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Trinity Curry
Stillman College, trinity.curry@stillman.edu

Jeffrey Krause
Dauphin Island Sea Lab, jkrause@disl.org

Ronald Baker
University of South Alabama, rbaker@disl.org

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SHORT COMMUNICATION

SMALL—SCALE VARIABILITY IN CARBON ISOTOPE RATIOS OF MICROPHYTOBENTHOS AND DISSOLVED INORGANIC CARBON IN A NORTHERN GULF OF MEXICO SALT MARSH[§]

Trinity Curry[1,2], Jeffrey W. Krause[2,3], and Ronald Baker[3,2*]
*[1]Stillman College, Tuscaloosa AL, 35401 USA; [2]Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island AL, 36528 USA; [3]Department of Marine Sciences, University of South Alabama, Mobile AL, 36688 USA; *Corresponding author, email: rbaker@disl.org*

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INTRODUCTION

Salt marshes are incredibly productive ecosystems, supporting a diversity of species including many of importance to fisheries (Baker et al. 2020). Early studies of marsh food webs attributed the high secondary production mainly to marsh macrophyte detritus (Teal 1962). Although less conspicuous than marsh macrophytes, aquatic producers such as microphytobenthos (MPB), phytoplankton, and epiphytic algae are highly productive (Sullivan and Moncreiff 1988) and these assemblages can make benthic communities net autotrophic (Forster and Kromkamp 2006, Cox et al. 2020). Isotope studies have revealed MPB make substantial contributions to marsh food webs (e.g., Currin et al. 1995, Sullivan and Currin 2000, Galvan et al. 2008). However, microalgal sources are difficult to both collect and isolate clean samples for isotopic analysis (Oakes et al. 2005, Bouillon et al. 2008), especially MPB which reside in surficial sediments. As a result, many isotope food web studies rely on limited replication to represent these sources in mixing models that estimate the contributions of each source to consumer diets (Currin et al. 2011).

There is growing evidence that the carbon isotopic values of MPB may show significant variation at small spatial and temporal scales (Currin et al. 2011, Deleon et al. 2019). Such variation may be driven by differences in growth rate and carbon demand (Currin et al. 2011), salinity variations that alter the $\delta^{13}C$ value of the dissolved inorganic carbon (DIC) pool from which they assimilate carbon (Fry 2002), or by local sources of remineralized carbon which may deplete the $\delta^{13}C$ of the DIC pool (Lin et al. 1991, Bouillon et al. 2008). If MPB carbon isotopic values show significant spatial or temporal variation at scales relevant to individual consumers and food web studies (Deleon et al. 2019), then the limited replication typically employed may be inadequate to represent realistic MPB isotopic variability in mixing models.

Bouillon et al. (2008) suggested that ^{13}C—depleted, remineralized mangrove carbon may drive spatial patterns in $\delta^{13}C$ values of aquatic producers via the DIC pool. They cited a 10 ‰ shift in seagrass $\delta^{13}C$ over ~4 km as "remarkable" and suggested similar patterns should be seen in MPB and phytoplankton once relevant data had been collected (Bouillon et al. 2008). Using a simple acetone extraction method to measure $\delta^{13}C$ of bulk extracted pigments as a proxy for the MPB community isotopic value (Demopoulos et al. 2008), Deleon et al. (2019) measured MPB $\delta^{13}C$ spanning more than 7.2 ‰ over 10's of meters in salt marshes of the northern Gulf of Mexico (GOM). These authors hypothesized this may have been due to remineralized marsh carbon from the *Juncus* marshes surrounding their study sites resulting in a ^{13}C—depleted DIC pool within the marsh creeks. The aim of the present study was to validate the spatial variation in MPB $\delta^{13}C$ measured by Deleon et al. (2019), and to test their hypothesis that DIC pool variation drives these isotopic shifts.

MATERIALS AND METHODS

Study Sites

Samples were collected from a tidal marsh creek and the adjacent open—water fringe of the Sawgrass Point salt marsh on Dauphin Island, AL, in the central northern GOM, USA (Figure 1). The region is subtropical and has a microtidal tide range

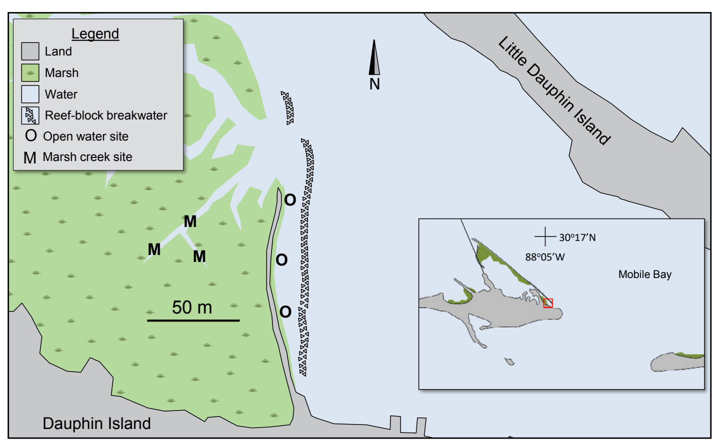


FIGURE 1. Sawgrass Point salt marsh study site at Dauphin Island, AL (United States) in the northern Gulf of Mexico (inset). MPB and DIC samples were collected from the outer fringe of the marsh (O) and a tidal creek within the marsh (M).

