Fluorescence characteristics of dissolved organic matter in the deep waters of the Okhotsk Sea and the northwestern North Pacific Ocean

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Abstract

Fluorescent dissolved organic matter (DOM), a fraction of chromophoric DOM, has been known to be produced in the deep ocean and has been considered to be bio-refractory. However, the factors controlling fluorescence properties of DOM in the deep ocean are still not well understood. In this study, we determined the fluorescence properties of DOM in the deep waters of the Okhotsk Sea and the northwestern North Pacific Ocean using excitation-emission matrix (EEM) fluorescence and parallel factor analysis (PARAFAC). One protein-like, two humic-like components, and one uncertain component, that might be derived from a fluorometer artifact, were identified by EEM-PARAFAC. Fluorescence intensity levels of the protein-like component were highest in the surface waters, decreased with depth, but did not change systematically in the bathypelagic layer (1000 m - bottom). Fluorescence characteristics of the two humic-like components were similar to those traditionally defined as marine and terrestrial humic-like fluorophores, respectively. The fluorescence intensity levels of the two humic-like components were lowest in the surface waters, increased with depth in the mesopelagic layer (200 - 1000 m), and then slightly decreased with depth in the bathypelagic layer. The ratio of the two humic-like components remained in a relatively narrow range in the bathypelagic layer compared to that in the surface layer, suggesting a similar composition of humic-like fluorophores in this layer. In addition, the fluorescence intensities of the two humic-like components were linearly correlated to apparent oxygen utilization (AOU) in the bathypelagic layer, suggesting that both humic-like components are produced *in situ* as organic matter is oxidized biologically. These findings imply that optical characteristics of humic-like fluorophores once formed might not be altered

further biologically or geochemically in the deep ocean. On the other hand, relationships of fluorescence intensities with AOU and Fe(III) solubility were different between the two humic-like components in the mesopelagic layer, suggesting different environmental dynamics and biogeochemical roles for the two humic-like components.

Keywords; Dissolved organic matter (DOM), Fluorescence, Excitation-emission matrix (EEM) fluorescence, parallel factor analysis (PARAFAC), Biological production, Deep water, Okhotsk Sea, northwestern North Pacific

1. Introduction

Dissolved organic matter (DOM) in the ocean constitutes one of the largest reduced carbon pools in the global carbon cycle (700 PgC; Hedges and Keil, 1995). Concentrations of dissolved organic carbon (DOC) are usually highest in the surface layer, decrease sharply with depth, and then do not change significantly in the mesopelagic (200 - 1000 m), bathypelagic (1000 – 4000 m), and abyssopelagic (4000 m - bottom) layers (Hansell, 2002). In terms of volume, however, the deep ocean occupies a major part of the marine system, and thus, the majority of DOM (~650 PgC) occurs in the deep ocean (Ogawa and Tanoue, 2003). It is therefore clearly important to better understand the composition and environmental dynamics of DOM in the deep sea.

In the deep ocean, DOC concentrations have been reported to slightly decrease along with deep ocean circulation, i.e., with increasing water mass age, indicating the removal of DOM in this layer (Hansell and Carlson, 1998). Radiocarbon measurements in bulk DOC showed "old" average apparent age (4000 - 6000 yrs; Williams and Druffel, 1987), however, "young" radiocarbon ages of neutral sugars in high molecular weight (HMW)-DOM were also reported, suggesting that an important fraction of deep sea DOM may be introduced by dissolution from large, rapidly sinking particles (Repeta and Aluwihare, 2006). In terms of quality, nuclear magnetic resonance analysis of HMW-DOM clarified that major chemical forms of HMW-DOM are similar to those of freshly produced organic matter (Benner et al., 1992; McCarthy et al., 1997; Aluwihare et al., 2002). However, contributions of humic substances, e.g., aromaticity in HMW-DOM, were greater in the deep HMW-DOM compared to the surface HMW-DOM (Benner et al., 1992; McCarthy et al., 1997; Aluwihare et al., 2002).

Chromophoric DOM (CDOM), the optically active fraction of DOM, occurs ubiquitously in the ocean and aquatic environments in general, and can be separated into two major classes, i.e., humic-like components and biogenic components such as protein-like fluorophores (Coble, 1996; Yamashita and Tanoue, 2003; 2009; Maie et al., 2006, 2007, 2008). Vertical profiles in the ocean of biogenic components in CDOM showed a pattern similar to those of DOC (Mopper and Schultz, 1993; Yamashita and Tanoue, 2009). Humic-like components in CDOM have been considered as a fraction of molecularly-uncharacterized components or humic-like substances (Hedges et al., 2000). Levels of humic-like components in CDOM, determined by absorption coefficients and fluorescence intensity, have been found to be lowest in surface waters, a trend commonly attributed to photodegradation. The components increase with depth in the mesopelagic, but show little or no gradient at greater depths (Hayase et al., 1988; Chen and Bada, 1992; Mopper and Schultz, 1993; Hayase and Shinozuka, 1995; Nelson et al., 2007; Yamashita et al., 2007; Yamashita and Tanoue, 2008; 2009). Thus, the contribution of humic-like components in CDOM to bulk DOM is larger in deep waters compared to surface waters. This view is also consistent with the differences in chemical forms of HMW-DOM between surface and deep waters.

