DISSOLVED ORGANIC MATTER DYNAMICS IN COASTAL AQUATIC SYSTEMS

by

MARIA L. LETOURNEAU

(Under the Direction of Patricia M. Medeiros)

ABSTRACT

Dissolved organic matter (DOM) is a critical component of aquatic environments and global carbon cycling; it has multiple sources including terrestrial runoff, riverine input, phytoplankton excretion, viral lysis, among others. These inputs have varying levels of contribution depending on temporal and spatial scales as well as environmental variables, making the characterization of the DOM increasingly complex. This dissertation used bulk (dissolved organic carbon - DOC), optical (chromophoric DOM - CDOM), molecular (FT-ICR MS) analyses as well as microbial incubation experiments to investigate changes in DOC concentration, DOM composition, and lability in coastal ecosystems in the southeastern U.S. In CHAPTER 2, changes in DOC concentration and DOM composition were analyzed monthly over a year at the Altamaha River and at the head of Sapelo Sound in coastal Georgia, USA. Results showed that river discharge was the primary driver that changed the DOM composition in both locations. In October 2016, the Georgia coast was hit by Hurricane Matthew, which increased the average DOC concentration by ~ 4 times and strongly augmented the terrigenous signature of

DOM. In CHAPTER 3, changes in DOM composition and bacterial processing were investigated at fifteen sites across a riverine-estuarine gradient system as part of the GCE-LTER domain over four seasons. The terrigenous-marine gradient in organic matter sources explained the most variation in DOM composition throughout the year. Increased microbial degradation rates were observed for DOM that had a stronger terrigenous character, especially for samples collected ~ 30 days after Hurricane Irma had impacted the studied area. Finally, in CHAPTER 4, changes in DOC concentration and DOM composition of ambient seawater were characterized after interaction with a loggerhead sponge, *Spheciospongia vesparium*, in the Florida Bay, USA. The sponge-microbial holobiont removed small, oxygen-depleted, nitrogen-rich compounds and the DOM composition was significantly different than that of the ambient seawater. Microbial incubations suggested that sponge exhalent seawater was less labile than ambient seawater, possibly due to holobiont removal of nitrogen-rich compounds. Overall, this dissertation illuminates the merits of combining different chemical analyses and microbial experiments to better uncover and understand the dynamics of different DOM pools across complex coastal environments.

INDEX WORDS: Oceanography; Biogeochemistry; DOM Composition; DOM Processing; FT-ICR MS; Altamaha River; Sapelo Sound; Doboy Sound; Estuary; Georgia Coastal Ecosystem LTER; Sponges; Porifera

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MARIA L. LETOURNEAU

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by

MARIA L. LETOURNEAU

Major Professor:	Patricia M. Medeiros
Committee:	Merryl Alber I. Jonathan Amster Adrian B. Burd Mary Ann Moran

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Dissolved organic matter (DOM) is a complex mixture containing thousands of different compounds (Moran et al., 2016) and is a critical link between the terrestrial and aquatic carbon cycles (Meyers-Schulte & Hedges, 1986). The marine DOM pool holds as much carbon as the atmospheric carbon pool (Hansell, 2013), showing its importance to global carbon cycling dynamics. The DOM pool is essential for the storage of atmospheric carbon (Hansell & Carlson, 1998, 2001; Hansell et al., 2009), plays an important role in elemental cycling (Pomeroy, 1974; Azam & Hodson, 1977), supports marine ecosystems (Williams, 1970; Pomeroy, 1974; Williams, 1981; Azam et al., 1983; Ducklow & Carlson, 1992), and is in dynamic flux between marine phytoplankton and bacterial communities (Couturier et al., 2016). Despite the relatively well-known importance of DOM, there is little known about its complex composition. The research presented in this dissertation aims to uncover some of the primary changes, and the drivers behind those changes, in DOM molecular composition across different aquatic settings.

Dissolved organic matter is operationally defined as organic matter that passes through a ~ 0.45 μm filter, and the organic matter that is retained is referred to as particulate organic matter, qualifying some bacteria, viruses or colloidal material/aggregates as DOM by the size requirement. The DOM pool consists mainly of

dissolved organic carbon (DOC), but also includes dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), and dissolved organic sulfur (DOS) compounds as well (Ferguson & Sunsa, 1984; Kiene et al., 2000; Malmstrom et al., 2004; Meon & Amon, 2004; Simon & Rosenstock, 2007). A portion of the dissolved organic matter pool can be further categorized as chromophoric dissolved organic matter (CDOM) which are compounds that absorb light in the ultraviolet region of the solar spectrum and undergo compositional and structural changes (Gonsior et al., 2009; Kujawinski et al., 2009). These compounds contain high amounts of aromatic material such as lignin, a structural compound in vascular plants (Sarkanen, 1971), and are usually large in size relative to other DOM compounds.

The complexity of the DOM pool is largely due to the wide array of sources of DOM, which can vary both by location, season, and environmental conditions (e.g., precipitation, droughts, etc.). The main inputs of DOM include both allochthonous and autochthonous sources. The major allochthonous sources include riverine input (Williams et al., 1969; Druffel et al., 1989; Bauer et al., 1992), terrestrial runoff (Aitkenhead-Peterson et al., 2003), and groundwater discharge (Baron et al., 1991). These sources of DOM are typically enriched with high amounts of lignin compounds (Hedges et al., 1994, 1997; Lehtonen et al., 2000), are large in size (Rocker et al., 2012), and historically thought to be recalcitrant in terms of their bioreactivity (Mantoura & Woodward, 1983; Álvarez-Salgado & Miller, 1998; Abril et al., 2002; Anesio et al., 2005; Farjalla et al., 2009). The autochthonous sources of DOM include phytoplankton excretion (Duursma, 1963), viral lysis (Wilhelm & Suttle, 1999; Middelboe, 2008), inefficient grazing of phytoplankton by zooplankton (Johannes & Webb, 1965; Jumars et al., 1989), as well as

microbial releases (Decho, 1990; Stoderegger & Herndl, 1998, 1999; Arnosti, 2011; Hmelo et al., 2011). The relative input of each of these sources to the DOM pool can vary on temporal as well as spatial scales, adding further complexity to understanding the DOM pool.

Dissolved OM has several removal processes as it is a key component in various aquatic environments, especially as it is utilized by microbial communities as a nutrient and carbon source (eg. Azam et al., 1983; Ducklow et al., 1986; Ouverney & Fuhrman, 2000; Reinthaler et al., 2006; Pomeroy et al., 2007). Marine DOM is a critical nutrient for microbial communities and its bioavailability can vary based on source (Goldman et al., 1987; del Giorgio & Cole, 1998; Goldman & Dennett, 2000) as well as the microbial community composition (Reinthaler & Herndl, 2005). In addition to microbial utilization, the other methods of DOM removal and transformations in aquatic environments include photodegradation, gel aggregation, and sorption onto particles. Photodegradation of marine DOM through absorption of UV light by chromophores in surface waters has been shown to break up high molecular weight DOM into more bioavailable low molecular weight DOM compounds (Kieber et al., 1989; Mopper et al., 1991; Moran & Zepp, 1997; Benner & Biddanda, 1998; Anderson & Williams, 1999). High molecular weight DOM can aggregate and assemble into physical and chemical gels that are able to sink through the water column (Verdugo, 2012). The final removal mechanism of aquatic DOM is the sorption of DOM onto sinking particles, which has been reported to account for the formation of 14% of the POC in the deep Pacific (Druffel & Williams, 1990).

Dissolved organic matter can also be categorized in terms of reactivity; the most readily utilized faction is termed labile and is used by microbes on timescales of hours or

days after production (Fuhrman, 1987; Amon & Benner, 1996; Rich et al., 1996; Carlson et al., 1999; Keil & Kirchman, 1999; Cherrier & Bauer, 2004; Halewood et al., 2012). Further down the reactivity scale is the semi-labile organic matter which can persist in the environment for weeks to years (Hansell & Carlson, 1998). Finally, refractory DOM is the least reactive and circulates through the global ocean on timescales of thousands of years (Williams & Druffel, 1987; Bauer, 2002).

There are a few hypotheses that attempt to explain the differences in lability of DOM. The microbial carbon pump is the process where microbial communities actively take up labile DOM compounds and produce refractory DOM compounds (Jiao et al., 2010, 2011). The labile compounds are metabolized by the microbes and the refractory products are shunted to a form that resists further biodegradation. The refractory nature of the microbial products could be explained by either the intrinsic stability hypothesis (Borch & Kirchman, 1999) where the DOM produced has a molecular composition that resists further degradation; or it could be explained by the molecular diversity hypothesis (Kattner et al., 2011) where the compounds produced are more diverse than the compounds taken up, further increasing molecular diversity and dilution of compounds that may be labile at higher concentrations. In other words, when the concentration of each substrate is diluted to a level below the chemoreceptive threshold of the microbial communities, the encounter rate for each substrate is too low, or when the energy demand to actively uptake each compound is greater than the energy received from the substrate.

One controversial hypothesis concerning the utilization of refractory DOM is the mechanism of priming. Priming, in terms of DOM utilization, is the idea that refractory DOM can be readily utilized by microbial communities when also supplied with a small

amount of bioavailable DOM (Guenet et al., 2010; Bianchi, 2011). While several studies have reported significant priming effects (Guenet et al., 2014; Hotchkiss et al., 2014; Bianchi et al., 2015; Steen et al., 2016), there are other studies that reported a distinct lack of the priming effect in aquatic systems (Bengtsson et al., 2015; Catalán et al., 2015; Dorado-García et al., 2016; Blanchet et al., 2017;), showing the ongoing debate over the relevancy of this effect. Despite the controversial nature of this hypothesis, terrestrial DOM, historically believed to be refractory (Wetzel, 1992; Moran & Hodson, 1994; Mann & Wetzel, 1995; Anesio et al., 2005; Farjalla et al., 2009), has recently been reported to have higher microbial utilization rates in coastal systems than previously thought (Volk et al., 1997; Buffam et al., 2001; Holmes et al., 2008; Fellman et al., 2009; Fellman et al., 2010; Ward et al., 2013; Wilson et al., 2013).

Estuarine areas are hotspots of DOM processing as the material moves through the estuary and to the coast. All three chapters of the research presented in this dissertation were completed in estuarine settings. CHAPTER 2 and CHAPTER 3 focus on the Georgia Coastal Ecosystem Long Term Ecological Research (GCE-LTER) domain which encompasses Sapelo Island and the surrounding tidal marsh areas. This estuarine system is characterized by a spatial salinity gradient driven by Altamaha River discharge levels (Wang et al., 2017). The Altamaha River is the third largest freshwater input to the Atlantic Ocean in North America (Schaefer & Alber, 2007), with discharge values averaging ~ 400 m^3/s with larger levels in the spring months due to increased local precipitation (Weston et al., 2009). Due to the dynamic and ever-changing nature of this system there are various inputs of DOM including marsh, terrigenous, as well as more marine-derived influences (Moran et al., 1991; Medeiros et al., 2015) which vary in their

relative contributions to the overall DOM pool both spatially and temporally within the domain. CHAPTER 4 analyzes the changes in DOM composition after interaction with a common marine sponge in the Florida Bay Estuary in the Florida Keys, USA. This system is relatively more marine than the GCE-LTER domain, however the Florida Everglades system provides large amounts of freshwater to the area from the north. The DOM in the Florida Bay estuarine system is mostly compromised of terrigenous and marine sources (Jaffé et al., 2004); it lacks the salt marsh signatures as seen in the GCE-LTER domain as studied in CHAPTER 2 and CHAPTER 3. The dynamics and transformations of different DOM pools in these coastal aquatic ecosystems is the basis of the research presented here.

Analyzing and understanding the changes behind DOM cycling across various aquatic environments has proven to be a difficult challenge for aquatic biogeochemists due to the complex and ever-changing composition of DOM. In this dissertation, I use both chemical techniques and microbial dark incubations in order to elucidate changes in the DOM pool over both spatial and temporal scales. Through the use of a total organic carbon (TOC) analyzer, I have measured the concentration of DOC in aquatic samples; using a UV-visible spectrophotometer, I have analyzed the changes in the CDOM composition through indices of aromaticity and molecular weight; and through Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS, aka ultra-high resolution mass spectrometry), I have analyzed the compositional changes of the molecular formulae in DOM extracts. In addition, microbial dark incubations were conducted in aquatic samples to assess DOM lability changes. This multi-technique approach has given me a better understanding of changes in concentration, composition,

and lability of DOM across various aquatic settings. In CHAPTER 2, I analyzed and uncover changes in concentration and composition over a year as well as their environmental drivers in the Altamaha River and at the head of Sapelo Sound in coastal Georgia, USA. The effects on organic carbon concentration and composition were also assessed following the passage of the Hurricane Matthew through the studied area. CHAPTER 3 took a more in-depth look at the DOM dynamics in the Altamaha-Doboy-Sapelo estuarine system and sampled across fifteen different sites within the system over four different seasons, including ~ 30 days after Hurricane Irma hit the GA coast. CHAPTER 4 used the same techniques described above to investigate DOM concentration, composition, and lability changes of ambient seawater after interacting with a loggerhead sponge in Florida Bay, USA. CHAPTER 5 summarizes all findings of this dissertation and suggests potential future work to further our knowledge of the dynamics of DOM across various aquatic environments.

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CHAPTER 2

DISSOLVED ORGANIC MATTER COMPOSITION IN A MARSH-DOMINATED ESTUARY: RESPONSE TO SEASONAL FORCING AND TO THE PASSAGE OF A HURRICANE[1]

[1]Letourneau, M.L. and Medeiros, P.M. 2019. *Journal of Geophysical Research: Biogeosciences,* 124. Reprinted here with permission of the publisher.

ABSTRACT

Dissolved organic matter (DOM) is a large and complex mixture of compounds with source inputs that differ with location, season, and environmental conditions. Here, we investigated drivers of DOM composition changes in a marsh-dominated estuary off the southeastern United States. Monthly water samples were collected at a riverine and estuarine site from September 2015 to September 2016, and bulk, optical, and molecular analyses were conducted on samples before and after dark incubations. Results showed that river discharge was the primary driver changing the DOM composition at the mouth of the Altamaha River. For discharge higher than ~ 150 m^3/s, dissolved organic carbon (DOC) concentrations and the terrigenous character of the DOM increased approximately linearly with river flow. For low discharge conditions, a clear signature of salt marsh-derived compounds was observed in the river. At the head of Sapelo Sound, changes in DOM composition were primarily driven by river discharge and possibly by summer algae blooms. Microbial consumption of DOC was larger during periods of high discharge at both sites, potentially due to the higher mobilization and influx of fresh material to the system. The Georgia coast was hit by Hurricane Matthew in October 2016, which resulted in a large input of carbon to the estuary. The DOC concentration was ~ 2 times higher and DOM composition was more aromatic with a stronger terrigenous signature compared to the seasonal maximum observed earlier in the year during peak river discharge conditions. This suggests that extreme events notably impact DOM quantity and quality in estuarine regions.

Keywords: DOM composition; hydrology; FT-ICR MS; Altamaha River and Estuary; Georgia

INTRODUCTION

Dissolved organic matter (DOM) is a key component of the carbon cycle in aquatic settings. This DOM pool is critical for bacterial production, biogeochemical transformations, and nutrient availability, and it influences bacterial and phytoplankton community structure and functions (Crump et al., 2009). The amount of carbon held in the marine DOM pool is comparable to the atmospheric carbon pool (Walther, 2013), which makes it an important part of the global carbon cycle. This DOM can come from many sources, each source contributing organic matter that is unique in its composition and concentration (Hopkinson, 1985), resulting in thousands of different molecular compounds (Moran et al., 2016) and attributing a highly complex and variable chemical makeup to its composition. Allochthonous inputs to the DOM pool include terrestrial runoff, river discharge, and groundwater flushing (Aitkenhead-Peterson et al., 2003), whereas autochthonous inputs include phytoplankton metabolism and excretion, viral lysis, and releases associated with zooplankton grazing (Nagata, 2000). These differing inputs vary over seasonal and spatial gradients and add an additional layer of complexity to the problem of quantifying and characterizing the DOM pool.

Coastal Georgia, USA, is a unique setting due to its short but ecologically diverse coastline. There are five large rivers in the area, the Savannah, Satilla, St. Marys, Ogeecheee, and the Altamaha River. The Altamaha River watershed covers an area of 36,718 km2 and is the third largest contributor of fresh water to the Atlantic Ocean from North America (Schaefer & Alber, 2007). Nearby saltmarsh areas along the estuary can vary substantially in their source inputs, DOM composition and processing (Moran et al., 1999). The Altamaha River has been shown to have a strong allochthonous terrestrial

signature and a general low dissolved organic carbon (DOC) bioavailability over a time scale of a few days (Wiegner et al., 2006). Medeiros, Babcock-Adams, et al. (2017) observed that part of the DOC from the Altamaha River can be exported all the way to the South Atlantic Bight shelf break, especially in late spring.

The transport of terrestrial material to aquatic systems is often enhanced following major rainfall events, where the export of DOM can account for the majority of the annual carbon export budget (e.g., Inamdar et al., 2006; Raymond & Saiers, 2010). Yoon and Raymond (2012) reported an export of 43% of the annual DOC flux in only 5 days in a forested watershed in New York following Hurricane Irene. Similarly, 3 days following the passage of the same hurricane, both DOC and chromophoric dissolved organic matter (CDOM) nearly tripled in the Neuse River estuarine system in North Carolina (Miller et al., 2016). Because of possible changes in hurricane activity in the future (Bender et al., 2010), it is important to have a better understanding of how storms currently affect coastal watersheds and the transport and processing of material in associated aquatic systems.

Through the use of ultrahigh resolution mass spectrometry, in particular Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), the chemical nature of the DOM has been investigated (e.g., Kujawinski et al., 2002; McIntyre et al., 1997; Sleighter & Hatcher, 2007) and compared over different seasons (Herzsprung et al., 2017; Medeiros, Seidel, Dittmar, et al., 2015; Singh et al., 2014) and locations (Kim et al., 2006; Koch et al., 2005; Seidel et al., 2015). Here, we used untargeted approaches to investigate the molecular composition of riverine and estuarine DOM in a typical marsh-dominated estuary off the U.S. East Coast. Using an untargeted approach is useful

because it is not known a priori which compounds (or classes of compounds) will dominate the changes in DOM composition in the system. We focused our analyses at the mouth of the Altamaha River and at the head of the Sapelo Sound estuary off Georgia (**Fig. 2.1**). While the Altamaha site is directly influenced by a river, Sapelo Sound is located 25 km to the north and is characterized by a larger influence of local precipitation (Wang et al., 2017) and marine inputs, which can potentially increase the complexity of patterns in DOM changes. We identified the DOM molecular signatures that are associated with the different organic matter sources as well as changes in DOM composition and bacterial processing that arose from variations in hydrological conditions over a year, including the passage of Hurricane Matthew. Comparing the evolutions of DOC concentration and DOM composition in these two sites with contrasting characteristics allows for investigating the relative contributions of multiple drivers, including variations in hydrology and microbial biodegradation, to compositional changes in this marsh-dominated system.

MATERIALS AND METHODS

Sample Collection

Monthly surface water samples were collected over the course of a year (September 2015 to September 2016) during high tide conditions at two locations, the Altamaha River (31.337°N, 81.449°W) and the head of Sapelo Sound (31.539°N, 81.423°W) in coastal Georgia, USA (**Fig. 2.1**). These two sampling locations were chosen due to their distinct characteristics in terms of riverine and marine influence, despite their geographical proximity. Temperature and salinity measurements were taken

at the time of sampling and are shown in Table A.1 in the supporting information. On 7 October 2016, approximately a month after the end of our year-long collection, Hurricane Matthew hit the coast of Georgia resulting in strong rainfall and in a storm surge of >2 m. Five days following the passage of the hurricane, a sample was collected at Sapelo Sound site after a change of water color was noticed in several parts of the estuary.

River discharge data were obtained from the U.S. Geological Survey (http://waterdata.usgs.gov) at the nearest monitoring station at Doctortown, GA, roughly 20 km upstream from the Altamaha River sampling site. Precipitation data were collected at Sapelo Island (**Fig. 2.1**) as part of the Georgia Coastal Ecosystem Long Term Ecological Research program. Water level measurements for Fort Pulaski, GA, located about 70 km to the north of Sapelo Island, were obtained from National Oceanic and Atmospheric Administration (https://tidesandcurrents.noaa.gov).

Sample Extraction and Dark Incubations

Immediately after collection, inorganic nutrients (20 μM Na_2PO_4; 50 μM NH_4Cl) were added to all samples prior to filtration in order to sustain microbial communities through long-term incubations (see below). To remove any photosynthetically active organisms, triplicate samples were filtered through 0.7 μm Whatman GF/F filters (precombusted at 450 °C for 5 hr) into acid-washed 1 L polycarbonate bottles. Samples referred to as T_0 were then filtered through prewashed 0.2 μm Pall Supor membrane filters into 60 mL amber bottles for DOC and CDOM analyses. Samples for DOC and CDOM analyses were immediately frozen (−20 °C) and refrigerated (4 °C), respectively, and analyzed within 5 days. The remaining filtrates of T_0 samples (~ 1 L) were acidified

to pH 2 (concentrated HCl), and DOM was extracted using solid phase extraction (SPE) with cartridges filled with a styrene divinyl benzene polymer (Agilent Bond Elut PPL) as described by Dittmar et al. (2008). The DOM extracts (SPE-DOM) from each month were eluted using methanol, concentrated using ultrapure nitrogen gas, and stored at −20 °C in the dark for FT-ICR MS analysis that were pursued at the end of the field sampling. Additional triplicate riverine and estuarine samples underwent in-lab dark incubations to track the temporal bacterial degradation. Samples were filtered through 0.7 μm Whatman GF/F filters and incubated during 2-, 5-, 10-, 20-, and 80-day intervals at temperature of collections. After incubations, samples were filtered through 0.2 μm Pall Supor membrane filters, collected, and stored for DOC and CDOM analyses as described previously. Samples collected in October 2015 and January, April, and July 2016 after the 80-day incubation (T_{80}) were also analyzed using FT-ICR MS.

Bulk DOC

Concentrations of DOC from both water samples and SPE-DOM (completely dried and resuspended in ultrapure water) were measured with a Shimadzu TOC-LCPH analyzer using potassium hydrogen phthalate as a standard for the DOC calibration curve. Prior to and alongside sample analysis, both internal blanks and Milli-Q water blanks were run on the instrument. Analytical accuracy and precision were tested against the Consensus Reference Material (Hansell, 2005) and were better than 5%. SPE extraction efficiency across all samples, defined as DOC concentration in the SPE extract versus DOC concentration in the original sample (Seidel et al., 2014), was 74 + 5% of the DOC. Bacterial utilization of DOC was determined as

$$\frac{DOC_{T0} - DOC_{T80}}{DOC_{T0}} \times 100 \quad (1)$$

where DOC_{T80} is the concentration of DOC after 80-day incubations were complete and DOC_{T0} is the initial concentration before incubations. Additional DOC measurements have been collected at the Altamaha River site from October 2000 to April 2009 as part of the Georgia Coastal Ecosystem Long Term Ecological Research monitoring efforts. The time interval between sampling during that period was 5.5 ± 6.6 days (Medeiros, Babcock-Adams, et al., 2017).

