

Distribution, degradation and bioavailability of dissolved organic matter in the East China Sea

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Abstract Water samples were collected from the East China Sea (ECS) in October 2015 to investigate the distribution, degradation and bioavailability of organic matter. Concentrations of dissolved organic carbon (DOC), total hydrolyzable amino acids (THAAs, including dissolved free, DFAA and combined fraction, DCAA), particulate amino acids (PAAs), and total dissolved carbohydrates (TDCHO, including monosaccharides, MCHO and polysaccharides, PCHO) were measured. DOC and TCHO concentrations exhibited similar distribution patterns

with high values occurring at nearshore stations, revealing the effects of terrestrial input and similar source and removal pathways of DOC and TCHO. The distributions of THAA, DCAA, and PAA displayed declining trends from the north to south of the ECS. Elevated THAA values simultaneously occurred in the center of the transect. The onboard incubation experiments with surface seawater from one station showed that the values of degradation index based on amino acids decreased with the increase of dissolved inorganic nitrogen (DIN) concentrations, indicating the mineralization of THAA to DIN during degradation process. TCHO-C% and THAA-C% are defined as the percentages of carbohydrates and amino acids in DOC, respectively. There were 21 stations suffering P limitation, implying that $\text{PO}_4^{3-}\text{-P}$ content was the key factor limiting the growth of phytoplankton. High TCHO-C% values were found at P-limited stations, indicating that phytoplankton preferentially produced carbohydrates when experiencing nutrient limitation. The difference of dissolved organic matter bioavailability between surface and bottom water were probably due to water stratification. Overall, the present study may have implications for the source, removal and bioavailability of organic matter in the ECS.

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Introduction

Dissolved organic matter (DOM) in the ocean is one of the largest dynamic reservoirs of organic carbon and nitrogen on earth (Hedges and Oades 1997). According to biological availability, the DOM can be classified operationally into three groups: labile DOM (LDOM), with a turnover time of days or weeks (Münster 1993; Søndergaard and Middelboe 1995; Amon and Benner 1996); semi-LDOM, which turns over on the time scale of months to years; and recalcitrant DOM, with lifetimes of years, decades, or longer (Carlson and Ducklow 1995; Hedges 1992; Jiao et al., 2010; Münster 1993). The most abundant components of the LDOM are carbohydrates and amino acids (Sundh 1992; Münster 1993; Biddanda and Benner 1997; Simon 1998). As structural and storage components, carbohydrates contribute to 15–30% of dissolved organic carbon (DOC) in marine surface waters (Pakulski and Benner 1994), and up to 50% of ultrafiltered DOC in marine surface waters (Benner et al. 1992). Generally, total dissolved carbohydrates (TDCHO) in marine systems include monosaccharides (MCHO), oligosaccharides, and polysaccharides (PCHO) (Borch and Kirchman 1997; Skoog and Benner 1997; Bhosle et al. 1998). The major sources of carbohydrates in marine environments include phytoplankton extracellular release (Biddanda and Benner 1997; Biersmith and Benner 1998), zooplankton excretion (Burney et al. 1979), bacterial release (Kawasaki and Benner 2006), river inputs and sediment resuspension (Hung et al. 2005; Arnosti and Holmer 1999). Carbohydrates can be transformed into proteins, lipids and other organic compounds to serve as carbon sources (Cuhel et al. 1984; Xiong et al. 2010). The contributions of amino acid carbon to DOC varied with sites and depths, but generally fell within 1–4% for surface waters and 0.4–0.8% at depths > 1000 m (Kaiser and Benner 2009; Yamashita and Tanoue 2003; Davis and Benner 2005). According to existing forms, amino acids in the ocean can be classified into dissolved amino acids and particulate amino acids (PAAs). During the degradation of organic matter, some amino acids are accumulated, whereas some decrease relatively, leading to the changes in the proportion of the individual amino acids. Thus, the contributions of amino acid carbon to organic carbon, relative proportion of individual amino acids, and degradation indices (DIs) based on

amino acids are used as indicators of decomposition and transport in marine environments (Cowie and Hedges 1994; Dauwe and Middelburg 1998; Dauwe et al. 1999; Keil et al. 2000).

In recent years, some studies on the origin, degradation and removal of DOM have been conducted, but most of these studies were only aimed at some specific forms. Few studies have been systematically done on different forms of amino acids. Our understanding of the sources, compositions, removal and bioavailability of DOM in the ocean is still limited (Amon et al. 2001; Hopkinson and Vallino 2005), because they are closely related to complex hydrogeologic, oceanic, and biochemical processes. Investigation of the bioavailability and diagenetic state of DOM at the molecular level in marginal area can contribute to a better understanding of the regional carbon cycle. However, a single incubation is insufficient to describe the full scenario. It is critical to combine incubation experiments with field investigations to quantify bulk carbon and the underlying transformation of DOM.

The East China Sea (ECS) is an important marginal area of the northwest Pacific Ocean and is noted for its high primary productivity and enormous discharge of river runoff into the sea, notably from the Changjiang River. The environment of the ECS has been greatly influenced by both fishery and land-based pollution over the past decades (Li and Daler 2004; Jiang et al. 2009). The continental shelf area of the ECS is a biologically highly productive region that is well known as an important fishery in the west Pacific. The ECS environment, especially coastal areas, are also significantly affected by terrestrial inputs. The ECS receives anthropogenic pollutants, including sewage, nutrients, and sediments, from river runoff. Nutrients are the dominant pollutant in the Changjiang Estuary and the adjacent ECS (Li and Daler 2004). Since 1960s, the application of chemical fertilizer to the Changjiang River Basin increased rapidly, and concentrations of dissolved inorganic nitrogen (DIN) and phosphate in the Changjiang water increased by a factor of 5 from the 1960s to the end of 1990s (Wang 2006; Li et al. 2007). In recent years excessive nutrients have led to severe eutrophication and increased the frequency of harmful algal blooms and seasonal hypoxia in the ECS adjacent to the Changjiang Estuary (Zhang et al. 2007; Zhou et al. 2008; Zhu et al. 2011).

To date, few studies have focused on the removal and control mechanism of organic matter in the ECS (Chen et al. 2016a; Hung et al. 2003; Zhu et al. 2014). In the present study, we conducted a systematic survey for the distributions and variations of amino acids, carbohydrates, and DOC in the surface water and water columns of the ECS. The aims of this study are to clarify the spatial variability of the concentrations of amino acids and carbohydrates; to evaluate the sources and degradation of DOM based on DI, carbohydrate and amino acid yields; and to assess the variations of DOM bioavailability via seawater incubation experiment.

Materials and methods

Study area

The ECS extends from Cheju Island in the north to the northern coast of Taiwan in the south with a mean depth of 72 m and a total surface area of approximately 770,000 km² (Liu et al. 2006). The ECS is heavily affected by several water masses, including the warm and nutrient poor Taiwan Warm Current (TWC), the nutrient rich and low salinity Changjiang Diluted Water (CDW), the Zhejiang–Fujian Coastal Current (ZFCC) and the Kuroshio Current (KC; Su 1998). As one of the largest rivers in the world, the Changjiang inputs large amounts of freshwater ($9.24 \times 10^{11} \text{ m}^3 \text{ year}^{-1}$), sediments ($4.86 \times 10^8 \text{ t year}^{-1}$), and abundant nutrients into the ECS (Tian et al. 1993; Zhang 1996). During summer, southwest winds prevail, and the CDW flows to the northeast towards the Tsushima/Korea Straits. In contrast, the northeast monsoon prevails in autumn, and the CDW flows southwest within a narrow band adjacent to the Zhejiang–Fujian coast (Beardsley et al. 1985; Liu et al. 2016). The KC, which was characterized by high temperature, high salinity, and oligotrophic water, flows along the eastern boundary of the ECS continental shelf (Su 1998).

