



# Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs

E. Parlanti \*, K. Wörz, L. Geoffroy, M. Lamotte

*Université Bordeaux I, LPTC, UMR 5472 CNRS, 351 crs de la Libération, 33405 Talence cedex, France*

---

## Abstract

Here we report on an investigation of the three-dimensional excitation-emission-matrix (EEM) fluorescence spectra of unconcentrated water samples collected in 1996, 1998 and 1999 at a site particularly propitious for macro-algae development. The degradation of these macro-algae was studied to determine the influence of their exudates on natural water EEM fluorescence spectra. This work demonstrates that biological activity is one of the major factors involved in the formation of the blue-shifted fluorescence band observed in marine waters ( $\beta$  component  $Ex/Em = 310\text{--}320\text{ nm}/380\text{--}410\text{ nm}$ ); our study also shows that fluorescence can be used to evaluate the biological activity both quantitatively and to determine its different phases. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Excitation-emission-matrix fluorescence, EEM; Coastal water; Dissolved organic matter, DOM; Biological activity; Macro-algae exudates; Humic matter

---

## 1. Introduction

Coastal environments represent complex systems submitted to high tidal conditions, involving the intensive exchange and mixing of fresh water inputs and seawater. The English Channel, in particular, is characterised by tidal hydrodynamics and meteorological forcing. The area studied, the Bay of Fresnaye in the Gulf of St Malo, is typical of relatively confined areas: its inshore currents allow living matter, nutrients and pollutants to be trapped, thereby increasing their residence times (Guegueniat et al., 1993). Due to autochthonous biological activity, as well as to terrestrial and anthropogenic inputs such as nitrates, phosphates, organic matter (pig and poultry manure), metals or pesticides, this bay is particularly propitious for macro-algae development and eutrophication.

Monitoring fluorescent dissolved organic matter (DOM) has often been used to distinguish between

water masses from various sources (Coble et al., 1990; Mopper and Schultz, 1993; Coble 1996; Matthews et al., 1996), to follow the distribution of water masses (Cabaniss and Schumann, 1987; Hayase et al., 1988; Chen and Bada, 1992) or, equally to study the mixing processes in coastal and estuarine waters (Berger et al., 1984; Laane and Kramer, 1990; De Souza-Sierra and Donard, 1991). Of particular interest are some reports on the observation of different positions of the emission spectra maxima for marine and continental water samples excited in the 308–325 nm range (Donard et al., 1989; Chen and Bada, 1992; Coble and Brophy, 1994; De Souza-Sierra et al., 1994).

Three-dimensional excitation-emission-matrix (EEM) spectroscopy can be used to identify fluorescent compounds present in complex mixtures, such as normal and tumour sera, for biochemical applications (Leiner and Wolfbeis, 1988). This technique has equally been applied recently to the study of seawater DOM (Coble et al., 1990, 1993; Mopper and Schultz, 1993; Coble, 1996). The reported variability in the EEM spectra (EEMs) clearly indicates that marine DOM can contain several types of chromophores, including protein-like and humic-like fluorophores, in various proportions.

---

\* Corresponding author. Tel.: +33-556-848-491; fax: +33-557-962-267.

E-mail address: e.parlanti@lptc.u-bordeaux.fr (E. Parlanti).

Conventional fluorometry has also been used to distinguish multiple chromophores in marine DOM. In an earlier study of the fluorescence spectra of different water samples excited in the 310–320 nm range, we show some evidence for the presence, in addition to humic substances fluorophores ( $\alpha$  component:  $Em_{max} \cong 430\text{--}450$  nm), of another class of fluorophores ( $\beta$  component:  $Em_{max} \cong 380\text{--}410$  nm, mainly observed in the marine and seaside estuarine samples) which have been proposed as tracers for marine water masses (De Souza-Sierra et al., 1994; Parlanti et al., 1997). Coble (1996), also reported the presence of marine humic material with blue-shifted fluorescence maxima (M-type) that could correspond to the  $\beta$  component.

The fluorescence spectra differences observed between continental and marine waters may result from the mixing of two very distinctive organic materials specifically produced in each type of aquatic medium. The question then arises of the origin of this  $\beta$  component. Indeed, although humic substances in freshwater and sea water have dissimilar sources and differ chemically (Hedges et al., 1992), studies of differences in their spectral properties in terms of their origins and composition are still being undertaken but have not yet led to any applications distinguishing between continental and marine DOM. In this paper, we report on a study of the EEM fluorescence spectra (EEMs) of both fresh and marine unconcentrated water samples collected from the Bay of Fresnaye in the Gulf of St Malo in 1996, 1998 and 1999. The aim of this work is to correlate, for a particular site, the fluorescence spectra variation of natural water with the variation over time of environmental conditions, particularly that of eutrophication. Moreover, we present the results of macro-algae degradation

studies used to determine how the macro-algae exudates influence the natural water EEM fluorescence spectra. We then compared the fluorescence spectra variations of green macro-algae degradation in pure water, artificial sea water and natural sea water. The green macro-algae, *Ulva lactuca*, used for these experiments were collected in June 1999 when they proliferated.

## 2. Materials and methods

### 2.1. Sampling

Thirty-five samples were collected at 10 stations (Fig. 1) from the Bay of Fresnaye in the Gulf of St Malo during several cruises in April and July 1996 (nine and seven samples, respectively), April and September 1998 (nine and ten samples respectively) at the F1, F2 sites (the Frémur river flowing into the Bay) and at M3 to M10 (marine water). Sea water samples were collected near the surface waters, using a GOFLO sampler. Fifteen other samples were also collected in February, April and June 1999: four fresh water samples [from the Frémur river (F1, F2) and from two other rivers flowing into the bay] and, at each site, one marine water sample (M2) was also taken. Immediately after sampling, all unconcentrated samples were filtered through pre-combusted Whatman GF/F glass fibre filters ( $0.70\text{ }\mu\text{m}$ ) and stored in the dark at  $4^\circ\text{C}$  for different periods, prior to analysis: these periods ranged from a minimum of 1 week to a maximum of one year. Even though bacteria can pass through such filters, the preservation of samples was checked and no evolution of DOM was observed.

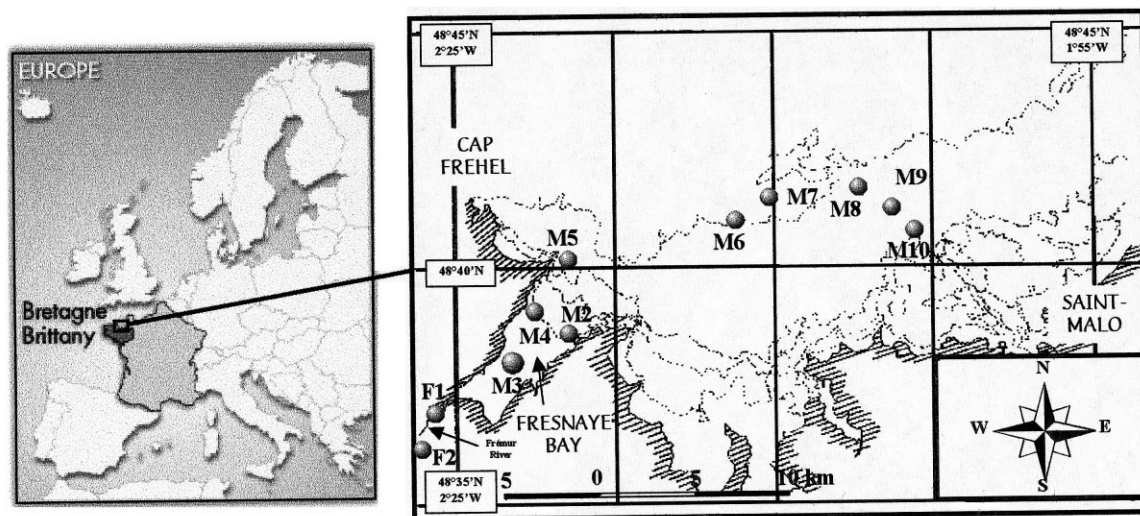


Fig. 1. Map of sites for the samples collected during 1996, 1998 and 1999 from the Bay of Fresnaye (Gulf of St Malo) in France.

## 2.2. Instrumentation and experimental conditions

The fluorescence spectra were recorded on two commercial spectrofluorometers with 450 watt xenon lamps, operated in ratio mode. The samples collected during the two 1996 cruises were run on a Fluorolog SPEX 212 fluorometer; all the other samples were analysed using a Fluorolog FL3-22 SPEX Jobin Yvon fluorometer. The two spectrofluorometers are equipped with double monochromators for both the excitation and the emission sides, giving them a low stray light level. Samples were contained in a 1 cm path length fused silica cell (Hellma), thermostated at 20°C.

EEM spectroscopy provides highly detailed information and the data can be analysed as excitation spectra, emission spectra or synchronous scan spectra. This technique reveals the complete photophysical system of the complex multi-chromophore macromolecular DOM even in the presence of smaller molecules (Matthews et al., 1996). The fluorescence EEM spectroscopy involved scanning and recording 17 individual emission spectra (260–700 nm) at sequential 10 nm increments of excitation wavelength between 250 and 410 nm. The bandwidths for both excitation and emission were 4 nm, with emission wavelength increment of 1 nm and integration time of 0.5 s. The spectra were obtained by subtracting Milli-Q (Millipore) water blank spectra, recorded in the same conditions, to eliminate water Raman scatter peaks. The 17 scans were used to generate three-dimensional contour plots of fluorescence intensity as a function of excitation and emission wavelengths. At the data collection intervals used in this study, EEMs result in discrete measurements of fluorescence intensity at 5627 excitation/emission wavelength pairs. To make the graphs readable, the topographic and contour EEM plots are presented in this paper with excitation and emission wavelength increments of 10 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 spectra recorded on the Fluorolog SPEX 212 fluorometer were electronically corrected for instrumental response (Ewald et al., 1983; De Souza-Sierra et al., 1994). However, this could not be done for the

Fluorolog SPEX FL3-22 fluorometer under current operational conditions. Consequently, for those samples collected during the 1998 and 1999 cruises, the excitation correction could not be applied to the spectra, and the emission correction was not valid below 300 nm. Nevertheless, the comparison of spectra obtained with the same instrument is correct here in noting that regardless of whether or not the corrections are made the spectra from a single instrument are internally consistent.