Chromophoric DOM

Absorbance measurements of water samples were taken at room temperature on an Agilent 8453 UV-visible spectroscopy system. Prior to sample measurement, blank calibrations were performed with Milli-Q water to achieve a baseline background reading. Absorbance was measured from wavelengths 190 to 1,100 nm and was converted to absorption coefficients as in D'Sa et al. (1999). Spectral slope ($S_{275\text{-}295}$) was calculated for the absorbance spectra between 275 and 295 nm as

$$\alpha_g(\lambda) = \alpha_g(\lambda_{ref})e^{-S(\lambda - \lambda ref)} \quad (2)$$

where $\alpha_g(\lambda)$ is the absorption coefficient of CDOM at each wavelength, λ_{ref} is a reference wavelength of 275 nm, and S is the slope fitting parameter (Helms et al., 2008; Spencer et al., 2008). Spectral slope has been shown to have a correlation with DOM molecular weight and a negative correlation with terrigenous DOM (Fichot & Benner, 2012; Helms et al., 2008). The ratio of absorbance at λ = 250 nm to λ = 365 nm ($a_g(250):a_g(365)$) was also calculated for each sample; this ratio is used as an inverse proxy for DOM aromaticity and molecular weight (Peuravuori & Pihlaja, 1997).

FT-ICR MS Analysis

The molecular composition of the DOM extracts (200 mg C/L in methanol) was analyzed on a 9.4 T FT-ICR MS with electrospray ionization (negative mode) at the National ICR Users' Facility at the National High Magnetic Field Laboratory (Florida State University, Tallahassee, FL). Sample processing was done as described in Vorobev et al. (2018). A total of 150 scans was accumulated for each sample. Each m/z spectrum was internally calibrated with respect to an abundant homologous alkylation series whose members differ in mass by integer multiples of 14.01565 Da (mass of a CH_2 unit) confirmed by isotopic fine structure (Savory et al., 2011), achieving a mass error of <0.4 ppm. Molecular formulae were assigned for masses in the range of 150 and 750 Da by applying the following restrictions: $^{12}C_{1\text{-}130}\,^{1}H_{1\text{-}200}\,O_{1\text{-}150}\,^{14}N_{0\text{-}4}\,S_{0\text{-}2}\,P_{0\text{-}2}$. Assignment of molecular formulae was performed by Kendrick mass defect analysis (Wu et al., 2004) with PetroOrg software (Corilo, 2015) and using the criteria described by Rossel et al. (2013). Only compounds with a signal-to-noise ratio of 6 or higher were used in the analysis to eliminate intersample variability based on peaks that were close to the limit of detection. The peak intensity of each molecular formula was normalized to the sum peak intensities of the total identified peaks in each sample. Peaks with molecular formulae assigned accounted on average for ~ 90% of the sum of the intensities of all peaks in the final spectra. Repeated analysis of several of these samples revealed that differences in DOM composition due to instrument variability were substantially smaller than variability between samples.

Statistical Analyses

The variability of DOM molecular composition at each location was analyzed using principal component (PC) analysis of the FT-ICR MS data. All peaks with molecular formulae assigned were used in the PC analysis. All modes shown here are significantly different (95% confidence level) from results obtained by pursuing a PC analysis of random processes that are spatially and temporally uncorrelated. This indicates that the signals in the modes described here are significantly greater than the level of noise (Overland & Preisendorfer, 1982). Spearman's rank correlation analysis (α level 0.05) was used to test correlations between environmental conditions, bulk (DOC concentrations and DOC biodegradation rates), and optical (spectral slope and $a_g(250):a_g(365)$) parameters as well as DOM molecular composition (FT-ICR MS). The Wilcoxon rank-sum test was used for comparisons between samples, as in Osterholz et al. (2016).

RESULTS AND DISCUSSION

The Altamaha River discharge is characterized by strong seasonality, generally peaking in March or April and reaching a seasonal minimum during fall (Medeiros, Babcock-Adams, et al., 2017). The peak in discharge in 2016 occurred a few months earlier in January and February (**Fig. 2.2a**), however, due to increased rainfall associated with the positive phase of the El Niño–Southern Oscillation (Hansen et al., 1997; Keener et al., 2010).

Seasonality of Riverine and Estuarine DOM Composition

Time series of DOC concentrations and of optical characteristics for the Altamaha River and for Sapelo Sound are shown in Figures 2.2 and 2.3, respectively. Concentrations of DOC at the Altamaha River ranged from 362 to 965 μM (**Table A.1**) and were strongly modulated by river discharge ($r = 0.87$). Peak concentrations were observed in winter and early spring, when river discharge was high (**Figs. 2.2a** and **2.2b** and **Table A.1**). The maximum correlation between river discharge and DOC concentrations occurred with no lag, indicating a rapid response to pulses in river flow. Climatologies of river discharge and DOC concentration at the Altamaha River, built using observations from 2000 to 2009, are also correlated ($r = 0.63$, $p < 0.05$), indicating that this is a robust pattern (**Fig. A.1**). Strong relationships between river discharge and DOC concentrations have been reported for other riverine and watershed systems (e.g., Raymond & Saiers, 2010; Ward et al., 2013).

The seasonal change in DOC concentration at the Altamaha River was accompanied by changes in DOM composition as determined by optical properties (**Figs. 2.2c–2.2f**). During high discharge conditions, both the $a_g(250):a_g(365)$ ratio and the spectral slope parameter ($S_{275\text{-}295}$) at the Altamaha River decreased ($r = -0.77$, $p < 0.05$ and $r = -0.86$, $p < 0.05$, respectively). Previous studies have revealed that the $a_g(250):a_g(365)$ ratio is related to changes in the aromaticity and molecular size of the DOM, with decreasing values indicating higher aromaticity and higher molecular size (Peuravuori & Pihlaja, 1997). Spectral slope ($S_{275\text{-}295}$) has been shown to be closely related to DOC-normalized lignin yields in rivers and thus to be a good tracer of terrigenous DOM (Fichot & Benner, 2012). Shallower slopes indicate a higher

terrigenous signature with a higher aromatic content and higher molecular weight (Del Vecchio & Blough, 2002; Helms et al., 2008). Concentration of DOC at the Altamaha River was correlated with both $S_{275\text{-}295}$ ($r = -0.82$, $p < 0.05$) and $a_g(250):a_g(365)$ ($r = -0.72$, $p < 0.05$; **Figs. 2.4a** and **2.4b**), suggesting that the increase in DOC concentration observed during high river flow conditions was at least in part related to terrigenous DOM input of high molecular weight aromatic compounds. The seasonal evolution of DOC concentration and DOM composition at the Altamaha River was consistent with the evolution reported for other riverine systems (e.g., Yukon River, Spencer et al., 2009; Kolyma River, Mann et al., 2012). Concentration of DOC and optical characteristics were not correlated with local precipitation ($p > 0.05$).

At the head of Sapelo Sound, both DOC concentration and DOM composition were also correlated with river discharge, as long as the sample collected in October 2016 shortly after the passage of Hurricane Matthew was not considered in the analysis. The magnitudes of the correlations between river discharge and DOC or optical parameters were somewhat lower than in the Altamaha River, ranging between 0.56 and 0.74 (**Fig. 2.3**). Similarly to results from the Altamaha River, high river discharge resulted in higher DOC concentrations, which were associated with a stronger terrigenous signature and with higher aromaticity and molecular weight (**Figs. 2.4c** and **2.4d**). Even though the Altamaha River is located farther south (see **Fig. 2.1**), Wang et al. (2017) showed that increased river discharge leads to decreased salinity over the entire estuarine area, including at Sapelo Sound. The lower correlation coefficients indicate that the control of DOM composition variability was more complex at Sapelo Sound, however, with factors other than river discharge presumably playing a larger role than at the Altamaha River.

Precipitation was not found to be correlated with DOC concentration or with optical properties at the head of Sapelo Sound. This is surprising, since salinity at that location has been shown to be correlated with precipitation data convoluted with a one-sided, exponentially decaying filter (Austin & Barth, 2002) with a decay scale of 26 days (Wang et al., 2017). Although tidal variation likely plays a role in the variability of DOM composition at the head of Sapelo Sound, that process cannot be resolved by our sampling, which was restricted to high tide conditions.

The sample collected at the head of Sapelo Sound in October 2016 was substantially different from those collected in previous months, indicating that the passage of Hurricane Matthew had a dramatic effect in the system. Despite the low river discharge at that time, DOC concentration reached ~ 3,700 μM, which is almost twice as high as the seasonal maximum that occurred earlier in the year in February and is approximately 4 times higher than the average concentration for the year (**Fig. 2.3**). Optical characteristics also indicated a considerable input of highly aromatic terrigenous material shortly after the passage of the hurricane, even though river discharge was at a seasonal minimum.

Molecular Characterization of DOM Composition

Composition of DOM was also investigated at the molecular level using FT-ICR MS analysis. Over 6,000 molecular formulae were assigned to the complex DOM mixture. PC analysis was used to identify the dominant modes of variability in DOM composition in the system. All initial samples (T_0) from each location were used in the

PC analysis, covering 13 months at the Altamaha River and 14 at the head of Sapelo Sound.

At the Altamaha River, the dominant PC accounted for 23% of the total variance in DOM composition in the system. PC 1 scores were highest from January to April and were lowest from June to September (**Fig. 2.5a**), which is consistent with the time variability in river discharge (see **Fig. 2.2**). PC 1 was correlated with $S_{275\text{-}295}$ ($r = -0.93$, $p < 0.05$; **Fig. 2.5d**), suggesting that PC 1 was related to the terrigenous character of the DOM. This is supported by analysis of a van Krevelen diagram of the loading of PC 1, which showed a tendency for high positive loadings (i.e., red dots in **Fig. 2.5b**) to cluster at low H/C ratios. Terrigenous DOM is generally enriched with formulae with low H/C ratios (Medeiros, Seidel, Ward, et al., 2015; Sleighter & Hatcher, 2008), which are indicative of more aromatic compounds (Kim et al., 2003; Koch & Dittmar, 2006, 2016). Thus, from January to April (i.e., when PC 1 scores are positive), the Altamaha DOM was enriched with compounds with more terrigenous characteristics, corroborating the results obtained based on optical analysis (**Fig. 2.2**).

At months when the PC 1 score is negative, the DOM at the Altamaha River was relatively enriched with molecular formulae with negative loading of PC 1 (i.e., blue dots in **Fig. 2.5b**). Marshes have been shown to be important sources of DOC to estuaries (e.g., Bauer et al., 2013; Moran & Hodson, 1994; Peterson et al., 1994). Medeiros, Seidel, Dittmar, et al. (2015) compared the DOM composition in water immediately before and after exposure to a nearby marsh to identify changes in DOM composition that were associated with the addition of new organic compounds and with transformation processes occurring in the marsh. The molecular formulae enriched after marsh exposure

(see Figure 4 in Medeiros, Seidel, Dittmar, et al., 2015) occupied a region in van Krevelen space similar to that occupied by formulae with negative loading of PC 1 (**Fig. 2.5b**). This suggests that during low discharge conditions, when PC 1 is negative, the DOM at the Altamaha River was imprinted with the signature of marsh-derived compounds. A quantitative assessment of this input can be obtained by selecting the 506 molecular formulae that were identified by Medeiros, Seidel, Dittmar, et al. (2015) as being enriched after marsh exposure and quantifying their contribution to the total intensity of the sum of all peaks with molecular formulae assigned for samples collected at the Altamaha River. The relative contribution of marsh-derived compounds at the Altamaha River (**Fig. 2.5c**) approximately mirrored the PC 1 score (**Fig. 2.5a**), suggesting that the increase in the relative abundance of formulae with negative loading of PC 1 (blue dots in **Fig. 2.5b**) from June to September was possibly related to inputs from salt marshes.

Collectively, these results indicated that during high river discharge conditions, the DOM at the Altamaha River became more aromatic due to the input of terrigenous material. During low discharge conditions, on the other hand, the terrigenous signature of the DOM decreased, and the relative importance of marsh-derived compounds increased. A scatterplot of river discharge versus PC 1 scores revealed a large change in the slope of the curve at about 150 m^3/s (**Fig. 2.6**). Marsh-derived compounds made an increasingly important contribution for the DOM composition when river flow was lower than that threshold. Indeed, marsh-derived compounds have been previously shown to imprint a distinct signature on the riverine DOM during drought conditions (Medeiros, Seidel, Dittmar, et al., 2015). For river discharge larger than ~ 150 m^3/s, the signature of marsh-

derived compounds was presumably overwhelmed by the input of terrigenous DOM. In that limit of high discharge, the terrigenous character of the DOM increased approximately linearly with river flow.

Two dominant modes of variability in DOM molecular composition were statistically significant ($p < 0.05$) at the head of Sapelo Sound (**Fig. 2.1**), and they each explained approximately the same fraction of the total variance (25% and 21% for PCs 1 and 2, respectively). We begin by presenting and discussing results from the second mode (**Figs. 2.7c** and **2.7d**), which are comparatively easier to interpret. The time series of PC 2 scores (**Fig. 2.7c**) was correlated with river discharge (especially if the sample influenced by Hurricane Matthew is neglected; **Fig. 2.8a**), and it was highly correlated with $S_{275\text{-}295}$ (**Fig. 2.8b**). The pattern captured by the loading of PC 2 (**Fig. 2.7d**) was typical of terrigenous/marine gradients in DOM composition, and it has been previously observed in river to ocean transects in this (Medeiros, Babcock-Adams, et al., 2017; Medeiros, Seidel, Gifford, et al., 2017) and in other river systems such as the Amazon River plume (Medeiros, Seidel, Ward, et al., 2015). It is also consistent with the pattern of variability observed along a river to ocean transect at the lower Chesapeake Bay, where H/C ratios were found to increase from more riverine to more oceanic samples (Sleighter & Hatcher, 2008). Thus, during high discharge conditions, DOM at the head of Sapelo Sound had a stronger terrigenous signature. Although river discharge remained low after the passage of Hurricane Matthew (**Fig. 2.3**), the storm resulted in a substantial input of terrigenous DOM to the system (**Fig. 2.7c**). In contrast to the Altamaha River site, however, the DOM at the head of Sapelo Sound had a more marine signature (instead of marsh-derived signature) when discharge was low. Time series of salinity

measured during the sampling period (**Table A.1**) indicated that salinity at the Altamaha River station hovered around zero year-round, indicating that the input of marine DOM should indeed be small. At Sapelo Sound, on the other hand, salinity varied from 10–13 during peak discharge to around 30–32 when river flow was at a minimum in early fall. This was consistent with a larger contribution of marine DOM during that period (**Figs. 2.7c** and **2.7d**).

Results from PC 1 at Sapelo Sound are more difficult to interpret. PC 1 scores were either approximately 0 or negative over the entire period, except during summer (May to August 2016) when they were positive (**Fig. 2.7a**). The time series was not correlated with river discharge or with precipitation. The pattern revealed by the loading of PC 1, shown color coded in a van Krevelen diagram (**Fig. 2.7b**), was different from the pattern typically observed in river to ocean transects (Medeiros, Babcock-Adams, et al., 2017; Medeiros, Seidel, Dittmar, et al., 2015; Medeiros, Seidel, Gifford, et al., 2017; Medeiros, Seidel, Ward, et al., 2015; see also **Fig. 2.7d**). This suggests that the mode was not related to the varying contribution of terrigenous vs marine sources to the estuarine DOM pool.

Several additional processes are known to transform the DOC pool and to result in changes in DOM composition in aquatic environments, including photochemical reactions (e.g., Chen et al., 2014; Medeiros, Seidel, Powers, et al., 2015; Stubbins et al., 2010), microbial degradation (e.g., Kujawinski et al., 2004; Moran & Zepp, 1997; Obernosterer & Benner, 2004; Seidel et al., 2015), inputs from phytoplankton (e.g., Landa et al., 2014; Medeiros, Seidel, Ward, et al., 2015), and flocculation (e.g., Hernes & Benner, 2003; Sholkovitz et al., 1978). The molecular signatures of DOM

transformations due to some of these processes have been previously identified using FT-ICR MS. For example, photodegradation is generally associated with the consumption of compounds with low H/C ratios and with the enrichment of compounds with high H/C and low O/C ratios (Medeiros, Seidel, Powers, et al., 2015; Seidel et al., 2015; Stubbins et al., 2010). Microbial biodegradation is also generally associated with the consumption of compounds associated with molecular formulae with high O/C and low H/C ratios, and with the enrichment in relative abundance of compounds associated with formulae with low O/C and high H/C ratios (Medeiros, Seidel, Ward, et al., 2015; Seidel et al., 2015). Thus, the pattern of transformation captured by PC 1 (**Fig. 2.7b**) was different from the pattern reported many times in the literature in multiple environments as characteristic of the transformation in DOM composition associated with photochemistry or microbial degradation. This suggests that photooxidation and microbial degradation were likely not the main drivers of the DOM transformation captured by PC 1.

Chlorophyll concentration, a proxy for phytoplankton abundance, was not measured simultaneously with the DOM analysis. However, historical observations at seasonal intervals (2014–2017) at the head of Sapelo Sound revealed that chlorophyll concentration was generally high during summer and was substantially lower during the remaining seasons (**Fig. A.2**). This time variability is somewhat similar to the time variability captured by PC 1 (**Fig. 2.7a**), suggesting that the pattern of DOM composition variability captured by mode 1 may be related to phytoplankton-derived DOM. Analysis of cultures grown in laboratory has revealed phytoplankton-derived DOM enriched with compounds with $H/C > 1$ and $O/C < 0.5$ (Landa et al., 2014). Additionally, analysis of DOM composition using FT-ICR MS in the Amazon River plume has revealed a pattern

in which samples characterized by high chlorophyll concentrations and high phytoplankton cell counts were enriched with compounds with H/C > 1 and low O/C, while samples with low chlorophyll concentrations and low phytoplankton cell counts were enriched with compounds with H/C > 1 and high O/C ratios (see Figure 2 in Medeiros, Seidel, Ward, et al., 2015). The loading of PC 1 (**Fig. 2.7b**) was consistent with that description. Thus, it is possible that summer algae blooms had a detectable impact driving variability in DOM composition at the head of Sapelo Sound. The interpretation of the process(es) that may have been responsible for the seasonal change in DOM composition captured by the first PC (**Figs. 2.7a** and **2.7b**) is characterized by high uncertainty. More detailed studies are thus needed to identify what is (are) the dominant mechanism(s) driving seasonal changes in the composition of the DOM at the head of Sapelo Sound.

Microbial Consumption of Riverine and Estuarine DOC

Dark incubations were pursued at both sites for all months to investigate microbial consumption of riverine and estuarine DOC. Concentration of DOC and optical parameters was measured in all cases. For October 2015 and January, April, and July 2016, FT-ICR MS analysis was also pursued at the end of the incubation after 80 days. No incubation was pursued for October 2016 following the passage of Hurricane Matthew. We note that variability in DOC consumption between the different months may be related both to DOM composition (i.e., to its lability) and to changes on microbial community composition.

Utilization of DOC, defined as the fraction of the DOC that is consumed during each incubation experiment (see equation (1)), was correlated with river discharge at the Altamaha River ($r = 0.65$, $p < 0.05$) and at Sapelo Sound ($r = 0.89$, $p < 0.05$; **Fig. 2.9**). Thus, microbial consumption of DOC at both sites was enhanced in months of high river discharge. Mann et al. (2012) observed that the contribution of humic-like fluorescence indicative of terrigenous DOM input was positively correlated with DOC utilization in an Arctic river. The increase in DOC in Arctic rivers during peak discharge is often young and rich in lignin (Raymond et al., 2007; Spencer et al., 2008), indicating that fresh terrestrial DOM can be highly biolabile possibly due to its short degradation history (Mann et al., 2012). To further investigate how river discharge influences microbial degradation in the Altamaha River and at Sapelo Sound, we computed the average change in DOC concentration and optical parameters during the incubations for high and for low discharge conditions. January to April 2016 were defined as high discharge months (discharge larger than average plus 1 standard error of the mean), while the remaining months (September to December 2015 and May to September 2016) were characterized by low discharge conditions. Consistent with **Fig. 2.9**, DOC concentration (expressed as a percentage of the initial concentration) decreased faster during high discharge conditions, especially at Sapelo Sound (**Figs. 2.10a** and **2.10d**). This suggests that increased discharge may have changed the DOM pool by mobilizing fresh terrestrial DOM from the soil and transported it into the coastal system (Vazquez et al., 2011). These changes were seen in both the Altamaha River and in the Sapelo Sound estuarine area, indicating a far-reaching effect of this environmental condition. Optical parameters were also different between high and low discharge conditions in both the Altamaha

River ($p < 0.05$) and in Sapelo Sound ($p < 0.05$). At both sites the spectral slope $S_{275\text{-}295}$ and the ratio $a_g(250):a_g(365)$ were lower during high discharge conditions, which was indicative of stronger terrigenous signature, higher aromaticity, and higher molecular size (Del Vecchio & Blough, 2002; Fichot & Benner, 2012; Helms et al., 2008; Peuravuori & Pihlaja, 1997).

It is interesting to note that $S_{275\text{-}295}$ and $a_g(250):a_g(365)$ remained approximately constant throughout the incubations (**Fig. 2.10**), with values at the initial condition (T_0) not being statistically different from values measured after 80 days (T_{80}) at the end of the incubation (Wilcoxon ran-sum test, $p > 0.05$). We also note that repeating the PC analysis of FT-ICR MS data described before (**Figs. 2.5a**, **2.5b**, and **2.7**) but including the samples collected at the end of the incubation (for October 2015 and January, April, and July 2016) produced nearly identical results for both sites (**Fig. A.3**). Moreover, for the months in which FT-ICR MS analyses were pursued at the end of the incubations, the PC scores for T_0 and T_{80} samples were not different from each other (Wilcoxon ran-sum test, $p > 0.05$). This indicates that any alteration in DOM composition that may have occurred during the incubations due to microbial degradation was smaller than the seasonal changes in DOM composition observed due to other processes (e.g., changes in hydrology, phytoplankton derived inputs). This is consistent with biodegradation not explaining the dominant patterns of DOM composition variability observed in the system (**Figs. 2.5a**, **2.5b**, and **2.7**). We note that a fraction of the decreased DOC measured during the experiments likely encompassed components of the DOM pool that cannot be detected by the techniques used here (Vorobev et al., 2018). For example, saccharides are

known to be labile (Kirchman et al., 2001; Rich et al., 1996), but they are not well retained by SPE.

Using our measurements of DOC and DOC utilization at the Altamaha River, we can estimate the total export of DOC and of biolabile DOC out of the system. Using historical DOC data from the Altamaha River from October 2000 to April 2009, Medeiros, Babcock-Adams, et al. (2017) calculated an average export of 69 Gg C/year. For the current sampling period, the total DOC export was larger at 108 Gg C/year. Medeiros, Babcock-Adams, et al. (2017) showed that DOC flux is highly correlated to discharge at the Altamaha River. Thus, the 50% increase in DOC export compared to the long-term average is likely related to increased river discharge during the study period associated with El Niño conditions (Sheldon & Burd, 2014). The residence time at the Altamaha River has been estimated to be about 5 days during low discharge conditions, decreasing to less than 2 days during high discharge (Wang et al., 2017). Since most of the DOC utilization occurred on time scales longer than that (**Fig. 2.10**), it is reasonable to assume that most of the biolabile DOC from the Altamaha River can be exported out of the system to the coastal ocean. If that is true, then the Altamaha River exported 18 Gg C/year of biolabile DOC to the shelf during our study period, or about 16.5% of the total DOC flux, which is comparable to the labile DOC flux as a percentage of total annual DOC flux out of other rivers (e.g., Holmes et al., 2008). If that fraction is approximately constant from year to year, then a climatological export of biolabile DOC can be estimated at 11 Gg C/year. Most of this biolabile DOC is presumably exported during spring, since river discharge, DOC concentration, and DOC lability all peak during that season.

