Spatiotemporal variation of dissolved carbohydrates and amino acids in Jiaozhou Bay, China*

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Abstract   Surface seawater samples were collected from Jiaozhou Bay, China, during six cruises (March–May 2010, September–November 2010) to study the distribution of dissolved organic matter including dissolved organic carbon (DOC), total dissolved carbohydrates, namely monosaccharides (MCHO) and polysaccharides (PCHO) and total hydrolysable amino acids. These included dissolved free amino acids (DFAA) and combined amino acids (DCAA). The goal was to investigate possible relationships between these dissolved organic compounds and environmental parameters. During spring, the concentrations of MCHO and PCHO were 9.6 (2.8−22.6) and 11.0 (2.9−42.5) µmol C/L, respectively. In autumn, MCHO and PCHO were 9.1 (2.6−27.0) and 10.8 (2.4−25.6) µmol C/L, respectively. The spring concentrations of DFAA and DCAA were 1.7 (1.1−4.1) and 7.6 (1.1−31.0) µmol C/L, respectively, while in autumn, DFAA and DCAA were 2.3 (1.1−8.0) and 3.3 (0.6−7.2) µmol C/L, respectively. Among these compounds, the concentrations of PCHO were the highest, accounting for nearly a quarter of the DOC, followed by MCHO, DCAA and DFAA. The concentrations of the organic compounds exhibited a decreasing trend from the coastal to the central regions of the bay. A negative correlation between concentrations of DOC and salinity in each cruise suggested that riverine inputs around the bay have an important impact on the distribution of DOC in the surface water. A significant positive correlation was found between DOC and total bacteria count in spring and autumn, suggesting bacteria play an important role in the marine carbon cycle.

Keyword: carbohydrates; amino acids; concentration; seasonal variation; biological parameter; Jiaozhou Bay

1 INTRODUCTION

Dissolved organic matter plays an important role in the global carbon cycle (Hedges, 1992). It is the main component in the cells of biological organisms in both terrestrial and marine ecosystems, and is also essential for the organisms' energy storage and transport media (Myklestad and Børsheim, 2007). In marine systems, dissolved organic matter supports heterotrophic activity (Pomeroy, 1974; Azam et al., 1983) and affects the penetration of light and exchange of gases in the sea surface (Bard et al., 1988). However, it is only possible to detect and analyze a small portion of the total dissolved organic matter, namely carbohydrates, proteins (amino acid) and lipids (Chester, 2000). A large fraction of humic substances that are abundant in aquatic systems simply can not be analyzed. Biomass, terrestrial inputs, grazing and microbial activities are important suppliers of dissolved organic carbon (DOC) in the water column.

Dissolved carbohydrates are often identified as the largest fraction of DOC in the ocean. They are the

primary products of the phytoplankton photosynthesis and can be released into the environment by phytoplankton, especially diatoms, under nutrient stress or toxic conditions, DOC decomposition, riverine inputs and sediment resuspension (Granum et al., 2002; Børsheim et al., 2005; Guéguen et al., 2006; Khodse et al., 2008; Wang et al., 2010; Youssef et al., 2014). Carbohydrates in the forms of dissolved monosaccharides (MCHO) and polysaccharides (PCHO), comprise up to 20%–40% of organic matter by weight of plankton, 13% of sinking particulate organic carbon, 15% of suspended organic carbon, 32% of DOC, and 13% of sedimentary organic carbon (Hernes et al., 1996; Skoog and Benner, 1997). However, carbohydrate production is affected by several factors, including algae growth, nutrient concentrations, phytoplankton species, and bacterial activity (Børsheim et al., 2005). As such, carbohydrate concentrations in seawater could be a useful tool in the investigation of the biogeochemical cycling of DOC in the marine environment (Wang et al., 2010).

Amino acids are also important components of the bulk DOC pool. Moreover, amino acids are the major constituents of organic nitrogen (ON), which accounts for ~15% of the bulk dissolved organic nitrogen (DON) pool (Keil and Kirchman, 1991; Hubberten et al., 1994). Dissolved amino acids in seawater exist in the forms of dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA). DCAA are the major forms of dissolved amino acids (Keil and Kirchman, 1993; Søndergaard and Middelboe, 1995) while DFAA are the minor forms. DFAA are a source of carbon and nitrogen for bacteria, and there is increasing evidence indicating that DFAA are also used as a nutrient by microalgae, particularly in low ON environments (Admiraal et al., 1987; Linares and Sundbäck, 2006).

To date, there are few studies about the distribution and concentrations of DOC, carbohydrates and amino acids in Jiaozhou Bay (Ji et al., 1983; Zhang et al., 2009). The abundance, distribution and composition of the dissolved organic matter and its seasonal variation in Jiaozhou Bay have not been described in detail. The aims of the current research were to characterize the composition of the DOC, dissolved carbohydrates and dissolved amino acids in the surface seawater, to identify the yields and seasonal variation of dissolved organic matter, and to investigate possible relationships between the constituents of dissolved organic matter and biogeochemical parameters.

2 MATERIAL AND METHOD

2.1 Study area

Jiaozhou Bay (35°58′–36°18′N, 120°04′–120°23′E) is a typical semi-enclosed water body, connecting with the Yellow Sea through a narrow mouth 2.5 km wide, with a surface area of 390 km² and an average depth of ~7 m. The maximum depth of the bay is ~70 m in the narrow mouth of the bay. It is affected by strong outer tidal currents (Zhang et al., 2006). It is surrounded by the city of Qingdao, which has a population of $7×10^6$. The bay has been influenced by industry, agriculture, anthropic behavior and increasing harbor construction. According to previous studies (Shen 2001; Zhang et al., 2006), industrial pollution and domestic sewage are the main causes of organic contamination and eutrophication in this area, with the northeast region of the bay the most severely affected. Because of the representative geographic location and ecologic conditions, Jiaozhou Bay is a suitable place to study the effects of human activities on the marine environment.

2.2 Sampling

Six cruises were conducted in Jiaozhou Bay in March, April, May, September, October and November 2010. The locations of sampling stations are shown in Fig.1. The spring cruises contained 19 sampling stations along five transects which were selected to describe the whole waters systematically. In autumn, weather conditions constrained the cruises to 15 sampling stations along five transects.

The surface water samples were collected from ~50 cm depth using a plexiglass sampler, which was rinsed three times prior to sampling. The water samples were immediately filtered through 47 mm Whatman GF/F glass-fiber filters (precombusted at 500°C for 3 h). The filtrate was divided into three parts for the analysis of dissolved carbohydrates, dissolved amino acids and DOC. These portions were collected into the precombusted 40 mL glass vials and stored at -20°C. The filters were stored at 4°C for the analysis of chlorophyll a (Chl-a). For the analysis of total bacteria count (TBC), 20 mL of seawater was fixed immediately using 2 mL formaldehyde solution with a final concentration of 2.5% in the sterilized bottle, and then stored at 4°C (Porter and Feig, 1980).

2.3 Determination of DOC, Chl-a and TBC

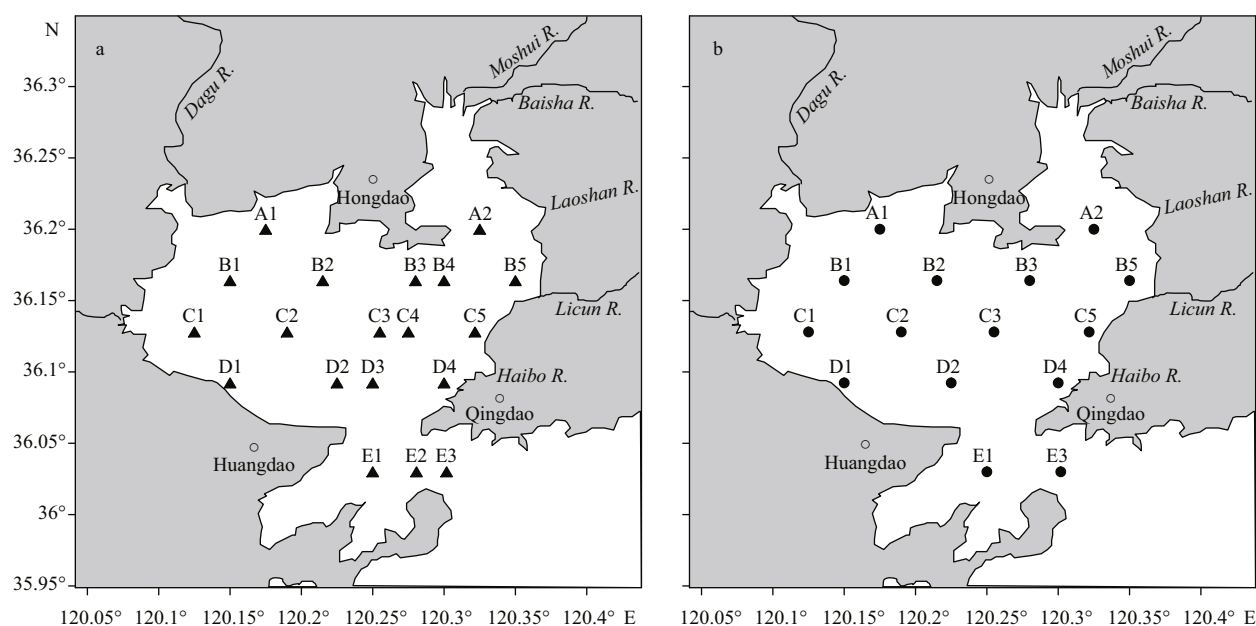Concentrations of DOC were determined by the

