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CARBON ISOTOPES ($\delta^{13}C$ AND $\Delta^{14}C$) IN SHELL CARBONATE, CONCHIOLIN, AND SOFT TISSUES IN EASTERN OYSTER (*CRASSOSTREA VIRGINICA*)

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ABSTRACT. Biogeochemical analyses of eastern oysters (*Crassostrea virginica*) are frequently included in environmental monitoring and paleoecological studies because their shells and soft tissues record environmental and dietary signals. Carbon isotopes in the mineral phase of the shell are derived from ambient bicarbonate and dissolved inorganic carbon (DIC), while organic carbon present in soft tissue is of dietary origin. Mineral-bound organic matter within the carbonate shell matrix ("conchiolin") is studied less frequently. The purpose of this study was to compare carbon isotope composition ($\delta^{13}C$ and $\Delta^{14}C$) of conchiolin to those of shell carbonates and soft tissues in eastern oysters and assess the extent to which conchiolin can provide insight into paleoecological records. Eleven oyster specimens were live-collected from Apalachicola Bay, USA, as well as a set of environmental samples (water, sediment, and terrestrial plants). Overall, the $\delta^{13}C$ values in all studied oyster tissue types record environmental signals related to carbon sources, with conchiolin being enriched in ^{13}C by an average of 2.3‰ relative to bulk soft tissues. $\Delta^{14}C$ values in oyster shell carbonates generally reflect the marine versus riverine source of DIC, while conchiolin $\Delta^{14}C$ values are impacted by variable relative contributions of young and old organic matter. Environmental samples indicate a significantly large difference in $\Delta^{14}C$ among sources, from –127‰ in particulate organic matter to approximately +15‰ in DIC. Conchiolin is significantly depleted in ^{14}C relative to other tissue types, by as much as 56.6‰, posing a major obstacle to the use of conchiolin as an alternative material for radiocarbon dating.

KEYWORDS: carbon cycling, environmental reconstruction, marine shell, shell.

INTRODUCTION

Biogeochemical analyses of mollusks are used frequently in environmental, paleontological, archaeological, and paleoenvironmental studies. Typically, such studies take advantage of the ecological and dietary signals that are recorded in the soft tissues of the animal or the climatic and environmental signals that are recorded in the carbonate mollusk shell. Mineral-bound organic matter within the carbonate shell matrix ("conchiolin") has, historically, been studied less frequently. The purpose of this study was

1. to compare carbon isotope signatures ($\delta^{13}C$ and $\Delta^{14}C$) in shell carbonate, conchiolin, and soft tissues in eastern oyster (*Crassostrea virginica*) from Apalachicola Bay, USA;
2. to assess the usefulness of oyster conchiolin as an ecological and dietary proxy analogous to soft tissue; and
3. to evaluate the potential of oyster conchiolin as an alternative material for radiocarbon (^{14}C) dating.

Eastern oyster is considered a "keystone species" for estuarine environments along the eastern coast of North America. It has been an important food resource for native peoples in the region for at least the past 8000 years, and has continued to be an important fishery and food source in historical and modern times. As such, oyster shell middens are a potentially powerful environmental and ecological archive that extend over the entire history of human occupation in eastern North America.

Shell carbonates are in isotopic equilibrium with seawater. $\delta^{13}C$ and ^{14}C in aquatic mollusk shells are typically assumed to reflect the carbon isotopic composition of the dissolved inorganic carbon (DIC) in the surrounding water, although a small proportion of shell carbon is obtained

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from metabolism (Tanaka et al. 1986; Gillikin et al. 2007; McConnaughey and Gillikin 2008). Stable carbon analyses of shell carbonates are used in paleothermometry and environmental reconstruction (Jones 1983; Kirby et al. 1998; Surge et al. 2003; Gillikin et al. 2005; Harding et al. 2010), and even for archaeological reconstructions of human foraging patterns (Andrus and Crowe 2000; Andrus and Thompson 2012). ^{14}C in biogenic carbonates are typically measured for ^{14}C dating, but also play an important role in modeling carbon cycling in the global ocean (e.g., Druffel 1997; Furla et al 2000).

$\delta^{13}C$ and ^{14}C of organic tissues of animals reflect the isotopic composition of the organic compounds at the base of their food web, with predictable levels of isotopic fractionation during metabolic processes (Peterson and Fry 1987). Thus, isotopic analyses of animal tissues can be used to investigate the sources of carbon and nitrogen supporting an ecosystem, e.g., terrestrial versus marine, or C_3 versus C_4 terrestrial plants (DeNiro and Epstein 1978; Rounick and Winterbourn 1986; Farquhar et al. 1989). Additionally, ^{14}C has been used to study the introduction of petro-based carbon into marine foodwebs (Chanton et al. 2012; Cherrier et al. 2014). Filter-feeding bivalves continuously sample local water column conditions through feeding. Carbon isotopes in bivalve soft tissues provide time-integrated information of feeding relationships and carbon flows through aquatic food webs (Peterson and Fry 1987; Post 2002; Fukumori et al. 2008).

