

Stable carbon isotopes as potential sea-level indicators in salt marshes, North Carolina, USA

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Abstract

We compared the use of $\delta^{13}\text{C}$ values and C:N ratios from salt-marsh sediments to reconstruct relative sea level (RSL) with an established approach using foraminifera. Analysis of bulk-organic sediment and plant samples collected along transects at two sites in North Carolina, USA demonstrates that sediment $\delta^{13}\text{C}$ values can be used to distinguish between *Spartina alterniflora*-dominated low marsh (C_4 photosynthetic pathway, $\delta^{13}\text{C}$ values from -17.6 ‰ to 16.1 ‰) and *Juncus roemerianus*-dominated high marsh (C_3 photosynthetic pathway, $\delta^{13}\text{C}$ values from -28.2 ‰ to -21.8 ‰) environments. *Juncus roemerianus* plants undergo little decompositional change in $\delta^{13}\text{C}$ (average 0.8 ‰), although there is a clear difference between *Spartina alterniflora* tissue and bulk-organic sediments (approximately 4 ‰). C:N ratios on bulk-organic sediment from freshwater upland and salt-marsh environments converge during early diagenesis, rendering them of little use in reconstructing RSL. The utility of $\delta^{13}\text{C}$ values as a sea-level indicator is limited by the elevational range of C_4 plants, making it difficult to recognize salt-marsh sub-environments and improve the precision of RSL reconstructions. Furthermore, *Juncus roemerianus*-dominated high marsh and freshwater upland sediments cannot be adequately distinguished with $\delta^{13}\text{C}$ values.

Keywords: salt marsh, carbon isotope ratios, sea level, foraminifera, North Carolina

1 Introduction

On low energy, temperate coasts, salt marshes exhibit a strong environmental gradient extending through marine, brackish and freshwater conditions that is driven by the frequency and duration of tidal inundation (e.g. Chapman, 1940, 1960). Vascular plants and microfaunas respond to this gradient by forming distinctive floral and faunal zones that reflect sub-environments along the gradient. This characteristic pattern of zonation can be used to estimate former relative sea level (RSL) by establishing the environmental origin of organic sediment preserved in estuaries, salt marshes and coastal lowlands (e.g. Shennan, 1992). RSL is reconstructed using sea-level indicators, which are physical, biological or chemical features possessing a systematic and quantifiable relationship to elevation in the tidal frame (Shennan, 1986, van de Plassche, 1986). The indicative meaning (Figure 1d) is the elevational range occupied by a sea-level indicator (indicative range) in relation to a contemporaneous tide level (reference water level).

Assemblages of salt-marsh foraminifera are widely used sea-level indicators that can accurately define zones within tidal environments with a precision of up to ± 0.05 m in high marsh settings (Scott and Medioli, 1978; Gehrels, 1994, 2000; Horton and Edwards, 2006). Foraminifera are frequently well preserved in salt-marsh deposits and their low diversity, high abundance assemblages (Sen Gupta, 1999; Gehrels, 2007) lend themselves to quantitative analysis. There are, however, potential difficulties with the application of salt-marsh foraminifera to reconstruct RSL. Interpretation of foraminiferal assemblages in subsurface sediments requires an understanding of the distribution and ecological preferences of modern foraminifera, including infaunal habitation (e.g. Goldstein and Harben, 1993; Ozarko *et al.*, 1997; Saffert and Thomas, 1998), seasonal population fluctuations (e.g. Murray and Alve, 2000) and micro-scale variability (patchiness) in the distribution of foraminifera (e.g. Hippensteel *et al.*, 2000). Post-depositional

modification of assemblages may also compromise paleoenvironmental reconstructions through selective removal of tests (e.g. Jonasson and Patterson, 1992; Goldstein and Watkins, 1999).

Potential alternative means to reconstruct RSL from salt-marsh sediments include the stable carbon isotopic composition ($^{13}\text{C}:^{12}\text{C}$ ratio, expressed as $\delta^{13}\text{C}$ in parts per mil (‰) compared to a standard reference sample, PDB) and the ratio of organic carbon to total nitrogen (C:N) in bulk-organic sediments (e.g. Wilson *et al.*, 2005; Lamb *et al.*, 2006, 2007). Salt-marsh plants using the C_4 photosynthetic pathway, create plant tissues with an average $\delta^{13}\text{C}$ composition of -12 ‰ (Schlesinger, 1997) and a range from -17 ‰ to -9 ‰ (Chmura and Aharon, 1995). In contrast, plants utilizing the C_3 photosynthetic pathway have a $\delta^{13}\text{C}$ signature of -34 ‰ to -23 ‰ (Chmura and Aharon, 1995). The distinction between these pathways offers a potential means to reconstruct RSL by identifying salt-marsh environments in organic sedimentary sequences (Chmura *et al.*, 1987). Reconstructions with increased precision are possible if salt-marsh zones (low and high marshes characterized by different proportions of C_3 and C_4 plants) can be recognized (Edwards, 2007). Such an approach requires locations where C_3 and C_4 plants have existed together with differing distributions for the period under consideration (Wilson *et al.*, 2005) and $\delta^{13}\text{C}$ is incorporated into organic sediments with little diagenetic change (e.g. Ember *et al.*, 1987; Fogel *et al.*, 1989, Goni and Thomas, 2000). Measured values of $\delta^{13}\text{C}$ and C:N record the balance between autochthonous and allochthonous inputs. The dominant autochthonous component in salt marshes is from vascular plants (Lamb *et al.*, 2006), whilst allochthonous sediment may be derived from fresh, brackish or marine environments as particulate or dissolved material. The contribution of allochthonous inputs (volume and provenance, relative to autochthonous materials) to measured sediment $\delta^{13}\text{C}$ and C:N values may be significant in salt-marsh environments (e.g. Lamb *et al.*, 2006; Gebrehiwet *et al.*, 2008).

