Combined effect of light exposure and microbial activity on distinct dissolved organic matter pools. A seasonal field study in an oligotrophic coastal system (Blanes Bay, NW Mediterranean)

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Abstract

A harmonic analysis of three years of data collected with fortnight to monthly frequency in the oligotrophic Bay of Blanes (NW Mediterranean) revealed that the water column mixing-stratification cycle dictated the seasonal build-up of the bulk and different coloured fractions of dissolved organic matter (DOM). Dissolved organic carbon (DOC) accumulated at late summer, reaching the annual maximum by early September, half a month later than water temperature. The seasonal cycle of the protein-like fluorescence ($F(280/350)$) was in phase with DOC, suggesting that reduced heterotrophic activity, due to severe P limitation, is likely the reason behind the late summer accumulation of these materials. The absorption due to conjugated carbon double bonds of DOM ($a_{CDOM}(254)$) reached the annual maximum by mid July, concomitant to the seasonal maximum of microbial activity, suggesting that biological production prevailed over photo-degradation of these compounds. On the contrary, the fluorescence of humic-like substances absorbing in the UV-A region ($F(340/440)$) presented the annual maximum in early February, coinciding with the seasonal maximum of autotrophic biomass, and the minimum in early August, because of the prevalence of photo-degradation over microbial production. The optical properties of DOM allowed distinguishing between three DOC pools of contrasting origin, photo- and bio-reactivity, in the oligotrophic NW Mediterranean Sea.

Keywords: CDOM, FDOM, Microbiology, Photochemistry, Mediterranean Sea, Blanes Bay.

1 Introduction

2 Coloured dissolved organic matter (CDOM) includes all dissolved organic
3 compounds that absorb ultraviolet and visible radiation. It is the major factor controlling
4 the attenuation of UV radiation in the ocean (Kirk, 1994), which affects both primary
5 and bacterial production (Herndl et al., 1993; Smith and Cullen, 1995). The absorption
6 properties of CDOM are due to the presence of conjugated carbon double bonds. When
7 they form aromatic rings, part of the absorbed light may be emitted as fluorescence
8 (Stedmon and Alvarez-Salgado, 2011). CDOM absorption coefficients and spectral
9 slopes have been used as tracers of the chemical structure and origin of DOM in aquatic
10 environments (Dahlén et al., 1996; Nelson et al., 2004; Helms et al., 2008). The C-
11 specific decadic absorption coefficient at 254 nm, $SUVA_{254}$, has been found to be a
12 good tracer for the aromaticity of aquatic humic substances (Weishaar et al., 2003) and
13 the ratio of absorption coefficients at 254 and 365 nm, $a_{CDOM}(254/365)$, and absorption
14 spectral slopes, have been used as indices of the average molecular weight of DOM
15 (Dahlén et al. 1996; Engelhaupt et al. 2003; Helms et al., 2008). The fraction of CDOM
16 that emits absorbed UV radiation as fluorescence is called fluorescent dissolved organic
17 matter (FDOM). The concatenation of emission fluorescence spectra collected at
18 different excitation wavelengths results in excitation emission matrices where different
19 peaks, characteristics of humic- and protein-like compounds, can be distinguished
20 (Coble et al., 1996). The peaks named as C, M and A are due to humic-like
21 fluorophores, while peaks T and B are due to protein-like fluorophores. Fluorescence
22 intensity measurements at these peaks can be used to follow the dynamics of different
23 DOM pools. The fluorescence to absorption coefficient ratio at 340 nm, a proxy of the
24 fluorescence quantum yield, has also been used as an aromaticity index (Birks, 1970;
25 Turro, 1991).

1 The main biogeochemical processes affecting the optical properties of DOM are:
2 photodegradation, which provokes a loss of absorption and fluorescence of humic-like
3 materials (e.g., Del Castillo et al., 1999; Moran et al., 2000; Nieto-Cid et al., 2006); and
4 biodegradation, which produces an increase of the absorption and fluorescence of
5 humic-like compounds and a decrease of the fluorescence of protein-like compounds
6 (e.g. Kramer and Herndl, 2004; Lønborg et al., 2010; Romera-Castillo et al., 2011a). In
7 some cases, natural UV light can produce an increase of the fluorescence and absorption
8 of both humic-like (Kieber et al., 1997) and protein-like compounds (Romera-Castillo et
9 al., in prep). The study of absorption and fluorescence, as well as the indices obtained
10 from them, allows to identify and somewhat quantify, the main processes affecting
11 DOM in aquatic systems. Despite the fact that fluorescence and absorption of DOM are
12 two parameters that can easily be measured, information about the temporal variability
13 of the CDOM and/or FDOM pools in time-series stations is still scarce (but see Nelson
14 et al., 1998; Para et al., 2010).