Stable isotopic analyses of conchiolin have been shown to record many of the same environmental and dietary signals as soft tissues (Watanabe et al. 2009; Kashiyama et al. 2010; Ellis et al. 2014). Conchiolin is tightly bound into the mineral structure, sheltered from the external environment and thus relatively protected from diagenetic alteration (Sykes et al. 1995). Ellis and colleagues (2014) described a 2‰ enrichment in $\delta^{13}C$ in oyster shell conchiolin over bulk soft tissue, suggesting that measurements of both are useful means of assessing carbon sources supporting estuarine production.

In this paper we present data supporting the 2‰ $\delta^{13}C$ conchiolin–tissue offset described by Ellis et al. (2014). ^{14}C measurements of conchiolin, shell carbonate, and bulk soft tissue of eleven oyster specimens indicate that the nearshore estuarine carbon reservoir is at near-equilibrium with atmosphere, complicating efforts to discern terrestrial from marine influences in shell ^{14}C. Nonetheless, preliminary data suggest that both $\delta^{13}C$ and $\Delta^{14}C$ in oyster conchiolin reflect variables that are expected to be useful for environmental and archaeological applications.

Oyster Biology

Eastern oysters thrive in a wide range of salinity and temperature regimes, inhabiting estuarine environments from the Gulf of Saint Lawrence to the Yucutan Peninsula. Oysters are sessile filter feeders, drawing water over gills with beating cilia. Suspended plankton, bacteria, detritus particles, etc., are trapped in the mucus of a gill, and from there are transported to the mouth, where they are eaten, digested, and expelled as feces or pseudofeces (Galstoff 1964; Newell and Jordan 1983). Gas exchange occurs via the gill plates, one pair on each side, and across their mantles. The mantle is a soft, bisymmetrical membrane that covers the visceral mass. The visceral mass consists of the esophagus, stomach, and intestine embedded in connective tissue. The mantle cavity is filled with seawater, an adaptation to life in the intertidal zone that permits the animal to survive many days of exposure (Galstoff 1964:68). The adductor muscle controls the opening and closing of two asymmetrical valves.

Adult oyster shells (Figure 1) are composed almost entirely of calcite, with aragonite present in the muscle scars and part of the ligament (Stenzel 1963). The shells are composed of an outer

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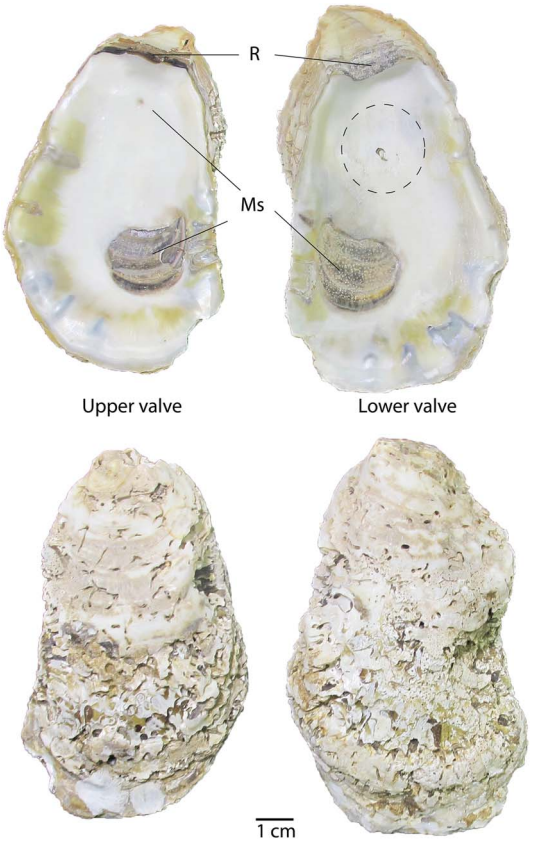

Figure 1 Live-collected *Crassostrea virginica* shells from Apalachicola Bay, USA, with aragonitic shell components (R = resilium; Ms = muscle scar) noted. Sample target area indicated with dashed circle.

prismatic layer and an inner foliated layer. The foliated layer has a plywood-like structure consisting of sheets of calcite separated by an organic matrix (Sikes et al. 1998; Lee and Choi 2007). The organic matrix, conchiolin, contributes typically less than 5% of oyster shell weight (Galstoff 1964:39) and functions in modulating biomineralization. Two models have been proposed for molluscan shell formation. The matrix model suggests that biomineralization occurs in a matrix of proteins secreted by the mantle. The cellular model posits that crystals are formed in haemocytes and then deposited at the mineralization front (Zhang et al 2012). New layers of shell form on the interior surface of the valves, covering previous layers and accreting new shell beyond the margins of previous layers, resulting in terrace-like growth increments (Carriker et al. 1980).

