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SHORT COMMUNICATION

IDENTIFYING STABLE ISOTOPE PATTERNS AMONG TAXA, SITES, AND ENVIRONMENTAL VARIABLES IN THE EASTERN MISSISSIPPI SOUND§

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INTRODUCTION

Estuaries are dynamic habitats at the interface of riverine, marine, and terrestrial habitats (Elliott and McLusky 2002). The interaction among these habitat types results in highly productive systems (Elliott and McLusky 2002, Wissel et al. 2005). Rivers bring substantial freshwater and associated organic matter and nutrients into these systems, helping to support species like shrimp, crabs, herring, and anchovies, which form important links in estuarine and coastal food webs (Nedwell et al. 1999, Wissel et al. 2005, Gillson 2011, Abrantes et al. 2013). The contributions of riverine and marine inputs often shape estuarine trophic dynamics and provide insights into conserving their ecological and socioeconomic values (Abrantes et al. 2013).

Stable isotope analysis provides a tool to quantify the nutritional influence of riverine and marine systems on estuaries and characterize organismal movement among rivers, estuaries, and marine habitats (Fry 2002, Wissel et al. 2005). In estuaries, stable carbon isotope values of organic materials from riverine catchments dominated by C3 are lower compared to marine sources (Fry 2002, Wissel et al. 2005, Abrantes et al. 2013), and stable carbon isotope values in estuaries increase along a salinity gradient. Stable nitrogen isotopes values increase with trophic level, so are frequently used to quantify trophic position (Post 2002, Ramirez et al. 2021). Usually less affected by salinity, stable nitrogen isotope values have been found to decrease with salinity (Fry 2002, Wissel et al. 2005).

Here, we determined trophic structure and nutrient input from associated freshwater sources using stable carbon and nitrogen isotope ratios from 3 sources throughout the eastern Mississippi Sound (EMSS) estuary along the northern Gulf of Mexico (nGOM): 1) biota (finfish, crustaceans, squid) tissues, 2) suspended particulate matter (seston), and 3) sediments. Although the EMSS is part of the highly productive nGOM coast and supports an economically important fishery, little work has isotopically described baselines and higher trophic levels across this region. This work tested 3 hypotheses. First, we predicted that isotope values in biota would reflect location–specific trophic structure from seston and sediment, with typical trophic enrichment (±1‰ for C, +2–4‰ for N; Post 2002, Caut et al. 2009). Secondly, we predicted that the variation of isotope values in biota and seston would be greater than in sediment, because fish and seston move through the estuary, potentially loosening spatial isotopic signatures. Third, we predicted that because freshwater inputs are along the northern part of EMSS, stable carbon isotope values would be lowest at northern sites and highest at southern sites, whereas nitrogen isotope values would not vary between more northerly and southerly sites.

MATERIALS AND METHODS

Study Site

The EMSS is a large embayment that is bordered by the contiguous Mississippi and Alabama coastlines to the north and Dauphin and Petit Bois Islands to the south (Figure 1A). There are a series of smaller sub–embayments along the Mississippi/Alabama coasts including, east–to–west, Heron Bay, Portersville Bay, and Grand Bay. Each embayment is fed by several small to mid–sized rivers that provide freshwater and associated nutrients to the EMSS estuary, except for Grand Bay, which only has direct freshwater inflow on the very western edge of the embayment. In addition to these sources of freshwater, the eastern end of EMSS opens to Mobile Bay, which is a major source of freshwater into the EMSS from east–to–west (Du et al. 2018). The primary habitats in EMSS include *Juncus roemerianus* marshes with fringes of *Spartina alterniflora*, oyster shell deposits, seagrass beds, and shallow, non–vegetated bottoms.