CONCLUSIONS

Bulk, optical, and molecular analyses of riverine and estuarine samples of a typical salt marsh-dominated estuary off the southeastern United States revealed that DOC concentration and DOM composition were constantly being altered by various processes throughout the year. River discharge strongly modulated changes in DOM composition at the Altamaha River. When discharge was higher than ~ 150 m^3/s, the Altamaha was characterized by higher DOC concentrations and DOM with a strong terrigenous character. At low discharge conditions, a clear imprint of marsh-derived compounds was observed in the river. At Sapelo Sound, the composition of DOM was also altered by river discharge through the entrainment of freshwater into narrow channels and streams that carve the estuarine area, showing the far-reaching effect of discharge on the DOM pool across this aquatic setting. Another seasonal pattern of variability in DOM composition was observed at the head of Sapelo Sound, which is possibly related to phytoplankton-derived inputs during summer. Higher DOC utilization by bacteria was observed during months of high discharge levels and DOM with a stronger terrigenous signature, potentially due to the higher mobilization and influx of fresh material to the system. Lastly, the effects of a severe weather event, Hurricane Matthew, were shown to have a great impact at Sapelo Sound. Concentrations of DOC were greatly increased, and DOM had a higher terrestrial and aromatic content directly following the hurricane compared to other months. This demonstrates that hurricanes can impact not only the DOC content but also the molecular composition of DOM potentially influencing its cycling in estuarine environments.

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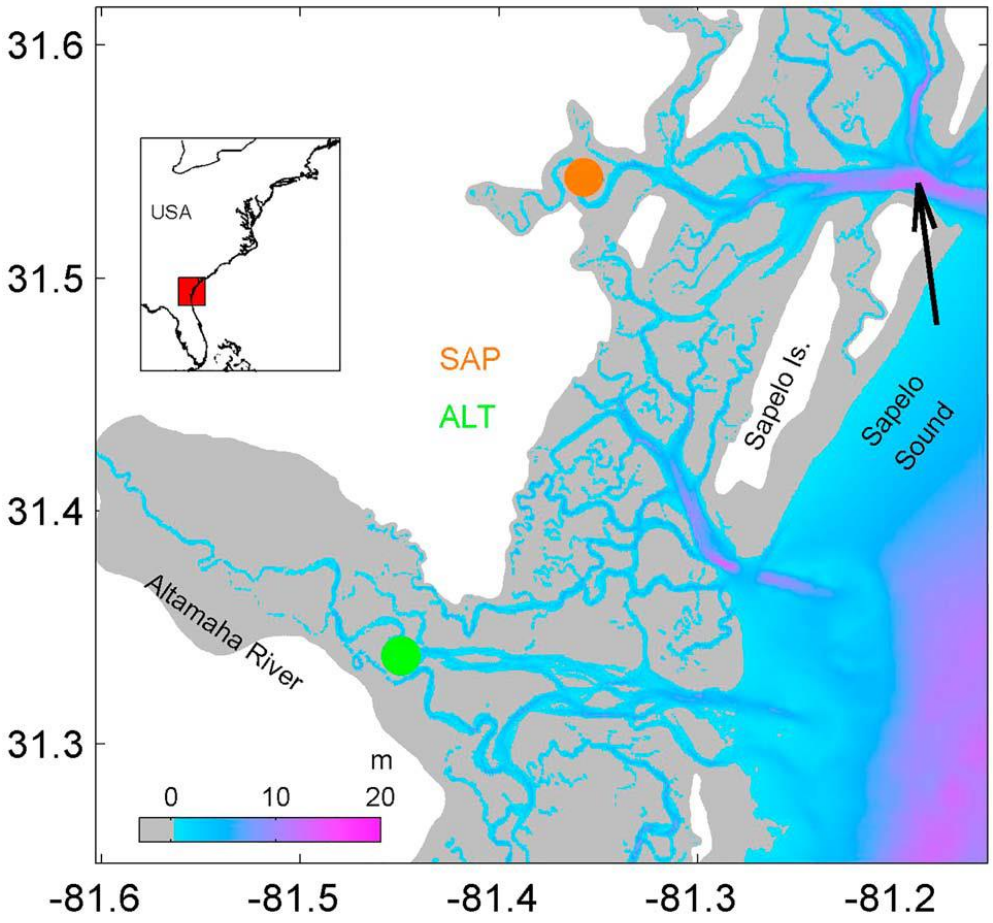

Fig. 2.1 Sampling location at the head of Sapelo Sound (orange circle) and at the Altamaha River (green circle). Salt marshes and uplands are shown in gray and white, respectively. Colors indicate bottom topography.

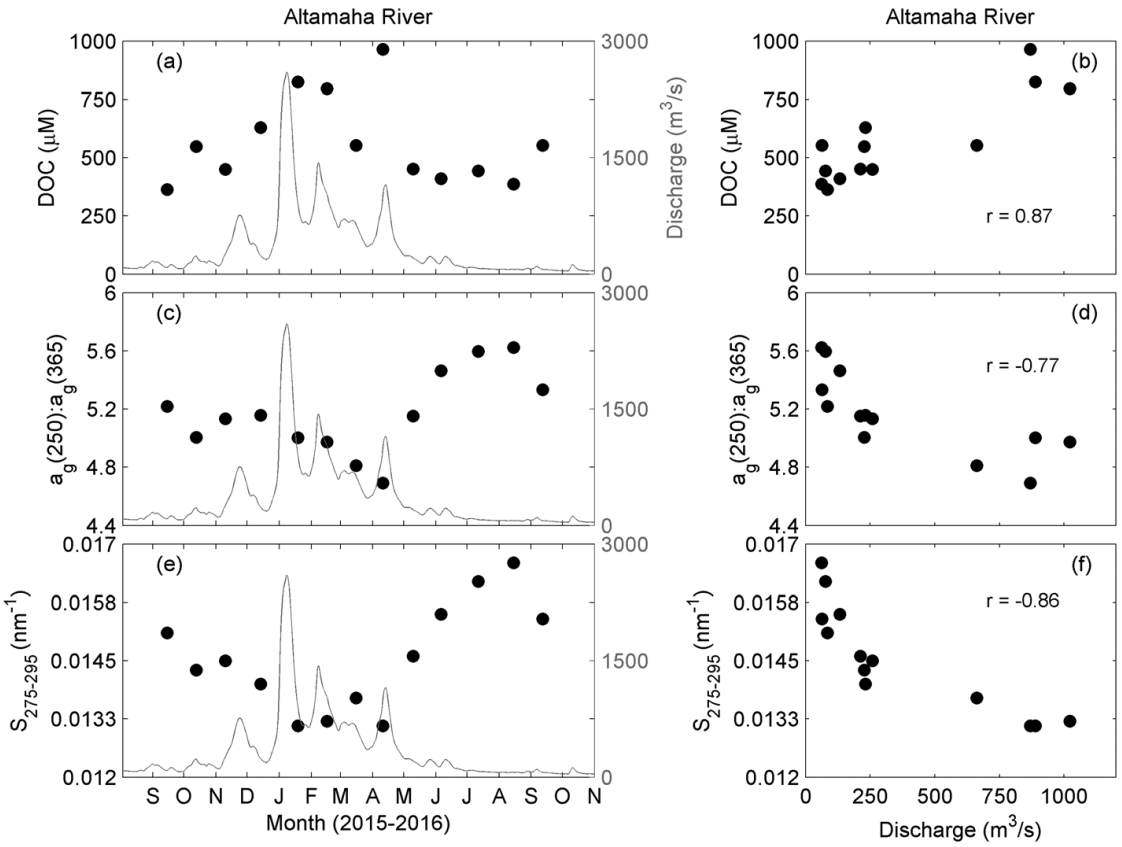

Fig. 2.2 Time series of (a) dissolved organic carbon (DOC) concentration, (c) ratio of absorbance at λ = 250 nm to λ = 365 nm ($a_g(250):a_g(365)$), and (e) spectral slope of absorbance spectra between λ = 275 nm and λ = 295 nm ($S_{275\text{-}295}$) at the Altamaha River. River discharge is shown in gray. Scatterplots and correlation coefficients between parameters and river discharge are shown on the right panels (b, d, and f).

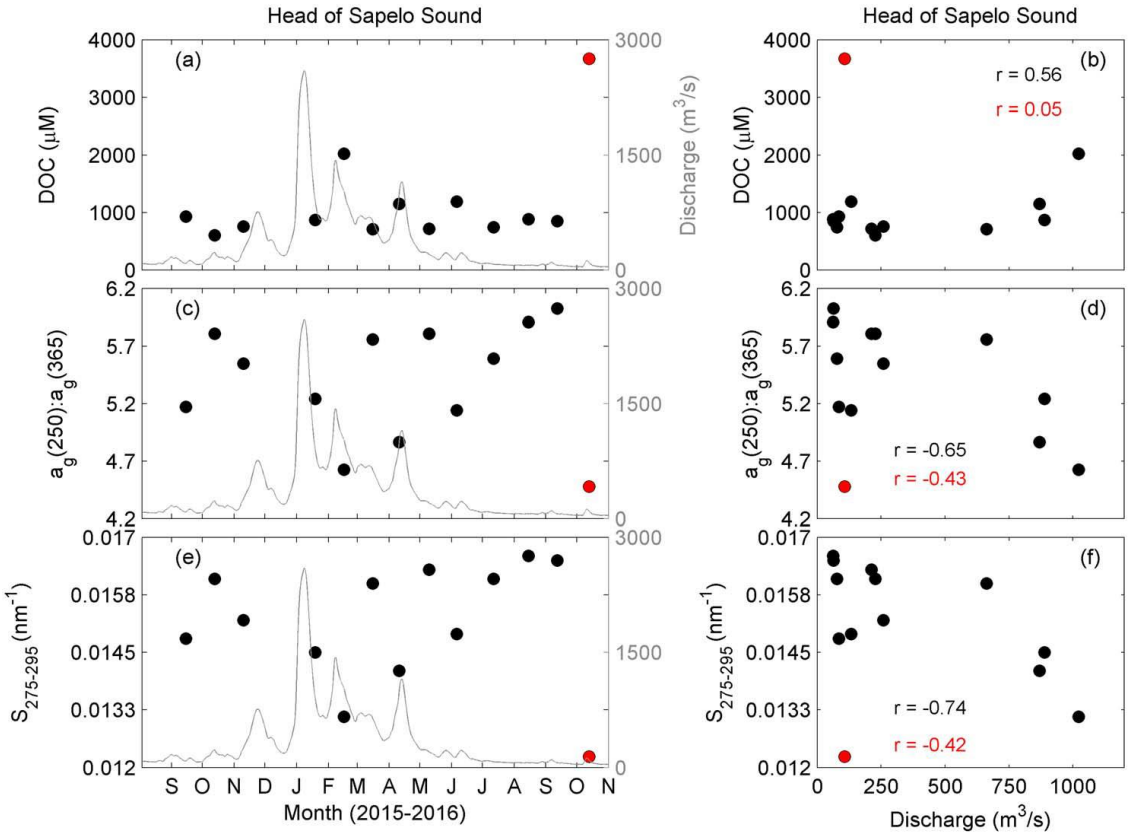

Fig. 2.3 Time series of (a) dissolved organic carbon (DOC) concentration, (c) ratio of absorbance at λ = 250 nm to λ = 365 nm ($a_g(250):a_g(365)$), and (e) spectral slope of absorbance spectra between λ = 275 nm and λ = 295 nm ($S_{275\text{-}295}$) at the head of Sapelo Sound. River discharge is shown in gray. Scatterplots and correlation coefficients between parameters and river discharge are shown on the right panels (b, d, and f). Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Correlation coefficients shown in red include sample collected shortly after the storm.

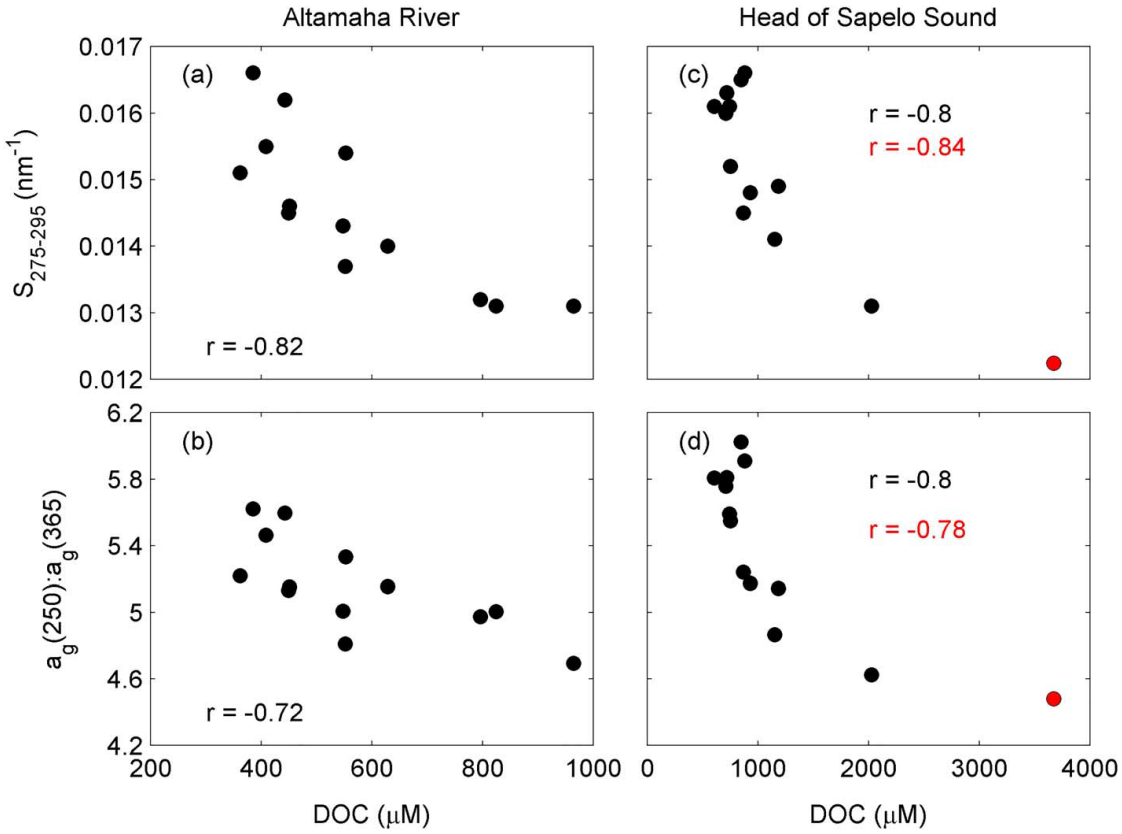

Fig. 2.4 Scatterplot of dissolved organic carbon (DOC) concentration and (a, c) spectral slope ($S_{275-295}$) and (b, d) ratio of absorbance at λ = 250 nm to λ = 365 nm ($a_g(250):a_g(365)$) for (left) Altamaha River and (right) head of Sapelo Sound. Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Correlation coefficients shown in red include sample collected shortly after the storm.

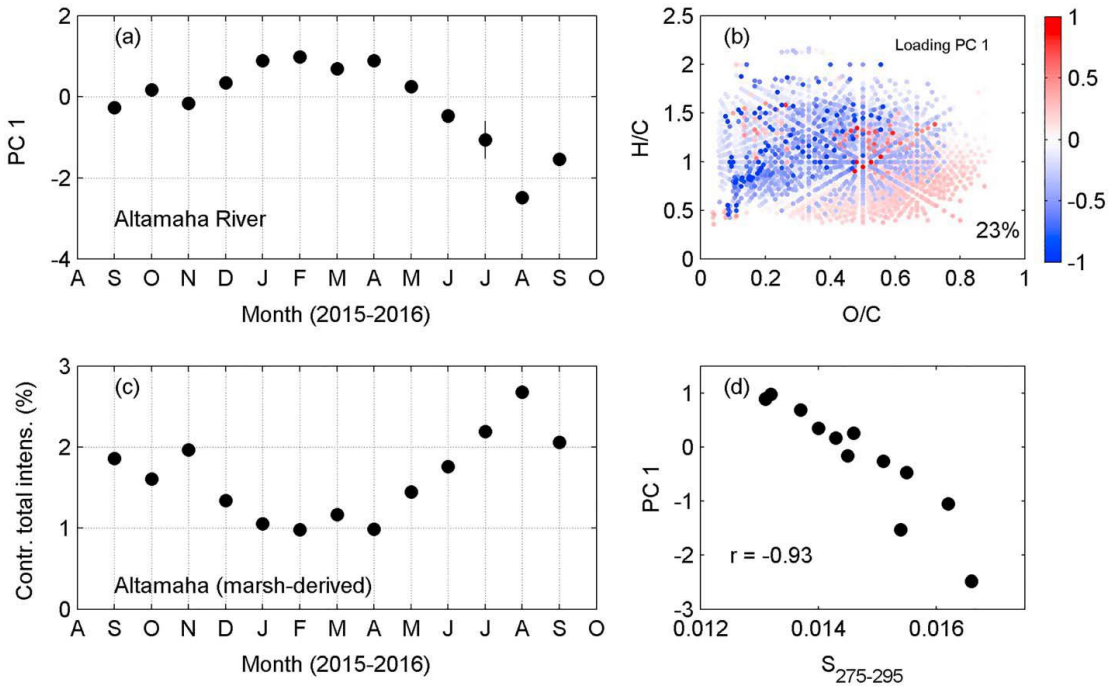

Fig. 2.5 Principal component (PC) analysis of dissolved organic matter (DOM) composition at the Altamaha River. (a) Time series of first principal component. (b) Van Krevelen diagram with loading of PC 1 color coded. (c) Percentage contribution of marsh-derived compounds to the sum of the magnitude of all peaks with molecular formula assigned in Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) spectra. Marsh-derived compounds identified by Medeiros, Seidel, Dittmar, et al. (2015). (d) Scatterplot of spectral slope $S_{275\text{-}295}$ and scores of PC 1 (from panel a).

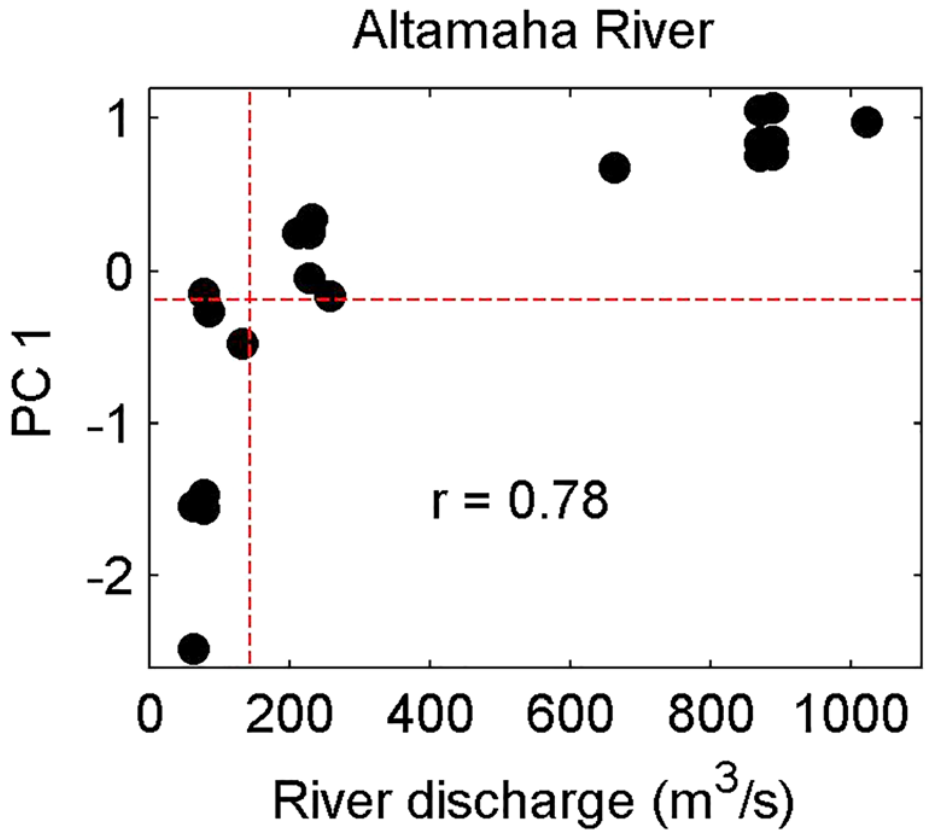

Fig. 2.6 Scatterplot of Altamaha River discharge and scores of first principal component (PC) of dissolved organic matter (DOM) composition at the Altamaha River (from Figure 2.5a). Red dashed lines emphasize point where large change in the slope of the curve is observed.

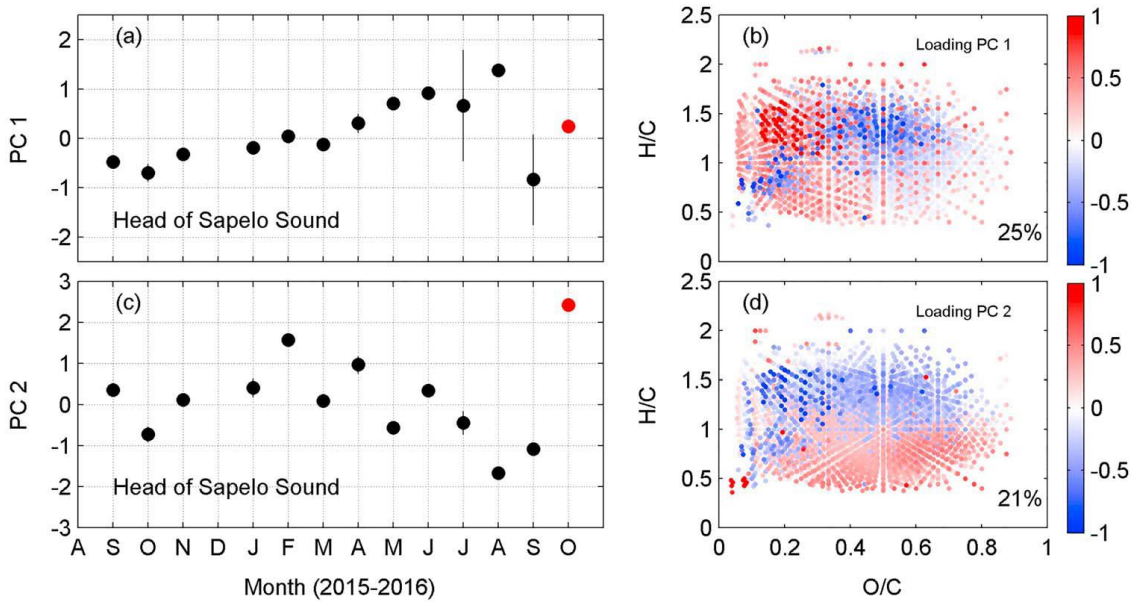

Fig. 2.7 Principal component (PC) analysis of dissolved organic matter (DOM) composition at the head of Sapelo Sound. Time series of (a) first and (c) second principal components. Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Van Krevelen diagrams with loading of (b) PC 1 and (d) PC 2 color coded.

