

## Article

## Dynamics of Humic and Non-Humic Substances in Estuaries of Coastal Wetlands

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### Abstract

Dissolved organic matter (DOM) that is transported from watershed soils to fluvial systems and then exported to coastal estuaries, supports the diversity and productivity of aquatic ecosystems. Wetlands are an important source of riverine DOM. Among DOM, humic substances (HS) are expected to carry trace elements, while non-humic substances (NHS) serve as a major source of nutrients, among their various functions. To understand the biogeochemical processes associated with DOM in estuaries, the dynamics of riverine HS and NHS as a function of salinity gradient were investigated for 3 rivers from coastal wetland watersheds, namely the Harney and Shark Rivers (Florida, USA) and the Judan River (Sarawak, Malaysia). The chemical characteristics of Judan River DOM in the upper estuary and at the mouth of the river were also compared. Both the HS and NHS concentration profiles in the Harney and Shark Rivers showed typical non-conservative patterns, and slowly decreased with increasing salinity. The rates of decreases were smaller in low salinity areas compared to those in high salinity areas, which suggested the supply of DOM, consisting of HS/NHS at a similar ratio to those in the river's watershed, likely derived from coastal vegetation (Mangroves). In contrast, the HS concentration profile for the Judan River was indicative of conservative mixing, and decreased with increasing salinity in a linear manner. However, the NHS concentration profile correlated positively with the degree of salinity, indicating that it originated from coastal sea water. In the Judan River, the degradation index of amino acids,  $\delta^{15}\text{N}$ , and the ratio of peptide/amide N to primary amine N were higher at the river mouth than at the upper estuary, suggesting that proteinaceous materials with smaller molecular weights are decomposed preferentially and/or that larger proteinaceous materials are supplied from the seawater.

**Keywords:** Dissolved organic matter, Estuaries, Humic substances, Non-humic substances.

### Introduction

Dissolved organic matter (DOM) transported from watershed soils into rivers, is estimated to be 0.25 Pg C y<sup>-1</sup> globally (Hedges et al., 1997). It is an important biogeochemical component for aquatic biota in estuarine and coastal regions as an energy and nutrient source (Hood et al., 2009; Laglera and van den Berg, 2009; Baken et al., 2011). These ecological functions are thought to depend largely on the composition of the DOM (Mopper and Kieber, 2002). Humic substances (HS) and non-humic substances (NHS) make up the bulk of DOM (Watanabe et al.,

2012). HS, a complex mixture of yellowish-brown to black colored amorphous organic materials, are compositionally and structurally different from NHS, compounds with known molecular structures of biochemical importance (Maie et al., 2006). HS are generally considered to be major carriers of trace metallic elements and account for 15–90% of the total DOC in streams (Day et al., 1991; Watanabe et al., 2012), with a mean of 40–60% for U.S. rivers from diverse biomes (Spencer et al., 2012). The proportion of DOC present as HS is smaller in estuarine and sea water, 2–20% (Druffel et al., 1992; Otero et al., 2003; Maie et al., 2006), suggesting that

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HS are produced in lower amounts and/or higher losses of HS occur in marine systems, compared to a larger source of NHS.

In estuarine environments, mixing with seawater increases ionic strength and occasionally pH, affecting the dynamics of riverine DOM (Moroi et al., 2012; Guéguen et al., 2016). In a classical study on the Amazon River, a decrease in humic acids, the acid insoluble fraction of HS, with increasing salinity was found (Sholkovitz et al., 1978), and the effect of polyvalent cations on this phenomenon was suggested (Sholkovitz and Copland, 1981). A greater extent of flocculation for allochthonous than for autochthonous DOM by amorphous iron hydroxide (Luider et al., 2003) and an increase in DOM precipitation due to interactions with Al with increasing pH (Nierop et al., 2002), were also suggested, based on model experiments. Uher et al. (2001) reported that sorption to suspended sediment was more important than flocculation as the mechanism responsible for the removal of colored DOM in areas of lower salinity in an estuary, based on both field and laboratory studies. No additional decreases in visible absorption with increasing salinity after the removal of particles were detected for Chinese river waters (Guo et al., 2007). On the contrary, the re-suspension of sediments and precipitated particulate matter can also be a source of colored DOM in estuaries (Yamashita et al., 2008; Shank et al., 2011) as well as drainage from adjacent terrestrial ecosystems (Tzortziou et al., 2008).

