Spring 5-2012

The Characterization of Organic Carbon from Sedimentary Cores from Zostera marina beds, Maquoit Bay, Casco Bay, Maine

Elizabeth Emily Sonshine
Bates College, esonshin@bates.edu

Follow this and additional works at: http://scarab.bates.edu/geology_theses

Recommended Citation
http://scarab.bates.edu/geology_theses/4

This Open Access is brought to you for free and open access by the Student Scholarship at SCARAB. It has been accepted for inclusion in Standard Theses by an authorized administrator of SCARAB. For more information, please contact batesscarab@bates.edu.
The Characterization of Organic Carbon from Sedimentary Cores from *Zostera marina* beds, Maquoit Bay, Casco Bay, Maine

Presented to
The Faculty of the Department of Geology
Bates College

In partial fulfillment of the requirements for the Degree of Bachelor of Science

by
Elizabeth Sonshine

Lewiston, Maine
April 6, 2012
Abstract

Seagrass beds are important ecosystems in nearshore environments. They provide nutrients and habitat for commercially important fish species, buffer against storm erosion, and are effective at sequestering carbon. Globally, seagrass beds are in a general state of decline due to human activities such as pollution, climate change, and dredging in the nearshore zone. Little is known about natural fluctuations in seagrass distribution through geologic time because long and unequivocal records of paleo-seagrass have yet to be produced. The purpose of this study is to use organic geochemical techniques (lipid biomarker concentrations and isotope composition) to determine if seagrass organic matter can be detected in sediment cores from Maquoit Bay, Casco Bay, Maine.

Maquoit Bay has extensive beds of the seagrass Zostera marina (hereafter referred to as eelgrass) and is located off of Casco Bay in the Gulf of Maine. Seven sediment cores (ranging between 20-50 cm in depth) were taken from Maquoit Bay using a livingstone corer. These cores were subsampled for organic geochemistry (bulk and higher plant leaf wax lipid carbon isotope composition), $^{239+240}$Pu chronology, and grain size determinations. Plutonium results indicate that the sediment cores represent the last 50 years of deposition. Within all of the analyzed cores, the $\delta^{13}$C of the bulk sediments ranged from -17‰ at the coretop to -22‰ deeper in the core. Given the multiple sources of carbon with varying $\delta^{13}$C values in the system, it is impossible to determine, with certainty, the degree to which eelgrass contributes to the total organic pool. Analysis of the lipid biomarker data indicates that eelgrass does contain higher plant leaf wax lipids (C24, C26, C28 fatty acids), as do the sediments in the core. High values of HPLWs have also been found at the surface of the core, which decrease with depth. Isotope analysis shows high C24 and C26, indicating a high even-over-odd predominance typical of HPLW lipids. Using a 2-end-member mixing model in conjunction with a source contribution plot, the organic carbon throughout the sediment core was characterized to determine that eelgrass (33%), phytoplankton (41%), bacteria (14%), and other C3 plants (12%) all contribute to the deposition of organic carbon in Maquoit Bay. This final analysis may provide an important proxy for eelgrass in other nearshore environments.
Acknowledgements

I would like to begin by thanking the Bates College Department of Geology. This department is full of incredibly nice people who teach the most interesting courses at Bates. Thank you to Dyk Eusden and Mike Retelle, who first made me fall in love with geology my sophomore year. I never would have chosen this path as my major if it hadn't been for the dozens of field trips I went on and the people I met.

It is my privilege to thank Beverly Johnson, without whom, this thesis would not exist. She taught me all the labwork I know, spent countless hours editing my drafts and showed great support for my endeavors in both geology and rowing. She is an incredibly kind and supportive advisor and I have had a wonderful time working with her this year.

I would like to thank all those involved in the NSF grant that I am only a small part of. It has been an incredible experience working on this project. I never would have realized how relevant and interesting this type of work it had it not been for this thesis. I would also like to thank to the Bates Student Research Fund for their great support of the entire Geology Department.

A special thanks to Phil Dostie, who helped me through some of my most difficult times during the thesis process. He answered late night questions regarding lab work and always put everything in perspective for me. I will be forever grateful for his tinkering talents and ability to fix many a broken freeze drier.

I would also like to thank all of the previous Bates graduates whose theses I studied and cited. Thank you for setting the bar so high and making the yearlong thesis in geology what it is today.

Thanks to all of my fellow geology majors for sharing many late nights in coram over the past three years. It's been wonderful getting to know you all.

Finally, I would like to thank my family. Mom and Dad, thanks for taking us on so many camping trips when we were younger. I never would have found geology if I hadn't been looking for an excuse to get outside. Daniel and David, you guys are the best. I love you all and I appreciate all the support you have given me throughout my time at Bates.
# Table of Contents

Abstract ........................................................................................................................................... ii  
Acknowledgements ........................................................................................................................... iii

**Introduction** ................................................................................................................................. 9  
1.1 Background and Importance of Eelgrass .................................................................................. 10  
1.2 Vulnerabilities ............................................................................................................................ 10  
1.3 Evidence for Past Shifts in Eelgrass Abundance ....................................................................... 11  
1.4 Stable Isotope Geochemistry ..................................................................................................... 12  
1.4.1 Stable Isotopes ...................................................................................................................... 12  
1.4.2 Delta Notation ........................................................................................................................ 12  
1.4.3 Fractionation ........................................................................................................................ 12  
1.4.4 C/N ......................................................................................................................................... 13  
1.5 Higher Plant Leaf Wax/Biomarker .............................................................................................. 14  
1.6 Biomarkers in Eelgrass: Review of Literature .......................................................................... 15  
1.7 \(^{239+240}\)Pu Dating .................................................................................................................. 15  
1.8 Purpose ....................................................................................................................................... 16  
1.9 Objectives ................................................................................................................................... 16  
1.10 Study Area ................................................................................................................................. 17  
1.10.1 Physical Characteristics of Maquoit Bay ............................................................................. 17  
1.10.2 Geology in Casco Bay .......................................................................................................... 18

**Methods** ...................................................................................................................................... 26  
2.1 Field Work in Maquoit Bay ......................................................................................................... 27  
2.2 Core Preparation/Logging/Sampling ......................................................................................... 27  
2.3 Grain Size ................................................................................................................................... 28  
2.3.1 Grain Size Analysis ................................................................................................................ 28  
2.4 Plutonium Dating ....................................................................................................................... 28  
2.5 Bulk Density and % Water Determinations .............................................................................. 29  
2.6 Isotopic Analysis ....................................................................................................................... 29  
2.7 Acidification ............................................................................................................................... 29  
2.8 Fatty Acid Extraction .................................................................................................................. 30  
2.8.1 Extraction of the Total Lipid Extract .................................................................................... 30  
2.8.2 Saponification ...................................................................................................................... 31  
2.8.3 Esterification ........................................................................................................................ 31  
2.8.4 Elemental Analyzer- Isotope Ratio Mass Spectrometer (detector) ........................................ 32
Results ................................................................. 40
3.1 Core Description .................................................. 41
3.1.1 Maquoit Bay Eelgrass Extent .............................. 41
3.1.2 Stratigraphy of Cores from Maquoit Bay ................. 41
3.2 Physical Data ....................................................... 42
3.2.1 %H₂O ............................................................... 42
3.2.2 Dry Bulk Density ............................................... 42
3.2.3 Grain Size Analysis ............................................. 42
3.3 Plutonium Dating .................................................. 43
3.4 Bulk Sediment Geochemistry .................................... 43
3.4.1 Chlorophyll ....................................................... 43
3.4.2 Organic Carbon and Nitrogen ............................... 43
3.4.3 C/N Ratios ....................................................... 43
3.4.4 δ¹³C of Bulk Sediments ...................................... 44
3.5 Eelgrass Geochemistry ............................................. 44
3.6 Lipid Biomarker Distribution .................................... 44
3.6.1 Total Fatty Acids Concentration ............................ 44
3.6.2 δ¹³C of Higher Plant Leaf Wax Fatty Acids ............... 45
3.6.3 Weighted Mean Isotopic Value of Higher Plant Leaf Waxes 45
Discussion ............................................................... 63
4.1 Stratigraphy ......................................................... 64
4.2 Chronology and Evidence of Mixing ........................... 64
4.2.1 Plutonium Analysis ............................................. 64
4.2.2 Chlorophyll ....................................................... 64
4.3 Early Diagenesis ................................................... 65
4.3.1 % Organic Carbon .............................................. 65
4.3.2 % Organic Nitrogen ............................................ 66
4.4 Eelgrass Data ........................................................ 67
4.4.1 Bulk Isotope Distribution in Eelgrass ..................... 67
4.4.2 Fatty Acid Methyl Ester Lipid distribution in eelgrass .. 67
4.5 Organic Matter Characterization Downcore .................. 68
4.5.1 C/N ................................................................. 68
4.5.2 δ¹³C of Bulk Sediments ....................................... 69
4.6 Organic Matter Characterization ............................... 69
4.6.1 Changes in Compound Specific Data Downcore .......................... 69
4.6.2 Weighted Mean Isotopic Value of Higher Plant Leaf Waxes .......... 70
4.6.3 Organic Carbon Cycling at Maquoit Bay- Terrestrial, Bacterial and Phyto-
plankton Contributions .......................................................... 71
4.6.4 Two-End-Member Mixing Modeling .................................... 72
4.6.5 Organic Carbon Characterization ........................................ 73
4.6.6 Implications ................................................................. 73

Conclusion ................................................................. 79
References ................................................................. 82

