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A. Huguet [a,b], L. Vacher [a,b], S. Relexans [a,b], S. Saubusse [a,b], J.M. Froidefond [c], E. Parlanti [a,b,*]

[a] Université de Bordeaux, UMR 5255, ISM-LPTC, 351 cours de la Libération, Talence F-33405, France
[b] CNRS, UMR 5255, ISM-LPTC, Talence F-33405, France
[c] Université de Bordeaux, UMR CNRS 5805, EPOC, 351 cours de la Libération, Talence F-33405, France

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ABSTRACT

The isolation, characterization and study of the properties of aquatic dissolved organic matter (DOM) still represent a challenge because of the heterogeneity, complexity and low concentration of organic material in natural waters. Based on its ability to interact with contaminants and thus to modify their transport and bioavailability, DOM is of interest for environmental purposes. The objective of this work was to better characterize DOM in the Gironde Estuary (southwestern France). The estuary represents an exchange zone between the continent and the Atlantic Ocean and conditions the transfer of organic and inorganic substances from the continental to the oceanic environment. Several samples were collected along the estuary during three cruises in 2002 and 2006. They were analysed using excitation–emission matrix (EEM) spectroscopy, a sensitive technique that allows direct analysis of water samples. Fluorescent DOM and dissolved organic carbon (DOC) did not behave conservatively in this estuarine system, i.e. the organic material did not undergo simple dilution from the upstream to the downstream part of the estuary. A seasonal variability in DOC content was pointed out, whereas few seasonal variations in DOM fluorescence were observed. DOM sources and processing in the estuary were further evaluated by determining two fluorescence indices – the humification index (HIX) and the index of recent autochthonous contribution (BIX). By applying these indices, the relative degree of humification (HIX) and autotrophic productivity (BIX) could be assessed. Based on the fluorescence and DOC results, the estuary was divided into three zones depending on salinity (S) and characterized by specific DOM: (i) A turbid zone of low salinity ($S < 5$) and high suspended particulate matter concentration with increase in the intensities of the α' and α fluorophores, characteristic of humic-like compounds. (ii) A mid-estuarine zone ($5 < S < 25$) characterized by low autotrophic productivity and containing strongly degraded organic material, as shown by the low values of BIX and high values of HIX. (iii) A higher salinity area ($S > 25$) characterized by increased autotrophic productivity and a marked marine influence, and associated with high and low values of BIX and HIX, respectively. The HIX and BIX indices were shown as useful tools for readily defining and classifying DOM characteristics in estuarine waters.

1. Introduction

The study of aquatic dissolved organic matter (DOM) is of significant environmental interest. In fact, it can interact with many organic or inorganic contaminants, such as metals, pesticides and polycyclic aromatic hydrocarbons (PAHs) resulting from anthropogenic activity and can directly influence their transport, stability and bioavailability (e.g. Akkanen et al., 2004; Hirose, 2007). The association of organic or inorganic species with DOM can contribute to a greater persistence of these contaminants in the environment, but can allow at the same time an increase or decrease in their toxicity towards living organisms (Suffet and MacCarthy, 1989). Therefore, it is essential to examine more precisely the nature of organic macromolecules in order to better understand the mechanisms implied in the processes of transport and transformation of these contaminants. The study of DOM is particularly important for coastal environments, which constitute exchange areas between continents and oceans and which allow the transfer of organic or inorganic substances from the continental to the oceanic environment.

DOM also plays a key role in bacterial production and microbial food web processes in coastal ecosystems. It acts as a substrate that supports heterotrophic bacterial activity (Carlson, 2002 and references therein). Heterotrophic bacteria are thus the major consumers of DOM in seawater (Azam and Hodson, 1977; Williams, 1981). The factors controlling its transfer, production, removal and accumulation in estuarine environments have therefore both biogeochemical and ecological significance. Determining its sources

* Corresponding author. Address: Université de Bordeaux, UMR 5255, ISM-LPTC, 351 cours de la Libération, Talence F-33405, France. Tel.: +33 540 008 491; fax: +33 540 002 267.
E-mail addresses: arnaud_huguet@yahoo.fr (A. Huguet), eparlant@u-bordeaux1.fr, e.parlanti@ism.u-bordeaux1.fr (E. Parlanti).

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(McCallister et al., 2006a,b) in coastal waters and the factors regulating its production, consumption and transformation (e.g. by photodegradation; Dalzell et al., 2009) are critical for understanding the carbon cycle in these complex environments.

The objective of this study was to follow the evolution of DOM during the mixing of fresh and marine waters in the Gironde Estuary (southwestern France). This was performed using spectrofluorometry (more precisely three dimensional excitation–emission matrix (EEM) spectroscopy). This has the advantage of being fast and non destructive, does not require sample pre-treatment and is sufficiently sensitive to detect fluorescent compounds at low concentration, as is the case for fluorescent DOM in coastal waters. Spectrofluorometry offers the possibility of easily following the evolution of fluorescent DOM (FDOM) from a qualitative and semi-quantitative standpoint. Three dimensional (3D) fluorescence spectra show significant differences as a function of the type and origin of samples (river, lake or sea water) and provide information on the type and relative concentration of fluorescent compounds making up DOM. Fluorescence spectroscopy has been used extensively in recent years to characterize and differentiate water masses (e.g. Mopper and Schultz, 1993; Coble, 1996, 2007) and to study their mixing in coastal and estuarine environments (e.g. Laane and Kramer, 1990; De Souza Sierra and Donard, 1991; Baker and Spencer, 2004; Boyd and Osburn, 2004; Callahan et al., 2004; Kowalczuk et al., 2003, 2005; Murphy et al., 2008).

2. Materials and methods

2.1. Sampling site

Samples were collected from the Gironde Estuary. The estuary (Fig. 1) extends over ca. 100 km, between KP 0 – Bordeaux – and KP 100 located at the mouth of the estuary, between La Pointe de Grave and Royan. KP (kilometric point) is the distance expressed in km from the city of Bordeaux (KP 0). Increasing KP is located downstream from Bordeaux while decreasing KP is located upstream from Bordeaux. The estuary can be divided into four main zones:

(i) The fluvial estuary, from the upstream limit of the influence of the dynamic tide in the Garonne and Dordogne Rivers (La Réole for the Garonne River (KP −70, i.e. 70 km upstream from Bordeaux) and Pessac-sur-Dordogne for the Dordogne River) to Le Bec d'Ambès (KP 25), the confluence of the two rivers.
(ii) The upstream estuary between Le Bec d'Ambès and Saint-Christoly (KP 65).
(iii) The downstream estuary between Saint-Christoly and La Pointe de Grave (KP 93).
(iv) The mouth of the estuary, downstream of La Pointe de Grave.