15 The Blanes Bay Microbial Observatory
16 (<http://www.icm.csic.es/bio/projects/icmicrobis/bbmo/>) is a reference station in the NW
17 Mediterranean Sea placed 1 Km offshore of the town of Blanes. These waters are
18 oligotrophic and phosphorous limited for most of the year (Lucea et al., 2005). Rain and
19 mixing episodes, frequent in autumn and early winter, are followed by a prolonged
20 period of high atmospheric pressure. The associated well irradiated and calm waters in
21 late winter act as the main seasonal trigger in the NW Mediterranean Sea, setting the
22 development of phytoplankton blooms (Duarte et al., 1999; Guadayol et al., 2009). This
23 station has been studied for a long period (e.g., Margalef, 1945; Gasol et al., 1995;
24 Duarte et al., 1999) with special emphasis on microbial plankton ecology (e.g. Alonso-
25 Sáez et al., 2008; Galand et al., 2010). The monthly sampling on this site since 1992 has

1 resulted in an invaluable database to examine and test environmental issues. In this
2 study, we took advantage of this data collection to investigate the seasonal cycles of
3 different sub-fractions of the DOM pool through their optical properties and the
4 physical and biogeochemical processes, photochemistry and microbial activity,
5 conditioning their variability.

7 **Materials and methods**

8 Survey area and sampling strategy

9 The Blanes Bay Microbial Observatory (NW Mediterranean Sea, 41° 40'N,
10 2°48'E), is located in the continental side of the shelf/slope front, between the
11 submarine Blanes Canyon, placed in the north, and the mouth of the La Tordera River
12 in the south. Bottom depth at the sampling station is 22 m.

13 Seawater samples for this project were taken with fortnightly to monthly
14 frequency from March 2008 to March 2011. They were collected from the surface layer
15 (0.5 m depth), pre-filtered through a 200 µm nylon mesh to remove larger
16 mesozooplankton and transported to the base laboratory (Barcelona) in acid-washed
17 L polycarbonate carboys within 2 hours. In situ water salinity and temperature were
18 measured with a CTD model SAIV A/S SD204. Total irradiance was recorded hourly
19 by a pyranometer in the nearby meteorological station of Malgrat de Mar (Catalan
20 Meteorological Service, SMC). The average total irradiance of three days before each
21 sampling date was used here.

23 Measured variables

24 For chlorophyll *a* determinations (Chl *a*), a sample volume of 150 mL was
25 filtered through Whatman GF/F filters and subsequently extracted in acetone (90% v/v)

1 in the dark at 4°C for 24 h. The concentrations were determined fluorometrically with a
2 Turner Designs fluorometer. For Chl *a* smaller than 3 µm we applied the same
3 procedure to 250 mL water sample previously filtered through polycarbonate filters
4 with a 3µm pore size.

5 Samples for carbon content, absorption and fluorescence measurements of DOM
6 were immediately processed after arriving into the laboratory. Samples were filtered
7 through Whatman GF/F filters using an acid-cleaned glass filtration system.
8 Approximately 10 mL of water were collected in pre-combusted (450°C, 12 h) glass
9 ampoules for dissolved organic carbon (DOC) determination. H₃PO₄ was added to
10 acidify the sample to pH < 2 and the ampoules were heat-sealed and stored in the dark
11 at 4°C until analysis. DOC was measured with a Shimadzu TOC-V CSH organic carbon
12 analyser. The system was standardized daily with potassium hydrogen phthalate. Each
13 ampoule was injected 3–5 times and the average area of the 3 replicates that yielded a
14 standard deviation <1% were chosen to calculate the average DOC concentration of
15 each sample after subtraction of the average area of the freshly-produced UV-irradiated
16 milli-Q water used as a blank. Reference materials provided by Prof. D. Hansell
17 (University of Miami) were analysed to test the performance of the analyser. A
18 concentration of $45.2 \pm 0.3 \mu\text{mol C L}^{-1}$ was obtained for the deep ocean reference
19 (Sargasso Sea deep water, 2600 m) minus blank reference materials. The nominal DOC
20 value provided by the reference laboratory is $45 \mu\text{mol C L}^{-1}$.

21 Samples for CDOM and FDOM determinations were measured by triplicate
22 immediately after filtration, between 2 and 3 h after collection. CDOM absorption was
23 measured in a Varian Cary spectrophotometer equipped with a 10 cm quartz cell.
24 Spectral scans were collected between 250 and 750 nm at a constant room temperature

of 20°C. Milli-Q water was used as blank. The absorption coefficient at all wavelengths,

$a_{\text{CDOM}}(\lambda)$ (in m^{-1}), was calculated as

$$a_{\text{CDOM}}(\lambda) = 23.03 \cdot [\text{Abs}(\lambda) - \text{Abs}(600-750)] \quad (1)$$

Where $\text{Abs}(\lambda)$ is the absorbance at wavelength λ , and $\text{Abs}(600-750)$ is the average

absorbance between 600 and 750 nm, which corrects for the residual scattering by fine

size particle fractions, micro-air bubbles or colloidal material present in the sample, or

refractive index differences between the sample and the reference (in m^{-1}). The carbon

specific absorption coefficient at 254 nm, SUVA_{254} (Weishaar et al., 2003), was

calculated dividing the decadic $a_{\text{CDOM}}(254)$ by the DOC concentration and expressed in

$\text{m}^2 \text{mol C}^{-1}$. Following Helms et al. (2008), CDOM spectral slopes were calculated over

two narrow wavelength ranges, $S(275-295)$ and $S(350-400)$, using linear regressions of

the natural log-transformed $a_{\text{CDOM}}(\lambda)$ spectra. In addition, the dimensionless $S(275-$

$295)/S(350-400)$ ratio, known as S_R , was also calculated.

