composition (Hornák et al., 2006; Teira et al., 2008). Consistently with these findings, we observed that *Gammaproteobacteria* grew well in the dark (spring only) and UV pre-treated water but their growth was much more pronounced in the UV treatments, suggesting that members of this group were positively affected by photo-altered DOM. Some members of the *Gammaproteobacteria* group has frequently shown low percentages of active cells incorporating labile substrates, such as glucose, amino acids or protein (del Giorgio and Gasol, 2008), suggesting that this broad phylogenetic group might include a great diversity of specialized bacteria, some of which could be successful using less bioavailable photo-altered DOM. By contrast, Piccini et al., (2009) found that *Beta-* and *Alphaproteobacteria* dominated the DOM degradation after UV exposure. This difference

in community response is probably connected with the higher contribution of autochthonous DOM and different initial bacterial community composition in our system compared with the coastal lagoon investigated by Piccini et al., (2009).

Our findings suggest that photo-altered DOM has a negative impact on the bacterial metabolism and favours the growth of *Gammaproteobacteria* in the Ría de Vigo. Caution should be taken to extrapolate these results, as the experiments were only conducted in winter and spring. We therefore suggest that future studies will take a more detailed approach towards understanding the impacts of photo-alteration on specific DOM sources and the subsequent effects on the bacterial community.

Acknowledgement

This study was funded by fellowships to C.L from the early stage training site ECOSUMMER (MEST-CT-2004-020501) and the Carlsberg Foundation. We thank the captain, crew, and technicians of R/V *Mytilus* and the members of the Department of Oceanography of the Instituto de Investigaciones Mariñas for the collaboration during the sampling program. Access to vessel time was provided by the RAFTING project (Impact of the mussel raft culture on the benthic-pelagic coupling in a Galician Ria, grant number: CTM2007-61983/MAR). E.T. was founded by a Juan de la Cierva and a Ramón y Cajal MEC-contract.

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Table 1. Concentrations of phosphate (DIP), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), dissolved humic-like substances (F(340/440)) and CDOM absorption coefficient at 340 nm ($a_{\text{CDOM}}(340)$) at the sampling site (Field) in the Ría de Vigo and after 3.5 days in the darkness (dark) or exposed to natural sunlight (UV). Initial concentrations of dissolved organic carbon (DOC) and nitrogen (DON) at the sampling site and in the incubations (day 0), at incubation day 4 (DOC_4 and DON_4) and the corresponding bioavailable fraction (BDOC and BDON). The F(340/440) /DOC ratio is shown for the sampling site and after 3.5 days exposure to dark and UV-light. Bacterial production (BP), electron transport system (ETS) activity, bacterial carbon demand (BCD) and growth efficiency (BGE) are shown for incubation days 0 (BP_0 , ETS_0 , BCD_0 , BGE_0) and 4 (BP_4 , ETS_4 , BCD_4 , BGE_4) and the integrated BCD (BCD_{Int}) and BGE (BGE_{Int}) over the incubation time. Values are averages $\pm$ standard deviation; –, not measured.

