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Factors controlling the geographical distribution of fluorescent dissolved organic matter in the surface waters of the Pacific Ocean

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Running head: FDOM in the surface waters of the Pacific

Keywords: dissolved organic matter (DOM); excitation emission matrix (EEM); parallel factor analysis (PARAFAC); geographical distribution; biological degradability; photochemical reactivity; Pacific Ocean

Abstract

Dissolved organic matter (DOM) in the epipelagic ocean is produced by marine organisms and consumed by microbes. Thus, the distributional patterns of DOM quantity and quality in surface waters are possibly related to marine ecological provinces. In this study, surface waters collected throughout the Pacific Ocean were used to investigate the geographical distributions of fluorescent DOM (FDOM) quantity and quality. An excitation-emission matrix and parallel factor analysis revealed two humic-like and two protein-like components. The levels of humic-like components showed clear meridional trends with some zonal variability and were highest and lowest in the northern high-latitude and the subtropical provinces, respectively. The photochemical reactivity, determined by the ratio of two humic-like components, was found to be lowest in the subtropical provinces, implying that the major factor controlling the geographical distribution of humic-like components is the degree of photobleaching. The distributional patterns differed between levels of two protein-like components, i.e., tryptophan-like and tyrosine-like. The ratio of tyrosine-like to tryptophan-like components was established as a possible indicator of microbial degradability, and the highest ratio occurred in subtropical provinces. A negative correlation was found between this ratio and the chlorophyll *a* concentration. Such geographical distributions of protein-like components imply that relatively recalcitrant protein-like components are distributed uniformly throughout the surface waters, but substantial contributions of reactive fractions occur in regions characterized by high biological production. Cluster analysis with the FDOM composition clarified that the

43 diagenetic states of DOM were similar and variable in the northern high-latitude and the
44 subtropical provinces, respectively.
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Introduction

Marine dissolved organic matter (DOM) constitutes one of the largest active carbon reservoirs on Earth, comparable in magnitude to the atmospheric CO₂ reservoir (Hansell et al. 2009). Biological processes are important in driving the DOM cycle. DOM in the ocean mainly originates from the activity of marine organisms (i.e., phytoplankton and heterotrophic bacteria exudation, viral cell lysis, protozoan grazing and zooplankton sloppy feeding) and is mainly consumed by heterotrophic bacteria (Nagata 2008). Thus, the geographical distribution of DOM in surface waters can be considered to be related to marine ecological provinces (Longhurst 1998) that are divided on the basis of the prevailing role of physical forcing as a regulator of phytoplankton distribution. The basin-scale distributions of DOM quantity, e.g., dissolved organic carbon (DOC) concentrations, have been described at major oceanic basins (e.g., Hansell et al. 2009; Ogawa et al. 2014). Hansell et al. (2009) noted that both ocean physics and biological processes shape the basin-scale distribution of DOC concentrations. In surface waters, higher concentrations of DOC are present in subtropical systems because of the slow accumulation of organic matter resistant to biological degradation with the vertical stratification of the upper water column, whereas lower concentrations are evident in subpolar seas, where low-DOC deep waters are more readily mixed to the surface (Hansell et al. 2009).

The reactive components of DOM with relatively short time scales (i.e., labile or semi-labile DOM) occupy minor fractions in bulk DOM (Nagata 2008); therefore, the DOC

concentration may not be adequate for evaluating the roles of DOM in microbial loops and biogeochemical cycles. The DOM quality, which represents the diagenetic state (or reactivity) of DOM, provides valuable insight into the biogeochemical roles of DOM. Recently, a meridional cruise of the Atlantic surface ocean determined the basin-scale distribution of DOM quantity and quality with biogeochemical parameters and elucidated that the DOC concentration was positively correlated with the bacterial abundance and productivity, which were in turn correlated with the temperature (Neogi et al. 2011; Koch and Kattner 2012; Flerus et al. 2012). In addition, the $\Delta^{14}\text{C}$ values and diagenetic states of DOM that were estimated from the molecular composition in solid-phase extracted DOM were found to be correlated with the bulk DOC concentration (Flerus et al. 2012). These results implied that freshly produced, semi-labile DOM contributed to higher concentrations of DOC in the tropical and subtropical surface waters of the Atlantic. By contrast, DOM in the subtropical regions of the Pacific surface ocean was characterized by higher DOC concentrations with lower microbial degradability than that in the equatorial and subarctic regions (Raimbault et al. 2008; Ogawa et al. 2014). Although recent studies have explained the meridional variability in DOM quantity and quality in relation to reactivity, our knowledge regarding how the DOM quality or reactivity varies across oceanic provinces, and thus the roles of DOM in microbial loops and biogeochemical cycles, remains limited.

Optical methods have been applied to evaluate basin-scale distributions of chromophoric DOM (CDOM) and fluorescent DOM (FDOM) (e.g., Yamashita and Tanoue

2008; Nelson et al. 2010; Catalá et al. 2015). In particular, the excitation-emission matrix (EEM) and parallel factor analysis (PARAFAC) have been applied to elucidate the variability in FDOM compositions in the deep ocean (Yamashita et al. 2010; Jørgensen et al. 2011; Catalá et al. 2015) and, more recently, in epipelagic meridional transects (Kowalczyk et al. 2013; Heller et al. 2013; Catalá et al. 2016). The fluorescent components in marine DOM can be distinguished into protein-like and humic-like components (Coble 1996). The levels of protein-like components generally decrease with depth (Yamashita et al. 2010; Catalá et al. 2015) and have been found to decrease during 72 h of microbial dark incubation in epipelagic waters (Lønborg et al. 2015), which implies that protein-like components are a biologically reactive fraction in DOM. The levels of humic-like components increase with depth irrespective of differences in ocean basins and are linearly correlated with apparent oxygen utilization in the deep ocean, which indicates that humic-like components are photochemically labile but produced in situ during the microbial oxidation of organic matter (e.g., Yamashita and Tanoue 2008; Catalá et al. 2015). The occurrence of terrigenous humic-like components has also been observed at ocean margins (e.g., Kowalczyk et al. 2013; Jørgensen et al. 2014; Yamashita et al. 2015). Helms et al. (2013) observed shifts in the peak position of humic-like fluorophores towards shorter wavelengths with the photobleaching of deep DOM. In addition, different decay rate constants with photobleaching have recently been observed among different humic-like components that were obtained by EEM-PARAFAC (Timko et al. 2015). Similarly, different microbial degradability between two protein-like components (i.e.,

tyrosine-like and tryptophan-like components) has been distinguished (Yamashita and Tanoue, 2004; Yamashita et al. 2015). Such compositional variability in FDOM with photo- and bio-degradation indicates that the FDOM composition represents the diagenetic state of DOM. Thus, the geographical distribution of FDOM compositions in surface waters across marine ecological provinces should provide new insights into the roles of DOM in microbial loops and biogeochemical cycles in the surface ocean; however, such observations have rarely been conducted.

In the present study, we conducted four research cruises with continuous surface water sampling throughout the Pacific Ocean (from 40°S to 68°N and from 134°E to 86°W; Fig. 1). The geographical distributions of the FDOM quantity and quality were determined by EEM-PARAFAC with high spatial resolution. The aim of the study was to clarify the geographical distribution of the FDOM quantity and quality in the surface waters of the Pacific Ocean to (1) establish the meridional and zonal (longitudinal) variability of the diagenetic status of DOM and (2) explore whether the diagenetic status of DOM can be described by marine ecological provinces (Longhurst 1998).

Materials and Methods

Observation and sample collection

Observations and sample collections were conducted by four *Hakuho Maru* cruises on December 1st 2011 – January 25th 2012 (KH-11-10), July 6th – August 14th 2012 (KH-12-3), December 11th 2013 – January 21st 2014 (KH-13-7), and June 23rd - August 11th 2014

(KH-14-3) through the equatorial to polar regions, which covered 12 of Longhurst's biogeographic provinces (Fig. 1). During the KH-11-10 cruise, samples were collected at 30°N/145°E, from 155.0°E to 158.1°W (near Hawaii) along the 23°N line, and from near Hawaii (18.0°N/154.8°W) to the subtropical province of the South Pacific (SPSG) through the PNEC and PEQD. The samples were collected from 46.9°N to 10°N along the 160°E line and along the route to Japan during the KH-12-3 cruise. The samples from the central Pacific (from 40°S to 68°N, near 180°E) in the Southern and Northern Hemispheres were collected during the KH-13-7 and KH-14-3 cruises, respectively. During the KH-14-3 cruise, samples were also collected along the route from the Chukchi Sea (BPLR) to Japan through the BERS, PSAG (W), and KURO (Fig. 1). The spatial distributions of sea surface temperature and salinity during observations are also shown in Supporting Fig. S1.

Surface water samples ($n = 123$) were collected from an underway pumping system from the hull of the ship (approximately 5 m below the surface). Samples from 5 m or 10 m at 64 observation stations were collected by a carousel multi-sampling system that was equipped with 12-L Niskin-X bottles. The chlorophyll *a* concentrations were determined by the fluorometric method (Welschmeyer 1994). The chlorophyll *a* concentrations in the surface water from the underway pumping system were continuously monitored with calibrated fluorescence sensors during the KH-11-10 cruise. Samples for FDOM analysis were filtered by using pre-combusted GF/F filters, and the filtrates were collected into pre-combusted glass vials with Teflon-lined caps after triple rinsing. The samples were then stored frozen in the dark until analysis. It was described that optical properties of

high-concentration terrestrial DOM changed after freeze/thaw (Spencer et al. 2007; Thieme et al. 2016). However, our preliminary experiments with seawater samples from surface and mesopelagic layers of the subtropical western North Pacific showed no significant effect of freeze/thaw on EEMs.