CDOM induced fluorescence (FDOM) was determined with a LS 55 Perkin

Elmer Luminescence spectrometer, equipped with a xenon discharge lamp, equivalent

to 20 kW for 8 μs duration. The instrument has 2 monochromators that ranged between

200 and 800 nm for excitation wavelengths and between 200 and 900 nm for emission

wavelengths. The detector was a red-sensitive R928 photomultiplier, and a photodiode

worked as reference detector. Slit widths were fixed to 10 nm for the excitation and

emission wavelengths and the scan speed was 250 nm min^{-1} . Measurements were

performed at a constant room temperature of 20°C in a 1 cm quartz fluorescence cell.

Single measurements at specific excitation-emission wavelengths (Ex/Em) were

performed. To compare with other studies, the wavelengths chosen for the Ex/Em pair

measurements were those previously established by Coble (1996): 280 nm/350 nm

(peak T) for protein-like substances; and 340 nm/440 nm (peak C) for humic-like

substances absorbing in the UV-A. The fluorescence of UV-radiated Milli-Q at those Ex/Em pairs was subtracted from all samples. Factory-set excitation and emission corrections of the instruments were used. Since the absorption coefficients of all samples at any wavelength were $< 10 \text{ m}^{-1}$, it was not necessary to correct for inner filters effects (Stedmon and Bro, 2008). Following Coble (1996), the fluorescence intensities were expressed in quinine sulphate units (QSU) by calibrating the instrument at Ex/Em: 350 nm/450 nm against a quinine sulphate dihydrate (QS) standard made up in 0.05 mol L^{-1} sulphuric acid. Fluorescence intensities of peaks T and C in QSU have been named as $F(280/350)$ and $F(340/400)$, respectively.

Finally, the quantum yield of fluorescence at excitation 340 nm, $\Phi(340)$ —i.e. the portion of the light absorbed by the DOM at 340 nm that is re-emitted as fluorescent light— was calculated by comparison to the fluorescence emission between 400 and 600 nm from the QS standard using the equation (Green and Blough, 1994):

$$\Phi(340) = \frac{F(400-600)}{a_{\text{CDOM}}(340)} \cdot \frac{a_{\text{CDOM}}(340)_{\text{QS}}}{F(400-600)_{\text{QS}}} \cdot \Phi(340)_{\text{QS}} \quad (2)$$

Where $a_{\text{CDOM}}(340)_{\text{QS}}$ is the absorption coefficient of the QS standard at 340 nm (in m^{-1}); $F(400-600)$ and $F(400-600)_{\text{QS}}$ are the average integrated fluorescence spectra between 400 and 600 nm at a fixed excitation wavelength of 340 nm (in QSU) of the sample and the QS standard, respectively; and $\Phi(340)_{\text{QS}}$ is the dimensionless fluorescence quantum yield of the QS standard, 0.54 according to Melhuish (1961).

Bacterial heterotrophic activity (BA) was estimated using the [^3H]-leucine (Leu) incorporation method (Kirchman et al., 1985) as in Alonso-Sáez et al. (2008). For each sample, triplicate or quadruplicate aliquots (1.2 ml) and one or two TCA killed controls were incubated with 40 nmol l^{-1} Leu for about 2 h at in situ temperature in the dark. The incorporation was stopped with the addition of 120 μl of cold TCA 50% and samples

were kept frozen at 20°C until processing, which was carried out by the centrifugation method. Finally, samples were counted on a Beckman scintillation counter 24 h after the addition of 1 ml of scintillation cocktail (Optiphase Hisafe2, Perkin-Elmer).

Harmonic analysis of the time series

To obtain the annual mean (b1), amplitude (b2) and diphas (b3) parameters defining the seasonal cycle of a variable (Y), a harmonic analysis of the annual component (period, 365 days) of each time series was performed using the Levenberg-Marquardt algorithms implemented in the STATISTICA software. We fitted the data of total irradiance, water temperature, salinity, DOC, Chl a, BA, $a_{CDOM}(254)$, $SUVA_{254}$, $S(275-295)$ and $S(350-400)$, S_R , $F(340/440)$, $F(280/350)$ and $\Phi(340)$ to the following trigonometric equation:

$$Y = b1 + b2 \cdot \cos\left(\frac{2\pi}{365} \cdot t + b3\right) \quad (3)$$

where t is the ordinal date (ranging from 1 to 365/366). The diphas parameter, b3, was expressed as the ordinal date at which each time series achieved the annual maximum value.

