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Importance of seagrass as a carbon source for heterotrophic bacteria in a subtropical estuary (Florida Bay)

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ABSTRACT

A stable carbon isotope approach was taken to identify potential organic matter sources incorporated into biomass by the heterotrophic bacterial community of Florida Bay, a subtropical estuary with a recent history of seagrass loss and phytoplankton blooms. To gain a more complete understanding of bacterial carbon cycling in seagrass estuaries, this study focused on the importance of seagrass-derived organic matter to pelagic, seagrass epiphytic, and sediment surface bacteria. Particulate organic matter (POM), seagrass epiphytic, seagrass (*Thalassia testudinum*) leaf, and sediment surface samples were collected from four Florida Bay locations with historically different organic matter inputs, macrophyte densities, and primary productivities. Bulk (observed and those reported previously) and compound-specific bacterial fatty acid $\delta^{13}C$ values were used to determine important carbon sources to the estuary and benthic and pelagic heterotrophic bacteria. The $\delta^{13}C$ values of *T. testudinum* green leaves with epiphytes removed ranged from −9.9 to −6.9‰. *Thalassia testudinum* $\delta^{13}C$ values were significant more enriched in ^{13}C than POM, epiphytic, and sediment samples, which ranged from −16.4 to −13.5, −16.2 to −9.6, and −16.7 to −11.0‰, respectively. Bacterial fatty acid $\delta^{13}C$ values (measured for br14:0, 15:0, i15:0, a15:0, br17:0, and 17:0) ranged from −25.5 to −8.2‰. Assuming a −3‰ carbon source fractionation from fatty acid to whole bacteria, pelagic, epiphytic, and sediment bacterial $\delta^{13}C$ values were generally more depleted in ^{13}C than *T. testudinum* $\delta^{13}C$ values, more enriched in ^{13}C than reported $\delta^{13}C$ values for mangroves, and similar to reported $\delta^{13}C$ values for algae. IsoSource mixing model results indicated that organic matter derived from *T. testudinum* was incorporated by both benthic and pelagic bacterial communities, where 13–67% of bacterial $\delta^{13}C$ values could arise from consumption of seagrass-derived organic matter. The IsoSource model, however, failed to discriminate clearly the fraction of algal (0–86%) and mangrove (0–42%) organic matter incorporated by bacterial communities. These results indicate that pelagic, epiphytic, and sediment surface bacteria consumed organic matter from a variety of sources. Bacterial communities incorporated consistently seagrass-derived organic matter, the dominant macrophyte in Florida Bay, but seagrass $\delta^{13}C$ values alone could not account fully for bacterial $\delta^{13}C$ values.

1. Introduction

Heterotrophic bacteria are important decomposers and transformers of primary production and provide an important link between detritus and the aquatic food web. In seagrass ecosystems, much of seagrass primary production is unavailable to animal consumers through direct ingestion and must undergo microbial reworking before it can enter the food web (Harrison, 1989). In addition to seagrass productivity, seagrass epiphytes, microphytobenthos, benthic macroalgae, and allochthonous terrestrial and marine inputs can contribute significantly to the organic matter pool of seagrass ecosystem. Hence, bacterial carbon demands in seagrass ecosystem can potential depend on one or a variety of carbon source(s). In general, benthic heterotrophic bacterial incorporation of organic matter in seagrass ecosystems is influenced by the ecological state of the seagrass system (Holmer et al., 2004) as well as the organic matter content of the sediment (Bouillon and Boschker, 2006). In addition, carbon use by benthic and pelagic bacteria is influenced by the bacterial community's location in the

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ecosystem and their proximity to autochthonous and allochthonous organic matter inputs (Boschker et al., 2005; Williams et al., 2009).

Stable carbon isotope ratios are used frequently to determine the contribution of autochthonous and allochthonous primary production to the ecosystem organic matter pool and food web when isotopically unambiguous carbon sources are present (Fry and Parker, 1979; Chasar et al., 2005; Behringer and Butler, 2006). Yet, the bulk (i.e. whole plant, mixed-sediment, and particulate organic matter) stable isotope approach cannot discriminate between bacterial carbon and that of the organic matrix encompassing the bacteria. Instead, a compound-specific approach must be taken to determine the carbon source(s) used by heterotrophic bacteria. Fatty acids are useful for compound-specific stable carbon isotope chemistry and the determination of bacterial carbon source because: (1) fatty acids are turned over rapidly upon cell death and represent recent production (Canuel and Martens, 1996; Boschker et al., 1999); (2) a subset of fatty acids (branched- and short, odd-chained fatty acids) are biomarkers for heterotrophic bacteria; and (3) the stable carbon isotope signatures of bacteria and their fatty acids respond rapidly (within 24–48 h) to changes in carbon input (Coffin et al., 1989). Bacterial-specific fatty acid concentrations correlate generally well with bacteria biomass. As such, bacterial-specific fatty acids have been used widely to determine the sources of carbon consumed by sediment/soil bacteria in terrestrial and aquatic systems (Burke et al., 2003; Bouillon and Boschker, 2006; Dai and Sun, 2007). As examples, using a bacterial-specific stable carbon isotope approach, seagrass was found to be an important carbon source to sediment bacteria in pristine ecosystems (Holmer et al., 2001, 2004; Jones et al., 2003), an important ecosystem structural component that traps allochthonous organic matter for sediment bacterial incorporation (Bouillon et al., 2004), and unimportant to sediment bacterial carbon cycles in more eutrophic ecosystems (Boschker et al., 2000; Holmer et al., 2004).

