$\delta^{15}N$ AS A POTENTIAL PROXY FOR ANTHROPOGENIC NITROGEN LOADING IN CHARLESTON HARBOR, SOUTH CAROLINA

by

TAYLOR N. PAYNE

C. FRED ANDRUS. COMMITTEE CHAIR
NATASHA T. DIMOVA
THOMAS S. TOBIN
MARTHA A. ZIERDEN

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ABSTRACT

Bivalve shell geochemistry can serve as a useful indicator of changes in coastal environments. There is increasing interest in developing paleoenvironmental proxies from mollusk shell organic components. Numerous studies have focused on how the $\delta^{15}N$ obtained from bivalve tissues and shells can be used to trace present-day wastewater input into estuaries. However, comparatively little attention has been paid to tracing the impact of anthropogenic nitrogen loading into estuaries over time. By measuring historic levels of $\delta^{15}N$ in the organic fraction of oyster shells (*Crassostrea virginica*) from archaeological sites around Charleston Harbor and comparing those levels to the $\delta^{15}N$ content of modern shells, it is possible to assess how nitrogen has fluctuated historically in the area. Whole-shell samples from the Woodland Period (~1400-800 BP), 18th, 19th, and 20th centuries, and modern controls were measured for %N and $\delta^{15}N$. $\delta^{15}N$ was found to not vary significantly with time. The highest $\delta^{15}N$ values came from shells dated to the mid and late 19th century. Mean modern $\delta^{15}N$ (8.6‰) were found to be similar to mean Woodland Period $\delta^{15}N$ (8.5‰). This is in contrast to studies done by Black (2014), but similar to a study done by Darrow et al. (2016). This information could help understand how large-scale anthropogenic nitrogen loading has affected coastal ecosystems over time and guide future remediation. Furthermore, this project will help refine and improve this novel proxy of past environmental conditions.

DEDICATION

This thesis is dedicated to Nick Jamroz.

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
EA	Elemental analyzer
IRMS	Isotope ratio mass spectrometer
Km^2	Kilometers squared
mg	Milligram
m	Meters
N	Nitrogen
N^{14}	Nitrogen-14
N^{15}	Nitrogen-15
POM	Particulate organic matter
Ppt	Parts per trillion
‰	Per mil
%	Percent
SCDNR	South Carolina Department of Natural Resources

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I. INTRODUCTION

Bivalve shells have been used extensively as proxies for environmental changes, primarily focusing on analyzing the mineral portion of the shell. (Gröcke and Gillikin, 2008; Andrus, 2011). As filter-feeders, it has been documented that bivalves produce organic matter as part of their shell structure which is composed of raw materials from the shell's diet and ambient water (Carmichael and Valiela 2004a; Carmichael and Valiela 2004b; Carmichael *et al.* 2008; Chase *et al.* 2001 Fertig *et al.* 2010; Black 2014; Kovacs *et al.* 2010). This characteristic has the potential to make them a useful proxy for evaluating anthropogenic nitrogen loading into estuaries (Carmichael *et al.* 2008; Fertig *et al.* 2010; Black 2014; Kovacs *et al.* 2010).

With the beginning of the industrial revolution in American starting from 1860 to the early 20^{th} century, widespread population growth and subsequent land use change has resulted in an increase in nitrogen loading into estuaries and other aquatic systems (Porter *et al.* 2013). This increased nutrient loading can cause eutrophic conditions in estuaries which can lead to a decline in the health of the ecosystem (Paerl, 1997). Without a significant decrease in anthropogenic nitrogen inputs, eutrophic conditions are likely to worsen in many estuaries (Bricker et al., 1999).

Few methods exist to evaluate the history of nitrogen in estuaries. One approach is to measure the N content of sediments from cores; however this can be problematic due to difficulties in dating the cores, nitrogen movement through bioturbation and other processes, and sediment loss through natural and anthropogenic actions such as dredging.

A novel method involves using the $\delta^{15}N$ values of mollusk shells as a proxy for wastewater input.

It has already been shown that nitrogen stable isotopes in the shells of bivalves record wastewater inputs into estuaries (O'Donnel *et al*. 2003; Carmichael *et al*. 2008; Kovacs *et al*. 2010; Fertig 2010). It is well established that the soft tissue of mollusks reflects ambient N, and it has recently been shown that the nitrogen content of the organic portion of the shell is nearly identical to the nitrogen content of the mollusk's soft tissue (Black, 2014). The present study is focused only on analyzing the $\delta^{15}N$ values of the organic portions of the shells since the reported offset in $\delta^{15}N$ values between the soft tissues and the organic matrix of the shell have been found to be negligible (Kovacs *et al*. 2010; Black 2014). By comparing the $\delta^{15}N$ content of modern shells to the $\delta^{15}N$ content of shells from historic and prehistoric archaeological sites it should be possible to develop a record nitrogen input into estuaries from sources that have $\delta^{15}N$ values distinct from natural reservoirs. For example, animal and human waste have more negative $\delta^{15}N$ values than other common N sources, thus increased sewage and farming inputs may be tracked over time. It should then be expected that lower levels of sewage and effluent from farming activities in preindustrial Charleston would lead to lower $\delta^{15}N$ values. For this study, modern and archaeological samples of the oyster *Crassostrea virginica* were collected from Charleston Harbor and analyzed for $\delta^{15}N$. Shells are a more useful proxy than the soft tissues for tracing wastewater inputs into estuaries over time, because they are more likely to be preserved in the archaeological and fossil record.

Unlike some previous studies, there is no other reliable record of wastewater input into Charleston Harbor over the past four centuries. Black (2014) was able to compare the $\delta^{15}N$ values obtained from archaeological and modern shells and relate those data to $\delta^{15}N$ values taken

from cores taken from Chesapeake Bay. Extensive dredging and related anthropogenic alteration in Charleston Harbor makes it difficult for a core to be used as a comparison. Charleston Harbor is also a smaller estuary than Chesapeake Bay and was more densely populated in the 18th century, which means that a greater percentage of shoreline was extensively developed earlier in time. Thus it seems possible that a different $\delta^{15}N$ history would be expected here than was detected in Chesapeake Bay.

a. Study Area: Charleston, South Carolina

The harbor is located on the eastern seaboard of the United States where the Ashley River, Wando River, and Cooper River drain into the Atlantic Ocean (see Figure 1). The harbor has a mean monthly salinity of 22.0 ppt (Kjerfve and Magill, 1990) and exhibits a semidiurnal tide with a mean range of 1.6m (Kjerfve and Magill, 1990).

Charleston, South Carolina was founded by British settlers in 1670 (Fraser Jr., 1989). Native Americans inhabited the area since the end of the Pleistocene (Waters et al., 2009), but at far lower population densities than were present after European settlement. Charleston's metropolitan area has a population of approximately 727, 689 (U.S. Census, 2014).

b. *Crassostrea virginica*

Crassostrea virginica, also known more commonly as the eastern oyster, is found throughout the North American Atlantic coast and can exist under wide range of environmental conditions (NOAA Fisheries Eastern Oyster Biological Review Team, 2007) (see Figure 3). They are among the most common mollusks found in Charleston Harbor (Stone et al., 2013). *Crassostrea virginica* can be found in the fossil record from the Cretaceous (Kirby, 1998).

