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Bioavailability of macroalgal dissolved organic matter in seawater

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ABSTRACT: The bioavailability of macroalgal dissolved organic matter (DOM) was examined by decomposition experiments using released DOM from *Ecklonia cava* Kjellman (Phaeophyceae) living in Oura Bay, Shimoda, Izu Peninsula, Japan. The samples used for the decomposition experiments were obtained by enclosing the plants in bags. Based on the reduction rates of the concentrations of dissolved organic carbon (DOC), the turnover times of the macroalgal DOC were calculated to be between 24 and 172 d, with monthly-seasonal timescales. These values were mostly higher than those of phytoplanktonic DOC in previous studies (<1 mo). The relatively longer turnover time probably reflects the bio-refractory property of the macroalgal DOM. In most of the experiments (except for June), fucans and humic-like material were the major constituents of the released DOM. The fucans appeared to be partly decomposed during the experiments, but the compositional changes in the neutral carbohydrates in these seasons were less definite than those in June. The fluorescent intensity of the humic-like material did not decrease with time, suggesting a refractory character. Macroalgae are likely important DOM producers in Oura Bay, because the daily DOM production of *E. cava* accounts for 1.5 to 34% of DOM stock in Oura Bay per day. The concentration and the distribution of DOC inside and outside the bay strongly suggests that the released DOM was extensively exported out of the bay. These facts indicate that the macroalgal DOM contributes to marine DOM pools in a wider area including the adjacent coastal region.

KEY WORDS: Decomposition experiment · *Ecklonia cava* · Bio-refractory DOM · Excitation-emission matrix · EEM · Neutral sugar

INTRODUCTION

Marine dissolved organic matter (DOM) constitutes one of the largest organic carbon pools (685 PgC) on the earth's surface (Carlson 2002). Since DOM is almost equivalent in stock to atmospheric CO_2 (730 PgC) (Prentice et al. 2001) and exceeds that of land vegetation (500 PgC) (Prentice et al. 2001), DOM has been considered an essential component in the marine carbon cycle. Its large stock implies that DOM is a significant component in marine biogeochemical cycles. However, little is known about the origin of marine DOM. Generally, its ultimate source has been considered to be mostly the products of marine autotrophs, since terrestrial DOM appears to constitute a negligible fraction of marine DOM (Meyers-Schulte & Hedges 1986, Hedges et al. 1992). Phytoplankton accounts for >95% of marine primary production (Field et al. 1998), with the contribution of marine macroalgae on global productivity being estimated at just under 5%

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(De Vooys 1979, Charpy-Roubaud & Sournia 1990, Field et al. 1998), because most of the macroalgal distribution is limited to coastal areas. These facts logically lead to the simple conclusion that most marine DOM originated from phytoplankton, with little attention being given to the contribution of macroalgae to marine DOM dynamics.

Macroalgae are essential components in coastal ecosystems, with their productivity often exceeding that of phytoplankton in a coastal region (Mann 1973). In addition to their role as primary producers, macroalgae release a large fraction of their photosynthetic products as DOM (20 to 40%) (Khailov & Burlakova 1969, Sieburth 1969, Abdullah & Fredriksen 2004). Previously, we studied the DOM released by the macroalga *Ecklonia cava* Kjellman (Laminariales Phaeophyceae), by an *in situ* semi-closed bag experiment, in which we covered all the blades of *E. cava* with a transparent bag (Wada et al. 2007). We monitored the time course of the DOM concentrations in the bag, and estimated the DOM release as 18 to 62% of the photosynthetic production. These studies suggest that the percentage of macroalgal photosynthetic products released as DOM can be several 10s. Considering that these values are higher than the values for phytoplankton (generally around 10%: Baines & Pace 1991, Carlson 2002), macroalgae likely make a considerable contribution to coastal DOM pools.

Bioavailability is one of the most important factors controlling the biogeochemical role of DOM (Benner 2002). For example, labile DOM (L-DOM) acts as an organic substrate for heterotrophic microbes, sustaining the starting point of the microbial food web (Azam et al. 1983). Although L-DOM is known to be only a minor fraction of the DOM in seawater (Carlson 2002), a considerable amount of carbon can flow via L-DOM with a short turnover time (Suttle et al. 1991). On the other hand, most marine DOM pools consist of refractory DOM (R-DOM), which is a resistant fraction against microbial attack and subsists in seawater for several hundreds to thousands of years (Williams & Druffel 1987, Carlson 2002, Ogawa & Tanoue 2003). These facts suggest that R-DOM acts as a massive organic carbon reservoir on the earth's surface. In addition to these 2 categories, semi-labile DOM (S-DOM), whose turnover time ranges between months and years, has recently become the focus of attention as playing a possible role as a carrier of organic carbon into the ocean depths (Carlson et al. 1994, Carlson 2002, Ogawa & Tanoue 2003).

The bioavailability of DOM appears to be closely related to its organic composition. Since some organic compounds emit characteristic fluorescences, recent studies have applied fluorescent analysis of DOM to evaluate its bulk chemical composition (Coble et al. 1998, Parlanti et al. 2000, Yamashita & Tanoue 2004). It is well known that macroalgae release a yellow-brownish material, suggesting the possibility that these compounds have a fluorescent signature (Craigie & McLachlan 1964, Swanson & Druehl 2002, Wada et al. 2007). We previously measured the fluorescent spectra of macroalgal DOM, and suggested that it contains humic-like material (Wada et al. 2007). Because humic substances make up one of the most refractory organic groups in seawater (Chen & Bada 1992, Ogawa 2000), it should be expected that macroalgal DOM has relatively refractory properties.

In the present study, we carried out decomposition experiments to examine the bioavailability of DOM derived from *Ecklonia cava* using the sample obtained in the DOM production experiment in our previous study (Wada et al. 2007); the data from the end of the DOM production experiment in the previous study and from the start of the decomposition experiment in the present study overlap. We measured the concentration of dissolved organic carbon (DOC) to verify the mineralization rate of macroalgal DOM. The neutral carbohydrate composition and fluorescent spectra were also measured to assess the relationship between the bioavailability and the organic composition of macroalgal DOM. Finally, based on the results in the present study, we estimated the contribution of macroalgae to marine DOM dynamics.

MATERIALS AND METHODS

Decomposition experiments. DOM samples used for decomposition experiments were obtained after DOM production experiments in our previous study (Wada et al. 2007) at Stn 1 in Oura Bay, Shimoda, Izu Peninsula, Japan (34° 39′ N, 138° 56′ E) (Fig. 1) in October 2003, April, June and December 2004, and May 2005. The procedures of the DOM production experiment are outlined in the following paragraphs.

