Using fecal sterols to assess dynamics of sewage input in sediments along a human-impacted river-estuary system in eastern China

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HIGHLIGHTS

- Multiple sedimentary sterols used for the sewage contamination assessment.
- Higher sewage input observed in the Xiaoqing River than the Laizhou Bay.
- Significant spatial instead of seasonal variation in fecal sterol concentrations observed.
- Seasonal variation of two fecal sterol-based ratios observed.

GRAPHICAL ABSTRACT

The upper left panel showing the formation of 5 α - and 5 β -stanols is mainly modified from Bull et al., 2002.

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ABSTRACT

Sedimentary fecal sterols and other sterol biomarkers, combined with bulk total organic carbon (TOC) and its stable carbon isotope were applied to characterize the sewage contamination across a ca. 280 km transect from the Xiaoqing River to the Laizhou Bay, a typical river-estuary system subjected to extensive anthropogenic stress due to rapid regional urbanization and industrialization in eastern China. Two sampling events were performed in both spring and summer seasons in the Laizhou Bay adjacent to the Xiaoqing River in order to assess the potential seasonal variation. Fecal sterols such as coprostanol and epicoprostanol, which are typical indicators of anthropogenic sewage input, displayed high concentrations of up to $63.2 \mu\text{g g}^{-1}$ dry weight (dw) and $13.1 \mu\text{g g}^{-1}$ dw, respectively. Results suggested that most of the stations along the Xiaoqing River were severely contaminated by fecal inputs with a decreasing trend from the river to the estuary that was mainly explained by the increasing distance from the diffuse sewage sources and the gradual dilution by sea water. Although there was no significant difference in fecal sterol concentrations between spring and summer in the Laizhou Bay, suggestive of no significant difference in sewage abundance, significantly higher average epicoprostanol/coprostanol and lower coprostanol/epicoprostanol ratios were observed in spring than summer, indicative of different sewage sources (e.g., human vs. non-human). Seasonal discharge and land-runoff, air temperature related to microbial activity differences and different extend of animal manure irrigation during agricultural planting could be additional

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reasons and need further investigation. Nevertheless, fecal sterol concentrations, distributions and diagnostic ratios should all be taken into consideration to better understand sewage inputs and source dynamics in river-estuary ecosystems.

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1. Introduction

Anthropogenic interference in the form of domestic sewage discharge to rivers and coastal areas has caused severe environmental problems such as eutrophication, oxygen depletion and contamination (NBSC, 2012; Pan and Wang, 2012). Most of the coastal cities in China are characterized by a rapid industrialization and urbanization since the 1980s. Therefore, the assessment of anthropogenic contamination in coastal areas is of great interest for both the environment and human health, considering the high population density in these areas (e.g., Förstner, 2006; NBSC, 2012; Canuel and Hardison, 2016).

Sewage input, a major type of anthropogenic contamination that causes declining water quality in aquatic ecosystems, can be assessed using molecular markers (e.g., Bull et al., 2002; Carreira et al., 2004; Muniz et al., 2015; Derrien et al., 2017). Sterols, a common type of molecular markers, can be used to determine sources and fate of both anthropogenic (e.g., fecal input; e.g., Bull et al., 2002) and natural organic matter (OM; e.g., marine and terrigenous; Volkman, 2005) in environments. The phytosterols, including stigmasterol (stigmasta-5,22E-dien-3 β -ol) and β -sitosterol (stigmasta-5-en-3 β -ol), are biomarkers of terrestrial organic matter (OM; Moreau et al., 2002). Brassicasterol (ergosta-5,22E-dien-3 β -ol) is mainly produced by marine phytoplankton (Volkman, 1986); cholesterol (cholest-5-en-3 β -ol) is generally attributed to a wide diversity of phytoplankton and zooplankton (e.g., Volkman, 2005). These two sterols (brassicasterol and cholesterol) are widely used as biomarkers of aquatic OM. In contrast, the fecal sterols such as coprostanol (5 β -cholestan-3 β -ol) and epicoprostanol (5 β -cholestan-3 α -ol) are usually considered to be derived from fecal pollution and urban wastewater inputs (Grimalt et al., 1990; González-Oreja and Saiz-Salinas, 1998; Bull et al., 2002; Carreira et al., 2004; Vane et al., 2010; Adnan et al., 2012; Abreu-Mota et al., 2014; Muniz et al., 2015; Rada et al., 2016). Coprostanol, a fecal sterol (5 β -stanol) detected predominantly in human feces, is preferentially produced in human guts by enzymatic reduction of cholesterol through anaerobic bacteria (Macdonald et al., 1983). Coprostanol comprises up to 60% of total sterols in human feces (Leeming et al., 1996). Similar to other sterols that are commonly hydrophobic, coprostanol is generally associated with particulate matter in sewage and is finally incorporated and preserved in the bottom sediments (Bartlett, 1987). Previous studies proposed coprostanol concentrations above a certain level to be indicative of sewage contamination in aquatic sediments, although various thresholds were applied, such as 0.1, 0.5 or 0.7 $\mu\text{g g}^{-1}$ dry weight (Grimalt et al., 1990; González-Oreja and Saiz-Salinas, 1998; Rada et al., 2016). However, Tse et al. (2014) suggested that coprostanol alone may not be sewage specific, since in situ anaerobic hydrogenation or microbial reduction processes can also produce coprostanol in sediments using cholesterol as the precursor (Fattore et al., 1996). Instead, a more robust assessment of sewage contamination can be conducted by investigating sterol-based ratios (e.g., Grimalt et al., 1990; Writer et al., 1995). For example, sterol-based diagnostic ratios have been applied to evaluate (i) whether the sewage was treated or not before being discharged (Mudge and Seguel, 1999), and (ii) the origins of the feces (human vs. animals; Venkatesan and Santiago, 1989; Leeming et al., 1996; Leeming et al., 1997; Bull et al., 2002).