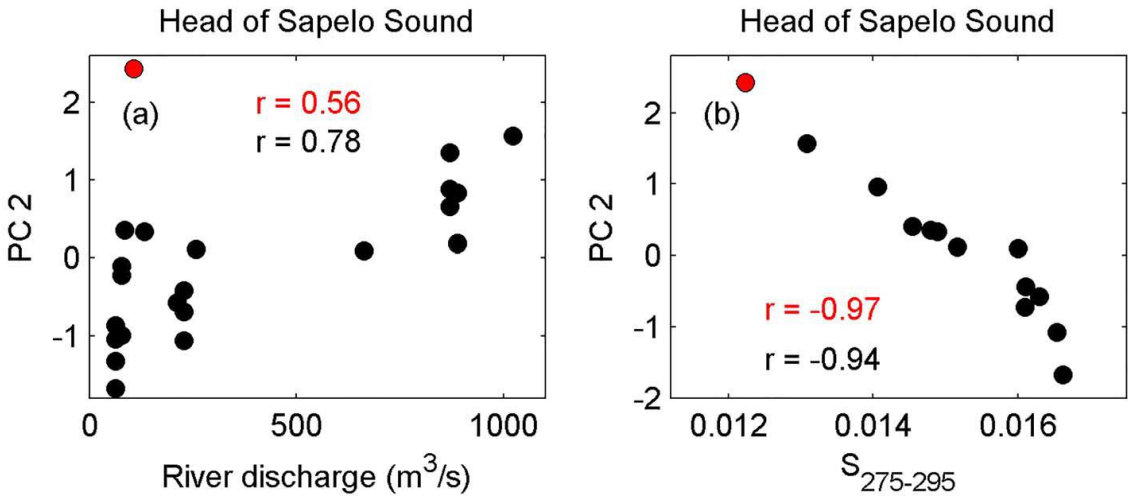

Fig. 2.8 (a) Scatterplot of river discharge and score of PC 2 (from Figure 2.7c) at the head of Sapelo Sound (b) Scatterplot of spectral slope $S_{275\text{-}295}$ and score of PC 2. Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Correlation coefficients shown in red include sample collected shortly after the storm. PC = principal component.

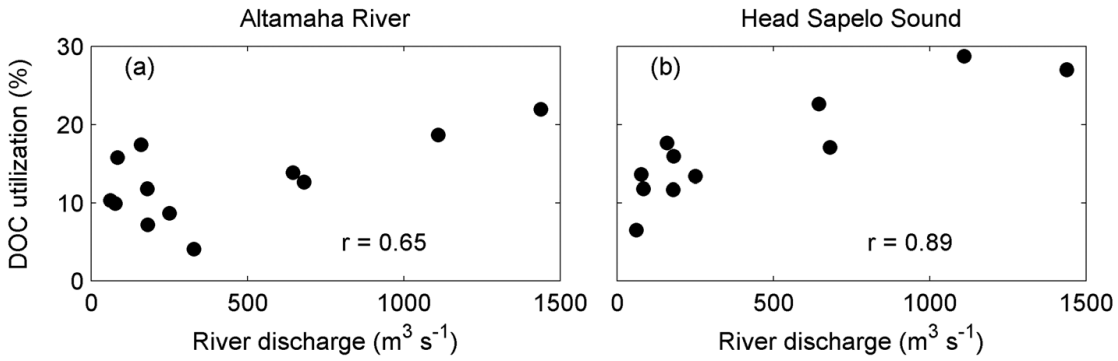

Fig. 2.9 Scatterplot of river discharge and dissolved organic carbon (DOC) utilization (as a fraction of the initial DOC concentration for each incubation; see equation (1)) for each month at the (a) Altamaha River and at the (b) head of Sapelo Sound.

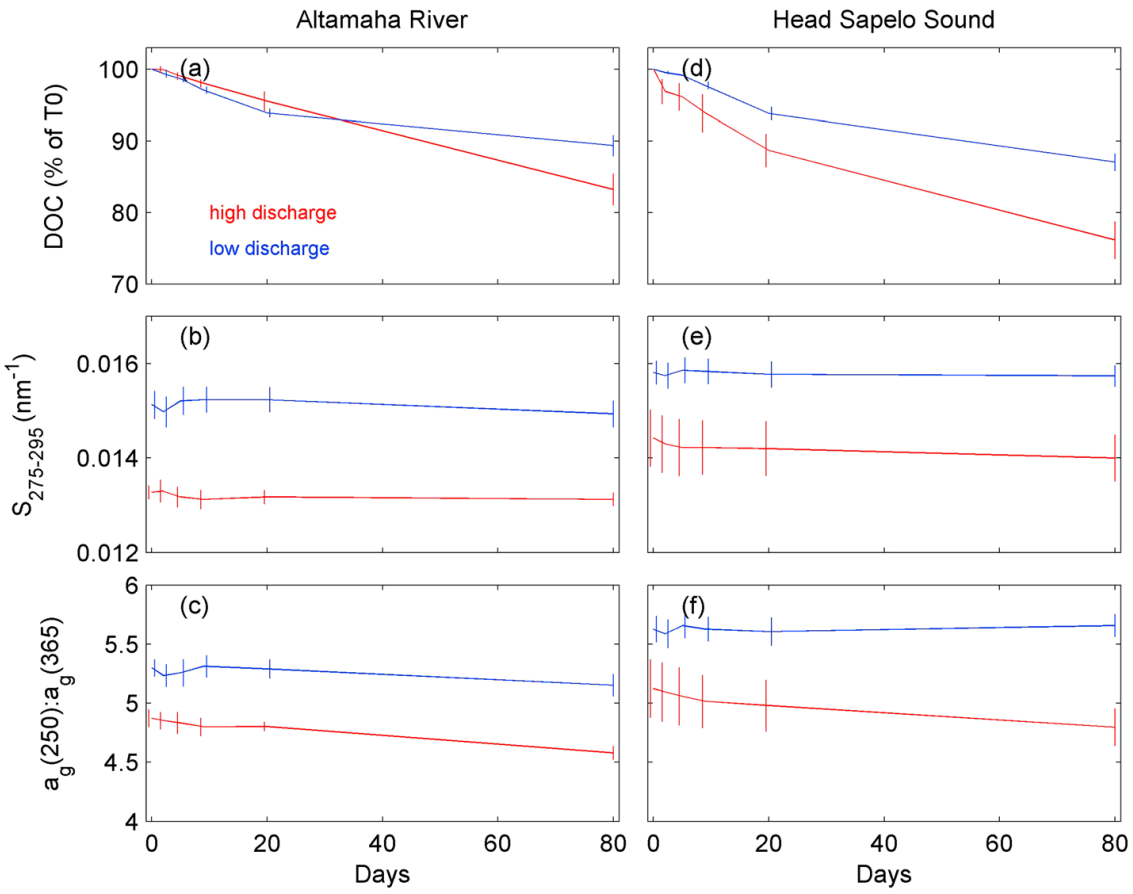

Fig. 2.10 Time series of (a, d) dissolved organic carbon (DOC) concentration, (b, e) spectral slope $S_{275\text{-}295}$, and (c, f) $a_g(250):a_g(365)$ during the course of incubations at the (left) Altamaha River and at the (right) head of Sapelo Sound. Values have been averaged for high (red) and low (blue) discharge conditions. Error bars are standard errors of the mean.

CHAPTER 3

SPATIO-TEMPORAL CHANGES IN DISSOLVED ORGANIC MATTER COMPOSITION ALONG THE SALINITY GRADIENT OF AN ESTUARINE COMPLEX IN THE SOUTHEASTERN U.S.[2]

[2]Letourneau, M.L., Schaefer, S.C., Chen, H., McKenna, A.M., Alber, M., and Medeiros, P.M. To be submitted to *Estuaries and Coasts*.

ABSTRACT

The interconnected estuarine complex of the Altamaha River and the adjacent Altamaha, Doboy and Sapelo Sounds functions as a hotspot for organic matter transformation as it is transported to the Atlantic Ocean. Here, we investigated how dissolved organic matter (DOM) composition changed both spatially and seasonally along the estuarine salinity gradient and how it influenced bacterial processing. Surface samples were collected during high tide at fifteen stations throughout the estuarine complex in April, July, October 2017 and January 2018. Bulk, optical, and molecular level analyses were conducted on initial samples and samples following dark incubations to assess DOM sources and transformation patterns in the system. The most important driver of change in DOM composition was found to be the terrigenous-marine gradient in organic matter sources. Six distinct clusters were identified throughout the system based on the terrigenous signature of the DOM pool. Bacterial consumption of dissolved organic carbon (DOC) was strongly influenced by DOM composition, with increased degradation rates for DOM that had a stronger terrigenous character. The passage of Hurricane Irma in September 2017 resulted in a large influx of terrigenous DOC to the system, likely due to inundation associated with storm surge and increased local precipitation. The storm also resulted in increased DOC biodegradation. These effects lasted for at least one month after the storm, revealing that hurricanes can have a large effect on DOM composition and cycling in coastal systems.

Keywords: DOM composition; FT-ICR MS; Altamaha River; Hurricane Irma; Georgia Coastal Ecosystems LTER

INTRODUCTION

Estuaries are important linkages between terrestrial and marine ecosystems and are key transition zones for carbon cycling. Dissolved organic matter (DOM) is an important component of the carbon pool, and its distribution and composition in estuaries are influenced by many factors namely an assortment of allochthonous sources, including riverine inputs, groundwater discharge, and terrestrial runoff, as well as autochthonous inputs such as phytoplankton excretion (Bianchi 2011). Spatial and temporal variations in these sources create DOM that is a heterogeneous mixture of aromatic and aliphatic compounds unique to each estuary (Bauer and Bianchi 2011).

Off the southeastern U.S., the Altamaha River is the primary source of freshwater to the entire central Georgia coast (Di Iorio and Castelao 2013). The Altamaha River watershed covers 36,718 km^2 and includes part of metro Atlanta, a major urbanized area (Schaefer and Alber 2007). It is characterized by large discharge (average ~ 400 m^3/s) with elevated values in the spring corresponding to higher precipitation levels in these months (Weston et al. 2009). The Altamaha River and estuarine complex, which is the focus of investigations by the Georgia Coastal Ecosystem Long Term Ecological Research (GCE-LTER) program, consists of the Altamaha, Doboy and Sapelo Sounds bordering the mainland and Sapelo Island, and fringing tidal marsh complexes throughout the area (**Fig. 3.1**). Salinity varies from 0 at the Altamaha River at the head of the Altamaha Sound to about 32 near the ocean, and its temporal variability has been shown to be correlated with river discharge (Wang et al. 2017), with system-wide freshening following peaks in river flow (Di Iorio and Castelao 2013). Ocean model simulations have revealed that the network of channels connecting the Altamaha, Doboy and Sapelo

Sounds can play an important role in water exchange between the adjacent sounds (Di Iorio and Castelao 2013) and in the spread of the low-salinity water from the Altamaha River throughout the estuarine complex (Wang et al. 2017). The residence time varies with river discharge, winds, and location, but it is around 2.5 to 8 days in Altamaha Sound, 5 days in Doboy Sound, and up to 2 weeks in Sapelo Sound (Wang et al. 2017).

Variation in Altamaha River discharge was shown to be the main driver controlling changes in the quantity and quality of the dissolved organic carbon (DOC) delivered to the estuary (Letourneau and Medeiros 2019). Another important source of DOC is derived from intertidal salt marshes, which are dominated by the salt marsh cord grass, *Spartina alterniflora*. The DOC released by this vascular plant can be highly labile, with 56-90% of the leached DOC being decomposed by bacteria within a month (Wang et al. 2014) and input of marsh-derived DOC to the system has previously been shown to be important (Moran et al. 1991; Medeiros et al. 2015a; Letourneau and Medeiros 2019).

Coastal Georgia is also susceptible to frequent high-intensity storm events formed in the equatorial North Atlantic Ocean. Severe weather events transfer large amounts of energy to the coast and can drastically alter the organic matter pool both in terms of quantity (Buffam et al. 2001; Inamdar et al. 2006; Yoon and Raymond 2012; Dhillon and Inamdar 2014) and quality (e.g. Miller et al. 2016; Letourneau and Medeiros 2019). For example, a meta-analysis of forested watersheds in the Northeastern U.S. showed that up to 86% of annual DOC loads can be exported during large precipitation events (Raymond and Saiers 2010), increasing the contribution of aromatic DOM compared to baseflow conditions (Yang et al. 2013; Osburn et al. 2019a). Severe storm events can mobilize

large amounts of terrestrially derived organic matter and can quickly transport this material downstream (Raymond et al. 2016; Letourneau and Medeiros 2019).

Characterizing changes in the molecular composition of DOM from different parts of the estuarine complex is important to better understand what changes occur in this organic matter pool and how they vary across spatial and temporal gradients. Here, we built on previous studies that investigated DOM changes in estuarine systems (e.g., Clark et al. 2008; Sleighter and Hatcher 2008; Clark et al. 2018; Hounshell et al. 2019; Letourneau and Medeiros 2019) and in other terrestrial/marine gradients (Ward et al. 2013; Medeiros et al. 2015b; Seidel et al. 2015) by characterizing DOM variability across the entire expanse of the salinity gradient during multiple seasons. We identified and tracked the input of terrigenous DOM from the Altamaha River as it was transported through the estuarine complex, and characterized the effects of Hurricane Irma on local changes in DOM quantity, quality and microbial lability.

MATERIALS AND METHODS

Sample Collection and Filtration

Surface water samples (~ 3-4 L) were collected during high tide at fifteen different stations throughout the Georgia Coastal Ecosystems LTER domain (**Fig. 3.1)** in April, July, October 2017, and January 2018. Immediately before filtration, inorganic nutrients (20 µM Na_2HPO_4; 50 µM NH_4Cl) were added to each sample in order to sustain microbial communities through long-term incubations. Samples were filtered through 2.7 µm Whatman GF/D filters (pre-combusted at 450° C for 5 hours) into acid-washed 1 L polycarbonate bottles to remove photosynthetically active organisms. Initial samples

were then filtered through pre-washed 0.2 µm Pall Supor membrane filters into triplicate 60 mL amber Nalgene bottles for dissolved organic carbon (DOC) and chromophoric dissolved organic matter (CDOM) analyses and were immediately frozen (-20°C) and refrigerated (4°C), respectively. The remaining filtrates (~ 1 L) were acidified to pH 2 (concentrated HCl), and DOM was extracted using solid phase extraction (SPE) with cartridges filled with a styrene divinyl benzene polymer (Agilent Bond Elut PPL) as described by Dittmar et al. (2008) prior to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) analysis. These samples characterized initial sampling conditions, i.e., $T_{initial}$. Additional samples were filtered through 2.7 µm filters and then dark-incubated (in triplicate) at the temperature of collection for 120 days. After the incubations were complete, these samples were filtered through 0.2 µm filters, collected into 60 mL bottles, and stored for DOC and CDOM analyses as previously described.

Altamaha River discharge data were obtained from the U.S. Geological Survey (http://waterdata.usgs.gov) at the nearest monitoring station at Doctortown, GA, roughly 20 km upstream from the GCE 7 sampling site in the Altamaha River.

Bulk Dissolved Organic Carbon

Concentrations of DOC from water samples were measured with a Shimadzu TOC-L_{CPH} analyzer with potassium hydrogen phthalate as a standard. Both internal and Milli-Q water blanks were tested before and interspersed within sample runs on the instrument. Accuracy and precision were tested against the reference material (Hansell 2005) and were better than 5%. SPE extraction efficiency across all samples was 70 $\pm$ 3% of the DOC. The percent DOC consumption was computed as:

$$\text{DOC Consumption} = \frac{\text{DOC}_{Tinitial} - \text{DOC}_{Tfinal}}{DOC_{Tinitial}} \text{ x } 100 \quad (1)$$

where $DOC_{Tinitial}$ is the concentration of DOC in the samples before incubations, and DOC_{Tfinal} is the concentration of DOC in samples after incubations.

Optical Analysis of Chromophoric DOM

Absorbance of chromophoric dissolved organic matter (CDOM) of room temperature samples was measured with an Agilent 8453 UV-visible spectroscopy system. Milli-Q water was used prior to sample measurement to complete blank calibrations to achieve a baseline background level. Absorbance was measured from wavelengths 190 to 1100 nm and was converted to absorption coefficients as in D'Sa et al. (1999). Spectral slope values were calculated for the 275 and 295 nm range with equation 2:

$$\alpha_g(\lambda) = \alpha_g(\lambda_{ref})e^{-S(\lambda-\lambda_{ref})} \quad (2)$$

where $\alpha_g(\lambda)$ is the absorption coefficient at each wavelength of CDOM, λ_{ref} is a reference wavelength of 275 nm, and *S* is the slope fitting parameter (Helms et al. 2008; Spencer et al. 2008). The spectral slope in the 275-295 nm range ($S_{275\text{-}295}$) has been shown to correlate with DOM molecular weight and negatively correlate to terrigenous DOM (Helms et al. 2008; Fichot and Benner 2012). An additional parameter for understanding CDOM characteristics includes $SUVA_{254}$. Weishaar et al. (2003) defined $SUVA_{254}$ as absorbance per unit carbon; larger values can be used as indicators of higher aromatic content. These optical indices were used to track CDOM compositional changes in the system.

FT-ICR MS Analysis

Molecular composition of the DOM in each initial ($T_{initial}$) sample (15 stations in each of the sampling months, April, July, October, and January) was analyzed with a 9.4 T Fourier transform-ion cyclotron resonance mass spectrometer (FT-ICR MS) at the National High Magnetic Field Laboratory in Tallahassee, FL (Kaiser et al. 2011; 2014) following Letourneau and Medeiros (2019). Samples were injected with negative electrospray ionization mode at concentrations of 200 mg C L^{-1} in methanol, and 150 scans were accumulated. Each mass spectrum was internally calibrated based on a "walking" calibration of highly abundant homologous alkylation series that differed in mass by multiples of 14.01565 Da (CH_2) confirmed by isotopic fine structure (Savory et al. 2011), achieving a mass error of < 0.5 ppm. The selected mass range of 150 to 750 Da was used to calculate masses by applying the following restrictions: $^{12}C_{1\text{-}130}\ ^{1}H_{1\text{-}200}\ O_{1\text{-}150}\ ^{14}N_{0\text{-}4}\ S_{0\text{-}2}\ P_{0\text{-}1}$. Molecular formulae assignments were performed by Kendrick mass defect analysis (Wu et al. 2004) with PetroOrg software (Corilo 2014) and the criteria described by Rossel et al. (2013). Mass spectral peaks with a signal-to-noise ratio of 6 or higher were used in the analysis and individual mass spectral peak intensity of each formula was normalized to the sum of peak intensities of the total identified peaks in each sample.

Statistical Analyses

Correlations between bulk (DOC concentrations), optical (spectral slope, $SUVA_{254}$), molecular composition (FT-ICR MS), and environmental conditions (e.g., salinity) were analyzed with Spearman's rank correlation analysis (α level 0.05). The Bray-Curtis dissimilarity coefficient was used to characterize the similarity of molecular

composition in samples both between seasons and throughout the estuarine area. Bray-Curtis values were then used in a cluster analysis to identify six groups of samples that were most similar to each other (Bray and Curtis 1957; Dittmar et al. 2007). We note that repeating the analysis using 5 or 7 clusters produced results similar to those reported here. The peak intensity of molecular formulae for all the sites in each cluster were averaged to obtain a combined spectrum for each cluster. A principal component (PC) analysis was then pursued on the combined spectra in order to better understand the main source of variation in DOM molecular composition between the six clusters. The loading of the PC for each molecular formula is plotted according to its molar ratio of hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) in what is referred to as a van Krevelen diagram. This is useful because the main chemical classes present in DOM have distinct molar ratios, and therefore cluster in specific regions of the van Krevelen diagram (Kim et al. 2003).

RESULTS AND DISCUSSION

The Altamaha River discharge reached its seasonal maximum in late January/early February 2017 (**Fig. 3.2, bottom panel**). The influence of seasonal variations in the Altamaha River discharge on the composition of the DOM delivered by the river to this system has been described in detail in Letourneau and Medeiros (2019). They showed that seasonal variation in river flow is the main control of DOM composition at the river mouth, with the terrigenous signature of the DOM increasing linearly with discharge. Here, sample collection started in April, about 3 months after the

peak in discharge, and we focused on identifying compositional changes throughout the estuarine complex not dominated by the seasonal variability in river flow.

The estuarine complex (**Fig. 3.1**), including the Altamaha River (STA 2, GCE 11, GCE 7) and Sound (GCE 8, GCE 9, GCE AL-2), Doboy (GCE 4, GCE 5, GCE 6, STA 11, GCE 10) and Sapelo Sounds (GCE 1, GCE 2, STA 18, GCE 3), was characterized by strong spatial salinity gradients throughout the year (**Fig. 3.2, top panel**). As expected, considering that the Altamaha River is the main source of freshwater to the system, salinity was consistently lower along the Altamaha Sound and increased in Doboy and Sapelo Sounds farther north. Temporal variability in salinity was quite small over most of the studied area, which was consistent with the small variability in river discharge observed during the sampling period (**Fig. 3.2, bottom panel**).

Bulk DOC and Optical Patterns

The concentration of DOC showed similar patterns within the system throughout the year. Across all seasons, the concentration of DOC was generally highest in the most upstream site in Sapelo Sound (**Fig. 3.3, Table B.1**). Increased DOC concentrations were also observed along the Altamaha River and the upstream half of the Altamaha Sound, while sites located closer to the ocean in all three sounds were characterized by lower concentrations. Temporally, DOC concentrations throughout the domain were highest in October 2017 and lowest in January 2018. At the head of Sapelo Sound (GCE 1; see **Fig. 3.1** for location), DOC concentration was about 60% higher in October 2017 (~ 1100 µM, **Table B.1**) compared to other seasons (average of ~ 700 µM).

The seasonal and spatial changes in DOC concentration throughout the system gradient were accompanied by changes in DOM composition as determined by optical properties. In all seasons the spectral slope ($S_{275\text{-}295}$) of CDOM absorption was lowest (indicating more terrigenous DOM; Del Vecchio and Blough 2002; Helms et al. 2008) in the Altamaha River and Sound sites and at the head of Sapelo Sound, and were highest (less terrigenous) in sites farther from the river (**Fig. 3.4**, **Table B.2**). The distribution of $SUVA_{254}$ revealed a pattern consistent with that of the DOC concentrations. Higher values, which are associated with a larger aromatic content (Weishaar et al. 2003), were observed along the Altamaha River, the upstream half of the Altamaha Sound, and near the uplands (**Fig. B.1, Table B.3**), which suggested a stronger contribution of terrigenous material to the DOM pool at these locations.