Sample Collection

Biota Tissues. Biota were collected from 14–16 June 2022 from 7 sites: Grand Bay, Portersville Bay, Heron Bay, Main Sound 1, Main Sound 2, Dauphin Island, and Petit Bois (Figure 1A). Biota were collected at all sites with an otter trawl that was 4.6 m wide with 3.8 cm mesh; trawls were pulled at ~2 km/h for 10–15 min. If an insufficient variety and number of species were captured on the first trawl, a second 15–minute trawl was performed. No additional trawls were conducted if an in-

§*The first author conducted this research as part of the Dauphin Island Sea Lab's Research Experience for Undergraduates in the coastal and nearshore marine science program.*

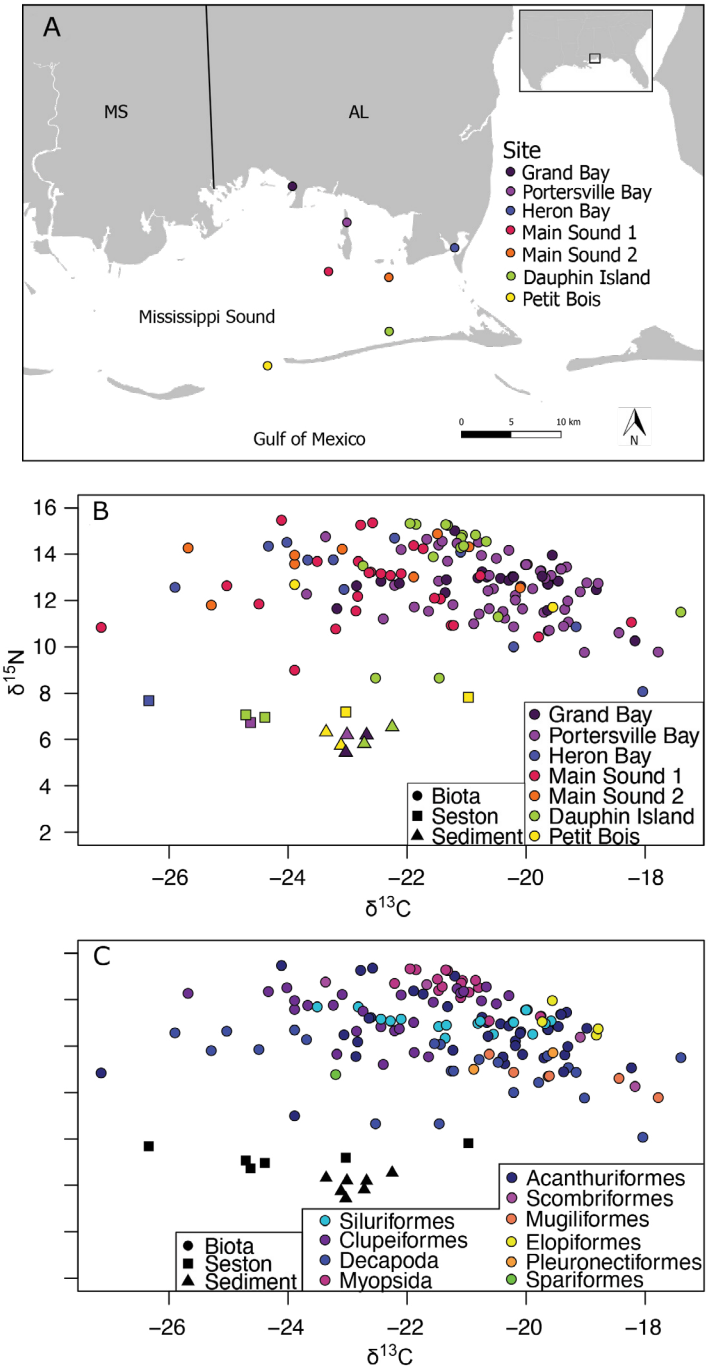

Figure 1. *Samples from the eastern Mississippi Sound, northern Gulf of Mexico in June 2022 for isotopic analysis. A. Study area with the specific sampling sites. B. Isotopic biplots of biota, seston, and sediment samples categorized by site. C. Isotopic biplots of biota, seston, and sediment samples categorized by taxonomic group.*