Bacterial-specific fatty acid stable carbon isotope analysis, however, has thus far focused only on sediment bacteria in seagrass ecosystems. Boschker et al. (2005) measured spatial patterns in bacterial-specific fatty acid stable isotope values for pelagic bacteria in the turbid, tidal Scheldt estuary, but similar studies have not taken place in seagrass ecosystems. Seagrass are known to release labile organic compounds from their roots/rhizomes and leaves, which in the sediment can fuel sulfate reduction (Holmer et al., 2001) and in the seagrass epiphytic community along with epiphytic primary production can support high rates of epiphytic bacterial production (Kirchman et al., 1984; Törnblom and Søndergaard, 1999; Williams et al., 2009). In the epiphytic and pelagic community, positive correlations between benthic primary production and bacterial production have been document (e.g. Moriarty and Pollard, 1982; Ziegler and Benner, 1999), but these correlations do not necessarily mean bacteria are reliant on seagrass for carbon. Primary production by micro- and macroalgae would also produce similar positive relationships. Bacterial-specific fatty acid analysis has the potential to resolve the above ambiguity, but to date, we know of no studies that have measured concomitantly pelagic, epiphytic, and sediment bacteria-specific carbon isotope values in seagrass ecosystems.

Over the past 25 years, Florida Bay, the study ecosystem, has experienced seagrass mass mortality, patchy seagrass die-off events, and episodic phytoplankton blooms (Robblee et al., 1991; Boyer et al., 1999; Fourqurean and Robblee, 1999; Rudnick et al., 2006; Koch et al., 2007). In addition, paleoenvironmental studies suggest significant increases in microbial organic matter inputs to Florida Bay sediment over the past decades (Xu et al., 2006a, 2007). Indirect evidence suggests that pelagic bacterial carbon demand is not strictly tied to seagrass (*Thalassia testudinum*) production and benthic bacteria communities likely metabolize both algal/cyanobacterial- (epiphytic and/or microphytobenthic) and seagrass-derived organic matter (Williams et al., 2009). In the current study, we employ bulk and bacteria-specific fatty acid stable carbon isotope methods to test directly the importance of seagrass-derived organic matter to pelagic, epiphytic, and sediment surface bacterial communities of Florida Bay. We hypothesize that stable carbon isotope values of heterotrophic bacteria in Florida Bay will reflect algal-derived organic matter with mixing from seagrass-derived organic matter.

2. Materials and methods

2.1. Study site description and sampling

Florida Bay is a shallow (average depth <2 m) subtropical seagrass estuary with a distinct wet (June–November) and dry (December–May) seasonal climate. Located at the southern tip of the Florida peninsula, the bay is bounded to the north by the Everglades wetlands, to the south and east by the Florida Keys, and to the west by the Gulf of Mexico (Fig. 1). Carbonate mudbanks and mangrove islands compartmentalize Florida Bay into semi-isolated basins, which have distinct benthic and pelagic characteristics (Fourqurean and Robblee, 1999; Williams et al., 2009). *Thalassia testudinum* is the dominant macrophyte in Florida Bay, but mangrove, phytoplankton, epiphytes, and benthic microalgae can contribute significantly to local productivity. All of which supply organic matter to benthic and pelagic communities of Florida Bay, which share characteristics of seagrass, microbial, and mangrove/terrestrial inputs (Maie et al., 2005; Xu and Jaffé, 2007; Xu et al., 2007).

On 28 May 2007, four seagrass beds in Florida Bay were visited to investigate the carbon source of pelagic, epiphytic, and sediment surface heterotrophic bacterial. Sampling stations were Little Madeira Bay, Duck Key, Bob Allen Key, and Whipray Basin (Fig. 1), corresponding to Florida Coastal Everglades Long-Term Ecological Research stations TSPh 7, 9, and 10, and Southeastern Environmental Research Center long-term monitoring station 13, respectively. Each seagrass bed was located in a distinct region of Florida Bay with different benthic and pelagic influences.

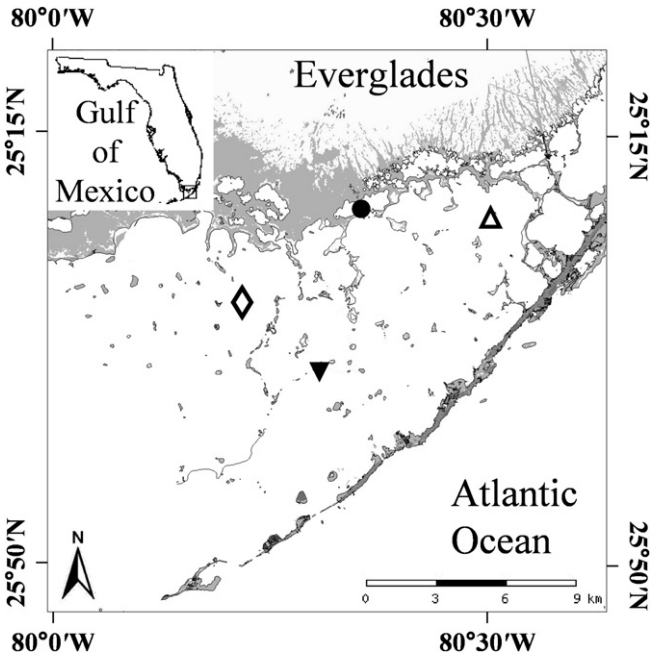

Fig. 1. Sampling stations in Florida Bay, south Florida; Little Madeira Bay (●), Duck Key (△), Bob Allen Key (▼), Whirpay Basin (◇).