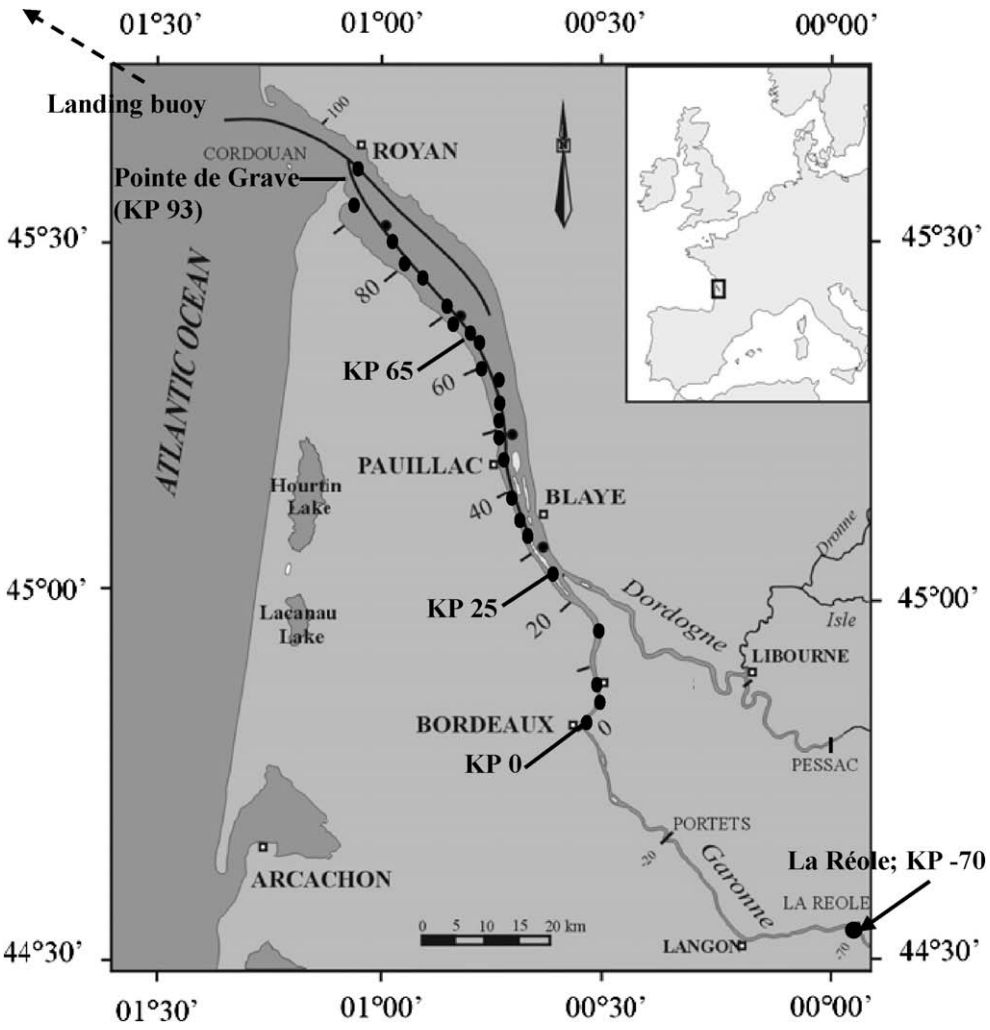

Fig. 1. Location of sampling sites in Gironde Estuary (Girox 1, February 2002; Girox 2, September 2002; OPTIC G3, September 2006; La Réole, February 2007). KP: kilometric point, distance in kilometers from the city of Bordeaux (KP 0); increasing KP is located downstream Bordeaux; KP 100 is the estuary mouth; KP −70 is the upstream limit of dynamic tide.

The catchment basin of the estuary, with a total area of ca. 79,000 km^2 and population density 75 inhabitants km^{-2}, is considered unpolluted. The estuary is macrotidal with a moderate flow rate (1000 m^3 s^{-1} average, with a maximum (1800 m^3 s^{-1}) in February and a minimum (400 m^3 s^{-1}) in August–September; Abril et al., 1999) and high turbidity. It is formed by the Garonne (65% of freshwater input) and Dordogne (35% of freshwater input) rivers (Saari et al., 2008). The discharge of the Dordogne is well regulated as a result of 29 dams of various sizes (0.14×10^9–8.8×10^9 m^3) associated with hydroelectric power plants along the river system (Maneux et al., 2001) and variation in annual mean is small (Schäfer et al., 2002). In contrast, the discharge of the Garonne River is more related to the natural hydrology of the whole drainage basin (e.g. wet/dry years, local events of high discharge, etc.) and water fluxes show greater temporal variation (Schäfer et al., 2002). The Gironde Estuary discharge also shows important annual variations, mainly depending on those of the Garonne River.

Water discharge for the Garonne and Dordogne rivers in 2002 and 2006 is presented in Fig. 2. Discharge variations of the Dordogne with the seasonal sampling conducted in this study were relatively small. Flow rates were equal to 240, 200 and 140 m^3 s^{-1} in February 2002, September 2002 and September 2006, respectively. Water flow of the Garonne was 380, 170 and 200 m^3 s^{-1} during the same periods.

The Gironde Estuary is characterized by a wide estuarine turbidity maximum (ETM), extending generally from Pauillac (KP 45) and, on one hand, Bordeaux in the Garonne River and, on the other hand, upstream of Libourne on the Dordogne River. It is one of the most turbid estuaries in Europe. During the winter floods, the ETM moves downstream. The ETM, which appears in estuarine systems, is created by the mixing of fresh water, rich in suspended particulate material (SPM) and nutrients, and marine waters. The particle residence time is up to 5 years. As for the SPM content of the ETM, this can reach 1 g L^{-1} at the surface (Kraepiel et al., 1997; Abril et al., 1999). The high volumes of SPM also limit the penetration of light, which in turn limits the production of phytoplankton. Thus, the organic nutrients in the estuary are rarely consumed, except at the estuary mouth. The system is nonetheless characterized by a strong biological production (Dauvin, 2008).

2.2. Sampling strategy

Samples were collected during three cruises by the R/V Côte d'Aquitaine. Collection of water samples was performed along a salinity gradient, in the ETM and in the downstream part of the estuary. Of these, 18 were collected during the first cruise (GIROX 1) in February 2002 and 20 on the second cruise (GIROX 2) in September 2002. During the third cruise (OPTIC G3) in September

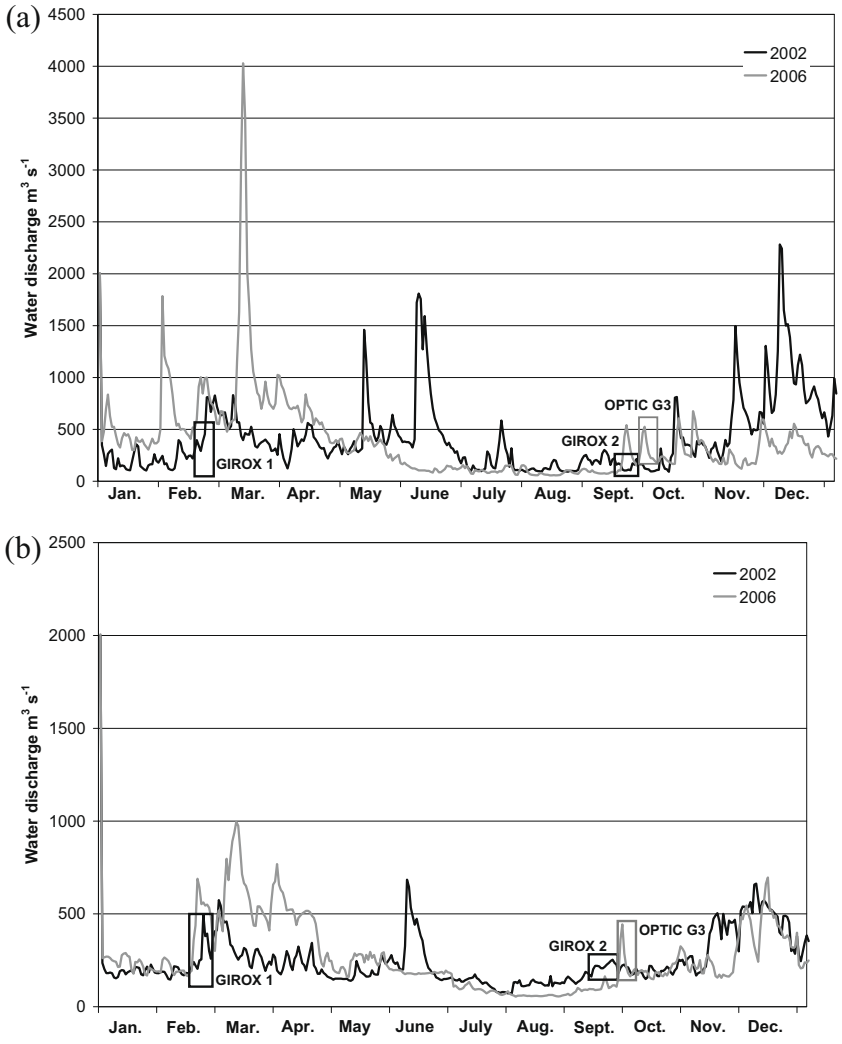

Fig. 2. Water discharge for the Garonne (a) and Dordogne (b) rivers in 2002 and 2006.

2006, 19 water samples were collected at different points in the estuary from KP 25 to the landing buoy located along the coasts (Fig. 1). A "stationary point" sampling was also carried out near KP 60: seven samples were collected at different times over a tidal cycle. For each sample, 0.5–1l water were collected. In February 2007, a freshwater sample was collected from the upstream part of the estuary, more precisely from the Garonne River (at La Réole, KP −70). The characteristics of all the samples (sampling location, salinity, SPM and DOC contents) are summarised in Table 1.

Samples were filtered under reduced pressure with precombusted Whatman glass fibre filters (GF/F; average porosity 0.7 μm) immediately after sampling and collected in glass bottles with Teflon caps. Water samples were stored in the dark at 4 °C for a few days prior to analysis. All glassware was thoroughly washed with non-ionic detergent (RBS 50) and abundantly rinsed with tap water and ultrapure water (Milli-Q, Millipore) before sampling. The cleanliness of the glassware was tested using fluorescence.

