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The origin and characteristics of dissolved organic carbon in the highly urbanized coastal waters of Tokyo Bay

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Abstract

In highly urbanized coastal waters, sewage treatment plant (STP) effluent comprises an increasingly large percentage of surface water supplies. However, the impacts of effluent organic matter on receiving riverine and coastal waters remain largely unknown. Therefore, this study investigates the spatial and temporal variations of dissolved organic carbon (DOC), biologically recalcitrant DOC (RDOC), and bioavailable DOC (BDOC) concentrations in Tokyo Bay and its watershed from June 2013 to April 2014. Fluorescent dissolved organic matter (FDOM) was also estimated with an excitation–emission matrix (EEM) combined with parallel factor analysis (PARAFAC). EEM–PARAFAC modeling resulted in six fluorescent components characterized as five humic-like and tryptophan-like substances. Degradation experiments showed that RDOC concentrations were higher than those of BDOC in all seasons and stations. Most FDOM were also biologically recalcitrant, especially remarkable for humic-like substances C1 (97.6 ± 9.2%), C2 (95.8 ± 12.7%), and C6 (99.8 ± 14.5%). Linear relationships between FDOM and salinity revealed that most of the FDOM in the bay were derived from STP effluent, and supplied minimally by phytoplankton.

Keywords FDOM · RDOC · Carbon cycling · Estuary · Sewage

1 Introduction

Coastal waters are complex aquatic systems that link the terrestrial and oceanic carbon cycles and influence the character and amount of dissolved organic carbon (DOC) delivered to open ocean. Several sources of DOC in coastal waters are derived from terrestrial, phytoplanktonic, and anthropogenic inputs. One such source is the influx of untreated domestic wastewater, significantly impacting DOC concentrations in urbanized coastal areas where sewage treatment plants (STPs) are not maintained (e.g., Mostofa et al. 2010). Organic matters derived from untreated domestic wastewater are mainly composed of proteins and household detergents, which decompose more efficiently than terrestrial organic matters like fulvic acid in estuaries and coastal areas (e.g., Mostofa et al. 2010; Parr et al. 2015).

In highly urbanized coastal waters with complete STP maintenance, STP effluent discharge is a significant source of anthropogenic DOC, nutrients, and other materials in place of untreated wastewater. At the STP, primary and secondary treatments are mainly performed. Primary treatment involves sedimentation and removal of suspended solids, while in the secondary treatment, organic matter is further removed by decomposition by bacteria. Advanced treatment, which removes the nutrients such as nitrogen and phosphorus by bacteria or the addition of coagulants and so on, is also conducted in some STPs. Previous studies have demonstrated the importance of STP effluent in delivering nutrients into coastal waters (Fisher et al. 2021; Testa et al. 2022). In particular, nutrient concentrations significantly decrease in coastal waters that are affected by STP effluent that has undergone advanced treatment (Kubo et al. 2019, 2020).

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In contrast, several reports on changes in DOC concentrations and quality are mainly estimated based on the content of fluorescent dissolved organic matter (FDOM) within the STPs (Baker 2001; Meng et al. 2013; Li et al. 2020). FDOM measurements have been conducted in many environments, since it is a relatively simple method of tracking changes to the bulk chemical composition of DOM (Coble 1996; Stedmon and Markager 2005a; Meng et al. 2013). DOC concentrations are lower in STP effluents than influents due to the sewage treatment process (Baker 2001; Baker and Spencer 2004), yet are frequently higher than in the upstream river waters (Griffith and Raymond 2011; Meng et al. 2013). For example, STPs in Yokohama and Shizuoka City have a DOC of $3,045 \pm 1,157$ µmol L^{-1} ($n = 7$) in STPs influent compared to the 385 ± 180 µmol L^{-1} ($n = 9$) in STPs effluent, a reduction of approximately 90% (Kubo, unpublished data). However, DOC concentrations in the upstream river water unaffected by sewage are at most 100 µmol L^{-1}, and hence, the impact of sewage effluent water is important for urban rivers. Several studies have investigated the change in FDOM within each treatment process at STPs (Baker 2001). Consequently, tryptophan-like substances decrease in STPs, while humic-like substances increase (Wang and Chen 2018). As a result, DOM in STP effluents is known to become aromatic and contain relatively non-biodegradable materials (Wang and Chen 2018). However, the dynamics of DOC and FDOM from STP effluents are unclear after entering coastal waters, especially since their decomposition properties have not been evaluated.

In Tokyo Bay, the amount of nutrient and bioavailable DOC (BDOC) loading into the bay has decreased with the progress in the construction of STPs since the 1970s (Kubo et al. 2015, 2019; Kubo and Kanda 2020). However, the removal of nutrients from STPs remains insufficient, since advanced-treated water in STPs in the watershed of the bay accounted for 26.3% of the total STP effluent in 2015 (Kubo et al. 2019). Therefore, phytoplankton blooms still occur throughout the year (Kubo et al. 2017).

The biologically recalcitrant DOC (RDOC) and BDOC concentrations were estimated by performing DOC degradation experiments (Kubo et al. 2015). The terrestrial RDOC derived from STP effluent was much more abundant than the RDOC derived from phytoplankton (Kubo et al. 2015). Meanwhile, BDOC was not correlated with either salinity or chlorophyll *a* (Chl *a*) concentration in the central bay, and its source was poorly understood. In any case, the input of RDOC derived from STPs is an essential source in the bay (Kubo and Kanda 2020). However, the composition of DOM derived from STPs and the decomposition properties of each component in the DOM remain largely unknown. Therefore, an evaluation of the environmental behavior of each FDOM component derived from STP effluents and their decomposition properties is warranted. Urbanization broadly alters watersheds and coastal ecosystems, yet the impact of inputs from the STP effluent on their quality, and the ultimate fate of FDOM in such ecosystems is poorly understood. Therefore, the dynamics of FDOM derived from STP effluents in Tokyo Bay, a highly urbanized coastal area, can clarify the dynamics of organic matter inputs associated with future urban development. This study describes the quality of DOM by measuring FDOM in Tokyo Bay and its decomposition characteristics through degradation experiments.

