

Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC)

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Abstract

The distributions of fluorescent components in dissolved organic matter (DOM) from Ise Bay, Japan, were determined by excitation emission matrix (EEM) fluorescence spectroscopy combined with parallel factor analysis (PARAFAC). Three terrestrial humic-like, one marine humic-like, and three non-humic-like fluorescent components were identified by PARAFAC, and the environmental dynamics of individual fluorescent components in the bay area were evaluated. The observed linear relationships between salinity and abundance of two of the three humic-like components in the bay area indicate a terrestrial origin and conservative mixing behavior of these components. On the other hand, nonconservative mixing for the other terrestrial and the marine humic-like components was observed, indicating that the sources of these were other than solely riverine inputs. Thus, in addition to riverine sources, this terrestrial humic-like component may receive inputs from biogeochemical reworking of terrestrial DOM and/or particulate organic matter, while the most likely sources for the marine humic-like component are estuarine biological activity and/or microbial reworking of plankton-derived DOM. From the spatial distributions in the bay area as well as their relationships with salinity, two of the non-humic-like components were suggested to be of autochthonous estuarine origin and likely represent biologically labile components. Microbial degradation processes were suggested to be important factors driving the dynamics of another non-humic-like component. This study exemplifies the potential applicability of EEM-PARAFAC in studies of fluorescent DOM dynamics in estuaries.

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Dissolved organic matter (DOM) is a major form of organic matter in aquatic environments and plays an important role in the global carbon cycle. In coastal environments, substantial terrestrial DOM inputs through river discharges are estimated to contribute 0.25×10^{15} g carbon (C) yr⁻¹ to the ocean carbon pool (Hedges et al. 1997). Together with the terrestrial DOM, rivers convey inorganic nutrients, which can result in intense primary productivity, and 30% of primary production in the world oceans is evident in coastal environments (Lalli and Parsons 1997). Such primary production leads to the production of autochthonous DOM. Thus, the dynamics of DOM in coastal environments can be complex, and the fate of terrestrial DOM and the production and degradation of autochthonous DOM need to be assessed separately

to better understand and estimate their relative contributions.

Excitation emission matrix (EEM) fluorescence techniques have been widely used to distinguish between allochthonous and autochthonous DOM sources in coastal environments (Coble et al. 1998; Del Castillo et al. 1999; Mayer et al. 1999). Two main fluorophore types, namely, protein- and humic-like fluorophores, have been reported and are easily distinguished based on their peak position in the EEM. The major source of humic-like fluorophores in coastal environments is river-discharged terrestrial humic substances, and consequently, a negative linear relationship between salinity and humic-like fluorescence intensity has often been reported (de Souza Sierra et al. 1997; Del Castillo et al. 1999; Jaffé et al. 2004). However, marine humic-like fluorophores can be distinguished from terrestrial humic-like fluorophores by EEM fluorescence (Coble 1996), and qualitative changes in the distributions of these have been reported in coastal environments (de Souza Sierra et al. 1997; Del Castillo et al. 1999). In contrast, the protein-like fluorophores have been considered to be derived from freshly produced DOM (Mayer et al. 1999; Yamashita and Tanoue 2003), and an increase in contribution of protein-like fluorophores to total fluorescence with increasing salinity was observed in coastal environments (Kowalczyk et al. 2003). In addition, linear relationships between the protein-like fluorescence intensity and concentrations of total hydrolyzable amino acids have been reported (Yamashita and Tanoue 2003). Thus, EEM fluorescence can estimate the dynamics of both allochthonous (humic-like fluorophores) and autochthonous (protein-like fluorophores) DOM. However, since the EEMs are often composed of various types of overlapping fluorophores, it may be difficult to properly evaluate DOM dynamics in coastal areas based solely on the EEM "peak picking" technique.

Recently, Stedmon et al. (2003) introduced parallel factor analysis (PARAFAC), a statistical modeling approach, to decompose EEMs into their individual fluorescent components and revealed five distinct DOM components in a Danish estuary and its catchment. Since the pioneering work of Stedmon et al. (2003), the combination of EEM and PARAFAC has been applied to characterize DOM extracted from soils (Ohno and Bro 2006), in terrestrial aquatic environments (Fulton et al. 2004; Cory and McKnight 2005; Hall et al. 2005), and in laboratory and mesocosm experiments (Stedmon and Markager 2005a; Stedmon et al. 2007). Consequently, Cory and McKnight (2005) reported on their findings of 13 fluorescent components using a model derived from DOM samples collected from diverse terrestrial aquatic environments. In coastal environments, Stedmon and Markager (2005b) identified eight components using a large data set ($n = 1,276$) for a Danish estuary and its catchment, showed differences in fluorophore composition among these environments, and evaluated the similarity or dissimilarity of the dynamics of individual fluorophores.

