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Microbial and photochemical reactivity of fluorescent dissolved organic matter in a coastal upwelling system

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Abstract

We observed significant changes in the dissolved oxygen content and the fluorescence of humic substances and dissolved aromatic amino acids after 24 h light and dark incubations in the coastal upwelling system of the Ría de Vigo under a wide variety of meteorologic and oceanographic conditions. Respiration rates were inversely correlated with the net production of humic fluorescence in the dark at a net rate of -0.027 ± 0.003 μg equivalents of quinine sulphate per μmol of O_2, suggesting that marine humics are a by-product of the bacterial respiration of dissolved organic matter (DOM). On the contrary, humic fluorescence consumption in the light minus dark incubations was positively correlated with the net production in the dark, indicating a rapid photodegradation of recently produced marine humic substances. Parallel incubation experiments demonstrated that daily photodegradation rates and residual humic fluorescence levels followed a seasonal pattern characterized by a marked autumn maximum. Finally, a significant linear correlation between the gross primary production (Pg) and the net production of aromatic amino acids fluorescence in the light pointed to the rapid consumption of dissolved protein-like materials at a net average rate of -1.4 ± 0.2 ppb equivalents of tryptophan per day, which accumulates in the water column only when Pg exceeds 80 ± 20 μmol kg^{-1} d^{-1}.

Marine dissolved organic matter (DOM) constitutes the main substrate for bacterioplankton growth and respiration (Azam and Cho 1987). DOM sources in estuarine and coastal waters include phytoplankton exudation, cell autolysis, and grazing pressure (Nagata 2000), as well as allochthonous organic matter of terrestrial and oceanic origin (Wollast 1993). The diversity of sources produces a myriad of different compounds, with a microbial reactivity ranging from hours for the dissolved free amino acids (DFAA), monosaccharides, and other labile molecules (Fuhrman 1987) to thousands of years for the most refractory humic compounds upwelled from the deep sea (Williams and Druffel 1987). The resistance of humic substances to microbial degradation contrasts with their susceptibility to photochemical decomposition (Benner and Biddanda 1998).

Fluorescence is a useful, simple, and quick technique to characterize and quantify two different classes of DOM: the labile DFAA (Yamashita and Tanoue 2003) and the recalcitrant humic substances (Coble et al. 1990). These two classes of compounds can be used to trace diverse biogeochemical processes such as labile organic matter production (Nieto-Cid et al. 2005), respiration (Chen and Bada 1992; Nieto-Cid et al. 2005), and photobleaching (Skoog et al. 1996; Moran et al. 2000). All these processes play a key role in the accumulation, recycling, and export of DOM in marine ecosystems (Carlson 2002). This work proposes using fluorescence to deal with the following three partially unresolved questions about the dynamics of marine DOM:

1. The origin of dissolved labile organic matter can be autotrophic, via extracellular release (Myklestad 1995) and cell lysis (Kirchman et al. 1993), or heterotrophic, via grazing losses (Storm et al. 1997). Rapid turnover maintains these compounds at nanomolar concentrations in the open ocean, but they support a large portion of the heterotrophic bacterial growth and respiration (Skoog et al. 1999). We propose using the fluorescence of dissolved aromatic amino acids ($FDOM_T$) to discriminate whether the accumulation of labile DOM in the marine environment is preferentially due to anabolic (related to primary production) or catabolic (related to respiration) processes.
2. The origin of marine humic substances is not completely resolved. An alternative to the classical polyphenol and melanoidin condensation models (Hedges 1978) and the photo-oxidation of lipids (Kieber et al. 1997) is the formation of humic substances as a by-product of microbial respiration (Brophy and Carlson 1989; Kramer and Herndl 2004). A fraction of the respired organic carbon is transformed into biologically refractory organic matter instead of CO_2 (Chen and Bada 1992; Hayase and Shinozuka 1995). We propose using the fluorescence of humic compounds ($FDOM_M$) to trace the daily production of humic substances during microbial respiration processes in the dark.

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3. Exposure to sunlight degrades high–molecular weight humic substances into smaller photoproducts that are mainly removed from the DOM pool by two pathways: through direct volatilization of carbon gases (CO, CO_2, SO_2, etc.) and through rapid bacterial utilization of labile photoproducts (Kieber et al. 1997). The relationship between photochemical and heterotrophic processes has been investigated during the last decade in different environments (Lindell et al. 1995; Moran et al. 2000; Obernosterer and Benner 2004; Kramer and Herndl 2004). Since photodegradation of DOM involves mainly humic substances, $FDOM_M$ is a suitable parameter to study this process. We then propose using $FDOM_M$ to test the quick photochemical degradation in the light of the humic material produced during bacterial respiration in the dark throughout a seasonal cycle. In our study case, a coastal upwelling system, attention is also paid to the photodegradation in the surface layer of the humic substances upwelled from the bottom layer.

Coastal upwelling systems are ideal to carry out this work, since microbial activity intensifies because of the enhanced entry of nutrients from the adjacent ocean (Wollast 1993). Pelagic and benthic processes are tightly coupled as the result of the reduced water column depth; the organic matter produced in the photic layer is rapidly processed by microheterotrophs in the aphotic layer and the sediments, and upwelling enhances the quick rise to the surface of the products and by-products of microbial degradation.

This study was performed in the northwest coast of the Iberian Peninsula, an area affected by a marked seasonal cycle of coastal winds (Nogueira et al. 1997). From April to October (the upwelling-favorable season), intermittent northerly winds of period 1–2 weeks cause Eastern North Atlantic Central Water (ENACW) to upwell over the shelf. From November to March (the downwelling-favorable season), southerly winds prevail and a marked downwelling front develops between the Iberian Poleward Current (IPC) carrying warm and salty subtropical surface and central water to our latitudes and the coastal water (Álvarez-Salgado et al. 2000). The study site is the Ría de Vigo, one of the Rías Baixas, four large, V-shaped coastal embayments in the northwestern Iberian shelf. These embayments, which behave as an extension of the shelf, are characterized by average flushing times of about 1 week (Álvarez-Salgado et al. 2000).