All blades of an individual *Ecklonia cava* were covered by a transparent bag containing ambient seawater, and the open end of the bag was tied up at the algal stipe by scuba divers. Two sample bags were usually set up to collect DOM originating from *E. cava* (1 bag in October 2003). In addition, 2 bags were filled with ambient seawater without *E. cava* for use as control samples. Water samples in the bags were collected at intervals of 6 to 54 h by divers using 100 ml glass syringes inserted through the sampling mouth, which was equipped with a valve. The samples were filtered through precombusted (450°C, 4 h) glass fiber filters (Whatman GF/F) immediately following collection, and the DOC concentrations and organic composition of DOM were analyzed.

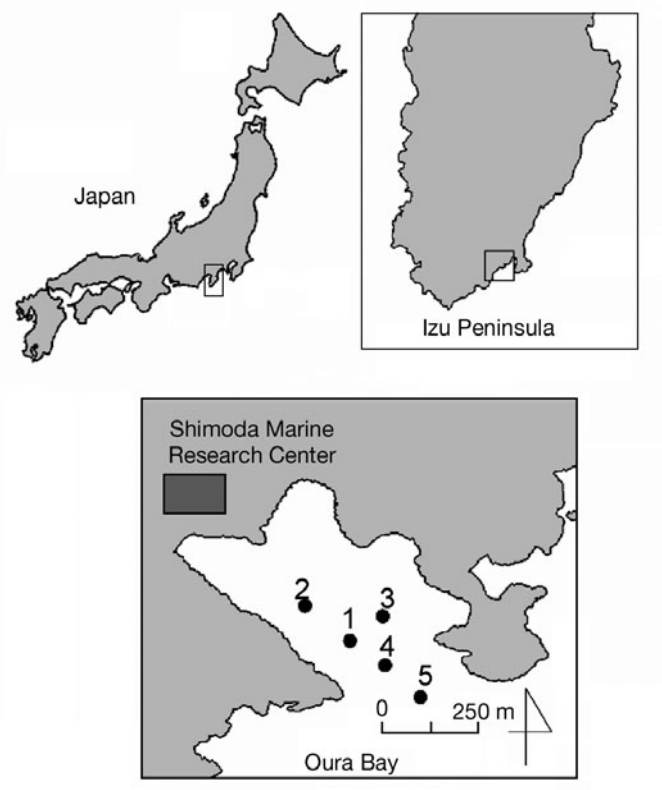


Fig. 1. Oura Bay. Numbers indicate locations of sampling stations

It is possible that most of the macroalgal DOM decomposed during the DOM production experiments (50 to 100 h). If microbial decomposition in the enclosed bag affected the DOM in the bag, then it is conceivable that the organic composition of the DOM changed with time, because heterotrophic bacteria preferentially uptake particular labile components in DOM as will be discussed later. However, we showed in our previous study (Wada et al. 2007) that the ratios of the concentrations of the released DOC and an organic constituent (humic-like material) were mostly constant during the DOM production experiments, suggesting little effect of bacterial degradation on the decomposition experiments in the present study.

For the decomposition experiments in the present study, the seawaters in the sample and control bags were recovered at the end of the production experiments, and were filtered through precombusted glass fiber filters. The filtrates were transferred into acid-cleaned 9 l polycarbonate bottles, which were then stored in the dark at 20°C for 30 d, except for April 2004 (50 d) and October 2003 and June 2004 (60 d). We will mainly discuss the time course 0 to 30 d, because the DOC concentrations varied little after 30 d as will be shown in 'Results'. The filtrate samples were bubbled via an air filter (0.45 µm pore size) to prevent an anoxic condition. We corrected the values of the DOC concentration (DOC_{cor}) by subtracting the DOC concentrations in the control bottles from those in the sample bottles.

Estimation of distribution area. The distribution area of *Ecklonia cava* in Oura Bay was calculated from an aerial photograph (Fig. 2) obtained in May 2003. An area survey showed that the dark region corresponds with the distribution area of *E. cava*, which was integrated using calculation software (Lia 32).

Collection of seawater. To measure the concentration of DOC in ambient seawater in Oura Bay, the surface and bottom (8 m depth) waters (April 6, 2004), or bottom (8 m depth) waters (August 18 and October 27, 2003, April 5, June 8 and December 13, 2004, and May 16, 2005) were collected with a glass syringe at Stn 2. Surface seawater samples were also collected with a plastic bucket at Stns 1, 2, 3, 4 and 5 in summer 2004 (July 15 and 27 and August 5 and 10). The seawaters were passed through GF/F filters immediately following collection, and the filtrates were stored at –20°C until analysis.

Fig. 2. Aerial photograph of Oura Bay. Area enclosed by yellow line is kelp forest

Analysis. The DOC concentrations were analyzed using a total carbon analyzer (Shimadzu TOC 5000A). To determine the monosaccharide composition of carbohydrates, the filtrates were acid-hydrolyzed in 1 N H_2SO_4 after desalination by electrodialysis (Microacilizer S-3, Asahi Chemical) and concentration by rotary evaporator. Desalination was achieved to 2.5 mS cm^{-1}, and the water volume was concentrated to around 20 ml, which was similar to a previous study (3.0 mS cm^{-1} and 20 ml; Hama & Yanagi 2001) in which the recovery of dissolved carbohydrates during these procedures was >95%. We used 1 ml from the concentrated samples for the following analysis. After hydrolysis, the liberated monosaccharides were converted into acetyl derivatives, and analyzed with a gas chromatograph (HP-6890) (Hama & Yanagi 2001). The 3-dimensional excitation-emission matrix (EEM) spectra were measured using a fluorescence spectrometer (Hitachi F-4500). A Milli-Q water blank of the EEM spectrum was subtracted to eliminate the water's Raman scatter peaks. The fluorescent intensity was normalized with quinine sulfate units (QSU). The characterization of the peak locations was based on Coble et al. (1998); the ranges of the wavelengths (excitation/emission: nm) of tyrosine- or protein-like, tryptophan- or protein-like, unknown, UV humic-like, visible marine humic-like and visible humic-like materials are 275/305 (peak B), 275/340 (peak T), 280/370 (peak N), 260/400–460 (peak A), 290–310/370–410 (peak M) and 320–360/420–460 (peak C), respectively. These peak ranges corresponded to those shown in other EEM studies (e.g. Coble 1996, Yamashita & Tanoue 2003). Thereafter, the highest QSU values in the rage of each peak were defined as the intensities of the peaks. Detailed analytical methods of DOC, neutral carbohydrate, and fluorescence are described in Wada et al. (2007).