Due to rapid urbanization in China, anthropogenic activities (e.g., sewage discharge) have induced severe contamination to the Chinese marginal seas, such as the Laizhou Bay (Zhang et al., 2009; Li et al., 2012). Laizhou Bay is a semi-closed system in the west of the Bohai Sea

and receives water discharge from the Xiaoqing River and the Yellow River directly. The Xiaoqing River is the second largest river draining into the Laizhou Bay, only secondary to the Yellow River. In contrast with the Yellow River that is dominated by natural OM (Tao et al., 2015), the Xiaoqing River is severely polluted since the drainage basin of the Xiaoqing River is characterized by high-density population and extensive agricultural farming (Jiang et al., 2017). Although this system has been widely studied in terms of water chemistry and sediment chemistry (e.g., dissolved organic matter, heavy metals and persistent organic pollutants) (Ren et al., 2014; Wang et al., 2016; Jiang et al., 2017; Jiao et al., 2017), our current knowledge on fecal contamination, owing to the intensive urbanization in the watershed, is limited. Surface sediments in the river-estuary system provide excellent opportunities to evaluate levels of anthropogenic contamination and their influence on coastal ecosystems (e.g., Andrews et al., 1998). With high sedimentation rates of up to 9.4 cm/yr (Li et al., 2012), fast accumulation of pollutants was detected in the Xiaoqing River mouth and Laizhou Bay (Zhang et al., 2009). The seasonal variations of the Xiaoqing River, in terms of water discharges, rain falls and hydrodynamic processes, could potentially affect the transport and deposition of sewage related OM (Liu et al., 2009; Gao, 2011; Yu et al., 2016).

In this study, surface sediments from spring and summer were collected along the Xiaoqing River–Laizhou Bay estuary system to (i) determine concentrations and sources of sedimentary sterols, (ii) assess sewage contamination levels in surface sediments, and (iii) examine spatial and seasonal influences on sedimentary fecal sterol distributions and possible controlling factors. Results from this study could potentially be relevant to sewage regulation and treatment in the future.

2. Materials and methods

2.1. Description of study sites

The Xiaoqing River is ca. 240 km and covers a drainage basin of ca. 2800 km². It flows through numerous large cities, such as Jinan, Zibo, Binzhou, Dongying and Weifang, and is comprehensively utilized for controlling flood, irrigation, and shipping, etc. The Xiaoqing River empties into the Laizhou Bay in the Bohai Sea. The population sizes of Jinan, Zibo, Binzhou, Dongying and Weifang cities are approximately 7.23 million, 4.71 million, 3.8 million, 2.13 million, and 9.28 million, respectively. The average annual rainfall rate in the Xiaoqing River basin is ca. 620 mm/yr with strong heterogeneity monthly. For example, July and August account for >50% of the annual total rainfall (Gao, 2011). The average air temperature in spring and summer are 5 °C and 27 °C, respectively, based on data from a weather monitoring station in Jinan City (Gao, 2011). In recent years, the Xiaoqing River has undergone serious pollution due to rapid urbanization and industrialization. According to a survey in 2014 (Jinan Environmental Quality Report), 68.8% of Jinan's industrial emission was discharged into the Xiaoqing River. There are approximately 20 key pollution sources distributed in the Xiaoqing River basin (Jinan Environmental Quality Report). Dissolved organic carbon (DOC) concentrations in the surface water of the Xiaoqing River mouth were from 5.1 to 10.1 mg L⁻¹ and 5.1 to 55.9 mg L⁻¹, and NH₄⁺-N concentrations from 2.5 to 9.3 mg L⁻¹ and 2.5 to 9.3 mg L⁻¹ in May and August of 2010, respectively (Cui et al., 2013), suggesting that the Xiaoqing River is heavily polluted.

2.2. Sample collection

A total of 35 surface sediment samples (ca. 0–4 cm) were collected from the Xiaoqing River–Laizhou Bay in early April (spring) and late August (summer) of 2014. Sampling sites were selected based on an optimum spatial coverage of this system (Fig. 1; Fig. S1). Specifically, samples from X1 to X11 were only collected in spring and operationally defined as spatial end members representative of the Xiaoqing River (with surface water salinity < 4 psu), whereas stations from L1 to L11, with salinity between 5.5 and 29.1 psu, were sampled in both spring (Lsp) and summer (Lsu) to identify potential seasonal variations. All the sediment samples were collected using a stainless-steel dredge sampler and immediately stored on ice during transportation to the laboratory and then freeze dried at -50°C before further analysis. During each sampling trip, water chemistry parameters, such as dissolved oxygen (DO) concentration, pH, salinity, and redox potential, were measured using portable YSI ProPlus equipment (USA).

2.3. Bulk parameter measurements

Freeze dried sediment samples (ca. 1 g) were homogenized and acidified using 0.1 M HCl solution to remove carbonates, rinsed with deionized water, and dried at 60°C . TOC measurements were performed using a Thermo Scientific FLASH2000 Series CNS Elemental Analyzer. Aliquots of the carbonate-free sediments were analyzed for $\delta^{13}\text{C}_{\text{org}}$ using a FLASH2000 Organic Elemental Analyzer coupled to a Thermo Fisher MAT-253 mass spectrometer. Samples were measured in duplicates and values were reported in average. A reference standard material (glycine) was analyzed every five measurements. The standard deviation of the replicate measurements was $\pm 0.2\%$. Sediment grain size was measured following the method described in Fan et al. (2015).

2.4. Extraction and fractionation

Freeze dried sediments (ca. 10 g) were Soxhlet extracted using mixed solvents of 100 ml dichloromethane and 50 ml methanol under dark conditions for 48 to 50 h. The total extracts were concentrated to ca. 4 ml by rotoevaporation followed by further dry-down under high purity N_2 gas. The total extracts were separated on a silica gel column into aliphatic, aromatic and polar fractions using *n*-hexane, benzene and methanol/dichloromethane (1:1, v/v), sequentially. Aliquots (50 μl) of the polar fractions (containing sterols) were then evaporated to dryness under high purity N_2 gas and derivatized at 60°C for 2 h using ca. 50 μl of BSTFA (*N,O*-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane). Finally, the derivatization products were dried under high purity N_2 gas and redissolved in *n*-hexane for gas chromatography–mass spectrometry (GC–MS) analysis.

2.5. GC–MS analysis

The GC–MS system for biomarker analysis (Agilent 7890-5977MS) was equipped with an automatic split/splitless injector (autosampler AS-2912). The electron impact ionization (EI) mode was used. A DB5-MS capillary column (60 m, 0.25 mm i.d.) was used, and the GC temperature was set under the following conditions: 60°C (held for 3 min), ramping at $3^{\circ}\text{C min}^{-1}$ to 300°C (held for 30 min). The injection was performed in splitless mode at 280°C . The mass spectrometer ion source was operated in electron impact (EI) mode at 70 eV. The GC–MS interface and the ion source temperatures were set at 300°C and 280°C , respectively. Ultra-high purity helium was used as the carrier gas, and the flow rate was set to 1.2 mL min^{-1} . Analysis was performed in total ion current (TIC) mode (50 to 600 m/z ; Arcega-Cabrera et al., 2014). Individual sterol was identified based on relative retention times (e.g., Isobe et al., 2002; Derrien et al., 2011), the NIST 2008 library, literature and the interpretation of the ion fragmentation patterns. Deuterated *n* hexatriacontaine was used as the internal standard (IS).