Thus, EEM-PARAFAC is a powerful tool in the assessment of DOM dynamics in aquatic ecosystems. However, still little is known about the dynamics of

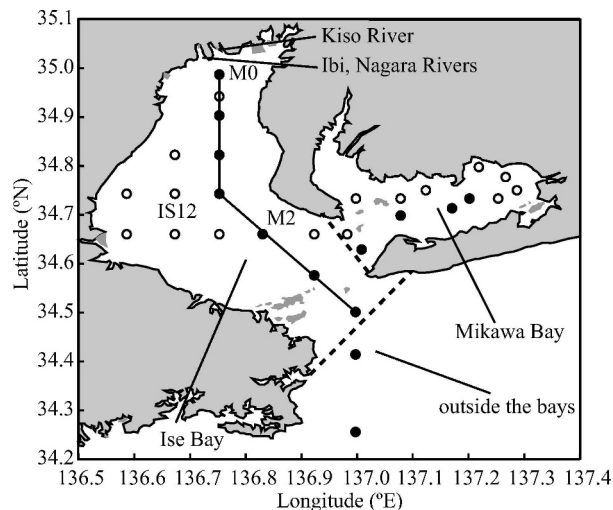


Fig. 1. Sampling locations in the present study. Sampling stations that conducted vertical and surface observations are indicated by closed and open circles, respectively. Sta. M0, M2, and IS12 indicate the sampling locations of sediment core for pore water. The distributions of fluorescent components along a transect, indicated by line in Ise Bay, are shown in Fig. 3.

individual fluorophores identified by PARAFAC in coastal environments.

The main objective of this study was the assessment of the dynamics of individual fluorophores using EEM-PARAFAC in coastal environments. To the best of our knowledge, the relationships between individual fluorescent components identified by PARAFAC and salinity are first described in the present study, and special attention was placed on establishing the biogeochemical factors controlling this relationship. Ise Bay, Japan, was chosen as a case study because of the well-established occurrence of both autochthonous and allochthonous fluorophores in this estuary (Yamashita and Tanoue 2003, 2004). We carried out EEM-PARAFAC on seawater samples obtained from the bay area covering a wide range of salinity and on pore-water samples extracted from sediments.

Methods

Sampling—The water samples were collected from Ise Bay and adjacent Mikawa Bay and outside of the bays (Fig. 1) during cruises of the T/S *Seisui-maru* (Mie University) on 16–18 July 2003 (SE03-12). The distribution of bulk humic-like fluorescence intensity for this sample set was previously reported (Yamashita and Tanoue 2004). Samples of surface water were collected in a plastic bucket, and those at different depths were collected using a conductivity–temperature–depth–rosette system equipped with Niskin bottles. Immediately after sampling, the samples were filtered through precombusted (450°C at 4 h) GF/F and 0.22- μm polyvinylidene difluoride filters (Durapore, Millipore) successively (Yamashita and Tanoue 2004). The filtrate was placed into precombusted glass bottles with Teflon-lined caps. The filtrates were stored in a

refrigerator (4°C) and EEM measurements were made in the laboratory within 5 d after collection.

To obtain pore-water samples, sediment cores were collected from Ise Bay during the cruise (Fig. 1). More detailed information on sampling locations and sedimentation rates can be found elsewhere (Lu and Matsumoto 2005). The cores were taken using a modified gravity core consisting of acrylic pipe, a sediment catcher, and a clear vent (Lu and Matsumoto 2005). Pore-water samples in this study were used only to contribute to the PARAFAC model. Details of the pore-water profiles will be published elsewhere.

EEM measurements and PARAFAC modeling—The measurement of EEM and normalization of fluorescence intensity were carried out according to the methods of Yamashita and Tanoue (2003, 2004). A single measurement of EEM was made on each sample using a fluorescence spectrophotometer (Hitachi F-4500). The EEMs were generated by scanning emission spectra from 225 to 500 nm at 1-nm intervals, with 5-nm increments of the excitation wavelength from 225 to 400 nm. The EEM of Milli-Q water was subtracted from that of each sample, and then EEMs were normalized to quinine sulfate units using $4 \mu\text{g L}^{-1}$ quinine sulfate monohydrate in a solution of $0.05 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$.

PARAFAC statistically decomposes the complex mixture of DOM fluorophores into components without any assumptions with regard to their spectra shape or their number (Stedmon et al. 2003). In addition to samples collected in July 2003 we used samples collected from the same estuarine (September and October 2004) to obtain a statistically significant number of samples for the PARAFAC modeling. The data set for PARAFAC modeling was composed of 156 estuarine water samples and 43 samples of pore water. For PARAFAC modeling, excitation wavelengths from 225 to 255 nm were deleted from each EEM because of the low precision of the fluorescence intensity at these wavelengths (Yamashita and Tanoue 2003). The analysis was carried out in MATLAB with the “N-way toolbox for MATLAB” (Andersson and Bro 2000), and split-half analysis (Stedmon et al. 2003; Cory and McKnight 2005) was used to validate the identified components.