Results

Annual cycles of environmental variables

The harmonic eq. 3 explained 86% of the variability (Table 1) of the time series of total irradiance in Blanes Bay (Fig. 1a). Maximum irradiances ($295 \pm 12 \text{ W m}^{-2}$) occurred by late June (day of the year 179 ± 4). Sea surface temperature followed also a well-defined annual cycle (Fig. 1b) that explains 90% of the variability of the time series with an average annual mean of $16.7 \pm 0.2 \text{ °C}$ (Table 1). The average annual minimum temperature, $11.4 \pm 0.5 \text{ °C}$, occurred by mid February when maximum winter

1 mixing happened, and the average annual maximum, 22.0 ± 0.5 °C, was recorded by
2 mid August (day of the year, 231 ± 3), i.e. 52 ± 7 days latter than the seasonal maximum
3 of irradiance, when summer stratification was highest. Conversely, the annual cycle
4 explained only 24% of the variability of the time series of salinity (Fig. 1c). Salinity was
5 quite stable around the average annual mean of 37.97 ± 0.04 , the amplitude being just
6 0.17 ± 0.05 , except during a few particular dates associated with rainwater episodes.
7 Maximum values were observed during autumn (mid November) and minimum during
8 spring (mid May).

9 The seasonal cycle of total chlorophyll *a*, as modelled with eq. (3), explained
10 41% of the variability of the time series (Fig. 1d; Table 1). The average annual mean
11 was 0.63 ± 0.05 mg m⁻³ with average maximum values of 1.04 ± 0.13 mg m⁻³ by late
12 February (day of the year, 54 ± 10) and minimum values of 0.22 ± 0.13 mg m⁻³ by late
13 August. A similar seasonal cycle was observed for the fraction of Chl *a* < 3 µm (Fig.
14 1e), but it explained 55% of the variability and reached the seasonal maximum
15 significantly earlier, by late January (day of the year, 28 ± 8). The portion of the Chl *a*
16 which was < 3 µm was maximum (64 ± 5 %) by late October (day of the year, 294 ± 20)
17 and minimum (45 ± 5 %) by late April (day of the year, 111 ± 20 ; Fig. 1e, solid line).
18 The fitting of bacterial activity to eq. (3) explained 36% of the variability, presenting an
19 average maximum of 70 ± 9 pmol leu L⁻¹ h⁻¹ by mid July (day of the year, 198 ± 13)
20 and a minimum of 8 ± 9 pmol leu L⁻¹ h⁻¹ by mid January (day of the year, 15 ± 13).

Annual cycles of the bulk and coloured fractions of DOM

DOC concentration and the optical properties of CDOM also followed statistically significant annual cycles that fit to eq. 3. Seasonality explained 55% of the variability of DOC. The average annual mean concentration of DOC was $79 \pm 2 \mu\text{mol C L}^{-1}$; it accumulated in Blanes Bay from the average annual minimum of $63 \pm 5 \mu\text{mol C L}^{-1}$ recorded by early March to the average annual maximum of $95 \pm 5 \mu\text{mol C L}^{-1}$ by early September (day of the year, 247 ± 9 ; Fig. 2a, Table 1), about half a month later than the maximum of sea surface temperature. Note that these are the typical annual concentrations but higher values, reaching $140 \mu\text{mol C L}^{-1}$, were sporadically recorded.

Fifty percent of the variability of the absorption coefficient at 254 nm, $a_{\text{CDOM}}(254)$, an indicator of the abundance of conjugated carbon double bonds (Lakowicz et al., 2006), was explained by eq. 3. Although $a_{\text{CDOM}}(254)$ also accumulated through the spring and summer, the average annual maximum absorption of $1.77 \pm 0.06 \text{ m}^{-1}$ (Fig. 2b) occurred by early August (day of the year, 217 ± 10), i.e. 30 ± 13 days before the annual maximum of DOC (Table 1). The diphasic of DOC and $a_{\text{CDOM}}(254)$, led to an annual cycle of SUVA_{254} that reaches the average annual maximum of $9.8 \pm 0.3 \text{ m}^2 \text{ mol C}^{-1}$ by early April (day of the year, 96 ± 22). SUVA_{254} is commonly used as an aromaticity index after Weishaar et al. (2003) found a good correlation between this variable and the percentage of aromaticity determined by ^{13}C -NMR spectroscopy. Other useful indices are the absorption coefficient ratio at 254 nm to 365 nm, $a_{\text{CDOM}}(254/365)$ (Fig. 2c), which is inversely correlated with the DOM average molecular weight (Dahlén et al. 1996; Engelhaupt et al. 2003) or the spectral slope ratio, S_R , which is directly correlated to the molecular weight and the photochemically induced shifts in molecular weight (Helms et al., 2008). In our study, both variables described statistically significant annual cycles that explained 16-21% of the total

variability of the time series and $a_{\text{CDOM}}(254/365)$ was in phase with the annual cycle of DOC and S_R with the annual cycles of $F(340/440)$ and $\Phi(340)$ (Table 1).

