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Lauren E. Alvaro

Florida Gulf Coast University, kitkat1027@gmail.com

John C. Lehrter

U.S. Environmental Protection Agency, jlehrter@disl.org

Mai Fung

University of South Alabama, mfung@disl.org

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SHORT COMMUNICATION

SPATIAL PATTERNS OF PARTICULATE ORGANIC CARBON CONCENTRATIONS AND ISOTOPIC SIGNATURES ACROSS A SALINITY GRADIENT IN A RIVER DOMINATED ESTUARY[§]

L.E. Alvaro¹, J. C. Lehrter^{* 2,3}, and M. Fung^{2,3}

¹Florida Gulf Coast University, 10501 FGCU Blvd S, Fort Myers, FL 33965 USA; ²University of South Alabama, 307 N University Blvd, Mobile, AL 36688 USA; ³Dauphin Island Sea Lab, University Programs, 101 Bienville Boulevard, Dauphin Island, AL 36528 USA; *Corresponding author, email: jlehrter@disl.org

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INTRODUCTION

In river-dominated estuaries, terrestrial and marine-derived particulate organic matter (POM) contribute to the base of highly productive food webs and drive biogeochemical processes that regulate carbon, oxygen, and nutrient dynamics (Peterson 1999). The POM from estuaries also contributes to the long-term burial of organic carbon in marine sediments and is significant in the global carbon cycle (Goñi et al. 1998). Pools of POM in the estuary consist of organic matter derived from terrestrial plants and soils delivered by rivers, local wetlands, and streams as well as from marine plants (predominantly phytoplankton) that thrive in estuarine and adjacent coastal waters where availability of nutrients and light promote phytoplankton blooms. Thus, the POM in an estuary is a mixture of terrestrial, marine, and other endmembers (e.g., source terms). A fundamental question to answer in order to understand carbon cycling and food webs in an estuary is: What are the dominant sources of organic matter across the estuarine salinity gradient from freshwater to seawater?

In this study, we investigated the spatial patterns of particulate organic carbon (POC) in the water column and sediments of Mobile Bay, AL across the salinity gradient. We used ¹³C stable isotope analysis to calculate the mixture of POC from terrestrial and marine (phytoplankton) endmembers (Fry 2006). We hypothesized that spatial patterns were mainly driven by river discharge to the estuary and subsequent spatial patterns of salinity and estuarine phytoplankton production. Since Mobile Bay is a river-dominated estuary, we expected that the upper and middle bay organic matter would primarily be derived from terrestrial sources with $\delta^{13}\text{C}$ of $< -26\text{‰}$, whereas lower parts of the bay, i.e. more distant from the freshwater input, would have more marine, phytoplankton-based organic matter with $\delta^{13}\text{C}$ of $\sim 21\text{‰}$ (Fry 2006). Similar results have been observed in other river-dominated systems such as the Louisiana shelf and Mississippi Sound (Sackett and Thompson 1963; Goñi et al. 1998).

MATERIALS AND METHODS

Study site

Mobile Bay, AL is a relatively large, shallow estuary with a surface area of 985 km² and a mean depth of 3 m (Dinnel et al. 1990). The bay is a river-dominated estuary with discharge from the Alabama and Tombigbee rivers. The mean combined discharge of these rivers was calculated to be 1,622 m³/s (USGS data: Alabama River site 02428400 and Tombigbee River site 02469761; 1971–2018), which was the 2nd largest river discharge to the Gulf of Mexico behind the Mississippi River. River discharge to the bay generally peaks during late winter to early spring and minimum discharge occurs from late summer to early fall. As a result of the large freshwater inputs, the bay also receives large sediment loads, estimated to be 3.6 million tons per year, of predominantly silt and clay (Isphording et al. 1996).