We investigated the use of $\delta^{13}\text{C}$ and C:N ratios to identify salt-marsh floral zones in bulk-organic sediments and reconstruct RSL in North Carolina, USA. Previous studies using $\delta^{13}\text{C}$ and C:N in salt-marsh deposits have used the technique to characterize paleoenvironments and infer vegetation change (e.g. Malamud-Roam and Ingram, 2004; Wilson *et al.*, 2005) or to provide qualitative information about the depositional environment of radiocarbon-dated samples (e.g. Chmura and Aharon, 1995; Törnqvist *et al.*, 2006, 2008). To date, this technique has not been used to quantitatively reconstruct RSL. The $\delta^{13}\text{C}$ and C:N values of surface sediments were measured along environmental and elevational gradients at two microtidal sites to consider the potential accuracy and precision of this approach. We compare $\delta^{13}\text{C}$ and C:N values from plants and surface sediments to better understand the effects of early diagenesis on salt-marsh sediments. To demonstrate the application of $\delta^{13}\text{C}$ in reconstructing RSL, we estimated the indicative meaning of 11 Holocene sea-level data points from North Carolina and compared the results to those from a foraminifera-based transfer function approach.

2 Study Area

The Atlantic coast of North Carolina is characterised by sounds separating the mainland from the Outer Banks barrier islands (Pilkey *et al.*, 2002). Water in the sounds reflects the balance between freshwater input from rivers and exchange of marine water with the Atlantic Ocean through inlets. The region is micro-tidal and wind-driven water levels regularly exceed the magnitude of astronomical tides. Salt marshes occupy the margins of the sounds where brackish conditions exist (Pilkey *et al.*, 2002). *Spartina alterniflora* forms narrow bands of low marsh (Adams, 1963; Brinson, 1991; Woerner and Hackney, 1997). *Juncus roemerianus* covers up to 77 % of salt-marsh area (Eleuterius, 1976) and dominates high marshes (Brinson, 1991). Other common species include *Distichlis spicata* and *Spartina patens*, which usually occur as patches in high marsh environments surrounded by *Juncus roemerianus*. At sites with low

salinity, *Spartina cynosuroides* occupies high marshes (e.g. Culver and Horton, 2005). The vertical zonation of salt-marsh plants is limited by the microtidal nature of the study area, but it is this microtidal regime together with continuous sequences of high marsh peat that provides an ideal setting for reconstructing changes in sea level. The study region is representative of a substantial proportion of back-barrier salt marshes on the Atlantic Coast of the USA.

We selected two sites on salt marshes at Oregon Inlet and Sand Point (Figure 1a) that represent different environmental conditions from a larger set of sites where Kemp *et al.* (2009) documented modern foraminiferal distributions. The Oregon Inlet site is a high salinity (26 psu; Williams *et al.*, 1973; Culver and Horton, 2005), back-barrier marsh located adjacent to the open inlet (Figure 1b). A 250 m long transect of 15 sampling stations (Figures 1b and 2) extended from a narrow (10 m wide) *Spartina alterniflora*-dominated low marsh to a large (240 m wide) high marsh, characterized by *Juncus roemerianus*, in association with *Iva frutescens* toward the upper edge of the marsh. The second site, Sand Point, is distal to an open inlet (Figure 1c), resulting in low salinity (8 psu; Schwartz and Chestnut, 1973). The marsh is up to 1500 m wide and displays a narrow (5 m), low marsh of *Spartina alterniflora* that is replaced inland by *Juncus roemerianus* and followed by a transition to freshwater upland vegetation. The transect included 21 sampling stations extending across the marsh into the surrounding upland (Figures 1c and 2). Similar to many salt-marsh shorelines in the Albemarle-Pamlico estuarine system, this site is experiencing significant erosion and the low marsh receives substantial inputs of reworked high-marsh sediment, which is likely several thousands of years old (Riggs, 2001). Therefore, low marsh environments were not sampled at this site. Tidal flat environments and pioneer stage salt marshes are unusual in North Carolina and were not present at either site.

Salt-marsh plants were collected from three additional sites (Figure 1a). Swan Quarter National Wildlife Refuge (NWR) is situated at the confluence of the Pamlico River with Pamlico Sound; it

is a large, low salinity salt marsh. Ocracoke is a narrow (150 m), higher salinity salt marsh on the Outer Banks. Cedar Island is one of the largest salt marshes in North Carolina (up to 6.7 km wide; Brinson, 1991) and is characterized by salinities between 15 and 20 psu.

3 Methods

We analyzed samples from transects at the Oregon Inlet and Sand Point sites to document the modern distribution of foraminifera and geochemical characteristics of salt-marsh sediment. The transects extended perpendicular to the shoreline from low marsh to freshwater upland environments. Positioning of samples reflected changes in elevation and vegetation. Sample elevations were measured by leveling to geodetic benchmarks (Figure 1b, c). The boundaries between floral zones were surveyed at as many as 40 locations at each site to estimate the elevational range of common plants. We measured the boundaries between a) freshwater upland and *Juncus roemerianus*, b) *Spartina alterniflora* and *Juncus roemerianus* and c) the lower limit of *Spartina alterniflora*. The program VDatum (Hess *et al.*, 2005) enabled conversion from orthometric to tidal elevations (expressed relative to local Mean Tide Level, MTL).

3.1 Sediment Grain Size and $\delta^{13}\text{C}$, C:N ratios and Total Organic Carbon of Modern Sediments and Plants

Surface sediment samples (0-1 cm) were collected for analysis along both transects. Additionally, at Sand Point, we collected 19 samples of *Juncus roemerianus* and two of *Distichlis spicata*. Plant material was also collected at Swan Quarter NWR (*Spartina alterniflora*, *Spartina cynosuroides* and *Spartina patens*), Ocracoke (*Distichlis spicata*, *Salicornia* sp., *Spartina alterniflora*, and *Spartina patens*) and Cedar Island (*Iva frutescens*, *Juncus roemerianus*, *Spartina alterniflora*, *Spartina cynosuroides* and *Spartina patens*). Sample grain

size was measured using laser diffraction after Allen and Thornley (2004) and Hawkes *et al.* (2007).

For measurement of $\delta^{13}\text{C}$ and C:N, sediment samples were treated with 5 % HCL for 18 hours, washed with deionised water, dried in an oven at 40 °C overnight and milled to a fine powder using a pestle and mortar. Plant samples were washed with deionised water to remove sediment particles, dried in an oven at 40 °C overnight and milled to a fine powder.

