## Response to Reviewers

We are grateful to the two reviewers for their thoughtful and critical comments, which we are confident will lead us to improve the paper considerably. Below, we repeat all of the comments by the reviewers and follow each comment by our response.

## Reviewer #1 Comments

**Reviewer Comment 1:** This manuscript provides valuable insights into the spatial drivers of benthic biomass and quantifies the substantial role of macrofauna in estuarine carbon fluxes. This is a well-structured and significant study that addresses critical knowledge gaps regarding the environmental drivers of benthic macrofauna biomass distribution and their role in estuarine carbon cycling. The manuscript leverages an extensive long-term dataset (8,128 samples) and robust statistical modeling (GAMs) to reveal spatial patterns in Chesapeake Bay. The focus on both respiration and calcification fluxes is particularly novel and highlights the underappreciated role of macrofauna in $CO_2$ production. While the methodology is sound and conclusions are largely well-supported, the linkage between the biomass and the carbon flux needs to be strengthened.

**Response:**

We appreciate the reviewer's overall positive view of our study as well as their view of a need to strengthen the linkage between biomass and carbon flux. We have addressed this need in our response to Reviewer Comment 2 below.

### Major Comments

**Reviewer Comment 2:** One key drawback is that this study lacks the link between the two research questions. In lines 191–195, although the two research questions raised in this study are significant, their relationship needs to be enhanced.

**Response:**

We appreciate this helpful observation. In the revised manuscript, we substantially restructured the introduction to begin with the impact of benthic macrofauna on estuarine carbon cycling through processes such as secondary production, calcification, and respiration. We then introduced the idea that different environmental variables can help predict benthic biomass. Because carbon fluxes are proportional to biomass, identifying consistent environmental drivers of biomass will provide a means to infer macrofaunal carbon cycling without relying on labor-intensive benthic sampling. This framing clarifies the connection between our two research questions, which we have reordered, and emphasizes how environmental managers and numerical modelers can use widely available environmental data to estimate benthic carbon fluxes.

**Reviewer Comment 3:** Additionally in this study, carbon flux is generally calculated from the macrofaunal community; It is highly expected to see a distribution map of carbon flux in the bay.

**Response:**

We agree that visualizing carbon flux distribution is important. However, because carbon fluxes in our study are calculated directly from biomass, their spatial distribution closely mirrors that of biomass. While we could have included a map of carbon fluxes in the Appendix for reference, we believe that would add limited new insight. Instead, we moved Figure A3 from the supplemental materials into the main text (Figure 4), with the addition of alkalinity, DIC, and $CO_2$ fluxes. This figure provides a clearer representation of the relationship between biomass and carbon flux and helps illustrate their relative magnitudes across space.

**Reviewer Comment 4:** Additionally, instead of showing the bivalve species in Figure 1, why not show the distribution of total macrofaunal biomass? And based on the biomass and the calculation to express the carbon in the bay area?

**Response:**

Thank you for this suggestion. We agree that showing total macrofaunal biomass would better support the aims of the study. We revised Figure 1 (now Figure 2) to display the distribution of total macrofaunal biomass rather than species-specific biomass, as species-level detail is already addressed in Figure 2 (now Figure 3). Our approach to visualizing carbon fluxes is addressed in our response to Reviewer Comment 3.

**Reviewer Comment 5:** The hypothesis that surface $NO_3^-$ acts as a proxy for allochthonous POC driving high biomass is compelling but requires stronger validation. Although Fig. 5 shows a correlation, direct evidence linking modeled/observed POC concentrations to biomass within the GAM framework is absent. The exclusion of ROMS-ECB POC due to poor validation weakens this claim.

**Response:**

We appreciate the reviewer's thoughtful critique. We acknowledge that the hypothesis linking $NO_3^-$ to allochthonous POC and thereby to macrofaunal biomass is currently speculative. Due to the limited validation of POC outputs in the ROMS-ECB model, we chose not to include them in our analysis. In the revised manuscript, while we briefly explored the hypothesis that $NO_3^-$ may act as a proxy for allochthonous POC, we clarified that this interpretation is speculative and will remove the figure showing the linear correlation between $NO_3^-$ and POC to avoid overstating the case. We also removed POC from the abstract to better reflect the tentative nature of this link.