Water column particulate organic matter sample collection and analysis

Water samples were collected at 15 Mobile Bay sites and 4 inner continental shelf sites (Figure 1A) during 26–28 June 2019. Samples were collected from the surface and bottom layers, i.e. about 0.5 m below the surface and 1 m above the bottom, respectively. Surface samples were obtained by grab sampling with a 200 mL bottle whereas bottom samples were collected with a 5 L horizontal Niskin bottle. For these discrete samples, salinity (S) in the surface layer was measured by conductivity with a handheld probe (YSI Pro20) and in the bottom layer by inserting the probe into the Niskin bottle. Vertical profiles of salinity were also obtained with a CTD (Seabird) at each site. Surface and bottom CTD salinity data were used to generate spatial maps of salinity distribution throughout the system (Figures 1B, 1C).

Water column samples for C and N composition and isotope analysis were filtered through pre-combusted (450°C for 2 h) 25 mm glass fiber filters (nominal pore size of 0.7 μm) and the volume filtered was recorded. Samples on the filters were

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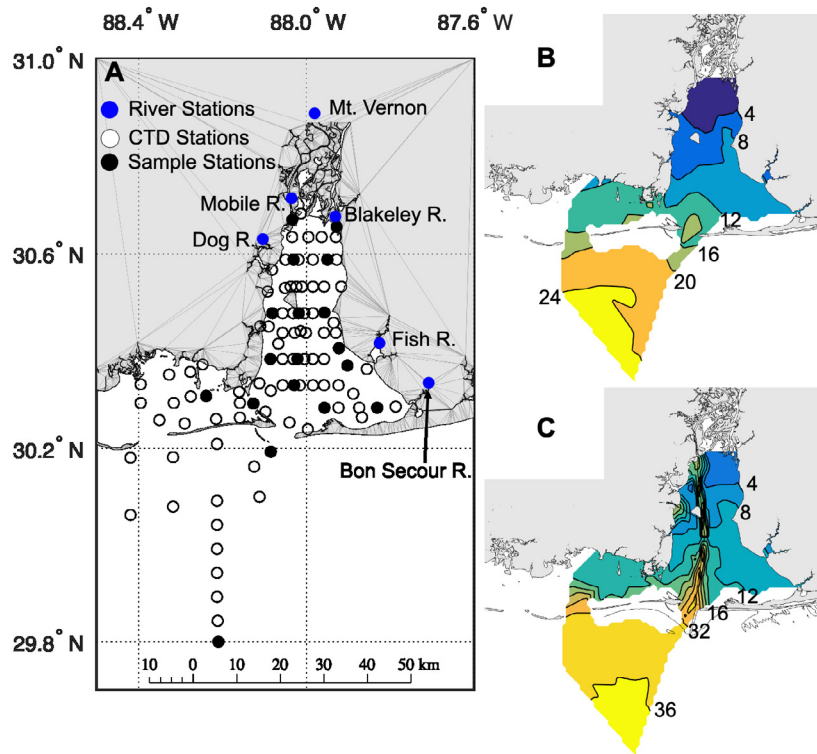


FIGURE 1. Sampling sites and salinity distribution in the Mobile Bay, AL and on the nearshore shelf. A. Map of sampling sites showing river sites (blue circle), CTD sites (open black circle), and CTD plus sediment and water column sampling sites (filled black circles). B. Salinity maps for the surface layer of the study area with labeled contours. C. Salinity maps for the bottom layer of the study area with labeled contours. Salinity data collected by CTD at all black circles in Figure A were used to generate salinity maps. Note the different color scale in upper (B) and lower (C) salinity maps.