Despite scatter, there was a general tendency for spectral slope values to increase with increasing salinity, which was observed throughout the entire system ($r = 0.78$, $p < 0.05$; **Fig. 3.5**), indicating that the terrigenous signature of DOM was correlated to freshwater content. In most instances, for a given salinity range the DOM composition in October 2017 had a more terrigenous character (lower spectral slope) compared to the DOM composition during other seasons. Thus, even though there was not a large change in river discharge or salinity (**Fig. 3.2**) in the system in October 2017 compared to the other sampling periods, the amount of DOC (**Fig. 3.3**) and the terrigenous signature of the DOC (**Fig. 3.4**) increased throughout the region. This suggests that the elevated concentration of terrigenous DOM in this month was not delivered to the system by the river. One possible explanation for the input is the passage of Hurricane Irma, which affected the study region about one month prior to the October 2017 sampling. Indeed,

higher concentrations of more terrigenous DOM following storm events have been observed in other systems previously (Hood et al. 2006; Hernes et al. 2008; Vidon et al. 2008; Wagner et al. 2019). Peak water level associated with the storm surge due to Hurricane Irma was 1.45 m past Mean Higher High Water on the Georgia coast (measured at Fort Pulaski, GA approximately 70 km to the north of the collection region) and 1.7 m higher than the predicted tides, which resulted in substantial flooding in many coastal islands (Cangialosi et al. 2018). As the water level retreated after inundation of coastal areas and marshes during the storm surge, it is possible that organic matter previously stored in those inundated areas was introduced into the system. This would result in a large input of terrigenous DOM to the estuary without significant changes in the observed salinity, which is consistent with our observations. Increased precipitation and the storm surge may also have increased the release of DOM from the marsh plant *S. alterniflora*, which is the dominant marsh plant species in the area (Wieski and Pennings 2014). *S. alterniflora* has been shown to release up to fifteen times more DOC when submerged in water than when above the waterline (Turner 1993). This effect may have also contributed to the large increase in DOC concentration observed throughout the studied area in October 2017 (**Fig. 3.3**).

Spatial and Temporal Variability in DOM Composition at the Molecular Level

We used cluster analysis of the FT-ICR MS data based on Bray-Curtis dissimilarity values (Bray and Curtis 1957; Dittmar et al. 2007) to identify six distinct groups of sampling sites within the estuarine salinity gradient according to their DOM composition (**Fig. 3.6**). Some groups were only seen in a single month, while others were

ubiquitous throughout the seasons. These six groups helped identify different regions in the estuary based on the molecular composition of the DOM found at each site and showed how these regions differed seasonally and spatially.

A principal component (PC) analysis of the combined spectrum for each cluster revealed the dominant mode of variability in DOM composition among the six clusters. The van Krevelen diagram of the dominant PC showed strong positive loadings (in red) for low H/C values, and strong negative loadings (in blue) in the higher H/C area (**Fig. 3.7**, right) indicating a pattern of changes in DOM composition that is typical of a gradient of terrigenous to marine organic matter contributions (Medeiros et al. 2015a,b; 2017a,b). While formulae with low H/C ratios are generally enriched in terrigenous DOM, marine DOM is generally enriched in formulae with high H/C ratios (Sleighter and Hatcher 2008). The PC scores for the dominant mode for each cluster were plotted in decreasing order (**Fig. 3.7**, left) using the same rainbow palette used in **Fig. 3.6**. Thus, clusters with positive scores (clusters 1 and 2; **Fig. 3.7**, left) were characterized by a stronger terrigenous signature, while clusters with negative scores (clusters 4-6) were characterized by a more marine signature. This indicated that stations along the estuary clustered according to their terrigenous-marine signature (**Fig. 3.6**) and that the analysis captured the distribution of the terrigenous material in the estuarine complex and how it mixed with marine DOM in each season.

In April 2017, most sites were characterized by having a large marine influence (clusters 5 and 6; **Fig. 3.6**). The only exception was the upper half of Altamaha Sound, which was dominated by terrigenous DOM (cluster 2). A similar pattern was observed in July 2017, with the exception that the signature of the terrigenous material could be

observed farther downstream along Altamaha Sound (cluster 3 at GCE 9; see **Fig. 3.1** for location) and at the mouth of Doboy Sound (cluster 4 at GCE 6). It is interesting that sites farther upstream of the mouth of Doboy Sound (GCE 5) or further offshore (GCE AL-2) in July 2017 had a larger marine influence (cluster 6) than the mouth itself (cluster 4). This may be due to the high degree of connectivity that exists between the Altamaha and Doboy Sounds (Wang et al. 2017), as terrigenous material introduced into the system by the Altamaha River was likely directly transported from the Altamaha Sound toward the mouth of Doboy Sound through the network of creeks and channels that connect those two sounds.

Consistent with the optical analyses described above (**Fig. 3.4**), in October 2017 the entire region was categorized into clusters representing terrigenous DOM (**Fig. 3.6**). In the Altamaha River sites and at the head of Sapelo Sound, in particular, the DOM had the strongest terrigenous signature observed throughout the study (cluster 1). The more marine clusters that were observed earlier in the year (clusters 4 to 6) were absent from the entire estuarine complex. Three months later, in January 2018, the terrigenous signature had decreased and some of the marine clusters were again observed, especially at sites with more oceanic influence.

Collectively, the analyses revealed that the Altamaha River and Sound were characterized by small temporal variability in DOM composition, with the terrigenous clusters being consistently present throughout the year. Since the terrigenous signature of the DOM introduced into the system by the river is linearly related to discharge (Letourneau and Medeiros 2019), this may be related to the low variability in river flow observed during the study (**Fig. 3.2**), which resulted in roughly the same hydrologic

conditions throughout the sampling months. In contrast, sites located in Doboy and Sapelo Sounds presented higher temporal variability in DOM composition, in part due to the large input of terrigenous DOM observed in October 2017.

The increased DOC concentrations (**Fig. 3.3**) and increased terrigenous signature of the DOM pool observed in October 2017 (**Figs. 3.4** and **3.6**) throughout the estuarine complex were presumably associated with the passage of Hurricane Irma one month before. At the head of the Altamaha Sound (GCE 7, see **Fig. 3.1**), long-term observations from the GCE-LTER project reveal that DOC concentrations were ~ 390 μM in August and early September, increased to ~ 1300 μM in September 15th shortly after the passage of Hurricane Irma, and progressively decreased to pre-storm concentrations (~ 410 μM) about 1.5 months later in early November. A similar increase in the terrigenous DOC content at the head of Sapelo Sound (GCE 1, see **Fig. 3.1**) was observed in October 2016 a few days after the passage of Hurricane Matthew, while the terrigenous DOC content in October 2015, when the estuarine complex was not influenced by hurricanes, was much lower (Letourneau and Medeiros 2019). CDOM and stable carbon isotope analyses in North Carolina's coastal waters following the passage of Hurricane Matthew in 2016 have indicated that the estuary was dominated by terrigenous DOM sources for several months after the storm (Osburn et al. 2019b).

A puzzling aspect for the Altamaha River and estuarine complex, however, is that the one-month delay between the passage of Hurricane Irma and the sampling in October 2017 is longer than the estimated residence time of the system. Modeling studies suggest that, for low river discharge and winds typical of fall conditions, the residence time at the head of the Altamaha Sound is around 6-8 days, and it is around 12-14 days at the head

of Sapelo Sound (Wang et al. 2017). A possible explanation is that, when estimating residence time, Wang et al. (2017) considered the time for a water parcel to leave the estuary for the first time, not accounting for subsequent reentries due to the tidal nature of estuaries (De Brauwere et al. 2011). Therefore, some of the terrigenous material introduced into the system following the storm may have been transported out of the estuary into the coastal ocean and then advected back into the system by tidal currents, increasing the effective residence time. Wang et al. (2017) residence time estimates are also valid for water parcels already in the estuarine channels, while there may have been a delay between the passage of the hurricane and the influx of the organic matter into the system (e.g., time associated with rainfall to flow as surface runoff or as groundwater into the estuarine channels, or for water to wash out from the marsh after the storm surge).

Spatial and Temporal Variability in Microbial Consumption of the DOC

Patterns of microbial DOC consumption throughout the study area varied seasonally, with lowest degradation being observed in April and highest in October 2017, one month after the passage of Hurricane Irma (**Fig. 3.8**). Microbial consumption of DOC was also generally higher in the Altamaha River sites and at the head of the Altamaha Sound as compared to Sapelo and Doboy Sounds. In the Altamaha Sound, consumption generally decreased toward the coast, which could be related to the degradation of the more labile DOM as it moved downstream (Fasching et al. 2014).

Microbial degradation of DOC is often related to DOM composition (Moran et al. 2016). Indeed, the percent consumption of DOC throughout the system was significantly negatively correlated to the spectral slope ($S_{275\text{-}295}$) of CDOM absorption (**Fig. 3.9**, left),

indicating higher biodegradation in samples characterized by DOM with stronger terrigenous signatures (i.e., lower $S_{275\text{-}295}$ values). This is consistent with results from analysis pursued at the molecular level. Correlation coefficients between the intensity of each molecular formula and the percentage of DOC consumed for each sample were plotted according to their molecular H/C and O/C ratios. The analysis revealed that increased DOC consumption occurred for samples relatively enriched in formulae characterized by low H/C ratios (shown in red in **Fig. 3.9**, right) and relatively depleted in formulae with high H/C ratios (shown in blue). The pattern is similar to that described previously as typical of gradients in terrigenous and marine sources of DOM (Medeiros et al. 2015a,b; 2017a,b; see also **Fig. 3.7**, right), indicating that larger DOC consumption was observed in samples with a stronger terrigenous signature.

Increased microbial consumption of DOC during periods when the DOM had a stronger terrigenous signature has been previously observed at the head of Sapelo Sound (GCE 1) and at an upstream site at the Altamaha River (GCE 7) (Letourneau and Medeiros 2019). Our results are consistent with those observations and reveal that this relationship extends for the entire estuarine complex (**Fig. 3.9**, left). Consumption of DOC was particularly strong in October 2017 following the passage of Hurricane Irma (**Fig. 3.8)**, presumably because of the input of fresh, labile, terrigenous DOM into the system. Enhanced aromatic carbon mineralization rates and higher lability of terrigenous DOM have been observed in other regions following large precipitation events (Fellman et al. 2009; Guo et al. 2014), as flooding of coastal wetlands can mobilize a large pool of labile DOM that is stored in forested wetlands (Osburn et al. 2019a). We note that increased DOC degradation when DOM had a stronger terrigenous signature (**Fig. 3.9**,

left) does not necessarily imply that most of the material degraded was terrigenous in nature. It is possible that additional (non-terrigenous) labile DOM was introduced into the system following Hurricane Irma, including components of the DOM pool that fall outside our analytical window (Vorobev et al. 2018).

CONCLUSIONS

The Altamaha River and estuarine complex around Sapelo Island off the U.S. East Coast is characterized by strong gradients in DOM composition and dynamics. During the study period, the Altamaha River provided large inputs of DOM with a strong terrigenous signature to the system. Dissolved organic matter decreased in both concentration and terrigenous content as it was transported downstream towards the Atlantic Ocean. The gradient from terrigenous to marine inputs was shown to be the most important driver in the variability of DOM composition across all seasons. While DOM in the Altamaha River and upstream half of the Altamaha Sound had a terrigenous signature year-round, DOM composition at Doboy and Sapelo Sounds presented large seasonal variability, with a clear marine signature during parts of the year. In all seasons, DOC consumption was influenced by DOM composition, with higher utilization observed for DOC with larger terrigenous content.

A large input of terrigenous DOC was observed in October 2017, presumably as a result of storm surge from Hurricane Irma which occurred ~ 30 days before the seasonal sampling. The apparent persistence of the hurricane influence on that time scale was surprising, considering the relatively shorter residence time of the system. Sampling the estuary with higher temporal resolution after the passage of strong storms would allow

for the time scale of influence of extreme events on DOM dynamics to be better quantified. The input of terrigenous material was likely associated with the storm surge and the inundation of coastal areas surrounding the estuarine complex, rather than being primarily delivered by the river. Since our sampling did not capture the period of peak river flow that occurred earlier in the year, it is not clear if the composition of the terrigenous DOM introduced into the system following the passage of Hurricane Irma (which we hypothesize was related to the storm surge) was different from the composition of the terrigenous DOM introduced seasonally into the system by the Altamaha River during high discharge conditions. Future studies focusing on that comparison on the molecular level would contribute to clarifying the role of extreme events on DOM composition and processing in estuaries. Lastly, more work is needed to better understand the factors leading to microbial preferences within the DOM pool and how these may vary depending on environmental conditions.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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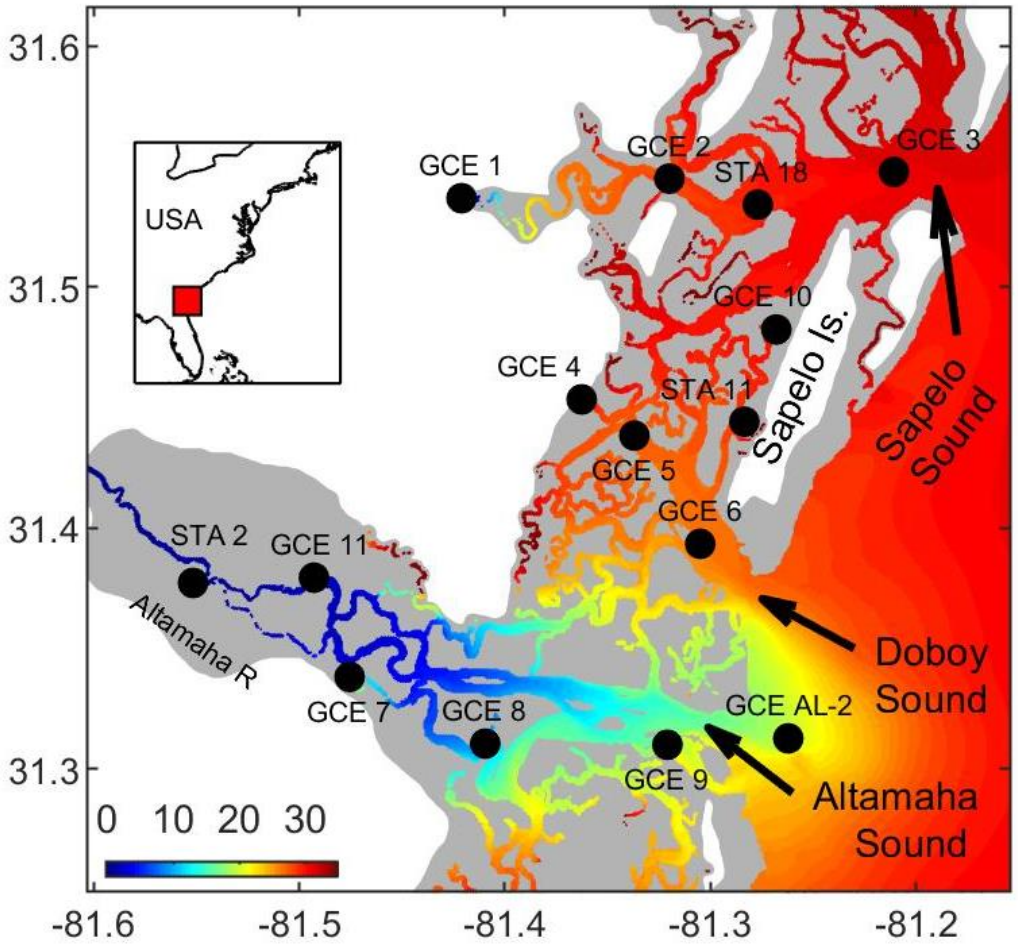

Fig. 3.1 Sampling locations along the Altamaha River and estuarine complex, which includes the adjacent Altamaha, Doboy and Sapelo Sounds. Uplands and salt marshes are shown in white and gray, respectively. Colors represent a one-year average of surface salinity from a numerical model simulation (Wang et al. 2017) for 2008, and are shown here to provide information on the typical distribution of freshwater in the system.

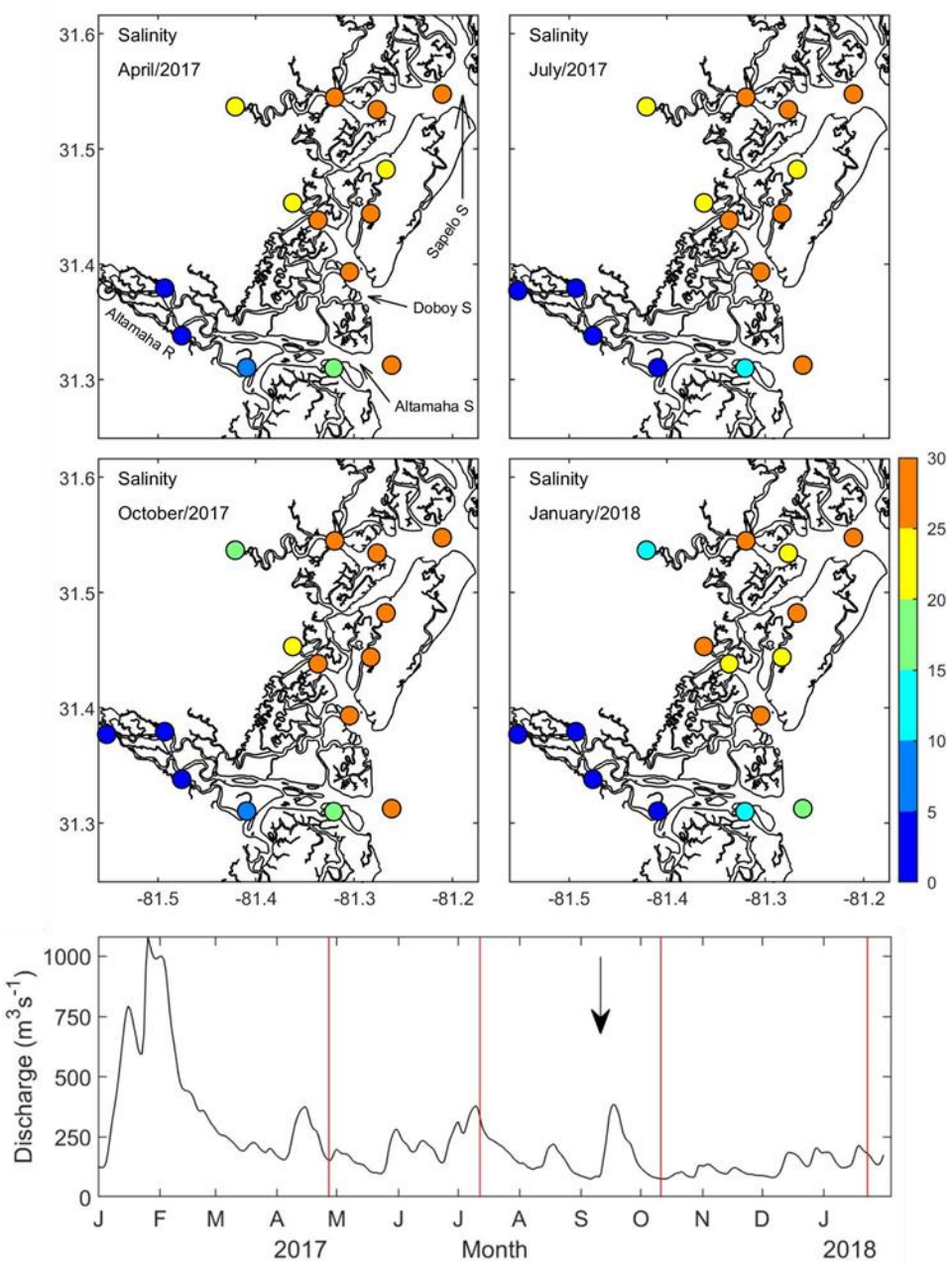

Fig. 3.2 (top) Surface salinity in April, July, October 2017, and January 2018; (bottom) time series of Altamaha River discharge at Doctortown, Georgia, USA. Vertical red bars indicate timing of sample collection. Black arrow indicates passage of Hurricane Irma.

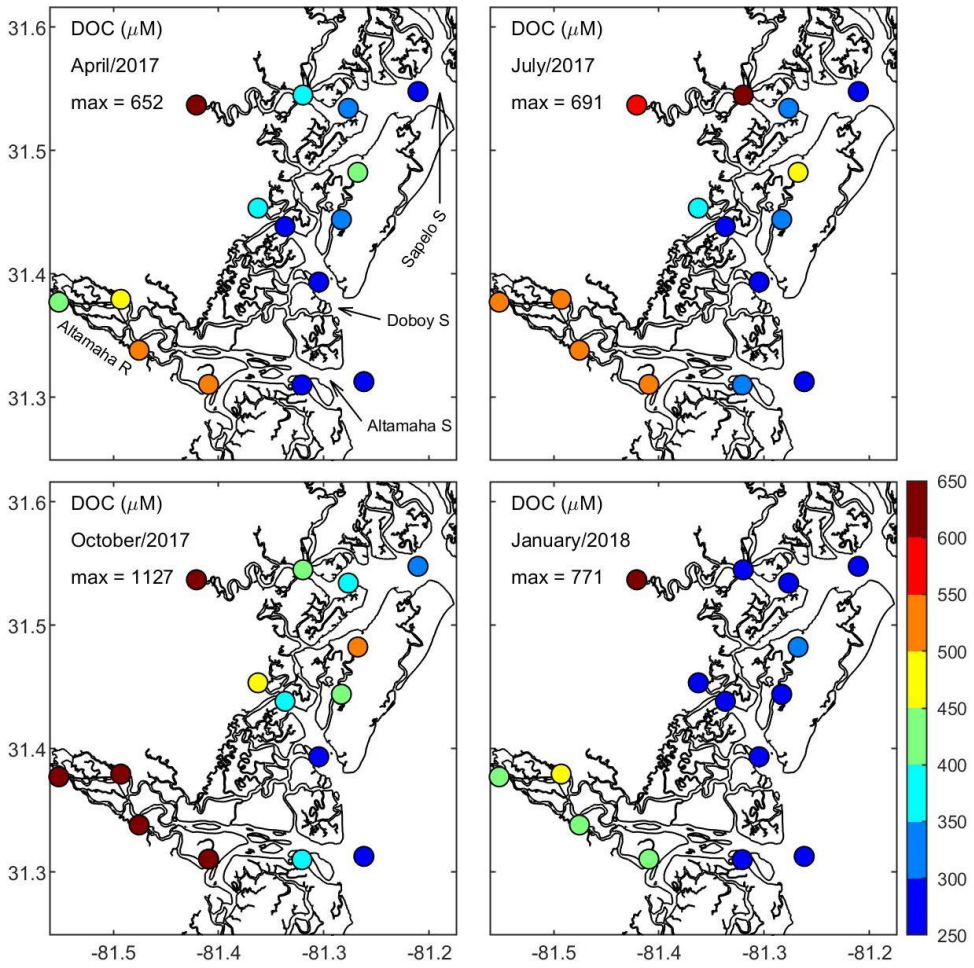

Fig. 3.3 Dissolved organic carbon (DOC) concentrations in April, July, October 2017, and January 2018. DOC higher than 650 μM is shown in red-brown to reveal as much as possible of the spatial variability in the system. Max = maximum DOC concentration.