2.3. EEM spectroscopy

Fluorescence spectra were recorded with a Fluorolog FL3-22 Jobin Yvon Fluorometer equipped with double monochromators both at the excitation and the emission sides. Samples were contained in a 1 cm path length fused silica cell (Hellma), thermostatted at 20 °C. Fluorescence EEM spectroscopy involved scanning and recording 17 individual emission spectra (260–700 nm) at sequential 10 nm increments of excitation wavelength between 250 nm and 410 nm as described by Parlanti et al. (2000). Experiments were run in ratio mode with 4 nm bandwidth for both excitation and emission, 0.5 s integration time and 1 nm emission wavelength increment (corresponding to a scan rate of about 6.5 nm min^{-1}). The photomultiplier voltage was set to 950 V. Spectra were obtained by subtracting ultrapure water blank spectra, recorded under the same conditions, to eliminate water Raman peaks. The 17 scans were used to generate 3D contour plots of fluorescence intensity as a function of excitation and emission wavelengths. To make the graphs readable, the topographic and contour EEM plots are presented with excitation and emission wavelength increments of 10 nm and 5 nm, respectively. Although the resolution in the plots is lower, the positions and intensities quoted in the text correspond to the original line spectra.

The ratio mode (sample/reference) selected as the acquisition mode produced spectra corrected for variations in lamp intensity with respect to time. The emission spectra were electronically corrected for instrumental response (Ewald et al., 1983; De Souza Sierra et al., 1994) but the emission correction was not valid below 300 nm. The excitation correction was not applied to the spectra. However, it should be noted that comparison of spectra obtained with the same instrument is acceptable, regardless of whether or not the corrections are made, because the spectra were obtained from a single instrument and are internally consistent.

Fluorescence spectra were calibrated by normalization to the integrated water Raman scattering band, yielding the Raman unit as a quantitative measure of the signal intensity (Determann et al., 1994; Nieke et al., 1997). Since signal intensities taken at specific wavelengths are normalized to intensities integrated over a wavelength interval, data are in units of inverse wavelength (nm^{-1}), denoted as "Raman units" (Nieke et al., 1997). Fluorescence spectra were normalized to the integrated intensities of the Raman scattering band of ultrapure water (Milli-Q, Millipore) samples. The water Raman scatter band was integrated from 380 nm to 420 nm at λ_{exc} 350 nm for 30 ultra pure water samples; the average value was used for normalization.

Table 1
Characteristics of water samples collected in Gironde Estuary during GIROX cruises (February and September 2002) and OPTIC G3 cruise (September 2006). SPM: suspended particulate matter.

KP/city	Salinity	DOC (μmol/L)	SPM (mg/L)
Girox 1			
KP0	0.2	233	2025
KP5	0.4	183	589
KP15	0.8	183	608
KP30	2.9	158	443
KP45	3.1	175	263
KP55	4.2	204	882
KP65	5.2	158	202
KP70	7	158	1314
KP75	9	129	540
KP80	13.5	125	108
	16.4	113	64
	20.5	108	37
	24	125	42
	25.4	92	51
	28	100	55
	30.3	88	72
	31.7	117	35
	33.8	83	24
Girox 2			
KP0	0.5	117	2250
KP7	0.6	125	1700
KP15	0.8	150	1750
KP30	1.3	121	1350
KP45	2.1	129	1050
	2.6	117	1600
	4.1	121	1300
	4.5	125	980
	5.4	125	590
	7.4	121	440
KP67	9.9	129	610
	12.7	133	440
	15	154	460
	17.6	125	235
	21	125	440
KP80	24	104	405
	25.9	100	137
	28.9	96	110
	31.5	83	55
	33	88	27.4

KP/city	Salinity	DOC (μmol/L)
Optic G3		
La Réole (KP −70)	0	155
	2	167
	2.2	162
	2.6	163
KP40	2.3	171
	3.5	157
Pauillac	4.7	167
	6.2	154
	12.4	160
	13	154
	11.1	156
	13.4	154
	21	147
KP80	25	128
	25.7	123
	26.2	127
	27.1	118
Royan	28.1	116
	33.1	102
Landing buoy	34.3	99
Stationary point		
KP60	12.9	150
KP60	13.4	160
KP60	9.8	158
KP60	8.2	162
KP60	6.4	152
KP60	7.9	159
KP60	9.6	154

Table 2
Major fluorescence bands for water, with notations used herein and nomenclature proposed by Coble (1996).

Band	Excitation max. (nm)	Emission max. (nm)	Compound type	Letter used by Coble (1996)
α	330–370	420–480	Humic substances	C
α'	230–260	380–480	Humic substances + recent materials	A
β	310–320	380–420	Autochthonous production	M
γ	270–280	300–340	Protein-like material	B

The analytical uncertainty in the measurement was calculated by recording the EEM spectrum of one water sample four times. The fluorescence intensities of the main fluorophores associated with DOM (Table 2) were measured after each data acquisition step. This experiment was carried out for three water samples from the estuary. The analyses showed that the analytical uncertainty associated with both measurement of fluorescence intensity and normalization to Raman units was less than 2%. The analytical uncertainty in the calculation of HIX and BIX does not exceed 2% and 1%, respectively.

In order to avoid an inner-filtering effect, the UV–visible absorbance spectrum of water samples was systematically recorded before analysis using spectrofluorometry. The fluorescence intensity is proportional to the concentration of fluorescent compounds only for low absorbance (< 0.1). Absorbance measurements were performed with a 1 cm path length fused silica cell (Hellma).The absorbance spectra were acquired with a Jasco V-560 spectrophotometer, between 210 nm and 700 nm, at 200 nm min^{-1} and with a slit width of 2 nm. The UV–visible absorbance spectrum of ultrapure water was subtracted from the water sample spectra. When the maximum absorbance (250 nm in this study) was higher than 0.1, samples were diluted with ultrapure water. The UV–visible absorbance spectrum was then recorded again to control the maximum absorbance value and define the dilution correction factor to apply to EEM spectra.

2.4. DOC and SPM

DOC was measured using a Shimadzu total organic carbon analyser (TOC-V CSN). The instrument was run in non purgeable organic carbon (NPOC) mode. The apparatus was calibrated using a standard solution of potassium hydrogen phthalate $C_6H_4(COOK)(-COOH)$, diluted to different concentrations according to the estimated DOC content of the samples. For each sample, the result is the mean of at least three satisfactory injections in terms of standard deviation (<0.1) and variation coefficient (<2%). The analytical uncertainty in the concentration of DOC is less than 4 μmol L^{-1}. Determination of SPM concentration was performed by filtration using dry pre-weighed filters (Whatman GF/F, 0.7 μm). The filters were dried to constant weight (45 °C). SPM concentration was only determined for the samples collected in 2002.

3. Results and discussion

All the samples (fresh, brackish and marine water samples collected from the Garonne River and from the Gironde Estuary) were analysed using 3D spectrofluorometry in order to observe the evolution of the fluorescence signal along the estuary. DOC and SPM concentrations were also determined. Absorption data are not discussed because they do not affect the interpretation of the results, all the more so as a linear relationship was observed between absorption and fluorescence (data not shown).

3.1. EEM plots

Few qualitative differences in fluorescent DOM were observed among the samples of variable salinity collected in the fluvial and upstream parts of the estuary during GIROX 1, GIROX 2 and OPTIC G3 cruises. As an example, Fig. 3 shows the contour EEM plots of eight different salinity samples collected in September 2006 (OPTIC G3 cruise) and February 2007 (La Réole). The bands in the EEM spectra are shown in Table 2, according to reports by several authors (Mopper and Schultz, 1993; De Souza Sierra et al., 1994; Coble, 1996, 2007; Parlanti et al., 2000; Stedmon et al., 2003). They are related to the type and relative concentration of fluorescent compounds present in water samples.

The spectra of the samples with salinity (S) 0, 2.6, 6.2, 13.4 and 21.0 are presented on the same intensity scale. There is almost no qualitative difference between the samples collected in the ETM (S = 2.6, 6.2 and 13.4) and those of higher salinity (S = 21.0, 25.7 and 28.1). The fluorophores α and α', associated with humic-like material, are predominant in the spectra of all the samples. Nevertheless, the spectrum of the sample with S = 2.6 is slightly more intense than those with S = 6.2 and 13.4. The spectrum of the freshwater sample (S = 0) is less intense than the samples from the ETM.

When the spectra of the samples with S = 21.0, 25.7 and 28.1 are compared, we observe that the fluorescence intensity, and therefore the FDOM concentration, decreases as salinity increases. This likely reflects dilution of the organic material in the downstream part of the estuary.

The spectrum of the marine sample (S = 34.3), shown with an intensity scale 20 times lower than that for the freshwater and ETM samples, is qualitatively different from the spectra of the other samples (Fig. 3). In this spectrum, the γ band from protein-like compounds can be observed at an excitation wavelength (λ_{exc}) of 280 nm and an emission wavelength (λ_{em}) of 340 nm. A broadening of the spectrum towards shorter emission wavelength for an excitation wavelength of 310 nm can also be observed. This shoulder is due to the presence of the β fluorophore associated with recently produced organic material (Parlanti et al., 2000).

