$\delta^{15}N$ IN MOLLUSK SHELLS AS A POTENTIAL PALEOENVIRONMENTAL PROXY FOR NITROGEN LOADING IN CHESAPEAKE BAY

by

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ABSTRACT

Crassostrea virginica is one of the most common oyster species in North America and is frequently found in archaeological sites and sub-fossil deposits, especially in the eastern US. Although there have been several sclerochronological studies on $\delta^{13}C$ and $\delta^{18}O$ in the shells of this species, little is known about $\delta^{15}N$ stored within the shells, which could potentially be a useful paleoenvironmental proxy to determine nitrogen loading and the subsequent anthropogenic impacts within an area.

In order to potentially serve as paleoenvironmental proxies for N loading, bivalve shells' organic matter needs to remain chemically unaltered. Since ancient peoples cooked most archaeological shells before depositing them in shell middens, it is necessary to determine if prehistoric cooking methods alter either %N or $\delta^{15}N$ stored within the shells. Twenty *C. virginica* oysters and twenty-two *Mercenaria spp.* clams were treated to five different prehistoric cooking methods: direct exposure to hardwood coals, roasting above hardwood coals, roasting in a dry oven, boiling in freshwater, or boiling in seawater. Each shell was bisected through the resilifer with one half treated with one of the five prehistoric cooking methods and the remaining half serving as a control. With the exception of roasting above the hardwood coals, prehistoric cooking methods do not significantly alter either %N or $\delta^{15}N$ within the shells. Those shells roasted above the coals were typically enriched in both %N and $\delta^{15}N$, which is likely an effect of smoke coming from the hardwood coals and infiltrating pore spaces within the outer layers of the shell.

Ninety archaeological *C. virginica* shells ranging in age from ~120 to 3,400 years old and thirty-two modern *C. virginica* shells were collected in Chesapeake Bay at the Smithsonian Environmental Research Center in Edgewater, Maryland. One valve from each shell was sub-sampled and the calcite powder was analyzed (without acidification pretreatment) using an EA-IRMS system equipped with a CO_2 trap to determine the %N and $\delta^{15}N$ content of the shells.

Comparison of %N and $\delta^{15}N$ in *C. virginica* shells from the six different time periods studied show relatively constant values from ~3,400 years ago to 1820 AD. Between 1820 and 1890, there are rapid increases in both %N and $\delta^{15}N$ in the shells, which continue to exponentially increase in value to the modern shells. The increases in %N and $\delta^{15}N$ are correlated with increased anthropogenic impact due to human population, sewage discharge, and urbanization in Chesapeake Bay at this time. Therefore, it is likely that *C. virginica* shells can be used as a paleoenvironmental proxy to measure the anthropogenic impact of a specific area over time.

However, the constant, relatively low %N and $\delta^{15}N$ values from ~3,400 years ago to 1820 AD compared to the increased N concentrations and enriched $\delta^{15}N$ shells from the modern periods could be influenced by diagenetic alteration of the shell after burial in the midden. It is possible that the shells are losing N and preferentially losing ^{15}N over time. More research is necessary to determine if bivalve shells are geochemically stable with regard to N over time or if diagenesis is likely to have occurred in these shells.

DEDICATION

This thesis is dedicated to my parents, Mae and Bobby Inscoe, and to the love of my life, Lewis Midkiff. They have provided unconditional love, support, patience, and advice, especially when I needed it the most throughout the years. I would not be the person I am today without them and I have nothing but absolute love and admiration for them. I am extremely thankful for all that they have done for me.

LIST OF ABBREVIATIONS AND SYMBOLS

$\delta^{15}N$	Delta notation $\delta^{15}N = \{(R_{sample}-R_{standard}/R_{standard})\}*1000$ where $R_{sample}={}^{15}N/{}^{14}N$ ratio of the sample, and $R_{standard}={}^{15}N/{}^{14}N$ ratio of the standard
$\delta^{13}C$	Delta notation $\delta^{13}C = \{(R_{sample}-R_{standard}/R_{standard})\}*1000$ where $R_{sample}={}^{13}C/{}^{12}C$ ratio of the sample, and $R_{standard}={}^{13}C/{}^{12}C$ ratio of the standard
EA	Elemental analyzer
IRMS	Isotope ratio mass spectrometer
SEM	Scanning electron microscopy
B2151	High organic content sediment isotope standard
USGS25	Ammonium sulfate $[(NH_4)_2SO_4]$ isotope standard
IAEA-N-2	Ammonium sulfate $[(NH_4)_2SO_4]$ isotope standard
VPDB	Vienna Pee Dee Belemnite
SERC	Smithsonian Environmental Research Center
CAF	Central Analytical Facility at The University of Alabama
N	Nitrogen
C	Carbon
CO_2	Carbon dioxide
$CaCO_3$	Calcium carbonate; calcite
POM	Particulate organic matter
H_2O	Water
‰	Per mil
%	Percent

σ	Standard deviation
mg	Milligram
mm	Millimeter
mV	Millivolt

ACKNOWLEDGMENTS

"Chemistry is, well technically, chemistry is the study of matter, but I prefer to see it as the study of change. Now just think about this. Electrons, they change their energy levels. Molecules change their bonds. Elements combine and change into compounds. But that's all of life, right? It's the constant, it's the cycle. It's solution, dissolution. Just over and over and over. It is growth, then decay, then transformation. It is fascinating, really."

-Walter White, Breaking Bad

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1. INTRODUCTION

Bivalve shell geochemistry is commonly used to determine ecological and environmental characteristics at the time the shell grew, such as sea surface temperature, salinity, and trophic structure (Epstein *et al*., 1953, Emiliani *et al.*, 1963, Grossman *et al*., 1986, Bailey *et al*., 1983, Jones *et al*., 1990**,** O'Donnell *et al*., 2003, and Rowell *et al*., 2010). The eastern oyster *Crassostrea virginica* has large environmental tolerances capable of surviving significant variations in temperature, salinity, and food supply. Since they are sessile filter feeders in constant physical contact with water (NOAA Fisheries Eastern Oyster Biological Review Team 2007), their soft tissues and shells are capable of recording environmental changes by incorporating organic materials from the surrounding water body (Dame *et al.*, 1989, and Carmichael *et al.*, 2004). Therefore, the $\delta^{15}N$ in the tissues reflects the ambient $\delta^{15}N$ in the ecosystem (McClelland *et al*., 1997). Anthropogenic nitrogen sources, specifically sewage and industrial pollutants, are typically isotopically heavier than non-anthropogenic sources (McClelland *et al*., 1997, Carmichael *et al*., 2008, and Fertig *et al*., 2010), so it is possible to trace nitrogen inputs into estuaries by studying the $\delta^{15}N$ in the tissues and shell material.

Unlike the soft tissue samples that were traditionally used in biological and geochemical studies of mollusks, shell material can potentially better serve as a proxy for past conditions due to the increased chance of preservation over time. Mollusk shell material accretes sequentially, sometimes in annual growth layers, which could potentially be used as a high temporal resolution proxy for historical nitrogen levels as well as potentially creating a time-series of nitrogen loading over the life of the organism (Jones *et al.*, 1990 and Carmichael *et al*., 2008).

The shell material is also never metabolized, so it can record the environmental conditions over a longer period of time than compared to soft tissues (O'Donnell *et al*., 2003 and Carmichael *et al.*, 2008).

There are numerous applications for a potential paleoenvironmental $\delta^{15}N$ proxy, such as monitoring pollution levels and marine primary productivity, and determining trophic hierarchies in ecosystems (Cabana *et al.*, 1996, Fry 2002, McKinney *et al*., 2002, O'Donnell *et al*., 2003, Watanabe *et al*., 2009, Fertig *et al*., 2010, Rowell *et al*., 2010, Bouillon *et al*., 2012, and Nerot *et al.*, 2012). Anthropogenic pollution, especially nitrogen, is a major concern in coastal estuaries (Fisher *et al*., 1991, McClelland *et al*., 1997, McKinney *et al*., 2002, and Brown *et al*., 2012). Increased nitrogen loading can potentially lead to eutrophication, which causes anoxic conditions in water bodies and die-off events of marine organisms (Cooper *et al*., 1993, Howarth, 2008, and Watanabe *et al*., 2009). In the last few decades, regulations governing N inputs have been introduced to affected industries (Van der Voet *et al*., 1996), but in order for these policies to be most effective, it is helpful to know base levels of nitrogen in the area. In most cases, however, base levels before anthropogenic influences began are unknown. If mollusk shells can serve as a paleoenvironmental proxy using $\delta^{15}N$, it would enable scientists to generate a base level of nitrogen by studying the record of nitrogen levels stored in the shells, as well as how nitrogen concentrations have fluctuated over time. In order to serve as a trustworthy environmental proxy, the mollusk samples will need to remain unaltered with respect to N after death and subsequent burial, both physically and geochemically.

The overall objective of this thesis project is to determine if *C. virginica* shells have the potential to serve as paleoenvironmental proxies for N loading in Chesapeake Bay during the late Holocene. In order for the shells to be capable of recording ecological and environmental

conditions of the bay, the shells need to be representative of both the organisms' soft tissues and the POM within the bay. The correlations between POM, different types of soft tissues, and the shell were assessed in modern *C. virginica* samples. To be considered a valid paleoenvironmental proxy, the shells must also remain unaltered, both physically and chemically. Therefore, additional studies were undertaken to determine the effects of prehistoric cooking methods on N content and N stable isotopes in two of the most common species of shells found in archaeological middens, *C. virginica* and *Mercenaria spp*. *C. virginica* shells obtained from archaeological middens in Chesapeake Bay were also analyzed over a period of ~3,400 years to determine if the shells were capable of recording increased N loading within the bay over time.

2. GEOGRAPHICAL LOCATION AND SAMPLES

Modern samples analyzed in this study, except those involved in the diagenesis experiments, were collected at the Smithsonian Environmental Research Center in Edgewater, Maryland (38°53' N 76°32' W) (Figure 1). Water, POM, and oyster samples were collected from six different locations within the Rhode River Estuary of Chesapeake Bay. The Smithsonian Institute collected archaeological samples while excavating shell middens in the bay. Modern water, POM, and oyster samples were collected as nearby as possible to archaeological sites.

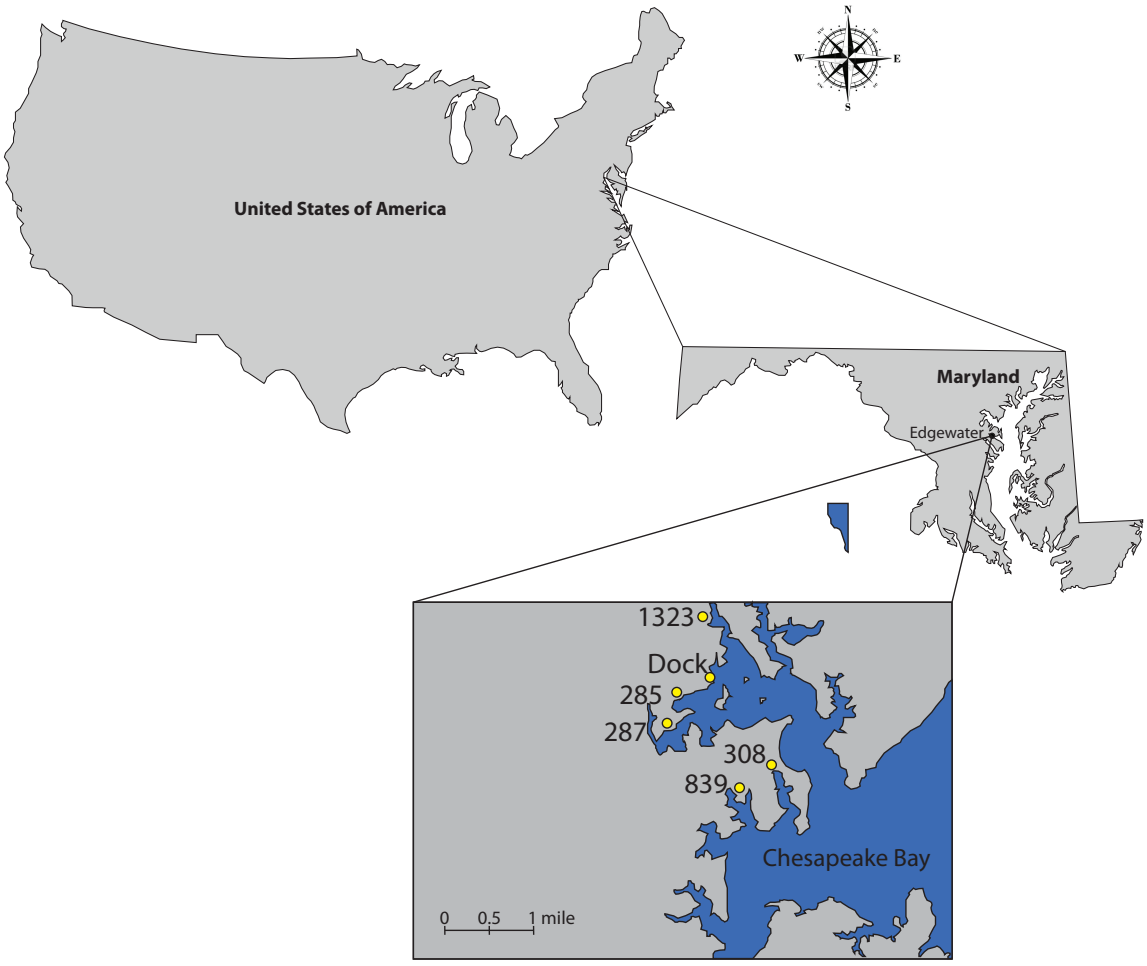

Illustration 1. Location of study area near Edgewater, MD. Sample locations are represented by their corresponding archaeological site number.

2a. RELATED ARCHAEOLOGICAL HISTORY OF THE CHESAPEAKE BAY REGION:

The oldest archaeological samples in this study date to the Early Woodland Period. During the Early Woodland, most people here lived in hunter-gatherer societies, with few tribes living sedentary, village dwelling lifestyles (Mouer 1991). For the most part, these hunter-

gatherers had intensive, seasonal occupations of areas, but there are several known large, permanent populations in the western parts of Chesapeake Bay (Custer 1994). Due to the lack of industrial technology and agriculture in addition to small population sizes, it is unlikely that human activities in Chesapeake Bay had a substantial impact on the chemical composition of the bay during this time period.

In the earlier portion of the Middle Woodland Period (i.e., AD 200 – 550), the people in Chesapeake Bay were still mostly hunter-gatherers, but the populations moved seasonally between small terrestrial camps of the bay and larger, more estuarine environments (Potter 1993). In the later portion of the Middle Woodland Period (i.e., after AD 550), these native civilizations were permanently settled near riverine and estuarine areas of the bay (Potter 1993). It was also during the Middle Woodland Period that native populations from the Middle Atlantic region began to migrate to Chesapeake Bay, significantly increasing the total population in the area (Fiedel 1994).

During the Late Woodland Period, there were settlements of large, permanent towns and the beginnings of horticulture in the area (Rice 2009). After 1300 AD, there was a rapid increase in population size as well as the amount of estuarine resources used. Although there were some settlements that had adapted horticulture at this point, most people on the western side of the bay did not begin to grow maize until the 16th century (Blanton *et al.,* 2005).

During the 16th century, European settlers began to claim land in the bay area. The colonial settlements cleared the forests and established large plantations on pristine agricultural land (Potter and Waselkov 1994). During the 19th century, industrialization rapidly increased in the northeastern US and continues today.

2b. SAMPLES:

Crassostrea virginica (Figure 2) is an oyster species that has large environmental tolerances capable of surviving significant variations in temperature, salinity, and food supply (NOAA Fisheries Eastern Oyster Biological Review Team 2007). This species encompasses a large geographical and geological range. *C. virginica* is commonly found throughout the North American coast from the Gulf of St. Lawrence to the West Indies (Kirby *et al.,* 1998) and is the dominate bivalve species in Chesapeake Bay (Surge *et al*., 2001). *C. virginica* shells are found from the Cretaceous to the present (Kirby *et al.,* 1998) and are commonly found in archaeological sites and museum collections (Andrus, 2012).

The growth, reproduction, and ecology of *C. virginica* have been researched extensively in mid-Atlantic estuaries (Harding *et al.,* 2010). The species is sessile, epifaunal, and immobile (NOAA Fisheries Eastern Oyster Biological Review Team 2007). While these oysters can live up to 25-30 years, shell growth rate diminishes with increasing age; the most rapid growth occurring in the first 5-6 years (NOAA Fisheries Eastern Oyster Biological Review Team 2007). *C. virginica* can live in waters up to 8 meters deep and is typically found attached directly to a hard substrate ((NOAA Fisheries Eastern Oyster Biological Review Team 2007).

C. virginica shells are primarily calcitic, but have a thin aragonite layer in the ligostracum and muscle scar (Stenzel, 1963). This species has previously been used for numerous stable isotope studies (e.g. Kirby *et al.,* 1998; Andrus and Crowe, 2000; Surge *et al.,* 2001; Harding *et al.,* 2010; Thompson and Andrus, 2011), and recently has been introduced as a possible paleoenvironmental proxy by analyzing the shell $\delta^{15}N$ (Carmichael *et al.*, 2004; Kovacs *et al.,* 2010; Fertig *et al.,* 2010).

Illustration 2. Image of disarticulated valves of *C. virginica*.

3. METHODS

3a. PREHISTORIC COOKING METHODS ANALYSIS

Twenty *C. virginica* oysters and twenty-two *Mercenaria spp*. clams were purchased from commercial seafood markets with known origins. Fourteen *C. virginica* samples were grown in Bayou La Batre, AL and six samples were grown off the Gulf Coast of Florida near the panhandle. Twelve *Mercenaria* samples were grown in Barlow Creek, VA and ten samples were grown in Sheraton, VA. Soft tissues were immediately removed and the shells were cleaned using DI water and a soft bottlebrush followed by sonification. The two shell valves were separated at the hinge and the left valves were bisected symmetrically through the resilifer using a slow speed diamond-wafering saw. One half of the valve was subjected to one of the following treatments for ~1 hour:

(1) Direct exposure to hardwood coals

(2) Roasting over hardwood coals

(3) Roasting in a dry oven at 150º C

(4) Boiling in freshwater

(5) Boiling in seawater

The remaining half of the valve was left untreated as an experimental control. An additional sample of each species was used a control sample in which both halves of the valve were left untreated to analyze for variability within the shell.