Oysters are filter feeders that exist primarily on a diet of phytoplankton and other suspended organic material (NOAA Fisheries Eastern Oyster Biological Review Team, 2007). The oyster shells can continue to grow at all stages of their life. The oyster's rate of shell growth is largely a function of temperature and food supply (Kennedy, 1996). However, growth rates slow with age (Quast et al, 1988). *Crassostrea virginica* can grow to around 20.6 to 35.5cm (Galtsoff, 1964) and can live up to 25 to 30 years (Martin, 1987). Their optimal temperature range is 20-30°C (NOAA Fisheries Eastern Oyster Biological Review Team, 2007). The shell of *Crassostrea virginica* is composed primarily of calcite, but portions of the muscle scar and ligostracum contain aragonite (Kirby, 1998).

Crassostrea virginica is found throughout the archaeological record on the east coast of North America from the Gulf of Mexico to the Canadian Maritime Provinces with its shells being a common feature in archaeological middens in much of this range. Due to its widespread occurrence, it is commonly used as a paleoenvironmental proxy (Andrus and Crowe, 2000; Thompson and Andrus, 2011; Black *et al*., 2016; Darrow *et al.,* 2016).

II. METHODOLOGY

Age (AD)	Number of Samples
610	4
1694	2
1730	8
1736	2
1740	5
1750	2
1760	4
1770	6
1790	5
1850	5
1880	3
1890	3
1920	3
1939	3
Modern	51
Total	**106**

Table 1: Approximate age and number of samples.

a. Field Methods

Modern oyster samples were collected from Charleston Harbor on December 8, 2014 and June 10, 2015. Live oysters were collected from the harbor during low tide while the oysters were exposed with help from the South Carolina Department of Natural Resources (SCDNR). Most of the beds sampled were planted by the SCDNR. Approximately 10-15 oysters were collected from five sites around the harbor (Figure 1). Oysters were collected by hand then stored on ice during transportation to The University of Alabama.

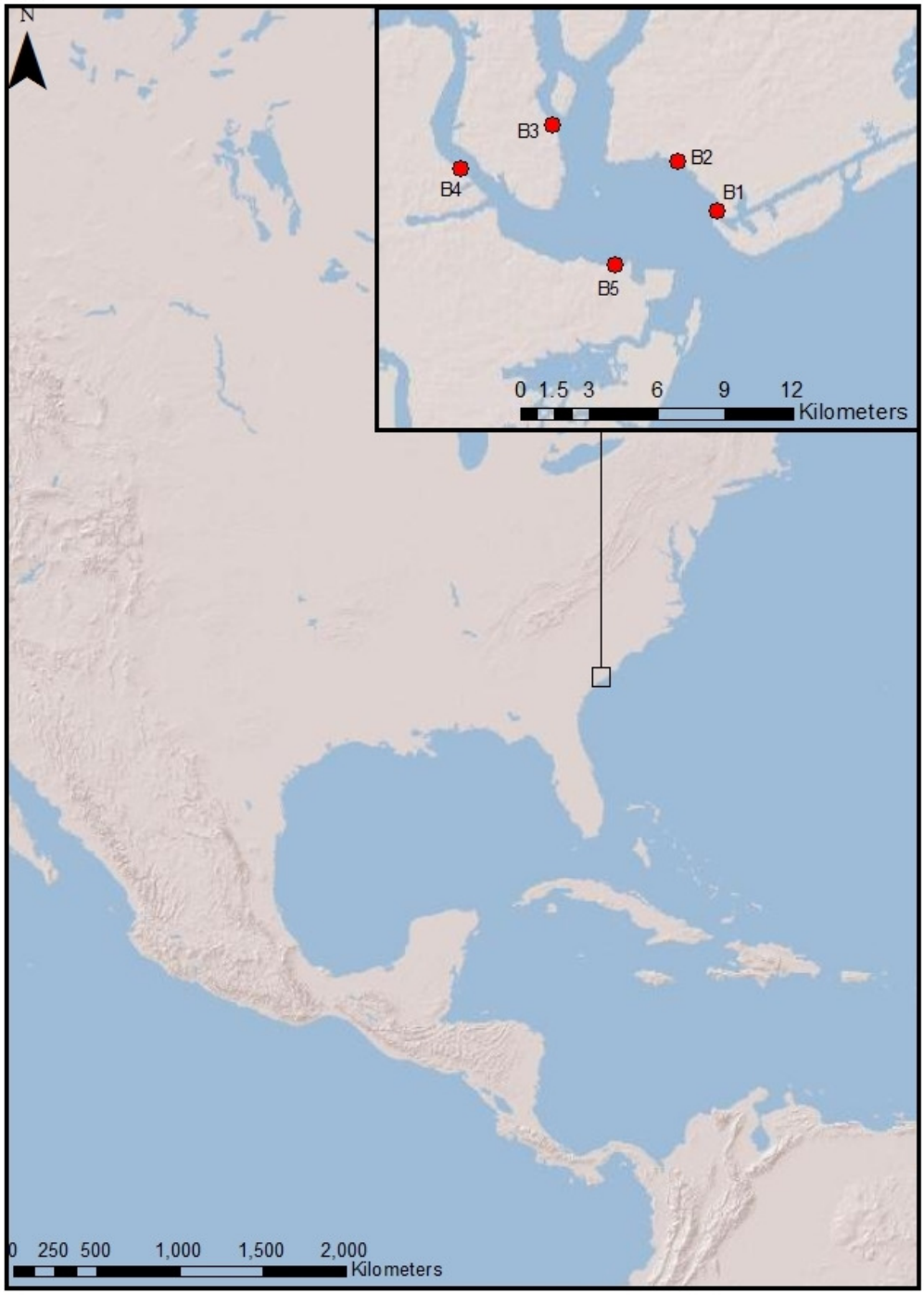

Figure 1: Map of Charleston, South Carolina. Red dots indicate sampling sites where modern samples of *Crassostrea virginica* (adapted from ArcGIS).

b. Archaeological Methods

Professional archaeology has been conducted in Charleston since the 1960s (Zierden and Reitz, 2016). The city contains a wide variety of sites including private residences, public gathering centers, and commercial sites. 106 shells were obtained from nine archaeological sites were excavated using standard excavation techniques (Herold, 1981; Zierden and Hacker, 1986; Zierden and Reitz, 2005 and 2007; Butler et al., 2012; Zierden et al. 2009). These sites included the Beef Market, Dock Street Theatre, the Exchange Building, South Adger's Wharf, 38Bk202, the Heyward-Washington House and Stables, the Aiken-Rhett House, 70 Nassau Street, and 48 Laurens Street (Figure 1). All of the sites used in this study are multicomponent sites and have been continuously occupied (Zierden and Reitz, 2016).

The Beef Market site is an 18th century market site located adjacent to the colonial City Hall (Zierden and Reitz, 2005). Charleston's Dock Street Theater has been occupied continuously over the past two and a half centuries and has undergone many renovations. Shell material collected from this site comes primarily from the structures colonial foundations (Zierden et al., 2009). The Exchange Building, located in the center of colonial Charleston, was the city's economic hub (Zierden and Hacker, 1986). South Adger's Wharf is the site of a portion of Charleston's original fortifications that dates back to the late 17th century (Butler et al., 2012). The Heyward-Washington House and associated stable is a domestic site that has been continuously occupied since the early 18th century (Zierden and Reitz, 2007). The Aiken-Rhett House is a domestic site that was occupied during the mid-19th century (Zierden, 2003). 70 Nassau Street is a domestic site that was occupied from the mid to late 19th century (Zierden et al., 2014).

