Estuarine, Coastal and Shelf Science 85 (2009) 507–514

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

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

Clayton J. Williams[a,1], Rudolf Jaffé[b], William T. Anderson[c], Frank J. Jochem[a,*]

[a] Marine Biology Program, Florida International University, 3000 NE 151 Street, North Miami, FL 33181, USA
[b] Environmental Geochemistry Laboratory, Southeast Environmental Research Center, and Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th Street, Miami, FL 33199, USA
[c] Southeast Environmental Research Center and Department of Earth and Environment, Florida International University, 11200 SW 8th Street, Miami, FL 33199, USA

ARTICLE INFO

Article history:
Received 30 January 2009
Accepted 18 September 2009
Available online 24 September 2009

Keywords:
stable isotopes
biomarker
carbon cycling
bacteria
seagrass
Florida Bay

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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[*] Corresponding author at: Algenol Biofuels, Inc., Florida Aquaculture & Field Research Station, 2805 B Road, Loxahatchee, FL 33470, USA.
E-mail address: frank@jochem.net (F.J. Jochem).
[1] Present address: Department of Biology, Trent University, 1600 West Bank Drive, Peterborough, ON K9J 7B8, Canada.

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doi:10.1016/j.ecss.2009.09.019

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.

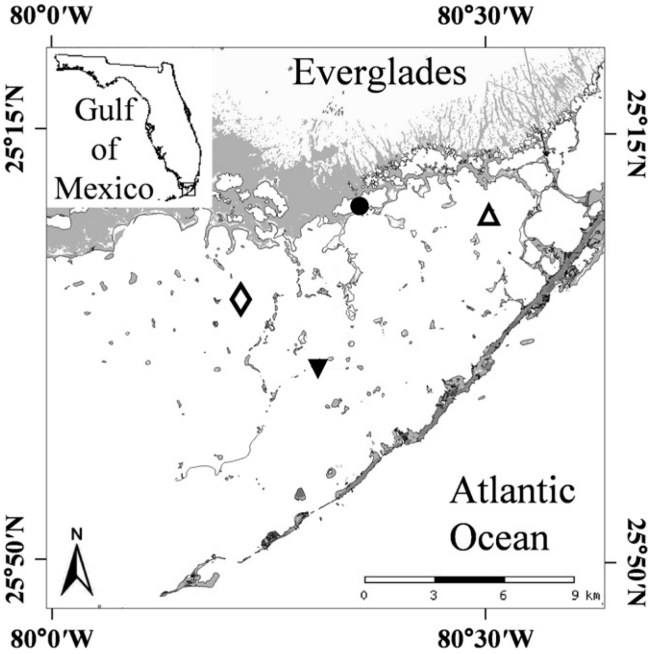

Fig. 1. Sampling stations in Florida Bay, south Florida; Little Madeira Bay (●), Duck Key (△), Bob Allen Key (▼), Whirpay Basin (◇).