## 2.3. Macro-algae degradation experiments

The green macro-algae, *Ulva lactuca*, used for these experiments were collected in June 1999 at the M2 sample site where they were developing excessively. The macro-algae were put in 1 l beakers of pure water (Milli-Q, Millipore), artificial sea water (without any kind of humic-like matter or other DOM constituents) or natural sea water (M2 sample, June 1999), to follow up their degradation. Samples were collected every day for the first week and then every 2 or 3 days over a period of 2 months. They were then immediately filtered through pre-combusted Whatman GF/F glass fibre filters (0.70 µm) and analysed by EEM spectroscopy.

The artificial sea water, used for this experiment, was prepared with the following salts for 1 kg of sea water: NaCl (23.476 g), MgCl<sub>2</sub> (4.981 g), Na<sub>2</sub>SO<sub>4</sub> (3.917 g), CaCl<sub>2</sub> (1.102 g), KCl (0.664 g), NaHCO<sub>3</sub> (0.192 g), KBr (0.096 g), H<sub>3</sub>BO<sub>3</sub> (0.026g), SrCl<sub>2</sub> (0.024 g), and NaF (0.003 g).

## 3. Results and discussion

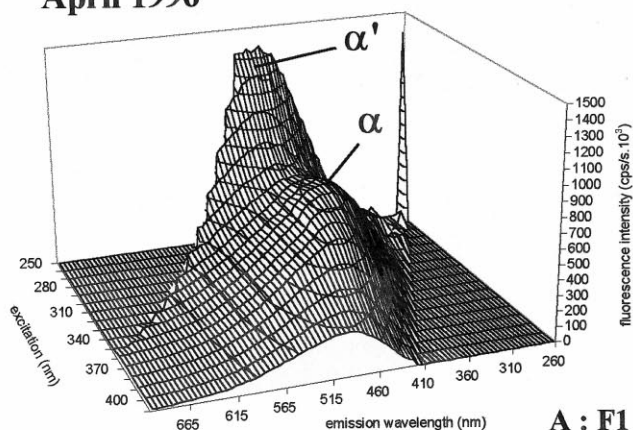
The fluorescence peaks discussed in this work, together with the wavelength ranges of their maxima and their assignments, are given in Table 1, in accordance with what is reported in the literature by several authors (Mopper and Schultz, 1993; De Souza-Sierra et al., 1994; Determann et al., 1994; Coble 1996; Parlanti et al., 1997). We did not observe the humic-like  $\alpha'$  maximum, since Mopper and Schultz (1993) used a shorter excitation wavelength (220 nm) and determined that maximum humic fluorescence intensity occurred at 230 nm excitation. Nevertheless, the peak  $\alpha'$  we observe

Table 1  
Major fluorescent components in bulk sea water<sup>a</sup>

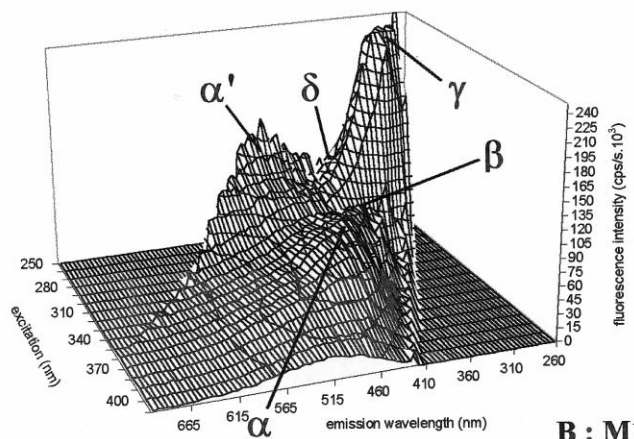
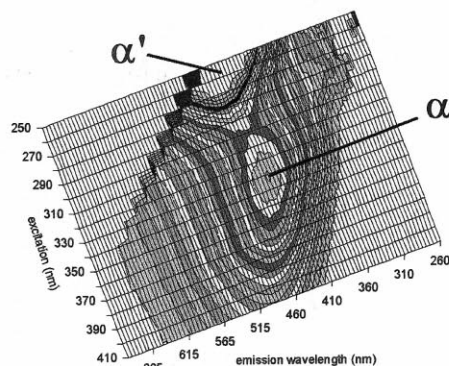
Peak	$E_{x_{max}}$ (nm)	$E_{m_{max}}$ (nm)	Component type	Letters used by Coble (1996)
$\alpha$	330–350	420–480	Humic-like	C
$\alpha'$	250–260	380–480	Humic-like	A
$\beta$	310–320	380–420	Marine humic-like	M
$\gamma$	270–280	300–320	Tyrosine-like, protein-like	B
$\delta$	270–280	320–350	Tryptophane-like, protein-like or phenol-like	T

<sup>a</sup> Correspondence with the fluorescence peaks reported in this paper, and with the symbols used by P.G. Coble in the literature.

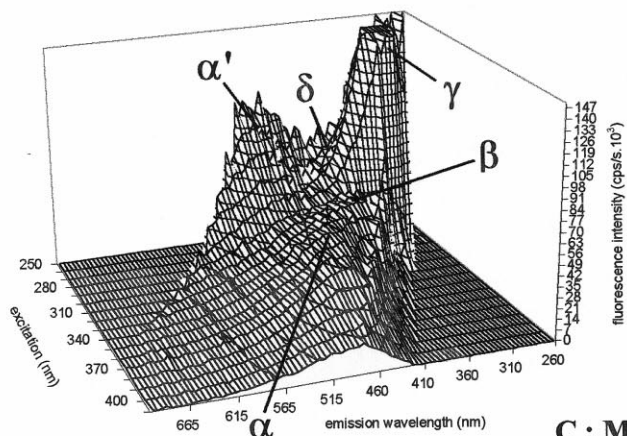
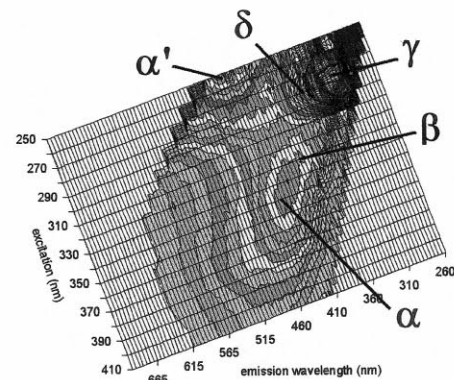
April 1996



**A : F1 – S 0.5 – DOC 6.5 mg/L**



**B : M10 – S 34.4 – DOC 1.7 mg/L**



**C : M6 – S 35.3 – DOC 1.9 mg/L**

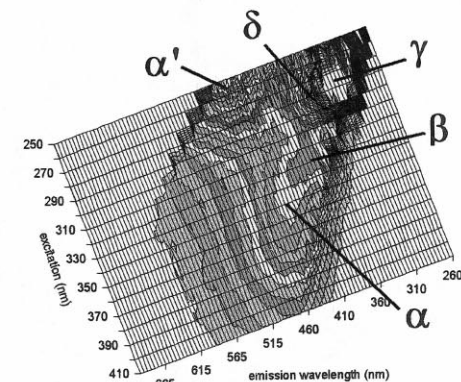


Fig. 2. Representative topographic and contour EEM plots for samples collected during the April 1996 cruise. A: fresh water (F1 sample), collected from the Frémur River flowing into the Bay; B and C: marine waters (M10 and M6 samples).