Standard archaeological field methods were used during the excavation of each site. Features and test units were excavated using shovels and trowels. Soil was screened through a ¼" mesh. Representative portions of shell deposits were retained from closed context and organically-rich proveniences (Herold, 1981; Zierden and Reitz, 2007; Butler et al., 2012; Zierden et al. 2009).

All of the proveniences were dated by stratigraphy, *terminus post quem*, and documented history of the property. Stratigraphy refers to the use of the law of superposition to date archaeological strata based on their relative position to each other (Zierden and Reitz, 2016). *Terminus post quem* is an archaeological dating principle whereby strata are dated using diagnostic artifacts. The earliest known artifact found in the strata is used in dating (Zierden and Reitz, 2016).

Samples ranged in age from 550-1939AD. Samples were stored in dry conditions until transportation to the University of Alabama. The samples were then separated into eight categories based upon the age of each site: Late Woodland (610 +/- 70 AD), Early 18th Century (1694-1730AD), Mid-18th Century (1740-1760AD), Late 18th Century (1770-1790AD), Mid-19th Century (1850-1860), Late 19th Century (1880-1890), Early 20th Century (1920-1939), and Modern (2014-2015). All collections were curated at The Charleston Museum and are on loan to The University of Alabama. Unused and partially-used shells were returned to the museum's permanent collections.

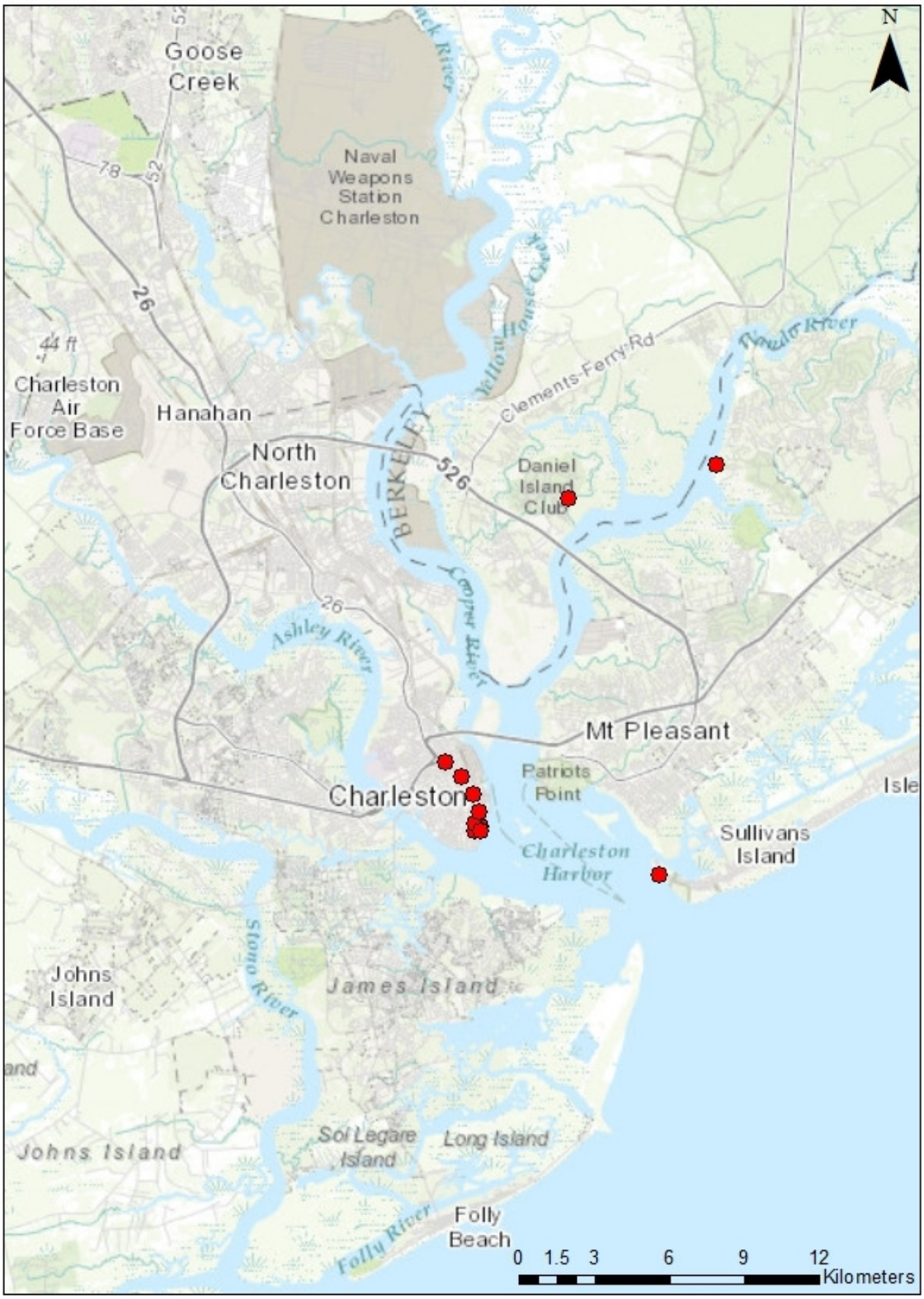

Figure 2: Map of archaeological sites in Charleston, South Carolina where samples of *Crassostrea virginica* were collected from (adapted from ArcGIS).

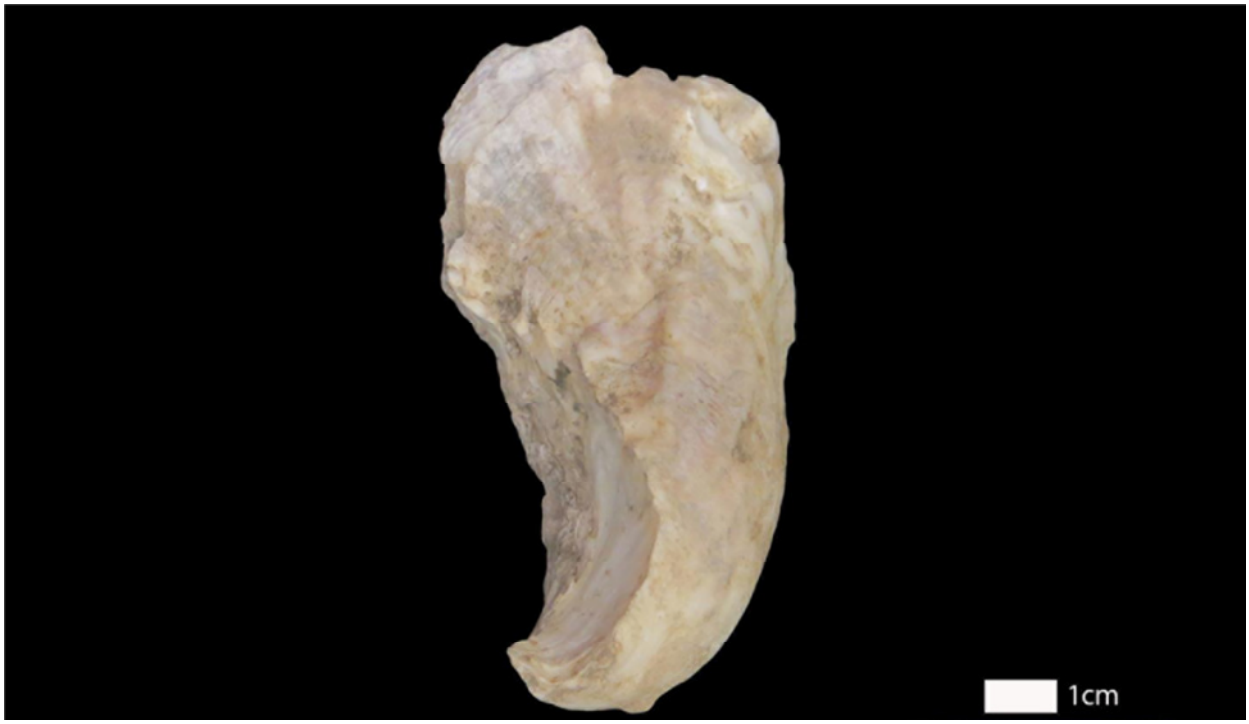

Figure 3: Left valve of *Crassostrea virginica*.