[§]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.

of 0.8 m. Dauphin Island lies at the entrance to Mobile Bay, which has the second highest discharge of freshwater (after the Mississippi River) to the GOM (Stumpf et al. 1993). Sawgrass Point marsh is dominated by needlerush *Juncus roemerianus*, which has a $\delta^{13}C$ value around –26 ‰ (Hackney and Haines 1980), and a thin fringe of smooth cordgrass *Spartina alterniflora* around the lower–elevation fringes. *Spartina alterniflora* $\delta^{13}C$ values are typically between –12 and –14 ‰ (Currin et al. 1995, Baker et al. 2013).

Sample Collection and Analysis

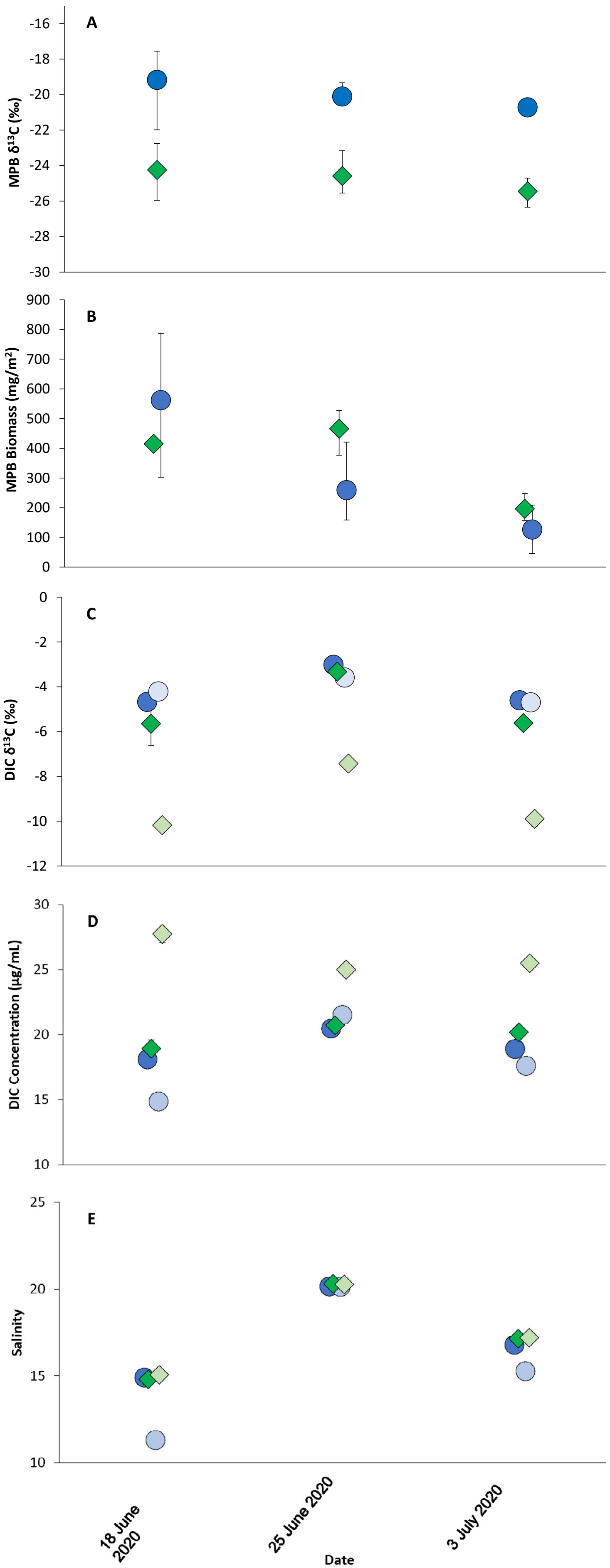
MPB samples were collected weekly from 18 June to 3 July 2020. On each occasion, 3 replicate samples were collected from each of 2 habitats; the open–water fringe on the outside of the marsh, and within shallow unvegetated waters of a tidal marsh creek within the marsh (Figure 1), for a total of 18 samples. In each habitat, samples of surficial sediments (upper 4 mm) were collected about 1 m from the edge of the marsh grass. The collection points were submerged at the time of sampling by 10–50 cm of water. Sediment for isotopic analysis was collected using a stainless steel bowl (50 cm dia.) to scoop sediment, raise it above the water, and gently pour off any collected water while minimizing disturbance of the surface layer of sediment. A clean spatula was then used to scrape surface sediments from undisturbed parts of the scoop. If the surface layer of the scooped sediment was excessively disturbed (e.g., exposure of the shallow anoxic layer), or insufficient undisturbed sediment was collected, additional scoops were made; however a single scoop provided a complete sample on most occasions. These sediment samples were placed on ice and frozen until processing. Biomass samples were collected with a syringe corer directly from the submerged sediment surface, and all sediment samples were processed for carbon isotopic analysis and biomass estimation (as sediment chlorophyll *a*, Chl *a*) as described by Deleon et al. (2019). Sediments were extracted with acetone, and the filtered acetone evaporated to leave a residue of pigments, primarily chlorophyll, for $\delta^{13}C$ analysis as a proxy for the isotopic value of the MPB community (Demopoulos et al. 2008, Baker et al. 2013, Deleon et al. 2019).