sufficient number of species were collected on the second trawl. Grand Bay was additionally sampled with 2 gill nets 198 m in length that were oriented perpendicular to each other. The first net had mesh sizes ranging from 11.5–15 cm, and the second net had mesh sizes 6.0–11.5 cm. Individuals that were taken for sampling were generally between 6–35 cm, except for *Elops saurus* and *Cynoscion nebulosus*, which had individuals > 35 cm. Up to 6 individuals of each species were reserved from each trawl or gillnet, placed on ice in the field and frozen at –20°C in the laboratory until dissection. Additional specimens were returned to the water. No elasmobranchs were collected. Samples were identified to species (Hoese and Moore 1998), sorted by size, and placed into taxonomic groups of order for fishes following Betancur–R et al. (2017) and class for invertebrates following Hopkins et al. (1989) and Kaplan and Kaplan (1999). A small portion of hypaxial muscle was dissected and removed from fish and Decapoda and a section of mantle was removed from Myopsida. Dissected tissues were transferred to a glass petri dish, and dried in an oven at 60°C for ~48 h.

Within 10 min after trawling or gillnetting at each site, we measured salinity using a YSI Pro2030 (Yellow Springs, OH, U.S.A.) at the surface (~1 m below the surface) and 1 m above the bottom if the water depth was ≥ 3 m. Many sites had water depths < 3 m and only one measurement was taken at the surface. As a result, all analyses using salinity were done with surface measurements because they were available for all sites.

Water and Sediment Samples. Water and sediment samples were collected at each site where biota were sampled on 25 June 2022 (Figure 1A). Water samples were taken using a horizontal water sampler with a 1.2 L capacity (LaMotte 1087 Horizontal Water Sampler, Chestertown, MD, USA), passed through a 200 µm filter to remove zooplankton, and stored in 1 L brown Nalgene bottles on ice. Water samples were taken 1 m below the surface, and for sites with water depths ≥ 3, we took a sample 1 m above the bottom. Samples were returned to the lab where they were vacuum filtered through 0.7 µm glass fiber filters. Water was pumped until filters were clogged with seston (74 – 632 ml of filtered water). Filters were dried in an oven at 60°C.

Sediment samples were collected from each site using a 15.25 x 15.25 x 15.25 cm dredge (Wildco Ekman dredge, Buffalo, NY, USA), which sampled the upper 1–4 cm of sediment. Two sediment samples were taken per site and stored in Ziploc bags on ice until they were returned to the lab. Sediment was transferred to glass petri dishes and dried at 60°C for ~48 h. Shell and other visible carbonate materials were removed by hand.

Stable Isotope Analysis

Dried tissue samples and sediments were homogenized using mortar and pestle, and samples (~1 µg tissue, ~25 mg sediment) were packed into 3 x 5 mm tin capsules. Filters containing seston were folded and each packed into an 8 x 5 mm tin capsule. All samples were sent to the Stable Isotope Facility at the University of California Davis and results are reported using the standard delta notation (δ) in parts per thousand (‰). The reference material was Vienna–Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Repeated analysis of in–house reference materials (bovine liver, glutamic acid, and nylon 6 for C and N) showed that precision (± sd) was ± 0.05‰ and 0.09‰ for C and N, respectively.

Statistical Analyses

We used ANOVAs to determine if there were differences in $\delta^{13}C$ and $\delta^{15}N$ values among sites and taxonomic groups. We further determined differences among individual sites and taxonomic groups using Tukey honest significant difference tests, which tests all pairwise differences and accounts for the probability of making type 1 errors. We used a general linear model

to determine if $\delta^{13}C$ and $\delta^{15}N$ values were affected by salinity. We ran a Pearson's correlation between $\delta^{13}C$ and CN ratios to determine if tissues high in lipid content (i.e., those with high CN ratios) affected $\delta^{13}C$ values. All analysis were done in R (R 2013).

