# Contribution of macroalgae to coastal dissolved organic matter pool

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ABSTRACT

Dissolved organic matter (DOM) in coastal environments has various origins; one of the most intensely studied sources is terrestrial DOM input via rivers. On the other hand, contributions from other significant DOM sources, such as macroalgae, to the coastal DOM pool have not been extensively studied. In the present study, to quantify the contribution of macroalgae to the DOM pool in the coastal environment, we first carried out a bag-covering experiment on a brown alga, *Ecklonia cava*, and identified fluorescent DOM components by parallel factor analysis of three-dimensional excitation-emission matrix spectra. Using the fluorescent DOM as an indicator, we evaluated the horizontal distribution of macroalgal DOM in the coastal area, showing that the fluorescent DOM component had a synchronous gradient with dissolved organic carbon (DOC) concentrations along the transect line from the coast to offshore. On the basis of the correlation between DOC and fluorescent DOM, we evaluated concentrations of DOC originating from macroalgae, accounting for up to 20% of total DOC concentrations. Such finding implies that macroalgae have measurable contribution to coastal DOM pool in spite of past disregard of their role as DOM source.

*Keywords*: Macroalgae, DOM, DOC, EEM, PARAFAC

## 1. Introduction

Dissolved organic matter (DOM) is an important component of marine ecosystems, because it has various ecological roles such as being the largest organic carbon reservoir on earth (Hedges 2002), serving as an energy source in the microbial loop (Azam et al. 1983; 1993), transporting organic carbon (Bauer and Druffel 1998), an absorbing ultraviolet radiation (Blough and Del Vecchio 2002) and interacting with chemical pollutants (Sanchez-Marin et al. 2010). These ecological functions of DOM are commonly found in various ecosystems including coastal area. In particular, coastal zone provides great value of ecological service compared with other ecosystems (Costanza et al 1997), thus study on the dynamics of coastal DOM will be an important issue. Marine organisms have been considered as an important DOM sources (e.g., Hama et al. 2004; Kitayama et al. 2007; Condon et al. 2010), but input of terrestrial DOM via rivers also constitutes a part of coastal/estuary DOM pools (Blough and Del Vecchio 2002; Cauwet 2002). In coastal environments, riverine input has been considered an important DOM source, and the contribution of terrestrial DOM has been estimated on local, regional and global scales (Meyer-Schulte and Hedges 1986; Opsahl and Benner 1997; Cauwet 2002). The DOM pool in the estuaries of large rivers is strongly affected by terrestrial DOM (Harvey and Mannino 2001; Goñi et al. 2003), but such environments are of limited extent in some coastal areas.

Macroalgae are one of the most important primary producers in coastal ecosystems where their productivity often exceeds phytoplankton and other benthic carbon fixer such as seagrass, coral and benthic microalgae (Mann 1973; Yokohama et al. 1987; Alongi 1998). It is well known that organic matter from macroalgal tissue supports various organisms such as mesograzers (Mann 1973; Itoh et al. 2007), suspension feeders of detrital macroalgae (Duggins et al. 1989; Duggins and Eckman 1997) and heterotrophic microbes (Mann 1988; Rieper-Kirchner 1989; Uchida 1996). In addition, production of DOM is known to be one of

the important fates of macroalgal production, and a large part of the photosynthetic products of macroalgae are released into ambient seawater as DOM (20-40%; Khailov and Burlakova 1969; Abdullah and Fredriksen 2004; Wada et al. 2007). Considering that these estimates are higher than other primary producers such as phytoplankton (around 10%) (Baines and Pace 1991; Hama and Yanagi 2001), macroalgae could be the most important DOM producer in coastal environments. However, the above studies on macroalgal DOM were based on examination with incubation method for macroalgae using closed bag or chamber set up in laboratory or natural environment, and the distribution of macroalgal DOM in the natural marine environment is unknown. Therefore, the contribution of macroalgae to the coastal DOM pool has not yet been studied.

To quantify the macroalgal DOM in seawater, an effective approach would be to use some organic component as an indicator of macroalgal DOM. In our previous study, collection of macroalgal DOM was achieved by covering bags on a brown alga *Ecklonia cava* Kjellman, which is a common species in the northwestern Pacific Ocean, and analyses of fluorescent spectra showed that fluorescent DOM (F-DOM) originating from *E. cava* contains humic-like fluorophores (Wada et al. 2007). Since macroalgal release of humic-like material with yellowish color had been known in various macroalgal species (Fogg and Boalch 1958; Craigie and McLachlan 1964; Hulatt et al. 2009), the distribution of macroalgal DOM might be evaluated based on that of the humic-like fluorophore. Although humic substances have also been used as an indicator of terrestrial DOM (Klinkhammer et al. 2000), our research site (Oura bay) has little riverine effect because a river does not feed directly into the bay and there is a rapid turnover rate of the seawater in the bay (detailed description is in material and methods, and discussion sections). Therefore the choice of this research site allows us to use the humic-like fluorophore as an indicator of macroalgal DOM in natural coastal environments.

In the present study, we have carried out bag-covering experiment according to Wada et al. (2007) using *E. cava* to identify the F-DOM component originating from macroalgae. *E. cava* is the dominant species in Oura bay, and their cover degree (composite community consisting of *E. cava* and other macroalgal species) is 50-90% (Biodiversity Center of Japan 2011). Using the identified F-DOM components as an indicator, we provide novel evidence of the macroalgal contribution to the coastal DOM pool by transect seawater sampling from nearshore algal beds to offshore waters. Since there are only small effects from riverine DOM into Oura bay, as shown by the analysis of estuary water from rivers around Oura bay, we can quantify the macroalgal DOM based on the distribution of F-DOM.

## 2. Materials and methods

### 2.1. Description of the research site

The bag-covering experiment was carried out at a central area in Oura bay (34° 39′N, 138° 56′E), and distribution of macroalgal DOM was evaluated on a transect line from the near algal bed in the bay to the offshore (34° 36′N, 138° 59′E) (Fig. 1). Oura bay is located on the coast of Shimoda city, Izu Peninsula, Japan, and the maximum depth is about 14 m (mean: about 8 m). It is the habitat for various macroalgae, e.g., *E. cava*, *Sargassum* spp. and *Eisenia bicyclis* (Yokohama et al. 1987; Mikami et al. 2006; Wada et al. 2008), and *E. cava* is the dominant species (Biodiversity Center of Japan 2011). On the transect line, St. 1 is the nearest station to an algal bed and St. 10 is about 7 km off the coast. The largest rivers around our research site are the Aono and Inozawa rivers, but these rivers do not flow directly into Oura bay.

### 2.2. Collection of dissolved organic matter derived from macroalgae

As described in Wada et al. (2007), SCUBA divers used a transparent bag with a stopcock to cover a whole blade of *E. cava* growing in Oura bay in November 2010 (duplicate individuals). Briefly, we covered all blades of an individual *E. cava* with a transparent bag containing ambient seawater, and the open end of the bag was tied up at the algal stipe. In addition to two bags covering *E. cava*, we set up another two bags without algae as control samples (duplicate). Duplicate samples were collected for each bag through the stopcock using a 100-ml glass syringe (reproducibility of DOC concentrations: 7.0 ± 9.1%) at each sub-sampling time (0, 4, 24, and 29 h). The seawater was filtered through pre-combusted (450°C, 4 h) glass-fiber filters (Whatman, GF/F) immediately after collection. The filtrate was transported in acid cleaned polycarbonate bottles, and stored at below -20°C until analysis.