2 Materials and methods

2.1 Study site and sampling

Tokyo Bay, with a mean water depth of 19 m and an area of approximately 920 km^2 in central Japan, is surrounded by the Tokyo metropolitan area, the largest megacity in the world, with a total population of approximately 31 million. The residence time of the bay water is estimated at 30–50 days (Okada et al. 2007). Freshwater discharges into the bay at a total discharge of 11 km^3 $year^{-1}$ (Matsumura and Ishimaru 2004). The watershed of the bay is highly urbanized, and the sewage maintenance rate is almost 100%. Therefore, approximately half of the freshwater inflow into the bay is STP effluent (Japan Sewage Works Association, https://www.jswa.jp/database/).

Freshwater samples were collected monthly from up and downriver of the Arakawa River, and effluent samples were collected from the Shibaura STP (Fig. 1) between June 2013 and April 2014. The Shibaura STP effluent, which is mainly conducted for secondary treatment that flows into the lower reaches of the Sumida River, diverged from Arakawa River. Freshwater samples were collected using a bucket, and observations were made either 1 or 2 days before the observations at Tokyo Bay. Surface seawater samples from Tokyo Bay were collected monthly from the inner bay to the bay mouth using a bucket from onboard the T/V Seiyo-maru of the Tokyo University of Marine Science and Technology from June 2013 to April 2014 at 13 stations (Fig. 1).

Water samples were transferred into 1-L polycarbonate bottles and filtered immediately through GF/F filters precombusted at 450 °C for three hours. After filtration, DOC and FDOM samples were transferred to polycarbonate bottles for freshwater samples to prevent cracking the bottle, and glass vials for seawater samples, and then frozen until analysis. To measure Chl *a* concentrations, chlorophyllous pigments were extracted with *N, N*-dimethylformamide using filters used for DOC filtration (Suzuki and Ishimaru 1990). Temperature and salinity were measured in the field using a YSI EC 300 (YSI/Nanotech Inc., Yellow Springs, OH, USA) at all stations.
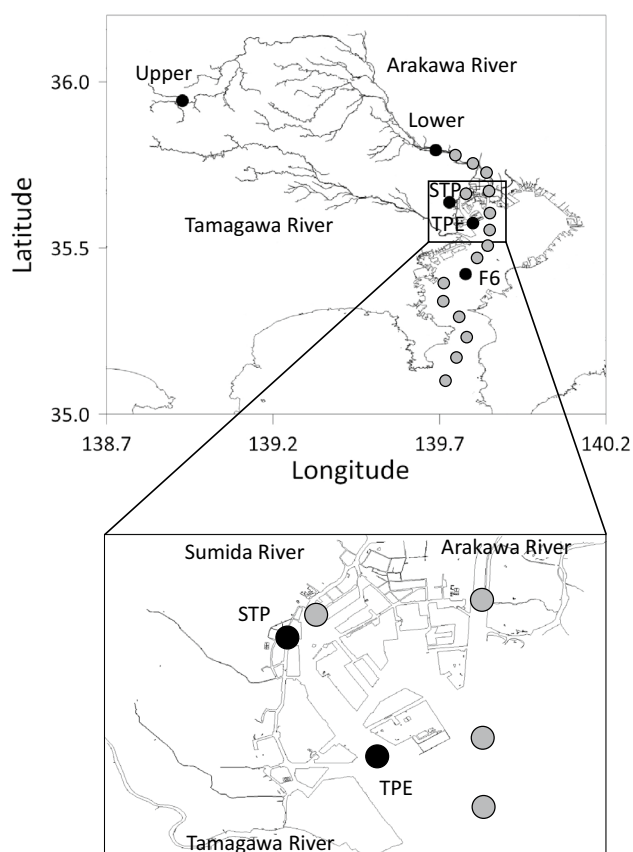
Fig. 1 Map of Tokyo Bay and watershed. The gray and black circles represent surface water sampling stations and biodegradation incubation experiment stations, respectively. STP and TPE indicate the sewage treatment plant effluent and the Tokyo Port Entrance, respectively

2.2 Sample analysis

DOC samples were measured using a Total Organic Carbon (TOC) analyzer (TOC-V$_{CSH}$, Shimadzu, Japan). Fluorescence EEMs were measured using a spectrofluorometer (FluoroMax-4, Horiba, Japan). The EEMs measured 250–550 nm excitation wavelengths at 5 nm intervals and 290–600 nm emission wavelengths at 2 nm intervals. The inner filter effect was corrected using an absorbance spectrum (McKnight et al. 2001) determined using a spectrophotometer (UV-1800, Shimadzu, Japan). EEM spectra were corrected to the area under the Raman scattering peak at 350 nm of excitation (Murphy et al. 2010) to normalize to Raman units (R.U.), and the Raman normalized MilliQ water EEM spectrum was subtracted from the EEM spectra of samples to remove water Raman scattering. EEMs were analyzed using parallel factor analysis (PARAFAC), a multivariate modeling technique that decomposes the FDOM fluorescence signature into individual fluorescence components (Stedmon and Bro 2008). PARAFAC

modeling was conducted using DOMFlour toolbox of the MATLAB software package.

FDOM indices, such as humification index (HIX), biological index (BIX), peak A:T, and fluorescence index (FI), were calculated to evaluate the optical properties of the samples. In addition, UV–visible absorbance indices of spectral slope ratio (SR) and specific ultraviolet absorbance at 254 nm (SUVA) were also calculated. The calculation method of each parameter was as follows.