EEM-PARAFAC

The water samples were thawed and allowed to stand until reaching room temperature prior to EEM measurements. The EEM was measured by using a spectrofluorometer (FluoroMax-4, Horiba) according to Tanaka et al. (2014). Briefly, emission scans from 290 to 600 nm at 2-nm intervals were acquired at excitation wavelengths between 250 and 450 nm at 5-nm intervals. The bandpass was set at 5 nm for excitation and emission. Fluorescence spectra were scanned with 0.25 s of integration time and acquired in S/R ratio mode. The excitation and emission correction files that were supplied by the manufacturers were applied to correct the specific instrument's components. The EEM of Milli-Q water was subtracted from the samples' EEMs. The fluorescence intensities were corrected to the area under the water Raman peak of Milli-Q water (excitation = 350 nm), which was analyzed daily, and calibrated to Raman Unit (RU) (Lawaetz and Stedmon 2009). The coefficient of variation of the area under the water Raman peak during analyses was 1.4%. The inner filter effect was not corrected because the absorption coefficient of epipelagic DOM is low (Catalá et al. 2016).

PARAFAC statistically decomposes EEMs into fluorescent components without any assumptions in terms of their spectra shape or their number, and fluorescent components that are obtained by PARAFAC can be treated quantitatively with the same units as EEMs, namely, RU (Stedmon et al. 2003). PARAFAC modeling was conducted in MATLAB (Mathworks) with the drEEM toolbox (Murphy et al. 2013). The EEMs of excitation wavelengths from 260 to 450 nm and emission wavelengths from 300 to 550 nm were used for PARAFAC modeling. Because a large gradient of fluorescence intensity was present in the dataset, each EEM was normalized to its total signal before it was processed by PARAFAC (Murphy et al. 2013). The model was validated through split-half validation and random initialization according to Murphy et al. (2013).

A cluster analysis with the FDOM composition that was derived from PARAFAC was conducted by R (version 3.2.1) to group observation stations throughout the Pacific Ocean according to their FDOM composition.

Results and Discussion

Fluorescent components

Four components were statistically obtained by using PARAFAC of the surface water samples (Fig. 2; Supporting Fig. S2). The spectral characteristics of the four components in this study were compared with those reported in earlier studies through an online repository of published PARAFAC components (Murphy et al. 2014). Two components were

statistically similar to components obtained by previous PARAFAC studies of the open ocean, as mentioned below.

The fluorescence characteristics of components 1 (C1) and 3 (C3) were similar to previously identified humic-like PARAFAC components. Although humic-like C1 and C3 can be categorized into two traditional types of humic-like fluorescence of terrestrial and marine (microbial) origin, respectively (Coble 1996), both components are produced in marine environments (Yamashita et al. 2010; Tanaka et al. 2014; Catalá et al. 2015). C1 was composed of two peaks with excitation maxima at <250 nm and 340 nm at 474 nm emission and was statistically similar to humic-like components found in the Atlantic Ocean (Kowalczyk et al. 2013), in the western North Pacific Ocean and the Japan Sea (Yamashita et al. 2010; Tanaka et al. 2014), and in the global ocean (Catalá et al. 2015). The fluorescence of C3 was blue-shifted relative to C1 and had fluorescence peaks at 390 nm emission with <250 nm and 295 nm excitation. The microbial humic-like C3 found in this study peaked at shorter excitation and emission wavelengths compared with microbial humic-like PARAFAC components previously reported in the open ocean (Yamashita et al. 2010; Kowalczyk et al. 2013; Catalá et al. 2015). Because EEMs in surface waters were only used for PARAFAC modeling in this study, characteristics of surface waters, possibly including greater photobleaching, may affect the peak position of microbial humic-like C3.

Two additional components were found in the EEM region, which corresponded to protein-like fluorophores (Coble 1996; Yamashita and Tanoue 2003). C2 had a peak at 280 nm excitation and 324 nm emission. Although the spectral characteristics of C2 were

statistically similar to tyrosine-like PARAFAC components found in the open ocean (Yamashita et al. 2010; Catalá et al. 2015), the peak position and spectral shape of C2 could be categorized as tryptophan molecules located within polypeptides (Lakowicz, 2006). Therefore, C2 was assigned to the tryptophan-like component. The peak of C4 was located at 260 nm excitation and 306 nm emission and was possibly a tyrosine-like component. It should be noted that the terrigenous propyl-phenols, namely tannin and lignin, have also been known to contribute to fluorescence in the protein-like regions (Maie et al. 2007; Hernes et al. 2009).

Geographical distribution of humic-like components in surface waters

The distributional patterns of humic-like C1 and C3 in the surface waters of the Pacific Ocean were almost the same, and the fluorescence intensity of C1 was linearly correlated with that of C3 (Fig. 3a), implying that the major factors controlling the levels of these two humic-like components in the surface waters were almost the same. The geographical distribution of the sum of the humic-like components (C1+C3) in the surface waters showed clear differences among oceanic provinces (Fig. 4a). The level of humic-like components was highest at high latitudes in the Northern Hemisphere, i.e., the BPLR and BERS, where substantial contributions of terrigenous humic-like fluorophores from Alaskan rivers and high biological production occur (Grebmeier et al. 2006; D'Sa et al. 2014; Tanaka et al. 2016). This level gradually decreased towards the south and reached its lowest value in the NPTG. Humic-like components increased from the NPTG to the

equatorial region, possibly because of equatorial upwelling characterized by relatively low temperature (Supporting Fig. S1) and high levels of chlorophyll *a* (Fig. 1). They then decreased again towards the SPSG, with some exceptions (at 15-19°S/85-97°W). The relatively high levels of humic-like components at 15-19°S/85-97°W, especially in the eastern part of the region, characterized by relatively low salinity (Supporting Fig. S1), were influenced by the Peru Oceanic Current (Wyrski 1967; Penven et al. 2005) and were thus possibly affected by coastal upwelling waters.

The pattern of geographical distribution of humic-like components in surface waters was opposite those of sea surface temperature and salinity (Supporting Fig. S1). Therefore, the levels of humic-like components could basically be categorized by latitude (Fig. 4b), although zonal differences were also evident, especially in the subtropical provinces (Fig. 4a). The levels of humic-like components in the western region of the NPTG (approximately 10-30°N/140-170°E), characterized as relatively high temperature (Supporting Fig. S1), were lower than those in the central region. The same trend was found through long-term CDOM observations by ocean color imagery (Morel et al. 2010). The levels of humic-like components in the eastern subtropical region of the SPSG (approximately 20-30°S/120°W), characterized as relatively high salinity (Supporting Fig. S1), were lower than those in the central region along 170°W. Again, this trend was the same as what was observed by satellites (Morel et al. 2007, 2010). Overall, the geographical distribution of humic-like components in the surface waters was similar to the overall trends of colored dissolved and detrital material (CDM) observed by ocean color

imagery. Siegel et al. (2005) observed that regions with shallow mixed layers and downwelling Ekman pumping have lower CDM values than regions with seasonally deep mixed-layer depths and upwelling Ekman fluxes. The authors suggested that photolysis driven by the mixed layer averaged light-dose of ultraviolet radiation is an important process that regulates the global CDM distribution (Siegel et al. 2005). Humic-like components, including both C1 and C3, have been found to degrade by sunlight (Helms et al. 2013; Timko et al. 2015), indicating that photobleaching is one of the major factors controlling geographical distribution of humic-like components.

The geographical distribution of humic-like components was similar to that of the chlorophyll *a* concentration (Figs. 1 and 4a). A significant positive correlation was evident between two parameters (Fig. 3b), implying the oceanographic and/or ecological linkages between these parameters. Bricaud et al. (2012) used ocean color imagery and observed that the spatial variations in CDM were correlated to those in the chlorophyll *a* concentration with no seasonal variability. Such relationships found through satellite observation were suggested to be related to (1) the production of CDOM with phytoplankton decay and/or (2) inputs of CDOM and nutrients from deep waters through vertical mixing or upwelling (Siegel et al. 2002; Bricaud et al. 2012). Thus, as previously suggested for CDOM distributions (Siegel et al. 2005; Nelson et al. 2010), input from deep waters, local production, and photobleaching may be important factors shaping the geographical distribution of levels of humic-like components in oceanic surface waters away from terrigenous influences, although terrigenous humic-like components might be

contributed to the area influenced by the Alaskan rivers (the shelves of the BERS and BPLR).

Geographical distribution of protein-like components in surface waters

The geographical distribution of tryptophan-like C2 was markedly different from that of tyrosine-like C4 (Figs. 4c and 4e). A negative correlation between the fluorescence intensities of these two protein-like components (Fig. 3c) indicated that the major factor controlling fluorescence intensity was different between tryptophan-like C2 and tyrosine-like C4 in the surface waters (see the section below for details). A weak but significant negative correlation was also observed between tryptophan-like and tyrosine-like components along a meridional transect of the Atlantic (Heller et al. 2013).