After the cooking experiments were complete, all samples were treated in an identical manner. Each sample was ground to a fine, homogenous powder using an agate mortar and pestle and was separated into two ~35 mg sub-samples for analysis. Each sub-sample was

packed into 5 x 9 mm tin capsules and analyzed for $\delta^{15}N$ and %N using a Costech ECS 4010 Elemental Analyzer (EA) coupled to a Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS) fitted with a carbon trap in The University of Alabama Department of Geological Sciences stable isotope laboratory without acid pre-treatment of the samples following Versteegh *et al*., (2011) and Hansen (2011). Multiple standards, including a high organic content sediment isotope standard (B2151), two ammonium sulfate [$(NH_4)_2SO_4$] isotope standards (USGS25 and IAEA-N-2), and acetanillide (C_8H_9NO), were analyzed with the samples to assess analytical precision and drift. $\delta^{15}N$ data are reported in parts per mil (‰) vs. air. Precision (1σ) was better than 0.09‰ based on analysis of multiple standards over a range of isotopic values.

3b. STABLE ISOTOPE ANALYSIS OF SOFT TISSES AND PARTICULATE ORGANIC MATTER

Twenty-two modern collected live oysters from the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland (38°53' N 76°32' W) (Figure 1) were stored on ice until sample preparation. Samples were collected by hand in shallow waters within a small area along the coast. The samples were planted as larvae along the hard substrate and were approximately five years old at collection. After thawing at room temperature, each organism was dissected and the left mantle, right mantle, adductor muscle, gills, and undigested stomach content were separated. The stomach content samples likely contained some soft tissue from the stomach. The samples were rinsed in DI water, freeze-dried in a Labconco 2.5 Plus lyophilizer at -85°C for ~72 hours, and ground to a fine, homogenous powder using an agate mortar and pestle. Between 1.0 and 1.2 milligrams (mg) of powdered sample were compacted in 5 x 9 mm tin capsules and each tissue was analyzed with two replicates in order to determine analytical precision.

The samples were analyzed for nitrogen and carbon content and stable isotopes using a Costech ECS 4010 Elemental Analyzer (EA) coupled to a continuous flow Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS) in The University of Alabama Department of Geological Sciences stable isotope laboratory. Multiple standards (a high organic content sediment isotope standard (B2151), two ammonium sulfate [$(NH_4)_2SO_4$] isotope standards (USGS25 and IAEA-N-2), and acetanillide (C_8H_9NO) were analyzed with the samples to assess analytical precision and drift. $\delta^{15}N$ data are reported in parts per mil (‰) vs. air and $\delta^{13}C$ data are reported in parts per mil (‰) vs. Vienna Pee Dee Belemnite (VPDB). Precision (1σ) was better than 0.08‰ based on analysis of multiple standards over a range of isotopic values.

Sixteen particulate organic matter (POM) samples were taken from four sites within Chesapeake Bay between 09/04/2013 – 09/06/2013. The sites were located near archaeological sites 287, 308, 1323, and off the SERC dock. Samples were collected by filtering ~250 mL of surface water from the bay through pre-combusted Whatman Gf/F glass fiber filters. Used filters were stored in sterile petri dishes on ice in the field. Before EA-IRMS analysis, the samples were dried at ~55°C for 72 hours. The used filters were cut approximately in half and one half was used for EA-IRMS analysis (to permit the samples to fit in the EA). Samples were packed in 9 x 10 mm tin capsules and were analyzed for nitrogen content and isotopes using the same analytical methods as the soft tissues. Precision (1σ) was better than 0.04‰ based on analysis of multiple standards over a range of isotopic values.

3c. STABLE ISOTOPE ANALYSIS OF SHELL MATERIAL

In order to determine the minimum amount of shell material necessary to obtain reliable $\delta^{15}N$ values, different ratios of pure calcite to isotope standard were analyzed. Two isotope

standards, acetanillide (C_8H_9NO) and IAEA-N-2 [$(NH_4)_2SO_4$] were used to cover a range of possible $\delta^{15}N$ values and to determine the point in which there was too little N to accurately measure in an EA-IRMS. The mass of the total mixture was held constant at ~35 mg, but the percentage of acetanillide ranged from 0.06% to 2.80% and IAEA-N-2 ranged from 0.05% to 2.65%. The amount of N in each sample ranged from 1.78 µg to 76.84 µg for acetanilide and 5.31 µg to 79.52 µg for IAEA-N-2. Each sample was packed into 5 x 9 mm tin capsules and analyzed for $\delta^{15}N$ and %N using a Costech ECS 4010 Elemental Analyzer (EA) coupled to a Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS) fitted with a carbon trap in The University of Alabama Department of Geological Sciences stable isotope laboratory. $\delta^{15}N$ data are reported in parts per mil (‰) vs. air. Precision (1σ) was better than 0.09‰ based on analysis of multiple standards over a range of isotopic values.

Ninety archaeological shell samples ranging in age from ~150 to 3400 years old and thirty modern shell samples were analyzed for %N and $\delta^{15}N$. The soft tissues were removed from the modern shells and the shells were cleaned using tap water and a soft bottlebrush followed by sonification. The shells were dried overnight at room temperature. The left valve of each shell sample was bisected along the longest axis using a slow-speed diamond-wafering saw. Each shell was subsampled using a hand held micro-drill. In order to create a lifetime average of the %N and $\delta^{15}N$, a transect was drilled in cross-section through the resilifer to a depth of approximately 2.0 mm (Figure 3). No carbonate powder from the exterior surface of any valve was sampled. The powdered samples were stored in 4.5 mL round-bottomed borosilicate vials until EA-IRMS analysis.

Illustration 3. Cross-section of *C. virginica* shell. Red line shows drilling transect through the ontogeny of the shell. Ruler for scale; The upper black boxes indicate one inch and the lower black boxes indicate one centimeter.

In order to obtain comparable peak sizes in mV between the modern and archaeological shell samples, different masses were used. Between 34-35 mg of powdered samples of modern shells were transferred to 5 x 9 mm tin capsules and 100-110 mg of archeological shells were transferred to 9 x 10 mm tin capsules. The samples were analyzed for nitrogen content and isotopes using the same analytical methods as above. $\delta^{15}N$ data are reported in parts per mil (‰) vs. air. Precision (1σ) was better than 0.09‰ based on analysis of multiple standards over a range of isotopic values.

One-way ANOVA and Tukey Post Hoc tests were completed on the shell samples based on time period to determine if there were any statistically significant differences between periods over time.

3d. SEM

Scanning electron microscope (SEM) images were taken of *C. virginica* shells at The University of Alabama's Central Analytical Facility (CAF). At least one sample from each time period was chosen randomly for SEM imaging to determine the amount and location of intercrystalline organic material within the shell. In preparation for analysis, the left valve was bisected along the longest axis using a slow-speed diamond-wafering saw. One half of the sectioned valves were embedded in epoxy, thin sectioned using a slow-speed diamond-wafering saw, and polished using increasingly finer wet abrasives, alpha aluminum oxide, and colliodal silica. The samples were cleaned in an ultrasonic bath between each step of polishing and were dried overnight at room temperature. The thin sections were etched in 10% hydrochloric acid for approximately two minutes and gold coated for 90 seconds. Images were taken by a JOEL 7000 Field Emission SEM and Phillips XL 30 SEM.

4. RESULTS

4a. PREHISTORIC COOKING METHODS ANALYSIS

The five different cooking methods had differing effects on the $\delta^{15}N$ of the cooked half of *C. virginica* shells (Appendix Table 10). Significance was determined based on similarity or difference relative to analytical precision (2σ) of whole, untreated control samples. Cooking on the coals did not significantly alter the $\delta^{15}N$ values. Roasting above the coals significantly increased the $\delta^{15}N$ values for four samples, but one sample was within 2σ of the control half. Roasting in a dry oven had no significant effect on the $\delta^{15}N$ values. Boiling in saltwater decreased the $\delta^{15}N$ values in two samples, but was within 2σ of the control in one sample. Boiling in freshwater decreased $\delta^{15}N$ values in three samples, but was within 2σ of the control in one sample.

Cooking Method	Sample	Cooked Half		Untreated Half	
		$\delta^{15}N$ (‰ AIR)	Weight Percent (%)	$\delta^{15}N$ (‰ AIR)	Weight Percent (%)
On Coals	O1	8.3	0.10	9.2	0.14
On Coals	O2	10	0.20	9.4	0.13
On Coals	O3	8.5	0.17	8.1	0.15
On Coals	O21	8.8	0.01	9	0.13
Above Coals	O4	9.3	0.04	8.7	0.19
Above Coals	O5	10.4	0.04	8.4	0.15
Above Coals	O20	11.2	0.05	9.6	0.09
Above Coals	O22	11.9	0.06	9.7	0.13
Above Coals	O23	10.6	0.07	9.4	0.09
Oven	O6	8.3	0.19	8.3	0.13
Oven	O7	8.2	0.11	8.9	0.10
Boiling in Saltwater	O8	8.4	0.16	9.8	0.15
Boiling in Saltwater	O9	7.9	0.16	9.3	0.09
Boiling in Saltwater	O24	9.4	0.13	9.6	0.15
Boiling in Freshwater	O10	7.6	0.19	8.4	0.12
Boiling in Freshwater	O11	7.8	0.11	8.9	0.10
Boiling in Freshwater	O12	8.3	0.23	10.1	0.15
Boiling in Freshwater	O25	9.5	0.11	9.4	0.11

Table 1. $\delta^{15}N$ and %N values for control and cooked halves of *C. virginica* valves.

No cooking method had a significant impact on the %N in the cooked half of the *C. virginica* shell. All samples were within 2σ of the control half with the exception of one sample roasted on the coal, which had a slight decrease in the %N value.

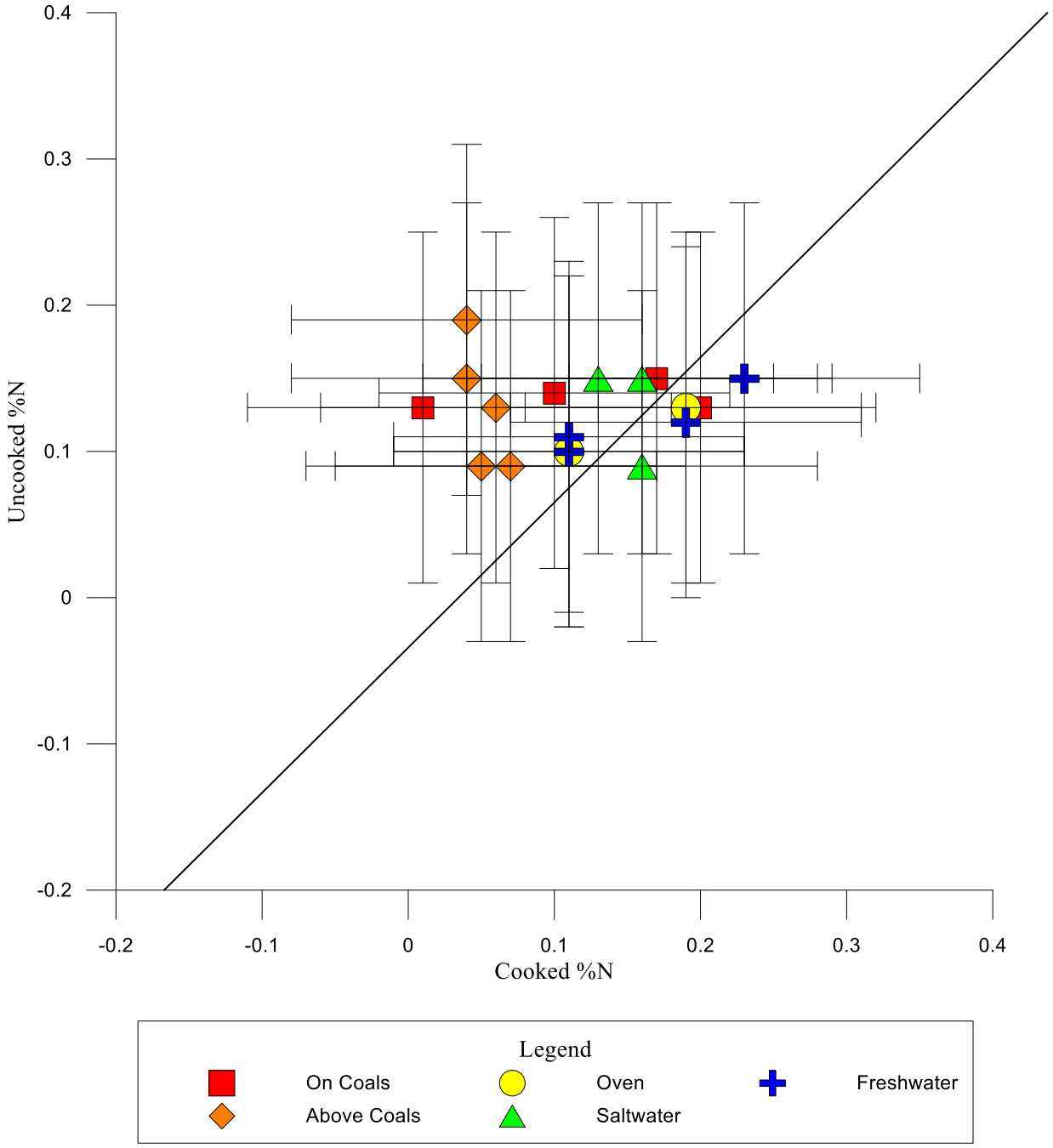

Figure 1. Plots of %N values for *C. virginica* samples treated with different prehistoric cooking methods. Y-axis is control samples. X-axis is cooked samples. The solid line represents a 1:1 ratio. Error bars depict 2σ of standard deviation within a single untreated shell (0.12%).

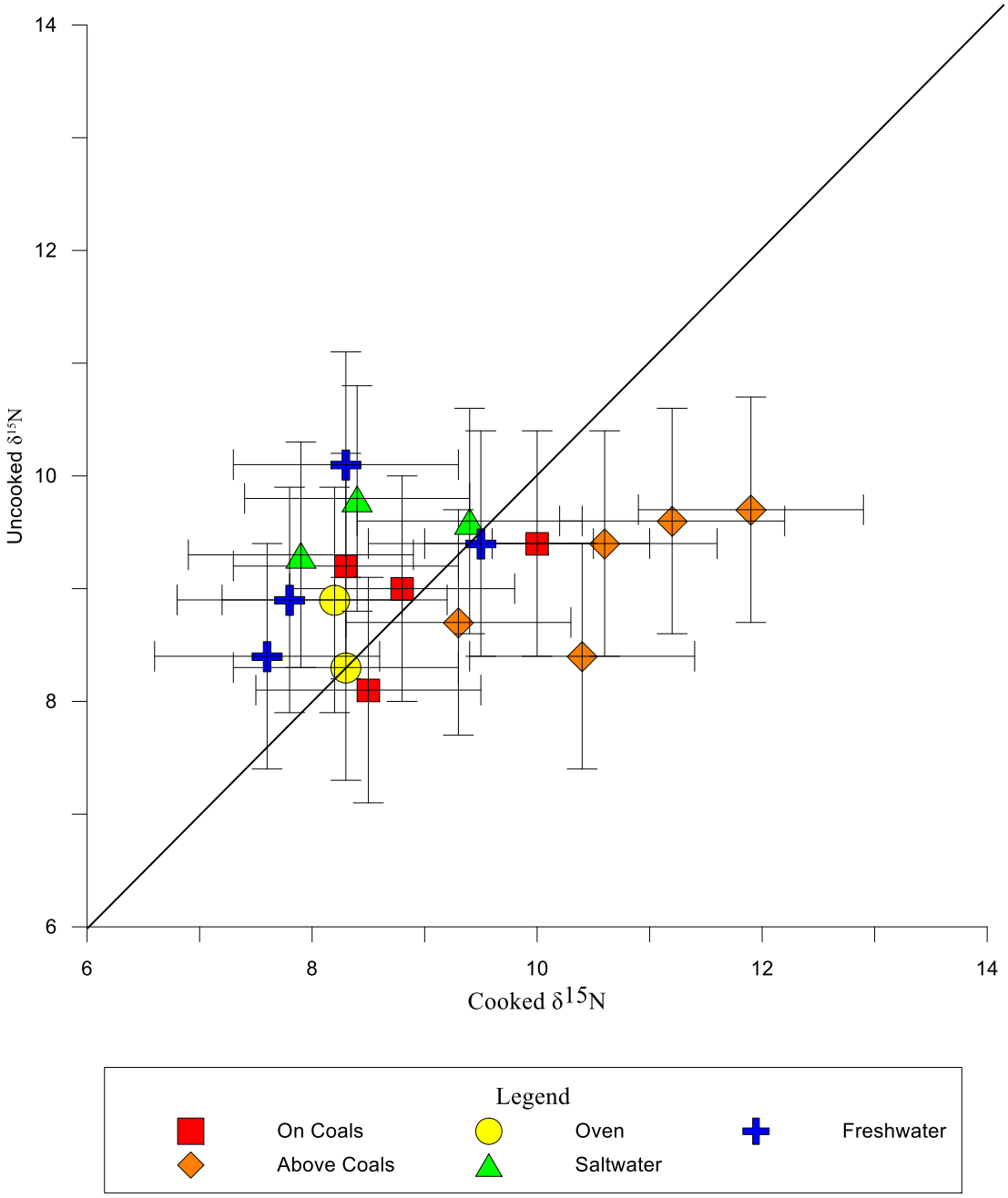

Figure 2. Plots of $\delta^{15}N$ values for *C. virginica* samples treated with different prehistoric cooking Pethods. Y-axis is control samples. X-axis is cooked samples. The solid line represents a 1:1 ratio. Error bars depict 2σ of standard deviation within a single untreated shell (1.0‰).