Fig.1 Locations of the sampling stations in Jiaozhou Bay during the cruises of spring (a, ▲) and autumn (b, ●) in 2010

method of catalytic high temperature oxidation using a total organic carbon analyzer (Shimadzu TOC-V$_{CPH}$, Shimadzu Co. Japan). After being acidified to pH 2–3, the samples were bubbled with pure nitrogen gas for 10 min to clear up the inorganic carbon component. Triplicates of 50 μL aliquots were injected by an autosampler into the furnace, which was filled with a normal sensitivity catalyst at 680°C. The combusted products were detected by the non-dispersive infrared gas analyzer (NDIR). The concentrations of DOC were determined based on a calibration curve using potassium hydrogen phthalate (Nacalai Tesque, Inc. Kyoto, Japan). The precision was estimated as the standard deviation of the aliquots and less than 2% of the mean.

Chl-*a* on the membrane was extracted with acetone for 24 h at 4°C, centrifuged for 10 min and then measured by a fluorescence spectrophotometer (F-4500, Hitachi Co. Japan) as per the method described in Parsons et al. (1984).

To estimate TBC, a 2-mL seawater sample was stained with DAPI (4′,6′-diamidino-2-phenylindole, final concentration 0.01%) for 5 min, and filtered through a 0.22-μm black polycarbonate filter (Whatman). The bacterial cells were counted in 10 randomly sights using 100× objective and an epifluorescence microscope (DMLA, Leica). Average cell number per sight was calculated and used to estimate TBC following the procedure from Porter and Feig (1980) and Parsons et al. (1984).

2.4 Determination of dissolved carbohydrates and amino acids

MCHO and PCHO in seawater were measured by 2,4,6-tripyridyl-s-triazine (TPTZ) spectrophotometric method (Myklestad et al., 1997) based on the absorption at 596 nm of the violent complex compound generated by Fe^{2+} and TPTZ resulting from the reduction of Fe^{3+} to Fe^{2+} by reducing sugars. An aliquot of 1 mL of seawater mixed with 1 mL solution of potassium ferrocyanide (0.7 mmol/L) in a test tube of 10 mL was heated at 100°C for 10 min in a boiling water bath. After being cooled in cold water, 1 mL ferric chloride solution (2.0 mmol/L) and 2 mL TPTZ solution (2.5 mmol/L) were added and the absorbance was measured at 596 nm with a UV-Visible Spectrophotometer (UV-2550, Shimadzu Co., Japan). The concentration of MCHO was determined under the standard calibration curve made by D-(+)-glucose.

The concentrations of total dissolved carbohydrates (TCHO) can be inferred by turning all combined sugars to monomeric sugars. In 5 mL sealed ampoules, acid hydrolysis was performed with 4 mL of seawater and 0.4 mL 1 mol/L HCl at 100°C for 20 h. Then the concentration of PCHO was equal to the difference between TCHO and MCHO([PCHO]=[TCHO]−[MCHO]). The concentration of carbohydrates was measured in μmol C/L by multiplying by a factor of 6 according to the molecular structure of glucose. The coefficient of variation for the method was 5%–10%

Table 1 Details of sampling stations in Jiaozhou Bay during the cruises of March, April, May, September, October and November 2010

Cruises				March		April		May		September		October		November	
Station	Longitude	Latitude	Depth (m)	T (°C)	Salinity	T (°C)	Salinity	T (°C)	Salinity	T (°C)	Salinity	T (°C)	Salinity	T (°C)	Salinity
A1	120.18°E	36.20°N	4	5.9	31.63	11.4	32.25	17.6	32.47	21.5	29.69	15.0	31.35	9.3	31.63
A2	120.33°E	36.20°N	2	6.1	31.42	11.9	31.50	17.7	30.06	21.8	27.81	13.6	29.96	10.0	29.85
B1	120.15°E	36.16°N	5	6.6	32.20	11.2	32.25	17.9	32.88	21.7	29.74	15.0	31.77	11.1	31.24
B2	120.22°E	36.16°N	5	6.3	31.88	11.2	32.11	18.8	32.48	22.2	29.63	14.9	31.27	10.1	31.00
B3	120.28°E	36.16°N	4	6.0	31.93	11.2	32.06	18.0	31.87	22.2	29.02	14.3	30.37	10.2	30.58
B4	120.30°E	36.16°N	3	6.4	32.12	10.8	32.02	17.8	32.27	-	-	-	-	-	-
B5	120.35°E	36.16°N	7	5.3	31.61	11.0	31.18	18.9	30.92	25.5	28.32	15.8	31.16	11.4	30.29
C1	120.13°E	36.13°N	8	6.5	32.06	12.2	32.28	16.5	32.28	22.2	28.44	13.8	30.91	11.9	31.92
C2	120.19°E	36.13°N	7	7.0	32.29	11.5	32.56	15.0	32.24	23.3	30.62	16.5	31.48	13.2	31.67
C3	120.26°E	36.13°N	11	6.7	32.33	11.6	32.37	15.0	32.77	24.0	30.92	17.1	31.46	12.9	31.98
C4	120.28°E	36.13°N	8	7.0	32.48	12.0	32.16	16.5	32.74	-	-	-	-	-	-
C5	120.32°E	36.13°N	6	5.8	32.20	10.0	31.84	17.5	31.46	-	-	16.1	30.92	12.3	31.41
D1	120.15°E	36.09°N	3	7.0	32.42	12.9	32.22	17.5	32.04	21.9	29.13	15.5	31.58	12.3	31.71
D2	120.23°E	36.09°N	10	6.3	31.82	11.2	32.33	15.1	32.02	23.6	30.13	17.4	31.16	13.3	32.00
D3	120.25°E	36.09°N	17	6.6	32.54	10.2	32.40	15.2	32.36	-	-	-	-	-	-
D4	120.30°E	36.09°N	8	5.6	32.25	10.0	32.43	16.0	31.66	23.3	30.72	16.4	31.98	12.4	31.40
E1	120.25°E	36.03°N	26	5.4	32.65	10.8	32.19	-	-	23.6	31.50	17.7	31.25	13.3	33.05
E2	120.28°E	36.03°N	33	5.2	32.47	10.8	32.47	-	-	-	-	-	-	-	-
E3	120.30°E	36.03°N	33	5.0	32.49	9.5	32.42	-	-	23.1	31.85	16.4	31.87	13.4	31.18

-: no observation.

and the detection limit was 2.4 µmol C/L.

The concentrations of DFAA and DCAA were determined by gradient elution of reversed-phase high performance liquid chromatography (HPLC) coupled with fluorescence detection method modified with *o*-phthalaldehyde-3-mercaptopropionic acid (OPA-MPA) to make water sample derivatives (Lindroth and Mopper, 1979). The HPLC was a Waters system (e2695) equipped with an online degasser, autosampler, quaternary pump, column heater and fluorescence detector (e2475). An aliquot of 20 µL of seawater sample that had pre-column derivatization was injected into the e2695 system so that amino acids could be separated by Agilent ZORBAX Eclipse column (150 mm×3.0 mm×3.5 µm). DFAA concentrations were determined by measuring the fluorescence intensity at E_x/E_m=340/450 nm under a standard amino acid mix (Sigma Chemical Company). To analyze for DCAA, a process of acidified hydrolyzation was conducted in 5 mL sealed ampoules. Then, 2 mL of seawater was acidified by 2 mL 12 mol/L HCl and 40 µL 0.2% (w/v) ascorbic acid at 110°C for 22 h under N_2. The hydrolysate was

evaporated to remove all acid from the samples at 60°C using a rotary vacuum evaporator, and redissolved in Milli-Q water for the derivation before HPLC analysis to determine the concentration of total hydrolysable amino acids (THAA), as per the method of Sugimura and Suzuki (1983). In this case, the concentration of DCAA was equal to the difference between THAA and DFAA concentrations (Witter and Luther III, 2002). The coefficient of variation for the method was 1%–10% and the detection limit was 0.01 µmol/L.

