Benthic macrofaunal carbon fluxes and environmental drivers of spatial variability in a

large coastal-plain estuary

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Abstract

    While the importance of carbon cycling in estuaries is increasingly recognized, the role of benthic macrofauna remains poorly quantified due to limited spatial and temporal resolution in biomass measurements. Here, we ask: (1) To what extent do benthic macrofauna contribute to estuarine carbon cycling via respiration and calcification? and (2) How well can routinely collected environmental variables predict their biomass? We analyzed data from 8,128 benthic samples collected from the Chesapeake Bay between 1995 and 2022 and estimated associated carbon fluxes using empirical relationships. We then used generalized additive models to relate observed and modeled environmental variables to the biomass. Biomass was highest in the upper main stem of the Bay (Upper Bay) and upper Potomac River Estuary, the largest tidal tributary of the Bay. In the Upper Bay, benthic macrofauna respired 18–45% of the estimated organic carbon supply. Calcification-driven alkalinity reduction reached 6.31 ± 2.84 mol m^{-2} yr^{-1} in the Potomac River Estuary, aligning with prior estimates of alkalinity sinks in the tributary and highlighting the potential importance of calcifying fauna in alkalinity dynamics. Estimated CO_2 production in the Upper Bay from benthic respiration and calcification (151 g C m^{-2} yr^{-1}) also exceeded observed air–sea CO_2 fluxes (74.5 g C m^{-2} yr^{-1}). Generalized additive models revealed that low salinity, moderate dissolved oxygen, and elevated nitrate best predicted high-biomass zones, with the three predictors explaining 52% of biomass deviance. These predictive relationships offer a pathway to estimate macrofaunal biomass and associated carbon fluxes in systems where direct biomass measurements are sparse. Our findings demonstrate that benthic macrofauna play a substantial and spatially structured role in estuarine carbon cycling. Incorporating their contributions into estuarine biogeochemical models will improve predictions of ecosystem responses to environmental and anthropogenic change.

1. Introduction

Benthic macrofauna are vitally important to estuarine ecosystems by improving water quality, producing and consuming organic matter, recycling nutrients, cycling pollutants, stabilizing and transporting sediment, and providing food for both human populations and estuarine organisms (Schratzberger & Ingels, 2018; Snelgrove, 1997; Wilson & Fleeger, 2023). Benthic macrofauna also play a role in estuarine carbon cycling through second production, respiration, and calcification. Secondary production refers to the consumption of organic matter by benthos to produce soft tissue biomass (Diaz & Schaffner, 1990; Dolbeth et al., 2012; Sturdivant et al., 2013). This biomass contributes to trophic transfer, as benthic organisms are consumed by predators or decomposers, with much of the associated organic carbon eventually being removed from the estuary through advection to the open ocean or burial in sediment (Diaz & Schaffner, 1990; Wilson & Fleeger, 2023). Respiration and calcification influence carbon cycling through their impact on the carbonate system, the chemical equilibrium governing the partitioning of inorganic carbon among its primary forms.

Impacts of respiration and calcification on the carbonate system are best understood via their impacts on two master variables, dissolved inorganic carbon (DIC) and total alkalinity (TA). DIC is a measure of the total concentration of inorganic carbon species in water, including CO_2, bicarbonate ion, and carbonate ion:

$$[DIC] = [CO_2] + [HCO_3^-] + [CO_3^{2-}]. \tag{1}$$

TA is the excess of proton acceptors over donors and reflects the capacity of a water body to buffer pH changes. For understanding impacts of biogeochemical processes, the form of TA known as the explicit conservative alkalinity (Glud, 2008; Wolf-Gladrow et al., 2007) is useful:

$$[TA] = [Na^+] + 2[Mg^{2+}] + 2[Ca^{2+}] + [K^+] + 2[Sr^{2+}] + \dots - [Cl^-] - [Br^-] - [NO_3^-] - \dots$$

→ In addition to shaping ecosystem structure and serving as indicators of environmental health, benthic macrofauna

88   –   TPO$_4$ +   TNH$_3$ –   2TSO$_4$ –   THF   –   THNO$_2$,

89   (2)

90   where the last five terms represent the total forms of phosphate, ammonia, sulfate, fluoride, and

91   nitrite, respectively.

92   Respiration, unlike secondary production, directly alters the carbonate system.

93   Respiration is the metabolic process by which organisms break down organic matter to release

94   energy. Aerobic respiration, produces carbon dioxide (CO$_2$), water, and energy with oxygen

95   serving as the terminal electron acceptor:

96    $$CH_2O + O_2 \rightarrow CO_2 + H_2O. \hspace{2cm} (3)$$

97   When oxygen becomes depleted, anaerobic respiration takes over, relying on alternative electron

98   acceptors such as nitrate, sulfate, or iron oxides. For benthic macrofauna, oxygen is essential for

99   respiration; anaerobic respiration in sediments is primarily mediated by microbes (Glud, 2008).

100   The CO$_2$ generated during aerobic respiration increases DIC (Equation 1). Although aerobic

101   respiration slightly decreases TA due to the regeneration of nitrate during organic matter

102   remineralization, the effect is small ($\approx$16 moles of NO$_3^-$ per 106 moles of CO$_2$; Redfield, 1963).

103   During calcification, benthic organisms, such as bivalves, take up calcium (Ca^{2+}) and

104   HCO$_3^-$ to form calcium carbonate (CaCO$_3$) shells, releasing CO$_2$ and water as byproducts:

105   $$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O. \hspace{2cm} (4)$$

106   Although calcification increases CO$_2$ in the water column, it results in a net decrease in DIC,

107   since two moles of HCO$_3^-$ are removed for every mole of CaCO$_3$ precipitated, while only one

108   mole of CO$_2$ is returned to the water column (Equation 1). The removal of Ca^{2+} also decreases

109   TA by twice the amount of DIC (Equation 2). Calcification is thermodynamically favored when

110   the calcium carbonate saturation state (Ω) exceeds 1.

111

125 When $\Omega < 1$ (undersaturation), dissolution is favored, leading to the release of Ca^{2+} and

126 CO_3^{2-} back into solution and increasing TA. Low Ω impairs shell formation and increases

127 mortality in juveniles, regardless of pH or $p\mathrm{CO_2}$ (Green et al., 2009; Thomsen et al., 2015;

128 Waldbusser et al., 2015).

129 Building on these conceptual and mechanistic insights, field and modeling studies have

130 revealed the potentially large influence of benthic macrofauna on estuarine carbon cycling,

131 particularly through respiration and calcification. A compilation of data from 20 estuaries

132 estimated that benthos (encompassing microbes, meiofauna, and macrofauna) respire

133 approximately 24% of the total input of organic carbon, defined here as the sum of inputs of

134 autochthonous (from primary production) and allochthonous (e.g., from rivers) organic carbon,

135 (Hopkinson & Smith, 2004). An excess of benthic respiration over primary production observed

136 in many estuaries implies significant allochthonous inputs (Hopkinson & Smith, 2004; Kemp et

137 al., 1997; Schwinghamer et al., 1986). Benthic macrofauna, particularly bivalves, substantially

138 contribute to benthic respiration and have been hypothesized to significantly decrease the

139 phytoplankton and suspended particulate concentrations in estuaries (Cerco & Noel, 2010;

140 Galimany et al., 2020; Nakamura & Kerciku, 2000; Newell & Ott, 2011).

141 In contrast to respiration, the impact of benthic calcification on estuarine carbon cycling

142 remains underexplored (Waldbusser et al., 2013). However, large alkalinity sinks in estuarine

143 tributaries suggest that calcifying organisms, such as bivalves, may play a substantial role (Najjar

144 et al., 2020). The combined CO_2 production from respiration and calcification may also

145 constitute a significant carbon flux to the atmosphere. In San Francisco Bay, this production is

146 estimated to occur at twice the rate of CO_2 uptake by primary production, while global

147 extrapolations indicate that these emissions may rival those from the world's lakes or planetary

210 volcanism (Chauvaud et al., 2003). As previously described, although both respiration and

211 calcification generate CO_2, they have opposite effects on DIC: respiration increases DIC,

212 whereas calcification reduces it. Thus whether calcifying benthos act as a net source or sink of

213 DIC depends on the relative magnitudes of these two processes.

214   To quantify the magnitude of these benthic contributions to carbon cycling, researchers

215 often estimate rates of secondary production, respiration, and calcification based on benthic

216 biomass. Because secondary production can be time-consuming and costly to measure, it is often

217 estimated using empirical relationships that relate it to benthic biomass, incorporating variables

218 such as taxon, average body mass, and water temperature (Brey, 1990; Chauvaud et al., 2003;

219 Dolbeth et al., 2012; Edgar, 1990; Schwinghamer et al., 1986; Sturdivant et al., 2013; Tumbiolo

220 & Downing, 1994). Calcification and respiration rates can be inferred from these secondary

221 production estimates using empirical relationships or proportional scaling (Chauvaud et al.,

222 2003; Schwinghamer et al., 1986).

223   Despite their importance to carbon cycling, benthic macrofauna biomass is rarely

224 measured due to high cost and logistical challenges (Pearson & Rosenberg, 1978; Snelgrove et

225 al., 2018). In contrast, environmental variables that influence benthic communities are typically

226 measured more frequently. To better incorporate benthic macrofaunal processes into carbon

227 budgets and numerical models, it is useful to assess how well these more readily available

228 environmental variables can serve as predictors of biomass.

229   There are many environmental influences on the distribution of benthic macrofauna.

230 Hypoxia (dissolved oxygen concentrations ≤ 2 mg L^{-1}) is a major stressor that reduces biomass,

231 alters community structure, and impairs key functions such as metabolism, bioturbation, and

232 calcification (Borja et al., 2008; Diaz et al., 1995; Diaz & Rosenberg, 2008; Levin et al., 2009;

239 Murphy et al., 2011; Rousi et al., 2019; Seitz et al., 2009; Woodland & Testa, 2020). Benthic

240 macrofauna are particularly vulnerable due to their proximity to the sediment–water interface,

241 immobility, and limited capacity to avoid low-oxygen zones (Dauer & Alden, 1995; Vaquer-

242 Sunyer & Duarte, 2008). Behavioral and physiological responses to hypoxia differ among taxa,

243 with some groups like bivalves showing greater tolerance to short-term events (Seitz et al., 2009;

244 Vaquer-Sunyer & Duarte, 2008; Woodland & Testa, 2020). Salinity also plays a central role in

245 structuring benthic communities, with species distributions reflecting distinct physiological

246 tolerances (Dauer, 1993; Holland et al., 1987; Little et al., 2017; Seitz et al., 2009; Sturdivant et

247 al., 2013). Ocean acidification has been shown to negatively impact calcifying taxa, particularly

248 mollusks, by reducing growth, survival, and development (Birchenough et al., 2015; Jakubowska

249 & Normant-Saremba, 2015; Jansson et al., 2013; Kroeker et al., 2013; Thomsen et al., 2015).

250 Sediment characteristics also influence benthic biomass and diversity. Finer grain sizes and

251 higher organic content tend to be associated with lower biomass and biodiversity, whereas sandy

252 sediments are generally more favorable (Dauer & Alden, 1995; Grebmeier et al., 2015; Seitz et

253 al., 2009; Woodland & Testa, 2020). Finally, food availability, primarily from phytoplankton

254 production, strongly governs benthic biomass (Ehrnsten et al., 2019; Hagy, 2002; Kemp et al.,

255 2005; Pearson & Rosenberg, 1978). However, the relationship is complex, as high primary

256 production can also lead to oxygen depletion that ultimately suppresses benthic communities

257 (Dauer et al., 2000; Kemp et al., 2005). In summary, dissolved oxygen, salinity, carbonate

258 chemistry, sediment composition, and food availability all serve as important environmental

259 predictors of benthic macrofauna biomass in estuarine ecosystems.

260 While numerous studies have examined the environmental drivers of benthic biomass in

261 estuaries, most have focused on relatively small spatial or temporal scales. Biomass, as opposed

263   to other metrics such as diversity and abundance, is particularly important because it is most

264   directly related to estuarine carbon cycling processes (Cerco & Noel, 2010; Snelgrove, 1999).

265   While benthic respiration has been widely quantified, estimates of benthic calcification remain

266   rare (e.g., Chauvaud et al., 2003; Waldbusser et al., 2013), limiting our understanding of benthic

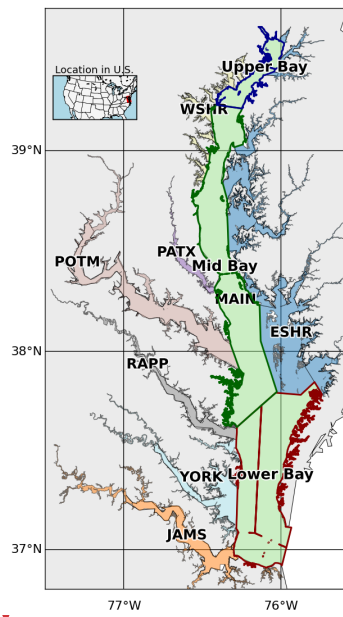
267   contributions to carbon and alkalinity dynamics. The Chesapeake Bay, a large, coastal-plain

268   estuary in the eastern United States (Fig. 1), provides a valuable case study due to its extensive

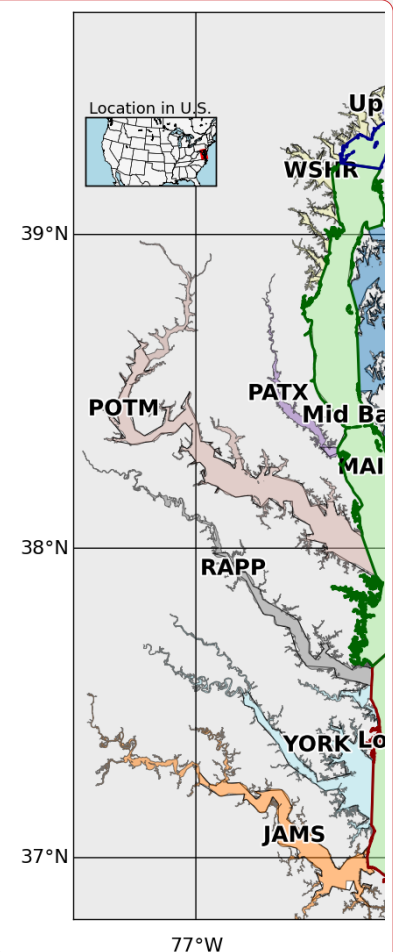
269   historical monitoring data and diversity of carbon and alkalinity dynamics across its several tidal

270   tributaries (Najjar et al., 2020). Although prior studies in the Bay have linked environmental

271   variables to benthic biomass (Seitz et al., 2009; Woodland et al., 2021), these studies either used

272   older datasets or focused on biomass primarily as a food source for higher trophic levels.

