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GULF AND CARIBBEAN

R E S E A R C H

VARIABILITY IN MICROPHYTOBENTHOS BIOMASS AND CARBON ISOTOPIC VALUES IN SHALLOW COASTAL WATERS OF THE NORTHERN GULF OF MEXICO[§]

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INTRODUCTION

Estuaries and inshore coastal waters of the northern Gulf of Mexico (GOM) are highly productive systems supporting diversity of life, including important fisheries species (e.g., Minello et al. 2003). Salt marshes and seagrass meadows are formed by conspicuous and high—biomass primary producers, long considered important at the base of coastal food webs (Teal 1962). However, the inconspicuous primary producers, phytoplankton and microphytobenthos (MPB, single—celled micro—algae on the sediment surface) are also important in these systems, having been shown to support a variety of consumers (Currin et al. 1995, 2011, Galvan et al. 2008). While disentangling MPB biomass and productivity rates is logistically challenging, there are many studies which suggest both phytoplankton and MPB represent a potentially large portion of primary production in these systems due to the rapid turnover rates (Sullivan and Moncreiff 1988, Blanchard et al. 2002).

Strong sedimentary physical and chemical gradients, dynamic shear and variable light (both driven by tides), and rapid turnover drives substantial spatial and temporal variability in MPB biomass (Barranguet et al. 1997, Currin et al. 2003, 2011, Kromkamp et al. 2006). Beyond the challenge of understanding the ephemeral nature of MPB biomass, their carbon isotopic values may change rapidly in response to changes in salinity which shift the $\delta^{13}C$ isotopic value of the dissolved inorganic carbon pool from which they derive their carbon for photosynthesis (Fry 2002, Currin et al. 2003). Stable isotopes are a powerful tool for inferring the importance of various primary producers in supporting secondary production in coastal food webs (e.g., Currin et al. 1995). Some isotope studies undertake extensive sampling to represent the MPB community available to consumers in their food web models (e.g., Currin et al. 2003, 2011, Galvan et al. 2008). However, perhaps due to the challenges of obtaining uncontaminated samples of MPB for isotopic analyses (Oakes et al. 2005), many other studies rely on a limited number of MPB samples to represent their isotopic value

(e.g., Baker et al. 2013).

To achieve a better and more comprehensive understanding of the role of MPB in isotopic studies and coastal food webs, a better understanding of the spatial and temporal variability in the biomass and their $\delta^{13}C$ isotopic values is needed (Currin et al. 2003). Such knowledge can help in the design of future food web studies. Therefore, the aim of this study was to assess short term (days to weeks) small to meso—scale (1's to 1000's m) variability in MPB standing biomass, and to provide a preliminary assessment of variability in $\delta^{13}C$ values of MPB.

METHODS

Study sites

Samples were collected from intertidal salt marshes and the subtidal waters of Mobile Bay and coastal Alabama, USA in June and July 2019, for a total of 19 sites sampled (Figure 1). This region is subtropical and has a microtidal
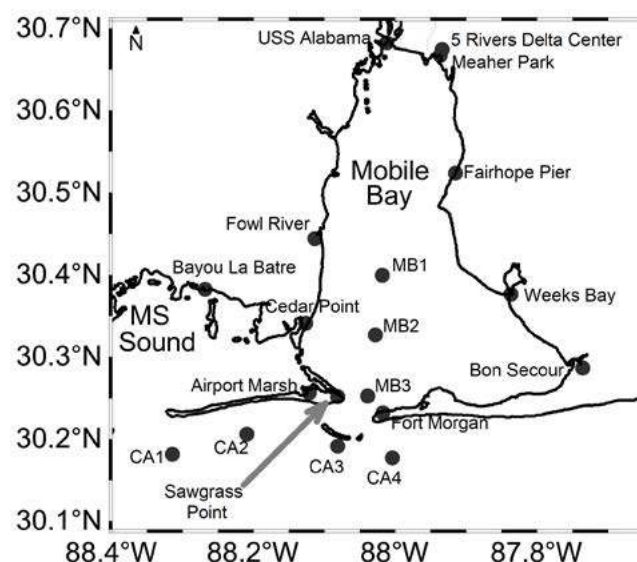

FIGURE 1. Field sites for benthic chlorophyll analysis in Mobile Bay (MB), Mississippi Sound and the coastal Alabama region (CA) of the northern Gulf of Mexico.

range of 0.8 m. Mobile Bay is fed by multiple rivers and discharges ~1800 m³/s into the northern GOM, making it second only to the Mississippi River for discharge to the Gulf (Stumpf et al. 1993).