HIX was estimated as the area under the emission spectra 434–480 nm divided by the peak area at the excitation 300–346 nm at excitation 255 nm (Zsolnay et al. 1999). This index indicated humic substance content or the extent of humification, and higher values show an increasing degree of humification. BIX was estimated as the ratio of emission intensity at 380 nm divided by 430 nm at excitation of 310 nm (Huguet et al. 2009). This index is an indicator of autotrophic productivity. High values (> 1) correspond to recently produced DOM of autochthonous origin. Peak A:T was estimated as the ratio of emission intensity at 450 nm at excitation 260 nm and emission intensity at 304 nm at excitation 275 nm (Hansen et al. 2016). This index is an indicator of the amount of recalcitrant and labile DOM. FI was estimated as the ratio of the emission intensity at 470 nm and 520 nm at excitation of 370 nm (McKnight et al. 2001). This index is an indicator of the terrestrial and microbial source of the DOM pool. SR was estimated as the spectral slope determined at 275–295 nm (S$_{275\text{-}295}$) divided by spectral slope determined at 350–400 nm (S$_{350\text{-}400}$) according to Helms et al. (2008). This indicator negatively correlates to DOM molecular weight material and decreases aromaticity. In addition, SUVA was estimated as an absorption coefficient at 254 nm divided by DOC concentrations (Weishaar et al. 2003). A higher SUVA value is associated with greater aromatic content.

Chl a samples were measured using a fluorometer (TD-700, Turner Designs, Sunnyvale, CA, USA).

2.3 Degradation experiment

Samples for the DOC degradation experiment (Fig. 1) were filtered through GF/F filters and then transferred to 500 mL glass bottles and stored at room temperature (20 °C) in total darkness according to the method of Kubo et al. (2015). Each degradation experiment was performed for 150 days using a single bottle. Although this experiment was conducted in single, our previous experiments in Tokyo Bay showed that standard errors of the results derived from triplicates were approximately 7% (Kubo et al. 2015), similar to those of Lønborg et al. (2009) (approximately 10%). Accordingly, comparable standard errors ($< 10\%$) would be contained in the experiments of this study. After the 150-day incubation, freshwater and seawater samples were dispensed

into polycarbonate bottles and glass vials, respectively, and frozen until analysis. Kubo et al. (2015) demonstrated that the results of the degradation experiments in Tokyo Bay were not affected by the addition of nutrients, and hence, nutrient addition was not performed. Samples were used for the analyses of DOC and FDOM. RDOC is defined as the concentration of DOC remaining at 150 days. BDOC was obtained by subtracting RDOC from the initial DOC (Lønborg et al. 2009).

2.4 Statistical analysis

Principal component analysis (PCA) was conducted using XLSTAT (version 2015). PCA procedure was performed with the data for temperature (T), salinity (Sal), Chl *a*, DOC, BDOC, RDOC, FDOM, and FDOM indices (HIX, BIX, SR, SUVA, peak A: T, and FI) data.

3 Results and discussion

3.1 Spatial and temporal variations of DOC in freshwater sites and Tokyo Bay

In the freshwater sites, DOC concentrations were low at the upper stations (41 ± 6 µmol L^{-1}; $n = 3$) and relatively high at the lower stations (218 ± 40 µmol L^{-1}; $n = 8$) and the STP effluent (382 ± 52 µmol L^{-1}; $n = 8$). There was no distinct seasonal variation in the concentration of DOC at all stations (Fig. 2). Across the observation months, the RDOC concentration was higher than the BDOC concentration in all stations (Fig. 2). The increase in DOC concentration downriver was mainly caused by the input of the STP effluent (Kubo et al. 2015).

The freshwater flowing into Tokyo Bay primarily consists of upriver water and STP effluent. The total discharge ratio of the upriver water to STP effluent is approximately 1:1 (Japan Sewage Works Association, https://www.jswa.jp/database/). Assuming this ratio and the data collected at the upper station (RDOC and BDOC concentrations were 33.2 ± 8.9 and 7.4 ± 2.9 µmol L^{-1}, respectively, $n = 3$) and in the Shibaura STP effluent (RDOC and BDOC concentrations were 310 ± 26 and 72 ± 41 µmol L^{-1}, respectively, $n = 8$) represent these two sources, the average concentrations of RDOC and BDOC in freshwater were 172 ± 27 and 40 ± 41 µmol L^{-1}, respectively.

These average RDOC and BDOC concentrations were comparable to that observed at the lower station (RDOC and BDOC concentrations were 160 ± 31 µmol L^{-1} and 59 ± 22 µmol L^{-1}, $n = 8$). Therefore, RDOC and BDOC were mainly supplied by the STP effluent due to these high concentrations compared to that of the upper station. However, BDOC concentrations at the lower station were often higher
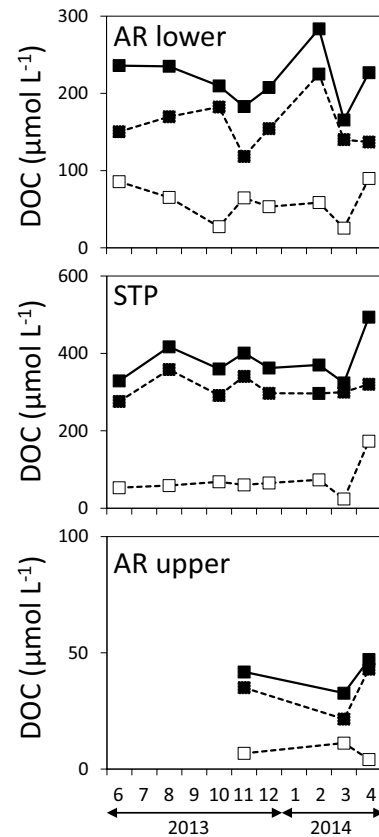
Fig. 2 Seasonal variations of DOC (black square and solid line), RDOC (black square and dashed line), and BDOC (white square and dashed line) at the lower station of the Arakawa River (AR lower), the sewage treatment plant effluent (STP), and the upper station of the Arakawa River (AR upper)

than that of STP effluents, implying the possibility of other sources, although the main factor was effluent from STPs.