The five cooking methods had different effects on the $\delta^{15}N$ values of the cooked half of the *Mercenaria spp.* valve (Appendix Table 9). Cooking on the coals slightly increased the $\delta^{15}N$ of the shell, but was typically within 2σ of the control half (one sample had slightly lower $\delta^{15}N$ than 2σ). Roasting above the coals differed in individual shells; three samples had more positive $\delta^{15}N$ values while two samples had lower $\delta^{15}N$ values. All five shells roasted above the coals were not within 2σ of the control half. Roasting in a dry oven also had different $\delta^{15}N$ values between individuals; one sample had significantly higher $\delta^{15}N$ values than the control, three samples were not different from the control half, and one sample had significantly lower $\delta^{15}N$ than the control half. Boiling in saltwater and freshwater had no significant effect on the $\delta^{15}N$ of the cooked half of the shell.

		Cooked Half		Untreated Half	
Cooking Method	Sample	$\delta^{15}N$ (‰ AIR)	Weight Percent (%)	$\delta^{15}N$ (‰ AIR)	Weight Percent (%)
On Coals	C1	10.7	0.07	10.3	0.10
On Coals	C2	10.4	0.07	9.9	0.09
On Coals	C3	10.9	0.07	10.2	0.09
On Coals	C21	9.4	0.02	10	0.07
Above Coals	C5	9.5	0.02	10.4	0.10
Above Coals	C6	9.3	0.01	10.3	0.08
Above Coals	C26	11.3	0.03	10.1	0.08
Above Coals	C29	10.7	0.03	9.7	0.07
Above Coals	C31	11.5	0.05	10.5	0.07
Oven	C7	9.5	0.11	10.1	0.06
Oven	C8	9.9	0.09	9.9	0.07
Oven	C9	10.5	0.07	9.4	0.15
Oven	C24	9.2	0.18	9.5	0.05
Oven	C27	10.2	0.08	10.1	0.06
Boiling in	C10	10.2	0.06	9.5	0.08
Boiling in Saltwater	C11	9.9	0.11	10.2	0.07
Boiling in Saltwater	C12	10.3	0.07	9.8	0.08
Boiling in Saltwater	C20	9.8	0.05	10	0.07
Boiling in Saltwater	C23	9.2	0.08	9.4	0.05
Boiling in Freshwater	C13	10	0.09	9.9	0.07
Boiling in Freshwater	C14	9.9	0.11	10.4	0.09
Boiling in Freshwater	C25	9.4	0.07	9.4	0.06
Boiling in Freshwater	C28	10.1	0.07	10	0.07

Table 2. $\delta^{15}N$ and %N values for *Mercenaria spp.* samples treated by prehistoric cooking methods.

The cooking methods also had differing effects on %N in the shells of *Mercenaria spp* (Appendix Table 9). Cooking on the coals typically had no effect on %N, but one sample was

lower in %N than the control half. Roasting above the coals significantly decreased the %N in four samples but was within 2σ for one sample. Roasting in the oven had differing effects on %N; two samples had significantly increased %N, one sample had significantly decreased %N, and three samples were within of the control half. Boiling in saltwater or freshwater had no significant effect on %N in the cooked half of the shell.

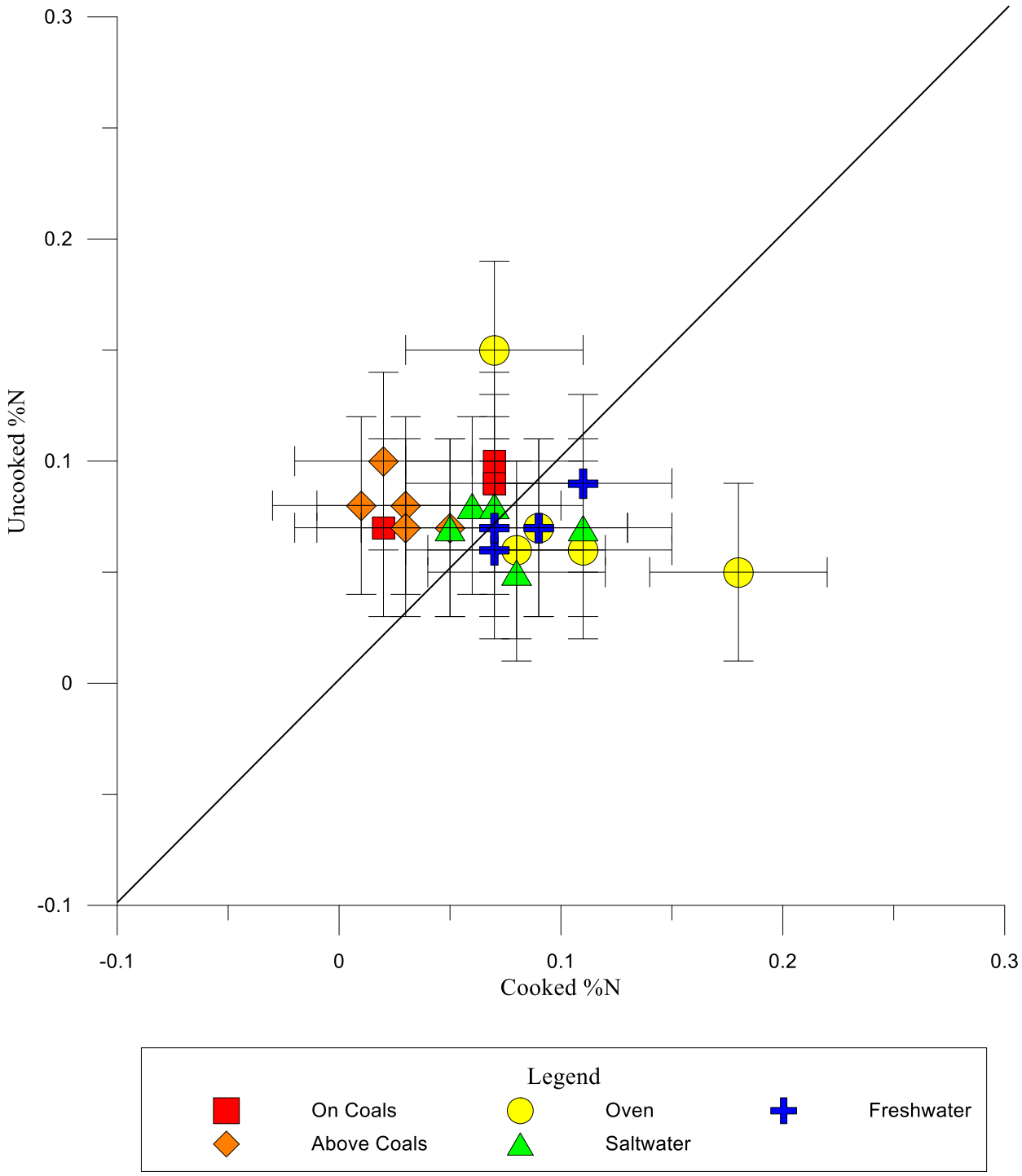

Figure 3. Plots of %N values for *Mercenaria spp.* samples treated with different prehistoric cooking methods. Y-axis is control samples. X-axis is cooked samples. The solid line represents a 1:1 ratio. Error bars depict 2σ of standard deviation within a single untreated shell (0.04%).

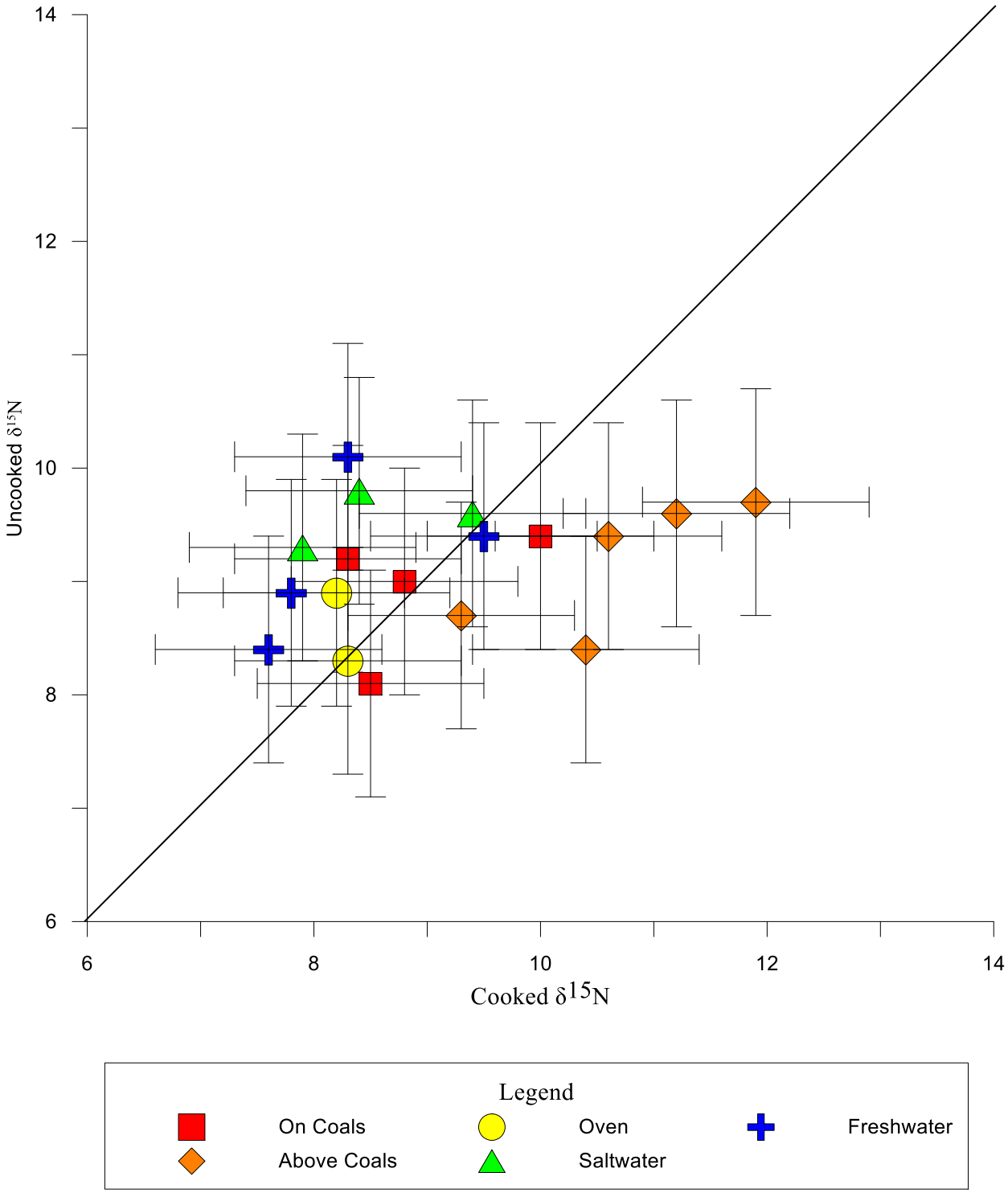

Figure 4. Plots of $\delta^{15}N$ values for *C. virginica* samples treated with different prehistoric cooking Pethods. Y-axis is control samples. X-axis is cooked samples. The solid line represents a 1:1 ratio. Error bars depict 2σ of standard deviation within a single untreated shell (0.62‰).

4b. STABLE ISOTOPE ANALYSIS OF SOFT TISSUES AND POM

$\delta^{15}N$, $\delta^{13}C$, %N, and %C values for the soft tissues of *C. virginica* differed by the tissue type (Appendix Table 11). $\delta^{15}N$ values for the tissues sampled (right mantle, left mantle, adductor muscle, gills, and undigested stomach contents) have an average of 13.12‰, 13.00‰, 13.99‰, 13.30‰, and 11.81‰. Average $\delta^{13}C$ values for the right mantle, left mantle, adductor muscle, gills, and undigested stomach contents are -25.95‰, -26.06‰, -24.09‰, -25.53‰, and -28.76‰. $\delta^{15}N$ values range from 12.7‰ to 13.8‰ for the right mantle, 11.6‰ to 13.4‰ for the left mantle, 13.3‰ to 14.4‰ for the adductor muscle, 12.9‰ to 13.8‰ for the gills, and 11.2‰ to 13.1‰ for the undigested stomach contents. $\delta^{13}C$ values range from -27.1‰ to -24.4‰ for the right mantle, -32.5‰ to -24.1‰ for the left mantle, -29.6‰ to -23.1‰ for the adductor muscle, -30.8‰ to -24.3‰ for the gills, and -33.9‰ to -25.8‰ for the undigested stomach contents (Figure 8).

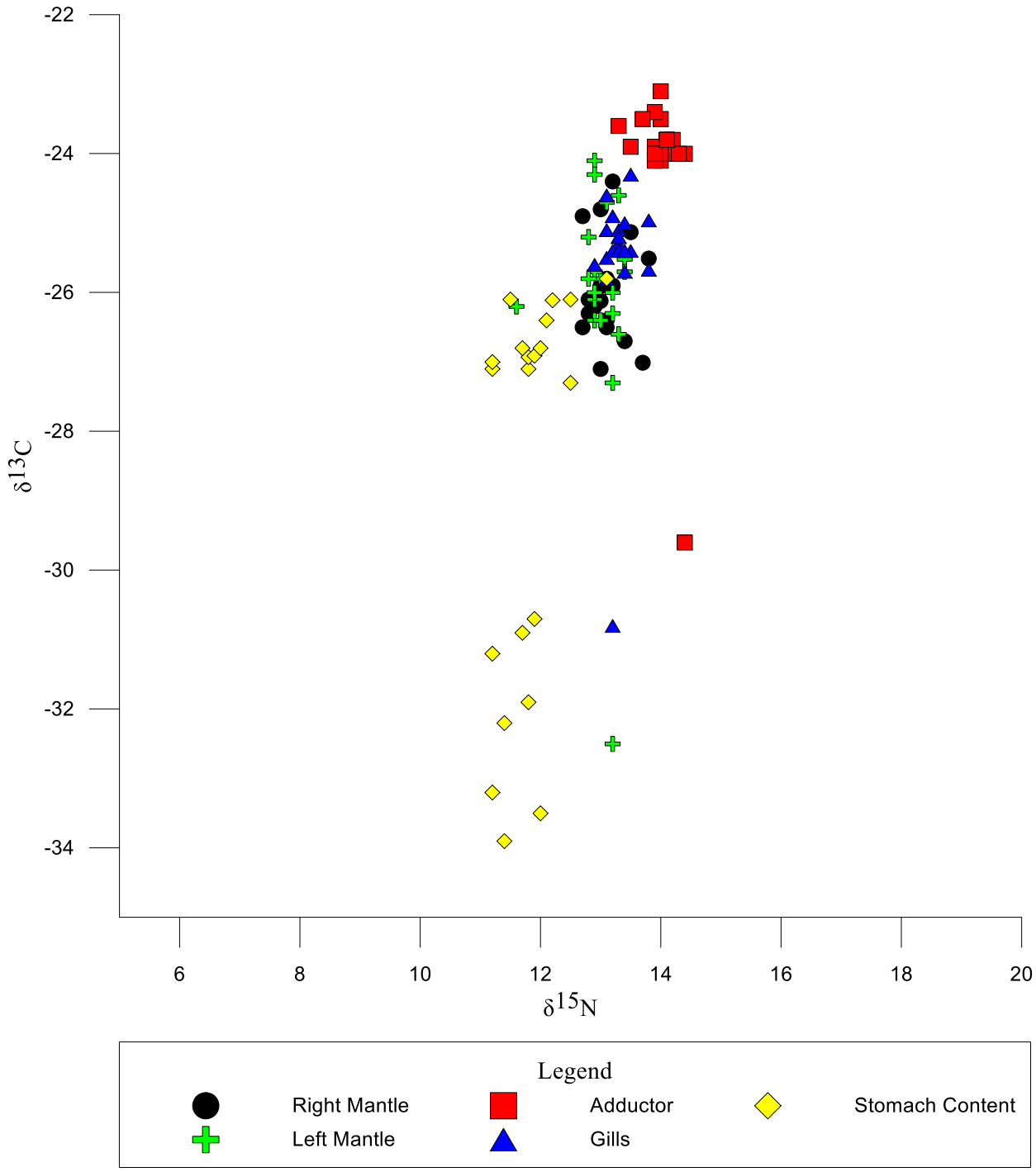

Figure 5. $\delta^{15}N$ and $\delta^{13}C$ plot for different *C. virginica* soft tissues. Y-axis is $\delta^{13}C$ and X-axis is $\delta^{15}N$.

%N values for the tissues sampled (right mantle, left mantle, adductor muscle, gills, and undigested stomach contents) have an average of 6.83%, 6.98%, 11.79%, 8.36%, and 7.49%. Average %C values for the right mantle, left mantle, adductor muscle, gills, and undigested stomach contents are 42.71%, 42.60%, 42.96%, 41.15%, and 44.83%. %N values range from 5.23% to 8.44% for the right mantle, 5.13% to 9.48% for the left mantle, 11.17% to 12.64% for the adductor muscle, 7.40% to 9.20% for the gills, and 6.58% to 9.51% for the undigested stomach contents. %C values range from 39.62% to 45.92% for the right mantle, 40.17% to 44.40% for the left mantle, 40.05% to 44.22% for the adductor muscle, 37.31% to 42.93% for the gills, and 41.77% to 47.24% for the undigested stomach contents (Figure 9).