Fig. 1. Sampling map of the Xiaoqing River–Laizhou Bay, eastern China.

(D'Anjou et al., 2012). Quantification was mainly based on Derrien et al. (2011), with some modifications. Briefly, a 5-point calibration curve (0.2 to $40 \mu\text{g ml}^{-1}$) was applied with a constant IS concentration at $10 \mu\text{g ml}^{-1}$. Coprostanol, cholesterol and stigmasterol were quantified using the calibration curve of coprostanol, cholesterol and stigmasterol, respectively. Epicoprostanol, cholestanol (5α -cholestan- 3α -ol), epicholestanol (5β -cholestan- 3α -ol), stigmastanol (stgmastan- 3β -ol) and coprostanone (5β -cholestan- 3 -one) were quantified using the calibration curve of coprostanol, whereas campesterol, sitosterol, brassicasterol, cholestenone and stigmastanone were quantified using the calibration curve of cholesterol, assuming same response factors (D'Anjou et al., 2012).

2.6. Quality assurance/quality control

A calibration of the GC–MS was conducted daily. A blank (pure *n*-hexane) and external standard (coprostanol, stigmasterol, and cholesterol) were analyzed first. The sample analysis was then performed only after the correct instrument response was verified. Procedural blanks were also checked to ensure no peaks coeluted with the peaks of the target sterol compounds. The extraction recoveries of $>70\%$ were considered acceptable. The precision for individual sterols was determined based on the analysis of a sediment sample in triplicate and standard deviation was found to be $<5.0\%$ (He et al., 2014).

2.7. Statistical analysis

All statistical analyses were performed using the Origin 8.0 and R software. The Pearson correlation and principal component analysis

(PCA) were performed using a Vegan package. For the PCA, the major sterols detected were considered as the variables and the samples as the individuals.

3. Results and discussion

3.1. Bulk parameters

The pH of the surface water spanned a small range from 7.44 to 8.18, 7.49 to 8.06 and 7.49 to 7.92 for the X, Lsp and Lsu samples, respectively, whereas the DO concentration varied considerably from 2.2 to 14.5 mg l^{-1} , 6.4 to 9.5 mg l^{-1} and 5.8 to 12.5 mg l^{-1} , respectively (Table 1). The redox potential of the surface water ranged from -27.2 to 198.0 mV , 109.1 to 213.2 mV and 31.7 to 56.2 mV . In general, the upstream Xiaoqing River was characterized by low DO concentrations, while the Laizhou Bay was characterized by low redox potential in summer.

The sediments of the Xiaoqing River–Laizhou Bay were predominantly composed of silt (between 40.9% and 77.8%; Table 1), denoting a region with relatively weak hydrodynamic conditions. The highest sand content (53.0%) was observed at station L5 in spring, close to the Xiaoqing River mouth, suggesting this location was subject to intense waves and tidal currents. However, in general, no obvious spatial trend was observed for clay, silt or sand contents. The TOC contents ranged from 0.04% (station L6) to 4.01% (station X8; Table 1). This large range of TOC contents generally reflected different inputs of OM, hydrodynamic sorting processes, and possibly post-depositional biodegradation (Meyers, 2003).

Table 1
Bulk parameters of both the surface water and surface sediments at each sampling station.

Station lists	Salinity (psu) ^a	pH ^a	Dissolved oxygen (mg l^{-1}) ^a	Redox potential (mV) ^a	TOC (%)	$\delta^{13}\text{C}$ (‰)	Clay (%)	Silt (%)	Sand (%)	Site description
X1	0.6	7.44	2.5	−27.2	3.13	−25.3	12.9	69.6	17.5	1
X2	0.7	7.50	2.4	166.8	1.61	−25.9	10.3	58.5	31.2	2
X3	0.7	7.54	2.2	155.3	1.60	−25.5	23.3	61.7	15.1	3
X4	0.9	7.78	3.1	−15.6	1.83	−26.0	7.1	51.7	41.3	
X5	0.8	7.70	7.0	164.3	0.76	−25.3	7.8	56.4	35.8	4
X6	1.6	7.56	6.1	116.4	1.09	−25.3	12.6	66.7	20.8	5
X7	1.0	7.76	7.0	103.5	1.12	−26.0	11.2	77.8	11.0	6
X8	2.4	7.47	6.0	150.5	4.01	−26.7	7.7	65.0	27.2	7
X9	1.7	7.85	8.5	149.0	0.87	−25.0	23.3	61.7	15.1	8
X10	3.6	8.18	14.5	198.0	0.66	−26.3	10.5	61.0	28.5	
X11	3.2	7.58	10.8	148.9	0.74	−26.5	10.6	58.1	31.3	9
L1sp	9.2	7.49	6.4	111.4	0.57	−26.2	7.7	47.3	45.0	10
L2sp	5.5	7.67	7.8	170.4	0.66	−25.9	9.0	55.4	35.6	11
L3sp	10.2	7.61	7.1	149.7	0.50	−25.4	13.3	60.0	26.8	
L4sp	12.7	7.55	6.8	153.3	0.25	−26.2	11.2	62.1	26.8	
L5sp	25.0	7.50	6.8	109.1	0.38	−24.6	6.1	40.9	53.0	
L6sp	26.9	7.86	8.3	135.6	0.51	−24.4	10.2	55.3	34.4	
L7sp	27.2	7.93	9.5	213.2	0.22	−23.5	16.4	66.7	16.9	
L8sp	26.4	8.02	9.4	183.4	0.17	−22.6	25.1	70.5	4.3	
L9sp	27.2	7.97	8.8	147.3	0.09	−23.1	17.4	66.8	15.8	
L10sp	27.7	8.06	9.5	166.7	0.15	−23.1	26.0	70.0	4.0	
L11sp	27.7	7.98	8.4	150.2	0.15	−23.1	16.1	69.3	14.7	
L1su	14.4	7.49	5.8	46.8	0.68	−25.5	N.A.	N.A.	N.A.	
L2su	16.2	7.47	6.1	56.2	0.26	−24.7	N.A.	N.A.	N.A.	
L3su	18.1	7.51	4.8	41.5	0.38	−25.5	N.A.	N.A.	N.A.	
L4su	21.7	7.56	6.5	50.1	0.38	−24.6	N.A.	N.A.	N.A.	
L5su	23.8	7.64	9.1	31.7	0.17	−24.1	N.A.	N.A.	N.A.	
L6su	27.7	7.92	12.5	35.4	0.04	−22.1	N.A.	N.A.	N.A.	
L7su	28.6	7.88	9.0	44.4	0.17	−22.9	N.A.	N.A.	N.A.	
L8su	28.9	7.79	8.7	43.7	0.23	−23.3	N.A.	N.A.	N.A.	
L9su	28.5	7.85	8.6	34.9	0.16	−23.0	N.A.	N.A.	N.A.	
L10su	28.6	7.78	8.7	39.0	0.19	−23.3	N.A.	N.A.	N.A.	
L11su	29.1	7.85	3.6	37.7	0.18	−23.6	N.A.	N.A.	N.A.	
L4-1su	27.2	7.81	8.4	50.1	0.13	−23.5	N.A.	N.A.	N.A.	
L4-2su	25.3	7.65	8.4	52.1	0.16	−22.5	N.A.	N.A.	N.A.	