Higher levels of tryptophan-like C2 in surface waters were evident at the BPLR, BERS, PSAG, the western part of the NPPF, and the Peru Oceanic Current (Fig. 4c). This distribution of tryptophan-like C2 was similar to those of chlorophyll *a* as well as humic-like components, although relatively high values were not evident at the equatorial region (Fig. 4d). High levels of C2 found at the shelves of the BERS and BPLR suggested that terrigenous tannin and lignin (Maie et al. 2007; Hernes et al. 2009) might contribute to tryptophan-like C2 in these regions. However, it has also been broadly recognized that the tryptophan-like component in the particulate fraction is related to recent primary production (Brym et al. 2014) and that phytoplankton excrete tryptophan-like fluorophores (Romera-Castillo et al. 2010). A positive correlation between tryptophan-like C2 and the

chlorophyll *a* concentration (Fig. 3d) implied that the tryptophan-like C2 observed in this study was primarily derived from tryptophan molecules, even in the shelves of the BERS and BPLR. A positive correlation was also observed between the tryptophan-like component and chlorophyll *a* concentration along a meridional transect of the Atlantic (Heller et al. 2013).

Higher levels of tyrosine-like C4 were observed in the subtropical provinces (NPTG and SPSG), but lower levels were found along the shelves of the BERS and BPLR (Fig. 4e). Similarly, the highest values of the tyrosine-like component were observed at the oligotrophic southern tropical gyre along a meridional transect of the Atlantic (Heller et al. 2013). The meridional distribution of tyrosine-like C4 was basically a mirror image of that of tryptophan-like C2 (Figs. 4d and 4f) as well as the chlorophyll *a* concentration (Figs. 1, 3e).

A extremely low level of both tryptophan-like C2 and tyrosine-like C4 was found at the eastern equator, where relatively high levels of humic-like components were evident (Fig. 4), which suggests that deep water that was characterized by low protein-like but high humic-like components contributed to surface waters through equatorial upwelling. The ranges of the fluorescence intensity of both the tryptophan-like and tyrosine-like components were narrower than those of the humic-like components, especially at the equator (PEQD and PNEC, except for a station at the eastern equator), the subtropical provinces (NPTG and SPSG), and the NPPF (Figs. 3 and 4). These results implied that semi-labile (relatively recalcitrant) protein-like components are relatively uniformly

distributed in the surface waters throughout the Pacific Ocean. Lønborg et al. (2015) conducted 72-h biodegradation incubations of epipelagic DOM that was collected from the eastern North Atlantic and found that $72\pm 9\%$ of the initial protein-like fluorescence did not degrade during these 72-h incubations. The authors hypothesized that protein-like fluorophores are composed of both labile dissolved free aromatic amino acids and simple peptides and that amino acid moieties are bounded to more complex and recalcitrant structures, which are not utilized after 72 h of incubation (Lønborg et al. 2015). Thus, the relatively high levels of tryptophan-like C2 that were found in the BPLR, BERS, PSAG, the western part of the NPPF, and the Peru Oceanic Current (Fig. 4c), where relatively high chlorophyll *a* was observed (Fig. 1), imply that freshly produced reactive protein-like components with greater primary production (Romera-Castillo et al. 2010; Brym et al. 2014) contributed to the background level of semi-labile fractions.

Surface DOM quality according to the fluorescent components

The relative contribution (%) of individual fluorescence components (e.g., Tanaka et al. 2016) and the ratios of specific fluorescence components (e.g., Murphy et al. 2008) have been used to evaluate environmental dynamics of DOM. Kowalczyk et al. (2013) applied the ratio of the sum of protein-like components to the sum of humic-like components ($I_{\text{protein}}/I_{\text{humic}}$) to an Atlantic meridional transect to determine the dominant fraction of DOM fluorescence. The distribution of $I_{\text{protein}}/I_{\text{humic}}$, namely, $(C2+C4)/(C1+C3)$, was also determined for the surface waters of the Pacific Ocean. The ratio was highest in the western

part of the NPTG, characterized as a high temperature region, and the eastern part of the SPSG, characterized as a high salinity region (Fig. 5a and Supporting Fig. S1). The lowest ratio was evident at the BPLR, possibly due to terrigenous contributions and/or low photobleaching of humic-like components, and at the PEQD because of the upwelling of subsurface water rich in humic-like components, as mentioned above. As such, although relatively large variability was found in the subtropical provinces, $I_{\text{protein}}/I_{\text{humic}}$ showed a meridional trend (Fig. 5b). A similar trend of $I_{\text{protein}}/I_{\text{humic}}$ in surface waters was also observed along the Atlantic meridional transect (Kowalczyk et al. 2013).

The relative contribution (%) of protein-like components to the total fluorescent components, which is similar to $I_{\text{protein}}/I_{\text{humic}}$, has been found to be positively correlated with the bioavailable fraction of DOC in freshwater environments (e.g., Balcarczyk et al. 2009). However, the relative contribution of protein-like components is not positively correlated with an indicator of DOM bioavailability (i.e., DOC-normalized yields of amino acids) in the saline waters of the Gulf of Mexico, possibly because of the complex effects of dilution, the photodegradation of terrestrial humic-like components, and the autochthonous production of protein-like components (Yamashita et al. 2015). The microbial degradability of DOC in the subtropical North Pacific was found to be less than that in the subarctic North Pacific (Ogawa et al. 2014); thus, the meridional distribution of $I_{\text{protein}}/I_{\text{humic}}$ observed in surface waters of the North Pacific seems to be opposite that of the microbial degradability of DOC. This opposite trend in the distributional pattern implies that

$I_{\text{protein}}/I_{\text{humic}}$ and the relative contribution of protein-like components cannot be used to evaluate the microbial degradability of DOM in the surface ocean.

Factors controlling the composition of humic-like components in surface waters

A linear relationship was evident between C3 and C1 for the surface waters (Fig. 3a), including the Chukchi Sea (BPLR) and the Bering shelf (eastern BERS), where terrigenous humic-like fluorophores were possibly present (D'Sa et al. 2014; Tanaka et al. 2016). The geographical distribution of the ratio of the two humic-like components (C3/C1) was determined to further evaluate the factors that control not only the level but also the composition of humic-like components (Figs. 5c and 5d). Higher values of C3/C1 were evident in the subtropical provinces (NPTG and SPSG), although large variability was observed in these provinces. Lower ratios were evident at higher latitudes in the Northern Hemisphere (BPLR, BERS, PSAG, and NPPF). In the shelves of the BERS and BPLR, terrigenous humic-like components from Alaskan rivers rich in traditional terrestrial humic-like C1 compared to marine humic-like C3 (Coble, 1996) may have contributed to the lower C3/C1 values. In oceanic surface waters away from terrigenous influences, low C3/C1 ratios with a high level of humic-like components were distributed at the basin of the BERS, PSAG, and PEQD (Figs. 4a and 5c), which are characterized by high chlorophyll *a* concentrations (Fig. 1) and high primary production (Longhurst 1998). Thus, the preferential production of C1 by phytoplankton might be a major factor that controls the composition of humic-like components in the oceanic surface waters. However,

blue-shifted humic-like fluorophore (corresponding to C3) is exuded from phytoplankton, whereas red-shifted humic-like fluorophore (corresponding to C1) is produced by bacteria (Romera-Castillo et al. 2011). Thus, high primary production would not be a major factor that shapes the geographical distribution of the composition of humic-like components.

The other factor that possibly affects the composition of humic-like components is photobleaching. Photobleaching shifts the excitation and emission maxima of terrigenous humic fluorophores towards shorter wavelengths (blue-shift) (e.g., Moran et al. 2000). Recently, Helms et al. (2013) conducted a photo-irradiation experiment with concentrated DOM from deep water (674 m) in the North Pacific and found that oceanic humic-like fluorophores also blue-shifted with photobleaching. Timko et al. (2015) also conducted photo-irradiation experiments with samples collected from various depths in the Sargasso Sea. These authors found that two humic-like PARAFAC components that have peaks at longer emission wavelengths (447 nm and 497 nm) showed significant photo-lability at all depths, whereas another humic-like component that had peaks at a shorter wavelength (404 nm) showed variable, but more limited, photo-reactivity. These experimental results imply that C1 would be preferentially lost; thus, C3/C1 would increase with photobleaching. The high C3/C1 ratios and low abundances of humic-like components observed in the subtropical provinces (Figs. 4a and 5c) consistently indicate that the major factor controlling the fate of locally produced humic-like components in the surface waters is photobleaching.

The highest C3/C1 ratios in the NPTG and SPSG indicate that the DOM in the subtropical provinces is highly photobleached and mostly resistant against photobleaching. Because the ratio of two humic-like components corresponding to C3/C1 in deep DOM is lower than that in surface DOM (Yamashita et al. 2010; Jørgensen et al. 2011; Tanaka et al. 2014), the relatively low values that occurred in the PEQD imply that subsurface water DOM, which is relatively photo-labile, may be distributed in surface water because of equatorial upwelling. The lowest C3/C1 ratios, which were observed at higher latitudes in the Northern Hemisphere (i.e., BPLR, BERS, PSAG, and NPPF), indicated that the DOM in these regions is photo-labile because of low annual insolation and the relatively short residence time of surface waters.