Chl *a* concentrations were sometimes high during spring and summer in the lower station, even though there was no correlation between Chl *a* and BDOC concentrations at the lower station (Figure S1). However, BDOC is possibly supplied by phytoplankton (e.g., He et al. 2010), because the residence time of Chl *a* and BDOC are likely different. Some studies assessed the influence of catchment land use on bulk DOC properties and export (Wilson and Xenopoulos 2009; Williams et al. 2010; Yamashita et al. 2011). Yamashita et al. (2011) reported a protein-like component, which was a proxy of BDOC, supplied by the riparian/hyporheic zone. Therefore, some BDOC may be derived from the riparian/hyporheic zone at the watershed of the bay.

The BDOC concentration was significantly higher in April 2014 compared to other seasons at the lower station and in the STP effluent, but this was probably caused by the input of untreated sewage water. There was heavy rainfall the day before the observation (65 mm day^{-1}), unlike other observation months with little precipitation 3 days

prior to observation (< 1 mm). Most sewerage systems in the watershed of the bay are combined systems, in which sewage and rainwater are discharged into a single sewer pipe. While all sewage is collected and treated at STPs on sunny and weak rainy days, untreated sewage is discharged directly from STPs into downstream rivers during heavy rain. Combined sewage systems can cause serious water pollution during combined sewer overflow events when combined sewage and surface runoff flows exceed the capacity of STPs. Therefore, the supply of BDOC derived from STPs may be underestimated due to limited observation data of runoff events.

In Tokyo Bay, DOC concentrations were high during spring and summer and low during autumn and winter (Figure 3). This pattern was mainly affected by the BDOC concentration changes, while RDOC concentrations were relatively constant throughout the year. At stations Tokyo Port Entrance (TPE) and F6, the BDOC concentrations were 63 ± 51 and 30 ± 16 μmol L^{-1} ($n = 7$), respectively. The BDOC derived from river input increased during spring and summer. At station TPE, a significant negative correlation was found between salinity and BDOC concentration ($R^2 = 0.83$ and $p < 0.001$, $n = 7$), although it was not significant at station F6. Therefore, BDOC at station TPE was mainly derived from STP effluents and the riparian/hyporheic zone. However, since BDOC undergoes decomposition in the bay, the correlation between salinity and BDOC was no longer observed at station F6. More DOC can be produced during high biological activity by phytoplankton photosynthesis (e.g., Søndergaard et al. 2000), but neither BDOC nor RDOC concentrations showed a significant correlation with Chl a concentration at stations TPE and F6 (t

test and $p < 0.05$) due to rapid bacterial uptake and utilization of BDOC, and less significant contribution of RDOC production.

Kubo et al. (2015) estimated the degradation rate constants (k; d^{-1}) of DOC in Tokyo Bay using a first-order exponential decay model with a constant RDOC pool using the Marquardt–Levenberg algorithm. The degradation rate constants at 20 °C in Tokyo Bay, the lower station, the upper Arakawa River, and the STP effluent were 0.151 ± 0.045, 0.047 ± 0.016, 0.064 ± 0.007, and 0.050 ± 0.022 day^{-1}, respectively. These values corresponded to 7, 21, 16, and 20 days of turnover time, respectively. Regarding Tokyo Bay, the k value was higher than that of the world's coastal waters (0.098 ± 0.067; Lønborg et al. 2009); in contrast, freshwater values were slightly lower. Based on the degradation rate constant, > 80% of the freshwater BDOC was degraded in the bay within the freshwater residence time (< 50 days).

The BDOC concentration was high at station TPE as well as at the lower station of the Arakawa River and the Shibaura STP in April 2014. The low salinity of the surface water suggested that the inner part of the bay was also affected by the untreated overflow water.

In coastal waters and estuaries where DOC degradation experiments were conducted (3 < salinity < 34), average DOC and RDOC concentrations have been reported as 170 ± 98 and 132 ± 79 μmol L^{-1}, respectively ($n = 171$; Lønborg et al. 2012), which is in line with the DOC and RDOC concentrations in the TPE in this study (164 ± 63 and 106 ± 20 μmol L^{-1}, respectively; $n = 7$). Although a few detailed observations of seasonal variations were conducted, Lønborg et al. (2009) reported a constant RDOC concentration (79 ± 5 μmol L^{-1}) throughout the year in

Fig. 3 Seasonal variations of DOC (black square and solid line), RDOC (black square and dashed line), BDOC (white square and dashed line), salinity (black square and solid line), and Chl a (green square and solid line) at the Tokyo Port Entrance (TPE) and F6 stations
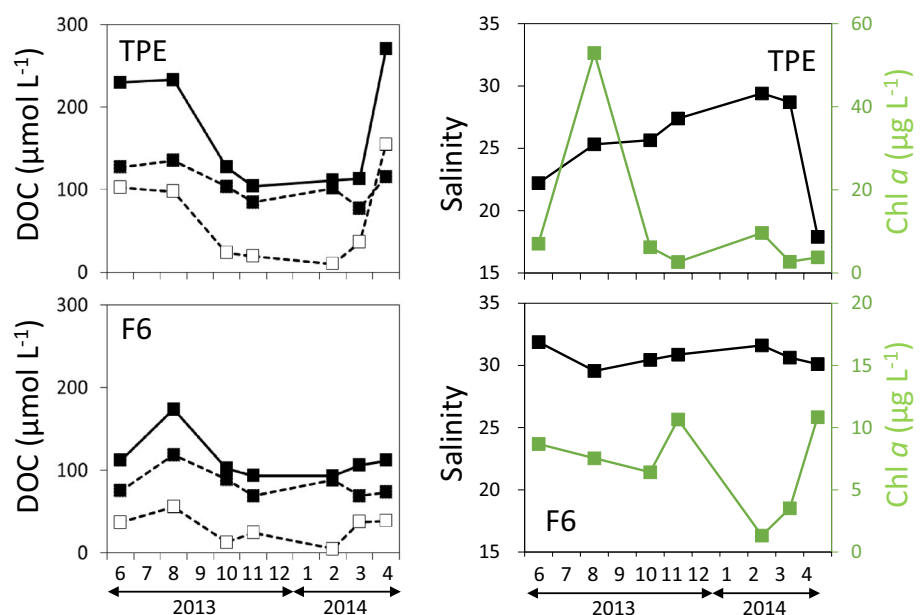
Loch Creran, West Scotland, similar to Tokyo Bay. In addition, Fukushima et al. (2001) showed that DOC and RDOC concentrations in Hiroshima Bay were 106 ± 17 and 81 ± 16 μmol L^{-1}, respectively; RDOC concentrations were constant throughout the year in Hiroshima Bay. These observations were comparable to station F6 in Tokyo Bay (112 ± 28 and 83 ± 16 μmol L^{-1}). The seasonal variations of DOC in Loch Creran and Hiroshima Bay were attributed to the supply of BDOC via active photosynthesis of phytoplankton, as in Tokyo Bay. These areas were relatively high salinity (28.7 ± 3.3 and 31.2 ± 1.7, respectively); hence, similar results were observed due to the large amount of RDOC mainly derived from open ocean water.