Material and methods

Sampling strategy—The middle segment of the coastal upwelling system of the Ría de Vigo (Fig. 1) was sampled about 1 h before sunrise, twice per week, during winter (18, 21, 25, and 28 February); spring (11, 15, 18, and 22 April); summer (15, 18, 22, and 26 July); and autumn (17, 19, 23, and 26 September) 2002. Samples were taken with a rosette equipped with twelve 10-liter PVC Niskin bottles with stainless-steel internal springs. Salinity and temperature

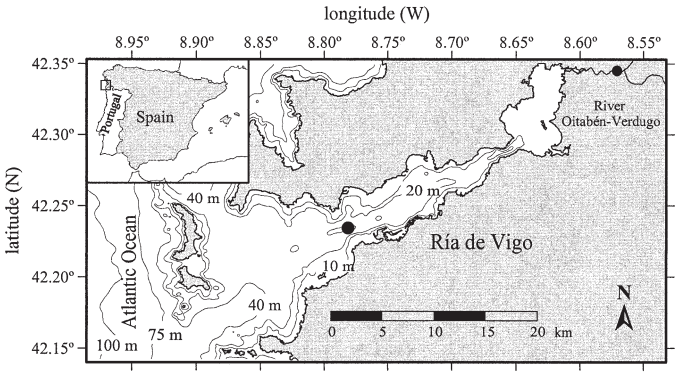


Fig. 1. Chart showing the situation of the sampling stations (filled circles) in the river Oitabén-Verdugo and the Ría de Vigo (NW Spain). The 10, 20, 40, 75, and 100 m isobaths are shown.

were recorded with an SBE 9/11 conductivity-temperature-depth probe attached to the rosette sampler. Conductivity measurements were converted into practical salinity scale values with the equation of UNESCO (1985). Water samples for the analyses of dissolved oxygen and DOM were collected from five depths: the surface (50% photosynthetic available radiation [PAR], average 2.1 ± 0.6 m); the depth of the 25% PAR (average 8 ± 2 m); the depth of the 1% PAR (average 16 ± 3 m); 27 ± 2 m; and the bottom (average 41 ± 1 m).

Dissolved oxygen (O_2)—Samples were collected into calibrated 110 mL glass flasks. After fixation, they were kept in the dark until analysis in the laboratory, 24 h later. O_2 was determined by Winkler potentiometric end-point titration using a Titrino 720 analyser (Metrohm) with a precision of ± 0.5 μmol kg^{-1}. The apparent oxygen utilization, AOU = O_2sat − O_2, was calculated using the algorithm proposed by Benson and Krause (UNESCO 1986) for oxygen saturation (O_2sat).

Dissolved organic carbon (DOC)—Samples for DOM were collected into 500-mL acid-cleaned flasks and filtered through precombusted (450°C, 4 h) 47 mm ø Whatman GF/F filters in an acid-cleaned glass filtration system under low N_2 flow pressure. Aliquots for the analysis of DOC were collected into 10-mL precombusted (450°C, 12 h) glass ampoules. After acidification with H_3PO_4 to pH < 2, the ampoules were heat-sealed and stored in the dark at 4°C until analysis. DOC was measured with a Shimadzu TOC-5000 organic carbon analyzer, as described in Nieto-Cid et al. (2005). The system was standardized daily with potassium hydrogen phthalate. The concentrations of DOC were determined by subtracting the average peak area from the instrument blank area and dividing by the slope of the standard curve. The precision of measurements was ± 0.7 μmol C L^{-1}. The accuracy of the DOC analysis was tested daily with the TOC reference materials provided by D. Hansell (University of Miami). We obtained an average concentration of 45.7 ± 1.6 μmol C L^{-1} (n = 26) for the deep ocean reference (Sargasso Sea deep water, 2,600 m) minus blank reference materials. The nominal value for

TOC provided by the reference laboratory is 44.0 ± 1.5 μmol C L^{-1}.

Fluorescence of dissolved organic matter (FDOM)—Samples for FDOM determination were filtered through precombusted (450°C, 4 h) 47 mm ø Whatman GF/F filters in an acid-cleaned glass filtration system, under low N_2 flow pressure, and preserved at 4°C until determination in the base laboratory with a Perkin Elmer LS 55 luminescence spectrometer within 1 h of sample collection. The instrument was equipped with a xenon discharge lamp, equivalent to 20 kW for 8 μs duration, and a 1-cm quartz fluorescence cell. Milli-Q water was used as a reference for fluorescence analyses, and the intensity of the Raman peak was checked regularly. Discrete excitation/emission (Ex/Em) pair measurements were performed at peaks M (marine humic substances, average Ex/Em 320/410 nm; $FDOM_M$) and T (aromatic amino acids, average Ex/Em, 280/350 nm; $FDOM_T$), specifically tryptophan (Trp). These fixed Ex/Em wavelengths were chosen to assure that we were measuring the same fluorophores in all samples and to allow comparison with other works. Basically, we determined the fluorescence at the wavelengths proposed by Coble et al. (1990) and confirmed by Nieto-Cid et al. (2005) in the Iberian upwelling system. Four replicate measurements were performed for each Ex/Em wavelength. A four-points standard curve was prepared daily with a mixed standard of quinine sulphate (QS) and Trp in sulphuric acid 0.05 mol L^{-1} (Nieto-Cid et al. 2005). The equivalent concentration of every peak was determined by subtracting the average peak height from the blank height and dividing by the slope of the standard curve. Fluorescence units were expressed in ppb equivalents of QS (ppb QS) for $FDOM_M$ and ppb equivalents of Trp (ppb Trp) for $FDOM_T$. The precision were ±0.1 ppb QS and ±0.6 ppb Trp, respectively.