Factors controlling the composition of protein-like components in surface waters

The abundance of protein-like components can be considered to be controlled by a balance between biological production and microbial consumption, because protein-like components in marine environments are mainly derived from aromatic amino acids, i.e., tryptophan and tyrosine molecules (Yamashita and Tanoue 2003; Yamashita et al. 2015). The different geographical distributions between tryptophan-like C2 and tyrosine-like C4 (Figs. 4c, and 4e) imply that the factors that controlled the fluorescence intensity were different between these two protein-like components. Different distributional patterns between tryptophan-like and tyrosine-like components were also observed in epipelagic waters (Heller et al. 2013; Catalá et al. 2016). Protein molecules that contain both

tryptophan and tyrosine molecules usually show only tryptophan fluorescence because of energy transfer (Lakowicz 2006). A previous study analyzed the fluorescence properties of surface DOM in the Sagami Bay and showed that only tryptophan-like fluorescence was evident in high-molecular-weight fractions, whereas tyrosine-like fluorescence was dominant in low-molecular-weight fractions (Yamashita and Tanoue 2004). High-molecular-weight DOM is more easily degraded by microbes than low-molecular-weight DOM (Amon and Benner 1994; Benner and Amon 2015). Recently, a weak but significant relationship was found between the ratio of tryptophan-like to tyrosine-like components and the DOC-normalized yields of amino acids (Yamashita et al. 2015), an indicator of the bioavailability of DOM (Davis and Benner 2007). In this study, the ratio of tyrosine-like C4 to tryptophan-like C2 ($C4/C2$) was established as a possible indicator for the diagenetic state of DOM against microbial degradation. Low values of $C4/C2$ indicate relatively high microbial degradability, and vice versa.

Figures 5e and 5f show the geographical and meridional distribution of $C4/C2$ in the surface waters, respectively. The geographical distribution of $C4/C2$ was a mirror image of that of the chlorophyll *a* concentration (Fig. 1). The negative correlation found between $C4/C2$ and the chlorophyll *a* concentration (Fig. 6) confirmed that low $C4/C2$ indicates fresh biologically available DOM, although the time scales that control $C4/C2$ and the chlorophyll *a* concentration may be different.

Despite the large variability, the highest $C4/C2$, which indicates a biologically recalcitrant nature, was found in the subtropical provinces (Figs. 5e and 5f). The $C4/C2$ in

surface waters of the subarctic provinces (PSAG) was lower than that in the subtropical provinces of the North Pacific, and such a trend was consistent with the result of microbial degradation experiments that were conducted with surface waters from the North Pacific (Ogawa et al. 2014). The C4/C2 in the PEQD and the eastern part of the SPSG were relatively low, accompanied by relatively high concentration of chlorophyll *a* (Figs. 1 and 5e), possibly because of biological productivity that was associated with upwelling. The lowest C4/C2 ratio was observed in the PSAG, BERS, and BPLR. Therefore, most of the bioavailable DOM was distributed within the PSAG, BERS, and BPLR, where high chlorophyll *a* concentration (Fig. 1) and biological production (Longhurst 1998) were evident.

Variability of the diagenetic state of surface DOM with marine ecological provinces

The composition of humic-like components (i.e., C3/C1) and protein-like components (i.e., C4/C2) showed meridional trends with some zonal variability, especially in the subtropical provinces (Fig. 5), indicating that the diagenetic state of DOM (or DOM reactivity) is linked, at least partially, with marine ecological provinces that are meridionally and zonally defined (Fig. 1; Supporting Fig. S1). Cluster analysis was conducted using DOM compositions that were obtained by EEM-PARAFAC, namely, C3/C1, C4/C2, and $I_{\text{protein}}/I_{\text{humic}}$, to confirm the linkage between the diagenetic state of DOM and marine ecological provinces. Five clusters were defined.

Figure 7 shows the geographical distributions of five clusters. Five clusters were sequentially separated in terms of photo-reactivity (C3/C1) and bio-degradability (C4/C2) (Fig. 8) and can be characterized as follows: low photo-reactivity and low bio-degradability (LpLb), low photo-reactivity and middle bio-degradability-1 (LpMb-1), low photo-reactivity and middle bio-degradability-2 (LpMb-2), high photo-reactivity and middle bio-degradability (HpMb), and high photo-reactivity and high bio-degradability (HpHb). The HpHb cluster was only present in the PSAG, BERS, and BPLR, which implies that the diagenetic states of DOM were relatively similar within and among these provinces. Two clusters, i.e., HpMb and HpHb, were present in the PEQD. The temporal and spatial variability of the upwelling possibly corresponds to the different clusters in the PEQD. The meridional transition of clusters (LpMb-2, HpMb, and HpHb) was observed at a transition zone province (NPPF), indicating that the diagenetic state of DOM possibly changed during the transition from subtropical to subarctic waters. Interestingly, all the clusters were observed in the subtropical provinces (NPTG and SPSG), although the major clusters were LpLb, LpMb-1, and LpMb-2. This variability in clusters in the subtropical provinces again indicates that the photo-reactivity and bio-degradability of DOM vary within the subtropical provinces, although subtropical provinces have been considered homogenous in terms of physical (i.e., homogenous temperature and salinity) and biogeochemical (i.e., extremely low chlorophyll *a* and nutrient concentrations) conditions (Longhurst et al. 1998; Sarmiento and Gruber 2008).

The LpLb was predominant in the western region of the NPTG, whereas LpMb-1 and LpMb-2 were predominant in the central region (Fig. 7). The LpLb was characterized by high $I_{\text{protein}}/I_{\text{humic}}$ and high C4/C2 (i.e., highly bio-degraded, low bio-reactive DOM) compared with LpMb clusters (Fig. 8). Thus, the DOM in the western NPTG, compared with that in the central NPTG, could be characterized by protein-like components that were abundant compared to humic-like components but highly bio-degraded. North Pacific Tropical Water (NPTW), which is characterized by maximum salinity, is distributed within the subsurface layer of the western NPTG (Suga et al. 2000). The high salinity of the NPTW is the result of high evaporation accompanied by large annual insolation, so the DOM in the NPTW can be characterized as highly photobleached. Thus, one reason for the low level of humic-like components in the western NPTG may be winter vertical mixing with the NPTW. The western NPTG has recently been characterized as a region with extremely low level of phosphate due to active dinitrogen fixation as enhanced by aeolian dust deposition (Hashihama et al. 2009). Thus, extremely low concentrations of phosphate and active dinitrogen fixation may restrict the consumption of semi-labile dissolved organic nitrogen, including the highly bio-degraded protein-like components in the western NPTG.

A zonal difference in cluster distribution was also evident in the SPSG. The LpMb-1 and LpMb-2 were mainly distributed in the central SPSG along the 180°E line, whereas all the clusters occurred in the eastern SPSG. Just like the PEQD, HpMb and HpHb were observed in the eastern part of the SPSG, characterized as relatively low salinity (Supporting Fig. S1), possibly because of the effect of upwelling alongside the Peru

Oceanic Current (Wyrski 1967; Penven et al. 2005). The LpLb cluster was located near Easter Island in the SPSG, which is defined as the clearest waters in the South Pacific (Morel et al. 2007). The highly bio-degraded nature of the LpLb (Fig. 8) was consistent with DOM with low biological degradability, as determined by neutral sugars and bacterial production in the region (Sempéré et al. 2008).

In summary, photochemically and biologically reactive DOM are distributed in provinces with upwelling deep waters and deep winter convection, which in turn cause higher biological activity. By contrast, photochemically and biologically recalcitrant DOM are basically distributed in subtropical provinces, which are characterized by lower biological activity and longer surface water residence times, that is, a greater insolation history. However, compared to provinces with upwelling deep waters and deep winter convection, the photochemical and biological reactivities of DOM were variable in the subtropical provinces, which have been considered oceanographically and ecologically homogenous.

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700

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710

Figure Legends

Figure 1. Map of Longhurst's marine ecological provinces that were crossed during four *Hakuho Maru* cruises. The black circles indicate the sampling locations. The color represents the chlorophyll *a* concentration ($\mu\text{g L}^{-1}$). BPLR, Boreal Polar; BERS, North Pacific Epicontinental Sea; PSAG, Pacific Subarctic Gyre (East and West); KURO, Kuroshio Current; NPPF, North Pacific Transition Zone; NPTG, North Pacific Tropical Gyre (East and West); WARM, Western Pacific Warm Pool; PNEC, North Pacific Equatorial Countercurrent; PEQD, Pacific Equatorial Divergence Province; SPSG, South Pacific Subtropical Gyre.

Figure 2. Excitation (solid line) and emission (dashed line) spectra of four PARAFAC components: C1 (a), C2 (b), C3 (c), and C4 (d).

Figure 3. Relationships between humic-like C1 and humic-like C3 (a), the sum of the humic-like components and the chlorophyll *a* concentration (b), tryptophan-like C2 and tyrosine-like C4 (c), tryptophan-like C2 and chlorophyll *a* concentration (d), and tyrosine-like C4 and chlorophyll *a* concentration (e). All the axes are shown on log scales, but correlations among the parameters were determined on linear scales.

Figure 4. Geographical and meridional distributions of the sum of humic-like C1+C3 (a, b), tryptophan-like C2 (c, d), and tyrosine-like C4 (e, f). The solid lines in the maps (a, c, e)

represent the boundaries of Longhurst's marine ecological provinces. The meridional distribution (open black circle) with the latitudinal average (solid blue line) are shown in (b), (d), and (f).

Figure 5. Geographical and meridional distributions of the ratio of the sum of the protein-like components (C2+C4) to the sum of the humic-like components (C1+C3), $I_{\text{protein}}/I_{\text{humic}}$ (a, b); the ratio of the humic-like components, C3/C1 (c, d); and the ratio of the tyrosine-like to the tryptophan components, C4/C2 (e, f). The solid lines in the maps (a, c, e) represent the boundaries of Longhurst's marine ecological provinces. The meridional distribution (open black circle) with the latitudinal average (solid blue line) is shown in (b), (d), and (f).

Figure 6. Relationship between the ratio of the tyrosine-like to the tryptophan components (C4/C2) and the chlorophyll *a* concentration on log scales. A correlation between the parameters was determined on linear scales.