Appendix A ............................................................... 87
## Table of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Shows the global distribution of <em>Zostera marina</em> (Short and Short, 2003)</td>
<td>19</td>
</tr>
<tr>
<td>1.2</td>
<td>Map of Casco Bay in the Gulf of Maine</td>
<td>20</td>
</tr>
<tr>
<td>1.3</td>
<td>Recovery of eelgrass in Maquoit Bay at the Mere Point site over one year from 2000 to 2001</td>
<td>21</td>
</tr>
<tr>
<td>1.4</td>
<td>Graph of the age of cod and flounder fish bones from Penobscot Bay in the Gulf of Maine as it is plotted against the heavy carbon isotope (δ^{13}C) values (Johnson, 2010)</td>
<td>22</td>
</tr>
<tr>
<td>1.5</td>
<td>Cross-sectional drawing of a simplified plant cuticle (modified after B.E. Juniper, Botany Schools, Oxford University, in Eglinton and Hamilton, 1967)</td>
<td>23</td>
</tr>
<tr>
<td>1.6</td>
<td>Schematic of sources and processes contributing to sedimentary organic matter at Maquoit Bay</td>
<td>24</td>
</tr>
<tr>
<td>1.7</td>
<td>Map of Maquoit Bay in Casco Bay, Maine (Neckles, et al, 2005)</td>
<td>25</td>
</tr>
<tr>
<td>2.1</td>
<td>Map of Maquoit Bay</td>
<td>35</td>
</tr>
<tr>
<td>2.2</td>
<td>is a visual of core 6 taken on June 28, 2011</td>
<td>36</td>
</tr>
<tr>
<td>2.3</td>
<td>shows the two chemical reactions involved in saponification (Lindelof, 2011)</td>
<td>37</td>
</tr>
<tr>
<td>2.4</td>
<td>Flow chart of methods for HPLW extractions of Fatty Acids (Lindelof, 2011)</td>
<td>38</td>
</tr>
<tr>
<td>2.5</td>
<td>shows the chemical reaction for esterification (Lindelof, 2011)</td>
<td>39</td>
</tr>
<tr>
<td>3.1</td>
<td>shows the downcore physical data for core 1</td>
<td>46</td>
</tr>
<tr>
<td>3.2</td>
<td>shows the downcore physical data for core 2</td>
<td>47</td>
</tr>
<tr>
<td>3.3</td>
<td>shows the downcore physical data for core 6</td>
<td>48</td>
</tr>
<tr>
<td>3.4</td>
<td>shows the $^{239+240}$Pu dating analysis downcore for core 6</td>
<td>49</td>
</tr>
<tr>
<td>3.5</td>
<td>shows the chlorophyll analysis downcore for core 6</td>
<td>50</td>
</tr>
<tr>
<td>3.6</td>
<td>shows the downcore bulk isotope geochemical data for core 1</td>
<td>51</td>
</tr>
<tr>
<td>3.7</td>
<td>shows the downcore bulk isotope geochemical data for core 2</td>
<td>52</td>
</tr>
<tr>
<td>3.8</td>
<td>shows the downcore bulk isotope geochemical data for core 6</td>
<td>53</td>
</tr>
<tr>
<td>3.9</td>
<td>shows the concentration of leaf wax lipids for carbon chain lengths from 20-30</td>
<td>54</td>
</tr>
<tr>
<td>3.10</td>
<td>shows the relative abundance of HPLW lipids for carbon chain lengths from 12-30</td>
<td>55</td>
</tr>
<tr>
<td>3.11</td>
<td>shows the concentration of HPLW lipids for carbon chain lengths from 24-30</td>
<td>56</td>
</tr>
<tr>
<td>3.12</td>
<td>shows a chromatogram of a sediment sample</td>
<td>57</td>
</tr>
<tr>
<td>3.13</td>
<td>shows the weighted mean average for the C22 and C24 carbon chain lengths</td>
<td>58</td>
</tr>
<tr>
<td>4.1</td>
<td>shows a map of Maquoit Bay with core stratigraphies</td>
<td>74</td>
</tr>
<tr>
<td>4.2</td>
<td>plots the δ^{13}C FAMEs show the average of C22 and C24 chain lengths downcore, Weighted Mean Isotopic value of HPLW of chain lengths from C22-C24, % of higher plant carbon derived from eelgrass according to the 2-end-member mixing model and the % organic carbon derived from eelgrass</td>
<td>75</td>
</tr>
<tr>
<td>4.3</td>
<td>shows a plot δ^{13}C for different carbon chain lengths from C22 to C30 downcore</td>
<td>76</td>
</tr>
<tr>
<td>4.4</td>
<td>is a normalized plot of terrestrial plant fatty acid, phytoplankton fatty acid and bacterial fatty acid contributions in Maquoit Bay</td>
<td>77</td>
</tr>
</tbody>
</table>
# Table of Tables

<table>
<thead>
<tr>
<th>Table Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>shows %H, O analysis for all cores.</td>
<td>59</td>
</tr>
<tr>
<td>3.2</td>
<td>shows dry bulk density analysis for all cores.</td>
<td>59</td>
</tr>
<tr>
<td>3.3</td>
<td>shows % silt and clay analysis for all cores.</td>
<td>60</td>
</tr>
<tr>
<td>3.4</td>
<td>shows $^{239+240}$Pu analysis for core 6.</td>
<td>60</td>
</tr>
<tr>
<td>3.5</td>
<td>shows % organic carbon analysis for all cores.</td>
<td>61</td>
</tr>
<tr>
<td>3.6</td>
<td>shows % organic nitrogen analysis for all cores.</td>
<td>61</td>
</tr>
<tr>
<td>3.7</td>
<td>shows C/N analysis for all cores.</td>
<td>62</td>
</tr>
<tr>
<td>3.8</td>
<td>shows $\delta^{13}$C analysis for all cores.</td>
<td>62</td>
</tr>
<tr>
<td>4.1</td>
<td>compares the bulk isotope data for an eelgrass sample and sediment core</td>
<td>78</td>
</tr>
<tr>
<td>4.2</td>
<td>The average $\delta^{13}$C values for core 6 by carbon chain length.</td>
<td>78</td>
</tr>
</tbody>
</table>
Introduction
1.1 Background and Importance of Eelgrass

Zostera marina or, eelgrass, is an angiosperm (flowering plant) that grows in shallow/subtidal and low intertidal areas across the globe (Figure 1.1). Eelgrass colonizes low intertidal and subtidal silty substrates at depths of 35 meters below the surface or less. It is the predominant species of seagrass in the Gulf of Maine (Short and Short, 2003). It provides nutrients, as well as, a good nursery habitat to commercially important species such as crustaceans, small fish and other marine organisms at crucial life stages (Short and Short, 2003; Neckles, et al., 2005). Eelgrass buries and sequesters carbon below the sediment, and exports detrital carbon to near shore and off shore systems via trophic transfer (Short and Neckles, 1999; Fourqurean and Schrlau, 2003; Kennedy et al., 2010). Eelgrass can also absorb energy from strong waves, protect coastal areas from storm surges and stabilize coastlines.

1.2 Vulnerabilities

Eelgrass systems are at risk due to environmental factors including anthropogenic activities, excess nutrients and sediments, mechanical destruction, biological events (disease), weather phenomena, climate change and sea level rise (Short and Neckles, 1999). Because eelgrass needs sunlight to survive, it is extremely vulnerable to increases in water column turbidity, suspended sediment, and/or excess nutrients and concurrent increases in phytoplankton can both contribute to loss of eelgrass (Short and Neckles, 1999; Wall, 2011). Changes in climate, sea level, disease and dredging can also have a very strong impact on eelgrass growth and reproduction (Short and Short, 2003; Neckles, et al., 2005). In addition, high algae growth outcompetes eelgrass for nutrients and can prevent further seagrass reproduction. It is often difficult to reestablish seagrass beds once a system has undergone eutrophication (Godet, 2008).

In the 1930’s, wasting disease killed ~90% of global seagrass beds, but there has been good recovery in the Gulf of Maine since (Godet, 2008) and there are many areas where eelgrass thrive throughout Casco Bay (Figure 1.2). At the time of the collapse, eelgrass beds from the French
coast almost completely disappeared from certain areas and are now only half as extensive as they once were. In the 1950’s eelgrass beds had still hardly recolonized but now there are some subtidal and intertidal zones that are rapidly expanding (Godet, 2008).

In certain settings, eelgrass has recovered well from mechanical destruction. Eelgrass aerial extent in Maquoit Bay decreased by 10% after a dredging event in 1999 (Neckles, et al., 2005). From 2000 to 2001, eelgrass had recovered a 23% increase in eelgrass extent (Figure 1.3). Although eelgrass recovery strongly depends on initial dragging intensity, there was considerably quick and substantial growth and revegetation of eelgrass in areas that were lightly dragged (Neckles, et al., 2005). Little is known about changes in eelgrass in Maquoit Bay prior to and since the late 1990’s.

1.3 Evidence for Past Shifts in Eelgrass Abundance

According to early navigation charts and anecdotal information from fishermen, there was 65% more eelgrass south of Cape Cod and 20% more eelgrass north of Cape Cod prior to European settlement than is currently found (Short and Short, 2003). The recent losses of eelgrass have been attributed to wasting disease, mechanical destruction and water column turbidity (Short and Neckles, 1999; Godet, 2008).