July 1996

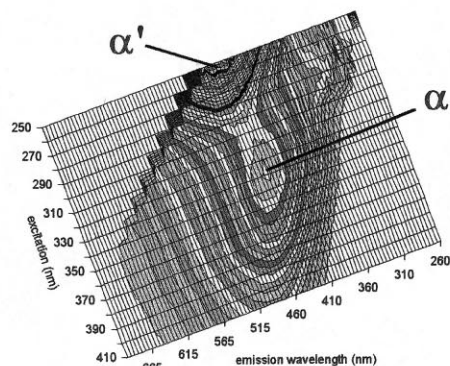
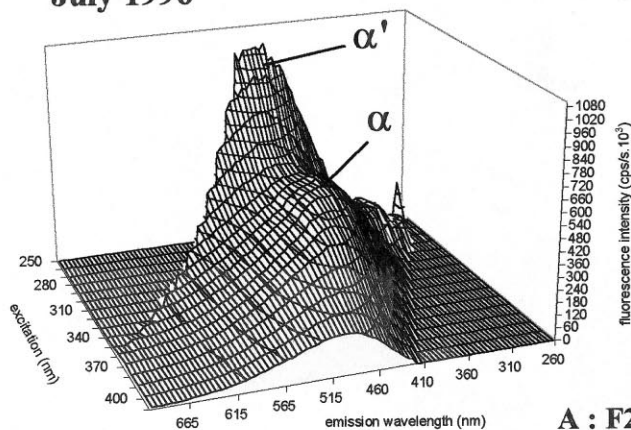
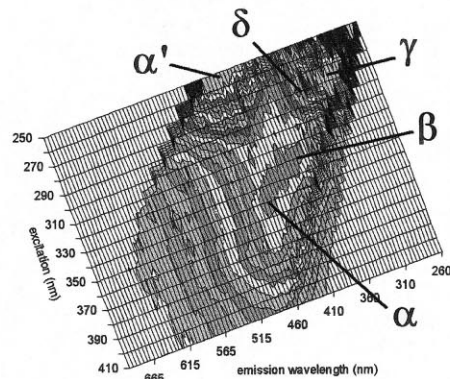
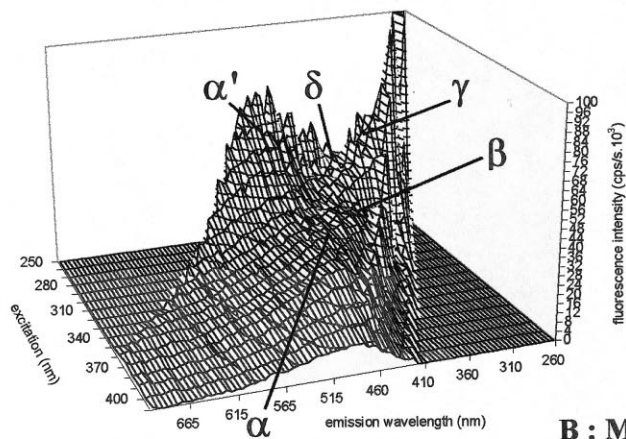
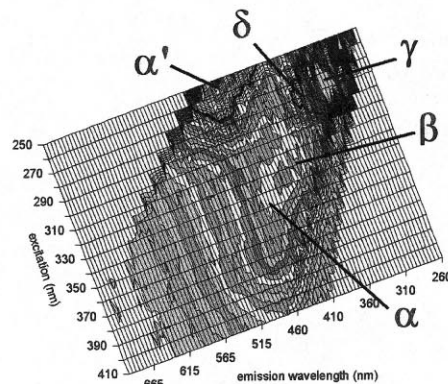
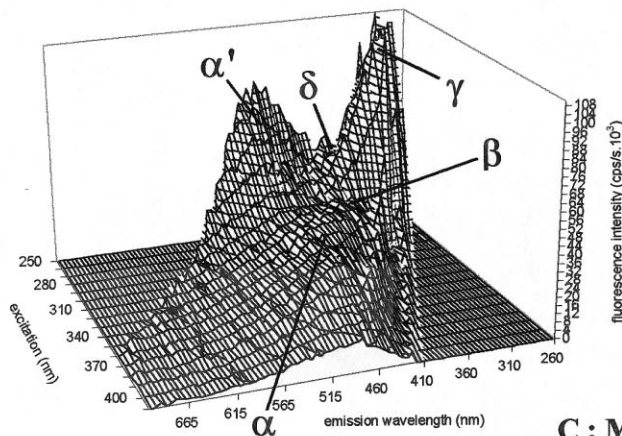
**A : F2 – S 0.3 – DOC 6.6 mg/L****B : M10 – S 34.9 – DOC <0.1 mg/L****C : M4 – S 35.0 – DOC 0.1 mg/L**

Fig. 3. Representative topographic and contour EEM plots for samples collected during the July 1996 cruise. A: fresh water (F2 sample), collected from the Frémur River flowing into the Bay; B and C: marine waters (M10 and M4 samples).

does give information about the composition of humic substances.

### 3.1. Samples collected during the 1996 cruises

The general fluorescence properties of the unconcentrated water samples collected in April and July 1996 (nine and seven samples respectively) from the Fresnaye Bay are illustrated in Fig. 2 (April) and Fig. 3 (July).

As previously reported during mixing processes between fresh and sea waters (Donard et al., 1989; De

Souza-Sierra et al., 1997; Parlanti et al., 1997), the fluorescence intensity of the  $\alpha$  peak decreased for marine waters, and in the 310–320 nm excitation range the fluorescence was blue-shifted. This blue-shift increases with salinity and distance from shore.

All the April water samples exhibited a maximum of fluorescence blue-shift to an extent never previously observed (by up to 70 nm) with respect to the maximum of the continental sample (collected from the river flowing into the Bay). These results are illustrated in Fig. 2 for two sea water samples which were compared with a fresh

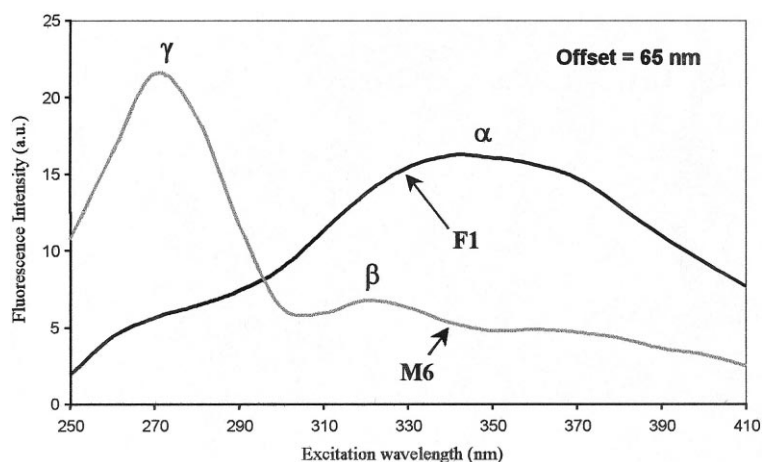


Fig. 4. Comparison of synchronous fluorescence spectra obtained with an offset of 65 nm for a fresh water sample (F1) and a marine water sample (M5), both collected in April 1996.

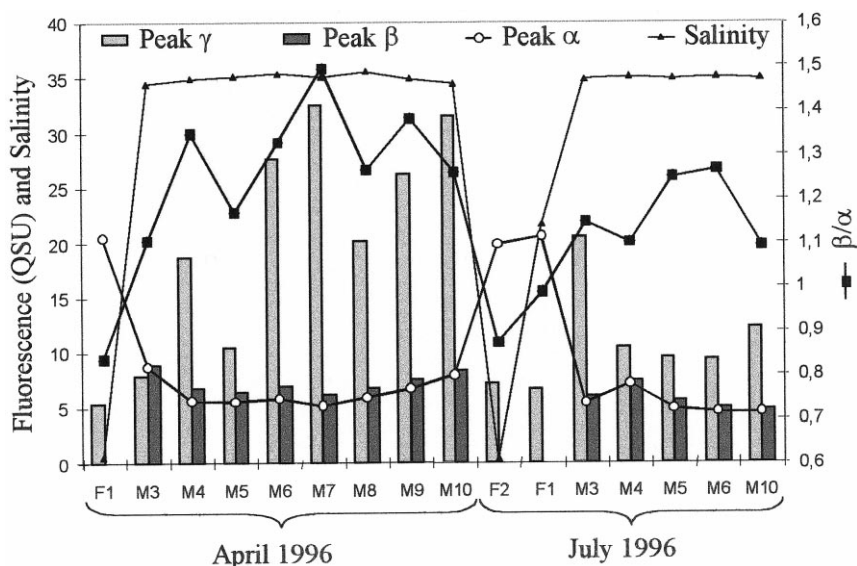


Fig. 5. Comparison of the fluorescence intensities (converted to quinine sulphate units) of the peaks  $\alpha$ ,  $\beta$  and  $\gamma$  for all the samples collected in 1996. Salinity, and the ratio of the fluorescence intensities of peak  $\beta$  versus peak  $\alpha$ , are superimposed on this pattern.

water sample. F1 is a continental sample, characterised by a typical fluorescence signature, with relative stability of the humic substances fluorescence maximum ( $\alpha$  peak  $Em_{max} \approx 460$  nm) which practically does not change with the excitation wavelength variation. M6 and M10 are sea water samples. Their contour EEM plots show both considerable blue shift at 320 nm ( $\beta$  component) as well as two other fluorescence bands at  $Ex/Em = 270$  nm/325

nm ( $\gamma$  peak) and at  $Ex/Em = 280$  nm/350 nm ( $\delta$  peak) attributable to proteinaceous material, suggesting recent biological activity (Mopper and Schultz, 1993; Determann et al., 1994, 1998; Seritti et al., 1994). The  $\delta$  peak is observed as a shoulder of the  $\gamma$  peak.

All the April marine samples presented very low concentrations in fluorescent DOM. The important blue-shift of their fluorescence maximum appears to result