Figure 7. Distribution of five clusters of the FDOM composition that was obtained from cluster analysis. The names of clusters were defined from Figure 8 as follows: LpLb (low photo-reactivity and low bio-degradability), LpMb-1 (low photo-reactivity and middle bio-degradability-1), LpMb-2 (low photo-reactivity and middle bio-degradability-2), HpMb

(high photo-reactivity and middle bio-degradability), and HpHb (high photo-reactivity and high bio-degradability).

Figure 8. Box and whisker plots of the ratio of the sum of the protein-like components to the sum of the humic-like components, $I_{\text{protein}}/I_{\text{humic}}$ (a); ratio of the humic-like components, C3/C1 (b); and the ratio of the tyrosine-like to the tryptophan components, C4/C2 (c) among five clusters; LpLb (low photo-reactivity and low bio-degradability), LpMb-1 (low photo-reactivity and middle bio-degradability-1), LpMb-2 (low photo-reactivity and middle bio-degradability-2), HpMb (high photo-reactivity and middle bio-degradability), and HpHb (high photo-reactivity and high bio-degradability).

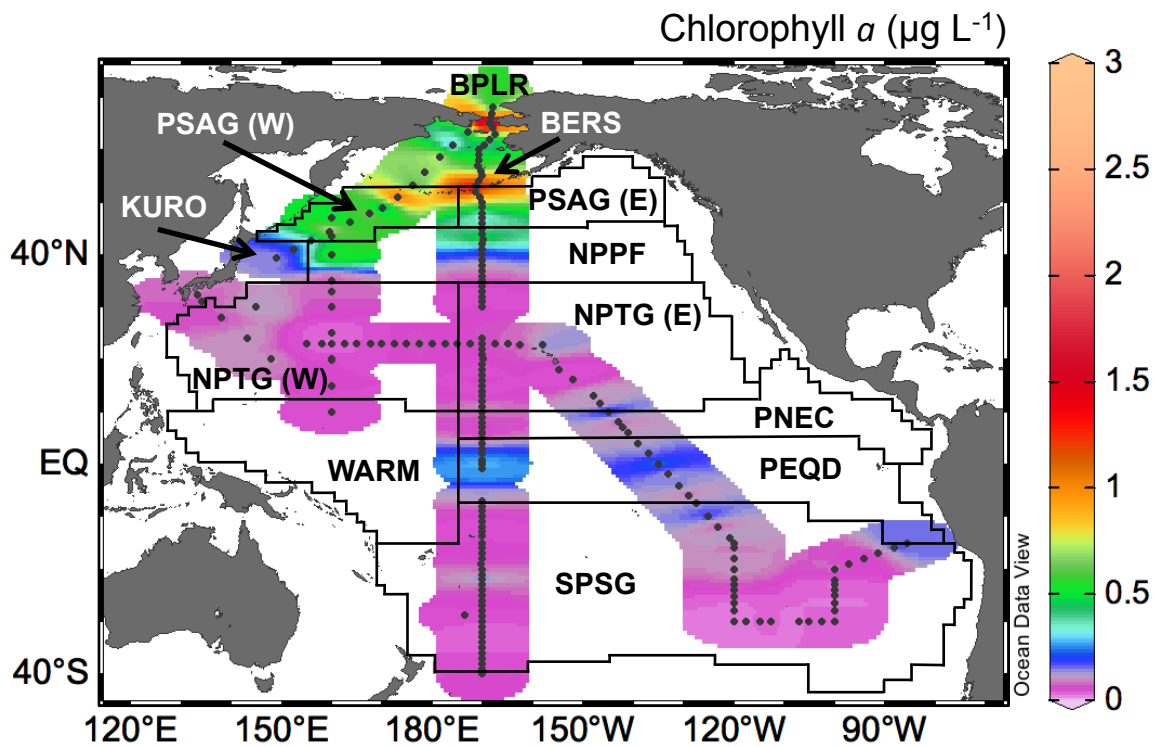


Figure 1. Map of Longhurst's marine ecological provinces that were crossed during four *Hakuho Maru* cruises. The black circles indicate the sampling locations. The color represents the chlorophyll *a* concentration ($\mu\text{g L}^{-1}$). BPLR, Boreal Polar; BERS, North Pacific Epicontinental Sea; PSAG, Pacific Subarctic Gyre (East and West); KURO, Kuroshio Current; NPPF, North Pacific Transition Zone; NPTG, North Pacific Tropical Gyre (East and West); WARM, Western Pacific Warm Pool; PNEC, North Pacific Equatorial Countercurrent; PEQD, Pacific Equatorial Divergence Province; SPSG, South Pacific Subtropical Gyre.

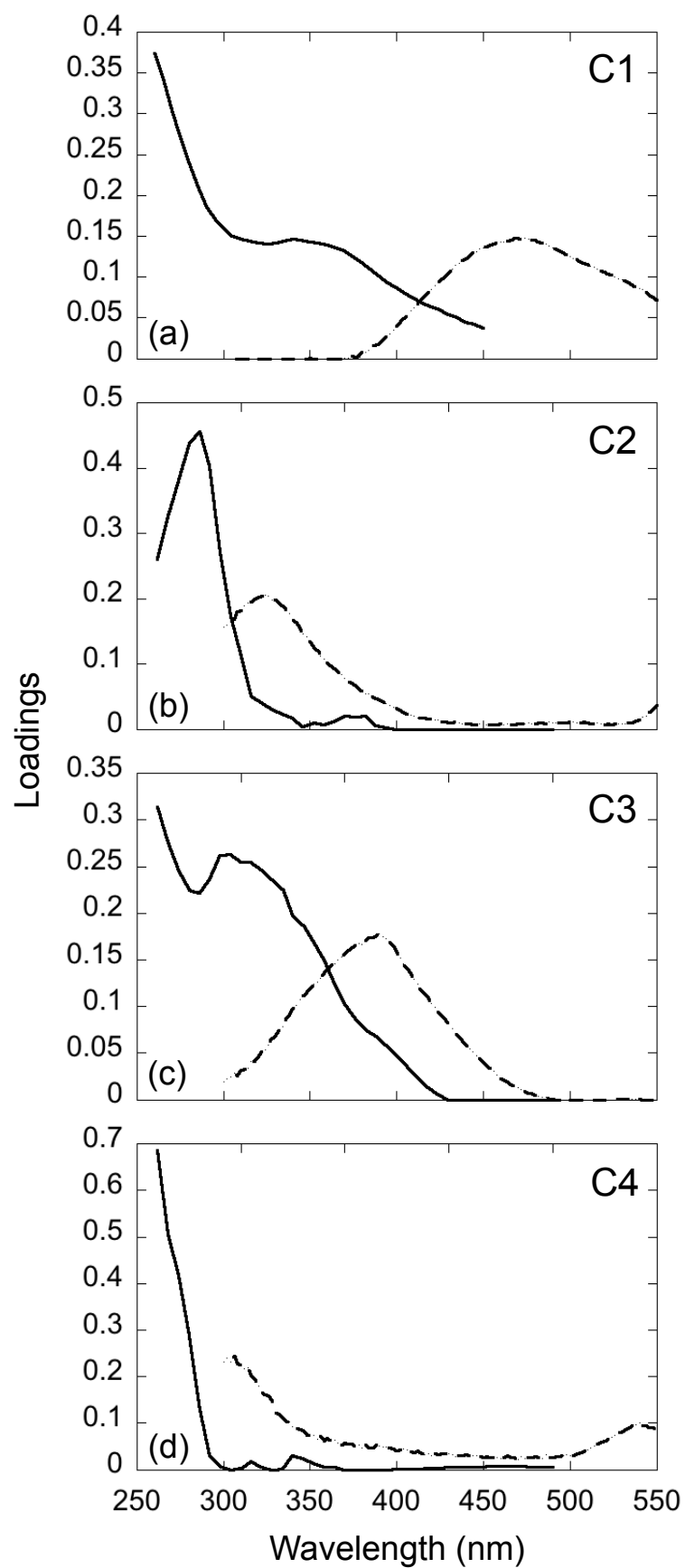


Figure 2. Excitation (solid line) and emission (dashed line) spectra of four PARAFAC components: C1 (a), C2 (b), C3 (c), and C4 (d).