3 RESULT

3.1 Hydrographic features

The variation of hydrographic parameters is given in Table 1. Water temperature in Jiaozhou Bay varied from 5.0 to 18.9°C in spring and from 9.3 to 24.0°C in autumn. The average salinity in the bay was 32.09 in spring and 31.43 in autumn. Higher salinity was recorded at stations E1 and E3 near the bay mouth connecting to the south Yellow Sea, whereas extremely low salinity was detected at station A2 near
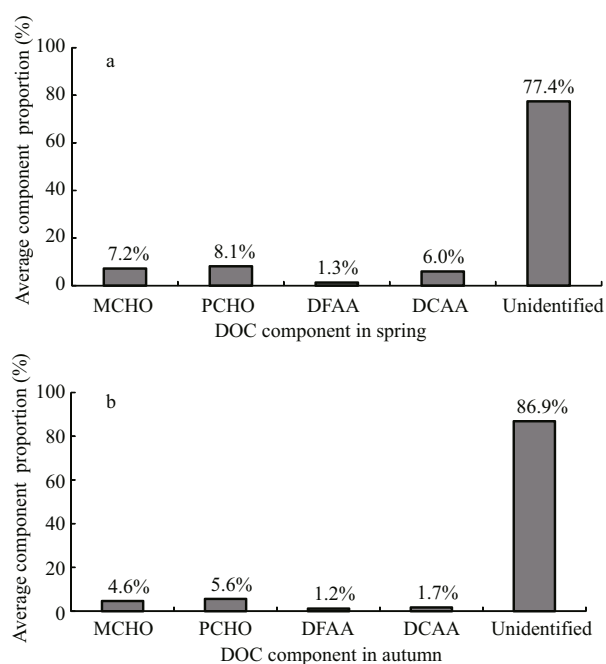
Fig.2 Average proportions of dissolved carbohydrates and amino acids of dissolved organic carbon (DOC) in surface waters of Jiaozhou Bay in spring and autumn

MCHO: dissolved monosaccharides; PCHO: dissolved polysaccharides; DFAA: dissolved free amino acids; DCAA: dissolved combined amino acids.

the Baisha River and Licun River estuary. The concentrations of Chl-*a* in the surface water ranged from 0.67 to 3.03 µg/L with an average of 1.35 µg/L in spring, while those in autumn ranged from 0.64 to 4.19 µg/L with an average of 1.29 µg/L.

Transects A (A1, A2) and B (B1–B5) were close to an extensive benthic shellfish farming region in the northern part of the bay, and exhibited low salinity, high temperature and high concentrations of Chl-*a*. Transect E (E1–E3) was located in the mouth area with high salinity, low temperatures and low concentrations of Chl-*a*.

3.2 Concentrations of DOC species

The concentrations of DOC ranged from 112 to 205 µmol C/L (mean: 132 µmol C/L) in spring and from 140 to 294 µmol C/L (mean: 193 µmol C/L) in autumn, respectively. The proportions of dissolved carbohydrates and amino acids of DOC in the surface water of Jiaozhou Bay in spring and autumn are shown in Fig.2. It clearly showed that PCHO, accounting for 8.1% of DOC, constituted the largest proportion of the detectable component in spring, followed by MCHO (7.2%), DCAA (6.0%), and DFAA (1.3%). In autumn, PCHO was again the major

component (5.6%), followed by MCHO (4.6%), DCAA (1.7%), and DFAA (1.2%). In general, dissolved carbohydrates and amino acids accounted for 22.6% of DOC in spring and 13.1% in autumn.

3.3 Concentrations of dissolved carbohydrates

In spring, the concentrations of MCHO in the surface water ranged from 2.8 to 22.6 (mean 9.6±2.1) µmol C/L. PCHO concentrations ranged from 2.9 to 42.5 (mean 11.0±3.4) µmol C/L. In autumn, the concentrations of MCHO ranged from 2.6 to 27.0 (mean 9.1±1.8) µmol C/L, while those of PCHO varied from 2.4 to 25.6 (mean 10.8±2.5) µmol C/L. These values are consistent with results obtained from other coastal and estuarine areas (Senior and Chevolot, 1991; Bhosle et al., 1998; Hung et al., 2001; Witter and Luther III, 2002; Amon and Benner, 2003; Wu and Yang, 2013).

The highest concentration of MCHO in spring (22.6 µmol C/L) was found at station B5 in the east of Jiaozhou Bay. This concentration is nearly three times higher than the lowest value (5.2 µmol C/L) observed at station E3 near the bay mouth. Meanwhile, in autumn, the highest concentration of MCHO (14.8 µmol C/L) was observed at station A1, and nearly two times higher than the lowest value (6.2 µmol C/L) observed at station E3. The highest concentrations of PCHO were observed at station B5 (32.5 µmol C/L) in spring and A2 (25.6 µmol C/L) in autumn, while the lowest mean values were both found at station E1 (2.9 µmol C/L in spring and 2.5 µmol C/L in autumn).

3.4 Concentrations of dissolved amino acids

The surface water concentrations of DFAA and DCAA at the 19 sampling stations in spring varied from 1.1 to 4.1 (mean 1.7±0.7) µmol C/L and 1.1 to 31.0 (mean 7.6±2.7) µmol C/L. These values are consistent with those observed from other coastal environments (Garrasi et al., 1979; Zhou et al., 1996; Sommerville and Preston, 2001). In autumn, the concentrations of DFAA varied from 1.1 to 8.0 (mean 2.3±0.7) µmol C/L and DCAA ranged from 0.6 to 7.2 (mean 3.3±1.7) µmol C/L. In spring, the highest concentration of DFAA was found at station A2. Station B4 exhibited the highest concentration of DCAA. In autumn, the highest concentrations of both DFAA and DCAA were found at station C5 near the Loushan and Licun River estuaries where many streams containing industrial and domestic sewage enter the bay. Fifteen kinds of amino acids were quantified in the surface
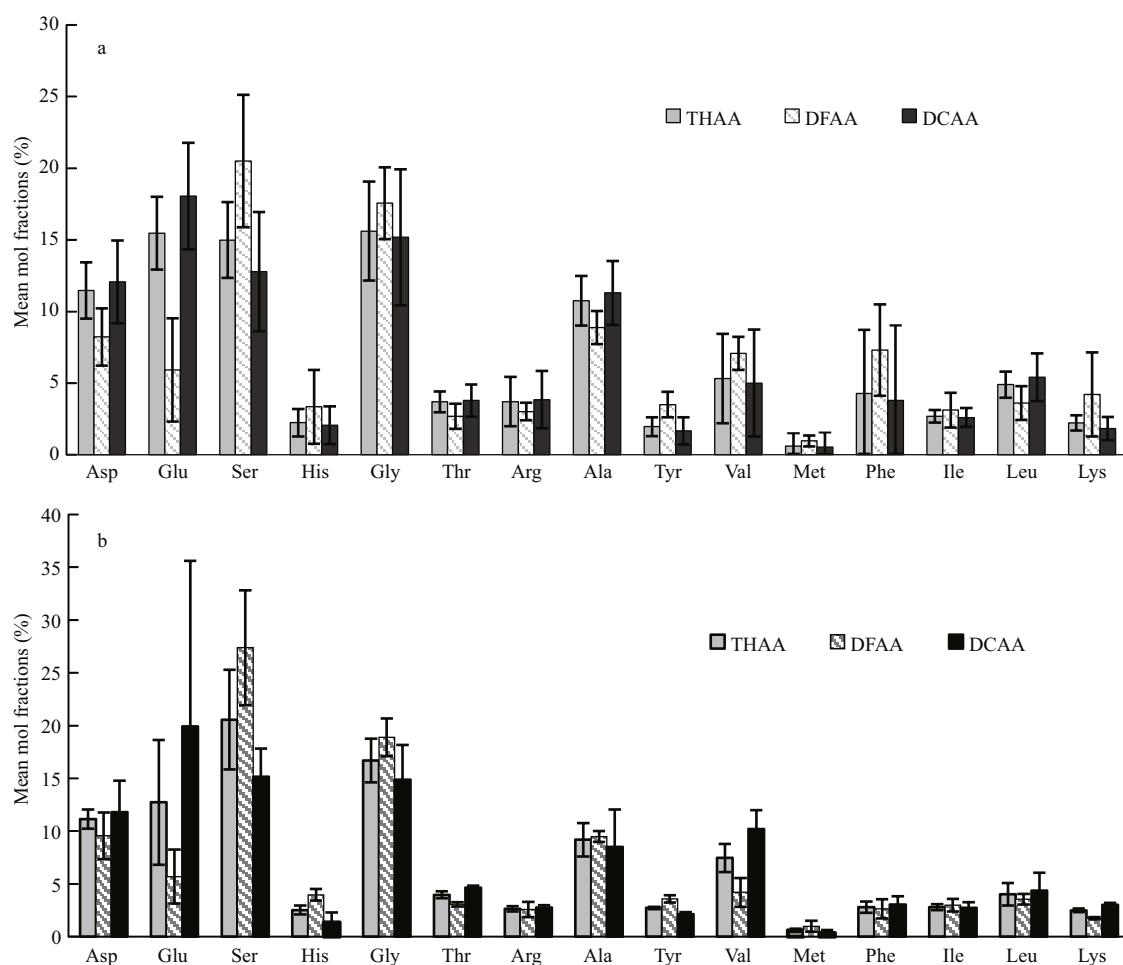
Fig.3 Average molar percentages of individual amino acids in total hydrolysable amino acids (THAA), DFAA and DCAA in spring (a) and autumn (b)