Johnson and students of Bates College have looked at carbon isotopes in fish bones in Penobscot Bay and found that fish diets have become significantly more depleted and more restricted in δ¹³C over the last 1300 years (Harris, 2010). The isotopic shift is greatest in shallow water species suggesting significant changes in shallow water ecosystems over the last 4500 years until 500 years ago. Johnson and colleagues attribute this isotopic shift to loss of eelgrass and eelgrass-derived nutrients (Figure 1.4). The most accelerated loss of eelgrass occurred in the last 400 years in Penobscot Bay (Harris, 2010).
1.4 Stable Isotope Geochemistry

1.4.1 Stable Isotopes

Isotopes are variations of the same element, each having differing numbers of neutrons. In carbon, each atom has 6 protons and 6 electrons, but the amount of neutrons can change, changing the mass of the atom. The three most common carbon isotopes are $^{12}\text{C}$, which has 6 neutrons and 98.89% abundance; $^{13}\text{C}$, which has 7 neutrons and 1.11% abundance; and $^{14}\text{C}$, which has 8 neutrons and trace abundance. $^{14}\text{C}$ is also an unstable isotope, and undergoes radioactive decay at a constant rate.

1.4.2 Delta Notation

The stable isotopic composition of a material is presented in delta notation, as the ratio of the heavy stable isotope to the light stable isotope in the sample relative to a standard. Delta notation is calculated as follows:

$$\delta_{\text{sample}} = 1000 \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1$$

where $R$ is $^{13}\text{C}/^{12}\text{C}$ and the standard for carbon is Vienna Pee Bee Bellemnite (VPDB) and. A high delta value means that the sample is enriched in the heavy isotope while a low delta value means that the sample is enriched in the light isotope.

1.4.3 Fractionation

Fractionation refers to the partitioning of isotopes in various phases of reactions and/or processes. Isotopic fractionation in most biochemical reactions arises when similar molecules of slightly different mass react at different rates. The degree of equilibrium fractionation is typically very small and can vary with temperature (Hoefs, 2004). However, kinetic fractionation only varies slightly and involves the separation of stable isotopes by mass during unidirectional processes (Farquhar, et al., 1989).

Plants actively incorporate CO$_2$ through photosynthesis and although all plants select for lighter carbon isotopes ($^{12}\text{C}$), some are better at taking up the heavier carbon isotope ($^{13}\text{C}$) than others. These plants are considered enriched in the heavy carbon isotope ($^{13}\text{C}$). One such plant is
eelgrass, which is more efficient in taking up the heavier carbon isotope relative to many other C3 plants. As a result, eelgrass has a more enriched $^{13}$C signal in comparison to other plants.

The three major primary producers in nearshore ecosystems (kelp, phytoplankton and eelgrass) all have very distinct carbon isotope values (Bird, et al., 1995). Typical stable carbon isotope values for the three main primary producers are $\delta^{13}$C Eelgrass = -11‰; $\delta^{13}$C Kelp = -17‰; and $\delta^{13}$C phytoplankton = -25‰ (Bird, et al. 1995; Canuel, et al., 1997; Kennedy, et al., 2010).

The isotope composition of sediments represents a mix of inputs with different proportions and different isotopic compositions including bacteria, phytoplankton, and other C3 plants. Therefore, it can be difficult, to identify the dominant sources of organic matter from bulk analyses alone. Pagani and colleagues (1999) also found that $\delta^{13}$C values of total organic carbon, as determined through bulk stable isotope analysis, do not accurately represent $\delta^{13}$C values in phytoplankton when working with sediment in marine settings because there are multiple sources of carbon contributing to this value. $\delta^{13}$C values of total organic carbon are extremely variable and the characterization of organic carbon is most precise when compound-specific isotope analysis of biomarkers is used (Pagani, et al., 1999; Pancost and Pagani, 2006).

1.4.4 C/N

C/N ratios in sedimentary organic matter can be used as tracers for source matter (Thorton and McManus, 1994; Meyers, 1999; Jaffé, et al., 2001) and help in identifying sediment with significant terrestrial input (Matson and Brinson, 1990). C/N ratios >15 have been attributed to source matter that is derived from terrestrial material (Meyers, 1999). Comparatively, a C/N ratio between 4 and 10 indicates that the source matter is primarily derived from aquatic materials such as phytoplankton (Libes, 1992; Meyers, 1999). Phytoplankton has low C/N ratios because it does not contain cellulose or other plant cellular wall materials within its structure that many other plants are made of (Meyers, 1997). In addition, C/N ratios for sediments typically increase with depth because nitrogen-rich compounds (proteins) degrade faster than nitrogen deficient compounds (carbohydrates and lipids) (Libes, 1992).
1.5 Higher Plant Leaf Wax/Biomarker

Lipid biomarkers are organic compounds that are synthesized by a specific source. Higher plant leaf waxes (HPLW) are a suite of biomarkers, which are synthesized by higher plants. HPLW fatty acids are long chain compounds of approximately 26-30 carbons with an even-over-odd predominance (higher ratios of even numbered carbons comprising the carbon chain, over odd numbers of carbon). HPLWs are the source of epicuticular wax lipids, which act as a barrier between plant cells and the outside environment (Figure 1.5). The vascular plant leaf waxes, form a protective coating on the surfaces of leaves to prevent physical damage and excessive water loss (Makou, et al., 2007).

The lipids in a sediment can be used to determine terrestrial input from higher plant sources and consist of a variety of long chain compounds including fatty acids that make up the HPLW (Eglinton and Hamilton, 1967). HPLW lipids are stable through time and do not break down due to their bulky structures (Logan, et al., 1995; Pancost and Pagani, 2006). HPLW have been found in Miocene sediment from lacustrine deposits, eelgrass beds and other areas.

Provided there are only 2 isotopically distinct sources of organic matter in the sediments, the isotopic composition of HPLWs can be incorporated into a 2-end-member-mixing model, which will give a rudimentary approximated of the amount of carbon derived from a specific source (Xu, et al., 2007). Applications of this approach include the tracking of organic matter in sediment. Kennedy, et al. (2010), used a 3-end-member mixing model to characterize the sources contributing to the organic matter in the sediment. In this study, the three end members included seagrass, phytoplankton and mangroves/terrestrial organic matter. Mixing models effectively determine a rough estimate for organic matter characterization but cannot account for every source of organic matter.
1.6 Biomarkers in Eelgrass: Review of Literature

Several recent studies have investigated the presence of lipid biomarkers in eelgrass (Hernandez, et al., 2001; Jaffé, et al., 2001; Jaffé, et al., 2006; Xu and Jaffé, 2007; Kennedy, et al., 2010). However, these studies traced organic matter using n-alkane lipid biomarkers rather than HPLW fatty acids. N-alkanes have a strong odd-over even predominance rather than the even-over-odd predominance that is characteristic of fatty acids. These applications have also been effective in distinguishing marine organic matter from terrestrial organic matter in an effort to determine environmental change.

1.7 $^{239+240}$Pu Dating

Plutonium is included in a group of elements known as transuranic elements, which have been distributed in the Earth’s surface environment due to anthropogenic activities over the past 60 years (Ketterer and Szechenyi, 2008). Transuranic elements are produced from fission bombs or nuclear reactors that produce electrical power. All of the plutonium on Earth is of anthropogenic origin, specifically from atmospheric weapons testing, atmospheric deposition of $^{239+240}$Pu has a well-known maximum at 1963. Following this date, there is sharp decrease in plutonium deposition that corresponds to the enactment of the Limited Test Ban Treaty (Ketterer and Szechenyi, 2008). $^{239+240}$Pu is typically analyzed in lake (Eberle, 2008) and estuarine (Sanders, et al., 2010) sediments.

The 1963 peak in plutonium values is a well-known chronostratigraphic marker in sediments (Sanders et al., 2010). Plutonium peak values can range between .1 bq/kg and 50 bq/kg depending on location, surface sediment mixing and other conditions (Eberle, 2008; Ketterer, et al., 2004). A gradual decrease in $^{239+240}$Pu concentrations to values near 0 is seen in core tops in ideal plutonium chronologies. $^{239+240}$Pu geochronology is widely and successfully used in marine sedimentation studies because plutonium is relatively immobile compared to $^{137}$Cs (another chronostratigraphic marker) under both fresh and saltwater conditions (Sanders et al., 2010).
Pu has great potential for examining sedimentation processes and is well-suited for saltwater conditions, such as Maquoit Bay (Sanders et al., 2010).

1.8 Purpose

The goal of my work is to characterize the organic matter in sediment cores from Maquoit Bay over the last several decades. I will use stable isotopes and lipid biomarkers to determine the percent of organic matter derived from eelgrass, phytoplankton, bacteria and C3 terrestrial plants.

1.9 Objectives

Living eelgrass will be extracted for HPLW fatty acids to determine the concentration of fatty acids in a pure sample. Sediment cores from Maquoit Bay (an eelgrass bed) will be analyzed for grain size, bulk sediment organic geochemistry, and the concentration and isotopic composition of HPLWs. Isotope data will be used to measure the amount of eelgrass organic matter in the sediment. The importance of eelgrass as a carbon sink will be determined through the characterization of the organic carbon in sediment from an eelgrass bed. In addition, a set of methods for the detection of eelgrass organic matter through time will be established.

Maquoit Bay is a somewhat complicated system with multiple sources of organic carbon to the sediment including phytoplankton, bacteria, eelgrass and C3 higher plants. The relative proportion of these sources varies with tidal flushing, river input, runoff and wind (Figure 1.6).

Given the natural range in δ13C eelgrass (-20‰ to -5‰; Kennedy et al., 2010), and δ13C phytoplankton (-28‰ to -21‰; Wissel et al, 2005) and the fact that there is some (albeit scant) kelp present in this system, a 2-end-member-mixing model has its limits. In order to obtain a more detailed understanding of the organic matter in the core, the lipids of higher plant leaf waxes will be extracted and identified.