### 2.3. Seawater sampling on the transect line from the near algal bed to offshore

We collected seawater samples (duplicate) at 1-m depth using a Niskin bottle at stations

1-10 from the near algal bed in Oura bay to the offshore region (Fig. 1) in October and December 2010 (Sts. 1-4, 6, and 10) and May 2011 (all stations). The conductivity, temperature and depth (CTD) were logged using a CTD probe (Idronaut, Ocean Seven 301). The seawater samples were filtered through a GF/F filter, and the filtrate was stored in polycarbonate bottles below -20°C until analysis.

**2.4. Collection of estuary waters**

In October and December 2010, and May 2011, we collected surface water from the estuary zone of 2 major rivers (Aono and Inozawa rivers) using a plastic bucket. These rivers are near Oura bay, but the mouths of these rivers are outside of the bay. We confirmed using a salinometer (Atago, Maste-AS/Millα) that the salinity of the sample was near zero. The samples were filtered through a GF/F filter, and the filtrate was stored in polycarbonate bottles below -20°C until analysis.

**2.5. Analysis**

The high temperature catalytic oxidation method using a total carbon analyzer (Shimadzu, TOC-V) was employed to measure the dissolved organic carbon (DOC) concentration after acidification of the sample with HCl (Wada and Suzuki 2011). The quantification limit (3σ limit) of DOC concentrations was 0.304 mg C $l^{-1}$. Analysis of F-DOM was performed using a fluorometer (Hitachi F-4500) in three-dimensional excitation-emission matrix (3D-EEM) scanning mode. The excitation and emission spectra was collected at 5- and 2-nm intervals and in the ranges 230-450 and 300-500 nm, respectively. The bandpass widths were 10 nm for both excitation and emission, and the scan speed was 2400 nm $min^{-1}$. The spectrum of milli-Q water was subtracted as the blank, and the fluorescent intensity was normalized using the value from a quinine sulfate solution (10 μg $l^{-1}$ in 0.1 N $H_2SO_4$ solution) at 350/450 nm as 10 quinine sulfate unit (QSU) (Wada et al. 2007).

Using the average values of 3D-EEM spectra between duplicate samples, we identified

components of F-DOM by parallel factor analysis (PARAFAC, Stedmon et al. 2003) with MATLAB (Mathworks) which statistically decomposed the spectra into each component. The evidence for the validity of PARAFAC model was provided with split-half analysis (Stedmon and Bro 2008) in which good agreement was obtained for excitation and emission loadings between two randomly divided datasets. For the samples collected from the bag covering on *E. cava*, three components, M1, M2 and M3 were identified by the PARAFAC analysis. This analysis was also applied for the seawater samples along the transect cruise, and four components, S1, S2, S3 and S4 were identified. The wavelengths (Ex/Em) at the top of the peaks were described in Table 1.

## 3. RESULTS

### 3.1. Collection of macroalgal DOM in the bag-covering experiment

At the start of the experiment, DOC concentrations in the bags with *E. cava* were 0.863-0.867 mg C $l^{-1}$, which were similar to those in the control bags (0.809-0.945 mg C $l^{-1}$). DOC concentrations in the bags with *E. cava* increased linearly with time, and the values at the end of the experiment were 2.25-2.33 mg C $l^{-1}$. These values were 2-3 times higher than those in the control bags (0.872-0.896 mg C $l^{-1}$), demonstrating that the increase in DOC in the bag was due to the release of DOM from *E. cava*. Contamination of DOC from the bag would be negligible, because the concentrations in the control bag were mostly constant throughout the experimental period as well as Wada et al. (2007). Wada et al. (2007) had also carried out five times bag-covering experiment in same way, and the DOC concentrations of the seawater in the control bag were constant for 54-102 hrs. Since the DOC concentrations partly depend on the water volume in the bag and the size of the algal body, we normalized them as DOC per dry weight (N-DOC) according to Wada et al. (2007) using the following equation:

$$\text{N-DOC (mg C g (dry weight)}^{-1}) = (\text{DOC}_{\text{sample}} - \text{DOC}_{\text{control}}) \times V / W$$

where $\text{DOC}_{\text{sample}}$ and $\text{DOC}_{\text{control}}$ are the DOC concentrations (mg C $l^{-1}$) in the sample and the control bags, respectively. $V$ and $W$ are the seawater volume (l) in the sample bags and the dry weight of the plant blades (g), respectively. The values of N-DOC were near zero at the start of the experiment (-0.011 mg C g (dry weight)$^{-1}$), and increased with time. At the end of the experiment, the values were 1.07-1.44 mg C g (dry weight)$^{-1}$ (Fig. 2a). Using the increase in N-DOC between 0 and 29 h, we calculated the DOC production rate per dry weight in November 2010 to be 0.893-1.20 mg C g (dry weight)$^{-1}$ $d^{-1}$, consistent with the results of Wada et al. (2007), in which similar values were estimated for a similar season (1.5 and 1.2 mg C $g^{-1}$ $d^{-1}$ in October 2003 and December 2004). The macroalgal DOM in the bag would be

partly consumed by heterotrophic bacteria, and our estimate of DOC production rate implies net value. However, we consider that bacterial activity has just negligible effect on the estimate of DOC production rate, because our previous study on bacterial decomposition had shown less availability of DOM originated from *E. cava* (decomposition rate: 0.58-3.4% $d^{-1}$) (Wada et al. 2008). Using PARAFAC analysis, we identified three F-DOM components, M1, M2 and M3 (Fig. 3a-c). All F-DOM components had a single emission maximum, and double excitation maxima. There are several studies on the spectral characteristics of F-DOM components in various aquatic environments (Table 1; Coble 1996; 2007), and our identification was based on the peak positions from those studies. The emission wavelength of the M1 component was 388 nm, and there were double excitation maxima at 240 and 290 nm. Although the peak at 240/388 nm has not yet been defined, another peak at 290/388 nm is similar to those of a marine humic-like fluorophore. The M2 component also has a single emission maximum and double excitation maxima. The peak at 255/452 nm resembles that of a humic-like fluorophore in the ultraviolet region and the peak at 350/452 is similar to a visible humic-like one. The peaks for the M3 component had relatively short wavelengths, 275/332 nm, which are similar to those from a protein-like fluorophore.