Asp: Aspartic acid; Glu: Glutamic acid; Ser: Serine; His: Histidine; Gly: Glycine; Thr: Threonine; Arg: Arginine; Ala: Alanine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Phe: Phenylalanine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine.

seawater of Jiaozhou Bay in spring and autumn (Fig.3). The major constituents (>60%) of both DFAA and DCAA in spring and autumn were serine, glycine, glutamic acid, alanine and aspartic acid. This is similar to previous studies in other areas (Meon and Kirchman, 2001; Zhang et al., 2009), illustrating that the basic constituents of amino acids do not change.

4 DISCUSSION

4.1 DOC species in Jiaozhou Bay

Chemically characterized organic matter, including dissolved carbohydrates and amino acids species, comprised 22.6% of the total DOC in Jiaozhou Bay in spring and 13.1% of DOC in autumn. This indicates that carbohydrates and amino acids were the predominant component of DOC in this area, which is in agreement with previous investigations (Pettine et

al., 1999; Görs et al., 2007; Chen et al., 2013).

In this study, the concentrations of MCHO might be overestimated by the TPTZ method. Compared with the HPAEC-PAD method, the TPTZ method has low detection limits and precision (<10%). As the TPTZ method was based on the reduction of Fe^{3+} ($K_3[Fe(CN)]_6$) by reducing sugars (Avigad, 1968), all PCHO and oligosaccharide molecules containing one reducing end would be determined as one monomer in the MCHO pool. If the average degree of polymerization of PCHO was known, the deviation could be determined. If the PCHO consisted mainly of large molecules in the seawater samples, the overestimation of MCHO would be smaller.

In spring, the mean ratio of MCHO to TCHO was 50.4% and PCHO to TCHO was 49.6%. In autumn, MCHO accounted for 49.3% of TCHO and PCHO 50.7%. This indicates that there was no significant

difference between MCHO and PCHO concentrations in the bay.

However, the average ratios of PCHO to MCHO were 1.7 in spring and autumn, demonstrating that the PCHO could make a major contribution to TCHO in the bay (Hung et al., 2001; Wu and Yang, 2013). On average, MCHO accounted for 6.1% of DOC and PCHO 7.3%. The contributions of TCHO to DOC were 15.3% in spring and 10.5% in autumn. Ratios of TCHO to DOC of >10% were detected in two-thirds of all surface water samples. Our observations demonstrated that dissolved carbohydrates accounted for the largest identified fraction of DOC in the surface water (Ji et al., 1983; Witter and Luther III, 2002; Zhang et al., 2009). The concentrations of MCHO had a significant negative correlation with the concentrations of PCHO (R=-0.219, n=53, P<0.01) in spring, which was consistent with other results reported for Jiaozhou Bay (R=-0.724, n=19, P<0.001) (Zhang et al., 2009) and the Arabian Sea (R=-0.806, P≤0.001) (Bhosle et al., 1998). The results showed that PCHO could be degraded by exoenzymes to MCHO (Myklestad and Børsheim, 2007).
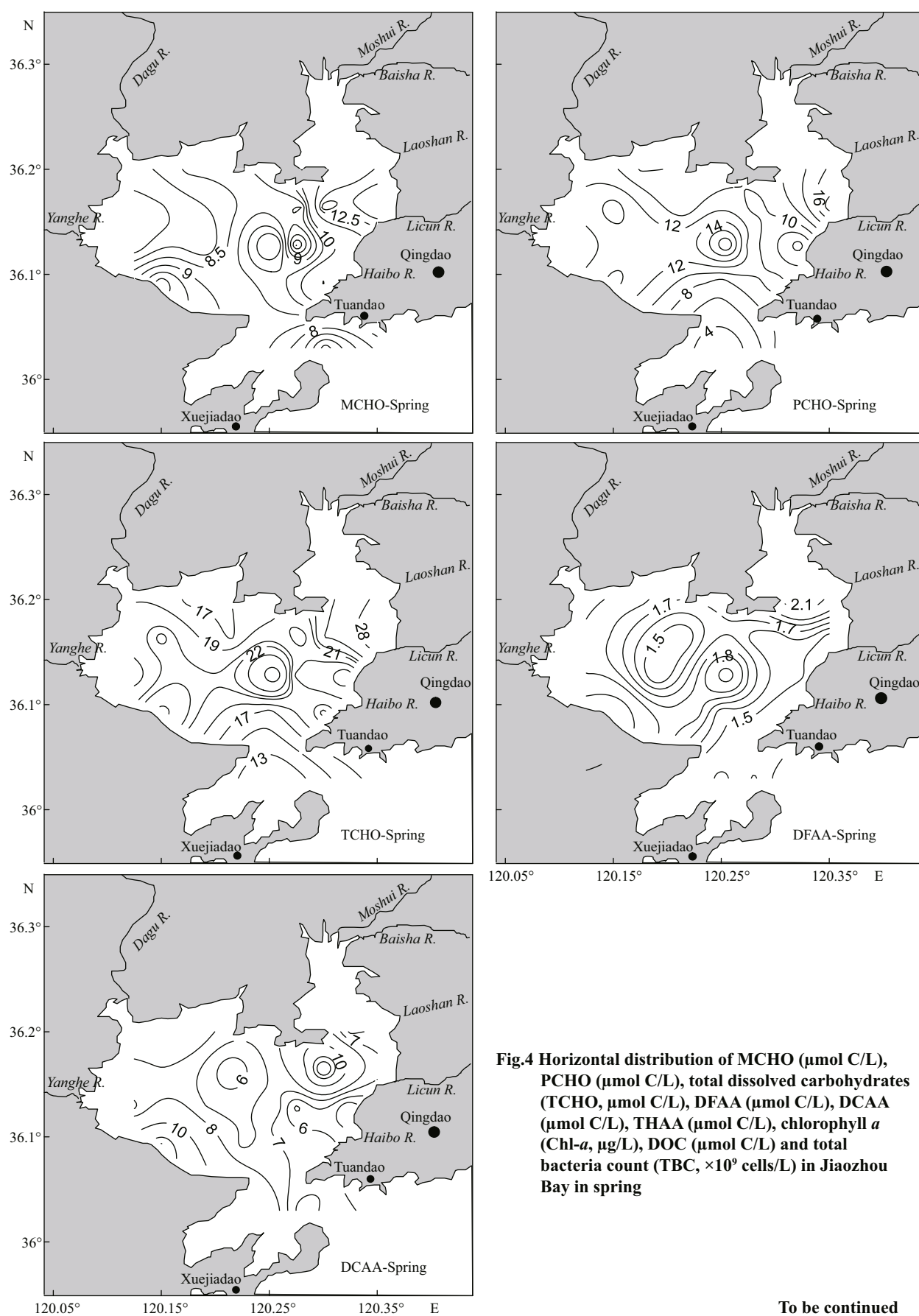
The average ratios of DCAA to THAA were 79.1% in spring and 58.9% in autumn, indicating that DCAA was the major form in the THAA pool. No correlation was found between the DFAA and THAA concentrations in spring, while a significant positive correlation was found between DFAA and THAA in autumn (R=0.649, n=45, P<0.001). From the composition patterns of dissolved amino acids (Fig.3), the molar C:N ratios in DFAA and DCAA in spring were both 4.0±0.2, while in autumn, the molar C:N ratios in DFAA was 3.9±0.2 and 3.6±0.2 in DCAA. This suggests that the average carbon chain length of the amino acids was longer than that (C:N=3.2±0.3) reported by of Görs et al. (2007). Glycine and serine were less utilized by some bacteria than other constituents in the THAA pools, while leucine, tyrosine and phenylalanine were depleted rapidly by microbial activities (Ietswaart et al., 1994; Yamashita and Tanoue, 2003; Görs et al., 2007). Accordingly, the high proportions of serine and glycine might demonstrate a strong degradation state in the organic matter pool of Jiaozhou Bay.