273

274   Figure 1: Segmentation of the mainstem of the Chesapeake Bay and its tidal tributaries. The

275   mainstem is divided into three regions, Upper Bay, Mid Bay, and Lower Bay. Acronyms denote

279   specific tributaries and sub-estuarine areas: ESHR refers to Eastern Shore tributaries, WSHR to

280   Western Shore tributaries, POTM to the Potomac River, PATX to the Patuxent River, RAPP to

281   the Rappahannock River, YORK to the York River system (including the Mattaponi and

282   Pamunkey Rivers), and JAMS to the James River.

283   In our study, we build on this previous work by addressing the following research

284   questions in the context of the Chesapeake Bay:

285   1) To what extent do benthic macrofauna contribute to estuarine carbon cycling through

286   respiration and calcification, and how might these contributions vary across space?

287   2) How well can environmental variables routinely collected in estuarine monitoring

288   programs predict benthic macrofauna biomass?

289   Answering these questions will help clarify the role of benthic macrofauna in estuarine carbon

290   cycling and improve the ability of monitoring programs and numerical models to account for

291   benthic processes using more accessible environmental data.

292   **2. Methods**

293   *2.1 Overview*

294   We examined historic benthic macrofauna biomass data collected annually by the

295   Chesapeake Bay Long-Term Benthic Monitoring Program (BMP) (Dauer et al., 2000; Llansó &

296   Zaveta, 2017). We applied empirical relationships linking biomass to secondary production,

297   respiration, and calcification to quantify the impact of benthic macrofauna on carbon cycling in

298   the Bay. To identify environmental predictors of benthic biomass, we compiled measurements of

299   bottom water temperature, salinity, dissolved oxygen, and sediment sand fraction measured by

300   the BMP at the same time and location as the benthic biomass samples. In addition to these

301   measured environmental variables, we utilized output from ROMS-ECB, which is a three-

302 dimensional estuarine carbon and biogeochemistry (ECB) model embedded into the Regional

303 Ocean Modeling System (ROMS; Shchepetkin & McWilliams, 2005) developed for the

304 Chesapeake Bay (St-Laurent & Friedrichs, 2024b). We identified correlations between these

305 environmental variables (observed and modeled) and benthic macrofauna biomass using

306 generalized additive models (GAMs).

307 *2.2 Benthic biomass data*

308   Details of the BMP data we used in our study are described elsewhere (Dauer, 1993;

309 Dauer et al., 2000; Dauer & Lane, 2010; Llansó & Scott, 2011; Llansó & Zaveta, 2017) and are

310 summarized here. The program began in 1984, but consistency in sampling started in 1995. The

311 sampling occurs mainly in the summer, between July 15 and September 30, at both fixed and

312 random stations, with the latter changing location every year. The sampling is conducted

313 separately by the states of Maryland (Llansó & Zaveta, 2017) and Virginia (Dauer & Lane,

314 2010), with slightly different protocols used in each state (see below). Our analysis spanned 1995

315 to 2022, the recent extent of available data, incorporating 28 years of data.

316   The Maryland monitoring program comprises 27 fixed and 150 random stations in the

317 upper Chesapeake Bay. The Virginia monitoring program comprises 21 fixed and 100 random

318 stations in the lower Chesapeake Bay. In Virginia, random stations were not sampled in 1995,

319 and fixed stations were not sampled in 2017 or 2018. Water depths greater than 12 m in the

320 mainstem part of the Bay in Maryland are not sampled because the bottom waters become anoxic

321 in the summer, resulting in azoic sediments. The tributaries are sampled only in the tidal zone;

322 areas with less than 1 m mean lower low water are considered non-tidal. Some locations, such as

323 oyster reefs and other hard substrates, are also not sampled due to gear unsuitability in such

324 habitats. To avoid seasonal biases, we excluded samples collected outside of the summer

325   sampling window of July 15–September 30, which occurred earlier in the study period. This

326   selection resulted in a dataset of 8128 samples across both states, or an average of 290 samples

327   per year, slightly less than the maximum of 298.

328   At each sampling station, the uppermost layers of sediment are collected. In Maryland,

329   the sites introduced in 1995 (two fixed sites and all random sites) are sampled with a Young

330   grab, which collects a surface area of 0.0440 m^2 to a depth of 0.10 m. For the other fixed sites in

331   Maryland, nearshore shallow sandy habitats of the mainstem and tributaries are sampled with a

332   modified box corer with a surface area of 0.0250 m^2 to a depth of 0.25 m. Muddy habitats and

333   deep-water habitats in the mainstem and tributaries of Maryland are sampled with a Wildco box

334   corer with a surface area of 0.0225 m^2 to a depth of 0.23 m. The fixed site in the Nanticoke River

335   is sampled with a Petite Ponar grab with a surface area of 0.0250 m^2 to a depth of 0.07 m. In

336   Virginia, fixed sites use a spade-type box-coring device with a surface area of 0.0182 m^2 to a

337   depth of 0.02 m; random sites use a Young grab with a surface area of 0.0400 m^2 to a depth of

338   0.10 m. One limitation of using multiple types of sampling gear is that differences in design,

339   such as penetration depth, sample volume, and sediment disturbance, can introduce variability in

340   estimates of benthic diversity, abundance, and biomass (Eleftheriou & Moore, 2013).

341   For both monitoring programs, the sampling contents are sieved through a 0.5 mm screen

342   to retain only benthic macrofauna. The macrofauna are identified at the lowest taxonomical

343   level. The specimens are dried on a pan for at least 24 hours to a constant weight, then a final

344   weight is measured. The specimens are then placed in a muffle furnace for 4 hours at 500 °C for

345   ashing, and the specimens are weighed again. The ash-free dry weight (AFDW), representing

346   only the soft-tissue biomass, is the difference between the dry and ashed weight. We converted

347   the AFDW in g per sample to biomass density B in units of g m^{-2} by dividing the AFDW by the

348   area of the sampling device. The data used in our subsequent statistical analysis include the

349   biomass density of individually selected species and classes and the total biomass density.

350   Benthic biomass is known to be highly patchy, with large variability even between

351   samples taken in close proximity, hence the common use of replicate sampling in field protocols.

352   To mitigate this noise and extract meaningful large-scale spatial patterns, we averaged across the

353   28-year time frame. All 8128 biomass samples collected from 1995 to 2022 were averaged onto

354   a uniform grid with cells each measuring 0.04° in longitude and 0.03125° in latitude (~3.5 km

355   per side). 1295 cells had at least one biomass measurement during the 28-year period. Across

356   these cells, the average number of samples was 6.19, the median was 3, and the maximum was

357   69. Tributaries and the upper Bay were sampled more densely than the mainstem (Appendix A

358   and Fig. A1). This "time-averaging" approach enabled clearer interpretation of spatial gradients

359   in biomass and strengthened the robustness of subsequent statistical analyses aimed at

360   identifying consistent environmental drivers. Some benthic biomass sampling stations fell

361   outside the Chesapeake Bay Program (CBP) water quality segmentation scheme, which was

362   originally developed to support monitoring and management analyses (Chesapeake Bay Program

363   Analytical Segmentation Scheme: Revisions, Decisions and Rationales, 1983–2003, 2004). The

364   CBP segments are defined at a relatively coarse spatial scale and often exclude narrow nearshore

365   environments and small tributary creeks. To maintain consistency with the spatial framework

366   used in coupled hydrodynamic–biogeochemical models, in our case ROMS-ECB, we excluded

367   these points from analysis. This filtering step ensured that all biomass observations were aligned

368   with the modeled domain, avoiding mismatches at the land–water interface. After this step, 1008

369   grid cells remained, of which 868 contained at least one biomass observation and the remainder

370   were empty cells.

371 ***2.3 Carbon flux estimations***

372   To estimate the impact of benthic macrofauna on carbon cycling, we applied empirical

373 equations from the literature to quantify secondary production, calcification, and respiration.

374 These equations were identified through an informal literature review using online tools (e.g.,

375 Google Scholar), with emphasis on studies relevant to soft-sediment bivalves, the dominant

376 macrofauna in the Chesapeake Bay. We prioritized relationships applicable to local taxa and

377 easily scalable to our biomass dataset. While multiple sources were available for secondary

378 production, equations for respiration and calcification were more limited; in those cases, we

379 selected models based on representative species that could be scaled from production estimates.

380 We used a range of published values for biomass-to-carbon conversion, secondary production,

381 and species-specific respiration and calcification ratios to estimate upper and lower bounds of

382 carbon fluxes. Additional details on uncertainty calculations are provided in the Appendix B.

383 *2.3.1 Secondary production*

384   We converted biomass to secondary production S (units of g C m^{-2} yr^{-1}). Multiple

385 approaches have been used for this conversion, and we used the average of three approaches to

386 be able to broadly quantify uncertainty in our estimates.

387   In the first approach, secondary production is given by:

388
$$S_1 = \alpha B_c \tag{5}$$

389 where α is the specific growth rate and B_c is carbon-based biomass (units of g C m^{-2}). We found

390 three studies that estimated α for benthic macrofauna. At the low end, a study of benthic

391 macrofauna in the Chesapeake Bay used α = 1.06 yr^{-1} (Wilson & Fleeger, 2023). At the high

392 end, a study of *C. fluminea* used α = 4.45 yr^{-1} (Chauvaud et al., 2003). An intermediate value of

393 α = 2 yr^{-1} was based on monthly observations of benthic macrofauna dominated by the

394 crustacean *Corophium volutator* and the bivalve *Limecola balthica* at an intertidal site in the

395 upper Bay of Fundy (Schwinghamer et al., 1986). A mean value of 2.50 yr^{-1} was used in

396 Equation 5. B_c is computed from biomass B (units of g m^{-2}) using:

397 $$B_c = r_c B \tag{6}$$

398 where r_c is the ratio of carbon mass to total mass in benthic organic matter. In a study on the

399 bivalve filter feeders *Rangia cuneata* and *Corbicula fluminea*, which dominate in the tidal fresh

400 and oligohaline waters of the Chesapeake Bay, r_c = 0.47 g C g^{-1} was used (Cerco & Noel,

401 2010). A slightly lower value of 0.41 g C g^{-1} was used in a study of native and introduced

402 bivalves in six North American freshwater systems (Chauvaud et al., 2003). A mean value of r_c

403 = 0.44 g C g^{-1} was used in our calculation.

404   In the second approach, temperature (T) dependence for bivalve secondary production

405 was added (Sturdivant et al., 2013):

406 $$S_2 = S_0 \left(\frac{B}{1 \text{ g m}^{-2}} \right)^{0.87} \left(\frac{T}{1 \text{ }^\circ\text{C}} \right)^{0.46} \tag{7}$$

407 where S_0 = 0.40 g C m^{-2} yr^{-1}. Note that the coefficient at the beginning of the equation differs

408 from that of Sturdivant et al. (2013) because of a change in units of B from mg to g m^{-2} and of S

409 from mg C d^{-1} to g C m^{-2} yr^{-1}. This equation has been shown to agree well with secondary

410 production and biomass measurements (Sturdivant et al., 2013).

411   Finally, in the third approach (Tumbiolo & Downing, 1994), depth (Z) dependence was

412 added:

413 $$\log_{10} \left(\frac{S_3}{1 \text{ g C m}^{-2} \text{ yr}^{-1}} \right)$$
$$= \beta_0 + b \log_{10} \left(\frac{B}{1 \text{ g m}^{-2}} \right) - m \log_{10} \left(\frac{M}{1 \text{ mg}} \right) + t \left(\frac{T}{1 \text{ }^\circ\text{C}} \right)$$
$$- z \log_{10} \left(\frac{Z}{1 \text{ m}} + 1 \right) \tag{8}$$

where $\beta_0 = 0.24, b = 0.96, m = 0.21, t = 0.03, z = 0.16$, and M is the maximum individual

body mass. Equation 8 was developed by Tumbiolo & Downing (1994) through multiple

regression analysis of 125 benthic invertebrate populations, including data from the Chesapeake

Bay. In their model, M refers to the maximum individual mass of the largest size class observed

within a population. It was identified as the second most important predictor of secondary

production after total biomass. Their analysis showed that species with greater maximum sizes

tend to have slower turnover rates and lower production-to-biomass ratios. In our application, we

treated M as a constant, based on the maximum individual mass of the bivalve $R.\ cuneata$

collected in a study in the Choptank River in the Chesapeake Bay, which was 5953 mg (Hartwell

et al., 1991). $R.\ cuneata$ is among the most abundant and largest benthic macrofaunal species in

our study area (Cerco & Noel, 2010; Hartwell et al., 1991).

In Equations 7 and 8, we used the bottom water temperature measured on the same day

the benthic samples were collected. This reliance on summer temperature values represents a key

limitation of our study, as it likely leads to overestimation of annual secondary production,

particularly for Equation 8. For example, Equations 7 and 8 both predict maximum summer

secondary production that is about 5 times larger than the minimum winter production, assuming

an annual temperature range of 1 ºC to 25 ºC. However, we note that the first approach for

secondary production (Equation 6) has no temperature dependence. Figures C1–C3 in Appendix

C provide a detailed comparison of secondary production across the three models and assess

temperature effects on Models 2 and 3.

2.3.2 Calcification

Benthic macrofaunal calcification C (g $CaCO_3$ m^{-2} yr^{-1}) was computed from secondary

production following Chauvaud et al. (2003):

443 $$C = r_s S \tag{9}$$

444 where r_s is the ratio of shell CaCO$_3$ mass production to tissue organic C mass production, which

445 has units of g CaCO$_3$ (g C)$^{-1}$. Based on samples of the bivalve *Potamocorbula amurensis* in the

446 northern San Francisco Bay, Chauvaud et al. (2003) found r_s to be 10 g CaCO$_3$ (g C)$^{-1}$. They

447 also cited a ratio of 15 g CaCO$_3$ (g C)$^{-1}$ for the bivalve *C. fluminea*, a more relevant species to

448 our study, but the reference is from unpublished data. We used the average of the two values,

449 12.5 g CaCO$_3$ (g C)$^{-1}$, which corresponds to a molar ratio of 1.5 mol CaCO$_3$ (mol C)$^{-1}$.