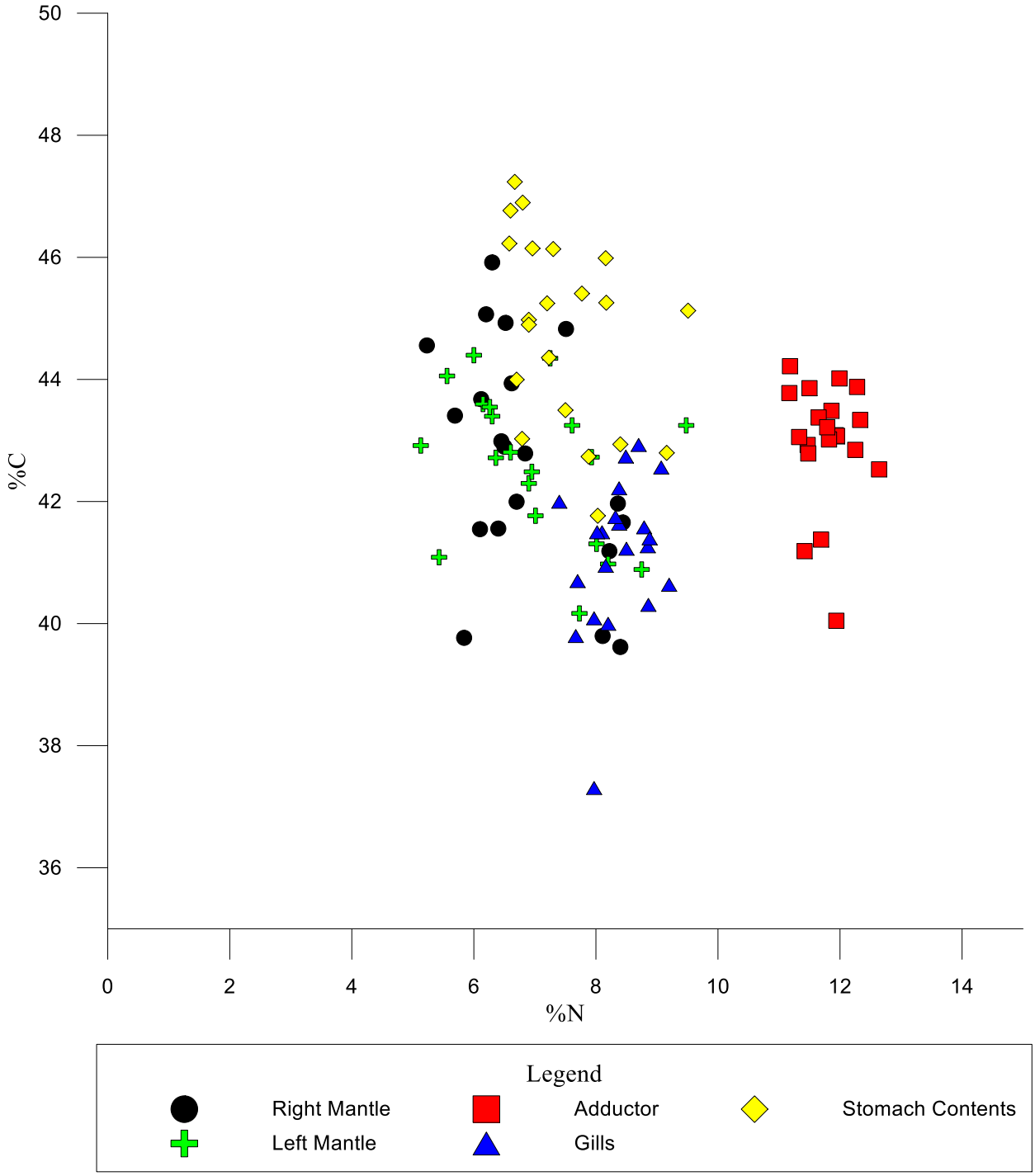

Figure 6. Weight percent of of *C. virginica* soft tissues. Y-axis is %C and X-axis is %N.

In most of the organisms studied, both $\delta^{15}N$ and $\delta^{13}C$ became more positive from undigested stomach contents < left mantle tissues < right mantle tissues < gills < adductor muscles. The %N values increased from the left mantle < right mantle < undigested stomach contents < gills < adductor muscle. The %C values increased from the gills < left mantle < right mantle < adductor muscle < undigested stomach contents.

$\delta^{15}N$ and %N values for POM samples differed with location within Chesapeake Bay (see Appendix Table 12). $\delta^{15}N$ values for the archaeological sites (1323, 287, and 308) averaged 6.0‰, 7.4‰, and 7.4‰. POM sampled from the dock over multiple days averaged 8.4‰ and ranged from 7.4‰ to 9.3‰ with a standard deviation of 0.7‰. A blank pre-combusted filter was also analyzed and had a $\delta^{15}N$ value of 6.4‰ but had an insignificant peak size of 486mV.

%N values of POM for the archaeological sites (1323, 287, and 308) averaged 0.44%, 1.03%, and 0.71%. POM sampled from the dock over multiple days averaged 0.93% and ranged from 0.59% to 2.03% with a standard deviation of 0.44%. The blank pre-combusted filter was 0.01%N; so all %N filters were adjusted to this value.

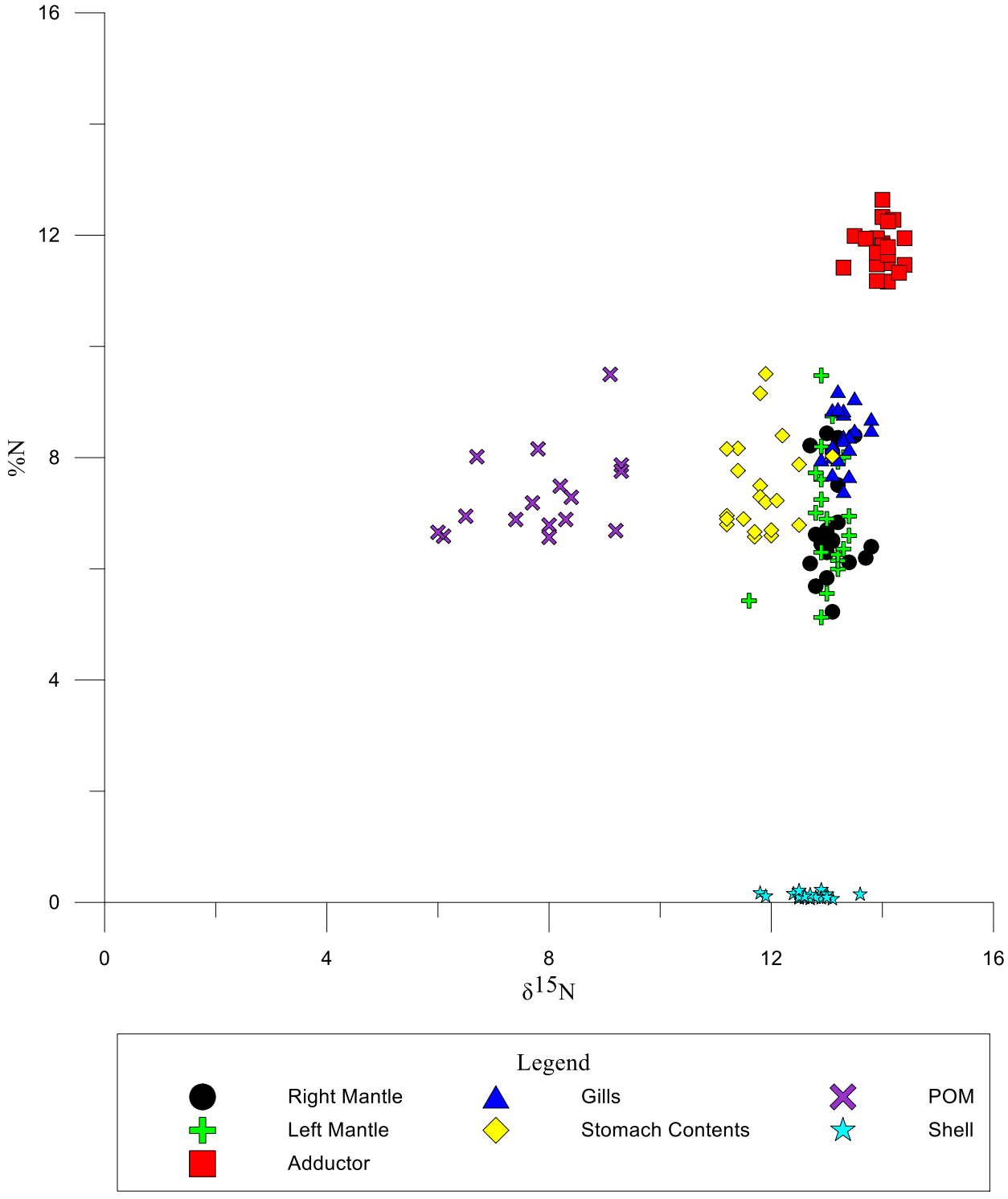

Figure 7. Plot of %N and $\delta^{15}N$ for different *C. virginica* soft tissues, POM, and shells. Y-axis is %N and X-axis is $\delta^{15}N$.

4c. STABLE ISOTOPE ANALYSIS OF SHELL MATERIAL

$\delta^{15}N$ and %N values for the mixture of pure calcite and isotope standards were relatively constant until a minimum amount of N was reached (Appendix Table 14). $\delta^{15}N$ values measured for the mixture of acetanilide and pure calcite have an average value of -0.6‰ and range from -1.0 to 2.1‰. %N values for the mixture of acetanilide and pure calcite have an average value of 0.12% and range from 0.01 to 0.23%. $\delta^{15}N$ values for the mixture of IAEA-N-2 and pure calcite have an average value of 19.3‰ and range from 15.9 to 19.9‰. %N values for the mixture of IAEA-N-2 and pure calcite have an average value of 0.14% and range from 0.03 to 0.24% (Figure 11).

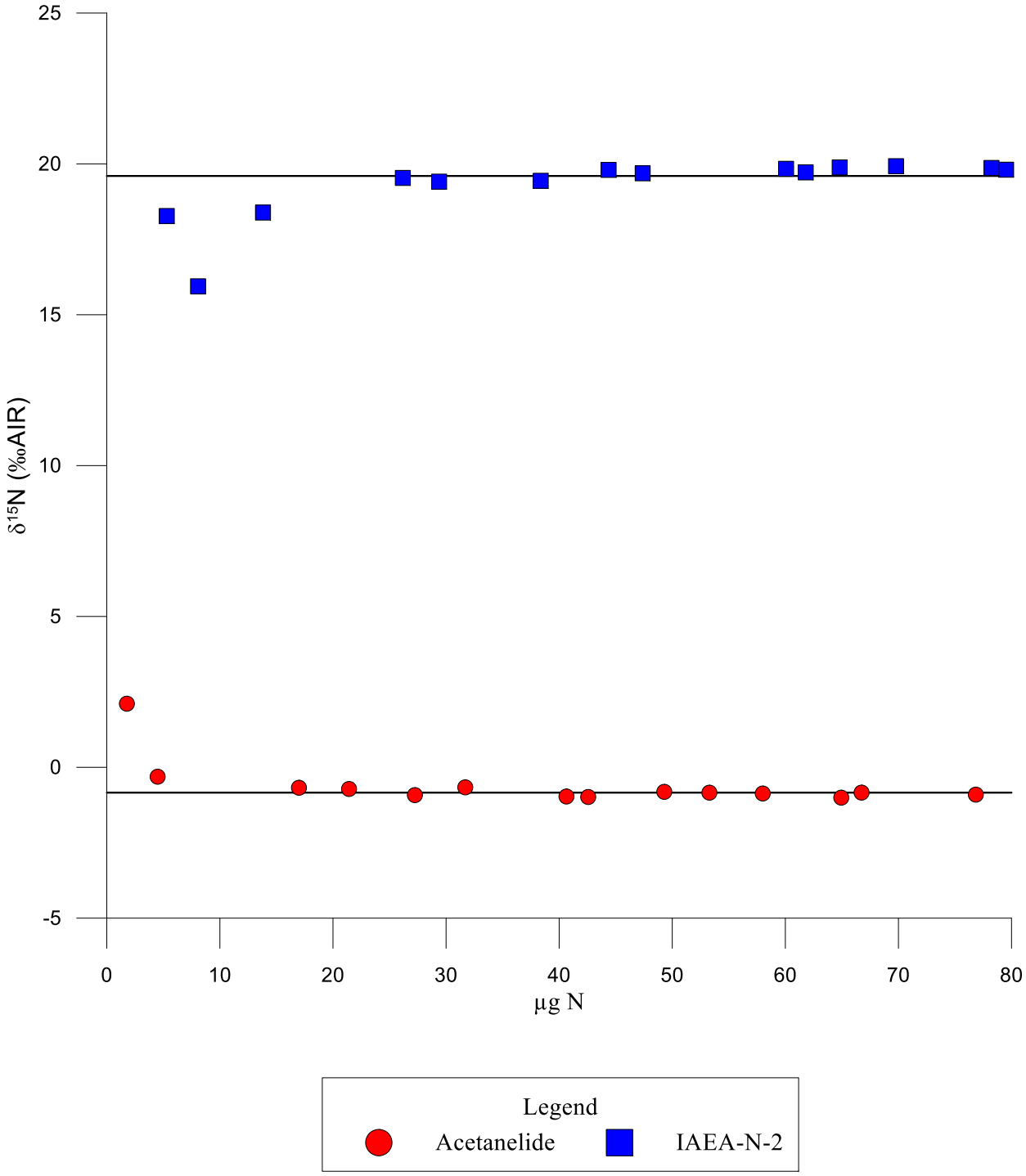

Figure 8. Calcite and isotope standard mixtures. Y-axis is $\delta^{15}N$ and X-axis is µg N.

Archaeological Site	Average $\delta^{15}N$	Minimum $\delta^{15}N$	Maximum $\delta^{15}N$
839	8.3‰	7.6‰	9.0‰
1323	9.3‰	7.9‰	11.4‰
308	8.8‰	8.2‰	9.9‰
287	8.3‰	6.5‰	8.9‰
285	8.2‰	7.0‰	8.9‰
Modern	12.6‰	11.8‰	13.6‰

Table 3. $\delta^{15}N$ values for the periods sampled (Archaeological sites 839, 1323, 308, 287, 285, and Modern).

Archaeological Site	Average %N	Minimum %N	Maximum %N
839	0.05%	0.02%	0.09%
1323	0.06%	0.03%	0.08%
308	0.02%	0.01%	0.05%
287	0.04%	0.02%	0.05%
285	0.03%	0.02%	0.05%
Modern	0.11%	0.05%	0.23%

Table 4. %N values for the periods sampled (Archaeological sites 839, 1323, 308, 287, 285, and Modern).

One-way ANOVA tests for $\delta^{15}N$ between time periods produced an F-value of 297.279 and a significance of 0.000 at α=0.05 (Appendix Tables 13 and 15). A Tukey Post Hoc test yielded results that show that archaeological site 308 is statistically significantly different from sites 285, later 1323 samples, and modern samples. Archaeological site 285 was statistically significantly different from sites 308, later 1323, and modern samples. Archaeological site 287 was statistically significantly different from later 1323 and modern samples. Archaeological site 839 samples were statistically significantly different from later 1323 and modern samples. Earlier 1323 samples were statistically significantly different from later 1323 samples and modern samples. Later 1323 samples were statistically significantly different from all other time periods. Modern shells were significantly different from all other time periods.

The F-value produced by a one-way ANOVA for %N was 34.779 with a significance of 0.000 at α=0.05. A Tukey Post Hoc test yielded results that show archaeological sites 308, 285,

and 287 were statistically significantly different only from the modern samples. 839 and 1323 samples were only statistically significantly different from the modern samples collected in the winter. The modern samples collected in the winter were statistically significantly different from all other time periods, including modern samples collected in the summer. Modern samples collected in the summer were statistically significantly different from all other time periods with the exception of 839 and 1323.

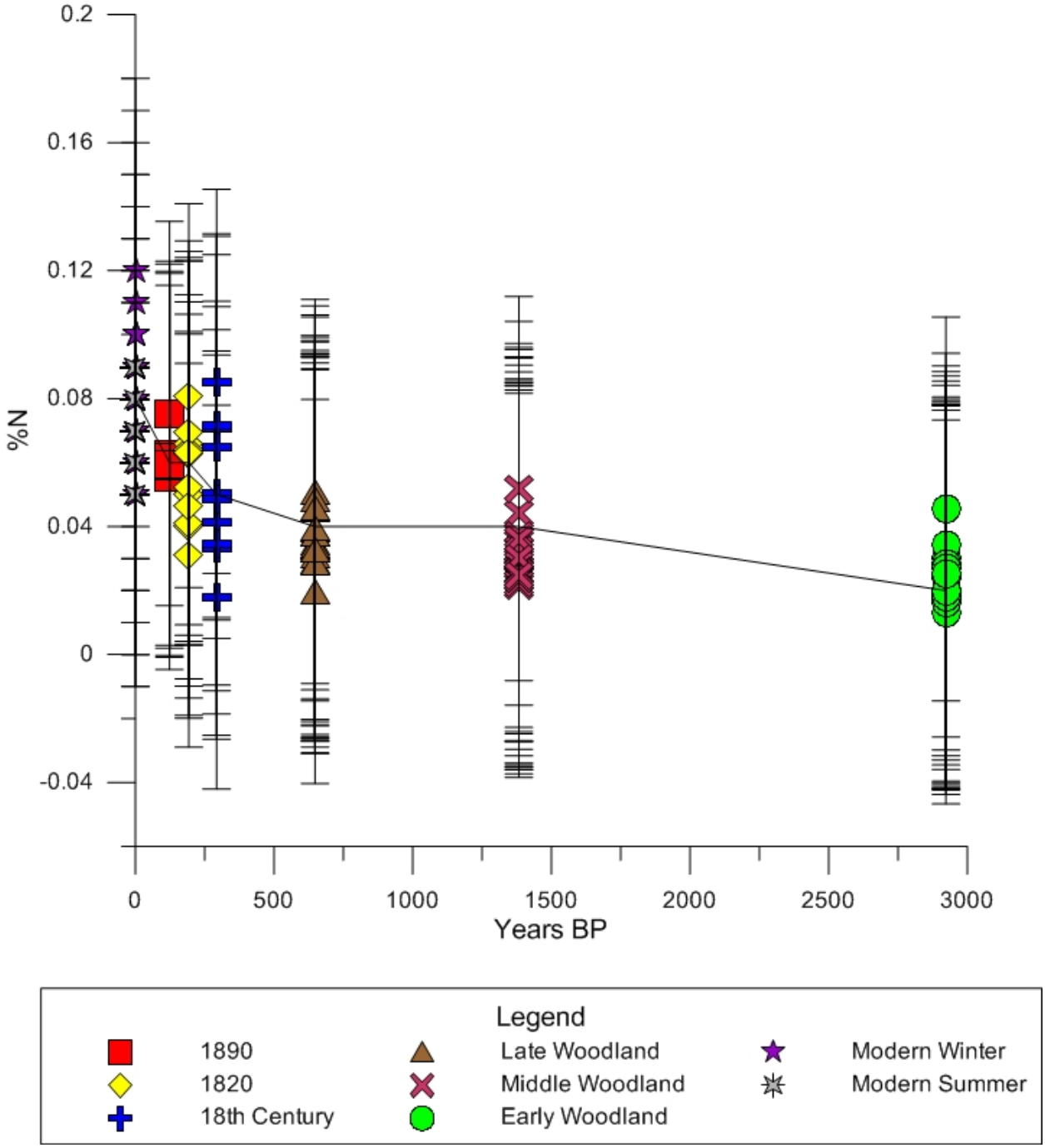

Figure 9. Plot of %N values for modern and archaeological *C. virginica* shells. Y-axis is %N and X-axis is years before present.