With respect to the M1 and M2 peaks, initial intensities in the bag with *E. cava* were 0.62-0.65 and 0.48-0.49 QSU, respectively. These values increased with time, and at the end of the experiment were 0.87-1.3, and 1.6-1.8 QSU, (Fig. 2b and c), which are about 2-3 times higher than those in the control samples (0.56-0.63 and 0.66-0.66 QSU at the end of the experiment). The intensity of the M3 peak also tended to increase with time, but at the end of the experiment (2.1-3.3 QSU) was just a little higher than those of the control samples (1.8-2.2 QSU) (Fig. 2d). The initial intensity of the M3 peak in the control bag was 1.9 times higher than that in the bag with *E. cava*. The reason is unclear, but one of the possible reasons is that protein in natural seawater was rapidly decomposed in the bag with *E. cava*. As shown

in a previous report (Sakami and Sugiyama 1994), bacterial community and their substrate preference on the surface of macroalgal body would be characteristic. Therefore, we considered that microbiota in seawater changes after covering bag on *E. cava*, and it might induce the alteration of decomposition process. While such alteration would have little effect on the dynamics of macroalgal DOM due to its refractory property (Wada et al. 2008), protein in natural seawater is highly labile and decomposed within short period (turnover time: 0.5-33 and 4-82 hours; Keil and Kirchman 1993 and 1999, respectively), leading the difference in the intensity of the M3 peak.

### 3.2. Distribution of DOM along transect line

The average value of the duplicate samples for the DOC concentrations in Oura bay (Sts. 1-4) in October, December and May were 0.85-0.94, 0.71-0.77 and 0.73-0.92 mg C $l^{-1}$, respectively, being higher than those for the offshore region (Sts. 5-10: 0.87-0.89, 0.72-0.73 and 0.72-0.76 mg C $l^{-1}$, respectively) (Fig. 4a). Application of PARAFAC analysis for 3D-EEM spectra showed four F-DOM components, S1, S2, S3 and S4, although the peak shape of S4 was unclear (Fig. 3d-g). The other components, S1, S2 and S3, had similar fluorescent properties with M1, M2 and M3 F-DOM components, respectively, based on the peaks shapes (Fig. 3) and the profiles of the spectral loadings of excitation and emission of the peaks (Fig. 5). In addition, we calculated Kendall's coefficient of concordance (W) using the values of loadings, and high values were obtained for all of the peaks (more than 0.97), showing significant similarity of spectral characteristics between macroalgal and marine DOM ($P<0.01$).

The peak intensities of S1 and S2 were higher in Oura bay (S1: 1.1-1.8, 0.76-0.85 and 1.0-1.7 QSU, and S2: 0.67-1.4, 0.44-0.52 and 0.73-1.3 QSU in October, December and May, respectively), and clearly decreased toward offshore (S1: 0.70-1.2, 0.63-0.66 and 0.72-1.0 QSU, and S2: 0.43-0.68, 0.34-0.34 and 0.47-0.74 QSU in October, December and May,

respectively) (Fig. 4b and c), showing that these F-DOM components originated near the coast of Oura bay. Intensities of S3 were also higher at the stations in Oura bay, but relatively high intensities were sometimes found in the offshore region (St. 6 in October and December 2010; St. 9 in May 2011) (Fig. 5d). Such a distribution suggests that the origins of the S3 component might be both in Oura bay and offshore (e.g., phytoplankton). The salinity of seawater samples were calculated from the CTD values of according to UNESCO 1978, and the values were 33.6-33.8, 33.3-34.4 and 33.9-34.5 in October, December and May, respectively (data not shown).

### 3.3. DOC concentrations in estuary waters

DOC concentrations in estuary waters from the Inozawa and Aono rivers were 0.55-0.86 and 0.66-0.97 mg C $l^{-1}$, respectively (Table 2), which are comparable or a little lower than those in coastal region (Sts. 1-10: 0.71-0.92 mg C $l^{-1}$).

## 4. Discussion

In the bag-covering experiment, the intensities of the M1 and M2 peaks, which correspond to marine humic-like and UV/visible humic-like materials (Coble 1996) increased with time (Fig. 2b and c), showing the release of humic-like material from *E. cava*. Considering that the macroalgal release of humic-like substances has been known using various algal species (e.g., *E. cava*, *Fucus vesiculosus*, *Laminaria hyperbrea*, *Ascophyllum nodosum*, *Chadophorasle* sp.) (Craigie and McLachlan 1964; Sieburth and Jensen 1969; Wada et al. 2007; Jiang et al 2010), the release of DOM with humic-like characteristics from *E. cava* is consistent with the previous findings.

The F-DOM components identified by seawater sampling along a transect line were S1, S2, S3 and S4, and two of them, S1 and S2, had similar spectral characteristics to M1 and M2, respectively (Fig. 3, 4). Such spectral similarities suggest that the organic composition of the coastal DOM pool partly reflects the production of macroalgal DOM. In the horizontal profiles of F-DOM components, the intensities of the S1 and S2 peaks were higher at the stations near the coast of Oura bay compared to those in the offshore region (Fig. 4b and c), showing the presence of sources of S1 and S2 near the coast of the bay. Since there is community area of *E. cava* near the coast of Oura bay (Wada et al. 2008; Biodiversity Center of Japan 2011), the origin of the S1 and S2 components is likely to be macroalgae in Oura bay. Unlike the S1 and S2 components, the intensity of S3 is sometimes higher in offshore region. We consider that the origin of the S3 component is not only macroalgae but also other source such as phytoplankton, being consistent with the previous reports that phytoplankton-derived protein has been found in various natural environments (Moncheva et al. 2003; Powell et al. 2005). The S4 peak had been identified by other previous studies (Murphy et al. 2008; Yamashita et al. 2010), and they suggested this peak would be an artifact in the fluorescent analysis. Therefore, we used the two peaks (S1 and S2) as the fluorescent tracer of macroalgal

DOM.

Another possible origin of the F-DOM components in the coastal area is riverine input, because terrestrial DOM also contains humic substances (Coble 1996; Stedmon et al. 2003). In the present study, we evaluated the contribution of terrestrial DOM based on the salinity at each station and the DOC concentrations of estuary water from the Aono and Inozawa rivers. The salinity values at Sts. 1-10 were in the ranges of 33.56-33.81, 33.30-34.42, and 33.85-34.45 in October, December and May, respectively, and the maximum difference in salinity between the seawater in Oura bay and in the offshore region was just 1.12, showing the small contribution from freshwater (up to 3% of water volume). In addition, DOC concentrations in the estuary water from Aono and Inozawa rivers were 0.66-0.97 and 0.55-0.86 mg C $l^{-1}$, respectively (Table 2), which are similar to those in the seawater along the transect line (0.85-0.94, 0.71-0.77 and 0.72-0.92 mg C $l^{-1}$ in October, December and May, respectively), showing that riverine input of terrestrial DOM has a negligible contribution to the DOM pool along the transect line. As a conclusion, we provide three piece of evidence in the present study, similarities of spectral characteristics of fluorescent components between macroalgal and coastal DOM, higher fluorescent intensities near algal bed, and negligible contribution of riverine DOM that show the origin of the F-DOM components, S1 and S2, is macroalgae in Oura bay.