The heterotrophic bacterial composition between freshwater and marine ecosystems changes along a salinity gradient (Bouvier and Giorgio 2002). It is generally accepted that marine bacterial communities preferentially utilize labile and recently produced autochthonous dissolved organic carbon (Cherrier et al. 1999). On the other hand, Benner and Opsahl (2001) showed that the DOC bioavailability is more

dependent on DOC quality rather than bacterial communities. The degradation experiments in this study may contain uncertainty, since degradation experiments were conducted using *in situ* bacterial communities. Further investigations remain warranted.

3.2 PARAFAC components and spectral indices

The PARAFAC of the EEM spectra ($n = 292$) identified six components (Fig. 4), as summarized in Table 1. Five components had broad emission and excitation spectra, with emission maxima at visible wavelengths typical of humic-like substances such as C1, C2, C4, C5, and C6. These five humic-like components were reported in other coastal environments (Yamashita et al. 2008, 2013; Kowalczuk et al. 2009). C2 was defined as a microbial humic-like component produced by microbial degradation in the terrestrial and aquatic environment, although this was first reported as a marine humic-like component (Cory and McKnight 2005; Yamashita et al. 2010). C1 and C2 were abundant in STP

Fig. 4 Variation of the six-component model and the spectral characteristics of each PARAFAC component. Black solid and dashed lines show the excitation and emission loadings, respectively. Contour plots of each component are also shown
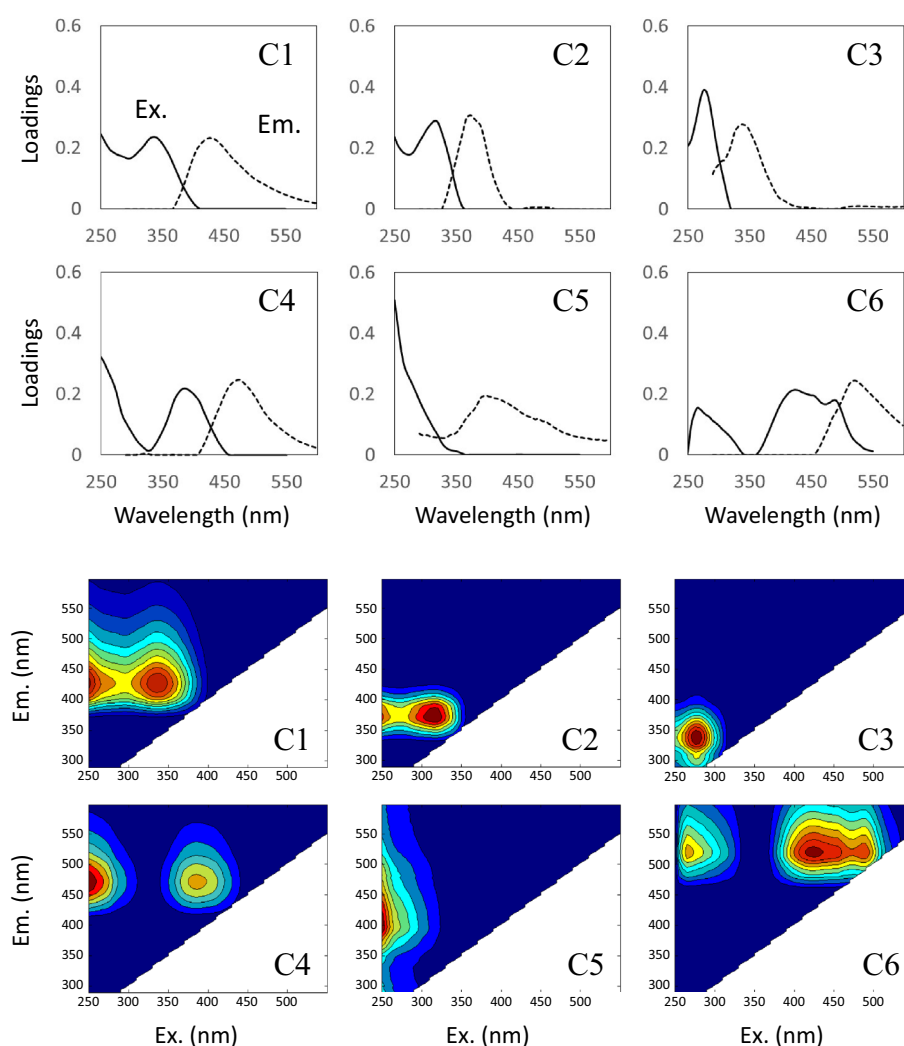
Table 1 Fluorescence characteristics of six components identified in this study compared with those previously identified