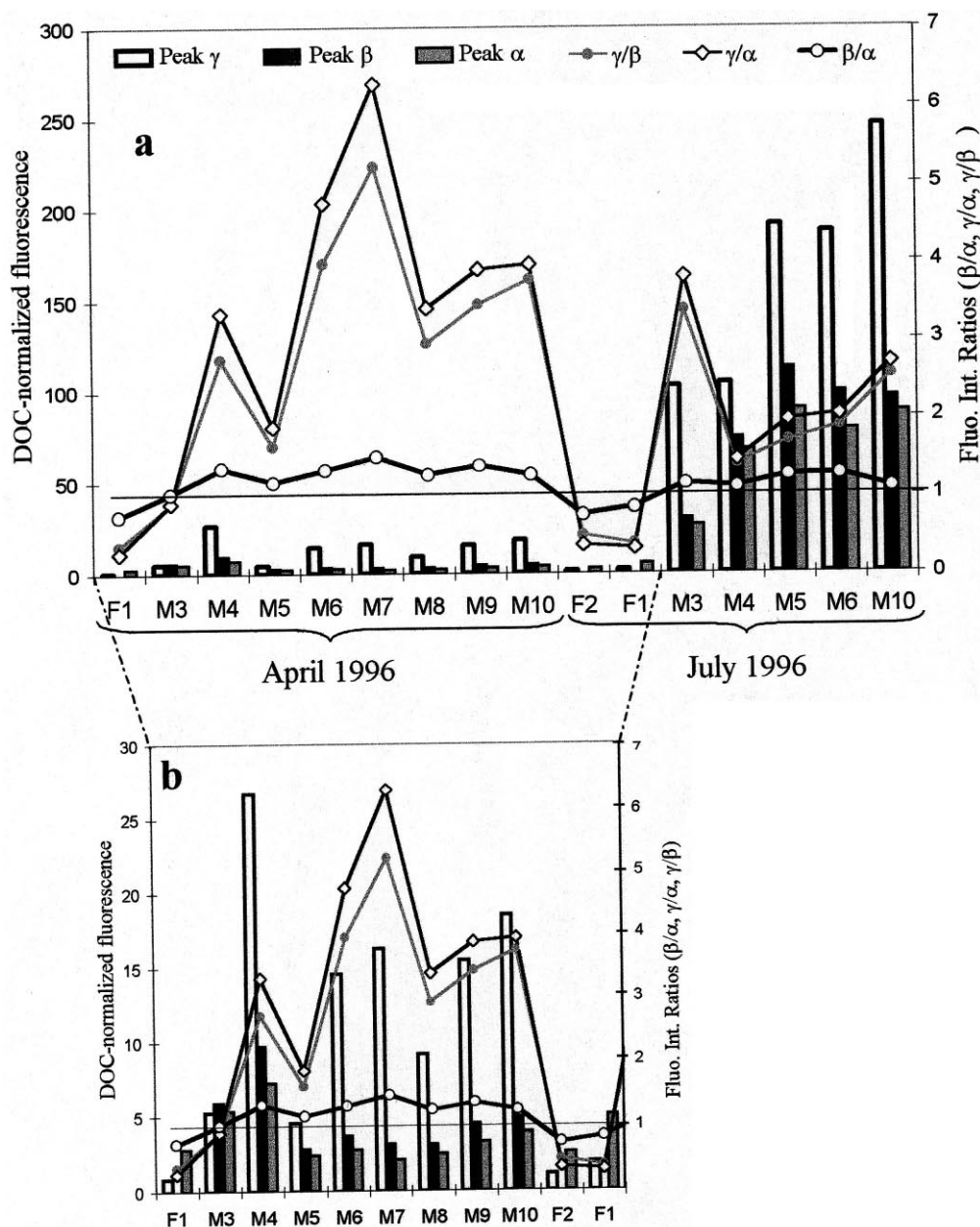
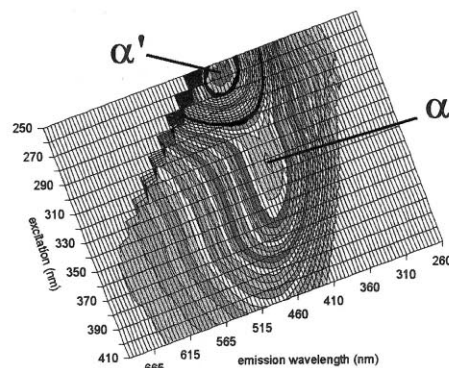
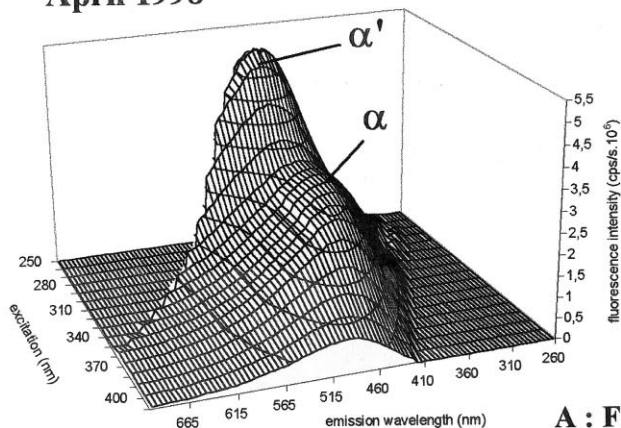


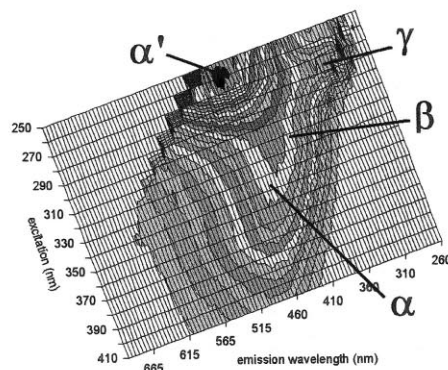
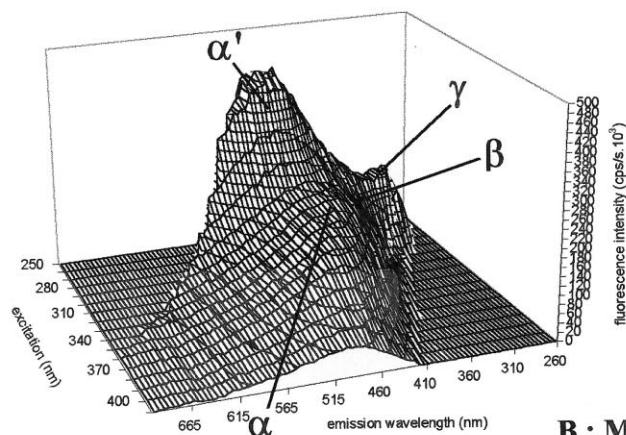
Fig. 6. (a): Comparison of the QS unit fluorescence values normalized by measured DOC concentrations for all the 1996 samples. The intensity of the  $\beta$  peak/ $\alpha$  peak,  $\gamma$  peak/ $\alpha$  peak and  $\gamma$  peak/ $\beta$  peak ratios are also plotted on this graph; (b) zoom of the  $\alpha$ ,  $\beta$  and  $\gamma$  peaks' distributions for a better visualisation of their intensities.



**April 1998**

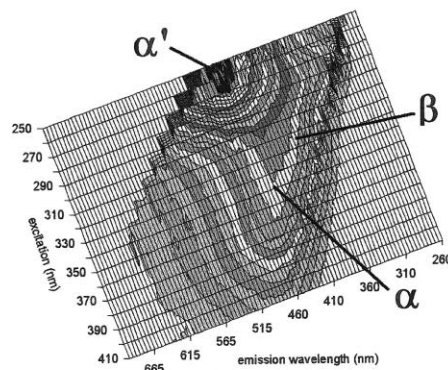
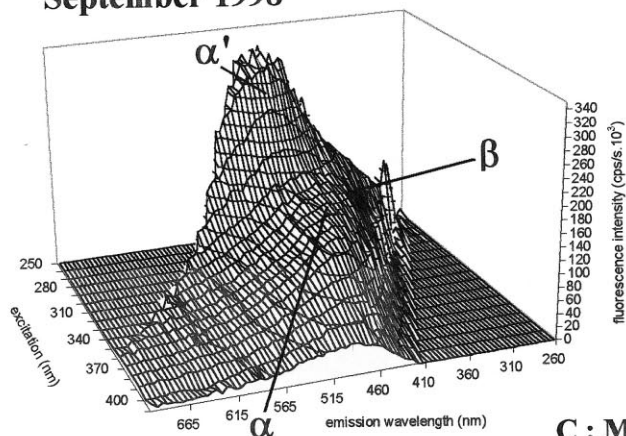


**A : F1 – S 0.6 – DOC 9.4 mg/L**



**B : M7 – S 34.7 – DOC 0.8 mg/L**

**September 1998**



**C : M4 – S 35.1**

Fig. 7. Representative topographic and contour EEM plots for samples collected during the April and September 1998 cruises. (A) fresh water (F1 sample, April 1998), collected from the Frémur River flowing into the Bay; (B) and (C) marine water samples (M7 in April 1998 and M4 in September 1998).



mostly from the contribution of the  $\beta$  component previously reported (De Souza-Sierra et al., 1994; Parlanti et al., 1997) but to an extent practically never observed before except by Coble (1996) in the Gulf of Maine. Throughout year 1996, but more particularly in April there was excessive macro-algae development and eutrophication, which could explain the profile of the  $\beta$  peak. Evidence of this  $\beta$  component is also clearly demonstrated by synchronous fluorescence, with an offset of 65 nm (Fig. 4). The  $\beta$  component is accompanied by a band due to protein-like fluorophores, thereby indicating the presence of compounds of recent biological origin. Taking into account both autochthonous biological resources and excessive green macro-algae development, these results tally with the hypothesis of a biological origin for the  $\beta$  component.

Globally, the samples collected in July (Fig. 3) presented fluorescence EEM shapes similar to those obtained in April. Nevertheless, the study of all the 1996 samples has shown a noticeable seasonal effect on the fluorescence properties of the DOM. Fig. 5 shows the variations in intensity of the  $\alpha$ ,  $\beta$  and  $\gamma$  peaks with salinity, in April and July 1996. The fluorescence intensities were transformed to equivalents of quinine sulphate (QS units) as previously reported (De Souza-Sierra et al., 1994). First of all, for the samples collected in July, we observed a decrease in the fluorescence intensity, especially for the  $\gamma$  peak, compared with samples collected in April. Furthermore, the  $\beta$  peak, although still well-defined, is however slightly less shifted than for the April marine samples. In April, the  $\beta$  component intensity is greater than the  $\alpha$  one for marine water samples,

whereas in July they are similar. As the sea water samples we investigated are coastal water samples, the  $\alpha$  peak is always great and contributes to the  $\beta$  component intensity. If we consider the ratio of the intensities between the  $\beta$  peak and the  $\alpha$  peak, we can see in Fig. 5 that its variation in April follows approximately the same trend as the  $\gamma$  peak. This result suggests that  $\beta$  and  $\gamma$  compounds have the same origin or are, at the very least, produced at the same time, which once again tallies with the hypothesis of a biological origin for the  $\beta$  component. On the other hand, in July, the distributions of the  $\gamma$  peak and the ratio of the  $\beta$  peak/the  $\alpha$  peak differ, leading us to think that the evolutions of the  $\beta$  and  $\gamma$  compounds differ over time.