In General, from the northeast to west, sediment organic matter content, sediment depth, phytoplankton biomass, and the *T. testudinum* density tend to increase (Fourqurean and Robblee, 1999 and references therein). The contribution of mangrove-derived organic matter to the pelagic and benthic carbon pools follows the opposite trend, decreasing gradually from the northeast mangrove fringe (i.e. Little Madeira Bay) toward the southwestern bay (Evans et al., 2006; Xu et al., 2006b; Xu and Jaffé, 2007). Still, all sites within the current study were located near mangrove islands and likely receive local inputs of mangrove detritus. Phytoplankton biomass is typically higher at Whipray Basin, but in recent history phytoplankton blooms were visible at all sites in the current study expect Little Madeira Bay (Rudnick et al., 2006; Koch et al., 2007; Williams et al., 2009). In 2006, a year prior to the current study, sediment bacterial abundance was higher than or similar to pelagic bacterial abundance, which were two orders of magnitude higher than epiphytic abundance (Williams et al., 2009). Bacterial production, however, was highest in sediment and epiphytic communities and lowest in the pelagic community. Spatially, epiphytic bacteria at Whipray Basin were more abundant and productive than at the other sites, although this difference was likely due to the increased density of seagrass at this site (Williams et al., 2009).

For the current study, particulate organic matter (POM), sediment surface, *T. testudinum*, and *T. testudinum* epiphytic samples were collected at each station for stable carbon isotopic analysis. Replicate integrated water samples were collected with an acid-washed PVC pipe with a rubber stopper. Care was taken not to disturb the bottom or to entrain seagrass blades in the integrated water sampler. To better isolate free living bacteria and bacteria attached to small particles, each integrated water sample was passed through a 20 μm mesh screen. Water samples were stored in 8 L amber HDPE or frosted Lowboy containers for transport back to the laboratory. Larger-size POM samples were collected with a 20 μm mesh plankton tow at each station. $POM_{>20\ \mu m}$ was rinsed into precombusted glass jars. Replicate sediment surface samples were collected with a 5 cm^3 syringe with the tip removed and stored in precombusted glass scintillation vials. Each replicate represented a pooled sediment surface (1 cm depth) sample of five independent sediment cores. *T. testudinum* short shoots were collected with epiphytes attached and kept moist in plastic bags. All samples, except for the integrated water samples, were placed on ice immediately after collection. Integrated water samples were kept shaded. All samples were transported back to the laboratory within 8 h of collection.

In the laboratory, 0.1–0.3 L of integrated water sample was collected unto 0.2 μm 47 mm diameter aluminum oxide Whatman Anodisc filters for bulk stable carbon isotope analysis. $POM_{0.2–20\ \mu m}$ from 6 L of the integrated water sample was collected serially on precombusted 47 mm diameter Whatmann GF/F filters and pre-rinsed 0.2 μm 47 mm diameter Whatman Nylon membrane filters for fatty acid extraction and compound-specific stable carbon isotope analysis. All filters were stored in aluminum foil envelopes. New and mature green leaves from 3 to 10 short shoots (depending on leaf size and visible epiphyte load) of *T. testudinum* were cleaned of epiphytic communities by gentle scrapping with a razor blade. *T. testudinum* green leaves were stored in aluminum foil envelopes. The epiphytic community was retained in precombusted glass scintillation vials for stable isotope analysis. All sample types (i.e. $POM_{0.2–20\ \mu m}$, $POM_{>20\ \mu m}$, *T. testudinum* green leaves, seagrass epiphytic, and sediment surface) were frozen at −20 °C, lyophilized, powdered, and stored at −20 °C until further processing.

2.2. Bulk and compound-specific stable isotope analysis

Bulk stable carbon isotope analysis was conducted for $POM_{0.2–20\ \mu m}$, *T. testudinum* green leaves, seagrass epiphytic, and sediment surface samples. All samples were treated with 1 N HCl and washed with deionized water to remove carbonates. Decarbonated samples were oven-dried (70 °C) and powdered before ^{13}C isotope analysis. Standard Elemental Analyzer Isotope Ratio Mass Spectrometry (EA-IRMS) methods were applied using a Finnigan MAT Delta C IRMS. All carbon isotope values are reported in the standard delta (δ) notation relative to the internal standard, Vienna Pee Dee belemnite (PDB):

$$\delta^{13}C(‰) = \left[\left(^{13}C/^{12}C\right)_{sample}/\left(^{13}C/^{12}C\right)_{PDB} - 1\right] \times 1000$$

Sample reproducibility for bulk carbon analysis was ±0.1‰ (±1 standard deviation, $n = 10$). Except for $POM_{0.2–20\ \mu m}$ samples ($n = 1$), stable carbon isotopic analyses were duplicated from independent samples. $POM_{0.2–20\ \mu m}$ could not be replicated because of sample loss.

Fatty acids were isolated from $POM_{0.2–20\ \mu m}$, $POM_{>20\ \mu m}$, *T. testudinum* epiphytic, and sediment surface samples. Due to sample loss, fatty acids from $POM_{0.2–20\ \mu m}$ are only reported for Little Madeira Bay and Whipray Basin. Total lipids were extracted from environmental samples with dichloromethane and methanol (3:2; v/v) by Soxhlet extraction for 24 h in the presence of activated copper to remove elemental sulfur (Jaffé et al., 2001, 2006). Total lipid extracts were concentrated by rotary evaporation, saponified with 1 N KOH, and acid and neutral lipid fractions separated. The acid fraction, which contains fatty acids and polar lipids, was incubated with Na_2SO_4 for 12 h to remove water and then concentrated by rotary evaporation. Fatty acids were converted to fatty acid methyl esters (FAMEs) by derivatization with freshly distilled diazomethane.