1, Caiyuan bridge of Jinan city and close to a sewage outlet; 2, Jinan city; 3 close to a dam; 4, Little bridge at Zibo city; 5, close to a hydrological monitoring station; 6, close to Jinjia dam, 7, Zibo city; 8, downstream of the Xiaoqing River; 9, Yangkou town; 10 Yangkou Bay; 11, close to an iron mine.

^a Parameters for surface water at each sampling station. N.A., data not available.

The $\delta^{13}\text{C}_{\text{org}}$ values in sediments ranged from -26.7‰ to -25.0‰ , -26.2‰ to -22.6‰ and -25.5‰ to -22.1‰ for the X, Lsp and Lsu samples, respectively, suggesting OM inputs from both terrestrial and marine sources (Pancost and Boot, 2004). The terrestrial OM is commonly characterized by lower $\delta^{13}\text{C}_{\text{org}}$ values (e.g., $\sim -27\text{‰}$), whereas the marine phytoplankton-derived OM has higher $\delta^{13}\text{C}_{\text{org}}$ values (e.g., $\sim -19\text{‰}$ to -21‰ ; Fry and Sherr, 1989). A general decrease of TOC contents and an increase of $\delta^{13}\text{C}_{\text{org}}$ values were observed at stations with higher salinity (or spatially the Xiaoqing River to the Laizhou Bay; Fig. 2), indicative of a natural gradient where terrestrial OM generally decreased when gradually being dominated by marine environment.

3.2. Sterol distributions and concentrations

Human/animal and plant sterols were identified in all sediment samples, indicating that a variety of sources contributed to the composition of the sedimentary OM in this area (Table 2). The typical mass chromatograms of the sterols, stenones and stanones detected were presented in Fig. 3. The highest total sterol concentration ($198.2 \mu\text{g g}^{-1}$) was observed at station X8 with cholesterol being the dominant and followed by β -sitosterol, campesterol, and stigmaterol.

Coprostanol was detected in all samples, with concentrations ranging from $0.03 \mu\text{g g}^{-1}$ dw (station L6su) to $63.3 \mu\text{g g}^{-1}$ dw (station X8), while epicoprostanol was from $0.02 \mu\text{g g}^{-1}$ dw (station L6su) to $13.10 \mu\text{g g}^{-1}$ dw (station X1). The highest coprostanol concentration detected in this study is much higher than various other coastal areas worldwide, such as some tropical estuaries in Brazil (Martins et al., 2014; Carreira et al., 2015), the black sea (Readman et al., 2005), Kuwait's marine areas (Saeed et al., 2015) and the Macao Estuary close to Zhuhai and Macao cities, southern China (Peng et al., 2002) (Table S1). Coprostanol is a major component in omnivore feces (Leeming et al., 1996), but occurs at trace levels in plant species (Moreau et al., 2002). Epicoprostanol is commonly converted from coprostanol by intensive microbial activities and is usually detected in digested sludge samples (e.g., Mccalley et al., 1981). Therefore, the presence of epicoprostanol in all samples suggested that the sewage has been microbially degraded or partially digested (Mccalley et al., 1981; Mudge and Seguel, 1999; Bull et al., 2002). This result is reasonable when considering that there are some

wastewater treatment systems in large cities in the Xiaoqing River watershed, such as Jinan and Zibo. Since sterols are hydrophobic, they preferentially adsorb onto fine suspended particles and are enriched in sediments with high TOC content (Writer et al., 1995; Froehner et al., 2009; Tolosa et al., 2014). The coprostanol concentrations correlated strongly with TOC contents ($r^2 = 0.87$, $P < 0.01$; Fig. 2c, d), in agreement with a previous study in Cienfuegos Bay, Cuba (Tolosa et al., 2014). As expected, station X8, located at a sewage discharge site near Zibo city (Fig. S1), had the highest coprostanol concentration and TOC content (4.01%). Station X1 near Jinan city displayed the second highest concentration of coprostanol, together with high TOC content (3.13%), as well. Station X1 was severely affected by domestic sewage and manure waste from both Tianqiao and Lixia district of the Jinan City (Wang et al., 2011). Additionally, the strong correlation between coprostanol concentration and TOC content indicated that: (i) fecal sterols and the major part of OM had the similar source in this region, and (ii) fecal sterols could potentially track sewage input to this area. Strong anaerobic diagenetic processes were also seen at stations X8 and X1, as indicated by high concentrations of stenones and stanones (Table 2; Nishimura, 1982; de Leeuw et al., 1993; Rushdi et al., 2006). This finding was consistent with the general low redox potential and DO concentration at station X1.