Fig. 6 shows QS unit fluorescence values of the  $\alpha$ ,  $\beta$  and  $\gamma$  peaks, normalized by the samples' dissolved organic carbon (DOC) concentrations. The ratios of the intensities of these peaks ( $\beta/\alpha$ ,  $\gamma/\alpha$ ,  $\gamma/\beta$ ) are also plotted on this figure.

We observe a significant difference in intensity between the samples collected in April and July 1996. Taking into account the already described biological activity in April, the lower values observed for all April marine samples may be explained by a high production of extracellular organic materials excreted by phytoplankton or algae, increasing the DOC quantity. These materials could be mostly polysaccharides, organic acids, amino acids and carbohydrates (Gillam and Wilson, 1983). These exudates may be used or degraded quickly, thus, in July 1996, the DOC is lower. Considering the greater intensities observed in Fig. 6 for July marine samples, we can conclude that this DOC is mostly constituted by fluorescent DOM.

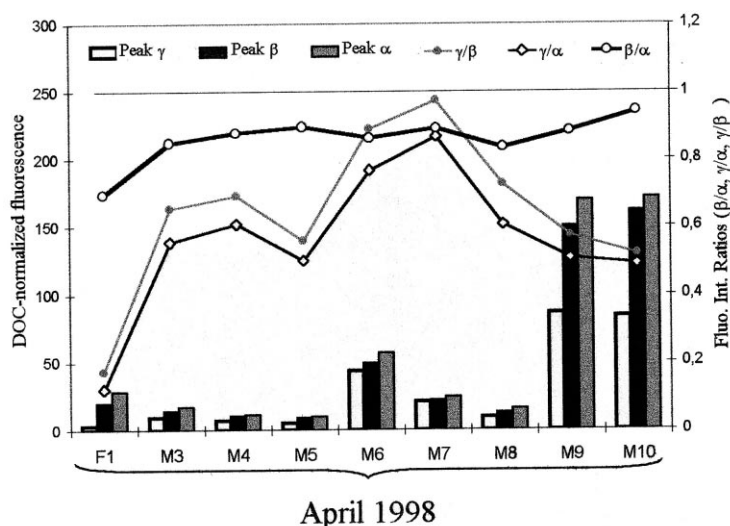


Fig. 8. Comparison of the QS unit fluorescence values normalized by measured DOC concentrations for the April 1998 samples. The intensity of the  $\beta$  peak/ $\alpha$  peak,  $\gamma$  peak/ $\alpha$  peak and  $\gamma$  peak/ $\beta$  peak ratios are also plotted on this graph.

The  $\beta$  peak/ $\alpha$  peak ratio curve follows, for April samples, the production of the  $\gamma$  peak. In July, no tendency can be observed, leading us to think once again that the evolutions of the  $\beta$  and  $\gamma$  compounds differ over time.

### 3.2. Samples collected during the 1998 cruises

The year 1998 was characterised in the bay, both in April and September, by a significant lower development of macro-algae. Fig. 7 illustrates the representative EEMs for samples collected in 1998. All fresh water and sea water samples exhibited the same fluorescence properties, respectively. For marine water samples we observed, in comparison with fresh water, only a slight blue shift in the excitation range of 310–320 nm for the  $\beta$  component, which can be seen as just a weak shoulder on the  $\alpha$  band. The protein-like fluorophore bands ( $\gamma$  and  $\delta$ ) were almost absent, and no correlation is

observed, for April samples, between these bands and the  $\alpha$  peak (Fig. 8). The  $\beta$  component was not so extensive as in 1996, but if we consider the lower macro-algae development, the formation of the  $\beta$  fluorophore seems once again to correlate with biological activity.

### 3.3. Samples collected during the 1999 cruises

Fig. 9 shows the EEMs of two samples (F2 and M2) collected in June 1999. Until June, all fresh waters samples gave the same fluorescence signature. In February 1999, fresh and sea water samples exhibited the same EEMs except as regards intensity. In April 1999 the M2 marine water EEM spectrum was the same as those obtained for the April 1998 sea water samples, corresponding to weak macro-algae development in the Bay. By June, the macro-algae had again developed excessively and were proliferating in the Bay. In the EEMs of the M2 sample

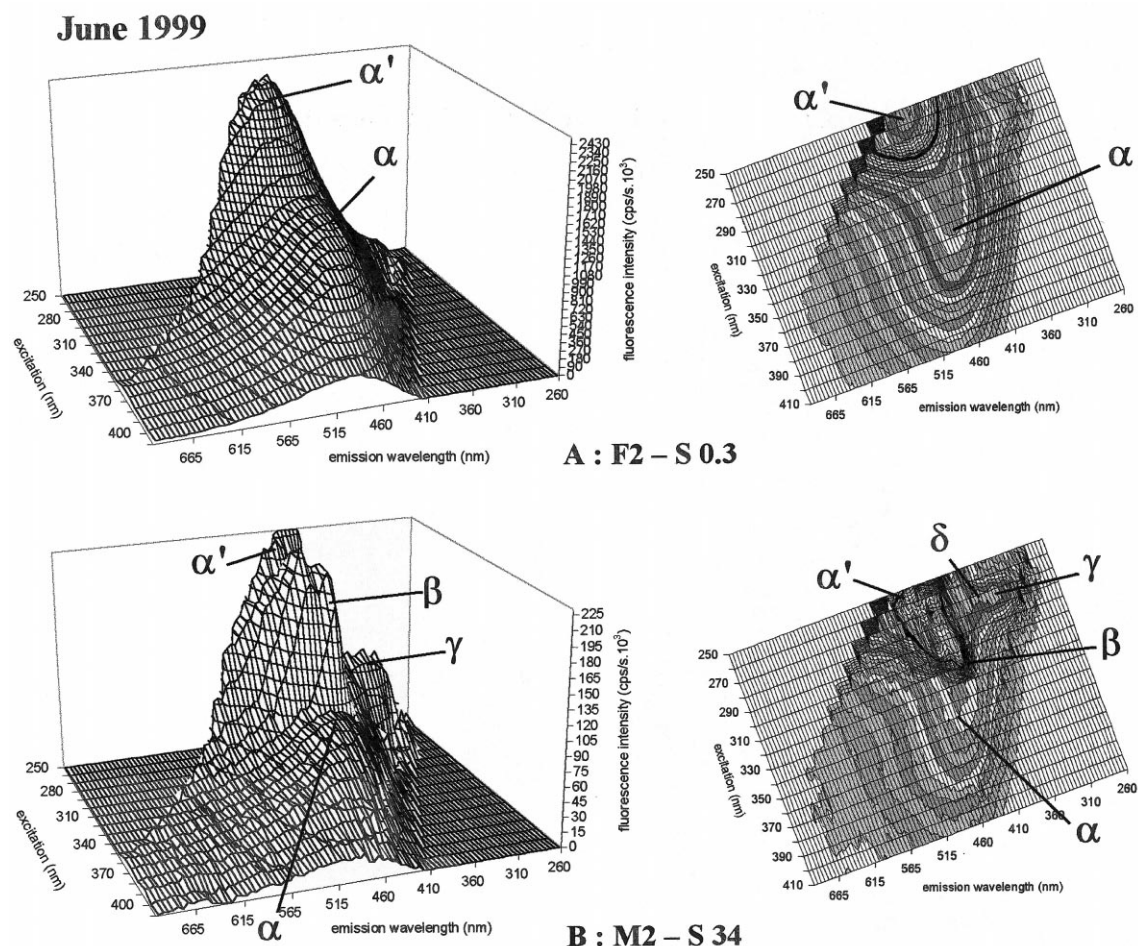


Fig. 9. Representative topographic and contour EEM plots for samples collected during the June 1999 cruise: (A) fresh water (F2 sample), collected from the Frémur River flowing into the Bay; (B) marine water (M2 sample).