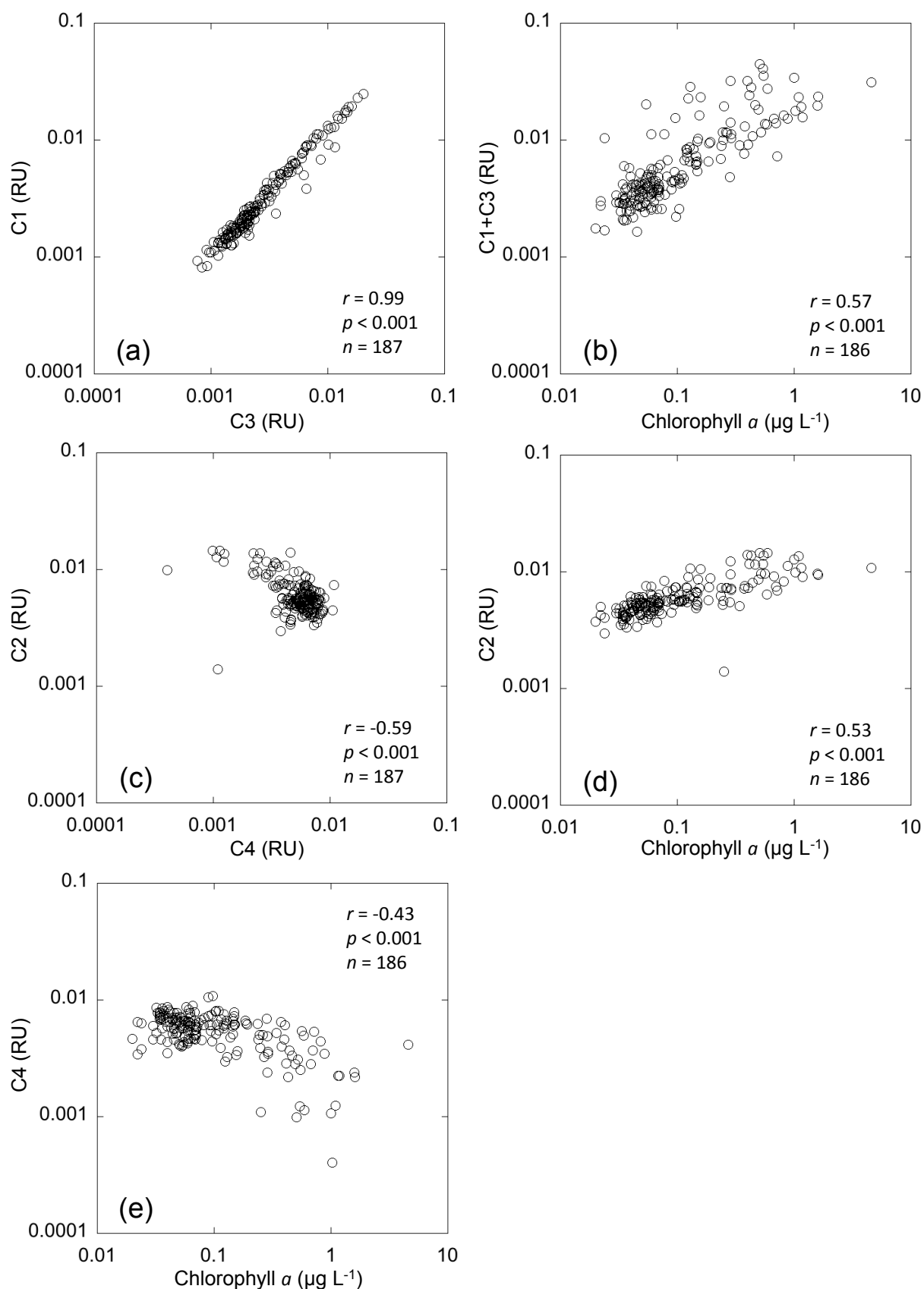


Figure 3. Relationships between humic-like C1 and humic-like C3 (a), the sum of the humic-like components and the chlorophyll *a* concentration (b), tryptophan-like C2 and tyrosine-like C4 (c), tryptophan-like C2 and chlorophyll *a* concentration (d), and tyrosine-like C4 and chlorophyll *a* concentration (e). All the axes are shown on log scales, but correlations among the parameters were determined on linear scales.

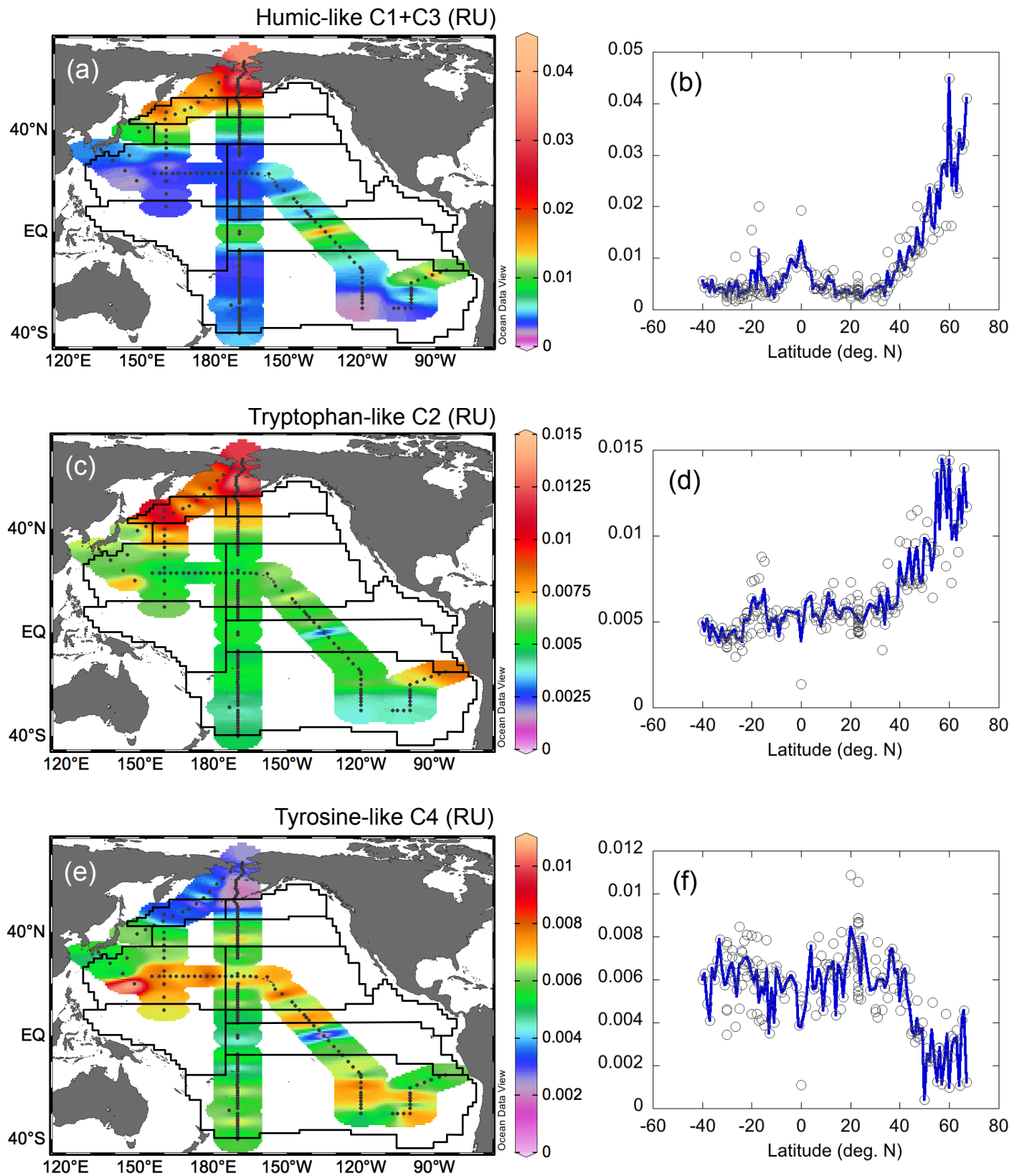


Figure 4. Geographical and meridional distributions of the sum of humic-like C1+C3 (a, b), tryptophan-like C2 (c, d), and tyrosine-like C4 (e, f). The solid lines in the maps (a, c, e) represent the boundaries of Longhurst's marine ecological provinces. The meridional distribution (open black circle) with the latitudinal average (solid blue line) are shown in (b), (d), and (f).