Biodegradation and photodegradation are also potential mechanisms that can lead to quantitative and qualitative changes in DOM in estuaries. These effects on the DOM concentration require a longer distance and a lower flow rate in the mixing zone, i.e., a longer residence time (Cifuentes and Eldridge, 1998). Photochemical processes have been reported to alter the molecular structure of estuarine DOM, including reducing the average molecular weight, aromaticity, and carboxyl group concentration (Dittmar et al., 2006; Dalzell et al., 2009; Helms et al., 2013).

The DOC concentration in river water is generally  $<10 \text{ mg L}^{-1}$  (Hope et al., 1994; Hood et al., 2005), but can be as high as  $40\text{--}60 \text{ mg L}^{-1}$  if wetlands are present in the watershed areas (Laudon et al., 2004; Billett et al., 2006; Moore et al., 2011). In wetland associated waters, it has been reported that  $40\text{--}90\%$  of the DOC is present in the form of HS (Watanabe

et al., 2012). The NHS concentration can also be significant in river waters associated with tropical and subtropical wetlands,  $2\text{--}10 \text{ mg L}^{-1}$  (Watanabe et al., 2012). Differences in the behavior of humic-like fluorescence and protein-like fluorescence in estuaries have been shown using excitation-emission matrix (EEM)-parallel factor analysis of fluorescence (PARACFAC) (Cawley et al., 2014; Guéguen et al., 2016). However, differences in the estuarine dynamics between dissolved HS and NHS have not been well documented. Direct quantification of HS and NHS as proxies for different DOM reactivity pools may be quantitatively more representative of the bulk DOC pool compared to fluorescence measurements.

The objective of this study was to evaluate the estuarine dynamics of HS and NHS in coastal wetland streams. For this purpose, water samples were collected from two rivers fringed with tidally submerged mangrove marshes (Florida, USA) and a river that flows through a peatland and then empties into the ocean (Sarawak, Malaysia) were analyzed. The two Florida rivers have a long flow channel before reaching the sea while the mixing zone of the Sarawak river is narrow. As such, it was expected that the type and intensity of factors that influence the dynamics of DOM in an estuary would be varied between those rivers. River water samples that were collected at different salinities were fractionated using DAX-8, a hydrophobic resin, which can estimate the changes in HS and NHS concentrations separately (Watanabe et al., 2012). The chemical characteristics of DOM in the Malaysian river were also compared between the upper estuary without being affected by seawater and at the river mouth.

## Materials and methods

### *Location of Everglades sampling sites*

The Everglades, located in the southern tip of the Florida Peninsula, in the USA, is among the largest subtropical wetland (Fig. 1a). The average annual temperature is  $25^\circ\text{C}$  and the average precipitation is 1521 mm. The Shark River begins in the northern boundary of the Everglades National Park and flows south through an inland freshwater marsh dominated by emergent wetland plants such as *Cladium* and *Eleocharis* and a high abundance of calcareous periphyton mats. The vegetation of the watershed

shifts to a tidally submerged mangrove marsh at the lower reach, and the river empties into the Gulf of Mexico. The Harney River diverges from the Shark River at ca. 16 km upstream from the estuary that is dominated by mangrove trees.

#### *Locations of Mukah sampling sites*

The Mukah area is located in the middle part of coastal area in Sumatra, Malaysia (Fig. 1b). The average annual precipitation, mean temperature, mean maximum temperature, and mean minimum temperature between 2007 and 2010 were 3854 mm, 26.4, 34.5, and 22.0°C, respectively. It has sporadic dry and wet seasons accompanied by monsoons. The Judan River flows through a mixed peat swamp forest ca. 13 km apart from the town of Mukah and empties into the South China Sea. The total length of the river is 23 km, and villagers living along the river use this river for transportation.

#### *Collection of water samples and preparation of DOM samples*

River water samples were collected at points with different salinities using a boat from the Judan River (15 samples) in August 2009 and from the Harney and Shark Rivers (19 samples including a sample from the Gulf of Mexico) in March 2010. Water samples were collected in 100-mL polypropylene bottles that had been prewashed with 0.5–1.0 M HCl and were transported to the laboratory in a cooler. Samples were filtered through combusted glass fiber

filters with a nominal pore size of 0.40  $\mu\text{m}$  (GB-140, ADVANTEC, Tokyo) before analysis.