Sample collection

The cruise was conducted aboard the R/V “Science No. 3” in the ECS in October 2015. Locations of 36 sampling stations are shown in Fig. 1. Surface water samples (< 5 m) and the profile samples at different

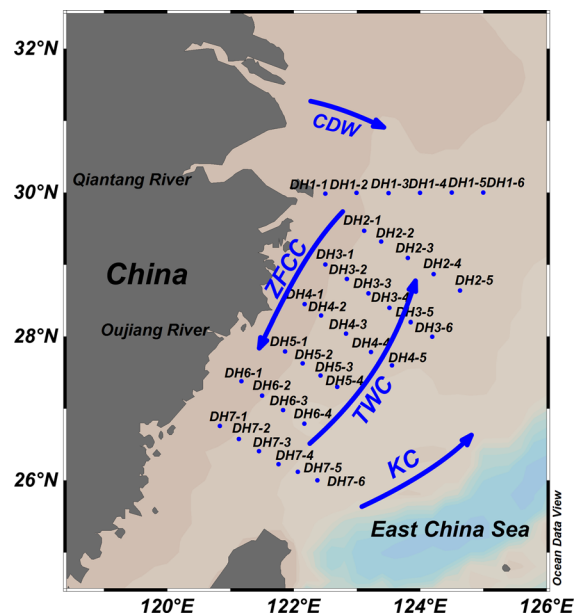


Fig. 1 Location of sampling stations in the East China Sea in October 2015. CDW Changjiang Diluted Water, KC Kuroshio Current, TWC Taiwan Warm Current, ZFCC Zhejiang–Fujian Coastal Current. The figure was generated using Ocean Data View software (Schlitzer 2014)

depths were collected using 12 L Niskin bottle samplers attached to the conductivity, temperature, and depth sensor (CTD) rosette system. Transect DH1, which is close to the Chanjiang Estuary, was chosen to investigate the vertical distribution of DOM. Seawater incubation experiment was carried out at station DH3–6. Detailed information about the sampling stations and hydrographic parameters are described in Table S1.

After collection, the samples were immediately filtered through 47 mm Whatman GF/F glass fiber filters (precombusted at 500 °C for 5 h) under low pressure. The filtrate was stored in acid-rinsed glass vials at -20 °C for the analyses of dissolved carbohydrates and dissolved amino acids. Samples for nitrogen species analyses were collected into preconditioned 60 mL high-density polyethylene bottles and stored at -20 °C. Samples for DOC analyses were collected in 20 mL glass vials and stored at 4 °C after adding two drops of 12 mol L^{-1} HCl. The filters were packed using aluminum foil and stored at -20 °C to analyze Chl-*a* and PAA. All glassware used during sampling and analysis were acid washed and combusted at 500 °C for 5 h. The samples were then

analyzed immediately after being brought to the land laboratory.

Incubation experiment

Incubation experiments were performed using seawater collected from station DH3–6. Up to 18 L surface water was filtered through an acid-cleaned Acropak 1000 Supor membrane 0.8/0.2 μm cartridge filter into polycarbonate carboys. Approximately 2 L surface water was filtered through 47 mm Whatman GF/F glass fiber filters (precombusted at 500 °C for 5 h) under low pressure to remove particulate matter and most photoplankton. 18 L 0.2 μm filtrate was inoculated with 2 L 0.7 μm filtrate in a polycarbonate carboy. The carboy was mixed well and incubated in the dark at room temperature for 71 days. Up to 20 L 0.2 μm filtrate was collected in a polycarbonate carboy as control. Prior to use, all carboys were washed in 5% HCl and rinsed with Milli-Q water three times.

Subsamples of incubation water were collected in duplicate at days 1–7, 9, 14, 19, 25, 32, and 71 during the incubation period to evaluate the changes in the concentrations of DOC, DIN (NO_3^- , NO_2^- , and NH_4^+), carbohydrates, bacteria abundance, and amino acids. Blank samples were collected at days 1, 4, 32, and 71 from control carboys as comparison.

Analytical methods

Amino acid concentrations were measured using *o*-phthaldialdehyde-3-mercaptopropionic acid derivatization method and separated by high-performance liquid chromatography (HPLC) with fluorescence detection (Lindroth and Mopper 1979; Chen et al. 2013). Analytical precisions for different amino acids were between 1.62 and 3.80% (relative standard deviation). The detection limits (signal-to-noise ratio of 3) for individual amino acids were between 0.096 nmol L^{-1} (Thr) and 0.450 nmol L^{-1} (His). Samples for total hydrolyzable amino acid (THAA) measurements were hydrolyzed with 6 mol L^{-1} HCl inside flame sealed ampoules at 110 °C for 22 h under N_2 , and then the hydrolysate was evaporated, and the dried hydrolysate was redissolved in Milli-Q water for derivatization before HPLC analysis. The DFAA concentrations were directly analyzed without

hydrolysis. The DCAA concentrations were equal to the difference between THAA and DFAA concentrations. For PAA analysis, freeze-dried filters were hydrolyzed with 6 mol L^{-1} HCl inside flame sealed ampoules at 110 °C for 24 h. The hydrolyzed samples were centrifuged to get the supernatant and then the supernatant was evaporated. The dried hydrolysate was redissolved in Milli-Q water for derivatization before HPLC analysis.

MCHO and TDCHO were determined using the 2,4,6-tripyridyl-striazine spectrophotometric method (Myklestad et al. 1997). For TDCHO concentrations, 4 mL seawater and 0.4 mL 1 mol L^{-1} HCl were added into ampoules, which were then sealed with flame and hydrolyzed at 100 °C for 20 h. Then, the hydrolysate was neutralized with 1 mol L^{-1} NaOH and measured according to MCHO. The concentration of PCHO was calculated by subtracting MCHO from TDCHO. The relative standard deviation between replicate samples was approximately 2–10%. The reagent blank absorbance was 0.01–0.03 and the detection limit was 2.2 $\mu\text{mol C L}^{-1}$ which is comparable to that of similar method ($2.4 \pm 0.4 \mu\text{mol C L}^{-1}$, Witter and Luther III 2002; $2.4 \mu\text{mol C L}^{-1}$, Myklestad and Børshheim 2007).

Chl-*a* retained on the filter was extracted with 10 mL 90% acetone for 24 h in the dark at 4 °C, centrifuged for 10 min (4000 r min^{-1}), and then measured using a fluorescence spectrophotometer (F-4500, Hitachi Co., Japan), based on the procedure by Parsons et al. (1984).

DOC concentrations were analyzed by catalytic high-temperature oxidation using a total organic carbon analyzer (Shimadzu TOC-V CPH, Japan). The Milli-Q water was used as blank. Potassium hydrogen phthalate was used to achieve a standard curve. Deep seawater references from Hansell Laboratory were used for quality control on a daily basis during sample analysis. The precision was measured as the mean standard deviation of the triplicate injections, which was less than 2%.

Concentrations of nutrients (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} and SiO_3^{2-}) were determined by a nutrient automatic analyzer (Seal Analytical AA3). The detail was described by Strickland and Parsons (1972). The precision for nutrients was better than 3%. The DIN concentration was calculated as the sum of the concentrations of NO_3^- , NO_2^- and NH_4^+ .

Samples for the enumeration of bacteria abundance were fixed with particle-free formaldehyde. Cells were filtered onto Whatman black-stained 0.22 µm polycarbonate filters, stained with 4'-6'-diamidino-2-phenylindole (DAPI, Porter and Feig 1980) and counted with a Leica DM4500B epifluorescence microscope (×400). At least 15 fields and 200 cells (per slide) were counted for each sample to calculate the average of bacterial abundance.

Degradation index (DI)

To directly compare the bioavailability of dissolved to particulate organic matter, the DI of dissolved [DI(d)] and particulate [DI(p)] amino acids were calculated using the same factor coefficients and the following formula proposed by Dauwe et al. (1999):

$$DI = \sum_i \left[\frac{\text{var}_i - \text{AVG}_i}{\text{STD}_i} \right] \times \text{fac.coef}_i,$$

where var_i is the mole percentage of amino acid_{*i*} in our data set, AVG_i and STD_i are the average and standard deviations of the mole percentage of amino acid_{*i*}, respectively, and fac.coef_i is the factor coefficient for amino acid_{*i*} from Table 1 in Dauwe et al. (1999).

Statistical analyses

Statistical tests discussed in the text [one-way analysis of variance (ANOVA), linear correlation, and principal component analysis (PCA)] were conducted with SPSS statistics packages. ANOVA was carried out to determine the significant difference between variables. PCA was performed using the mole percentage of individual amino acids as the original data matrix. Unless otherwise stated, all test results refer to 95% confidence levels.

Results

Horizontal and vertical distribution of DOC and carbohydrate species

The average TCHO concentration measured in this study was $8.15 \pm 3.21 \mu\text{mol C L}^{-1}$, lower than those measured by Wang et al. (2013) in the Bohai Sea and Yellow Sea but higher than those recorded in open

oceans (Pakulski and Benner 1994). Within the TCHO pool, the average concentration of PCHO ($5.23 \pm 2.93 \mu\text{mol C L}^{-1}$) was approximately twice higher than that of MCHO ($2.92 \pm 2.05 \mu\text{mol C L}^{-1}$) (Table 1). Overall, DOC and TCHO concentrations exhibited similar distribution patterns with high values at nearshore stations (Figs. 2, 3). A significant correlation was observed between DOC and TCHO concentrations ($R = 0.460$, $n = 36$, $P < 0.01$). The MCHO/PCHO ratio ranged from 0.09 to 2.93, with an average of 0.80 ± 0.72 , indicating that PCHO is the dominant component of the carbohydrate species. No significant correlation was observed between the Chl-*a* and TCHO concentrations ($P > 0.05$). The result coincided well with the previous studies reported by Wang et al. (2006) for the western Arctic Ocean and He et al. (2015) for the ECS in the same season.