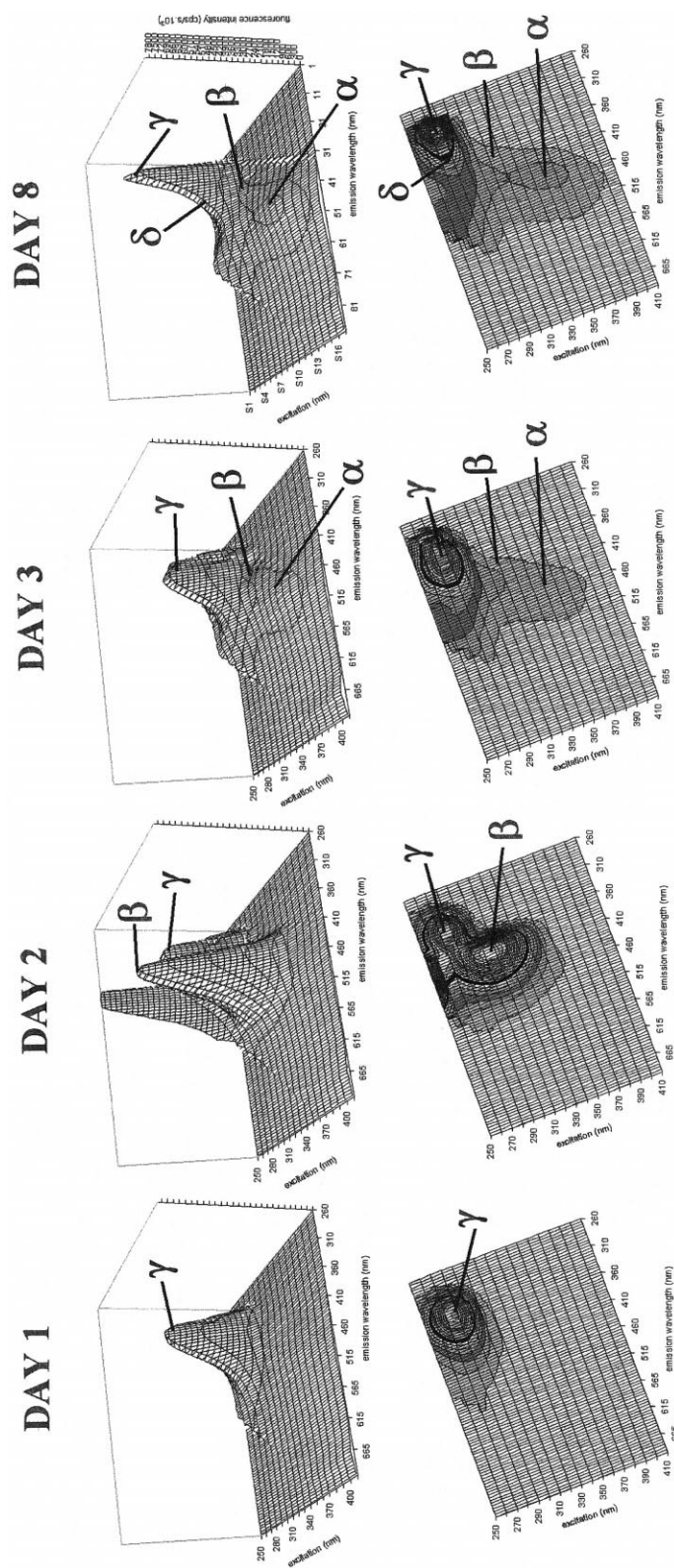


Fig. 10. Topographic and contour EEM plots for macro-algae degradation samples collected after 1, 2, 3 and 8 days. Experiment conducted in pure water.

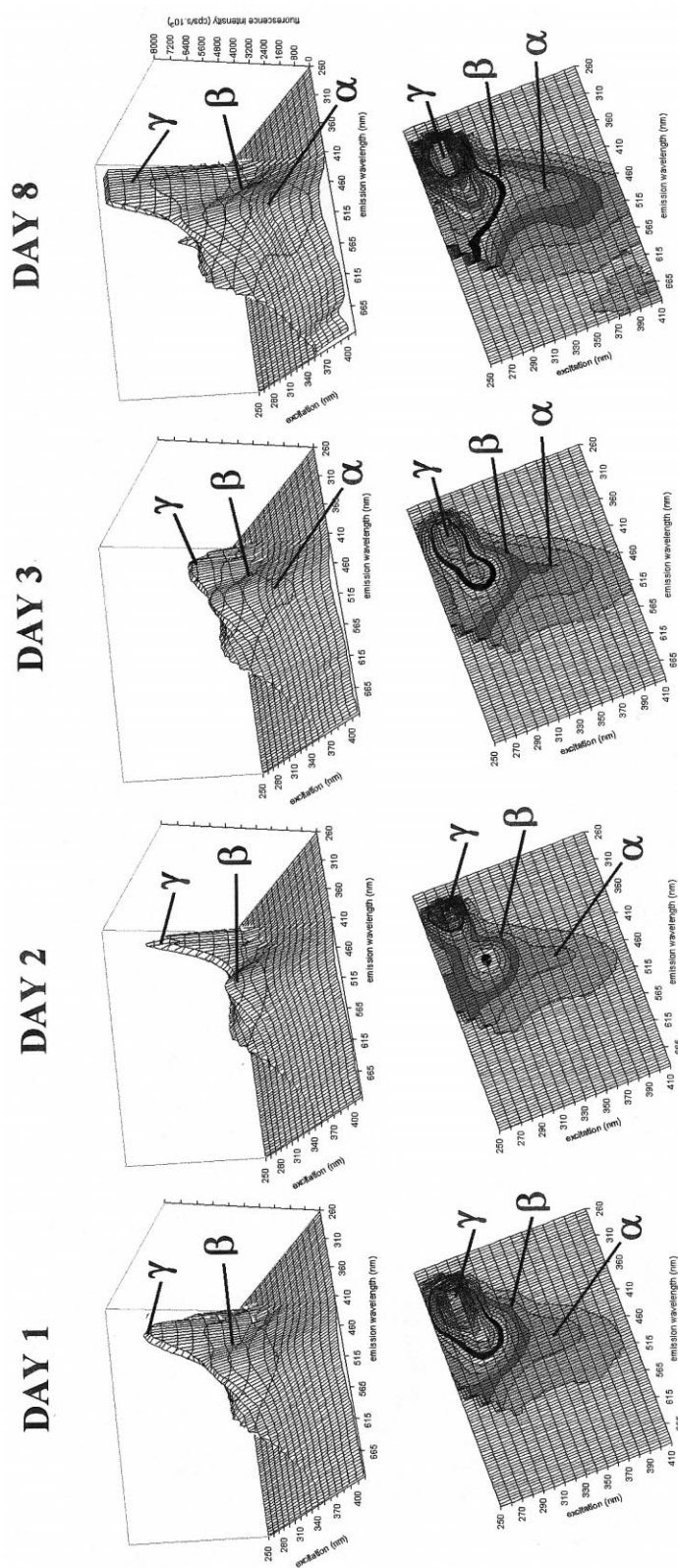


Fig. 11. Topographic and contour EEM plots for macro-algae degradation samples collected after 1, 2, 3 and 8 days. Experiment conducted in natural sea water (M2 sample).

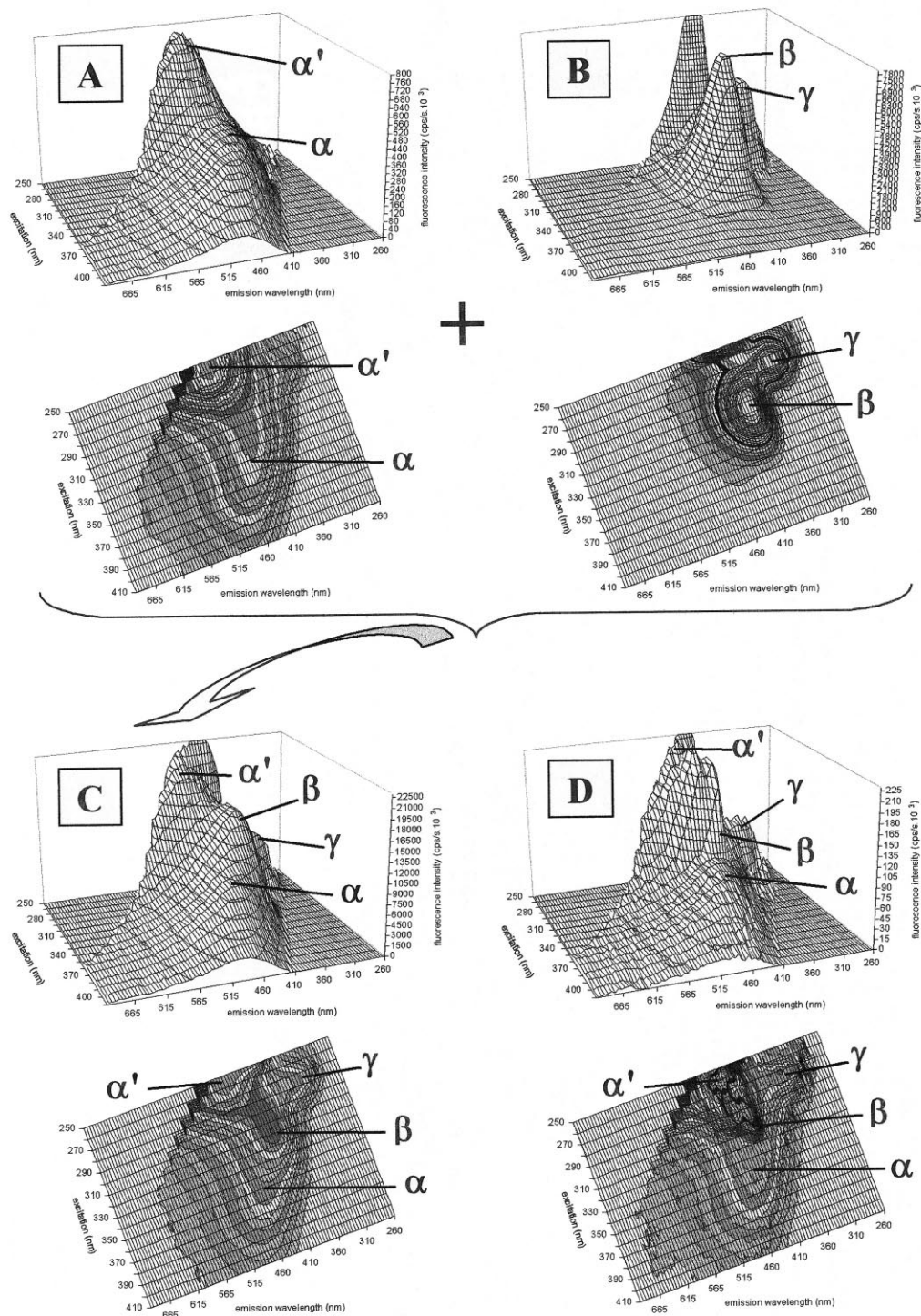


Fig. 12. The addition of EEMs of A (M2 sample, collected in February 1999) and B (2 day macro-algae degradation in pure water). The resulting topographic and contour EEM plots C are compared to D (M2 sample, collected in June 1999).

another fluorescence band occurred with a maximum at  $Ex/Em=300/415$  nm that could be related to the  $\beta$  component. This peak was not observed for all the other samples collected in 1999. This band, very high in intensity relative to the  $\alpha$  and  $\gamma$  peaks, may represent very freshly released algae exudates in their native form before transformation, and may well constitute the  $\beta$  component generally observed in marine waters.