As coprostanol is commonly associated with sewage discharge, its concentration has been used to indicate the level of sewage contamination (Grimalt et al., 1990; Takada and Eganhouse, 1998; González-Oreja and Saiz-Salinas, 1998; Rada et al., 2016). For example, Grimalt et al. (1990) suggested that coprostanol concentrations above $0.1 \mu\text{g g}^{-1}$ dw were indicative of sewage contamination, whereas 'significant' sewage contamination was defined as the level above $0.5 \mu\text{g g}^{-1}$ dw (González-Oreja and Saiz-Salinas, 1998). Nevertheless, Rada et al. (2016) used $0.7 \mu\text{g g}^{-1}$ dw as the threshold of sewage contamination. In our case, concentrations $> 0.7 \mu\text{g g}^{-1}$ dw were conservatively considered as sewage contamination. Based on this criterion, all X stations, 4 of 11 Lsp stations and 3 of 13 Lsu stations displayed obvious sewage contamination. In fact, most of the X stations were located near sewage sources from local cities or towns. In contrast, all other stations with lower concentrations of coprostanol were located in the Laizhou Bay, possibly due to seawater dilution. It clearly suggested that seawater

Fig. 2. Bulk physicochemical parameters and coprostanol concentrations of surface sediments from the Xiaoqing River–Laizhou Bay: scatter plots of (a) TOC versus salinity, (b) $\delta^{13}\text{C}$ versus salinity, (c, d) TOC versus coprostanol concentrations.

Table 2

Sterol biomarker for surface sediments from the Xiaoqing River–Laizhou Bay.

Station lists	Sum of sterols	Coprostanol	Epicoprostanol	Epicholestanol	Cholestanol	Cholesterol	Brassicasterol	Coprostanone	Cholestanone	24 Ethylcoprostanol	Cholestenone	Stigmasterol	Stigmastanol	Stigmastanone	Campesterol	Sitosterol
X1	158.343	47.513	13.096	4.886	29.067	8.820	0.621	3.340	3.626	24.434	0.527	1.380	11.556	0.122	1.775	7.580
X2	126.275	27.003	5.530	1.304	8.360	37.411	1.845	4.655	2.946	12.341	0.701	4.192	3.399	0.231	5.149	11.208
X3	99.45	22.547	4.527	2.449	9.118	23.877	1.121	4.765	2.447	11.263	0.737	2.300	4.006	0.383	3.374	6.536
X4	18.606	3.436	0.524	0.200	1.479	5.588	0.357	0.496	0.371	2.224	0.136	0.976	0.849	0.075	0.976	0.919
X5	56.757	11.227	2.152	0.898	4.355	16.012	1.430	2.108	1.277	5.257	0.455	3.226	2.110	0.172	3.226	2.852
X6	32.937	4.131	1.167	0.377	2.225	10.530	1.138	0.846	0.697	2.332	0.345	3.631	0.849	0.075	3.226	1.368
X7	27.773	4.161	2.076	0.692	2.610	4.290	0.839	1.208	1.289	2.560	1.865	1.189	1.466	0.551	1.189	1.788
X8	195.865	63.262	7.299	3.433	6.063	21.764	2.442	7.884	6.087	13.162	46.769	2.650	1.835	3.668	4.318	5.229
X9	17.516	1.064	0.169	0.054	1.236	2.493	0.230	0.691	0.680	1.497	0.297	1.312	4.498	0.171	0.900	2.224
X10	22.446	2.301	0.285	0.095	2.173	4.034	0.937	0.317	0.509	1.657	0.352	2.310	2.001	0.331	1.854	3.290
X11	27.736	3.793	1.949	0.65	2.377	4.637	0.815	1.014	1.040	2.439	1.586	1.703	1.428	0.431	1.064	2.810
L1sp	17.199	1.558	0.794	0.265	1.571	3.962	0.849	0.286	0.280	1.368	0.239	1.165	1.055	0.134	1.311	2.362
L2sp	28.853	3.063	1.153	0.818	3.052	6.063	1.794	0.670	0.353	1.928	0.260	2.151	1.617	0.071	1.579	4.281
L3sp	15.872	1.905	0.282	0.094	1.649	2.979	0.750	0.436	0.638	1.149	0.349	1.169	1.072	0.189	1.062	2.149
L4sp	35.145	3.610	0.930	1.027	4.133	8.134	2.574	0.525	0.433	2.052	0.486	2.520	1.895	0.169	2.267	4.390
L5sp	6.339	0.327	0.085	0.028	0.719	2.404	0.452	<LQ	<LQ	0.303	<LQ	0.462	0.410	<LQ	0.389	0.760
L6sp	8.788	0.676	0.186	0.124	0.977	2.608	0.565	0.145	0.204	0.463	0.136	0.520	0.511	0.031	0.565	1.077
L7sp	2.352	0.043	0.041	0.039	0.272	0.604	0.163	<LQ	<LQ	<LQ	<LQ	0.165	0.168	<LQ	0.163	0.694
L8sp	5.465	0.144	0.035	<LQ	0.745	1.642	0.470	<LQ	<LQ	0.101	<LQ	0.264	0.689	<LQ	0.470	0.905
L9sp	2.839	0.064	0.043	0.047	0.309	0.831	0.214	0.019	0.038	<LQ	<LQ	0.159	0.166	<LQ	0.214	0.735
L10sp	6.602	0.114	0.077	0.075	0.578	1.850	0.427	<LQ	<LQ	<LQ	<LQ	0.435	0.505	<LQ	0.427	2.114
L11sp	5.025	0.091	0.051	0.068	0.577	1.446	0.534	0.025	0.040	0.117	<LQ	0.269	0.295	<LQ	0.343	1.169
L1su	64.035	4.553	2.169	1.855	9.336	10.154	2.025	1.785	1.437	4.118	1.189	4.601	5.675	0.724	4.845	9.569
L2su	16.82	0.710	0.432	0.144	1.961	4.511	0.619	0.279	0.288	0.623	0.226	1.025	1.488	0.107	1.545	2.862
L3su	18.885	0.331	0.153	0.085	0.785	8.827	1.198	0.261	0.229	0.380	0.152	1.404	0.522	0.052	2.318	2.188
L4su	35.574	3.567	1.385	1.291	6.538	5.660	1.132	0.793	0.741	2.954	0.606	1.786	3.798	0.252	1.723	3.348
L5su	4.678	0.178	0.053	0.014	0.470	1.776	0.576	<LQ	<LQ	0.124	<LQ	0.464	0.283	<LQ	0.225	0.515
L6su	2.059	0.029	0.021	<LQ	0.222	1.148	0.065	<LQ	<LQ	0.020	<LQ	0.067	0.156	<LQ	0.061	0.270
L7su	4.072	0.073	0.079	0.076	0.763	1.406	0.302	0.017	0.067	<LQ	<LQ	0.181	0.279	<LQ	0.299	0.530
L8su	6.352	0.081	0.055	0.099	1.111	1.656	0.529	0.026	0.087	0.122	<LQ	0.292	0.810	<LQ	0.446	1.038
L9su	10.504	0.21	0.162	0.264	0.210	4.340	0.900	0.056	0.125	0.216	0.173	0.484	0.831	0.059	0.902	1.572
L10su	2.675	0.048	0.036	0.053	0.416	0.683	0.211	0.018	0.026	<LQ	<LQ	0.146	0.315	<LQ	0.201	0.522
L11su	5.636	0.041	0.032	0.061	0.711	2.728	0.543	0.024	0.050	<LQ	<LQ	0.250	0.274	<LQ	0.326	0.596
L4-1su	5.852	0.083	0.067	0.087	0.695	1.986	0.245	<LQ	<LQ	<LQ	<LQ	0.373	<LQ	<LQ	0.319	1.997
L4-2su	7.784	0.139	0.087	0.084	0.638	3.582	0.645	<LQ	<LQ	0.108	<LQ	0.402	0.222	<LQ	0.595	1.282

Note: all the units are $\mu\text{g g}^{-1}$ dry weight.