Kennedy, et al., (2010) looked at δ13C values, organic carbon and total nitrogen concentrations for seagrass and underlying sediments from 88 locations around the world.
Seagrass values ranged between -20‰ to -5‰ whereas the associated sediment ranged between -26.6‰ to -7.3‰. Kennedy, et al., (2010) used a 3 source model and calculated that 50% of the organic carbon in the surface sediment from a seagrass bed was derived from seagrass. With a 3-source-mixing model, two isotopic ratios can be compared with 3 sources of organic matter. However, there are several weaknesses in Kennedy’s study. It is impossible to account for all of the sources of organic matter using a 3-end-member-mixing model. In addition, the regression between $\delta^{13}$Cseagrass and $\delta^{13}$Csediment is quite low, with an $r^2$ value of 0.22, indicating a very weak trend. Also, microbial processes at the surface of the sediment were not mentioned in the study. Finally, Kennedy only studied the surface sediment (top 5 cm) from an eelgrass bed. Thus, the data do not accurately represent the potential for modification of organic matter downcore or the volume of sequestered carbon throughout an eelgrass bed.

Although Kennedy and colleagues determined that 50% of the organic carbon in the surface sediment of seagrass meadows is derived from the seagrass, compound specific isotope analysis will provide more specific information on composition of organic matter in seagrass sediments.

1.10 Study Area

1.10.1 Physical Characteristics of Maquoit Bay

Maquoit Bay is a finger embayment located in the eastern part of Casco Bay off the Gulf of Maine (Figure 1.7). The Gulf of Maine (GoM) is a well-mixed and productive marginal shelf basin in the northwest Atlantic (Johnson, 2007). It is a long, narrow and shallow estuary with an eelgrass bed that has wide, muddy tidal flats and low flushing rates. The flushing time in Maquoit Bay is 6 days on average and can range from 5-15 days depending on stream flow and other conditions (Heinig and Campbell, 1992). These slow flushing rates may be the cause of high productivity in the area since phytoplankton are retained for longer periods of time in waters that are shallow, warm, fertile, and ideal for algae growth (Heinig and Campbell, 1992).

The bottom of the bay is predominantly soft mud throughout, with eelgrass beds on the
northern and western shores (Larsen, et al. 1983; Heinig and Campbell, 1992; Neckles, et al., 2005). The two marine primary producers in the subtidal zone are eelgrass and phytoplankton. Maquoit Bay covers 1013 hectares, and is approximately 5 km from the town of Brunswick, Maine (Neckles, et al., 2005). In addition, a small salt marsh exists along the northern margins of the bay.

There are very high rates of sediment turnover at high and low tide in Maquoit Bay. Clammers walk and drive through the mud flat, mixing sediments. Horsehoe crabs and clams also mix surface sediments. As a result, the first 5-10 inches of sediment are very well mixed. Other animals include deeper dwelling worms, clams and bivalves.

Maquoit Bay is a unique site because although there has been a global decrease in seagrass, Maquoit Bay currently has a very healthy eelgrass bed.

1.10.2 Geology in Casco Bay

The bedrock in Casco Bay is composed almost entirely of Ordovician metamorphic gneiss and schist. The Casco Bay Group preserves the clearest record of multiple folding (Maine Geologic Survey, 2008). The bedrock was formed under medium to high grade metamorphism and is largely composed of late Ordovician sedimentary basins (Maine Geological Survey, 2002).

The surface geology of the edge of Maquoit Bay is composed of till and overlying fine grained glaciomarine sediments, which make up the Presumpscot Formation (Maine Geological Survey, 2002).
Figure 1.1 Shows the global distribution of *Zostera marina* (Short and Short, 2003).
Figure 1.2 Map of Casco Bay in the Gulf of Maine. The widespread extent of eelgrass in the area from 2005 can be seen in purple (Eelgrass 2005 layer provided by the Maine Office of Geographic Information Systems)
Figure 1.3 Recovery of eelgrass in Maquoit Bay at the Mere Point site over one year from 2000 to 2001. Eelgrass extent is shown in black and gray (Neckles, et al, 2005).
Figure 1.4 Graph of the age of cod and flounder fish bones from Penobscot Bay in the Gulf of Maine as it is plotted against the heavy carbon isotope ($\delta^{13}C$) values. The blue box highlights the extreme shift in $\delta^{13}C$ in flounder bones over the last 500 years, likely due to loss of eelgrass (Johnson, 2010).
Figure 1.5 Cross-sectional drawing of a simplified plant cuticle. The epicuticular wax layer contains HPLW and compounds of interest (modified after B.E. Juniper, Botany Schools, Oxford University, in Eglinton and Hamilton, 1967).
Figure 1.6 Schematic of sources and processes contributing to sedimentary organic matter at Maquoit Bay.
Figure 1.7 Map of Maquoit Bay in Casco Bay, Maine with specific study sites pointed out, including Mere Point (MP), Little Flying Point (LFP), Bunganuc East (BE) and Bunganuc West (BW) (Neckles, et al, 2005).
Methods
2.1 Field Work in Maquoit Bay

A total of 7 sediment cores were collected from Maquoit Bay on June 21, 2011 and June 28, 2011 using a Livingston corer (Figure 2.1). On June 21, 2011, this author and Phil Dostie walked 1 mile out from the boat launch at the northern end of Maquoit Bay and took 1 sediment core during low tide. However, this sediment core was compromised during extraction and will not be presented further in this thesis. On June 28, 2011, this author and Phil Dostie took 6 additional sediment cores off the side of a motorboat during high tide. It was calm and sunny on both days, with minimal wind. Core depths varied widely, from 20 cm to 40 cm long and were extracted from areas that were emergent during low tide. Core 1 was extracted from a water depth of .55 m, while core 2 and core 6 were extracted from a water depth of .75 m. Cores were stored at 40°C until they were split on June 30, 2011. After cores were split in half and sampled, they were returned to storage at 4°C.

2.2 Core Preparation/Logging/Sampling

All of the cores were extruded first and then split in half using a fishing wire. One half was used for subsampling and the other half was archived and kept in the core refrigerator. All cores were photographed and the Munsell Color Chart was used to record variations in color. Changes in grain size, macrophytes and shell content were also recorded and stratigraphic columns were drawn in Sigma Plot. Four cores (6/21/11-1; 6/28/11-1; 6/28/11-2; 6/28/11-6) were subsampled using a 2cc constant volume sampler every 5 centimeters. Three of the cores (6/28/11-1; 6/28/11-2; 6/28/11-6) were sampled again at the same depths (Figure 2.2) for a total of 4cc across, to collect samples to be run in the IRMS for organic geochemistry and grain size analysis. Cores to be sampled more extensively were chosen based on length of the core and water depth at which the cores were extracted.
2.3 Grain Size

Bagged samples were weighed out using a Mettler-Toledo balance and then placed in the freezer. Once frozen, samples were placed in the freeze drier under a vacuum. The mass of the dried samples was recorded to determine a total dry weight, subtracting out the mass of the plastic bag.

2.3.1 Grain Size Analysis

One half of the sample (1cc every 5 cm down core) of cores 6/28/11-1; 6/28/11-2 and 6/28/11-6 was used to analyze grain size. Samples were wet sieved at 40-mesh (.0625mm) to determine the percent sand vs. percent silt and clay in the samples. Percent silt and clay was calculated using the following equation:

\[
\% \text{ Silt and Clay} = \left( \frac{\text{total weight} - \text{sand weight}}{\text{total weight}} \right) \times 100
\]

2.4 Plutonium Dating

Core 6 was chosen to be analyzed for plutonium dating because it is the longest core from the greatest depth. The second “archived” half of core 6 from June 28, 2011 was labeled as “Split B” and was subsampled every centimeter down the entire length of the core because a continuous record is required for accurate plutonium analysis. These samples were also freeze-dried and a known mass of sediment was ground using a mortar and pestle. The mortar and pestle were washed in between each sediment sample using soap and hot water followed by three rinses of de-ionized water. Twenty 2 cm³ samples from the top 20 centimeters were placed into 20 mL glass vials and sent to Michael Ketterer at Northern Arizona University for \(^{239+240}\text{Pu}\) dating. The rest of the samples from core 6 were stored in plastic Ziploc bags after the freeze-drying process was complete.
2.5 Bulk Density and % Water Determinations

The %H$_2$O was determined using the following equation:

\[
\%H_2O = \frac{(\text{Wet weight} - \text{Dry weight})}{\text{Dry weight}} \times 100
\]

The dry bulk density was determined using the following equation:

\[
\text{Dry Bulk Density} = \frac{\text{Dry mass}}{2 \text{cm}^3}
\]

2.6 Isotopic Analysis

Samples were de-salted prior to isotopic analysis to prolong the life of the oxidation column in the EA-IRMS. Sediment samples of 2 cm$^3$ in size were mixed with 150 mL e-pure water, stirred and left to sit for 1-2 days. Water was then decanted off and the beakers were placed in the drying oven (Precision 51221132) at 60°C for 1-2 days or until completely dry.

Because carbonate $\delta^{13}$C values are extremely enriched relative to the organic carbon $\delta^{13}$C values, the carbonate must be removed prior to isotope analysis. Large chunks of carbonate (shell fragment) were picked out by hand using tweezers. Samples were scraped out of the beaker and ground with a mortar and pestle. About half of the grinded sample went through the acidification process described below.