The DOC concentrations in Oura bay were 0.85-0.94, 0.71-0.77 and 0.73-0.92 mg C $l^{-1}$ in October, December and May, respectively, which were generally higher than the values in the offshore region (0.88 ± 0.017, 0.72 ± 0.0046 and 0.74 ± 0.013 mg C $l^{-1}$) (Fig. 5a), consistent with the profiles of the F-DOM components, S1 and S2 (Fig. 4b and c). In the case of the data from the stations in Oura bay (Sts. 1-4), the DOC concentrations and the intensities of the S1 and S2 peaks were significantly correlated ($r^2$=0.706 and 0.600, $P<0.01$), but this was insignificant for the samples in offshore region (Sts. 5-10) (Fig. 6). Considering that the

S1 and S2 peaks originated from macroalgal DOM as discussed above, the spatial variation of the DOC concentrations in Oura bay is strongly influenced by macroalgal DOM. Our analysis based on the horizontal distribution of DOM is useful in Oura bay, but it might be difficult to adopt such approach in other coastal area with significant source of terrestrial DOM. In such area with terrestrial DOM, we consider that analysis of organic component which is typical of macroalgal DOM is effective. Considering that macroalgal DOM has characteristics which contains mucous polysaccharides (Wada et al. 2007), analysis of carbohydrate composition might allow us to make the distribution of macroalgal DOM more clear.

The slopes of the regression curve relating DOC concentrations and the intensities of the S1 and S2 peaks were 0.213 and 0.207, respectively (Fig. 6a and b), and they represent the ratios of the DOC concentration and intensities of F-DOM components of macroalgal DOM. Using these values, we can calculate the macroalgal DOC concentration in seawater in Oura bay according to following equation,

$$\text{M-DOC} = S \times (FI_{bay} - FI_{offshore})$$

where S is the slope of the regression curve, and $FI_{bay}$ is the fluorescent intensities at each stations (Sts. 1-4) in the bay, and $FI_{offshore}$ is the average value of the fluorescent intensities in the offshore region (Sts. 5-10). Based on the intensities of S1 and S2, we calculated the concentration of macroalgal DOC to be 0.025-0.19 and 0.022-0.18 mg C $l^{-1}$, respectively, accounting for 3.5-20% (S1) and 2.7-19% (S2) of total DOC concentrations (Fig. 7). These estimates imply the novel evidences that macroalgae have measurable contribution (up to 20% of total DOC concentrations) to the coastal DOM pool and that macroalgae are one of the major factors controlling the spatial variation in DOC concentrations.

Contribution of macroalgae to the coastal DOM pool would depend on the macroalgal production of DOM and physical mixing of seawater between inside and outside of the bay. DOM production of macroalgae has been reported for various species (Khailov and

Burlakova 1969; Sieburth 1969; Abdullah and Fredriksen 2004; Wada et al. 2007), and the macroalgal species in Oura bay (e.g., *Ecklonia cava, Eisenia bicyclis, Sargassum spp*.) were commonly found in North Pacific regions and congeneric species are globally distributed (De Vooys 1979). In addition, the productivity of macroalgae in Oura bay (around 1000 g C $m^{-2}$ $y^{-1}$; Yokohama et al. 1987) is similar to those in other regions (Mann 1973; Alongi 1998), suggesting that the capacity of DOM production of macroalgae in Ouar bay could be comparable with other coastal ecosystems.

In addition to the capacity of DOM production, the turnover rate of seawater in the bay is also an important factor controlling the macroalgal contribution to the DOM pool, because macroalgae are sessil organisms in coastal areas and the DOM produced by macroalgae would be dispersed by mixing of the water mass. In order to estimate an approximate indication of the turnover rate of seawater in Oura bay, we placed a CTD probe at a depth of 1 m near St. 2 after a hard rain in December 2010 (precipitation 33.5 mm within one day). Because Oura bay is shallow (depth <14 m), the seawater in the bay was measurably diluted by rainwater, and the salinity decreased to 33.4 immediately after the rain. The salinity increased with time due to exchange with seawater and stabilized at 33.7 after 12 h (data not shown), showing that the turnover rate of water in the bay is 2 per day (2 $d^{-1}$). Since the turnover rate would be variable due to water current and landform, we cannot simply compare the value in Oura bay to those in other local bay. However, similar timescales were also reported in other local coastal area (e.g., Gokasho bay: 0.15-3.45 $d^{-1}$, Bora bay: 2.4-16 $d^{-1}$; Uchida et al. 1998, Casareto et al. 2000), supporting our idea that macroalgal contribution to the coastal DOM pool could be found in other coastal environments.

## 5. Conclusion

In the present study, we examined the applicability of F-DOM component as the indicator of macroalgal DOM based on three different evidences (similarity of fluorescent

characteristics, high intensity near algal bed and negligible contribution of riverine input). In addition, we estimated the concentrations of DOC derived from macroalgae using the correlation between bulk DOC concentration and intensity of F-DOM component. The concentrations of macroalgal DOC account for up to 20% of bulk DOC concentrations, implying that macroalgae would have measurable contribution to the coastal DOM pool.

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Figure legends

Figure 1 Location of study area.

Figure 2 Time course of N-DOC and fluorescent intensities in bag-covering experiment. Ordinate shows N-DOC (mg C $g^{-1}$) or fluorescent intensities normalized as QSU. Abscissa is the time after covering bag (h). The panels of the values of (a) N-DOC, and the intensities of (b) M1, (c) M2 and (d) M3 were shown. Filled and open squares are bag with and without *E. cava*, respectively. Plot is the average values and error bars are range between duplicate data.

Figure 3 Fluorescent components identified by PARAFAC analysis. Ordinate and abscissa are excitation and emission wavelengths (nm), respectively. Contour line shows the fluorescent intensities. Fluorescent components of macroalgal DOM and DOM in seawater samples are (a) M1, (b) M2 and (c) M3, and (d) S1, (e) S2, (f) S3 and (g) S4, respectively.

Figure 4 Horizontal distribution of DOC concentrations and intensities of fluorescent components. Ordinate shows the DOC concentrations (mg C $l^{-1}$) or fluorescent intensities normalized as QSU. Abscissa is the sampling station number. The horizontal distributions of (a) DOC concentrations, and fluorescent intensities of (b) S1, (c) S2 and (d) S3 were shown. Open square, open triangle and filled diamond show the distributions in October and December 2010, and May 2011, respectively. Error bars of the data on DOC concentrations were range of duplicate samples. We did not show the error bar in the data of fluorescent components, because the PARAFAC analysis was carried out on the average of duplicate analysis of EEM spectra.

Figure 5 Profiles of loadings of each peak. Ordinate and abscissa are intensity (QSU) and wavelength (nm), respectively. Open and filled circles mean the peaks of macroalgal and seawater DOM, respectively. Each panel shows the loadings of excitation of M1 and S1 (a), M2 and S2 (b) and M3 and S3 (c), and emission of M1 and S1 (d), M2 and S2 (e) and M3 and S3 (c), respectively.

Figure 6 Relationship between DOC and fluorescent intensities. Ordinate and abscissa are DOC concentrations and fluorescent intensities, respectively. The relationships of DOC and S1 and S2 in Oura bay (Sts. 1-4) were shown in (a) and (b), and those in offshore region (Sts. 5-10) were shown in (c) and (d), respectively.