Metabolic balance of the water column—Daily photosynthetic production (Pg) and respiration (R) rates of the plankton community were estimated by the oxygen light-dark bottle method (Strickland and Parsons 1972). Samples collected in 10-liter Niskin bottles were transferred to black polyethylene carboys. Five levels were sampled: 50%, 25%, and 1% of surface light, and two more depths below (27 ± 2 and 41 ± 1 m). The carboys were gently shaken before sampling to prevent sedimentation of the particulate material. Series of eleven 110 mL Winkler bottles composed of triplicate initial and quadruplicate light and dark subsamples were filled for each depth. Each series of light and dark subsamples were incubated for 24 h (starting within 1 h of the sunrise) at the original light and temperature conditions in incubators placed in the terrace of the base laboratory. Dissolved oxygen was determined by Winkler potentiometric end-point titration.

Theses incubators were also used to follow the changes in $FDOM_M$ and $FDOM_T$ under the same conditions. Series of nine 250 mL all-glass flasks compose of triplicate initial, dark and light subsamples were filled. In this case, samples were filtered before analysis through precombusted (450°C, 4 h) 47 mm ø Whatman GF/F filters in an acid-cleaned glass filtration system under low N_2 flow pressure. Fluorescence of initial, dark-, and light-filtered samples was analyzed with the Perkin Elmer LS 55 spectrofluorometer.

Photodegradation of humic substances—Incubation experiments to follow the natural sunlight photodegradation of humic substances in riverine and sea (surface and bottom) water were conducted. Riverine samples were collected once per week in the Soutomaior bridge, in the upstream limit of fresh water-seawater interface of the River Oitabén-Verdugo (Fig. 1). The salinity of riverine samples was <2. Surface and bottom waters of the middle ría also were incubated once per week. Samples were filtered (1) through precombusted (450°C, 4 h) 47 mm ø Whatman GF/F filters (nominal pore size, 1 μm), and (2) through 47 mm ø Gelman Laboratory Supor-200 membrane filters (nominal pore size, 0.2 μm) in an acid-cleaned glass filtration system under low N_2 flow pressure. The filtrates were collected in 250-mL quartz incubators and placed in the terrace of the base laboratory, exposed to 100% natural sunlight. $FDOM_M$ was measured several times during the incubation (0, 1, 3, 7, and 15 d).

Results

The mean profile of microbial respiration rates (R) presented two significant maxima ($p < 0.005$) at the surface and bottom layers, with average values of 6.2 and 1.8 μmol kg^{-1} d^{-1}, respectively (Fig. 2a). The largest variability was observed in the photic layer, especially at the depth of 25% PAR. In the case of the Pg, a surface maximum was observed (average 37 μmol kg^{-1} d^{-1}; Fig. 2b). R and Pg ranged from 0 to 15 μmol kg^{-1} d^{-1} and from 0 to 105 μmol kg^{-1} d^{-1}, respectively. These wide ranges resulted from sampling a wide variety of meteorologic (coastal wind and freshwater runoff regimes) and hydrographic (stratification and homogenization regimes) conditions. The water column–integrated R showed a marked seasonal trend: low values during winter and spring, and significantly higher ($p < 0.001$) during the summer and autumn (Fig. 2c). On the contrary, the water column–integrated Pg did not show any significant seasonal pattern (Fig. 2d). The intraperiod variability (timescale of one half week) was of the same magnitude than the interperiod variability (seasonal timescale). Average ± SD water column–integrated Pg expressed in carbon units was 2.7 ± 2.1 g C m^{-2} d^{-1} if a O_2 : C molar ratio of 1.4 is assumed (Fraga 2001). On average, about 40% of Pg was respired in the water column: three fifths in the photic layer and two fifths in the aphotic layer. The remaining 60%, the so-called new or export production (Quiñones and Platt 1991), is available for export to the adjacent shelf, sinking to the sediments or transferred to a higher trophic level. The ratio of new to total production (Pg-R) : Pg, ranged from 0.6 to 0.8 during winter, spring, and summer (autotrophic phase), but it dramatically decreased to −0.2 during autumn (heterotrophic phase). This conspicuous seasonal pattern has been previously described by Moncoiffé et al. (2000).

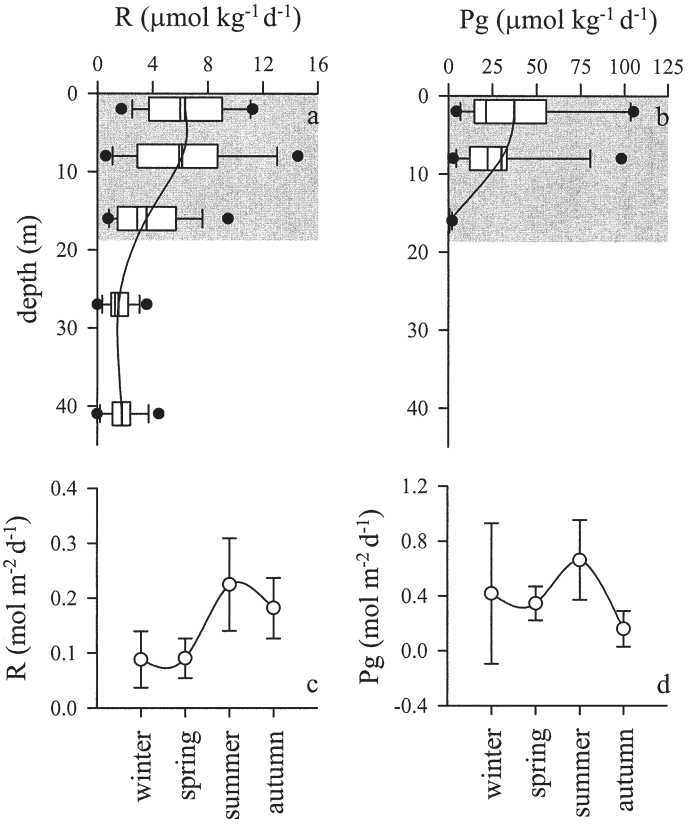


Fig. 2. Box and whisker plot of (a) respiration and (b) gross primary production rates in the middle Ría de Vigo. Fifty percent of the data are included within the limit of the boxes, and the caps represent the 10th and 90th percentiles. Solid lines represent the average profiles. Panels (c) and (d) show the average and standard deviation of the seasonal evolution of the water column–integrated respiration and gross primary production rates, respectively. The grey-shaded area shows the photic layer.