2.7 Acidification

Approximately 3-4 mg of each sample was weighed out on weigh paper and transferred to plastic centrifuge tubes. 12 mL of diluted phosphoric acid (3.3M $\text{H}_3\text{PO}_4$) was added to the tubes, which were inverted multiple times and allowed to sit for approximately 5 hours. The samples were then centrifuged at 2,100 RPM (Rotations per Minute) for 3 minutes using the Centra CL2 Thermo IEC centrifuge. The acid was then decanted from the tubes. This process was completed 2 more times for a total of 3 gentle acid rinses. In order to remove the acid, 12 mL of e-pure water was added to the tubes, the samples were shaken by hand, centrifuged (at the same settings as the
acid) and the water was decanted off. Samples did not sit between e-pure water rinses. Samples were rinsed a total of 3 times. The acidified samples were placed in the freezer and after 2 hours, or until frozen, were placed in the freeze drier for 1-2 days, or until completely dry. Finally, samples were transferred into 4 mL glass vials and labeled as ground and acidified.

Some portion (samples ranged in mass from .3 to .5 grams) of these samples was weighed out into a 5.0 mm X 9.0 mm tin cups and folded into a compact shape using forceps. Samples were stored until run through the EA-IRMS.

### 2.8 Fatty Acid Extraction

#### 2.8.1 Extraction of the Total Lipid Extract

Six sediment samples from Core 6, one batch of dried eelgrass and one blank were extracted for higher plant leaf wax analysis. Each sample contained a range in depth to maximize potential lipid concentration. Graphs of lipid concentrations at specific depths actually represent the following ranges in depth: 1) 0-5 cm, 2) 12-15 cm, 3) 21-24 cm, 4) 26-29 cm, 5) 31-34 cm, and 6) 36-39 cm.

The first step involves soxhlet extraction of sediments. Sediment samples ranging from 18 to 33 grams in mass were placed into pre-muffled glass fiber thimbles and put into a soxhlet extractor. 8 round bottom flasks filled with 300 mL of DCM:MeOH (2:1 by volume) and 3 boiling chips were attached to the bottom of the soxhlet apparatus for continuous extraction of lipids. The top of the soxhlet extractors were attached to a series of condensers connected to a chiller. The system was left for 2 days after which the round bottom flasks and fiberglass filters were removed.

The solution of DCM, methanol and lipids from each sediment sample was added to a 1 L separatory funnel with approximately 75 mL of 5% NaCl solution to increase the partitioning coefficient between MeOH and DCM. The separatory funnels were shaken vigorously for 90 seconds each to cause emulsion and left to separate for 24 hours. The bottom layer, which includes the DCM and lipids, was drained from the separatory funnel into the previously used round bottom flasks. Approximately 25 mL of DCM was added to the aqueous phase (NaCl + H₂O +
MeOH) of each sample’s separatory funnel, shaken vigorously, and then left to sit for another 24 hours. The organic phase was then drained and pooled with the other organic phases.

This process was repeated once more for a total of 3 extractions of the aqueous phase. NaSO₄ was added to the organic phase to absorb the portion of H₂O that still exists in the organic solution. Samples were then run through the rotovap (Buchi Rotovapor R-3000) until they were almost completely evaporated, transferred to 4 mL glass vials using DCM, and labeled as the total lipid extract (TLE).

2.8.2 Saponification

Saponification refers to the cleaving of large leaf wax lipid molecules into n-alkanes and n-fatty acids and involves reacting lipids in potassium hydroxide (KOH) and methanol (MeOH). During this process, a water molecule is used to break a chemical bond and cleave the molecule into a carboxylate salt and an n-alcohol (Figure 2.3)

0.5N KOH/MeOH was added to the Total Lipid Extract. The sample was refluxed in a VWR Scientific Products Standard Heatblock for 2 hours at 100°C and then transferred using hexane and MeOH. Samples were centrifuged at 2,100 RPM (Rotations Per Minute) for 3 minutes and extracted 3 times with hexane. Samples were labeled as “TLE-Acids” and contain the compounds of interest. A flow chart is used to keep track of the hexane extractions involved in retrieving TLE-Acids from the total lipid extracts (Figure 2.4).

Approximately 20 drops of 6N HCl was added to react with the aqueous phase of the samples. Samples were refluxed in the heating block for 2 hours at 100°C and then transferred again using hexane and MeOH. Samples were centrifuged at 2,100 RPM (Rotations Per Minute) for 3 minutes, extracted 3 times with hexane and labeled as “TLE-Neutrals”.

2.8.3 Esterification

TLE Acids can be converted into a more volatile form, or fatty acid methyl esters (FAME) through esterification (Figure 2.4). The TLE Acids are reacted with methanol in the presence of a catalyst (BF₃) to form FAMEs that can then be analyzed with the gas chromatograph.

500 µL of 3% BF₃/CH₃OH was added to the acid fraction of the samples and refluxed in a
heating block for 2 hours at 100°C. Hexane and 5% NaCl solution were added and the top layer of hexane was drawn off. A total of 3 extractions were performed and the hexane rinses were combined into a 100 mL pear shaped flask. NaSO$_4$ was added to the organic phase to absorb the portion of H$_2$O that still exists in the solution. Samples were run through the rotovap until they were almost completely evaporated. The FAMEs from the final stage were then transferred to GC autovials with 3 X 1 mL hexane rinses and evaporated completely with N$_2$ gas. Samples were then diluted with 500 µL of hexane and run through the Gas Chromatograph- Flame Ionization Detector (GC-FID).

**2.8.4 Elemental Analyzer- Isotope Ratio Mass Spectrometer (detector)**

The ThermoFinnigan Delta V Advantage Stable Isotope Ratio Mass Spectrometer can measure the relative abundances of carbon and nitrogen isotopes in a sample simultaneously.

Samples are combusted and then carried in a helium stream through a chromatographic column. In the ion source, electrons are released under high vacuum where a tungsten filament is heated. The electrons are accelerated and enter the ionization box where they impact sample gas and form positively charged particles. The resulting ion beam is repelled towards a flight tube and focused to form a thin beam (Sulzman, 2007). The beam enters the mass analyzer where a magnetic field separates the lighter isotope (beam bends more) from the heavier isotope (beam bends less). The beams produced are broad peaks that are captured in Faraday cups of the ion detector. The ion current flowing through the resistor creates a voltage when ions collide, which is fed into the computer system. The computer converts the relative signal strength to a ratio and then a delta value in parts per thousand relative to a standard (Sulzman, 2007). Analysis by the EA-IRMS provides values for % organic carbon, % nitrogen, $\delta^{13}$C and $\delta^{15}$N. Once the data have been compiled, C/N molar ratios can also be calculated.

**2.9 GC-FID and Compound Concentrations**

The Gas Chromatograph- Flame Ionization Detector (Agilent Technologies 6890N Network GC System GC-FID) is used to quantify the abundance of a compound. In the GC-
FID, samples are injected into the gas chromatograph and are vaporized and carried through a heated column by a carrier gas (helium). The compounds separate according to their boiling points as the oven temperature increases and pass through the flame ionization detector, which records when each compound in a sample passes through and the relative concentration of each compound (Fessenden and Fessenden, 1984).

The method used was titled BJLIPID.M. Running conditions were as follows: initial 55°C hold for 5 minutes. Ramp 1: 15°C/min to 180°C. Hold 0 minutes. Ramp 2: 8°C/min to 310°C. Hold for 25 minutes. Inlet temperature: 250°C. Detector temperature: 300°C. 1 uL of sample was injected through a rubber septum where it was vaporized and carried through the 60 m heated column by helium gas.

Once the abundance of compounds is determined from the GC-FID, a calibration curve is constructed to convert the area under the curve into a concentration (ng/g dry weight) and relative % per sample. The calibration curve in this study was based on the following equation: \( y = 1184.55X - 33.633 \) and had an \( r^2 \) value equal to 0.9168.

The Gas Chromatograph-Combustion- Isotope Ratio Mass Spectrometer (Thermo Electron Trace GC Ultra- Finnigan GC Combustion III- Delta V Advantage Isotope Ratio Mass Spectrometer GC-C-IRMS) was used to determine \( \delta^{13}C \) values of the FAMEs. The instrument operates similarly to the EA-IRMS, however, the column in the GC-IRMS allows compounds to combust and separate according to their boiling points as the oven temperature increases. As a result, compounds come off of the column separately accurate identification of the samples.
2.10 2-End-Member Mixing Model

$\delta^{13}C$ values of the sedimentary FAMEs can be used in a 2-end-member mixing model to determine the amount of carbon present in the sediment from two different sources, provided there are 2 isotopically distinct sources of organic matter in the sediments. The following equation is used to determine the percent carbon derived from eelgrass:

$$(\delta^{13}C \text{ Eelgrass FAMEs})(X) + (\delta^{13}C \text{ Terrestrial FAMEs})(1-X) = \delta^{13}C \text{ Sediment FAMEs}$$

Where $X$ is the fraction of carbon from eelgrass; $(1-X)$ is the fraction of carbon from terrestrial input; $\delta^{13}C$ Eelgrass = -12‰; $\delta^{13}C$ Terrestrial/Phytoplankton = -24‰ and $\delta^{13}C$ Sediment = -18‰.
Figure 2.1 Map of Maquoit Bay. The extent of eelgrass is visible in purple and the GPS locations of core extractions (6/28/11-1; 6/28/11-2; 6/28/11-6) that were subsampled and are pointed out with orange and white dots. Core 6 is shown in orange because all of the lab methods referenced below apply to this core. The eelgrass extent from 1997 is shown in a white transparent overlay and the eelgrass from 2005 is shown in purple (Eelgrass 2005 and 1997 layers provided by the Maine Office of Geographic Information Systems)
Figure 2.2 is a visual of core 6 taken on June 28, 2011. The core was subsampled 4cc across, every 5 cm downcore.
Figure 2.3 shows the two chemical reactions involved in saponification (Lindelof, 2011).
Figure 2.4 Flow chart of methods for HPLW extractions of Fatty Acids (Lindelof, 2011).
Figure 2.5 shows the chemical reaction for esterification (Lindelof, 2011).
Results
3.1 Core Description

3.1.1 Maquoit Bay Eelgrass Extent

According to calculations completed in GIS, eelgrass extent in Maquoit Bay has increased by 53% percent in Maquoit Bay between 1997 and 2005 (Figure 2.1). Although seagrass extent is decreasing globally, eelgrass in Maquoit Bay seems to be thriving and even expanding.

