ORIGINAL RESEARCH ARTICLE

Factors regulating the compositions and distributions of dissolved organic matter in the estuaries of Jiaozhou Bay in North China

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Summary   Water samples collected from the Jiaozhou Bay every two months between April 2016 and February 2017 were analyzed for dissolved organic carbon (DOC), particulate organic carbon (POC), total dissolved carbohydrates (TCHO), total hydrolyzed amino acids (THAA), and chromophoric dissolved organic matter (CDOM) to explore the biogeochemical processes of dissolved organic matter (DOM) in anthropogenic estuarine and coastal environments. In addition, nutrients, chlorophyll *a* and COD (chemical oxygen demand) in these samples were also analyzed. All parameters exhibited temporal and spatial variations: POC 0.13—22.40 mg/L (average 1.75 mg/L), DOC 0.98—32.75 mg/L (average 5.04 mg/L), COD 0.23—7.58 mg/L (average 1.67 mg/L), TCHO 0.34—14.09 μM (average 3.18 μM), THAA 0.89—8.30 μM (average 4.04 μM), and the absorption coefficient a(355) of CDOM 0.23—16.35 m^{-1} (average 3.09 m^{-1}). The temporal and spatial variations in the concentrations of TCHO, THAA, and DOC implied that the DOM in the study areas had a relatively higher biochemical activity. The canonical correspondence

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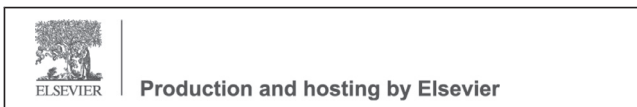
analysis (CCA) and maximal information coefficient (MIC) revealed that seasonal variations in temperature and the phosphate concentration were the dominant factors regulating the DOM distributions in Jiaozhou Bay, while riverine inputs and *in situ* reproduction mainly controlled the DOM compositions.

1. Introduction

Dissolved organic carbon (DOC) is the largest reducing carbon pool in the ocean. The distributions and compositions of DOC regulate not only the regional primary production (Markager and Vincent, 2000; Wang and Chen, 2018) but also the diagenesis of bioactive elements, such as heavy metals (Chen et al., 2003; Shank et al., 2005), and the activities of marine microorganisms (Jiao et al., 2010). Dissolved amino acids and carbohydrates have been identified as major components of DOM and serve as important nitrogen and carbon sources for phytoplankton (Veuger et al., 2004) and bacteria in the ocean (Liang et al., 2001; Middelboe et al., 1995). These biogenic organic compounds are more labile and primarily represent the DOM characteristics.

DOM in estuaries is derived from terrigenous riverine inputs, *in situ* production, and sediment re-suspension (Castillo et al., 1999; Chen and Bada, 1992; Cheng et al., 2008; Nelson et al., 1998; Rochelle-Newall and Fisher, 2002). Rivers deliver DOM at a flux of 0.20—0.21 Pg C yr^{-1} globally (Battin et al., 2009; Hedges and Keil, 1995). Riverine DOM is relatively stable and resistant to degradation by microorganisms (Hudson et al., 2010; Mantoura and Woodard, 1983). However, mixed DOM derived from anthropogenic materials and local production (such as carbohydrates and amino acids) in estuarine systems is capable of being biochemically utilized (Bronk and Glibert, 1993; Fellman et al., 2010; Stepanauskas et al., 1999). Therefore, riverine DOM might provide significant carbon and nitrogen sources for estuarine microbes and algae (Middelboe et al., 1995). On the other hand, DOM degrades and releases dissolved inorganic carbon (DIC) and inorganic nutrients, leading to environmental problems such as eutrophication in the estuary and coastal areas (Seitzinger and Sanders, 1997). Thus, the sources, compositions, properties, and potential functions of DOM in coastal ecosystems must be discerned.

Jiaozhou Bay is a semi-closed bay located on the west coast of the Yellow Sea in the western Pacific Ocean. From estuaries to central Jiaozhou Bay, DOM concentrations in the waters decrease significantly by 2.6—4.2-fold (Jiang et al., 2007; Kong et al., 2016; Shen et al., 2006; Yang et al., 2010). Thus, the ambient rivers provided a substantial amount of organic carbon to Jiaozhou Bay (Yang et al., 2010). The rapid industrialization and civilization in coastal Qingdao City have exerted substantial effects on the DOM distribution and composition through the discharge of rivers into the bay, which in turn, directly and indirectly, affect the biochemical processes of DOM in the estuaries and Bay (Xi et al., 2018). More than a dozen rivers flow into Jiaozhou Bay, with substantial differences in the natural profiles and backgrounds among these rivers. Haibo River, a small river flowing into east Jiaozhou Bay, represented a major source of DOM in east Jiaozhou Bay, but little DOM accumulated in ambient waters because of strong hydrodynamic and tidal effects (Jiang et al., 2007). Dagu River, the largest river flowing into the northwest bay, carried considerable amounts of humic-like materials into Jiaozhou Bay (Ji et al., 2006). Meanwhile, the variation in the levels of humic-like materials was significantly correlated with the variation in the carbohydrate levels in the estuary of Dagu River (Ji et al., 2006), and a similar correlation was discovered among the levels of carbohydrates, amino acids and nutrients in the bay (Shi, 2015). However, researchers have not clearly determined how these relationships and interactive processes of DOM in the estuaries are impacted by human activities. To date, no studies have been conducted to verify how these rivers control the DOM cycle in Jiaozhou Bay.

Water samples were collected from the bay every two months between Apr. 2016 and Feb. 2017, and DOC, carbohydrate, amino acid and CDOM concentrations in the water samples were analyzed to identify the key factors that regulate DOM variations in the estuaries of Jiaozhou Bay. The specific goals of this study are (1) to describe the variations in the levels of total organic carbon and DOM components, (2) to clarify the characteristics and sources of DOM, (3) to discern the multiple interactions among DOM components and physiochemical factors, and (4) to estimate the impact of relative runoff on DOM transport from each river to the bay.