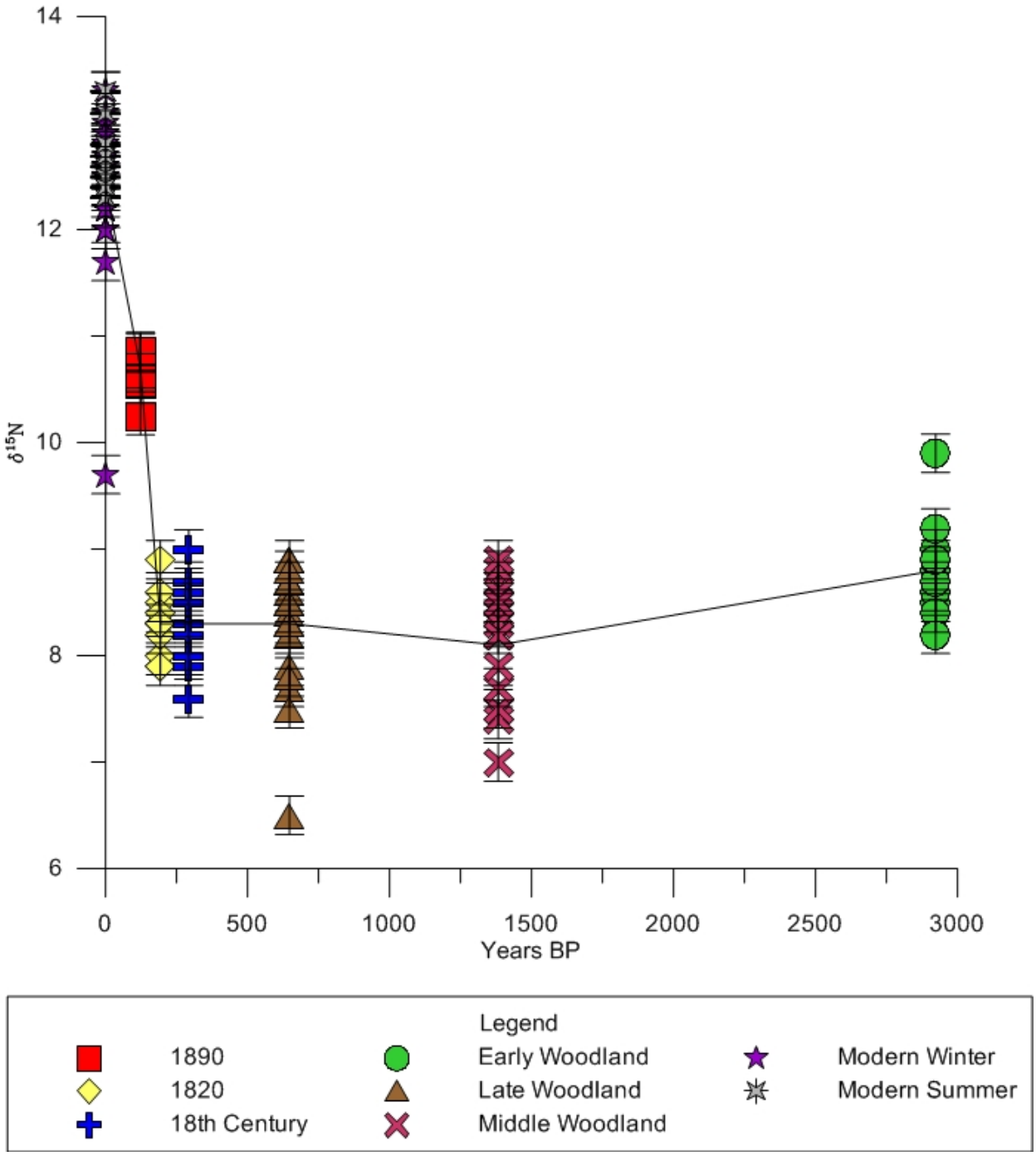

Figure 10. Plot of $\delta^{15}N$ values for modern and archaeological *C. virginica* shells. Y-axis is $\delta^{15}N$ and X-axis is years before present.

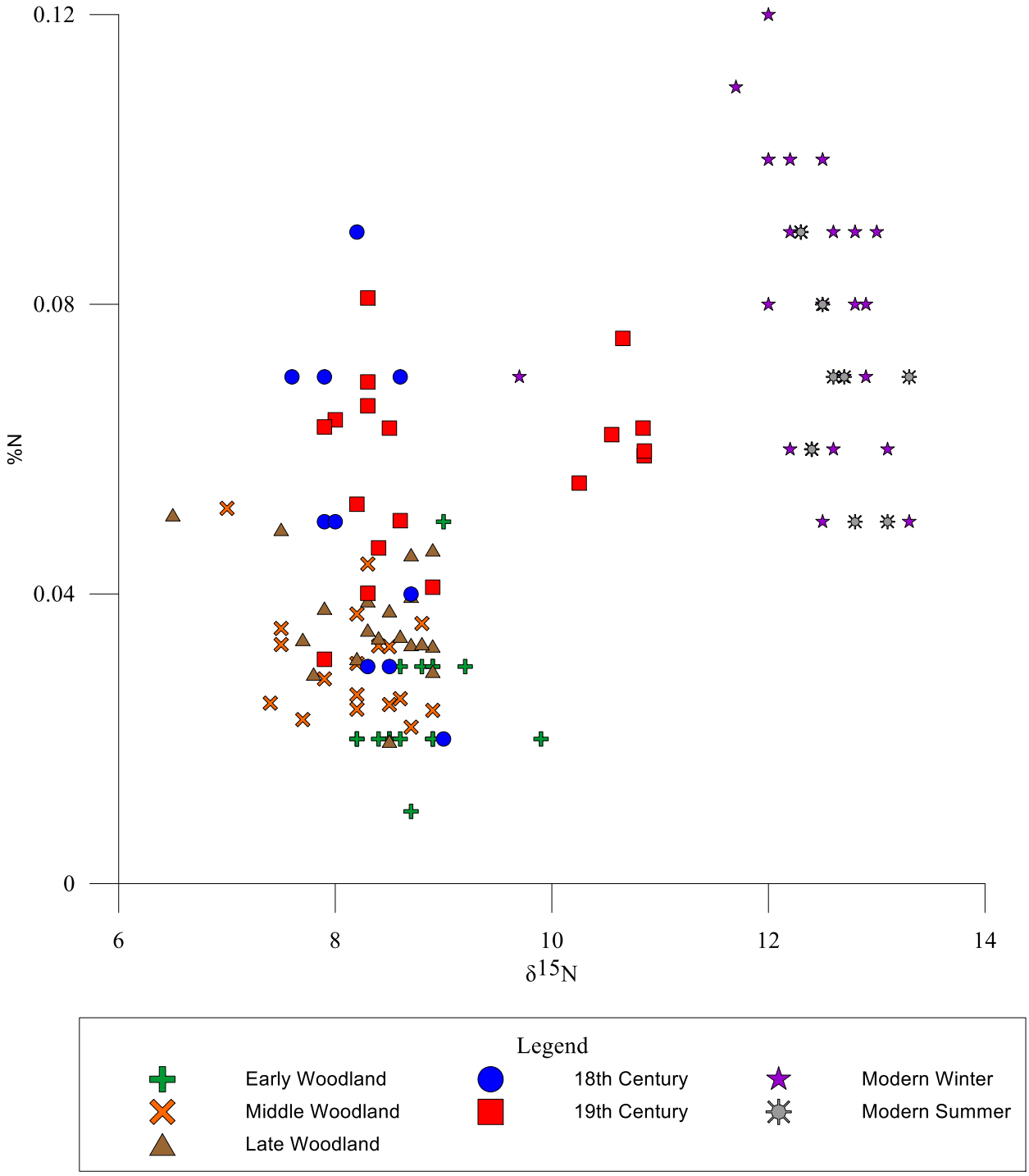

Figure 11. Plot of %N and $\delta^{15}N$ in modern and archaeological *C. virginica* shells. Y-axis is %N and X-axis is $\delta^{15}N$.

4d. SEM

The intercrystalline organic matter within the shell matrix is developed as an envelope enclosing individual calcite crystals and was qualitatively measured in regard to whether or not it completely covered the crystal, and if so, a relative thickness of the organic matter envelope. SEM images show little differentiation between the amount of intercrystalline organic matter between modern and archaeological samples within the resilier of the oyster shell. However, there are relative differences in the amount of organic matter in different locations within the shell, i.e., resilifer and margin. It appears that there is more organic matter closer to the edge of the shell than in more interior regions.

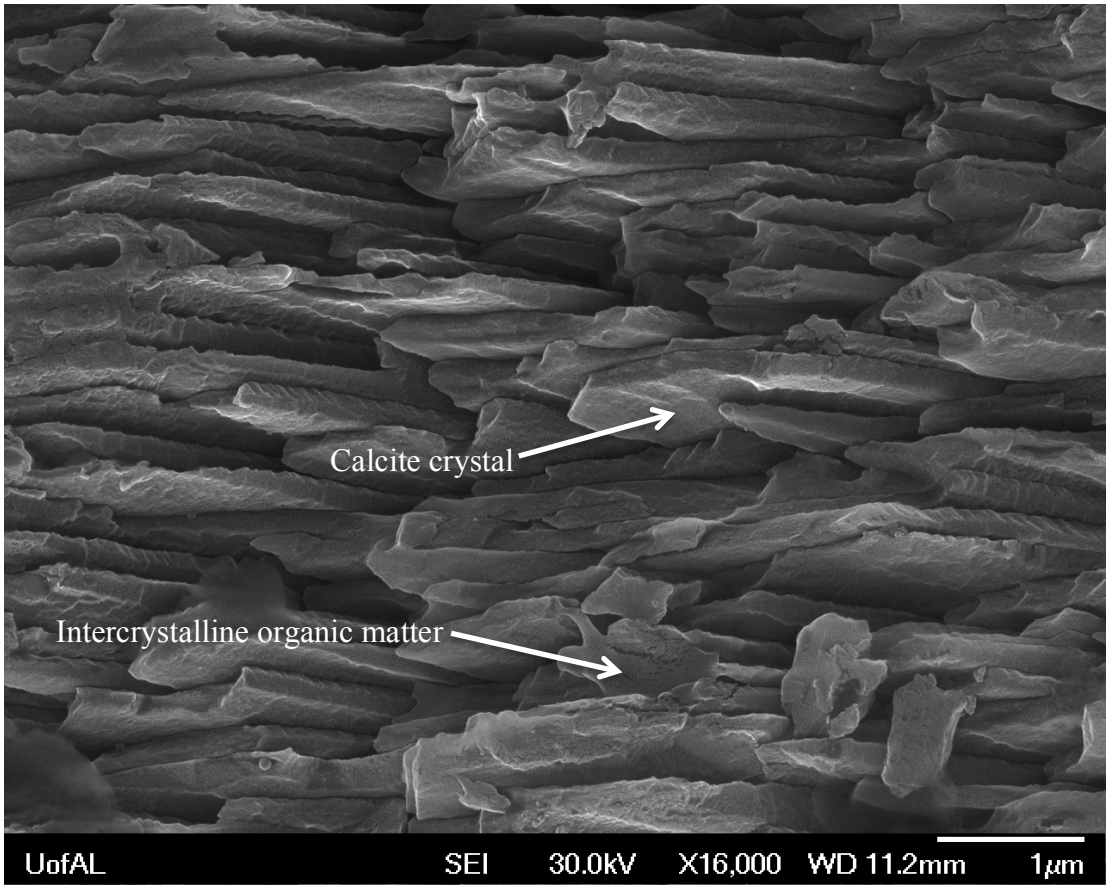

Illustration 4. SEM image of modern sample of *C. virginica* showing degree of covering of calcite crystals by intercrystalline organic matter.

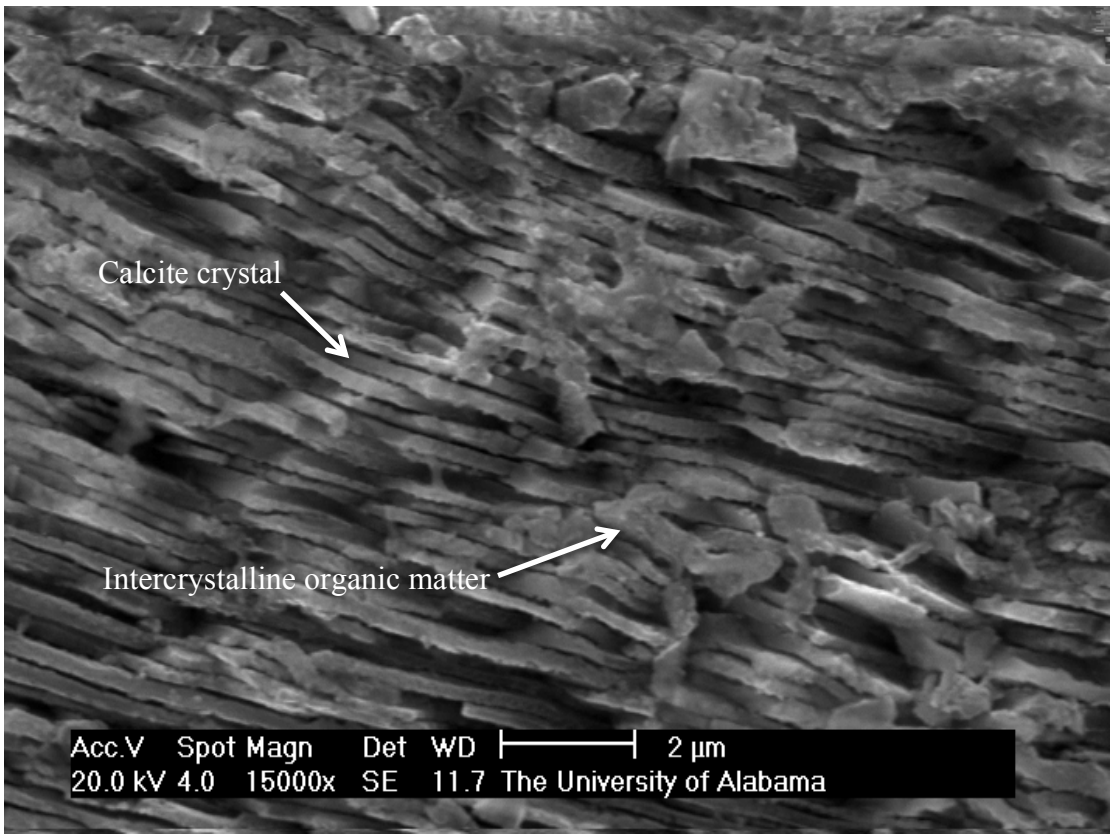

Illustration 5. SEM image of ~ 3400 year old sample of *C. virginica* showing degree of covering of calcite crystals by intercrystalline organic matter.

5. DISCUSSION

5a. PREHISTORIC COOKING METHODS ANALYSIS

Assessing the potential alteration of $\delta^{15}N$ in the cooked samples is important to paleoenvironmental reconstructions using archaeological samples because direct evidence of cooking methods are not typically found in archaeological sites, yet some methods could impact shell chemistry. The elemental and isotopic properties of both *C. virginica* and *Mercenaria spp.* are stable under the tested cooking methods, with the exception of roasting above the coals. While the two bivalve species differed slightly in the amount of $\delta^{15}N$ enrichment or depletion that occurred from cooking the shells, the majority of samples were within 2σ of the control sample. Therefore, there is not a significant difference in the $\delta^{15}N$ values between the control and cooked samples for the sample cooked directly on the coals, roasting in a dry oven, and boiling in fresh and salt water. Therefore, most archaeological shells can potentially be used as a paleoenvironmental proxy using %N and $\delta^{15}N$.

However, the samples that were cooked above the coals all showed a significant difference ($>2\sigma$) from the control samples. Both *C. virginica* and *Mercenaria spp*. showed an increased concentration of %N and a depletion of ^{15}N in the cooked half of the sample. Currently, there are no published studies on the effect of smoke on the elemental and isotopic concentration of N in carbonates, but there are numerous studies of the effect on smoke on $\delta^{13}C$ in plants (Czimczik *et al.,* 2002, Krull *et al.,* 2003, and Das *et al*., 2010). It is likely that $\delta^{15}N$ in carbonates behaves similarly to $\delta^{13}C$ in vegetation as it is burned and it is the smoke that alters the %N and $\delta^{15}N$ measured in the shell, not an alteration of the carbonate itself. Since these

shells were crushed whole, it is possible that the smoke from the coals had infiltrated pore spaces and deposited N on the exterior of the shell.