RESULTS

Decomposition of organic carbon

The DOC_{cor} concentrations decreased with time in every experiment and those on Day 30 accounted for 29 to 86% of initial values (Fig. 3, Table 1), with the highest declining rate in June. Since it is apparent that long-term (60, 50 and 60 d in October, April and June, respectively) decomposition experiments showed little variation in DOC_{cor} concentrations after 30 d, an exponential model was fitted to the DOC_{cor} concentrations between 0 and 30 d (r^2 = 0.986, 0.608, 0.984, 0.957 and 0.982 in October, April, June, December and May, respectively) (Fig. 3). All relationships between DOC_{cor} concentrations and time were significant ($p < 0.05$) (Fig. 3).

$$DOC_{cor}(t) = DOC_{cor}(0) \times e^{-kt} \quad (1)$$

where DOC_{cor} (0) and DOC_{cor} (t) are the DOC_{cor} concentrations on Day 0 and Day t, respectively. The

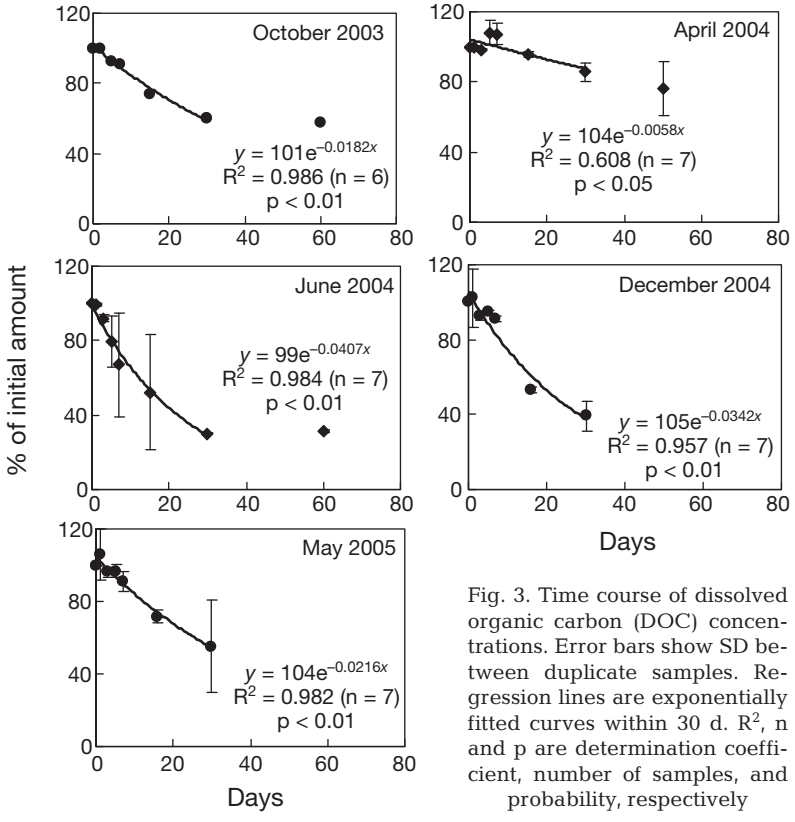


Fig. 3. Time course of dissolved organic carbon (DOC) concentrations. Error bars show SD between duplicate samples. Regression lines are exponentially fitted curves within 30 d. R^2, n and p are determination coefficient, number of samples, and probability, respectively

Table 1. Concentrations and remaining proportions of DOC_{cor} and decay constants. SDs are given between duplicate samples. Remaining proportion of DOC_{cor} is the value on Day 30 divided by that on Day 0. Decay constants were calculated by fitting an exponential curve (see 'Results: Decomposition of organic carbon'). DOC_{cor}: corrected concentration of dissolved organic carbon

Sampling month	DOC_{cor} concentration Day 0	Day 30	Remaining proportion of DOC_{cor} at Day 30 (%)	Decay constant (k-value: d^{-1})
Oct 2003	7.5	4.5	59.7	0.0182
Apr 2004	19.0 ± 0.4	16.3 ± 1.3	85.7 ± 5.3	0.0058
Jun 2004	356.4 ± 0.08	103.5 ± 0.03	28.9 ± 1.0	0.0407
Dec 2004	1.6 ± 0.3	0.6 ± 0.2	39.2 ± 8.1	0.0342
May 2005	15.2 ± 19.6	10.9 ± 14.7	55.1 ± 25.5	0.0216

k-values are calculated to be 0.0182, 0.0058, 0.0407, 0.0342 and 0.0216 d^{-1} in October, April, June, December and May, respectively. The higher *k* value in June reveals the relatively bio-labile character of the released DOM. Turnover times (reciprocals of the *k*-values) are calculated as 55, 172, 24, 32 and 46 d in October, April, June, December and May, respectively, having monthly-seasonal timescales.

Neutral carbohydrate composition of DOM

The concentrations of 8 monosaccharides (rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose) were determined as the neutral carbohydrate components of DOM. The results for neutral carbohydrates on Days 0, 7 and 30 are summarized in Table 2 (data on Day 0 correspond to that at the end of the DOM production experiment in the previous study, Wada et al. 2007). The total concentrations of the neutral carbohydrates (sum of the 8 monosaccharides) of DOM on Day 0 were 1.08, 1.06 ± 0.29, 20.41 ± 13.02, 0.37 ± 0.06 and 0.67 ± 0.84 mgC l^{-1} in October, April, June, December and May, respectively, which accounted for 3.7 to 14.5% of the total DOC concentrations (Wada et al. 2007). In October, April, December and May, the major component of the neutral carbohydrates was fucose, accounting for 35.9 to 43.9% of the total. In the sample obtained in June, however, the major monosaccharide was mannose (87.4% of the total neutral carbohydrates) (Wada et al. 2007).

Overall, the total concentrations of the neutral carbohydrates of DOM decreased with time. The concentrations on Day 30 were 0.61, 0.69 ± 0.15, 13.21 ± 1.42, 0.07 and 0.98 mgC l^{-1} in October, April, June, December and May, respectively (Table 2), which accounted for 20 to 79% of the concentrations on Day 0. Except in June, the proportions of the total neutral carbohydrates in the DOC concentrations tended to slightly decrease with time. In June, the value increased with time from 5.4 ± 2.4% on Day 0 to 12.7 ± 1.9% on Day 30.

Except in June, the proportion of fucose contents among total neutral carbohydrate of DOM tended to decrease and the values on Day 30 ranged from 13.8 to 42.4% (Table 2). Rhamnose, xylose, mannose, galactose and glucose together accounted for 56.4 to 70.5% of the total concentrations on Day 30, with ribose and arabinose being merely minor components (Table 2).