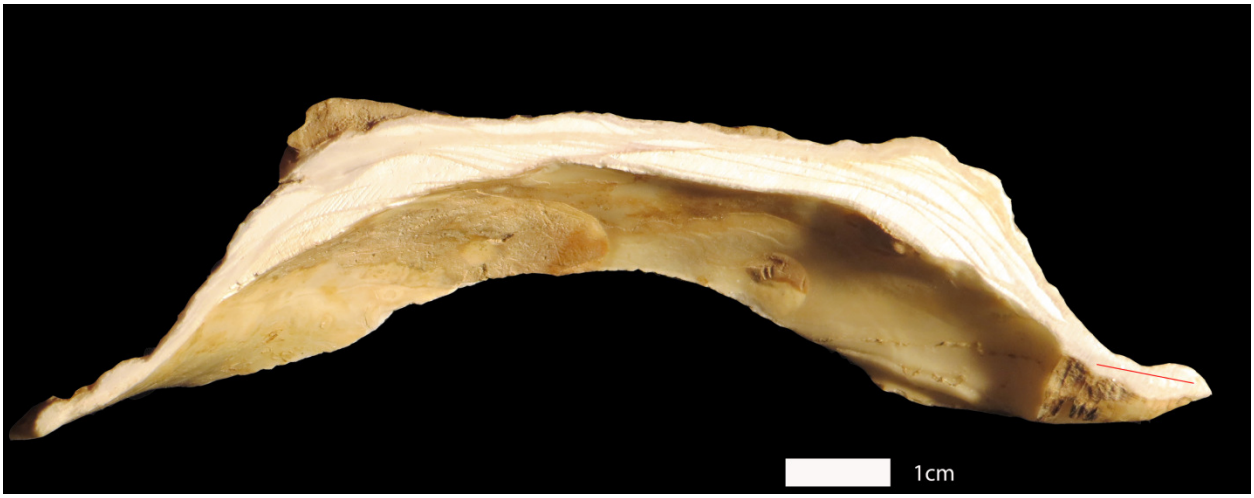

Figure 4: Cross-section of a left valve of *Crassostrea virginica*. A trench was drilled across the hinge portion of the shell (indicated by red line).

c. Lab Methods

Samples were thawed after icing and transport, and then the soft tissue of each sample was removed with a knife (Figure 3). The left valve was bisected symmetrically along the hinge using a trimming saw. Samples were left to dry overnight. Shell material was then extracted from the hinge area using a variable-speed drill (Figure 4) using a carbide bit. Shell material was weighed and placed into 5x10mm tin capsules for analysis. Approximately 40mg of shell material was collected from modern samples, and 120mg was collected from archaeological samples. Samples were then analyzed for %N and δ^{15} N using a Thermo Delta V isotope ratio mass spectrometer (IRMS) coupled via continuous helium flow with a Costech ECS 4010 Elemental Analyzer (EA) in The University of Alabama Stable Isotope Laboratory. A carbon dioxide trap was attached to the IRMS to filter out carbon. δ^{15} N data are reported in parts per mil (‰) vs. air.

Two standards were used to calibrate %N measurements: B2151 High Organic Content Sediment 5gm (0.62%) and Acetanilide 135.17gm (10.36%). IAEA-N-2 Ammonium Sulfate (20.3 ± 0.2 ‰) and B2151 High Organic Content Sediment (4.42‰) were used as the isotope standards.

III. RESULTS

$\delta^{15}N$ and %N values of *Crassostrea virginica* fluctuated with time (Figures 5 and 6). The average values of $\delta^{15}N$ for the varying time periods (late Woodland, early 18th Century, mid-18th Century, late 18th Century, mid-19th Century, late 19th Century, early 20th Century, and modern) are 8.6‰, 9.5‰, 9.3‰, 9.6‰, 9.9‰, 10.0‰, 9.1‰, and 8.7‰ (Table 1). $\delta^{15}N$ values range from 8.4‰ to 9.0 ‰ for late Woodland, 8.8‰ to 10.3‰ for the earl 18th Century, 8.9‰ to 10.0‰ for the mid-18th Century, 9.2‰ to 10.1‰ for the late 18th Century, 9.6‰ to 10.7‰ for the mid-19th Century, 8.5‰ to 10.7‰ for the late 19th Century, 8.4‰ to 10.4‰ for the early 20th Century, and 7.6‰ to 9.8‰ for the modern shells (Table 1). Average %N values were .04% for the late Woodland, .07 % for the early 18th Century, .06% for the mid-18th Century, .07% for the late 18th Century, .06% for the mid-19th Century .08% for the late 19th Century, .12% for the early 20th Century, and .10% for the modern samples (Table 2). %N values ranges vary from .03% to .05% for the late Woodland, .06% to .11% for the early 18th Century, .04% to .07% for the mid-18th Century, .06% to .09% for the late 18th Century, .06% to .09% for the mid-19th Century, .07% to .1% for the late 19th Century, .1% to .17% for the early 20th Century, and .08% to .16% for modern samples (Table 2).

A one-way ANOVA test was performed on $\delta^{15}N$ values between time periods. The test produced a P-value of 3.6969×10^{-7} and an F-value of 8.5733 which suggests that there is a significant amount of variation in $\delta^{15}N$ values between time periods (Appendix 2). A two-tailed T-Test assuming unequal variances was then performed between each time period group (Appendix 4). The test produced results that suggest the $\delta^{15}N$ values of the early 18th Century,

mid-18th Century, late 18th Century, and mid-19th Century were significantly different than values from the Woodland period. The test shows that $\delta^{15}N$ values from the mid-18th Century, late 18th Century, and late 19th Century are significantly different than values from the modern samples. The test also suggests that there is a significant difference between $\delta^{15}N$ values from the late 19th Century and values from the early 20th Century. A one-way ANOVA test was also performed on modern $\delta^{15}N$ values between different locations (Appendix 3). A p-value of 1.82e-36 suggests that there is no significant difference in $\delta^{15}N$ values of oysters collected from the five modern beds in Charleston Harbor.