Gas chromatographic (GC) – mass spectrometry (MS) analysis of FAME was performed with a Hewlett-Packard 5973 GC/MS using a DB-5MS (30 × 0.25 mm i.d., film thickness 0.25 μm) capillary column. The GC oven conditions were: hold at 60 °C for 1 min, ramp at 6 °C min^{-1} to 300 ∼C, hold at 300 °C for 10 min (Jaffé et al., 2001, 2006). FAMEs were identified by m/z mass spectra libraries, reference standards, and chromatographic retention characteristics. Fatty acids are reported in standard shorthand nomenclature (Ratledge and Wilkinson, 1988). Straight-chain fatty acids are indicated as total number of carbon atoms followed by the number of double bonds separated with a colon, where the position of the first double bond nearest to the methyl end is indicated with "ω". Branched-chain fatty acids are reported as branch position (unknown = br, anteiso = a, iso = i) followed by the total number of carbon atoms and colon-separated number of double bonds.

Compound-specific $\delta^{13}C$ analysis was conducted with a Hewlett-Packard 6890 GC coupled to a Finnigan Mat Delta Plus IRMS under the above column and GC conditions. FAMEs are reported in $\delta^{13}C$ notation as above. GC-IRMS reproducibility of the internal standard (19:0ME) was ±0.6‰ (±1 SD, $n = 34$). FAMEs were converted to fatty acids by correcting for the one carbon methyl group addition that occurred during derivatization (Boschker et al., 1999):

$$\delta^{13}C_{fatty\ \ acid}(‰) = \left[\left(\delta^{13}C_{FAME} \times C\#_{FAME}\right) - \left(\delta^{13}C_{ME} \times C\#_{ME}\right)\right] / C\#_{fatty\ \ acid}$$

Analysis of variance (ANOVA) determined significant ($\alpha = 0.05$) differences between stable carbon isotopic values. Games–Howell post-hoc test was used for pairwise comparison.

2.3. Methodological considerations

To allow interpretation of $\delta^{13}C$ values of bacterial fatty acids and to determine bacterial carbon sources, two methodological and one

biological issue(s) require consideration. First, $\delta^{13}C$ values were determined from the total fatty acid pool, which in addition to phospholipid derived fatty acids can include storage and detrital fatty acids not associated with live bacterial biomass. In general, microbial fatty acids in the sediment surface are degraded within days, but bacterial biomarker degradation rates are more variable and can proceeded at a slower rate than algal biomarkers (Canuel and Martens, 1996). However, bacterial production is high in the sediment surface (Williams et al., 2009) and *in situ* production of bacterial fatty acids might artificially reduce degradation rate measurements for bacterial biomarkers (Canuel and Martens, 1996). In addition, bacterial cell counts determined for Florida Bay pelagic, epiphytic, and sediment surface communities correlated well with i- and a15:0 bacterial fatty acid concentrations (Authors' unpublished data). Due to these observations, it is unlikely that detrital fatty acids made up a significant proportion of the total bacterial fatty acid pool. Fatty acid $\delta^{13}C$ values of bacterial biomarkers were considered representative of recent *in situ* carbon production.

Second, kinetic isotope fraction can affect FAME $\delta^{13}C$ values derivatized with diazomethane (Rieley, 1994). To test for these isotope effects, the total error of i- and a15:0 $\delta^{13}C$ measurements was calculated following the recommendations of Rieley (1994), which accounts for error associated with instrument variability and kinetic fractionation during derivatization. Using derivatized standards that ranged from 12C to 22C in size, the total error associated with bacterial fatty acid $\delta^{13}C$ values was on average $\pm 1.0‰$, which was slightly greater than the GC-IRMS analytical error of $\pm 0.6‰$. Similarly, Monson and Hayes (1982) did not report a significant kinetic isotope effect on $\delta^{13}C$ values due to diazomethane derivatization. Kinetic isotope fractionation during diazomethane derivatization did not influence strongly bacterial fatty acid $\delta^{13}C$ values determined during the current study.

Finally, $\delta^{13}C$ values of whole bacteria are isotopically similar to that of their carbon source, but bacterial fatty acids can be from 1 to >20‰ more deplete in ^{13}C than whole bacteria and their carbon source (Monson and Hayes, 1982; Boschker et al., 1999; Teece et al., 1999; Burke et al., 2003; Fang et al., 2006). This isotope depletion is due to metabolic fractionation of source carbon during fatty acid synthesis, which is dependent on the complexity and identity of the carbon substrate and metabolic pathway used to synthesize fatty acids (Monson and Hayes, 1982; Teece et al., 1999; Fang et al., 2006). However, for a mixed bacteria community growing on complex substrates, such as organic matter found in seagrass/coastal ecosystems, metabolic fractionation during fatty acid synthesis approaches −3‰ (Bouillon and Boschker, 2006). For field-based estimates, bacterial fatty acid $\delta^{13}C$ values were $-3.7 \pm 2.1‰$ more depleted in ^{13}C than bacterial carbon source (Bouillon and Boschker, 2006), which agreed well with previous lab-based fractionation measurements of −5.6‰ (Boschker et al., 1999) and −3.0‰ (Monson and Hayes, 1982). In the current study, fatty acid $\delta^{13}C$ values were converted to whole bacteria $\delta^{13}C$ values assuming a −3.0‰ fractionation because this fractionation factor was similar to field-based estimates and was determined for diazomethane-derivatized bacterial fatty acids.