3.1.2 Stratigraphy of Cores from Maquoit Bay

Sediments preserved in core 1 (Figure 3.1) consist of two separate soft mud matrix units. The lower unit, a very dark greenish black, (2.5/10Y) extends from the bottom of the core (44 cm) to 30 cm and is solely comprised of the soft mud matrix. The upper unit (2.5/10Y) extends through the top 30 cm of the core and contains large shell fragments as well as shreds of eelgrass blades, roots and rhizomes.

Sediments preserved in Core 2 (Figure 3.2) consist almost entirely of a soft mud matrix with various amounts of whole shells and crushed shell hash. From 43 cm to the bottom of the core at 52 cm, the core consists of a crushed shell hash matrix. Sediment between 18.5 and 43 cm consist solely of the soft mud matrix, and is a very dark greenish gray (3/5GY). Finally, sediment between 0 and 18.5 cm consist of the soft mud matrix (2.5/10GY) and whole shell fragments of *Mya arenaria*.

Sediments preserved in Core 6 (Figure 3.3) consist almost entirely of a soft mud matrix as well (3/5GY), with layers of shell fragments of *Mya arenaria* and shell hash. From 36 cm to the bottom of the core at 41.5 cm there are larger shell fragments within a matrix of soft mud and some shell hash. The shell fragments are absent from 31-36 cm and the unit only consists of the soft mud matrix. From 26.5 to 31 cm, the soft mud matrix begins to gather shell fragments again from mussels and clams. Finally, the top 26.5 cm of the core are made up of the soft mud matrix with eelgrass roots and rhizomes throughout the top 5 cm and a nimartime worm that was found at 24 cm.
3.2 Physical Data

3.2.1 %H₂O

Although the three cores vary slightly in their specific values for % H₂O, overall values among the three cores range from 82-24% water (Table 3.1). Most often, the cores showed similar trends, with high %H₂O at the top of the core and gradually decreasing with depth.

3.2.2 Dry Bulk Density

Dry bulk density is inversely correlated to %H₂O within the three cores (Table 3.2). As %H₂O increases, density typically decreases. Density values ranged from .8-1.5 g/cm³ among the three cores. Again, the lower values were typically found at the top of the cores, with density gradually increasing with depth. These trends are expected because as sediment becomes more condensed and compact with increasing pressure, density increases.

3.2.3 Grain Size Analysis

Grain size varies moderately throughout the length of Core 1 (Figure 3.1 and Table 3.3). The majority of the core contains approximately 80% silt and clay and 20% sand particles, but there is about 90% silt and clay at the bottom of the core. From 25-35 cm there is a decrease to 50% silt and clay particles and then a gradual return back to around 80% silt and clay at the top of the core.

Grain size is extremely stable throughout most of the length of Core 2 (Figure 3.2). From 40 cm to the bottom of the core at 52 cm, grain size increases abruptly and the core is comprised of approximately 30% silt and clay and 70% sand particles. Whereas, the top 40 cm of the core contains consistent values of approximately 65% silt and clay and 35% sand.

Finally, Core 6 (Figure 3.3 had a relatively stable trend in grain size downcore. Grain size ranged between 55-65% silt and clay throughout the core, with a larger percentage of silt and clay at the bottom of the core and a smaller percentage of silt and clay at the top of the core.
3.3 Plutonium Dating

The sediment in the top 20 cm of Core 6 from Maquoit Bay contains stable concentrations of plutonium between 0.5 and 1.0 bq/kg (Table 3.4, Figure 3.4). The average concentration of 239+240Pu throughout the top 20 cm is .55 bq/kg + .13 bq/kg. There are no significant changes in 239+240Pu trends downcore.

3.4 Bulk Sediment Geochemistry

3.4.1 Chlorophyll

Chlorophyll values for core 6 ranged from .02 to .09 ug/gram, with an average value of .05 ug/gram. The eelgrass sample had a chlorophyll value of .22 ug/gram. (Figure 3.5)

3.4.2 Organic Carbon and Nitrogen

The %OC values (Table 3.5) in core 1 range from 1.7 %OC and 1.9 %OC (Figure 3.6) and %N mimics the same pattern as %OC. The values are stable downcore with a range between .09 %N and .18 %N (Table 3.6).

The %OC values for core 2 (Figure 3.7) range from .1 %OC and 2.8 %OC with low values in the shell hash unit and higher values in the soft mud unit. In core 2, %N parallels the pattern seen in %OC. It displays relatively stable values of %N ranging between .04 and .2 %N.

The %OC in core 6 (Figure 3.8) decreases over 5 to 20 cm. Values for %OC range between 1.9 %OC at the top of the core and .2 %OC towards the bottom of the core. %N shows a similar pattern for core 6, with values ranging between .2 %N and .02 %N.

3.4.3 C/N Ratios

In core 1 (Figure 3.6), C/N ratios range between 12.6 and 14 and are relatively stable throughout the core (Table 3.7). In core 2 (Figure 3.7), C/N ratios range between 12.5 and 19.6. This increased value is from a core that was closer to the edge of the bay, and is likely receiving increased higher plant input. C/N values were not available for the bottom 10 cm of the core, due to the fact that the concentrations of nitrogen were too low for the IRMS to analyze. Areas of the
core where values exceed 15 indicate stronger signals of terrestrial plant material. These areas exist from 10-15 cm depth and 30-40 cm depth. Core 6 (Figure 3.8) showed stable C/N ratios ranging between 11.2 and 13.7.

### 3.4.4 δ¹³C of Bulk Sediments

Terrestrial plants have a signal around -27‰ and phytoplankton has a signal around -25‰. On the other end of the spectrum, pure eelgrass has a signal around -12‰. δ¹³C values (Table 3.8) for organic matter in core 1 range from -17.5‰ to -20‰ with peaks at 20 cm and 25 cm (Figure 3.6).

In core 2 (Figure 3.7), δ¹³C values for organic matter range from -17.9‰ to -20.8‰, with a significant peak from 20 to 30 cm depth. The average value for δ¹³C in core 2 was -19.7‰.

In core 6 (Figure 3.8), δ¹³C values for organic matter range between -18.1‰ and -20.7‰. At 40 cm, there is a decrease to a value of -20‰. The average value for δ¹³C in core 6 was -19.1‰.

### 3.5 Eelgrass Geochemistry

In pure eelgrass, %OC values are around 36% and %N values are around 1.7. Pure eelgrass has a δ¹³C value of -9.5‰ and a C/N ratio of 25. Pure eelgrass is significantly more concentrated in organic carbon and organic nitrogen. In addition, δ¹³C for eelgrass fatty acids are enriched by approximately 10‰ in comparison to the eelgrass bulk isotope analysis.

### 3.6 Lipid Biomarker Distribution

#### 3.6.1 Total Fatty Acids Concentration

In core 6, the total fatty acid concentration is lowest at the bottom of the core and increases gradually upcore (Figure 3.9). The sediment from the top 5 cm of the core contains the highest concentration of C24 with 35.6 ng/g. This section of sediment displays a trend of increasingly concentrated values for even-numbered carbon chain lengths. However, the top 5 cm of sediment are most concentrated at C24 and concentration decreases gradually as the carbon
chain length increases. The top 5 cm also has a greater relative abundance in the even-numbered carbon chain lengths. However, relative abundance increases from C20 to C24 and then it decreases gradually from C24 to C30. Therefore, the top 5 cm shows a peak in concentration and relative abundance in the C24 carbon chain length.

The rest of the core depths parallel these trends for both concentration and relative abundance. In addition, values for relative concentration decrease gradually with depth, with the exception of 21-24 cm, which has lower concentrations for all carbon chain lengths. Values for relative abundance differ slightly with depth, with the exception of 12-15 cm, which has lower relative abundance for all carbon chain lengths (Figure 3.10). Relative percent v. carbon chain length also shows high abundances of cell membrane lipids (smaller carbon chain lengths of C16-C20) which break down quickly until the HPLW lipids are apparent (C22-C24).

Even-over-odd predominance of carbon chain lengths is also clear in the concentrations of leaf wax lipids after samples were run through the GC-FID. (Figure 3.11). As expected concentrations of leaf wax lipids in the modern eelgrass plant tissue were greater than in the sediment. Chromatograms of the eelgrass and sediment samples (Figure 3.12) also show the strong even-over-odd predominance that is expected from leaf wax lipids.

3.6.2 $\delta^{13}$C of Higher Plant Leaf Wax Fatty Acids

$\delta^{13}$C of the C24 carbon chain length ranges between -21.1‰ and -23.6‰ throughout core 6. Sediment from the bottom of the core and from 21-24 cm is the most depleted while sediment from the top of the core is the most enriched. Generally, there are only slight fluctuations in $\delta^{13}$C upcore.