The mean DOC profile showed a significant decrease with depth ($p < 0.001$) from an average surface value of 77 μmol L^{-1} C to a bottom value of 68 μmol L^{-1} C (Fig. 3a). Maximum variability occurred at the surface. This trend has been previously described by Doval et al. (1997) and Nieto-Cid et al. (2005). The $FDOM_M$: DOC ratio showed a slightly, but significant, increase ($p < 0.001$) with depth, from 5.0 to 5.5 ppb QS (ppm C)$^{-1}$ (Fig. 3b). The $FDOM_T$: DOC ratio presented higher values in the upper layer, with a subsurface maximum (15.2 ppb Trp [ppm C]$^{-1}$ at the 25% PAR depth), and a large variability in the whole water column (Fig. 3c). DOC and $FDOM_M$: DOC displayed marked seasonal cycle in the lower layer (<1% PAR); the observed values were significantly higher ($p < 0.001$ and $p < 0.005$, respectively) during the autumn, with average values of 74.0 μmol L^{-1} C and 7.8 ppb QS (ppm C)$^{-1}$, respectively (12% and 70% increase compared with the winter minimum; Fig. 3d,e). Seasonal variability of DOC and $FDOM_T$: DOC in the upper layer (>1% PAR) showed a similar pattern, with a significant increase ($p < 0.001$ and $p < 0.005$, respectively) from winter to autumn (78.2 μmol L^{-1} C and 18.2 ppb Trp [ppm C]$^{-1}$; 8% and 39% increase compared with the winter minimum, respectively; Fig. 3d,f). In summary, Fig. 3d–f indicates a seasonal accumulation of DOC with a marked enrichment in protein fluorescent material in the upper layer and humic fluorescent material in the lower layer.

Microbial reactivity—The net production of humic fluorescence measured in the dark incubations, $FDOM_M$ (dark), correlated significantly ($r^2 = 0.54$, $n = 79$, $p > 0.001$) with R (Fig. 4a). The slope of this linear regression equation, 0.027 ± 0.003 ppb QS (μmol kg^{-1})$^{-1}$, indicates the rate of humic fluorescence production to dissolved oxygen consumption by microbial respiration. It is noticeable the significant production of humic substances in an incubation time as short as 24 h.

The fluorescence of aromatic amino acids measured in the dark and light incubations correlated worst with respiration and primary production rates. The only significant correlation ($r^2 = 0.29$, $n = 46$, $p < 0.001$) was found between the net production obtained in the light incubations, $FDOM_T$ (light), and gross primary production, Pg (Fig. 4b). The slope of 0.018 ± 0.004 ppb Trp (μmol kg^{-1})$^{-1}$ indicates the rate of aromatic amino acids to dissolved oxygen production by the phytoplankton community. The origin intercept, −1.4 ± 0.2 ppb Trp d^{-1}, pointed to $FDOM_T$ consumption quicker than production, except for Pg exceeding 80 ± 20 μmol kg^{-1} d^{-1}.

It is opportune to test if these regression parameters from an incubation bottle (10^{-1} liters) could be extrapolated to the volume of a ría (10^{12} liters). In order to study the relationship between dissolved oxygen and humic fluorescence in the ría after removal of the water mass mixing effect, a regression analysis involving two conservative parameters was used. The variability of any nonconservative parameter due to the mixing of the three water masses that meet in the ría (subtropical ENACW, subpolar ENACW, and coastal water) can be estimated by means of a multiple linear regression of the nonconservative parameter with salinity and temperature (Nieto-Cid et al. 2005). Only samples with AOU > 0 can be used (<1% PAR, R > Pg) because S and T are conservative and O_2 is not exchanged with the atmosphere in these samples. The multiple regression of $FDOM_M$ with S, T, and dissolved oxygen for sample with AOU > 0 was

$$FDOM_M(\pm 0.6) = 78(\pm 19) - 2.1(\pm 0.5)S + 0.55(\pm 0.08)T - 0.029(\pm 0.003)O_{2C}$$

$$r^2 = 0.83, n = 56, p < 0.001$$

The dissolved oxygen of the samples was referred to the oxidation state of nitrate (O_{2C}) to compare the oxygen consumption to the N-nutrient production independently of the nitrogen form involved in the process (Fraga 2001). Because 0.5 mole of oxygen is necessary to oxidize 1 mole of nitrite to nitrate and 2 moles of oxygen are required to