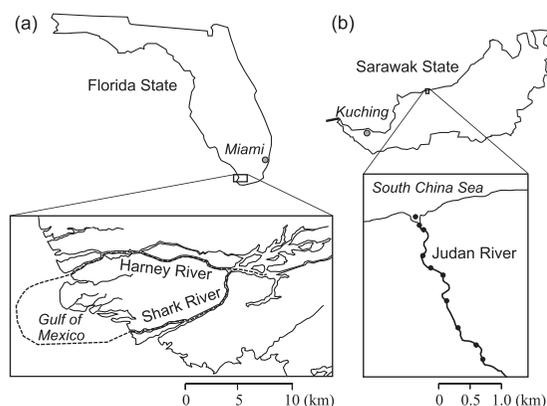
Two 40-L Judan River water samples were also collected at the river mouth and at 2.5-km upstream from the river mouth on the same day. These samples were filtered through a combusted glass fiber filter (15 cm $\phi$ ; GB-140) and then through hydrophilic polyvinylidene difluoride membranes (nominal pore size, 0.22  $\mu\text{m}$ ) that were mounted on a Pellicon-2 Mini tangential flow ultrafiltration system (Millipore, Billerica, MA, USA). The filtrate was desalted by removing molecules with <1,000 Da using the Pellicon-2 Mini equipped with regenerated cellulose membranes. Both types of membranes were washed with 0.5 M HCl and 0.1 M NaOH solutions beforehand, and the water samples were cooled on ice water during the entire filtration procedures. The concentrated DOM sample was filtered through a PTFE membrane (nominal pore size, 0.45  $\mu\text{m}$ ; ADVANTEC) and then freeze-dried.

#### *DOC, HS, and NHS concentrations in river water samples*

The DOC concentration in the water samples was measured using a dissolved carbon analyzer (TOC-V<sub>CPH</sub>, Shimadzu, Kyoto, Japan) after adjusting the pH to around 3 with 6 M H<sub>2</sub>SO<sub>4</sub> and CO<sub>2</sub> was excluded by sparging with N<sub>2</sub>.

For the determination of HS and NHS, an aliquot (40 mL) of a water sample was adjusted to pH 1.5 with 6 M H<sub>2</sub>SO<sub>4</sub> and passed through a column ( $\phi$ 10 mm) packed with 3 mL of prewashed Supelite DAX-8 (Supelco, Bellefonte, PA, USA). The resin was then washed with 3 column volumes of water (pH 1.5). The eluate and washings were combined to make up the NHS fraction. The DOC concentration in the NHS fraction was measured using TOC-V<sub>CPH</sub> after sparging with N<sub>2</sub>. The same procedures were also conducted using ultra pure water (DOC < 0.05 mg L<sup>-1</sup>) to obtain a blank value. The HS concentration was estimated from the difference between the total DOC and the NHS C concentration, taking the rate of dilution into consideration.

The relationship between DOC, HS, or NHS concentration and salinity was investigated using regressing analysis. The significance levels at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.005$  were expressed using \*, \*\*, and \*\*\*, respectively.



**Fig. 1.** Site maps of (a) Harney and Shark Rivers and (b) Judan River. Closed circles in (b) indicate points where water samples were collected. Dotted line in (a) indicates the path for collecting water samples. Smaller islands in the Gulf of Mexico are omitted.

### Chemical characteristics of Judan River DOM samples from the upper estuary and river mouth

Hydrolyzable amino acid composition was measured as follows: a 1 mg sample was reacted with 1 mL of 6 M HCl containing 10 mg mL<sup>-1</sup> of phenol in a sealed, evacuated ampoule by heating at 110°C for 24 h. The soluble fraction was collected by filtration, dried, and dissolved in buffer solution consisting of 0.0167 M sodium citrate and 0.167 M citric acids with 0.15 M sodium chloride, 5 mL L<sup>-1</sup> ethanol, and 100 μL L<sup>-1</sup> caprylic acid. Amino acids in the sample solution were quantified using an amino acid analyzer (JLC/500V, JEOL, Tokyo) with the ninhydrin color reaction and the external standard method. The amino acid-based degradation index (DI; Dauwe et al., 1999) was calculated using the following equation:

$$DI = \sum_i \left[ \frac{\text{var}_i - AVE \text{var}_i}{SD \text{var}_i} \right] \times \text{fac.coef}_i \quad (1).$$

where var<sub>*i*</sub> is the mol% of amino acid *i* in the sample, and AVEvar<sub>*i*</sub>, SDvar<sub>*i*</sub>, and fac.coef<sub>*i*</sub> are the average, standard deviation, and factor coefficient of amino acid *i* of the dataset obtained from particulate organic matter in various diagenetic states, respectively. Higher and lower DI values suggest the lower and higher degrees of diagenetic reworking of the proteinaceous components, respectively.