Results

We collected and analyzed a total of 143 biota samples from Grand Bay (n = 32 gillnet, 21 trawl), Portersville Bay (n = 25), Heron Bay (n = 26), Main Sound 1 (n = 10), Main Sound 2 (n = 11), Dauphin Island (n = 16), and Petit Bois (n =2). We collected biota samples from 10 taxonomic groups, including Decapoda (22 individuals), Myopsida (18 individuals), Acanthuriformes (42 individuals), Clupeiformes (27 individuals), Elopiformes (4 individuals), Mugiliformes (6 individuals), Spariformes (1 individuals), Pleuronectiformes (2 individuals), Scombriformes (4 individuals), and Siluriformes (17 individuals). See Supplemental Table S1 for a detailed list of which species were captured at which sites. We analyzed seston samples from 4 sites; Portersville Bay (n = 1, number of filters analyzed), Heron Bay (1), Petit Bois (2), and Dauphin Island (2) and sediment samples from 4 sites, Grand Bay (n = 2, number of sediment samples analyzed), Portersville Bay (1), Main Sound 1 (2), and Dauphin Island (2). Total C and/or N levels were too low in other seston and sediment samples from other sites to obtain accurate estimates, which had thresholds of 100 and 20 µg for C and N, respectively.

Isotopic values followed fairly predictable patterns. The range of $\delta^{13}C$ values for seston and sediment samples fell within the range of $\delta^{13}C$ values for biota but on average were lower in comparison (Figure 1B). Seston samples had greater variation in $\delta^{13}C$ compared to sediments (Figure 1B; Supplemental Table S2), and average C:N values of seston were 6.56 (Supplemental Table S2). Variation in $\delta^{13}C$ values from biota was uncorrelated with C:N ratios and lipid content likely didn't affect $\delta^{13}C$ values (t_{141} = 1.435, p = 0.153, r = 0.12). The $\delta^{15}N$ values in most biota were 3–8‰ above seston and sediment values (Figure 1B; Supplemental Table S2). Overall, $\delta^{13}C$ and $\delta^{15}N$ values overlapped considerably among sites (Figure 1B), and no sites occupied distinct isotopic spaces for both elements for either baselines (sediment and seston) or biota (Figure 1B). The $\delta^{13}C$ values differed among sites ($F_{7, 135}$ = 7.985, p < 0.001). Biota from Grand Bay and Portersville Bay to the northwest had higher $\delta^{13}C$ values than Heron Bay to the east or the Main Sound (1 and 2), with intermediate values found near the islands to the south (Figure 2A). There were no differences in $\delta^{15}N$ values among sites ($F_{7, 135}$ = 1.635, p = 0.131) (Figure 2B). We found greater differences in $\delta^{13}C$ and $\delta^{15}N$ values among fish taxonomic groups than among sites (Figures 1B, 1C, 2C, 2D), with some groups like Elopiformes, Mugiliformes, Myop-