2. Material and methods

2.1. Sampling sites and methods

Water samples were collected from 10 sites in Jiaozhou Bay every two months from April 2016 to February 2017 (Fig. 1): 2 sites R1 (N36°04′06″, E120°17′73″) and R2 (N36°05′37″, E120°19′41″) at piers and 8 sites in the estuaries. Rivers flowing into the east bay include Haibo (R3, N36°06′73″, E120°19′39″), Licun (R4, N36°09′39″, E120°21′35″), Banqiaofang (R5, N36°11′07″, E120°21′95″), and Loushan (R6, N36°12′32″, E120°21′61″) Rivers, which run through or near Qingdao City. Baisha River (R7, N36°14′55″, E120°18′97″) flows into the north bay and Yang River (R9, N36°07′58″, E120°06′01″) into the west bay, which run through the rural area. The largest river, the Dagu River (R8, N36°10′85″, E120°07′46″), flows into the northeast bay and the Lianwan River (R10, N36°00′26″, E120°11′24″) covers a newly developed area.

Table 1 Methods on physicochemical parameters.

Parameters	Methods	Maximum of relative error [%]	References
COD	Basic potassium permanganate method	1.5	P.R. China National Standard (2007)
SS	Gravimetric method	0.6	P.R. China National Standard (2007)
NO_3^-—N	Cadmium copper reduction method	0.1	Grasshoff et al. (2007)
NO_2^-—N	Diazotization titration method	0.6	Grasshoff et al. (2007)
NH_4^+—N	Indophenol blue spectrophotometric method	0.7	Grasshoff et al. (2007)
PO_4^{3-}—P	Molybdenum blue method	4.3	Grasshoff et al. (2007)
Chl-a	Three-color spectrophotometry method	0.3	Jeffrey and Humphrey (1975)
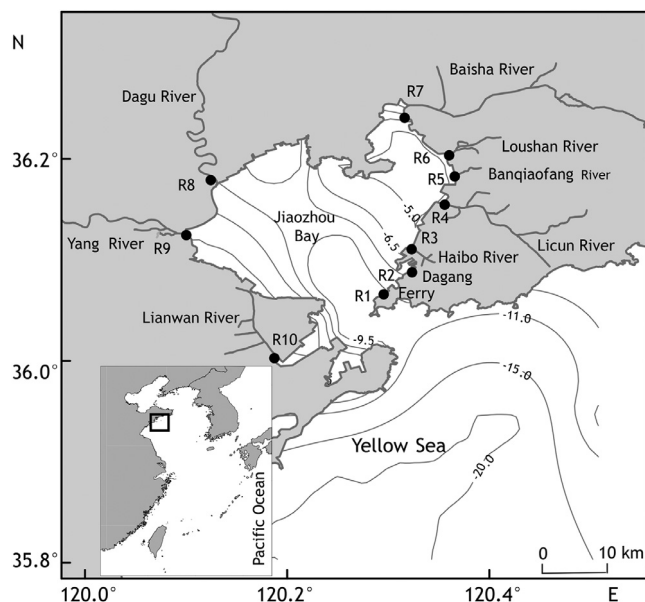
Figure 1 Study area and sampling sites at the estuaries of Jiaozhou Bay.

Samples were collected at a depth of 20 cm under the water surface, because of the shallow depth in the estuaries ($<$ 5 m). Water samples were filtered through pre-treated fibers (Whatman GF/F (Qingdao, Ocean University of China, China), combusted under 450°C for 4 h) and were stored frozen until the analyses of DOC, TCHO, THAA, CDOM, and dissolved nutrient levels. The frozen filters were used to analyze the levels of particulate organic carbon (POC) and chlorophyll a (Chl-a). Unfiltered water samples were stored in a refrigerator until the chemical oxygen demand (COD) analysis. Temperature (T), salinity (S), pH and the dissolved oxygen (DO) level were monitored simultaneously using a multi-parameter water quality monitor (HQ40d, Hach, Ocean University of China, China).

2.2. Analytical methods

2.2.1. Physicochemical parameters
The levels of COD, Chl-a, suspended particle (SS) and nutrients (NO_2^-—N, NO_3^-—N, NH_4^+—N and PO_4^{3-}—P) were measured using the methods listed in Table 1. The dissolved inorganic nitrogen (DIN) concentration is the sum of the NH_4^+—N, NO_2^-—N and NO_3^-—N concentrations. The con-

centrations of NO_2^-—N, NO_3^-—N, NH_4^+—N and PO_4^{3-}—P were measured using an automatic analyzer (QuAAtro, Salt Analytical GmbH, Ocean University of China, China) (Grasshoff et al., 2007). The maximum relative error is listed in Table 1 and was calculated from the corresponding triplicate measurements.

2.2.2. POC and DOC
The POC concentration was measured in acidified filter samples using an elemental analyzer (PerkinElmer 2400 series-2, UC Davis, US), with a relative error of less than 0.3% in duplicate measurements.

The DOC concentration of water samples was measured using catalytic high temperature oxidation with a total organic carbon analyzer (TOC-L, Shimadzu, Ocean University of China, China). Low carbon water and standard seawater (provided by Hansell Laboratories, University of Miami, USA) were used as the blank and to construct a standard curve for instruments. The relative error was less than 3% for duplicate measurements.