Such vertical distribution profiles of humic-like components in CDOM suggest that the biogeochemical roles of CDOM in deep waters can be more important than those in surface waters. For example, vertical profiles of the humic-like fluorescence intensity were related to those of Fe(III) solubility in the mesopelagic and bathypelagic layers but not in the surface layer, suggesting that humic-like components in CDOM are major factor in controlling the Fe(III) solubility and the dissolved Fe concentration in the deep

ocean through their complexation with Fe(III) as natural organic ligands (Tani et al., 2003; Takata et al., 2004; 2005; Kitayama et al., 2009).

Recently, the basin scale distribution of the humic-like fluorescence intensity was examined for the Pacific Ocean (Yamashita and Tanoue, 2008), finding a continuous increase in fluorescence intensity in the bathypelagic and abyssopelagic layers from the Southern Ocean to the northern North Pacific, while a decrease in DOC concentration was evident (Hansell and Carlson, 1998). A strong linear relationship between the fluorescence intensity and apparent oxygen utilization (AOU) was also reported in these layers for the Pacific (Yamashita and Tanoue, 2008). Since AOU increases with oxygen consumption during organic matter oxidation processes, it is apparent that bio-refractory humic-like components in CDOM are produced *in situ* as organic matter is oxidized biologically. In addition, Yamashita and Tanoue (2008) reported variations of AOU-fluorescence intensity relationships between the bathypelagic (+ abyssopelagic) and mesopelagic layers and suggested that the cause leading to these different relationships was the distribution of different water masses having different pre-formed CDOM levels between these layers.

Previous studies assumed that fluorescence properties were not significantly different across water depths and oceanic regions, and therefore, the levels of humic-like components in CDOM were determined by the fluorescence intensity of single excitation-emission pairs (e.g., 320 nm excitation and 420 nm emission) (Hayase et al., 1988; Chen and Bada, 1992; Hayase and Shinozuka, 1995; Yamashita et al., 2007; Yamashita and Tanoue, 2008). Thus, it is still unclear whether optically similar DOM is produced *in situ*

and accumulated in the deep ocean, or if fluorescence characteristics change with increasing AOU.

In the present study, CDOM in surface and deep waters of the Okhotsk Sea and the northwestern North Pacific Ocean were characterized by excitation-emission matrix (EEM) fluorescence with parallel factor analysis (PARAFAC). The PARAFAC methodology was successfully applied to decompose EEMs into a number of fluorescence components (Stedmon et al., 2003; Stedmon and Markager, 2005a; Cory and McKnight, 2005; Murphy et al., 2008; Jaffé et al., 2008; Yamashita et al., 2008). The main objective of the present study was to examine the dynamics of the CDOM components obtained through EEM-PARAFAC. Special attention was paid to clarify the similarity/dissimilarity of fluorescence characteristics for the deep ocean, an area ideally suited to study the effects on DOM composition by microbial activity in the absence of photochemical interferences.

2. Materials and methods

Seawater samples were collected from surface to bottom layers at 4 stations in the Okhotsk Sea and northwestern North Pacific Ocean (Fig. 1) during May and June 2000. In this paper, we defined depths from 200 to 1000 m and from 1000 m to the bottom as the mesopelagic and bathypelagic layers, respectively. Sampling and filtration procedure were descried in detail elsewhere (Tani et al., 2003). Samples were filtered through 0.22 μm filter and kept frozen (< -20 ˚C) on board. In the laboratory, frozen samples were thawed and used for measurements of Fe(III) solubility and fluorescence intensity at 320 nm excitation and 420 nm emission (Tani et al., 2003). Aliquots for EEM measurements

were taken in low density polyethylene tubes and then re-frozen. These tubes were pre-cleaned using the same procedure as used for trace metal sampling (Bruland et al., 1979). The effects of freezing on EEMs of oceanic DOM are not clear. However, using samples from central North Pacific surface and deep layers, we determined differences in humic-like fluorescence intensity at 320 nm excitation and 420 nm emission before and after freezing, finding that differences were -0.1±1.7% (n = 9) relative to fresh samples analyzed prior to any freezing or thawing cycles. The freezing effects on protein-like fluorophores found in marine DOM samples are also unknown. However, a recent study on the effects of freezing and thawing on DOM fluorescence documented up to ±50% change in fluorescence intensity for the humic and protein peaks in EEMs of riverine DOM that had been filtered prior to analysis with 1.2 μm GF/C filters (Spencer et al. 2007). It is likely that in the latter study, some of the changes in fluorescence upon freezing may be attributed to rupture of microbial cells or particulate origin, which would not be expected in our study where 0.22 μm filters were used for sample preparation.

The results of bulk fluorescence intensity at 320 nm excitation and 420 nm emission, Fe(III) solubility, temperature, salinity, sigma-theta, nutrients, and AOU at 4 stations can be found elsewhere (Tani et al., 2003).