STUDY AREA

The Apalachicola estuary (Figure 2) has supported a productive oyster industry since the mid-19th century. However, oysters have been the center of the local economy for thousands of years in this region, and this legacy is written on the landscape. Over 60 ancient oyster shell midden sites are known along the bayshore and barrier islands of the Apalachicola

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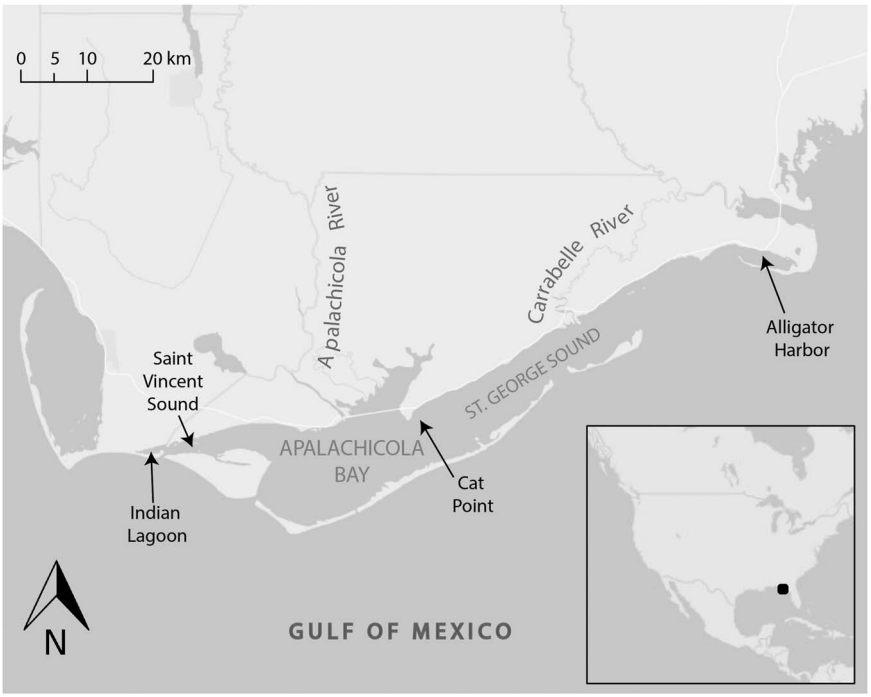

Figure 2 Map of the study area showing location of four sampling localities in Apalachicola Bay, USA.

estuary, the earliest dating to ca. 3000 BP (White 2014). Oysters are one of the most abundant archaeological materials in this region, making them a potentially important environmental archive.

The Apalachicola Bay system is a shallow, river-dominated estuary located in the panhandle of Florida, USA. The Apalachicola River is the dominant source of freshwater to the system, with maximum river flow occurring in the late winter to early spring months, highly correlated with the wet season period for the area. Hydrology in the system is influenced by riverine discharge, current flows, and wind. Spatially, the system varies physiochemically in current velocities, salinity, and color, whereas temperature is generally uniform. Bottom water salinities tend to be lowest at Cat Point, as low as ~7‰ during periods of high discharge, where the bar is most impacted by river flow and discharge (Livingston et al. 2000). Increased salinities are found at sites farther from the river, typically around 20‰ in Indian Lagoon and in excess of 30‰ at Alligator Harbor (Sastry 1963). Thus, the sampling localities were classified as "river-influenced" (Saint Vincent Sound and Cat Point) and "marine-influenced" (Indian Lagoon and Alligator Harbor).

While the Apalachicola River dominates the salinity regime of the system, the river, floodplain, neighboring swamps, and the estuary, together act as significant sources of organic matter. Transport of terrestrial organic matter to the Apalachicola Bay system is influenced by periodic inundation of its floodplain. The Apalachicola floodplain is characterized by bottom-land hardwood and cypress forests, which contribute an increased and substantial amount of organic carbon to the bay system during periods inundation (Elder and Cairns 1982). These carbon loads are introduced into the system by the river and neighboring swamps, such as Tate's Hell

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Swamp (Livingston et al. 2000). Within the bay system, the distribution of dissolved organic matter (DOM) is regulated by hydrodynamic forces (i.e. wind, river, and tides). Generally, however, terrestrial organic matter predominates proximal to the Apalachicola River mouth. Here, secondary producers, such as oysters, depend on terrestrial organic matter to a greater degree, relative to organisms growing in other locations of the bay system where estuarine primary production is a more important food source (Chanton and Lewis 2002).

MATERIALS AND METHODS

Live *C. virginica* samples were harvested in December of 2016 from four sites in the northern Gulf of Mexico, within the Apalachicola Bay system: Indian Lagoon, St. Vincent Sound, Area 1622 (Cat Point), and Alligator Harbor (Figure 2). In April 2017, additional oyster and environmental samples (water, sediment, and terrestrial plants) were collected from Alligator Harbor for comparison.