The results obtained for all the samples collected during 1996, 1998 and 1999 indicate that salinity and distance from shore are not the only factors controlling the presence and importance of the  $\beta$  component. Coble (1996), considering different natural water types from several sampling sites, drew the same conclusions. Our investigations of the fluorescence signal variation during 3 years of sampling at the same sites demonstrate that biological activity may largely contribute to the presence or absence of the  $\beta$  peak. To reach a better understanding of a hypothetical mechanism whereby material of marine organisms are transferred to the DOM pool, we analysed the degradation of the green macro-algae, *Ulva lactuca*, collected in June 1999.

#### 3.4. Degradation, in the laboratory, of green macro-algae

In the laboratory, we followed up the fluorescence spectra variation with macro-algae degradation in pure water, in artificial sea water and in natural sea water. Here we report on the results obtained for the pure and natural water experiments. The fluorescence of the algae degradation products during the first week is illustrated in Fig. 10 for the experiment in pure water, and in Fig. 11 for that in natural water (M2 — June 1999). The same fluorescence intensity scale was used for all these EEM plots, allowing an easy visual comparison of the different peaks' relative intensities. In the first stages of algae degradation we observed the appearance first of protein-like fluorophores (peak  $\gamma$ :  $Ex$  270–280 nm/ $Em$  320–350 nm) and then of a fluorescence band corresponding to the  $\beta$  component ( $Ex$  300–310 nm/ $Em$  400–410 nm). The latter quickly disappeared in favour of a

blue-shift in the excitation range of 310–320 nm corresponding to a shoulder of the  $\alpha$  peak. It is also important to notice the appearance of the  $\alpha$  peak consecutive to the disappearance of the  $\beta$  peak. Another point is the continual decrease in the  $\gamma$  peak, suggesting that these compounds may be used to produce the fluorophores giving the other peaks' fluorescence signature. Nevertheless, the increase in intensity of the  $\beta$  peak is greatly superior to the intensity decrease observed for the  $\gamma$  peak, which suggests two possible interpretations. The first hypothesis could be that the  $\gamma$  peak fluorophores may continue to being produced while they are being transformed. The second hypothesis could be that the  $\gamma$  peak fluorophores alone may be protein-like material and that the  $\beta$  peak may have another origin, such as bacterial fluorescence, even though Determann et al. (1998) concluded that bacteria do not produce significant fluorescence signal at excitation wavelengths higher than 300 nm. Tanoue et al (1995) reported that bacterial membranes may be a possible source of a major dissolved protein in sea water. They suggested a pathway for the production of DOM whereby enzyme-resistant biopolymers survive and accumulate in the sea. Even if the  $\beta$  peak and the  $\gamma$  peak fluorophores have different origins, at one stage at least of their formation their evolutions seem to be correlated. Further algae incubation experiments under control conditions are needed to draw more definite conclusions. It has already been suggested that the protein fluorescence is directly attributable to planktonic activity in surface waters (Matthews et al., 1996). We have also noted the production of the  $\beta$  component by phytoplankton in *Isochrysis galbana* incubation studies (Parlanti et al., 1997). This work, however, demonstrates that the  $\gamma$  and  $\beta$  peaks may be produced by macro-algae exudates and perhaps also at least the  $\alpha$  peak.

Degradation experiments in artificial and natural sea waters gave exactly the same results and were practically similar to those conducted in pure Milli-Q water. We can easily imagine that bacterial and enzymatic activities differ in pure and salty waters, thus giving rise to the

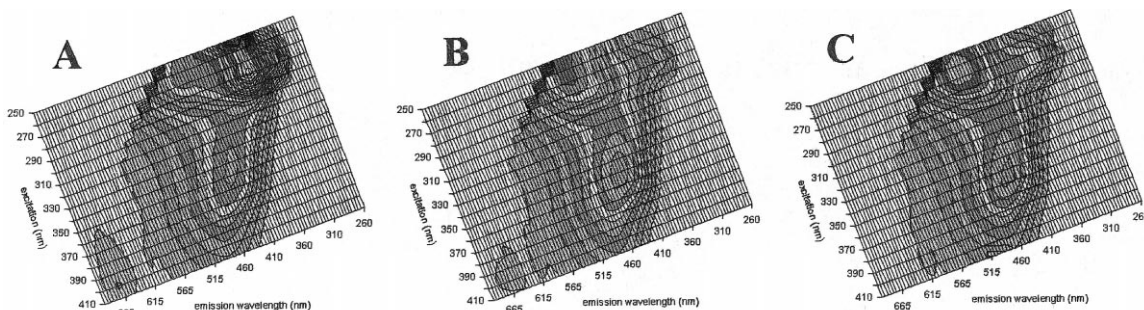


Fig. 13. Topographic and contour EEM plots for macro-algae degradation samples collected after 2 months for the experiments conducted in pure water (A), in artificial sea water (B), and in natural sea water (C).

slight differences we observed in Fig. 11: the shorter excitation wavelength of the  $\beta$  component (290 nm instead of 300–310 nm) and a relatively lower proportion of this fluorescence band. The  $\alpha$  peak formation seems to follow the same pattern as in pure water experiments. The trend observed for the different peaks' evolution is that the  $\gamma$  peak decreases with the increase of the  $\beta$  peak and that the  $\alpha$  peak appears last. The  $\gamma$  fluorophores again seem to be produced in great amounts after one week. The global fluorescence signal exhibited after a period of 1 week's degradation is similar to those generally observed in marine waters when the biological activity is intense.

We combined the EEMs showing the formation of the  $\beta$  peak after 2 days' degradation in pure water (Fig. 12B) with the EEMs of the M2 sample collected in February 1999 (Fig. 12A) when its fluorescence pattern was the same as that of the fresh water collected at the same period. The M2 sample's fluorescence intensity was multiplied by a factor determined by the ratio of the two samples' absorbance values. We can see in Fig. 12 that the EEMs obtained exhibited a fluorescence pattern very close to that of the M2 sample collected in June 1999, and more generally, to those observed in sea waters submitted to conditions of intense biological activity.

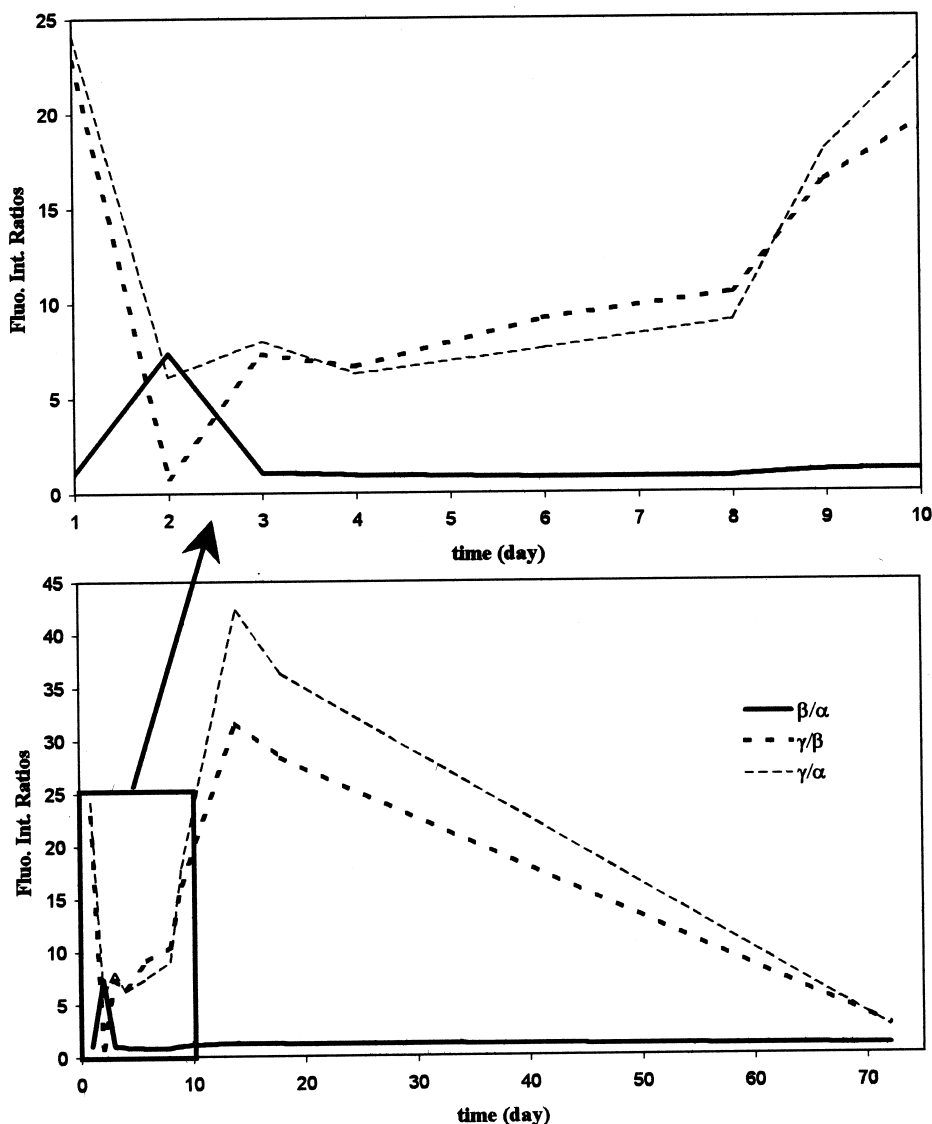


Fig. 14. Intensities of the  $\beta$  peak/ $\alpha$  peak,  $\gamma$  peak/ $\alpha$  peak and  $\gamma$  peak/ $\beta$  peak ratios plotted versus time for the algae degradation in pure water experiment.