$^{13}\text{C}/^{12}\text{C}$ analyses were performed on plant and sediment samples by combustion in a Costech Elemental Analyser coupled on-line to an Optima dual-inlet mass spectrometer. $\delta^{13}\text{C}$ values were calculated to the VPDB scale using a within-run laboratory standard (cellulose, Sigma Chemical prod. no. C-6413) calibrated against NBS-19 and NBS-22. C:N ratios were analysed on the same instrument and the ratios were calibrated through an acetanilide standard. All C:N values are expressed on a weight ratio basis. Replicate analysis indicated a precision of <0.1 ‰ (1 SD) for $\delta^{13}\text{C}$ and C:N measurements.

3.2 Modern Foraminifera

We collected surface sediment samples (0-1cm) for foraminiferal analysis. Samples were stored in buffered ethanol and stained with rose Bengal (Walton, 1952) to allow identification of live individuals. Samples were sieved to isolate the foraminifera-bearing fraction between 63 μm and 500 μm . Each sample was divided into eight aliquots and a minimum of 200 individuals was counted from a known fraction of the sediment (Scott and Hermelin, 1993; de Rijk, 1995). Salt-marsh foraminifera typically form low diversity assemblages and counts of 200 are representative of the target population (Fatela and Taborda, 2002). Assemblage zones were identified by unconstrained, hierarchical cluster analysis (Kemp *et al.*, 2009).

4 Results

4.1 Elevational Distribution of Vascular Plants

Leveling of floral boundaries provided estimates of the local vertical range of *Spartina alterniflora* and *Juncus roemerianus*. Despite differences in salinity and tidal range, the elevational distribution of plants at Oregon Inlet and Sand Point is similar. At Oregon Inlet, *Spartina alterniflora* occupies mean elevations between -0.09 m and 0.22 m MTL; *Juncus roemerianus* is found from 0.14 m to 0.42 m MTL. At Sand Point, *Spartina alterniflora* was dominant at elevations between -0.10 m and 0.11 m MTL. *Juncus roemerianus* was the dominant plant at elevations ranging from 0.10 m to 0.35 m MTL, but was absent from sites above approximately 0.35 m MTL, where it was replaced by freshwater upland vegetation.

4.2 $\delta^{13}\text{C}$ and C:N ratios of Modern Salt-Marsh Sediments

At Oregon Inlet, surface sediments in the *Spartina alterniflora* low marsh between 0 m and 28 m along the transect exhibited $\delta^{13}\text{C}$ values from -17.6 ‰ to -16.2 ‰ (average -17.0 ‰) and C:N ratios between 14.8 and 16.1 (Figure 2). These samples had an average total organic carbon (TOC) of 7.9 % (4.0 % to 10.7 %) and grain size concentrated in the silt-sized (3.9 μm to 62.5 μm) fraction (47 % to 71 %, average median grain size, d_{50} , of 22 μm). Surface sediments in *Juncus roemerianus* environments (28 m to 258 m along the transect) had $\delta^{13}\text{C}$ values between -27.1 ‰ and -24.5 ‰ (average -26.3 ‰) and C:N ratios from 14.5 to 22.5 (Figure 2). TOC in these samples averaged 8.3 % (1.7 % to 15.8 %) and grain size was concentrated in the silt-sized fraction (12 % to 71 %, median grain size of 49 μm).

At Sand Point, surface sediment samples from 0 m to 454 m along the transect in a *Juncus roemerianus* marsh yielded $\delta^{13}\text{C}$ values from -28.2 ‰ to -21.8 ‰ (average -26.3 ‰) and C:N ratios between 11.1 and 41.3 (Figure 2). These samples had an average TOC of 24.6 % (14.5

% to 41.3 %) and a median grain size of 35 μm (16 % to 74 % silt-sized). Samples at the inland end of the transect (454 m to 471 m) in a freshwater upland had $\delta^{13}\text{C}$ values between -28.8 ‰ and -26.6 ‰ (average -27.8 ‰) and C:N ratios of 41.6 to 48.8. TOC in these samples averaged 39.2 % (11.1 % to 48.8 %), the median grain size was 83 μm with 26 % to 70 % of clastic sediment in the silt-sized fraction.

4.3 $\delta^{13}\text{C}$ and C:N Ratios of Modern Salt-Marsh Plants

The $\delta^{13}\text{C}$ and C:N composition of seven common salt-marsh plants is shown in Figure 3. From 17 specimens at Sand Point, $\delta^{13}\text{C}$ values for *Juncus roemerianus* ranged from -28.1 ‰ to -24.9 ‰ (average -26.6 ‰), C:N ratios for this species varied from 31.8 to 70.3 (average 45.9). Three examples of *Distichlis spicata* (two from Sand Point and one from Ocracoke) had an average $\delta^{13}\text{C}$ content of -15.0 ‰ (-16.2 ‰ to -13.1 ‰) and average C:N ratio of 48.3 (28.0 to 82.6). A *Salicornia* sp. plant from Ocracoke yielded a $\delta^{13}\text{C}$ value of -27.8 ‰ and a C:N ratio of 58. An *Iva frutescens* plant from Cedar Island had a $\delta^{13}\text{C}$ value of -28.3 ‰ and C:N ratio of 84.9. Three samples of *Spartina alterniflora* had $\delta^{13}\text{C}$ values from -13.1 ‰ to -12.6 ‰ (average -12.9 ‰) and C:N ratios that averaged 139 (117.4 to 174.5). Two *Spartina cynosuroides* plants (Cedar Island and Swan Quarter) had $\delta^{13}\text{C}$ values of -12.1 ‰ and -12.8 ‰ with C:N ratios of 70.7 and 343.2. Three *Spartina patens* plants (one each from Cedar Island, Ocracoke and Swan Quarter NWR) displayed $\delta^{13}\text{C}$ values between -13.6 ‰ and -12.9 ‰ and C:N ratios of 202.8 to 310.8.

4.4 Elevational Distribution of Modern Foraminifera

At Oregon Inlet, 12 species of foraminifera were recorded in 15 samples along a 250 m transect (0.02 m to 0.42 m MTL; Figure 4). Between 0 m and 28 m (low marsh, *Spartina alterniflora* zone), the dominant species was *Miliammina fusca* (up to 78 %). High abundances of *Arenoparrella mexicana* (up to 80 %) define the assemblage between 38 m and 223 m. Vegetation in this part of the marsh consisted of mono-specific stands of *Juncus roemerianus*.