**Minor Comments**

**Reviewer Comment 6:** Line 29: It is a little confusing that "(1995–2022)" behind the "Bay." Does this mean the samples were collected during 1995–2022?

**Response:**

Yes, the reviewer is correct. We revised the sentence for clarity to indicate that the dataset includes samples collected between 1995 and 2022 (now Line 3).

**Reviewer Comment 7:** Line 52: "As adults?" Typo?

**Response:**

We appreciate this comment. "As adults" is not a typo, but we agree that the specification is unnecessary and could confuse readers. We removed the phrase for clarity (Still Line 52).

**Reviewer Comment 8:** Line 72: Try not to use "the most."

**Response:**

We revised this phrasing to avoid the use of superlatives and improve clarity and precision.

**Reviewer Comment 9:** Table formatting: Please delete the vertical lines in tables.
**Response:**
Thank you for the formatting suggestion. We removed all vertical lines from the tables in the revised manuscript to align with journal standards. However, we retained some vertical lines in Table 2 (now Table 5) to preserve the clarity of variable groupings, which would otherwise be confusing.

## Reviewer #2 (Ludovic Pascal) Comments

**Reviewer Comment 1:** First of all, I want to acknowledge the extensive work of the authors. All the comments in this review are intended to improve clarity and enhance the manuscript's impact for a broad readership.
**Response:**
We appreciate the reviewer's positive comment and are grateful for their suggestions to make the manuscript clearer and more impactful. Responses to specific comments about clarity and impact are given below, particularly in the responses to Reviewer Comments 4, 6, 7, 8, 10, 22, 29, 36, and 42.

**Reviewer Comment 2:** Ajayi et al. aim to demonstrate how benthic macrofauna biomass in Chesapeake Bay relates to environmental drivers and contributes to estuarine carbon fluxes. They use an impressive long-term dataset in association with empirical conversion factors to estimate respiration and calcification fluxes. Notably, their CO2 production estimates, driven by both respiration and calcification, exceed measured outgassing at the air–sea interface. However, without a clearer discussion of carbonate chemistry and alkalinity, this result risks being over-interpreted.
**Response:**
We appreciate that the reviewer has noted a need for a clearer discussion of carbonate chemistry and our specific responses are given following Reviewer Comments 5, 12, 13, 14, 15, and 27.

**Reviewer Comment 3:** Overall, the manuscript provides valuable insight by highlighting an underexplored component of estuarine carbon budgets. The focus on macrofauna-mediated calcification fluxes is a novel and an important contribution.
**Response:**
We appreciate the reviewer's positive comment about our contribution.

**Reviewer Comment 4**: To strengthen the study, I recommend to structure the narrative more smoothly, particularly in the abstract and introduction to clearly link ecological contest, knowledge gaps and research objectives
**Response:**
Our revised abstract follows a clearer narrative flow, moving from motivation, to research questions, to key results, and finally to broader implications. Please also see our response to Comment 2 of Reviewer 1 and Comment 8 of Reviewer 2.

**Reviewer Comment 5:** To strengthen the study, I recommend expanding the theoretical framework to include a more detailed section on carbonate chemistry, bioturbation and early diagenesis

**Response:**
We agree with the reviewer that the manuscript would benefit from a more comprehensive theoretical framework addressing carbonate chemistry, bioturbation, and early diagenesis, particularly in relation to the role of benthic macrofauna in carbon cycling. In the revised manuscript, we expanded the background to include a more detailed discussion of these processes and their relevance to estuarine biogeochemistry. To accommodate this addition without exceeding length constraints, we streamlined and generalized the section on environmental drivers of benthic macrofauna. This revision better frames carbon cycling as the central theme of the study while still highlighting key ecological drivers of biomass distribution.