Subtidal sites were chosen to capture both north–to–south and east–to–west variability in Mobile Bay, including one station in Mississippi Sound, a coastal lagoon system immediately to the west of Mobile Bay (Figure 1). Sites near the shoreline (n = 10) were accessed by piers or docks, each

TABLE 1. *Mean benthic chlorophyll (± standard deviation) for the surface sediments collected in Mobile Bay (MB), Mississippi Sound, and coastal Alabama (CA). Date of collection and site bottom depth are also reported. Benthic chlorophyll values for Sawgrass Point and Airport Marsh denote mean of all time points, variability over time and substrate type are found in Figure 2.*

Station	Date (MM-DD-YY)	Bottom Depth (m)	Benthic Chlorophyll (mg/m2)
MB1	07-18-19	12	2.1±0.6
MB2	07-18-19	15	13.0±10.4
MB3	07-18-19	16	20.1±3.8
CA3	07-18-19	8	33.7±4.9
USS Alabama	07-19-19	2	23.3±1.8
5 Rivers Delta Center	07-19-19	2	16.3±0.5
Fowl River	07-19-19	2	2.5±0.2
Bayou La Batre	07-19-19	2	34.0±14.1
Cedar Point	07-19-19	2	17.3±1.5
Fort Morgan	07-19-19	2	42.2±0.4
Bon Secour	07-19-19	2	4.0±0.6
Weeks Bay	07-19-19	2	42.5±0.9
Fairhope Pier	07-19-19	2	5.9±0.2
Meaher Park	07-19-19	4	10.4±1.3
CA4	07-25-19	13	34.0±5.4
CA2	07-25-19	11	20.3±2.4
CA1	07-25-19	17	10.6±0.3
Sawgrass Point	June-July 19	Intertidal	37.2
Airport Marsh	June-July 19	Intertidal	29.0

had shallow depths (<2 m), and most of the sediment was sand and silt; these sites were sampled 19 July 2019 (Table 1). Three sites were selected within the Central Mobile Bay shipping channel, which is dredged to maintain at least 15 m depth for commercial maritime traffic; sampling occurred on 18 July 2019 aboard the *R/V Alabama Discovery*. Outside Mobile Bay, 4 coastal Alabama sites were sampled (depth 12–15 m) on 25 July 2019, aboard the same vessel; stations ranged from the west end of Dauphin Island and east to the Fort Morgan Peninsula (Alabama, USA).

The two intertidal salt marsh sites on Dauphin Island, Sawgrass Point (SGP) and Airport Marsh (AP) (Figure 1), are dominated by *Juncus roemerianus* with fringes of *Spartina alterniflora*. Drainage creeks within each marsh have mud substrate while outer shorelines are predominantly sand. Salt marsh sediment samples were collected weekly at each marsh site for 4 weeks during June 2019. At each site, sam-

ples were collected from both sand and mud intertidal substrates directly adjacent to marsh vegetation (1–5 m). Three replicate collection points in each habitat type were separated by 10–20 m; substrate proximity meant that adjacent sand and mud replicates were also separated by 10–20 m. Properties among substrate type were compared using a Students t test (calculated using Microsoft Excel).

Among all sites, metadata and hydrographic data (temperature, salinity, irradiance) were collected either in the overlying waters or at the tidal edge. For AP and SGP, the collected hydrographic data were compared to the nearby Dauphin Island Station of Alabama's Real–Time Coastal Observing System (ARCOS, www.arcos.disl.org). The Dauphin Island ARCOS station is 0.47 km from the SGP, and 5 km (by water) from AP. Point measures of physical parameters corresponded well with continuous data from the station; hence, these data on water level and salinity were used to contextualize the physical conditions during the sampling period (Figure 2A).

Subtidal collections

Subtidal sediment was collected using 2 approaches. For shipboard work, a 4–spot multi–corer was deployed. Depending on the site, additional deployment was sometimes necessary to ensure one core with an intact sediment water interface, optimal penetration (~10–20 cm), and a proper seal (no visible air bubbles escaping). Bottom water was gradually removed from the cores, then the upper 2 cm of material extruded, subsampled, and processed. For dockside and pier sampling, surface sediments (0–2 cm) were collected by dropping a surface–corer or Petite Ponar Grab. Material was subsampled and processed in the laboratory for MPB biomass; no isotope analyses were done for this material.