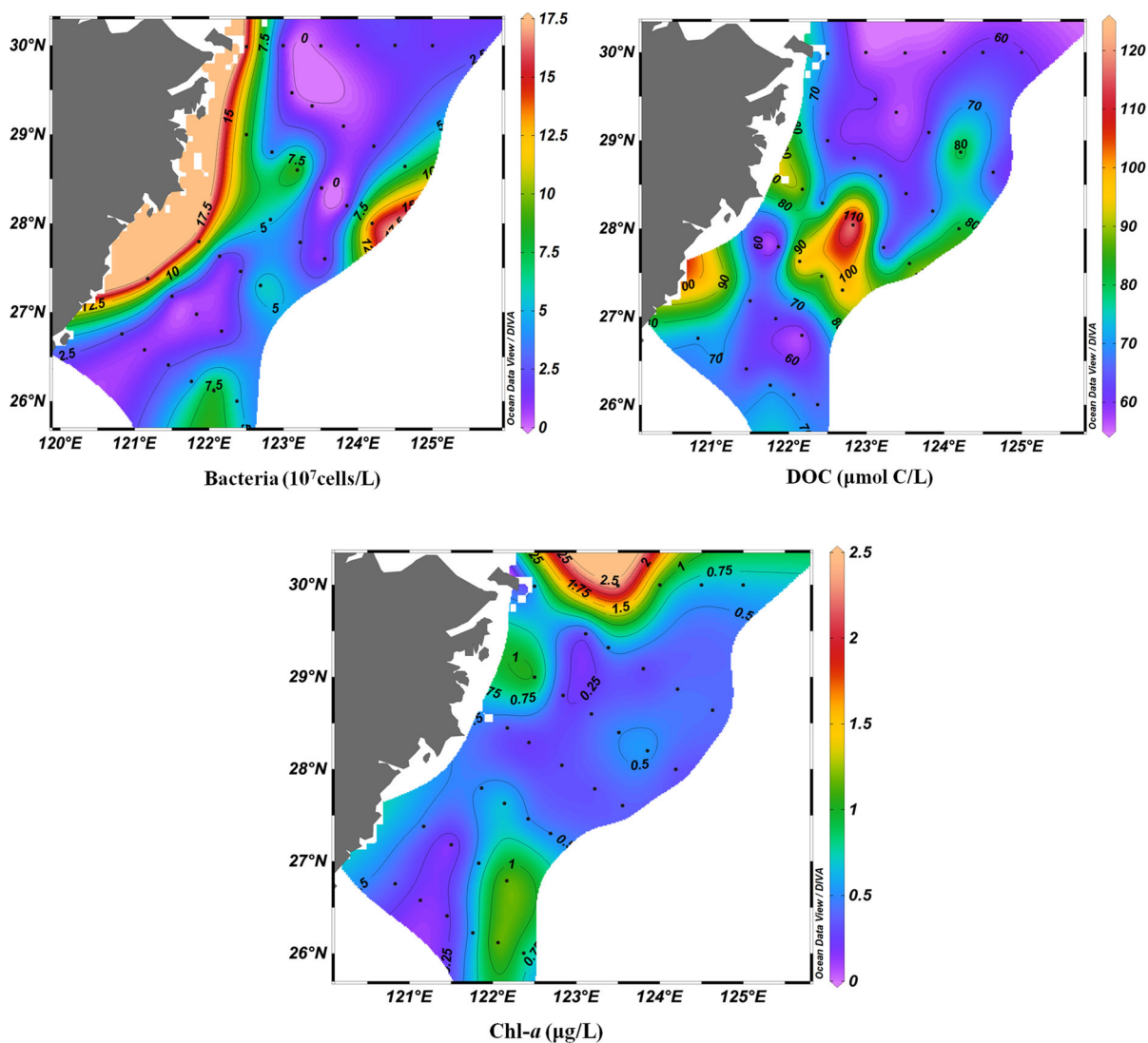
Transect DH1 was dramatically affected by the ZFCC and CDW and was thus chosen to evaluate the vertical variations of DOM. As shown in Fig. 4, the vertical distribution of DOC was similar to that of TCHO along transect DH1, displaying a declining trend from the surface layer to the bottom layer, with high values in surface water at nearshore sites (such as DH1–1). High DOC concentrations were mostly observed within the upper 20 m of the sea.

Distributions and compositions of THAA and PAA

In this study, the THAA concentrations ranged from 0.80 to $1.76 \mu\text{mol L}^{-1}$ in the surface water, which were comparable with those reported by Chen et al. (2016a) in the ECS. Overall, THAA concentrations displayed a declining trend from the northern to southern part of the ECS and high THAA values simultaneously occurred in the middle of the transect (Fig. 5). For instance, the average concentration of THAA was approximately $1.3 \mu\text{mol L}^{-1}$ at transect DH1. This value was obviously higher than that at transect DH7 ($\sim 1.1 \mu\text{mol L}^{-1}$). Similar to the situation of THAA, high DCAA concentrations were observed at the northern and central parts of the study area (Fig. 5). In this investigation, DFAA concentrations in the surface water were generally low, with an average of $0.35 \pm 0.17 \mu\text{mol L}^{-1}$. In addition, no distinct distribution pattern of DFAA was observed in the study area. The concentrations of PAA in the surface water exhibited a similar distribution pattern to Chl-*a* with high values in the northern area and low

Table 1 Concentrations of DOC, Chl-*a*, carbohydrate, amino acids and yields (%) of TCHO and THAA in the surface water of the East China Sea in October 2015

	DOC ($\mu\text{mol C L}^{-1}$)	Chl- <i>a</i> ($\mu\text{g L}^{-1}$)	Carbohydrate ($\mu\text{mol C L}^{-1}$)			Amino acid ($\mu\text{mol L}^{-1}$)				TCHO-C%	THAA-C%
			TCHO	MCHO	PCHO	DFAA	DCAA	THAA	PAA		
Minimum	55.50	0.15	2.27	0.44	1.01	0.12	0.53	0.80	0.01	3.28	3.78
Maximum	121.42	2.39	16.48	7.89	13.80	1.23	1.41	1.76	0.85	19.35	11.64
Mean	70.53	0.60	8.15	2.92	5.23	0.35	0.86	1.21	0.41	11.64	7.15
SD	15.00	0.46	3.21	2.05	2.93	0.17	0.24	0.24	0.20	4.05	1.83

**Fig. 2** Horizontal distribution of bacterial abundance, DOC, and Chl-*a* in the surface water of the East China Sea in October 2015