Time Period	Highest Value	Lowest Value	Mean
Early Woodland	9.0	8.4	8.6
Early 18th Century	10.3	8.7	9.5
Mid-18th Century	10.0	8.9	9.3
Late 18th Century	10.1	9.2	9.6
Mid-19th Century	10.7	9.4	9.9
Late 19th Century	10.7	8.5	10.0
Early 20th Century	10.4	8.4	9.1
Modern	9.8	7.7	8.7

Table 2: $\delta^{15}N$ values from each time period sampled. Table shows mean, highest, and lowest $\delta^{15}N$ values.

Time Period	Highest Value	Lowest Value	Mean
Early Woodland	.05	.03	.04
Early 18th Century	.11	.04	.07
Mid-18th Century	.10	.04	.06
Late 18th Century	.09	.06	.07
Mid-19th Century	.09	.04	.06
Late 19th Century	.10	.06	.08
Early 20th Century	.17	.10	.12
Modern	.16	.07	.10

Table 3: Weight Percent N values from each time period. Table shows mean, highest, and lowest %N values.

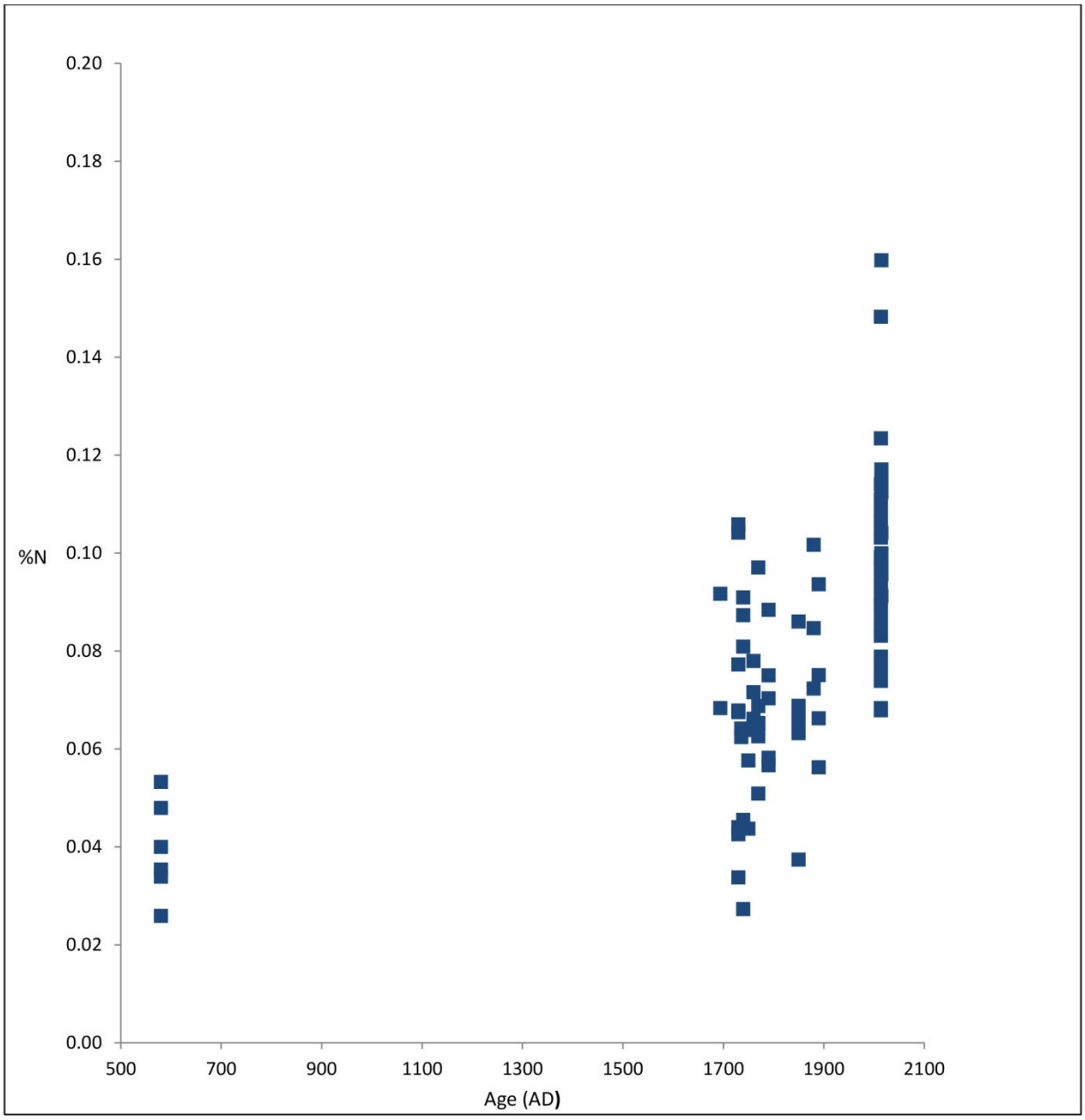

Figure 5: Plot of %N values for modern and archaeological *C. virginica* shells. Y-axis is Weight Percent Nitrogen and the X-axis is the age of the sample.