2.2.3. Relative concentrations of CDOM
The absorption spectrum of CDOM was scanned from 190 nm to 800 nm at 1 nm intervals using a UV—Vis spectrophotometer (UV-2550, Shimadzu, Ocean University of China, China) with 1 cm quartz cuvette. Pure water (Milli-Q) was used as blank reference. The absorbance value at every wavelength was calculated by subtracting the absorbance value at 700 nm to deduce the difference in refractivity between seawater and pure water and the baseline drift of scattering induced by fine particles (Castillo et al., 1999). The relative error was less than 4.24% for triplicate measurements, and the photometric accuracy of our instrument was ± 0.001 absorbance unit or an equivalent absorption coefficient of 0.23 m^{-1}. The absorption coefficient was calculated using the following equation (Kirk, 1994):

$$a(\lambda) = \frac{1}{L} 2.303 A(\lambda), \tag{1}$$

where $a(\lambda)$ represents the light absorption coefficient of CDOM at wavelength λ (m^{-1}), $A(\lambda)$ represents the corrected absorbance at λ, λ represents the wavelength (nm), and L represents the cuvette length (m). Samples were measured in duplicate.

2.2.4. Carbohydrates
Concentrations of dissolved monosaccharides (MCHO) and total dissolved carbohydrates (TCHO) were measured using
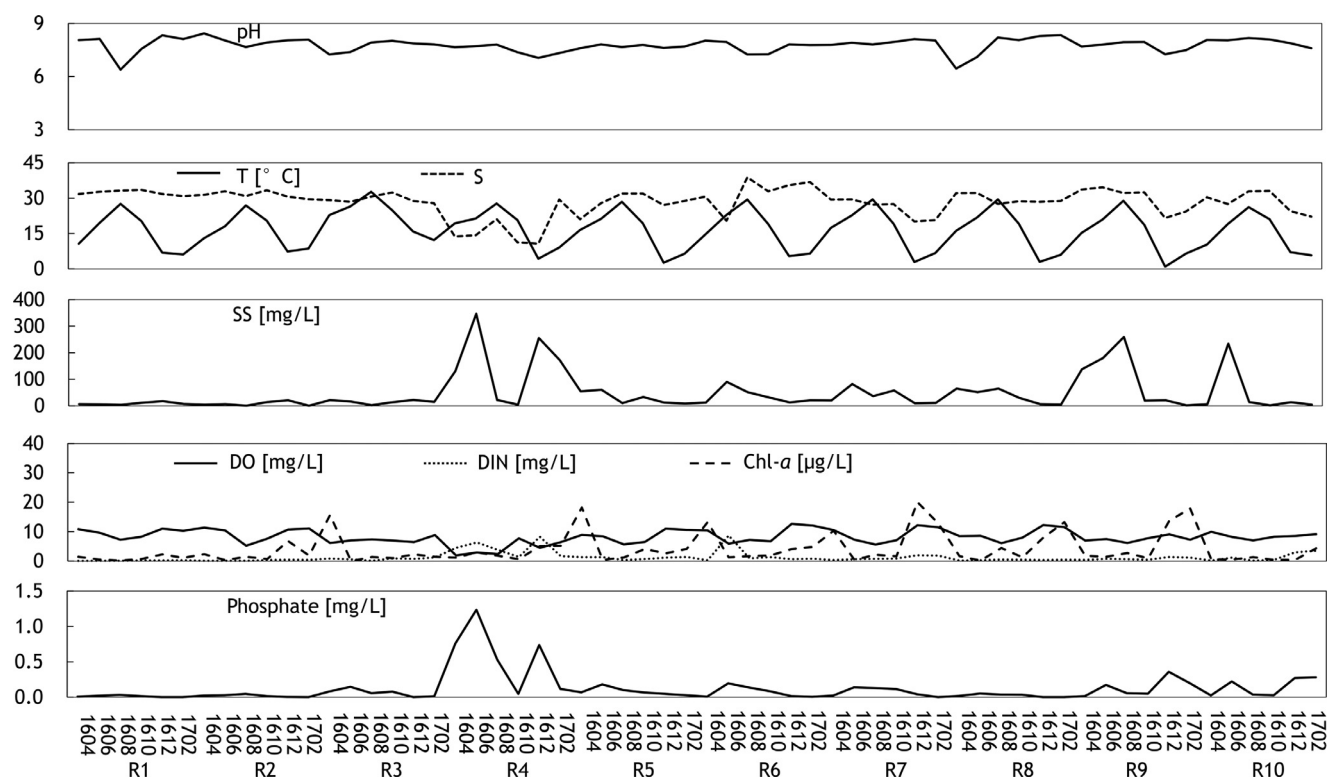
Figure 2 Temporal and spatial variations of temperature (*T*), salinity (*S*), pH, suspended particle (SS), dissolved oxygen (DO), dissolved inorganic nitrogen (DIN), phosphate and chlorophyll *a* (Chl-*a*) at the estuaries of Jiaozhou Bay.

the 2, 4, 6-tripyridyl-s-triazine (TPTZ) method (Myklestad and Børsheim, 2007). The MCHO sample was first heated to 100°C for 10 min, and then reacted with 1 mL of $FeCl_3$ (0.7 mg/L) and 2 mL of TPTZ (2.0 mg/L) to form a violet-colored complex with a maximum absorption at 596 nm (UV-2550, Shimadzu). Pure water (Milli-Q) was used as blank. TCHO was hydrolyzed by 1.2 M H_2SO_4 at 100°C for 2 h, neutralized with NaOH, and then subjected to the steps described for the MCHO measurement. The detection limit was 0.22 μM and the relative error was less than 5.44% for triplicate measurements. Concentrations of polysaccharides (PCHO) were calculated as the difference between TCHO and MCHO concentrations.