This was confirmed by plotting the emission spectra at λ_{exc} 310 nm of the samples with S = 0, 2.6, 13.4 and 34.3 (Fig. 4). The blue shift in the emission spectrum at λ_{exc} 310 nm (due to fluorophore β) for the marine sample (S = 34.3) is clear. The β fluorophore was initially attributed to humic-like material of marine origin (Coble et al., 1990). Later, several studies showed that it was responsible for the hypsochromic shift between marine samples and freshwater samples during fluorescence analysis (De Souza Sierra et al., 1994, 1997; Parlanti et al., 1997). It was also demonstrated that its presence was related to the biological activity in coastal marine environments (Parlanti et al., 2000), in agreement with the present results.

3.2. Evolution of fluorescence signal and DOC content along the Gironde Estuary

The fluorescence intensity of the four main bands associated with fluorescent DOM (α', α, β and γ) was determined for all the samples from the Gironde Estuary and the Garonne River. These four fluorescence bands are observed in spectral domains varying as a function of the nature and the origin of the samples analysed. This is why the fluorescence intensity of the α', α, β and γ bands was determined at fixed excitation wavelengths in order to remove the variability in the position of the fluorescence maxima and to compare more easily the results obtained with different water samples. Thus, the maximal fluorescence intensity of the α' band was measured at λ_{exc} 260 nm and that of the α band at λ_{exc} 370 nm. The fluorescence intensity of the γ band was determined

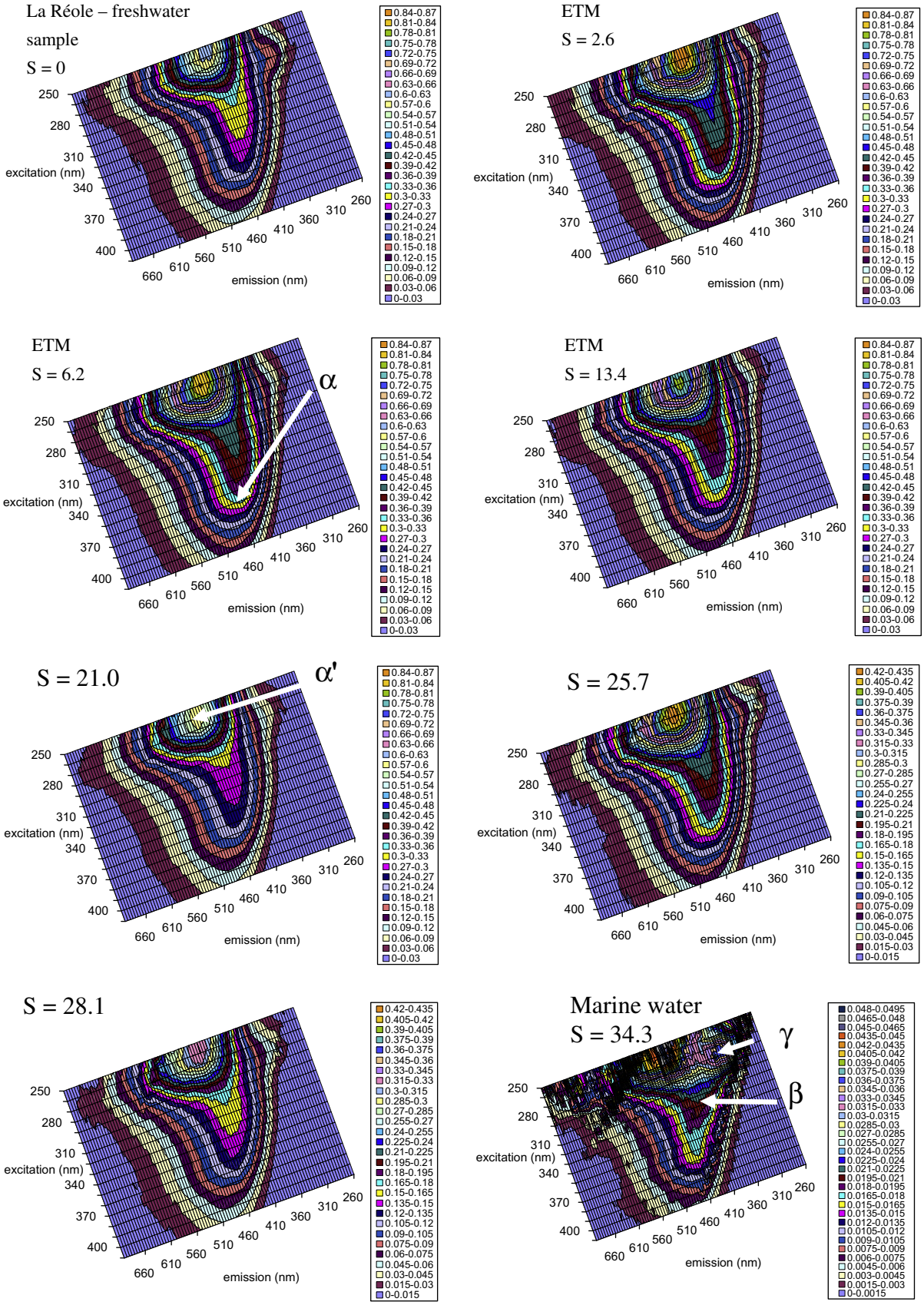

Fig. 3. Contour excitation–emission matrix (EEM) plots of EEM fluorescence spectra of eight samples of different salinity (*S*): *S* = 0 (La Réole; February 2007); *S* = 2.6; 6.2; 13.4; 21.0; 25.7; 28.1 and 34.3 (Gironde Estuary, Optic G3, September 2006). N.B. identical intensity scale for samples with salinity 0–21; scale two times lower for the samples with salinity 25.7 and 28.1 and 20 times lower for sample with *S* = 34.3. Fluorescence intensities are expressed in Raman units (nm^{-1}).

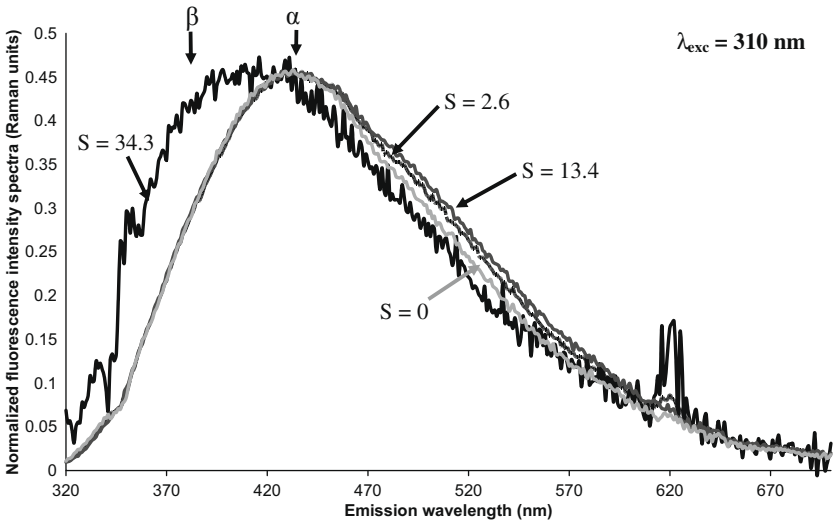

Fig. 4. Normalized emission spectra (at excitation wavelength 310 nm) of samples with salinity 0 (La Réole, February 2007), 2.6, 13.4 and 34.3 (Gironde Estuary, Optic G3, September 2006).

at λ_{exc} 280 nm and λ_{em} 330 nm. As for the fluorescence intensity for the β peak, this was measured at $\lambda_{exc}/\lambda_{em}$ 310/400 nm.

The α and α' bands are systematically present in the EEM fluorescence spectra, which is not the case for the γ and β bands (Fig. 3). The compounds responsible for the fluorescence of β and γ type bands may be present in the samples, but at a concentration such that their fluorescence signals are hidden by that of the humic substances. The fluorescence intensity of the γ and β bands was measured for all the samples, in order to follow the distribution of these two fluorophores and to determine their contribution to the total fluorescence signal.

The fluorescence intensities of the α', α and β bands are higher for the samples collected in September 2006 than for those collected in February and September 2002 (Fig. 5), in agreement with higher DOC concentrations (Table 1). Nevertheless, similar variations in fluorescence intensity as a function of salinity are observed for the three sampling cruises.