450 *2.3.3 Respiration*

451   The ratio of respiration to secondary production, expressed as energy fluxes, was derived

452 from an empirical relationship between benthic macrofauna biomass and respiration in the Bay-

453 of-Fundy study referenced earlier (Schwinghamer et al., 1986):

454 $$\log_{10}\left(\frac{R}{1\ \text{kcal m}^{-2}\ \text{yr}^{-1}}\right) = \alpha_0 + s \log_{10}\left(\frac{S}{1\ \text{kcal m}^{-2}\ \text{yr}^{-1}}\right) \tag{10}$$

455 where $s = 0.993$ and $\alpha_0 = 0.367$. Since s is nearly 1, the relationship simplifies to:

456 $$R = 10^{\alpha_0} S = 2.33S \tag{11}$$

Deleted: 12

457 Assuming that the conversion between carbon and energy is the same for S and R, this equation

458 can be directly applied to these processes expressed as carbon fluxes, consistent with our other

459 carbon flux estimates. Further, combining the relationships of calcification and respiration with

460 secondary production on a molar basis allows a best estimate of the molar ratio of respiration to

461 calcification: 2.33/1.5 = 1.55.

462 *2.3.4 Impact on the carbonate system*

463   From the estimated benthic macrofaunal calcification (C) and respiration (R), we

464 calculated TA and DIC fluxes (units of mol m^{-2} yr^{-1}):

$$F_{\text{TA}} = \frac{-2C}{M_{\text{CaCO}_3}} \tag{12}$$

$$F_{\text{DIC}} = \frac{R}{M_{\text{C}}} - \frac{C}{M_{\text{CaCO}_3}} \tag{13}$$

where M_{CaCO_3} and M_{C} are the molar masses of CaCO$_3$ and C, respectively, and the sign

convention is positive from the benthic fauna to the water column. Both calcification and

respiration will lead to increases in the concentration of aqueous CO$_2$, and so there is a CO$_2$ flux

from benthic fauna that can be decomposed into CO$_2$ fluxes resulting from these two processes:

$$F_{\text{CO}_2} = F_{\text{CO}_2}^{\text{TA}} + F_{\text{CO}_2}^{\text{DIC}} \tag{14}$$

It has been estimated, assuming air–sea CO$_2$ equilibrium, that every mole of CaCO$_3$ precipitated

leads to the evasion of 0.6–1 mole of CO$_2$ to the atmosphere, depending on the temperature,

salinity, atmospheric pCO$_2$, and initial alkalinity (Chauvaud et al., 2003; Frankignoulle et al.,

1998; Ware et al., 1992).

In light of strong departures from air–sea CO$_2$ equilibrium in estuaries, including the

Chesapeake Bay (Herrmann et al., 2020), we instead estimate the production rate of CO$_2$ using

buffer factors, ζ_{TA} and ζ_{DIC}, which are defined as the fractional change in [CO$_2$] divided by the

fractional change in [TA] and [DIC], respectively; e.g., $\zeta_{\text{TA}} = (\Delta[\text{CO}_2]/[\text{CO}_2])/(\Delta[\text{TA}]/[\text{TA}])$.

This approach also allows us to estimate the CO$_2$ flux from respiration. We computed the buffer

factors in three steps. First, we used output from ROMS-ECB (described in more detail later in

the Methods Section 2.4) for the years 1995–2022 and calculated long-term averages of bottom

[TA], [DIC], temperature, salinity, and pressure (estimated from water depth) at each model grid

cell. These values were used as input to PyCO2SYS (Humphreys et al., 2022) to calculate the

baseline [CO$_2$]. Second, we individually perturbed [TA] and [DIC] by 1% ($\Delta[\text{TA}]$ and $\Delta[\text{DIC}]$)

487 while holding all other variables constant and recalculated $[CO_2]$ to get $\Delta[CO_2]$. Third, we

488 computed ζ_{TA} and ζ_{DIC} using the definition of the buffer factors.

489   To relate the fluxes to the buffer factors, we use the flux form of the buffer factors:

$$\zeta_{TA} = \frac{F_{CO_2}^{TA}/[CO_2]}{F_{TA}/[TA]} \qquad (15)$$

$$\zeta_{DIC} = \frac{F_{CO_2}^{DIC}/[CO_2]}{F_{DIC}/[DIC]} \qquad (16)$$

492 These flux forms take the definitions of the buffer factors and replace ratios of concentration

493 changes (e.g., $\Delta[CO_2]/\Delta[TA]$) with ratios of fluxes (e.g., $F_{CO_2}/F_{CO_2}^{TA}$). Using Equations 15 and 16

494 in Equation 14 yields an expression for the CO_2 flux from benthic fauna to the water column:

$$F_{CO_2} = [CO_2]\left(\frac{\zeta_{TA}F_{TA}}{[TA]} + \frac{\zeta_{DIC}F_{DIC}}{[DIC]}\right). \qquad (17)$$

496 *2.3.5 Spatial analysis and comparison to other carbon fluxes*

497   To place benthic macrofauna contributions to estuarine carbon cycling in a broader

498 spatial context, we averaged fluxes over 10 regions based on aggregated Chesapeake Bay

499 Program segments (Fig. 1): the major tidal tributaries (the Patuxent, Potomac, Rappahannock,

500 York, and James River Estuaries), the Western and Eastern Shores, and the Upper, Mid, and

501 Lower (mainstem) Bay. Additionally, we computed averages over the mainstem Bay and the

502 whole Bay. For each region, we calculated benthic respiration and bivalve calcification rates

503 based on observed biomass, using the empirically derived equations just described. We then

504 compared these fluxes to other components of the estuarine carbon budget using published

505 values for primary production, allochthonous organic carbon inputs, oyster calcification, and air–

506 sea gas exchange.

510   First, we assessed the extent to which benthic macrofauna remineralize organic carbon

511   inputs by comparing our calculated respiration rates to net primary production (NPP) and

512   external particulate organic carbon (POC) loads. Primary production and POC values were

513   obtained from prior studies (Herrmann et al., 2015; Kemp et al., 1997; Najjar et al., 2018; Zhang

514   & Blomquist, 2018) and scaled to match the spatial extent of our biomass data. In the Upper Bay,

515   we calculated organic carbon inputs by distributing the Susquehanna River's POC load over the

516   combined surface area of the oligohaline and tidal fresh segments (CB1 and CB2, (Olson, 2012).

517   This approach assumes that the majority of the annual POC load from the Susquehanna River

518   remains concentrated in the Upper Bay and is respired rather than transported downstream, an

519   assumption supported by Canuel & Hardison (2016). For comparison, we also distributed

520   Susquehanna River POC inputs across the entire mainstem. For brevity, we restricted our

521   tributary-level comparison to the Potomac River Estuary, where the full organic carbon load was

522   distributed across the surface area of the entire tidal tributary (Fig. 1).

523   Second, we compared our soft-sediment bivalve calcification estimates to published

524   oyster calcification rates from (Fulford et al., 2007). Oyster ash-free dry weight was converted to

525   live weight using a 10:1 ratio (Mo & Neilson, 1994), and a calcification rate of 2 mg $CaCO_3$ per

526   g live weight per day was applied (Waldbusser et al., 2013). Because Eastern oyster (*Crassostrea*

527   *virginica*) populations in the Chesapeake Bay were historically at least two orders of magnitude

528   higher than current levels (Fulford et al., 2007; Newell, 1988), we also scaled published oyster

529   calcification rates up by a factor of 100 to estimate potential historical contributions. This

530   comparison allowed us to place present-day bivalve calcification into the broader context of

531   ecosystem function.

532   Third, we evaluated whether riverine calcium supply could limit observed calcification

533   by estimating annual calcium fluxes from the Susquehanna and Potomac Rivers using non-tidal

534   USGS data from 1995 to 2022 and the Weighted Regression on Time, Discharge, and Season

535   (WRTDS) method (Hirsch et al., 2010). Assuming a 1:1 molar ratio between Ca^{2+} and $CaCO_3$

536   formation, we estimated the maximum potential calcification that could occur if all incoming

537   calcium were used for shell production in adjacent estuarine zones.

538   Finally, we compared CO_2 generated from respiration and calcification (Equation 17) to

539   published estimates of air–sea CO_2 exchange (Chen et al., 2013; Herrmann et al., 2020).

540   The estimated carbon fluxes reveal important spatial patterns, but their interpretation

541   should be tempered by uncertainty in several underlying assumptions. While secondary

542   production models are relatively well-calibrated across estuarine systems, the calculations for

543   calcification and respiration are more sparse and less empirically constrained. Our propagated

544   uncertainty in calcification and respiration incorporates variation in biomass-based carbon

545   content, multiple conversion approaches from biomass to secondary production, and calcification

546   parameters derived from two different bivalve species. Although we used different methods to

547   estimate calcification and propagated these uncertainties accordingly, only one approach was

548   available for respiration.

549   We estimated uncertainties in F_{DIC} and F_{TA}, which represent the propagated uncertainties

550   from respiration and calcification alone, with no additional sources of uncertainty included in

551   those calculations. However, we did not calculate formal uncertainties in F_{CO_2} due to the

552   complexity involved. Additional uncertainty in F_{CO_2} arises from the use of annually averaged,

553   model-derived values for salinity, temperature, depth, DIC, and TA. Further uncertainty stems

555   from the method used to calculate ζ from TA and DIC perturbations. While these additional

556   sources contribute to the overall uncertainty in F_{CO_2}, the uncertainties in F_{DIC} and F_{TA} are likely

557   to remain the dominant contributors.

558   The resulting error bars in the various carbon fluxes reflect these methodological

559   uncertainties, offering a plausible range rather than precise values. Thus, the benthic macrofaunal

560   carbon fluxes presented here should be interpreted with some caution, with more confidence

561   given to the broad spatial patterns and less to the absolute values.

562   *2.4 Environmental predictors of benthic biomass*

563   From the BMP, we used bottom temperature, salinity, and dissolved oxygen data, which

564   are measured with a YSI 660 Sonde or Hydrolab DataSonde 4a in Maryland and a YSI 85 Model

565   meter in Virginia 1 m above the sediment surface. Extreme outliers, defined as data points that

566   fall beyond three times the interquartile range from the first or third quartile, were removed. As a

567   result, one value for dissolved oxygen and one for water temperature were excluded from the

568   analysis. The salinity zones are also characterized at each site as tidal fresh (<0.5 ppt),

569   oligohaline (0.5–5 ppt), low mesohaline (5–12 ppt), high mesohaline (12–18 ppt), and polyhaline

570   (>18 ppt); these zones are relatively geographically fixed (with some changes in 2011 due to

571   Hurricane Irene and Tropical Storm Lee) based on long-term averages of salinity (Llansó, 2002;

572   Llansó & Zaveta, 2017). Sediment sand fraction was measured by first collecting two 120 ml

573   benthic grab sub-samples. Sand particles are separated by wet-sieving through a 63-μm stainless

574   steel sieve. Sand fraction is recorded after drying and weighing the samples. The bottom water

575   quality and sediment composition data were time-averaged using the same scheme described

576   earlier for the benthic biomass data.

577   ROMS-ECB output was used to characterize environmental variables not measured by

578   the BMP that could be relevant to predicting the benthic macrofauna biomass distribution.

579   ROMS-ECB uses 20 terrain-following vertical levels and a uniform horizontal resolution of 600

580   m (St-Laurent & Friedrichs, 2024b). We compiled daily averaged output at various grid points

581   from 1995 to 2022 corresponding to the locations of each of the 8128 benthic biomass samples.

582   We selected the ROMS output at the nearest ROMS grid point to each BMP sample location for

583   each variable of interest, described below. Several ROMS-ECB environmental variables are

584   directly linked to primary production, but the timing of peak primary production does not always

585   align with periods of greatest food availability for benthic organisms. To capture ecologically

586   meaningful variation in benthic–pelagic coupling, we calculated both seasonal and annual

587   averages for each variable. Seasonal groupings reflect key ecological periods: spring (March–

588   May), which includes the diatom bloom that delivers fresh organic material to the benthos, and

589   summer (June–August), characterized by stratification, hypoxia, and intense remineralization.

590   Fall (September–November) and winter (December–February) were also included to represent

591   background seasonal variability. We then time-averaged the seasonal and annual means as

592   described earlier. Coverage dropped from 868 to 846 cells after adding ROMS-ECB outputs,

593   largely where the model masks shallow/tributary cells or lacks complete seasonal data.

594   We used ROMS-ECB output, as opposed to data from the Chesapeake Bay Water Quality

595   Monitoring Program (WQMP), which has measured water quality variables since 1984 at over

596   100 tidal stations (Chesapeake Bay Program, n.d.). However, the stations are at different

597   locations than the BMP stations, requiring interpolation to correlate these data with benthic

598   biomass. Other studies have opted to use kriging, a method used to spatially interpolate surface

599   water quality data, to increase the spatial resolution. Kriging has been found to outperform the

standard inverse distance weighting tools typically used in the Chesapeake Bay (Murphy et al., 2015). One study evaluated the kriging of surface WQMP data for July 2007 using 117–123 data points (Murphy et al., 2015). In cross-validation, one measured sample is removed, and the interpolation is then performed at that location; the interpolated value is then compared to the observed value. Cross-validation of temperature, salinity, and dissolved oxygen yielded root-mean-square errors (RMSE) of 0.75 °C, 1.1 ppt, and 1.2 mg L^{-1}, respectively. In contrast, ROMS-ECB output was evaluated with over 500,000 WQMP data points at multiple depths and locations from 1985 to 2021 (St-Laurent & Friedrichs, 2024a). The RMSEs for temperature, salinity, and dissolved oxygen were 1 °C, 1.9 psu, and 1.5 mg L^{-1}, respectively. Although these RMSEs are slightly higher than those from kriging, the evaluation was much more robust, and the long time series evaluation is more relevant to our time-averaging technique over 28 years. In addition, we wanted to utilize bottom water quality data, as these variables are measured closer to the location of benthic macrofauna. However, cross-validation for kriging was only performed at the surface (Murphy et al., 2015), possibly due to the challenges of accounting for bottom topography in spatial interpolation.