C. virginica were collected and returned to the lab, where the oysters were shucked. The soft-tissue was rinsed 3 times with DI water, freeze dried and pulverized in a Spex Mill. A subsection of the soft-tissue was treated with cold, 1 N HCl to remove any carbonate salts, centrifuged, rinsed with DI an additional 3 times, and freeze dried. The acid-treated soft tissue samples were then loaded into Pyrex tubes with excess CuO and combusted at 575°C to produce CO_2.

The shells were washed exhaustively in DI water with sonication. Each rinse cycle lasted 15 minutes, after which the water was decanted, new DI water added and sonication commenced. The rinsing procedure was repeated until the water was clear and burrowing shrimp were not observed under microscopic evaluation. The inner surface of valves was sampled by carefully scraping the nacre near the hinge using a Dremmel tool, avoiding the ligament. The inorganic and organic fractions were then isolated.

The inorganic shell fraction was sampled by reacting approximately 10 mg of powdered shell with 100% H_3PO_4 in evacuated reaction vessels to produce CO_2. A separate subsection of 50 mg of powdered shell was treated for analysis of the insoluble organic shell fraction, which consists predominately of conchiolin. Powdered shell was acidified with 12 N HCl, thereby removing the carbonate phase and soluble organic molecules, including acid-soluble proteins, soluble amino acids, and carbohydrates. The acidified samples were neutralized by rinsing voluminously with Milli-Q water. The samples, consisting of insoluble organic matter, were then transferred to Pyrex tubes where they were dried and sealed. The samples were combusted at 575°C with excess CuO to produce CO_2. In cases with extremely low yields (< 4 μmol CO_2), the procedure was repeated with approximately 150 mg of shell to increase sample size. In these cases (UGAMS-27953, -27955, and -27956), the average $\delta^{13}C$ and $\Delta^{14}C$ values are reported.

Environmental samples included water, sediment, and terrestrial plants from the marine-influenced Alligator Harbor locality. A1-L water sample was split for separate analyses of DIC and suspended organic matter. Approximately 50 mL of water was acidified in 85% H_3PO_4 in an evacuated flask to recover CO_2 from DIC in the seawater. The remaining water sample was filtered through pre-baked glass fiber filters to recover suspended organic matter in the 1–20 μm range, the size particles typically ingested by oysters (Baldwin and Newell 1995). The precipitate was treated with cold, 1 N HCl to remove any carbonate salts. The sediment sample was filtered to isolate the 1–20 μm fraction, and treated with 1N HCl at 80°C for one hour to remove carbonates. Plant samples were freeze-dried, pulverized in a Spex mill, and treated with 1 N HCl to remove carbonates. Dried subsamples of the water filtrate, sediment, and plant tissues were loaded into quartz tubes with excess CuO and combusted at 900°C to produce CO_2.

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CO_2 samples were cryogenically purified from the other reaction products and the sample $^{13}C/^{12}C$ ratios were measured using a MAT-253 stable isotope ratio mass spectrometer and expressed with respect to VPDB, with an error of less than 0.1‰. CO_2 samples were catalytically converted to graphite (Cherkinsky et al. 2010), and graphite $^{14}C/^{13}C$ ratios were measured in 2017 using the NEC 500 kV Tandem Pelletron accelerator mass spectrometer at the Center for Applied Isotope Studies, University of Georgia, USA. The sample ratios were compared to the ratios measured from oxalic acid I (NBS SRM 4990). Size-matched standards were prepared for conchiolin samples (Cherkinsky et al. 2013). For carbonate samples, Carrara marble (IAEA C1) was used as the background, and travertine (IAEA C2) was used as a secondary standard. ^{14}C values are reported according to $\Delta^{14}C$ notation (Stuiver and Polach 1977), corrected for natural isotope fractionation using the isotope ratio mass spectrometer value.

Statistical tests were performed in RStudio Version 0.98.507. Pearson's product-moment correlation tests were used to test for correlations in isotopic values among tissue types. Welch Two-sample t-tests were used to determine whether the mean isotopic values of river-influenced and marine-influenced sampling localities are equal.

RESULTS AND DISCUSSION

Stable Carbon Isotopes ($\delta^{13}C$)

Stable carbon isotope values for shell carbonates, conchiolin, and soft tissues are presented in Table 1 and Figures 3 and 4. Shell carbonate $\delta^{13}C$ values span a narrow range, from –2.6‰ to –5.8‰, which is comparable to the DIC value from Alligator Harbor (Table 2), and within

Table 1 Carbon isotope values of *Crassostrea virginica* tissues from Apalachicola Bay, USA.