Fig. 7 Total DOC concentrations and proportion of M-DOC in total DOC concentrations at the stations in Oura bay. Number on the Abscissa is No. of each station. Line plot is the total DOC concentrations (mg C $l^{-1}$), and bar is the proportions of M-DOC in total DOC concentrations (%). Filled and open bars are the values calculated using the intensities of S1 and S2, respectively.

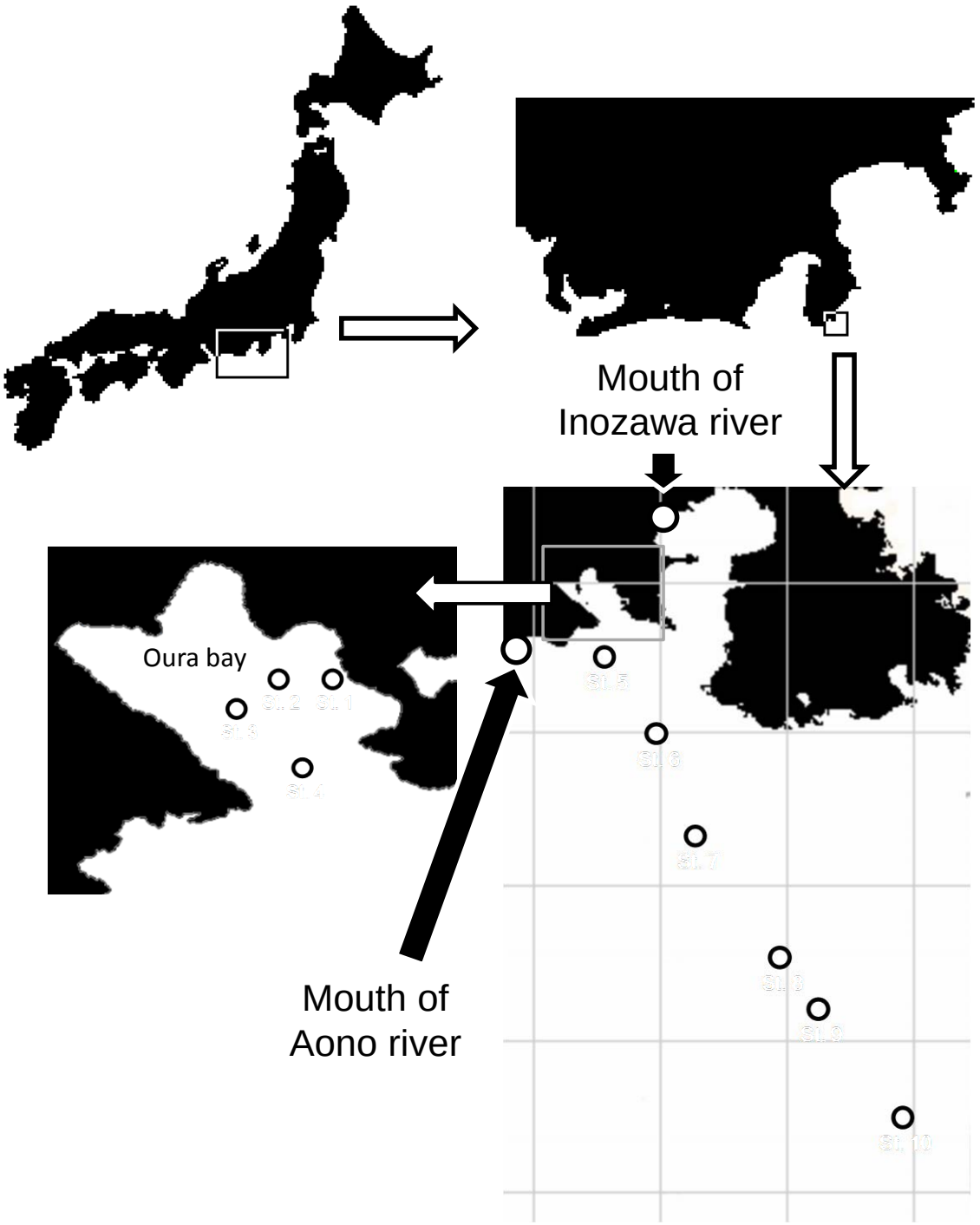


Fig. 1 Location of study area.

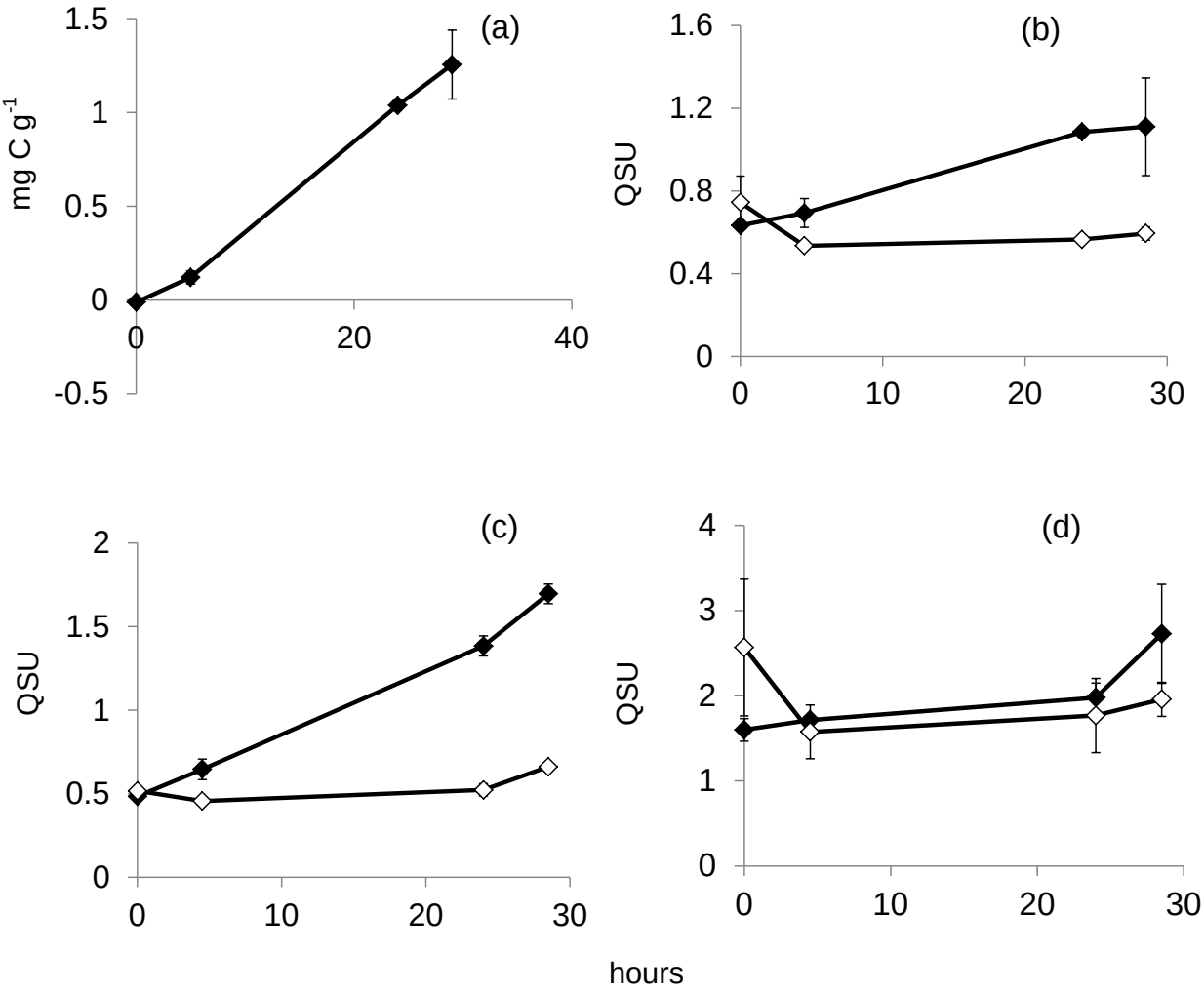


Fig. 2 Time course of N-DOC and fluorescent intensities in bag-covering experiment.