Results and discussion

Fluorescent components—Seven fluorescent components were identified by PARAFAC using 199 EEMs of multiple samples collected from the bay area (Fig. 2). All of the fluorescent components had single emission maxima with single or multiple excitation maxima. Spectral characteristics of the components identified in the present study were very similar to those of DOM in other aquatic environments previously identified by PARAFAC (Stedmon et al. 2003; Cory and McKnight 2005; Stedmon and Markager 2005b). The excitation and emission pairs of the main peak positions for each of the components are summarized in Table 1 and compared to those found in earlier studies. From the fluorescence characteristics, the components

could be distinguished into terrestrial humic-like components (components 1, 2, and 3), marine humic-like (component 6), and non-humic-like components (components 4, 5, and 7).

An intense excitation maximum for component 1 occurred below 260-nm at 458-nm emission (Fig. 2; Table 1), and was similar to the humic-like fluorophore in the ultraviolet region (peak A), as defined by Coble (1996) and Coble et al. (1998). This component was also similar to the terrestrial fluorescent component identified through PARAFAC by other authors (Table 1). The spectral characteristics of component 2 were characterized by a peak at 345-nm excitation and 433-nm emission wavelengths, similar to the humic-like fluorophore in the visible region (peak C; Coble 1996; Coble et al. 1998). Component 2 also resembled component 4, reported by Stedmon and Markager (2005b), which was ubiquitous in all environments studied by these authors, and terrestrial unknown component 1, reported by Cory and McKnight (2005). Component 3 comprised two peaks with an emission maximum at 479 and 390 nm and 270-nm excitation. This peak has not been traditionally defined (Coble 1996; Coble et al. 1998) but was similar to a terrestrial reduced quinone-like component (SQ1) reported by Cory and McKnight (2005) as well as a ubiquitous component 2 reported by Stedmon and Markager (2005b).

There were also two excitation maxima (at 325 nm and below 260 nm) observed in the EEM of component 6 (Fig. 2). The emission maximum at 385 nm could be categorized as the previously defined peak M, assigned to marine humic-like fluorophores (Coble 1996; Coble et al. 1998). The spectral features were also similar to a reported microbial derived component (C3; Cory and McKnight 2005) and were similar to a dominant component reported in wastewater (component 6; Stedmon and Markager 2005b).

Using the EEM-PARAFAC, we obtained three components (components 4, 5, and 7) in the excitation and emission wavelength range, in which non-humic-like fluorophores have been previously reported (Coble 1996; Coble et al. 1998; Yamashita and Tanoue 2003). Two protein-like fluorophores (i.e., tyrosine- and tryptophan-like fluorophores) have been identified by EEM (Coble 1996; Mayer et al. 1999; Yamashita and Tanoue 2003). Three aromatic amino acids (i.e., tyrosine, tryptophan, and phenylalanine) emit ultraviolet fluorescence, and the excitation and emission spectra of tyrosine and phenylalanine are similar (Lakowicz 1999). The quantum yields of tyrosine are one order of magnitude higher than that of phenylalanine (Lakowicz 1999), while levels of tyrosine and phenylalanine in seawater have been reported on the same order of magnitude (Dittmar et al. 2001; Yamashita and Tanoue 2003). Therefore, the two protein-like fluorophores observed in these EEMs have been assigned as tyrosine- and tryptophan-like fluorophores, and not phenylalanine-like fluorophore (Yamashita and Tanoue 2003). Similarly, previous EEM-PARAFAC studies have identified two protein-like components as tyrosine- and tryptophan-like fluorophores (Cory and McKnight 2005; Stedmon and Markager 2005a,b).

In the present study, component 7 exhibited a peak at 270-nm excitation and 299-nm emission (Table 1) and was