ROMS-ECB simulates many variables, and we considered the subset that might be good predictors of benthic biomass: bottom POC, bottom total suspended solids (TSS), and surface NO_3^-. POC was considered because a large fraction of POC represents food for benthic macrofauna. TSS was considered a metric of suspended inorganic material, which can inhibit filter-feeding organisms (Grant & Thorpe, 1991). Photosynthesis is largely limited by NO_3^- in the Chesapeake Bay (Zhang et al., 2021); because phytoplankton productivity and the subsequent sinking of POC is an important source of organic matter to the benthos, NO_3^- could be a predictor of benthic biomass. ROMS-ECB output was evaluated for robustness with WQMP data

623   using Spearman's rank correlation coefficient, R_s, which measures the strength of the association

624   between two variables (Hauke & Kossowski, 2011). In our analysis of benthic biomass

625   predictors, we included only variables with R_s above 0.7, which generally indicates a strong

626   association (Akoglu, 2018). NO_3^- was used as it had $R_s = 0.77$, whereas POC and TSS were not

627   as they had $R_s = 0.26$ and 0.24, respectively (St-Laurent & Friedrichs, 2024a).

628   We also considered potentially good predictors that could be computed from ROMS-

629   ECB output: surface oxygen supersaturation (ΔO_2) and bottom aragonite saturation state (Ω_{arag}).

630   ΔO_2 can be used as a tracer for net ecosystem production (Herrmann et al., 2020) and hence may

631   indicate organic matter availability to the benthos. $\Delta[O_2]$ is equal to O_2 minus the saturation

632   concentration, which was computed from temperature and salinity (Garcia & Gordon, 1992).

633   Positive ΔO_2 values are favored during net autotrophy (photosynthesis exceeds respiration),

634   while negative values are favored during net heterotrophy (respiration exceeds photosynthesis);

635   temperature change and transport can also create non-zero ΔO_2. Ω_{arag} could predict benthic

636   biomass because bivalve calcification is expected to depend on this metric (Thomsen et al.,

637   2015). PyCO2SYS (Humphreys et al., 2022) was used to derive the solubility product and

638   carbonate ion concentration from alkalinity, DIC, temperature, salinity, and water depth. We

639   examined ΔO_2 and Ω_{arag} in the subsequent statistical analysis because the ROMS-ECB output

640   variables used to compute them all had $R_s > 0.7$. Although calcium measurements were not

641   available for model evaluation, it is highly correlated to salinity, though deviations may occur at

642   low salinity (Beckwith et al., 2019).

643   *2.5 Statistical modeling of benthic biomass*

644   We used GAMs to evaluate how bottom water quality and biogeochemical model outputs

645   predict the spatial distribution of benthic macrofaunal biomass. GAMs are data-driven statistical

648 models that have been widely applied in coastal ecology since the late 20th century (Guisan et

649 al., 2002; Smith et al., 2023). They model additive, smoothed relationships between predictor

650 and response variables, making them well-suited for capturing the non-linear, non-monotonic

651 patterns often observed in ecological datasets (Grüss et al., 2014; Guisan et al., 2002; Hastie &

652 Tibshirani, 1987; Wood, 2017). GAMs also quantify the strength and relative contribution of

653 each predictor (Guisan et al., 2002) and have demonstrated predictive performance comparable

654 to or exceeding other modeling approaches (Drexler & Ainsworth, 2013). We used the mgcv

655 package in R and applied restricted maximum likelihood (REML) to optimize smoothness

656 (Wood, 2011; Wood, 2017). This approach is particularly effective with large datasets like ours,

657 enabling robust estimation of spatial patterns in biomass across a broad geographic region (Grüss

658 et al., 2014; Wood, 2011). Although GAMs can handle non-normal distributions of the response

659 variable (Guisan et al., 2002), we found the best model fit by applying a natural logarithmic

660 transformation to the biomass. A small constant (0.0001 g m^{-2}) was added to the biomass values

661 before applying the natural logarithmic transformation to account for zeros in the data.

662   As described earlier, we initially identified eight environmental variables: O_2, salinity,

663 total depth, water temperature, sand fraction, NO_3^-, Ω_{arag}, and $\Delta[O_2]$. To avoid multicollinearity,

664 which can distort GAM results (Grüss et al., 2014; Grüss et al., 2018; Guisan et al., 2002), we

665 used Pearson's correlation coefficients (r) to evaluate the linear correlation between the predictor

666 variables. When two variables were highly correlated ($r > 0.7$) (Dormann et al., 2013), we

667 retained the variable that was more directly measurable and broadly understood across estuarine

668 systems. Based on this assessment, we excluded Ω_{arag} and $\Delta[O_2]$ due to their strong correlation

669 with other variables and retained the remaining six variables for GAM analysis. While necessary

670 for model stability, this approach may limit the explanatory power of the GAM by excluding

671   ecologically meaningful predictors. The full list of candidate variables and their inclusion status

672   is provided in Table 1.

673   Table 1: Predictor variables from the Chesapeake Bay Benthic Monitoring Program and ROMS-

674   ECB compiled from 1995–2002. For ROMS-ECB output, daily values were used to calculate

675   annual averages and seasonal averages for spring (March–April), summer (July–August), fall

676   (September–November), and winter (December–February)

Dataset	Location	Variable	Units	Processing	Used in GAMs?
BMP	Bottom	O_2	mmol m^{-3}	Removed extreme outliers	Yes
BMP	Bottom	Salinity	ppt	None	Yes
BMP	Bottom	Total depth	m	None	Yes
BMP	Bottom	Water temperature	ºC	Removed extreme outliers	Yes
BMP	Bottom	Sand fraction	%	None	Yes
ROMS-ECB	Surface	NO_3^-	mmol m^{-3}	Annually and seasonally averaged	Yes
ROMS-ECB	Bottom	POC	mmol m^{-3}	Annually and seasonally averaged	No, model evaluation gave poor results

ROMS-ECB	Bottom	TSS	mg L^{-1}	Annually and seasonally averaged	No, model evaluation gave poor results
ROMS-ECB	Bottom	Ω_{arag}		Calculated from alkalinity, DIC, temperature, water depth, and salinity using PyCO2SYS. Annually and seasonally averaged	No, highly correlated with salinity
ROMS-ECB	Surface	$\Delta[O_2]$	mmol m^{-3}	Calculated from oxygen, salinity and temperature using Gracia & Gordon (1991, 1992). Annually and seasonally averaged	No, highly correlated with salinity and NO$_3^-$

677   We used Akaike's Information Criterion (AIC), a statistical measure that has been

678 increasingly used in ecology, to evaluate which combination of parameters results in the best

679 model fit (Symonds & Moussalli, 2011). AIC is calculated using the number of fitted parameters

680 in the model, the maximum likelihood estimate, and the residual sum of squares (Symonds &

681 Moussalli, 2011). Among the candidate models, the one with the lowest AIC value offers the

682 best balance between explanatory power and simplicity, requiring the fewest necessary

683   parameters. We chose the assemblage of predictor variables in our model that minimized AIC.

684   We generally used annually averaged predictor variables from the ROMS-ECB output because

685   using seasonal averages had a negligible difference on the AIC. To assess the relative influence

686   of each predictor variable, we used Akaike weights, which indicate the probability that a given

687   model is the best among those considered. We ranked models by AIC and summed the Akaike

688   weights for all models containing each predictor variable. Higher Akaike weights indicate

689   stronger support for a variable's inclusion in the best-fitting models. Interaction terms between

690   predictor variables were examined, but their inclusion did not significantly improve the AIC

691   values.

692   **3. Results**

693   ***3.1 Spatial distribution of benthic macrofauna biomass***

694   The time-averaged (1995–2022) summer benthic macrofauna biomass exhibits strong

695   spatial variability across the Chesapeake Bay, with higher concentrations in the tidal fresh and

696   oligohaline zones, and lower concentrations in the Mid Bay and lower sections of many

697   tributaries (Figure 2, 3a). The arithmetic mean of all biomass measurements based on the full

698   data set, $N = 8128$ is 7.93 g m^{-2} (median = 0.98 g m^{-2}), whereas the spatial mean across all grid

699   cells with at least one biomass measurement ($N = 1295$) is 7.16 g m^{-2} (median = 1.39 g m^{-2}). The

700   sampling scheme oversamples the tidal tributaries (Fig. A1), where biomass density is higher,

701   inflating the arithmetic mean. The standard deviation of the full dataset is 28.7 g m^{-2}, indicating

702   that the distribution is heavily skewed to the right, with the highest sample reaching up to 722 g

703   m^{-2}. The standard deviation of the time-averaged data is 18.0 g m^{-2}, considerably smaller than

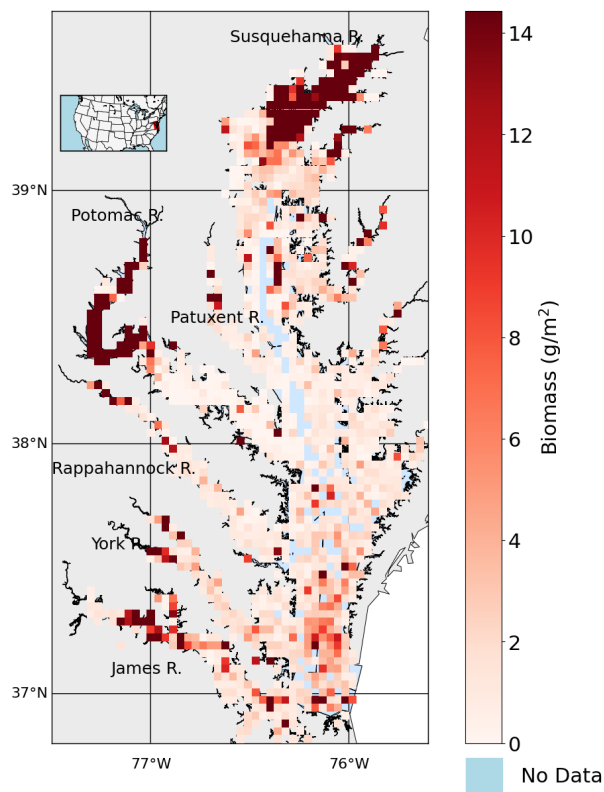
704   the full data set since it does not include temporal variability, but is nevertheless still skewed to

705   the right, with the highest gridded value of 220 g m^{-2}. Most of the high-biomass density zones

706 (>30 g m^{-2}) are concentrated in the tidal fresh and oligohaline sections of the mainstem and

707 Potomac River Estuary. The other tributaries have higher biomass in the tidal fresh and

708 oligohaline and zones compared to the other salinity zones. In the Mid Bay and lower sections of

709 many tributaries, biomass is very low.

710

711 Figure 2: Average summer biomass density from 1995 to 2022 from the Chesapeake Bay

712 Benthic Monitoring Program. Biomass values are shown using a red color scale, white represents

713 0 g m^{-2}, light blue indicates open water grid cells with no data, and gray represents land areas

714 with no data.

715   The distribution of the biomass into salinity zones for multiple taxonomic groups and

716   species is shown in Fig. 3b–f. Bivalves dominate biomass in all salinity zones, except the

717   polyhaline, where they are comparable to polychaetes. Bivalves comprise 88.0% of the benthic

718   biomass, and polychaetes comprise 7.3%. At a taxonomic species level, the bivalve *R. cuneata*

719   comprises 66.1% of the biomass, followed by the bivalves *C. fluminea* (8.0%) and *L. balthica*

720   (7.5%). Appendix D provides a more detailed spatial analysis of species distribution. The high-

721   biomass zones in the Upper Bay, Potomac River Estuary, and James River Estuary are

722   dominated by *R. cuneata*, mostly in the oligohaline zone (Figs. 3c & D1). *C. fluminea* also

723   dominates in relatively higher quantities in the tidal fresh zone of the Potomac River Estuary and

724   the tidal fresh zone of the Upper Bay (Figs. 3d & D1). In general, *R. cuneata* has a higher

725   biomass density in the tidal fresh than *C. fluminea* because *R. cuneata* is present in all tidal fresh

726   zones. *L. balthica* is distributed in multiple salinity zones; it is highest in the lower mesohaline

727   and is the dominant species in the lower mesohaline zone of the Upper Bay and Patuxent River

728   Estuary (Figs. 3e & D1). The biomass density of *L. balthica* in the lower mesohaline (<2 g m^{-2})

729   is significantly lower than that of *C. fluminea* and *R. cuneata* in the tidal fresh and oligohaline

730   zones. Polychaetes are the species that dominate over the largest geographic area, throughout the

731   polyhaline and part of the higher mesohaline (Figs. 2, 3f, & D1). However, their biomass density

732   is low relative to bivalves (<2 g m^{-2}). Bivalves are sparse throughout the higher mesohaline and

733   polyhaline zones.