Another explanation for the addition of %N and ^{15}N in the cooked shells could be caused by the loss of hydrophobic lipids. Within increasing temperatures, lipid molecules are degraded while hydrophilic proteins remain unaltered (Robbins and Ostrom, 1995), which could lead to increased %N and $\delta^{15}N$ concentrations within the shell after cooking. Additional tests should be completed to determine if there is any significant alteration of %N and $\delta^{15}N$ between the exterior and interior of a bivalve shell after cooking above the coals.

Regardless of the process that altered the elemental and isotopic composition of the shells burned above coals, the changes in %N and $\delta^{15}N$ could prevent their use as paleoenvironmental proxies. However, those shells that were roasted directly on and above the coals had a noticeable alteration of color (white to dark grey after roasting), and became chalky with a brittle texture. Since there is currently no way to independently decipher where archaeological shells were placed in the fire, burnt shells should not be used for isotopic analysis. Only shells that appear to be in an unaltered physical condition (i.e., non-chalky, white shell and purple ligament scar) should be used in any elemental or isotopic study involving N in archaeological shells.

5b. STABLE ISOTOPE ANALYSIS OF SOFT TISSUES

We sought to build upon previous studies (e.g. Carmichael *et al.,* 2008, Carmichael *et al*., 2004, Fertig *et al.,* 2010, and Fertig *et al*., 2009) in an attempt determine whether there is a correlation between the N stable isotopes in POM, individual types of soft tissues, and shells. There is a relative trend present in this study that shows slightly increasing $\delta^{15}N$ values from the undigested stomach contents to the mantle and gills, and then to the adductor muscle. The

differences between these values are likely due to the differences between the metabolic activities within these tissues. Those tissues that metabolize more slowly and therefore have a longer turnover rate, such as the adductor muscle, will incorporate more ^{15}N than those tissues that metabolize more quickly (Fertig *et al*., 2010 and Lorrain *et al*., 2002). Due to the isotopic fractionation during digestion and waste elimination, we should expect a 3-4‰ difference between each trophic step (Vander Zanden and Rasmussen, 2001, Minigawa and Wada, 1984, and De Niro and Epstein, 1981)), which we see between the POM samples and soft tissue and shell samples.

Due to the methodology used in this study to analyze the shells (no acid pretreatment and use of a carbon trap in the EA), there are no %C or $\delta^{13}C$ data for POM or shell material, but the differences between soft tissue samples are still useful to determine the isotopic fractionation between tissue types. It should also be noted that the sampling of the undigested stomach contents likely contains some of the organism's stomach tissues as well and is not a pure measure of the undigested food within the stomach. As we did not have stable isotope values of the potential food sources in Chesapeake Bay, we cannot quantitatively evaluate the diet of *C. virginica* in detail, but we can obtain a general idea of the isotopic fractionation between tissues. Unlike the enrichment of $\delta^{15}N$ over trophic levels, $\delta^{13}C$ in the soft tissues generally reflects the $\delta^{13}C$ content of the diet with little or no change in isotopic ratios (Lorrain *et al*., 2002, Fry and Sherr, 1984, and DeNiro and Epstein, 1978), and different energy sources have distinct values (Vander Zaden and Rasmussen, 2001).

$\delta^{13}C$ also shows an increasing trend in values from the undigested stomach content to the adductor muscle, but it differs slightly from that of $\delta^{15}N$. Numerous studies have shown that variations in $\delta^{13}C$ result from differences in the biochemical content of the tissues, especially the

lipid content. Lipids are typically depleted in ^{13}C in comparison to other biochemical components (Lorrain *et al.,* 2002, Thomson *et al*., 2000, and Focken and Becker, 1998). Therefore high lipid contents in soft tissues typically have a light isotopic $\delta^{13}C$ signal. This inverse relationship is shown as the tissues with a higher lipid composition (i.e., mantle) have a lighter $\delta^{13}C$ composition than tissues with a lower lipid composition (i.e., adductor muscle). Another possibility for the differences in $\delta^{13}C$ content between tissue types could reflect isotopic routing (Gannes *et al*., 1998), where specific tissues have different allocations of dietary elements (Lorraine *et al.,* 2002).

%N and %C values of the tissue types show less of a trend than the isotopic values do. They are more heavily clustered and show only slight differences between the tissue types. %N increases from the shell < mantle < undigested stomach contents < gills < adductor muscle. It is possible that the low amount of N in the mantle is due to physiological effects of shell building; the species might use the N within the mantle and incorporate it within the shell matrix as it is deposited. Since the gills and adductor muscle have higher %N values than the undigested stomach contents, it is likely that these tissues are storing excess N over time. However, it has been shown that $\delta^{15}N$ values of soft tissues in *C. virginica* are independent of the total nitrogen concentration of the water body (Fertig *et al*., 2009), so the elemental composition of the shells are unlikely to be used in conjunction with $\delta^{15}N$ values as environmental proxies. %C values increase from gills < mantle and adductor muscle < undigested stomach contents. Since the %C of the tissues were all lower than the undigested stomach contents, it is likely that the organism is using the C from its food supply to maintain its tissues and deposit the shell material.

Stable N isotope analyses in mollusks are typically performed on the soft tissues of the organism, but due to the rapid decomposition and unlikely preservation of the tissue over time, it

would be preferential to study the shells of these organisms. $\delta^{15}N$ in the shell is directly proportional to that in the soft tissues of *C. virginica*. Therefore, $\delta^{15}N$ can be considered to be almost equal between the soft tissues and shell of *C. virginica*. However, the $\delta^{15}N$ of the adductor muscle is typically 1-2‰ higher than the shell, so it might be useful to use multiple organs when analyzing the $\delta^{15}N$ content of soft tissues. O'Donnell *et al.* (2003) determined that the differences between the isotopic ratios in soft tissues and shell materials may be related to differences in the proteins within the shell, so it might also be beneficial to perform compound-specific isotopic analyses on both shell and soft tissues to determine if these small differences are due to proteins or other lipids.

This portion of the study showed that the $\delta^{15}N$ in shell organic matter of *C. virginica* could be a proxy of the soft tissues in $\delta^{15}N$ analyses. This conclusion corroborates other similar studies (e.g., O'Donnell *et al.,* 2003; Carmichael *et al*., 2008). Analyzing the shells not only allows for easier sample handling, but it can extend the possibility of isotopic analyses into museum and archaeological samples as well. Furthermore, since shell materials are not metabolically active, time-series reconstruction may be possible by sequentially measuring accreted shell. It is assumed that changes in the stable isotope ratios are negligible in decomposition and diagenesis of organic matter (Macko and Ostrom, 1994), but further experiments of the stability of $\delta^{15}N$ in shells over time need to be established to fully use mollusk shells as a paleoenvironmental proxy.

5c. STABLE ISOTOPE ANALYSIS OF SHELL MATERIAL

In order to obtain accurate $\delta^{15}N$ values from powdered shell samples, it was necessary to determine the minimum amount of carbonate powder and subsequently the mass of N required

for analysis. While most of the isotopic standards measured across various sizes were relatively close to the accepted $\delta^{15}N$ value of the standard, samples at or below 850 mV in peak size showed a significant shift in the measured $\delta^{15}N$ value of the sample. It is recommended that in future studies using similar methods and instrumentation, samples should have a peak amplitude of at least 1000 mV in order to be considered valid measurements. However, this value is machine specific and future studies should test their specific IRMS system to determine the minimum acceptable mV value for analysis. In order to reach this minimum amplitude in peak size, it was necessary to have at least 16 μg N in a powdered shell sample for *C. virginica*.

In order to determine if *C. virginica* shells can be used as a paleoenvironmental proxy, $\delta^{15}N$ and %N were studied in Early, Middle, and Late Woodland Period shells as well as 18th century, 19th century, and modern shells. Both %N and $\delta^{15}N$ values over time produced a roughly exponential curve with higher concentrations of N and more ^{15}N enriched shells occurring in the 19th century and modern shells. %N remains more constant than $\delta^{15}N$, but increases ~0.03% from the 18th century to modern shells. $\delta^{15}N$ values remain relatively constant from the Early Woodland until the first part of the 19th century (i.e., 1820) but then increase ~2‰ from 1820 to 1890. There was another substantial increase in $\delta^{15}N$ values (~2‰) between the 1890 shells and the modern collected shells, which is correlated to a 10-fold increase in reactive N species globally from 1860 to 1990 (Galloway *et al*., 2004). However, %N is less robust as a paleoenvironmental proxy to determine anthropogenic induced effects than $\delta^{15}N$ due to the smaller variations within %N values over time and greater analytical uncertainty.

These results mirror a sediment study in Chesapeake Bay over the last ~2700 years, with the exception of when $\delta^{15}N$ began to rapidly increase within the bay (Bratton *et al*., 2003). Bratton *et al*. (2003) observed significant increases in $\delta^{15}N$ from 1750 – 1800 whereas we

determined a later time period of enriched ^{15}N content. This is likely due to sampling location, however, since Bratton *et al*.'s study location was closer to the Susquehanna River, which is a larger source of N for Chesapeake Bay (Howarth, 2008) than our study location. A study location farther north in the bay would also not have as long of a period to mix with estuarine waters before deposition, so it could potentially record higher $\delta^{15}N$ ratios. Another possibility for the timing differences between this study and Bratton *et al.* (2003) is due to the dating methods used. While this study solely used ^{14}C from associated terrestrial carbon to date the shells, Bratton *et al*. (2003) used pollen stratigraphy, ^{14}C of shell material within the cores, and the short-lived radioisotopes ^{137}Cs and ^{210}Pb within recently deposited sediment (Cronin *et al*., 2000). Since pollen was primarily used to relatively date the sediment, it is possible that the absolute dates obtained by ^{14}C analysis in the shells could permit larger error in the dating due to reworking and bioturbation of the soil.

Due to the relatively constant %N and $\delta^{15}N$ values through time until 1820 and the dramatic increase in %N and $\delta^{15}N$ values after, it is interpreted that this exponential increase shows increased anthropogenic inputs into Chesapeake Bay during the 1800s. During the Woodland Periods, it is unlikely that the hunter-gathering and small settlement lifestyles of the native population had any significant impact on the N loading of the bay. While there was urban development during the 18th century, it is also unlikely that the lack of technological advances and use of larger scale agriculture had any effect on the bay. However, there are currently no published studies on how elevated concentrations of N within a water body affects %N in either bivalve soft tissues or shell material. More studies are necessary to determine if increased concentrations of N within a water body can affect the amount of N deposited within the shell matrix or if the N concentration within shell is biologically limited.

However, during the 19th century, population sizes and industrialization in the northeast US grew rapidly, which likely had a profound impact on the ecological health of the bay. Between 1830 and 1880, over 80% of the forest surrounding Chesapeake Bay had been cleared, which in combination with deep-plowing methods for agriculture, greatly increased the sediment accumulation in the bay (Bratton *et al.*, 2003, Cooper and Brush, 1993). Beginning in the 1840s, nitrogen rich commercial fertilizers, especially the South American guano, were frequently used in agricultural settings (Cooper 1995). During this time, the population size nearby Chesapeake Bay nearly doubled and the increased amounts of sewage discharge and erosion caused by plowing (Cooper and Brush, 1993) likely increased the N inputs and $\delta^{15}N$ values in the bay. The significant decrease in oyster populations in the bay at this time also contributed to the failing health of the bay due to decreased filtration rates in the bay (Kemp *et al*., 2005). Therefore, it is likely that %N and ^{15}N were able to accumulate within the POM and sediment of the bay. Consequently, we would expect a substantial shift in $\delta^{15}N$ values during the 19th century due to increased amounts of sewage and eroded soil entering the bay, which was reproduced in the results of shell experiments.

It is likely, however, that the $\delta^{15}N$ values within the shells underestimate N loading within Chesapeake Bay due to N sources with lighter isotopic signatures, especially synthetic fertilizer use within the last century, mixing with isotopically heavier anthropogenic sources of N in the bay. It is also possible that changes in the diet of *C. virginica* has changed over time due to anthropogenic impacts to nutrient availability and trophic structure, which would affect both %N and $\delta^{15}N$ values within the shell material as well. Trophic level changes typically produce a 3-4‰ increase between primary producers and their consumers (Vander Zanden and Rasmussen,

2001), so any significant changes in the type and amount of POM within the bay could produce changes within the %N and $\delta^{15}N$ content within *C. virginica.*

However, another possible explanation for the apparent trends in %N and $\delta^{15}N$ could be an indication of diagenetic alteration over time with shells losing total N and a preferential loss of ^{15}N. Since the ancient shells were deposited in a shell midden, it is possible that the surrounding soil and groundwater was able to leach N from the shells over time. Nonetheless, it appears contradictory to the kinetics of stable isotopes to lose heavier isotopes first since they have less energy than lighter isotopes.

A preliminary experiment for this thesis was conducted to determine the potential effects of diagenesis in shells collected from Sapelo Island, Georgia that range in age from modern to ~550 years (see Appendix Table 1). The results of this study indicate that $\delta^{15}N$ values within the shell matrix were relatively constant over time. However, there was a significant decrease in %N from modern to ancient shells. While these results could indicate the diagenetic loss of N over time, it is also possible the sampling methods used incorporated more of the N-rich periostracum in some of the samples since the shells were ground whole instead of sampling from the interior of the shell. Unfortunately, there are currently no published studies on the effects of diagenetic alteration of N in carbonate shells, so more research is necessary to determine how N is affected by diagenesis and if it is chemically possible to lose ^{15}N enriched isotopes first.

5d. SEM

With such small sample sizes and large differences in the amount and location of intercrystalline organic matter within the *C. virginica* shells from the same time period, it is difficult to determine if diagensis occurred in the shells. There appears to be a large variation in

the amount of organic matter between shells of the same time period, but there is no apparent trend in the amount of organic matter over time in the modern and archaeological shells. This could potentially be due to differences in the amount of organic matter in the water column while the organism was still living, especially since the archaeological shells could have been collected from several locations before they were deposited into one shell midden.

Since there is more organic matter near the edge of the resilifer in comparison to the middle of the shell, the differences in the amount of organic matter could also be related to the ontogentic age and growth pattern of the oyster. While the age of the organism is known for the modern shells, it cannot be easily determined for the archaeological shells. It is possible that the older organisms incorporate less organic matter in the shell over time than the faster growing, younger organisms.

Due to the scope and nature of this thesis project, additional SEM research by others is recommended to determine a qualitative amount and location of intercrystalline organic matter within bivalve shells to determine if diagenesis, and loss of organic matter, is occurring in archaeological shells. A larger age range and more samples per time period is recommended to determine the variation in organic matter between shells from the same time period as well as the variation between shells over multiple time periods. Another research recommendation includes SEM analyses of modern shells from multiple locations to determine if the amount of POM within the water column has an effect on the amount of organic matter stored within the shell. Additional recommended analyses would include Ramen and thermogravimetric analyses to obtain more quantitative results.

6. CONCLUSION

Because *Crassostrea virginica* is one of the most common North American oyster species, and they are frequently found in shell midden sites in archaeological sites, they have the potential to serve as important geochemical paleoenvironmental proxies. In order to determine the fluctuation of N over time as human populations and subsequently N pollution rapidly increased, obtaining a base level of N before human impact is vital. Since little is currently known about base levels of N in prehistoric environments, using a geochemical proxy such as *C. virginica* shells could greatly benefit paleoenvironmental reconstructions. $\delta^{15}N$ of shell material of *C. virginica* is nearly identical to the $\delta^{15}N$ values of the soft tissues of the organism, so the shells can be used to interpret environmental conditions farther back into the Holocene or earlier.

Since most prehistoric shells are found in shell middens in archaeological sites, care is necessary to ensure that no taphonomic or diagenetic processes have altered the geochemistry of the shell. Most prehistoric cooking methods, with the exception of cooking above coals, have no significant alteration on %N or $\delta^{15}N$ within the shells. However, since there is no clear pattern of the fractionation of $\delta^{15}N$ due to cooking above the coals, shells that have obviously been burned should not be used in future studies due to the possibility of $\delta^{15}N$ alteration due to the smoke from the coals.

Specifically looking at %N and $\delta^{15}N$ values in *C. viriginica* shells from Chesapeake Bay, there are significant changes in the geochemical composition of the shells over time. Both %N and $\delta^{15}N$ remain relatively constant from ~3,400 years ago until 1820, but these values

exponentially increase after 1890 to the live-collected shells. While there is a possibility this data is a sign of diagenetic loss of N and ^{15}N over time, it can also be interpreted that the sharp increases in both %N and $\delta^{15}N$ between 1820 and 1890 are due to the industrial revolution and the subsequent increases from 1890 to 2013 are due to an exponential increase in human population, sewage discharge, erosion, and other anthropogenic pollution sources in Chesapeake Bay over the last two centuries.

More research is necessary to determine whether the changes in %N and $\delta^{15}N$ over time are due solely to increased anthropogenic impacts in the area or if there is diagenetic alteration where the shells are not only losing N over time, but preferentially losing ^{15}N as well. It would be beneficial to know the location and amount of intercrystalline and intracrystalline organic matter in the shell as well, so that diagenetic alteration can be ruled out as a possibility in ancient shells. It is also recommended to reproduce this study in other locations, especially locations where there are shells that represent more continuous time periods or locations where significant anthropogenic impacts began at an earlier age.

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APPENDIX

Table 5:

$\delta^{15}N$ and %N Data for Preliminary N Over Time Experiment.

Twenty-one shells were all collected in Sapelo Island, Georgia and range in age from modern to ~500 years old. The shells were ground whole to a fine, homogeneous powder and analyzed using the EA-IRMS methods discussed above. The data indicate a relatively constant δ15N values over time, but show a significant decrease in %N from the modern shells to those ~550 years old. This could be an indicator of possible diagenesis and subsequent loss of N within the shell matrix over time.