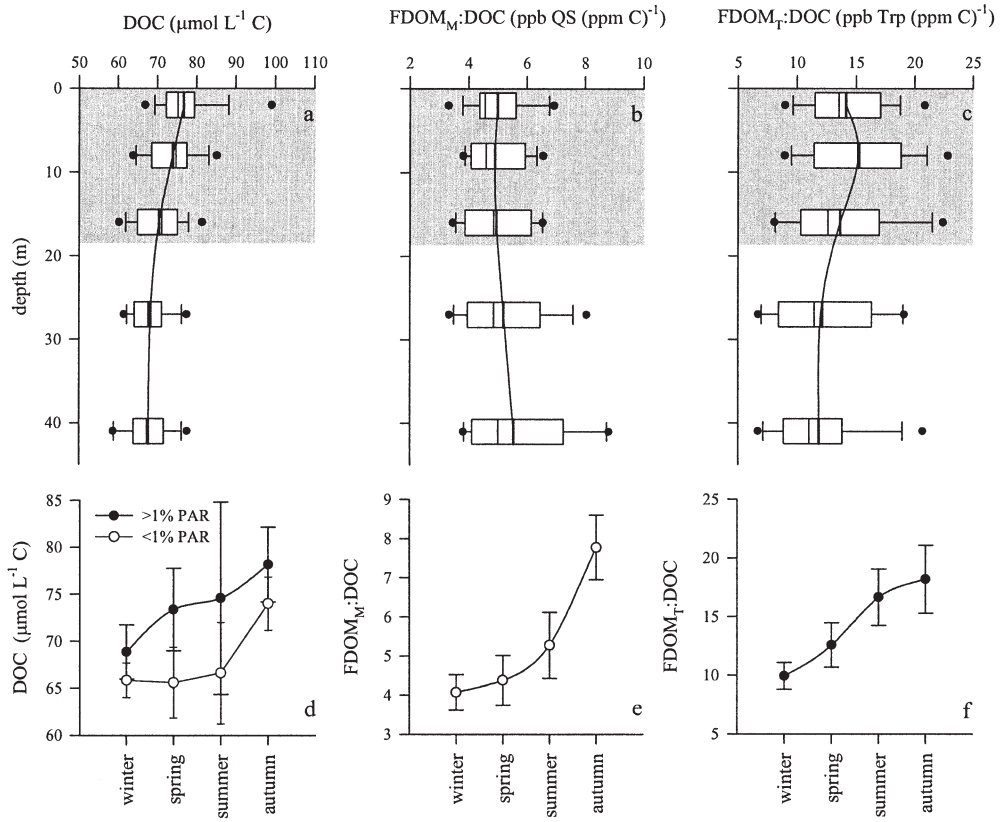


Fig. 3. Box and whisker plot of (a) DOC, (b) $FDOM_M$: DOC, and (c) $FDOM_T$: DOC profiles for the middle Ría de Vigo. Fifty percent of the data are included within the limit of the boxes, and the caps represent the 10th and 90th percentiles. Solid lines represent the average profiles. Plots (d), (e), and (f) show the average and standard deviation of seasonal evolution of DOC, $FDOM_M$:DOC, and $FDOM_T$: DOC, respectively. The grey shaded area shows the photic layer.

oxidize 1 mole of ammonium to nitrate,

$$O_{2C} = O_2 - \frac{1}{2} \times NO_2^- - 2 \times NH_4^+ \qquad (1)$$

The resultant slope, -0.029 (± 0.003) ppb QS (μmol kg^{-1} O_2)$^{-1}$, is not significantly different from the incubation experiments. The opposite sign is because respiration involves the utilization of oxygen—it proves the validity of the incubation approach at the ecosystem level.

For the case of $FDOM_T$, the correlation with primary production was observed in the upper layer (>1% PAR), where O_2 exchanges with atmosphere, and S and T do not behave conservatively. Therefore, validation of the incubation approach at the ecosystem level is not possible for the fluorescence of aromatic amino acids.

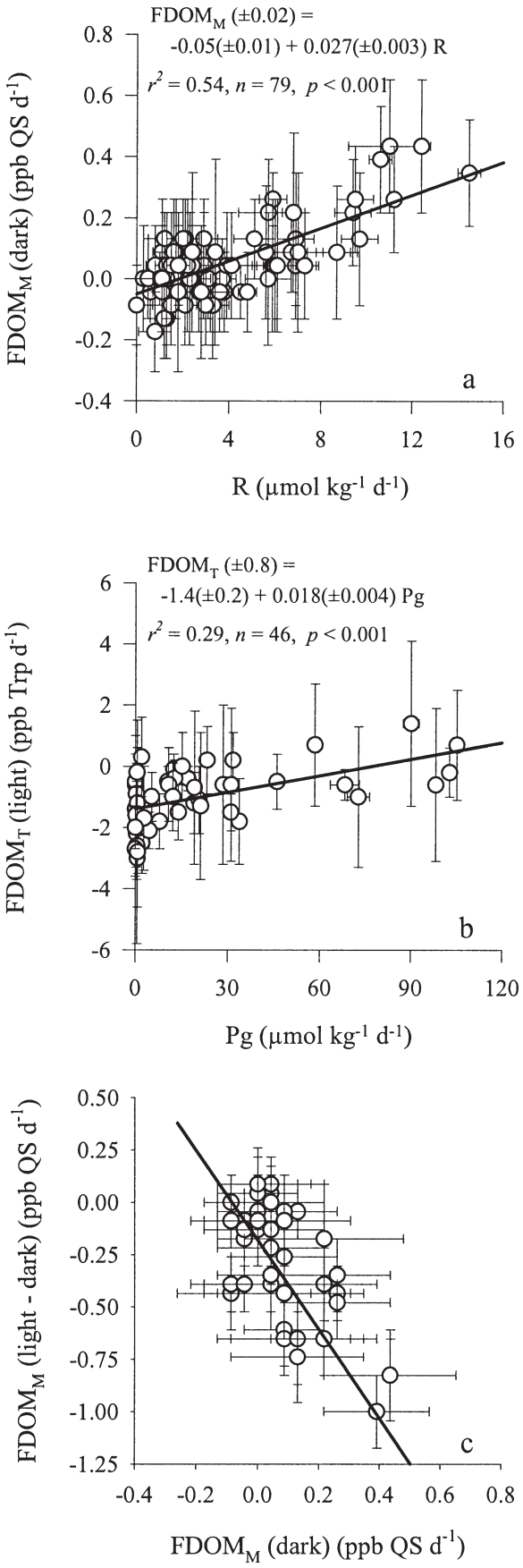
Photochemical reactivity—The difference between the net production of humic fluorescence in the light and dark incubations is the material consumed by photobleaching during 1 d. A significant linear relationship ($r^2 = 0.50$, $n = 46$, $p > 0.001$) was observed between the bacterial production and the photochemical consumption of humic fluorescence (Fig. 4c) in such a way that the humic material produced in the dark, either in the aphotic layer or during the night, is rapidly photodegraded in the light.