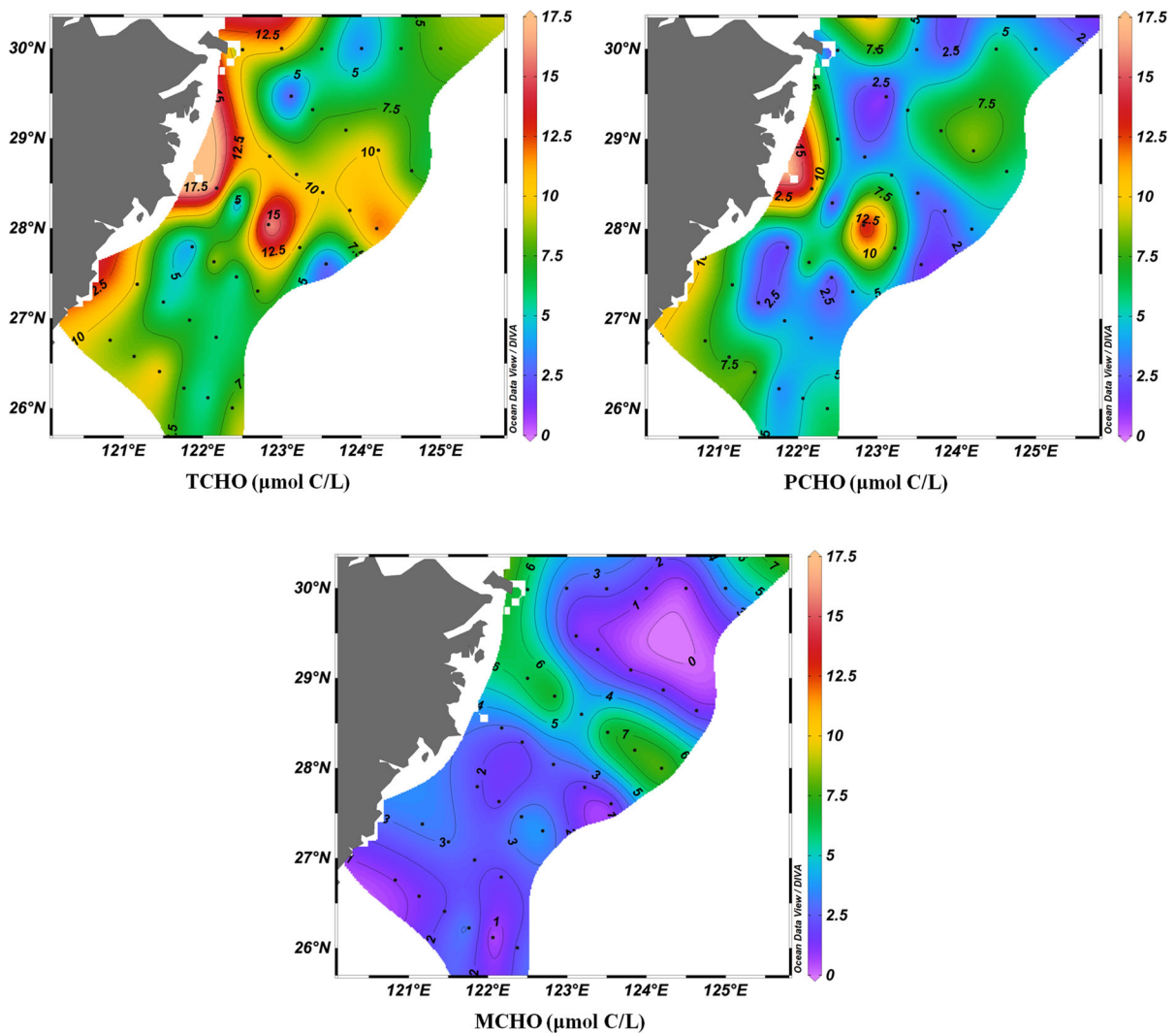


Fig. 3 Horizontal distribution of total dissolved carbohydrates (TCHO), polysaccharides (PCHO) and monosaccharides (MCHO) in the surface water of the East China Sea in October 2015

values in the southern region (Figs. 2, 5). Similar to the situation of DOC and TCHO, vertical distributions of THAA showed a decreasing trend from the surface layer to the bottom layer. However, high values were not observed in the surface water near the Qiantang River (e.g., DH1–1).

The major constituents of DFAA, DCAA, and PAA pool were Asp, Glu, Ser, Gly, Ala, and Leu, accounting for approximately 60% of the total amino acids (Fig. S1), which is similar to previous studies on the ECS and other coastal waters (Cowie and Hedges 1992, 1996; Meon and Kirchman 2001; Yang et al. 2009; Chen et al. 2013, 2016a).

Microbial incubation experiment at a typical station DH3–6

Results for the microbial incubation experiment are shown in Fig. S2. No obvious variations of each chemical parameter within the blank control were found throughout the incubation. This finding indicated that most heterotrophic bacteria were removed after filtration via 0.2 μm membrane. Bacterial abundance peaked to 2.83×10^7 cell L^{-1} after short-term (4–5 d) incubation, and then decreased gradually and remained at 1×10^7 cell L^{-1} in the long-term (71 days) incubation. DOC concentrations decreased

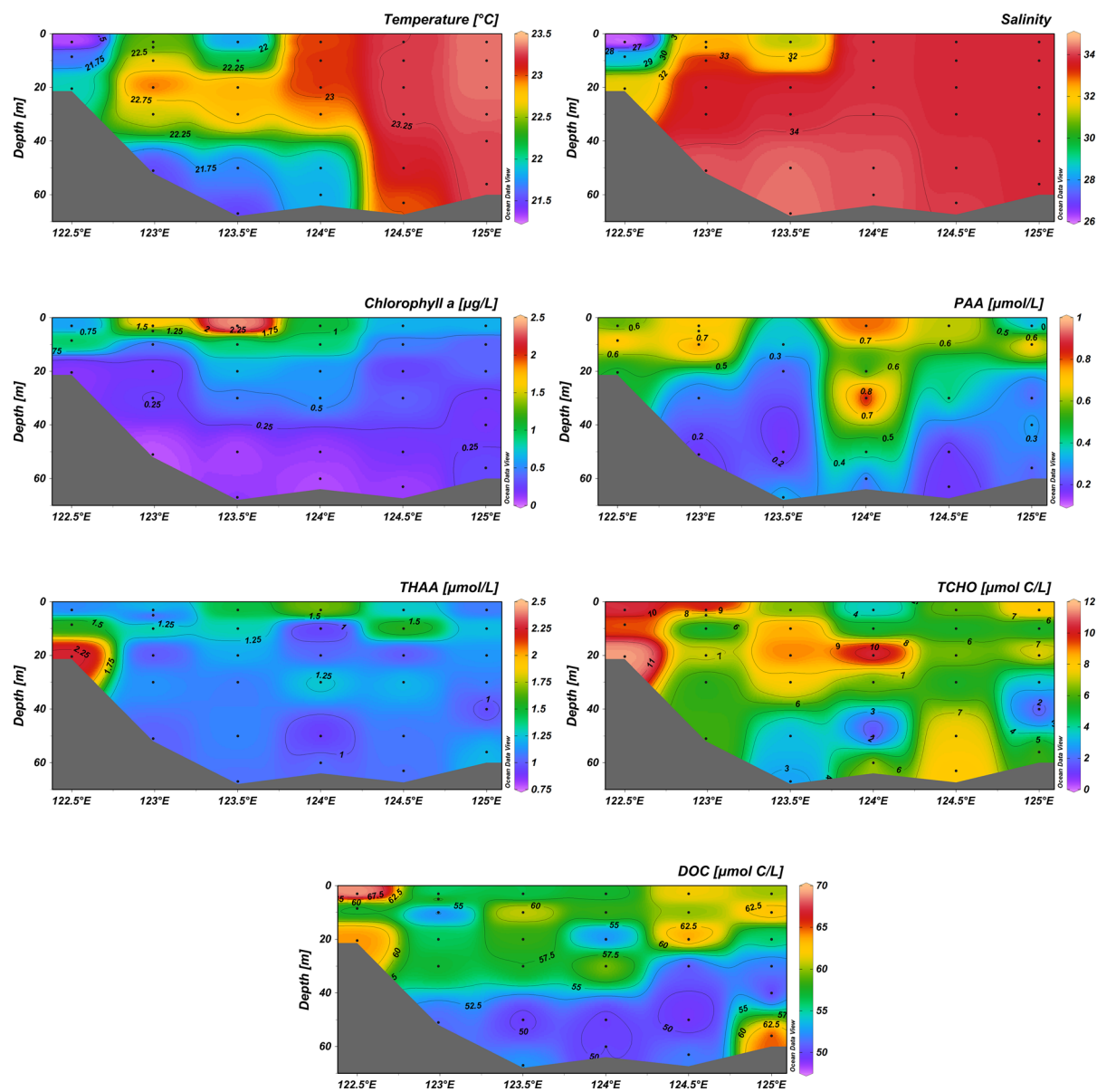


Fig. 4 Vertical distribution of temperature, salinity, Chl-*a*, PAA, THAA, TCHO, and DOC concentrations at transect DH1

from 72.9 to 60.23 $\mu\text{mol C L}^{-1}$, with a decreasing amplitude of 17.4% after 4 days, and then showed minor variations. Eventually, DOC concentrations decreased to 59.14 $\mu\text{mol C L}^{-1}$ at day 71.

As the major constituent of DOC, TCHO concentrations exhibited an obvious declining trend with a decreasing amplitude of 39.9%. MCHO and PCHO concentrations were highly variable within the first 5 days. After then PCHO concentrations decreased

largely over the long term incubation. The MCHO/PCHO ratio ranged from 0.1 to 0.9. THAA and DCAA dropped in first 4 days, then increased and peaked to 1.52 and 1.47 $\mu\text{mol L}^{-1}$ respectively at day 7. Both DFAA and DCAA displayed a decreasing trend during the long-term (71 days) incubation. DIN concentrations showed an increasing trend from 1.94 to 3.00 $\mu\text{mol L}^{-1}$. Correlation analysis revealed that both of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ linearly increased over