Component	Excitation (sub) (nm)	Emission (nm)	Characterization	A similar fluorophore in other studies
C1	< 250 (335)	430	Humic-like substance Sewage derived substance	C4 in Stedmon and Markager (2005a) G1 in Murphy et al. (2011)
C2	315 (< 250)	374	Marine humic-like substance Microbial humic-like substance	Peak M in Coble (1996) Cory and MaKnight (2005)
C3	275	338	Tryptophan-like substance	Peak T in Coble (1996)
C4	< 250 (390)	474	Humic-like substance	Peak A in Coble (1996) C1 in Stedmon and Markager (2005b)
C5	< 250	398	Humic-like substance	Peak A in Coble (1996) C2 in Stedmon and Markager (2005b)
C6	425 (265)	522	Photodegradation-like substance Terrestrial humic-like substance	C7 in Stedmon and Markager (2005b)

effluents (Coble 1996; Murphy et al. 2011). C4 can also be associated with the non-biodegraded DOM in the STP effluent or with microbial products (Meng et al. 2013). C3 was indicative of the presence of a tryptophan-like substance of peak "T" from Coble (1996). In coastal waters, a significant source of the protein-like substance was thought to be from autochthonous production by phytoplankton (Yamashita et al. 2008), but also come in large supply from domestic wastewater and STP effluents, similar to humic-like substances (Meng et al. 2013; Parr et al. 2015). Fluorescence intensities of all components, except C5, were high in the STP effluent throughout the year (Figure S2).

Meanwhile, the fluorescence intensity of C5 was high at the lower station of the Arakawa River rather than that of the STP effluent. Therefore, a source other than the supply from STP was considered a significant factor.

HIX and SUVA are indices of humic substance and aromatic contents, respectively. These were relatively high in the STP effluent (HIX: 3.18 ± 0.31; SUVA: 2.62 ± 0.25, $n = 8$) and the upper station (HIX: 2.53 ± 0.70; SUVA: 3.72 ± 2.08, $n = 3$), progressively decreasing from the freshwater site to Tokyo Bay (Figure S3). Since the HIX had low values in freshwater areas and Tokyo Bay (HIX < 4), FDOM was mainly derived from biological and aquatic bacteria (Parlanti et al. 2006). BIX is an indicator of autotrophic productivity. BIX was relatively constant in the STP effluent (1.82 ± 0.20, $n = 8$) and surface bay water (1.85 ± 0.25, $n = 167$), which corresponded to recently produced DOM of autochthonous origin (> 1; Figure S3). Low HIX (< 4) and high BIX (> 1) of DOM derived from STP suggested that bacterial-derived FDOM was produced due to organic matter degradation at STPs. SR, which is negatively correlated to the molecular weight and aromaticity of DOM (Helms et al. 2008), was low in the STP effluent (0.84 ± 0.05, $n = 8$) and the upper station (0.89 ± 0.11, $n = 3$). SR progressively increased from the freshwater site to Tokyo Bay (Figure S3), suggesting a decrease in the average molecular weight with increasing salinity. This was the mixing effect of river water with high molecular weight and low SR and of open ocean water with low molecular weight and high SR. In addition, photodegradation of DOM could also convert high-molecular-weight organic matter into lower ones (Catalá et al. 2015).

RDOC concentrations correlated with each component of PARAFAC except for C5 ($R^2 > 0.9$, $n = 33$). Therefore, the components, except for C5, were relatively biologically recalcitrant to the degradation and proxy of RDOC, especially C1, C2, and C6. This agreed that RDOC concentrations showed a strong negative linear relationship with salinity (Fig. 5) and no differences before and after the degradation experiment (Fig. 6). Conversely, BDOC did not show good correlation with each component ($R^2 < 0.25$, $n = 33$). As the BDOC in natural systems contains a complex mixture of organic compounds with changing lability, BDOC was simultaneously decomposed and supplied by biological activity. However, there was a good correlation between BDOC and C3 components in Tokyo Bay water ($R^2 = 0.8$, $n = 14$). The tryptophan-like C3 is not only of terrestrial origin but also produced by phytoplankton and microbial metabolism (Romera-Castillo et al. 2010; Fukuzaki et al. 2014), and hence may be effective as a proxy for BDOC in the bay, especially for BDOC generated in the bay. However, the results of the degradation experiments in the bay are relatively scarce, and therefore, detailed observations remain warranted.

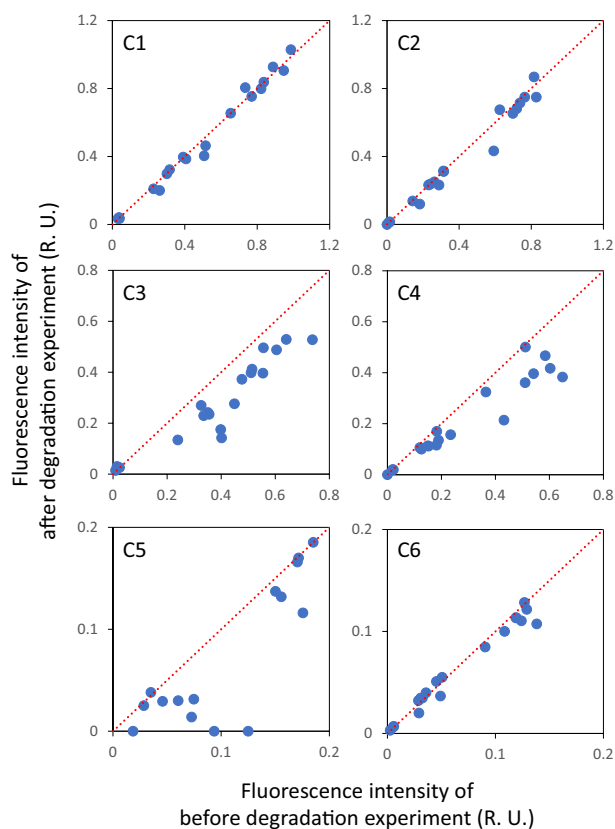### 3.3 Relationships of salinity with DOC and FDOM