The landward edge of the transect was represented by *Haplophragmoides wilberti* (57 %) and was associated with *Juncus roemerianus* and *Iva frutescens*.

Three elevation-dependent ecological zones were recognized at Oregon Inlet (Figure 4). Zone OI-1 extended from 0.02 m to 0.23 m MTL and was defined by high abundances of *Miliammina fusca*. The *Arenoparrella mexicana*-dominated part of the marsh formed zone OI-2 (0.23 m to 0.35 m MTL). Foraminiferal assemblages dominated by *Haplophragmoides wilberti* at elevations from 0.35 m to 0.42 m MTL constituted zone OI-3.

At Sand Point, 16 species of foraminifera were recognised at 21 stations across an elevational range from 0.10 m to 0.53 m MTL (Figure 4). Between 0 m and 73 m, the dominant species was *Ammonoastuta inepta* (up to 36 %). From 73 m to 137 m, *Miliammina fusca* (18 % to 59 %) was dominant, *Ammonoastuta inepta* (up to 25 %) was an important species between 137 m and 429 m. From 429 m to 448 m, *Jadammina macrescens* was the dominant species (up to 35 %) in association with *Ammonoastuta inepta* (up to 29 %). All of these intervals were associated with *Juncus roemerianus*. Seven samples, positioned between 448 m and 471 m in the freshwater upland contained no foraminifera. Based on foraminiferal assemblages, three elevation-dependent ecological zones were recognized at Sand Point. Between 0.10 m and 0.18 m MTL the dominant species was *Miliammina fusca* (zone SP-1). High proportions of *Ammonoastuta inepta* defined zone SP-2 (0.18 m to 0.30 m MTL). A *Jadammina macrescens*-dominated zone (SP-3) extended from 0.23 m to 0.35 m MTL. The samples in which foraminifera were absent were positioned above 0.35 m local MTL.

5 Discussion

5.1 Modern Distribution of Plants and Foraminifera

Salt-marsh plant communities form elevation-dependent, floral zones that are correlated to the frequency and duration of tidal inundation (e.g. Chapman, 1940; Redfield, 1972). This relationship has been exploited to reconstruct former RSL by identifying the floral zone represented by peat preserved in coastal sedimentary sequences (e.g. Shennan, 1986). We identified two salt-marsh zones on the basis of vascular plants. The low marsh at Oregon Inlet was vegetated by *Spartina alterniflora* (-0.10 m to 0.14 m MTL) and high marshes at Oregon Inlet and Sand Point were characterized by *Juncus roemerianus* (0.10 m to 0.42 m MTL). The limited number and narrow range of elevational zones are a factor of micro-tidal regimes at Oregon Inlet (0.35 m range) and Sand Point (0.17 m range).

In areas with larger tides, salt-marsh floral zones exist over an expanded elevational range. On the Atlantic coast of the USA, McKee and Patrick (1988, and references therein) showed that the average elevational range of *Spartina alterniflora* is 0.93 m (77 % of tidal range) but may be as much as 2.4 m (86 % of local tidal range). Adams (1963) showed a strong positive relationship between tidal range and the elevational extent of *Spartina alterniflora* at sites in North Carolina (up to 0.9m; 53 % of the tidal range). Similarly, larger tides were associated with an expanded elevational range of *Juncus roemerianus* (Adams, 1963). In the Cape Fear estuary of North Carolina, Woerner and Hackney (1997) recorded *Juncus roemerianus* occupying an elevational range of 0.65 m in a region with a tidal range of 1.43 m. Considerable variation in the elevational distribution of salt-marsh plants exists between sites and attests to the importance of local-scale factors (such as salinity and substrate) in determining floral zonation (e.g. McKee and Patrick, 1988; Woerner and Hackney, 1997). Gehrels (1994) noted differences in the elevational preference of plants between sites and argued that measurements of the elevational range of plant species (including our mean elevational ranges for *Spartina alterniflora* and *Juncus roemerianus*) should be considered a minimum because study of additional sites is likely to expand the observed range.

Foraminifera are more precise sea-level indicators than salt-marsh plants (e.g. Scott and Medioli, 1978, 1980; Gehrels, 1994), although direct comparisons in North America are few. At four sites in Maine, Gehrels (1994) showed that plants occupied wider and more variable elevational zones than salt-marsh foraminifera and concluded that the most precise (narrowest elevational ranges) foraminiferal assemblages were those from high marsh environments at the transition to freshwater upland. Foraminifera at Oregon Inlet and Sand Point also occupied narrower elevational ranges (as small as 0.07m, OI-3) than plants. In contrast, Patterson *et al.* (2000) concluded that the elevational range of plant and foraminiferal zones in British Columbia were similar. Jennings and Nelson (1992) showed that tidal flat, low marsh and high marsh zones could be delimited using either plants or foraminifera.

5.2 Sediment $\delta^{13}\text{C}$ Values as Sea-Level Indicators in North Carolina

Previous investigations of sediment $\delta^{13}\text{C}$ from salt marshes have alluded to the possibility of reconstructing RSL (e.g. Johnson *et al.*, 2007). The use of $\delta^{13}\text{C}$ and C:N values as sea-level indicators requires that they can be assigned an indicative meaning and identify salt-marsh zones in sedimentary sequences. Although there is not a simple linear correlation between salt-marsh surface elevation and bulk sediment $\delta^{13}\text{C}$ (Figure 5), there is a strong relationship between sediment $\delta^{13}\text{C}$ and plant species making it possible to distinguish between sediments derived from *Spartina alterniflora* (C_4) and *Juncus roemerianus* (C_3). Three low-marsh samples of surface sediment dominated by *Spartina alterniflora* at Oregon Inlet had $\delta^{13}\text{C}$ values between -17.6 ‰ and -16.2 ‰. In contrast, *Juncus roemerianus*-dominated environments at Oregon Inlet and Sand Point were characterized by sediment $\delta^{13}\text{C}$ values between -28.2 ‰ and -21.8 ‰ (average -26.3 ‰). This difference has been identified elsewhere in North Carolina and at other locations on the Atlantic and Gulf coasts of the USA. In the Pamlico River estuary of North Carolina, Craft *et al.* (1988) differentiated between sediments derived from C_4 plants, *Juncus*