3. Results

Thalassia testudinum green leaf $\delta^{13}C$ values differed significantly ($F_{3,12} = 215.9$, $p < 0.001$) from the $\delta^{13}C$ values of $POM_{0.2–20\ \mu m}$, epiphytic, and sediment surface samples and ranged from −9.9 to −6.9‰ (Tables 1 and 3). Bulk $\delta^{13}C$ values of $POM_{0.2–20\ \mu m}$, epiphytic, and sediment surface ranged from −16.4 to −13.5, −16.2 to −9.6, and −16.7 to −11.0‰, respectively. Spatially and across communities, bulk $\delta^{13}C$ values at Bob Allen Key were significantly enriched over other stations ($F_{3,12} = 63.2$, $p < 0.001$, Table 1).

Table 1
Mean (standard deviation, $n = 2$) bulk $\delta^{13}C$ values (‰; PDB) for pelagic (particulate organic matter, $POM_{0.2–20\ \mu m}$), seagrass epiphytic, sediment surface, and seagrass (*Thalassia testudinum*) communities. Standard deviation was not calculated for the pelagic community due to sample loss.

Station	Pelagic $\delta^{13}C$ (‰)	Epiphyte $\delta^{13}C$ (‰)	Sediment $\delta^{13}C$ (‰)	*T. testudinum* $\delta^{13}C$ (‰)
Little Madeira Bay	−14.8	−14.3 (1.4)	−16.5 (0.3)	−8.4 (0.2)
Duck Key	−16.4	−16.2 (0.1)	−14.4 (0.6)	−8.8 (0.1)
Bob Allen Key	−13.5	−9.8 (0.3)	−11.1 (0.2)	−7.0 (0.2)
Whipray Basin	−15.1	−14.2 (0.4)	−14.1 (0.7)	−9.9 (0.1)

Bacterial fatty acid $\delta^{13}C$ values ranged from 10.9‰ more depleted to 1.6‰ more enriched in ^{13}C than bulk $\delta^{13}C$ values. Due to sensitivity differences between GC-MS and GC-IRMS and incomplete GC resolution, $\delta^{13}C$ values were not measured for all bacterial fatty acids observed within each community. Most notably, $\delta^{13}C$ values for 18:1ω7, an important bacterial biomarker (Kaneda, 1991), could not be baseline resolved. Bacterial fatty acids (br14:0, 15:0, i15:0, a15:0, br17:0, and 17:0) that produced good peak intensities without peak overlap are reported in Table 2. These fatty acids were representative of common fatty acids synthesized mainly by heterotrophic bacteria and have been used frequently to determine the contribution of different organic matter sources to bacterial carbon cycles (Boschker et al., 1999; Jones et al., 2003; Dia and Sun, 2007).

Overall, bacterial fatty acid $\delta^{13}C$ values ranged from −25.5 to −8.2‰. Pelagic bacterial fatty acid $\delta^{13}C$ values for $POM_{0.2–20\ \mu m}$ and $POM_{>20\ \mu m}$ were similar and, hereafter, are designated inclusively as pelagic. The i15:0 and a15:0 $\delta^{13}C$ values differed significantly between stations ($F_{3,30} = 24.4$, $p < 0.001$) but did not differ significantly between communities. A statistically significant interaction between station and community type indicated that i15:0 and a15:0 $\delta^{13}C$ values were enriched in ^{13}C at Bob Allen Key within epiphytic and sediment surface communities ($F_{6,30} = 4.8$, $p = 0.002$). Pelagic i15:0 and a15:0 $\delta^{13}C$ values were, however, similar between stations. Statistical analysis for br14:0, 15:0, br17:0 and 17:0 $\delta^{13}C$ values was not possible due to missing values caused by poor peak resolution but visual assessment of these data suggested a similar pattern as found for i15:0 and a15:0 (Table 2). Whole bacterial $\delta^{13}C$ values were significantly more depleted in ^{13}C than *T. testudinum* $\delta^{13}C$ values ($F_{3,87} = 16.2$, $p < 0.001$; Table 3, Fig. 2). Seagrass $\delta^{13}C$ values were only similar to epiphytic and sediment surface bacterial $\delta^{13}C$ values at Bob Allen Key. Excluding Bob Allen Key, bulk $POM_{0.2–20\ \mu m}$, epiphytic, and sediment surface $\delta^{13}C$ values were not significantly different from pelagic, epiphytic, and sediment surface bacterial $\delta^{13}C$ values (Tables 2 and 3).

4. Discussion

Pelagic and benthic organic matter in Florida Bay is derived primarily from red mangrove (*Rhizophora mangle*), seagrass (*T. testudinum*), and microbial (i.e. seagrass epiphytes, microphytobenthos, and phytoplankton) sources (Frankovich and Zieman, 1994; Maie et al., 2005; Xu et al., 2006b; Xu and Jaffé, 2007). In the current study, sediment surface and $POM_{0.2–20\ \mu m}$ $\delta^{13}C$ values were similar to epiphytic $\delta^{13}C$ values, positioned between depleted mangrove and enriched *T. testudinum* $\delta^{13}C$ values (Tables 1 and 3). These results concur with previous reports in Florida Bay, except at Little Madeira Bay. POM and sediment surface organic matter samples collected previously during the wet season revealed

Table 2
Mean (standard deviation, $n = 2$) bacteria-specific fatty acid $\delta^{13}C$ values (‰; PDB) for pelagic (particulate organic matter, $POM_{0.2–20\ \mu m\ \&\ >20\ \mu m}$), *Thalassia testudinum* epiphytic, and sediment surface communities. Standard deviation was not calculated for some fatty acids due to sample loss or chromatographic peak overlap. nd, values not determined due to chromatographic peak overlap or absence of specific fatty acid.