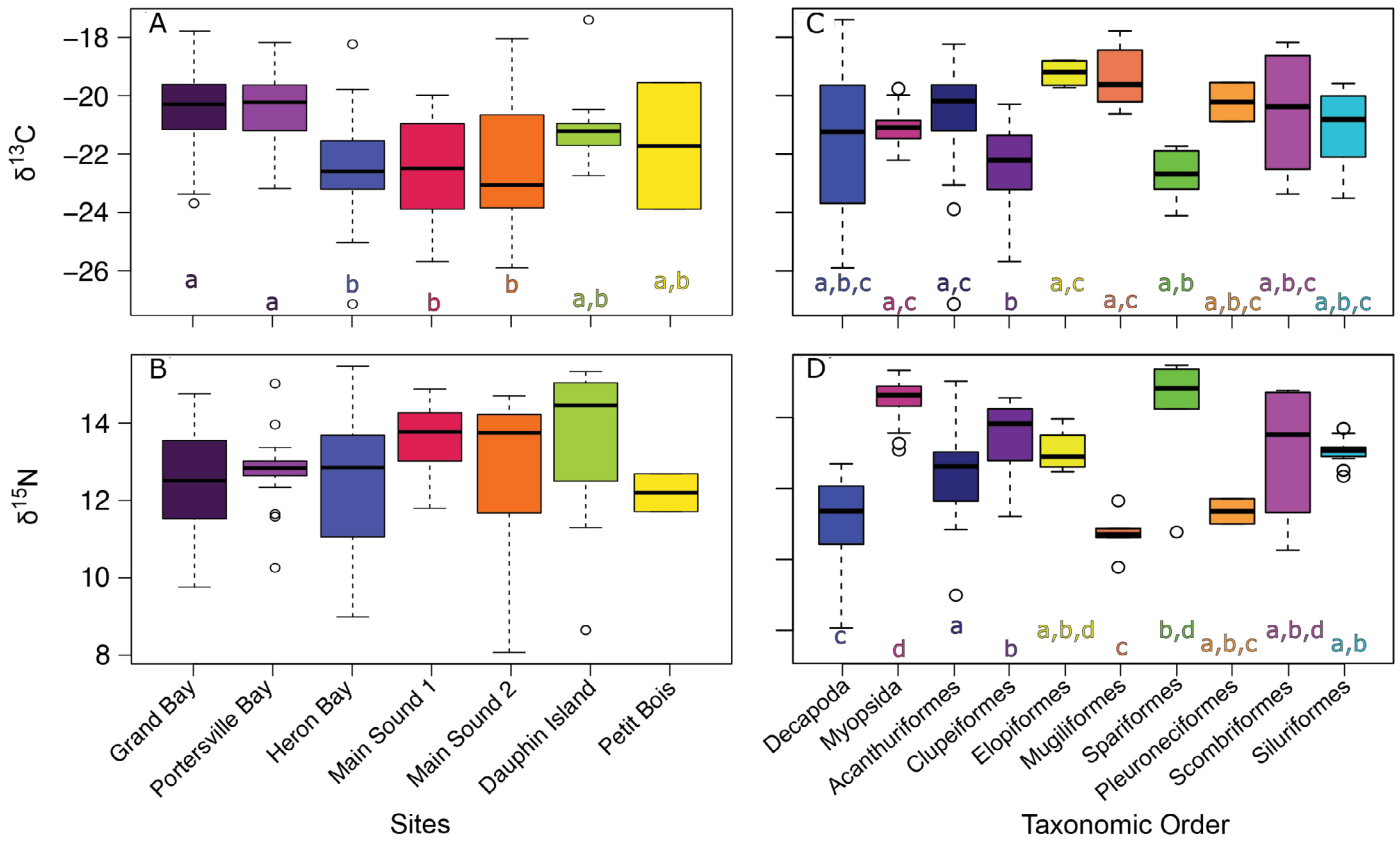

Figure 2. *Boxplots of isotopic values in biota samples from the eastern Mississippi Sound, northern Gulf of Mexico in June 2022. Solid bars represent medians, lower and upper boxes are 25% and 75% quartiles, whiskers are minimum and maximum without outliers and circles represent outliers. A. $\delta^{13}C$ values separated by site. B. $\delta^{15}N$ values separated by site. C. $\delta^{13}C$ values separated by taxonomic group. D. $\delta^{15}N$ values separated by taxonomic group. Lowercase letters represent significant differences among groups, ANOVA and Tukey Honest Significant Differences Post-hoc test, p < 0.05.*

sida, Perciformes, and Pleuronectiformes occupying distinct isotopic spaces (Figure 1C, 2C, 2D).

Salinity affected $\delta^{15}N$ values but not $\delta^{13}C$ values in biota samples. The $\delta^{13}C$ values of biota were unaffected by salinity ($F_{1, 141} = 1.97$, $p = 0.16$, $r^2 = 0.01$; Figure 3A), but $\delta^{15}N$ values increased marginally with salinity ($F_{1, 141} = 3.56$, $p = 0.06$, $r^2 = 0.02$; Figure 3B), and neither relationship was predictive.