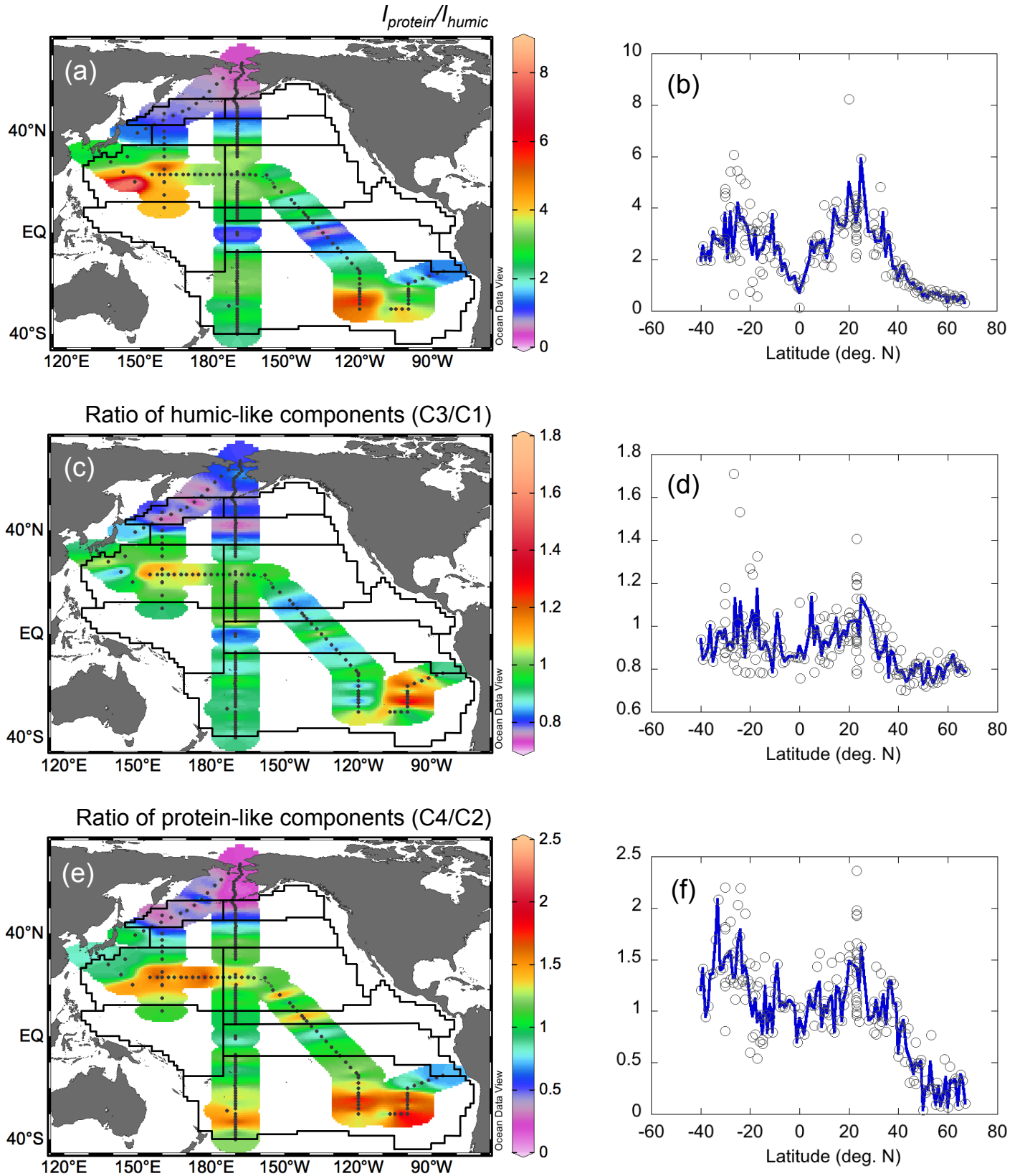


Figure 5. Geographical and meridional distributions of the ratio of the sum of the protein-like components (C2+C4) to the sum of the humic-like components (C1+C3), $I_{\text{protein}}/I_{\text{humic}}$ (a, b); the ratio of the humic-like components, C3/C1 (c, d); and the ratio of the tyrosine-like to the tryptophan components, C4/C2 (e, f). The solid lines in the maps (a, c, e) represent the boundaries of Longhurst's marine ecological provinces. The meridional distribution (open black circle) with the latitudinal average (solid blue line) is shown in (b), (d), and (f).

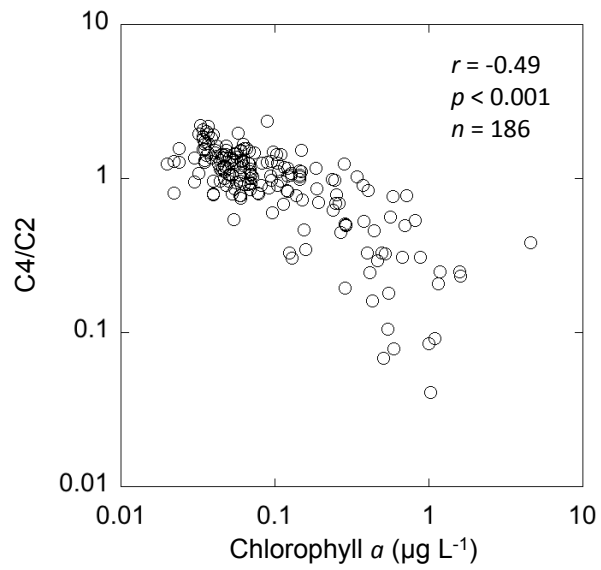


Figure 6. Relationship between the ratio of the tyrosine-like to the tryptophan components ($C4/C2$) and the chlorophyll a concentration on log scales. A correlation between the parameters was determined on linear scales.

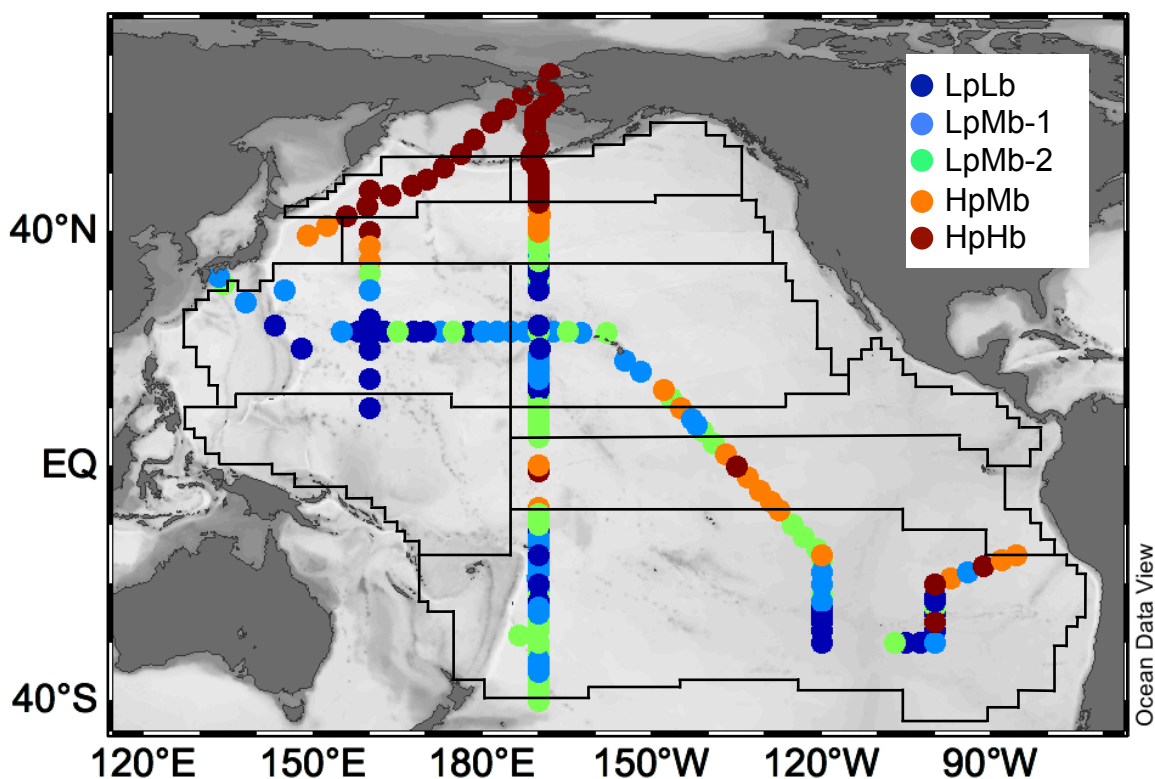


Figure 7. Distribution of five clusters of the FDOM composition that was obtained from cluster analysis. The names of clusters were defined from Figure 8 as follows: LpLb (low photo-reactivity and low bio-degradability), LpMb-1 (low photo-reactivity and middle bio-degradability-1), LpMb-2 (low photo-reactivity and middle bio-degradability-2), HpMb (high photo-reactivity and middle bio-degradability), and HpHb (high photo-reactivity and high bio-degradability).