Fig. 13 shows the EEMs obtained after a 2 month degradation period in pure water (A), in artificial sea water (B) and in natural water (C). We can see on plots B and C that the  $\beta$  and  $\gamma$  peaks have almost completely disappeared in favour of the  $\alpha$  peak, giving fluorescence properties very close to humic-like matter characteristics. In the case of the experiment conducted in pure water a slight difference can be observed with the  $\gamma$  peak and also the  $\delta$  peak being very well-defined and intense. Nevertheless, the  $\alpha$  peak is also as well-defined as in the two other experiments with sea water.

Fig. 14 shows the data, from the algae degradation in pure water study, plotted as time courses. The increase in intensity of the  $\beta$  peak/ $\alpha$  peak ratio the second day of the experiment is observed with the relative decrease of the  $\gamma$  peak, whereas the third day is characterized by a decrease of the  $\beta$  peak/ $\alpha$  peak ratio and an increase in the  $\beta$  peak/ $\alpha$  peak ratio intensity. We, however, cannot conclude if the  $\beta$  peak is produced from the transformation of at least a part of the  $\gamma$  peak compounds, or if these components have different precursors. They are nevertheless the two consecutive first products of the macro-algae degradation. The second step of the degradation is observed in Fig. 14 after 2 weeks: a great increase in the relative proportion of the  $\gamma$  peak is accompanied by a slight increase in the  $\beta$  peak/ $\alpha$  peak ratio intensity. Afterwards, a continuous decrease is observed for the three ratios and their evolutions tend to converge after 72 days, which tallies with the final formation of the  $\alpha$  peak.

Our results may be interpreted as follows: organic materials freshly transferred from algae to the DOM pool may be partly remineralized, but a fraction of the degraded products may be transformed into humic-like matter through successive condensation reactions or structural rearrangements, giving rise to the successive different fluorescence bands we observed and, finally, to the production of humic-like and/or marine-like material. The fluorescence can then be used to estimate the biological activity and the different stages of the humification processes.

#### 4. Conclusion

Our comparison of the fluorescence spectra variations of natural water with the variation over time of environmental conditions and particularly of eutrophication, at a particular site propitious to macro-algae development, can be summarised as follows.

1. The  $\beta$  component is observed together with a band due to protein-like fluorophores indicative of the presence of compounds of recent biological origin.

2. The specifically large contribution of the  $\beta$  component in the fluorescence of waters collected in a coastal zone characterised by an important biological activity

(Gulf of Saint-Malo) has been observed especially when conditions were propitious to excessive green macro-algae development.

3. These results and those of green macro-algae degradation experiments, clearly demonstrate that biological activity is one of the major factors controlling the  $\beta$  component formation, which could originate from variously degraded algae exudates.

4. Organic materials freshly transferred from algae to the DOM pool contribute to the successive different fluorescence bands we observed and, finally, to the production of humic-like and/or marine-like material.

5. The  $\beta$  and  $\gamma$  peaks could be used as markers to estimate the biological activity of coastal zones, not only in a quantitative way but also to estimate the different stages of the biological production.

6. This biogeochemical process of DOM production is moreover environmentally important if we consider that contaminants are largely adsorbed on the macro-algae surface. These results will help to improve our understanding both of contaminant binding and speciation, as well as of their dispersion at the interface continent/ocean.

#### Acknowledgements

This work was supported by grants from the Groupe De Recherche Manche. We wish to thank the crew of the ship of the Museum d'Histoire Naturel in Dinard on which most of the sampling presented in this study was performed. We are also most grateful to Dr. Daniel Bentley from the Institut National des Sciences et Techniques de la Mer (INTECHMER) in Cherbourg, France, who provided us with all the 1999 water and macro-algae samples.

#### References

- Berger, P., Laane, R.W.P.M., Ilahude, A.G., Ewald, M., Courtot, P., 1984. Comparative study of dissolved fluorescent matter in four West European Estuaries. *Oceanology Acta* 7, 309–314.
- Cabaniss, S.E., Shumann, M.S., 1987. Synchronous fluorescence spectra of natural waters: tracing sources of dissolved organic matter. *Marine Chemistry* 21, 37–50.
- Chen, R.F., Bada, J.L., 1992. The fluorescence of dissolved organic matter in seawater. *Marine Chemistry* 37, 191–221.
- Coble, P.G., 1996. Characterisation of marine and terrestrial DOM in seawater using excitation-emission spectroscopy. *Marine Chemistry* 51, 325–346.
- Coble, P.G., Brophy, M.M., 1994. Investigation of the geochemistry of dissolved organic matter in coastal waters using optical properties. *SPIE, Ocean Optics XII* 2258, 377–389.
- Coble, P.G., Green, S.A., Blough, N.V., Gagosian, R.B., 1990. Characterisation of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature* 348, 432–435.

- Coble, P.G., Mopper, K., Schultz, C.A., 1993. Fluorescence contouring analysis of DOC Intercalibration Experiment samples: A comparison of techniques. *Marine Chemistry* 41, 173–178.
- De Souza-Sierra, M.M., Donard, O.F.X., 1991. Simulation of fluorescence variability in estuaries. In: *Oceanological Acta, Proc. of the International Colloquium on the Environment of Epicontinental Seas*, vol. Sp. 11, Lille, 20–22 March 1990, 275–284.
- De Souza-Sierra, M.M., Donard, O.F.X., Lamotte, M., Belin, C., Ewald, M., 1994. Fluorescence spectroscopy of coastal and marine waters. *Marine Chemistry* 47, 127–144.
- De Souza-Sierra, M.M., Donard, O.F.X., Lamotte, 1997. Spectral identification and behaviour of dissolved organic fluorescent material during estuarine mixing processes. *Marine Chemistry* 58, 51–58.
- Determann, S., Reuter, R., Wagner, P., Willkomm, R., 1994. Fluorescence matter in the eastern Atlantic Ocean: part 1. Method of measurement and near-surface distribution. *Deep-Sea Research* 41, 659–675.
- Determann, S., Lobbes, J.M., Reuter, R., Rullkötter, J., 1998. Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. *Marine Chemistry* 62, 137–156.
- Donard, O.F.X., Lamotte, M., Belin, C., Ewald, M., 1989. High-sensitivity fluorescence spectroscopy of Mediterranean waters using a conventional or pulsed laser excitation source. *Marine Chemistry* 27, 117–136.
- Ewald, M., Belin, C., Berger, P., Weber, J.H., 1983. Corrected fluorescence spectra of fulvic acids isolated from soil and water. *Environmental Science and Technology* 17, 501–504.
- Gillam, A.H., Wilson, M.A., 1983. Application of  $^{13}\text{C}$ -NMR spectroscopy to the structural elucidation of dissolved marine humic substances and their phytoplanktonic precursors. In: Christman, R.F., Gjessing, E.T. (Eds.), *Aquatic and Terrestrial Humic Materials*. Ann Arbor Science Publishers, Michigan, pp. 25–36.
- Guegueniat, P., Salomon, J.C., Wartel, M., Cabioch, L., Fraizier, A., 1993. Transfer pathways and transit time of dissolved matter in the Eastern English Channel indicated by space-time radiotracers measurements and hydrodynamic modelling. *Estuarine, Coastal and Shelf Science* 36, 477–494.
- Hayase, K., Tsubota, H., Sunada, I., Goda, S., Yamazaki, H., 1988. Vertical distribution of fluorescent organic matter in the North Pacific. *Marine Chemistry* 25, 373–381.
- Hedges, J., Hatcher, P., Ertel, J., Meyers-Schulte, K.A., 1992. Comparison of dissolved humic substances from seawater with Amazon counterparts by  $^{13}\text{C}$ -NMR spectrometry. *Geochimica & Cosmochimica Acta* 56, 1753–1757.
- Laane, R.W.P.M., Kramer, K.J.M., 1990. Natural fluorescence in the North Sea and its major estuaries. *Netherlands Journal of Sea Research* 26, 1–9.
- Leiner, M.J.P., Wolfbeis, O.S., 1988. Biochemical applications of 3-dimensional fluorescence spectrometry. In: J.R. Lakowicz (Ed.), *Time Resolved Laser spectroscopy in Biochemistry*. SPIE Proc., Vol. 909, pp. 134–138.
- Matthews, B.J.H., Jones, A.C., Theodorou, N.K., Tudhope, A.W., 1996. Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs. *Marine Chemistry* 55, 317–332.
- Mopper, K., Schultz, C.A., 1993. Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. *Marine Chemistry* 41, 229–238.
- Parlanti, E., Belin, C., Texier, H., Ewald, M. & Lamotte, M. 1997 Interpretation of the fluorescence signature of the marine and continental waters in the Gironde and Seine Estuaries. In: Drozd, J., Gonet, S.S., Senesi, N., Weber, J. (Eds.), *The Role of Humic Substances in the Ecosystems and in Environmental Protection*, pp. 603–609.
- Seritti, A., Morelli, E., Nannicini, L., Del Vecchio, R., 1994. Production of hydrophobic fluorescent organic matter by the marine diatom *Phaeodactylum tricornutum*. *Chemosphere* 28, 117–129.
- Tanoue, E., Nishiyama, S., Kamo, M., Tsugita, A., 1995. Bacterial membranes: possible source of a major dissolved protein in seawater. *Geochimica & Cosmochimica Acta* 59, 2643.