The fluorescence of humic-like substances absorbing in the UV-A region of the spectrum, $F(340/440)$, followed an annual cycle that explained 38% of the variability of the time series and was out of phase with $a_{\text{CDOM}}(254)$ and DOC. $F(340/440)$ evolved from an average annual maximum of 0.62 ± 0.04 QSU by early February (day of the year, 35 ± 10) to a minimum of 0.38 ± 0.04 QSU by early August (Fig. 2d). The time course of the fluorescence quantum yield at 340 nm, $\Phi(340)$, another aromaticity index (Birks, 1970; Turro, 1991), followed also a statistically significant seasonal cycle that explained 39% of the variability of the time series and was significantly in phase with $F(340/440)$ (Table 1). The average annual mean value of $\Phi(340)$ was $0.63 \pm 0.02\%$, ranging from an average annual maximum of $0.76 \pm 0.05\%$ to an average annual minimum of $0.50 \pm 0.05\%$. Conversely, the fluorescence of protein-like substances (Fig. 2e) followed an annual cycle that explained 29% of the variability of the time series and was in phase with DOC, reaching the annual maximum by early September (day of the year, 255 ± 16 ; Table 1).

Deseasonalized time series

The studied variables were deseasonalised by subtracting the annual cycles modelled with eq. 3 (dotted lines in Figures 1 and 2) from the original times series (open dots in Figures 1 and 2). The deseasonalised time series of the fluorescence of the humic-like peak C, $\Delta F(340/440)$ (Fig. 3a), was generally opposite to the deseasonalised time series of salinity, ΔSal (Fig. 3b). In fact, a significant negative linear correlation ($R = -0.62$, $p < 0.001$, $n = 40$) existed between $\Delta F(340/440)$ and ΔSal (Fig. 4a). ΔSal was also negatively correlated with the aromaticity index $\Phi(340)$ ($R =$

-0.52, $p < 0.05$, $n = 31$, Fig. 4b). On the other hand, the deseasonalised time series of the protein-like peak-T, $\Delta F(280/350)$ (Fig. 3c), and that of the bulk DOC, ΔDOC (Fig. 3d), followed the same trend, presenting a significantly positive linear correlation ($R = 0.63$, $p < 0.001$, $n = 38$, Fig. 4c).

Discussion

The standard error of the values modelled with eq. (3) (SD res in Table 1) was about one order of magnitude larger than the analytical error of the measurement (Anal. err in Table 1) for all study variables except $a_{\text{CDOM}}(254/365)$ and $\Phi(340)$, which were of the same magnitude. Therefore, the part of the variability not explained by the harmonic model ($1 - R^2$) was essentially due to environmental variability at time scales of frequency higher than the annual cycle modelled by eq. 3. For the particular case of $a_{\text{CDOM}}(254/365)$ and $\Phi(340)$, the harmonic model was as good as the accuracy of the determination of these variables.

The Blanes Bay Microbial Observatory is representative of oligotrophic coastal ecosystems which sporadically receive nutrients and terrestrial carbon inputs during stormy periods (Guadayol et al., 2009). The annual cycle of sea surface temperature traces the seasonal evolution from winter mixing to summer stratification characteristic of temperate latitudes. The temperature range obtained in this work agrees with that reported elsewhere (Duarte et al., 1999). The average annual maximum concentration of Chla was composed of 50% of chlorophyll in phytoplankton $> 3\mu\text{m}$ and a 50 % in phytoplankton $< 3\mu\text{m}$. This maximum occurred at the time of the average annual minimum of temperature as previously found by Guadayol et al. (2009). The development of this late winter phytoplankton bloom is one of the seasonal characteristics of Mediterranean phytoplankton communities (Duarte et al., 1999).

Stratification of the water column throughout the spring and summer isolates the water of Blanes Bay from the subsurface offshore waters, preventing the flux of new inorganic nutrients into the bay, which leads to severe oligotrophic conditions (Alonso-Sáez et al., 2008). Nutrient concentrations during the summer season of the studied years were around $0.03 \mu\text{mol L}^{-1}$ for phosphate, $0.3 \mu\text{mol L}^{-1}$ for inorganic nitrogen, and $0.1 \mu\text{mol L}^{-1}$ for silicate (data not shown). The typical annual range of DOC concentrations recorded during the study period had previously been observed in Blanes Bay (Lucea et al., 2005; Alonso-Sáez et al., 2008; Vila-Reixach et al., 2012) as well as in other temperate marine ecosystems relatively unaffected by terrestrial inputs (Nelson et al., 1998; Álvarez-Salgado et al., 2001). The summer accumulation of DOC of $32 \pm 9 \mu\text{mol C L}^{-1}$ observed in Blanes Bay is similar to that reported for the tropical station of the Hawaii Ocean Time Series ($40 \mu\text{mol C L}^{-1}$, <http://hahana.soest.hawaii.edu/hot/>) but doubles that of the Bermuda Atlantic Time Series Study site ($15 \mu\text{mol C L}^{-1}$, Nelson et al., 1998).