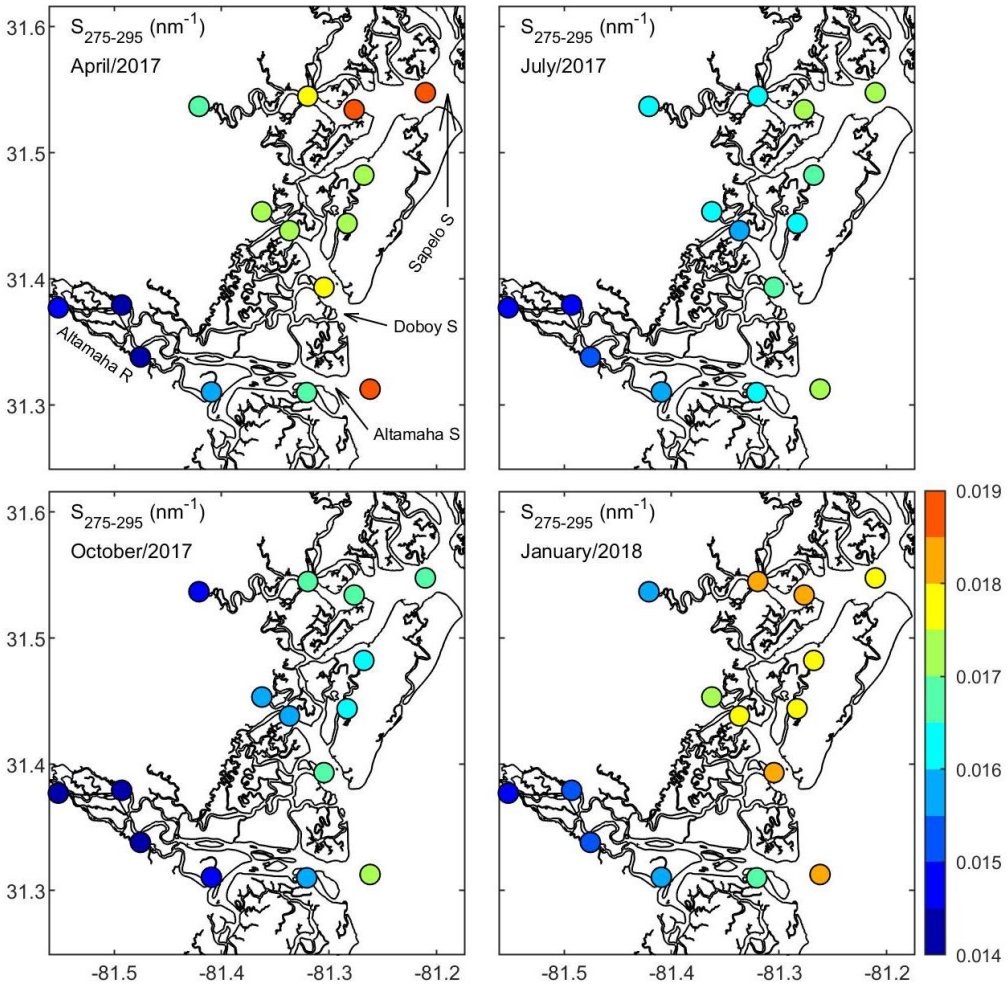

Fig. 3.4 Spectral slope of CDOM absorbtion between 275 and 295 nm ($S_{275\text{-}295}$) in April, July, October 2017, and January 2018. Low $S_{275\text{-}295}$ indicates higher terrigenous content.

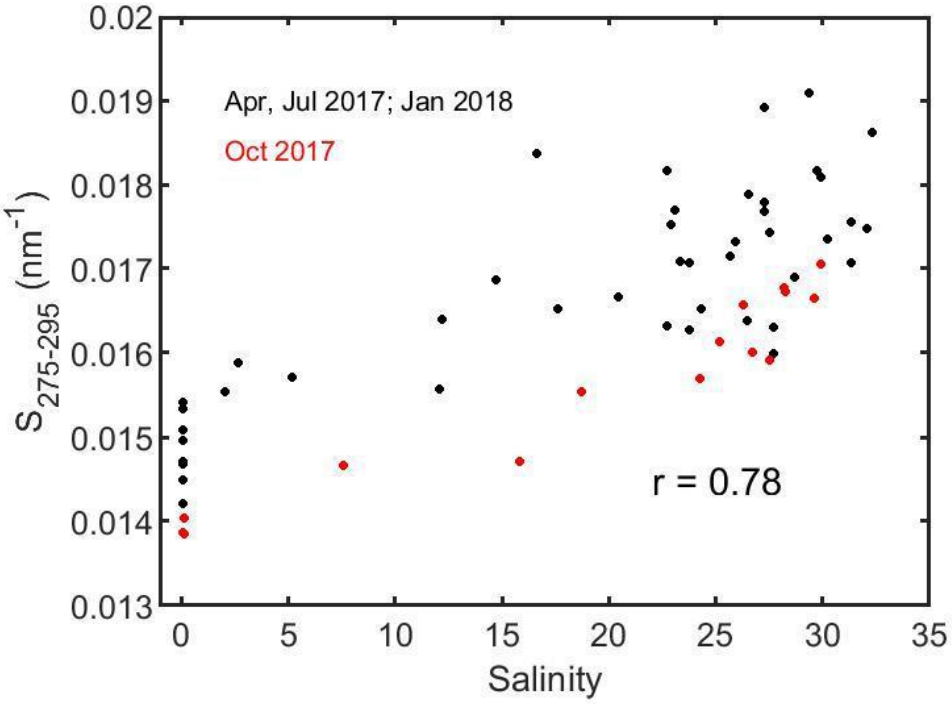

Fig. 3.5 Scatterplot of salinity versus spectral slope of CDOM absorbtion ($S_{275\text{-}295}$) for all samples. Samples collected in October 2017 after the passage of Hurricane Irma are shown in red. The correlation coefficient (*r*) is also shown.

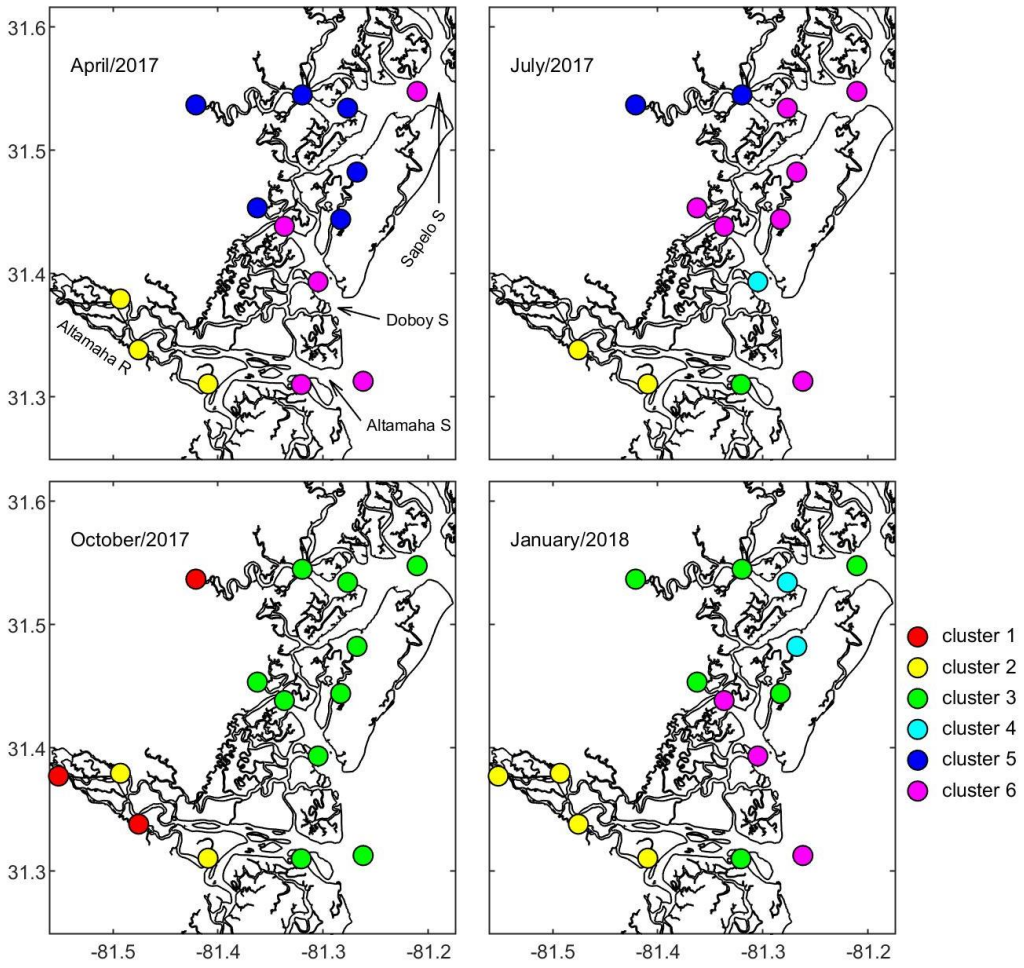

Fig. 3.6 Cluster analysis based on FT-ICR MS data in April, July, October 2017, and January 2018. Samples shown with the same colors across the different panels are characterized by similar DOM compositions. Clusters are numbered from 1 to 6, according to their terrigenous signature (see Fig. 3.7) using a rainbow palette. Cluster 1 captures samples with the strongest terrigenous signature.

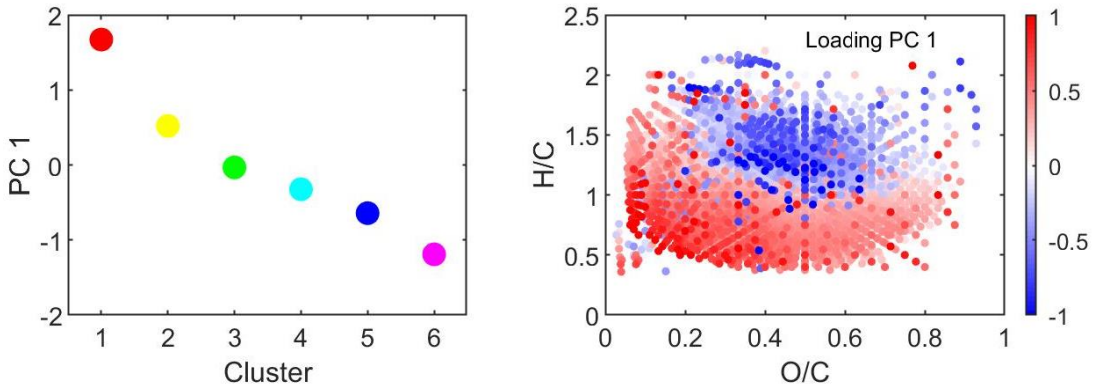

Fig. 3.7 Principal component analysis of DOM composition based on combined FT-ICR MS spectra for the different clusters identified in Fig. 3.6. (left) PC 1 scores for the various clusters identified are shown, using the same color scale used in Fig. 3.6. (right) van Krevelen diagram with loadings of PC 1.

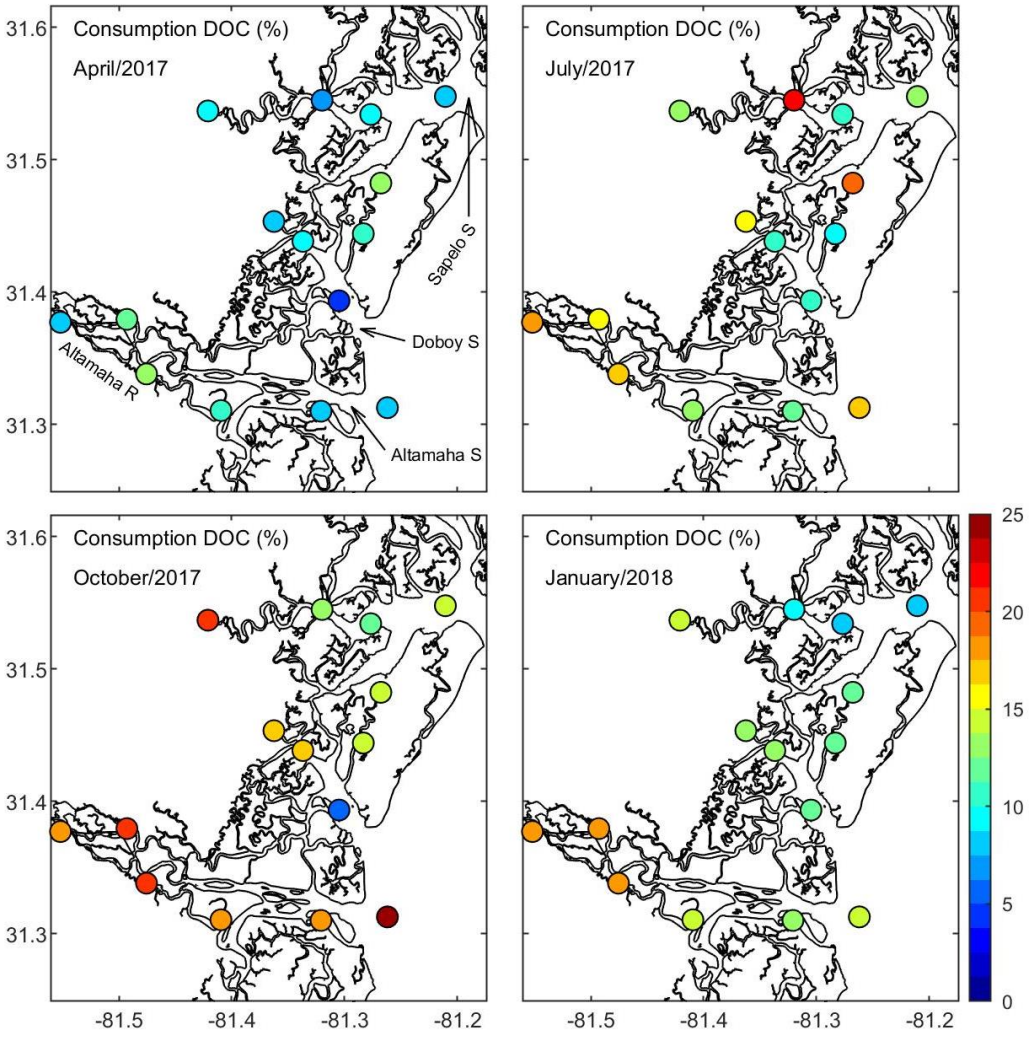

Fig. 3.8 Percent consumption of DOC throughout the estuarine area in April, July, October 2017, and January 2018, calculated with Eq. 1.

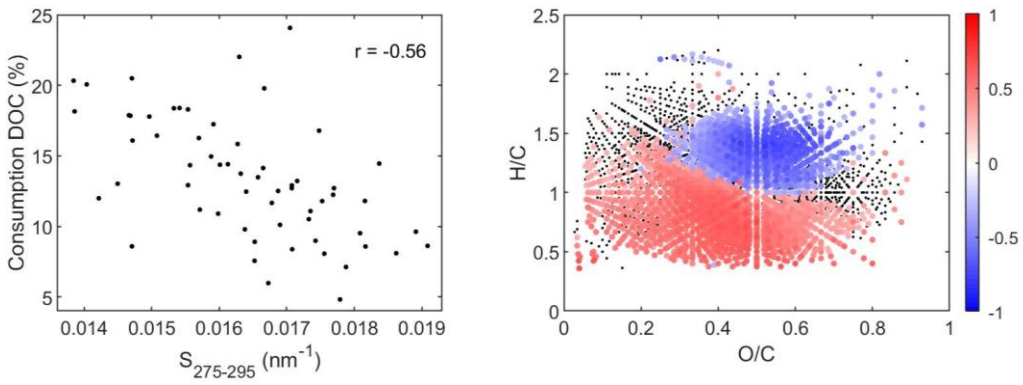

Fig. 3.9 (left) Scatterplot of spectral slope of CDOM absorbtion ($S_{275\text{-}295}$) versus consumption of DOC, with the correlation coefficient (r) also shown; (right) van Krevelen diagram color coded with the correlations between relative abundance of each molecular formulae and consumption of DOC. High DOC consumption occurs when the DOM is enriched with the formulae shown in red and depleted with formulae in blue. Black dots show formulae whose correlations with DOC consumption are not statistically significant.

CHAPTER 4

MOLECULAR COMPOSITION AND BIODEGRADATION OF LOGGERHEAD SPONGE *SPHECIOSPONGIA VESPARIUM* EXHALENT DISSOLVED ORGANIC MATTER[3]

[3]Letourneau, M.L., Hopkinson, B.H., Fitt, W.K., and Medeiros, P.M. To be submitted to *Coral Reefs*

ABSTRACT

Marine sponges are critical components of the marine reef environment, particularly in the Florida Keys, USA, due to their high filtering capacity, wide abundance, and alteration of biogeochemical cycling. Sponges have been shown to actively remove dissolved organic carbon (DOC) from ambient seawater through filtration and produce particulate organic carbon through a process termed the sponge loop. Although studies have shown that the sponge-microbial holobiont can actively consume DOC, there is little information on the molecular composition of the DOC that is inhaled and the transformations the organic matter undergoes as it is released. Here, we characterize dissolved organic matter (DOM) composition in the sponge-microbial holobiont exhalent seawater of a loggerhead sponge (*Spheciospongia vesparium*) and in the ambient seawater collected in Florida Bay (USA), as well as the microbial responses to each pool of DOM through dark microbial incubations. The results indicate that the sponge-microbial holobiont removed 6% of the seawater DOC, actively utilizing compounds that were low in carbon and oxygen content, yet high in nitrogen content relative to the ambient seawater. Over a 5-day incubation period, the microbial community was able to access ~ 7% of DOC from the ambient seawater but only 1% of DOC from the sponge exhalent seawater, suggesting a decrease in lability, possibly due to holobiont removal of nitrogen-rich compounds. These results could have far reaching implications for the Florida Keys hard bottom community and further shows that the sponge holobiont can actively utilize DOM and influence carbon cycling in reef ecosystems.

Keywords: DOM composition; Biodegradation; FT-ICR MS; Loggerhead sponge; Florida Bay.

INTRODUCTION

Sponge-microbial holobionts are key components of coral reef communities due to their high filtering capacity and ability to influence biogeochemical cycling on the reef (Ribes et al. 2005; Rix et al. 2017). Corals and macroalgae release up to 50% of their fixed carbon (Tanaka et al. 2008; Haas et al. 2010) of which up to 80% is in the form of dissolved organic matter (DOM) (Wild et al. 2004), which can be taken up by various sponge species on the reef community (Yahel et al. 2003; de Goeij et al. 2008a; de Goeij et al. 2013; Mueller et al. 2014). Sponges have been observed to take up dissolved organic carbon (DOC) and transform it into particulate organic carbon (POC), which is then accessible to higher trophic levels through the sponge loop (de Goeij et al. 2013). At least some of the DOM is processed by filter-feeding cells of the sponge through pinocytosis without mediation of resident bacteria (Achlatis et al. 2019). Through the sponge loop, sponges participate in reef biogeochemical cycling as a sink and modifier of DOM (de Goeij et al. 2013).

Different species of sponges in various reef environments have shown affinity for DOC uptake and utilization. Previous studies have shown that several species of encrusting sponges (de Goeij et al. 2008a,b; Mueller et al. 2014; Rix et al. 2017; McMurray et al. 2018) and several species of massive sponges (Yahel et al. 2003; McMurray et al. 2016, 2018; Hoer et al. 2017) all take up and rely on DOC to meet the

majority (up to 60 – 90%) of their carbon demand (Yahel et al. 2003; McMurray et al. 2016; Wooster et al. 2019), making DOM turnover by sponges important ecologically.

Sponge microbiomes have also been shown to remove not just DOC, but also inorganic and organic nitrogen from ambient seawater. Sponge-microbial holobionts are able to simultaneously perform competing nitrogen cycling pathways (e.g., nitrification and denitrification; Hoffmann et al. 2009; Schläppy et al. 2010; Fiore et al. 2015) playing critical roles in biogeochemical cycling of benthic ecosystems. Although there have been fewer studies on organic nitrogen, it appears that several sponge species actively take up nitrogen in its organic form as well (de Goeij et al. 2013). These studies indicated that nitrogen, in various forms, is an important aspect of sponge holobiont metabolism and is removed from the seawater by sponge-microbial holobionts.

While it has been demonstrated that DOC is a major component of the sponge-microbial holobiont metabolism, there have been few studies to characterize sponge derived DOM (de Goeij et al. 2008b; Fiore et al. 2017). Several metabolites, including 4-hydroxybenzoic acid, glycerol-3-phosphate, 5-methylthioadenosine, and pantothenic acid were identified as likely removed by the sponge holobiont, whereas several nucleosides and riboflavin were significantly correlated with exhalent samples, suggesting a release by the sponge microbial holobiont (Fiore et al. 2017).

The research presented here aims to build upon previous studies by using an untargeted approach to characterize how the sponge microbiont can modify the DOM pool in the Florida Bay ecosystem, where sponges are dominant contributors to biomass (McMurray et al. 2015). We specifically focus on *Spheciospongia vesparium*, the most abundant sponge species in the Florida Keys reef ecosystem, which has a round, squat

morphology and distinct oscula for expelling filtered water (Weisz et al. 2008, 2010). Using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), the chemical composition of DOM in the ambient seawater in Florida Bay was compared to the exhalent seawater from *Spheciospongia vesparium* to characterize the resulting changes in DOM composition. We also quantified changes in DOC lability associated with seawater filtration by the sponge-microbial holobiont, an important step to better constrain and quantify the influence of sponges on carbon cycling in these ecosystems.

METHODS

Study site

Sampling was conducted in Buttonwood Sound in Florida Bay, USA, during July 2017. Florida Bay is a sub-tropical lagoon between mainland Florida and the Florida Keys; it is the largest estuary in Florida, valuable for recreation and fisheries, and adjacent to the sensitive habitats of the Florida Keys National Marine Sanctuary and Everglades National Park (Wall et al. 2012). The Bay is made up of many shallow basins with depths ranging from 1 to 3 m surrounded by mangrove islands and coastal lagoons (Melo and Lee 2012).

The dominant benthic suspension feeders in Florida Bay are sponges, especially the loggerhead sponge *Spheciospongia vesparium* (Butler et al. 1995; Lynch and Phlips 2000). Loggerhead sponges are fully heterotrophic, with a high abundance of microbial endosymbionts (Weisz et al. 2008). Sponges, along with octocorals and solitary hard corals, are a key component of the Florida Bay benthic community, providing structural habitat for juvenile octopus, stone crabs, and spiny lobster (Butler et al. 1995). Since the

late 1980s, Florida Bay has been affected by a series of ecological disruptions, including sponge die-offs, blooms of the cyanobacteria *Synechococcus spp*., and seagrass mortality (Wall et al. 2012). In the early 1990s, there were a series of widespread sponge die-offs in Florida Bay that affected > 40% of the loggerhead sponges and > 70% of other sponge species (Butler et al. 1995).

Water sampling and filtration

A total of 10 L of exhalent seawater was collected directly above the osculum of a large (~ 40 cm diameter), healthy, actively pumping loggerhead sponge (*S. vesparium*; **Fig. 4.1**). Exhalent seawater was collected using a peristaltic pump at a slower rate (~ 0.003 L s^{-1}) than the average pumping rate of the sponge (0.17 L s^{-1} L^{-1} sponge, Fiore et al. 2017). At approximately the same time (< 10 min), an additional 10 L of ambient seawater was collected in the vicinity of but away (~ 10 m) from the influence of the sponge exhalent seawater. Immediately after collection, sponge exhalent and ambient seawater samples were taken to the University of Georgia Key Largo Marine Research Laboratory (25.101°N, 80.438°W), filtered sequentially through Whatman GF/D filters (pre-combusted at 450°C for 5 h; nominal 2.7 μm pore size) and 0.2 μm Pall Supor membrane filters into acid-washed 1 L polycarbonate bottle triplicate sets. Fifty mL aliquots of the filtrate from each sampling site were set aside in separated beakers for the preparation of microbial inocula. For that, the 0.2 μm filters from both sites (exhalent and ambient seawater) were aseptically cut into pieces and equal areas of the filters were pooled into 50 mL aliquots of 0.2 μm filtrate from each of the two sites and stirred for 30 minutes. The resulting filtrates, now containing microbes from both sites, were added

back to the respective triplicate set of bottles. This ensured that functional capabilities of the microbes were similar during all incubations and changes in DOM composition could therefore be attributed predominantly to the different initial composition of the DOM pools. A triplicate set from each site was immediately filtered (0.2 μm) in order to characterize the initial condition for each set of samples. Aliquots (~ 50 mL) were stored frozen (-20^{o}C) and refrigerated (4^{o}C) for DOC and chromophoric DOM (CDOM) measurements, respectively. The remaining filtrates were acidified to pH 2 (using HCl) and DOM was extracted using solid phase extraction (SPE) cartridges (Agilent Bond Elut PPL) as in Dittmar et al. (2008) for FT-ICR MS analysis. We refer to those samples as T_0. The remaining triplicate sets were incubated in the dark at the temperature measured at the time of collection for 5 days. At day 5, samples were filtered (0.2 μm) and processed for DOC and FT-ICR MS analyses as described above. These samples are referred to as T_5.