**Reviewer Comment 6**: To strengthen the study, I recommend clarifying methodological choices and quantifying uncertainties, especially because of the use of empirical conversion factors, and the decision to time-averaged the data.
**Response**:
We appreciate the reviewer's attention to these important methodological details. In the revised manuscript, we more clearly explain our rationale for time-averaging and explicitly describe how we quantified uncertainty, particularly given the use of empirical conversion factors. These clarifications are also addressed in more detail in our responses to Reviewer Comments 19, 20, 25, and 43 and the corresponding revisions to the methods and discussion sections.

**Reviewer Comment 7:** To strengthen the study, I recommend to improve the conclusion to highlight the key findings and the novel insights. In the present form the conclusion is confusing and undermines the confidence in methodology and thus in the findings
**Response:**
We appreciate the reviewer's feedback to communicate our findings more clearly. In response, we revised the Conclusion section to provide a clearer, high-level summary of the key findings and novel insights. To address concerns about clarity and confidence in the methodology, we moved detailed discussion of methodological assumptions and limitations to the Methods and Discussion sections, allowing the Conclusion to focus on synthesis rather than caveats. Additionally, we added a forward-looking paragraph that outlines future research directions, including the importance of assessing temporal variability.

**Reviewer Comment 8**: The abstract lacks clarity and structure. It doesn't clearly present the context or motivation for the study, and the objectives are not well defined. Instead, it quickly shifts to methodological details and a dense list of results, without a coherent narrative linking background, approach, and conclusions. A more structured format would greatly improve its clarity and impact.
**Response:**
Our revised abstract follows a clearer narrative flow, moving from motivation, to research questions, to key results, and finally to broader implications. We also believe that swapping research questions 1 and 2 (see response to Comment 4) created a stronger structure for the introduction and the overall paper. This reordering allows us to begin with the potential impacts of benthic macrofaunal biomass on carbon cycling, highlight the limitations caused by sparse biomass measurements, and then introduce the use of GAMs to identify environmental proxies for biomass. These proxies could, in turn, serve as indirect indicators of benthic macrofaunal contributions to carbon cycling.

**Reviewer Comment 9:** Finally, I think the manuscript is not yet mature enough and requires major revisions. Please note that I am not involved in any of the papers I suggest in this review. Below are more detailed comments on the manuscript.
**Response:**
We appreciate the reviewer's frank appraisal of the manuscript, their suggestions for additional papers to consider, and their very detailed and extensive comments on the manuscript, all of which led us to improve the manuscript considerably.

**Introduction**

**Reviewer Comment 10**: The introduction is difficult to follow and, as in the abstract, lacks a coherent narrative. It reads more like a sequence of examples leading to two objectives. Subheadings are unnecessary, they disrupt the flow and create redundancy.
**Response**:
We appreciate this helpful observation. As discussed in response to Comment 4, we substantially restructured the introduction to begin with the impact of benthic macrofauna on estuarine carbon cycling through processes such as secondary production, calcification, and respiration. We then introduced the idea that different environmental variables can help predict benthic biomass. Because carbon fluxes are proportional to biomass, identifying consistent environmental drivers of biomass will provide a means to infer macrofaunal carbon cycling without relying on labor-intensive benthic sampling. To streamline this narrative, we condensed the discussion of environmental drivers by focusing on overarching conclusions from multiple studies rather than listing individual results. This framing clarifies the connection between our two research questions and emphasize how environmental managers and numerical modelers can use widely available environmental data to estimate benthic carbon fluxes.

**Reviewer Comment 11**: Line 63: Define hypoxia clearly. What do you mean by "extremely low dO2"?
**Response**:
Thank you for pointing this out. In the revised manuscript, we defined hypoxia using the widely accepted ecological threshold for marine and freshwater systems: dissolved oxygen concentrations $\leq 2$ mg/L (Diaz & Rosenberg, 2008; Levin et al., 2009). We revised the text at Line 63 (now line 173) to clearly reflect this definition.