	br14:0 $\delta^{13}C$ (‰)	i15:0 $\delta^{13}C$ (‰)	a15:0 $\delta^{13}C$ (‰)	15:0 $\delta^{13}C$ (‰)	br17:0 $\delta^{13}C$ (‰)	17:0 $\delta^{13}C$ (‰)
Pelagic						
Little Madeira Bay	−18.2 (2.5)	−20.5 (2.6)	−21.4 (1.8)	−23.5 (2.8)	−18.7	−21.5 (1.4)
Duck Key	−18.6	−20.0	−21.6	−24.0	−20.0	−21.6
Bob Allen Key	nd	−16.4	−18.2	nd	nd	nd
Whipray Basin	−19.2 (0.8)	−16.7 (0.8)	−18.4 (1.4)	−21.2 (1.9)	−17.4	−18.2 (0.8)
Epiphytic						
Little Madeira Bay	−21.7	−17.2 (3.5)	−22.5 (3.8)	−21.3 (3.4)	nd	nd
Duck Key	nd	−19.4	−18.9	−22.9	−15.7	nd
Bob Allen Key	−13.5	−10.2 (0.7)	−10.1 (2.7)	nd	nd	nd
Whipray Basin	nd	−19.2	−24.1	−20.9	−19.5	nd
Sediment						
Little Madeira Bay	−18.3	−20.9 (0.5)	−20.7 (0.8)	−19.9 (0.9)	−18.6	−18.7
Duck Key	nd	−19.1 (0.6)	−18.7 (0.8)	−17.5	−13.9	nd
Bob Allen Key	−16.5 (2.7)	−14.2 (2.1)	−14.7 (1.5)	−13.75	−14.7 (3.1)	−13.0
Whipray Basin	−21.3 (0.1)	−20.2 (0.1)	−20.1 (0.6)	−19.4	−19.5 (3.0)	nd

a strong mangrove/terrestrial-derived $\delta^{13}C$ signature (i.e. $\delta^{13}C$ near −28‰) at Little Madeira Bay, which was not observed in the current late dry season study (Jaffé et al., 2001; Evans et al., 2006; Xu et al., 2006b). In the current study, sediment surface, $POM_{0.2–20\ \mu m}$, and epiphytic $\delta^{13}C$ values were similar to or more enriched in ^{13}C than a range of $\delta^{13}C$ values reported for benthic macroalgae, microphytobenthos, and phytoplankton (Table 3; Fry and Wainright, 1991; Currin et al., 1995 and references therein; Kieckbusch et al., 2004; Behringer and Butler, 2006). Spatially, bulk organic matter $\delta^{13}C$ values were significantly enriched at Bob Allen Key, which likely reflected a previously observed enrichment in dissolved inorganic carbon $\delta^{13}C$ values at this site (Lutz, 1997) rather than a divergence in organic matter composition from the other sites.

Bacterial $\delta^{13}C$ values ranged from −24.1 to −5.2‰, suggesting that bacteria used a mixture of algae/cyanobacteria, seagrass, and mangrove organic matter sources (Table 3, Fig. 2). Mean ± 1 SD bacterial $\delta^{13}C$ values were well within the reported range for epiphytes, macroalgae, microphytobenthos, and phytoplankton (Fry and Wainright, 1991; Currin et al., 1995; Kieckbusch et al., 2004), but similar $\delta^{13}C$ values would have been produced if bacteria consumed mangrove- and seagrass-derived organic matter in roughly equal parts in the absence of algal-derived organic matter. To resolve better the feasible contributions of Florida Bay carbon sources to heterotrophic bacteria in Florida Bay, a mixing model approach (IsoSource, Phillips and Gregg, 2003) was taken using all possible combinations of mangrove, seagrass, and algal carbon contributions to bacteria.

Average estimates of mangrove (−28.0‰), seagrass (−8.5‰), and algal (−18.0‰) $\delta^{13}C$ values were used to determine the fraction of bacterial $\delta^{13}C$ values explained by each carbon source. Mangrove,

Table 3
Potential carbon sources for heterotrophic bacteria of Florida Bay. Reported $\delta^{13}C$ values (‰; PDB; range and/or mean ± standard deviation depending on availability of reported data) of organic matter and primary producers in Florida Bay and related ecosystems.

Carbon source	$\delta^{13}C$ (‰) PDB	Reference
Mangrove: *Rhizophora mangle*		
Yellow leaves, Florida Bay	−28.7 ± 4.4	Fourqurean and Schrlau, 2003
Leaves and wood, south Florida	−30.8 to −25.2	Fry and Smith, 2002
Particulate organic matter		
0.2–20 μm, Florida Bay	−16.4 to −13.5	This study
0.1–50 μm, Florida Bay	−25.3 to −14.4	Evans et al., 2006
0.45–200 μm, Florida Bay	−18.0 to −16.0	Behringer and Butler, 2006
>64 μm, Florida Bay	−21.1 to −14.6	Chasar et al., 2005
Pelagic marine phytoplankton	−21.7 to −15.0	Fry and Wainright, 1991
Seagrass: *Thalassia testudinum*		
Green leaves, Florida Bay	−9.9 to −6.9, −8.5 ± 1.1	This study
Green leaves, south Florida	−13.5 to −5.2	Fourqurean et al., 2005
Epiphytes, Florida Bay	−16.2 to −9.6	This study
Epiphytes, Florida Bay	−17.4 to −15.1	Chasar et al., 2005
Macroalgae		
Laurencia spp., Florida Bay	−16.0 to −13.0, −14.9 ± 1.1	Behringer and Butler, 2006
Penicillus spp., south Florida	−16.7 ± 0.5	Kieckbusch et al., 2004
Halimedia spp., south Florida	−18.9 ± 3.3	Kieckbusch et al., 2004
Microphytobenthos		
Benthic diatoms, Georgia marsh	−17.9 to −16.2	Haines, 1976
Benthic microalgae and cyanobacteria	−20.6 to −8.5, −14.9 ± 3.2	Currin et al., 1995 and references therein
Sediment organic matter		
0–1 cm, Florida Bay	−16.7 to −11.0	This study
0–2 cm, Florida Bay	−25.9 to −11.2	Jaffé et al., 2001; Behringer and Butler, 2006; Xu et al., 2006a