Just before the EEM measurement, samples were thawed and allowed to stand until reaching near room temperature. The measurements of EEM were carried out using a fluorescence spectrometer (Hitachi F-4500) according to Yamashita and Tanoue (2003). The specific instrumental components were corrected for excitation and emission according to protocols recommended by the manufacturer. 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. Several post acquisition steps were involved in the correction of EEMs. First, the EEM of Milli-Q water, which was freshly produced and measured every day, was subtracted from the EEM of each sample. Second, fluorescence intensities were corrected to the area under the water Raman peak (excitation = 350 nm), analyzed daily, and then were converted to quinine sulfate unit (QSU) using a calibration with quinine sulfate monohydrate in a solution of 0.05 mol L^{-1} H_2SO_4. Inner filter corrections were not applied for these samples because of the extremely low absorbance for open ocean samples (Nelson et al., 2007; Yamashita and Tanoue, 2009).

PARAFAC modeling for EEM data has been described in detail elsewhere (Stedmon et al., 2003; Stedmon and Bro, 2008). The modeling was carried out in MATLAB with the DOMFluor toolbox (Stedmon and Bro, 2008) using 80 EEMs obtained from surface to deep waters. The EEM wavelength ranges used were 260-400 nm and 300-500 nm for excitation and emission, respectively. The determination of the correct number of components was primarily achieved by the split half analysis and random initialization (Stedmon and Bro, 2008). In this study, the fluorescence intensities of each PARAFAC component were reported as QSU.

3. Results and discussion

3.1. EEM fluorescence of DOM in the deep ocean

Typical EEM spectra obtained from surface and deep waters are presented in Fig. 2. Both EEMs showed high levels of fluorescence intensity at around 275 nm excitation and 305 nm emission. This peak corresponds to the tyrosine-like fluorophore or peak B

(Coble, 1996; Yamashita and Tanoue, 2003) and showed higher fluorescence intensity in surface waters compared to deep waters. While a detectable fluorescence signal was found for tyrosine-like fluorophores, tryptophan-like fluorophore were not observed in samples from this study region. Such fluorescence characteristics of protein-like fluorophores have been previously identified in the oceanic surface and deep waters (Mopper and Schultz, 1993; Yamashita and Tanoue, 2003; 2004a).

Both, surface and deep water EEMs also showed a high fluorescence intensity at < 260 nm excitation and 400 - 500 nm emission, and the fluorescence intensity of this peak was higher in deep waters compared to surface waters (Fig. 2). This peak corresponds to the humic-like peak A (Coble, 1996) and has been reported to be present from surface to deep waters in the open ocean (Mopper and Schultz, 1993; Coble, 1996). Low levels of this peak in the surface water compared to deep water were also found in the Sargasso Sea (Mopper and Schutlz, 1993). In addition to peak A, the region at 290-350 nm excitation and 380-400 nm emission presented another peak in both surface and deep waters (Fig. 2). According to Coble (1996), this peak corresponds to the marine humic-like peak M and/or humic-like peak C. This peak was red-shifted in the deep water compared with that in the surface water (Fig. 2). Even though only a few studies applied EEMs to deep ocean DOM (Mopper and Schultz, 1993; Coble, 1996), similar red-shift, i.e., dominance of marine humic-like fluorophore (peak M) in the surface water and the replacement to humic-like fluorophore (peak C) with depth, have been suggested (Coble, 1996).

3.2. PARAFAC components

EEMs showed differences in fluorescence characteristics of DOM between the surface and deep waters (Fig. 2), however, it is hard to evaluate such differences applying the peak picking technique. PARAFAC was, therefore, applied for quantitative evaluation of differences in EEMs. A four component PARAFAC model was validated and selected as most suitable for this dataset (Fig. 3). Spectral characteristics of components identified in the region studied were similar to those previously found in other aquatic environments (Table 1). The selection of the four component model does not mean that the EEMs only contained four different fluorophores, but that these selected four components were the most representative fluorescent groups in this dataset.

Component 1 was comprised of two excitation maxima (< 260 nm and 370 nm) with an emission maximum at 466 nm and was categorized as mixture of the traditional humic-like peaks A and C which were usually representative fluorophores in terrestrial environments and low salinity waters in coastal environments (Coble, 1996; Conmy et al., 2004). The spectral features were also similar to a terrestrial humic-like component found in the surface water from coastal to oceanic regions (C3/P3; Murphy et al., 2008). On the other hand, this component is also similar to a component reported as ubiquitous in coastal regions (4; Stedmon and Markager, 2005a). A similar component was also identified in an Antarctic lake, which lacks a higher plant contribution from its watershed (Cory and McKnight, 2005). Thus, component 1 showed similar spectral characteristics with terrestrial (higher plant derived) fluorophores and fluorophores related to microbial reworking of organic matter.

There was an intensive peak at 325 nm excitation and 385 nm emission for component 2. This peak was similar to the marine humic-like peak M traditionally defined (Coble,

1996) and was similar to marine humic-like components found in the PARAFAC model from coastal environments (C6; Yamashita et al., 2008) as well as surface waters from coastal to oceanic regions (Murphy et al., 2008). Again, a similar component was also identified in an Antarctic lake (C3; Cory and McKnight, 2005), and was found to be prominent in wastewater samples (6; Stedmon and Markager, 2005a), thus providing evidence that this component is associated with recent biological activity. Therefore, this component could be characterized as representing a group of humic-like fluorophores strongly associated with recent biological activity.