In June, the proportion of mannose among total neutral carbohydrates of DOM was 10 ± 1.8% on Day 30, considerably lower than the value on Day 0 (87.4%). In contrast to the substantial decrease in the mannose content, glucose became the major component (58.0 and 51.2% of total neutral carbohydrates on Days 7 and 30, respectively) (Table 2). Other components such

Table 2. Monosaccharide composition at 0 and 30 d. Values are percentages of each monosaccharide in total neutral carbohydrates, except for CHO conc, which shows the sum total of the 8 monosaccharides concentrations. SDs are given between duplicate samples. Rha, Fuc, Rib, Ara, Xyl, Man, Gal and Glc indicate rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose, respectively. CHO: carbohydrate. DOC: dissolved organic carbon. nd: not detected

Month	Day	Rha (% of total CHO)	Fuc (% of total CHO)	Rib (% of total CHO)	Ara (% of total CHO)	Xyl (% of total CHO)	Man (% of total CHO)	Gal (% of total CHO)	Glc (% of total CHO)	CHO conc. (mgC l^{-1})	CHO:DOC (%)
Oct	0 d	10.3	35.9	nd	2.1	7.2	11.0	19.3	14.3	1.08	12.7
	7 d	10.3	28.3	nd	1.5	6.9	13.6	22.3	17.1	0.82	10.4
	30 d	7.2	29.7	1.08	1.6	6.0	15.7	19.3	19.4	0.61	9.7
Apr	0 d	5.4 ± 0.15	43.9 ± 4.2	nd	nd	11.2 ± 0.8	8.5 ± 1.6	24.0 ± 4.7	7.0 ± 0.2	1.06 ± 0.29	5.3 ± 1.4
	30 d	4.3 ± 0.28	42.4 ± 7.7	nd	1.2 ± 0.2	6.8 ± 2.3	9.3 ± 3.4	17.3 ± 4.5	18.7 ± 6.1	0.69 ± 0.15	4.0 ± 0.6
Jun	0 d	nd	nd	nd	nd	nd	87.4 ± 0.9	nd	12.6 ± 0.9	20.41 ± 13.02	5.4 ± 2.4
	7 d	1.4 ± 0.7	4.1 ± 1.0	0.9 ± 1.2	1.6 ± 1.4	1.3 ± 0.02	29.9 ± 16.9	2.8 ± 0.5	58.0 ± 19.2	18.61 ± 9.19	7.8 ± 1.1
	30 d	7.4 ± 3.1	12.6 ± 5.8	5.5 ± 1.7	nd	1.6 ± 2.3	10.3 ± 1.8	11.4 ± 1.9	51.2 ± 9.2	13.21 ± 1.42	12.7 ± 1.9
Dec	0 d	7.8 ± 2.6	39.5 ± 2.3	1.2 ± 0.01	2.4 ± 0.1	7.5 ± 0.9	11.4 ± 1.0	18.0 ± 0.7	12.5 ± 2.9	0.37 ± 0.06	14.5 ± 0.7
	7 d	10.0 ± 0.03	32.8 ± 2.1	nd	2.3 ± 0.03	7.6 ± 0.8	12.1 ± 1.6	18.8 ± 0.8	15.8 ± 1.4	0.24 ± 0.15	8.2 ± 1.9
	30 d	10.1	13.8	9.9	5.7	9.9	14.3	14.2	22.0	0.07	3.9
May	0 d	7.3 ± 2.1	43.1 ± 17.3	nd	nd	15.8 ± 6.7	7.8 ± 8.0	13.7 ± 0.5	12.4 ± 14.3	0.67 ± 0.84	3.7 ± 0.7
	30 d	5.9	36.1	nd	nd	4.0	7.7	23.9	22.4	0.98	3.9

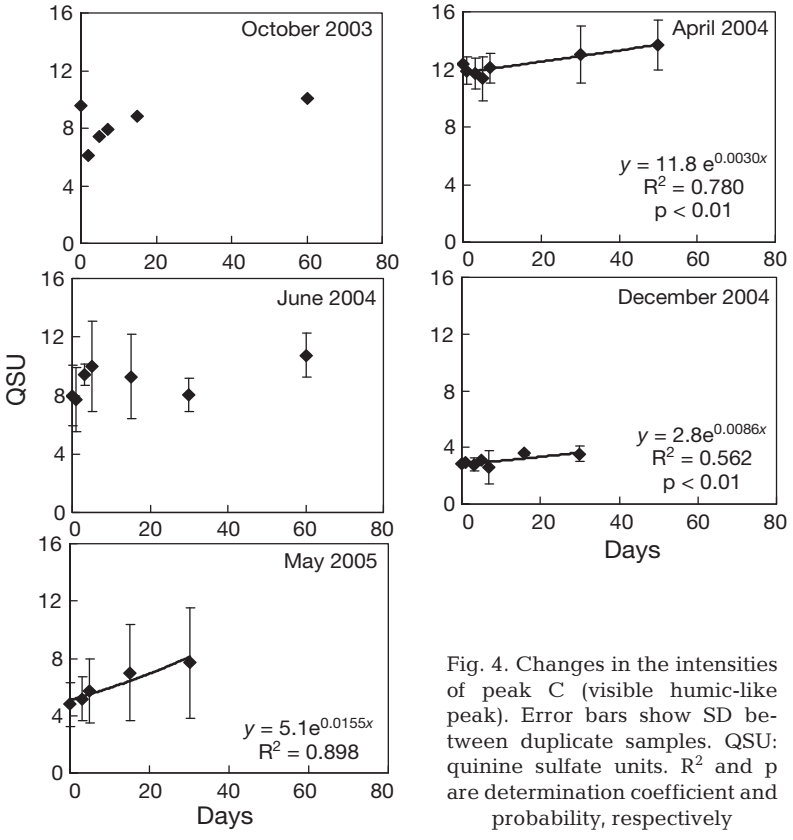


Fig. 4. Changes in the intensities of peak C (visible humic-like peak). Error bars show SD between duplicate samples. QSU: quinine sulfate units. R^2 and p are determination coefficient and probability, respectively