δ<sup>13</sup>C and δ<sup>15</sup>N were measured using an isotope mass spectrometer connected to an elemental analyzer (DELTA Plus-NC2500, Thermo Finnigan, San Jose, CA, USA).

N1s spectra of DOM samples were recorded on an X-ray photoelectron spectrometer (AXIS 165, Shimadzu, Japan) using a monochromatic Al Kα X-ray source (1486.6 eV) at an analyzer pass energy of 40 eV, an electric current of 30 mA, and a voltage of 10 kV. A finely powdered sample (ca. 1 mg) was fixed on the surface of a metallic sample block by means of Scotch double-sided non-conducting tape. Spectra were recorded for each visible line at 0.05 eV per step. The time for one scan was 298 ms, and 64 scanned data sets were accumulated. Correction of binding energy was made relative to the C-C/C-H signal at 285.0 eV in C1s spectra that were obtained simultaneously. The spectra were deconvoluted into three Gaussian curves with peak centers at 399.0±0.1 eV (aromatic N including imine, heterocyclic C=N, and aromatic amine), 400.4±0.1 eV (amide/peptide N including pyrrole, secondary and tertiary amines, and imide), and 402.3±0.1 eV (primary amine N

including other protonated N), and the proportions of the three N groups in the total N were estimated from the relative areas surrounded by Gaussian curves and base line with respect to the spectral area (VISION-2, Shimadzu; Abe and Watanabe, 2004).

## Results

### Everglades site

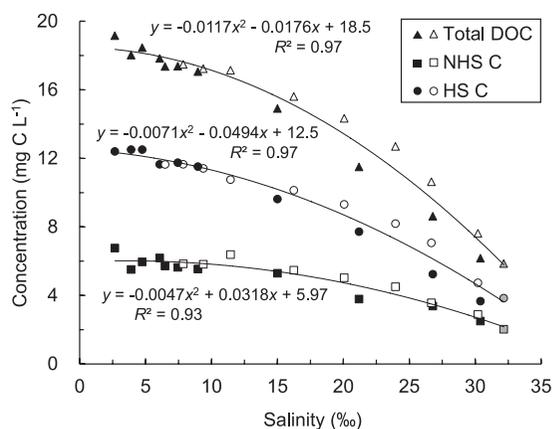
The DOC concentration in the Florida samples decreased from 19.2 to 5.9 mg C L<sup>-1</sup> with increasing salinity from 2.7 to 30 (Fig. 2). There was a negative correlation ( $r = 0.97^{***}$ ) between the DOC concentration and salinity and a higher determination constant, 0.97, was observed in regression to the quadratic function:

$$y = -0.0117x^2 - 0.0176x + 18.5 \quad (2).$$

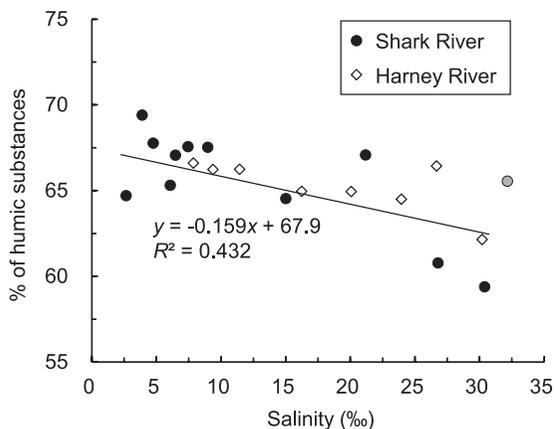
Negative correlations ( $r = 0.93^{***}$ – $0.97^{***}$ ) with higher determination constants of 0.94 and 0.97 in the regression to the quadratic function were also observed for the relationship between the HS or NHS concentration and salinity (Fig. 2). During the mixing with seawater, the proportion of HS in the DOM decreased as evidenced by the following equation (Fig. 3):

$$y = -0.159x + 67.9 \quad R^2 = 0.43^{***} \quad (3).$$

However, HS still comprised 59% of the total DOC in the sample from the Gulf of Mexico (salinity, 32.2). As such, the coastal sea in the Florida bay was considered to have a much higher %HS compared to the Open Ocean.