In this study, the remaining unidentified organic matter was mostly humic substances, which generally accounted for about 5%–15% of DOC in marine systems (Benner, 2002). It might contain fatty acids, phenols, vitamins and amines, accounting for 3.6%–12.7% of DOC (Zhang, 2004).

4.2 Horizontal distribution of carbohydrates and amino acids in Jiaozhou Bay
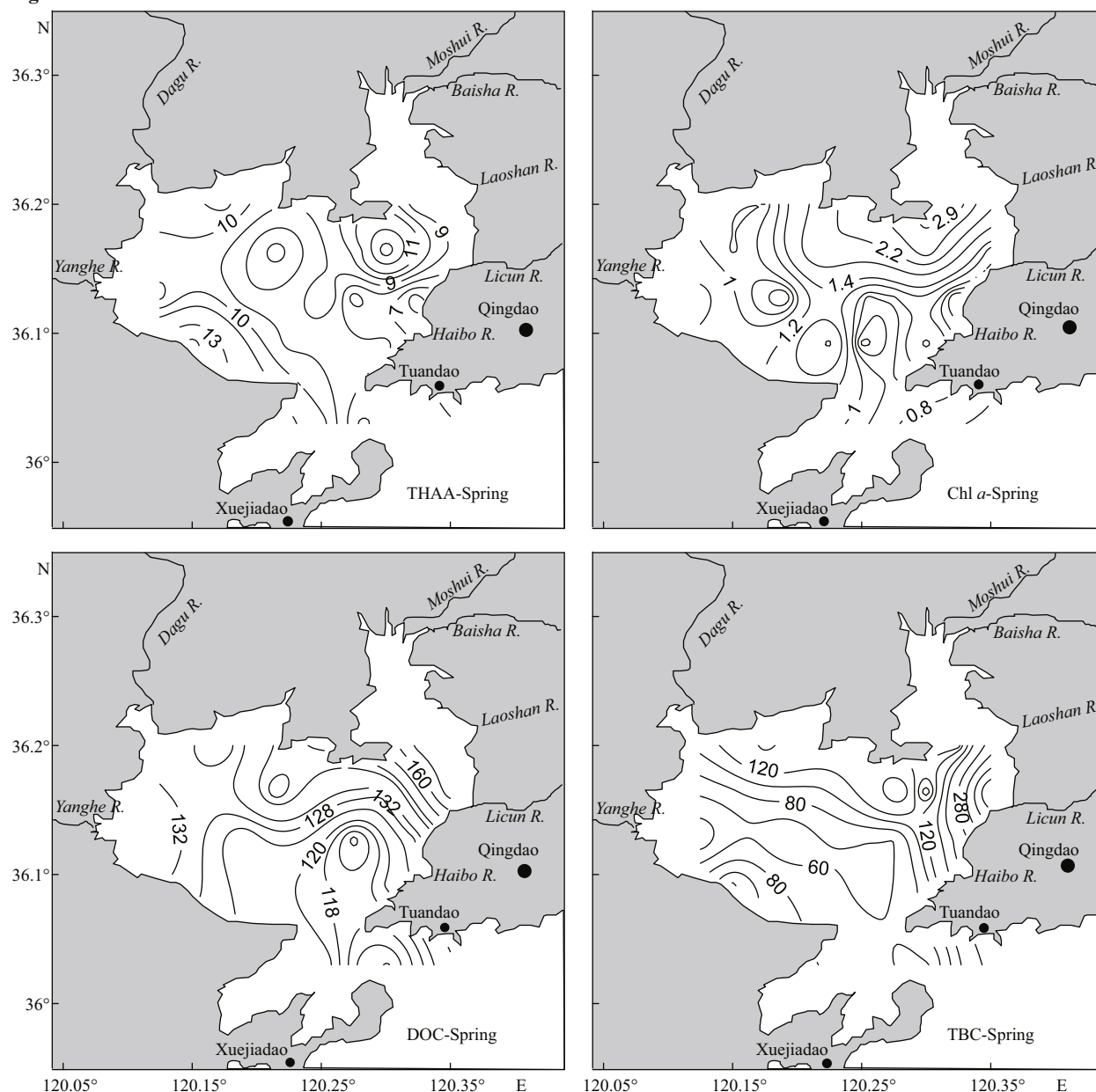
The horizontal variation of the mean concentrations of dissolved carbohydrates, amino acids, DOC, Chl-*a* and TBC is shown in Fig.4 (spring) and Fig.5 (autumn). MCHO, PCHO and TCHO generally showed a gradually decreasing trend from the coastal waters to the central bay, with low values observed in the bay mouth region. The similarity of their distribution might be caused by the following reasons. First, the east shore of the bay is heavily populated and industrialized. Five small rivers at the east shore, including Haibo River, Licun River, Loushan River, Baisha River and Moshui River, are the discharge channels of industrial wastes and domestic sewage from urban area where the annual discharge of industrial wastes was from 151.70×10^6 t/a in 1997 up to 316.11×10^6 t/a in 2007 (Sun et al., 2011). As the principal organic components of industrial wastewater are carbohydrates, proteins, lipids and other small quantities of synthetic organic chemicals (Rawat et al., 2011), higher concentrations of MCHO (>8.5 µmol C/L), PCHO (>9.0 µmol C/L) and TCHO (>22.0 µmol C/L) in spring were found in the east coast of the bay. Similarly high concentrations were also found in autumn, with MCHO >7.0 µmol C/L, PCHO >10.0 µmol C/L and TCHO >18.0 µmol C/L. Second, there are a number of aquaculture farms at the west and northeast coast. As seen in Fig.5, the concentrations of MCHO, PCHO and TCHO were relatively high in the central bay. Tidal currents move in a north-south direction and the east-west exchange is minimized by this tidal pattern (Liu et al., 2005; Yang et al., 2010). Water exchange in the coastal area is also restricted, especially in the east coastal waters (Chen et al., 1982). The southern Jiaozhou Bay water is replaced by the Yellow Sea water, which has lower concentrations of carbohydrates (unpublished data), which decreases the concentrations of carbohydrates in the bay mouth. Thus, carbohydrates were concentrated along the west and east coasts.

The concentrations of amino acids showed a similar decreasing trend from coastal waters to the central bay and from the central bay to the bay mouth. The riverine pollution from urban areas in the northwest coast had an important influence on the dissolved amino acids concentrations. Higher concentrations of DFAA (>1.6 µmol C/L), DCAA (>7.5 µmol C/L in spring and >3.0 µmol C/L in autumn) and THAA (>8.5 µmol C/L) were noted in

Fig.4 Horizontal distribution of MCHO (μmol C/L), PCHO (μmol C/L), total dissolved carbohydrates (TCHO, μmol C/L), DFAA (μmol C/L), DCAA (μmol C/L), THAA (μmol C/L), chlorophyll _a_ (Chl-_a_, μg/L), DOC (μmol C/L) and total bacteria count (TBC, ×10⁹ cells/L) in Jiaozhou Bay in spring

Fig.4 Continued

the industrial regions in the east coast which were heavily polluted with large amounts of waste water (151.70×10⁶ t/a in 1997 to 316.11×10⁶ t/a in 2007) (Shen, 2001; Liu et al., 2005; Sun et al., 2011). In contrast, much lower amino acids concentrations, comparable with values reported for the open sea (Chen et al., 2013), were found at the bay channel with fast water exchange between the bay and Yellow Sea (Liu et al., 2005). According to the nutrient concentrations and composition in Jiaozhou Bay, annual primary production could reach as high as 503±184 mg C/(m²·d) in 1993 (Yao et al., 2007),

which resulted in eutrophication in the bay. Thus, the concentrations of dissolved amino acids in the bay were higher than those measured in the bay mouth.