Water samples were collected for analysis of DIC concentration and its $\delta^{13}C$ signature. Replicate DIC water samples were collected from the same 3 open water and 3 marsh sites sampled for MPB at both the late–incoming/high tide, and late–outgoing/low tide on each sampling date, for a total of 36 samples. Both high and low tide samples were collected to account for any shifts in the DIC $\delta^{13}C$ caused by potential salinity differences between high and low tide (Fry 2002). For each replicate, water was drawn into a 60 mL syringe through a 20 cm tube from the mid–water column, avoiding any surface films or plumes of suspended sediment. The tube was then inserted to the base of an untreated 12 mL Exetainer vial, which was gently filled and overflowed with approximately triple the vial volume of water, ensuring no bubbles and minimal turbulence during drawing or filling. The vials were sealed with no headspace. Within about 2 hours of collection 100 µL of saturated $HgCl_2$ was added to prevent any biological activity that may alter the DIC $\delta^{13}C$ between collection and analysis (Taipale and Sonninen 2009). Isotope and DIC concentration analyses were conducted at the University of California Davis Stable Isotope Facility. The DIC analysis is conducted on a GasBench II system interfaced to a Delta V Plus IRMS (Thermo Scientific, Bremen, Germany), and replicate samples had a standard deviation of 0.05 ‰. The MPB $\delta^{13}C$ are analyzed on an EA–IRMS system using a PDZ Europa ANCA–GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), and replicate samples had a standard deviation of 0.04 ‰. Both systems use VPDB as the $\delta^{13}C$ standard.

Results and Discussion

Marsh creek MPB were consistently depleted in $\delta^{13}C$ compared to MPB from the outer fringe of the marsh by an average of 4.76 ± 0.17 ‰ (mean ± 1 se), and the most enriched marsh creek sample was lighter than the most depleted open fringe sample (Figure 2A). The greatest daily range between individual marsh creek and open water replicates was 8.4 ‰ (18 June) for samples separated by 50–100 m (minimum range 6.12 ‰, 3 July), and over the 3 weeks MPB $\delta^{13}C$ spanned 8.79 ‰. These findings are consistent with the spatial patterns recorded by Deleon et al. (2019), who found marsh creek MPB $\delta^{13}C$ values were consistently depleted compared to open fringe MPB, with values spanning over 7.2 ‰ at the same site. In both 2019 (Deleon et al. 2019) and 2020 (this study) spatial differences between marsh creek and fringing sites were greater than temporal variation among weeks, suggesting temporally consistent small–scale spatial variation between marsh creek and open water fringing sites. During both summers we anticipated the potential for significant salinity fluctuations to drive temporal patterns in MPB $\delta^{13}C$ (Fry 2002), however no such events occurred in either year.

Bouillon et al. (2008) considered a range in seagrass $\delta^{13}C$ of 10 ‰ over 4 km as "remarkable." Our measurements of MPB $\delta^{13}C$ varying consistently close to 5 ‰ between marsh creeks and adjacent outer fringes at 2 marsh sites over 2 summers, and up to 8.4 ‰ over 50 m on a single day (Deleon et al. 2019, this study), are themselves remarkable. At this scale, even moderately mobile primary consumers may integrate MPB production with widely varying carbon isotopic values during individual foraging forays in and around marsh systems. Conversely, predators consuming sedentary prey that feed on MPB may have widely varying carbon isotopic values depending on exactly where they forage in the marsh seascape. The standing biomass of MPB was similar between marsh creek and open fringing sites (Figure 2B) and to other regional values (e.g., Sullivan and Moncrief 1988). This suggests the patterns seen in MPB $\delta^{13}C$ are not driven by variations in MPB productivity between creeks and adjacent open waters. Tidal creeks can make up a significant proportion of a salt marsh, and MPB production within these creeks may be particularly important for small and juvenile consumers occupying these sheltered habitats (Galvan et al. 2008). If so, then the small–scale spatial variation in MPB isotopic values reported here may have



significant implications for food web studies (see below).