**Reviewer Comment 12**: Lines 62–78: The section on O2 is superficial and lacks nuance, especially regarding changes in organism behavior and metabolism under hypoxic conditions. You may want to look at Levin, Diaz, Rabalais or Rosenberg work. Rabouille and Lansard work are also good references for the influence of hypoxia on carbonate chemistry. There are other recent works focusing on macrobenthic community structure and activity changes under hypoxic conditions.
**Response**:
While we agree that the original section was somewhat superficial, we would like to clarify that its primary focus was on the role of dissolved oxygen in shaping benthic macrofauna biomass distributions. We addressed the influence of hypoxia on carbonate chemistry, including anaerobic respiration, in the section of the introduction that discusses carbon fluxes. That said, we revised the dissolved oxygen section to provide a more generalized synthesis of its ecological

effects, rather than focusing on individual studies. We reviewed all the references suggested and integrated several of them into the revised introduction to strengthen the conceptual framing.

**Reviewer Comment 13:** Lines 119–167: The absence of any mention of bioturbation is surprising, given its importance in sediment metabolism and its strong influence of carbonate chemistry.
**Response**:
We appreciate this important observation. While our study does not explicitly quantify bioturbation rates, we agree that bioturbation is a key mechanism through which benthic macrofauna influence sediment carbon dynamics and carbonate chemistry. In the revised manuscript (Lines 112–123) , we incorporated a brief discussion noting that bioturbation, including particle reworking and burrow ventilation, enhances organic matter decomposition, modifies redox gradients, and influences carbonate dissolution and precipitation processes. These processes are part of the broader influence of macrofaunal activity that we aim to capture through biomass-based estimates of respiration and calcification. Acknowledging this mechanism will help clarify how benthic fauna contribute to carbon fluxes beyond the direct empirical relationships used in our modeling framework.

**Reviewer Comment 14:** Lines 129–131: This describes aerobic respiration only. Anaerobic respiration and its impacts on carbonate chemistry should be included.
**Response:**
We appreciate this suggestion. Our study focuses specifically on macrofauna-driven carbon fluxes, which are fundamentally linked to aerobic respiration, as benthic macrofauna require oxygen to survive and actively contribute to sediment metabolism. In areas where oxygen is depleted and anaerobic respiration dominates, macrofaunal biomass declines sharply, limiting their role in biogeochemical cycling. Nevertheless, we agree that it is important to situate our analysis within the broader context of sediment carbon cycling. In the revised manuscript (Lines 87–94), we briefly acknowledged key anaerobic pathways and their contributions to DIC and alkalinity production while clarifying that our modeling framework focuses on macrofauna-mediated aerobic processes that are most relevant under suboxic to normoxic conditions.

**Reviewer Comment 15:** The carbonate chemistry section oversimplifies key processes. There is no mention of carbonate saturation state, and alkalinity is only briefly mentioned. Anaerobic respiration plays a key role in DIC and alkalinity dynamics under hypoxic conditions.
**Response:**
We appreciate this thoughtful comment. While carbonate saturation state ($\Omega$) and its role in benthic calcification is discussed later in the manuscript, we agree that introducing this concept earlier would help frame the relevance of calcification within the broader carbonate system. In the revised manuscript, we briefly define $\Omega$ (Lines 101–110) and note its connection to carbonate precipitation and dissolution dynamics as a way to better orient the reader prior to the more detailed discussion.

We also acknowledge that alkalinity is a key component of carbonate chemistry and its inclusion is important for understanding how macrofaunal processes influence buffering capacity and $CO_2$ dynamics. We revised the carbonate chemistry section to introduce alkalinity explicitly (Lines 75–81), noting how both biological and geochemical processes, including respiration and calcification, affect alkalinity in estuarine sediments. As addressed in our response to Comment 14 we additionally include a brief acknowledgment that anaerobic respiration can influence DIC and alkalinity production, especially under hypoxic conditions. In the results section, we convert edbenthic macrofaunal calcification and respiration fluxes into estimates of DIC and total alkalinity to quantify their impact on carbonate chemistry.

**Reviewer Comment 16**: Line 142: This is a strong claim supported by a single reference. Glud (2008) provides a more comprehensive review and may offer a better-supported estimate.
**Response:**
We thank the reviewer for this observation. The statement in line 142 was based on results from Rodil et al. (2022), which report elevated benthic oxygen consumption in a specific estuarine setting. However, we agree that this may overstate the generality of the finding when presented in isolation. In the revised manuscript, we will revise the language to make it clear that the result is site-specific and not necessarily representative of broader patterns.