RDOC and DOC concentrations showed a strong negative linear relationship with salinity ($p < 0.0001$), although BDOC concentrations did not show significant linear relationships ($p = 0.021$) (Fig. 5). RDOC was mainly supplied from the STP effluent because RDOC concentrations of the STP effluent were remarkably higher than that of the
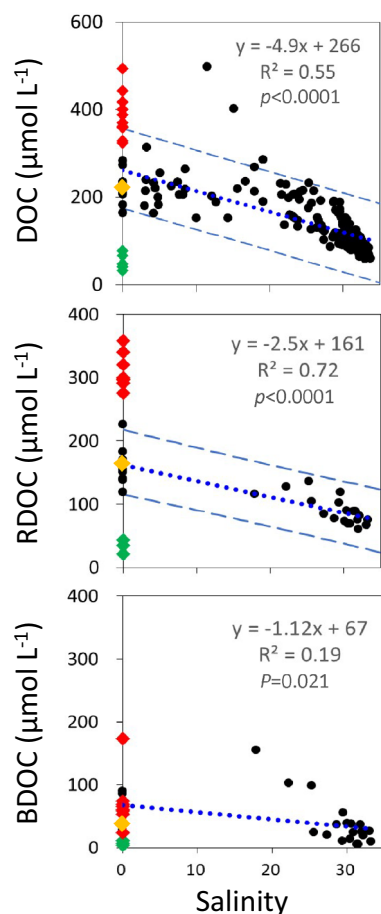
Fig. 5 Variation of DOC, RDOC, and BDOC against salinity in Tokyo Bay and river water. Black circles indicate data of Tokyo Bay and river water. Red, green, and yellow diamonds indicate the STP effluent, upper river water, and average value of STP effluent and upper river water, respectively. Dashed lines represent the 95% confidence interval of the linear regression
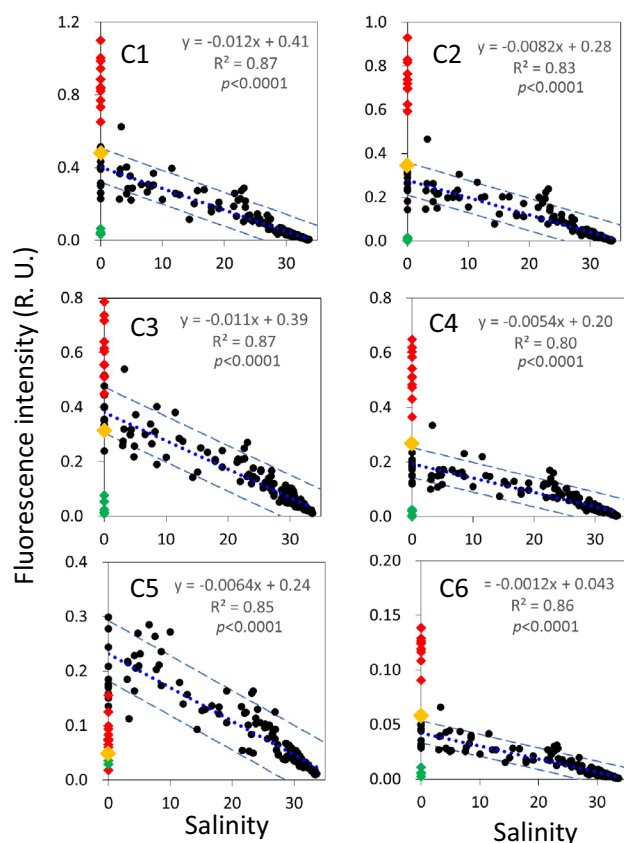

Fig. 6 Variation of each fluorescence intensity before and after the degradation experiment. The 1:1 line represents the expected relationship at a constant fluorescence intensity during the degradation experiments

water from the upper station of the Arakawa River. Therefore, RDOC concentrations were primarily controlled by mixing STP effluent and open ocean water due to the conservative distribution against salinity (Fig. 5). In this study, RDOC was defined as the DOC remaining after 150 days of incubation. Although a relatively long incubation period (> 40 days) is recommended for such culture experiments (Lønborg et al. 2012), the value of end-members would vary based on the RDOC definition.

Assuming that the incubation experiments in this study were completed within 40 days and degraded at the degradation rate constants of Kubo et al. (2015), the RDOC end-member value (yellow diamond of RDOC in Fig. 5) would be equivalent to approximately 10.5 µmol L^{-1} higher than that of this study. However, this error, which falls within the confidence interval of the regression line, did not significantly change our results.

DOC might have been derived from sediment resuspension in shallow brackish waters due to the high DOC concentration of pore water at the estuary of Tamagawa River, which was the watershed of the bay (Yasui et al. 2016). Although a negative linear relationship of DOC with salinity suggests a small effect, further studies are needed, especially in runoff events where resuspension is more likely to occur.

Each fluorescent component also demonstrated a strong negative linear relationship with salinity as with RDOC concentrations (Fig. 7). A decreasing trend in fluorescence intensity from the nearshore to offshore in Tokyo Bay indicates that most fluorescent components are also supplied mainly from the STP effluent. In addition, the intercept of the regression line and the end-members estimated from the STP effluent and the upper station of Arakawa River water corroborated each other (Table S1). The fluorescence intensity of each component was not significantly different between the value of the lower station and the averaged value of the upper station and the STP effluent, except for C5 (t test and $p > 0.05$). Furthermore, the high fluorescence intensity of the STP effluent suggested that FDOM was also mainly supplied by STP effluents and

Fig. 7 Variation of each fluorescence intensity against salinity in Tokyo Bay and river water. Black circles indicate data of Tokyo Bay and river water. Red, green, and yellow diamonds indicate the STP effluent, upper river water, and average value of STP effluent and upper river water, respectively. Dashed lines represent the 95% confidence interval of the linear regression

transported to the open ocean without decomposition in the bay. However, for the C5 component, there was a large discrepancy between the end-member values assumed from the upstream water of the Arakawa River and the STP effluent.