2.2.5. Amino acids
Concentrations of dissolved free amino acids (DFAA) were analyzed using liquid chromatography (HPLC) with the modified o-phthaldialdehyde-3-mercaptopropionic acid derivatization method (Kaiser and Benner, 2005). Total hydrolyzed amino acids (THAA) in the water samples were first hydrolyzed with HCl (6.0 M) at 110°C for 22 h in ampule bottles sealed under N_2 to form a hydrolysate. Then, the hydrolysates were evaporated and dissolved in pure water (Milli-Q). The separation of AAs was performed using a ZORBAX Eclipse AAA instrument (3.5 μm, 3.0 × 150 mm). The concentrations of fluorescent derivatives were measured at an excitation wavelength of 330 nm and emission wavelength of 450 nm. Concentrations of dissolved combined amino acids (DCAA) were calculated from the difference between THAA and DFAA concentrations. Standards for 18 amino acids were obtained from Sigma-Aldrich: as-

partic acid (Asp), serine (Ser), tryptophan (Trp), glutamic acid (Glu), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), cysteine (Cys), tyrosine (Tyr), valine (Val, methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu) and phenylalanine (Phe). The relative error of the amino acid concentrations was less than 2.80% for duplicate samples.

2.3. Statistical analysis

A canonical correspondence analysis (CCA) was performed using CANOCO 5 to study the relationship between environmental variables (T, S, pH, SS, DO, DIN, PO_4^{3-} and Chl-*a*) and DOM (Braak and Šmilauer, 2012). Potential correlations between DOM and physicochemical parameters were examined by calculating the maximal information coefficient (MIC) using the MINE package in R (Reshef et al., 2011). Then, the matrix of MIC values >0.51 was used to visualize the network associations between physicochemical parameters and DOM using Cytoscape software version 3.3.0 (Shannon et al., 2003).

3. Results and discussion

3.1. Physicochemical parameters in the estuaries of Jiaozhou Bay

The variations in T, S, pH, SS, DO, DIN, PO_4^{3-} and Chl-*a* in the waters of Jiaozhou Bay are shown in Fig. 2. T varied
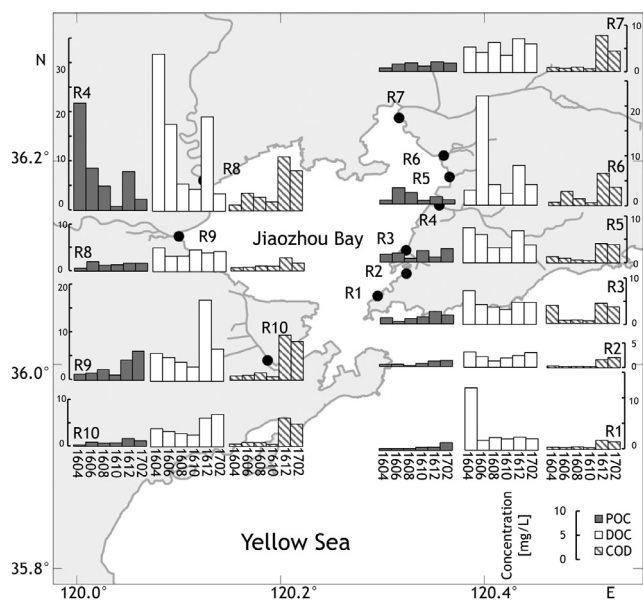
Figure 3 Temporal and spatial variations of POC, DOC and COD at the estuaries of Jiaozhou Bay.

Figure 4 Monosaccharide and polysaccharide concentrations at the estuaries of Jiaozhou Bay.

seasonally, while pH changed little with the season at all sites. With the exception of the Licun River (R4) between April and June, DO concentrations were relatively abundant (greater than 5 mg/L). The SS, DIN, PO_4^{3-}—P and Chl-a levels varied spatially and temporally in large ranges. Higher nutrient concentrations were observed in the Licun River (R4), and higher Chl-a concentrations were observed in the northwest bay.

3.2. Temporal and spatial variations in POC and DOC concentrations

The concentrations of POC, DOC and COD in the estuaries of Jiaozhou Bay varied spatially and temporally in the range of 0.13—22.40 mg/L (average 1.75 mg/L), 0.98—32.75 mg/L (average 5.04 mg/L), and 0.23—7.58 mg/L (average 1.67 mg/L), respectively (Fig. 3). The DOC concentration accounted for 52.43—97.29% (average 77.00%) of the total organic matter. Higher POC, DOC and COD concentrations were distributed in the northeast bay, consistent with a previous investigation (Zhang et al., 2013). Meanwhile, the DOC concentration was significantly and positively correlated with the POC concentration ($r = 0.8303$, $n = 60$, $P < 0.01$). Except for the obviously high values in the Licun River (R4), higher DOC and POC concentrations were recorded in December and April than in other months. The concentrations of POC and DOC at the Ferry (R1), the Dagang (R2), and the Haibo River (R3) were maintained at similar levels compared with the levels measured 10 years ago (Jiang et al., 2007). The COD concentration is used to indicate the total level of organic matter in many estuary and coastal waters in China (Zhu et al., 2002). This study applied COD as reference parameter, which was significantly and positively correlated with the combined DOC and POC concentrations ($r = 0.8303$, $n = 60$, $P < 0.01$).
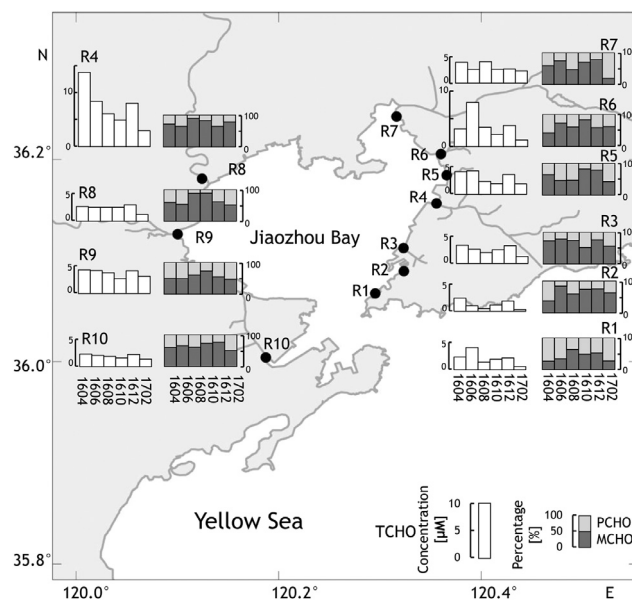
3.3. Temporal and spatial variations in DOM components