Component 3 did not appear as a peak in EEM and has not been commonly reported in previous PARAFAC studies (Cory and McKnight, 2005; Stedmon and Markager, 2005a; Stedmon et al., 2007; Yamashita et al., 2008; Yamashita and Jaffé, 2008). Only one study, using three fluorometers (i.e., SPEX FluoroLog-2, SPEX FluoroMax-2, and Varian Cary Eclipse), identified similar component by PARAFAC modeling for samples from surface waters in coastal to oceanic transects (P4; Murphy et al., 2008). This component has an uncertain origin, and is likely an analytical artifact of the fluorometer (Murphy et al., 2008). These results suggest that the peak A region can be affected by fluorometer artifacts and that caution is needed in the interpretation of EEMs for especially low CDOM level samples such as oceanic waters. Thus, based on the above, this component was not discussed further in this study.

Only one component was found in the region of protein-like fluorophores, in contrast to many previous studies that have identified up to four protein-like components (Cory and McKnight, 2005; Stedmon and Markager, 2005a; Murphy et al., 2008; Yamashita and Jaffé, 2008; Yamashita et al., 2008). Fluorescence characteristics of component 4

were almost identical to those of free tyrosine (Yamashita and Tanoue, 2003) and were similar to those of a tyrosine-like component found in previous PARAFAC studies (Cory and McKnight, 2005; Stedmon and Markager, 2005a; Murphy et al., 2008; Yamashita et al., 2008). The other common protein-like component, i.e., the tryptophan-like component, was not observed in this study. The dominance of tyrosine-like fluorophore in oceanic waters was also observed previously (Mopper and Schultz, 1993; Yamashita and Tanoue, 2003).

3.3. Vertical profiles of PARAFAC components

Levels of tyrosine-like component 4 were highest in the surface waters, decreased with depth, and then did not change systematically towards the bottom (Fig. 4). Similar vertical characteristics were also observed for protein-like fluorophores in EEMs at the Sargasso Sea (Mopper and Schultz, 1993) and the Sagami Bay, Japan (Yamashita and Tanoue, 2004a). Yamashita and Tanoue (2003) found the linear relationships between tyrosine-like fluorescence intensities and tyrosine concentrations as well as concentrations of total hydrolyzable amino acids from coastal to oceanic environments. Even though such relationships were not studied for the deep ocean, the vertical profiles of dissolved amino acids in the open ocean (Kaiser and Benner, 2008; 2009) were similar to those found for protein-like component 4 in this study. Such similarity of vertical characteristics between dissolved amino acids and tyrosine-like component 4 suggest that tyrosine-like component 4 is likely derived from tyrosine-containing molecules in the open ocean.

Vertical profiles of humic-like components 1 (corresponding to a mixture of the traditionally-defined peaks A and C, Table 1) and 2 (corresponding to the traditionally-defined peak M, Table 1) were similar to each other (Fig. 4). The lowest fluorescence intensity of humic-like components were found in the surface waters, followed by an increase with depth, maximizing at 600~1000 meters, and then showing a slight decrease with depth. In the mesopelagic layer, however, fluorescence intensity gradients for component 1 were greater than those for component 2. Vertical profiles of both humic-like components were almost identical to those of fluorescence intensity at 320 nm excitation and 420 nm emission at the same stations (Tani et al., 2003). The lowest levels of both humic-like components in the surface layer may have resulted from the degradation of humic-like fluorophores in the photic zone. This is most likely due to photodegradation since it is well known that, while humic-like fluorescence intensity remarkably decreases during photo-irradiation (Mopper et al., 1991; Chen and Bada, 1992; Nieto-Cid et al., 2006) it tends to increase during microbial degradation of organic matter (Yamashita and Tanoue, 2004; Nieto-Cid et al., 2006).

Ratios of humic-like component 1 to component 2, corresponding to the ratio of the traditionally-defined peak C/peak M (Table 1), were lowest (1.0~1.2) in the surface waters, increased with depth, and then remained in a relatively narrow range (1.4~1.5) in the bathypelagic layer (Fig. 4). Vertical characteristics indicated that humic-like components 1 and 2 were present at similar levels in the surface water, but humic-like component 1 was enriched compared to component 2 in the bathypelagic layer. The red-shift of the humic-like fluorophores in the deep water compared to the surface water (Fig. 2) could be explained by the abovementioned change in ratio, i.e., the change in humic-

like composition, with depth (Fig. 4). The shift of peak position from peak C (corresponding to component 1) to peak M (corresponding to component 2) was also observed during photodegdataion but not during biodegradation experiments using coastal waters (Moran et al., 2000). Thus, the observation of a low ratio for the surface waters in conjunction with the lowest fluorescence intensities of humic-like components suggest that component 1 may be more photo-reactive compared to component 2.