roemerianus (-26 ‰) and forest (-27 ‰). Goni and Thomas (2000) identified sediments from forest (-28.8 ‰ to -27.5 ‰), *Juncus* (-23.8 ‰ to -21.4 ‰) and *Spartina* (-20.1 ‰ to -17.3 ‰) environments in South Carolina. A study by Gebrehiwet *et al.* (2008) in Georgia, showed a clear difference in $\delta^{13}\text{C}$ values for sediments from *Spartina alterniflora* and *Juncus roemerianus* zones. In Louisiana, Chmura *et al.* (1987) reported $\delta^{13}\text{C}$ values of -27.8 ‰, -22.1 ‰ and 16.2 ‰ for freshwater upland, intermediate and salt-marsh sediments, respectively. DeLaune (1986) recorded sediments derived from *Spartina alterniflora* in Louisiana as having an average $\delta^{13}\text{C}$ value of -16.5 ‰. Choi *et al.* (2001) showed that *Juncus roemerianus* salt-marsh sediments in Florida had an average $\delta^{13}\text{C}$ value of -27 ‰. In Massachusetts, Middleburg *et al.* (1997) distinguished between sediments from *Spartina*-dominated and more brackish environments.

Although $\delta^{13}\text{C}$ values from bulk-organic sediments differentiate *Spartina alterniflora* and *Juncus roemerianus* environments, $\delta^{13}\text{C}$ values associated with other plants on North Carolina salt marshes must be considered (Figure 3). Three specimens (two from Sand Point and one from Ocracoke) of *Distichlis spicata* (a high marsh, C_4 plant) yielded an average $\delta^{13}\text{C}$ value of -15.0 ‰ (-16.1 ‰ to -13.1 ‰), which is comparable to measurements from other studies (e.g. Ungar, 1991; Chmura and Aharon, 1995). *Spartina patens* is also a C_4 plant with a patchy distribution in high marshes (Adams, 1963). Three examples (from Cedar Island, Ocracoke and Swan Quarter) had an average $\delta^{13}\text{C}$ value of -13.2 ‰. According to Chmura and Aharon (1995) this species has $\delta^{13}\text{C}$ values of -13.6 ‰ to -11.7 ‰. *Spartina cynosuroides* is a low salinity, high marsh plant (C_4); two examples from Cedar Island had $\delta^{13}\text{C}$ values of -12.6 ‰ and -12.8 ‰. Chmura and Aharon (1995) listed *Spartina cynosuroides* as having a $\delta^{13}\text{C}$ range of -13.6 ‰ to -12.0 ‰. *Distichlis spicata*, *Spartina cynosuroides* and *Spartina patens* have plant tissue $\delta^{13}\text{C}$ values that are comparable to that of *Spartina alterniflora* but they are found in high rather than low marshes. The problem that the wide distribution of these species presents for RSL reconstruction in the southeastern USA was highlighted by Chmura and Aharon (1995), who

recognized that high and low marsh C₄ plants may be indistinguishable on the basis of plant or sediment $\delta^{13}\text{C}$ and C:N values. Regional differences in the distribution of C₄ plants and $\delta^{13}\text{C}$ values must be considered before making paleoenvironmental interpretations (Chmura and Aharon, 1995).

In the same way, overlap of sediment $\delta^{13}\text{C}$ values from high marsh (*Juncus roemerianus*) and freshwater upland zones at Oregon Inlet and Sand Point makes differentiating them difficult. The average $\delta^{13}\text{C}$ of bulk sediments differs only slightly (-26.3 ‰ for *Juncus roemerianus* and -27.8 ‰ for freshwater upland zones) and their ranges overlap. Whereas *Juncus roemerianus* sediments displayed $\delta^{13}\text{C}$ values from -28.2 ‰ to -21.8 ‰, freshwater upland sediments ranged from -28.1 ‰ to -26.8 ‰. Several studies have distinguished these environments using sediment $\delta^{13}\text{C}$ values (e.g. Chmura *et al.*, 1987; Craft *et al.*, 1988; Chmura and Aharon, 1995; Goni and Thomas, 2000), but results from this study show that this distinction is difficult.

5.3 Bulk Sediment and Vegetation $\delta^{13}\text{C}$, C:N

We investigated whether surface sediments adequately represented the dominant type of vegetation at the time of accumulation by comparing $\delta^{13}\text{C}$ and C:N values of *Juncus roemerianus* plants to the surface sediment they were collected from at Sand Point (Figure 6). The average difference between plant and surface sediments was 0.8 ‰, although a single sample recorded a difference of 4 ‰ (Figure 6a). This change is not sufficient to cause samples from *Juncus roemerianus* zones to be mis-classified (figure 3) and is similar to differences reported elsewhere (e.g. Chmura *et al.*, 1987). In North Carolina, Craft *et al.* (1988) concluded that the $\delta^{13}\text{C}$ of *Juncus roemerianus* sediments (-23.8 ‰) was similar to plant material (-26.0 ‰). Goni and Thomas (2000) showed little difference between $\delta^{13}\text{C}$ from *Juncus roemerianus* plants (-24.9 ‰) and sediments (-23.8 ‰). Similarly, Johnson and Calder (1973) and Hackney

and Haines (1980) reported little difference between sediment and plant $\delta^{13}\text{C}$ values in *Juncus roemerianus* zones.