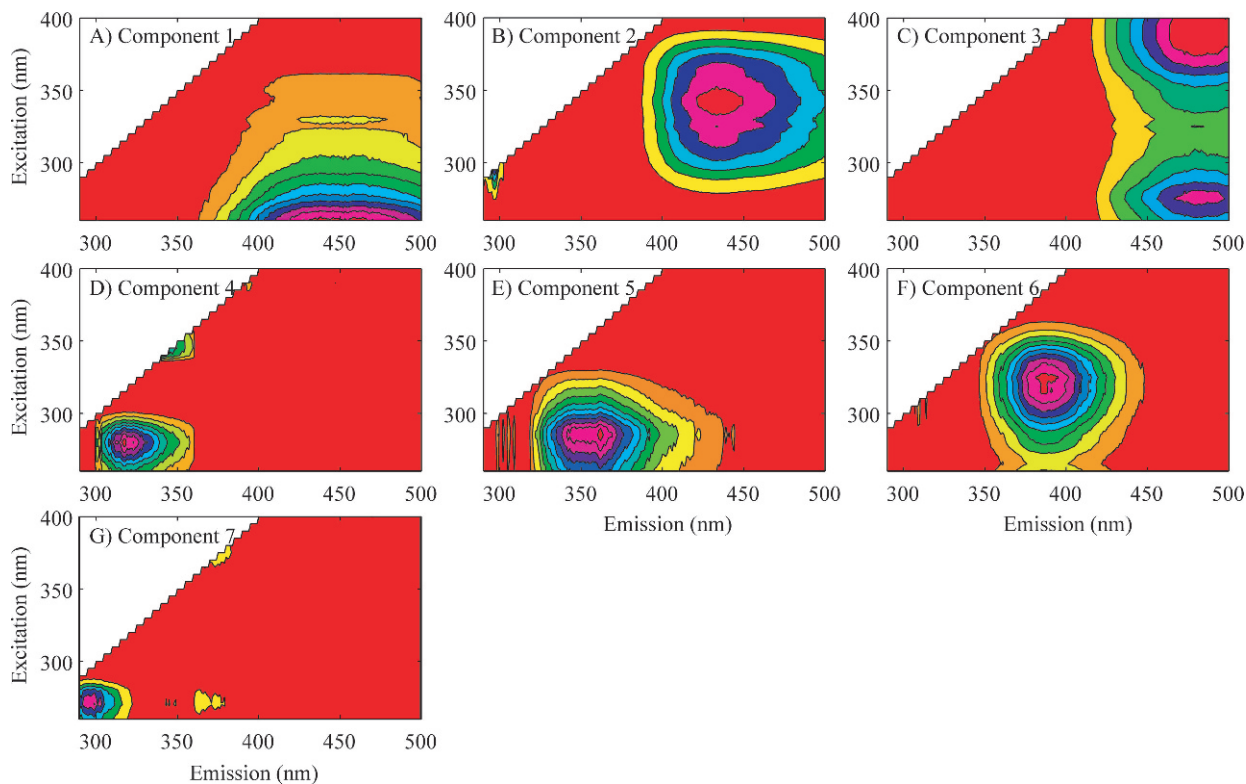


Fig. 2. The EEM contours of the seven fluorescent components identified by PARAFAC.

similar to the tyrosine-like fluorophore (peak B; Coble 1996; Coble et al. 1998) as well as to authentic tyrosine in aqueous solution (Lakowicz 1999; Yamashita and Tanoue 2003). Fluorescence characteristics of component 4, with a peak at 280-nm excitation and 318-nm emission, seem to correspond to the tryptophan-like fluorophore (peak T; Coble 1996; Coble et al. 1998), even though the emission maxima was blue-shifted from authentic tryptophan in aqueous solution (Lakowicz 1999; Yamashita and Tanoue 2003). Tryptophan-like fluorophores shifted to shorter emission wavelength were also found concurrently with tyrosine-like fluorophores in the ultrafiltered DOM of the Florida coastal Everglades (Maie et al. 2006). In addition to aromatic amino acid residues in DOM, polyphenols such as gallic acids and tannins exhibited such fluorescence characteristics (Maie et al. 2007). The fluorescence characteristics of component 4 were also similar to those of tannins, showing a peak at excitation/emission of 275/313 nm (Maie et al. 2007, 2008).

Lastly, the fluorescence characteristics of the third non-humic-like component (component 5; excitation/emission = 285/362 nm; Table 1) was very similar to peak N reported by Coble et al. (1998), but could also result from a combination of peak N and the tryptophan-like fluorophore T (Coble et al. 1998). An almost identical component to component 5 was also identified in DOM from a Danish estuary and its catchment (Stedmon et al. 2003).

Spatial distribution of fluorescent components in the bay—
The spatial distributions of salinity and individual fluores-

cent components along a transect from the head to the mouth of Ise Bay are shown in Fig. 3. A low-salinity water lens spread over the upper part (5 m) of the water column from the head to the mouth of Ise Bay was observed. The distributional patterns of two humic-like components, components 1 and 2, were similar. The levels of fluorescence intensity of these components were high at the head of bay and decreased toward the mouth of bay and were high in the surface water but decreased with depth. There was no evidence in the profile for sources from sediment pore waters, since levels of fluorophores were generally lowest in the bottom of the water column, with the possible exception of the sampling location at 34.58°N, where some contribution of sediment pore water seems possible. The relative abundances of humic-like and non-humic-like components with respect to the total components in pore water were basically larger and smaller, respectively, compared to those in seawater. The distributional patterns of components 3 and 6 were also similar to those of the aforementioned components (Fig. 3), although the higher abundance of component 6 in surface waters stretched out further into the estuary than did component 3. Considering that component 6 is presumably of autochthonous, marine origin, this pattern is not unexpected.