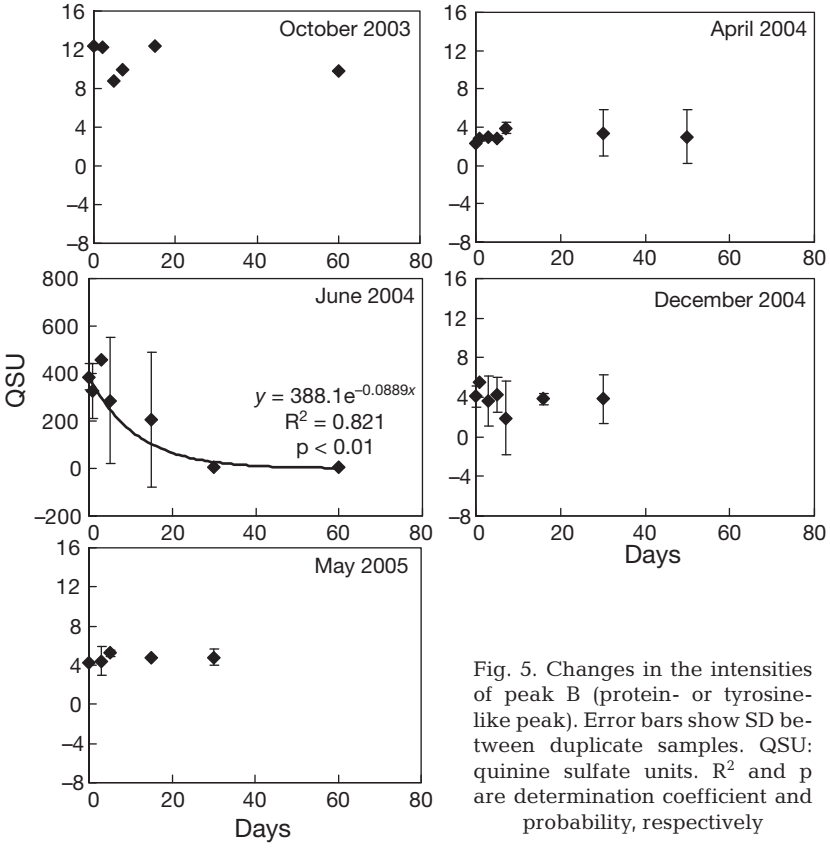


Fig. 5. Changes in the intensities of peak B (protein- or tyrosine-like peak). Error bars show SD between duplicate samples. QSU: quinine sulfate units. R^2 and p are determination coefficient and probability, respectively

as rhamnose, fucose, ribose, xylose and galactose were 1.6 to 12.6% on Day 30, which reduced them to minor components compared with glucose.

EEM spectra

Six peaks in the EEM spectra (peaks B, T, N, A, M and C, corresponding to tyrosine- or protein-like, tryptophan- or protein-like, unknown, UV humic-like, visible marine humic-like and visible humic-like materials, respectively) were identified based on the definition of Coble et al. (1998). EEM spectra of natural seawater showed that in October, April, December and May, on Day 0, a relatively high emission around the visible humic-like peak (peak C) was observed, but the other peaks were almost absent. In June, the spectrum was much different from those in other months; a characteristic high peak was found around the tryptophan- or protein-like peak (peak T). These results obtained on Day 0 correspond to the data from the end of the DOM production experiment in the previous study (Wada et al. 2007).

On Day 30 (in October: Day 60), the intensities of peak C (humic-like peak) were 10, 13 ± 2.0, 3.6 ± 0.57, 7.7 ± 3.9 QSU in October, April, December and May, respectively, and they were slightly higher than the values on Day 0 (9.5, 12.4, 2.9 ± 0.08 and 4.8 ± 1.5 QSU; Wada et al. 2007). Although time courses also indicate that the QSU values of peak C (humic-like peak) tended to increase with time in most cases (Fig. 4, Table 2), the relationships were sometimes weak (not significant in October, and $p < 0.1$ in December). Other peaks, B (tyrosine- or protein-like), T (tryptophan- or protein-like), N (unknown), A (UV humic-like) and M (visible marine humic-like), did not show definite changes in October, April, December and May (Figs. 5 to 9).

In June, however, the intensity of peak T (tryptophan- or protein-like) declined drastically from 386.0 ± 56.2 QSU on Day 0 to 4.0 ± 2.6 QSU on Day 60 (Fig. 6), when an exponential

model was fitted to the time course of QSU values ($r^2 = 0.82$, $p < 0.01$) of peak T (tryptophan- or protein-like peak) (Table 3). The *k*-value was 0.0889, being more than twice the *k*-value of the DOC_{cor} (0.0407) in June. In June, similar variations were observed for other peaks (B [tyrosine- or protein-like], N [unknown], A [UV humic-like] and M [visible marine humic-like]) (Figs. 5 to 9) but this is probably due to the effect of the broadness of peak T (tryptophan- or protein-like peak), whose base overlapped with other peaks (Wada et al. 2007).

Integration of DOM pool and DOM production of *Ecklonia cava* in Oura Bay

DOC concentrations of the surface seawaters were 1.10 ± 0.10 (0.97 to 1.31) mgC l^{-1} in summer 2004 (July and August) (Fig. 10). These values were multiplied by the seawater volume in Oura Bay (1.9×10^9 l), and the DOC pool in Oura Bay was calculated to be 1.8×10^3 to 2.5×10^3 gC (average: $[2.1 \pm 0.087] \times 10^3$ gC). Although this calculation was based on the values obtained in summer, there was little difference from the DOC concentrations in other seasons observed at Stn 2 (0.91 ± 0.07 mgC l^{-1}, Fig. 10). The difference between the average value along the Oura Bay in summer season and that at Stn 2, including the values in other seasons (0.18 mgC l^{-1}), is not enough to affect the estimation of the DOC pool in Oura Bay.

The daily DOC production rates per blades dry weight of *Ecklonia cava* were estimated as 1.2×10^{-4} to 58×10^{-4} gC g dry wt^{-1} d^{-1} (Wada et al. 2007). We multiplied these values by blades dry weight per unit distribution area of *E. cava* in Oura Bay (4.8×10^2 to 21×10^2 g dry wt m^{-2}, as reported by Yokohama et al. 1987) to calculate daily DOC production rates per distribution unit area (0.25 to 5.8 gC m^{-2} d^{-1}). Additionally, we multiplied these values by integrated distribution area (12×10^4 m^2) of *E. cava* kelp forest in Oura Bay esti-

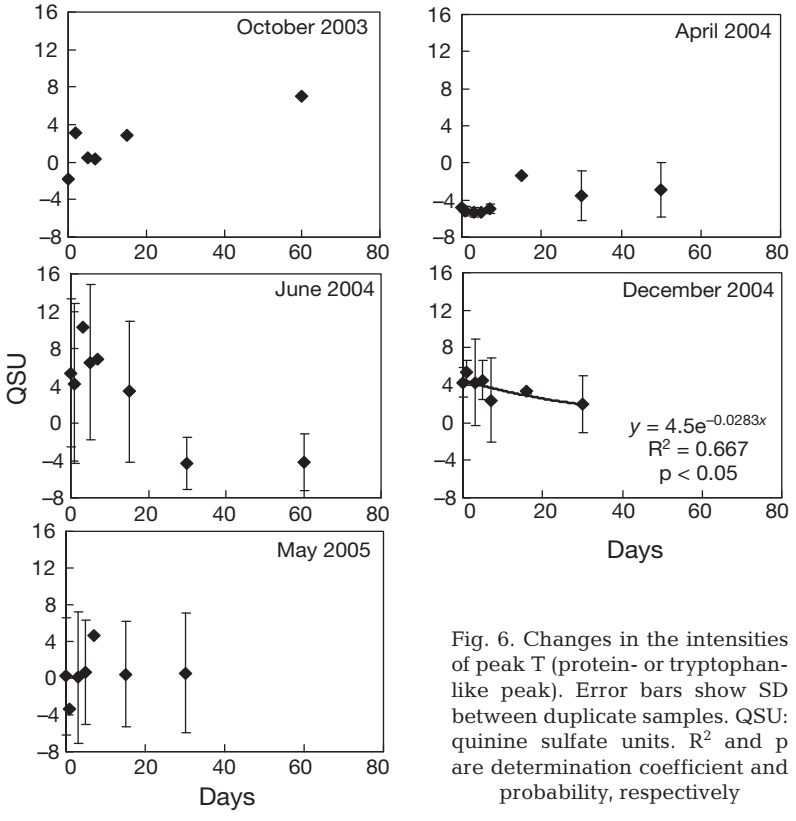


Fig. 6. Changes in the intensities of peak T (protein- or tryptophan-like peak). Error bars show SD between duplicate samples. QSU: quinine sulfate units. R^2 and p are determination coefficient and probability, respectively