To strengthen the context, we included Glud (2008) in the revised manuscript (Lines 133–135), which provides a more comprehensive and globally integrated estimate of benthic oxygen dynamics across marine environments, including both photic and aphotic zones. This allows us to more appropriately situate the findings of Rodil et al. within the broader range of known oxygen fluxes and improve the robustness of the statement.

**Reviewer Comment 17**: The paper clearly assumes readers are familiar with carbonate chemistry. For further background on DIC and alkalinity production/consumption, the authors may find the following recent syntheses helpful:

- https://doi.org/10.1029/2019RG000681 (Middelburg et al. 2020)
- https://doi.org/10.5194/bg-13-5379-2016 (Rasmann et al. 2016; not a review but I find the carbonate chemistry processes well described in this paper)

**Response:**
Thank you for this helpful suggestion. We agree that providing additional background on carbonate chemistry would improve accessibility for a broader readership. In the revised manuscript, we added a concise overview of the key components of the carbonate system, including the relationships among dissolved inorganic carbon (DIC), alkalinity, carbonate saturation state (Ω), and pH.

**Reviewer Comment 18:** Line 162: "Other characteristics" is vague—please specify.
**Response:**
Thank you for pointing this out. In the revised manuscript (Now Line 160), we removed the phrase "other characteristics" since the relevant factors, biomass, taxonomic identity, average body mass, and water temperature**,** are already explicitly listed and are the primary variables used in the referenced empirical models. This revision improves clarity and prevents potential confusion about which additional traits are being referred to.

**Reviewer Comment 19**: Lines 163–167: This is unclear. Do you mean calcification and respiration rates are inferred from secondary production estimates? What is the associated uncertainty?
**Response:**
We appreciate the reviewer's comment and recognize the need to clarify this aspect of the methodology. In the revised manuscript (Lines 156–164), we make the text clearer that respiration rates and calcification rates are inferred from secondary production estimates. While

these relationships are not associated with formal statistical uncertainty, we used a range of published values for biomass-to-carbon conversion, secondary production models, and species-specific calcification ratios, allowing us to estimate upper and lower bounds for carbon fluxes.

**Methods**

**Reviewer Comment 20**: Line 200: Clarify what "time-averaged" means in this context.
**Response:**
Thank you for this helpful comment. In the revised manuscript, we clarified that "time-averaged" refers to averaging all benthic biomass observations collected within each grid cell from 1995 to 2022 to generate a single long-term mean value per cell. The averaging was conducted after assigning individual observations to spatial grid cells, and in this context, the terms "time-averaged" and "gridded" are used nearly interchangeably. To avoid confusion, we removed the initial reference to time-averaging and instead introduce the concept more fully in the Methods section, where it can be explained in appropriate detail (Lines 284–304).

**Reviewer Comment 21:** Line 209: Provide more detail on how the literature review was conducted.
**Response:**
Thank you for the suggestion. In the revised manuscript, we clarified that the literature review was conducted informally using online search tools (e.g., Google Scholar) to identify empirical relationships that could be used to estimate secondary production, respiration, and calcification from benthic biomass. We prioritized studies focused on bivalves, which represent the dominant macrofauna in the Chesapeake Bay, and selected conversion relationships that were both relevant to local species and straightforward to apply to our dataset. While there was broader literature available for secondary production (with some values supported across multiple studies), fewer options existed for respiration and calcification. In those cases, we selected relationships derived from species commonly observed in the Bay and that could be easily scaled from production estimates. We revised the manuscript to include this rationale and approach. We expanded the literature review at the beginning of Section 2.3 (Carbon Flux Estimations), rather than including it in the Overview.

**Reviewer Comment 22**: Overall, the methods section could be streamlined and made clearer
**Response:**
We streamlined the Methods section by removing the portion on residual analysis from the GAM results, shortening the explanation of how GAMs work, and moving the uncertainty calculations to the Appendix. Additionally, we swapped Research Questions 1 and 2 to improve the logical flow and clarity of each subsection.