The fluorescence intensity of C5 was significantly higher at the lower station in contrast to the averaged value of the upper station and the STP effluent (t test and $p < 0.05$). Therefore, C5 is likely derived from a source other than that of the STP effluents and the upper river water. Previous studies have indicated that C5 may be organic matter derived from andosols (Chen et al. 2010; Yamashita et al. 2010), which are widely distributed in the watershed of the bay (Saigusa and Matsuyama 1998) and could be provided by groundwater inflow from riverbeds. Therefore, C5 may likely have been supplied by a different source than that of STPs and upstream waters. Observations from upstream to downstream of the Arakawa River on October 12 and 13, 2013 confirmed that C5 was supplied from midstream to downstream of the Arakawa River, although the other

components were primarily supplied from downstream (Figure S4).

Figure 6 shows the fluorescence intensity of each component before and after the degradation experiment ($n = 19$), which showed no difference in the before and after the degradation experiment for C1 ($97.6 \pm 9.2\%$), C2 ($95.8 \pm 12.7\%$), and C6 ($99.8 \pm 14.5\%$), suggesting that these were biologically recalcitrant components. Conversely, the fluorescence intensities of C3 ($83.4 \pm 37.6\%$), C4 ($77.2 \pm 13.5\%$), and C5 ($65.2 \pm 37.8\%$) slightly decreased after the degradation experiment, suggesting that they were semi-RDOC components compared to C1, C2, and C6. Most of the components tended to be transported out of the bay with little decomposing in the bay, where the water residence time is approximately 30–50 days (Okada et al. 2007). However, C3, C4, and C5 may be decomposed in the open ocean due to semi-recalcitrant characteristics. Therefore, C1, C2, and C6 can be used as proxies for biologically RDOC, but C2, C3, and C4 may be applicable as proxies of biologically semi-recalcitrant DOC. As no photodegradation experiments were conducted in this study, the effects of DOM photodegradation are unknown in the bay, even though DOC in rivers, coastal waters, and sewage treatment effluents has been reported to be photodegradable (e.g., Guéguen et al. 2016; Yin et al. 2021). Especially, Meng et al. (2013) reported that anthropogenic humic substances in STP influents are more susceptible to photodegradation than terrestrial organic substances. However, some organic matter may be already photodegraded before the effluent enters the coastal waters, because primary treatment at the STP is mainly conducted outdoor. In any case, future evaluations should be undertaken to quantify lability caused by the light, since the impact of photodegradation of DOC in the bay is unknown.

3.4 Relation of DOC and PARAFAC components to water quality

PCA was applied to the results of the river water, STP effluent, and Tokyo Bay water samples (Fig. 8). Salinity, temperature, DOC, RDOC, BDOC, FDOM optical parameters (six PARAFAC components and HIX, BIX, peak A:T, and FI), SUVA, and SR were used for the PCA. The first two PCA axes explained 69.9% of the total variance in the data set (PC1 = 55.8% and PC2 = 14.1%). Variables corresponding to recalcitrant and/or humic substances (RDOC, HIX, and SUVA) were closely associated with PC1 and represent most DOC. The PC1 axis showed strong positive loadings on DOC, RDOC, and FDOM (C1–C4 and C6, except for C5). These parameters were strongly influenced by the STP effluents and were also positively correlated with HIX and SUVA, suggesting humic-like with high aromaticity substances. Data on the STP effluent typically provided the highest DOC and RDOC concentrations, HIX, and
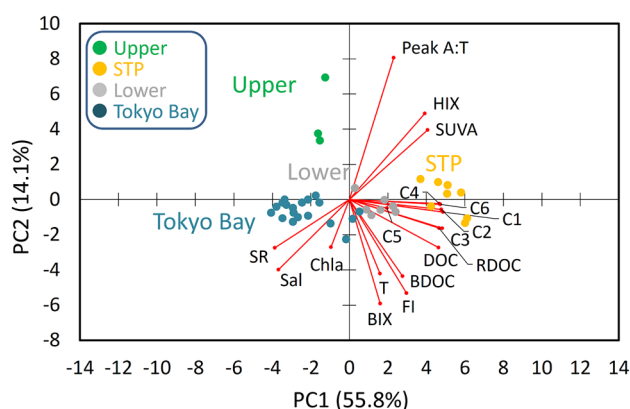
Fig. 8 Plots of loadings and scores of physicochemical variables in Tokyo Bay, river, and STP effluent. T and Sal indicate water temperature and salinity, respectively. HIX, BIX, peak A:T, and FI are indices of FDOM, while SR and SUVA are UV–visible absorbance spectra indices

SUVA (Figure S3). Meanwhile, variables corresponding to bioavailable and autochthonous substances (BDOC, FI, and BIX) were closely associated with PC2. The PC2 axis showed negative loadings on BDOC, BIX, FI, and Chl *a*, although BDOC concentrations were not significantly correlated with Chl *a* concentrations in the samples from Tokyo Bay. Therefore, in addition to the STP effluent, BDOC was supplied by bacteria in the bay, because high values of BIX in the bay (> 1; Figure S3) correspond to a predominantly autochthonous origin of DOM and the presence of DOM that was freshly released into the water (Huguet et al. 2009). The PCA result showed that each water sample from the STP and the lower station was more clustered. In contrast, the samples of Tokyo Bay were relatively scattered. The TPE samples during spring and summer, when salinity was low (22.8 ± 3.1; April−September), were close to the plots of the lower station, and hence, the TPE during spring and summer might have been more strongly influenced by freshwater and DOC derived from terrestrial origin. The results of the TPE samples during autumn and winter (28.5 ± 0.8; October–March) were close to those in central Tokyo Bay samples.

In highly urbanized coastal waters of Tokyo Bay with complete sewage maintenance, the STP effluent was a significant source of recalcitrant substances with humic-like characteristics and high aromaticity for coastal waters. Much of the bioavailable substances with tryptophan-like substances were not only supplied by STP effluent but also produced in the bay. Therefore, most of the organic matter supplied from STPs is RDOC. In addition, a carbon budget estimation in Tokyo Bay performed using the box model indicated that RDOC was mainly provided from STPs and was discharged out of the bay with little degradation (Kubo and Kanda 2020). In highly urbanized coastal waters with

complete sewage maintenance, the STP effluent is a significant source of recalcitrant substances with humic-like characteristics and high aromaticity for coastal waters.

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