Salt marsh collections

Multiple sediment cores were collected from undisturbed sediments at each collection point to quantify MPB biomass and stable isotopic ratios. Cores for biomass quantification were collected using a 3 mL syringe, with the surface 1 cm retained for analysis, providing a standardized sampling area (~2 cm²) and integration depth among sites. Stable isotope samples were collected using a 60 mL syringe and the surface 2–4 mm retained for analysis. Multiple isotope cores were necessary to obtain ~50 mL of sediment for processing.

Sample processing

The MPB biomass was quantified as chlorophyll *a* (Chl). The sediment was transferred into glass vials cleaned using Micro–90® solution followed by 10% HCl and liberally rinsed in deionized water. Chl pigments were extracted in 10 mL of 90% acetone (HPLC grade) in the dark for 24 hours at −20°C. After extraction, the solvent was decanted into a cuvette and Chl/phaeopigments quantified using a standard acidification method (Holm–Hansen et al. 1965) on a Trilogy Fluorometer (Turner Designs, USA). Benthic
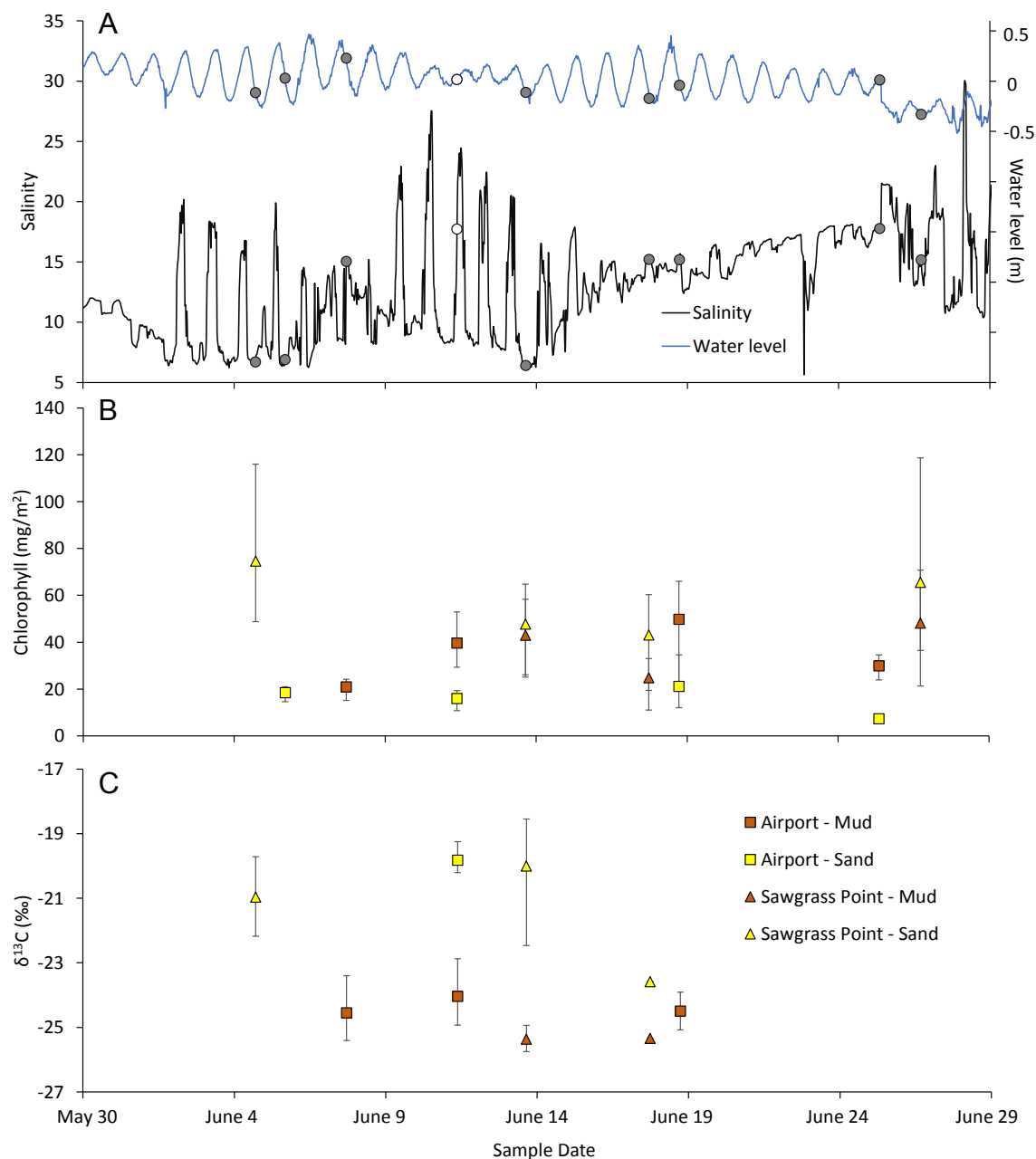
FIGURE 2. *Spatio-temporal variability in intertidal microphytobenthos (MPB) at salt marshes on Dauphin Island, AL. A. Variability in salinity and water level (relative to mean during study period) from the ARCOS Dauphin Island Station. Circles indicate the salinity and water level at the time of sampling; gray and white circles indicate the substrate was submerged or exposed, respectively, during sampling. B. Mean MPB chlorophyll biomass. Bars indicate range among replicates separated by 10-20 m. C. Mean MPB δ13C isotopic values. Bars indicate range among replicates separated by 10-20 m.*