734

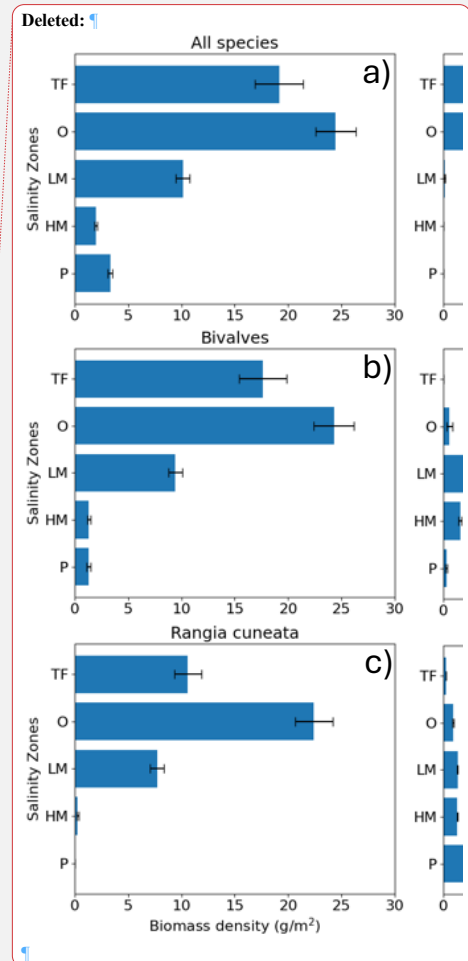
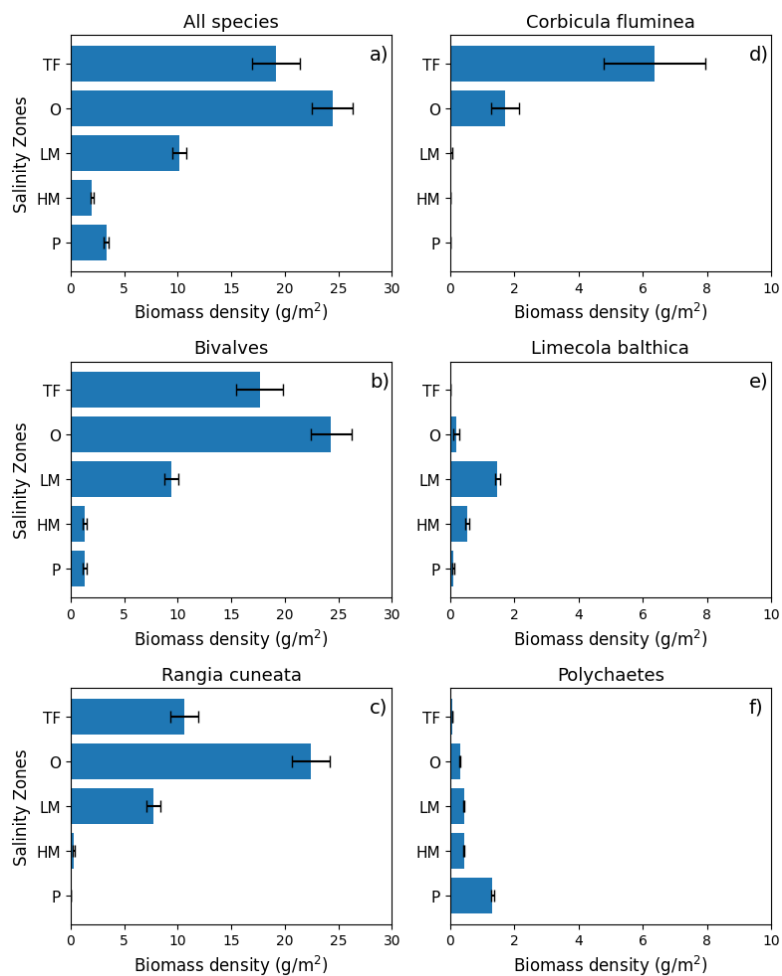



Figure 3: Average summer benthic biomass density of multiple classes and species in each

salinity zone from 1995 to 2022. The salinity zones are determined by the BMP based on the

long-term average of salinity in each geographic zone: TF – Tidal Freshwater (0–0.5 ppt), O –

Oligohaline (0.5–5 ppt), LM – Low Mesohaline (5–12 ppt), HM – High Mesohaline (12–18 ppt),

and P – Polyhaline (≥18 ppt). Averages and standard errors (bars) are computed using the full

743 data set, not the gridded values. Note the difference in horizontal scale between the left and right

744 columns.

3.2 Carbon flux estimates

746 Average benthic macrofaunal carbon and alkalinity fluxes vary across the 10 regions of

747 the Bay (Fig. 4). Among the different fluxes, which are all presented on a molar basis in Figure

748 4, F_{TA} has the largest magnitude, followed, in decreasing order, by F_{CO_2}, respiration (R/M_C),

749 calcification (C/M_{CaCO3}), and F_{DIC}. We first discuss calcification, respiration, F_{TA}, and F_{DIC}.

750 Calcification is 1.55 times respiration (best estimate), as a result of the molar ratio of these

751 processes (Section 2.3.3). F_{TA} is always negative, as expected because calcification is an

752 alkalinity sink, and twice the magnitude of calcification (Equation 12). The best estimate of F_{DIC}

753 was mostly positive, though it could be positive or negative because calcification is a DIC sink

754 and respiration is a DIC source (Equation 13). The best estimate of F_{DIC} ends up being positive

755 because respiration exceeds calcification. Using the best estimate of the molar

756 respiration:calcification ratio (1.55) and Equations 12 and 13, it can be shown that $-F_{TA}/F_{DIC} =$

757 3.6, consistent with the results in Fig. 4. Estimated uncertainties (Appendix B) were

758 approximately 45% for calcification, 40% for respiration, 135% for F_{DIC}, and 45% for F_{TA}. The

759 uncertainty for F_{DIC} being greater than 100% admits the possibility of negative DIC fluxes.

760 F_{CO_2} is always positive because benthic macrofauna remove TA from and add DIC to the

761 water column. The magnitude of F_{CO_2} can be understood by first noting that it is proportional to

762 F_{TA} and F_{DIC}, with proportionality constants of $[CO_2]\zeta_{TA}/[TA]$ and $[CO_2]\zeta_{DIC}/[DIC]$,

763 respectively (Equation 17; note that $F_{TA} < 0$ and $\zeta_{TA} < 0$, leading to $F_{CO_2} > 0$). In the open ocean,

764 using mean conditions of $pCO_2 = 400$ μatm, [TA] = 2300 μmol kg^{-1}, temperature = 15 °C, and

765 salinity = 35, we can use carbonate system equilibria to estimate $[CO_2] \sim 15$ μmol kg^{-1}, $[DIC] \sim$

771   2100 µmol kg^{-1}, and $-\zeta_{TA} \sim \zeta_{DIC} = 11$, which leads to F_{CO_2} about 7–8% of $-F_{TA}$ and F_{DIC}. Here,

772   we are seeing F_{CO_2} comparable in magnitude to F_{TA} and F_{DIC} because Bay waters tend to have

773   higher $[CO_2]$ and ζ_{DIC} and lower $[TA]$ and $[DIC]$ than what is observed in open-ocean waters, as

774   we show in Appendix E and Fig. E1. We observe much larger F_{CO_2} in tidal fresh (6–18 mol m^{-2}

775   yr^{-1}) compared to oligohaline (3–7 mol m^{-2} yr^{-1}) waters (Fig. 4), despite comparable values of

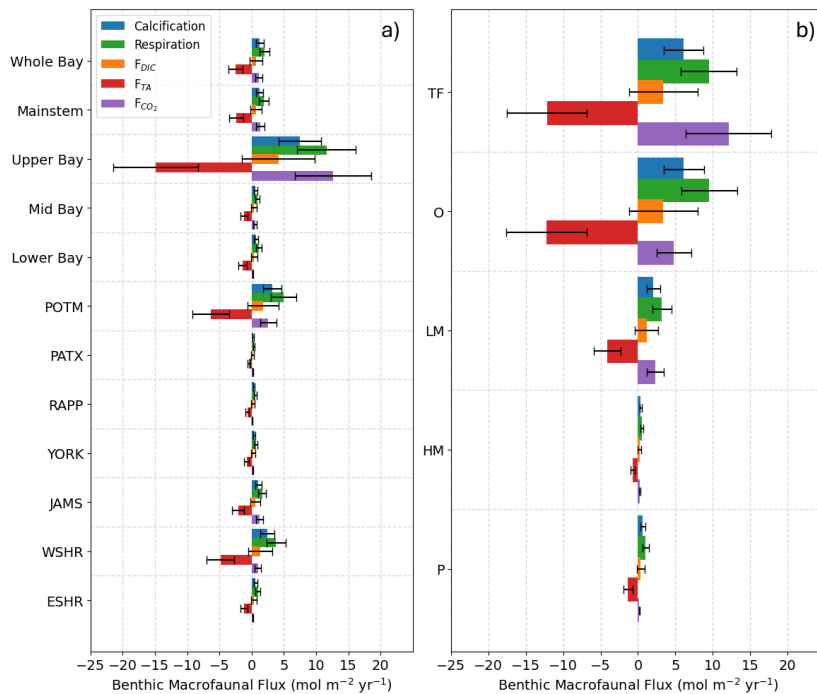
776   F_{DIC} and F_{TA}. These differences in F_{CO_2} between tidal zones are primarily driven by $[CO_2]$,

777   which is much higher in the tidal fresh due to a lower TA:DIC ratio (Fig. E1l). The magnitudes

778   of the buffer factors are also greater in lower salinity waters (Figs. E1g & E1h), decreasing

779   slightly in the polyhaline. Polyhaline values (13–16) are comparable to oceanic estimates of 9–15

780   (Egleston et al., 2010).

781

782 Figure 4: Average benthic macrofaunal fluxes by (a) Bay region and (b) salinity zone. Tributary

783 and sub-estuarine acronyms: ESHR – Eastern Shore tributaries; WSHR – Western Shore

784 tributaries; POTM – Potomac River; PATX – Patuxent River; RAPP – Rappahannock River;

785 YORK – York River system (including Mattaponi and Pamunkey Rivers); JAMS – James River.

786 Salinity zone abbreviations: TF – Tidal Freshwater (0–0.5 ppt), O – Oligohaline (0.5–5 ppt), LM

787 – Low Mesohaline (5–12 ppt), HM – High Mesohaline (12–18 ppt), P – Polyhaline (≥18 ppt).

788 Error bars represent uncertainty ranges (Appendix B).

789   In multiple segments of the Bay, benthic macrofaunal respiration rates (R) are

790 comparable to the total available organic carbon supply, defined here as the sum of net primary

791 production (NPP) and riverine POC loads (Table 2). In the Upper Bay, benthic macrofaunal

792 respiration rates are within the range of NPP alone. When combined with POC loads, our

793 estimates suggest that benthic macrofauna respire approximately f = 18–45% of the total

794 available organic carbon, where:

795
$$f = \frac{R}{NPP + POC} \tag{18}$$

796
$$\frac{\Delta f}{f} = \sqrt{\left(\frac{\Delta R}{R}\right)^2 + \left(\frac{\Delta NPP}{NPP}\right)^2} \tag{19}$$

797 Here, ΔR and ΔNPP are the standard errors of R and NPP, respectively. This formulation is

798 consistent with the uncertainty propagation approach described in Appendix B.

799   In the Potomac River Estuary, respiration rates exceed external organic carbon loads,

800 although NPP estimates for this region were unavailable. By contrast, in the mainstem and across

801 the Bay as a whole, benthic respiration rates are much smaller than NPP, accounting for only 3–

802 8% of the total inputs of organic carbon to the mainstem. These percentages were calculated

803 using the same approach as in Equations 18–19, but substituting mainstem benthic macrofaunal

804 respiration, NPP, and POC inputs.

805 Table 2: Riverine POC load, net primary production (NPP), and benthic respiration compiled

806 from the listed studies compared with benthic macrofaunal respiration from our study. POC load

807 estimates from Zhang & Blomquist (2018) were based on USGS river monitoring data combined

808 with flow-weighted sediment and organic carbon concentrations. For POC loads we specifically

809 used values from Table S5 of Zhang & Blomquist (2018), which reports long-term average true-

810 condition annual loads. Susquehanna loads were distributed across the mainstem and Upper Bay,

811 Potomac River loads were distributed across the Potomac, and total watershed load was

812 distributed across the whole Bay. These loads were divided by the surface area of the

813 corresponding segment area (Chesapeake Bay Program, 2004) NPP values from Harding et al.

814 (2002) were derived from depth-integrated in situ ^{14}C-uptake incubations across multiple cruises,

815 scaled using statistical models. Benthic respiration values from Kemp et al. (1997) were

816 estimated by combining seasonal in situ measurements of sediment oxygen consumption with

817 sulfate reduction rates, together accounting for microbial and macrofaunal respiration.

Region	Carbon Flux	Value (g C m^{-2} yr^{-1})	Reference
Whole Bay	Riverine POC load	12.0	Zhang & Blomquist (2018)
	Benthic macrofaunal respiration	24 ± 9	This study
Mainstem	Riverine POC load	18.8	Zhang & Blomquist (2018)
	NPP	385 ± 17	Harding et al. (2002)
	Benthic respiration	163.1	Kemp et al. (1997)
	Benthic macrofaunal respiration	22 ± 9	This study
Upper Bay	Riverine POC load	257.7	Zhang & Blomquist (2018)
	NPP	182 ± 27	Harding et al. (2002)

	Benthic respiration	44.3	Kemp et al. (1997)
	Benthic macrofaunal respiration	139 ± 55	This study
Potomac	Riverine POC load	31.9	Zhang & Blomquist (2018)
	Benthic macrofaunal respiration	59 ± 24	This study

819   Benthic macrofauna also contribute substantially to the calcium budget of the Bay. We

820   estimate that calcification by benthic macrofauna monitored in the BMP is slightly lower than

821   historical (pre-decline) estimates of the Eastern oyster (*Crassostrea virginica*) calcification in the

822   mainstem Bay but exceeds post-decline estimates by about 7 to 19 times in the Upper Bay (Table

823   3). This range was calculated by taking the upper and lower bounds of our measured calcification

824   rates (mean ± uncertainty) and dividing each by the post-decline oyster calcification rate.

825   Evaluating the role of bivalve calcification in utilizing calcium further underscores its

826   biogeochemical significance. Relative to annual riverine calcium fluxes, benthic macrofauna

827   would use ~22–58% of the available calcium in the Upper Bay and nearly all in the Potomac

828   River. These percentages were likewise calculated by taking the lower and upper bounds of our

829   measured calcification rates (mean ± uncertainty) and dividing each by the corresponding

830   maximum potential riverine calcium input, which was treated as fixed.

831   Table 3: Historic oyster calcification rates and estimated maximum potential calcification based

832   on riverine calcium input, compared with calcification rates from this study. Riverine calcium

833   load from the Susquehanna River was distributed across the Upper Bay and across the mainstem,

834   calcium load from the Potomac River was distributed within the Potomac segment, and the sum

835   of all riverine calcium inputs was distributed across the Whole Bay.

Region	Calcification Rate	Value (g CaCO$_3$ m^{-2} yr^{-1})	Reference
Whole Bay	Riverine Ca load	135	USGS
	Benthic macrofauna	142 ± 62	This study
Mainstem	Riverine Ca load	213	USGS

	Oysters	139	Fulford et al. (2007); Waldbusser et al. (2013)
	Benthic macrofauna	120 ± 54	This study
Upper Bay	Riverine Ca load	1861	USGS
	Oysters	57	Fulford et al. (2007); Waldbusser et al. (2013)
	Benthic macrofauna	747 ± 329	This study
Potomac	Riverine Ca load	262	USGS
	Benthic macrofauna	316 ± 142	This study

836   The role of benthic macrofaunal metabolic processes in the carbon budget is particularly

837   pronounced when the effects of calcification and respiration are combined to estimate CO_2

838   production. This CO_2 flux exceeds the amount of outgassing estimated in the Upper Bay and the

839   mainstem overall (Table 4).