3.3.1. Carbohydrates
TCHO concentrations in the estuaries of Jiaozhou Bay varied with a range of 0.34—14.09 µM (average 3.18 µM) (Fig. 4). Generally, TCHO concentrations were higher in June and December, but lower in October and February. MCHO concentrations were approximately two-fold higher than the PCHO concentrations, accounting for 23.33—93.95% of the TCHO content (average 64.60%). Moreover, higher MCHO and PCHO concentrations were observed in the Licun River (R4), whereas lower concentrations were observed in the Ferry (R1) and Dagang (R2) Rivers. The TCHO concentration exhibited a similar distribution to the POC and DOC concentrations, as it was higher in the estuaries of northeast bay.

3.3.2. Amino acids
THAA concentrations in the estuaries of Jiaozhou Bay ranged from 0.89—8.30 µM (average 4.04 µM) (Fig. 5). DCAA accounted for 33.72—97.60% of the THAA (average 83.42%), and its concentration was 4 times higher than the DFAA concentration. Similar to the POC, DOC and TCHO concentrations, THAA concentrations at the Licun River (R4) were higher than at other stations, while THAA concentrations at the Ferry (R1) and Dagang (R2) Rivers were lower than at other stations. THAA concentrations varied temporally, with larger ranges observed in April, June and August than in October and December.

3.3.3. CDOM
The absorption coefficient is often used to quantify the relative concentrations of organic matter based on light absorption (Peacock et al., 2013), which exhibits a positive linear correlation with the fluorescence characteristics (Rochelle-Newall and Fisher, 2002; Vignudelli et al., 2004). The relative concentrations of CDOM in coastal waters are
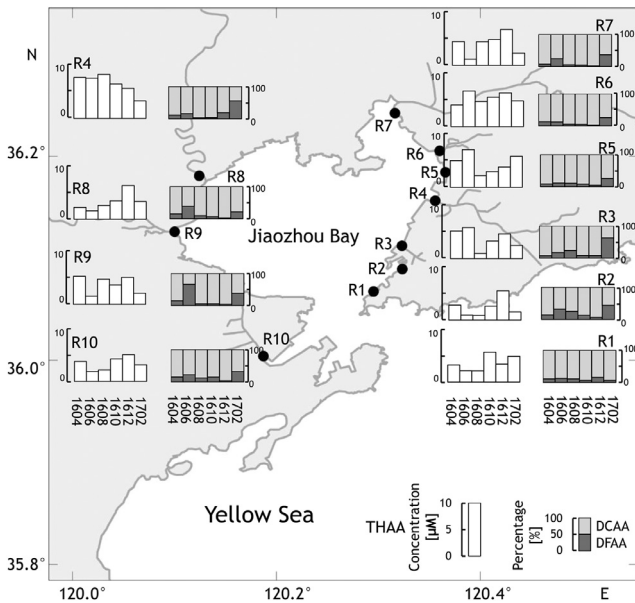
Figure 5   Concentrations of dissolved free (DFAA) and associated (DCAA) amino acids at the estuaries of Jiaozhou Bay.
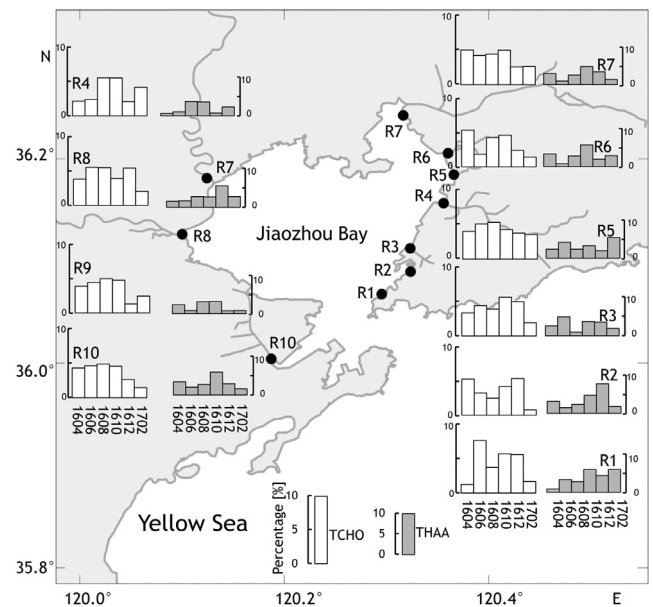

Figure 7   Percentages of carbohydrates and amino acids to DOC at the estuaries of Jiaozhou Bay.
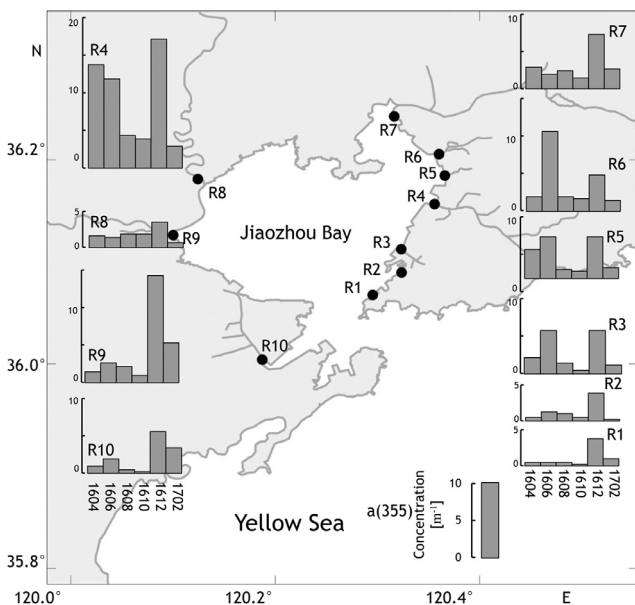