Chl data were expressed as area—integrated measurements (i.e., mg/m²).

The MPB isotopic samples were processed similarly. Acetone extracts from surface sediments provide a quick and simple proxy for MPB community $\delta^{13}C$ (Demopolous et al. 2008, Baker et al. 2013) and avoid some of the biases of targeting specific components of the MPB community, such as motile diatoms (Oakes et al. 2005). We added 10 mL of 90% acetone (HPLC grade) to 10 mL of wet sediment, and then samples were shaken and extracted for 24 hours at −20°C. Post extraction, the acetone was filtered through a pre—combusted glass—fiber filter (1.5 µm retention), evaporated, and the extracted pigments dried at 60°C. Salt crystals were removed from the dry pigments by adding deionized water, decanting immediately once the salt crystals had dissolved, and re—drying. This process was used to obtain ≥2 mg of extracted material (primarily Chl, Oakes et al. 2005), which was then encapsulated and analyzed for $\delta^{13}C$ at the University of California Davis Isotope facility.

Results and Discussion

MPB biomass variability

The MPB biomass varied by a factor of 20 among the subtidal sites not including the salt marshes (Table 1). Benthic Chl from the upper 2 cm ranged from 2.1–42.2 mg/m² among these sites, and averaged 29.0 ± 3.0 and 37.2 ± 4.9 mg/m² (mean ± S.E.) for AP and SGP, respectively, over the sampling duration (Table 1). These subtidal values are comparable to previous Mississippi Sound studies in salt marsh and sandy seagrass benthic Chl (Sullivan and Moncreiff 1988, Daehnick et al. 1992) and also the shallow (<10 m) Louisiana Shelf (Grippo et al. 2010). Compared to other published data from deeper and clearer waters in

the southeastern United States (compiled by Cahoon 1999), the benthic Chl values observed outside of the salt marshes were low. For example, in 15 m waters in Onslow Bay (North Carolina) benthic Chl ranged from 16–88 mg/m², and in Gray's Reef (Georgia) biomass ranged from 200–800 mg/m² (Cahoon 1999 and references therein). The benthic Chl reported here captured ~50% of the biomass within the upper 6 cm (deeper Chl data not shown). However, even if our sampling design underestimated benthic Chl by a factor of two, this would not change the inference that benthic Chl in the northern GOM appears to be relatively low compared to the broader southeastern United States region.

Mobile Bay and Mississippi Sound are shallow and have significant freshwater discharge; hence, the high turbidity in these waters may limit benthic productivity. For the Mobile Bay shipping channel, surface–water photosynthetically active radiation exceeded 700 µE/m²/s but attenuated to 1 µE/m²/s by 4–10 m (bottom 12–16 m). Among coastal Alabama sites, the 1 µE/m²/s isolume was between 9–11 m. For both subregions (Mobile Bay, Coastal Alabama), these irradiances at the sediment water interface would not support any measurable primary production (MacIntyre et al. 1996).