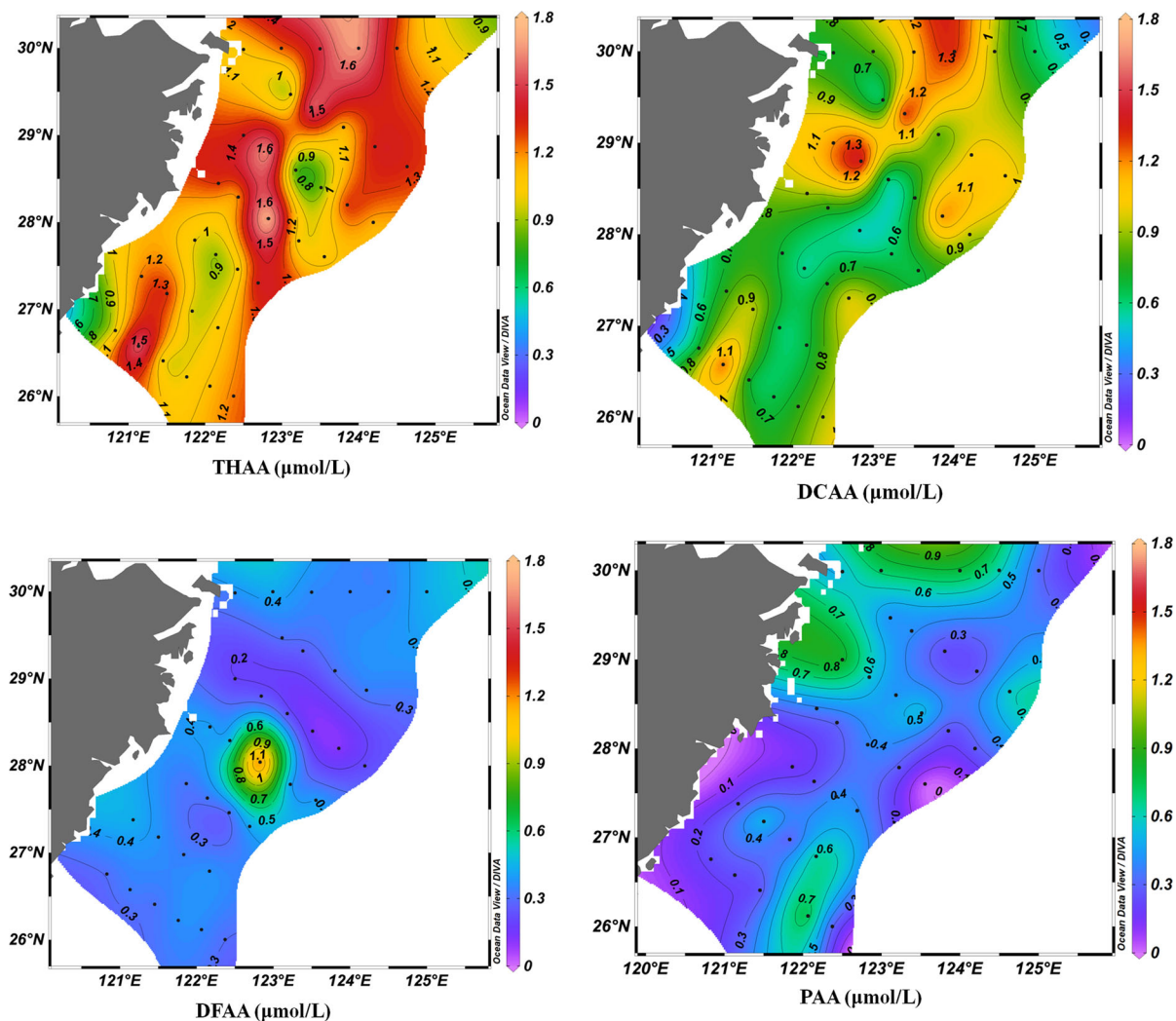


Fig. 5 Horizontal distribution of total hydrolyzable amino acids (THAAs), dissolved combined amino acids (DCAAs), dissolved free amino acids (DFAAs), and particulate amino acids (PAAs) in the surface water of the East China Sea in October 2015

time ($P < 0.05$). The DI values calculated in this incubation showed a declining trend from 0.30 to -0.67 (Fig. S2). TCHO-C% and THAA-C% are defined as the percentages of carbohydrates and amino acids in DOC, respectively. THAA-C% and TCHO-C% decreased from 7.12 and 12.98 to 5.89 and 9.44%, respectively in the long-term (71 days) incubation.

Amino acid and carbohydrate yields and degradation index

In this study, TCHO comprised $11.64 \pm 4.65\%$ of the bulk DOC and varied from 3.28 to 19.35% in the

surface water. High TCHO-C% occurred in the coastal area near the Qiantang River mouth and the central area. In comparison, the low values of TCHO-C% were observed at offshore stations in the south and north of the study area. THAA-C% varied from 3.78 to 11.64%, with an average of $7.17 \pm 1.83\%$. Overall, THAA-C% values of the northern area were relatively higher than those of the southern area (Fig. 6). TCHO-C%, THAA-C% and Chl-*a* displayed similar vertical distribution patterns, with high levels occurring within the upper 20 m. THAA-C% in this study were higher than those measured in the ECS in July 2011 (Chen et al. 2016a) and in the Bohai Sea in April 2010 (Chen

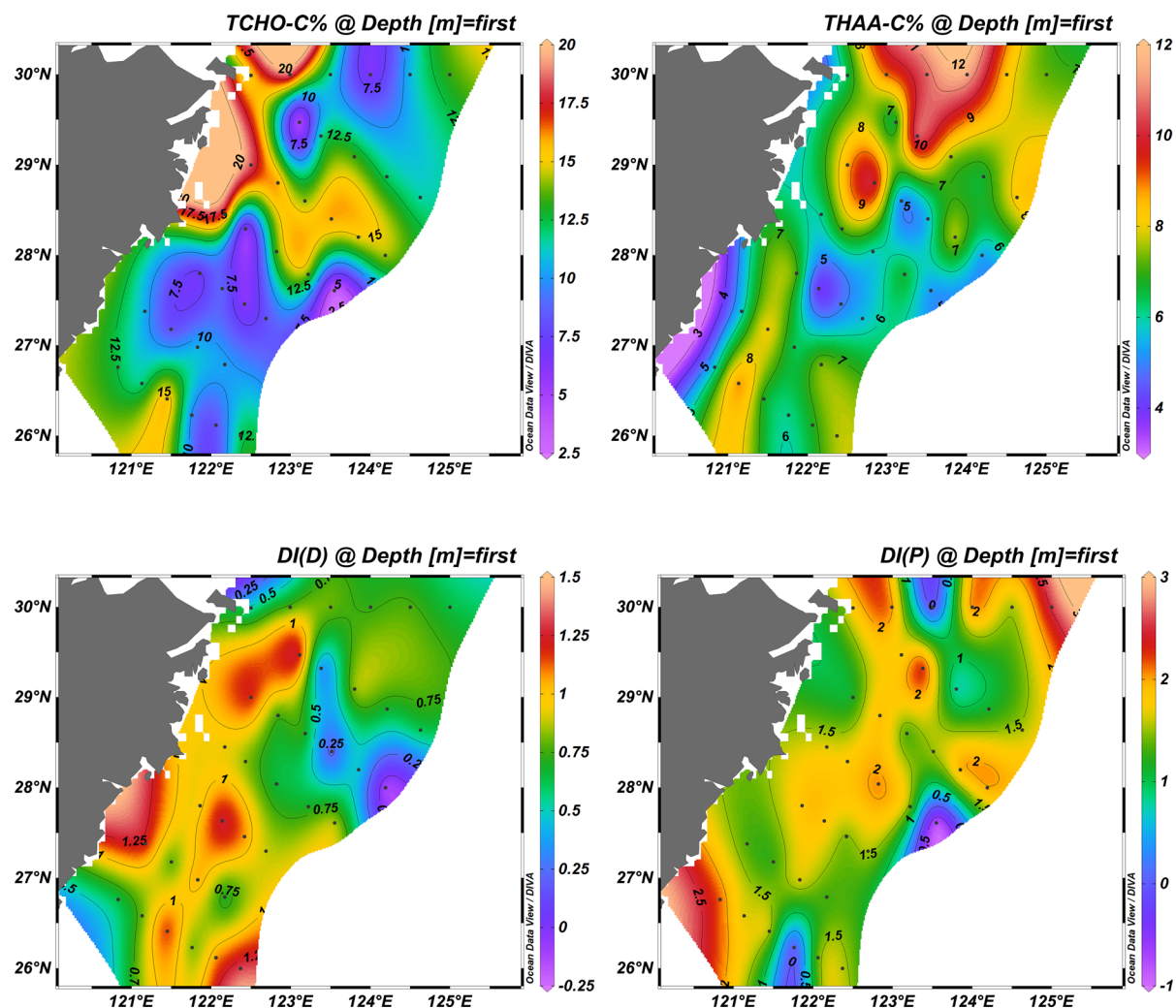


Fig. 6 Horizontal distribution of carbon-normalized yields of carbohydrate (TCHO-C%), carbon-normalized yields of amino acid (THAA-C%), degradation index of dissolved [DI(d)] and particulate [DI(p)] amino acids

et al. 2013) but lower than those in the South Yellow Sea in May 2010 (Chen et al. 2016b).