3.4. Fluorescence characteristics of in situ-produced CDOM

Strong evidence for the microbial production of humic-like CDOM during organic matter degradation processes has been provided by several authors (Chen and Bada, 1992; Hayase and Shinozuka, 1995; Nelson et al., 1998; 2007; Nieto-Cid, et al., 2005; Yamashita et al., 2007; Yamashita and Tanoue, 2008) and through *in vitro* experiments (Parlanti et al., 2000; Rochelle-Newall and Fisher, 2002; Nelson et al., 2004; Yamashtia and Tanoue, 2004b; Stedmon and Markager, 2005b; Nieto-Cid et al., 2006). Figure 5 shows relationships between AOU and fluorescence intensities of humic-like components 1 and 2 in the mesopelagic and bathypelagic layers. In the bathypelagic layer, the relatively simple water mass distribution, primarily supplied from Circumpolar Deep Water (Matsumoto, 2007), allowed for a better examination of relationship between AOU and fluorescence intensities (Yamashita and Tanoue, 2008). Therefore, in this study, *in situ*-produced humic-like fluorophores are discussed for the bathypelagic layer.

Fluorescence intensities of both humic-like components were linearly related to AOU in the bathypelagic layer (Fig. 5; [Comp. 1] = 0.0029 × [AOU] + 0.45, R^2 = 0.82, n = 16, $p < 0.001$; [Comp. 2] = 0.0018 × [AOU] + 0.36, R^2 = 0.69, n = 16, $p < 0.001$). The *in situ*

production of bio-refractory humic-like fluorophores were indicated from the linear relationship between AOU and fluorescence intensity at 320 nm excitation and 420 nm emission in the bathypelagic layer from the Southern Ocean to northern North Pacific where AOU ranged from 130 to 310 μmol kg^{-1} (Yamashita and Tanoue, 2008). The AOU range in the bathypelagic layer observed here (175 to 301 μmol kg^{-1}) was slightly narrower than that described in the Pacific Ocean (Yamashita and Tanoue, 2008), but still similar, suggesting that the relationships between AOU and fluorophores for the bathypelagic layers in the Okhotsk Sea and the northwestern North Pacific Ocean are comparable to that for the Pacific bathypelagic layer.

The red-shifted humic-like fluorophore (peak C) has been well known to be a terrestrial humic-like fluorophore, i.e., the dominant fluorophore in terrestrial environments (Coble, 1996). However, the linear relationships between AOU and humic-like components 1 and 2 in the bathypelagic layer suggest that both component 1 (corresponding to traditional peak C) and component 2 (corresponding to traditional peak M) are produced *in situ* in the deep ocean. PARAFAC components with fluorescence features similar to the traditional peak C were found from an Antarctic lake where no higher plant sources are evident (Table1; Cory and McKnight, 2005). The microbial production of all humic-like PARAFAC components including components corresponding to the traditionally-defined peak C, was reported for mesocosm experiments (Stedmon and Markager, 2005b). Results from such PARAFAC-based studies suggest that fluorophores showing similar fluorescence characteristics to component 1 can originate from either terrestrial or microbial sources of organic matter. It should be noted that the traditionally-defined peak C is often used as a tracer for

terrestrial CDOM in coastal environments (e.g., Chen et al., 2004; Conmy et al., 2004). Thus, further studies are necessary to clarify the dynamics of fluorophores corresponding to component 1 for the wide range aquatic environments.

In addition, ratios of humic-like component 1 to component 2 were in a relatively narrow range (Fig. 4) and were not linearly related to AOU in the bathypelagic layer ($[C1/C2] = 0.0004 \times [AOU] + 1.35$, $R^2 = 0.07$, $n = 16$, $p > 0.01$). Such results suggest that the relative proportion of *in situ*-produced fluorophores did not change with increase in AOU in the deep ocean. Similar optical properties of *in situ*-produced humic like CDOM were also suggested from the uniform distribution of the ratio of fluorescence intensity to absorption coefficient in the bathypelagic layer along a transect from the equatorial to northern North Pacific (Yamashita and Tanoue, 2009). Thus, similarities of optical characteristics of humic-like CDOM in the bathypelagic layer suggest that the composition of *in situ* produced humic-like CDOM may not be significantly altered biologically and geochemically.

3.5. Fluorescence characteristics and its environmental implications in the mesopelagic layer

Plots between AOU and component 1 in the mesopelagic layer had a similar linear relationship with that in the bathypelagic layer (Fig. 5). On the other hand, relationships between AOU and component 2 in the mesopelagic layer largely deviated from those in the bathypelagic layer (Fig. 5). Yamashita and Tanoue (2008) found different AOU-fluorescence relationships between the mesopelagic and bathypelagic layers in the Pacific Ocean and indicated that differences in levels of pre-formed fluorophores yield different

relationships. Thus, relationships between AOU and component 1 and 2 suggested that pre-formed levels of component 1 were similar between the mesopelagic and bathypelagic layers, but those of component 2 in the mesopelagic layer were high compared to the bathypelagic layer.

The water mass in the bathypelagic layer is supplied primarily from Circumpolar Deep Water (Matsumoto, 2007), but major water mass in the mesopelagic layer are derived from North Pacific Intermediate Water (NPIW) in the Oyashio region and the Okhotsk Sea Intermediate Water (OSIW) which contribute to the formation of the NPIW (Yasuda, 2004). The Amur River discharges into the Okhotsk Sea, and as a consequence, high levels of terrestrial biomarker as well as terrestrial CDOM were found in the NPIW for the central North Pacific (Hernes and Benner, 2002; Yamashita and Tanoue, 2009). Therefore, high levels of pre-formed component 2 in the mesopelagic layer might be the result from terrestrial environments, discharged through the Amur River, and thus adding to the fraction formed during OSIW and NPIW.