**Fig. 2.** Changes in the concentrations of total dissolved organic carbon, humic and non-humic substance in the Harney and Shark Rivers along the salinity gradient. Closed and open symbols indicate samples from the Harney and Shark Rivers, respectively. Gray symbols are data for the Gulf of Mexico sample.



**Fig. 3.** Relationship between the proportion of humic substances in total dissolved organic matter and salinity for the Harney and Shark Rivers. Gray symbol indicates the sample from the Gulf of Mexico.

#### *Mukah site*

At the sampling sites in the Judan River, the DOC concentration decreased from 45–46 mg C L<sup>-1</sup> at sites with salinity of 0.03–0.11 to 33 mg C L<sup>-1</sup> at the river mouth with salinity of 5.1, showing the following negative correlation with salinity (Fig. 4):

$$y = -2.14x + 45.3 \quad R^2 = 0.94^{***} \quad (4)$$

This relationship indicates that conservative mixing was the dominant process for the change in DOC concentration. The HS C concentration also showed a linear decrease with the salinity (Fig. 4).

The NHS concentration (Fig. 4) increased slightly with increasing salinity ( $P < 0.05$ ), and the decrease in DOC concentration can be attributed only to that in HS concentration as follows:

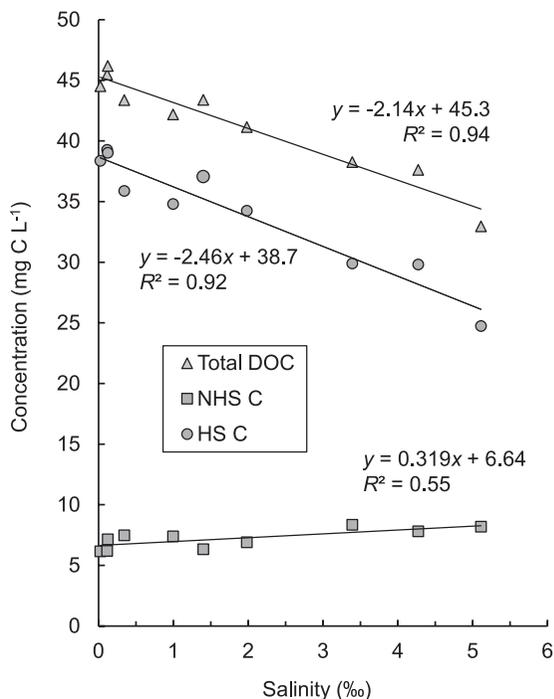
$$y = -2.46x + 38.7 \quad R^2 = 0.93^{***} \quad (5)$$

where  $x$  and  $y$  indicate the salinity and HS concentration (mg L<sup>-1</sup>), respectively. This result indicates a larger difference in the DOM composition between river water and coastal seawater.

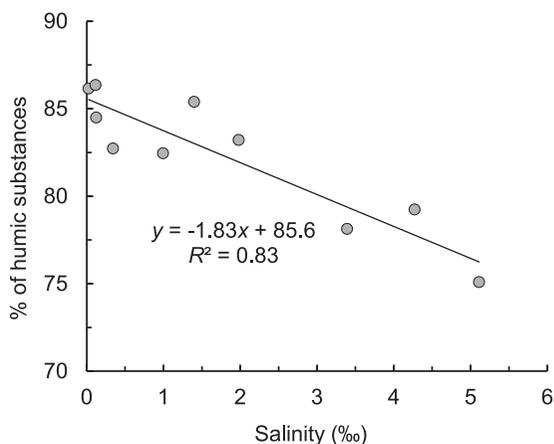
The change in the proportion of HS in the DOM with changing salinity (Fig. 5) could be expressed as:

$$y = -1.83x + 85.6 \quad R^2 = 0.83^{***} \quad (6)$$

Assuming that the salinity of the coastal water is distributed within the range of 32–34, the proportion of DOC present in the form of HS is estimated 23.4–27.0% using equation 6. This value tends to be higher than previously reported values for the proportion of HS C in total DOC for seawater (Druffel et al., 1992; Otero et al., 2003).



**Fig. 4.** Changes in the concentrations of total dissolved organic carbon, humic and non-humic substance in the Judan River along the salinity gradient.



**Fig. 5.** Relationship between the proportion of humic substances in total dissolved organic matter and salinity for the Judan River.