dried in an oven at 60°C and stored in a desiccator until analysis. Prior to stable isotope analysis, filters were encapsulated in tin capsules. The C and N concentrations and $^{13}\text{C}/^{12}\text{C}$ ratios (‰) were analyzed at the Stable Isotope Facility at the University of California (Davis, CA) following standard protocols (Levin and Currin 2012). Surface and bottom water samples for chlorophyll *a* (chl*a*) analysis were also collected at each site. The chl*a* samples were analyzed fluorometrically. Briefly, samples were filtered through 25 mm glass fiber filters and the volume filtered was recorded. Filters were then folded and stored in the dark at −20°C. Upon analysis, filters were placed in 90% methanol with ammonium acetate buffer (2% vol:vol) for 24 hours at −20°C to extract chl*a*. Extracted chl*a* was analyzed on a fluorometer at excitation and emission wavelengths of 436 and 685 nm, respectively (Turner Designs Trilogy, part number 7200, with the chl*a* extracted non-acidification module, part number 7200–046).

Sediment organic matter sample collection and analysis

Sediment samples were collected between 26–28 June 2019 at the same 15 Mobile Bay and 4 inner continental shelf sites as the water samples (Figure 1). Sediment samples were collected using a Van Veen grab in triplicate at each site and the top 1 cm was removed through sub-sampling for analysis. In addition, sediments were collected on 14 June 2019 from sites on 6 rivers

that flow into the bay: 1) Mobile River at Mt. Vernon just after the confluence of the Alabama and Tombigbee rivers, 2) Mobile River at the head of the bay, 3) Blakeley River at the head of the bay, 4) Dog River, 5) Fish River, and 6) Bon Secour River (Figure 1A). At river sites, about 1 cm of the surface sediments were collected using a syringe at depths of about 0.5 m. Sediment samples were dried at 60°C and homogenized with a mortar and pestle. Inorganic carbon (mainly small shells) was removed from the sediment samples through acidification with concentrated HCl (EPA 2012). After re-homogenization with mortar and pestle, samples were encapsulated into tin capsules and shipped to the Stable Isotope Facility for determination of organic C and N concentration and ^{13}C isotopic analysis.

Data Analysis and Isotope Mixing Model

For sediment samples in the bay and on the shelf, triplicate values for a site were averaged and the mean values were used in subsequent data analyses. Standard errors of the triplicates were on average < 10% of the mean. Pearson correlation analysis was used to describe patterns between salinity, POC, POC:PON (particulate organic nitrogen) (C:N, mol:mol), and chl*a*. Model II regression analyses with these variables were also conducted. Model II regression was implemented because all variables were measured and had error, the variables have different units, and they may be controlled by variables not measured in this study. Statistical analyses were conducted in Matlab (MathWorks, Inc.) with Model II regression analysis performed using the lsqfitgm.m Matlab script (Pelzer 2019).

Observations and the model II regression results were used to specify endmembers in an isotope mixing model (Fry 2006) that calculated the fraction of freshwater versus marine source contributions to the organic matter pool for each water sample. Endmembers in the mixing model were determined from Figure S1. The freshwater endmember ($S = 0$) was assigned a $\delta^{13}\text{C}$ value of −31‰ S based on the minimum surface and bottom water observations and the regression calculated intercept for the surface samples (Figure S1). Given the uncertainty in the estimated $\delta^{13}\text{C}$ of surface water POC at $S = 35$, we calculated the marine endmember by taking the mean of the surface water and sediment values (−20.5‰ and −21.5‰, respectively), which resulted in −21‰ for the marine endmember. Then, the fraction of a sample derived from the freshwater endmember (f_{fresh}) was calculated by $f_{\text{fresh}} = (\delta_{\text{sample}}^{13\text{C}} - \delta_{\text{marine}}^{13\text{C}}) / (\delta_{\text{fresh}}^{13\text{C}} - \delta_{\text{marine}}^{13\text{C}})$, where $\delta_{\text{sample}}^{13\text{C}}$ was the $\delta^{13}\text{C}$ of a sample and $\delta_{\text{fresh}}^{13\text{C}}$ and $\delta_{\text{marine}}^{13\text{C}}$ were the freshwater and marine $\delta^{13}\text{C}$ endmembers (i.e., −31‰ S and −21‰ S). Finally, the marine fraction (f_{marine}) was calculated as $f_{\text{marine}} = 1 - f_{\text{fresh}}$. The endmember values and the $\delta^{13}\text{C}$ of samples were then used to calculate the percent contribution of organic matter types with the mixing model.