3.6.3 Weighted Mean Isotopic Value of Higher Plant Leaf Waxes

Values ranged from -20.5 to -23.8 throughout core 6. In addition, the eelgrass sample had a weighted mean isotopic value of -19.0. It is clear that the modern tissue has a higher weighted mean than the sediment samples and is thus more enriched in the higher plant leaf wax lipids (Figure 3.13).
Figure 3.1 shows the downcore physical data for core 1, including %H\(_2\)O, Bulk Density and % Silt and Clay.
Figure 3.2 shows the downcore physical data for core 2, including %H$_2$O, Bulk Density and % Silt and Clay.
Figure 3.3 shows the downcore physical data for core 6, including %H₂O, Bulk Density and % Silt and Clay.
Figure 3.4 shows the $^{239+240}\text{Pu}$ dating analysis downcore for core 6.
Figure 3.5 shows the chlorophyll analysis downcore for core 6.
Figure 3.6 shows the downcore bulk isotope geochemical data for core 1, including %OC, %N, δ¹³C, and C/N.
Figure 3.7 shows the downcore bulk isotope geochemical data for core 2, including %OC, %N, δ\(^{13}\)C, and C/N.
Figure 3.8 shows the downcore bulk isotope geochemical data for core 6, including %OC, %N, $\delta^{13}$C, and C/N.
Figure 3.9 shows the concentration of leaf wax lipids in sediment and eelgrass samples for carbon chain lengths from 20-30.
Figure 3.10 shows the relative abundance of HPLW lipids in sediment for carbon chain lengths from 12-30.
Figure 3.11 shows the concentration of HPLW lipids in sediment samples for carbon chain lengths from 24-30.
Figure 3.12 shows a chromatogram of a sediment sample that has been run through the GC-FID with carbon chain length numbers identified.
Figure 3.13 shows the weighted mean average for the C22 and C24 carbon chain lengths.
Table 3.1 shows %H₂O analysis for all cores.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1</th>
<th>Core 2</th>
<th>Core 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>77.30</td>
<td>75.63</td>
<td>82.36</td>
</tr>
<tr>
<td>5</td>
<td>80.68</td>
<td>73.44</td>
<td>78.45</td>
</tr>
<tr>
<td>10</td>
<td>63.00</td>
<td>61.70</td>
<td>74.23</td>
</tr>
<tr>
<td>15</td>
<td>49.67</td>
<td>69.33</td>
<td>47.68</td>
</tr>
<tr>
<td>20</td>
<td>50.24</td>
<td>56.46</td>
<td>40.26</td>
</tr>
<tr>
<td>25</td>
<td>59.63</td>
<td>50.90</td>
<td>47.09</td>
</tr>
<tr>
<td>30</td>
<td>73.28</td>
<td>56.21</td>
<td>45.69</td>
</tr>
<tr>
<td>35</td>
<td>82.02</td>
<td>64.83</td>
<td>37.08</td>
</tr>
<tr>
<td>40</td>
<td>76.18</td>
<td>77.47</td>
<td>33.19</td>
</tr>
<tr>
<td>45</td>
<td>25.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>24.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2 shows dry bulk density analysis for all cores.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1</th>
<th>Core 2</th>
<th>Core 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.22</td>
<td>0.96</td>
<td>0.83</td>
</tr>
<tr>
<td>5</td>
<td>1.07</td>
<td>1.11</td>
<td>0.74</td>
</tr>
<tr>
<td>10</td>
<td>1.06</td>
<td>1.10</td>
<td>1.05</td>
</tr>
<tr>
<td>15</td>
<td>1.31</td>
<td>1.00</td>
<td>1.33</td>
</tr>
<tr>
<td>20</td>
<td>1.03</td>
<td>1.26</td>
<td>1.45</td>
</tr>
<tr>
<td>25</td>
<td>1.06</td>
<td>1.35</td>
<td>1.41</td>
</tr>
<tr>
<td>30</td>
<td>0.91</td>
<td>1.09</td>
<td>1.17</td>
</tr>
<tr>
<td>35</td>
<td>0.93</td>
<td>1.14</td>
<td>1.46</td>
</tr>
<tr>
<td>40</td>
<td>1.05</td>
<td>1.00</td>
<td>1.56</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>1.37</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3 shows % silt and clay analysis for all cores.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1</th>
<th>Core 2</th>
<th>Core 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.09</td>
<td>64.63</td>
<td>59.85</td>
</tr>
<tr>
<td>5</td>
<td>83.20</td>
<td>72.44</td>
<td>51.60</td>
</tr>
<tr>
<td>10</td>
<td>71.17</td>
<td>67.34</td>
<td>63.83</td>
</tr>
<tr>
<td>15</td>
<td>76.66</td>
<td>69.63</td>
<td>53.53</td>
</tr>
<tr>
<td>20</td>
<td>86.78</td>
<td>78.37</td>
<td>47.90</td>
</tr>
<tr>
<td>25</td>
<td>58.76</td>
<td>65.27</td>
<td>53.86</td>
</tr>
<tr>
<td>30</td>
<td>75.24</td>
<td>71.86</td>
<td>66.44</td>
</tr>
<tr>
<td>35</td>
<td>83.14</td>
<td>66.17</td>
<td>69.03</td>
</tr>
<tr>
<td>40</td>
<td>87.57</td>
<td>74.28</td>
<td>54.88</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>30.22</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>31.92</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4 shows $^{239+240}\text{Pu}$ analysis for core 6.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>bq/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>1.5</td>
<td>0.71</td>
</tr>
<tr>
<td>2.5</td>
<td>0.69</td>
</tr>
<tr>
<td>3.5</td>
<td>0.46</td>
</tr>
<tr>
<td>4.5</td>
<td>0.64</td>
</tr>
<tr>
<td>5.5</td>
<td>0.63</td>
</tr>
<tr>
<td>6.5</td>
<td>0.62</td>
</tr>
<tr>
<td>7.5</td>
<td>0.45</td>
</tr>
<tr>
<td>8.5</td>
<td>0.58</td>
</tr>
<tr>
<td>9.5</td>
<td>0.73</td>
</tr>
<tr>
<td>10.5</td>
<td>0.76</td>
</tr>
<tr>
<td>11.5</td>
<td>0.63</td>
</tr>
<tr>
<td>12.5</td>
<td>0.61</td>
</tr>
<tr>
<td>13.5</td>
<td>0.41</td>
</tr>
<tr>
<td>14.5</td>
<td>0.32</td>
</tr>
<tr>
<td>15.5</td>
<td>0.46</td>
</tr>
<tr>
<td>16.5</td>
<td>0.42</td>
</tr>
<tr>
<td>17.5</td>
<td>0.61</td>
</tr>
<tr>
<td>18.5</td>
<td>0.40</td>
</tr>
<tr>
<td>19.5</td>
<td>0.33</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>Core 1</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td>0</td>
<td>1.65</td>
</tr>
<tr>
<td>5</td>
<td>1.85</td>
</tr>
<tr>
<td>10</td>
<td>1.69</td>
</tr>
<tr>
<td>15</td>
<td>1.57</td>
</tr>
<tr>
<td>20</td>
<td>1.13</td>
</tr>
<tr>
<td>25</td>
<td>1.29</td>
</tr>
<tr>
<td>30</td>
<td>1.97</td>
</tr>
<tr>
<td>35</td>
<td>1.63</td>
</tr>
<tr>
<td>40</td>
<td>1.74</td>
</tr>
<tr>
<td>45</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.5 shows % organic carbon analysis for all cores.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1</th>
<th>Core 2</th>
<th>Core 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.14</td>
<td>0.12</td>
<td>0.17</td>
</tr>
<tr>
<td>5</td>
<td>0.17</td>
<td>0.23</td>
<td>0.19</td>
</tr>
<tr>
<td>10</td>
<td>0.16</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>15</td>
<td>0.13</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>20</td>
<td>0.10</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>25</td>
<td>0.11</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>30</td>
<td>0.18</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>35</td>
<td>0.14</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>40</td>
<td>0.15</td>
<td>0.11</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3.6 shows % organic nitrogen analysis for all cores.
### Table 3.7 shows C/N analysis for all cores.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1</th>
<th>Core 2</th>
<th>Core 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.45</td>
<td>12.54</td>
<td>11.83</td>
</tr>
<tr>
<td>5</td>
<td>12.64</td>
<td>14.51</td>
<td>11.74</td>
</tr>
<tr>
<td>10</td>
<td>12.63</td>
<td>15.11</td>
<td>12.07</td>
</tr>
<tr>
<td>15</td>
<td>14.01</td>
<td>18.08</td>
<td>13.53</td>
</tr>
<tr>
<td>20</td>
<td>13.43</td>
<td>13.24</td>
<td>12.82</td>
</tr>
<tr>
<td>25</td>
<td>14.03</td>
<td>13.54</td>
<td>13.44</td>
</tr>
<tr>
<td>30</td>
<td>13.04</td>
<td>15.98</td>
<td>11.23</td>
</tr>
<tr>
<td>35</td>
<td>13.91</td>
<td>16.53</td>
<td>13.77</td>
</tr>
<tr>
<td>40</td>
<td>13.21</td>
<td>19.63</td>
<td>12.72</td>
</tr>
</tbody>
</table>