The dissolved organic materials traced by $a_{\text{CDOM}}(254)$ and peak T accumulated also in Blanes Bay during the summer but $a_{\text{CDOM}}(254)$ reached its annual maximum about one month before peak T that, in turn, was in phase with DOC. Therefore, the optical properties of CDOM allowed distinguishing the accumulation of two DOC pools which differ in their potential bioavailability. Among the by-products of microbial respiration there are compounds with conjugated carbon double bonds that can be traced by $a_{\text{CDOM}}(254)$ since microorganisms produce fluorescent humic-like substances absorbing at around 260 nm and emitting at around 460 nm (Romera-Castillo et al., 2011a). This fluorescent maximum is within the range established by Coble (1996) for peak-A (Ex/Em 260/400-460). The annual minimum of $a_{\text{CDOM}}(254)$ was recorded in winter likely because of mixing with the less coloured offshore waters. In this sense,

Vila-Reixach et al. (2012) have recently shown that strong NW winds blowing from November to May, promote the offshore export of the DOC rich waters in the Bay. On the other hand, these authors found that DOC accumulated during spring and summer because of the increasing stratification and the prevalence of SE winds, which favours DOC accumulation into the Bay. Moreover, there is a dominance of net production associated to microbial respiration over photochemical degradation in the stratified surface layer. In fact, the annual cycles of $a_{CDOM}(254)$ and bacterial activity were in phase, i.e. the annual maximum of both variables coincided in time. Our bacterial activity maximum also coincided with the bacterial respiration maximum previously reported in the same area (Duarte et al., 2004). Reduced photochemical degradation of substances absorbing at 254nm also contributed to the summer accumulation of $a_{CDOM}(254)$ because the largest losses of fluorescence and absorbance occur at the same wavelengths at which the compounds are irradiated (Del Vecchio and Blough, 2002) and few photons of $\lambda < 295$ nm reach the Earth's surface.

The fact that the annual cycle of peak T was in phase with DOC suggests that the DOC accumulated in Blanes Bay contained free and combined amino acids. In this sense, it is known that maintenance of the algal photosynthetic machinery after inorganic N and P exhaustion is accompanied by excretion of DOM and especially carbohydrates (Norman et al., 1995). P limitation in the Mediterranean could lead to the accumulation of protein materials of little use under P starvation, despite the fact that it would be labile in other (P replete) environments. Therefore, despite the potential lability of the exuded materials, P limitation would prevent their utilization by heterotrophic bacteria leading to DOC accumulation in summer (Thingstad et al., 1997). DOM accumulation is a common process that has also been evidenced in other oligotrophic aquatic environments (e.g., Sargasso Sea, Thingstad et al., 1997; Siegel et

al., 2002) as well as in experimental mesocosms (Norman et al., 1995). The accumulation of protein-like substances in summer was also observed in Florida Bay (Jaffé et al., 2008). Another plausible reason for the protein-like fluorescence maximum recorded in summer could be that photo-degradation of humic substances releases amino acid moieties or changes its position in the protein throughout conformational changes of the macromolecule, exhibiting higher fluorescence in that new form (Lakowicz, 2006). This has been hypothesized in an experimental study where it was reported an increase of peak T after exposure of open ocean ultrafiltered DOM to sunlight (Romera-Castillo, et al., in prep.).

The annual cycle of the coloured and fluorescent humic-like substances that absorb natural light at wavelengths >300 nm, i.e. in the UV-B and UV-A range of the spectrum, is the result of the prevalence of consumption by photodegradation, over production by microbial respiration, of these materials (Coble, 2007). This pattern has been previously observed in other marine ecosystems relatively unaffected by continental runoff such as the oligotrophic BATS site (Nelson et al., 1998; 2004) or the eutrophic Iberian coastal upwelling system (Nieto-Cid et al. 2006; Romera-Castillo et al., 2011*b*) and it is the reason behind the relatively low values reported in the literature for the absorption coefficient (Nelson et al., 2010) and the induced fluorescence (Yamashita and Tanoue, 2008) of CDOM in surface ocean waters. Therefore, we hypothesize that the annual maximum accumulation of peak C, $F(340/440)$, was observed in winter as a result of three processes. First, the winter mixing of the waters into the bay with the more fluorescent waters offshore the bay. Given that peak-C is degraded by sunlight, its fluorescence intensity increases significantly with depth. Therefore, the deeper the winter mixed layer the higher the fluorescence of peak-C. The winter mixed layer in the offshore waters deepens to around 100 m (D’Ortenzio et al.,

2005) whereas maximum depth at the Blanes Bay Microbial Observatory is 22 m. Second, the production of peak C by the community associated to the Chl *a* < 3 μm , which peaked in late January, in phase with *F*(340/440). Note that cyanobacteria and micromonas produce fluorescent DOM that peaks at *F*(340/440) (Romera-Castillo et al., 2011a) and the smaller size fraction of phytoplankton in Blanes Bay is mainly composed on cyanobacteria, during summer, and micromonas, during winter (unpub. data). And third, photobleaching during spring and summer in the stratified waters of the bay. In fact, the seasonal minimum of *F*(340/440) (day 218 ± 10) occurred in between the seasonal maxima of total irradiance (39 ± 11 days before) and temperature (14 ± 10 days after), i.e. in between the seasonal maxima of UV radiation and water column stratification.