RESULTS

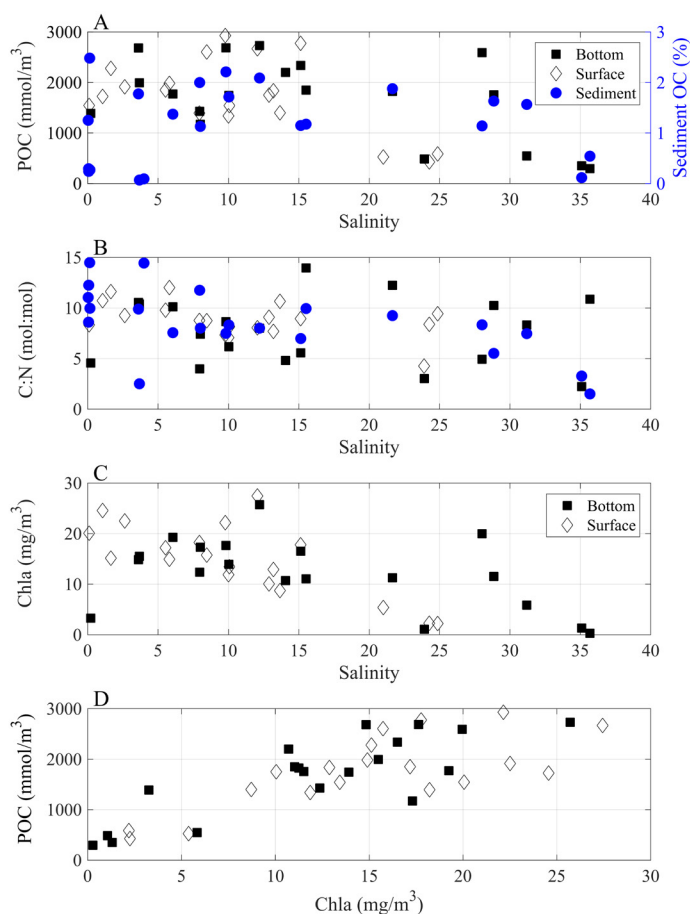


FIGURE 2. Salinity – property plots in the Mobile Bay, AL. A. Particulate organic carbon (POC) concentration in the water column and % organic carbon (OC) in the sediments in relation to salinity. B. C:N in relation to salinity. C. Chlorophyll a (Chla) in relation to salinity. D. Relationship between Chla and POC.

In June 2019, the salinity gradient from the bay to inner shelf ranged from 0.1 to 24.9 in surface water and from 0.2 to 35.7 in bottom water (Figure 1B and 1C, respectively). POC ranged from 295 to 2,926 mmol/m³. POC had a significant ($p < 0.05$) negative correlation with salinity of $r = -0.53$ for both surface and bottom water samples (Figure 2A). However, POC did not vary monotonically with salinity. POC concentrations were relatively high (mean POC = 1,892 mg/L) but variable for salinities of 0–10, were highest at salinities of 10–15 (mean POC 2,210 mg/L) and decreased significantly at salinities > 15 (mean POC = 1,130 mg/L). Sediment organic carbon (OC) ranged from 0.07 to 2.48% (reported as percentage of sediment dry weight) and did not exhibit a significant relationship with salinity ($p = 0.940$; Figure 2A). The C:N ratio also decreased as salinity increased (Figure 2B). Surface water and sediment C:N exhibited a significant relationship with salinity ($p = 0.037$ and $p = 0.002$, respectively), while bottom water C:N did not exhibit a significant relationship ($p = 0.766$).