	14/02/2008			24/04/2008		
	Field	Dark	UV	Field	Dark	UV
DIP ($\mu\text{M P}$)	0.42 ± 0.01	0.38 ± 0.06	0.41 ± 0.08	0.09 ± 0.01	0.05 ± 0.03	0.09 ± 0.05
NO_3^- ($\mu\text{M N}$)	5.77 ± 0.01	5.74 ± 0.09	5.71 ± 0.20	3.94 ± 0.07	3.69 ± 0.02	3.75 ± 0.03
NO_2^- ($\mu\text{M N}$)	0.33 ± 0.01	0.33 ± 0.10	0.29 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.11 ± 0.01
NH_4^+ ($\mu\text{M N}$)	2.62 ± 0.11	3.19 ± 0.20	2.69 ± 0.31	2.43 ± 0.13	2.57 ± 0.61	2.52 ± 0.47
F(340/440) (ppb QSU)	1.60 ± 0.04	1.67 ± 0.10	1.06 ± 0.04	2.92 ± 0.05	2.97 ± 0.5	1.35 ± 0.04
$a_{\text{CDOM}}(340)$ (m^{-1})	0.23 ± 0.01	0.22 ± 0.03	0.17 ± 0.02	0.64 ± 0.01	0.65 ± 0.02	0.30 ± 0.02
DOC ($\mu\text{M C}$)	73 ± 1	76 ± 2	76 ± 2	85 ± 1	85 ± 1	85 ± 1
DOC_4 ($\mu\text{M C}$)	–	65 ± 1	72 ± 1	–	73 ± 1	72 ± 2
BDOC ($\mu\text{M C}$)	–	12 ± 3	4 ± 2	–	11 ± 2	13 ± 4
DON ($\mu\text{M N}$)	4.5 ± 0.4	4.3 ± 0.3	4.6 ± 0.3	6.1 ± 0.2	6.1 ± 0.3	6.0 ± 0.4
DON_4 ($\mu\text{M N}$)	–	2.6 ± 0.4	2.9 ± 0.6	–	4.8 ± 0.4	4.6 ± 0.2
BDON ($\mu\text{M N}$)	–	1.7 ± 0.6	1.7 ± 0.9	–	1.4 ± 0.7	1.4 ± 0.6
F(340/440)/DOC (ppb QSU (ppm C) $^{-1}$)	1.8 ± 0.1	1.8 ± 0.1	1.2 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	1.3 ± 0.1
BP_0 ($\mu\text{M C day}^{-1}$)	–	0.40 ± 0.03	0.08 ± 0.01	–	0.50 ± 0.01	0.02 ± 0.01
BP_4 ($\mu\text{M C day}^{-1}$)	–	0.04 ± 0.01	0.01 ± 0.01	–	0.22 ± 0.01	0.03 ± 0.01
ETS_0 ($\mu\text{M C day}^{-1}$)	–	1.01 ± 0.14	0.34 ± 0.05	–	5.02 ± 0.02	0.88 ± 0.10
ETS_4 ($\mu\text{M C day}^{-1}$)	–	1.78 ± 0.23	0.24 ± 0.16	–	5.72 ± 0.64	3.82 ± 0.11
BCD_0 ($\mu\text{M C day}^{-1}$)	–	1.41 ± 0.10	0.42 ± 0.05	–	5.52 ± 0.03	0.89 ± 0.01
BCD_4 ($\mu\text{M C day}^{-1}$)	–	1.82 ± 0.01	0.25 ± 0.01	–	5.94 ± 0.64	3.84 ± 0.01
BCD_{Int}	–	15.07 ± 0.81	7.45 ± 0.04	–	23.11 ± 1.09	15.22 ± 0.50
BGE_0 (%)	–	28 ± 5	18 ± 3	–	9 ± 1	2 ± 1
BGE_4 (%)	–	2 ± 1	1 ± 1	–	4 ± 1	1 ± 1
BGE_{Int}	–	7 ± 1	6 ± 1	–	5 ± 1	3 ± 1

575 Table 2. Abundance ($\times 10^5$ cells ml^{-1}) and relative abundances (in brackets) of *Eubacteria* (EUB), *Roseobacter* (ROS), *Betaproteobacteria* (BETA),
 576 *Gammaproteobacteria* (GAMMA), SAR86 and *Bacteroidetes* (CFB) at days 0 (Dark₀, UV₀), 1 (Dark₁, UV₁) and 2 (Dark₂, UV₂) in the Dark and UV
 577 treatments. Values are means $\pm$ SD; –, not measured.