In general, however, the dominance of component 1 (corresponding to peak C; Table 1) compared to component 2 (corresponding to peak M; Table 1) would be expected for terrestrial environments, as mentioned above, but not for deep ocean waters. Such apparent contradiction might be explained by photodegradation of humic-like fluorophores. The higher photo-labile property of component 1 compared to component 2 was suggested by vertical profiles of their ratio (Fig. 4). The blue-shift of peak position was also found for humic-like fluorophores in estuarine DOMs during photodegradation (Moran et al., 2000), implying that peak C is more photo-reactive compared to peak M. Cory et al. (2007) also found that a PARAFAC component similar to component 1 was

more photodegradable than other PARAFAC components. Thus, the component 1 produced in the surface waters might be photodegraded readily, and thus, photodegradation processes might keep component 1 at low levels in the surface layer, irrespective of oceanic region differences. A fraction of component 1 might also be riverine derived and could be selectively photo-degraded compared to component 2 in the coastal margin of the Okhotsk Sea.

Different distributional patterns between components 1 and 2 in the mesopelagic layer (Fig. 5) suggested differences in the biogeochemical roles between the two humic-like components. Figure 6 shows the relationships between Fe(III) solubility and fluorescence intensity of components 1 and 2 in the mesopelagic layer. Linear relationships between Fe(III) solubility and humic-like fluorescence intensity were found in the deep ocean, suggesting that humic-like components in CDOM may be a major factor controlling the Fe(III) solubility and the dissolved Fe concentration in the deep ocean through the complexation of Fe(III) as natural organic ligands (Tani et al., 2003; Takata et al., 2004; 2005; Kitayama et al., 2009). Interestingly, Fe(III) solubility linearly correlated with levels of component 1 ($R^2 = 0.81$, $n = 20$, $p < 0.001$), but not with those of component 2 ($R^2 = 0.29$, $n = 20$, $p > 0.01$). Such differences in relationships implies that component 1 is likely to be important fluorophores as ligand for Fe(III) compared to component 2. Such differences in interactivity of trace metals with DOM fluorophores have previously been reported (Yamashita and Jaffé, 2008). In addition, extremely high concentrations of dissolved and particulate Fe in the OSIW and the NPIW compared to other water masses were found in the same region (Nishioka et al., 2007). These results suggest that component 1 may be an important contributor to Fe(III) solubility and, subsequently, may

regulate the high concentration of dissolved Fe in the mesopelagic layer of the North Pacific Ocean.

4. Conclusions

The *in situ* production of bio-refractory humic-like fluorophores was demonstrated based on linear relationship between humic-like fluorescence intensity and AOU as well as nutrient concentrations in the mesopelagic and bathypelagic layers (Hayase et al., 1988; Chen and Bada, 1992; Hayase and Shinozuka, 1995; Yamashita et al., 2007; Yamashita and Tanoue, 2008). To clarify the similarity/dissimilarity of humic-like fluorophores in the deep ocean, EEM-PARAFAC was for the first time applied to DOM in the deep ocean. PARAFAC statistically decomposed EEMs into one protein-like and two humic-like components at the Okhotsk Sea and the northwestern North Pacific Ocean.

The spectral characteristics of one of the humic-like components were similar to the microbial/marine humic-like fluorophore (peak M), while those of the other were similar to the terrestrial humic-like fluorophore (peak C) as traditionally assigned. The vertical profiles of the two humic-like components were similar and showed lowest levels in the surface waters, peaked in the mesopelagic layer, and decreased slightly with depth in the bathypelagic layer. Levels of both humic-like components were linearly correlated to AOU in the bathypelagic layer, implying that both humic-like components are *in situ* produced during microbial degradation processes of organic matter. In addition, the ratio of the two humic-like components remained in a relatively narrow range and was not related to AOU variations in the bathypelagic layer, suggesting that *in situ* produced

humic-like fluorophores once formed might not be altered biologically or geochemically along this layer.

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Figure captions

Figure 1. Sampling locations in the Okhotsk Sea (Stns A and B) and the northwestern North Pacific Ocean (Stns C and D).

Figure 2. Examples of EEMs from the surface water (20 m at Stn A) and the deep water (1500 m at Stn C).

Figure 3. Spectral characteristics and validation of four component model by PARAFAC. The grey lines in right columns show the results of split half validation.

Figure 4. Vertical profiles of two humic-like components (components 1 and 2), one protein-like component (component 4), and ratio of two humic-like components in the Okhotsk Sea (Stns A and B) and the northwestern North Pacific Ocean (Stns C and D). The mesopelagic layer (200 -1000 m) was shaded.

Figure 5. Relationships between AOU and humic-like components 1 and 2 in the mesopelagic and bathypelagic layers. Solid lines indicate linear relationships between AOU and humic-like components 1 and 2 in the bathypelagic layer, respectively.