Chla concentrations were highest at salinity < 15 (mean chla = 16.2 $\mu\text{g/L}$) and lowest at salinity > 15 (mean chla = 6.55 $\mu\text{g/L}$). Surface and bottom water chla exhibited a decreasing pattern ($p < 0.04$) as salinity increased ($r = -0.75$ and $r = -0.48$,

respectively; Figure 2C). POC and chla were also significantly positively ($p < 0.001$) correlated with one another in surface ($r = 0.78$) and bottom ($r = 0.82$; Figure 2D) water indicating that variability in phytoplankton biomass was associated with POC variability, which is expected when phytoplankton comprise a significant fraction of the organic matter in the water column.

The $\delta^{13}\text{C}$ in sediments were most variable at the 6 river sites, where values varied from -29.7‰ at Bon Secour River to -25.1‰ at the Mobile River site at the head of the bay. The Mt. Vernon site at the beginning of the Mobile delta river system had a $\delta^{13}\text{C}$ of -27.5‰ and the Blakeley River site closer to the head of the bay had a $\delta^{13}\text{C}$ of -26.1‰ . The other smaller river tributaries of Dog River and Fish River had $\delta^{13}\text{C}$ of -27.8‰ and -26.6‰ , respectively. At the marine endmember, sediment $\delta^{13}\text{C}$ were -21.9‰ and -21.5‰ at the 2 sites with bottom water salinity > 35 . The $\delta^{13}\text{C}$ of water column POC ranged from -30.9‰ to -24.0‰ . For water column and sediment $\delta^{13}\text{C}$, there were strong and significant positive relationships ($p < 0.001$) between salinity and $\delta^{13}\text{C}$ (Figure S1). For the freshwater endmember, the calculated intercept of the regression between salinity and surface water $\delta^{13}\text{C}$ yielded -31‰ (Figure S1). At the marine endmember, while we did not have surface water observations at $S = 35$, we calculated the $\delta^{13}\text{C}$ based on the surface water regression to be -20.5‰ (Figure S1). The observed sediment $\delta^{13}\text{C}$ values at $S = 35$ was about -21.5‰ . From

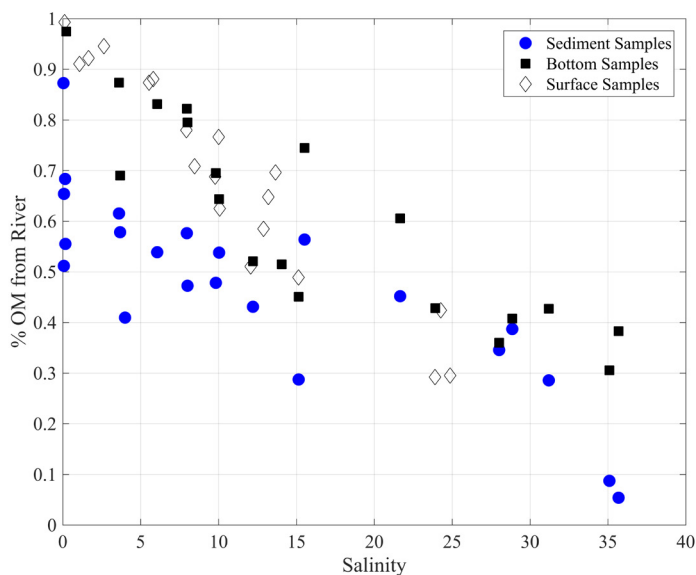


FIGURE 3. Changes in the estimated percentage of organic matter (OM) derived from freshwater (i.e., river) sources for sediment samples as related to salinity in the Mobile Bay, AL.

calculations based on the isotope mixing model at salinity < 10 the fraction contribution from freshwater organic matter ranged from 41 to 87% (Figure 3). The fraction of freshwater organic matter decreased significantly with salinity thereafter to minima of $< 10\%$ at a salinity of 35. In contrast, the calculated fractions of freshwater organic matter in the surface and bottom layers of the water column were much greater at salinity

< 10, approaching 100% at $S = 0$ and retaining 30–35% freshwater organic matter in the bottom layer at $S = 35$ (Figure 3).