		$\delta^{13}C$ (‰)			$\Delta^{14}C$ (‰)		
Locality	UGAMS #	Shell carbonate	Conchiolin	Soft tissue	Shell carbonate	Conchiolin	Soft tissue
Marine-influenced							
Indian Lagoon	27953	–3.58	–22.03	–23.48	19.2 ± 2.9	–21.5 ± 4.9	21.3 ± 2.9
	27954	–3.71	–19.87	–23.54	16.3 ± 2.9	13.7 ± 4.5	24.1 ± 2.9
Alligator Harbor	27959	–2.64	–18.79	–19.85	9.8 ± 2.9	1.2 ± 4.0	1.9 ± 2.8
	27960	–2.64	–17.47	–19.86	11.7 ± 2.9	–9.1 ± 5.0	9.7 ± 2.7
	29129	–2.75	–19.08	–22.54	7.0 ± 2.8	–48.9 ± 3.6	6.7 ± 2.9
	29130	–2.85	–20.32	–22.05	9.9 ± 2.8	–46.7 ± 3.1	3.7 ± 2.9
	29131	–2.56	–20.28	–23.04	6.0 ± 2.8	–10.7 ± 3.0	9.0 ± 2.8
Average		–2.96	–19.69	–22.05	11.4	–17.4	10.9
SD		0.47	1.44	1.59	4.8	23.4	8.5
River-influenced							
St Vincent Sound	27955	–5.53	–23.40	–25.12	–16.1 ± 2.8	–50.7 ± 15.2	–10.0 ± 2.8
	27956	–5.75	–23.63	–25.42	–11.9 ± 2.8	–55.3 ± 7.8	–10.1 ± 2.8
Cat Point	27957	–5.43	–22.31	–24.26	–12.1 ± 2.8	–10.8 ± 4.1	–7.2 ± 2.8
	27958	–5.04	–22.28	–25.61	–5.4 ± 2.9	–11.6 ± 4.6	0.3 ± 2.8
Average		–5.44	–22.91	–25.10	–11.4	–32.1	–6.8
SD		0.30	0.71	0.60	4.4	24.2	4.9
		**	**	*	**		*

Note: Statistically significant difference in means at $p < .05$ (*) and $p < .001$ (**).

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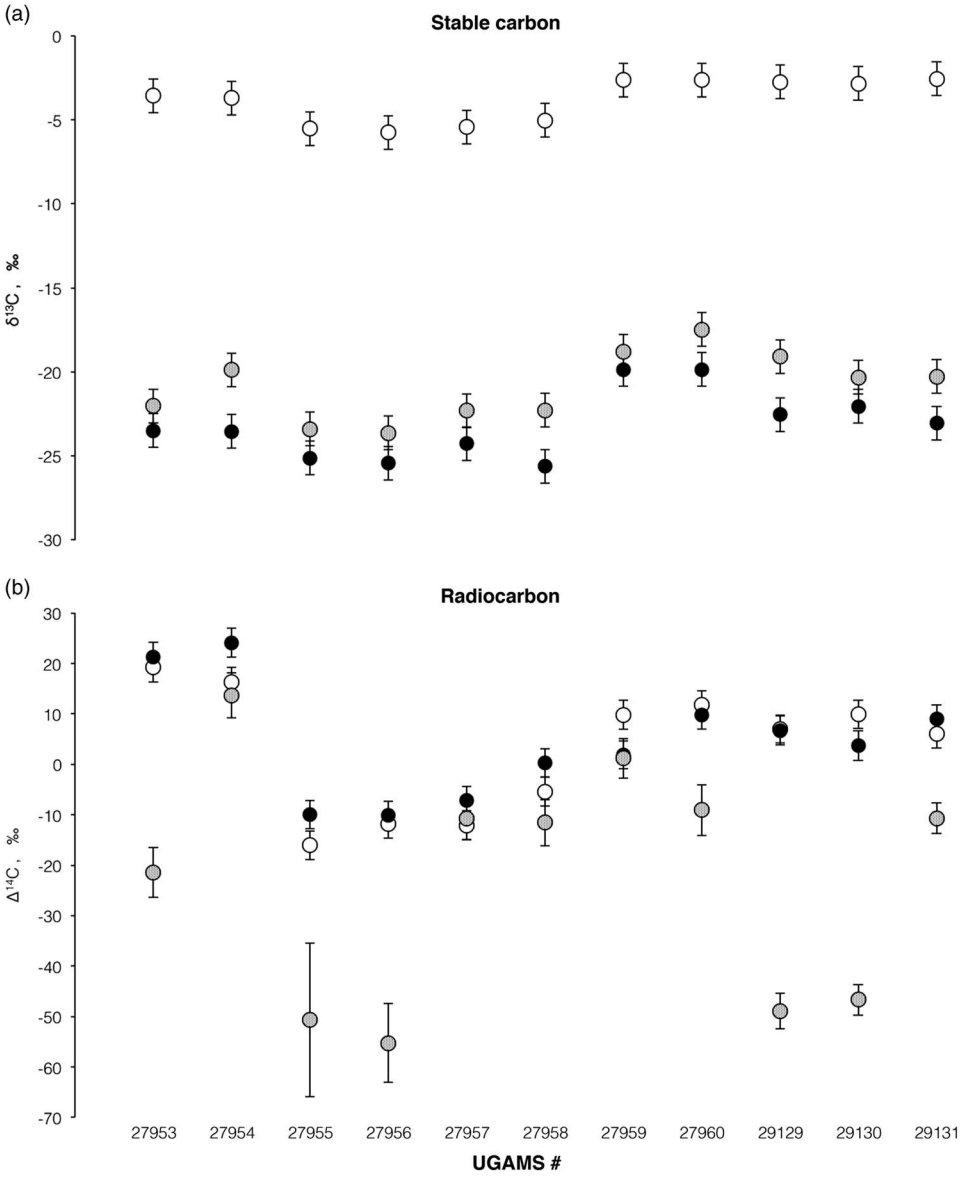