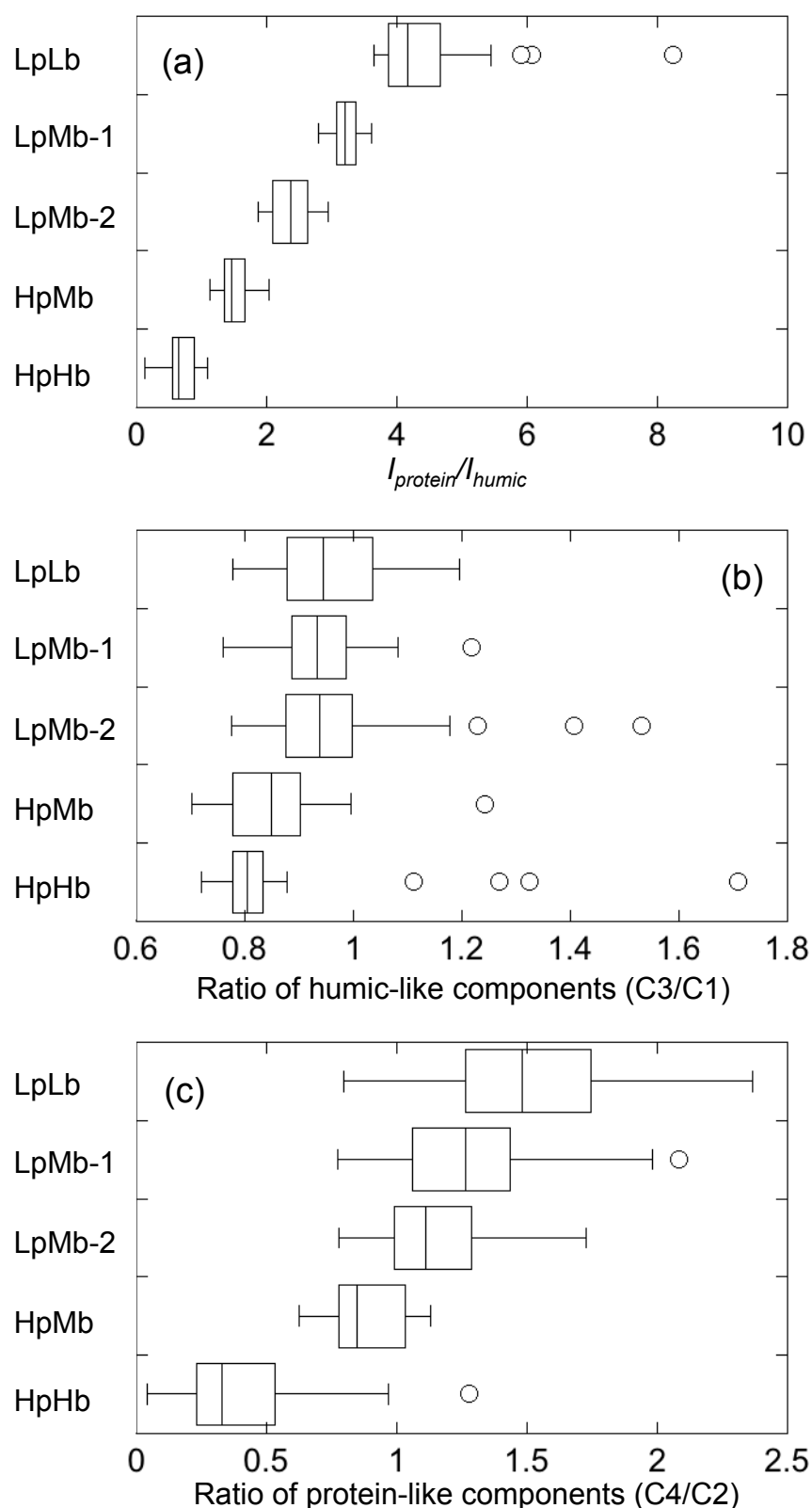


Figure 8. Box and whisker plots of the ratio of the sum of the protein-like components to the sum of the humic-like components, $I_{\text{protein}}/I_{\text{humic}}$ (a); ratio of the humic-like components, C3/C1 (b); and the ratio of the tyrosine-like to the tryptophan components, C4/C2 (c) among five clusters; LpLb (low photo-reactivity and low bio-degradability), LpMb-1 (low photo-reactivity and middle bio-degradability-1), LpMb-2 (low photo-reactivity and middle bio-degradability-2), HpMb (high photo-reactivity and middle bio-degradability), and HpHb (high photo-reactivity and high bio-degradability).

Supporting Information

For the research article:

Factors controlling the geographical distribution of fluorescent dissolved organic matter in the surface waters of the Pacific Ocean

Youhei Yamashita, Fuminori Hashihama, Hiroaki Saito,
Hideki Fukuda, and Hiroshi Ogawa

Contents:

Supporting Figure S1-S2

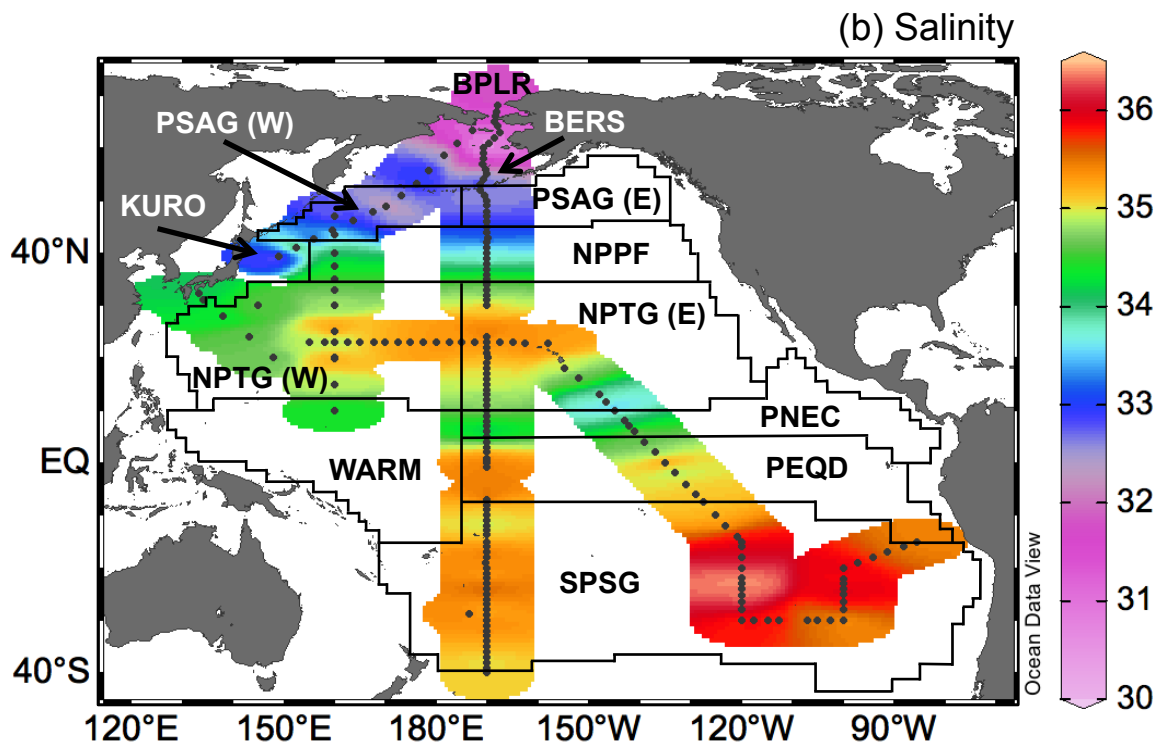
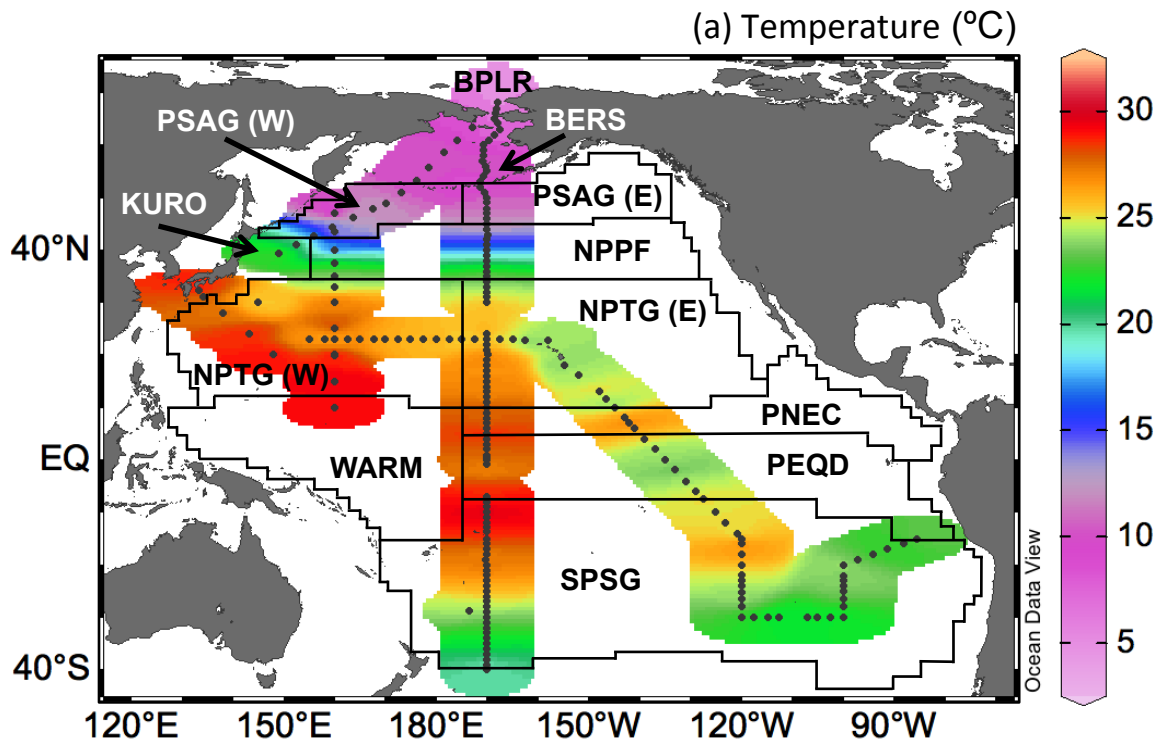


Figure S1. Spatial distributions of sea surface temperature (a) and salinity (b) during observations.

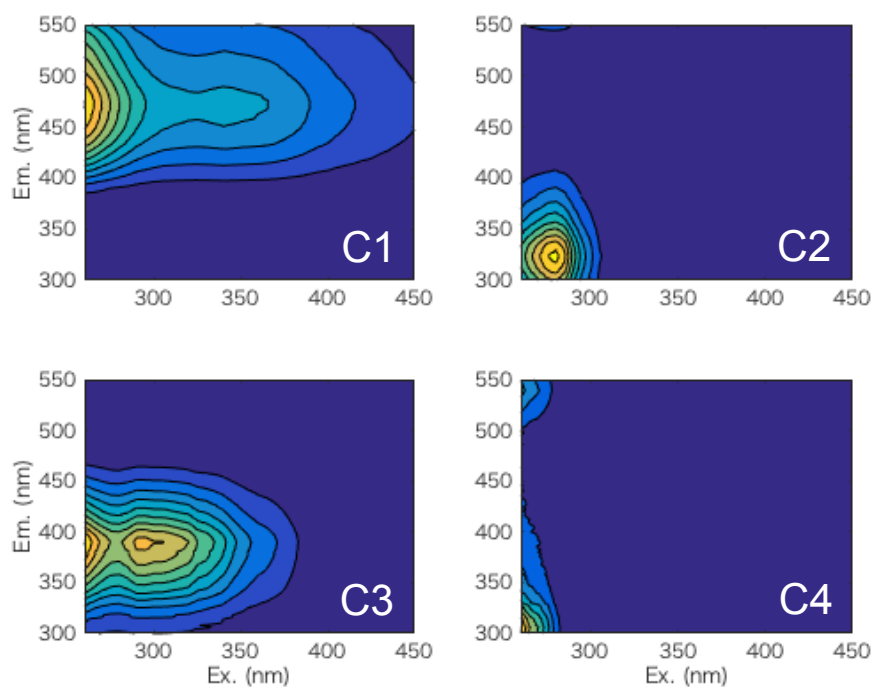


Figure S2. EEMs of four components obtained by parallel factor analysis (PARAFAC).