In contrast, there is a clear difference between $\delta^{13}\text{C}$ of *Spartina alterniflora* plants and surface sediments (Figure 6c). At Oregon Inlet, low marsh, *Spartina alterniflora* sediments had $\delta^{13}\text{C}$ values from -17.6 ‰ to -16.2 ‰ (Figures 2 and 6c), whilst plants (from Ocracoke, Swan Quarter NWR and Cedar Island) had less depleted values ranging from -13.1 ‰ to -12.6 ‰ (Figures 3 and 6c). *Spartina alterniflora*-derived sediments commonly have more negative $\delta^{13}\text{C}$ values than living plant tissue from this species. In North Carolina, Craft *et al.* (1988) showed that *Spartina alterniflora* plants had an average $\delta^{13}\text{C}$ value of -12.0 ‰, whilst corresponding surface sediments had an average $\delta^{13}\text{C}$ value of -18.4 ‰. Goni and Thomas (2000) reported changes of 4.0 ‰ to 6.8 ‰ between *Spartina alterniflora* tissue and sediment. Chmura *et al.* (1987) found that sediments were depleted by up to 4 ‰ in comparison with plant material. In Georgia, Fogel *et al.* (1989) recorded sediments that were up to 5.5 ‰ more depleted than *Spartina alterniflora* plants. Changes in $\delta^{13}\text{C}$ values between plants and bulk-organic sediment are probably not sufficiently large to cause *Spartina alterniflora* environments to be misidentified and assigned an erroneous indicative meaning (Figures 3 and 5).

Differences between plant and sediment $\delta^{13}\text{C}$ values may be caused by decomposition during accumulation of organic material (Lamb *et al.*, 2006). According to Ember *et al.* (1987), *Spartina alterniflora* values for plant tissues and bulk sediments are different due to fractionation of organic carbon within the living plant and the subsequent preferential breakdown of cellulose over lignin. Lignin $\delta^{13}\text{C}$ is typically more depleted than cellulose $\delta^{13}\text{C}$ or the $\delta^{13}\text{C}$ from the plant as a whole (Vane *et al.*, 2003; Lamb *et al.*, 2006). During decomposition, lignin is preferentially concentrated in surface sediments resulting in a relative depletion of sediment $\delta^{13}\text{C}$ (Chmura *et al.*, 1987; Chmura and Aharon, 1995; Mallamud-Roam and Ingram, 2001). Alternatively, $\delta^{13}\text{C}$ of

bulk sediments may differ from plant tissue due to allochthonous carbon inputs. Sources include fluvial or marine particulate and dissolved organic carbon (Lamb *et al.*, 2006). Low marsh settings are most likely to be inundated by astronomical tides and storms, whilst high marsh settings are more likely to receive inputs of terrestrial organic carbon. However, Chmura *et al.* (1987) concluded that local plants were the main source of organic carbon in salt marshes and that sediment $\delta^{13}\text{C}$ values were representative of the dominant vegetation. On longer timescales, two studies in the San Francisco Bay estuary (Byrne *et al.*, 2001; Malamud-Roam and Ingram, 2004) showed that salt-marsh zones could be reliably identified in sediments more than 3000 years after deposition, whilst Lamb *et al.* (2007) compared downcore $\delta^{13}\text{C}$ values in sediments up to 3300 years old to foraminiferal assemblages and concluded that there was little fractionation.

C:N ratios are strongly influenced by post-depositional processes. At Sand Point, organic sediment C:N ratios were lower than those recorded in plants (Figure 6b). Goni and Thomas (2000) demonstrated the tendency for forest, *Juncus*- and *Spartina*-derived sediments to have comparable C:N ratios despite large differences in the C:N ratio of source vegetation. The similarity in bulk sediment C:N ratios is a consequence of nitrogen retention coupled with carbon loss through oxidation of plant material during decomposition (Ember *et al.*, 1987; Chmura *et al.*, 1987). The size of this change makes it difficult to reconstruct the salt-marsh zone in which bulk-organic sediments formed because C:N ratios from different zones converge during early diagenesis, rendering them unsuitable for reconstructing former RSL.

5.4 Implications for Reconstructing Holocene Relative Sea Level

We examined the utility of sediment $\delta^{13}\text{C}$ as a sea-level indicator by reconstructing RSL from published sea-level data. Horton *et al.* (2009) compiled a database of validated North Carolina Holocene sea-level index points and data points that constrain the maximum and minimum

elevation of RSL (freshwater and marine limiting points). We considered a sub-sample of eight sea-level index points and three freshwater limiting points. Estimates of the indicative meaning were based on the elevational range of modern salt-marsh plants and recognition of these floral zones from the analogy between modern and fossil sediment $\delta^{13}\text{C}$ values (Table 1; Figure 7).

Sediment $\delta^{13}\text{C}$ values from -26.7‰ to -21.8‰ were considered to represent a *Juncus roemerianus* floral zone. Freshwater upland zones are identified by sediment $\delta^{13}\text{C}$ values more depleted than -28.2‰ . Sediments with intermediate values could be from either zone and are assigned an indicative meaning reflecting this uncertainty. These values may only be appropriate for the study region (Chmura and Aharon, 1995) and additional studies from meso- and macrotidal regions incorporating high, low and pioneer marshes and tidal flat environments would be appropriate and necessary for efforts to reconstruct RSL from other regions. From leveling of floral zone boundaries at Oregon Inlet and Sand Point we assigned *Spartina alterniflora* zones an indicative meaning of $0.02\text{ m MTL} \pm 0.12\text{ m}$ and *Juncus roemerianus* zones were assigned an indicative meaning of $0.26\text{ m MTL} \pm 0.16\text{ m}$. In the case of C_4 plants, sediment $\delta^{13}\text{C}$ values cannot distinguish between specific plants, therefore we gave them an indicative meaning of $0.16\text{ m MTL} \pm 0.26\text{ m}$, because they can occupy the full elevational range of salt-marshes in North Carolina. For comparison, alternative estimates of the indicative meaning were produced using a transfer function based upon the modern distribution of foraminifera in the Albemarle-Pamlico estuarine system (Kemp *et al.*, 2009). This statistical technique has become common-place for reconstructing RSL from salt-marshes (Gehrels, 2007).