DISCUSSION

Heavy rainfall and runoff during spring 2019 resulted in elevated inputs of freshwater and organic matter from the river systems to the bay. During 21 December 2018 – 21 June 2019 (winter and spring), the mean combined discharge was elevated at $2,711 \text{ m}^3/\text{s}$ as compared to the winter–spring climatological mean of $2,324 \text{ m}^3/\text{s}$ from 1971–2018 (calculated from USGS data: Alabama River site 02428400 and Tombigbee River site 02469761). A general pattern of low salinity (< 10) surface water throughout most of the bay was indicative of the elevated river discharge. There were general patterns of decreasing POC concentration, C:N, and chl *a* concentration with increasing salinity whereas $\delta^{13}\text{C}$ increased with increasing salinity. The high variability in the POC, C:N, and chl *a* may be attributed to a maximum at a salinity range of 10–15, which is where the chl *a* peaked. In previous work, chl *a* maxima were also commonly observed in this salinity range (Pennock et al. 1994). While this peak suggests that the salinity relationships to chl *a* were nonlinear, for this analysis we used simple linear correlations to demonstrate broad changes across the salinity gradient.

The near linear decrease in POC $\delta^{13}\text{C}$ in the surface and bottom layers versus salinity suggests that salinity, as expected, was a primary driver of water column POC source contribution. The decreasing C:N along the salinity gradient also suggests organic matter was transitioning from terrestrial to marine sources and supports the interpretation of the increasing $\delta^{13}\text{C}$ along the salinity gradient being due to greater marine phytoplankton influence (Hedges et al. 1997). Spatial patterns of sediment $\delta^{13}\text{C}$, however, do not conform to the patterns presented in the water column POC at low salinity. At low salinity (< 15), the sediment $\delta^{13}\text{C}$ values have a large deviation of $\delta^{13}\text{C}$ up to 2.5‰ from the surface and bottom POC $\delta^{13}\text{C}$. At higher salinity (> 15), the sediment $\delta^{13}\text{C}$ are intermediate between the predicted surface $\delta^{13}\text{C}$ at $S = 35$ and the observed bottom water $\delta^{13}\text{C}$, which suggests that the surface sediment $\delta^{13}\text{C}$ was sourced from organic carbon in the water column at these locations. Thus, based on the low salinity in the bay, most of Mobile Bay sediments have a substantial $\delta^{13}\text{C}$ deviation from $\delta^{13}\text{C}$ in the water column.

The deviation between the water column and the sediment $\delta^{13}\text{C}$ at low salinity may support alternative hypotheses that sediment resuspension and/or other organic matter source endmembers may be important in driving sediment patterns in the bay. As Mobile Bay is relatively shallow, it is prone to wind-driven sediment resuspension. Resuspension events are particularly common during the passage of winter cold fronts with strong north winds, and may also occur during tropical storm events or during periodic strong thunderstorms and associated winds. Mixing of sediment, homogenization, and subsequent redeposition could explain the $\delta^{13}\text{C}$ deviation between sediments and water column.

Other possible explanations and mechanisms may also contribute to the observed $\delta^{13}\text{C}$ deviation. For instance, we only

sampled this pattern once during June 2019. Salinity in the bay changes as a function of river discharge, which is seasonally variable. Thus, during low discharge periods, salinity at the head of the bay can increase substantially and has been observed to be > 25 in the bottom water of the Mobile River during low river discharge (Pennock et al. 1994). It is possible that the surface sediment $\delta^{13}\text{C}$ reflects a longer seasonal to annual time scale that may include excursions of high salinity during which more marine derived organic matter may be deposited. On average, though, salinity in the upper and middle bay is < 15 (Pennock et al. 1994), so it does not seem likely that periodic high salinity excursions could result in the large observed $\delta^{13}\text{C}$ deviation and the calculated high marine organic matter contribution in this region.