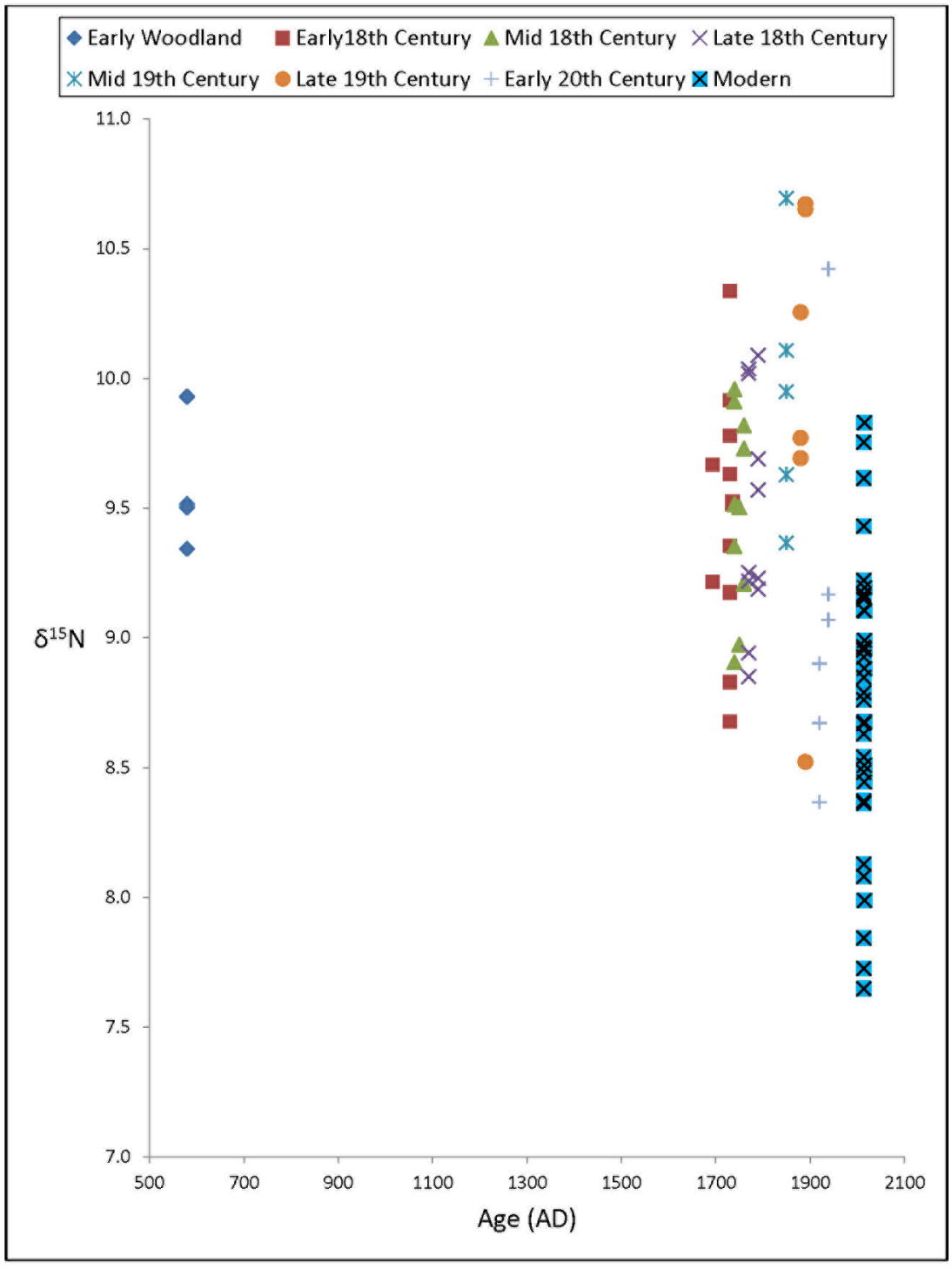

Figure 6: Plot of $\delta^{15}N$ values for modern and archaeological samples of *C. virginica*. Y-axis is $\delta^{15}N$ of each sample reported in parts per mil. X-axis is age of the samples.

IV. DISCUSSION

$\delta^{15}N$ values obtained from *Crassostrea virginica* fluctuated slightly with time. Post hoc two-tailed t-tests confirm that there was no significant difference in $\delta^{15}N$ values from the late Woodland period and $\delta^{15}N$ from 2015 samples (Appendix 4). Shells from the late Woodland period have the lowest mean $\delta^{15}N$ of 8.6 ‰ which is the measurement that most resembles $\delta^{15}N$ of modern shells from Charleston Harbor. Late Woodland Native American populations in South Carolina tended to live in small villages (King and Stephenson, 2016). These low-density population centers combined with a lack of large-scale agriculture means that anthropogenic nitrogen inputs into the harbor would have been low during the Woodland period.

This trend in low $\delta^{15}N$ changes with the arrival of European colonists to Charleston in around 1670 (Fraser, 1989). Starting in the early 1700s there is an increase in mean $\delta^{15}N$ from 8.6 ‰ to 9.5 ‰ (Table 1). The increase in $\delta^{15}N$ may be attributed to several factors including an increase in the human population and the introduction of large-scale agriculture to the Charleston area. An increased human presence in the area leads to a larger amount of human waste that is being flushed into the harbor. The introduction of large-scale agriculture can have a significant impact on $\delta^{15}N$. The waste produced by domesticated animals represents a potential new source of $\delta^{15}N$. Animal waste can also be introduced into the system as fertilizer for crops. The increase in $\delta^{15}N$ in the 18th century can be explained by the use of manure-based fertilizer in conjunction with an increase in human and livestock populations in the 18th century.

After the early 18th century, there does not appear to be another significant increase in $\delta^{15}N$ as determined by the application of two-tailed t-tests. Mean $\delta^{15}N$ values increased from

9.6‰ in the late 18th century to 9.9‰ in the mid-19th century (Table 1). However, using a two-tailed t-test it was determined that this increase was not significant. The t-test did determine that $\delta^{15}N$ values from the mid-18th century are significantly different from $\delta^{15}N$ values from the early Woodland. The population of Charleston County grew from approximately 57, 480 in 1800 to 72, 805 in 1850 (Frasier, 1989). With the population increasing by 20% in a span of 50 years, nitrogen inputs into the harbor from human and animal waste should have drastically increased. Low mean $\delta^{15}N$ values could also be a function of trends in land use. Manure was not widely used as a fertilizer until the mid-19th century partly due to the lack of livestock in the area. Instead, vegetable matter that sometime included burned shells, clay, grasses, brines etc., was often used (Mathew, 1992). The use of nutrient-poor material as fertilizer was one of several factors that lead to agriculture being limited in the Charleston area (Matthew, 1992). With comparatively less agricultural activities occurring, it is possible that the main source of anthropogenic nitrogen loading into the harbor was human effluent.

It's also possible that the small change in shell $\delta^{15}N$ is not completely representative of Charleston Harbor, but was affected by shells brought to market in Charleston from other nearby habitats. As Charleston was, and remains, the main human population center of the overall region, any oyster from these nearby estuaries would presumably contain lower $\delta^{15}N$ values than contemporaneous shells in Charleston Harbor. Therefore the reported $\delta^{15}N$ shell values in this thesis may be best viewed as the minimum likely $\delta^{15}N$ value from oysters in the region and there may have been areas with higher $\delta^{15}N$ values that were not harvested.

Mean $\delta^{15}N$ increased slightly from 9.9 ‰ in the mid-19th to 10.0‰ in the late 19th century (Table 1). Even though Charleston was decimated during the Civil War, there is another large population increase in Charleston County from 72, 805 in 1850 to 102, 800 in 1880 (U.S.

Census Bureau). It is possible that the mean $\delta^{15}N$ from the late 19th century is being significantly altered by an anomalous $\delta^{15}N$ measurement of 8.5‰ (Appendix 1). It is also possible that lower $\delta^{15}N$ are caused by the collapse of Charleston's economy immediately following the Civil War (Fraser, 1989). However, by the late 1860s due to several economic stimuli, the city's economy had largely recovered (Fraser, 1989).

Shells from the early 20th century had a mean $\delta^{15}N$ of 9.1 ‰ which is the lowest mean $\delta^{15}N$ of any of the other time periods besides the late Woodland (Table 1). After an earthquake devastated Charleston on August 31, 1886, the county's population plummeted from 102, 800 to 59, 903 (U.S. Census, 1890). It took over 30 years for the population of Charleston to reach pre-earthquake levels. By 1920 the population had increased back to around 108, 450 (U.S. Census, 1920). This huge decrease in the area's population would have had a significant effect on nitrogen flux into the harbor. The fewer people in the area, the less waste they will produce thereby decreasing nitrogen loading into Charleston Harbor. By the 20th century there is also a shift away from an agriculturally-based economy (Fraser, 1989). With less agricultural activity going on in the area, then less nitrogen would be discharged into the harbor from fertilizer and animal waste.

Modern $\delta^{15}N$ values have a mean of 8.7‰ which is the second lowest $\delta^{15}N$ mean measured (Table 1). Using the application of two-tailed t-tests, there was a significant decrease in $\delta^{15}N$ from the late 19th century to the present. This trend is different from what has been seen in previous studies (Black, 2014; Darrow et al. 2016). While the population of Charleston County did grow from 108, 450 in 1920 to 350,209 in 2010 (U.S. Census, 2010), there are several possible reasons for the decline in $\delta^{15}N$. From 1865 to 1895 sewer lines were installed in Charleston that emptied into the Ashley and Cooper Rivers (Fairey, 2014). Wastewater was

discharged into the harbor until 1971 when the Plum Island Wastewater Treatment plant began operating (Fairey, 2014). With wastewater no longer being discharged into the harbor, $\delta^{15}N$ would decrease significantly. Another possible cause of low $\delta^{15}N$ is the introduction of synthetic fertilizer. For most of human history, crops were fertilized using some form of organic compost or manure. The use of synthetic fertilizer became widespread in the United States starting in the 1960s (Keeney and Hatfield, 2008). Synthetic fertilizers fix nitrogen in the atmosphere into a biologically available form (Keeney and Hatfield, 2008). Because atmospheric nitrogen has a $\delta^{15}N$ value of approximately 0 ‰, nitrogen inputs from non-point synthetic fertilizer runoff cannot be directly detected with oyster shell $\delta^{15}N$ data alone (Black, 2014).