Figure 6   Relative concentrations of CDOM (a(355)) at the estuaries of Jiaozhou Bay.

indicated by the absorption coefficient at a wavelength of 355 nm (a(355)) (Green and Blough, 1994). The relative concentrations of CDOM in the estuaries of Jiaozhou Bay ranged from 0.23—16.35 m^{-1} (average 3.09 m^{-1}) (Fig. 6). Spatially, higher concentrations were observed in the Licun River (R4), while lower concentrations were observed in the Ferry (R1) and Dagang (R2) Rivers. Temporally, higher concentrations were observed in December, while lower concentrations were recorded in August and October. This finding might correlate with the dilution caused by local precipitation (Ren et al., 2018). Although the relative concentrations of CDOM in estuaries varied temporally and spatially, they were all

comparable to the relative concentrations of CDOM in the Liaohe estuary and reed wetland of Liaohe (Li et al., 2009; Zhang et al., 2016). The relative concentrations of CDOM in Haibo River (R3) measured in the present study are comparable with previous results (Jiang et al., 2007).

3.4. Contributors to the standing stock of DOM

The percentages of TCHO and THAA in DOC (TCHO-C% and THAA-C%) reflected the levels of activity and degradation of DOM components, and they were associated with labile organic matter, such as fresh plankton DOM (Davis et al., 2009). In general, higher TCHO-C% and THAA-C% indicate higher bioactivity (Davis et al., 2009). TCHO-C% and THAA-C% in the estuaries of Jiaozhou Bay ranged from 5.17—31.21% (average 15.87%) and 1.51—10.77% (average 4.05%), respectively (Fig. 7). The temporal variations were larger than the spatial variations. Specifically, TCHO-C% was higher (approximately 2 times) in August and October than in February. THAA-C% was higher in October, followed by December, which was approximately 2 times higher than the average values recorded in other months. The seasonal variations indicated that DOM was more active in summer and autumn than in winter in the estuaries of Jiaozhou Bay. Both the absolute DOC concentrations and relative bioactivity of DOM in the estuaries of Jiaozhou Bay were higher than in the estuaries and bays of the Adriatic Sea, Galveston Bay and Delaware estuary, which are also significantly impacted by human activities (Hung et al., 2001; Pettine et al., 2001; Witter and Luther, 2001; Yang et al., 2010).

The estuarine DOM originated from both riverine inputs and *in situ* primary production. Researchers have postulated that the humic-like materials are mainly derived from terrestrial input (Castillo et al., 1999), while Chl-*a* usually indicates *in situ* primary production, and glycine (Gly) traces the bacterial activities (Davis et al., 2009). The analysis of the linear relationship (Table 2) revealed significant posi-

Table 2 Correlation between concentrations of TCHO and THAA and relative contents of some organic matters.

	Humus-like	Protein-like	Glycine [Gly]	Chl-a
TCHO	0.790[a]	0.793[a]	0.628[a]	0.022
THAA	0.578[a]	0.525[a]	0.795[a]	0.160

[a] Showed significant correlation. ($p < 0.01$, $n = 60$, 2-tailed).

tive correlations between TCHO and THAA concentrations and the fluorescence intensities of humic-like materials, microbial protein-like materials (Ren et al., 2018), and Gly (simultaneous results), but no significant relationship was observed with Chl-a levels. Thus, riverine organic matter and bacterial degradation products primarily contribute to the standing stocks of TCHO and THAA. However, the insignificant relationships between TCHO/THAA and Chl-a indicated that primary production had little contribution to the standing stocks of TCHO and THAA. In previous studies, PCHO was mainly derived from the extracellular release by phytoplankton (Biersmith and Benner, 1998), and TCHO was positively correlated with phytoplankton growth and primary production (Kerhervé et al., 2002). The higher concentrations of Chl-a in the study area, ranging from 0.17 to 23.63 μg/L (averaging at 4.04 μg/L), inferred vigorous local phytoplankton productivity. Marine bacterial production activities preferentially use newly produced organic carbon (Bronk and Glibert, 1993). Therefore, we speculated that DOM produced through primary production in the study area was preferentially used by bacteria, and only a small amount was retained in the standing stock of DOM in the estuaries, leading to the insignificant relationships between TCHO and THAA with Chl-a in the study areas.

3.5. Factors regulating the DOM distributions

A canonical correspondence analysis (CCA) was used to discern the influences of environmental factors, including physical (T, S, pH and SS), chemical (DO, DIN and PO_4^{3-}) and biological (Chl-a) factors, on the OM (POC, DOC, TCHO, THAA and CDOM) distributions in the study estuaries. Correlation coefficients were higher between DOM characteristics and the first two axes (CCA1 and CCA2) of environmental factors (0.855 and 0.713) (Fig. 8), suggesting that close interactions existed between DOM characteristics and environmental factors. At the same time, the correlation coefficients of the first and second axes were as low as 0.0034 among DOM parameters, and even close to zero among the environmental factors, suggesting few interactions existed among the DOM parameters and separate environmental factors. The contributions of the first and second axes accounted for 78.4%. Thus, the CCA results were reliable to reveal the effects of environmental factors on DOM distributions.