840   Table 4: Air–sea gas exchange from the listed studies compared with total benthic macrofaunal

841   CO_2 flux calculated in our study.

Region	CO_2 Flux	Value (g C m^{-2} yr^{-1})	Reference
Whole Bay	Benthic macrofauna	13 ± 10	This study
Mainstem	Air–sea exchange	14.5 (outgassing)	Herrmann et al. (2020)
	Benthic macrofauna	16 ± 13	This study
Upper Bay (CB1–CB3)[a]	Air–sea exchange	74.5 (outgassing)	Herrmann et al. (2020)
(CB1 & CB2)[a]	Benthic macrofauna	151 ± 121	This study

842   [a] CB1–CB3 refer to Chesapeake Bay Program segmentation units (Chesapeake Bay Program,

843   2004): CB1 = tidal fresh, CB2 = oligohaline; CB3 = mesohaline.

844   ***3.3 Correlation of environmental variables with biomass***

845   The GAMs analysis, based on gridded, time-averaged biomass data, revealed key drivers

846   of benthic biomass both at the community level (all taxa combined) and for specific taxonomic

847   groups and species. The results for total benthic biomass (all taxa), polychaetes, and the bivalve

848   species *R. cuneata*, *C. fluminea*, and *L. balthica* are shown in Table 5. The bivalve group was

849   excluded from the table because its results were nearly identical to total benthic biomass results.

850   For total benthic biomass, the predictor variables (O_2, salinity, total depth, water temperature,

851   sand fraction, and NO_3^-) explain 54.9% of the deviance in biomass. For individual taxa, the

852   predictive capability of GAMs increases, with 73.7% of *R. cuneata* biomass deviance explained

853   by the predictor variables. For total benthic biomass, dissolved oxygen emerged as the strongest

854   predictor (with the highest summed Akaike weight), followed by total depth, salinity, and NO_3^-.

855   Although water temperature and sand fraction were included in the model, they had relatively

856   little influence. For individual taxa, dissolved oxygen became less influential, especially for *R.*

857   *cuneata* and *C. fluminea*. Salinity generally increased in influence as a predictor variable for

858   species-specific models, most notably for *C. fluminea*. NO_3^- also generally increased in influence

859   as a predictor variable for species-specific models, with very high influence as a predictor of *C.*

860   *fluminea* biomass.

861   Table 5: The best-fitting generalized additive model (GAM) for each taxa assemblage,

862   determined by minimizing Akaike's Information Criterion (AIC). $N = 846$ (number of grid boxes

863   with data) for all models. Summed Akaike weights indicate the relative importance of each

864   predictor variable across all models considered, with higher values suggesting stronger support

865   for a variable's inclusion in the best-fitting models. "N/A" indicates that the variable was not

866   included in the best model. Some variables may have high summed Akaike weights even if they

867   are not in the best-fitting model. For example, in the *R. cuneata* model, O_2 has a summed Akaike

868   weight of 0.571, while water temperature has 0.032. In the *C. fluminea* model, O_2 has 0.384, and

869   total depth has 0.24.

Taxa	Deviance Explained	Summed Akaike Weight

		O_2	Salinity	Total Depth	Water temperature	Sand fraction	NO_3^-
All taxa	54.9%	0.536	0.032	0.400	0.000	0.000	0.031
Polychaetes	50.0%	0.457	0.173	0.266	N/A	0.000	0.104
R. cuneata	73.7%	N/A	0.161	0.183	N/A	0.024	0.059
C. fluminea	65.9%	N/A	0.212	N/A	0.122	0.078	0.132
L. balthica	58.4%	0.888	0.000	0.112	N/A	0.000	0.000

870   The relative effects of O_2, salinity, NO_3^- and total depth on benthic biomass varied

871   considerably, as shown in the partial plots of Figure 5. Macrofauna biomass reaches its lowest

872   values at low dissolved oxygen, the only section of all the partial plots that reach 0 g m^{-2}.

873   Biomass is generally higher at shallower depths, although the effect is small. Biomass is highest

874   at both low and high salinities and high surface NO_3^-. In summary, biomass is highest at

875   moderate dissolved oxygen, shallow depths, low or high salinities, and high NO_3^-.

876

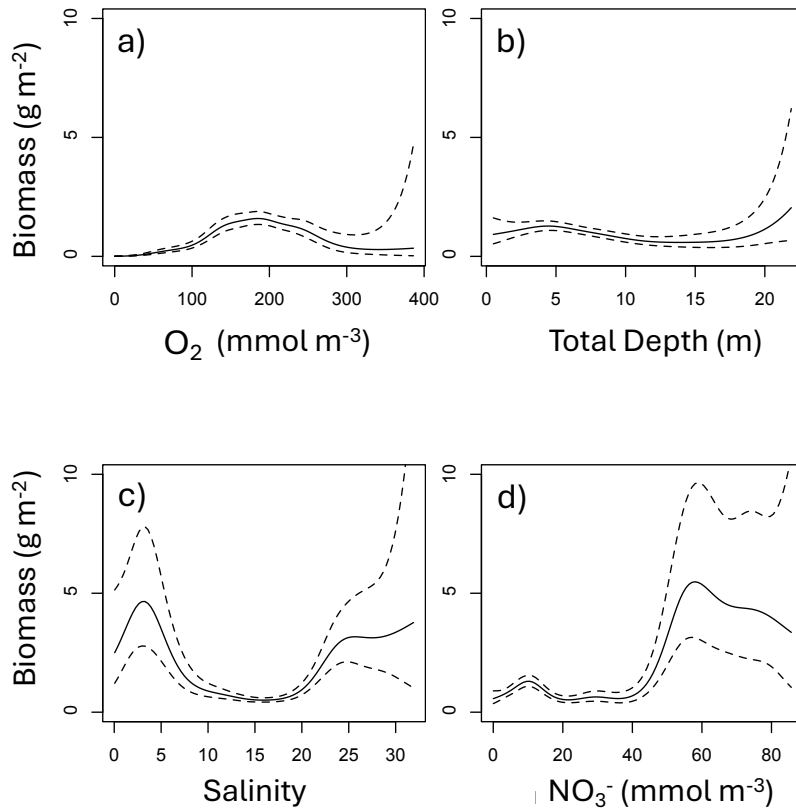



Figure 5: Partial plots of the effect of significant predictor variables on total biomass: (a) bottom

dissolved oxygen, (b) total depth, (c) bottom salinity, and (d) surface nitrate. Water temperature

and sand fraction were included in the model but omitted from display as they did not

significantly improve the model fit. The solid black line shows the response and the dashed lines

show the 95% confidence interval. The natural log transformation was removed from biomass to

enhance viewing of the partial plots. The x-axis was cut off at the upper quartile plus three

interquartile ranges in order to remove extreme outliers from the plots.

884   The model fit evaluation, presented in Appendix F and Fig. F1, reveals some systemic

885   biases. The residuals (observed values minus model-fitted values) are slightly skewed to the left

886   (Fig. F1b), meaning the model is more likely to overestimate biomass. The response vs. fitted

887   value scatter plot shows a nearly linear relationship (Fig. F1a). The map of residuals (Fig. F1c)

888   shows that there is also an underestimation in the model fit at the high biomass zones, especially

889   in the Upper Bay.

890   **4. Discussion**

891   ***4.1. Benthic macrofaunal contributions to the Chesapeake Bay carbon budget***

892   The higher benthic biomass in less saline waters of the Chesapeake Bay suggests a

893   significant role for respiration in the carbon budget in this region. The proportion of the total

894   input of particulate organic carbon that is respired by benthic macrofauna estimated in our study

895   in the Upper Bay (18–45%) is similar to the 14 to 40% range estimated for bivalve filter feeders

896   in tidal fresh and oligohaline waters by Cerco & Noel (2010). In the Upper Bay, where the

897   benthic macrofauna respiration is comparable to NPP, if all the NPP were respired solely by

898   benthic macrofauna, net ecosystem production would be zero. However, given the consideration

899   of additional respiration by other organisms (e.g., microbes and pelagic zooplankton) the

900   allochthonous POC from the Susquehanna River must be a critical source of organic carbon

901   driving net heterotrophy in this region. These findings reinforce the earlier conclusion that high

902   biomass zones in the Upper Bay are sustained by allochthonous organic inputs. The Potomac

903   River Estuary, however, presents a less conclusive case, partly due to the lack of available NPP

904   estimates. In this estuary, where benthic macrofaunal respiration exceeds the estimated POC

905   flux, additional carbon sources, such as autochthonous NPP or tidal wetland outwelling, may be

906   contributing to carbon metabolism. Notably, Herrmann et al. (2015) estimated that the amount of

907   organic carbon exported through wetland outwelling is approximately equal to half of that

908   exported by streamflow in Mid-Atlantic estuaries, highlighting its potential significance in

909   supporting heterotrophic processes in the Potomac.

910   Benthic macrofaunal respiration plays a smaller role in the rest of the Bay, where

911   autochthonous organic carbon dominates. For both the mainstem and the whole Bay, the

912   estimated primary production far exceeds the average estimated benthic macrofaunal respiration .

913   Kemp et al. (1997) estimated benthic respiration by combining sulfate reduction and sediment

914   oxygen consumption rates, which together account for both microbial and macrofaunal

915   respiration. Their results suggest that benthic respiration is a smaller fraction of NPP in the Mid

916   and Lower Bay, compared to the Upper Bay. This may reflect the relatively modest role of

917   macrofaunal respiration in these lower-biomass regions, despite higher autochthonous production

918   and organic carbon delivery to the sediments. Our estimates of benthic macrofaunal respiration

919   as a fraction of the total input of organic carbon to the mainstem (3–8%) is slightly lower than

920   estimates derived by Hopkinson & Smith (2004) and Rodil et al. (2022). Hopkinson & Smith,

921   (2004) estimated that approximately 24% of total available organic carbon is respired by the

922   benthos, while Rodil et al. (2022) found that benthic macrofauna account for roughly 40% of

923   total benthic respiration. Taken together, these studies suggest that around 10% of the total input

924   of organic carbon is expected to be respired by benthic macrofauna. Although the Bay-wide

925   fraction is small, macrofaunal respiration may still dominate locally in specific hotspots.

926   Unlike the extensive research on estuarine benthic respiration, benthic macrofaunal

927   calcification has received relatively little attention. Yet our findings suggest it may play a

928   significant role in the Chesapeake Bay carbon budget. As shown in Table 3, bivalves sampled

929   through the BMP program contribute substantially to calcification, surpassing calcification by

present-day Eastern oyster populations but comparable to that of historic populations in the

mainstem Bay. The importance of bivalve calcification is further emphasized when considering

calcium dynamics. To place bivalve calcification in context, we assumed that all riverine calcium

inputs were available for shell production. This is an upper-bound estimate because oceanic

calcium greatly exceeds riverine fluxes (Beckwith et al., 2019). While not a full calcium budget,

the comparison highlights how large the demand from calcification could be relative to the much

smaller riverine supply. Alkalinity fluxes further highlight the biogeochemical significance of

calcification. For the whole Bay, our estimated alkalinity consumption from benthic macrofaunal

calcification of 2.52 ± 1.13 mol m^{-2} yr^{-1} (6.90 ± 3.10 mmol m^{-2} day^{-1}) accounts for more than half

of the Bay-wide alkalinity sink estimated by Waldbusser et al. (2013) at 12 mmol m^{-2} day^{-1}. In

the Potomac River Estuary, our estimated alkalinity sink due to benthic calcification

(6.31 ± 2.84 mol m^{-2} yr^{-1} or 17.29 ± 7.78 mmol m^{-2} day^{-1}) is comparable to the alkalinity sink of

22 ± 1 mmol m^{-2} day^{-1} estimated by Najjar et al. (2020), who proposed that *C. fluminea* was

primarily responsible. Our findings, highlighting contributions from both *R. cuneata* and *C.

fluminea*, support their hypothesis and emphasize the substantial role of benthic calcification in

modulating alkalinity in estuaries.

Benthic macrofauna may play a major role in CO_2 outgassing, as indicated by our

estimated CO_2 fluxes from benthic macrofauna in the Upper Bay and upper tributaries, which

exceed typical CO_2 fluxes at the air–sea interface. Our benthic macrofaunal CO_2 generation

estimates for the Upper Bay (151 g C m^{-2} yr^{-1}), where bivalve biomass is concentrated, exceed

those reported by Chauvaud et al. (2003), who estimated 55 ± 51 g C m^{-2} yr^{-1} of CO_2 production

from calcification and respiration by the bivalve *P. amurensis* in northern San Francisco Bay.

Our results support their hypothesis that benthic calcifiers can serve as major CO_2 generators in

estuaries. Not all of this CO_2 generated would be outgassed. However, the combination of a TA

sink and DIC source from the bivalves leads to elevated pCO_2, enhancing the potential for

increased CO_2 outgassing (Middelburg et al., 2020). Given the high heterotrophy in the Upper

Bay and upper tributaries, as well as the balance between benthic macrofaunal respiration and

the total input of organic carbon to the Potomac River Estuary, it is conceivable that benthic

macrofauna are major contributors to CO_2 outgassing in these regions.

Although our empirical framework presented here captures the dominant patterns of

benthic secondary production, it does not explicitly incorporate seasonal temperature variability.

Because we applied summer temperatures associated with biomass, our production estimates

likely represent the upper end of expected values. A simple sensitivity analysis comparing

summer and winter conditions suggests that production, and by extension calcification and

respiration, may be closer to 60% of the values reported here under mean annual cycles.

Importantly, this potential bias is encompassed within our uncertainty bounds and therefore does

not affect our overall conclusions.

4.2. Environmental controls on benthic biomass distribution

Bivalve species distribution within the Bay is primarily driven by salinity tolerances.

Both the GAMs analysis (Table 5) and the biomass densities associated with salinity zones (Fig.