The distributional patterns of the non-humic-like components 4 and 5 were similar to those of humic-like components 3 and 6 (Fig. 3), with relatively high concentrations in surface waters extending far into the estuary, particularly for component 4. The highest concentrations of component 4 were observed at the middle salinity range, as discussed below, in which high levels of chlorophyll *a*

Table 1. Characteristics of the seven components identified in the present study compared with those previously identified.

Component	Excitation maximum (nm)	Emission maximum (nm)	Coble et al. (1998)	Stedmon and Markager (2005b)*	Cory and McKnight (2005)	Tentative source assignment and description of dynamics in the bay area (in this study)
1	<260	458	A‡	1 (Ter)	Q2	Humic-like component Terrestrial origin
2	345	433	C	4 (ubiquitous)	C1	Humic-like component Terrestrial origin
3	390 (275)	479	—	2 (ubiquitous)	SQ1	Humic-like component Terrestrial origin, biogeochemical processing of terrestrial POM
4	280	318	T	7 (Trp-like)	Trp-like†	Tryptophan-like component Blue-shifted from authentic tryptophan; autochthonous, biologically labile component
5	285	362	T or N	—	—	Non-humic-like component Peak N assigned by Coble et al. (1998) or combination of peak N and tryptophan-like fluorophore. Autochthonous, biologically labile component
6	325 (<260)	385	M	6 (Ant)	C3‡	Marine humic-like component Biological and/or microbial origin
7	270	299	B	8 (Tyr-like)	Tyr-like	Tyrosine-like component Degradation processes may be important for dynamics

POM, particulate organic matter.

* Ter and Ant represent origin of terrestrial and anthropogenic, respectively.

† Were identified in the Antarctic data set only (Cory and McKnight 2005), indicating microbial origin.

‡ Component names as defined in Coble et al. (1998).

were evident (data not shown). This distribution indicates that components 4 and 5 are most likely produced as a result of high biological production in the upper part of the water column in the bay area. In contrast, component 7 did not show any consistent horizontal or vertical changes in the bay area (Fig. 3). Such distributional characteristics indicated that the major fraction of component 7 did not originate from riverine inputs but also was not derived from the high biological production zone in the bay area. It is important to notice that component 7 is also the least abundant of all the identified DOM components from Ise Bay.

Changes in levels of fluorescent components with salinity—Figure 4 shows the relationships between salinity and fluorescence intensity of individual fluorescent components in Ise Bay. The relationships were focused on Ise Bay because the geophysical and biological conditions of Mikawa Bay differ from those of Ise Bay (Yamashita and Tanoue 2004). Since ~85% of freshwater inflow to Ise Bay is derived from the Ibi, Kiso, and Nagara Rivers located at the head of Ise Bay (Fujiwara et al. 1996), terrestrial derived fluorescent components in Ise Bay were evaluated based on the relationships between salinity and fluorescence intensity.

Judging from the relationships between salinity and levels of individual fluorescent components, dynamics of fluorescent components were divided into the following four types.

Type I: The fluorescence intensity of components 1 and 2 linearly decreased along the salinity gradient (Fig. 4A,B), even though one sample, obtained at the station nearest the head of the bay (salinity of 26.7 in Fig. 4), deviated from this linear relationship and thus was excluded for the calculations shown below. The strong linear relationships between salinity and component 1 ([fluorescence intensity] = $-0.32[\text{salinity}] + 12.7$; $R^2 = 0.91$, $n = 39$, $p < 0.001$) and component 2 ([fluorescence intensity] = $-0.12[\text{salinity}] + 5.02$; $R^2 = 0.92$, $n = 39$, $p < 0.001$) indicated conservative mixing of riverine components 1 and 2 in this estuarine environment.

Type II: The behavior of the other two humic-like components (i.e., components 3 and 6; Table 1) with salinity were quite different compared to the Type I components (Fig. 4C,F). High and low fluorescence levels of these two components were usually found at low- and high-salinity sites, respectively, which indicates that substantial amounts of these components in the bay area would be derived from riverine inputs. However, the relationships between salinity and these components could not be explained by conservative mixing. Most data points of Type II components in the bay area would deviate toward high fluorescence intensities (i.e., above the conservative mixing line), as compared to the Type I components (Fig. 4). Such deviation above the conservative mixing line indicated that these components have sources within the bay area in addition to riverine inputs or are being generated through biogeochemical processing of

