Reviewer #1 General Comments

The author presents a study in which he uses mass balance and ecosystem metabolism data to generate carbon (C) sources and transformations in the James, Mattaponi, and Pamunkey River Estuaries. He found that the C inputs differed between rivers and season based on watershed characteristics and discharge. Contrary to his prediction, highest retention of organic C occurred during periods of relatively high discharge. These systems were net heterotrophic, though there was some contribution from autotrophy that varied by river and season. Finally, the author applied a bioenergetics model to estimate the proportion of organic C removed by catfish, bald eagles, and osprey.

This is a nice study that will be well received by the readers of this journal. The study is a thorough examination of the C cycle in terms of external (river inputs, tidal exchange) versus internal (metabolism) drivers in influencing the forms and fluxes of C in the study systems. The manuscript is well written, clear, and well organized, and I think this is a very strong and interesting dataset. For the James River, they have a relatively complete, impressive C budget dataset that spans 10 years. For the Mattaponi and Pamunkey Rivers, the dataset is less complete and spans only 2 years. But the systems are different enough that it is worth including the analysis of the less-sampled Mattaponi and Pamunkey Rivers for comparison. There are a lot of display items (2 tables + 11 figures + supplemental material), but they all seem to serve a purpose, so I don't recommend dropping any. Overall, I am comfortable with the conclusions and support publication of this manuscript with minor edits, as detailed below.

Specific Comments

Lines 70-89 Other studies to consider for this section of tidal freshwater zones:

Xu, X., H. Wei, T. Light, S. Melton, K. Holt, G. Barker, A. Salamanca, B. Hodges, K. Moffett, J. McClelland, A.K. Hardison. 2021. Tidal freshwater zones as hotspots for biogeochemical cycling. Estuaries and Coasts44:722-733. DOI: 10.1007/s12237-020-00791-4.

Jones, A.E., A.K. Hardison, B. R. Hodges, J.W. McClelland, K. B. Moffett. 2019. An expanded rating curve model to estimate river discharge during tidal influences across the progressive-mixed-standing wave spectrum. PLoS ONE14(12):e0225758,doi:10.1371/journal.pone.0225758.

Jones, A.E., B.R. Hodges, J.W. McClelland, A.K. Hardison, and K.B. Moffett. 2017. Residence time-based classification of surface water systems. Water Resources Research, 53:5567-5584, doi:10.1002/2016WR019928.

Author's Response: thank you for the suggestions. I have added the Xu et al. (2021) and Jones et al. (2017) papers.

Line 82 and elsewhere: Is there a reason why you refer to your systems as the James Estuary and not the James River Estuary? (Similar for the Mattaponi and Pamunkey River Estuaries)

Author's Response: For brevity and clarity, I use the terms James River and James Estuary to refer to the non-tidal and tidal segments, respectively.

Line 157 How did you determine the "constant fraction" the ungauged discharge was relative to the Fall Line discharge?

Author's Response. Text revised to clarify this point: "We estimated the local (ungauged) runoff as a constant fraction of the daily Fall Line discharge based on the proportion of catchment area represented by tributaries entering below the Fall Line."

Line 214 Define GPP and ER abbreviations.

Author's Response. Text modified according to Reviewer's suggestion: "Previously published estimates of Gross Primary Production (GPP) and Ecosystem Respiration (ER) were used to assess internal C transformations for the James and Pamunkey (Bukaveckas et al. 2020)."

Line 234 Define PQ and RQ abbreviations.

Author's Response. Text modified according to Reviewer's suggestion: "Oxygen-based values were converted to C assuming a photosynthetic quotient of 1.2 and a respitory quotient of 1."

Line 312-325 Refer more often to Fig. 4 and Table 2 throughout this text. (Also, please do this in the subsequent paragraphs explaining Figs. 5, 6.)

Author's Response: I can appreciate that there might be some confusion over which statements were made in relation to Figures 4, 5 and 6 vs. Supplemental Figures 1, 2 and 3. I have added some additional references to the figures to clarify.

Line 435 Replace "reveled" with "revealed"

Author's Response. Text modified according to Reviewer's suggestion: "An analysis of C dynamics in the upper portions of the James, Mattaponi and Pamunkey estuaries revealed differences in dominant forms of C and variable responses to changes in river discharge."

Lines 438-440 Explain briefly which rocks in the Mountain and Piedmont regions contribute substantially to DIC runoff.

Author's response: My main point here is that the predominantly sandy soils of the Coastal Plain yield little DIC and therefore the rivers that derive a greater proportion of their runoff from the Coastal Plain (Pamunkey & Mattaponi) have lower DIC in comparison to the James. Limestone deposits occur in the Piedmont and Mountain regions, but I feel that the current text adequately conveys the main point, without going into the regional geology. **Line 446 and elsewhere** Since you are the sole author of this manuscript, you may not want to use the "we" pronoun.

Author's Response: I have removed we's throughout the manuscript except in a few places where "we" can be taken as referring to both the author and the readers (e.g., "Here we focus on predation by bald eagles and osprey as there are census data...").

Lines 484-487 Your findings suggest the inland waters function as pipes during high discharge periods. This is counterintuitive, as one would expect particulates to not be able to settle during high discharge relative to lower discharge. Can you expand on this concept? Are your data an exception to a relatively well-established rule established from other systems? What mechanism in your system might be at play?