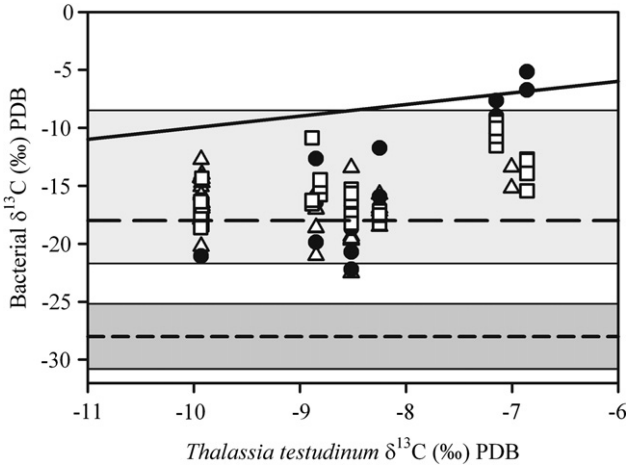

Fig. 2. Similarity between pelagic (△), epiphytic (●), and sediment surface (□) bacterial-specific $\delta^{13}C$ values and *Thalassia testudinum* $\delta^{13}C$ values. To allow direct comparison between bacterial-specific $\delta^{13}C$ values and *T. testudinum*, fatty acid $\delta^{13}C$ values for br14:0, 15:0, i15:0, a15:0, br17:0, and 17:0 were corrected for a metabolic fractionation of −3‰ from carbon source. Expected bacterial $\delta^{13}C$ values when bacteria consumed only seagrass-derived organic matter (solid line) are indicated. $\delta^{13}C$ values used in the IsoSource mixing model with the reported $\delta^{13}C$ value range for estuarine and coastal ecosystems (Table 3) are displayed for algae/cyanobacteria (long-dash line, light gray shaded area) and mangroves (short-dash line, dark grey shaded area).

seagrass, and algal carbon sources contributed 0–42, 13–57, and 0–86% (1–99%-ile range), respectively, of pelagic bacterial carbon, 0–32, 32–67, and 0–67% of epiphytic bacterial carbon, and 0–33, 30–66, and 0–69% of sediment surface bacterial carbon. The IsoSource results revealed a consistent seagrass carbon influence on heterotrophic bacterial $\delta^{13}C$ signatures. However, the feasible contributions of algal- and mangrove-derived organic matter to heterotrophic bacterial $\delta^{13}C$ values included zero and the mixing model did not discriminate clearly between mangrove- and algal-derived organic matter. The bacterial-specific stable carbon isotopic results support the importance of seagrass as a carbon source to Florida Bay bacterial communities, but 33–87% of bacterial $\delta^{13}C$ signatures were not explained by seagrass $\delta^{13}C$ values, indicating that bacteria incorporated additional carbon sources (Fig. 2).

Across seagrass ecosystems, sediment bacterial $\delta^{13}C$ signatures and carbon preference depend on the nutrient status and the availability and quantity of organic matter sources in the ecosystem (Bouillon et al., 2004; Holmer et al., 2004; Bouillon and Boschker, 2006). For example, in the oligotrophic Lower Laguna Madre estuary, Texas, sediment bacterial i15:0 and a15:0 $\delta^{13}C$ values reflected $\delta^{13}C$ values of *T. testudinum*, indicating that benthic bacterial carbon cycles were coupled to seagrass production (Jones et al., 2003). In seagrass beds located in the Gazi Bay, Kenya, sediment bacterial i15:0 and a15:0 $\delta^{13}C$ values reflected that of mixed carbon sources, influenced by allochthonous, mangrove-derived organic matter as well as local seagrass and microbial inputs (Bouillon et al., 2004). In Gazi Bay and Laguna Madre seagrass beds as well as those in Florida Bay, sediment bacterial $\delta^{13}C$ values were similar to sediment TOC $\delta^{13}C$ values, suggesting that bacteria at times consume broadly the organic matter available in the bulk carbon pool.

The %TOC content of sediment also influences the sediment bacterial $\delta^{13}C$ values and the ability to discriminate between carbon sources (Bouillon and Boschker, 2006). Across multiple ecosystems, bacterial-specific isotope values reflected that of mixed carbon sources when sediment %TOC was between 1 and 10% and that of the dominant and enriched carbon source when the sediment had >10 and <1% TOC, respectively (Bouillon and Boschker, 2006). However, sediment bacterial-specific $\delta^{13}C$ values in seagrass beds did not always follow expectations based on sediment %TOC alone. Florida Bay sediment had %TOC content ranging from 1.1 to 8.0% and sediment surface $\delta^{13}C$ values representative of mixed carbon sources. Similarly, bacterial-specific $\delta^{13}C$ values reflected that of mixed carbon sources.