**Reviewer Comment 23**: Lines 234–244: Could differences in sampling gear affect community diversity, abundance, or biomass? You could test this by comparing overlapping stations.
**Response**:
Thank you for this important comment. Differences in gear design, such as penetration depth, sample volume, and sediment disturbance, can influence estimates of diversity, abundance, and biomass. This limitation is now noted explicitly in the revised text (Line 272–274), and we cited Eleftheriou & Moore (2013), who discuss gear-related sampling effects on benthic macrofauna.

**Reviewer Comment 24**: Lines 251–253: The mention of GAM analysis is confusing without further detail. Consider removing or relocating.
**Response**:
Thank you for the suggestion. In the revised manuscript, we will remove the mention of the GAM analysis at this point in the methods and instead introduce it later in the section where it can be more fully explained in context.

**Reviewer Comment 25:** The rationale for time-averaging is unclear. Was variability around the average considered in your analysis? Temporal variability or stability in environmental conditions may be ecologically relevant.
**Response:**
Thank you for raising this important point. We revised the methods section (Lines 284–304) to clarify that we used time-averaged environmental conditions to capture long-term spatial gradients most relevant to benthic macrofaunal biomass. Benthic biomass itself is highly variable, even samples taken near the same location can differ substantially, which is why replicate sampling is common. To reduce this inherent noise and enable more robust statistical analyses, we applied time-averaging to both biomass and environmental data. This approach allowed us to highlight persistent spatial patterns and better identify consistent environmental drivers across segments. We acknowledge that this averaging removes ability to assess temporal variability, which may also influence benthic processes, and we noted this in the conclusion as an important avenue for future research.

**Reviewer Comment 26**: Section 2.4: The inclusion of spring and fall environmental data needs clarification. The logic behind this choice is not well explained.
**Response:**
Thank you for this comment. In the revised manuscript, we clarified that seasonal environmental data (spring, summer, fall, and winter) were included to align with ecologically meaningful time periods for benthic–pelagic coupling. For example, the spring diatom bloom delivers fresh organic material that fuels benthic production, while summer conditions reflect stratification, hypoxia, and high remineralization rates. Fall and winter may represent baseline conditions. By using seasonal groupings rather than annual or arbitrary averages, we aimed to capture the temporal windows most relevant to benthic macrofaunal biomass and their environmental drivers.

**Reviewer Comment 27:** Line 327: carbonate saturation state (Ω) should be introduced earlier, ideally in the Introduction.
**Response:**
Thank you for this comment. As noted in our response to Reviewer Comment 15 we revised the manuscript to introduce carbonate saturation state (Ω) earlier in the carbonate chemistry section.

**Reviewer Comment 28**: Line 346: Were the GAMs in this study not based on environmental variables? Please clarify.
**Response:**
Thank you for this observation. We agree that the original wording was unclear. The GAMs are indeed based on environmental variables, and we revised the sentence to explicitly state that fact.

**Reviewer Comment 29:** The statistical analysis section is overly detailed. Focus less on the mechanics of each test and more on the reasoning behind the six predictor variables.
**Response:**
Thank you for this helpful feedback. We agree with your comment, and in the revised manuscript, we streamlined the statistical analysis section to focus more on the rationale for selecting the six environmental predictors used in the GAMs. Specifically, we moved the section on residuals to the Supplement and condensed the description of GAMs, removing excessive detail about collinearity and model mechanics.

**Results**

**Reviewer Comment 30**: Lines 501–516: If the distribution is heavily skewed, why use the average instead of the median?
**Response:**
Thank you for this thoughtful observation. In the revised manuscript, we will clarify that we report mean biomass to remain consistent with previous studies that have investigated relationships between benthic biomass and environmental drivers in the Chesapeake Bay, including Woodland et al. (2021) and Seitz et al. (2009) both of which reported mean biomass. However, we agree that due to the skewed nature of the data, median values can provide a more representative measure. Therefore, we will include both mean and median values in the revised manuscript to give a more complete picture of biomass distributions.