Sample	Age	$\delta^{15}N$	%N
O26	550	10.4	0.02
O41	550	8.1	0.03
O59	550	7.6	0.03
O94	550	9.5	0.04
A54	20	10.3	0.07
JC	20	7.9	0.06
M517	20	10.1	0.16
MT34	20	10.3	0.09
SC7	20	7.7	0.06
O1	Modern	9.2	0.14
O2	Modern	9.4	0.13
O3	Modern	8.1	0.15
O4	Modern	8.7	0.19
O5	Modern	8.4	0.15
O6	Modern	8.3	0.13
O7	Modern	8.9	0.10
O8	Modern	9.8	0.15
O9	Modern	9.3	0.09
O10	Modern	8.4	0.12
O11	Modern	8.9	0.10
O12	Modern	10.1	0.15

Table 6:

Shell Sample Height, Width, and Mass

Sample	Height	Width (mm)	Mass (g)
RR.M.1	113.5	57.78	67.01
RR.M.2	83.85	58.45	43.40
RR.M.3	128.36	63.85	61.20
RR.M.4	121.51	58.51	53.94
RR.M.5	95.46	52.43	34.80
RR.M.6	110.02	68.64	64.90
RR.M.7	120.40	67.88	68.52
RR.M.8	121.03	76.91	42.70
RR.M.9	82.98	52.18	29.40
RR.M.10	93.09	61.93	32.13
RR.M.11	109.08	56.57	32.80
RR.M.12	93.83	50.14	25.40
RR.M.13	115.85	64.41	44.22
RR.M.14	106.67	67.92	92.80
RR.M.15	126.36	53.59	49.21
RR.M.16	117.30	58.13	20.74
RR.M.17	92.69	60.50	33.60
RR.M.18	114.47	72.04	58.83
RR.M.19	109.72	65.41	49.42
RR.M.20	121.34	77.11	62.70
RR.M.21	121.37	67.18	52.31
18AN1323.7	97.00	54.60	46.05
18AN1323.7	112.90	64.10	69.92
18AN1323.7	86.00	59.50	49.75
18AN1323.7	87.60	63.50	46.05
18AN1323.7	70.80	62.20	34.45
18AN1323.7	79.80	61.50	53.89
18AN1323.7	77.10	53.10	35.56
18AN1323.7	76.30	51.60	19.64
18AN1323.7	85.70	42.10	31.25
18AN1323.7	85.80	55.00	46.60
18AN1323.7	90.30	56.70	36.78
18AN1323.7	79.80	44.40	33.13
18AN1323.7	80.80	55.50	32.78
18AN1323.7	61.20	44.30	15.50
18AN1323.1	110.5	73.60	83.53
18AN1323.1	90.30	63.10	67.03
18AN1323.1	92.60	61.80	39.21
18AN1323.1	84.00	60.80	56.13
18AN1323.1	83.30	74.50	53.89

18AN1323.1	74.40	63.30	29.15
18AN839.1	119.02	81.15	100.27
18AN839.2	107.63	56.53	58.54
18AN839.3	94.69	61.30	40.50
18AN839.4	78.20	42.57	21.79
18AN839.5	88.15	58.70	43.19
18AN839.6	74.54	47.33	30.25
18AN839.7	91.40	56.13	48.60
18AN839.8	110.48	58.79	75.86
18AN839.9	90.95	57.51	42.66
18AN839.10	114.59	62.62	86.28
18AN287.1	77.22	58.65	55.55
18AN287.2	87.99	54.54	41.09
18AN287.3	66.53	43.83	29.98
18AN287.4	79.26	57.13	45.77
18AN287.5	87.96	52.87	50.65
18AN287.6	83.31	73.40	61.27
18AN287.7	88.58	55.40	31.07
18AN287.8	54.57	43.76	25.59
18AN287.9	91.21	52.14	43.43
18AN287.10	88.47	57.55	22.56
18AN287.11	112.46	78.22	69.76
18AN287.12	88.93	59.63	40.54
18AN287.13	81.61	62.07	32.77
18AN287.14	76.30	49.07	25.25
18AN287.15	85.27	58.92	24.78
18AN287.16	100.04	67.78	52.28
18AN287.17	89.86	55.68	42.87
18AN287.18	109.48	54.38	32.03
18AN287.19	90.97	69.88	59.27
18AN287.20	109.98	61.73	51.51
18AN285.1	74.46	47.75	21.01
18AN285.2	66.28	44.36	24.51
18AN285.3	72.18	63.68	36.08
18AN285.4	75.53	51.03	40.54
18AN285.5	108.49	66.70	90.18
18AN285.6	58.17	46.33	16.37
18AN285.7	83.84	53.00	27.83
18AN285.8	61.73	37.33	15.58
18AN285.9	72.81	66.23	27.36
18AN285.10	79.96	57.25	50.26
18AN285.11	67.76	47.36	26.40
18AN285.12	66.53	42.21	11.89
18AN285.13	72.07	61.18	43.54

18AN285.14	69.85	49.47	20.73
18AN285.15	77.17	52.76	23.08
18AN285.16	69.88	46.56	27.28
18AN285.17	78.07	55.32	27.57
18AN285.18	87.88	54.77	53.13
18AN285.19	77.16	53.91	30.27
18AN285.20	87.18	57.31	36.95
18AN308.1	83.47	54.45	23.01
18AN308.2	66.79	36.72	7.31
18AN308.3	69.03	53.23	28.68
18AN308.4	77.93	57.56	19.19
18AN308.5	111.75	68.65	56.65
18AN308.6	84.03	56.69	26.42
18AN308.7	91.33	50.67	21.78
18AN308.8	69.69	44.19	11.82
18AN308.9	81.85	56.99	51.30
18AN308.11	70.22	59.27	30.06
18AN308.12	74.85	46.31	15.24
18AN308.13	78.10	41.89	13.40
18AN308.14	79.64	55.82	39.10
18AN308.15	86.84	57.79	77.26
18AN308.16	83.55	52.69	19.27
18AN308.17	74.55	38.69	12.46
18AN308.18	65.65	47.49	25.39
18AN308.19	86.30	53.43	33.73
18AN308.20	99.53	53.83	43.89
18AN308.21	77.50	64.64	36.08

Table 7:

Inventory of Archaeological Samples

Site	Time Period	Unit	Level	Count
18AN287	Late Woodland	1-S	LEVEL 1- UPPER	10
18AN287	Late Woodland	1	LEVEL 1- LOWER	10
18AN1323	19th century	1	LEVEL 1	6
18AN1323	19th Century	1	LEVEL 7	14
18AN285	Middle Woodland	1	Level 6	10
18AN285	Middle Woodland	1	Level 7	10
18AN839	18th Century	Bulk Sample 1	2-15 cmbs	10
18AN308	Early Woodland	1	4	10
18AN308	Early Woodland	1	3	10

Table 8:

Radiocarbon Data from Archeological Shells

Site	#	Provenience	Material[1]	$d^{13}C$	^{14}C Age	Calibrated Age (AD/BC, 2σ)[2]
18AN285	285c	Unit 1c.s., 70-71 cmbs	*C.v.*	-3.56	1890 ± 25	AD 560-700
18AN287	287a	Unit 2, 66cmbs	*C.v.*	-3.4	985 ± 25	AD 1410-1520
	287b	Unit 1, 55-58 cmbd	*C.v.*	-4.22	990 ± 30	AD 1400-1520
	287c	20 cmbs in creek exposure	*C.v.*	-3.86	1110 ± 25	AD 1310-1430
	287d	Unit 1, 65cmbd	C.v.	-4.1	1120 ± 30	AD 1300-1430
	287e	Unit 1 South, Level 1	*C.v. t.*	-4.91	1390 ± 25	AD 1050-1220
	287f	Unit 1 South, Level 1	*C.v. t.*	-4.54	1510 ± 45	AD 890-1150
18AN308	308a	Unit 1, 35cmbd, pair1	Char	-25.65	2760 ± 20	970-840 BC
	308b	Unit 2, 31cmbd, pair2	Char	-24.81	2900 ± 20	1190-1010 BC
	308c	C.S. 1, 42-43 cmbd, bottom of deposit	*C.v.*	-2.81	3150 ± 20	930-780 BC
	308d	Unit 1, 35cmbd, pair1	*C.v.*	-3.17	3210 ± 20	1000-820 BC
	308e	Unit 2, 31cmbd, pair2	*C.v.*	-3.96	3230 ± 20	1030-840 BC
	308f	20cmbs in creek exposure	*C.v.*	-.354	3240 ± 25	1060-840 BC
	308g	STP2, 33cmbs	*C.v.*	-1.83	3250 ± 20	1070-860 BC
	308h	C.S. 1, 16-18cmbd, top of deposit	*C.v.*	-3.74	3250 ± 20	1070-860 BC
	308i	AN308	*C.v.*	-2.23	3360 ± 30	1250-990 BC
18AN839	854a	Eroding exposure, 10cmbs	*C.v.*	-3.53	510 ± 25	AD 1840-modern
	854b	Bulk Sample 1, 12-14 cmbs	*C.v.*	-5.03	605 ± 25	AD 1720-modern
18AN1323	1323a	Base of Unit 69-70 cmbs	*C.v*	-3.98	465 ± 30	AD 1890-modern
	1323b	Top of Unit, 10-13 cm	*C.v.*	-4.97	550 ± 25	AD 1820-modern

[1] *C.v.= Crassostrea virginica*. *C.v.t.= Crassostrea virginica* shell temper. Char=Charcoal.

[2] All dates calibrated using OxCal 4.1 (Bronk Ramsey ; Reimer et al. 2009) and applying a standard reservoir correction of 118 ± 21 years for all marine shells.

Table 9: Prehistoric Cooking Methods Data for *Mercenaria spp.*

		Cooked Half		**Untreated Half**	
Cooking Method	Sample	$\delta^{15}N$ (‰ AIR)	Weight Percent (%)	$\delta^{15}N$ (‰ AIR)	Weight Percent (%)
On Coals	C1	10.7	0.07	10.3	0.10
On Coals	C2	10.4	0.07	9.9	0.09
On Coals	C3	10.9	0.07	10.2	0.09
On Coals	C21	9.4	0.02	10	0.07
Above Coals	C5	9.5	0.02	10.4	0.10
Above Coals	C6	9.3	0.01	10.3	0.08
Above Coals	C26	11.3	0.03	10.1	0.08
Above Coals	C29	10.7	0.03	9.7	0.07
Above Coals	C31	11.5	0.05	10.5	0.07
Oven	C7	9.5	0.11	10.1	0.06
Oven	C8	9.9	0.09	9.9	0.07
Oven	C9	10.5	0.07	9.4	0.15
Oven	C24	9.2	0.18	9.5	0.05
Oven	C27	10.2	0.08	10.1	0.06
Boiling in	C10	10.2	0.06	9.5	0.08
Boiling in Saltwater	C11	9.9	0.11	10.2	0.07
Boiling in Saltwater	C12	10.3	0.07	9.8	0.08
Boiling in Saltwater	C20	9.8	0.05	10	0.07
Boiling in Saltwater	C23	9.2	0.08	9.4	0.05
Boiling in Freshwater	C13	10	0.09	9.9	0.07
Boiling in Freshwater	C14	9.9	0.11	10.4	0.09
Boiling in Freshwater	C25	9.4	0.07	9.4	0.06
Boiling in Freshwater	C28	10.1	0.07	10	0.07

Table 10: Prehistoric Cooking Methods Data for *Crassostrea virginica*

		Cooked Half		Untreated Half	
Cooking Method	**Sample**	**$\delta^{15}N$ (‰ AIR)**	**Weight Percent (%)**	**$\delta^{15}N$ (‰ AIR)**	**Weight Percent (%)**
On Coals	O1	8.3	0.10	9.2	0.14
On Coals	O2	10	0.20	9.4	0.13
On Coals	O3	8.5	0.17	8.1	0.15
On Coals	O21	8.8	0.01	9	0.13
Above Coals	O4	9.3	0.04	8.7	0.19
Above Coals	O5	10.4	0.04	8.4	0.15
Above Coals	O20	11.2	0.05	9.6	0.09
Above Coals	O22	11.9	0.06	9.7	0.13
Above Coals	O23	10.6	0.07	9.4	0.09
Oven	O6	8.3	0.19	8.3	0.13
Oven	O7	8.2	0.11	8.9	0.10
Boiling in Saltwater	O8	8.4	0.16	9.8	0.15
Boiling in Saltwater	O9	7.9	0.16	9.3	0.09
Boiling in Saltwater	O24	9.4	0.13	9.6	0.15
Boiling in Freshwater	O10	7.6	0.19	8.4	0.12
Boiling in Freshwater	O11	7.8	0.11	8.9	0.10
Boiling in Freshwater	O12	8.3	0.23	10.1	0.15
Boiling in Freshwater	O25	9.5	0.11	9.4	0.11

Table 11. Isotopic and Weight Percent Data for Modern Oyster Tissues

Sample	$\delta^{15}N$	%N	$\delta^{13}C$	%C
RR.M.1.G	13.8	8.70	-25.7	42.93
RR.M.1.RM	13.7	6.20	-27.0	45.07
RR.M.13.RM	13.8	6.40	-25.5	41.56
RR.M.17.RM	13.5	8.40	-25.1	39.62
RR.M.18.S	12.2	8.40	-26.1	42.94
RR.M.2.S	11.8	7.30	-26.9	46.14
RR.M.4.S	11.9	7.20	-26.9	45.25
RR.M.6.G	13.8	8.50	-25.0	41.23
RR.M.6.LM	13.4	6.60	-25.5	42.81
RR.M.6.RM	13.0	6.30	-26.1	45.92
RR.M.7.G	13.3	7.40	-25.1	42.00
RR.M.1.LM	13.2	5.99	-27.3	44.40
RR.M.1.S	11.8	7.47	-27.1	43.50
RR.M.15.S	12.0	6.72	-26.8	44.00
RR.M.2.G	13.1	8.06	-25.8	41.50
RR.M.5.S	11.2	6.81	-27.1	46.90
RR.M.7.LM	13.0	6.88	-25.8	42.30
RR.M.7.RM	13.0	6.70	-25.9	42.00
RR.M.8.G	13.1	7.70	-25.8	40.70
RR.M.8.RM	13.1	6.48	-26.4	42.90
RR.M.8.S	11.2	6.88	-27.0	44.90
RR.M.9.G	13.1	8.20	-24.6	40.00
RR.M.13.G	13.4	7.67	-25.0	39.80
RR.M.16.G	13.3	8.85	-25.2	41.27
RR.M.20.G	13.2	7.97	-24.9	40.09
RR.M.20.LM	12.9	5.56	-26.4	44.06
RR.M.21.S	12.1	7.23	-26.4	44.36
RR.M.3.S	11.5	6.90	-26.1	44.98
RR.M.4.RM	12.9	6.45	-26.2	42.99
RR.M.5.G	13.1	8.02	-25.1	41.50
RR.M.5.LM	12.9	5.13	-26.1	42.92
RR.M.5.RM	12.8	5.69	-26.1	43.41
RR.M.10.G	13.5	9.07	-24.3	42.56
RR.M.10.LM	13.3	8.01	-24.6	41.31
RR.M.12.RM	13.0	8.44	-24.8	41.66
RR.M.15.G	13.4	8.38	-25.7	42.22
RR.M.15.RM	13.2	6.68	-26.6	44.37
RR.M.16.LM	13.2	7.93	-26.3	42.73
RR.M.18.G	13.2	8.88	-25.4	41.40
RR.M.19.G	13.3	8.32	-25.4	41.75
RR.M.19.RM	13.2	6.84	-25.9	42.79

RR.M.21.G	13.4	8.16	-25.4	40.95
RR.M.8.LM	12.9	6.30	-26.4	43.40
RR.M.10.RM	13.2	8.36	-24.4	41.97
RR.M.11.A	14.2	12.28	-23.8	43.88
RR.M.11.S	11.7	6.58	-26.8	46.23
RR.M.13.LM	13.4	6.95	-25.7	42.49
RR.M.14.RM	13.1	6.52	-25.9	44.93
RR.M.15.RM	13.1	6.27	-26.7	45.02
RR.M.16.A	14.0	11.82	-24.0	43.02
RR.M.5.A	13.5	11.99	-23.9	44.02
RR.M.6.A	14.1	11.50	-23.8	43.86
RR.M.8.A	13.9	11.18	-24.1	44.22
RR.M.10.A	14.0	12.33	-23.1	43.34
RR.M.11.LM	12.9	7.25	-26.0	44.35
RR.M.11.RM	13.2	7.51	-25.9	44.83
RR.M.12.A	13.9	11.48	-23.4	42.79
RR.M.13.A	13.9	11.95	-23.9	43.07
RR.M.18.RM	12.8	6.62	-26.3	43.94
RR.M.20.A	14.1	11.79	-23.8	43.22
RR.M.3.G	13.3	8.38	-25.3	41.64
RR.M.4.A	13.9	11.93	-24.1	43.09
RR.M.4.LM	12.9	7.61	-26.4	43.25
RR.M.9.LM	12.9	9.48	-24.3	41.77
RR.M.14.G	13.3	8.79	-25.2	41.58
RR.M.14.LM	13.2	6.26	-26.0	43.55
RR.M.17.G	13.1	8.86	-25.5	40.31
RR.M.17.LM	12.9	8.20	-25.7	40.98
RR.M.18.A	14.1	11.65	-23.8	43.38
RR.M.19.A	14.3	11.33	-24.0	43.06
RR.M.2.A	14.0	11.86	-24.1	43.49
RR.M.2.RM	13.1	5.23	-26.5	44.56
RR.M.3.A	14.1	11.17	-24.0	43.78
RR.M.9.A	13.3	11.42	-23.6	42.16
RR.M.9.RM	12.7	8.22	-24.9	41.19
RR.M.1.A	14.0	12.64	-23.5	42.53
RR.M.12.LM	13.1	8.75	-24.7	40.89
RR.M.15.A	13.7	11.94	-23.5	40.05
RR.M.16.RM	13.1	8.11	-25.8	39.80
RR.M.19.LM	12.8	7.73	-25.2	40.17
RR.M.20.S	12.1	6.79	-26.1	43.03
RR.M.3.LM	12.9	5.43	-26.2	41.09
RR.M.4.G	13.1	7.97	-25.6	37.31
RR.M.6.S	11.7	8.03	-25.8	41.77
RR.M.12.G	13.2	9.2	-30.8	40.64