Figure 6. Relationships between Fe(III) solubility and humic-like components 1 and 2 in the mesopelagic layer.

Table 1. Characteristics of four components derived from PARAFAC model compared with those previous studies[a]

Component	Excitation maximum (nm)	Emission maximum (nm)	Coble (1996)	Stedmon and Markager (2005a)[b]	Cory and McKnight (2005)	Yamashita et al. (2008)[d]	Murphy et al. (2008)[d]
1	<260 (370)	466	A/C	4 (Ter/Aut)	SQ2[c]	-	C3/P3 (Ter)
2	325 (<260)	385	M	6 (Ant)	C3[c]	C6 (Mar)	C2/P1 (Mar)
3	<260	-	-	-	-	-	P4
4	275	306	B	8 (Tyr-like)	Tyr-like[c]	C7 (Tyr-like)	C1/P5 (Tyr-like)

[a] symbols and acronyms used for PARAFAC components previously reported could be found in cited literature.

[b] Ter, Aut and Ant means origin of terrestrial, autochthonous and anthropogenic, respectively.

[c] were identified in the Antarctic data set only (Cory and McKnight, 2005), indicating microbial origin.

[d] Ter and Mar means terrestrial and marine humic-like components, respectively.

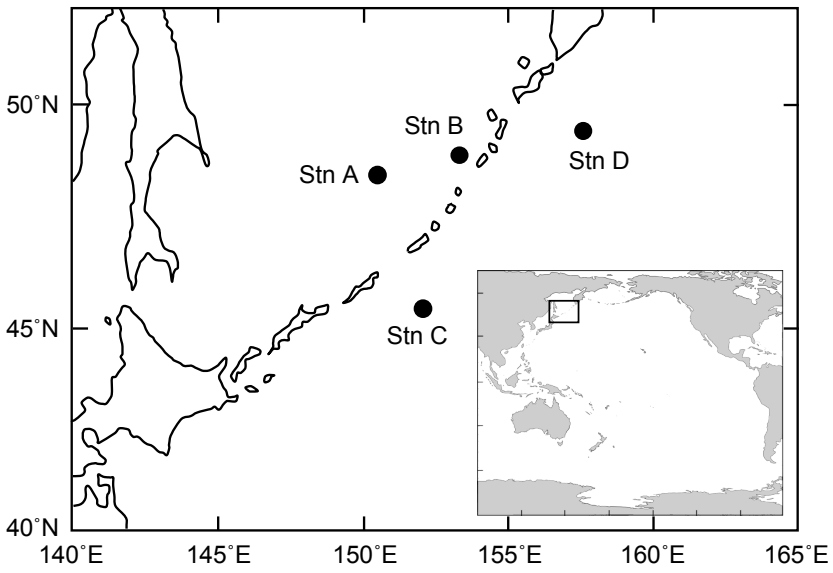
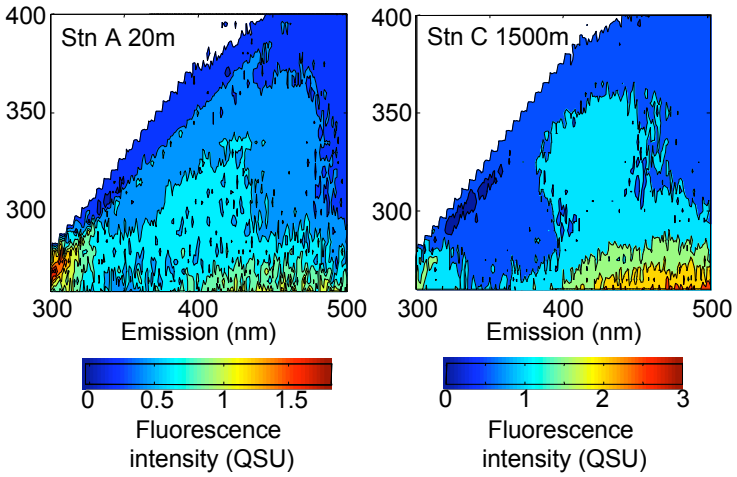