**Reviewer Comment 31**: Figure 1: This figure could be misleading as it only shows species with the highest time-averaged biomass, potentially obscuring relative changes in the biomass of other species.
**Response:**
Thank you for this observation. To avoid confusion and improve clarity, we revised Figure 1 (now Figure 2) to display only total macrofaunal biomass using a single color scale. Because Figure 2 (now Figure 3) already presents species-level differences across the estuary, it is not necessary for Figure 1 (now Figure 2) to show species-specific biomass. This revision will help ensure the figure supports the broader spatial patterns of biomass without potentially obscuring trends in less dominant taxa. We also addressed a similar comment for Reviewer #1 Comment #4.

**Reviewer Comment 32**: Lines 523–540: Was the shell biomass removed from biomass measurements?
**Response:**
Thank you for raising this question. Biomass in our dataset is reported as ash-free dry weight (AFDW), which excludes shell material and therefore reflects only the soft tissue mass. We clarified this more explicitly the first time AFDW is introduced to ensure this point is clear to all readers (Lines 279–281).

**Reviewer Comment 33:** Figure 2: The x-axis shows "O", but the caption says "OH", and the same issue occurs with "P".
**Response:**

Thank you for pointing this out. We will correct the x-axis labels to match the caption for clarity and consistency (Now Figure 3).

**Reviewer Comment 34**: Line 554: What are the predictor variables?
**Response:**
Thank you for this comment. While the predictor variables are listed in Table 2 (Now Table 5), we agree it would improve clarity to explicitly state them in the text. In the revised manuscript (Line 766), we listed the six environmental predictors used in the GAMs at this part of the results section.

**Reviewer Comment 35**: Lines 607–624: This section describes the methodology, not results.
**Response:**
Thank you for this helpful comment. We agree that portions of this section included methodological details more appropriate for the Methods section, and we have revised the manuscript accordingly. Key calculation steps and assumptions have been moved to the Methods. However, this section also includes the results of our empirical calculations, which we believe are important to retain in the Results to support interpretation and comparison with published values.

**Reviewer Comment 36**: The overall structure of the results is difficult to follow, as sometimes full dataset is used, and at other times only the average gridded values are presented. There is also some redundancy in the presentation.
**Response**:
Thank you for this helpful observation. In the revised manuscript, we calrified at each point in the Results section whether the full, time-averaged/gridded, or regionally averaged (by Chesapeake Bay segment) dataset is being used. The methodology for both types of averaging iw now described more clearly in the Methods. In the Results section, we ensure it is always explicit which dataset is being referenced. We also edited the flow of the text to reduce redundancy.

**Discussion**

**Reviewer Comment 37**: Lines 702–704: Many species can survive hypoxic conditions. See, for example, the work of Levin.
**Response:**
Thank you for this suggestion. In the revised manuscript, we now clarify that our focus is specifically on benthic *macro*fauna, which are often more sensitive to hypoxia and less likely to survive prolonged low-oxygen conditions. The original phrasing referred to “benthic fauna,” which was too general and potentially misleading. This distinction is nowexplicitly noted in the Introduction, and we added the word “often” to acknowledge that some taxa are tolerant of hypoxia. To further address the reviewer’s concern, we clairfy that the primary reason for macrofaunal sensitivity to hypoxia is their relative immobility (especially for infaunal , macroinvertebrates), while their general physiological sensitivity, compared to more tolerant taxa such as meiofauna, is a secondary factor.

**Reviewer Comment 38**: Lines 733–787: The rationale here is unclear. The long paragraph begins with NO3 and then moves to DO2 and POC. However, the latter two variables were excluded from the model due to poor results or collinearity. This raises questions about the overall quality of the model.

**Response:**

Thank you for this helpful comment. We agree that the rationale in this section requires clarification and we significantly shorten the paragraph to reflect that much of the discussion is speculative.

We clarify that $NO_3^-$ is included in the GAMs, while $\Delta[O_2]$ and POC are not. $\Delta[O_2]$ was excluded from the GAMs due to high collinearity with salinity and dissolved oxygen, highlighting a known limitation of GAMs, where ecologically meaningful variables may be dropped when they are highly correlated with others. We point out this limitation more explicitly in the Methods.