3) showed that benthic biomass, dominated by bivalves, was much higher at lower salinities. The

strong influence of salinity on benthic fauna distribution within estuaries has long been

recognized (Cain, 1975; Hopkins et al., 1973). This relationship is illustrated in our study by the

preference of the most abundant species, *R. Cuneata*, *L. balthica*, and *C. fluminea*, for less saline

water. *R. Cuneata* is widely known to need less saline waters, optimally between 1 and 15 ppt, to

survive (Hopkins et al., 1973). *C. fluminea* prefers freshwater environments (Phelps, 1994; Sousa

976 et al., 2008). *L. balthica* has a range of tolerances but abundance declines below 5 ppt (Jansson et

977 al., 2015). In our study, *L. balthica* is more concentrated between 5 and 13 ppt. While salinity

978 determines which species dominate, other factors influence their relative abundances within

979 salinity zones.

980 Within mesohaline zones, summer hypoxia appears to be driving extremely low benthic

981 biomass. In our GAMs analysis, an association existed between low biomass and extremely low

982 bottom dissolved oxygen values. Among the different salinity zones, low biomass is associated

983 with low dissolved oxygen in the high mesohaline, where we had the lowest biomass densities.

984 Long-term exposure to hypoxia (<2 mg L^{-1} or 62.5 mmol m^{-3}) is often fatal to benthic

985 macrofauna (Diaz et al., 1995; Seitz et al., 2006; Seitz et al., 2009) and many benthic

986 communities (approximately 50%) only recover on an annual timescale (Diaz et al., 1995). In

987 other words, regions that suffer frequent hypoxia often see lower levels of benthic biomass even

988 when hypoxic conditions are not occurring. In the Chesapeake Bay, hypoxia is most likely to

989 occur in the deeper, mesohaline regions (Frankel et al., 2022; Zheng & DiGiacomo, 2020),

990 providing evidence that our suppressed values of benthic macrofauna at this salinity zone could

991 be due to mass mortality from hypoxia. This pattern is particularly evident where biomass is

992 extremely low through the Mid Bay and lower Potomac River Estuary, regions typically

993 associated with summer hypoxia (Sturdivant et al., 2013).

994 Across the Bay, the relatively narrow range of summer bottom water temperatures likely

995 explains why temperature has an insignificant effect on the spatial distribution of benthic

996 biomass. In our GAMs analysis, summer bottom water temperature was not significantly

997 correlated with benthic biomass. Water temperature has been considered an important driver of

998 benthic biomass (Marsh & Tenore, 1990; Seitz et al., 2006; Seitz et al., 2009; Testa et al., 2020),

999 with low temperature specifically creating mass mortality of *R. cuneata* during winter and spring

1000 (Tuszer-Kunc et al., 2020). Higher temperature, however, will drive early and larger seasonal

1001 hypoxia in the Chesapeake Bay (Hinson et al., 2022; Irby et al., 2018; Ni et al., 2019). So,

1002 temperature could still indirectly affect benthic biomass through its effect on dissolved oxygen.

1003 Therefore, while temperature many not directly drive spatial patterns of biomass, it could be

1004 more relevant to temporal changes in biomass.

1005 Although NO_3^- is a strong predictor of benthic biomass, the mechanisms are likely

1006 complex and not necessarily linked to autochthonous primary production. In upper estuarine

1007 zones, high NO_3^- concentrations are often associated with elevated suspended solid loads, which

1008 limit light and may inhibit autotrophy (Brush et al., 2020b). Yet these same areas show strong

1009 heterotrophy, suggesting that respiration exceeds local production. Prior studies have shown that

1010 POC in upper estuaries contains substantial terrigenous inputs (Canuel & Hardison, 2016), and

1011 respiration of this organic matter likely contributes to heterotrophy in these regions (Kemp et al.,

1012 1997; Testa et al., 2020). This pattern raises the possibility that NO_3^- may serve as a proxy for

1013 allochthonous POC delivery. Supporting this, USGS data compiled by Zhang & Blomquist

1014 (2018) indicate that tributaries with high NO_3^- also tend have high loads of POC. However,

1015 further study is needed to test this hypothesis, and the observed NO_3^-–POC correlation should

1016 not be interpreted as causal.

1017 Our model explained roughly half of the deviance in biomass, indicating that

1018 environmental predictors captured key bottom-up controls. However, our model did not account

1019 for top-down factors such as predation, which may also play a significant role in shaping

1020 biomass distributions, particularly in an estuary with a densely populated watershed and

1021 substantial fishing pressure.

Conclusion

This study quantifies estuarine-scale benthic macrofaunal biomass and associated carbon fluxes, including secondary production, respiration, and calcification, across the Chesapeake Bay. The results demonstrate that benthic macrofauna play a substantial and spatially structured role in estuarine carbon cycling, particularly in upper estuarine zones where biomass is highest. In these regions, benthic macrofauna respiration is a significant fraction of total available organic carbon inputs, up to 45%, while calcification accounts for a large portion of the observed alkalinity sink. These contributions vary across the salinity gradient, shaped by both environmental drivers and taxonomic composition.

Patterns observed in this study suggest that benthic biomass and its associated carbon fluxes are influenced by gradients in salinity, oxygen, and organic matter inputs. The dominance of bivalves in low-salinity zones has particularly strong biogeochemical implications due to the role of bivalves in secondary production and calcification. Notably, NO_3^- emerged as a strong predictor of benthic biomass, not necessarily due to enhanced autotrophic production, but potentially reflecting allochthonous organic matter inputs that drive heterotrophic metabolism. The significant contributions of benthic communities to alkalinity consumption and DIC release highlight their potential to alter estuarine carbon chemistry in ways that depend on both biological composition and environmental conditions.

To build on these results, future work should examine how benthic biomass and associated carbon fluxes vary over time in response to both natural variability and anthropogenic change. Predicting future responses is especially challenging due to the high-frequency variability in estuarine systems, which can obscure long-term signals. Understanding how POC inputs, particularly allochthonous sources, change seasonally and interannually will be critical, as

1045   will clarifying the extent to which NO_3^- serves as a proxy for those inputs. Long-term monitoring

1046   and modeling efforts that link changes in watershed nutrient loading, land use, and climate with

1047   benthic community dynamics will be essential for forecasting ecosystem responses and for

1048   developing more accurate, responsive biogeochemical models. These predictive relationships

1049   between benthic biomass and environmental conditions could also support management and

1050   restoration planning. By identifying areas where oxygen, salinity, or sediment improvements are

1051   most likely to enhance benthic productivity, the models provide a framework for prioritizing

1052   restoration efforts and assessing ecosystem responses to changing conditions.

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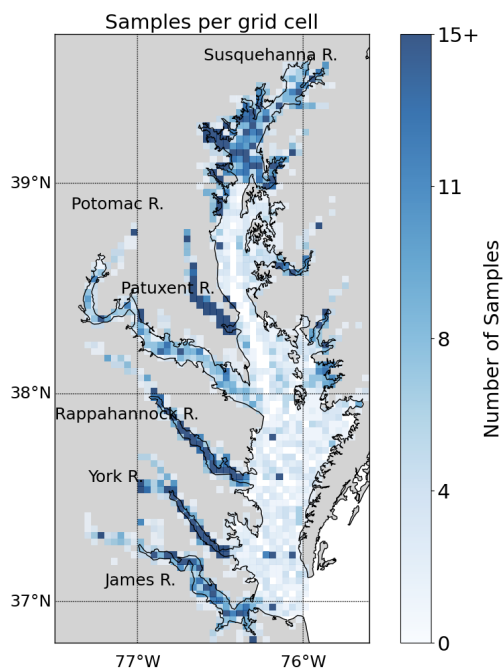
1072 **Appendix A**

1073 *Biomass Sampling Density*

1074   This appendix shows the spatial distribution of sampling effort for the BMP from 1995 to

1075 2022. Fig. A1 illustrates the number of samples collected in each grid cell, highlighting areas of

1076 higher and lower sampling density across the Chesapeake Bay and its tributaries.

1077

1078 Figure A1: Number of benthic samples collected in each grid cell in the summer (July 15 to

1079 September 30) from 1995 to 2022 from the Chesapeake Bay Benthic Monitoring Program. The

1080 grids are approximately square, measuring 3.5 km by 3.5 km. Each grid cell contains a time-

1081 average of each measurement collected in the cell

1082 **Appendix B**

1083 *Carbon flux uncertainties*

1084   This appendix explains the methodology for propagating uncertainty in the benthic

1085   macrofaunal carbon flux estimates. It outlines the parameter ranges used, the calculations for

1086   uncertainty bounds, and the assumptions underlying these estimates. These details support the

1087   uncertainty ranges shown in figures and tables in the Results section. Uncertainty estimates were

1088   propagated through empirical equations using standard error propagation techniques (Taylor,

1089   2022) assuming uncorrelated inputs and approximating relative errors when only a range of

1090   values was available.

1091   Uncertainty for carbon-based biomass (B_c) is derived from the two different r_c values:

$$\frac{\Delta B_c}{B_c} = \frac{\Delta\, r_c}{r_c} \tag{B1}$$

1092

1093   where $\Delta\, r_c = 0.03$ g C g^{-1}, half the range of possible r_c values.

1094   The uncertainty for the first approach for estimating secondary production (S_1) is derived

1095   from B_c and α:

$$\frac{\Delta S_1}{S} = \sqrt{\left(\frac{\Delta\, \alpha}{\alpha}\right)^2 + \left(\frac{\Delta\, B_c}{B_c}\right)^2} \tag{B2}$$

1096

1097 $\Delta\, \alpha$ is half the range of possible α values and equals 1.695 yr^{-1}.

1098   Uncertainty in secondary production (S) arises from the multiple equations used:

$$\frac{\Delta S}{S} = \sqrt{w\left(\frac{\Delta S_1}{S_1}\right)^2 + 2w\varepsilon} \tag{B3}$$

1099

1100   where each model is equally weighted ($w = 1/3$). Only Model 1 (Equation 5) includes an explicit

1101   source of uncertainty, while Models 2 and 3 (Equations 7 and 8) do not. Therefore a small error

1102   term $\varepsilon = 0.001$ was assumed for those two models.

1103   Calcification (C) uncertainty is derived from r_s and S:

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$$\frac{\Delta C}{C} = \sqrt{\left(\frac{\Delta r_s}{r_s}\right)^2 + \left(\frac{\Delta S}{S}\right)^2} \tag{B4}$$

where r_s has one value for *C. fluminea* and one for *P. amurensis*. $\Delta r_s = 2.5$ g CaCO$_3$ (g C)$^{-1}$ and

is half the range of possible r_s values.

 The uncertainty in respiration (R) is derived solely from S:

$$\Delta R = 2.33 \Delta S \tag{B5}$$

 The uncertainties in the fluxes of alkalinity and DIC (F_{TA} and F_{DIC}) are also derived from

C and R:

$$\Delta F_{TA} = \frac{-2\Delta C}{M_{CaCO_3}} \tag{B6}$$

$$\Delta F_{DIC} = \sqrt{\left(\frac{\Delta R}{M_C}\right)^2 + \left(\frac{\Delta C}{M_{CaCO_3}}\right)^2} \tag{B7}$$

where M_{CaCO_3} and M_C are the molar masses of CaCO$_3$ and C, respectively.

 Finally, we propagate the error in the flux of carbon dioxide (F_{CO_2}) from the errors in F_{TA}

and F_{DIC}:

$$\Delta F_{CO_2} = \sqrt{\left(\frac{[CO_2]\zeta_{TA}\,\Delta F_{TA}}{[TA]}\right)^2 + \left(\frac{[CO_2]\,\zeta_{DIC}\Delta F_{DIC}}{[DIC]}\right)^2} \tag{B8}$$

We do not consider errors in the buffer factors, [CO$_2$], [DIC], and [TA] in ROMS.

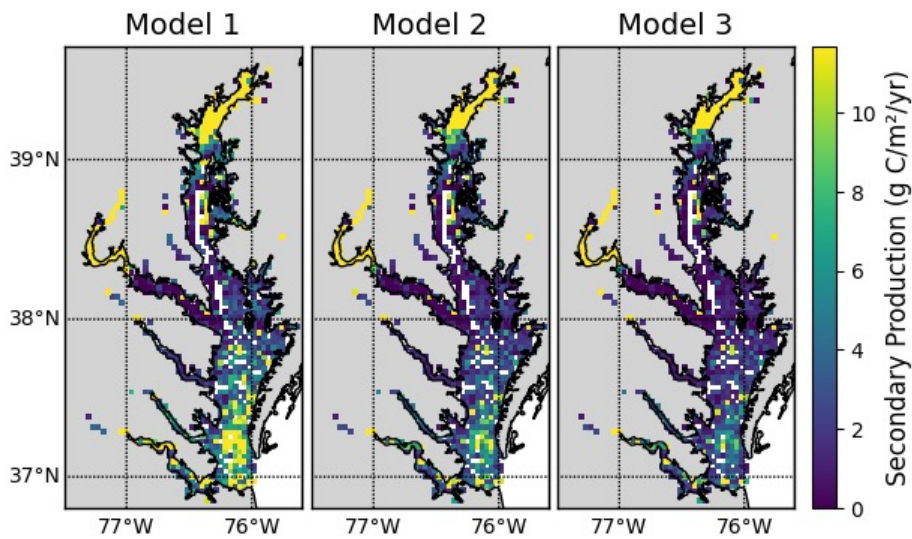
Appendix C

Secondary production model comparison

 This appendix details the comparison of secondary production estimates from the three

approaches described in Section 2.3.1. Seasonal and spatial variations are shown to illustrate the

1125 influence of temperature and other parameters on production estimates. These comparisons

1126 provide context for interpreting model differences presented in the main text.

1127

1128 Figure C1: Long-term average secondary production across the Chesapeake Bay for each of the

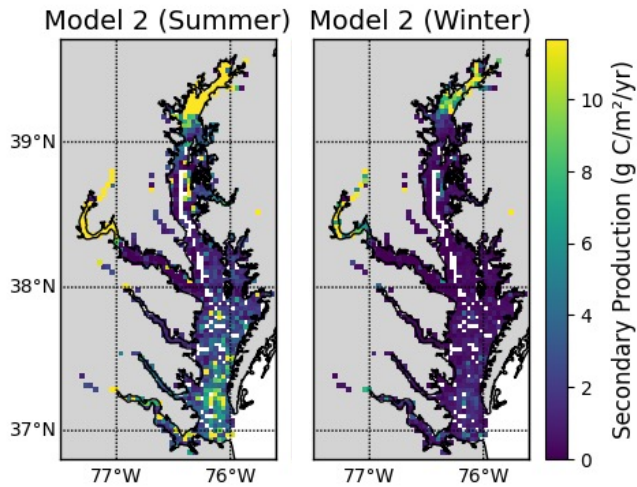
1129 three secondary production models.