Fig. 3. Examples of typical GC–MS key ion plots for sterols (m/z 129), stanols (m/z 215) and triterpenoids (m/z 218) in sediment samples from the Xiaoqing River–Laizhou Bay: (a) X1 spring; (b) L11 summer; (c) L1 spring; (d) L1 summer. Triangles and circles denote n -alkanoic acids and n -alkanols, respectively.

dilution may be an important factor to consider in environments strongly dominated by marine organic carbon inputs.

3.3. Evaluation of sterol ratios and sewage contamination

Diagnostic ratios based on selected sterols were further calculated and applied to identify the presence of fecal contamination and differentiate sources of fecal matter (Table 3, Fig. S2; Writer et al., 1995; Fattore et al., 1996; Patton and Reeves, 1999; Carreira et al., 2004; Furtula et al., 2012 and references therein; Derrien et al., 2017 and references therein). In detail, a set of six ratios used in this study (Table 3) were classified into three groups to indicate sewage vs. non-sewage (R1 and R2), human vs. non-human sewage (R3, R4 and R5) and treatment vs. non-treatment sewage (R6).

The ratio of coprostanol/(coprostanol + cholesterol) (R1) was used to indicate the presence of sewage in aquatic ecosystems (e.g., Carreira et al., 2004; Rushdi et al., 2006; Martins et al., 2007). Based on R1, X2 to X5 and X8 stations appeared to be contaminated by sewage. In contrast, according to the coprostanol threshold value of $0.7 \mu\text{g g}^{-1}$ dw, all X stations (X1–11), L1sp to L4sp, L1su, L2su and L4su appeared to be contaminated by sewage. The coprostanol/(cholesterol + cholesterol) ratio (R2) is also used to elucidate the contribution from sewage vs. non-sewage sources with a criterion of $R2 > 0.06$ (Writer et al., 1995). At stations where these compounds were detected, R2 were higher than 0.06 for all X stations, 6 Lsp stations (L1sp to L6sp), and 4 Lsu stations (L1su, L2su, L4su and L5su), in agreement with high concentrations of coprostanol at these stations (Table 2) and the seawater dilution from the river to the Laizhou Bay spatially. Therefore, it is apparent, based on coprostanol concentrations and diagnostic ratios (R1 and R2), that the Xiaoqing River and the estuary region are severely contaminated by sewage.

The ratio of coprostanol/epicoprostanol (R3) is largely used to differentiate human sources and other mammalian feces (Fattore et al., 1996). R3 ranged from 0.92 to 8.67, with higher values at X stations than Lsp and Lsu stations, pointing to a strong human waste input instead of other mammalian feces (Fattore et al., 1996). R3 can be further

modified into (coprostanol + epicoprostanol) / (coprostanol + epicoprostanol + cholesterol) (R4) to compensate for any potential microbial conversion of coprostanol to epicoprostanol (Grimalt et al., 1990). Similar to R3, R4 has been applied as an indicator of human vs. non-human inputs with a criterion of >0.7 for human inputs (Bull et al., 2002). Based on this criterion, most of the X stations were confirmed to be dominated by human sewage input (see the picture of X8 in Fig. S1 as an example). Similarly, coprostanol/cholestanol (R5) ranged between 0.06 and 10.43 and higher ratios are generally associated with inputs from human sewage other than algae. The high ratios (0.86 to 10.43) of R5 found in most of the X stations and selective spring stations in Laizhou Bay (e.g., L1sp to L4sp, L6sp, and L9su) implied a higher human sewage input than algal input (e.g., Shah et al., 2007), especially in spring. This is consistent with high coprostanol concentrations at most of these stations mentioned earlier. Additionally, the ratio of the 5 β -phytosterols to the sum of the 5 β -phytosterols, coprostanol and epicoprostanol [$5\beta\text{C}_{28+29} / (5\beta\text{C}_{28+29} + 5\beta\text{C}_{27})$] has been applied to assess the influence of livestock operations (Rushdi et al., 2006). This ratio spanned from 0 to 0.55 (averaged as 0.29 ± 0.16), suggesting that the livestock waste was not the most dominant input in this ecosystem, which agreed with the dominance of human feces as suggested by R3, R4 and R5. Even though, the presence of herbivores sourced contaminants in this system is confirmed by the ratio of [(coprostanol) / (coprostanol + 24 ethylcoprostanol)] that ranged between 0.40 and 0.69 (Bull et al., 2002). The overall low average value of $5\beta\text{C}_{28+29} / (5\beta\text{C}_{28+29} + 5\beta\text{C}_{27})$ is in contrast to a study of Mesopotamian marshlands in Iraq, where higher values (0.86 ± 0.12 to 0.91 ± 0.13) were associated with the herbivore waste (Rushdi et al., 2006). Interestingly, the upper Xiaoqing River close to Jinan City and Zibo City (X1 to X8) were affected by abundant manure discharge and consisted $>58\%$ of the total manure load from the entire watershed (Gao, 2011). However, from the diagnostic ratios (R3 to R5), the human fecal related sewage contamination from the cities should be more dominant than that of the animal manure in the Xiaoqing River.

The ratio of epicoprostanol/coprostanol (R6), although reciprocal to R3, is considered to be indicative of the level of treatment or age of the

Table 3
Sterol ratios indicative of sewage contamination.