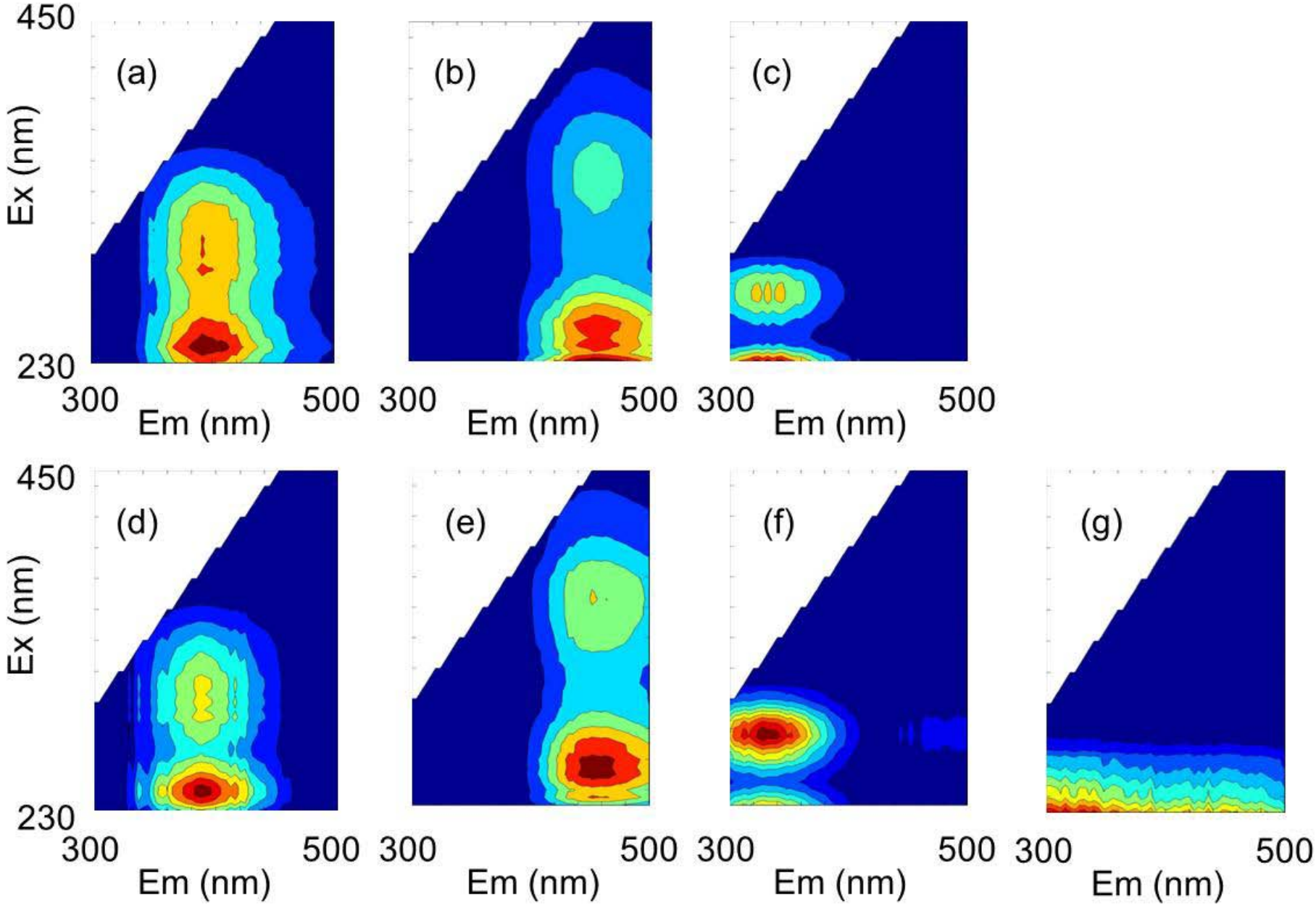


Fig. 3 Fluorescent components identified by PARAFAC analysis.