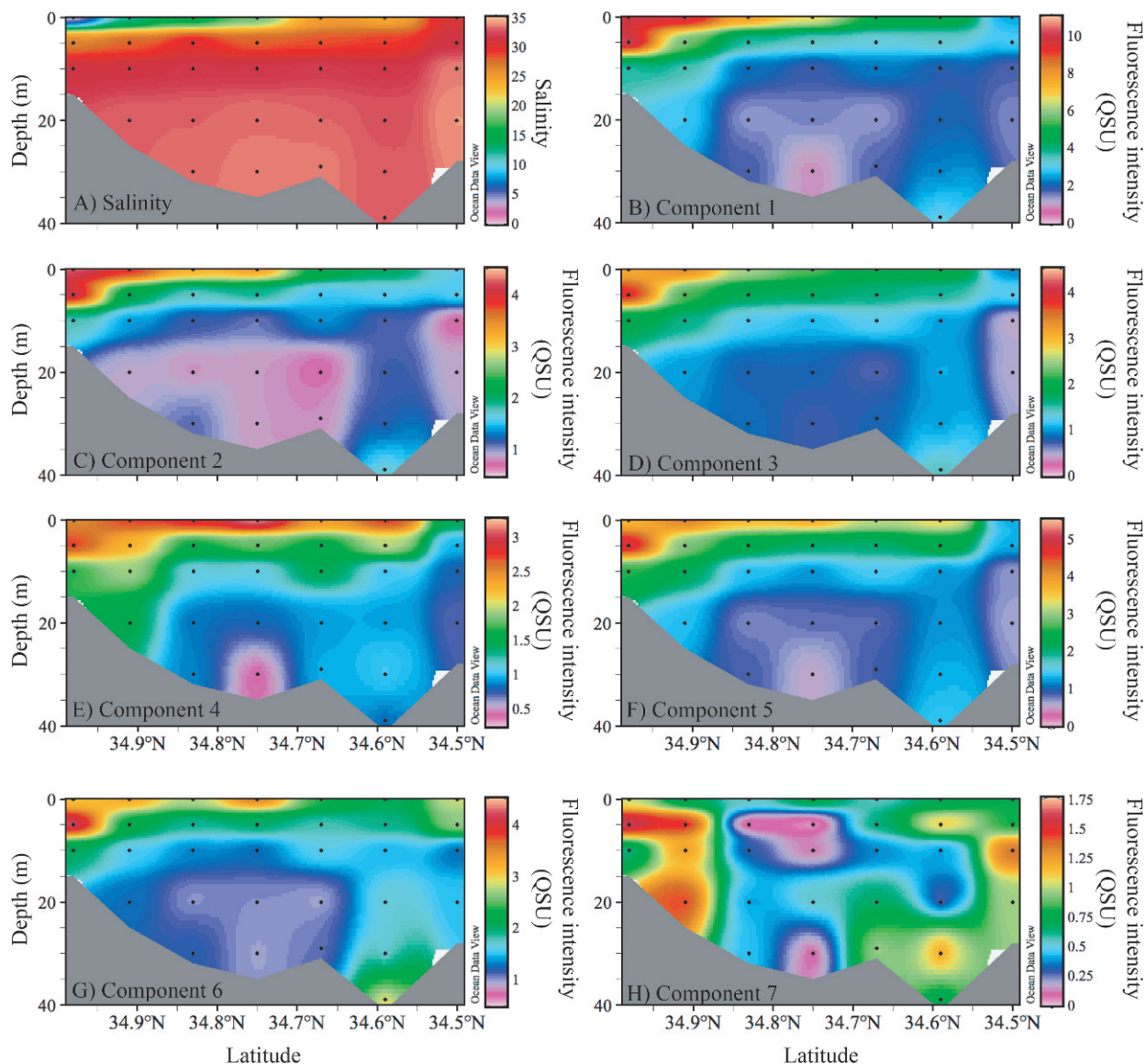


Fig. 3. Distributions of salinity and seven fluorescent components along a transect in Ise Bay. The scales of the color bar were different among fluorescent components. Distributional patterns were performed with Ocean Data View (Schlitzer 2004).

terrestrial organic matter during transport across the Ise Bay.

Photobleaching has been reported as a dominant removal process of terrestrial fluorescent DOM (Blough and Del Vecchio 2002), and nonconservative behavior of terrestrial fluorescent DOM as a result of photobleaching has been observed in coastal environments (Vodacek et al. 1997). In the present study, the relationships between salinity and Type I humic-like components showed that none presented appreciable deviations below the conservative mixing line, but in contrast, Type II humic-like components deviated above the conservative mixing line (Fig. 4). This indicates that if terrestrial component 3 is at least in part derived from photo- or biodegradation of components 1 and/or 2, the reactivity of the latter is rather low. Quantitatively, however, the abundance of component 1 plus component 2 is about four times that of component

3, indicating that even a limited, in-estuary processing of components 1 and 2 could contribute to the production of component 3 in the mid-estuary, where both light penetration and microbial activity are expected to be higher than in the river. This generation of component 3 seemingly occurs without affecting notably the overall concentration of the parent components.