Discussion

The large overlap in $\delta^{13}C$ and $\delta^{15}N$ values suggests considerable mixing of organic matter and biota across the EMSS. This overlap occurred from baselines (seston and sediment) through biota. Although isotope values of seston at a site can change in hours to days via tidal cycles and freshwater discharge and values from biota can change in weeks or months depending on isotopic incorporation rates (Carmichael and Valiela 2005), isotope values from sediment are integrated at that location over longer periods of time (Barth et al. 2017). The small variation in sediment isotope values provide strong support for even mixing across the estuary. The mixing of $\delta^{13}C$ and $\delta^{15}N$ of baselines and biota across the EMSS is likely driven by a combination of environmental factors. Freshwater sources along the northern EMSS coast are small and tidal and may not provide enough riverine nutrients to significantly lower $\delta^{13}C$ values. Furthermore, $\delta^{13}C$ values didn't increase with salinity. Instead, the slight variation we see in $\delta^{13}C$ values from biota may actually be driven by connectedness to freshwater from Mobile Bay. Sites with lower $\delta^{13}C$ values from biota (Heron Bay, Main Sound 1, Main Sound 2, Dauphin Island) are close to Mobile Bay, and the sites with the highest $\delta^{13}C$ values from biota are the farthest away (e.g., Portersville Bay and Grand Bay). Our data suggest that the greatest sources of riverine nutrients into EMSS come from the Mobile–Tensaw river system rather than adjacent contiguous land runoff (Du et al. 2018). Additionally, the weeks before sampling had well below average precipitation and river levels, creating potential conditions for saltwater incursion across the EMSS (Coogan et al. 2021). In fact, mean C:N of seston was 6.56, near the Redfield Ratio of 106/16 (6.63), suggesting that plankton were primarily of marine origin (They et al. 2017). These environmental conditions likely drive the mixing of organic matter along a gradient associated with connectedness to Mobile Bay outflow.

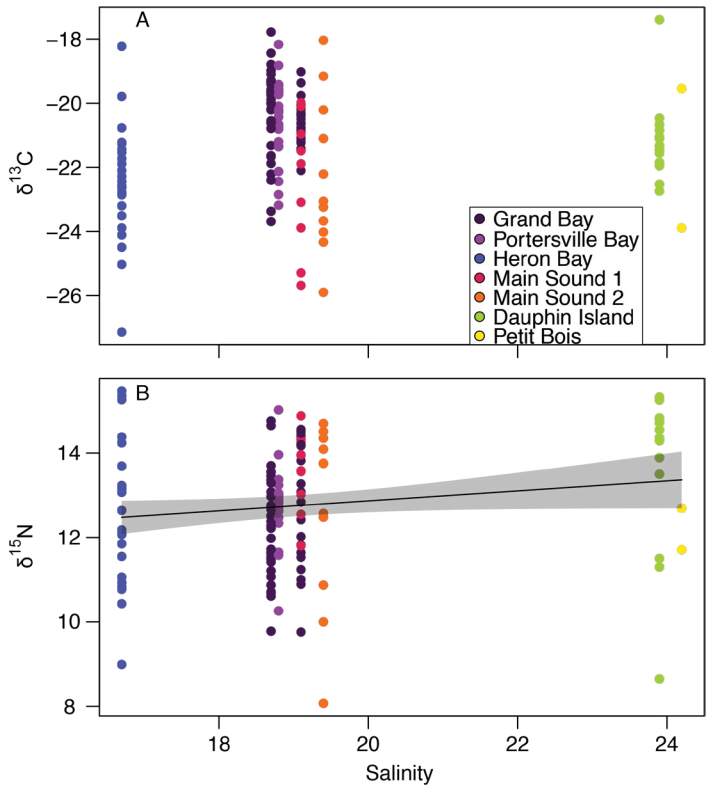

Figure 3. *Influence of salinity on biota isotopic values. A. $\delta^{13}C$ values. B. $\delta^{15}N$ values. Regression line with 95% confidence intervals, p = 0.06, r2 = 0.02.*