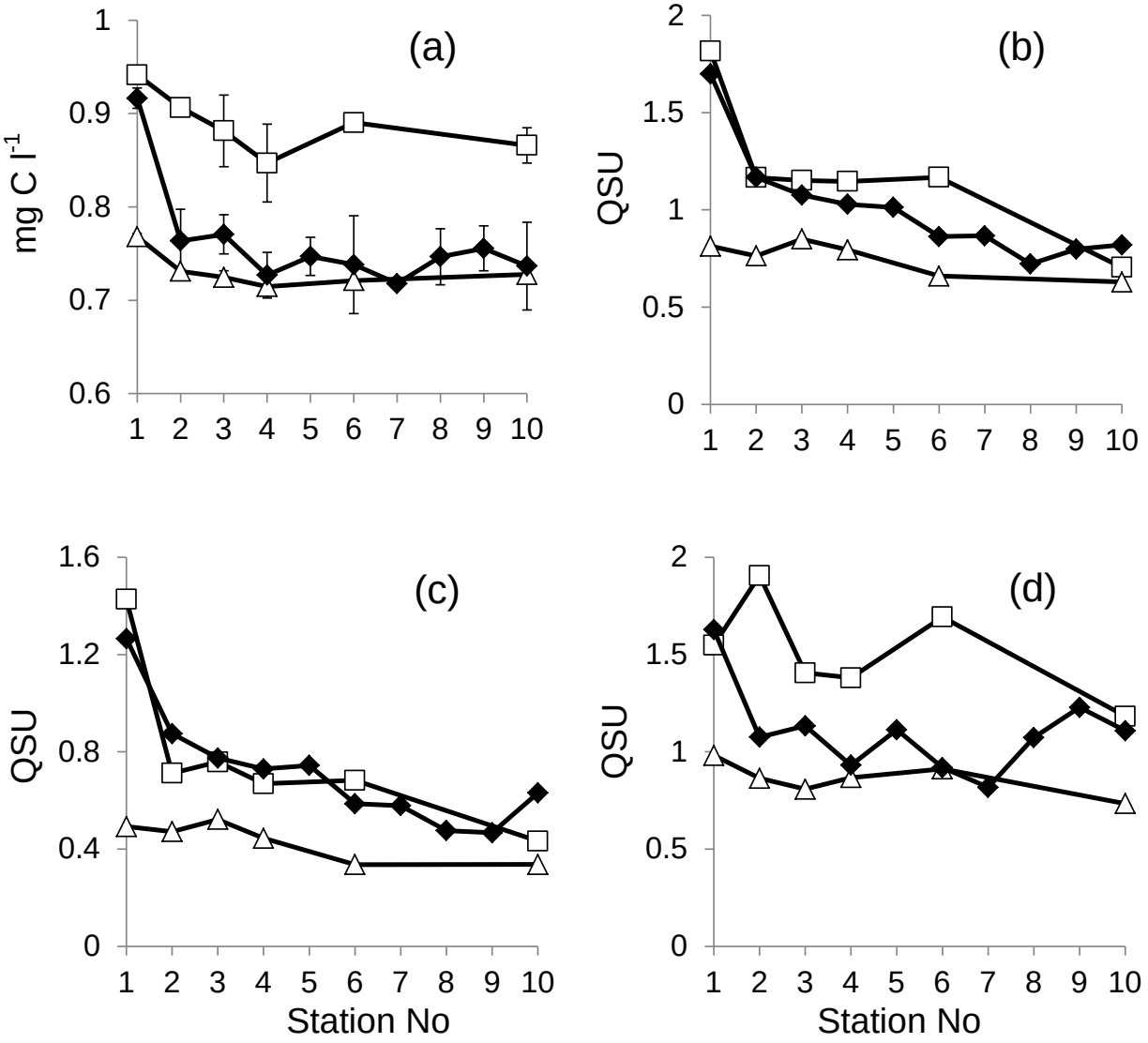


Fig. 4 Horizontal distribution of DOC concentrations and intensities of fluorescent components.