The 39% reduction of *F*(340/440) in Blanes Bay from the annual maximum in early February to the annual minimum in early August was close to those reported in the Middle Atlantic Bight (~30% , Vodacek and Blough, 1997) and in the eutrophic coastal system of the Ría de Vigo (40-50%, Nieto-Cid et al., 2006). Photobleaching of humic-like materials produces a dramatic loss of aromaticity and a decrease of the molecular weight of the irradiated materials (Moran and Zepp, 1997; Osburn et al., 2001). Coherently, our annual cycles of the aromaticity indices SUVA_{254} and $\Phi(340)$ and the average molecular weight indices $a_{\text{CDOM}}(254/365)$ and S_R agree with the photodegradation of aromatic and high molecular weight compounds during summer to generate colourless, more aliphatic and lower molecular weight organic products, as well as CO and CO₂ (Moran and Zepp, 1997). Therefore, the fraction of CDOM that reaches maximum concentrations by mid-September seems to be of relatively lower average molecular weight and aromaticity than the CDOM accumulated during the winter. Although labile compounds are formed during photodegradation processes

(Moran and Zepp, 1997; Obernosterer et al., 1999), the previously referred inability of the microbial loop led to the accumulation of these materials in the stratified surface layer.

Although direct comparison of spectral slopes is not an easy task because different wavelength ranges were used by different authors, it can be reported that the absorption spectral slopes obtained in Blanes Bay are relatively high when compared with the values reported for surface waters of the Gulf of Lions (Ferrari, 2000) and for the Bay of Marseille (Para et al., 2010), both in the NW Mediterranean Sea, or for an eutrophic coastal embayment as the Ría de Vigo (Romera Castillo et al., 2011*b*). In Blanes, as in Marseille, absorption spectral slopes are relatively lower during winter than in summer (Para et al., 2010) as a consequence of a photo-degradation increase during summer, characteristic of temperate latitudes. Continental CDOM inputs tend to decrease spectral slopes, while photodegradation processes tend to rise them (Del Vecchio and Blough, 2004; Nelson et al., 2010). Since natural irradiation is not much different between both Mediterranean and Atlantic studied sites, with average values of around $160 - 180 \text{ W m}^{-2}$ (Ruiz et al., 2008; www.meteogalicia.es), the reason behind the relatively higher values of *S* in Blanes Bay is likely because it has less terrestrial influence than the Bay of Marseille or the Ría de Vigo. The former is close to the major source of freshwater and terrigenous particles of the NW Mediterranean Sea (Rhône River; Sempere et al., 2000) and the Ría de Vigo directly receives Oitaben-Verdugo river waters with an average annual flow of $15 \text{ m}^3 \text{ s}^{-1}$ (Nogueira et al., 1997).

The annual mean $\pm$ amplitude of the fluorescence quantum yield at 340 nm found in Blanes Bay, $0.63 \pm 0.12\%$, is within the range of values reported for the Gulf of Lions but lower than the average recorded in the more eutrophic Rhône river plume (0.96% ; Ferrari, 2000) and Ría de Vigo ($0.91 \pm 0.30\%$). Laboratory experiments have

shown that photobleaching produces a decrease of the fluorescence quantum yield, while microbial degradation produces an increase (De Haan, 1993; Lønborg et al., 2010). Therefore, the differences in $\Phi(340)$ values found in oligotrophic and eutrophic sites can be explained as a consequence of higher microbial degradation activities in the latter sites. Moreover, the terrestrial materials associated to the Rhône River plume could also contribute to increase $\Phi(340)$, since terrestrial organic matter presents a higher degree of aromaticity than marine organic matter does (Repeta et al., 2002; Benner, 2003).

The seasonal cycle of the fluorescent humic-like substances is disrupted by the episodic arrival of continental waters to the sampling site, which can be traced by a salinity decrease (Fig. 1c). Lower salinities are usually accompanied by higher values of $F(340/440)$ (Fig. 2d); in fact, a significant negative correlation was observed between the deseasonalised times series of salinity and those of $F(340/440)$ (Fig. 4a) and $\Phi(340)$ (Fig. 4b). This is, again, because continental waters are richer in humic-like fluorescent compounds with respect to marine waters (Coble, 1996; Repeta et al., 2002; Benner, 2003). The $F(340/440)$ measured at the mouth of River Tordera, placed south of the sampling site, on 15/05/2008 was as high as 53 QSU while DOC and total inorganic N values were of $263 \mu\text{mol L}^{-1} \text{C}$ and $90.3 \mu\text{mol L}^{-1}$, respectively (unpub. data). Furthermore, continental runoff also transports new inorganic nutrients to the sampling site that can contribute to enhance microbial activity, which is known to also produce humic-like substances (Nieto-Cid et al., 2006; Lønborg et al., 2010; Romera-Castillo et al., 2010, 2011a). Coherently, the major determining factors for the interannual differences in microplankton metabolism are the variability in rainfall and continental runoff (Satta et al., 1996; Guadayol et al., 2009). The fact that the marked salinity decrease observed in June 2008 was not accompanied by a large increase of $F(340/440)$

1 could be due to photodegradation of continental humic substances during water
2 advection to the sampling site and/or a delay in the stimulation of microbes by nutrients
3 transported by continental waters.