The general trend is a decrease in fluorescence intensity with salinity, which indicates a decrease in the concentration and thus the dilution of fluorescent DOM. However, the fluorescence vs. salinity relationship is not linear. According to fluorescence intensity variations, the estuary can be divided into three zones: the fluorescence intensity of the α', α and β bands increases with salinity between 0 and 2, remains constant for salinity between 2 and 8/10 and then decreases linearly (dashed lines in Fig. 5). Therefore, the mixing of water masses in the Gironde Estuary cannot be described by a simple two end member mixing model. Such a simple dilution would have resulted in a straight line, meaning that neither loss nor uptake occurred during the mixing in the estuary. Non-conservative behaviour has been reported during the mixing of fresh and marine water in an estuary (Stedmon and Markager, 2003). This results in concave or convex curves, according to addition of or depletion in organic matter. Fig. 5 therefore points to an addition of fluorescent DOM during the mixing of fresh and marine water in the Gironde Estuary, whatever the season.

The DOC concentrations of the September 2006 samples are globally higher than those of the samples collected in September 2002 (Table 1), in agreement with the higher fluorescence intensities observed in 2006 (Fig. 5). Comparison of the results from the

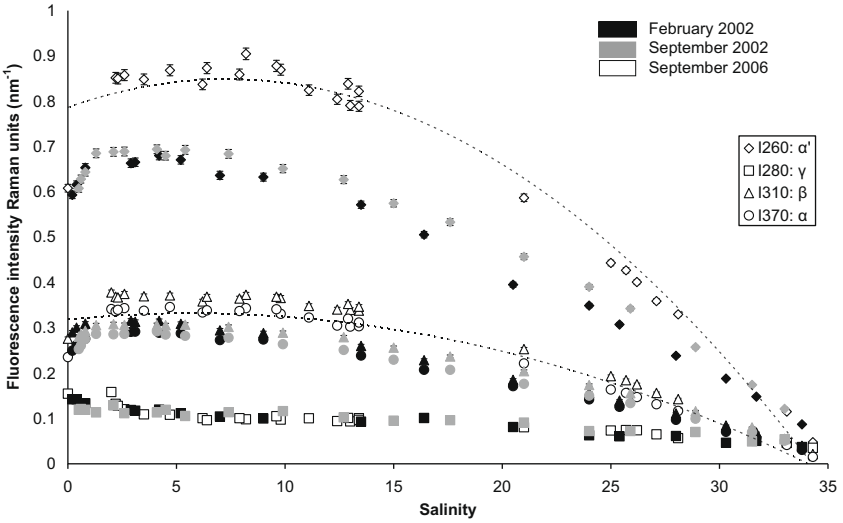

Fig. 5. Fluorescence intensity vs. salinity (Girox 1, February 2002; Girox 2, September 2002; Optic G3, September 2006; La Réole, February 2007). The analytical uncertainty associated with both measurement of fluorescence intensity and normalization to Raman units is lower than 2%.

three sampling cruises shows two particular types of behaviour of DOC as a function of season. During the winter (February 2002), the organic carbon does not undergo a conservative dilution and seems to show depletion in the middle of the estuary. In this zone of the estuary, which acts as a sink of DOC, the decrease in DOC content as a result of the dilution is increased by the phenomenon of consumption of organic material. In September 2002 and 2006, variations in DOC (Table 1) and fluorescence intensity (Fig. 5) vs. salinity are almost similar: DOC remains almost constant from S 0 to 15 and then decreases approximately linearly. During the summer season, addition of DOC is thus observed instead of a simple dilution, which explains the relatively constant DOC values for $S < 15$. The middle zone of the estuary constitutes a DOC production area.

The same DOC seasonal trend was observed by Miller (1999) in the Tamar Estuary (UK), with DOC consumption during winter in the medium salinity area and DOC production in this zone from June to October. In the same way, Abril et al. (2002) observed an addition of DOC in the Gironde, Sado (Portugal) and Ems (Netherlands) estuaries for the June, September and July 1997 periods, respectively. The non-linear trend observed for fluorescent DOM and DOC content vs. salinity during the summer season is also similar to results obtained by Klinkhammer et al. (2000) for the Columbia River Estuary (USA).

The seasonal variations in DOC content vs. salinity in the Gironde Estuary might be explained by exchange between the particulate and dissolved phases resulting from decomposition of organic matter (Middelburg and Herman, 2007). Physical phenomena, such as flocculation or desorption of dissolved compounds, previously associated with particulate material, may also lead to these seasonal DOC trends (Miller, 1999; Abril et al., 2002). Physical phenomena result from changes in the physicochemical conditions occurring during the mixing of fresh and marine water.

The presence of an ETM throughout the year, as is the case in the Gironde Estuary, may favour this type of phenomenon, because of the high SPM content and the large particle-attached bacterial population generally observed in turbid estuaries (Crump et al., 1998). These bacteria actively participate in the decomposition of labile organic matter (Abril et al., 2002). Salinity changes in the ETM in response to tidal mixing, influencing the absorption and desorption of organic material at particle surfaces (Abril et al., 2002). A part of the DOC that is formed or released as a result of these complex phenomena can contribute to the increase in the fluorescence signal observed during the mixing of fresh and marine water in the Gironde Estuary.

A relatively good correlation between fluorescence intensity and DOC content was observed for the September 2006 samples (Fig. 6a) as reported in several studies (Vodacek et al., 1995,

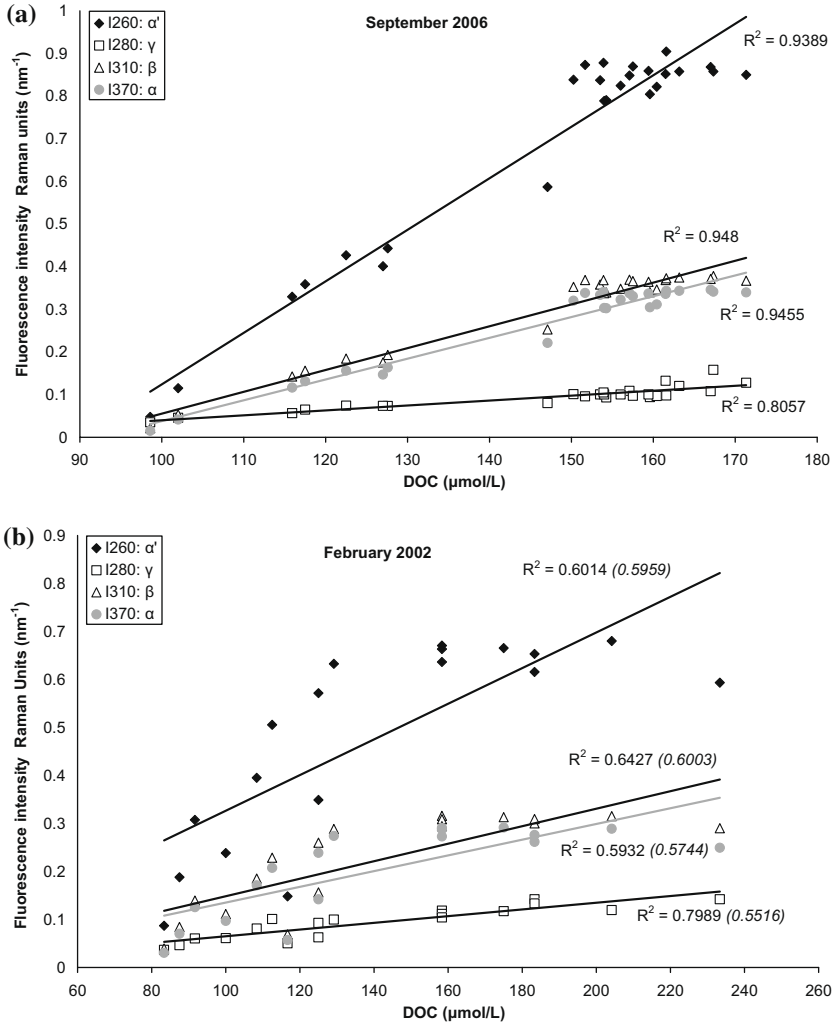

Fig. 6. Fluorescence intensity as a function of DOC concentration for water samples collected in September 2006 (a) and February 2002 (b). Correlation coefficients for the September 2002 samples are provided in brackets next to those obtained for the February 2002 samples.

1997; Chen, 1999; Klinkhammer et al., 2000). This correlation is lower for the samples collected in February and September 2002, as shown in Fig. 6b for the February 2002 samples. Indeed, a relationship between DOC and fluorescent DOM does not systematically exist and was not observed, for example, in the case of marine water samples (Nelson et al., 1998; Ferrari, 2000). This may be due to the fact that fluorescent organic material represents only a part of the DOC and that the sources and sinks of coloured organic material are not necessarily the same as those of the entire DOM (Blough and Del Vecchio, 2002).