RR.M.12.S	11.4	8.17	-32.2	45.26
RR.M.13.S	12	6.6	-33.5	46.77
RR.M.14.S	11.7	6.67	-30.9	47.24
RR.M.16.S	11.4	7.77	-33.9	45.41
RR.M.17.S	11.8	9.16	-31.9	42.8
RR.M.19.S	11.2	8.16	-33.2	45.99
RR.M.2.LM	13.2	6.15	-32.5	43.6
RR.M.7.A	14.4	11.47	-29.6	42.93
RR.M.7.S	11.2	6.96	-31.2	46.15
RR.M.9.S	11.9	9.51	-30.7	45.13
RR.M.10.S	12.5	7.88	-27.3	42.74
RR.M.14.A	14.4	11.95	-24	43.08
RR.M.17.A	13.9	11.69	-24	41.38
RR.M.20.RM	13.0	5.67	-27.1	43.8
RR.M.21.A	14.1	12.25	-23.8	42.85
RR.M.21.LM	13.3	6.36	-26.6	42.72
RR.M.21.RM	13.4	6.12	-26.7	43.68

Table 12: Isotopic and Weight Percent Data for Filtered POM Samples

Sample	**Date Sampled**	**Time Sampled**	**$\delta^{15}N$**	**%N**
Blank	N/A	N/A	6.4	0.00
D1.1.F	9/4/2013	7:43 PM	8.2	0.72
D1.2.F	9/4/2013	7:51 PM	8.4	0.61
D2.1.F	9/5/2013	9:30 AM	7.4	0.65
D2.2.F	9/5/2013	9:41 AM	7.7	0.60
287.1.F	9/5/2013	11:41 AM	8.0	0.85
287.2.F	9/5/2013	11:47 AM	6.7	1.23
308.1.F	9/5/2013	1:41 PM	6.5	0.67
308.2.F	9/5/2013	1:49 PM	8.3	0.76
D3.1.F	9/5/2013	4:56 PM	9.1	0.89
D3.2.F	9/5/2013	5:01 PM	9.3	2.04
D4.1.F	9/6/2013	9:16 AM	8.0	0.82
D4.2.F	9/6/2013	9:19 AM	7.8	1.12
1323.1.F	9/6/2013	10:24 AM	6.1	0.46
1323.2.F	9/6/2013	10:28 AM	6.0	0.43
D5.1.F	9/6/2013	1:47 PM	9.2	1.24
D5.2.F	9/6/2013	1:53 PM	9.3	0.75

Table 13: Isotopic and Weight Percent Data for Modern and Archaeological Shells

Sample	$\delta^{15}N$	%N
839.1	9	0.02
839.2	8.7	0.04
839.3	8.3	0.03
839.4	8.6	0.07
839.5	8.5	0.03
839.6	8.2	0.09
839.7	7.6	0.07
839.8	7.9	0.05
839.9	8	0.05
839.10	7.9	0.07
1323.1.1	10.9	0.06
1323.1.2	11.4	0.06
1323.1.3	11.1	0.06
1323.1.4	11.3	0.06
1323.1.5	11.1	0.08
1323.1.6	11.4	0.06
1323.7.1	8.5	0.06
1323.7.2	7.9	0.03
1323.7.3	8	0.06
1323.7.4	8.2	0.05
1323.7.5	8.3	0.07
1323.7.7	8.4	0.05
1323.7.8	7.9	0.06
1323.7.9	8.3	0.08
1323.7.10	8.6	0.05
1323.7.11	8.3	0.07
1323.7.12	8.3	0.04
1323.7.13	8.9	0.04
308.1	8.9	0.03
308.2	8.9	0.02
308.5	9.9	0.02
308.7	9.2	0.03
308.8	8.2	0.02
308.9	8.9	0.03
308.11	8.5	0.02
308.12	8.6	0.03
308.13	8.6	0.02
308.14	9	0.05
308.15	8.7	0.01
308.16	8.2	0.02
308.17	8.8	0.03
308.18	8.4	0.02

308.19	8.5	0.02
308.21	8.9	0.02
287.1L.11	7.7	0.03
287.1L.12	8.9	0.03
287.1L.13	6.5	0.05
287.1L.14	8.9	0.03
287.1L.16	7.9	0.04
287.1L.17	8.5	0.02
287.1L.18	8.3	0.04
287.1L.19	8.2	0.03
287.1L.20	7.8	0.03
287.1U.1	8.4	0.03
287.1U.2	8.7	0.05
287.1U.3	8.3	0.04
287.1U.4	7.5	0.05
287.1U.5	8.6	0.03
287.1U.6	8.9	0.05
287.1U.7	8.5	0.04
287.1U.8	8.7	0.04
287.1U.9	8.8	0.03
287.1U.10	8.7	0.03
285.6.1	8.7	0.02
285.6.2	7.5	0.03
285.6.3	8.4	0.03
285.6.4	7.4	0.02
285.6.5	7.5	0.04
285.6.6	8.3	0.04
285.6.7	7.7	0.02
285.6.8	8.5	0.03
285.6.9	7.9	0.03
285.7.11	8.2	0.02
285.7.13	7	0.05
285.7.14	8.2	0.04
285.7.15	8.8	0.04
285.7.16	8.2	0.03
285.7.17	8.9	0.02
285.7.18	8.6	0.03
285.7.19	8.2	0.03
285.7.20	8.5	0.02
M.1	11.8	0.17
M.2	12.9	0.23
M.3	12.5	0.08
M.4	12.5	0.1
M.5	11.9	0.11
M.6	12.7	0.07

M.7	13.1	0.06
M.8	13.6	0.15
M.9	12.9	0.08
M.10	12.5	0.1
M.11	13	0.15
M.12	12.5	0.16
M.13	12.5	0.08
M.14	13	0.09
M.15	12.6	0.09
M.16	12.8	0.09
M.17	12.4	0.16
M.18	12.5	0.11
M.19	12.7	0.14
M.20	12.6	0.11
M.21	12.5	0.21
M.22	12.1	0.14
M2.1	12.4	0.1
M2.2	12.7	0.07
M2.3	13.3	0.07
M2.4	12.3	0.09
M2.5	12.3	0.09
M2.6	13.1	0.05
M2.8	12.8	0.05
M2.9	12.1	0.11
M2.10	12.6	0.13
M2.11	12.5	0.08

Table 14: Isotopic and Weight Percent Data for Calcite and Isotope Standard Analysis

Sample	µg N in sample	$\delta^{15}N$	%N
A.1	76.84	-0.9	0.23
A.2	66.74	-0.8	0.19
A.3	64.94	-1.0	0.19
A.4	58.00	-0.9	0.17
A.5	53.28	-0.8	0.16
A.6	49.30	-0.8	0.15
A.7	40.64	-1.0	0.12
A.8	42.57	-1.0	0.13
A.9	31.70	-0.7	0.10
A.10	27.24	-0.9	0.09
A.11	21.42	-0.7	0.06
A.12	16.99	-0.7	0.05
A.14	4.50	-0.3	0.02
A.15	1.78	2.1	0.01
I.1	79.52	19.8	0.24
I.2	78.23	19.9	0.24
I.3	69.79	19.9	0.21
I.4	64.80	19.9	0.19
I.5	60.07	19.8	0.18
I.6	61.80	19.7	0.19
I.7	47.38	19.7	0.16
I.8	44.37	19.8	0.14
I.9	38.37	19.4	0.12
I.10	29.38	19.4	0.09
I.11	26.18	19.5	0.12
I.13	13.81	18.4	0.04
I.14	5.31	18.3	0.04
I.15	8.07	15.9	0.03

ANOVA

15N (AIR)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	424.365	7	60.624	297.279	.000
Within Groups	21.412	105	.204		
Total	445.777	112			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: 15N (AIR)
Tukey HSD

(I) Age (Year)	(J) Age (Year)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
-910	630	.6236*	.1552	.003	.144	1.104
	1365	.4572	.1532	.067	-.017	.931
	1720	.4925	.1820	.133	-.071	1.056
	1820	.4625	.1725	.140	-.071	.996
	1890	-2.4375*	.2162	.000	-3.106	-1.769
	2012	-3.8557*	.1484	.000	-4.315	-3.397
	2013	-3.8475*	.1820	.000	-4.411	-3.284
630	-910	-.6236*	.1552	.003	-1.104	-.144
	1365	-.1664	.1485	.951	-.626	.293
	1720	-.1311	.1781	.996	-.682	.420
	1820	-.1611	.1683	.979	-.682	.359
	1890	-3.0611*	.2129	.000	-3.720	-2.403
	2012	-4.4793*	.1435	.000	-4.923	-4.035
	2013	-4.4711*	.1781	.000	-5.022	-3.920
1365	-910	-.4572	.1532	.067	-.931	.017
	630	.1664	.1485	.951	-.293	.626
	1720	.0353	.1764	1.000	-.510	.581
	1820	.0053	.1665	1.000	-.510	.520
	1890	-2.8947*	.2115	.000	-3.549	-2.241
	2012	-4.3129*	.1414	.000	-4.750	-3.875
	2013	-4.3047*	.1764	.000	-4.850	-3.759
1720	-910	-.4925	.1820	.133	-1.056	.071
	630	.1311	.1781	.996	-.420	.682
	1365	-.0353	.1764	1.000	-.581	.510
	1820	-.0300	.1934	1.000	-.628	.568
	1890	-2.9300*	.2332	.000	-3.651	-2.209
	2012	-4.3482*	.1722	.000	-4.881	-3.815
	2013	-4.3400*	.2020	.000	-4.965	-3.715
1820	-910	-.4625	.1725	.140	-.996	.071
	630	.1611	.1683	.979	-.359	.682

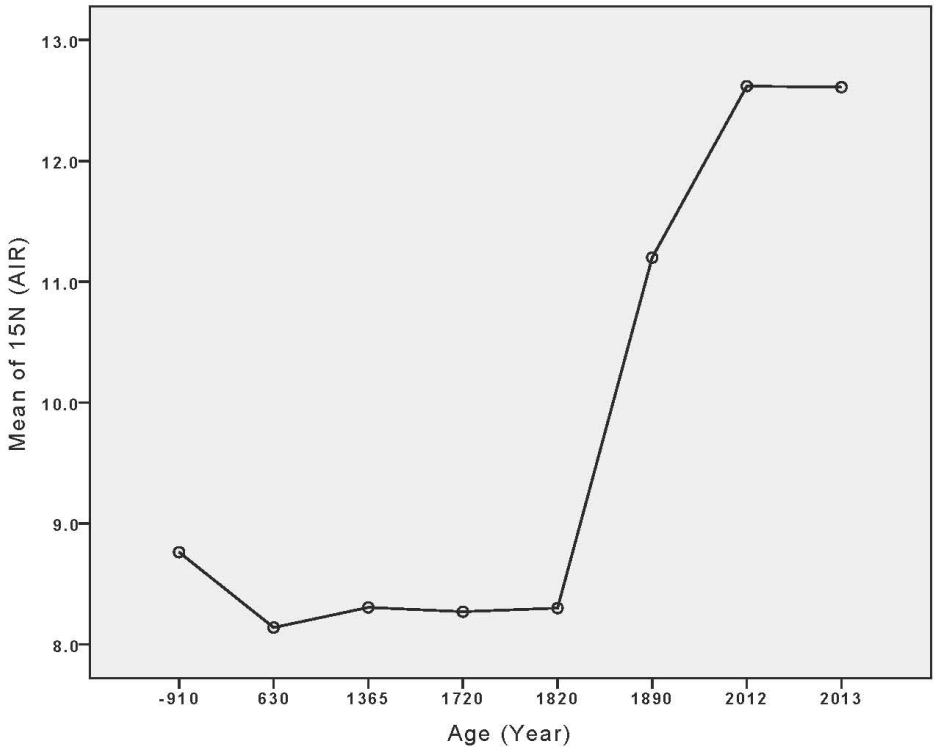
Multiple Comparisons

Dependent Variable: 15N (AIR)
Tukey HSD

(I) Age (Year)	(J) Age (Year)	Mean Difference (I–J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	1365	-.0053	.1665	1.000	-.520	.510
	1720	.0300	.1934	1.000	-.568	.628
	1890	-2.9000*	.2258	.000	-3.598	-2.202
	2012	-4.3182*	.1621	.000	-4.819	-3.817
	2013	-4.3100*	.1934	.000	-4.908	-3.712
1890	-910	2.4375*	.2162	.000	1.769	3.106
	630	3.0611*	.2129	.000	2.403	3.720
	1365	2.8947*	.2115	.000	2.241	3.549
	1720	2.9300*	.2332	.000	2.209	3.651
	1820	2.9000*	.2258	.000	2.202	3.598
	2012	-1.4182*	.2080	.000	-2.061	-.775
	2013	-1.4100*	.2332	.000	-2.131	-.689
2012	-910	3.8557*	.1484	.000	3.397	4.315
	630	4.4793*	.1435	.000	4.035	4.923
	1365	4.3129*	.1414	.000	3.875	4.750
	1720	4.3482*	.1722	.000	3.815	4.881
	1820	4.3182*	.1621	.000	3.817	4.819
	1890	1.4182*	.2080	.000	.775	2.061
	2013	.0082	.1722	1.000	-.525	.541
2013	-910	3.8475*	.1820	.000	3.284	4.411
	630	4.4711*	.1781	.000	3.920	5.022
	1365	4.3047*	.1764	.000	3.759	4.850
	1720	4.3400*	.2020	.000	3.715	4.965
	1820	4.3100*	.1934	.000	3.712	4.908
	1890	1.4100*	.2332	.000	.689	2.131
	2012	-.0082	.1722	1.000	-.541	.525

*. The mean difference is significant at the 0.05 level.

Means Plots



ANOVA

Weight Percent (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.138	7	.020	34.779	.000
Within Groups	.060	105	.001		
Total	.198	112			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Weight Percent (%)

Tukey HSD

(I) Age (Year)	(J) Age (Year)	Mean Difference (I–J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
-910	630	-.0073561	.0081844	.986	-.032670	.017958
	1365	-.0128682	.0080824	.754	-.037867	.012130
	1720	-.0284483	.0096022	.071	-.058147	.001251
	1820	-.0321009*	.0090964	.014	-.060236	-.003966
	1890	-.0389038*	.0114030	.020	-.074172	-.003635
	2012	-.0983862*	.0078264	.000	-.122593	-.074180
	2013	-.0605686*	.0096022	.000	-.090268	-.030870
630	-910	.0073561	.0081844	.986	-.017958	.032670
	1365	-.0055122	.0078348	.997	-.029745	.018721
	1720	-.0210923	.0093947	.334	-.050150	.007965
	1820	-.0247448	.0088772	.109	-.052201	.002712
	1890	-.0315477	.0112289	.104	-.066278	.003183
	2012	-.0910301*	.0075705	.000	-.114445	-.067615
	2013	-.0532125*	.0093947	.000	-.082270	-.024155
1365	-910	.0128682	.0080824	.754	-.012130	.037867
	630	.0055122	.0078348	.997	-.018721	.029745
	1720	-.0155801	.0093060	.704	-.044363	.013203
	1820	-.0192326	.0087833	.366	-.046399	.007933
	1890	-.0260355	.0111547	.285	-.060537	.008465
	2012	-.0855180*	.0074601	.000	-.108592	-.062444
	2013	-.0477004*	.0093060	.000	-.076483	-.018917
1720	-910	.0284483	.0096022	.071	-.001251	.058147
	630	.0210923	.0093947	.334	-.007965	.050150
	1365	.0155801	.0093060	.704	-.013203	.044363
	1820	-.0036525	.0101991	1.000	-.035198	.027893
	1890	-.0104554	.0123006	.990	-.048500	.027590
	2012	-.0699379*	.0090846	.000	-.098036	-.041840
	2013	-.0321203	.0106526	.062	-.065068	.000828
1820	-910	.0321009*	.0090964	.014	.003966	.060236
	630	.0247448	.0088772	.109	-.002712	.052201
	1365	.0192326	.0087833	.366	-.007933	.046399
	1720	.0036525	.0101991	1.000	-.027893	.035198
	1890	-.0068029	.0119100	.999	-.043640	.030034
	2012	-.0662854*	.0085483	.000	-.092725	-.039846
	2013	-.0284678	.0101991	.108	-.060013	.003078
1890	-910	.0389038*	.0114030	.020	.003635	.074172
	630	.0315477	.0112289	.104	-.003183	.066278
	1365	.0260355	.0111547	.285	-.008465	.060537
	1720	.0104554	.0123006	.990	-.027590	.048500
	1820	.0068029	.0119100	.999	-.030034	.043640
	2012	-.0594825*	.0109707	.000	-.093414	-.025551
	2013	-.0216648	.0123006	.647	-.059710	.016380
2012	-910	.0983862*	.0078264	.000	.074180	.122593
	630	.0910301*	.0075705	.000	.067615	.114445

Multiple Comparisons

Dependent Variable: Weight Percent (%)

Tukey HSD

(I) Age (Year)	(J) Age (Year)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	1365	.0855180*	.0074601	.000	.062444	.108592
	1720	.0699379*	.0090846	.000	.041840	.098036
	1820	.0662854*	.0085483	.000	.039846	.092725
	1890	.0594825*	.0109707	.000	.025551	.093414
	2013	.0378176*	.0090846	.002	.009719	.065916
2013	-910	.0605686*	.0096022	.000	.030870	.090268
	630	.0532125*	.0093947	.000	.024155	.082270
	1365	.0477004*	.0093060	.000	.018917	.076483
	1720	.0321203	.0106526	.062	-.000828	.065068
	1820	.0284678	.0101991	.108	-.003078	.060013
	1890	.0216648	.0123006	.647	-.016380	.059710
	2012	-.0378176*	.0090846	.002	-.065916	-.009719

*. The mean difference is significant at the 0.05 level.

Means Plots