#### *Comparison of DOM between an upper estuary and the river mouth (Mukah site)*

Table 1 indicates the yield and composition of acid-hydrolysable amino acids in the Judan DOM samples prepared from river water collected in the upper estuary and at the river mouth. Sum of the

yield of amino acids was 1.2 and 1.0 mg N g<sup>-1</sup> in the upper estuary and river mouth samples, respectively. The dominant amino acids included glycine, aspartic acid, and alanine, followed by threonine, glutamic acid, and lysine. These amino acids are typically the dominant ones that are found in DOM samples when analyzed using a similar methodology (Duan and Bianchi, 2007; Ylla et al., 2011; Watanabe et al., 2014). Although there were no remarkable differences in the yield or composition of amino acids between the two samples, the DI was higher in the upper estuary sample (-0.58) than in the sample from the mouth of the river (-0.70).

The  $\delta^{13}\text{C}$  values (Table 2) for the two Judan DOM samples were similar to each other, -29.3 and -29.0‰, which can be attributed to C<sub>3</sub> plants in the mixed peat swamp forest and palms along with the

**Table 1** Amino acid contents ( $\mu\text{g N g}^{-1}$ ) and degradation index (DI) in Judan River DOM at the upper estuary and river mouth

Amino acid	Upper estuary	River mouth
Aspartic acid	125	116
Threonine	89	76
Serine	84	71
Glutamic acid	87	76
Glycine	219	208
Alanine	101	91
Cysteine	7	6
Valine	63	54
Methionine	5	4
Isoleucine	32	27
Leucine	41	35
Tyrosine	16	14
Phenylalanine	19	17
Lysine	90	70
Histidine	65	50
Arginine	54	52
Proline	76	63
Total	1171	1033
DI	-0.58	-0.70

**Table 2** Composition of N functional groups in Judan River DOM, as estimated from XPS N1s spectra.

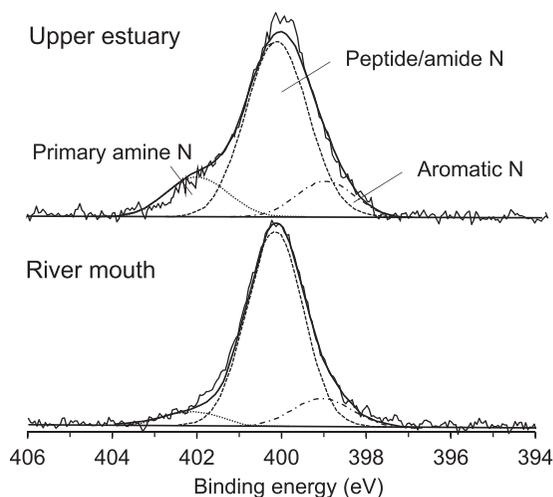
Site	Primary amine N (%)	Peptide/amide N (%)	Aromatic N (%)
Upper estuary	15.7	71.4	12.9
River mouth	6.4	82.4	11.2

river as major DOM sources. The  $\delta^{15}\text{N}$  value for the DOM (Table 2) at the river mouth (0.36‰) was higher than that in the upper estuary (-0.55‰).

The XPS N1s spectrum (Fig. 6) of the upper estuary DOM sample was characterized by a prominent shoulder at around 402 eV compared to that of the river mouth DOM sample. The proportion of amide/peptide N in the total N, as estimated from the XPS N1s spectra, was larger in the river mouth sample than in the upper estuary sample, 82% vs 71%, while the proportion of primary amine N in the total N was in reverse order, 6% vs 16%, with a similar proportion of aromatic N in the total N, 11% and 13% (Table 2).

## Discussion

Previous studies (Jaffé et al., 2004, Cawley et al., 2014; Maie et al., 2014) suggested that the coastal mangrove marsh along the Harney Rivers serves as an important source of DOM based on the non-conservative behavior of HS-like and protein-like fluorescence intensities. The findings reported here



**Fig. 6.** XPS N1s spectra of dissolved organic matter in the upper estuary and at the mouth of the Judan River.

indicate that a significant amount of HS and NHS is supplied from mangrove fringe and that the DOC concentration profiles in the Harney and Shark Rivers are controlled at least by two factors, i.e., mixing with sea water and contribution from the mangrove fringe. Equation 3 indicates that HS supplied from watershed areas accounted for 68% of the DOM in the upper stream than the mixing zone with sea water. The small decrease in the proportion of HS suggests that the DOM contributed by mangroves was also enriched in HS.