Cell counts

Triplicate samples, preserved in 0.1% glutaraldehyde solution (Hopwood 1969), were prepared for flow cytometry to measure cell counts before and after the incubations of ambient and sponge exhalent seawater. Bacterial counts were obtained using a CytoFLEX S (Beckman Coulter, Hialeah, Florida) flow cytometer at the Cytometry Shared Resource Library at the University of Georgia. Replicate samples were analyzed with Milli-Q water between each sample in order to keep flowlines clean. Cell counts were quantified by staining cells with SYBR Green-I, as described by Marie et al. (1997).

Bulk DOC and Chromophoric DOM

Concentrations of DOC from initial and post-incubation samples of ambient and sponge exhalent seawater were measured with a Shimadzu TOC-L_{CPH} analyzer with potassium hydrogen phthalate as a standard. Milli-Q water blanks were tested before sample analysis and interspersed between sample runs on the instrument. Accuracy and precision were tested against deep-sea reference material (Hansell 2005) and were better than 5%. Biodegradation was determined through

$$\frac{\mathrm{DOC}_{T0} - \mathrm{DOC}_{T5}}{DOC_{T0}} \times 100 \qquad (1)$$

where DOC_{T0} was the concentration of DOC in the samples before incubations, and DOC_{T5} was the concentration of DOC in samples after five-day incubations.

UV-visible absorbance scans for chromophoric DOM (CDOM) were made on a single-beam spectrophotometer (Agilent UV-VIS 8453) using a 1 cm quartz cuvette, and absorption coefficients were computed as in D'Sa et al. (1999). Milli-Q water was used prior to sample measurement to complete blank calibrations to achieve a baseline background level. In order to track CDOM compositional changes, the ratio of absorptivity at 250 nm to 365 nm (a_{250}:a_{365}) was calculated; higher values can be used as indicators of lower aromaticity and a higher proportion of small molecules (Peuravuori and Pihlaja 2007).

FT-ICR MS

Molecular composition of the DOM of triplicate T_0 and T_5 ambient seawater and sponge exhalant samples were analyzed with a 9.4 T Fourier transform-ion cyclotron resonance mass spectrometer (FT-ICR MS) at the National High Magnetic Field

Laboratory in Tallahassee, FL following Letourneau and Medeiros (2019). Samples were injected at concentrations of 50 mg C L^{-1} in methanol with negative electrospray ionization mode and 150 scans were accumulated. Each mass spectrum was internally calibrated based on a "walking" calibration of highly abundant homologous alkylation series that differed in mass by multiples of 14.01565 Da confirmed by isotopic fine structure (Savory et al. 2011), achieving a mass error of < 0.5 ppm. The restrictions $^{12}C_{1\text{-}130}$ $^{1}H_{1\text{-}200}$ $O_{1\text{-}150}$ $^{14}N_{0\text{-}4}$ $S_{0\text{-}2}$ $P_{0\text{-}1}$ were used to calculate masses from the mass range of 150 to 750 Da. Molecular formulae assignments were performed by Kendrick mass defect analysis (Wu et al. 2004) with PetroOrg software (Corilo 2014) and the criteria described by Rossel et al. (2013). The peak intensity of each formula was normalized to the sum peak intensities of the total identified peaks in each sample and compounds with a signal-to-noise ratio of 6 or higher were used in the analysis.

Statistical analyses

The variability of DOM molecular composition for sponge exhalent and ambient seawater was analyzed using principal component (PC) analysis of the FT-ICR MS data. All peaks with molecular formulae assigned were used in the PC analysis. All modes shown here are significantly different (95% confidence level) from results obtained by pursuing a PC analysis of random processes that are spatially and temporally uncorrelated. This indicates that the signals in the modes described here are significantly greater than the level of noise (Overland and Preisendorfer 1982). The Wilcoxon rank-sum test was used for comparisons between samples, as in Osterholz et al. (2016). Loadings from the PC analysis were plotted on van Krevelen diagrams according to their

molecular hydrogen-to-carbon (H/C) and oxygen-to-carbon ratios (O/C) for each molecular formula.

RESULTS AND DISCUSSION

Initial DOC concentration and DOM composition

The sponge-microbial holobiont actively removed dissolved organic carbon (DOC) as compared to the ambient seawater samples. The average DOC concentration for the ambient seawater samples was 564 ± 2 µM, whereas the average concentration for the exhalent water was 529 ± 3 µM (**Table 4.1**), showing a 6.2% reduction in DOC concentration associated with removal by the sponge-microbial holobiont. Observed removal values were consistent with DOC removal previously reported for several species of sponges, which ranges from 0% to 24% of ambient seawater. However, DOC removal for *S. vesparium* was not statistically different from zero in a previous study (Hoer et al. 2017).

Analysis at the molecular level revealed that the removal of DOC by the sponge-microbial holobiont transformed the composition of the DOM pool, with compounds relatively enriched in sponge exhalent DOM occupying a different region of the van Krevelen diagram compared to compounds relatively enriched in ambient seawater (**Fig. 4.2**). In particular, compounds with relative abundance enriched in ambient seawater (and thus depleted in sponge exhalent DOM; shown in blue in **Fig. 4.2**) were characterized by a low number of carbon and oxygen atoms and by an increase in the number of nitrogen atoms (**Fig. 4.3**). This suggests that small carbon compounds, compounds with low oxygen content, and nitrogen-rich compounds may have been preferentially removed

from the ambient seawater by the sponge-microbial holobiont, actively transforming the carbon pool. This finding was corroborated by the lower values of the a_{250}:a_{365} ratio for the sponge exhalent compared to the ambient seawater samples (**Table 4.1**). As the sponge-microbial holobiont removed small, less aromatic compounds, the sponge exhalent DOM was characterized by a higher proportion of large molecules and higher aromatic content. Removal of low molecular weight DOM compounds has been previously reported for two sponge species in Florida Bay (Fiore et al. 2017).

The preferential depletion in relative abundance of nitrogen-containing compounds in exhalent seawater is particularly interesting. While only 20% of the compounds enriched in sponge exhalent DOM contained at least one nitrogen atom, about 50% of the compounds enriched in ambient seawater had at least one nitrogen (**Fig. 4.3c**). The uptake of nitrogen-containing compounds by the microbial-sponge holobiont was also revealed by total dissolved nitrogen (TDN) concentrations (**Table 4.1**), with sponge exhalent TDN being slightly less concentrated than ambient seawater ($p > 0.05$). This preferential uptake of nitrogen-containing compounds may have ecological significance in the Florida Keys hard-bottom environment. *S. vesparium* is the most dominant member of the sponge community in Florida Bay and is estimated to make up 58% of the community biomass (Stevely et al. 2010). In the early 1990s, widespread sponge mortality events in the Florida Keys caused sponge biomass to decline by up to 90% in some locations (Butler et al. 1995). The direct cause of these mortality events was not determined, but the loss of these sponges, the dominant suspension feeders in the system, was accompanied by widespread phytoplankton and cyanobacteria blooms (Peterson et al. 2006). The large phytoplankton blooms are thought to have been caused

by the reduction in sponge grazing following the large-scale mortality event, since the five most common species of sponges in Florida Bay (including *S. vesparium*) have been shown to graze upon multiple plankton species, including the cyanobacteria *Synechococcus elongatus*, the diatom *Cyclotella choctawhatcheeana*, and the dinoflagellate *Prorocentrum hoffmanianum* (Peterson et al. 2006). Our results suggest that mortality of these sponges in the 1990s not only decreased grazing pressure, but may have also reintroduced nitrogen-containing organic compounds that sponges had been previously removing from the ambient seawater. To the extent that photochemical reactions can release bioavailable nitrogen from organic nitrogen (e.g., Bushaw et al. 1996; Vähatalo and Zepp 2005), sponge mortality/abundance may directly influence nutrient concentrations in these systems through their alteration of potentially bioavailable nitrogen-rich compounds.

Microbial responses through dark incubations

Many previous studies have shown that DOM composition plays a key role controlling microbial degradation of DOC (e.g. Moran et al. 2016). Given the changes in DOM composition observed in sponge exhalent samples associated with the depletion in relative abundance of small organic compounds with a low number of carbon and oxygen atoms and rich in nitrogen, it is possible that the interaction of sponges with the DOM pool may also alter the lability of that carbon pool. To test this, we analyzed the microbial degradation of each DOM pool through 5-day dark incubations.

At first glance, the general patterns of DOM composition transformations observed during the incubations were somewhat similar to each other, with molecular

formulae that had their relative abundance depleted or enriched during the incubations occupying approximately the same location in van Krevelen space (**Fig. 4.4**). In both cases, there was a tendency for preferential depletion of compounds characterized by high O/C and low H/C ratios, and preferential enrichment of compounds with low O/C and high H/C ratios. This is consistent with changes in DOM composition associated with microbial degradation observed in other coastal systems (e.g., Medeiros et al. 2015, 2017).

However, analysis of changes in DOC concentrations between the two incubations revealed statistically significant differences in DOC consumption. For the dark incubations of ambient seawater, 6.7% of the DOC contained in the pre-incubation sample was consumed during the 5-day long incubation (**Table 4.1**). For the incubation with sponge exhalent water, on the other hand, microbial DOC consumption was lower at 1%. This suggests a difference in DOC lability between the two samples, with ambient seawater DOC being more labile than sponge exhalent DOC. Similarly, TDN also had an increased reduction during incubation of ambient seawater compared to incubation of sponge exhalent seawater (**Table 4.1**). To investigate if this is consistent with the observed changes in DOM composition at the molecular level, we compared the compounds with relative abundance depleted during the incubations (blue dots in **Fig. 4.4**) with the compounds with relative abundance depleted after filtration by the sponge (blue dots in **Fig. 4.2**). Approximately 60% of the molecular formulae associated with compounds with relative abundance depleted after filtration by the sponge also had their relative abundance depleted during incubation of ambient seawater (blue dots in **Fig. 4.4a**). This suggests that as the sponge filtered ambient seawater, the holobiont may have

preferentially removed compounds that were microbially labile. This is also consistent with microbial incubations pursued in other coastal environments, which have revealed that compounds preferentially targeted by bacteria often had a higher number of nitrogen heteroatoms compared to the average DOM pool (Vorobev et al. 2018). Since about half of the molecular formulae associated with compounds with relative abundance depleted during filtration by the sponge contained nitrogen (**Fig. 4.3**), it is possible that many microbially labile compounds were removed in the process. On the other hand, the fraction of the molecular formulae with relative abundance depleted during filtration by the sponge-microbial holobiont that also had abundance depleted during the incubation with sponge exhalent DOM (blue dots in **Fig. 4.4b**) was much smaller at 4%. This indicated that the microbial communities interacted differently with the DOM after it had been filtered by the sponge-microbial holobiont. It is therefore possible that the sponge-microbial holobiont removed a large fraction of the compounds that were labile to bacteria during filtration, and the DOM pool left behind in the sponge exhalent samples was more recalcitrant, resulting in reduced DOC degradation for incubations of sponge exhalent seawater (**Table 4.1**).

Differences in DOC consumption may have been related to differences in DOM composition as discussed above, but they may also have been influenced by changes in microbial community. The initial samples for incubations of both ambient seawater and sponge exhalent water had bacterial abundances of around 6×10^4 bacteria/mL (**Table 4.1**). The average bacterial abundance for the final ambient seawater samples was 2.4×10^5 bacteria/mL, whereas the average bacterial abundance for the final sponge exhalent samples was 1.3×10^5 bacteria/mL (**Table 4.1**). This indicated that the bacterial

community in the ambient seawater increased in density by about 3.4 x 10^4 cells/mL/day, whereas the bacterial community in the sponge exhalent samples only increased by about 1.4 x 10^4 cells/mL/day, a difference of almost two and a half times. Thus, it is possible that components of the DOM pool that are important to sustain the microbial community were removed during filtration by the sponge holobiont, resulting in lower bacterial growth rates in sponge exhalent water. The resulting increased bacterial abundance during the incubation of ambient seawater could at least partially explain the larger DOC consumption in that case compared to the incubation of sponge exhalent water.

In summary, we found that the *S. vesparium* holobiont actively took up about 6% of the DOC from the surrounding ambient seawater, resulting in a decrease in relative abundance of compounds with low carbon numbers, low oxygen content, and with high nitrogen content as compared to the ambient seawater. The microbial communities interacted differently with sponge-microbial holobiont exhalent and ambient seawater over 5-day dark incubations. While the microbial community in the sponge exhalent samples was only able to utilize 1% of the DOC in the samples, in the ambient seawater samples there was an almost 7% biodegradation, revealing a decrease in lability of DOC after being exhaled by the sponge-microbial holobiont. Analyses at the molecular level confirmed that several of the compounds whose relative abundance decreased during filtration by the sponge-microbial holobiont were preferentially degraded by bacteria, suggesting that the *S. vesparium* holobiont may have removed labile compounds leaving behind the more recalcitrant fraction of the DOM pool. This may have far-reaching implications in carbon and nitrogen cycling in this area, as well as throughout the Florida Bay hard-bottom community. This study is one of the first to examine the changes in

DOM composition before and after interaction with *S. vesparium*, as well as the microbial community's interaction with the DOM in ambient seawater samples as compared to sponge holobiont exhalent DOM. Further studies are necessary determine how these findings vary for other species of marine sponges, especially those known for removing larger amounts of DOC from seawater.

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Figure 4.1 Collection of exhalent seawater directly above the osculum of a *Spheciospongia vesparium* located in Florida Bay (USA).

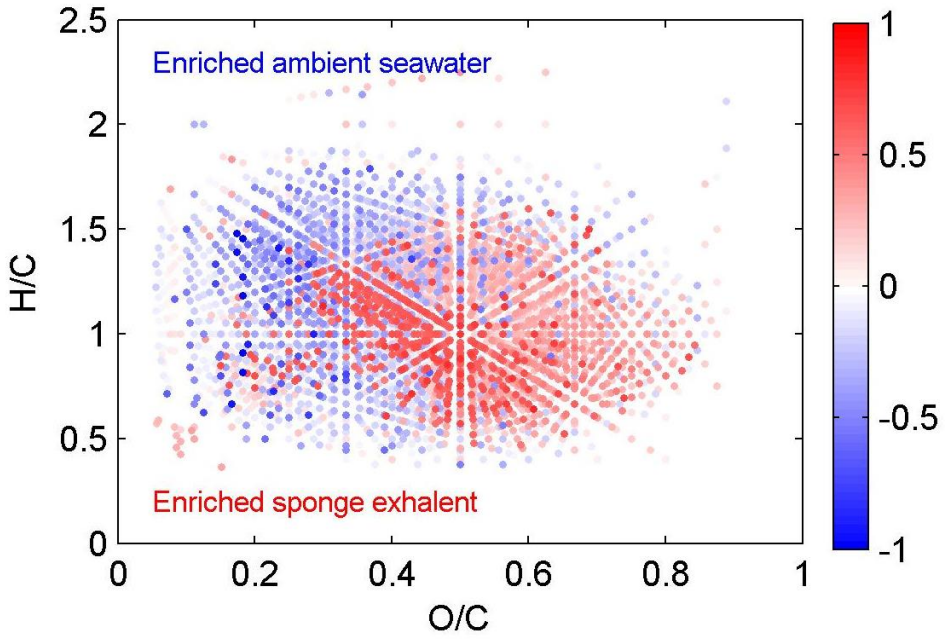

Figure 4.2 Van Krevelen diagram showing loadings of dominant principal component of ambient seawater and sponge exhalent water DOM composition. Molecular formulae shown in red were relatively enriched in sponge exhalent water, while formulae shown in blue were enriched in ambient seawater (and thus depleted in sponge exhalent water).

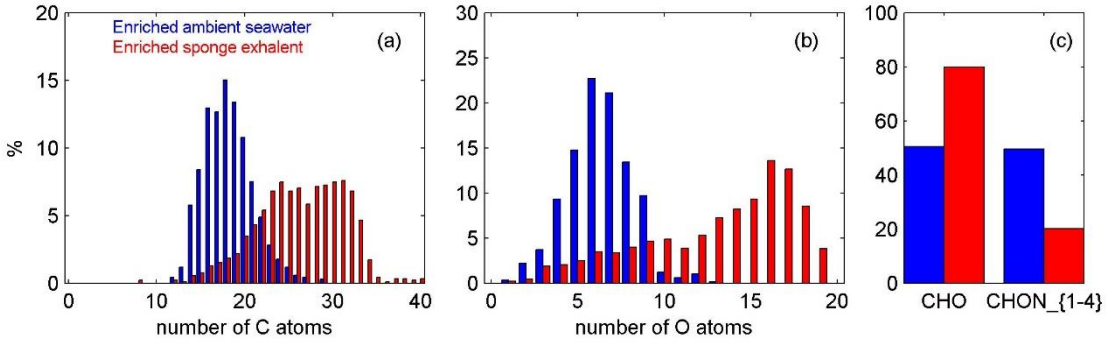

Figure 4.3 Histograms of number of (**a)** carbon, (**b**) oxygen, and (**c**) nitrogen in compounds with relative abundance enriched in sponge exhalent (red) and in ambient seawater (blue) samples.

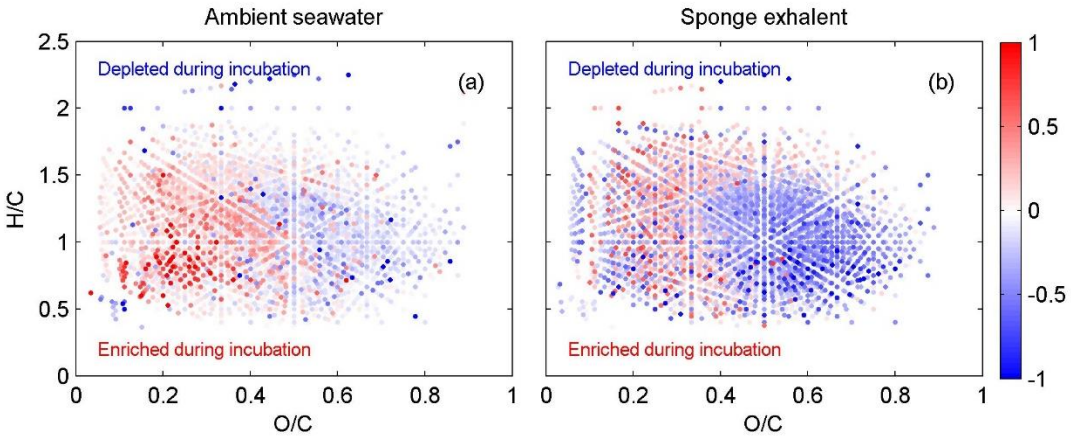

Figure 4.4 Van Krevelen diagram showing loadings of dominant principal component of DOM transformation during dark incubations of (**a**) ambient seawater and (**b**) sponge exhalent seawater.

Table 4.1 Chemical and biological variables for loggerhead sponge exhalent and ambient seawater samples.

Sample Name	Time Point (days)	DOC (μM)	DOC Consumption (%)	TDN (μM)	a_{250}:a_{365}	Microbial Abundance (Bacteria/mL)
Ambient Seawater	0	564.3 ± 2.1	-	47.6 ± 0.1	8.8 ± 0.4	$6.7 \times 10^4 \pm 9.8 \times 10^3$
Ambient Seawater	5	526.3 ± 1.5	6.7	44.3 ± 0.2	-	$2.4 \times 10^5 \pm 3.2 \times 10^4$
Sponge Exhalent	0	529.3 ± 3.4	-	46.5 ± 0.2	8.0 ± 0.2	$6.1 \times 10^4 \pm 1.8 \times 10^4$
Sponge Exhalent	5	524.2 ± 1.7	0.96	45.1 ± 0.1	-	$1.3 \times 10^5 \pm 3.0 \times 10^4$

CHAPTER 5

CONCLUSIONS

Dissolved organic matter (DOM) is a complex pool of thousands of compounds with various sources, concentrations, and reactivities. This dissertation focuses on better understanding the broad changes in organic matter concentration, composition and lability across various aquatic environments through the use of untargeted organic chemical analyses, namely dissolved organic carbon (DOC), optical indices of chromophoric dissolved organic matter (CDOM), and ultrahigh resolution mass spectrometry (FT-ICR MS), as well as microbial incubations. The research presented here aims to answer three main questions: 1) How does DOM composition change in response to seasonal forcing over the course of a year in a riverine site and in a marsh-influenced estuarine site? 2) What are the spatial-temporal changes in DOM composition across a salinity gradient within a complex estuarine system and how do these compositional changes influence microbial degradation? 3) How is the DOM composition and lability of ambient seawater altered as it is filtered through a marine sponge?

Changes in DOC concentration and DOM composition were investigated on a monthly basis over the course of a year in the Altamaha River and at the head of the Sapelo Sound Estuary (Georgia, USA) in CHAPTER 2. The Altamaha River discharge levels were found to be the primary driver changing the DOM composition in the

Altamaha and Sapelo Sound sites. When discharge levels were higher, DOC concentrations were higher, terrigenous content and aromaticity of the DOM pool and biodegradation rates were increased. This research showed that when Altamaha River discharge levels were under ~ 150 m^3 s^{-1}, there was a clear signature of marsh-derived compounds in the river; at discharge levels above ~ 150 m^3 s^{-1}, terrestrially-derived compounds were the dominant component of the DOM pool, masking the signal of the marsh compounds. Finally, this research showed that Hurricane Matthew (which hit the studied area a month after our year-long collection) nearly doubled the seasonal maximum DOC concentration observed earlier in the year and added large amounts of highly aromatic DOM into the system. This demonstrates that hurricanes can impact not only the DOC content, but also the molecular composition of DOM potentially influencing its cycling in estuarine environments.

The DOM compositional changes in a riverine-estuarine gradient were further investigated in CHAPTER 3. Fifteen sites were sampled quarterly over a year in the Altamaha River and the Altamaha, Doboy and Sapelo Sounds interconnected system, and the DOC concentration, DOM composition, and lability were analyzed with respect to both temporal and spatial changes. This research showed that DOC was more concentrated at the head of the Sapelo Sound and at upstream sites in the Altamaha River, it decreased in both concentration and terrigenous content as it was transported downstream towards the Atlantic Ocean. Cluster analysis was used on the DOM molecular data and 6 clusters were identified throughout the system based on the terrigenous signature of the DOM pool, allowing us to visualize the seasonal movement of the terrigenous matter throughout the riverine-estuarine system. While DOM in the

Altamaha River and upstream half of the Altamaha Sound had a terrigenous signature year-round, DOM composition at Doboy and Sapelo Sounds presented large seasonal variability, with a clear marine signature during parts of the year. October 2017 was consistently shown to be the month with the highest DOC concentrations, had the highest aromatic content, and had the highest microbial utilization rates. This was hypothesized to be due to Hurricane Irma, which impacted the area ~ 30 days before the October sampling, which could have flushed a large amount of fresh, terrigenous DOM to the system. For all collections, terrigenous content was significantly positively correlated to microbial utilization, showing that the more aromatic the DOM character, the more consumed it was by the microbial community. Findings presented in CHAPTER 3 represent an important contribution to those interested in the dynamics of terrigenous organic material and showed the longer lasting effects of a hurricane on DOM concentration, composition, and processing within a riverine-estuarine complex.