4.3 Temporal variation of dissolved carbohydrates and amino acids in Jiaozhou Bay

The average concentrations of dissolved carbohydrates and amino acids in spring and autumn in the surface water are shown in Fig.6. Because of the accumulation of organic matter in summer, the average concentration of DOC in autumn was 1.5 times higher than that in spring. No significant
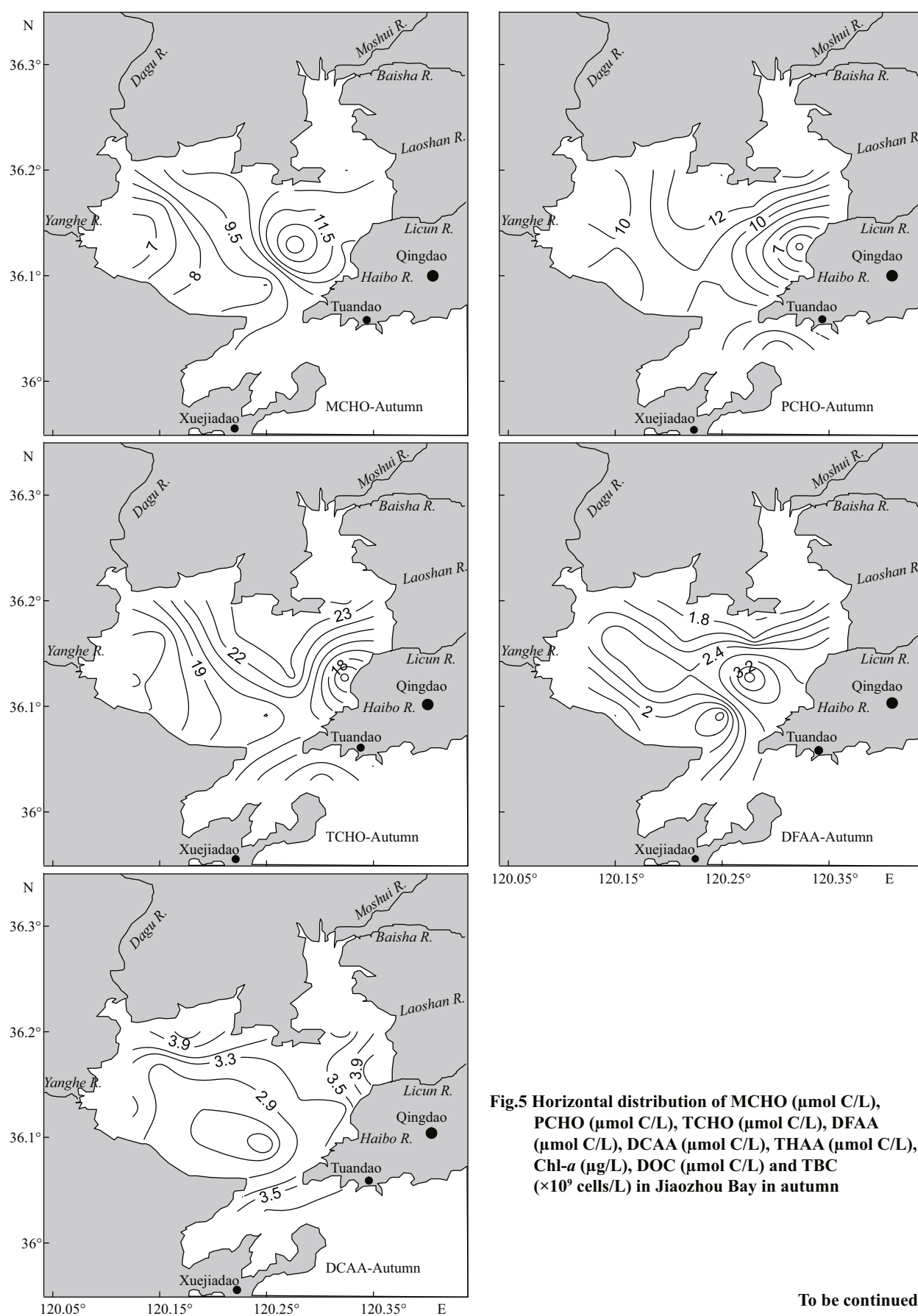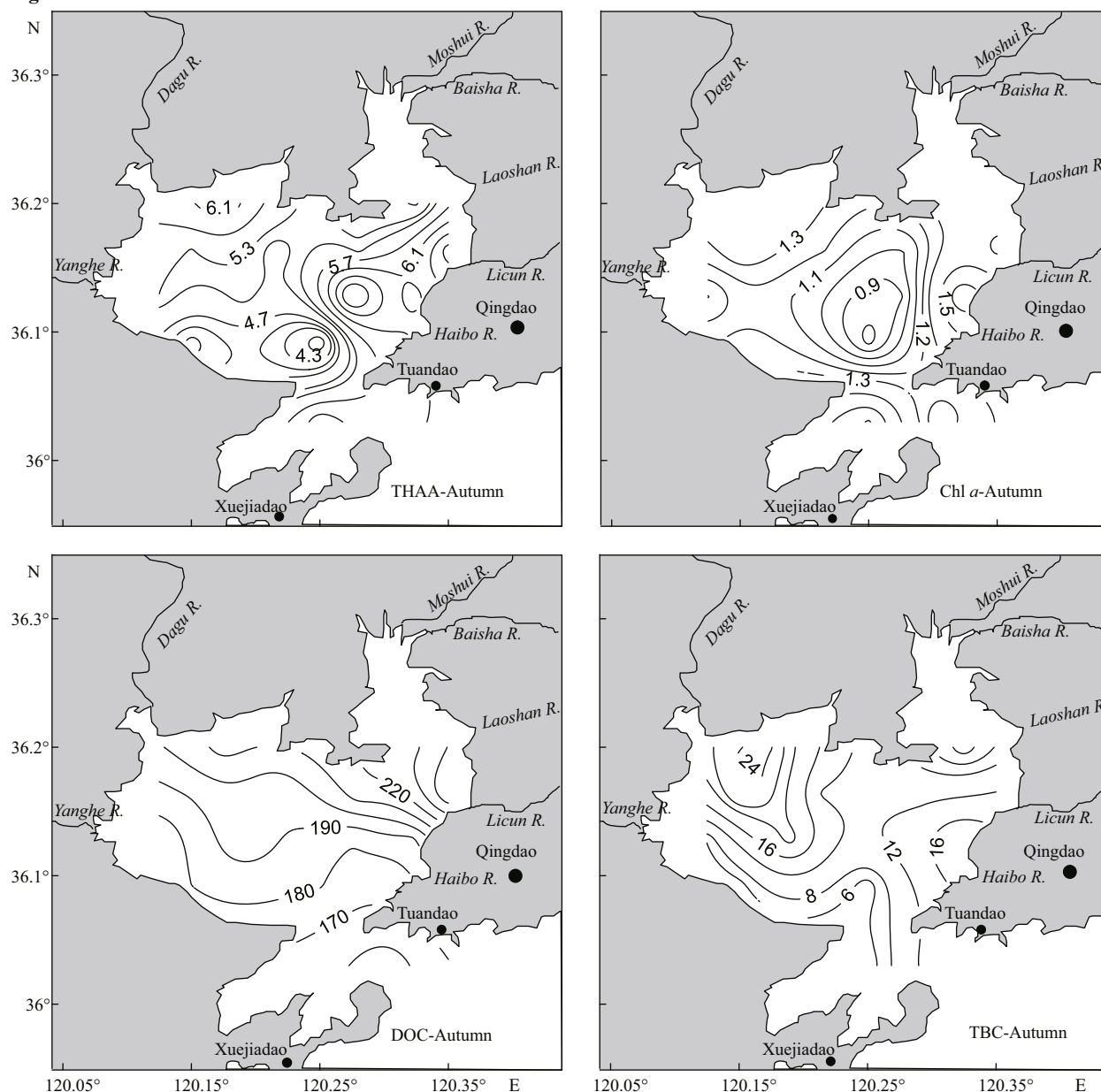
Fig.5 Horizontal distribution of MCHO (μmol C/L),
PCHO (μmol C/L), TCHO (μmol C/L), DFAA
(μmol C/L), DCAA (μmol C/L), THAA (μmol C/L),
Chl-*a* (μg/L), DOC (μmol C/L) and TBC
(×10⁹ cells/L) in Jiaozhou Bay in autumn

Fig.5 Continued

seasonal difference appeared in MCHO, PCHO and TCHO concentrations in the bay. However, the average concentrations of TCHO in spring at stations B5 (near Licun River) and C3 (near Yanghe River) were much higher than the values in autumn, which might be due to seasonal variation in riverine inputs.