Relationships between the variables in this study (concentrations of DOC, HS C, and NHS C and %HS) and optical properties that were investigated by Cawley et al. (2014) and Maie et al. (2014) (ultraviolet (UV) and fluorescence spectrometric variables) were analyzed for the Harney and Shark Rivers (Table 3). The  $A_{254}$  and fluorescence intensities showed a strong positive correlation, not only with DOC and HS C concentrations, but also with the NHS C concentration, probably because the composition of the DOM supplied from the mangrove fringe was similar to that in the Harney and Shark River waters, in terms of HS/NHS. A relatively low correlation coefficient between optical properties and %HS was likely due to a small variation in %HS throughout the entire DOC range, while the lack of a significant correlation between specific UV absorbance (SUVA) and %HS suggests that the ratio of chromophoric structures to non-colored moieties in a HS molecule were varied.

Maie et al. (2014) reported that DOM in the Judan

River mixed conservatively with those in estuarine water based on DOC,  $A_{254}$ , and fluorescence data. A similar pattern was also observed in the case of a river in Sumatra that was affected by a coastal peatland, in which the DOC concentration decreased from 61 to 7.2 mg C L<sup>-1</sup> (maximum salinity, 26.7; Alkhatib et al., 2007). The findings of the present study indicate that the decrease in DOC concentration with increasing salinity in the Judan River is dependent on the HS concentration (Fig. 4). When the present results were compared with optical properties of DOM reported by Maie et al. (2014), the HS C concentration and %HS correlated positively with the  $A_{254}$ , SUVA, and terrestrial fulvic acid-like (F3) and humic acid-like (F4) fluorescence intensities and negatively with the protein-like fluorescence intensity (F6). However, estimation of the HS C or DOC concentration in the Judan River based on fluorescence intensity does not appear to be reliable, because the fluorescence intensity could vary with pH (Maie et al., 2014).

The increase in NHS C concentration with increasing salinity in the Judan River suggests that NHS is produced in the estuary. There was a negative correlation between  $A_{254}$  and the NHS C concentration (Table 4), which can be explained by a two-endmember mixing model; a high HS concentration for the freshwater endmember and a high NHS concentration for the marine endmember. Possible sources of NHS in estuaries include (photo) degradation products derived from estuarine sediment organic matter (Pisani et al., 2011) and the

**Table 3** Correlation coefficients between DOC, HS C, and NHS C concentration or %HS and  $A_{254}$ , SUVA, or some of the fluorescence peak intensities (F1 to F5) in the Harney and Shark Rivers

Variable <sup>†</sup>	DOC (mg L <sup>-1</sup> )	HS C (mg L <sup>-1</sup> )	NHS C (mg L <sup>-1</sup> )	%HS
$A_{254}$	0.933***	0.921***	0.933***	0.578**
SUVA	-0.372	-0.395	-0.312	-0.314
F1 (Ex <260 nm / Em 376 nm)	0.973***	0.970***	0.955***	0.622***
F2 (Ex <260 nm / Em 454 nm)	0.959***	0.956***	0.938***	0.611***
F3 (Ex <260 nm / Em 416 nm)	0.968***	0.963***	0.953***	0.604***
F4 (Ex 275 nm / Em >500 nm)	0.936***	0.923***	0.940***	0.559*
F5 (Ex 300 nm / Em 342 nm)	0.968***	0.967***	0.943***	0.641***

$A_{254}$ , Absorbance at 254 nm; SUVA,  $A_{254}/\text{mg C}$ ; F1, microbial synthesized HS-like peak; F2, terrestrial HS-like peak (photo-refractory or photodegradation product); F3, terrestrial fulvic acid-like peak; F4, terrestrial humic acid-like peak; F5, protein-like materials peak.

<sup>†</sup>Cited from Cawley et al. (2014) and Maie et al. (2014).

\*, \*\*, and \*\*\* indicate significance at  $P < 0.05$ , 0.01, and 0.005, respectively.