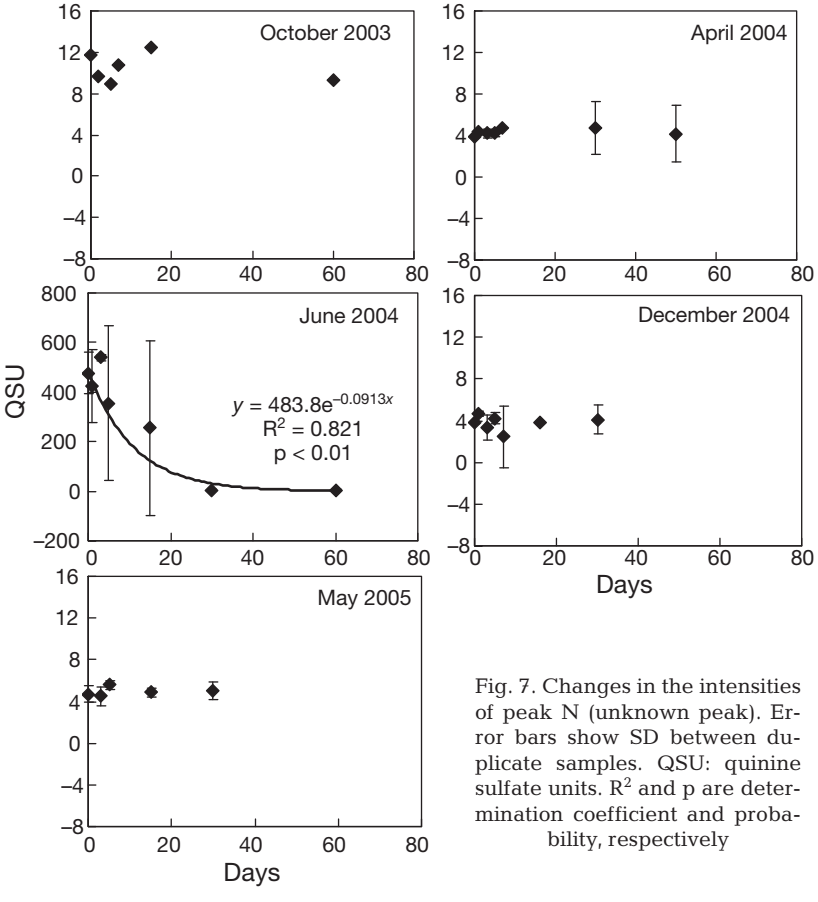
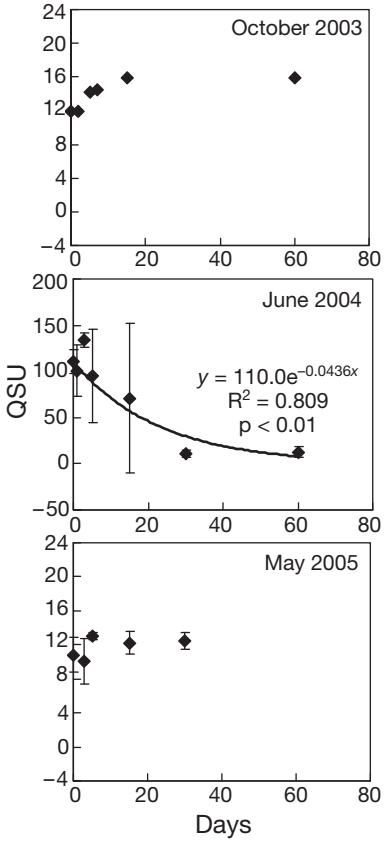


Fig. 7. Changes in the intensities of peak N (unknown peak). Error bars show SD between duplicate samples. QSU: quinine sulfate units. R^2 and p are determination coefficient and probability, respectively



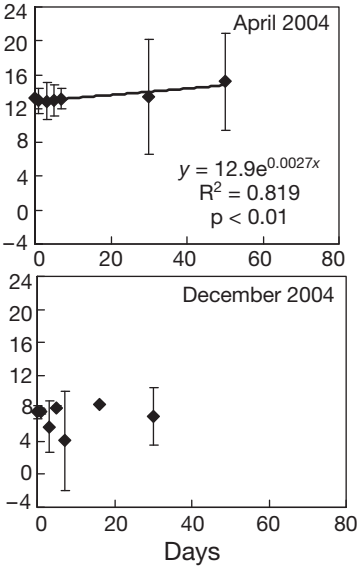
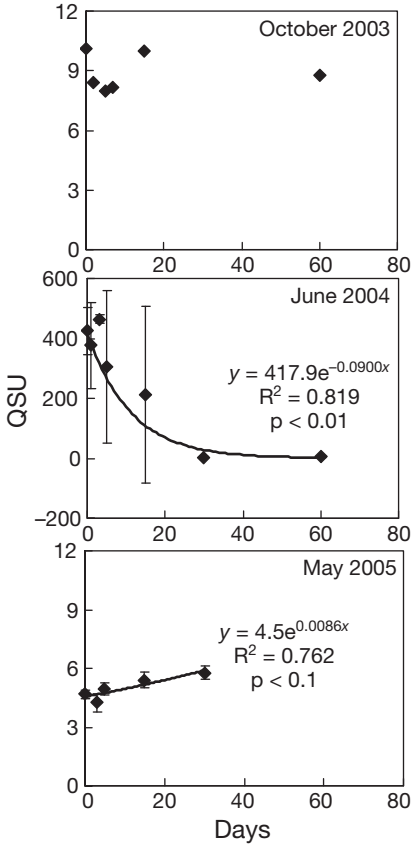


Fig. 8. Changes in the intensities of peak A (UV humic-like peak). Error bars show SD between duplicate samples. QSU: quinine sulfate units. R^2 and p are determination coefficient and probability, respectively



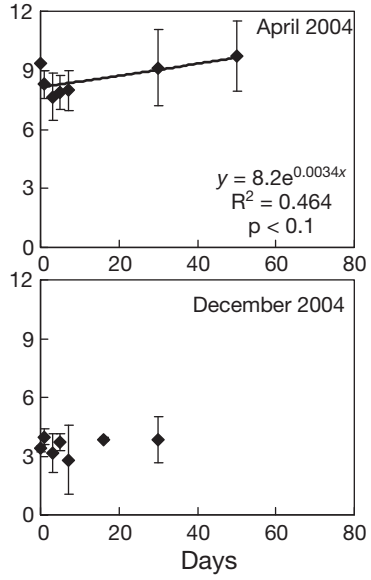


Fig. 9. Changes in the intensities of peak M (visible marine humic-like peak). Error bars show SD between duplicate samples. QSU: quinine sulfate units. R^2 and p are determination coefficient and probability, respectively

mated from an aerial photograph (Fig. 2). The sum total of the daily DOC production rates by *E. cava* in Oura Bay were calculated as 3.1×10^4 to 72×10^4 g C d^{-1}, accounting for 1.5 to 34% d^{-1} of the DOC pool in the bay.