Figure 3 (a) Stable carbon and (b) ^{14}C isotope ratios of shell carbonate (white), conchiolin (gray), and soft tissue (black) of *Crassostrea virginica* specimens from Apalachicola Bay, USA.

the range of DIC values previously reported by Chanton and Lewis (1999) for Apalachicola Bay (–1‰ to –15‰). Soft tissue $\delta^{13}C$ values range from –19.8‰ to –25.6‰, similar to the isotopic values reported by Chanton and Lewis (1999) for organic sediment, plankton, and particulates (–22‰ to –32‰), as well as those reported in Table 2. $\delta^{13}C$ values of conchiolin and soft tissue are strongly correlated ($R = 0.90$, $p < 0.001$), with conchiolin enriched in ^{13}C by an average of +2.3‰ relative to soft tissue. These findings are consistent with the 2‰ offset reported for this same species by Ellis and colleagues (2014). The 2‰ depletion in tissue relative to conchiolin may be indicative of an increased concentration of lipids, which tend to be more

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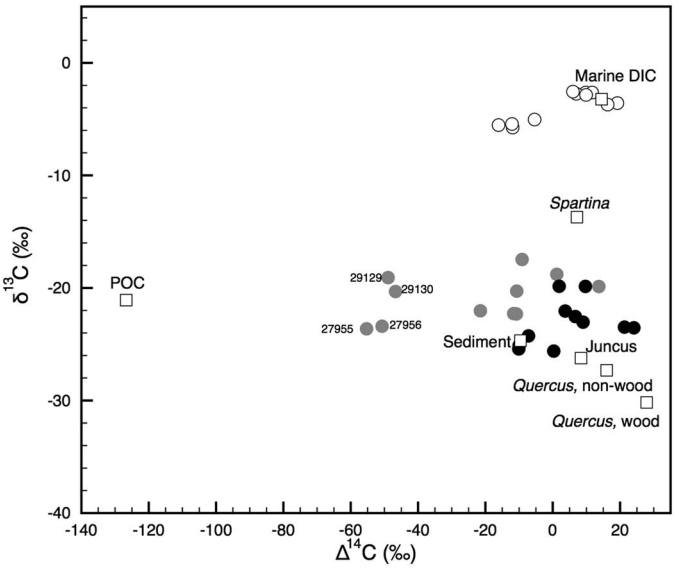

Figure 4 $\delta^{13}C$ versus $\Delta^{14}C$ of shell carbonate (white), conchiolin (gray), and soft tissue (black) of *Crassostrea virginica* specimens from Apalachicola Bay, USA.

Table 2 Alligator Harbor environmental samples.

UGAMS #	Material	$\delta^{13}C$ (‰)	C:N	$\Delta^{14}C$	±
29132	Sediment	–24.68	10.3	–9.6	2.8
29133	Plant, *Spartina*	–13.71	28.8	7.2	2.8
29134	Plant, *Juncus*	–26.23	48.7	8.4	2.8
29135_NW	Plant, *Quercus* non-wood	–27.32	—	16	2.8
29135_W	Plant, *Quercus* wood	–30.17	22.3	27.9	2.8
29136_DIC	Water DIC	–3.23	—	14.5	2.8
29136_F	Water POC	–21.08	9.8	–126.7	2.8

depleted in ^{13}C as compared to other biomolecules, in the soft tissue relative to the conchiolin. In this study, the stable carbon isotope of conchiolin represents only the insoluble fraction of the shell organic matter, as soluble components (e.g. carbohydrates, hydrophilic amino acids, and acid-soluble proteins) were excluded during the acidification process. As such, the offset between tissue and total conchiolin (i.e. soluble and insoluble fractions) of *C. virginica* may differ from the observed 2‰ offset found in this study and will be assessed further in future studies. Given our findings, however, oyster conchiolin may prove useful as a proxy for oyster soft tissue, providing insight into diet and environmental influences, in cases where soft tissues are not available for study.