Compared with THAA-C%, DI was more effective at intermediate stages of organic matter degradation. In this study, DI(d) ranged from -0.13 to 1.32 , with a mean of 0.80 ± 0.31 in the surface water of the ECS. DI(d) values in the northern and southern regions were higher than those in the central area. Moreover, DI(d) displayed a declining trend from nearshore to offshore stations. For instance, high DI(d) levels (> 1) were observed at DH2-1, DH3-1, DH5-2, DH5-3, DH6-1, DH6-3, DH7-3, DH7-5, and DH7-6. DI(p) averaged 1.51 ± 0.70 and ranged from -0.90

to 2.56 . One-way ANOVA showed that DI(p) was significantly higher than DI(d) (one-way ANOVA, $F = 30.46$, $P < 0.001$), indicating that PAA was relatively fresher than dissolved amino acids.

As shown in Fig. 7, vertical distribution of DI(d) showed a decreasing trend from the surface layer to the bottom layer. Furthermore, low DI(d) values were found in the surface water at station DH1-1 close to the Qiantang River mouth. This finding may be due to the highly degraded DOM delivered by terrestrial runoff. Vertical distribution of DI(p) was similar to that of DI(d). However, low DI(p) levels were measured at the middle layer of 20 m. According

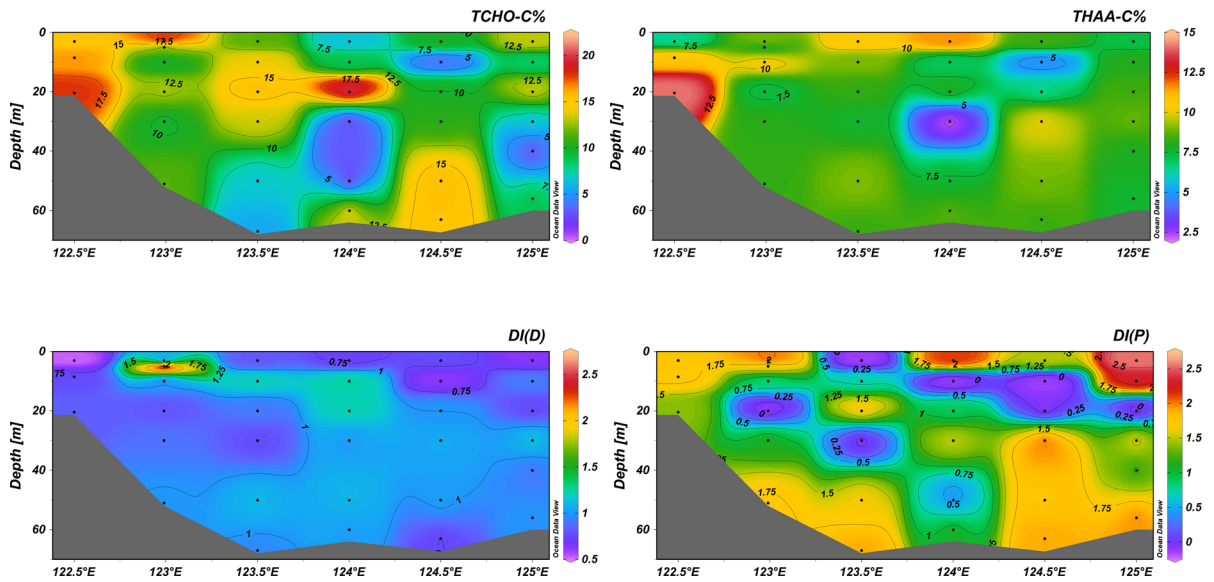


Fig. 7 Vertical distributions of carbon-normalized yields of carbohydrate (TCHO-C%), carbon-normalized yields of amino acid (THAA-C%), degradation index of dissolved [DI(d)] and particulate [DI(p)] amino acids

to the temperature and salinity diagram (Fig. 4), a thermocline and halocline occurred at 20 m depth because of TWC intrusion. This phenomenon blocked the sedimentation of highly degraded particulate matters from the surface water to the underlying waters, thereby leading to a region with low DI(p) values above the spring layer.

Discussion

Distributions of DOM and its control in the water column

The carbohydrate accumulated in seawater originates predominantly from phytoplankton primary production, but the elevated TCHO levels were not found in samples with high levels of Chl-*a*. For instance, the elevated TCHO concentration was 15.98 and 16.48 $\mu\text{mol C L}^{-1}$ at station DH4-1 and DH4-3, respectively, while the Chl-*a* concentration ($\sim 0.3 \mu\text{g L}^{-1}$) was relatively low at the two stations. The absence of significant correlation between the Chl-*a* and TCHO concentrations ($P > 0.05$) may suggest that the in situ phytoplankton production is not the key factor for controlling TCHO concentrations in the surface water at this period. As for the

physical process driving the observed distribution pattern of DOC and TCHO, there was neither correlation between salinity and DOC nor correlation between salinity and TCHO. These phenomena may result from a complex mechanism, more than one simple factor. Previous studies suggested that the in situ phytoplankton production coupled with microbial and grazing activities and water mass transport often controls the carbohydrate concentrations in seawater (Strom et al. 1997; Hopkinson et al. 2002; Guo et al. 2004; Chen et al. 2013). In addition, carbohydrates, especially PCHO, are considered to be highly bioactive and preferentially utilized by microbes (Amon et al. 2001; Wang et al. 2010). Furthermore, the contributions of different phytoplankton species to Chl-*a* are diverse, and the ability of some algal species to produce MCHO and PCHO is also different (Biddanda and Benner 1997). All those influencing factors led to the absence of significant correlation between the TCHO and Chl-*a* concentrations.

High DOC concentrations were mostly observed within the upper 20 m of the sea in this study. According to the temperature and salinity diagram (Fig. 4), a thermocline and halocline occurred in the 20 m layer of the nearshore because of the invasion of TWC surface water and prevented the vertical

exchange between the surface and bottom waters. As a consequence, surface water with high DOC concentrations cannot exchange with the bottom water, which is responsible for the difference of DOC concentrations between the surface and bottom layers. Moreover, as the major constituent of DOC, TCHO originates mostly from phytoplankton extracellular releases. The difference of phytoplankton biomass between the surface and underlying water is another important reason.

Although amino acids are largely plankton-derived (Cowie and Hedges 1992), there were neither significant correlation between Chl-*a* and THAA concentrations nor between Chl-*a* and PAA concentrations during this study, suggesting that amino acid concentrations were not mainly influenced by phytoplankton biomass. The variations of THAA and PAA may result from a group of processes including phytoplankton production, biological degradation, ocean currents, and terrestrial inputs. In this investigation, a significant correlation between DCAA and THAA concentrations was observed ($R = 0.756$, $P < 0.01$, $n = 36$), implying that DCAA is the major component of THAA. DFAA concentrations showed no distinct distribution pattern in the study area. This finding may be because DFAA are the most labile component of marine organic matter and have fast turnover rates in seawater.

The relative levels of the individual amino acids may be influenced by their varying rates of production and utilization. Asp, Glu, Ser, Gly, Ala, and Leu were the major constituents of DFAA, DCAA, and PAA pool. One-way ANOVA showed no significant differences among DFAA, DCAA, and PAA compositions ($P > 0.05$), indicating that different forms of amino acids may have the same sources or removal pathways. Diatoms were the dominant algae in the water of ECS in autumn, occupying more than 70% of total phytoplankton biomass (Wang et al. 2008). Moreover, Ala, Ser, Asp, and Gly are major compositions of frustules (Kirchman and Hodson 1984). Nguyen and Harvey (1997) found preferential preservation of Gly in microbial decomposition of diatom *Thalassiosira weissflogii*. Cowie and Hedges (1996) investigated the effect of zooplankton digestion on THAA in *T. weissflogii*, and found that Gly was significantly preserved relative to other amino acids. The high proportions of Gly and Ala may be attributed to their comparatively minor food value to micro- and

macroconsumers because of their short chain length and probably because it can be synthesized from many other amino acids in heterotrophic metabolism.

Insight from the microbial incubation experiment

In the present study, the seawater incubation experiment was conducted at station DH3–6 to investigate variations of the DOM concentrations and compare the effectiveness of different DIs. The elevated bacterial abundance resulted in a significant decrease in DOC concentrations from the first to the fourth day of incubation. Overall, DOC concentrations displayed a significant declining trend during the long-term (71 days) incubation, indicating that DOC was utilized by heterotrophic bacteria. The obvious declining trend of TCHO concentrations revealed that TCHO, a major labile fraction of DOC, was preferentially utilized by heterotrophic bacteria as a carbon source. No significant variation trend was found in MCHO/PCHO ratio, indicating that MCHO and PCHO were not selectively consumed by heterotrophic bacteria.