Figure 1. Yamashita et al



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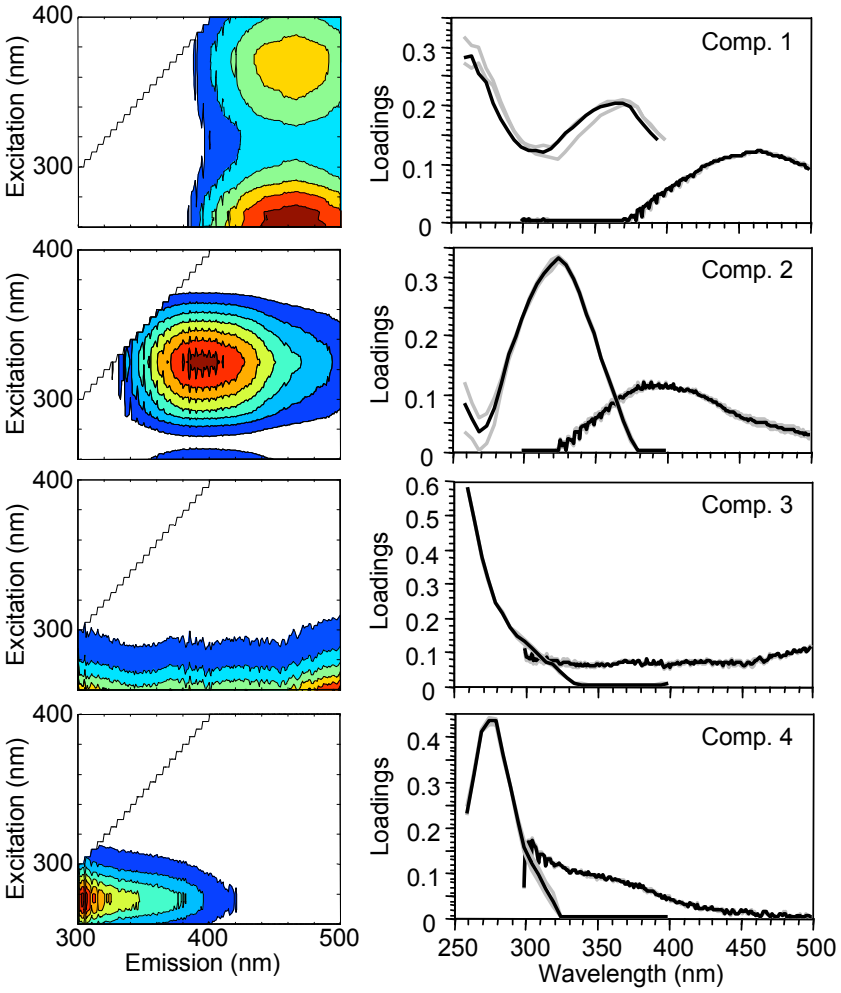
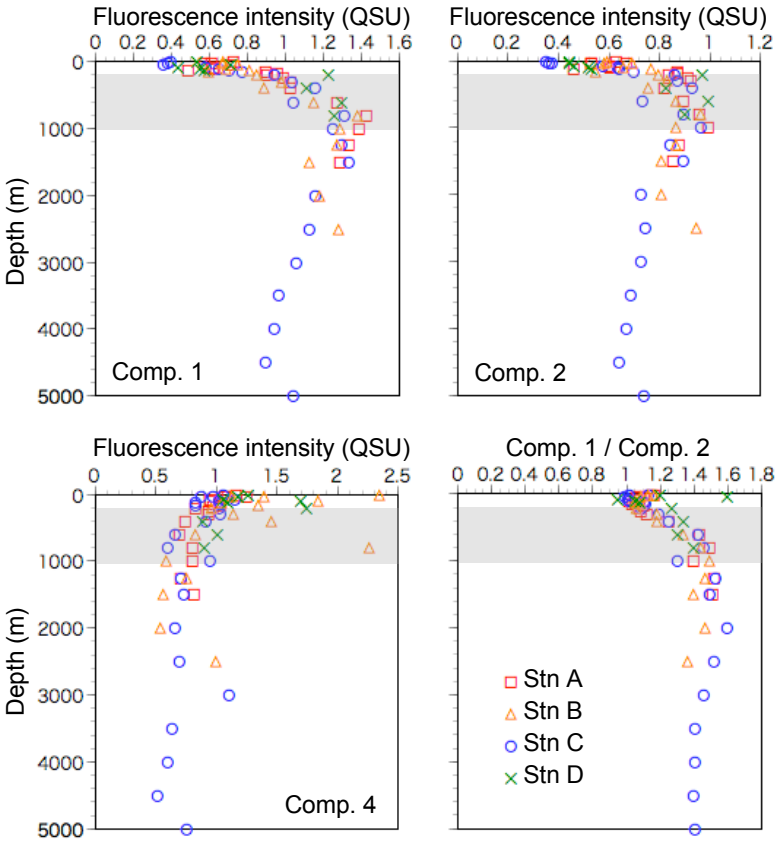


Figure 3. Yamashita et al



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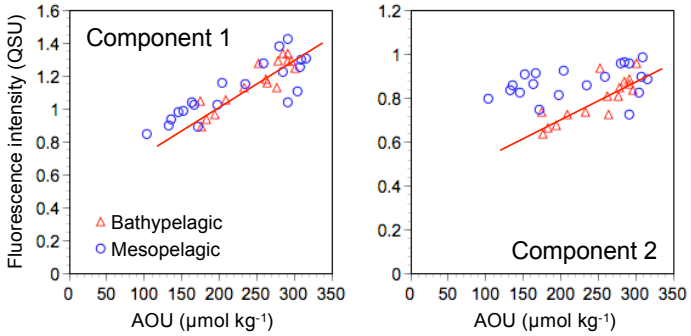

Figure 5. Yamashita et al

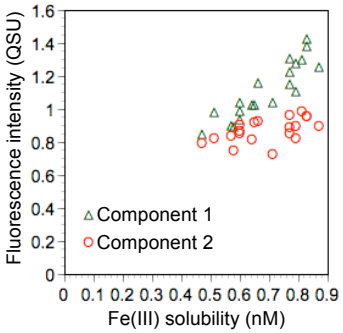

Figure 6. Yamashita et al