In all three oyster tissue types, stable carbon isotopes are significantly more depleted in oysters from river-influenced sites compared to those from marine-influenced sites (Table 1). The depleted ^{13}C signal closer to the Apalachicola River derives from the varied carbon sources in estuaries. River water DIC is isotopically lighter (more depleted in ^{13}C) than marine DIC

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because it is derived from weathered carbonate minerals and mineralized organic matter that has undergone prolonged degradation, while DIC in seawater is near isotopic equilibrium with atmospheric CO_2 (Mook and Tan 1991). DIC isotopic composition within Apalachicola Bay co-varies with salinity, ranging from riverine-like values (–12‰ to –15‰) during high river flow to marine-like values (–0.6‰ to –0.8‰) during low-flow periods (Chanton and Lewis 1999), with DIC being a major control on the isotopic composition of estuarine phytoplankton (Chanton and Lewis 1999). Similarly, organic carbon in estuaries varies in stable carbon isotopic composition as a consequence of the various sources of organic carbon. The $\delta^{13}C$ signal of rivers is usually relatively depleted, reflecting contributions from C_3 terrestrial plant detritus, whereas marine organic carbon is primarily derived from phytoplankton and is relatively more enriched in ^{13}C. The stable carbon isotope composition of the soft tissue and conchiolin reveal how differences in diet and available carbon are recorded in oyster tissues. The soft tissue of oysters collected from areas with a large riverine influence (27955, 17956, 27957, and 27958) are more depleted, with a carbon isotope composition of approximately –25‰, relative to the soft tissue of oysters collected from more distal sites, for which stable isotope composition ranged from –23 to –19‰. The stable carbon isotope composition of the conchiolin, likewise, is more depleted in oysters growing nearer to the river (–22.91 ± 0.71‰) relative to the oysters growing in marine dominated sites (–19.69 ± 1.44‰). While soft tissues from oysters have been used to infer the relative contribution of various carbon sources in estuarine diets (e.g. Chanton and Lewis 2002), here we show the potential use of conchiolin as a proxy for interpreting diet and relative growing location in paleoecological studies.

Radiocarbon ($\Delta^{14}C$)

Interpreting carbon sources of ^{14}C in oyster tissues is complicated because the ^{14}C isotopic compositions of the various carbon reservoirs have been rapidly changing since the post-industrial period, with the surface ocean and atmospheric carbon reservoirs at near-equilibrium values in some locations (Druffel et al. 2016). Although environmental samples were not collected at each oyster sampling locality, the environmental samples presented in Table 2 indicate that modern terrestrial plants range from +7‰ to +28‰, indistinguishable from surface water DIC at Alligator Harbor, suggesting near-equilibrium conditions at this locale. Shell carbonate and oyster soft tissues from the marine-influenced sites strongly resemble these environmental samples in terms of $\Delta^{14}C$ values, and there is little difference between shell carbonate and organic soft tissue within individuals (Figure 3).

While evidence suggests that the ^{14}C signal at marine sites is being influenced by carbon reservoir equilibrium, at river dominated sites ^{14}C in oysters seems to be more strongly influenced by riverine transport. The Apalachicola River passes through Miocene- and Oligocene-age limestones, carrying ^{14}C-depleted DIC to the river-influenced regions of the bay (Hadden and Cherkinsky 2015, 2017). Ground water discharges from the Upper Floridan aquifer (UFA) and intermediate system to the Apalachicola River are another possible source of ^{14}C-depleted DIC. The measured ^{14}C activities are very low in waters from the UFA, with corresponding ^{14}C ages of 8000 to >47,300 ^{14}C yr (Plummer and Sprinkle 2001). These signals are preserved in oyster shell carbonates, with river-influenced oyster specimens being significantly depleted in ^{14}C relative to marine-influenced specimens (Table 1). An avenue for future research will be to compare conchiolin and shell carbonate ^{14}C in ancient specimens, when marine and atmospheric reservoirs were separated in $\Delta^{14}C$ space.

Although carbonate transport may indirectly affect the $\Delta^{14}C$ of conchiolin and oyster soft tissue, it is expected that diet would have a more direct influence on the isotopic signature of these fractions. Conchiolin is in every case depleted in ^{14}C compared to soft tissues (Table 1), although

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the offset varies from as little as 0.7‰ to as much as 55.6‰. There is no statistical difference between river- and marine-influenced locales, in terms of the average $\Delta^{14}C$ values or in terms of the magnitude of the conchiolin–soft tissue offset. A biplot of $\delta^{13}C$ and $\Delta^{14}C$ (Figure 4), however, suggests that dietary sources may be driving the variation in $\Delta^{14}C$ observed in *C. virginica*. Four oysters, 29129, 29130, 27955, and 27956, exhibit more depleted $\Delta^{14}C$ values, approaching the highly depleted value observed for POC in the 1–20 μm size range. This suggests that these organisms were incorporating a larger degree of POC-derived carbon into the conchiolin matrix. The consistently more depleted conchiolin $\Delta^{14}C$ values, relative to soft tissue, suggests isotopic routing may be occurring, wherein POC-derived carbon, which is more depleted in ^{14}C, is utilized for conchiolin development while carbon from younger organic matter is utilized for biosynthesis of soft tissues. Isotopic routing occurs when isotopes constituting dietary components are differentially routed to specific tissues and body compartments (Gannes et al. 1997).