Several studies showed that DI, TCHO-C% and THAA-C% can effectively indicate the degradation state of organic matter (Cowie and Hedges 1994; Dauwe and Middelburg 1998; Dauwe et al. 1999; Keil et al. 2000). For example, the DI values generally covered a range from -1.5 to 2 . Commonly, relatively low DI values indicate a high degree of degradation and low DOM bioavailability. TCHO-C% and THAA-C% generally decreased with increasing degradation. The declining trend of DI, THAA-C% and TCHO-C% values calculated in this long-term (71 days) incubation indicated the increasing degradation state and decreasing bioavailability of surface DOM.

To further understand the internal relations among the compound concentrations, PCA was used to reveal the relationship in the incubation system. As shown in Fig. 8a, DIN and its main constituents on PC1 were positively correlated with incubation time, whereas DOC, DFAA, DCAA, MCHO and PCHO exhibited negative correlations. This result indicated that DOM was consumed by heterotrophic bacteria and accumulated DIN. According to PC2, bacterial biomass showed highly negative loadings. By contrast, DFAA and DCAA exhibited highly positive loadings.

To illustrate the changes in the compositions of amino acids, PCA was used to examine the

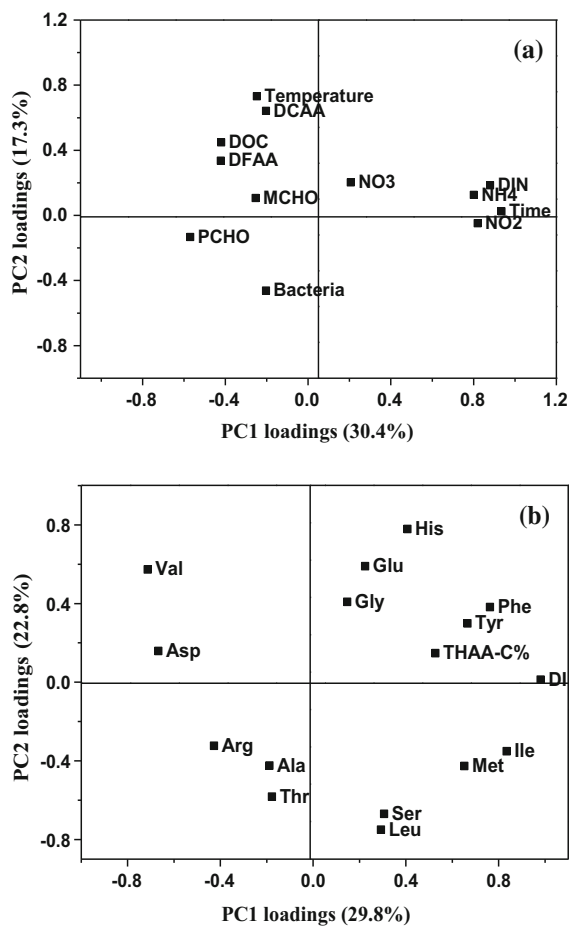


Fig. 8 Principal component loadings for different compounds and environmental factors (a), and for THAA and degradation indices in the surface water (b)

compositional differences. As shown in Fig. 8b, for the PC1, DI, THAA-C%, Ile, Phe, Met, and Tyr exhibited highly positive loadings (> 0.5). By comparison, Val and Asp showed negative loadings. The result revealed that Ile, Phe, Met, and Tyr contents decreased with increasing degradation of organic matter (especially organic carbon), whereas Val and Asp increased. Similarly, for the PC2, Val, His, and Glu exhibited highly positive loadings. Conversely, Leu, Thr, Met, and Ser showed highly negative loadings. The result indicated the removal of Val, His, and Glu and the accumulation of Leu, Thr, Met, and Ser during organic matter degradation. Amino acids are the major carbon and nitrogen sources for microbes. Some amino acids are accumulated and

others are consumed because of the selective utilization by microbes (Yamashita and Tanoue 2003).

Evaluation of bioavailability of DOM in the ECS

Abundant nutrients and terrestrial organic matters are transported into the ECS by the riverine input and human activities, leading to the high primary productivity ($437 \text{ mg C m}^{-2} \text{ day}^{-1}$) in the ECS (Liu et al. 2015). Various phytoplankton and bacteria biomass caused the difference in DOM bioavailability in the study area. A DOM decomposition experiment conducted by Amon et al. (2001) demonstrated that bacteria played a crucial role in controlling both the concentration and composition of marine DOM by selectively removing bioactive components and by leaving behind biorefractory components. In general, high TCHO-C% and THAA-C% indicate high bioavailability of DOM. The stations with high bioavailability often display dramatic autotrophic and heterotrophic activities and high circulation rate of biogenic elements (Shen et al. 2016).

The stations with higher TCHO-C% and THAA-C% levels than average are illustrated in Fig. 9 (hot spot). Overall, the elevated THAA-C% and TCHO-C% levels were measured in samples with high Chl-*a* concentrations. In addition, the higher mole percentage of Gly and Ser which were probably associated with the species and biomass of phytoplankton (Hecky et al. 1973; Wang et al. 2008) was found in this investigation. All these results indicated that bioavailable organic matters in seawater were derived mainly from phytoplankton. Similarly, previous study conducted by Shen et al. (2016) at the northern region of the Gulf of Mexico revealed that high THAA-C% values are generally found in the regions with enhanced phytoplankton biomass. However, there was neither significant correlation between THAA-C% and Chl-*a*, nor between TCHO-C% and Chl-*a* in the study area. Carbohydrates and amino acids are major carbon, nitrogen and energy sources for marine microbes and exhibit high turnover rates (Suttle et al. 1991; Keil and Kirchman 1992; Reinthaler et al. 2008). Microbial degradation (Amon et al. 2001) and photodegradation (Kovac et al. 1998) are responsible for the decrease of the bioavailability of organic matter. The absence of these correlations suggests that phytoplankton production coupled with microbial degradation and photodegradation may be responsible

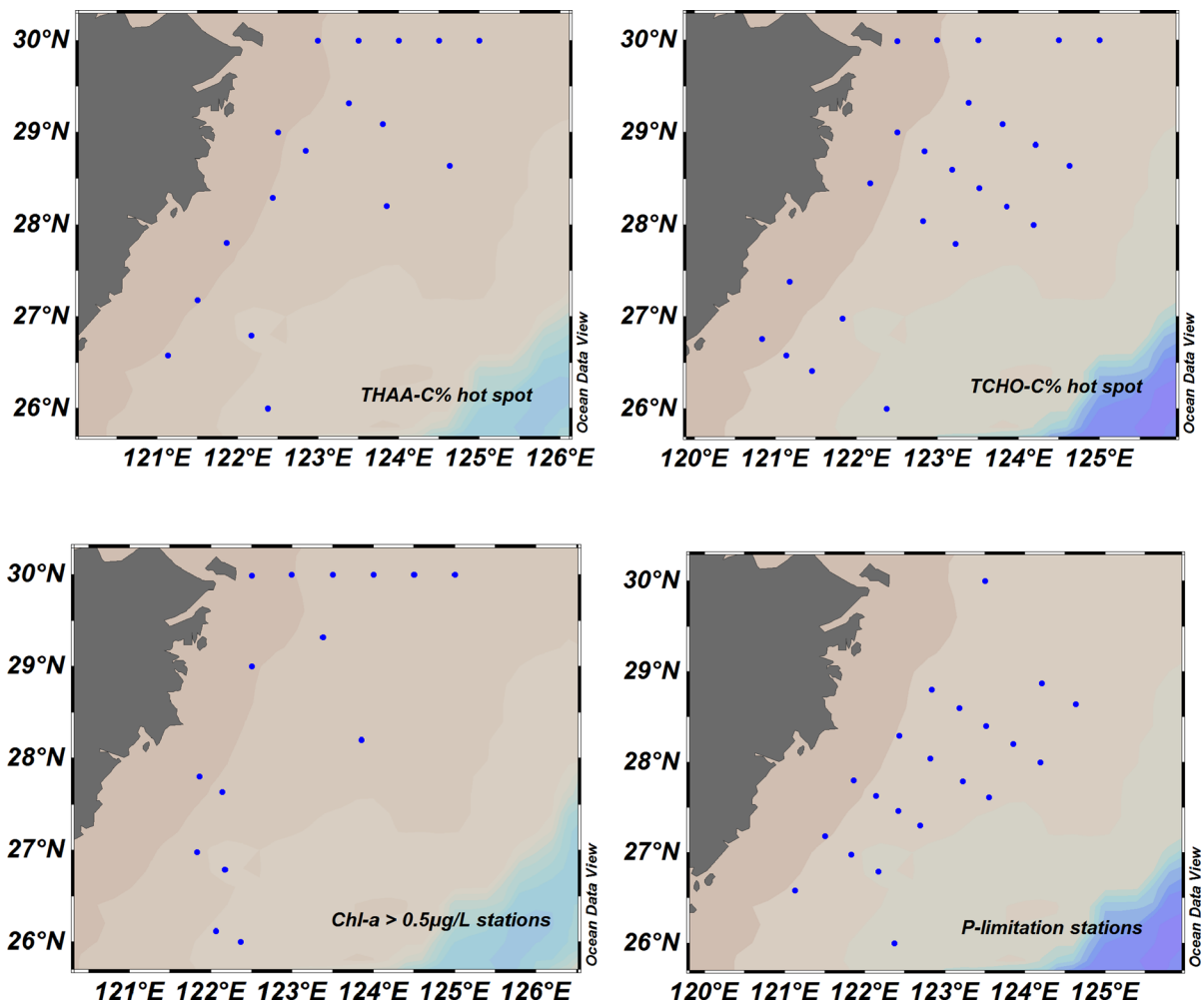


Fig. 9 Stations with high levels of carbon-normalized yields of amino acid (THAA-C%), carbon-normalized yields of carbohydrate (TCHO-C%), Chl-*a*, and P-limitation stations