DISCUSSION

Decomposition of DOC

Bioavailability is an important factor controlling the dynamics of marine DOM (Benner 2002, Carlson 2002). L-DOM, the fraction that is rapidly consumed by heterotrophic bacteria, drives the microbial food web (Azam et al. 1983), while R-DOM resists bacterial attack, and acts as a carbon reservoir in seawater (Carlson 2002, Ogawa & Tanoue 2003). The bioavailability of macroalgal DOM has rarely been studied, even though macroalgae are important primary producers in coastal environments (Mann 1973, Yokohama et al. 1987, Jeffrey & Hayes 2005). In the present study, we estimated the turnover times of macroalgal DOC to be 24 to 172 d (*k* values: 0.0058 to 0.0407 d^{-1}). Concerning the DOC derived from phytoplankton, several estimates have been made. Hama et al. (2004) estimated the turnover time of phytoplanktonic DOM to be 30 d based on the declining rate of released DOM using the ^{13}C isotope tracer method. The turnover times of DOC in the spring phytoplankton bloom in the North Atlantic were estimated at 2.8 to 40 d (*k* values: 0.025 to 0.36 d^{-1}) based on the decrease in DOC concentration in incubated samples (Kirchman et al. 1991). Amon & Benner (1994) reported the turnover times as 6 and 26 d for high-molecular-weight (>1 kDa) and low-molecular-weight (<1 kDa) DOM of the surface water from the northern Gulf of Mexico, respectively. The results of these studies imply that the turnover of DOC originating from phytoplankton is <1 mo, and the present study strongly suggests that macroalgal DOM is relatively bio-refractory in character compared with phytoplanktonic DOM.

Table 3. r^2 values for the relationship between fluorescent intensity and time. Excitation/emission wavelengths are given for each peak (after Coble et al. 1998). Positive and negative relationships are indicated by (+) and (–), respectively. nf: not fitted (the intensities of peak B [tryptophan- or protein-like peak] could not, except in December, be fitted to an exponential curve because of the negative values). n: number of data in each season. ns: not significant

Month	Peak B (275/305)	Peak T (275/340)	Peak N (280/370)	Peak A (260/400–460)	Peak M (290-310/370–410)	Peak C (320-360/420–460)
Oct 2003 (n = 5)	nf	r^2 = 0.0971 ns	r^2 = 0.0969 ns	r^2 = 0.4454 ns	r^2 = 0.0001 ns	r^2 = 0.3358 ns
Apr 2004 (n = 7)	nf	r^2 = 0.0674 ns	r^2 = 0.0162 ns	r^2 = 0.8192 $p < 0.01$ (+)	r^2 = 0.464 $p < 0.1$ (+)	r^2 = 0.7798 $p < 0.01$ (+)
Jun 2004 (n = 7)	nf	r^2 = 0.8212 $p < 0.01$ (–)	r^2 = 0.8208 $p < 0.01$ (–)	r^2 = 0.8093 $p < 0.01$ (–)	r^2 = 0.819 $p < 0.01$ (–)	r^2 = 0.2686 ns
Dec 2004 (n = 7)	r^2 = 0.667 $p < 0.05$ (–)	r^2 = 0.0102 ns	r^2 = 0.0061 ns	r^2 = 0.0117 ns	r^2 = 0.1045 ns	r^2 = 0.5622 $p < 0.1$ (+)
May 2005 (n = 5)	nf	r^2 = 0.1299 ns	r^2 = 0.0387 ns	r^2 = 0.2829 ns	r^2 = 0.7619 $p < 0.1$ (+)	r^2 = 0.8982 $p < 0.05$ (+)

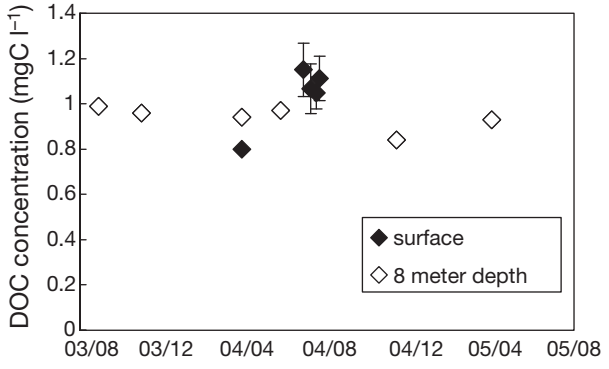


Fig. 10. Dissolved organic carbon (DOC) concentrations in Oura Bay. ◆, ◇: values of waters at surface and bottom (8 m depth), respectively. Surface seawaters were collected in April, July and August 2004. Bottom seawaters were collected in August and October 2003, April, June and December 2004, and May 2005. Error bars show SD among the values at Stns 1, 2, 3, 4 and 5. Dates on the *x*-axis are given as yy/mm

It is possible that the longer turnover time of macroalgal DOM partly reflects its particular organic composition. We previously measured the monosaccharide composition of carbohydrates in the DOM released from *Ecklonia cava*, and suggested that the macroalgal dissolved carbohydrates are mainly comprised of mucopolysaccharides (Wada et al. 2007). The release of mucopolysaccharides was suggested based on their high fucose contents on Day 0 (35.9 to 43.9% of total neutral carbohydrate), because fucose is a major component of the mucopolysaccharides referred to as fucans (Marais & Joseleau 2001, Bilan et al. 2004, 2006, Wada et al. 2007). The contributions of fucose to the total neutral carbohydrates on Day 30 were slightly lower than the value on Day 0. However, the proportions of fucose on Day 30 (29.7, 42.4, 13.8 and 36.1% of total neutral carbohydrate in October, April, December and May, respectively) were still higher than those of other monosaccharide components (Table 2), suggesting that a considerable proportion of fucans resisted microbial degradation for 30 d.