Station lists	R1 ^a	R2 ^a	R3 ^a	R4 ^a	R5 ^a	R6 ^a
X1	0.62	1.25	3.63	0.68	1.63	0.28
X2	0.76	0.59	4.88	0.80	3.23	0.20
X3	0.71	0.68	4.98	0.75	2.47	0.20
X4	0.70	0.49	6.56	0.73	2.32	0.15
X5	0.72	0.55	5.22	0.75	2.58	0.19
X6	0.65	0.32	3.54	0.70	1.86	0.28
X7	0.61	0.60	2.00	0.70	1.59	0.50
X8	0.91	2.27	8.67	0.92	10.43	0.12
X9	0.46	0.29	6.31	0.50	0.86	0.16
X10	0.51	0.37	8.07	0.54	1.06	0.12
X11	0.61	0.54	1.95	0.71	1.60	0.51
L1sp	0.50	0.28	1.96	0.60	0.99	0.51
L2sp	0.50	0.34	2.66	0.58	1.00	0.38
L3sp	0.54	0.41	6.76	0.57	1.16	0.15
L4sp	0.47	0.29	3.88	0.52	0.87	0.26
L5sp	0.31	0.10	3.83	0.36	0.45	0.26
L6sp	0.41	0.19	3.64	0.47	0.69	0.27
L7sp	0.14	0.05	1.06	0.23	0.16	0.94
L8sp	0.16	0.06	4.18	0.19	0.19	0.24
L9sp	0.17	0.06	1.47	0.26	0.21	0.68
L10sp	0.16	0.05	1.47	0.25	0.20	0.68
L11sp	0.14	0.04	1.76	0.20	0.16	0.57
L1su	0.33	0.23	2.10	0.42	0.49	0.48
L2su	0.27	0.11	1.64	0.37	0.36	0.61
L3su	0.30	0.03	2.16	0.38	0.42	0.46
L4su	0.35	0.29	2.58	0.43	0.55	0.39
L5su	0.27	0.08	3.34	0.33	0.38	0.30
L6su	0.12	0.02	1.37	0.18	0.13	0.73
L7su	0.09	0.03	0.92	0.17	0.10	1.09
L8su	0.07	0.03	1.47	0.11	0.07	0.68
L9su	0.50	0.05	1.30	0.64	1.00	0.77
L10su	0.10	0.04	1.33	0.17	0.11	0.75
L11su	0.05	0.01	1.29	0.09	0.06	0.77
L4-1su	0.11	0.03	1.24	0.18	0.12	0.81
L4-2su	0.18	0.03	1.59	0.26	0.22	0.63
Lsp av. ^b	0.32	0.17	2.97	0.38	0.55	0.45
Lsu av. ^c	0.21	0.08	1.72	0.29	0.31	0.65
P value ^d	0.11	0.07	0.04	0.16	0.10	0.04

Bold fonts indicate the value exceed the traditional criteria.

^a R1 Coprostanol / (coprostanol + cholestanol), indicator for sewage vs. biogenic source, >0.7 is the criteria (Grimalt et al., 1990); R2 Coprostanol / (cholesterol + cholestanol), indicator sewage vs. non-sewage, >0.06 is the criteria (Writer et al., 1995); R3 Coprostanol/Epicoprostanol, indicator for human vs. non-human, >1.5 is the criteria (Venkatesan and Santiago, 1989); R4 (Coprostanol + epicoprostanol) / (coprostanol + epicoprostanol + cholestanol), indicator of human vs. non-human, >0.7 is the criteria (Bull et al., 2002); R5 Coprostanol/cholestanol human vs. algae sterols, >0.5 is the criteria (Leeming et al., 1996); R6 Epicoprostanol/coprostanol, indicator for untreated vs. treated sewage, <0.2 is the criteria (Mudge and Seguel, 1999).

^b Lsp av. denotes the average values of L1sp to L11sp.

^c Lsu av. denotes the average value of L1su to L11su.

^d P value denotes significant Student's *t*-test comparing the average values of Lsp stations and Lsu stations.

fecal material (Mccalley et al., 1981; Mudge and Seguel, 1999; Venkatesan and Santiago, 1989). R6 ranged between 0.12 and 1.09, implying contributions from both untreated and treated sewage. Many X stations (X4, X5, X8 to X10) had R6 ratios lower than 0.2, suggestive of a substantial portion of the untreated sewage at these stations (Mudge and Seguel, 1999; Furtula et al., 2012). Overall, our results suggested that the sewage input in this region, although partially untreated, is dominated by human feces and is seconded by herbivores feces.

3.4. Allocation of sewage contamination by principal component analysis

The principal component analysis (PCA) has been shown to be a powerful tool assessing OM source, including the sewage contamination assessment (e.g., Derrien et al., 2012). Similar to other studies (Adnan et al., 2012; Frena et al., 2016; Speranza et al., 2018), PCA was performed using major sterols that were consistently detected (sterols that were <0.5% abundance or not detected in partial samples were excluded), including coprostanol, epicoprostanol, cholestanol, cholesterol,

brassicasterol, stigmasterol, stigmasterol, campesterol, and β -sitosterol. The relative abundance (%) of each individual sterol was used as variables for PCA (Derrien et al., 2015). The first two components explained 67.6 (PC1) and 18.8% (PC2) of the total variance, respectively (Fig. 4). Most of the compounds showed positive loadings along PC1 axis, with the exception of brassicasterol that had a negative loading. Along PC2, all the sewage indicators such as coprostanol and epicoprostanol had positive loadings, whereas the dominant biogenic sterols such as cholesterol, β -sitosterol, stigmasterol, campesterol and brassicasterol all displayed negative loadings. Therefore, PC1 seems to differentiate algal sources from terrestrial and anthropogenic sources, whereas PC2 seems to differentiate sterols (mainly precursors) from stanols (mainly by-products). Based on scores on PC1 and PC2, the X stations were generally separated from all other stations, whereas the Lsp stations were not separated from the Lsu stations, even though the Lsp stations had higher average PC1 and PC2 scores (−0.34 and −0.01, respectively) than those of the Lsu stations (−0.45 and −0.06; Fig. 4). With higher average PC1 and PC2 scores, most of the X stations showed contamination from sewage, which is consistent with fecal sterol concentrations and calculated diagnostic ratios (Tables 2, 3, Fig. S2). For instance, X1 and X8 had the highest PC1 and PC2 scores, consistent with positive loadings of fecal sterols (coprostanol and epicoprostanol) on PC1 and PC2. The stations located in the Xiaoqing River mouth (X9 to X11) or Laizhou Bay (L5 to L11) had significantly lower PC1 scores than the upstream X stations (X1 to X3), probably due to the dilution of sewage by seawater and/or the effects of marine currents at these stations. In addition, the Lsp and Lsu stations had lower PC1 scores than the X

Fig. 4. Principal component analysis showing loadings and scores for two main components of sediment samples collected from the Xiaoqing River–Laizhou Bay. Copr. stands for coprostanol, Epi-copr. epicoprostanol, Cholesta. Cholestanol, Choleste. Cholesterol, Stigma. Stigmasterol, Stigmasta. Stigmastanol, Camp. Campesterol, Sito. β Sitosterol.

stations, suggesting relatively stronger algal inputs in the estuarine stations than the river stations.