Sea-level index points 1 to 3 (table one) had $\delta^{13}\text{C}$ values between -25.5‰ and -21‰ and represent *Juncus roemerianus* zones. Foraminiferal data provided estimated reference water levels (RWL) between 0.20 m and 0.29 m MTL with an average indicative range (IR) of ± 0.05

m. Index point 4 was identified as being from either a *Juncus roemerianus* or freshwater upland zone because it had an intermediate $\delta^{13}\text{C}$ value of -26.67‰ . However, foraminifera indicated that it formed in brackish rather than freshwater conditions and was assigned an indicative meaning of $0.19\text{ m MTL} \pm 0.05\text{ m}$. Sea-level index points 5 to 8 had $\delta^{13}\text{C}$ values typical of C_4 vegetation (Table 1) and were assigned an indicative meaning spanning the entire elevational range of salt-marsh vegetation (Figure 7; Table 1). Foraminifera associated with these samples estimated RWLs from 0.12 m to 0.25 m MTL with an average IR of $\pm 0.05\text{ m}$ (Figure 7; Table 1). The RWL estimated for sea-level index points using $\delta^{13}\text{C}$ values are not significantly different from transfer function estimates. Foraminifera are more precise sea-level indicators that are able to estimate the indicative meaning of samples from all salt-marsh zones. In contrast, sediment $\delta^{13}\text{C}$ values are restricted to describing broad changes in RSL by identifying sea-level index points (with large uncertainties) derived from salt marshes dominated by C_4 vegetation.

Freshwater limiting points are derived from supra-tidal environments and provide an important constraint on the upper limit of former RSL (Shennan, 2007). We considered three samples that were defined as freshwater limiting points by Horton *et al.* (2009) on the basis of diatom and pollen assemblages and an absence of foraminifera (Mallinson *et al.*, 2005; Culver *et al.*, 2008). Using this microfossil evidence, we recognized samples 9, 10 and 11 as freshwater limiting points (Table 1, Figure 7). The reported $\delta^{13}\text{C}$ values of these samples were -28.0‰ , -27.21‰ and -26.35‰ . Due to the difficulty in separating freshwater upland and *Juncus roemerianus*-dominated zones, samples with $\delta^{13}\text{C}$ values between -28.5‰ and -26.0‰ may represent either zone and were assigned an indicative meaning reflecting this uncertainty (Table 1, Figure 7).

5.5 Refinement of $\delta^{13}\text{C}$ and C:N as Sea-Level Indicators

A key issue in using bulk-organic sediment $\delta^{13}\text{C}$ and C:N values to reconstruct sea-level is the difficulty in identifying salt-marsh zones dominated by a particular plant species. We have

shown that it is possible to recognize sediments derived from C₃ or C₄ zones and that the application of this approach is limited by the broad elevational tolerance of C₄ plants and problems distinguishing between freshwater upland and salt-marsh C₃ plant zones. Measured values of δ¹³C and C:N in bulk sediment incorporate the influence of allochthonous material and plant decomposition, which may distort the signal from the dominant plant community.

One approach that might overcome these difficulties is to analyze δ¹³C values from a specific fraction of the sediment, which may include organic components from only the dominant plant community. For example, some components may consist of the fraction that is soluble in common organic solvents (lipids, pigments, flavonoids) or the insoluble fraction such as lignin, tannins and structural polysaccharides (Stephenson *et al.*, 2005, 2008). Molecular level studies of lipids and major cell wall polymers (e.g. lignin, tannins and structural polysaccharides) may be potential indicators of plant source and vegetation zones (Benner *et al.*, 1991; Goni *et al.*, 2000; Herenes, 2001). In particular, lignin is well preserved in salt-marsh sediments (Vane *et al.*, 2003, 2005, 2006; Lamb *et al.*, 2007). Analytical pyrolysis, tetra alkyl chemolysis, and alkaline CuO oxidation are frequently applied to cleave bonds within the lignin structure and yield monomers that can be separated and identified by gas-chromatography mass-spectrometry (GC/MS). Assuming the distribution and quantity of monomers can be related to plant species, they may be used to identify the dominant type of vegetation in sediments (Goni *et al.*, 2000; Vane, 2003)

A second possible approach is to analyze sediment δ¹³C values retained in particular organic compounds, namely *n*-alkanes. Compound-specific isotopic analysis (CSIA) of long chain *n*-alkanes *n*C₂₁-*n*C₃₃ in salt-marsh sediments has been used to distinguish specific plant communities in northeastern USA (Wang *et al.*, 2003 in Massachusetts; Tanner *et al.*, 2007 in Maine). Gas chromatography isotope-ratio mass spectrometry (GC-IRMS) of individual *n*-

alkanes extracted from living plants showed that the nC_{27} and nC_{29} homologues were abundant in all plants and could distinguish *Spartina alterniflora* and *Spartina patens* (C_4) from C_3 plants such as *Juncus* spp. based upon a 3.0 to 6.4 ‰ minimum difference (Tanner *et al.*, 2007). However, other homologues such as nC_{31} were not present in all plants and are variable in absolute concentration; high concentrations of compounds are generally considered a prerequisite for GC/IRMS analysis. Although detailed molecular techniques show considerable promise as tools to identify vegetation zones, they are presently more timing consuming and costly than carbon isotope analysis of bulk-organic sediment.

6 Conclusions

We investigated the use of bulk-organic sediment geochemistry as a means of identifying floral zones that could be used as sea-level indicators. $\delta^{13}C$ values and C:N ratios were measured along modern transects of surface sediment at Oregon Inlet and Sand Point (North Carolina, USA) and compared to elevational zones defined by plants and foraminiferal assemblages. Sediment $\delta^{13}C$ values do not have a simple linear correlation with elevation but can distinguish zones dominated by *Spartina alterniflora* (C_4 photosynthetic pathway, $\delta^{13}C$ values from -17.6 ‰ to -16.2 ‰) and *Juncus roemerianus* (C_3 photosynthetic pathway $\delta^{13}C$ values from -28.2 ‰ to -21.8 ‰). The elevational range of the two floral zones at these sites reflects the microtidal nature of the region. Comparison between the geochemical characteristics of *Juncus roemerianus* plants and surface sediments revealed that there is little change in $\delta^{13}C$ values during diagenesis (average 0.8 ‰). Based on three samples, the difference between *Spartina alterniflora* plants and sediment was approximately 4 ‰. In contrast, C:N ratios are significantly altered during early diagenesis, causing low marsh, high marsh and freshwater upland sediments to have similar values.

The elevational distribution of common salt-marsh plants was used to estimate the indicative meaning of sea-level index points using sediment $\delta^{13}\text{C}$ values to identify floral zones. The use of sediment $\delta^{13}\text{C}$ values as independent sea-level indicators is complicated in North Carolina by C_4 plants (such as the low marsh species *Spartina alterniflora* and high marsh species including *Distichlis spicata*, *Spartina cynosuroides* and *Spartina patens*) occupying the full elevational range of salt-marsh environments. Uncertainty in differentiating between sediments derived from these plant species on the basis of $\delta^{13}\text{C}$ values results in a large indicative range. Furthermore, *Juncus roemerianus*-dominated sediments from high marsh settings cannot be adequately distinguished from the sediment of freshwater upland zones.