1130   The models have similar spatial patterns (Fig. C1), which are similar to the pattern of

1131 benthic biomass (Fig. 2). Secondary production is generally higher in Model 1 than in Models 2

1132 and 3, which can be seen most clearly in the means, which are 17.92, 8.31, and 7.59 g C m^{-2} yr^{-1}

1133 in Models 1, 2, and 3, respectively.

1134

1135 Figure C2: Seasonal sensitivity of Model 2 to bottom temperature inputs.

1136   Fig. C2 and C3 compare secondary production between summer and winter scenarios.

1137 The summer scenario uses summer bottom water temperatures measured concurrently with

1138 biomass samples, while the winter scenario assumes a temperature of 1 °C. Fig. C2 shows the

1139 results for Model 2, which has a mean secondary production of 8.31 g C m⁻² yr⁻¹ in summer and

1140 1.89 g C m⁻² yr⁻¹ in winter. Fig. C3 shows the results for Model 3, which has a mean secondary

1141 production of 7.59 g C m⁻² yr⁻¹ in summer and 1.44 g C m⁻² yr⁻¹ in winter. For both models,

1142 production in summer is about 5 times that of winter, as expected from the rough estimates in the

1143 Methods section. Assuming spring and fall are halfway between winter and summer, and

1144 weighting the seasons equally, the annually averaged secondary production rate would be about

1145 60% of the summer rate.

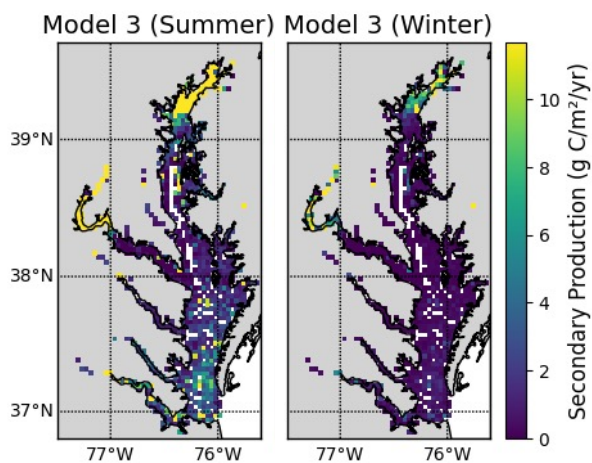



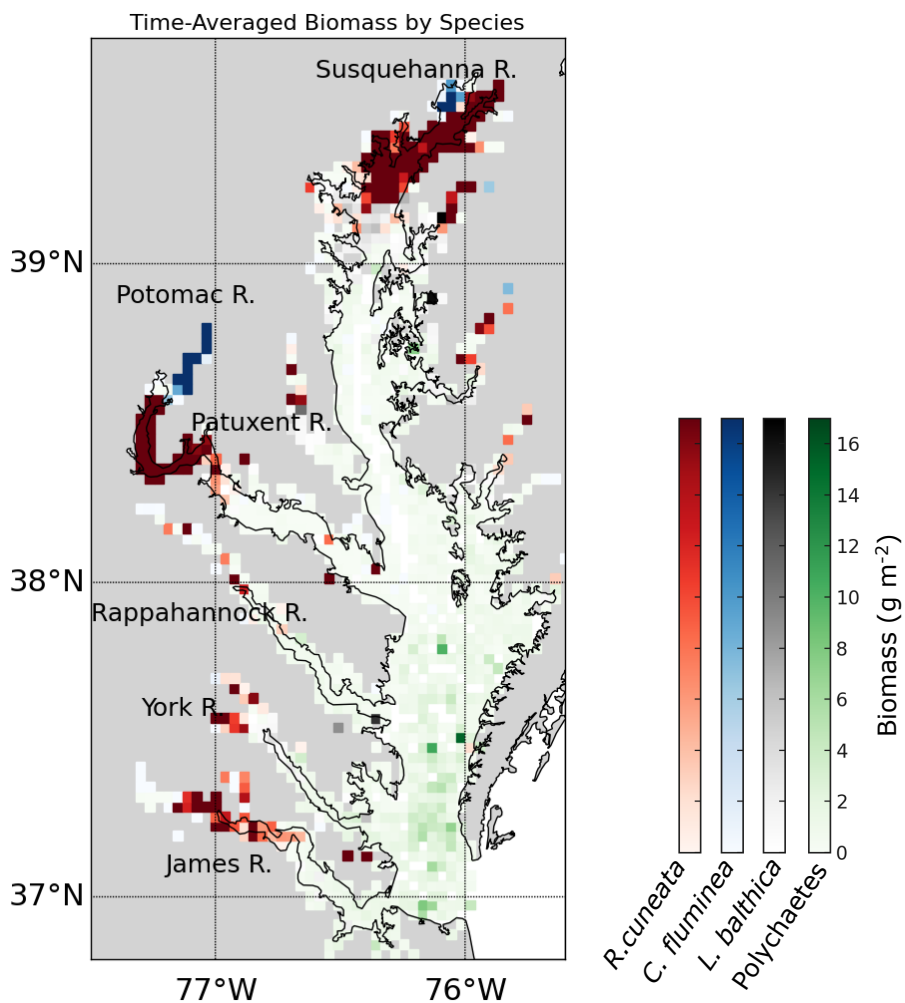
Figure C3: Seasonal sensitivity of Model 3 to bottom temperature inputs.

Appendix D

Species biomass distribution

  This appendix expands on the biomass distribution results in Section 3.1 by showing

detailed spatial patterns for individual species and taxonomic classes. Fig. D1 complements the

summary figures in the main text, highlighting species-specific habitat associations.

1153

1154 Figure D1: Average summer biomass density from 1995 to 2022 from the Maryland and Virginia

1155 Benthic Monitoring Program. Each color corresponds to a specific bivalve species (*M. balthica,*

1156 *C. fluminea, or R. cuneata*) or polychaetes. The color shown on the map is the species with the

1157 highest time-averaged biomass in that grid cell.

1158 **Appendix E**

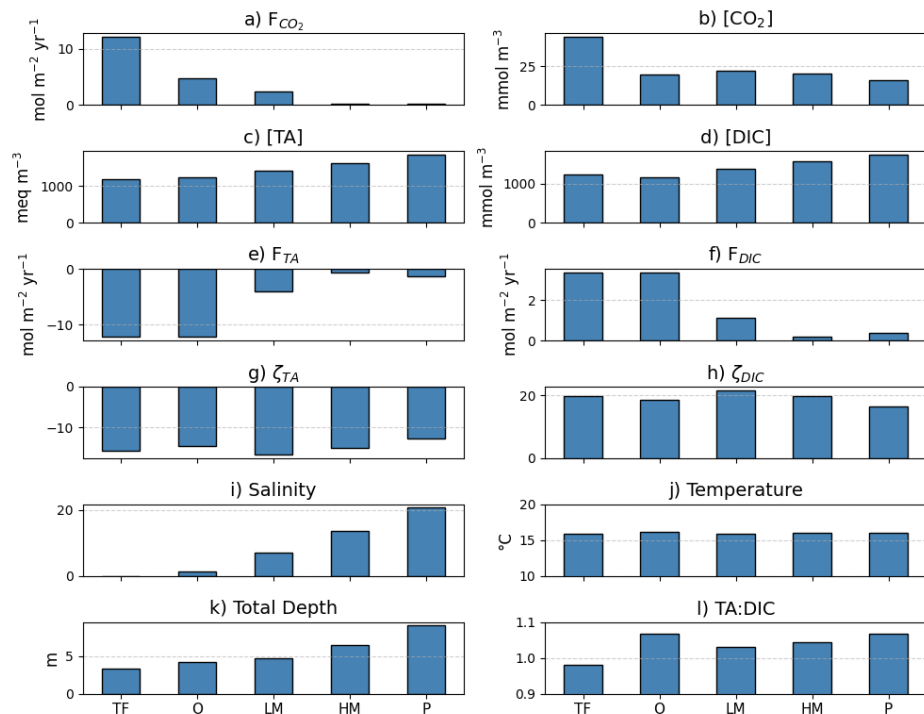
1159 *Carbon flux-related parameters*

1160   To illustrate estuarine variability in carbonate system drivers, we summarized a suite of

1161 flux and water column properties across tidal salinity zones of the Chesapeake Bay (Fig. E1).

1162 The patterns highlight strong gradients from tidal fresh through polyhaline regions. Notably

1163 $[CO_2]$ is elevated in tidal fresh waters, underscoring the combined influence of salinity, TA, and

1164 the TA:DIC ratio on estuarine carbonate chemistry.

1166 Figure E1: Carbon flux-related parameters averaged within salinity zones of Chesapeake Bay.

1167 $[CO_2]$ was calculated using PyCO2SYS; temperature, salinity, total depth, [TA] and [DIC] were

1168 obtained from ROMS model output. F_{TA} and F_{DIC} were calculated following Equations 12–13,

1169 ζ_{TA} and ζ_{DIC} from Equations 15–16, and F_{CO_2} from Equation 14.

1173 **Appendix F**

1174 *GAMs evaluation*

1175   This appendix presents diagnostic plots and statistics for the generalized additive models

1176 described in Section 2.5. Residual maps, histograms, and fitted-vs-observed plots (Fig. F1) are

1177 included to evaluate model performance and identify patterns in prediction errors.

1178   A good model would have points on a modeled vs. observed scatterplot fall close to a 1:1

1179 line and have the departures from that line (residuals) be close to normally distributed. Although

1180 GAMs can handle non-normal distributions of the response variable (Guisan et al., 2002), we

1181 found the best model fit by applying a natural logarithmic transformation to the biomass. A small

1182 constant (0.0001 g m^{-2}) was added to the biomass values before applying the natural logarithmic

1183 transformation to account for zeros in the data. The resulting biomass was normally distributed

1184 after applying the natural logarithm function, so the GAM was fitted with a Gaussian distribution

1185 with an identity link function.

1186

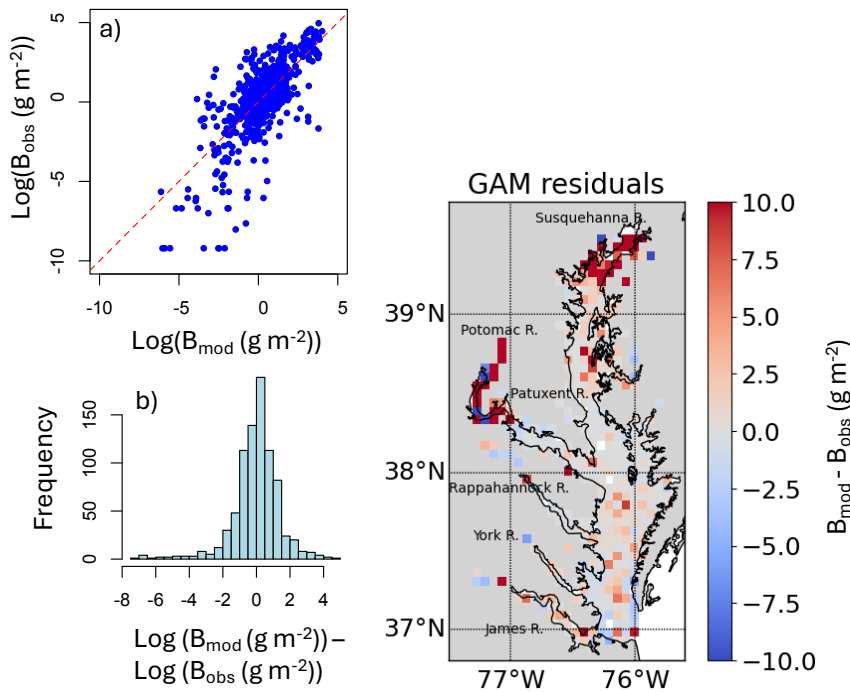



Figure F1: Evaluation of the total biomass GAM, which includes O_2, salinity, total depth, NO_3^-, sand fraction, and water temperature as predictor variables. "Log" refers to the natural logarithm. (a) Modeled vs. observed values. (b) Histogram of biomass residuals (modeled minus observed values, $B_{mod} - B_{obs}$). (c) Spatial distribution of the residuals.

Data Availability

All data used in this study are publicly available and have been described in the Methods section.

Water quality data were obtained from the *Chesapeake Bay Water Quality Monitoring Program* (https://datahub.chesapeakebay.net/WaterQuality), and benthic data were sourced from the *Chesapeake Bay Long-Term Benthic Monitoring Program*

1198 (https://www.baybenthos.versar.com/data.htm). Model output used in this study is publicly

1199 available at https://www.seanoe.org/data/00882/99441/.

1200

1201 **Author Contribution**

1202 SA led the research, conducted data analysis, interpreted results, and wrote the initial and

1203 subsequent drafts of the manuscript.

1204 RN conceptualized the project, defined overarching research goals, secured financial support,

1205 and contributed substantially to manuscript drafts through critical reviews, commentary, and

1206 revisions.

1207 ER provided significant insights from her expertise on the physiological responses of marine

1208 invertebrates to environmental variables, influencing the scope and direction of the study. She

1209 also substantially contributed to manuscript revisions through critical reviews and commentary.

1210 RW contributed significant expertise on benthic biomass distribution in Chesapeake Bay and

1211 generalized additive modeling techniques, shaping the analytical framework of the study. He

1212 provided critical feedback and revisions during manuscript preparation.

1213 MF provided ongoing feedback throughout the research process, particularly regarding the

1214 integration of modeling information and interpretation of results, and contributed critical

1215 commentary on multiple presentations of the work.

1216 PS consistently provided feedback and contributed modeling expertise, assisting with data

1217 interpretation and manuscript revisions through critical reviews and commentary.

1218 SD developed the original empirical models linking benthic macrofaunal biomass to carbon

1219 fluxes, laying foundational methodological contributions to the study.

1220

Competing Interests

The authors declare that they have no conflict of interest.

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