In addition to DOM fluorescence and DOC concentration, the ratio of fluorescence intensity to DOC concentration varies with salinity (Fig. 7). Similar patterns can be observed for the four ratios. Values of, and variations in, fluorescence per unit carbon are consistent between seasons, although slightly lower values of fluorescence/DOC are observed for September 2002. The proportion of fluorescent organic material within the DOC pool is almost constant for salinity lower than 15, as suggested by the relatively constant values of fluorescence intensity (Fig. 5) and DOC concentration (Table 1) for these salinities. The relatively constant FDOM/DOC ratio for $S < 15$ (except for the α' ratio in September 2002) may be related to the fairly constant values of fluorescence intensity observed at high DOC concentration in Fig. 6.

At higher salinity ($S > 15$), DOM fluorescence as a proportion of DOC decreases almost linearly (Fig. 7). This decrease, particularly marked for fluorophore α', could be the result of photo-transformation of FDOM to non-fluorescent material as surface water is transported to sea. A linear decrease in the FDOM/DOC ratio at higher salinity was indeed observed by Callahan et al. (2004) for the Pearl River Estuary (China). These authors performed photodegradation experiments which showed fluorescence depletion within 48 h, which supported FDOM transformation to non-fluorescent compounds through photobleaching in high salinity water of the Pearl River Estuary. A similar phenomenon could take place in the high salinity area ($S > 15$) of the Gironde Estuary. Nevertheless, FDOM photodegradation may be limited in September 2002 as a result of the relatively high SPM content of the Gironde Estuary up to $S = 25$ (Table 1). Indeed, due to tidal influence, residence times for both water (0.5–3 months) and suspended matter (12–24 months) are long in the estuary (Jouanneau and Latouche, 1981). This leads to a well developed ETM with high SPM concentration (from several hundred mg l^{-1} to several g l^{-1}), where mineralization exceeds primary production as a result of extreme light limitation (Etcheber et al., 2007). The dilution of riverine water with marine water could also account for the decrease in the FDOM/DOC ratio in the high salinity area of the estuary. Mixing of fresh and marine water with different fluorescence properties could reduce the riverine signal and partly control DOM optical properties (Del Castillo et al., 2000). Both photodegradation and mixing of marine and riverine end members could explain the profile of the FDOM/DOC ratio vs. salinity curve.

In the low salinity area of the estuary, DOC could be generated from SPM via photochemical processes. Indeed, Kieber et al. (2006) performed a series of photolysis experiments using simulated sunlight in the presence and absence of estuarine bottom sediments and observed that DOC photoproduction from resuspended sediments increased proportionally with the percent organic carbon content of the added sediment. Photodissolution of particulate organic carbon was also observed by Mayer et al. (2006) after irradiation of Mississippi River suspended sediments in distilled water. Finally, photoproduction of DOC from river sediments was recently demonstrated by Riggsbee et al. (2008). These results suggest that sunlight interaction with SPM could potentially be a DOC source in the Gironde Estuary, more particularly in the upstream region ($S < 15$) where the SPM concentration is relatively high (Table 1). During the summer season, DOC was roughly constant from salinity 0 to 15 and then behaved conservatively (Table 1). The DOC input observed for $S < 15$ could thus be partly due to photochemical generation of DOC from SPM. The phenomenon probably takes place at the water surface only, as a result of the high SPM content which limits penetration of light in the water column.

Results from our study are consistent with previous studies describing the complexity of studying DOM in estuarine environments due to the variability in freshwater flow, anthropogenic input, autochthonous production and removal processes (e.g. Callahan et al., 2004; Guo et al., 2007). All these studies demonstrate the difficulties associated with studying carbon cycling in estuarine environments. Indeed, several parameters (seasonal variability, various freshwater sources, anthropogenic input, autochthonous production, removal processes) can influence DOM distribution (Callahan et al., 2004).

3.3. Fluorescence intensity ratios

In order to more easily follow the modification of fluorescent DOM in the estuary, intensity ratios of all the bands were calculated relative to the α band. The variation in the intensity ratios $I\alpha'/I\alpha$, $I\beta/I\alpha$ and $I\gamma/I\alpha$ vs. salinity shows that the ratios reveal

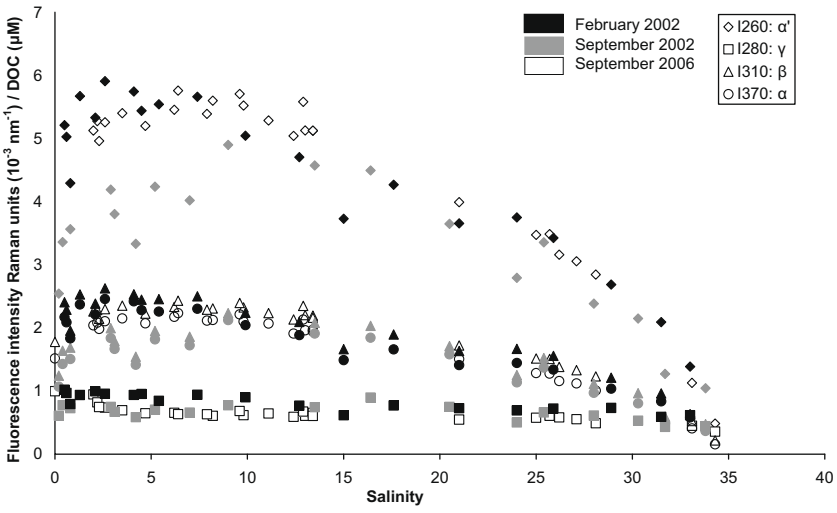

Fig. 7. Fluorescence intensity per unit DOC vs. salinity (Girox 1, February 2002; Girox 2, September 2002; Optic G3, September 2006; La Réole, February 2007).

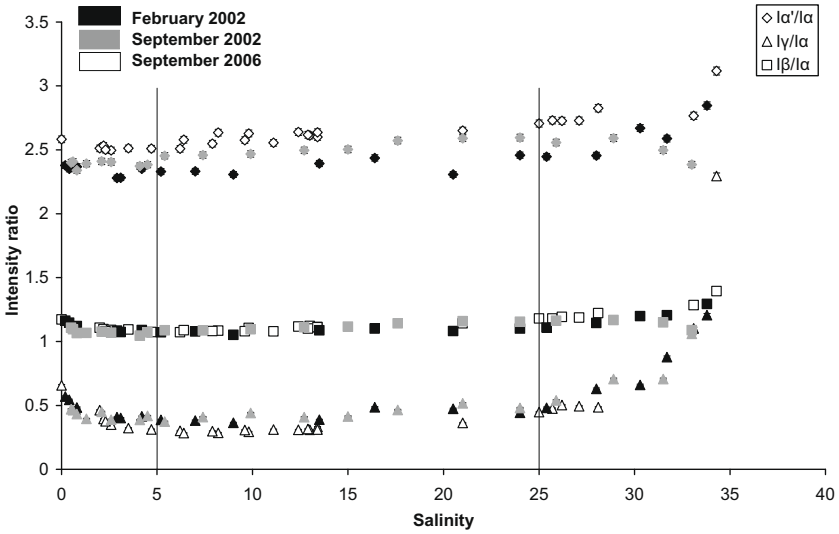

Fig. 8. Fluorescence intensity ratio between different bands ($I\alpha'/I\alpha$, $I\beta/I\alpha$ and $I\gamma/I\alpha$) vs. salinity (Girox 1, February 2002; Girox 2, September 2002; Optic G3, September 2006; La Réole, February 2007). Vertical lines delimit estuary zones where DOM characteristics differ. The analytical uncertainty for the calculation of the ratios does not exceed 1%, i.e. the error bars are not visible on the chart.

seasonal variability (Fig. 8). Except for a slight initial decrease between S 0 and 3, especially marked for February 2002 samples, the $I\alpha'/I\alpha$ ratio for the samples collected in February 2002 and September 2006 increases all along the estuary, especially for S above 25. For the September 2002 samples, the ratio decreases for S higher than 25. The $I\alpha'/I\alpha$ ratio appears to be useful for distinguishing the α' and α fluorophores, which are both associated with humic- and fulvic-like material, and are simultaneously present in the fluorescence spectra of natural waters. The qualitative evolution of the respective intensities of these two bands along the estuary is relatively similar (Fig. 5). Therefore, the variation in the intensity ratio of the α' and α fluorophores as a function of salinity (Fig. 8) shows that the two fluorophores are clearly distinct and that there is an increase in the concentration of fluorophore α' vs. fluorophore α in the upstream part of the estuary for the February 2002 and September 2006 water samples. In contrast, the concentration of the α' fluorophore decreased relative to the α fluorophore in the samples collected in September 2002. The $I\beta/I\alpha$ ratio showed the same evolution as the $I\alpha'/I\alpha$ ratio.