4 Absorption and fluorescence spectroscopy measurements of CDOM at the
5 Blanes Bay Microbial Observatory have allowed distinguishing between three DOM
6 pools of different origin and photo- and bio-lability. We show that the optical properties
7 of DOM, which are simple, fast and relatively inexpensive to measure, can be
8 incorporated in monitoring sampling programs as a complement to DOC measurements
9 as they provide useful information about the physical and biogeochemical processes
10 controlling the stocks and the reactivity of the DOM pool.

11

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Variable	Annual mean (b1)	Amplitude (b2)	Seasonal max.(b3) (day of the year)	R^2	n	p -level	SD res	Anal. err
Irradiance (W m^{-2})	182 ± 5	113 ± 7	179 ± 4	0.86	43	< 0.001	31	9
Temperature ($^{\circ}\text{C}$)	16.7 ± 0.2	5.2 ± 0.3	231 ± 3	0.90	43	< 0.001	1.2	0.01
Salinity	37.97 ± 0.04	0.17 ± 0.05	321 ± 19	0.24	43	< 0.001	0.23	0.01
Total Chla (mg m^{-3})	0.63 ± 0.05	0.41 ± 0.08	54 ± 10	0.41	42	< 0.001	0.32	0.05
Chla $< 3 \mu\text{m}$ (mg m^{-3})	0.33 ± 0.02	0.21 ± 0.03	28 ± 8	0.55	38	< 0.001	0.12	0.05
BA ($\text{pmol leu L}^{-1} \text{ h}^{-1}$)	39 ± 5	31 ± 8	198 ± 13	0.36	31	< 0.001	27	3.5
DOC ($\mu\text{mol L}^{-1}$)	79 ± 2	16 ± 3	247 ± 9	0.55	38	< 0.001	11	1
$a_{\text{CDOM}}(254)$ (m^{-1})	1.59 ± 0.02	0.18 ± 0.03	217 ± 10	0.50	31	< 0.001	0.11	0.01
SUVA ₂₅₄ ($\text{m}^2 \text{ mol C}^{-1}$)	8.9 ± 0.2	0.9 ± 0.3	96 ± 22	0.25	29	< 0.024	2.5	0.2
$a_{\text{CDOM}}(254/365)$	14.8 ± 0.6	2.3 ± 0.9	249 ± 23	0.21	31	< 0.005	2.8	2.7
$S(350-400)$ (nm^{-1})	0.0165 ± 0.0004	0.0019 ± 0.0006	210 ± 15	0.29	31	< 0.001	0.0020	0.0004
$S(275-295)/S(350-400)$	2.02 ± 0.05	0.17 ± 0.07	21 ± 23	0.16	31	< 0.015	0.26	0.07
$F(340/440)$ (QSU)	0.50 ± 0.02	0.12 ± 0.03	35 ± 10	0.38	41	< 0.001	0.10	0.01
$F(280/350)$ (QSU)	1.09 ± 0.05	0.27 ± 0.07	255 ± 16	0.29	41	< 0.001	0.28	0.03
$\Phi(340)$ (%)	0.63 ± 0.02	0.13 ± 0.03	23 ± 12	0.39	31	< 0.001	0.11	0.08

Table 1. Fitting parameters ($\pm$ standard error) of eq. 3 for the time series of the studied variables: annual mean (b1), amplitude (b2) and diphas (b3). Coefficients b1 and b2 are expressed in the units of each variable and b3 is expressed in days of the year. R^2 , determination coefficient; n , number of data of each time series; p -level, significance of the linear fitting for the measured versus the modelled data; SD res, standard error of the residuals of the measured minus the modelled data; Anal. err, analytical error of the experimental determination of each variable.

Figure captions

Fig. 1. Time series of a) total irradiance, b) temperature, c) salinity, d) chlorophyll *a* and e) chlorophyll *a* < 3 μm . Modelled values (dotted line) and measured values (open dots). Solid line in plot e) corresponds to modelled values of % Chla < 3.

Fig. 2. Time series of a) DOC, b) $a_{\text{CDOM}}(254)$, c) $a_{\text{CDOM}}(254/365)$, d) peak-C or $F(340/440)$ and e) peak-T or $F(280/350)$. Modelled values (dotted line) and measured values (open dots).

Fig. 3. Time series of the deseasonalized a) peak-C or $F(340/440)$, b) salinity, c) peak-T or $F(280/350)$ and d) DOC.

Fig. 4. Deseasonalized time series of a) peak-C or $F(340/440)$ versus salinity, b) $\Phi(340)$ versus salinity, and c) peak-T or $F(280/350)$ versus DOC.

Fig. 1

Fig. 2

Fig. 3

Fig. 4