3.5. Sewage input dynamics and their controlling factors

On a broad scale, a strong spatial variation of sewage contamination was revealed by significantly higher coprostanol concentrations and distinctly different sterol-based ratios between the Xiaoqing River (X stations) and the Laizhou Bay (Lsu and Lsp; Fig. S2). Furthermore, a clear spatial variation was observed along the Xiaoqing River, as supported by our PCA results and biomarker data. As mentioned earlier, the highest coprostanol concentrations and TOC contents were found at X8 and X1 stations. These two stations were highly associated with sewage discharge from large cities in Shandong province (Fig. S1), suggesting point source inputs may explain extraordinary patterns commonly observed along rivers.

The Xiaoqing River showed generally higher biomarker concentrations (e.g., coprostanol) in the upstream (X1–X8) than the downstream (X9–11). Although fluctuation has been observed among stations in each segment of the river, as displayed in biomarker data, the much higher sewage contamination seen in the upstream can largely be explained by human related fecal inputs due to the presence of many cities that host >23 million people, overall high people density (>699 people per km²), and to a less extend by animal manure irrigation during agricultural planting (Zhang et al., 2008) in the upstream.

In addition to the spatial variation, a seasonal variation has been detected in the Laizhou Bay (L1 to L11) based on selected sterol-derived ratios (Fig. S2). For example, the average ratio of coprostanol/(cholesterol + cholestanol) in spring samples was more than twice of Lsu samples (avg. 0.17 vs 0.08; $P = 0.07$), indicative of relatively higher sewage input in spring than in summer. In addition, the mean ratio of R1 and R2 were significantly higher and lower in spring than in summer, respectively, suggesting relatively higher human derived and less treated sewage input in spring than in summer (Table 3, Fig. S2). Similarly, temporal variation of sewerage contamination was found in an aquifer under the dry and rainy events, which is likely associated with sewage transported by surface runoff (Arcega-Cabrera et al., 2014). However, the seasonal variation is not observed in the PCA when considering the major sterols, which may be caused by the complex input of plant sterols (e.g., stigmasterol, campesterol), algal sterol (brassicasterol) and non-source specific sterols (e.g., cholesterol). Furthermore, it should be careful that the seasonal variation observed based on diagnostic ratios could be caused by many other factors, such as river discharge and the consequent hydrodynamic sorting (Wang et al., 2014), temperature difference (about 20 °C difference in average between spring and summer) and related microbial community structure and activities (Yla et al., 2012). The latter one may likely cause preferential degradation of sterols or conversion reactions. Nevertheless, further research is required to explore the seasonal differences in this system.

3.6. Further considerations

There are some inconsistencies in specific sterol ratios in the Xiaoqing River–Laizhou Bay system (Table 3). For example, although epicoprostanol/coprostanol ratio and coprostanol/epicoprostanol ratio were significantly different between spring and summer, no significant difference was observed between these two seasons for the coprostanol/cholestanol ratio, the coprostanol/cholesterol ratio, the coprostanol/(cholestanol + cholesterol) ratio, and coprostanol concentrations. These inconsistencies could be caused by limited empirical observations and high variation in the estuarine hydrodynamics (e.g., Han et al., 2012; Canuel and Hardison, 2016), which requires further investigation. Therefore, the coupling of geochemical analysis with hydrogeological studies should be performed in the future for a better and comprehensive understanding of OM transport (Canuel et al., 2012). For instance, Wang et al. (2015) observed preferential

absorption of biomarkers onto specific particles based on density fractionation in the Yangtze River Estuary and the adjacent shelf. In addition, when considering tremendous amount of sewage inputs as implied by fecal sterol concentrations, their diagnostic ratios and high deposition rates (Li et al., 2012), seasonal monitoring is recommended for evaluating environmental qualities and waste control strategy in this estuarine ecosystem. Especially, as stated in this study, point sources (X1 and X8) could be an important sewage contributor to the Xiaoqing River and require further regulation based on environmental policies and economic necessity.

With growing evidence of the adverse effects of emerging anthropogenic influences on estuarine ecosystems (Kennish, 1991; Paerl et al., 2006), it is necessary to combine multiple techniques and develop new approaches to accurately assess sewage contamination and sources (Canuel and Hardison, 2016). In addition, the usefulness of sterol ratios for sewage contamination evaluation and source tracking may also depend on factors other than seasonality and may even depend on estuary-specific processes. This highlights the necessity to deepen our understanding of the multiple processes influencing sterol ratios across a wide range of systems, which will strengthen the use of sterols as reliable, stable and meaningful indicators of fecal input on a broader scale.

4. Conclusions

The sewage input was determined for the first time in sediments collected from the Xiaoqing River–Laizhou Bay using sterol biomarkers. Source tracing of the sediments has revealed a widespread occurrence of human/animal and plant sterols. In general, the significant correlation observed between the coprostanol concentrations and TOC suggested that this biomarker reflects sewage inputs in this area. Sterol analysis is a reliable tool for sewage contamination assessment, but multiple sterol-derived ratios should be considered. Based on coprostanol concentrations, the diagnostic ratios between selected sterols and PCA analysis, most of the riverine stations and a few of the estuarine stations showed signatures of sewage contamination. With the high variability of environmental conditions in this estuary, a precise evaluation of seasonal variation is hard to assess at this point; however, the distinction between the spatial distributions of sterol biomarkers and different patterns of sterol ratios between seasons were observed. The spring season was characterized by relatively higher sewage input and less biogenic input.

This present study demonstrated the importance of organic geochemical studies in spatially different environments and different seasons; these studies are necessary for an integrated view of OM sources and transportation mechanisms. Additionally, environmental policies are required to reduce anthropogenic impacts and promote the health of this estuarine ecosystem. Furthermore, the results obtained in this study provided a reference example for future monitoring of other typical river–estuary systems, especially in coastal areas of eastern China.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.04.314>.

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