Within the salt marshes, benthic Chl was more variable temporally and on small spatial scales than observed among all other sampled sites (Figure 2). For example, at SGP, small–scale spatial variability was high in both mud and sand sediments, particularly for sand where benthic Chl ranged from 6.3–119 mg/m² (Figure 2B). This range encompassed the entire range observed (Figure 2B). Among both sites and all time points, the mean benthic Chl did not significantly differ by substrate (t–test, p = 0.98). These data suggest that other factors such as vertical migration of MPB (Barranguet et al. 1998) or N limitation (Sullivan and Currin 2000), may drive high patch variability over small spatial scales.

Carbon isotopic variability

Stable carbon isotope ratios of MPB acetone extracts were both spatially and temporally variable at salt marsh sites (Figure 2C). Among the 22 samples analyzed, $\delta^{13}C$ values varied by 7.2‰ from −25.75‰ for SGP mud (June 13) to −18.54‰ for sand just tens of meters away at the same site and day (Figure 2C). Similarly, samples from AP on June 11 varied by 5.6‰ between sand and mud substrate. The MPB samples from sandy locations were enriched by 2–5‰ over samples from muddy locations collected at the same site and day. Temporal variability was not as pronounced as among–habitat variation, and 9 out of the 10 samples from sand substrates were more enriched than the most enriched sample from mud substrates. Within–habitat spatial variation was also high, varying almost 4‰ on sand at SGP (June 13) and more than 2‰ at AP on mud (June 11).

The striking difference in $\delta^{13}C$ values between nearby sand and mud substrates may be driven by the depletion of the dissolved inorganic carbon (DIC) pool in waters on the marsh surface through remineralization of isotopically light *J. roemerianus* biomass. Currin et al. (2003) reported the depletion of MPB $\delta^{13}C$ values of 3–5‰ on the vegetated marsh surface compared to MPB from nearby unvegetated habitats, and suggested reduced primary production due to marsh shading and the uptake of depleted DIC originating from remineralized marsh detritus could account for this depletion. In our study system, both mud and sand sites were unshaded open water sites adjacent to the marsh vegetation, equivalent to the creek bank or flat sites of Currin et al. (2003). However our mud collection points were drainage creeks within the marsh, while sand collection points were in more well–mixed waters on the outer fringe of the marshes and separated from the marsh platforms by a berm. As such, the remineralization of isotopically light *J. roemerianus* biomass may explain the range in MPB carbon value reported here. The taxonomic composition of the MPB community can also influence the $\delta^{13}C$ values (Currin et al. 2011). Identifying the taxonomic composition of MPB in our samples was beyond the scope of the present study, but taxonomic variation may have contributed to some of the spatial and temporal variation in isotopic values.

Studies assessing the importance of MPB production for coastal food webs often use limited replication to represent the $\delta^{13}C$ values of this source in their models (discussed in Currin et al. 2003). Based on our moderate sample size from 2 marsh sites over 4 weeks, there is substantial $\delta^{13}C$ variability within the MPB Chl extracts. Further replication is required to assess the significance of this variability. However, if this magnitude of variability is typical of shallow coastal waters more generally (e.g., Currin et al. 2003), our findings suggest that some previous estimates of the contributions of MPB production to consumer diets may contain significant uncertainty.

The isotopic analysis of acetone extracts as a proxy for MPB community $\delta^{13}C$ values proved to be a simple and quick approach in the present study. Despite simplicity, the validity of using this method for providing MPB source estimates for food web mixing models (e.g., Demopolous et al. 2008, Baker et al. 2013) is uncertain. Demopolous et al. (2008) found no difference in $\delta^{13}C$ values for acetone extracts and whole algal material for epiphytic algae; however, it is possible that the extraction process may fractionate $\delta^{13}C$ and provide values that deviate from those obtained from the whole MPB cells ingested by consumers (Oakes et al. 2005). Future work should investigate this method further before its widespread use to provide data for food web mixing models. Assuming any fractionation between the MPB community and the acetone extracts was constant among samples in the present study, then our data suggests the potential for significant small scale spatial and temporal variability in $\delta^{13}C$ values of MPB that should be considered when designing future isotopic food web studies.

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