In instances where microfossil assemblages are absent from the fossil record or have undergone significant post-depositional modification, $\delta^{13}\text{C}$ values may help distinguish the sediments of former high and low marshes. Refined analysis using $\delta^{13}\text{C}$ values from specific fractions of sediment may increase the possible precision of RSL reconstructions from geochemical proxies preserved in bulk-organic sediments by recognizing particular plant species.

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Table One

The indicative meaning of sea-level data estimated from $\delta^{13}\text{C}$ values and foraminifera

No.	Type	Lab Code	$\delta^{13}\text{C}$ (‰)	Description	$\delta^{13}\text{C}$ RWL (m MTL)	IR (\pm m)	Foraminifera RWL (m MTL)	IR (\pm m)
1	SL Index Point	OS-43066	-24.80	<i>Juncus</i>	0.26	0.16	0.20	0.04
2	SL Index Point	OS-43068	-25.50	<i>Juncus</i>	0.26	0.16	0.21	0.05
3	SL Index Point	OS-43069	-21.90	<i>Juncus</i>	0.26	0.16	0.29	0.06
4	SL Index Point	OS-58897	-26.67	<i>Juncus</i> /Fresh	Above 0.05	-	0.19	0.05
5	SL Index Point	OS-58896	-14.08	C ₄	0.16	0.26	0.17	0.05
6	SL Index Point	OS-58712	-13.40	C ₄	0.16	0.26	0.25	0.05
7	SL Index Point	OS-58711	-13.28	C ₄	0.16	0.26	0.18	0.05
8	SL Index Point	OS-58710	-13.78	C ₄	0.16	0.26	0.12	0.05
9	Fresh Limiting	Beta-168060	-28.00	<i>Juncus</i> /Fresh	Above 0.10	-	Above 0.35*	-
10	Fresh Limiting	OS-36098	-27.21	<i>Juncus</i> /Fresh	Above 0.10	-	Above 0.35*	-
11	Fresh Limiting	OS-36100	-26.35	<i>Juncus</i> /Fresh	Above 0.10	-	Above 0.35*	-

Interpretation of Holocene sea-level index points and freshwater limiting points. For each sample, a Reference Water Level (RWL) and Indicative Range (IR) were estimated using sediment $\delta^{13}\text{C}$ values and a foraminifera-based transfer function. *The RWL assigned to freshwater limiting points reflects the quantitative diatom evidence (with an absence of foraminifera) reported in Horton *et al.* (2009).

Figure Captions

Figure 1: (A) Location of the Oregon Inlet and Sand Point sites in coastal North Carolina. Foraminifera, $\delta^{13}\text{C}$ and C:N were measured in samples along two transects (dashed lines, X to Y) at (B) Oregon Inlet and (C) Sand Point. Sample elevations were established by leveling to geodetic benchmarks. (D) The components of *indicative meaning*, which defines the relationship between a sea-level indicator and elevation in the tidal frame. HAT = Highest Astronomical Tide, MHHW = Mean Higher High Water, MTL = Mean Tide Level.

Figure 2: Pattern of $\delta^{13}\text{C}$, C:N, Total Organic Carbon (TOC) and grain size of bulk-organic sediment along modern transects at Oregon Inlet and Sand Point. Median grain size (d_{50}) in μm is shown by dots and percentage clay by volume is shown by vertical grey bars. Solid circles show samples collected in *Juncus roemerianus* environments; at Oregon Inlet samples from the *Spartina alterniflora* (SA) low marsh are shown as hollow circles; at Sand Point these represent samples from the freshwater upland.

Figure 3: $\delta^{13}\text{C}$ and C:N values measured from modern specimens of common salt-marsh plants and bulk-organic sediment in North Carolina. Grey boxes represent the range of values thought to be representative of C_3 and C_4 plants (after Lamb *et al.*, 2006). Dashed lines are the average $\delta^{13}\text{C}$ of C_4 (-12 ‰; Schlesinger, 1997) and C_3 (-27 ‰; Choi *et al.*, 2001) plants.

Figure 4: (A) Distribution of samples, vegetation and dominant salt-marsh foraminifera along modern transects at Oregon Inlet and Sand Point. Only species that dominant foraminiferal zones are presented. Solid circles show samples collected in *Juncus roemerianus* environments; at Oregon Inlet samples from the *Spartina alterniflora* (SA) low marsh are shown as hollow circles; at Sand Point these represent samples from the freshwater upland. Elevation-dependent ecological zones of foraminifera at Oregon Inlet (OI) and Sand Point (SP) defined by cluster analysis are presented for each site.

Figure 5: (A) The relationship between surface sediment $\delta^{13}\text{C}$ values and elevation at Oregon Inlet and Sand Point. Samples are differentiated on the basis of the floral environment from which they were collected. (B) The indicative meaning assigned to *Juncus roemerianus* (Jr), *Spartina alterniflora* (Sa), C_4 and freshwater upland vegetation zones based on levelling of the boundaries between floral zones. The elevational ranges are a minimum estimate.

Figure 6: Comparison of plant and bulk sediment $\delta^{13}\text{C}$ values (A) and C:N ratios (B) from a *Juncus roemerianus*-dominated salt marsh at Sand Point. (C) Non-paired samples of *Spartina alterniflora* plants (open circles; Ocracoke, Swan Quarter and Cedar Island) and sediments (filled circles; Oregon Inlet).

Figure 7: Indicative meanings of eight sea-level index points and three freshwater limiting points from a database of North Carolina Holocene sea-level data. Estimates of indicative meaning were made on the basis of sediment $\delta^{13}\text{C}$ values (grey boxes) and a foraminifera-based transfer function (white boxes). Hashed boxes represent the inference made from diatom evidence that the samples were formed in a freshwater upland environment. Arrows represent show that estimates associated with freshwater limiting points are minimums. MTL = Mean Tide Level.

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