The ratio of PCHO to MCHO could be used as a tool to examine monthly variation of carbohydrates dynamics in seawater. In our study, the ratios of PCHO to MCHO ranged from 0.7 to 5.7 (mean: 1.6) in spring and from 0.1 to 5.7 (mean: 1.7) in autumn. These ratios are consistent with those of previous measurements in other areas (1.1 to 14.1 in the

Gerlache Strait, Pakulski and Benner, 1994; 1.9 to 5.0 in Darβ-Zingst Bodden Chain, Görs et al., 2007). According to previous findings PCHO is produced by phytoplankton photosynthesis (Pakulski and Benner, 1994; Børsheim et al., 2005). In our research, the ratios of PCHO to MCHO in the bay increased from 1.3 in April to 1.8 in May, while Chl-*a* concentrations increased from 8.18 μg/L to 10.92 μg/L in April/May, because of the increasing release of PCHO by phytoplankton in connection with the spring bloom. The average PCHO/MCHO ratios decreased from 2.0 in September to 1.4 in October, which might be explained by the degradation of PCHO and the
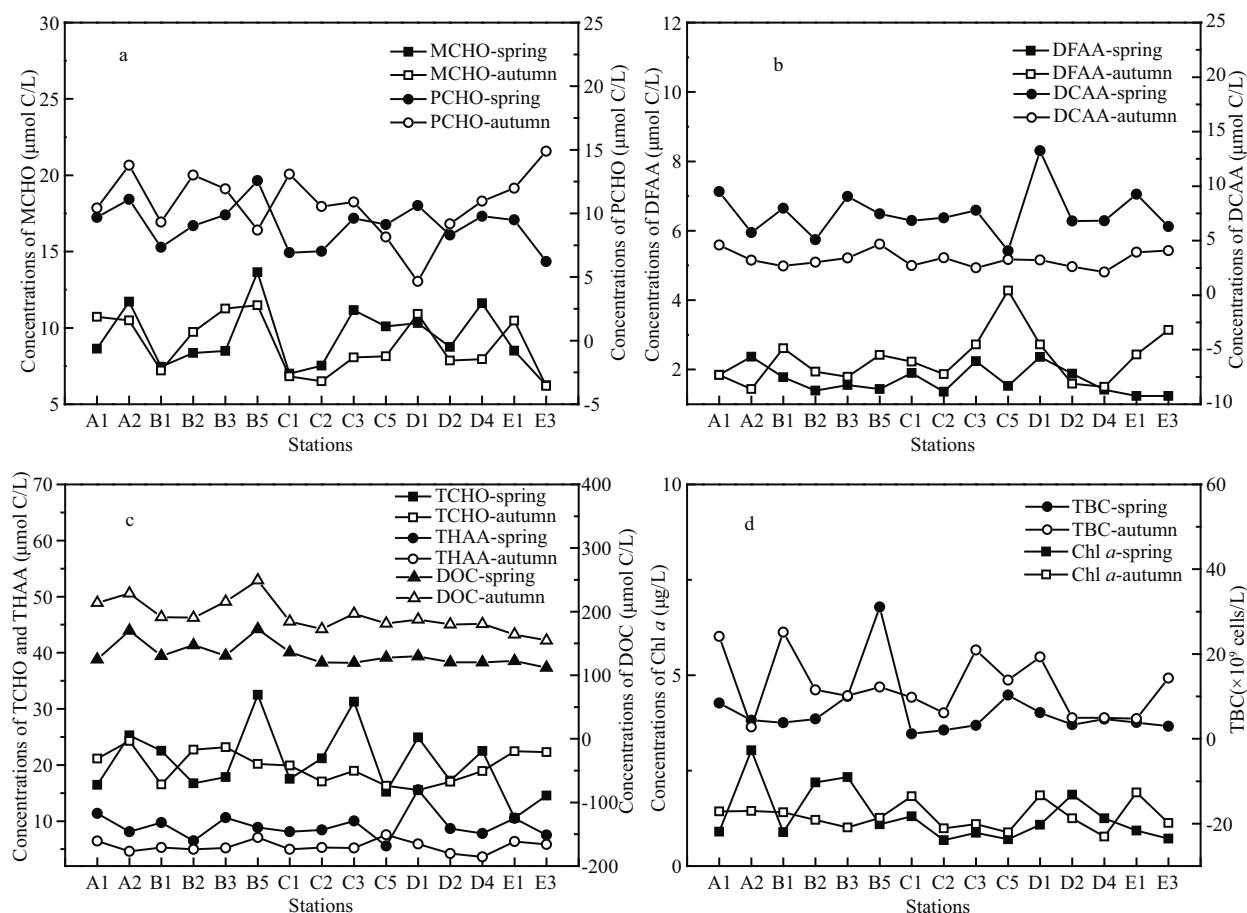
Fig.6 Temporal variation of the average values of (a) the concentrations of MCHO and PCHO, (b) the concentrations of DFAA and DCAA, (c) the concentrations of TCHO, THAA and DOC, and (d) TBC and the concentrations of Chl-*a* in Jiaozhou Bay

concomitant increase in MCHO concentrations in the surface water, because PCHO can be easily degraded to monomers by extracellular enzymes cleaving one sugar monomer and oxidation/reductions involving iron and free radicals (Myklestad and Børsheim, 2007).

The mean concentrations of DCAA and THAA in autumn were lower than those observed in spring, while autumn DFAA concentrations were higher. The average concentration of DFAA in autumn was almost 1.5 times higher than that in spring. The distribution of amino acids is controlled by biological processes and terrestrial runoff. To further understand the seasonal variation of amino acids, the variation of TBC was tracked. TBC had two peaks: one in spring (15.1×10^9 cells/L, station A1 in April) and one in autumn (36.6×10^9 cells/L, station D1 in September). On average, TBC was almost three times higher in autumn than in spring, owing to net degradation of organic matter. DFAA is considered to be the primary nitrogen source for bacteria in many aquatic systems

(Keil and Kechman, 1991; Chen et al., 2013). Following the summer algal bloom, the average concentration of Chl-*a* in autumn was 1.2 times higher than that in spring (Liu et al., 2003), indicating that phytoplankton photosynthetic production might play an important role in seasonal variation in DFAA concentrations. The seasonal variation in the concentrations of carbohydrates and amino acids was mainly associated with phytoplankton and bacteria communities.

4.4 Relationships among dissolved carbohydrates, amino acids and general biological parameters

Linear correlation analyses were carried out between MCHO, PCHO, TCHO, DFAA, DCAA, THAA, DOC and environmental parameters to investigate the biogeochemical factors influencing the distribution of dissolved organic matter (Table 2 and Table 3). The average Chl-*a* concentration in spring was slightly higher than that obtained in this bay in 2009 (Zhang et al., 2009). There was a significant

Table 2 Linear correlation analyses among MCHO, PCHO, TCHO, DFAA, DCAA, THAA, DOC, Chl-*a* and TBC of Jiaozhou Bay in spring

		PCHO	TCHO	DFAA	DCAA	THAA	DOC	Chl-*a*	TBC
MCHO	*R*	-0.219**	0.295*	0.492**	0.093	0.074	0.242	0.035	0.180
	P	0.005	0.032	0.000	0.510	0.597	0.132	0.804	0.196
PCHO	*R*		0.868**	0.043	0.041	0.103	0.212	0.048	-0.017
	P		0.000	0.758	0.768	0.462	0.190	0.732	0.906
TCHO	*R*			0.293*	0.088	0.139	0.308	0.065	0.076
	P			0.033	0.532	0.322	0.053	0.644	0.591
DFAA	*R*				-0.195	-0.070	-0.149	0.025	-0.065
	P				0.161	0.618	0.358	0.860	0.642
DCAA	*R*					0.990**	-0.015	0.283*	-0.073
	P					0.000	0.928	0.040	0.605
THAA	*R*						-0.023	0.270	-0.089
	P						0.888	0.050	0.525
DOC	*R*							0.085	0.583**
	P							0.600	0.000
Chl-*a*	*R*								-0.093
	P								0.509

* correlation significant at the 0.05 level (two-tailed); ** correlation significant at the 0.01 level (two-tailed).

Table 3 Linear correlation analyses among MCHO, PCHO, TCHO, DFAA, DCAA, THAA, DOC, Chl-*a* and TBC of Jiaozhou Bay in autumn

		PCHO	TCHO	DFAA	DCAA	THAA	DOC	Chl-*a*	TBC
MCHO	*R*	0.233	0.648**	-0.183	0.285	0.092	0.225	0.022	-0.087
	P	0.132	0.000	0.241	0.064	0.559	0.147	0.888	0.607
PCHO	*R*		0.892**	-0.490**	0.003	-0.343*	-0.257	-0.125	-0.107
	P		0.000	0.001	0.987	0.024	0.096	0.426	0.529
TCHO	*R*			-0.469**	0.135	-0.226	-0.097	-0.087	-0.121
	P			0.002	0.389	0.145	0.537	0.577	0.474
DFAA	*R*				-0.220	0.649**	0.156	0.300**	0.391*
	P				0.146	0.000	0.305	0.046	0.014
DCAA	*R*					0.599**	0.396**	0.225	0.221
	P					0.000	0.007	0.137	0.175
THAA	*R*						0.435**	0.422**	0.489**
	P						0.003	0.004	0.002
DOC	*R*							0.342*	0.564**
	P							0.021	0.000
Chl-*a*	*R*								0.368*
	P								0.021

* correlation significant at the 0.05 level (two-tailed); ** correlation significant at the 0.01 level (two-tailed).