An example of isotopic routing is found during the biosynthesis of proteins. Proteins are composed of a combination of essential and non-essential amino acids. While essential amino acids must be acquired from dietary amino acids, non-essential amino acids are resynthesized with atoms derived from all dietary fractions (i.e. lipids, carbohydrates). When dietary sources are protein-limited, organisms are found to incorporate a greater degree of carbon from carbohydrates and non-proteinaceous molecules into their synthesized proteins (Kennedy 1988). However, when a protein-rich diet is available, the isotopic contribution of carbohydrates and lipids to protein resynthesis may be underrepresented (Ambrose and Norr 1993). As a result, proteins synthesized predominately from protein-rich diets are going to differ isotopically from proteins synthesized predominately from dietary polysaccharides. This isotopic difference arises when biochemical constituent classes have different isotopic compositions (Degens 1969). Isotopic routing may occur in *C. virginica* because of nutritional differences in dietary sources. These differences can be gleaned by comparing the C/N ratio of several possible dietary sources in the Apalachicola Bay system (Table 2). The C/N ratio of POC is 9.8, indicating a greater contribution of nitrogenous compounds. POC, then, may contain and therefore contribute a greater degree of proteinaceous biomolecules, facilitating the biosynthesis of a predominately proteinaceous conchiolin matrix. On the other hand, plant detritus, consisting of a relatively greater degree of polysaccharides and carbohydrates, may provide a greater proportion of the organic carbon utilized in biosynthesizing the more molecularly diverse soft tissues.

Aside from diet and isotopic routing, sorption of oil and humic materials may also play a role in the $\Delta^{14}C$ variations observed between tissue and the acid-insoluble conchiolin matrix in *C. virginica.* There is potential for sorption and incorporation of sediments, dissolved organic matter, and particulate organic matter (Griffin and Druffel 1989) during shell secretion and biomineralization. In this scenario, in lieu of dietary routing of organic carbon to the conchiolin matrix, organic carbon is directly adsorbed and bound within the shell matrix during shell accretion. A potential source of carbon to the particulate and dissolved organic carbon pools are hydrocarbons. These hydrocarbons may originate from natural oil seeps, which release oil into the Gulf of Mexico at a rate of 2.2–30 m^3 1000 km^{-2} d^{-1} (Macdonald et al. 1993). These hydrocarbons are depleted in ^{13}C and ^{14}C relative to other biomolecules and sorption onto the shell matrix would result in a more depleted signal in the shell relative to the tissue. It is likely that the isotopic compositions measured in the shell organic matrix represent a combination of dietary incorporation and adsorption of particulate organic matter into the shell organic matrix.

Genomic, transcriptomic, and proteomic analyses show unique adaptations of oysters to sessile life in a highly stressful intertidal environment (Zhang et al 2012). Air exposure, carbon limitation, and temperature and salinity stress may also induce physiologic and metabolic

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responses that manifest in isotopic fractionation in shell organic carbon. Whatever the cause, the large and variable depletion of ^{14}C in modern oyster shell conchiolin with respect to other tissues suggests a potential problem with ^{14}C dating more ancient samples.

CONCLUSIONS

In this study we set out to investigate potential uses of conchiolin in addressing paleoecological and archaeological questions. Conchiolin, an organic-rich matrix, should more readily reflect the $\Delta^{14}C$ of dietary sources than the $\Delta^{14}C$ signal of aquatic carbonate pools. As such, we hypothesized that conchiolin may help overcome the difficulties encountered when ^{14}C dating shell. In this study we report near-equilibrium conditions between surface water DIC, terrestrial plants, and macrofauna at Alligator Harbor. We found that while conchiolin did seem to reflect the $\Delta^{14}C$ signal of *C. virginica* diet, the disparate $\Delta^{14}C$ values in food sources complicates the interpretation of ^{14}C ages. However, based on the following findings, we propose using a combined multi-isotope approach for recreating diet and hydrologic dynamics using conchiolin, as follows:

- Environmental $\delta^{13}C$ signals are retained in all three oyster tissue types studied here, largely reflecting a marine versus riverine influence.
- Shell carbonates and soft tissue record a marine- versus riverine- source of ^{14}C.
- Conchiolin is significantly depleted in ^{14}C with respect to soft tissue as a result of significant influence of particulate organic matter resulting from either isotopic routing, adsorption of ^{14}C-depleted material, or a combination of these processes.
- The magnitude of the offset may reflect variations in metabolism, possibly related to stress responses.

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