Deleon et al. (2019) hypothesized that the spatial pattern in MPB carbon isotopic values may be due to remineralized marsh carbon depleting the δ^{13}C of the DIC pool within the marsh creeks. Depletion of the DIC pool by remineralized wetland biomass has been demonstrated over much larger spatial scales in mangrove systems (Lin et al. 1991, Bouillon et al. 2008). Our δ^{13}C measurements of the DIC reveal that at the sites sampled for MPB, the marsh creek DIC at low tide was consistently depleted compared to open water DIC (high and low tide combined), by an average of 5.03 ± 0.48 ‰ (Figure 2C). The maximum range between marsh creek and open water replicates was between 4.47 and 6.23 ‰ over the 3 sampling occasions, with the total range in DIC δ^{13}C spanning 7.43 ‰. The DIC concentration was higher in water samples from the marsh creek at low tide (Figure 2D). The δ^{13}C values of *Juncus* (–26 ‰) and *Spartina* (–13 ‰) are both depleted compared to the DIC of the flooding tide (–3 to –5 ‰, Figure 2C), meaning the addition of remineralized carbon from decomposition of either marsh plant would deplete the DIC pool. Together these findings suggest depleted carbon is added to the DIC pool while the water is on the marsh surface.

The greatest differences in DIC δ^{13}C (Figure 2C) occurred in the absence of any substantial changes in salinity (Figure 2E). On 18 June and 3 July the open water low tide salinities were lower than the other samples from those dates (Figure 2E), but this did not correspond to a shift in the DIC in those water samples (Figure 2C). There is some evidence that overall DIC δ^{13}C tracked salinity as expected (Fry 2002), with the most enriched DIC values on 25 June when salinities were highest, and depleted values on 18 June and 3 July when salinities were lower (Figures 2C, E). The low tide marsh—creek water samples are from water that spent the 5—10 h prior to sampling on the flooded marsh surface over the previous high tide. This suggests that the depletion of the DIC pool occurs on the marsh surface and is consistent with the hypothesis that remineralized marsh carbon drives the patterns observed in MPB carbon isotopic values (Bouillon et al. 2008; Deleon et al. 2019).

Our study was limited to a single marsh location, and further work is needed to confirm if similar or greater variation in MPB δ^{13}C is a widespread phenomenon. Salt marshes across the northern GOM are microtidal, and the hydrology of our site is pretty typical of the region (Minello et al. 2012). Re-

FIGURE 2. *Spatial and temporal patterns in microphytobenthos (MPB), dissolved inorganic carbon (DIC) and salinity at Sawgrass Point marsh in Dauphin Island, AL. A. δ^{13}C of MPB in a tidal marsh creek (green diamonds) and adjacent outer fringe of the marsh (blue circles). B. MPB biomass (symbols as per A); C. δ^{13}C of the DIC in waters collected at the same marsh and open water sites at high (dark filled symbols) and low (light symbols) tides. D. DIC carbon concentration (symbols as per C). E. Salinity during DIC sample collection (symbols as per C). In A-D data are mean values, bars indicate range for n = 3 samples in each case. Data in E are individual point measures at the time of DIC sample collection. For several points the range was small, and bars are hidden by the symbol. Symbols for each date are slightly offset for visibility.*

gional variation in tidal amplitude or other water movement and mixing forces may modify the effects of local sources of remineralized carbon, by diluting or concentrating the effects, such that the magnitude and spatial scale of variation is likely to vary among regions. However, the underlying drivers of variability reported here and elsewhere (Bouillon et al. 2008) appear to be widespread phenomena. As such we cautiously offer some more general implications. The standard deviation of the 18 MPB samples in the current study was 2.77 ‰. Such large standard deviations for source values appear to be rare in published isotope mixing models, suggesting many food web studies may have significant uncertainty in their source contribution estimates. Because MPB $\delta^{13}C$ tends to be intermediate between other sources in salt marsh food webs (Baker et al. 2013), many food web models probably erroneously assign variable MPB contributions to more extreme sources, thereby underestimating the importance of MPB to marsh food webs. To improve our understanding of the contributions of various production sources to estuarine food webs, it is important that we gain a clearer understanding of the extent of spatial and temporal variation in aquatic producer isotopic values in different regions, especially for rapid—turn over production sources like MPB and phytoplankton.

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