Photobleaching experiments showed a loss of the humic fluorescence of the samples with the incubation time, which fitted to the exponential decay function:

$$C = (C_0 - C_f) \exp(-kt) + C_f \qquad (2)$$

where C_0 is the initial concentration of humic compounds, C_f is the humic compounds resistant to photobleaching, and k is the exponential decay constant.

The parameters of Equation 2 for the riverine samples in winter, spring, summer, and autumn (Fig. 5a–d, respectively) were contrasting. It was found that the variability of C_0 and C_f followed the same pattern: they increased from a winter-spring minimum to an autumn maximum (Fig. 5e). The values of k suggested also a progressive increase of the decomposition rate from winter to autumn (Fig. 5f). Because incubations were made under natural light conditions, k depended not only on the incubation time but also on the contrasting incident light intensity, which increases from winter to autumn. Corrected k values to an average incident light are also shown in Fig. 5f; a constant decomposition rate of $\sim$20% d^{-1} was observed from winter to summer, followed by a threefold increase in autumn,



when 65% of humic fluorescence was bleached per day. However, the percentage of photobleached material [(C_0 − C_f) : C_0×100] was maximum in winter-spring (68%) and minimum in autumn (57%).

Bottom and surface seawater samples from the ría displayed the same exponential decay pattern (Fig. 6a–d), characterized by lower initial and final concentrations (Fig. 6e) but with similar incident light–corrected decomposition rates than riverine samples (Fig. 6f). The values of C_0, C_f, and k were comparable from winter to summer, but autumn presented again the largest discrepancies: higher fluorescence and decomposition rates. It should also be noted that bottom and surface k values coincided in winter, but these values were spacing out with the increase of stratification in the water column. The decomposition rates became larger for the bottom (20–90% d^{-1}) than for the surface water (20–55% d^{-1}). Despite this, the percentage of photodegraded material during the incubations was similar at both depths. Values ranged from 44% in winter to 59% in summer for the bottom samples, whereas the percentages varied from 42% in summer to 50% in winter for the surface samples.

Discussion

Microbial production of labile and recalcitrant DOM—A significant positive correlation has been found between the fluorescence of marine humic substances and the concentration of inorganic nutrients in the water column of different open oceans (Hayase et al. 1987; Chen and Bada 1992) and coastal marine systems (Chen and Bada 1992; Hayase and Shinozuka 1995; Nieto-Cid et al. 2005). All of these authors concluded that marine humic substances and nutrient salts should have the same origin: the mineralization of settling organic particles. In the case of Nieto-Cid et al. (2005), they were the first to separate the physical (water masses mixing) and biogeochemical components of the fluorescence of humic substances and nutrient salts to test a significant positive correlation between biogeochemical components. Therefore, they were all implicitly assuming that marine humic substances should be a by-product of bacterial respiration processes (Brophy and Carlson 1989; Kramer and Herndl 2004). Other models, such as the condensation of poly-phenols (Hedges 1978) or the photooxidation of lipids (Kieber et al. 1997) would restrict to estuaries, where poly-phenols of terrestrial origin can be abundant, and the surface ocean layer, where the UV radiation is able to oxidize triglycerides and fatty acids of planktonic origin.

Net production rates of humic fluorophores in dark incubations of natural plankton populations had not been

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Fig. 4. X-Y plots of (a) net FDOM$_M$ production in the dark incubations versus respiration, (b) net FDOM$_T$ production in the light incubations versus gross production, and (c) net FDOM$_M$ production in the light minus dark incubations versus respiration. Solid lines represent the corresponding linear regression lines.

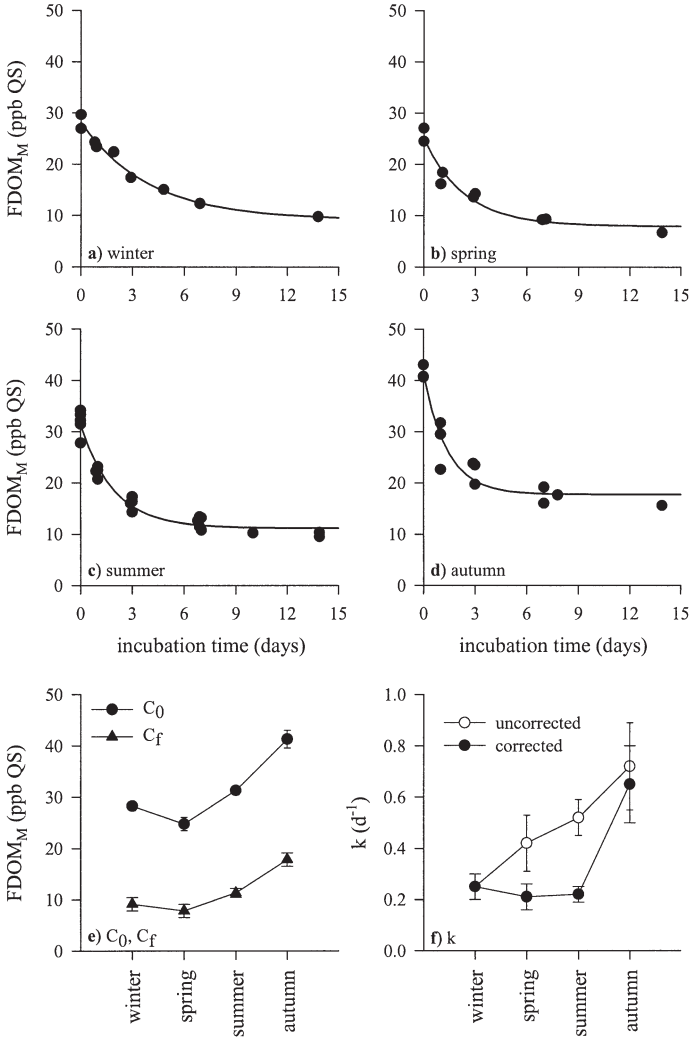

Fig. 5. $FDOM_M$ course during the incubation time for (a) winter, (b) spring, (c) summer, and (d) autumn riverine samples. The solid line represents the fit to an exponential decay function: $C = (C_0 - C_f) \exp(-kt) + C_f$. Seasonal variation of the estimated (e) C_0 and C_f, and (f) the constant (k) uncorrected and corrected for incident light intensity.