In addition to sediment %TOC content, bacteria depend on seagrass as a carbon source in pristine ecosystems but shift their carbon preference in impacted seagrass systems with increased organic matter and nutrient inputs (Holmer et al., 2004). In Mediterranean seagrass beds, sediment bacterial $\delta^{13}C$ values were generally similar to seagrass stable carbon isotope signatures, except at anthropogenically impacted sites, where bacteria $\delta^{13}C$ values were similar to that of seston and benthic microalgae (Holmer et al., 2004). In two oligotrophic, tropical (Ban Pak Klok, Thailand) *Cymodocea rotundata* and *Thalassia hemprichii* seagrass beds, sediment bacterial $\delta^{13}C$ values resembled that of the seagrass (i.e. $\delta^{13}C$ values near $-12‰$), though bulk sediment TOC $\delta^{13}C$ values were around $-22‰$ (Holmer et al., 2001). In contrast, in four relatively impacted European *Zostera marina* beds, sediment bulk and bacterial $\delta^{13}C$ values were similar to that of benthic macroalgae, and seagrass was not an important carbon source for benthic bacteria (Boschker et al., 2000).

Florida Bay is considered a historically pristine seagrass ecosystem, which has experienced some recent signs of eutrophication, seagrass mortality and phytoplankton blooms (Robblee et al., 1991; Fourqurean and Robblee, 1999; Koch et al., 2007). In the current study, low to intermediate contribution of seagrass carbon to sediment bacterial $\delta^{13}C$ signatures seem to be in line with this increase in eutrophication of Florida Bay. Eutrophication is further evident by a reported gradual long-term increase in sediment nutrient content in Florida Bay (Orem et al., 1999) and, over the past few decades, an increase in microbial-derived organic matter inputs to Florida Bay sediment (Xu et al., 2006a, 2007). On the whole, however, sediment bacterial $\delta^{13}C$ values in Florida Bay fit the %TOC (Bouillon and Boschker, 2006) and pristine state (Holmer et al., 2004) models for bacterial carbon preference in seagrass/coastal ecosystems. Thus, sediment bacteria in Florida Bay might have: (1) incorporated carbon from the mixed organic matter pool somewhat indiscriminately; or (2) responded to increased algal and/or allochthonous production by reducing the amount of seagrass carbon they incorporated in favor of alternative carbon sources. Given that the IsoSource model could not discriminate clearly between alternative carbon sources, we cannot conclude which of the above scenarios is more likely to occur in Florida Bay.

Previous published reports of bacterial $\delta^{13}C$ values in seagrass ecosystems have not included pelagic and epiphytic communities. In one investigation of pelagic bacterial-specific $\delta^{13}C$ values in the tidal Scheldt estuary, pelagic bacterial $\delta^{13}C$ values resembled allochthonous-derived organic matter in the turbid upper estuary and phytoplankton-derived organic matter in less turbid lower estuary (Boschker et al., 2005). These bacterial $\delta^{13}C$ values also corresponded to bulk POM $\delta^{13}C$ values, suggesting that pelagic bacteria incorporated carbon mainly from the dominant carbon source. In the current study, pelagic, epiphytic, and sediment bacterial $\delta^{13}C$ values resembled an algal/cyanobacterial or mixed carbon source(s) and were similar across sites and community type despite documented spatial differences in primary and secondary productivity and nutrient concentrations in Florida Bay (Fig. 2; Boyer et al., 1999; Fourqurean and Robblee, 1999; Frankovich and Zieman, 1994; Williams et al., 2009).

Multivariate analysis of benthic and pelagic nutrient and bacterial activity data suggested that seagrass-derived organic matter was linked to epiphytic, sediment, and to a lesser extent pelagic bacterial carbon demand, while phytoplankton biomass was linked strongly and weakly to pelagic and sediment, respectively, bacterial carbon demand (Williams et al., 2009). However, the IsoSource mixing model results were inconclusive and did not confirm our hypothesis that algal/cyanobacterial-derived organic matter made up a significant contribution to pelagic as well as benthic bacterial carbon demands. Seagrass-derived organic matter was a consistent source of carbon to all bacterial communities, but 0–86 and 0–69% of pelagic and benthic, respectively, bacterial $\delta^{13}C$ signatures could have resulted from bacterial incorporation of algal/cyanobacterial-derived carbon (Fig. 2). As such, pelagic and epiphytic bacterial-specific $\delta^{13}C$ values did not help resolve further if the observed bacterial carbon signatures were caused by relatively high abundance of multiple carbon sources, a trophic shift in Florida Bay away from a pristine state toward an impacted ecosystem, or a combination of both conditions.

5. Conclusion

Pelagic, epiphytic, and sediment surface bacteria-specific $\delta^{13}C$ values were similar to each other and to that of the bulk organic matter pool, suggesting that these communities responded similarly to changes in the ecosystem state of Florida Bay or that each community had similar access to the organic matter pool despite their distinct location within the estuary. Seagrass-derived (*Thalassia testudinum*) organic matter made up 13–67% bacterial-specific $\delta^{13}C$ signatures. However, isotopic overlap of the mixed carbon pool prevented the clear separation of mangrove- and algae/cyanobacterial-derived carbon contributions to pelagic and benthic bacterial communities. In future work, difficulties with mixing

model isotope overlap might be overcome by determining more exact algal/cyanobacterial and mangrove $\delta^{13}C$ values at each station and applying these spatiotemporally specific values to the IsoSource model rather than using average estimates of alternative carbon sources. The results of the current study fit well within the pristine state (e.g. Holmer et al., 2004) and organic matter content (e.g. Bouillon and Boschker, 2006) views of bacterial carbon incorporation in seagrass and coastal ecosystems. Bacterial-specific stable carbon isotopic signatures might be a continued sign of ecosystem deterioration in Florida Bay and/or arisen through broad bacterial incorporation of the organic matter sources available within the ecosystem. Overall, pelagic, epiphytic, and surface sediment bacterial communities consumed consistently seagrass-derived organic matter and a mixture of mangrove- and/or algal-derived organic matter to meet their carbon demands.

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