Alternatively, the relatively high abundance of component 3 found in the middle estuarine region (Figs. 3D, 4C) indicates that it may in part be derived from the dissolution of terrestrial particulate organic matter (POM). According to Sugimoto et al. (2006), high inputs of terrestrial POM to the bay area during times of high discharge have been observed in Ise Bay, and terrestrial POM loaded into the bottom layer can be pushed out into the bay by the enhanced estuarine circulation and uplifted to the middle depth layer. Dissolution of such POM may thus contribute

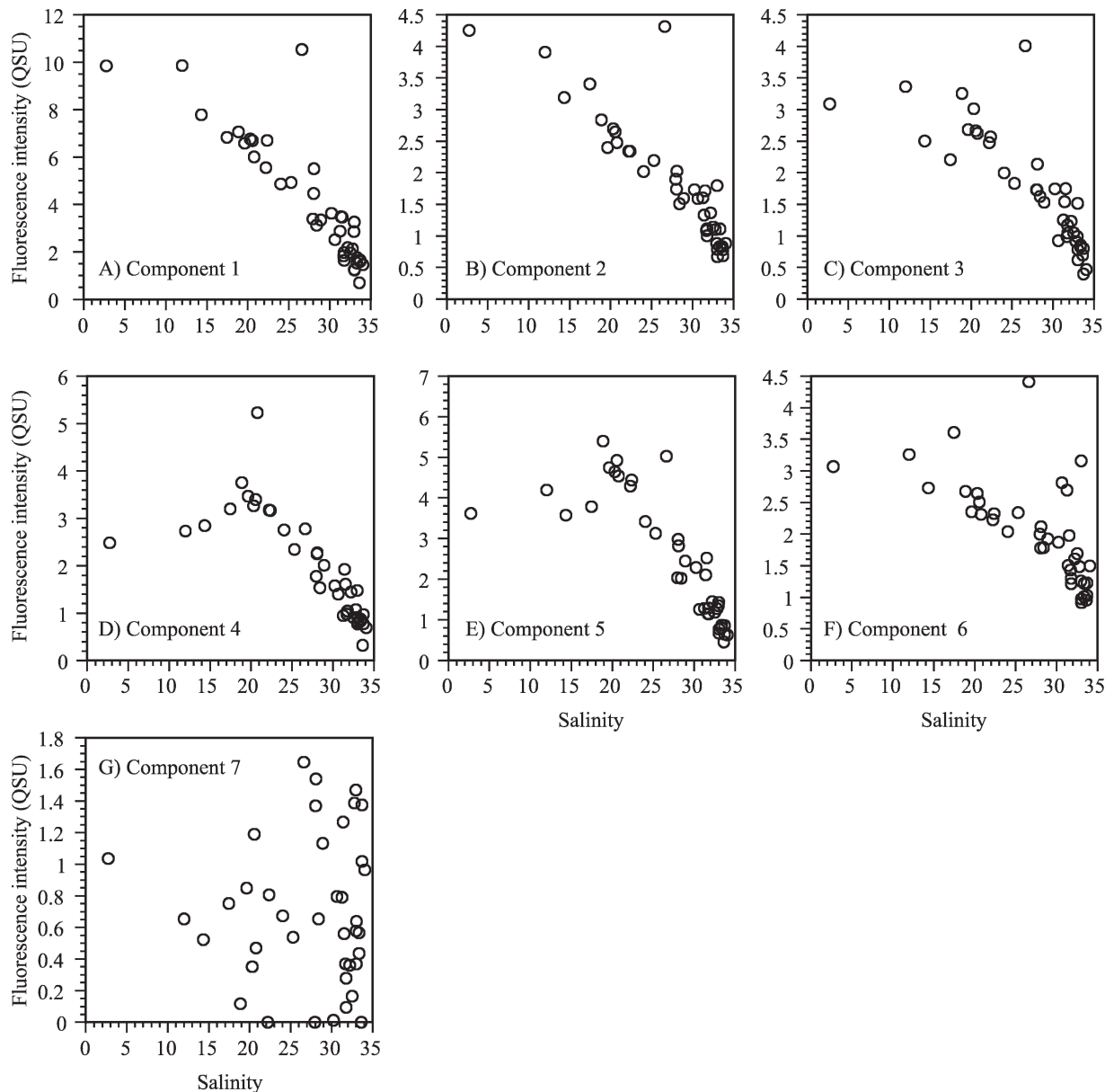


Fig. 4. Plots between salinity and fluorescence intensity of seven fluorescent components in Ise Bay.

to the mid-estuarine production of component 3. The high relative abundance of component 3 with respect to the total fluorescence intensity of humic-like components (components 1, 2, and 3) found in the pore water of surface sediments (data not shown), in which photodegradation should be insignificant, indicates that the more likely source of component 3 in the bay area seems to be dissolution of terrestrial POM or microbial alteration of terrestrial components rather than photoalteration of terrestrial components.