14/02/2008								24/04/2008						
	EUB	ROS	SAR11	BETA	GAMMA	SAR86	CFB	EUB	ROS	SAR11	BETA	GAMMA	SAR86	CFB
Dark ₀	2.3 $\pm$ 0.1 (63 $\pm$ 10)	0.2 $\pm$ 0.1 (6 $\pm$ 1)	0.5 $\pm$ 0.1 (13 $\pm$ 2)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	0.1 $\pm$ 0.1 (4 $\pm$ 2)	–	1.7 $\pm$ 0.1 (48 $\pm$ 5)	6.5 $\pm$ 0.1 (92 $\pm$ 6)	1.3 $\pm$ 0.1 (18 $\pm$ 2)	0.2 $\pm$ 0.1 (3 $\pm$ 1)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	2.3 $\pm$ 0.1 (32 $\pm$ 9)	0.2 $\pm$ 0.1 (2 $\pm$ 1)	2.6 $\pm$ 0.1 (37 $\pm$ 7)
Dark ₁	21.1 $\pm$ 0.1 (100 $\pm$ 8)	5.5 $\pm$ 0.1 (26 $\pm$ 2)	2.2 $\pm$ 0.1 (10 $\pm$ 2)	0.2 $\pm$ 0.1 (1 $\pm$ 1)	4.0 $\pm$ 0.1 (19 $\pm$ 2)	0.3 $\pm$ 0.1 (1 $\pm$ 1)	6.3 $\pm$ 0.1 (30 $\pm$ 4)	11.1 $\pm$ 0.1 (99 $\pm$ 5)	3.3 $\pm$ 0.1 (30 $\pm$ 5)	0.8 $\pm$ 0.1 (7 $\pm$ 2)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	1.4 $\pm$ 0.1 (12 $\pm$ 1)	0.3 $\pm$ 0.1 (2 $\pm$ 1)	6.0 $\pm$ 0.1 (54 $\pm$ 10)
Dark ₂	15.5 $\pm$ 0.1 (100 $\pm$ 5)	2.1 $\pm$ 0.1 (14 $\pm$ 4)	0.3 $\pm$ 0.1 (2 $\pm$ 1)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	2.9 $\pm$ 0.1 (18 $\pm$ 5)	0.3 $\pm$ 0.1 (2 $\pm$ 1)	3.6 $\pm$ 0.1 (23 $\pm$ 3)	7.6 $\pm$ 0.1 (91 $\pm$ 9)	1.7 $\pm$ 0.1 (21 $\pm$ 4)	0.3 $\pm$ 0.1 (3 $\pm$ 1)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	1.2 $\pm$ 0.1 (15 $\pm$ 3)	0.1 $\pm$ 0.1 (2 $\pm$ 1)	2.9 $\pm$ 0.1 (35 $\pm$ 6)
UV ₀	2.3 $\pm$ 0.1 (63 $\pm$ 10)	0.2 $\pm$ 0.1 (6 $\pm$ 1)	0.5 $\pm$ 0.1 (13 $\pm$ 2)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	0.1 $\pm$ 0.1 (4 $\pm$ 2)	–	1.7 $\pm$ 0.1 (48 $\pm$ 5)	6.5 $\pm$ 0.1 (92 $\pm$ 6)	1.3 $\pm$ 0.1 (18 $\pm$ 2)	0.2 $\pm$ 0.1 (3 $\pm$ 1)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	2.3 $\pm$ 0.1 (32 $\pm$ 9)	0.2 $\pm$ 0.1 (2 $\pm$ 1)	2.6 $\pm$ 0.1 (37 $\pm$ 7)
UV ₁	18.6 $\pm$ 0.2 (98 $\pm$ 20)	6.3 $\pm$ 0.1 (33 $\pm$ 4)	1.5 $\pm$ 0.1 (8 $\pm$ 2)	0.2 $\pm$ 0.1 (1 $\pm$ 1)	9.0 $\pm$ 0.1 (47 $\pm$ 3)	0.2 $\pm$ 0.1 (1 $\pm$ 1)	3.7 $\pm$ 0.1 (20 $\pm$ 4)	13.4 $\pm$ 0.3 (98 $\pm$ 6)	4.0 $\pm$ 0.1 (29 $\pm$ 7)	0.7 $\pm$ 0.1 (5 $\pm$ 2)	0.2 $\pm$ 0.1 (2 $\pm$ 1)	4.5 $\pm$ 0.1 (33 $\pm$ 4)	0.2 $\pm$ 0.1 (2 $\pm$ 1)	2.9 $\pm$ 0.1 (21 $\pm$ 4)
UV ₂	16.8 $\pm$ 0.1 (100 $\pm$ 3)	5.4 $\pm$ 0.1 (32 $\pm$ 6)	0.7 $\pm$ 0.1 (4 $\pm$ 2)	0.3 $\pm$ 0.1 (2 $\pm$ 1)	6.8 $\pm$ 0.1 (41 $\pm$ 3)	0.2 $\pm$ 0.1 (1 $\pm$ 1)	2.2 $\pm$ 0.1 (13 $\pm$ 3)	8.7 $\pm$ 0.1 (94 $\pm$ 9)	1.2 $\pm$ 0.1 (13 $\pm$ 3)	0.1 $\pm$ 0.1 (1 $\pm$ 1)	0.4 $\pm$ 0.1 (5 $\pm$ 1)	4.5 $\pm$ 0.1 (48 $\pm$ 7)	0.2 $\pm$ 0.1 (1 $\pm$ 1)	1.4 $\pm$ 0.1 (15 $\pm$ 4)

578

579 **Figure legend.**

580 Fig. 1 Time course of a, b) bacterial production (BP), c, d) bacterial abundance (BA), e, f) electron
581 transport system (ETS) activity and g, h) bacterial growth efficiency (BGE) during the 4 days
582 incubations. Error bars represent standard deviations.

583

584 Lønborg et al., Fig. 1