As indicated by the correlation between the first axis (CCA1) and environmental factors, the DOM composition is influenced by T, PO_4^{3-}, DO, SS, pH, DIN, Chl-a and S in order of higher to lower significance. According to the correlation between the second axis (CCA2) and environmental factors,
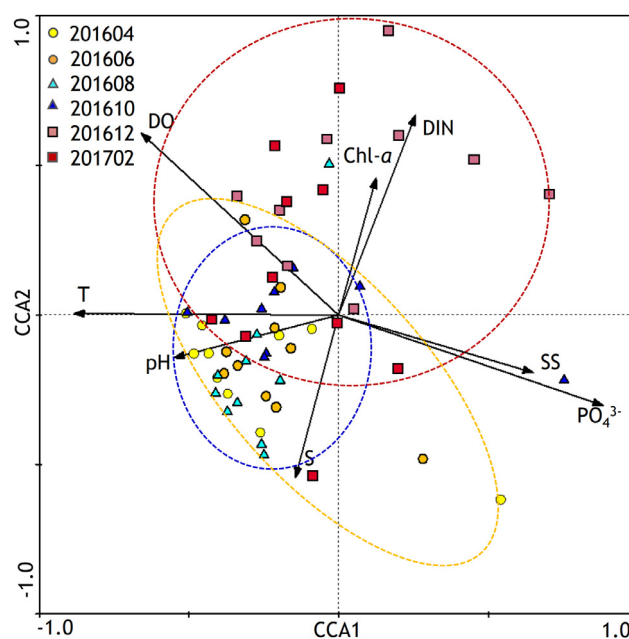

Figure 8 CCA analysis between DOM and environmental factors at the estuaries of Jiaozhou Bay. (DOM composition is represented by POC, DOC, TCHO, THAA and CDOM, while environmental factors are represented by T, pH, DO, S, Chl-a, SS, DIN and PO_4^{3-}. The length and direction of arrow line represent the importance of factor and relationship to DOM composition. The dots in one dash circle represent the similar group of DOM composition.)

the DOM composition is influenced by DIN, DO, S, Chl-a, PO_4^{3-}, SS and pH in order of higher to lower significance. The CCA results shown in Fig. 8 suggest important effects of T and the PO_4^{3-} concentration in April, June, August and October, DIN and PO_4^{3-} concentrations in December, and the DIN concentration in February. In general, T and PO_4^{3-} concentrations were the primary factors affecting the spatial and temporal distributions of DOM in the Jiaozhou Bay, followed by DO and SS concentrations. DO and Chl-a concentrations were also key factors controlling the spatial and temporal distributions of amino acids and carbohydrates. Moreover, S, DIN and PO_4^{3-} concentrations were the factors that effectively regulated the spatial and temporal distributions of POC, DOC and classified DOM components.

T not only represents a direct factor regulating the degradation rate of organic matter but also controls biological metabolism by regulating biological activity (Hudson et al., 2010; Mantoura and Woodard, 1983). As a primary factor, T exhibited significant temporal variations with seasons in the study area. The important functions of S (negative correlation), DIN and PO_4^{3-} (positive correlation) in regulating DOM implied that the riverine materials were the major sources of DOM in Jiaozhou Bay. The results from the CCA analysis are consistent with the findings described in Section 3.4 and Table 2. Namely, DO and Chl-a are the key factors controlling the spatial and temporal distributions of amino acids and carbohydrates. We inferred that the in situ primary production generated a certain amount of DOM, which contained greater concentrations of the active components amino acids and carbohydrates.
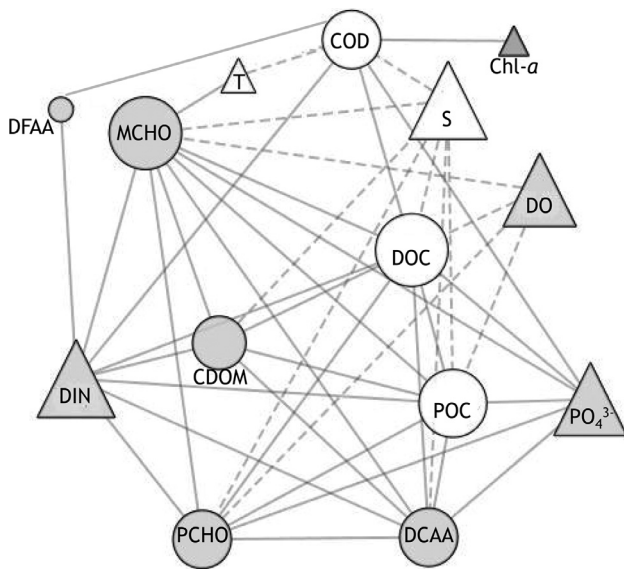
Figure 9 Maximal information coefficient (MIC) analysis of DOM components and physicochemical factors at the estuaries of Jiaozhou Bay. (The triangle is the environmental parameter, the circle is the dissolved organic parameter; solid line represents the positive correlation, dotted line represents negative correlation, and there is no line connecting between the parameters without correlation or with non-significant correlation; the size of the circle is proportional to the influence of each factor in the water environment.)