The trend in $\delta^{15}N$ over time in Charleston oyster shells is similar to results from coastal Mississippi (Darrow et al. 2016), who detected no significant change in $\delta^{15}N$ between ancient shells ranging in age from 500 to 2100 years old and modern shells from the same area. This relative stability was attributed to a comparative lack of change in land use of the area and low overall population densities even in the modern era.

The trend in $\delta^{15}N$ over time in Charleston oysters contrasts with the findings from Black (2014), who measured an exponential increase in $\delta^{15}N$ beginning at the start of the 20th century. One possible cause of this difference is due to population density. The area around the Chesapeake Bay, including the cities of Baltimore, Richmond, and Washington, has a much higher populations historically than Charleston, albeit over a much larger drainage area. Higher populations around Chesapeake Bay means there are more sources of wastewater inputs into the bay than in Charleston Harbor. The area around Chesapeake Bay also has experienced higher levels of industrialization and agriculture than Charleston Harbor (Bratton et al., 2003; Zimmerman and Canuel, 2000).

Another possible cause of the difference in $\delta^{15}N$ is the size and flushing rates of the harbors. Chesapeake Bay has an area of approximately 11,600km^2. Charleston Harbor is approximately 20.7 km^2. While the hydrodynamics of both estuaries are complex and variable over time and space, in general with Chesapeake Bay being much larger than Charleston Harbor, the flushing rate of the Bay is probably significantly slower, this effects of anthropogenic inputs on $\delta^{15}N$ would be greater (e.g see discussion in EPA, Nutrient Criteria Technical Guidance Manual: Estuarine and Coastal Waters 2001).

Even though this study has shown that $\delta^{15}N$ values of the harbor are relatively low, Charleston Harbor still exhibits modest eutrophication conditions (Bricker et al., 2001). One possible cause of eutrophication is inputs of nitrate into the harbor derived from synthetic fertilizer runoff. As it has been previously discussed, $\delta^{15}N$ values do not take into account nitrogen from man-made fertilizer runoff. However, the use of synthetic fertilizer can increase denitrification of nitrogen in the soil which can increase $\delta^{15}N$ in groundwater (Ogawa et al., 2001). Another possible source is atmospheric deposition of nitrate and/or phosphate. It has been shown that nutrient levels in Charleston Harbor are significantly affected by the atmospheric deposition of several compounds including fluoride, chloride, phosphate, nitrate, and sulfate (Lindner and Frysinger, 2007). It is probable that both of these factors are acting in tandem to create the modest eutrophic conditions in the harbor.

Before this method is to be trusted as a reliable proxy for baseline levels of nitrogen, it needs to be determined whether shells are preferentially losing N^{15} over time after deposition. After burial, as the shells decompose, they lose organic material, which is where most of the nitrogen contained in the shells is located. This is evident in the loss of weight percent nitrogen (%N) over time. %N was measured in modern and ancient shells and compared to %N values of

shells from Chesapeake Bay (Black, 2014). %N of modern and ancient shells from Charleston Harbor approximately follow the same trend as %N of shells from Chesapeake Bay (Figure 4). Some %N values from Charleston Harbor were slightly higher than %N values from the Chesapeake during the 18^{th} century. This is most likely a function of differences in depositional environments. There is some concern that as the shells lose organic matter, they preferentially lose N^{15} over N^{14} (Black, 2014). Based upon the principles of stable isotope geochemistry, when nitrogen fractionates from the organic material into a different phase, N^{14} should preferentially fractionate over N^{15}, because it is the lighter isotope. If this is the case, then $\delta^{15}N$ values are may be made to seem heavier through diagenesis in ancient shells. This is not a likely explanation for the Chesapeake Bay study where $\delta^{15}N$ values from ancient shells are significantly lower than modern $\delta^{15}N$ from modern shells (Black, 2014). However, this cannot be entirely discounted in the present study since some ancient $\delta^{15}N$ values are higher than modern $\delta^{15}N$ values. Further research needs to be conducted to understand the fate of N^{15} in shells after deposition to better demonstrate the utility of this proxy.

V. CONCLUSION

$\delta^{15}N$ values from *Crassostrea virginica* shells collected from Charleston Harbor vary with time. The highest $\delta^{15}N$ values are from the mid and late 19th century. The lowest values are from the Late Woodland and modern time periods. This is in contrast to a study done by Black (2014) and similar to Darrow et al. (2016). The lowest $\delta^{15}N$ values are from the early Woodland and the present. These low values possibly correspond to low anthropogenic nitrogen inputs from wastewater. Low modern $\delta^{15}N$ values can be explained by the introduction of sewage treatment plants and changes in land use. The highest $\delta^{15}N$ values occur during the mid to late 19th century that corresponds with a rapid rise in the population of the area. Mean modern $\delta^{15}N$ is 8.7‰, and mean early Woodland $\delta^{15}N$ is 8.6‰. This indicates that $\delta^{15}N$ values in Charleston Harbor are close to natural, pre-colonial levels. While wastewater inputs may be minimal, Charleston Harbor still shows some signs of eutrophication that may be caused by a combination of atmospheric deposition of nutrients and fertilizer runoff.

$\delta^{15}N$ values may be affected by the loss of nitrogen contained in the inter-crystalline organic matrix during burial caused by. Further studies need to be conducted to determine if shells preferentially lose N^{15} after deposition. It is also possible that the size of the estuary plays a role in $\delta^{15}N$ values. Larger estuaries may have slower flushing rates, allowing for the accumulation of N^{15}. This has the potential to increase or decrease $\delta^{15}N$ depending on the size of the estuary. Flushing rates needed to be taken into account when using $\delta^{15}N$ as a paleoenvironmental proxy.

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APPENDIX I:

Age, weight percent nitrogen and d15N of shell samples.