Research in CHAPTER 4 moved away from coastal Georgia and investigated the DOM compositional changes relating to interactions with a marine sponge species in Florida Bay, USA. The marine loggerhead sponge, *Spheciospongia vesparium*, was shown to actively remove about 6% of the DOC from the ambient seawater and markedly alter its DOM composition. The sponge-microbial holobiont preferentially took up small, oxygen-depleted, nitrogen-rich compounds from the ambient seawater DOM pool. Over a 5-day incubation period, the microbial community was able to access ~ 7% of DOC from the ambient seawater but only 1% of DOC from the sponge exhalent seawater, suggesting a decrease in lability, possibly due to the removal of nitrogen-rich compounds by the sponge-microbial holobiont. In addition, the sponge exhalent DOM sustained lower

bacterial cell counts than the ambient seawater. These results could have far reaching implications for the Florida Bay hard bottom community and further shows that sponges actively utilize DOM and influence carbon cycling in reef ecosystems.

This dissertation provides useful insights on the various applications of both bulk and molecular analyses to better understand complex DOM compositional changes across various aquatic settings. Bulk and optical analyses, as well as dark microbial incubations, showed general patterns of concentrations, quality, and lability of the DOM. Ultrahigh resolution mass spectrometry helped uncover the molecular level changes in DOM samples and understand the drivers behind the compositional changes. Bringing these techniques together allowed for a full and rich assessment of the various changes in the DOM pool. This dissertation has advanced the knowledge of DOM in coastal areas by improving our understanding of the impact of river discharge on the composition of DOM, the impacts of severe weather events on estuarine DOC concentrations, DOM composition, and microbial lability, as well as the effects of loggerhead sponge filtration on the DOM molecular composition. The body of work presented here has shown that the variability of the DOM in the Altamaha-Doboy-Sapelo estuarine complex is largely due to the terrestrial-marine gradient, driven by Altamaha River discharge values. Microbial degradation in this system is correlated with terrestrial content, with higher lability seen in more terrestrial samples. And the loggerhead sponge microbial holobiont was shown to be able to preferentially take up specific DOM compounds, leaving the exhalent water with a different molecular composition than ambient seawater.

Future work is needed to better understand several factors that were outside of the scope of this dissertation. Tidal cycles are pivotal forces within estuaries; more studies

aimed at understanding the effects of tidal cycles on DOM composition within estuarine domains would add a great deal of insight and depth to this body of work. Additionally, a higher temporal resolution of sampling before and following severe weather events would better constrain the short and long-term changes in DOM composition. This dissertation revealed DOM compositional changes directly following Hurricane Matthew (CHAPTER 2) and ~ 30 days following Hurricane Irma (CHAPTER 3) across an estuarine system; however a study that compiles samples both directly following a severe weather event and at several timepoints on weekly and monthly scales would be a natural next step in this avenue of research. Finally, more work is needed to better understand how various environmental conditions effect microbial preferences and lability within the DOM pool.

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APPENDIX A.

SUPPLEMENTARY INFORMATION FOR CHAPTER 2

DISSOLVED ORGANIC MATTER COMPOSITION IN A MARSH-DOMINATED ESTUARY: RESPONSE TO SEASONAL FORCING AND TO THE PASSAGE OF A HURRICANE

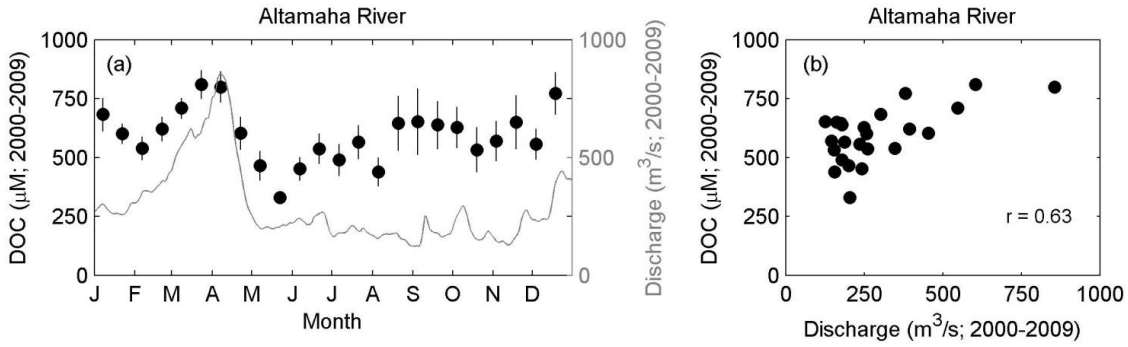

Figure A.1 (a) Time series of historical (2000-2009) DOC concentration (black symbols) and discharge (gray) at the Altamaha River; (b) Scatterplot and correlation coefficient between historical DOC concentration and river discharge.

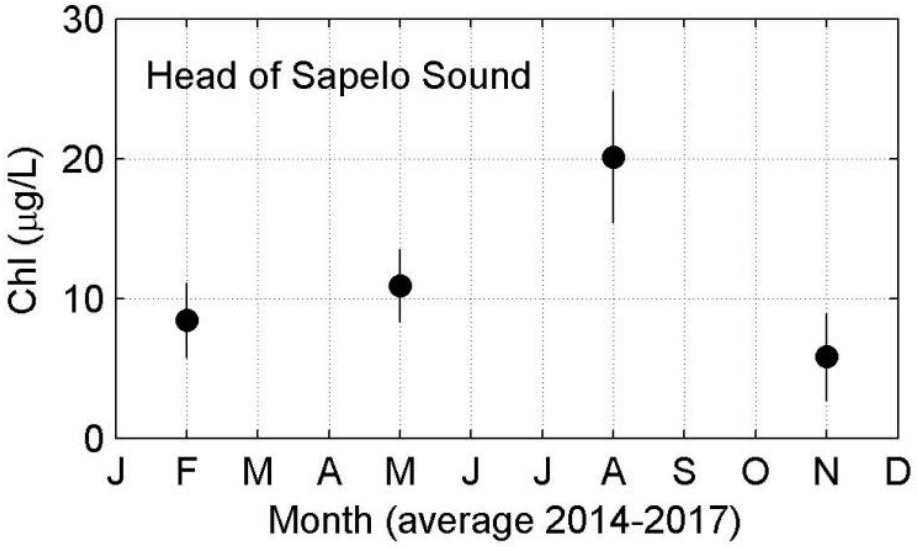

Figure A.2 Average concentration of chlorophyll a at the head of Sapelo Sound from 2014-2017.

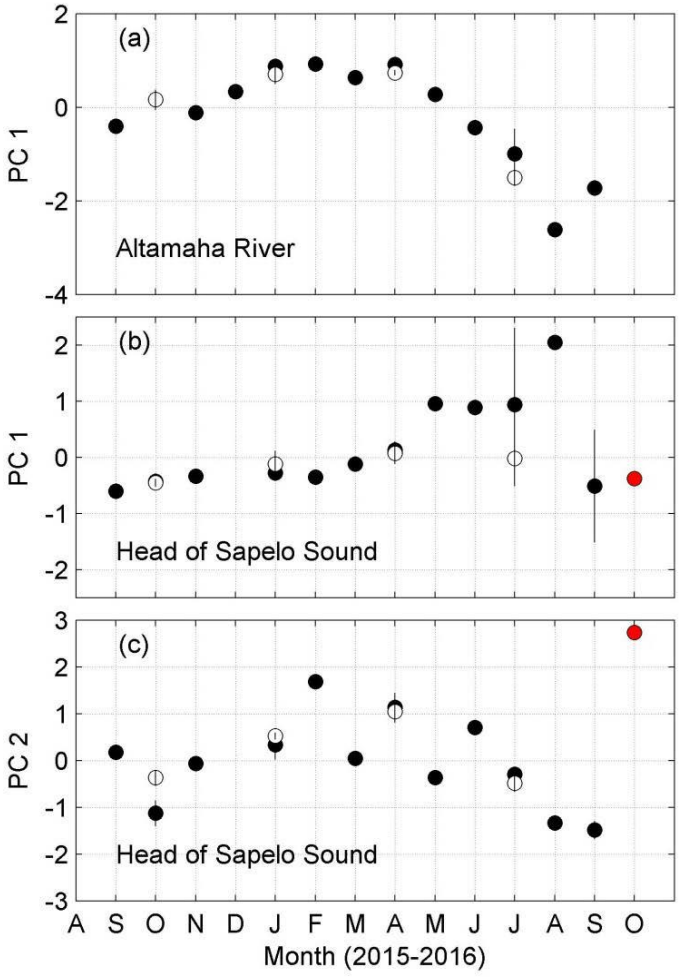

Figure A.3 Principal component analysis of DOM composition at the Altamaha River and at the head of Sapelo Sound, including T_0 (solid symbols) and T_{80} samples (open symbols). (a) Time series of first principal component at the Altamaha River. Time series of (b) first and (c) second principal components at the head of Sapelo Sound. In some cases T_{80} samples overlay T_0 samples (e.g., October 2015 in panel a).

Table A.1 Physical and chemical variables at sampling sites collected over one year in the Altamaha River and Sapelo Sound.

Collection date	Water Temperature (°C)	Salinity	DOC (µM)	Spectral Slope ($x10^{-3}$ nm^{-1})	a250:a365	Discharge (m^3 s^{-1})
Altamaha River						
September 15, 2015	26.1	2.4	362 ± 14	15.10 ± 0.05	5.22 ± 0.02	84
October 13, 2015	22.5	0.1	548 ± 15	14.27 ± 0.07	5.01 ± 0.05	229
November 10, 2015	21.8	0.1	449 ± 4	14.53 ± 0.05	5.13 ± 0.05	253
December 14, 2015	15.8	0.0	615 ± 10	13.96 ± 0.03	5.15 ± 0.07	207
January 19, 2016	9.7	0.0	794 ± 15	13.12 ± 0.07	5.00 ± 0.03	909
February 16, 2016	11.0	0.0	782 ± 15	13.23 ± 0.03	4.97 ± 0.03	1135
March 15, 2016	21.0	0.0	541 ± 15	13.73 ± 0.04	4.81 ± 0.02	672
April 10, 2016	18.9	0.0	965 ± 10	13.10 ± 0.03	4.69 ± 0.09	860
May 9, 2016	22.8	0.0	454 ± 6	14.56 ± 0.03	5.15 ± 0.09	214
June 5, 2016	28.7	0.0	405 ± 5	15.46 ± 0.01	5.46 ± 0.08	132
July 11, 2016	28.7	2.7	423 ± 8	16.20 ± 0.05	5.60 ± 0.07	77
August 14, 2016	31.9	2.2	366 ± 11	16.57 ± 0.06	5.62 ± 0.03	63
September 11, 2016	29.6	1.8	536 ± 14	15.37 ± 0.02	5.33 ± 0.04	69
Head of Sapelo Sound						
September 15, 2015	26.0	22.7	825 ± 18	14.80 ± 0.06	5.16 ± 0.02	-
October 13, 2015	23.0	27.1	574 ± 19	16.10 ± 0.03	5.81 ± 0.04	-
November 10, 2015	21.1	23.7	773 ± 12	15.12 ± 0.05	5.55 ± 0.03	-
December 14, 2015	18.8	29.0	324 ± 10	16.82 ± 0.06	6.78 ± 0.04	-
January 19, 2016	10.0	13.8	876 ± 12	14.54 ± 0.04	5.24 ± 0.02	-
February 16, 2016	14.0	7.3	1985 ± 13	13.09 ± 0.01	4.62 ± 0.01	-
March 15, 2016	23.0	19.9	709 ± 19	16.01 ± 0.01	5.76 ± 0.01	-
April 10, 2016	20.0	16.9	1152 ± 11	14.06 ± 0.04	4.87 ± 0.04	-
May 9, 2016	24.4	23.3	715 ± 7	16.29 ± 0.02	5.81 ± 0.02	-
June 5, 2016	30.7	22.3	987 ± 14	14.89 ± 0.04	5.14 ± 0.09	-
July 11, 2016	32.5	25.8	739 ± 8	16.11 ± 0.03	5.59 ± 0.06	-
August 14, 2016	32.7	31.5	723 ± 16	16.63 ± 0.02	5.91 ± 0.05	-
September 11, 2016	30.6	27.8	834 ± 15	16.54 ± 0.03	6.02 ± 0.07	-
October 12, 2016 - Hurricane Matthew	21.8	0.9	3672	12.24	4.48	-

APPENDIX B.

SUPPLEMENTARY INFORMATION FOR CHAPTER 3

SPATIO-TEMPORAL CHANGES IN DISSOLVED ORGANIC MATTER COMPOSITION ALONG THE SALINITY GRADIENT OF AN ESTUARINE COMPLEX IN THE SOUTHEASTERN U.S.

Table B.1 Seasonal dissolved organic carbon (DOC) concentrations at sampling sites in the Altamaha River, Sapelo and Doboy Sounds

Sampling site*	DOC (µM)				Area
	April 2017	July 2017	October 2017	January 2018	
GCE 1	652.3 ± 3.6	598.9 ± 3.5	1126.5 ± 10.2	771.2 ± 1.5	Sapelo Sound
GCE 2	368.9 ± 9.9	690.8 ± 2.5	435.9 ± 1.0	273.7 ± 1.5	Sapelo Sound
STA 18	325.8 ± 8.1	317.2 ± 1.6	387.6 ± 5.0	258.6 ± 2.4	Sapelo Sound
GCE 3	247.0 ± 5.5	250.0 ± 1.2	307.6 ± 2.7	218.5 ± 4.2	Sapelo Sound
GCE 4 STA 12	364.7 ± 4.3	390.2 ± 7.7	470.7 ± 5.7	278.3 ± 2.7	Doboy Sound
GCE 5 STA 9	296.1 ± 1.6	296.7 ± 4.9	374.9 ± 7.5	254.1 ± 1.9	Doboy Sound
GCE 6	246.5 ± 2.3	213.1 ± 4.8	250.6 ± 3.9	206.6 ± 8.0	Doboy Sound
STA 11	329.9 ± 9.5	309.6 ± 7.7	420.5 ± 1.1	261.8 ± 1.4	Doboy Sound
GCE 10	410.8 ± 7.6	466.8 ± 7.8	521.5 ± 5.3	307.4 ± 4.1	Doboy Sound
STA 2	431.3 ± 5.3	534.7 ± 5.6	650.8 ± 8.2	448.3 ± 8.4	Altamaha River
GCE 11 STA 4	488.6 ± 8.3	536.2 ± 1.1	776.3 ± 11.1	456.0 ± 3.1	Altamaha River
GCE 7 STA 3	503.4 ± 5.4	525.0 ± 5.3	753.3 ± 10.0	447.2 ± 5.3	Altamaha River
GCE 8 STA 6	513.6 ± 9.1	528.3 ± 6.0	819.5 ± 1.2	444.0 ± 2.1	Altamaha River
GCE 9	283.6 ± 7.1	308.1 ± 4.1	378.3 ± 7.0	298.1 ± 4.8	Altamaha River
GCE AL-2	225.4 ± 2.5	203.6 ± 9.1	231.4 ± 7.8	187.4 ± 9.7	Altamaha River

* See Fig. 3.1 for location.

Table B.2 Seasonal spectral slope ($S_{275-295}$) values at sampling sites in the Altamaha River, Sapelo and Doboy Sounds.

Sampling sites*	$S_{275-295}$ (x 10^{-3} nm^{-1})				Area
	April 2017	July 2017	October 2017	January 2018	
GCE 1	16.52 ± 0.36	16.38 ± 0.38	14.71 ± 0.07	15.57 ± 0.08	Sapelo Sound
GCE 2	17.89 ± 0.48	16.30 ± 0.12	16.58 ± 0.09	18.09 ± 0.09	Sapelo Sound
STA 18	18.91 ± 0.16	17.35 ± 0.02	16.78 ± 0.05	18.19 ± 0.54	Sapelo Sound
GCE 3	19.09 ± 0.35	17.08 ± 0.49	16.65 ± 0.05	17.56 ± 0.10	Sapelo Sound
GCE 4 STA 12	17.09 ± 0.33	16.27 ± 0.07	15.70 ± 0.06	17.16 ± 0.20	Doboy Sound
GCE 5 STA 9	17.43 ± 0.42	16.07 ± 1.37	15.92 ± 0.07	17.71 ± 0.34	Doboy Sound
GCE 6	17.81 ± 0.68	16.91 ± 0.63	16.73 ± 0.19	18.16 ± 0.66	Doboy Sound
STA 11	17.33 ± 0.09	16.38 ± 0.20	16.01 ± 0.49	17.53 ± 0.38	Doboy Sound
GCE 10	17.08 ± 0.18	16.67 ± 0.10	16.13 ± 0.06	17.71 ± 0.61	Doboy Sound
STA 2	14.72 ± 0.74	14.68 ± 0.04	13.87 ± 0.19	14.96 ± 0.28	Altamaha River
GCE 11 STA 4	14.23 ± 0.53	14.71 ± 0.13	13.84 ± 0.05	15.33 ± 0.05	Altamaha River
GCE 7 STA 3	14.50 ± 0.61	15.09 ± 0.56	14.04 ± 0.09	15.41 ± 0.15	Altamaha River
GCE 8 STA 6	15.71 ± 0.05	15.54 ± 0.90	14.66 ± 0.02	15.88 ± 0.10	Altamaha River
GCE 9	16.52 ± 0.25	16.40 ± 0.02	15.54 ± 0.06	16.87 ± 0.06	Altamaha River
GCE AL-2	18.62 ± 0.14	17.48 ± 0.03	17.06 ± 0.36	18.37 ± 0.26	Altamaha River

* See Fig. 3.1 for location.

Table B.3 Seasonal specific UV absorbance at 254 nm ($SUVA_{254}$) values at sampling sites in the Altamaha River, Sapelo and Doboy Sounds.

Sampling sites*	$SUVA_{254}$ (L mg C^{-1} m^{-1})				Area
	April 2017	July 2017	October 2017	January 2018	
GCE 1	7.55 ± 0.41	5.71 ± 0.39	8.01 ± 0.25	6.96 ± 0.07	Sapelo Sound
GCE 2	7.05 ± 0.30	7.70 ± 0.23	6.69 ± 0.07	5.82 ± 0.03	Sapelo Sound
STA 18	6.83 ± 0.13	6.12 ± 0.08	6.44 ± 0.12	5.70 ± 0.38	Sapelo Sound
GCE 3	6.20 ± 0.27	6.14 ± 0.02	6.13 ± 0.20	5.59 ± 0.05	Sapelo Sound
GCE 4 STA 12	7.61 ± 0.12	6.66 ± 0.01	7.11 ± 0.04	6.00 ± 0.15	Doboy Sound
GCE 5 STA 9	7.34 ± 0.05	6.97 ± 0.94	6.66 ± 0.17	5.78 ± 0.12	Doboy Sound
GCE 6	6.59 ± 0.28	5.93 ± 0.20	5.91 ± 0.11	5.14 ± 0.21	Doboy Sound
STA 11	7.32 ± 0.24	6.82 ± 0.21	6.85 ± 0.02	5.78 ± 0.05	Doboy Sound
GCE 10	6.87 ± 0.48	6.19 ± 0.10	6.99 ± 0.19	5.84 ± 0.26	Doboy Sound
STA 2	9.11 ± 0.70	8.13 ± 0.09	8.63 ± 0.12	7.13 ± 0.13	Altamaha River
GCE 11 STA 4	8.85 ± 0.68	8.16 ± 0.05	8.45 ± 0.32	6.88 ± 0.16	Altamaha River
GCE 7 STA 3	8.97 ± 0.05	8.10 ± 0.08	8.29 ± 0.13	6.86 ± 0.19	Altamaha River
GCE 8 STA 6	7.82 ± 0.42	7.34 ± 0.06	7.68 ± 0.06	6.33 ± 0.05	Altamaha River
GCE 9	6.52 ± 0.07	6.72 ± 0.07	6.81 ± 0.39	5.87 ± 0.11	Altamaha River
GCE AL-2	5.99 ± 0.07	5.37 ± 0.11	5.65 ± 0.04	5.02 ± 0.23	Altamaha River

* See Fig. 3.1 for location.

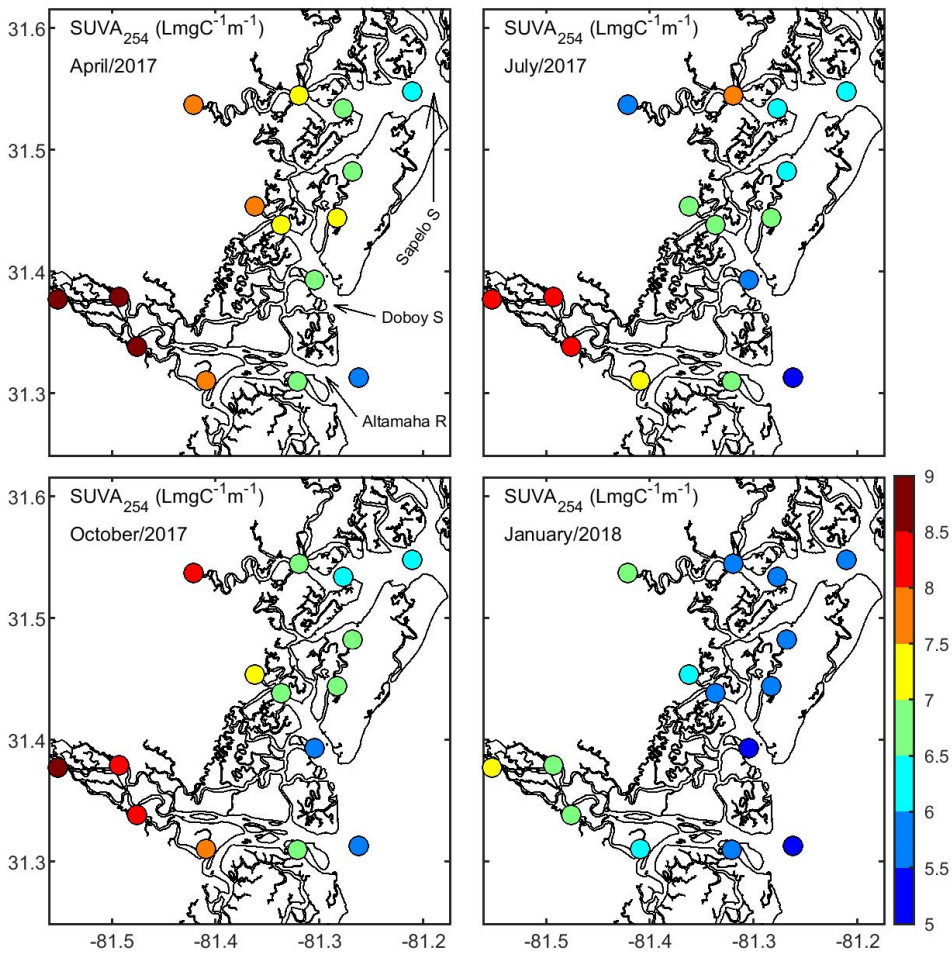

Figure B.1 $SUVA_{254}$ in April, July, October 2017, and January 2018