**Table 4** Correlation coefficients between DOC, HS C, and NHS C concentration or %HS and  $A_{254}$ , SUVA, or some of the fluorescence peak intensities (F2 to F6)<sup>‡</sup> in the Judan River

Variable <sup>†</sup>	DOC (mg L <sup>-1</sup> )	HS C (mg L <sup>-1</sup> )	NHS C (mg L <sup>-1</sup> )	%HS
$A_{254}$	0.959***	0.950***	-0.721*	0.894***
SUVA	0.814***	0.806***	-0.603	0.794**
F2 (Ex <260 nm / Em 466 nm)	0.413	0.353	0.029	0.260
F3 (Ex 295 nm / Em 406 nm)	0.858***	0.844***	-0.606*	0.814***
F4 (Ex <260 nm / Em >530 nm)	0.953***	0.953***	-0.765**	0.924***
F6 (Ex 305 nm / Em 448 nm)	-0.883***	-0.862***	0.587	-0.790**

$A_{254}$ , Absorbance at 254 nm; SUVA,  $A_{254}/\text{mg C}$ ; F2, terrestrial HS-like peak (photo-refractory or photodegradation product); F3, terrestrial fulvic acid-like peak; F4, terrestrial humic acid-like peak; F6, ubiquitous HS-like peak.

<sup>†</sup>Cited from Maie et al. (2014). <sup>‡</sup>Peaks corresponding to F1 and F5 in Table 3 were lacking.

\*, \*\*, and \*\*\* indicate significance at  $P < 0.05$ , 0.01, and 0.005, respectively.

incorporation of microbially-derived DOM (Abdulla et al., 2010). It has been reported that DOM becomes smaller in molecular size (Brinkmann et al., 2003), has less color (Cao et al., 2016), and is more bioavailable (Kieber et al., 1989) after undergoing photodegradation. The decrease in the UV absorption of the estuarine DOM brought by photochemical reactions was larger than the decrease in DOC concentration (Cao et al., 2016), possibly due to the destruction of C=C bonds in conjugated systems. Such structural alterations might weaken the adsorption of DOM, which is carried backward from the coastal area, to DAX-8.

The Judan DOM samples from the upper estuary and river mouth had different chemical characteristics. The amino acid-based DI of the river mouth sample was also smaller compared to the values reported for 4 DOM samples from the two rivers in Mukah, -0.04 to -0.53 (Watanabe et al., 2014). An increase in  $\delta^{15}\text{N}$  from 0.7‰ to 5~6‰ was also reported for suspended matter in an estuary of a Sumatra river (Alkhatib et al., 2007). The large proportion of primary amine N in the total N observed in the upper estuary sample is typical of DOM from rivers in the Mukah area, while the less conspicuous shoulder at around 402 eV was major for Everglades DOM samples including those from the Shark River (Watanabe et al., 2014). Assuming that the amide/peptide N and primary amine N are derived mainly from peptide bonds and terminal amino groups in peptide chains, respectively, the average molecular weight of proteinaceous components was lower in samples from the upper estuary than those from the river mouth. Hence, we

conclude that smaller proteinaceous materials were preferentially decomposed or larger proteinaceous materials were supplied as NHS in the estuary. Watanabe et al. (2014) speculated that peptides were protected from decomposition by binding to HS, which were present in high levels in the Mukah rivers. If HS were degraded by photo-breaching, the proteinaceous materials bound to HS would be released.

According to Maie et al. (2006),  $\delta^{15}\text{N}$ , DI, and % primary N in the DOM samples isolated from a low and high salinity sites of the Shark River were 3.50% vs 3.38%, -0.4 vs -0.2, and 5.1% vs 2.8%, respectively. As such, the trends observed in the Judan River were not consistent with those from the Shark River. Although this is not very surprising, further research to confirm the observed trends as general processes in tropical rivers associated with peatland may be of value.

## Conclusions

Dynamics of riverine HS and NHS in an estuary showed different patterns between samples from the Harney and Shark Rivers and the Judan River. In the Harney and Shark Rivers, the concentrations of both HS and NHS showed a non-conservative decrease with a smaller rate in areas of low salinity. This indicates that the supply of DOM consisting of HS/NHS, the proportions of which were similar to those in the rivers from coastal vegetation (Mangroves), resulted in DOM that contained a high proportion of HS in the coastal sea. In the Judan River, the NHS concentration was increased, indicating that NHS

was supplied from coastal sea water. Although the proportion of DOM that is present as NHS in the Judan River was very small, the NHS concentration was still similar to or higher than that in the Harney and Shark Rivers. Thus, the productivity of NHS in the coastal sea in Mukah area appears to be high. A comparison of the chemical characteristics of purified DOM samples suggest that the higher molecular weight proteinaceous materials that were formed by recycling N contributes to the NHS pool supplied from coastal sea water. On the contrary, the concentration of HS decreased conservatively, suggesting that none of the mechanisms that enhance the removal of HS were not operative during their passage through a short channel to the river mouth, even if the HS concentration was high.

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