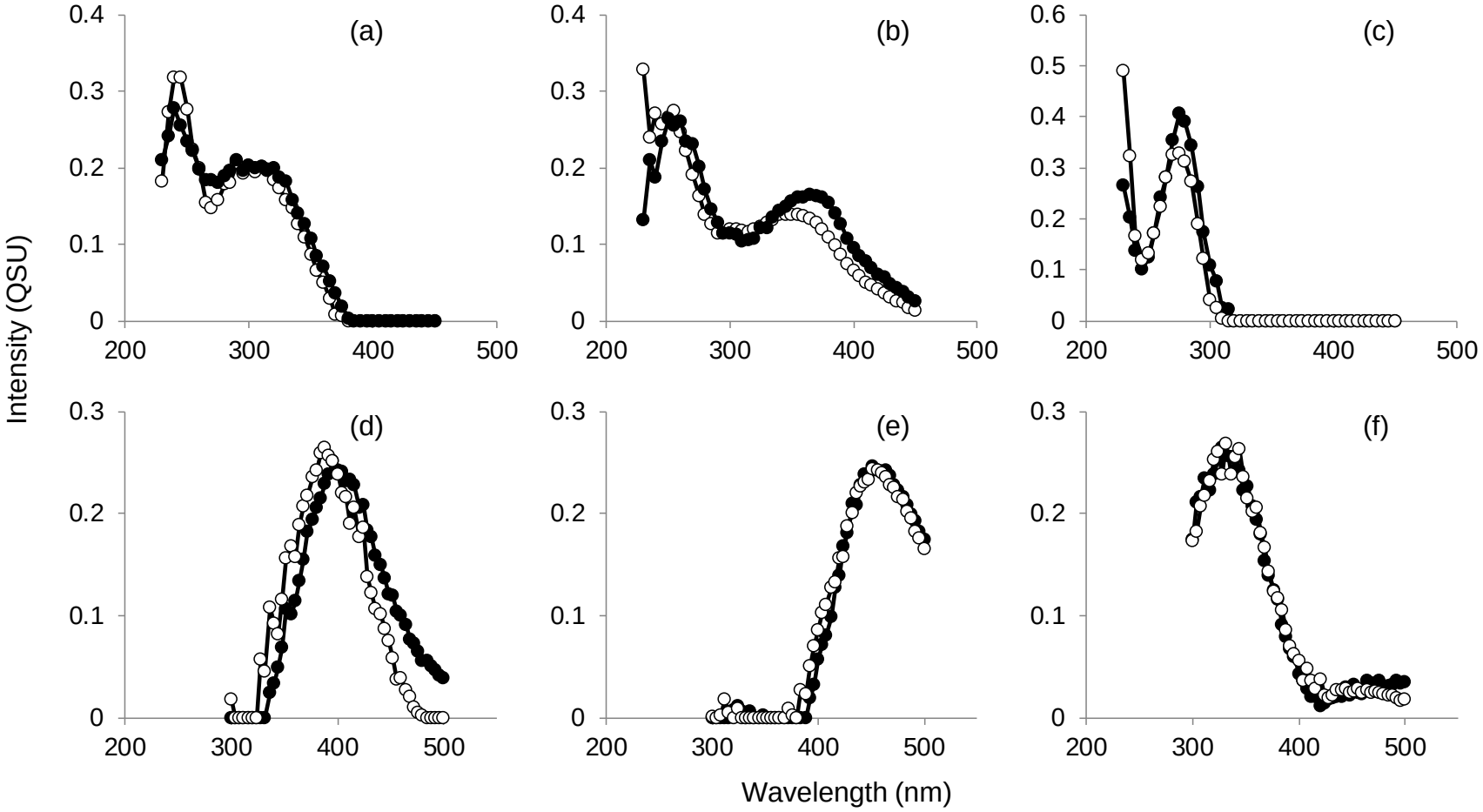


Fig. 5 Profiles of loadings of each peak

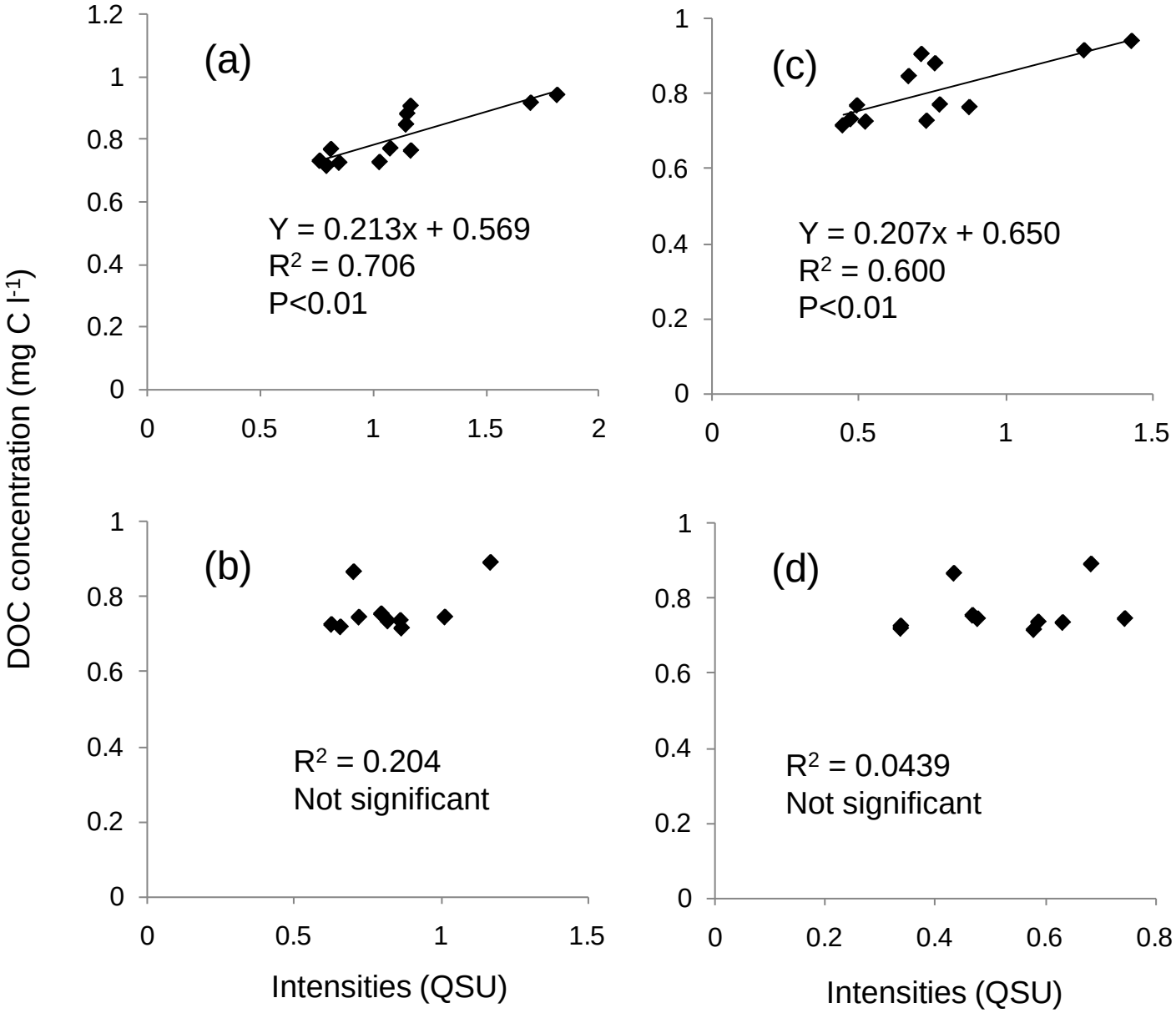


Fig. 6 Relationship between DOC and fluorescent intensities.

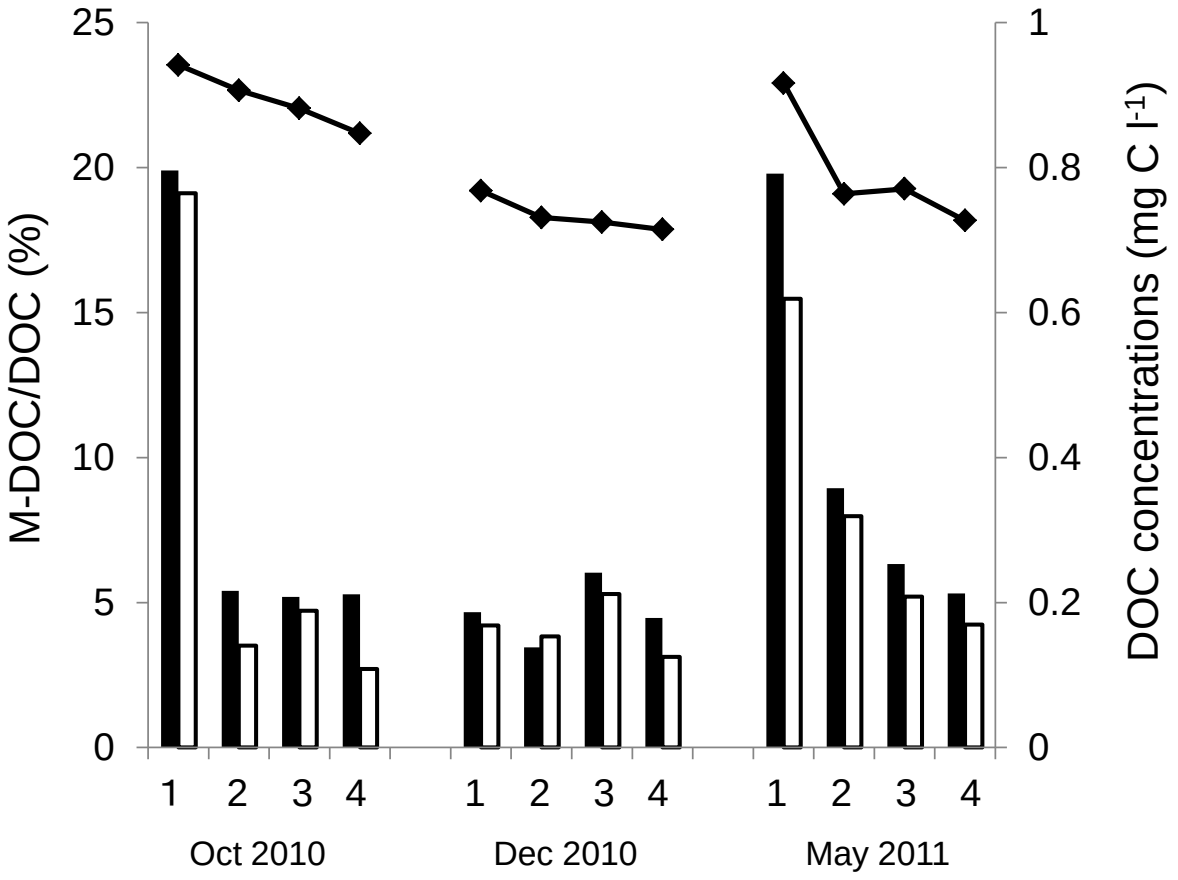


Fig. 7 Total DOC concentrations and proportion of M-DOC in total DOC concentrations at the stations in Oura bay.

Table 1 Wavelengths (Ex/Em: nm) at the top of each peak and previous report (Coble 1996).

| Peak | Wavelengths | | |
|---|---|---|---|
| M1 | 240/388 & 290/388 | | |
| M2 | 255/452 & 350/452 | | |
| M3 | 275/332 | | |
| S1 | 240/392 & 290/392 | | This study |
| S2 | 250/452 & 365/452 | | |
| S3 | 275/328 | | |
| S4 | Not clear | | |
| B | 275/310 | Tyrosine-like, Protein-like | |
| T | 275/340 | Tryprophan-like, Protein-like | |
| A | 260/380-460 | Humic-like | Coble (1996) |
| M | 312/380-420 | Marine humic-like | |
| C | 350/420-480 | Humic-like | |

Table 2 DOC concentrations (mg C $l^{-1}$) in estuary of Aono and Inozawa rivers.

| | October 2010 | December 2010 | May 2011 |
|---|---|---|---|
| Aono river | 0.711 | 0.664 | 0.965 |
| Inozawa river | 0.553 | 0.616 | 0.858 |
| Oura Bay (average of Sts. 1-4) | 0.885 | 0.719 | 0.819 |