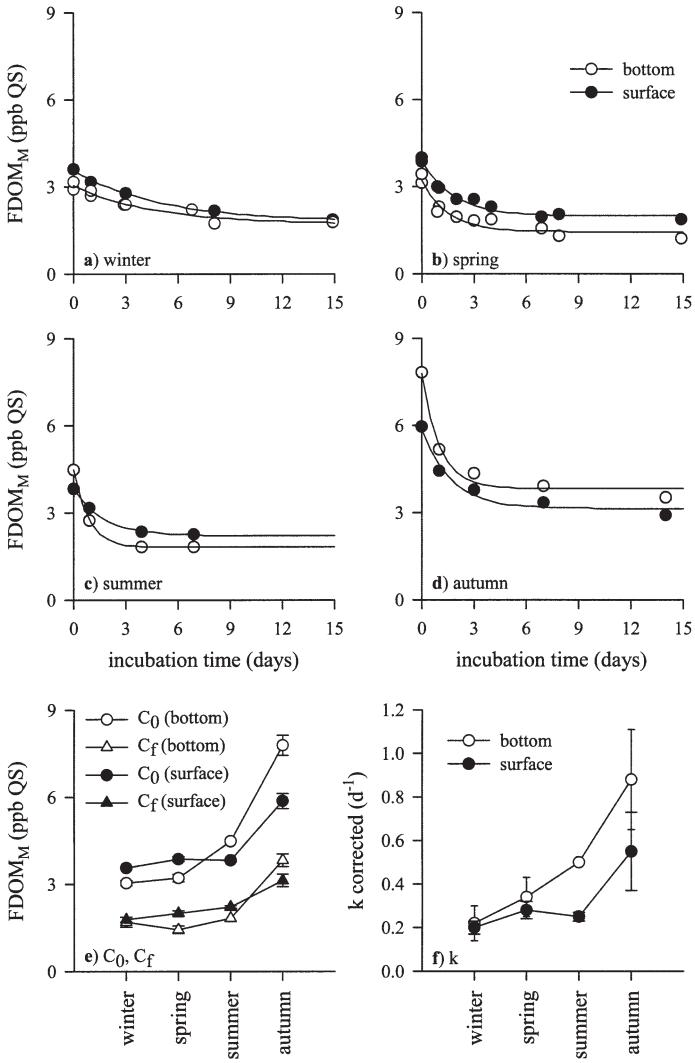

Fig. 6. $FDOM_M$ course during the incubation time for (a) winter, (b) spring, (c) summer, and (d) autumn seawater samples, surface, and bottom. The solid line represents the fit to the exponential decay function $C = (C_0 - C_f) \exp(-kt) + C_f$. Seasonal variation of the estimated (e) C_0, C_f, and (f) k corrected for incident light intensity.

obtained until very recently, by Kramer and Herndl (2004) and Yamashita and Tanoue (2004). These authors found that about 25% of the humic-like fluorescence intensity in the surface waters of Ise Bay (Japan) was produced in situ. A degradation experiment using natural plankton from Ise Bay demonstrated the rapid production of humic substances in tandem with plankton degradation within the short timescale of 1 d in the dark. The production rate was as high as 2.97 ppb QS d^{-1} during the initial 12 h, was reduced to 1.04 ppb QS d^{-1} during the initial 3 d, and then increased gradually at 0.07 ppb QS d^{-1}. Following a similar approach, Kramer and Herndl (2004) concentrated and resuspended, in artificial seawater, bacterioplankton of the coastal North Sea. They observed a steady increase of the fluorescence of humic substances at a rate of 0.15 $\pm$ 0.03 ppb QS d^{-1} until day 9 of the incubation. Thereafter, fluorescence remained constant until the end of the experiment on day 21.

In this work, we incubated during 1 d unaltered natural marine samples collected at different depths in the euphotic and aphotic layers of a coastal upwelling system under a wide variety of meteorologic and oceanographic conditions. The results confirm the degradation experiment conducted by Yamashita and Tanoue (2004) in the estuarine system of Ise Bay, and Kramer and Herndl (2004) in the coastal North Sea, consistently suggesting that in situ production of marine humic fluorophores play an important role in the dynamics of DOM in coastal environments. Daily production rates of humic fluorescence in the coastal upwelling system of the Ría de Vigo, <0.5 ppb QS d^{-1}, were much lower than in the experiment of Ise Bay and similar to those found in the coastal North Sea. Kramer and Herndl (2004) observed that whereas the $FMOD_M$: DOC ratio of the bacterial-derived DOM varied from 3 to 7 ppb QS (ppm C)$^{-1}$, the marine bulk

DOM from the North Sea, Adriatic Sea, and North Atlantic was <1.5 ppb QS (ppm C)$^{-1}$. Note that the $FMOD_M$: DOC ratio for the samples of the Ría de Vigo varied from 4 to 8 ppb QS (ppm C)$^{-1}$ (Fig. 3), further reinforcing the hypothesis of a bacterial origin of the humic fluorescence.