Biota had larger isotopic ranges compared to seston and sediment, likely because biota have a greater movement capacity and more variation in the isotopic discrimination among species. Many biota are fish species that move from estuaries to riverine or marine habitats (Sackett et al. 2007, Shipley et al. 2021), which can result in intermediate isotope values between the estuary and end members outside of it, such as from C4 plants like seagrass and *Spatina* spp. or from C3 plant species like *Juncus*. Additionally, variation in isotopic discrimination among trophic levels is a source of isotopic variation at higher trophic levels. Discrimination of $\delta^{13}C$ is highly variable, influenced by dietary quality and composition (Caut et al. 2009, Stephens et al. 2022), and can amplify isotopic variation through food webs (Kadye et al. 2020, Stephens et al. 2022). Although $\delta^{15}N$ was highest in species from higher trophic levels in our study (e.g., Clupeiformes, Elopidae, Myospida, and Spariformes), considerable variation of discrimination in $\delta^{15}N$ exists within and among trophic levels and this variation can amplify differences in isotope values throughout the food web (Post 2002, Ramirez et al. 2021). Understanding this variation in sources is important for determining how it affects our ecological inferences derived from isotopic values (Kadye et al. 2020, Ramirez et al. 2021).

Isotopic discrimination is often similar within taxonomic and ecologically similar groups, resulting in similarity within groups even in an isotopically well mixed estuary. For example, $\delta^{15}N$ discrimination is driven by the mode of nitrogenous waste production, which is related to phylogenetic relationships and the environment organisms inhabit (i.e., aquatic vs terrestrial) (Post 2002). Taxonomically related groups are likely to have similar functional roles in their ecosystems (Kürten et al. 2013). For example, groups with higher $\delta^{15}N$ values like Myopsida, Clupeiformes, and Elopidae consist of many species that feed relatively high on the food chain (Post 2002), whereas Decapoda, Mugiliformes, and Pleuronectiformes had relatively low $\delta^{15}N$ values and consist of species that generally feed at lower trophic levels (Post 2002). Groups with large ranges or intermediate $\delta^{13}C$ values (e.g., Acanthuriformes, Clupeiformes, Decapoda, Scombriformes, Silurformes) are often generalist species that feed on a wide range of dietary items that span a large isotopic variation (Bearhop et al. 2004, Martínez del Rio et al. 2009).

Elopidae and Mugiliformes are more pelagic, highly mobile species that may have spent more time in marine habitats before moving into the estuary, resulting in a legacy of tissues with high $\delta^{13}C$ values (Levesque 2011, Myers et al. 2020). Thus, the variation in isotopic values among fish and Decapoda in this study are likely driven by a combination of isotopic discrimination and ecological effects.

Our study highlights the potential complexity of trophic dynamics in estuaries when isotopes are well mixed from baselines through biota, yet isotopes varied in ecologically meaningful ways. These results represent a snapshot during specific environmental conditions; under different environmental conditions, i.e., high precipitation and freshwater inflow or variation in wind and tidal dynamics, the isotopic dynamics will differ (Fry 2002). Estuaries are at the interface of many environmental boundaries, and conditions at any given time are the product of the interactions of those environmental factors. The isotopic dynamics will necessarily reflect a combination of these environmental factors and behavior of estuarine species in the weeks and months prior to sampling. Even when an estuary is in a well—mixed state, we found that isotopes were still capable of describing ecological and trophic relationships within the community and major sources of riverine input, especially when the conditions prior to sampling were considered. In comparison to studies in the region that demonstrated more isotopic variation (Dillon et al. 2015), our study provides evidence that estuaries are complex and can be isotopically restructured depending on the input from riverine or marine sources, and points to the need for additional study to resolve the drivers of trophic dynamics in this complex system.

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