correlation ($R=0.283$, $n=53$, $P<0.05$) between the DCAA and Chl-*a* concentrations in spring. In addition, the concentrations of DFAA and THAA in autumn also appeared to be significantly correlated with the concentrations of Chl-*a* (Table 3). However, no correlation was observed in each month between carbohydrates components (MCHO, PCHO, TCHO) and Chl-*a*, implying that phytoplankton biomass was
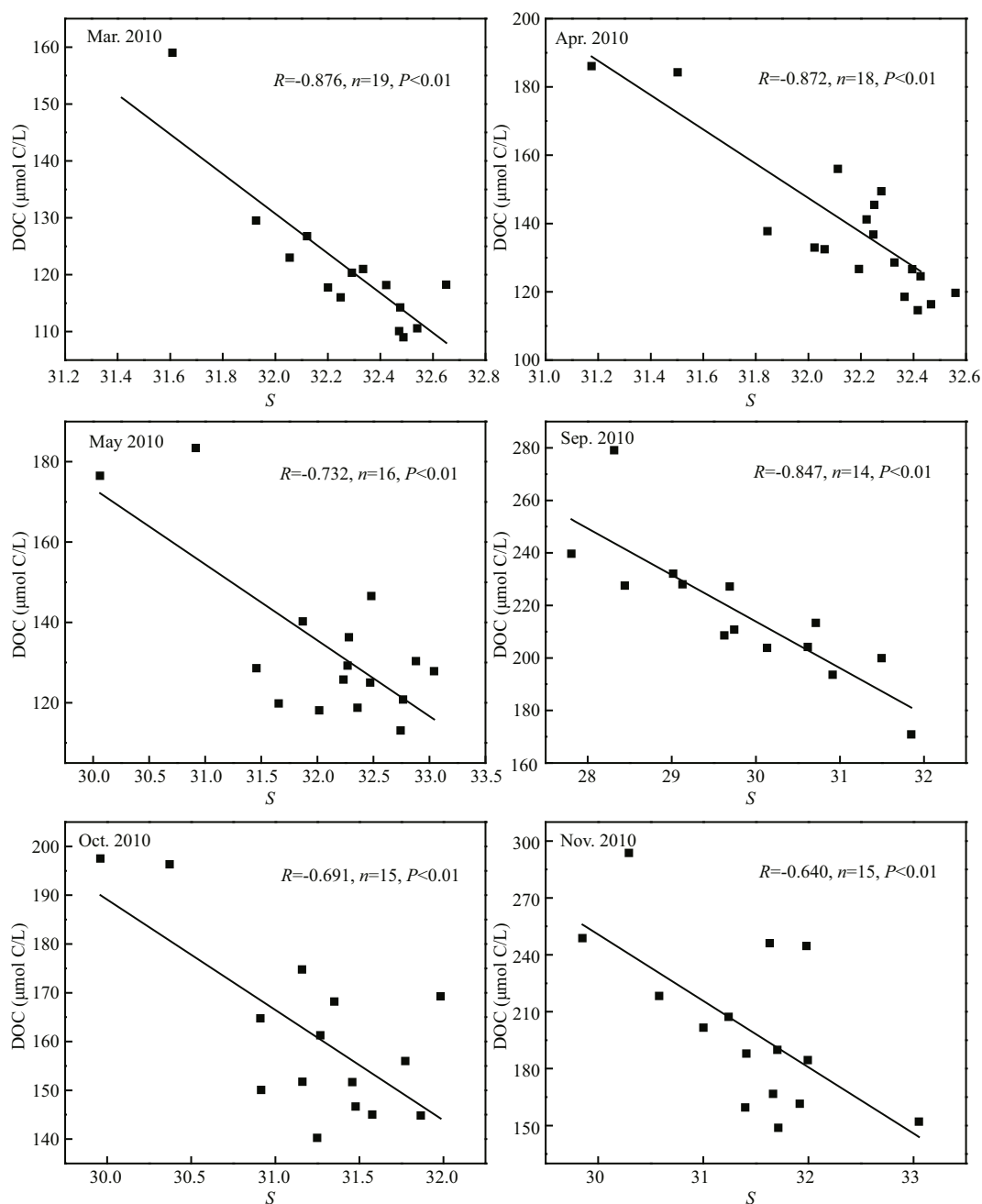
Fig.7 Relationships between the concentrations of DOC and seawater salinity in the Jiaozhou Bay during six cruises

not a major factor affecting the concentrations of organic matter in Jiaozhou Bay. Hung et al. (2001) reported that Chl-*a* concentration had significant linear correlations with MCHO and PCHO concentrations in Galceston Bay, indicating that MCHO was mostly produced in situ by phytoplankton. Previous studies showed that the concentrations of carbohydrates and amino acids were influenced by biological and chemical factors, including the nutrients, the species of plankton and bacterial activities.

DOC data collected in each cruise were plotted against seawater salinity in Fig.7. There was a significant negative correlation between the concentrations of DOC and salinity in each cruise. Although the correlation coefficients were lower in October and November of 2011 than in the other months, the negative relationship indicates that the riverine inputs around the bay could be an important source of DOC.

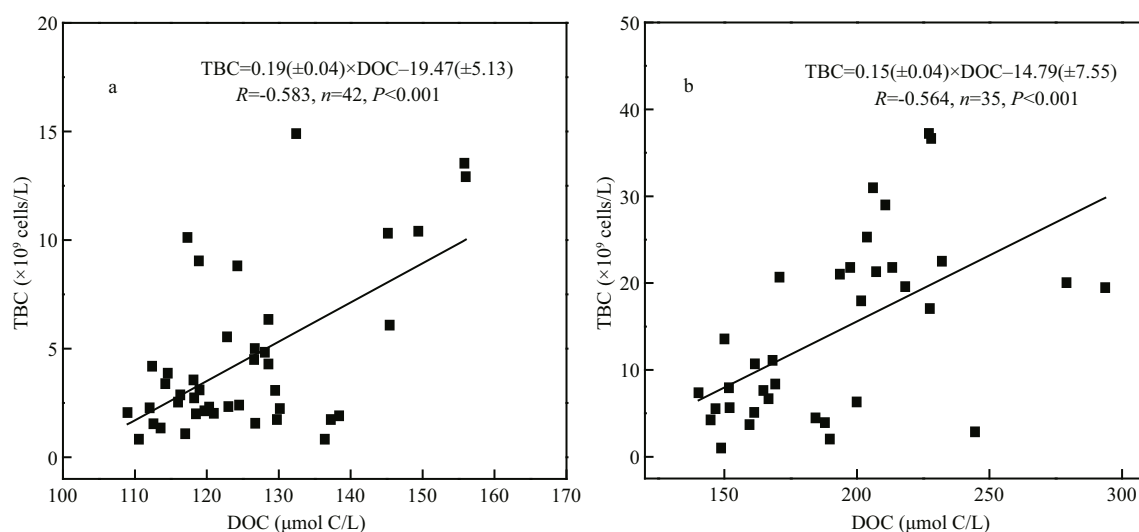Many studies have demonstrated that bacterial production and degradation can impact the regional

Fig.8 Relationships between the concentrations of DOC and TBC in Jiaozhou Bay in the spring (a) and autumn (b) of 2010

and global carbon cycle (Carlson et al., 1994; Hansell and Carlson, 1998; Shiah et al., 2000; Amon et al., 2001; Khodse and Bhosle, 2001). TBC datasets are plotted against DOC in Fig.8. The significant positive relationships seen between DOC and TBC during spring and autumn in Jiaozhou Bay are further evidence that bacterial activity might have a significant impact on the carbon cycle in marine systems. A significant relationship between DOC and TBC was also found in the Mandovi estuary (Khodse et al., 2010). Marine bacterioplankton play a crucial role in the production and consumption of leftover dissolved organic substance that would otherwise be exported, and act as both producers and decomposers in seawater.

5 CONCLUSION

Spatiotemporal variation of MCHO, PCHO, DFAA and DCAA in Jiaozhou Bay was investigated. Our study showed that the influences of terrestrial inputs, human activities and tidal patterns result in distribution patterns of carbohydrates and amino acids in Jiaozhou Bay that gradually decrease from coastal waters to the central bay and from the central region to the bay mouth. High concentrations of carbohydrates and amino acids were generally noted in an industrially polluted area. Seasonal variations were not obvious in MCHO, PCHO and TCHO concentrations. The temporal variation in the ratios of PCHO to MCHO might be attributed to the joint action of monthly changes of Chl-*a* and TBC. The concentrations of DFAA in autumn were generally higher than those in spring, while DCAA and THAA concentrations in the

surface water displayed higher values in spring. The concentrations of DOC in autumn were slightly higher than those observed in spring. In addition, the DOC concentrations were significantly correlated with the abundance of bacteria in the study area. The present study showed that phytoplankton biomass and bacterial activities might play a significant role in controlling the seasonal variation of DOC concentrations. Salinity correlated negatively with the concentrations of DOC, suggesting that riverine inputs also had an important impact on the distribution of DOC in Jiaozhou Bay.

6 ACKNOWLEDGEMENT

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