POC, on the other hand, was excluded not because of model performance, but because ROMS-modeled POC does not meet our quality threshold (Spearman $r_s < 0.7$). While we briefly explore the hypothesis that $NO_3^-$ may act as a proxy for allochthonous POC, we now clarify that this interpretation is speculative and will remove the figure showing the linear correlation between $NO_3^-$ and POC to avoid overstating the case. We also remove POC from the abstract to better reflect the tentative nature of this link.

**Reviewer Comment 39:** Line 761: Could NO3- be used as a proxy for freshwater input?

**Response:**

We agree that surface $NO_3^-$ could serve as a partial proxy for freshwater input, particularly in non-tidal riverine zones where nitrate concentrations are primarily influenced by terrestrial sources. However, within estuarine tributaries, the relationship between $NO_3^-$ and freshwater input becomes more complex. Biological uptake of nitrate varies along the salinity gradient and is strongly influenced by light availability, water column stratification, and phytoplankton dynamics. As a result, $NO_3^-$ concentrations can become decoupled from freshwater delivery in downstream tidal regions. For the sake of brevity, we will not include this clarification in the revised text but appreciate the opportunity to note it here.

**Reviewer Comment 40:** Lines 828–840: The changes in the oyster population should be mentioned in the methods section. Do you think that the decline in the population could influence your results, especially since you compare them to older literature?

**Response:**

Thank you for this helpful comment. As noted in the Methods section, our analysis is based on benthic grab samples that do not capture oyster reef habitats, so oysters were not included in our biomass or calcification estimates. Their mention in the Discussion is intended to provide ecological and historical context for interpreting the role of calcifying bivalves in estuarine carbon cycling.

We now clarify in the Methods that oyster populations in the Chesapeake Bay have declined by more than two orders of magnitude and explicitly state that our comparison to oyster calcification reflects historical (pre-decline) levels. In the results, we now compare soft-tissue bivalve calcification to historical oyster reef calcification by multiplying by 100, rather than using present-day values. We believe this better illustrates the potential scale of calcification from bivalve communities under more robust oyster population scenarios.

**Reviewer Comment 41:** The discussion of calcification discussion is relatively brief, despite it appearing to be a central focus of this article.
**Response:**
We agree that the discussion of calcification can be expanded, especially given its central role in our study. In the revised manuscript, we add alkalinity and DIC calculations to the Methods and Results sections and compare our alkalinity estimates from bivalves to values reported in the literature. We also elaborate on the potential biogeochemical significance of calcification by discussing how reductions in alkalinity and increases in DIC may increase $CO_2$ outgassing. These additions help strengthen the link between benthic biomass, carbonate chemistry, and broader carbon cycling dynamics.

**Conclusion**

**Reviewer Comment 42:** The conclusion should not primarily focus on the limitations. It gives the impression that you lack confidence in your results. Limitations should be addressed in the methods section, with an evaluation of the associated uncertainties.
**Response:**
As mentioned in Comment 4, we moved detailed discussion of methodological assumptions and limitations to the Methods and Discussion sections, allowing the Conclusion to focus on synthesis rather than caveats. We also expand the discussion of uncertainty in the methods and discussion.

**Reviewer Comment 43:** Overall**,** my main concern lies with the uncertainty in the modeling. The authors did not adequately assess or estimate the significance of these uncertainties (although the equations for uncertainty assessment are provided in the methods).
**Response:**
Thank you for raising this important point. In the revised manuscript, we move the detailed mechanics of uncertainty propagation to the Appendix for clarity, while placing greater emphasis in the Methods and Discussion sections on how uncertainties were calculated, their implications, and our degree of confidence in the resulting estimates. Although formal uncertainties were not reported for many of the empirical relationships we use, we estimate and propagate uncertainty by drawing on the range of published equations at each stage, from biomass to biomass-based carbon, from biomass to secondary production, and from secondary production to calcification and respiration. We then evaluate our confidence in the resulting flux estimates by comparing them to values reported in prior studies. This revised framing will help clarify the basis for our uncertainty estimates and demonstrate how our results align with independent literature, thereby strengthening the robustness of our conclusions.

**References**

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science*, 926–929. https://www.science.org

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