Additionally, Peterson (1999) found that spatial and time related changes in $\delta^{13}\text{C}$ values of dissolved inorganic carbon can lead to variation in the $\delta^{13}\text{C}$ values of estuarine phytoplankton. Phytoplankton photosynthesis fractionates $\delta^{13}\text{C}$ at around -20‰ as it takes up inorganic carbon from the dissolved inorganic carbon (DIC) pool. Thus, since the marine DIC pool has a $\delta^{13}\text{C}$ of about zero (Fry 2006), the marine phytoplankton have a $\delta^{13}\text{C}$ of -21‰ to -20‰ . The DIC pool in freshwater that phytoplankton are taking up for photosynthesis likely has a $\delta^{13}\text{C}$ of about -8‰ to -9‰ , as observed in a coastal river in Texas (Zeng et al. 2011). Thus, if phytoplankton growing at the freshwater endmember of Mobile Bay were using DIC with a similar $\delta^{13}\text{C}$, they would have a $\delta^{13}\text{C}$ in the range of -30‰ to -28‰ . At intermediate salinity of 15–18, the phytoplankton would grow on DIC with $\delta^{13}\text{C}$ of about -4‰ (assuming $\delta^{13}\text{C}$ DIC of the ocean is ~ 0 (Fry 2006) and that mixing is conservative) and would have a $\delta^{13}\text{C}$ of around -24‰ . Thus, a peak in phytoplankton biomass at mid-salinity can result in an increase in $\delta^{13}\text{C}$, which represents growth on DIC from both terrestrial and marine endmembers. The relationship between chl *a* and POC confirms that increasing phytoplankton biomass is associated with increasing POC. Hence, in the water column, phytoplankton production at specific salinities may contribute to isotopic signatures. However, if this were the dominant source, we would expect the water column and surface sediment $\delta^{13}\text{C}$ to be similar.

A final possibility we considered is that there are additional endmember organic matter sources that may be significant. For example, there are substantial areas of submerged aquatic vegetation (SAV) in the lower delta and upper bay (Vittor and Associates 2016). Organic matter derived from macroalgae has an average isotope value of about -15 (Fry 2006) and thus exports of SAV organic carbon could contribute to the $\delta^{13}\text{C}$ deviation. However, a high $\delta^{13}\text{C}$ such as -15 was not reflected in the water column $\delta^{13}\text{C}$, where we would expect that the elevated signal be observed at least in the bottom water if there was substantial export and transport of SAV or some other carbon source. Again, it may be possible that our sampling effort did not capture the time scale at which SAV, or other potential endmembers, could contribute to the sediment.

Future work should examine these patterns during different seasons and different river discharge regimes and should

also evaluate $\delta^{13}\text{C}$ of other potential endmembers, such as the SAV in the delta. Research is needed to understand how resuspension events physically mix and broadcast organic matter throughout the bay and the time scales over which these events control organic matter patterns. Next steps may also include evaluating how the patterns described here contribute to water column and sediment metabolism and ultimately to water quality problems such as the development and maintenance of hypoxia in this system.

In conclusion, we presented the patterns of water column and sediment organic carbon based on sampling during June 2019. Based on the $\delta^{13}\text{C}$ results, it was clear that the water col-

umn POC pattern was driven by the salinity distribution in the bay. However, the sediment $\delta^{13}\text{C}$ had a pattern that indicated the surface sediment organic carbon pool was not directly tracking the POC in the water column. Based on the endmember mixing model, the sediments throughout the bay had large percentages of marine organic matter. Sediment resuspension events may be one mechanism that could achieve the apparent mixing of marine organic matter all the way to the head of the bay. It seems likely that the sediment organic matter pool reflects longer seasonal to annual scale processes such as wind-driven resuspension.

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