3.6. The impacts of physicochemical factors on DOM components

The maximal information coefficient (MIC) was applied to clarify the interactions among OM components (POC, DOC, MCHO, TCHO, DFAA, DCAA and CDOM) and environmental factors (T, S, DO, DIN, $PO_4{}^{3-}$ and Chl-a). Based on the results (Fig. 9), DOC and MCHO concentrations had the most weight and they were representative characteristics of the local DOM. S was the most important physicochemical factor and was negatively correlated with the levels of DOC, MCHO and other DOM components. Based on the MIC, we concluded that riverine discharge dominated the existing DOM components in the studied estuaries. Although T was a primary factor contributing to the spatial and temporal distributions of DOM, it exerted a small effect on MCHO and COD concentrations. Thus, T exerted minor effects on the compositions and properties of DOM. According to the results of the statistical analysis, Chl-a had a small effect on COD and did not directly affect other DOM components. Therefore, the primary products of phytoplankton had little contribution to the existing DOM compositions, which was also supported by the CCA results. Nutrients (DIN and $PO_4{}^{3-}$) had higher weights and were positively correlated with DOM components, and DO also had a higher weight but was negatively correlated with DOM components. Thus, we speculated that DO played an important role in oxidizing DOM components in the studied estuaries and was simultaneously responsible for the release of nutrients. CDOM had a lower weight among the DOM components, and was not significantly correlated with physiochemical factors. Therefore, CDOM was derived from the similar sources or mutual transformation with other components of DOM, but was less impacted by physicochemical factors and was maintained at relatively stable levels.

Based on the CCA and MIC results, T, S, DO, DIN, $PO_4{}^{3-}$ and Chl-a principally regulate the DOM distributions and composition. Then, numerical equations between these 5 parameters and DOM composition were established using a multiple regression analysis to further address the potential contribution of every principal parameter to the DOM concentration. The equations are listed below:

$$POC = -0.118T - 0.075S - 0.567DO - 0.222DIN + 7.506PO_4^{3-} \\ - 0.001Chl - a + 9.855, \tag{2}$$

$$DOC = -0.088T - 0.040S - 0.248DO + 1.438DIN + 8.002PO_4^{3-} \\ + 0.048Chl - a + 6.783, \tag{3}$$

$$CDOM = -0.119T - 0.167S - 0.242DO + 0.164DIN \\ + 4.125PO_4^{3-} + 0.038Chl - a + 10.966, \tag{4}$$

$$TCHO = 0.018T - 0.068S - 0.138DO + 0.351DIN + 3.258PO_4^{3-} \\ + 0.022Chl - a + 5.007, \tag{5}$$

$$THAA = -0.010T - 0.029S + 0.033DO + 0.325DIN \\ + 1.488PO_4^{3-} + 0.031Chl - a + 4.077. \tag{6}$$

$PO_4{}^{3-}$ ranks first in increasing the DOM concentration for every unit increase in its concentration. This finding is consistent with the relative absence of $PO_4{}^{3-}$ in Jiaozhou Bay, as well as most estuaries in China (Gong et al., 2015). DIN ranks second and plays an important role in regulating the DOM concentration, but exerted an obviously weaker effect than $PO_4{}^{3-}$ for every unit increase. As the primary limiting element, any increase in the $PO_4{}^{3-}$ concentration would stimulate the primary productivity in the bay. This finding was confirmed by the positive relationship between $PO_4{}^{3-}$ and Chl-a concentrations, as well as the positive relationship between Chl-a and DOM concentrations. However, the lower correlation coefficient between Chl-a and DOM concentrations suggested that primary production had a lower contribution to the standing pool of DOM. This result is consistent with the contribution of the Chl-a concentration to the DOM standing stock described in Section 3.4. DO has a similar contribution to DIN, and presents a negative correlation with the concentrations of DOM components, except THAA. DO is largely produced via primary productivity in oceans and is also consumed as an oxidant during the degradation of organic matter. Both of these processes are intricately linked with DOM production and elimination. The relationship between DO and DOM concentrations implies that degradation rather than primary production has a greater contribution to DOM components in the studied estuaries. T and S rank at lower levels in terms of contributions to the DOM concentration for every unit increase. However, this result does not contradict the important roles of T and S in

determining the DOM composition and distribution identified using the CCA and MIC analyses described above. When the units of every parameter are considered, T and S ranged from 6.01—21.95°C and 10.68—33.50, which exhibited much wider variations than the absolute concentrations of PO_4^{3-} and DIN throughout the year. Thus, T and S play key roles in determining the DOM composition and distribution when the wide variations of T and S are considered in the studied areas.

4. Conclusions

Jiaozhou Bay is a typical semi-closed bay at the edge of the western Pacific Ocean and is significantly regulated by anthropogenic activities. An investigation of data from one year showed that the concentrations of POC, DOC and COD in the study area were relatively higher than in comparable estuaries and bays, ranging from 0.13—22.40 mg/L (average 1.75 mg/L), 0.98—32.75 mg/L (average 5.04 mg/L), and 0.23—7.58 mg/L (average 1.67 mg/L), respectively. In addition to the significant positive relationship between DOC and POC concentrations, higher concentrations of these components were observed in December and April temporally, and in Licun River spatially. TCHO and THAA concentrations did not exhibit the same patterns as the POC and DOC concentrations, because higher concentrations were observed in summer and autumn but lower concentrations were recorded in winter. Meanwhile, wider temporal variations in TCHO and THAA concentrations were observed than in the spatial variations. Furthermore, the variations in TCHO-C% and THAA-C%, including both absolute and relative high concentrations, implied that DOM with higher bioactivity was observed in the study area, particularly in the period between summer and autumn.

According to the CCA and MIC results, the variations in T controlled the spatial and temporal distributions of DOM in the estuaries of Jiaozhou Bay, while the materials derived from rivers and bacteria dominated the existing DOM compositions. Phytoplankton contributed many of the bioactive organic components, such as amino acids and carbohydrates, but they accounted for a minor fraction of the existing DOM. MCHO and THAA were typical characteristic components, reflecting variations and processes of DOM in the estuaries of Jiaozhou Bay. The relative contributions of POC, DOC and CDOM displayed similar patterns in the Jiaozhou Bay estuaries, while TCHO and THAA concentrations varied due to their higher bioactivities.

Acknowledgments

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