Identifier	AGE (AD)	Weight Percent N (%)	d15N (% AIR)
38C1025.2	580	0.04	9.9
38C1025.3	580	0.04	9.3
38CH1025.7	580	0.03	9.5
38CH1025.8	580	0.05	9.5
SAW.1	1694	0.07	9.2
SAW.2	1694	0.09	9.7
HW.6.1	1730	0.04	9.2
HW.65A.1	1730	0.07	9.9
HW.65A.6	1730	0.07	9.4
HW.F65.1	1730	0.10	8.7
HW.F65.3	1730	0.11	9.6
HWS.128.1	1730	0.04	10.3
HWS.3	1730	0.03	9.8
HWS.3.3	1730	0.08	8.8
DT.1	1736	0.06	9.5
DT.2	1736	0.06	9.5
86C.Z3.1	1740	0.09	9.5
86C.Z3.2	1740	0.03	9.9
86C.Z3.3	1740	0.09	9.4
86C.Z3.4	1740	0.08	8.9
86C.Z3.5	1740	0.05	10.0
38BK202.1	1750	0.06	9.0
38BK202.2	1750	0.04	9.5
86C.F6.1	1760	0.07	9.2
86C.F6.3	1760	0.06	9.8
86C.F6.4	1760	0.07	9.7
86C.F6.5	1760	0.08	9.2
E.6.1	1770	0.06	9.3
E.F2.1	1770	0.05	8.9
EB.1	1770	0.10	9.2
EB.EP6.1	1770	0.07	8.9
EB.F2.1	1770	0.07	10.0
EB.P.1	1770	0.07	10.0
BM.1	1790	0.08	10.1
BM.F35.1	1790	0.06	9.7
BM.F35.2	1790	0.06	9.2
BM.Fs107.1	1790	0.07	9.2
BM.Fs107.2	1790	0.09	9.6

HWK.V.1	1850	0.09	10.7
HWK.V.2	1850	0.04	9.4
HWK.XX.1	1850	0.07	9.9
AR.3.1	1850	0.07	10.1
AR.5.1	1850	0.06	9.6
NS.1	1880	0.07	10.3
NS.2	1880	0.08	9.7
NS.3	1880	0.10	9.8
HWS.122.1	1890	0.06	10.7
HWS.122.2	1890	0.08	10.7
HWS.3.1	1890	0.07	8.5
LN.39.6.1	1920	8.90	0.1
LN39.6.2	1920	8.37	0.2
LN39.6.3	1920	8.67	0.1
489L.5	1939	10.42	0.1
48L.2	1939	9.17	0.1
48L.6	1939	9.07	0.1
B2.D.1	2014	0.08	9.2
B2.D.2	2014	0.11	9.0
B2.D.3	2014	0.09	8.5
B2.D.4	2014	0.09	9.1
B2.D.5	2014	0.08	9.8
B4.D.10	2014	0.11	8.8
B4.D.11	2014	0.07	9.2
B4.D.5	2014	0.15	8.5
B4.D.6	2014	0.09	9.1
B4.D.7	2014	0.11	7.8
B4.D.9	2014	0.10	9.6
B5.D.2	2014	0.11	8.7
B5.D.3	2014	0.12	9.2
D.1.1	2014	0.10	8.8
D.1.1	2014	0.10	8.8
D.1.5	2014	0.10	8.1
D.1.5	2014	0.10	8.1
D.2.1	2014	0.08	8.1
D.2.1	2014	0.08	8.1
D.2.5	2014	0.09	8.8
D.2.5	2014	0.09	8.8
D.3.2	2014	0.09	8.6
D.3.2	2014	0.09	8.6
D.3.4	2014	0.07	8.9
D.3.4	2014	0.07	8.9
D.3.6	2014	0.07	7.7
D.3.6	2014	0.07	7.7
D.3.7	2014	0.11	7.6
D.3.7	2014	0.11	7.6
D.3.8	2014	0.08	8.4
D.3.8	2014	0.08	8.4
D.5.1	2014	0.07	9.0
D.5.1	2014	0.07	9.0

D.5.3	2014	0.08	9.4
D.5.3	2014	0.08	9.4
D.5.6	2014	0.10	8.4
D.5.6	2014	0.10	8.4
B1.J.1	2015	0.10	9.1
B1.J.1	2015	0.10	9.1
B1.J.2	2015	0.12	9.0
B1.J.2	2015	0.12	9.0
B1.J.5	2015	0.16	8.0
B2.J.2	2015	0.11	8.4
B3.J.1	2015	0.09	9.8
B3.J.1	2015	0.09	9.8
B3.J.2	2015	0.10	8.5
B3.J.2	2015	0.10	8.5
B3.J.5	2015	0.10	8.7
B3.J.6	2015	0.10	9.2
B4.J.1	2015	0.11	8.9
B4.J.2	2015	0.10	9.0

APPENDIX II:
One-way ANOVA tests for $\delta^{15}N$ and %N between different time periods

ANOVA $\delta^{15}N$

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.39275389	6	2.565458982	8.573261789	3.36969E-07	2.214192795
Within Groups	23.93916384	80	0.299239548			
Total	39.33191773	86				

ANOVA %N

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.025871803	6	0.004311967	12.85590731	4.08833E-10	2.214192795
Within Groups	0.026832597	80	0.000335407			
Total	0.0527044	86				

APPENDIX III:
One-way ANOVA tests for $\delta^{15}N$ between modern oyster bed locations

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	632.5331978	1	632.5331978	592.0627	1.82E-36	3.973897
Within Groups	76.92156622	72	1.068355086			
Total	709.454764	73				

APPENDIX IV:
Positive Two-Tailed T-Tests for $\delta^{15}N$

	Woodland	*Early 18th Century*
Mean	8.6026	9.468686643
Variance	0.0974753	0.210879392
Observations	5	12
Hypothesized Mean Difference	0	
df	11	
	-	
t Stat	4.498417459	
P(T<=t) one-tail	0.00045178	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.00090356	
t Critical two-tail	2.20098516	

	Early 18th Century	*Modern*
Mean	9.468686643	8.728136096
Variance	0.210879392	0.304303202
Observations	12	51
Hypothesized Mean Difference	0	
df	19	
t Stat	4.826711775	
P(T<=t) one-tail	5.86055E-05	
t Critical one-tail	1.729132812	
P(T<=t) two-tail	0.000117211	
t Critical two-tail	2.093024054	

	Late 18th Century	*Modern*
Mean	9.552930513	8.728136
Variance	0.136068938	0.304303
Observations	5	51
Hypothesized Mean Difference	0	
df	6	
t Stat	4.527976223	
P(T<=t) one-tail	0.001991849	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.003983699	
t Critical two-tail	2.446911851	

	Woodland	*Late 18th Century*
Mean	8.6026	9.552930513
Variance	0.0974753	0.136068938
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	-4.397188639	
P(T<=t) one-tail	0.001147683	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	0.002295367	
t Critical two-tail	2.306004135	

	Mid-18th Century	*Modern*
Mean	9.349021962	8.728136
Variance	0.218533882	0.304303
Observations	8	51
Hypothesized Mean Difference	0	
df	10	
t Stat	3.403278654	
P(T<=t) one-tail	0.003366938	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.006733877	
t Critical two-tail	2.228138852	

	Woodland	*Mid-19th Century*
Mean	8.6026	9.94139416
Variance	0.0974753	0.260439673
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-5.003902946	
P(T<=t) one-tail	0.000779207	
t Critical one-tail	1.894578605	
P(T<=t) two-tail	0.001558413	
t Critical two-tail	2.364624252	

	Mid-18th Century	*Early 20th Century*
Mean	9.94139416	9.098849333
Variance	0.260439673	0.502821667
Observations	5	6
Hypothesized Mean Difference	0	
df	9	
t Stat	2.285581946	
P(T<=t) one-tail	0.024060581	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.048121162	
t Critical two-tail	2.262157163	

	Late 19th Century	*Modern*
Mean	9.95	8.728136096
Variance	0.687	0.304303202
Observations	6	51
Hypothesized Mean Difference	0	
df	6	
t Stat	3.520377814	
P(T<=t) one-tail	0.006256408	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.012512815	
t Critical two-tail	2.446911851	

APPENDIX V:
Plot of %N values for modern and archaeological *C. virginica* shells.