for controlling the bioavailability of DOM in the surface water of the ECS. By contrast, elevated TCHO-C% levels mostly occurred in nutrient-poor or nutrient-limited waters. Nutrients limit the growth of phytoplankton, leading to reduced production of proteins. However, photosynthesis still proceeds, resulting in preferential production of carbohydrates over proteins (Mykkestad and Haug 1972; Jiang et al. 2012). Dortch and Whitlege (1992) proposed a standard of nutrient limitation to estimate whether the growth of phytoplankton was limited by nutrient in study area. They put forward that N limitation was $\text{DIN/P} < 10$, and $\text{DIN} < 1.0 \mu\text{mol L}^{-1}$, P limitation was $\text{DIN/P} > 30$, and $\text{P} < 0.20 \mu\text{mol L}^{-1}$, and Si limitation was $\text{Si/P} < 3$, $\text{Si/DIN} < 1$, and

$\text{Si} < 2.0 \mu\text{mol L}^{-1}$. According to the standard, the nutrient data in this study were processed, and there were 21 stations suffering P limitation, accounting for 58% of the total stations, without N and Si limitation in the study area. This result indicated that the $\text{PO}_4^{3-}\text{-P}$ content was the key factor to limit the growth of phytoplankton. In this light, elevated TCHO-C% levels were found at transects DH3 and DH4, which experienced P limitation in our study. Our data provided further evidence that when phytoplankton experienced nutrient limitation there was an excess production of carbohydrates. Differences in phytoplankton biomass and nutrient distributions as well as the P-limiting condition may lead to the different

distribution patterns between THAA-C% and TCHO-C% in the ECS.

Amon and Benner (2003) indicated that TCHO-C% higher than 4% is representative of freshly derived phytoplankton DOM, whereas TCHO-C% lower than 2.5% are indicative of degraded DOM or terrestrial DOM. In this investigation, low TCHO-C% values (< 4%) were found in four samples at the surface layer of DH2–1 and DH4–5 and 50 m depth of DH1–4 and DH1–6. Moreover, the TCHO-C% values of other samples were higher than 4%. Some literature data for yield of amino acids in sea areas are described in Table 2. Kaiser and Benner (2009) pointed out that THAA-C% decreased rapidly with depth. Previous studies conducted by Yamashita and Tanoue (2003) revealed that THAA contributions to DOC are high in the bay and coastal areas, reduced toward the oceanic area.

In this investigation, THAA-C% values were relatively higher than those (~ 1%) measured in shelf Chukchi Sea and deep ocean water by Davis and Benner (2005), indicating that the bioavailability of organic matters in the ECS during autumn was relatively high. The primary production in the ECS in autumn (~ 527 mg C m⁻² day⁻¹) (Song et al. 2008) was lower than that in the Chukchi Sea in summer (~ 783 mg C m⁻² day⁻¹) (Hill and Cota 2005), but higher than that in Canada Basin (~ 324 mg C m⁻² day⁻¹) (Hill and Cota 2005). The THAA concentration and THAA-C% values in the ECS in autumn were higher than those in the Chukchi Sea. Compared with the ECS, the higher primary production in the Chukchi Sea did not co-occur spatially with the greater THAA concentrations and THAA-C% values, indicating the rapid utilization of this most reactive component of DOC and an

Table 2 Literature data for carbon-normalized yields of amino acid and nitrogen-normalized yields of amino acid in some sea areas

Locations	Sample isolation	Depth range (m)	AA-C%	AA-N%	Sampling periods	References
North Pacific	UDOM	10	10.2*	17.4*	April 1991	McCarthy et al. (1996)
Sargasso Sea	UDOM	2	14.7*	28.7*	May 1992	McCarthy et al. (1996)
Gulf of Mexico	UDOM	10	8.7*	16.6*	August 1991	McCarthy et al. (1996)
Bermuda	Unfiltered	20	0.9	–	June 2001	Kaiser and Benner (2009)
Hawaii	Unfiltered	20	1.2	–	November 1999	Kaiser and Benner (2009)
Northwestern Pacific	GF/F	Upper 400	0.9–6.9	–	18–26 April 2001	Yamashita and Tanoue (2003)
Chukchi Sea	GF/F	Surface layer	1.7	8.8	8 May–11 June, 17 July–21 August 2002	Davis and Benner (2005)
Canada Basin	Unfiltered	Below 1000	0.8	7.4	8 May–11 June, 17 July–21 August 2002	Davis and Benner (2005)
North Sea	–	Sediment	8–28	25–60	August 1994, May 1996	Dauwe and Middelburg (1998)
Knebel Vig, Denmark	–	Sediment	24	53	July 4, 1994	Lomstein et al. (1998)
	–	Porewater	9.2	26.8		
The South Yellow Sea	GF/F	Subsurface water	7.76	–	May 2010	Chen et al. (2016b)
Bohai Sea, China	GF/F	Subsurface water	6.7	–	April 2010	Chen et al. (2013)
East China Sea	GF/F	Surface layer	3.1	6.0	7–16 July 2011	Chen et al. (2016a)
East China Sea	GF/F	Surface layer	7.15	25.79	October 2015	This study

UDOM ultrafiltered (> 1000 Da) dissolved organic material

*AA-C% is expressed as mg amino acid per 100 mg OC, AA-N% is on a weight basis

uncoupling between production and consumption processes. The wide shelves of the Arctic Ocean receive about 10% of annual global riverine discharge and 25 Tg of terrigenous DOC each year (Aagaard and Carmack 1989; Opsahl et al. 1999). Previous work in the estuaries of Ob and Yenisei and the adjacent Kara Sea showed the riverine DOM was largely unreactive and refractory (Köhler et al. 2003). Due to the low primary production, the DOC and THAA concentrations and THAA-C% in deep waters of the Canada Basin were much lower than those in the surface waters of the ECS. In general, deep-water DOC has old average radiocarbon age (Williams and Druffel 1987; Druffel et al. 1992) and is resistant to biological utilization (Barber 1968). It can be inferred that the deep-water DOM (> 1000 m) in Canada Basin was much more refractory than the surface-water DOM in the ECS. Phytoplankton production coupled with microbial degradation and terrestrial input may be responsible for diverse bioavailability of DOM in different sea areas.

Conclusions

The present study described the horizontal and vertical distributions of DOC, amino acids, and carbohydrates at seven transects of the ECS in October 2015. Spatial distribution trends of amino acid and carbohydrate concentrations in surface waters of the ECS during the study period may result from a group of processes including phytoplankton production, biological degradation, ocean currents, and terrestrial inputs. A positive correlation was observed between the DOC and TCHO concentrations, revealing similar source and removal pathways of DOC and TCHO. Incubation experiments showed that TCHO is preferentially utilized by heterotrophic bacteria as a carbon source. Mole fraction of Ile, Phe, Met, and Tyr decreased during the degradation of organic matter, whereas Val and Asp increased, reflecting selective consumptions of individual amino acids. The DI, THAA-C% and TCHO-C% values showed decreasing trends, indicating that heterotrophic bacterial activities largely affect the DOM bioavailability. Phytoplankton production coupled with microbial degradation and photodegradation may be responsible for controlling the bioavailability of DOM in the ECS. The elevated TCHO-C% levels were found at P-limited stations in our study.

Consistent with previous results, this study also revealed that when phytoplankton experienced nutrient limitation there was an excess production of carbohydrates. The $\text{PO}_4^{3-}\text{-P}$ content was the key factor limiting the growth of phytoplankton in the study area. Further work is needed to carry out a continuous research on the processes of DOM transformation and degradation using bacterial biomarkers such as D-AAs. Moreover, the mechanism of selective consumption of individual amino acids also merit further study.

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