Author's Response: the notion that inland waters act as pipes during high discharge is more applicable to rivers and streams than to lakes, and here we show that estuaries act more like lakes than rivers. Text added to clarify this point: "The counter-intuitive finding that peak retention occurs during periods of high transport (when "pipe" conditions might be expected to prevail) is based on a consideration of the fate of both dissolved and particulate organic matter, as the former largely passes through, while the latter is highly retained. The retention of particulate matter reflects the underlying hydrodynamics of estuaries, and lakes, where the rapid dissipation of fluvial forces promotes high retention of particulate matter during periods of elevated discharge."

Lines 490-500 Your data suggested use of a lower exchange coefficient (1 to 1.5 m/d; section 2.7), and you ended up using a value ~4x higher (4.3 m/d) based on values published by Raymond and colleagues. But in this section of the discussion, you refer to another study where Raymond used a value closer to the low value (1.1 m/d), so you then suggested that might be more appropriate to get your values closer to the Raymond et al. 2000 air-water fluxes. It seems to me like you should have stuck with your data-driven value (1 to 1.5 m/d) in the first place? This issue warrants further explanation in the methods and discussion.

Author's Response: the lower exchange value (1.1 m/d) was used in an earlier paper by Raymond (2000), whereas in later papers (2017) he advocates for using a higher exchange coefficient in rivers and estuaries. My use of the higher exchange coefficient is consistent with current thinking that turbulence in rivers and estuaries is the more important factor affecting boundary layer conditions than is wind speed (i.e., coefficients derived from wind speed alone underestimate atmospheric exchange). I have moved some of the text from Methods to Discussion to clarify this point. "Raymond et al. (2000) used what they considered a conservative exchange coefficient (1.1 m d-1). More recent studies have adopted higher exchange coefficients, particularly for systems where tidal and fluvial forces likely play a greater role in determining boundary layer conditions than are predicted from wind-based models. Wind speeds are low in the upper segments of these estuaries because the prevailing winds (SSW) are nearly perpendicular to the long axis of the channel, which runs mostly east-west. Turbulence generated by strong tidal forces in shallow channels likely plays a greater role in influencing boundary conditions for gas exchange (Raymond and Cole 2001; Borges et al. 2004). These conditions support the use of higher exchange coefficients than would be derived from wind speed alone."

Lines 533-535 What characteristics of the Susquehanna River and Chesapeake Bay mainstem make them net autotrophic?

Author's Response. Text added: "In the case of Chesapeake Bay, it may be that much of the terrestrial organic matter (or at least, the POC fraction) is captured in the tributaries (as shown in this paper), thereby favoring a prevalence of autochthony over allochthony, and GPP in excess of R."

Line 548 Insert "times" after "residence"

Author's Response. Text modified according to Reviewer's suggestion: "This is likely a consequence of tidal conditions, which allow for longer water residence time compared to non-tidal rivers."

Line 584 Insert "of" before "POC"

Author's Response. Text modified according to Reviewer's suggestion: "These estimates can be refined to better reflect availability for consumers by discounting GPP by 40% to reflect loss via autotrophic respiration (Ruegg et al. 2021) and taking into account the fraction of POC and DOC that is retained ($28 \pm 3\%$)."

Bukaveckas Response to Reviewer #2 Comments

Reviewer #2 General Comments

The paper discussed Sources and transformation of C to understand external (river inputs & tidal exchange) vs. internal (metabolism) in upper segments of the James, Pamunkey and Mattaponi Estuaries. The contrast in the qualitative and quantitative capacities of different carbon pools in the three studied estuaries, despite that they flow adjacent to each other and share almost similar carbon sources in their catchment, is unique and essential considering the modified carbon cycle under changing global climate condition. The manuscript provides new insight to the modified carbon cycling along the tidal freshwater regions of selected tributaries of Chesapeake Bay, is well-written and the data quality is good. I think the readers of this journal will benefit from the information contained in this paper. I therefore recommend publication of the paper after minor revision listed below.

Author's Response: thank you for your comments.

Reviewer #2 Specific Comments

Introduction: The relative fraction of area covered under each estuaries during the study is not clear, whether it represent the entire estuarine contribution?

<u>Author's Response</u>. Text revised to clarify this point: "The study reach within the James Estuary is the tidal fresh segment, which extends 88 km from the Fall Line (Richmond, VA) to the confluence with the Chickahominy River, and accounts for ~50% of the length of the estuary. Study reaches for the Pamunkey and Mattaponi Estuaries encompassed the tidal fresh and oligohaline segments, extending 86 km to their confluence with the York Estuary."

Methods: Information on the data collection frequency and use for the model is missing.

<u>Author's Response</u>. Text revised to clarify this point: "Fall Line samples were collected at approximately monthly intervals, with supplemental samples collected during periods of high discharge. Approximately 200 measurements of DOC and POC were obtained at each of the gauging sites over the 10-year span (Table 1), along with continuous measurements of river discharge. For the James, the USGS data were supplemented by measuring DIC and Cl at the Fall Line at 1-2 week intervals during 2012-2019 (189 samples collected)."

Summary: The relevance and global significance of the study in terms of tropical and non-tropical context.

<u>Author's Response</u>. I devote some attention to the global significance of tidal freshwater systems in the third paragraph of the Introduction ("Tidal freshwaters are a common feature of river-dominated estuaries throughout the world..."). For the closing point of the summary, I opted to focus on the importance of integrating mass balance, metabolism and food web studies as I felt this was a key novel aspect of the paper. I regret that I do not have specific insights to offer regarding the functioning of tropical vs. non-tropical estuaries, though I agree that this is an important research topic.