The $I\alpha'/I\alpha$ intensity ratio has been commonly used to characterize the relative evolution of the α' and α fluorophores (Coble et al., 1990; 1996; De Souza Sierra et al., 1994). The ratio was described as an indicator of the young or mature character of humic material in natural water samples (e.g. Coble, 1996). The α fluorophore is associated with more degraded and older material, i.e. with more humified material. The fact that, for the February 2002 and September 2006 samples, $I\alpha'/I\alpha$ increases more distinctly for salinity higher than 25 could indicate that FDOM in this area is relatively more recent than in the rest of the estuary. Flow rates of the Dordogne and Garonne Rivers were lower in September 2002 than in February 2002 and September 2006 (Fig. 2). These variations in water discharge probably had an impact on terrigeneous material input in the Gironde Estuary. This could explain the decrease in the $I\alpha'/I\alpha$ ratio observed in the downstream part of the estuary for the September 2002 samples.

$I\gamma/I\alpha$ decreases in the area of low salinity ($S < 3$) and then stays relatively constant up to S 20 before increasing for higher salinity, whatever the season. The γ fluorophore is associated with labile compounds of protein or bacterial origin (Yamashita and Tanoue, 2003; Cammack et al., 2004). Therefore, it gives information about the recent autochthonous character of DOM and on the productivity in the aquatic environment.

The constant and relatively low value (<0.5) of $I\gamma/I\alpha$ for salinity lower than 20 (Fig. 8) seems to show that primary production is limited in a major part of the estuary, mainly because high levels of turbidity limit light in the water column (Irigoien and Castel, 1997). In fact, the SPM concentration, measured for the 2002 water samples, is relatively high in the low salinity area of the estuary, which is characteristic of the turbidity maximum zone (Table 1). In February 2002, the ETM was located in an area of salinity lower than 10. In September 2002, the ETM was much more extensive. This was characterized by high turbidity for S 0–5 and extending nearly to $S = 25$, with SPM concentration of about 400 mg/l. The decrease in the ratios $I\gamma/I\alpha$ and $I\beta/I\alpha$ in the low salinity area is likely due to the increase in the α' and α band intensities in this zone of high turbidity.

In the downstream region of the estuary, where the SPM concentration is lower than in the ETM (Table 1), the primary production is more intense (Irigoien and Castel, 1997). This higher productivity is supported by the increase in $I\gamma/I\alpha$ for $S > 30$. Variation in $I\gamma/I\alpha$ with salinity suggests that the productivity was as intense in February 2002 as in September 2002 and 2006. The climatic conditions were indeed particularly mild in February 2002 and were conducive to the development of winter phytoplanktonic blooms (Labry et al., 2001; Gohin et al., 2003). This could explain the unusually high productivity observed during the 2002 winter in the downstream part of the estuary.

Together, the fluorescence and DOC results suggest that the Gironde Estuary can be divided into three main areas, where the DOM characteristics differ. The low salinity ($S < 5$) region is the most turbid portion of the ETM, as shown by the high SPM concentration (Table 1). An increase in the intensities of the α' and α fluorophores, characteristic of humic-like compounds, is observed in this zone (Fig. 5), as well as a slight decrease in $I\gamma/I\alpha$ (Fig. 8). This decrease is partly due to the increase in the intensity of the α band.

The second area corresponds to salinity between 5 and 25. The ETM is present in waters of salinity up to 10 in winter and up to 25 in summer. In this zone, primary production is relatively low. There is an addition of DOC in this part of the estuary in summer, likely due to the degradation, or modification, of organic material already partially degraded, or to the transfer of macromolecular compounds associated with particles from the particulate to the dissolved phase as proposed by Klinkhammer et al. (2000). In winter, a decrease in DOC content is observed, possibly resulting from

flocculation of terrestrial organic material as a result of salinity effects. Other phenomena, such as decomposition and mineralization of DOC, may also explain the organic carbon impoverishment in the middle of the estuary.

The third area ($S > 25$) reflects a stronger marine influence and primary production is more intense. This is supported by the higher $I\gamma/I\alpha$ values (Fig. 8). In this part of the estuary, the production and decomposition of organic material contribute to the variation in the $I\alpha'/I\alpha$ ratio (Fig. 8), i.e. to a change in the relative concentration of the α' and α fluorophores, associated with humic-like material. It should be noted that the three zones of the estuary determined here are similar to those described by Callahan et al. (2004) for microbial production and photo-transformation of chromophoric DOM and DOC along the Pearl River Estuary (China).

3.4. Fluorescence indices

We have shown that measurements of the fluorescence intensities and intensity ratios allow the monitoring of fluorescent DOM modification and evolution. Fluorescence spectra can also be used to assess the origin and transformation degree of DOM through calculation of fluorescence indices. Three fluorescence indices were determined: the f_{450}/f_{500} index (McKnight et al., 2001), the humification index (HIX, Zsolnay et al. 1999) and the index of recent autochthonous contribution (BIX).

3.4.1. The f_{450}/f_{500} index

McKnight et al. (2001) introduced a fluorescence index, f_{450}/f_{500}, the ratio of fluorescence intensity at the emission wavelength 450 nm to that at 500 nm at λ_{exc} 370 nm, to discriminate the sources of DOM. They reported that f_{450}/f_{500} is about 1.9 for aquatic and microbial sources and about 1.3 for terrestrial and soil sources. Values of f_{450}/f_{500} for the Gironde Estuary samples range between 1.15 and 1.22 (Fig. 9) and do not vary seasonally. They are similar to those obtained by Battin (1998) for a blackwater river in South Venezuela and are lower than the terrestrial threshold value of this index determined by McKnight et al. (2001). The low values of f_{450}/f_{500} in the Gironde Estuary suggest that terrestrial organic matter dominates, even in the high salinity area of the estuary. This is not consistent with the increase in the $I\gamma/I\alpha$ ratio (Fig. 8) and the stronger marine influence in this part of the estuary and suggests limitations in applying the f_{450}/f_{500} index to estuarine environments.

The calculation of the index is based on the shift in fluorescence emission spectra towards longer wavelength with increasing aromaticity (Senesi, 1990). Nevertheless, emission wavelength maxima also increase with molecular weight (Berger et al., 1984; Alberts et al., 2002). Flocculation of humic substances with increasing salinity was thus observed by several authors (McCarthy et al., 1996; Van Heemst et al., 2000; Benner and Opsahl, 2001) and may lead to a bathochromic shift (i.e. shift to longer emission wavelength) in estuarine sample spectra for excitation wavelength between 340 and 370 nm. A bathochromic shift was indeed observed at λ_{exc} 370 nm for all the high salinity water samples collected in the Gironde Estuary. This red shift has been reported (De Souza Sierra et al., 1994; Coble, 1996) but its exact origin is still unknown. It could be due to the flocculation of high molecular weight humic compounds with increasing salinity. Parlanti et al. (2000) performed macro algae degradation experiments and observed a fluorescence maximum red-shifted compared to maxima generally observed for the α band. The bathochromic shift for high salinity samples could thus also be caused by the overlap of the α band with another type of fluorophore only found in marine waters or present at too low a concentration in continental samples compared to other fluorophores.

Since several phenomena may lead to variations in fluorescence emission maximum at λ_{exc} 370 nm, it seems complicated to use the f_{450}/f_{500} index to unambiguously determine the predominant source of DOM in bulk estuarine water samples. Jaffé et al. (2004) applied this parameter to characterize mangrove samples and reached almost similar conclusions. These authors also underlined the potential effect of UV irradiation on the evolution of this index. The f_{450}/f_{500} index was developed for the source assessment of humic substances (more particularly fulvic acids). It can not be as effective for water samples such as estuarine ones whose organic material originates from a variety of sources and that can contain significant amounts of non-humic substances.