In contrast to component 3, the nonconservative distribution of component 6 would more likely be the result of microbially derived DOM processing, or it may result from direct biological inputs, as this component clearly seems to be of autochthonous nature (Coble et al. 1998). Rochelle-Newall and Fisher (2002) reported that

phytoplankton communities are not a direct source of humic-like fluorophores but that bacteria can produce humic-like fluorophores using nonfluorescent organic matter derived from phytoplankton. Away from river-dominated coastal environments, the production of humic-like fluorophores during microbial oxidation processes has been suggested based on observed relationships between levels of fluorescence intensity and nutrients as well as apparent oxygen utilization in the ocean interior (Chen and Bada 1992; Yamashita et al. 2007). As additional support for a microbial source, the fluorescence characteristics of component 6 were similar to those of C3, assigned by Cory and McKnight (2005) as having a microbial origin.

Type III: The behaviors of two of the non-humic-like components (i.e., components 4 and 5) with changes in

salinity were similar to each other (Fig. 3). Fluorescence levels of these two components were relatively high at the low-salinity range and gradually increased along the salinity gradient to reach a maximum at the middle salinity range (salinity < 20). From there on, they sharply decreased with increasing salinity. Like the Type II humic-like components, these distributional patterns indicate a combination of the riverine and estuarine sources of these components in the bay area. Their mid-salinity maximum indicates a planktonic source in the estuary, with highest productivity in the mid-estuary and dilution or degradation thereafter.

From the fluorescence characteristics (Table 1; Fig. 2), component 5 seems to be similar to peak N or a combination of the peak N and tryptophan-like fluorophore, both of which have been considered to be a labile component produced as a result of biological production in marine environments (Coble et al. 1998; Yamashita and Tanoue 2003). Thus, distributional patterns (Figs. 3, 4) and fluorescence characteristics (Table 1; Fig. 2) of component 5 collectively indicated that it was freshly produced and thus may be biologically labile.

Since the fluorescence characteristics of component 4 were similar to those of tannins (Maie et al. 2007, 2008) as well as to a blue-shifted tryptophan-like fluorophore (Maie et al. 2006), it is difficult to determine the precise origin of component 4 based only on the fluorescence characteristics. However, the relationship with salinity as well as the transect distribution of component 4 were almost identical to those of component 5 (Figs. 3, 4), indicating that the source of component 4 may be associated with biological production and not with terrestrial polyphenols, such as tannins. Thus, component 4 could be considered a blue-shifted tryptophan-like fluorophore produced in the water column by biological activity.

Type IV: No systematic changes in fluorescence intensity of component 7 were observed along the salinity gradient, and both high and low abundances were observed at salinity values above 26 (Fig. 4). The tyrosine-like fluorophores have been considered to be derived from tyrosine residues in low-molecular-weight DOM, which might be highly biodegraded and biorefractory (Yamashita and Tanoue 2003). The tryptophan-like components (i.e., components 4 and 5) were assigned to be of autochthonous origin and may be biologically labile components, indicating that component 7 might be a remnant of the degradation of autochthonously produced DOM.

In summary, three terrestrial humic-like components, one marine humic-like component, and three non-humic-like components were identified by EEM-PARAFAC, and their dynamics were evaluated based on distributional patterns as well as relationships with salinity in the bay area. The origin and basic dynamics of the seven components evaluated in the present study were summarized in Table 1. The distributions of the three terrestrial humic-like components were strongly controlled by riverine inputs. Components 1 and 2 behaved conservatively with changes in salinity and were assigned to a terrestrial origin,

while component 3, also of terrestrial origin, was suggested to increase in abundance along the estuary through biogeochemical processing of terrestrial POM. The autochthonous character of the marine humic-like component 6 was distinguished from that of the other three humic-like components and is possibly derived from estuarine biological activity and/or microbial processing of such biologically produced DOM. The three non-humic-like components and the marine humic-like component were suggested to have an autochthonous origin. Components 4 and 5 are potentially biologically labile components, and their degradation may be a determining factor in the generation of component 7 in the bay area. The present study shows that the terrestrial and marine origin of humic-like components can be distinguished by EEM-PARAFAC, even in coastal environments where terrestrial humic substances are dominant. Thus, the present study proves that the application of EEM-PARAFAC is an important tool for better evaluating the dynamics of allochthonous and autochthonous DOM in complex coastal environments.

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