The predominance of fucose in the carbohydrates of the macroalgal DOM implies that fucose content is a useful biomarker of macroalgal DOM. The monosaccharide composition of DOM in coastal and pelagic waters has been determined and the contribution of fucose to total carbohydrates ranged from 10 to 20% (Sakugawa & Handa 1985, Aluwihare et al. 1997, Aluwihare & Repeta 1999). These values are generally lower than that obtained for DOM derived from *Ecklonia cava*, indicating that fucose content of carbohydrates is able to be available to estimate the contribution of macroalgal DOM. However, no study has examined the monosaccharide composition of dissolved carbohydrates in algal beds. Systematic field observations such as transect sampling from algal beds to offshore regions may provide evidence of the distribution of macroalgal DOM.

On the other hand, a highly bio-labile character has been suggested for the carbohydrates of phytoplanktonic DOM in previous studies. Hama et al. (2004) suggested that glucose and/or its polymer in phytoplanktonic DOM was decomposed within 3 d. Since phytoplankton release glucan as a major constituent of the dissolved carbohydrate (Hama & Yanagi 2001, Granum et al. 2002, Hama et al. 2004), the carbohydrate of phytoplanktonic DOM turns over within a relatively short time compared with those of macroalgal DOM. Thus, it is quite possible that the properties of released carbohydrate constitute one of the important factors that determine the relatively recalcitrant characteristics of macroalgal DOM.

Another possible factor preventing rapid microbial utilization is that macroalgal DOM contains a humic-

like substance. The refractory character of such a substance in marine environments was suggested in previous studies using ^{14}C-age, in which the humic fraction in marine DOM has a longer ^{14}C-age than that in bulk DOM (Bauer et al. 1992, Druffel et al. 1992). Chen & Bada (1992) estimated that the timescales of humic materials would be from 100s to 1000s of years, based on the vertical and horizontal distributions of marine DOM fluorescence. In addition to these field observations, laboratory studies have indicated the inferior bioavailability of humic substances. Moran & Hodson (1990) and Lara & Thomas (1995) carried out incubation and decomposition experiments using a batch culture of diatoms and a natural bacterial population, and showed that the humic fraction extracted by XAD resins is relatively recalcitrant over a long period.

Several studies showed that macroalgae produce UV-absorbing humic-like material (Craigie & McLachlan 1964, Sieburth & Jensen 1969, Swanson & Druehl 2002, Wada et al. 2007). Considering that macroalgae produce phenolic compounds that absorb UV (Swanson & Druehl 2002, Shibata et al. 2006), the phenolic compounds might be the constituents of the humic-like material of macroalgae. Since phenolic compounds appear to be produced for defense against attack by other organisms (Van Alstyne 1988, Paul et al. 2006), we would expect that the humic-like material in macroalgal DOM would be resistant against microbial attack as well. In the present study, we actually monitored the time course of the intensity of the humic-like peak (peak C) and found that it did not diminish (Fig. 4), in contrast to the concentration of DOC_{cor} (Fig. 3) during the experiments. Consequently, these results indicate that the humic-like material of macroalgal DOM is also resistant to bacterial decomposition.

An exceptionally short turnover time of DOC was noticed in June (Table 1). The organic composition of DOM obtained in this experiment was definitely different from that in other samples. The major monosaccharide was mannose, and a protein-like peak (peak T) was present with characteristic high fluorescence on Day 0 in June, suggesting the releases of reserved carbohydrate (mannitol) and intracellular protein (Wada et al. 2007). The mannose contribution to total neutral carbohydrate decreased with time (Table 2), an indication of the highly bio-labile property of mannitol as well as of the reserved carbohydrate of phytoplankton (Hama et al. 2004). The protein-like peak (peak T) in the EEM spectra also decreased drastically in the early stage of decomposition, and had a relatively higher *k*-value compared with DOC_{cor} (Fig. 6, Table 1), and this finding is consistent with previous studies on the selective decomposition of proteinaceous amino acids in seawater (Rosenfeld 1979, Cowie & Hedges 1994). These changes in the concentration and composition of the biomolecules strongly suggest that the relatively short turnover time of DOC in June was primarily due to the exceptional release of intracellular material into the ambient seawater.

The bioavailability of macroalgal DOM has rarely been studied. The long turnover times of DOC together with the results of compositional analyses in the present study suggest that macroalgae release relatively bio-refractory DOM. Considering the monthly-seasonal timescales of the turnover times, macroalgal DOM would accumulate in the water column and, as a result, should constitute a considerable fraction of coastal DOM pools.

Implications of macroalgae in DOM dynamics

Macroalgae fractionate a large proportion of photosynthetic products into the DOM fraction (Khailov & Burlakova 1969, Sieburth 1969, Abdullah & Fredriksen 2004, Wada et al. 2007), but their impact on oceanic DOM pools has seldom been estimated. In the present study, based on an aerial photograph (Fig. 2), we estimated the macroalgal impact on the DOM pool in Oura Bay, and found that the daily DOM production of *Ecklonia cava* accounts for 1.5 to 34 % d^{-1} of the DOC pool in the bay.

Based on both the very high level of macroalgal DOM inputs to the DOC pool (1.5 to 34 % d^{-1}) and the fact that *Ecklonia cava* releases relatively refractory DOM, one may suppose that the released DOM accumulates as a constituent of the DOM pool, resulting in the increase in DOC concentrations in Oura Bay. However, the seasonal determination of DOC concentrations (August and October 2003, April, June and December 2004, and May 2005, detailed results not shown) indicates that the concentrations of DOC around the *Ecklonia* sp. forest in Oura Bay remain low (Fig. 10). Such concentrations are lower than those found in general coastal waters (Cauwet 2002) and roughly comparable to those of surface seawater in oligotrophic waters (Benner 2002). This indicates that the exchange of water mass between the inner and outer Oura Bay may be what keeps the concentrations at such a low level. The relationship between precipitation and salinity in Oura Bay (Shimoda Marine Research Center 2004) supports this possibility. The salinity in the bay is usually maintained at around 34 to 35 psu, but it declines to around 33 psu just after a heavy rain. However, the salinity level recovers rapidly (mostly within a day) to around its usual value, probably due to the rapid export of low-salinity water out of the bay, and the import of the high-salinity waters. Such a rapid recovery of salinity indicates that the res-

idence time of the seawater in Oura Bay is within one day. Given that brief residence time, most macroalgal DOM should be exported out of the bay. In other words, macroalgal DOM should make some significant contribution to DOM pools in coastal regions and open oceans.

The present study suggested that macroalgal DOM has a relatively bio-refractory property compared with phytoplanktonic DOM. Considering this together with the large DOM production rate of macroalgae (Khailov & Burlakova 1969, Sieburth 1969, Abdullah & Fredriksen 2004, Wada et al. 2007) compared with phytoplankton (Baines & Pace 1991, Carlson 2002), macroalgae presumably has a significant contribution as a producer of relatively bio-refractory DOM, which plays an important biogeochemical role in oceanic environments (Carlson 2002, Ogawa & Tanoue 2003). Further studies on the fate of organic carbon produced by macroalgae would underline the biogeochemical importance of macroalgae.

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