3.4.2. HIX and BIX

Two alternative fluorescence indices are proposed for studying the complex DOM dynamics in estuarine systems: the HIX and BIX indices. Since humification is associated with an increase in the C/H ratio (Stevenson, 1982) and with a resulting shift to longer emission wavelength (Senesi, 1990), the HIX index was introduced by Zsolnay et al. (1999) on the basis of the location of the emission spectra in order to estimate the degree of maturation of DOM in soil. HIX is the ratio H/L of two spectral region areas from the emission spectrum scanned for excitation at 254 nm. These two areas are calculated between emission wavelengths 300 nm and

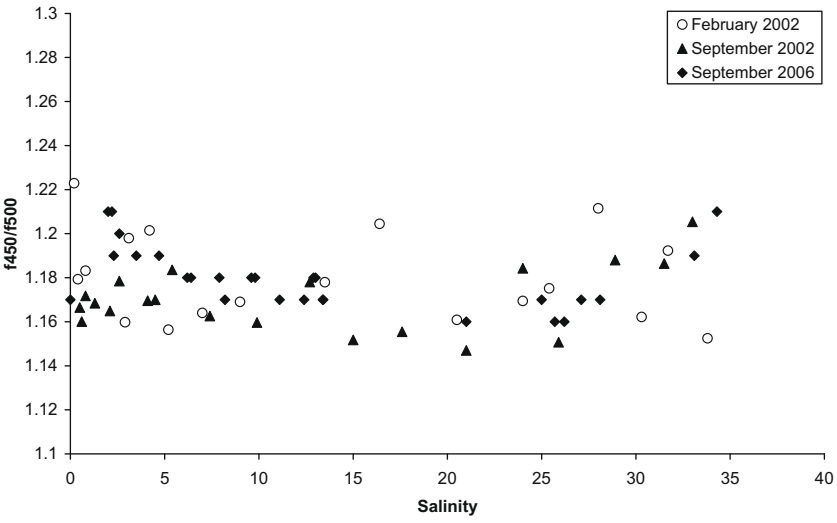

Fig. 9. The f_{450}/f_{500} index vs. salinity (Girox 1, February 2002; Girox 2, September 2002; Optic G3, September 2006; La Réole, February 2007).

345 nm for L and between 435 nm and 480 nm for H. When the degree of aromaticity of DOM increases, the emission spectrum (at λ_{exc} 254 nm) is red shifted, which implies that the H/L ratio, and thus the HIX index, increases. High HIX values correspond to maximal fluorescence intensity at long wavelength and thus to the presence of complex molecules like high molecular weight aromatics (Senesi et al., 1991). To the best of our knowledge, this is the first time that the use of the HIX index is reported for samples from natural aquatic systems. We have already measured HIX for samples from the Gironde, Loire and Seine Estuaries (France) and the Mediterranean Sea (Vacher, 2004) and determined that high values (between 10 and 16) were the sign of strongly humified organic material, mainly of terrestrial origin, whereas low values (<4) were associated with autochthonous OM.

We also introduce a new fluorescence index (BIX) to determine the presence of the β fluorophore, characteristic of autochthonous biological activity in water samples. Its calculation is based on the broadening of the emission fluorescence spectrum due to the presence of the β fluorophore at λ_{exc} 310 nm. It can be better visualised in the fluorescence spectra of water samples with various salinity values (S = 0, 2.6, 13.4 and 34.3) collected in the estuary (cf. Fig. 5). BIX is calculated at λ_{exc} 310 nm, by dividing the fluorescence intensity emitted at λ_{em} 380 nm, corresponding to the maximum of intensity of the β band when it is isolated, by the fluorescence intensity emitted at λ_{em} 430 nm, which corresponds to the maximum in the α band. An increase in BIX (i.e. the intensity ratio $I_{em}380/I_{em}430$) is related to an increase in the concentration of the β fluorophore. BIX was determined for the same estuarine and marine samples as for HIX (Vacher, 2004) and we observed that high values of BIX (>1) correspond to a predominantly autochthonous origin of DOM and to the presence of OM freshly released into water, whereas a lower DOM production in natural waters will lead to a low value of BIX (0.6–0.7).

The HIX and BIX fluorescence indices were calculated for all the samples and are plotted vs. salinity in Fig. 10. It can be seen first that there is no significant seasonal difference between the HIX values, except in the low salinity area. The values are indeed lower for the February 2002 samples than for the September 2002 and 2006 samples (in the area of salinity 0–5 in 2002 and 0–15 in 2006). This can be explained by the presence of the ETM and the higher SPM content in this part of the estuary in September than in February (see Table 1).

The HIX and consequently the degree of humification of DOM progressively increases (from 10 to about 16) in the area of low salinity (S < 7). Then, the values stay relatively high (higher than 10) up to a salinity of about 25, which seems to indicate the presence of strongly humified compounds in this part of the estuary. These results confirm those obtained from the $I\gamma/I\alpha$ ratio, relatively low (<0.5) for a salinity lower than 25 (Fig. 10). Therefore, the region of the estuary with salinity between 5 and 25 seems to be characterized by highly modified organic material. Maximal HIX values are reached in the ETM (salinity between 2 and 15), in agreement with the SPM content in the estuary (Table 1).

For salinity higher than 25, HIX clearly decreases down to only 2.5 for the most saline sample (S = 34.3), which indicates the presence of organic material of biological origin with a low degree of humification in the downstream part of the estuary. Calculation of the $I\gamma/I\alpha$ ratio, which significantly increases for salinity higher than 30 (Fig. 8), leads to the same conclusion. Therefore, there is clearly a change in OM source in this area and a removal of terrestrial material, perhaps by photodegradation, as suggested by the linear decrease in the FDOM/DOC ratio for S > 15 (Fig. 7). Earlier studies have furthermore reported coloured DOM removal due to photobleaching in the high salinity area of large estuaries and shelf regions (Vodacek et al., 1997; Del Castillo et al., 1999).

The BIX values (Fig. 10) stayed relatively constant (between 0.6 and 0.7) for salinity values lower than about 25, and then strongly increased up to 0.9. These results confirm those obtained after the calculation of the intensity ratios and of HIX, i.e. the upstream part of the estuary is characterized by a lower biological productivity, compared to the marine environment (S > 25), where the primary production is higher. It can also be noticed that no significant seasonal difference exists between the BIX values of the samples, even though BIX is slightly lower for the February 2002 samples than for the September 2002 and 2006 samples in the area of salinity higher than 20.

The BIX and HIX indices show a greater range of values (Fig. 10) than the f_{450}/f_{500} index (Fig. 9) for the Gironde Estuary samples and could be more adapted to the study of DOM processing and transport in estuarine systems than f_{450}/f_{500}. We determined the HIX and BIX indices for all the samples as well as for a large number of samples from the Gironde, Loire and Seine Estuaries and the Mediterranean Sea (Vacher, 2004; Parlanti et al., 2006). This allowed us to draw up a scale of values for each index (Table 3).

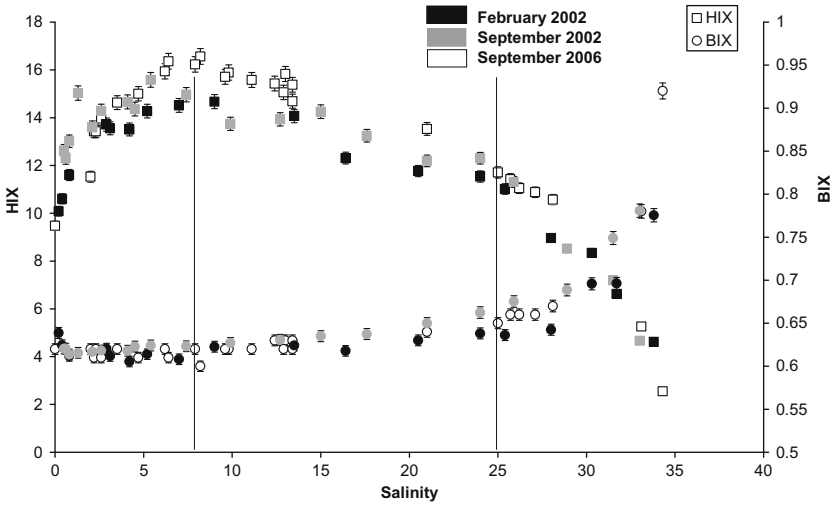

Fig. 10. Humification index (HIX) and index of recent autochtonous contribution (BIX) vs. salinity (Girox 1, February 2002; Girox 2, September 2002; Optic G3, September 2006; La Réole, February 2007). Vertical lines delimit estuary zones where DOM characteristics differ. The analytical uncertainty for the calculation of HIX is at most 2% and that for calculation of BIX does not exceed 1%.

Table 3
DOM characteristics associated with the range of values obtained for HIX and BIX from samples in this study and those from the Seine, Loire and Gironde estuaries and in the Mediterranean Sea (Vacher 2004; Parlanti et al. 2006).

HIX values	DOM characteristics
>16	Strong humic character/important terrigeneous contribution
6–10	Important humic character and weak recent autochthonous component
4–6	Weak humic character and important recent autochthonous component
<4	Biological or aquatic bacterial origin
BIX values	**DOM characteristics**
0.6–0.7	Low autochthonous component
0.7–0.8	Intermediate autochthonous component
0.8–1	Strong autochthonous component
>1	Biological or aquatic bacterial origin

The work showed that HIX and BIX are complementary tools and are particularly suitable for determining DOM origin and aging in coastal environments in a simple and fast manner.

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