An added value of our work is the possibility to compare, for the first time in the literature, the daily production of marine humic fluorescence with the daily consumption of dissolved oxygen in the dark. Therefore, the significant positive correlation between the net production of humic fluorescence and the net consumption of dissolved oxygen constitutes field evidence of the fast production of humic substances as a by-product of microbial respiration processes in the marine environment in general, and a costal upwelling system in particular. The local or universal validity of the resultant ratio of marine humic fluorescence production to dissolved oxygen consumption, 0.027 ± 0.003 ppb QS (μmol kg^{-1})$^{-1}$, has to be tested in further studies in other marine environments.

Yamashita and Tanoue (2004) also observed a continuous decrease in the fluorescence of dissolved amino acids in tandem with the production of marine humic substances during a 71-d dark incubation of the natural plankton of Ise Bay. In agreement with these authors, we obtained an average net consumption rate of protein-like fluorescence of 1.4 ± 0.2 ppb Trp d^{-1}. This rate is the origin intercept of the significant positive correlation observed between the net production of protein-like fluorophores and the net production of dissolved oxygen in the light. This relationship indicates that the production of dissolved amino acids is probably linked to phytoplankton exudation at a rate of $18 \pm 4\ 10^{-3}$ ppb Trp (μmol kg^{-1})$^{-1}$, and it is rapidly consumed by bacteria in such a way that accumulation of protein-like fluorescence after a 1-d incubation only occurs when primary production exceeds 80 ± 20 μmol O_2 kg^{-1} d^{-1} (i.e., about 0.7 ± 0.2 g C m^3 d^{-1} if a O_2:C molar ratio of 1.4 is assumed; Fraga 2001).

Bioavailability of marine humic substances by photobleaching—Photochemical processes have been identified as a potentially important mechanism for the degradation of terrestrial (e.g., Moran et al. 2000) and marine (e.g., Mopper and Zhou 1990; Obernosterer and Herndl 2000) humic substances. Photochemical degradation of DOM is usually followed by the exponential decrease of the concentration of DOC or the absorbance of DOM at wavelengths ranging from 350 to 450 nm during the course of incubation experiments under laboratory or natural UV light conditions. Less attention has been paid to fluorescence techniques for evaluating photodegradation rates, although in marine systems DOC concentrations and chromophoric DOM levels are low compared with freshwater and estuarine systems. In addition, fluorescence allows following the decomposition of specific fluorophores rather than the bulk DOM (Pullin and Cabaniss 1997). Incubation experiments to derive DOM photodegradation rates following the decrease of humic fluorescence under natural light conditions during short time periods (1–2 weeks) are scarce in the literature. In fact, to our knowledge, only Skoog et al. (1996) carried out this type of experiment at several depths in the Baltic Sea on a single day in May 1992. They found higher photodegradation rates (0.60–1.25 d^{-1}) and similar percentages of photobleached material (50–56%) as in the Ría de Vigo for incubation times of 4 d. We observed maximum photodegradation rates during the autumn, at the time when bacterial mineralization became the dominant process either in terrestrial and marine systems. Therefore, the contribution of freshly produced humic substances to DOC is maximum during the autumn, producing highest values of k. The photosensitivity of the material or the quality of natural light (Del Vecchio and Blough 2002) could enhance photodegradation during this period. Unfortunately, there are no studies following the evolution of photodegradation rates during a seasonal cycle to compare with our results.

Increased availability of DOM to bacteria exposed to natural UV light has been observed in lakes (e.g., Lindell et al. 1995; De Lange et al. 2003) and coastal waters (e.g., Miller and Moran 1997; Obernosterer and Herndl 2000). However, Tranvik and Bertilsson (2001) indicated that photochemical transformation of DOM can either reduce or enhance bacterial utilization; whereas humic substances are predominantly transformed into labile forms, algal-derived DOM is transformed into compounds of decreased bacterial substrate quality. The same argument was used by Benner and Biddanda (1998) to explain why the effects of sunlight exposure on bacterial utilization of DOM was negative for surface samples (15–115 m) and positive for deep samples (150–1,000 m) of the Gulf of Mexico. According to these authors, surface DOM was enriched in phytoplankton-derived material, whereas the concentration of marine humic substances increased with depth. Accordingly, Obernosterer and Benner (2004) observed that although photodegradation enhanced the biodegradation of terrigenous humic substances, this effect was not detected for plankton-derived material.

In this work, we have obtained a significant negative correlation between the bacterial production and the photochemical decomposition of humic fluorophores. It suggests rapid photodegradation of recently produced marine humic substances that, in turns, would enhance bacterial activity as a consequence of their transformation into compounds of increased bacterial substrate quality.

Finally, we have also concluded that the humic fluorophores of the bottom layer were photodegraded faster than the humic fluorophores of the surface layer during the summer and autumn stratification periods. The increase of photodegradation rates with depth was also observed by Skoog et al. (1996) in the Baltic Sea. In a coastal system such as the Ría de Vigo, upwelling promotes the quick rise of bottom waters to the surface layer and continental inputs are quite scarce (Álvarez-Salgado et al. 2000). Therefore, the humic material of the surface layer is the same material as at the bottom layer after partial photodegradation. Although it has been previously shown that the deep sea DOM is more photoreactive than photobleached surface material (Mopper and Zhou 1990; Mopper and Kieber 2000), the timescale of the upwelling process needed to photodegrade

the deep sea material is decades to centuries. On the contrary, the humic material produced during bacterial respiration in the bottom layer of the Ría de Vigo is advected to the surface layer in just 1 week (Álvarez-Salgado et al. 2000), where intense photodegradation takes place. In this sense, a tight coupling between photochemical and microbial degradation processes during an upwelling episode is expected. Photochemical degradation of marine humics will produce gases such as CO_2 and low–molecular weight carbonyl compounds (Mopper and Kieber 2000) able to stimulate the bacterial activity of the surface layer. Therefore, the humic substances produced by the bacterial activity in the bottom layer will enhance, in about 1 week, the bacterial activity of the surface layer after exposure to sunlight.

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