### Table 3.8 shows δ¹³C analysis for all cores.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1</th>
<th>Core 2</th>
<th>Core 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-19.49</td>
<td>-18.74</td>
<td>-18.53</td>
</tr>
<tr>
<td>5</td>
<td>-18.48</td>
<td>-18.42</td>
<td>-18.15</td>
</tr>
<tr>
<td>10</td>
<td>-19.23</td>
<td>-20.01</td>
<td>-18.67</td>
</tr>
<tr>
<td>15</td>
<td>-20.07</td>
<td>-20.67</td>
<td>-18.55</td>
</tr>
<tr>
<td>20</td>
<td>-19.04</td>
<td>-17.89</td>
<td>-18.76</td>
</tr>
<tr>
<td>25</td>
<td>-17.58</td>
<td>-18.28</td>
<td>-18.82</td>
</tr>
<tr>
<td>30</td>
<td>-18.27</td>
<td>-20.41</td>
<td>-19.59</td>
</tr>
<tr>
<td>35</td>
<td>-19.04</td>
<td>-20.42</td>
<td>-20.69</td>
</tr>
<tr>
<td>40</td>
<td>-19.49</td>
<td>-20.26</td>
<td>-20.11</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>-20.65</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>-20.81</td>
<td></td>
</tr>
</tbody>
</table>
Discussion
4.1 Stratigraphy

A map of Maquoit Bay shows the stratigraphies of the cores that were taken, as well as their relative depths (Figure 4.1). One interpretation is that the shell fragment layers from core 6 are correlated to the shell hash layer in core 2, which was completely compressed before it reached core 1 (Figure 4.1). However, another more realistic interpretation is that the shell layers are all localized and cannot be correlated from one site to another. Since there are coarser grains in shallower waters in Maquoit Bay, the shallower waters are likely experiencing higher levels of energy, forced by semi-diurnal tidal fluctuations at the site.

4.2 Chronology and Evidence of Mixing

4.2.1 Plutonium Analysis

The plutonium analysis from the top 20 cm of core 6 from Maquoit Bay ranged between 0.32 and 0.76 bq/kg. There was no peak in $^{239+240}$Pu in the core that could be attributed to a rise in atmospheric deposition of $^{239+240}$Pu between 1952-1963, and a decrease to 0 post-1963. The $^{239+240}$Pu profile suggests that the surface sediment in Maquoit Bay is well mixed, likely due to bioturbation, or has been deposited since 1963 (Ketterer et al., 2004). It is suspected that a combination of these processes is in play in these sediments, where bioturbation is most important in the upper 10 cm.

The minimum rate of deposition (lower limit) therefore, would be 20 cm in 60 years, which is .33 cm/year sedimentation rate. However, it is conceivable that deposition rates in estuarine systems such as Maquoit Bay are even faster than 0.33 cm/year as found in some mudflats in France, where sedimentation rates of 15-18 cm/year have been found (Deloffre, J., et al., 2007). Because better control on sedimentation rates does not currently exist, the data from the sediment cores will continue to be presented in terms of depth.

4.2.2 Chlorophyll

Due to the fact that samples were analyzed for chlorophyll using a method modified from
Holm-Hansen et al. (1965), it is not prudent to compare absolute chlorophyll concentration to those published in Tietjen’s study of New England estuaries (1965). However, downcore trends and Fo:Fa ratios are useful in thinking about chlorophyll diagenesis and decomposition.

Chlorophyll is a pigment that is generally not stable through time or in oxic settings. The presence of chlorophyll in sediments represents a combination of input (via primary productivity) and decomposition. It can be transported to the site of deposition or it can be formed \textit{in situ}. The Fo:Fa ratios are useful in evaluating the amount of chlorophyll decomposition that has occurred in the sediments. In Maquoit Bay, a large portion of plant detritus from the roots and rhizomes of eelgrass, along with some phytoplankton, are deposited in the sediment, upon which bacteria and other organisms feed.

According to Lorenzen (1965), Fo:Fa ratios of 1.35 or less indicate that 50% or more of the chlorophyll is in a degraded form. All Fo:Fa ratios taken from sediment in Maquoit Bay range between 1.04 and 1.13, suggesting that a major fraction of the chlorophyll from Maquoit Bay sediment is in a degraded form (in agreement with Tietjen, 1965). The Fo:Fa value for eelgrass is 1.7 and is similar to values for living plants found in several estuaries in New England (Tietjen, 1965).

Chlorophyll concentrations peak at the surface of the sediment and again at 28 cm depth. Since chlorophyll should degrade with time, the peak in chlorophyll might be indicative of a period of rapid deposition where organic matter was accumulating at a greater rate than it was breaking down. Alternatively, it may represent a peak in chlorophyll production at the site.

\section*{4.3 Early Diagenesis}

\subsection*{4.3.1 \% Organic Carbon}

Organic matter within a sediment core is dependent on the balance between organic inputs and outputs. Organic carbon inputs include deposition of plants, detritus, bacteria, and phytoplankton that may have been produced \textit{in situ} or may have been transferred to the site in varying amounts. Sedimentary organic carbon can be removed via decomposition by bacteria.
within the sediments. Since aerobic bacteria require oxygen to decompose material, degradation of organic matter is rapid in the oxic zone (Meyers, 1997). This aerobic sediment layer (oxic zone) is only a few millimeters thick over much of Maquoit Bay (Heinig and Campbell, 1992). In general, higher rates of decomposition occur at the surface of the oxic sediment where aerobic bacteria thrive, while lower rates of decomposition occur further downcore as conditions become anoxic and anaerobic bacteria exist.

For steady state organic inputs and diagenesis, the %OC concentration is expected to decrease exponentially with depth (Berner, 1980). All of the cores analyzed from Maquoit Bay show trends of higher values for %OC in the surface and decreasing values with depth. In some cases (core 6), the decrease in %OC appears exponential, suggesting steady state inputs and decomposition. In other cases (core 2), the %OC in the surface sediment remains high and might represent an active organic matter accumulation zone, or an area where the accumulation rate of organic carbon is equal to or greater than the decomposition rate of organic carbon. The increase in %OC within the surface sediment also indicates thorough bioturbation of the top 5 cm of sediment.

Below the top 5 cm of the sediment cores, organic carbon concentrations decrease, implying that decomposition becomes more significant. In other cases, a change in %OC correlates with stratigraphy. Aerobic bacteria dominate the top few mm of sediment and gradually decrease downcore until the environment becomes anoxic, anaerobic bacteria replace aerobic bacteria, and stable/consistent %OC values are present. However, these stable %OC values are not present at the bottom of core 2, where there is a sharp decrease in %OC at the bottom 7 cm. This is likely due to the distinct shift in stratigraphy downcore where soft mud transitions to dense shell hash and a higher energy environment produces increased deposition of organic matter.

4.3.2 % Organic Nitrogen

The percent organic nitrogen throughout core 6 parallels the trends seen in %OC since they are both subject to the same diagenetic processes. %N values are higher at the surface and decrease with depth. Because the major sources of nitrogen in organic matter are amino acids and
proteins, it is not surprising that %OC and %N parallel one another. Values for %N are smaller than %OC because phytoplankton provides more organic carbon to the system than organic nitrogen. The average atomic ratio of C to N to P in marine phytoplankton is 106 to 16 to 1 (Libes, 1992). This ratio exhibits the relatively low concentrations of nitrogen relative to carbon in seawater.

4.4 Eelgrass Data

4.4.1 Bulk Isotope Distribution in Eelgrass

Bulk isotope data of pure eelgrass varies greatly in comparison to the average downcore bulk isotope data for sediment in Maquoit Bay. Comparing pure eelgrass and sediment bulk isotope data (%OC, %N, $\delta^{13}C$ and C/N) explains the connections between the sediment and an input of organic matter (Table 4.1). The geochemical values of pure eelgrass are visible in the geochemistry of the bulk sediment, as one of four sources.

Fatty acid $\delta^{13}C$ values are approximately 9.5‰ depleted compared to bulk isotope values in eelgrass (Table 4.1). A range of 8‰ to 12‰ depletion in $\delta^{13}C$ relative to bulk tissue data was exhibited in lipids from tree leaves in the UK (Lockheart et al., 1996) and a range of 3‰ to 5‰ depletion in $\delta^{13}C$ relative to bulk tissue data was exhibited in eelgrass lipids (Canuel, et al., 1997). This is expected since precursor compounds are isotopically depleted (DeNiro and Epstein, 1977). Therefore, bulk isotope data for the modern tissue and sediment will have more enriched values than the lipid data, due to biochemical fractionation during lipid synthesis (DeNiro and Epstein, 1977; Lockheart, et al., 1996; Makou et al., 2007). These published values parallel the depleted values from fatty acids in comparison to bulk tissue from eelgrass samples in Maquoit Bay.

4.4.2 Fatty Acid Methyl Ester Lipid distribution in eelgrass

The concentration of fatty acids in eelgrass is different from the concentration typically found in higher plants. This distribution is different from other C3 terrestrial plants, which are dominated by the C26 chain length and contain substantial concentrations of carbon chain lengths through C32 (Eglinton and Hamilton, 1963). Additional analyses of eelgrass are
necessary to determine the expected variability in HPLW composition. The fatty acid methyl ester lipid composition of the eelgrass sample had an average value of -19.0‰ ± .4‰ for the C22 and C24 chain lengths. In addition, the weighted mean isotopic value of higher plant leaf waxes is -19.0‰ for eelgrass. Other studies looking at eelgrass biomarkers have analyzed long chain n-alkanes, rather than fatty acids (Xu and Jaffé, 2007). Since long chain n-alkanes are characteristic of having an odd-over-even carbon chain length predominance, the concentrations and carbon chain lengths from long chain n-alkanes cannot be compared to the concentrations and carbon chain lengths from fatty acids. In addition, other biomarkers for seagrasses have been discovered (Xu and Jaffé, 2007) but are beyond the scope of this thesis.

4.5 Organic Matter Characterization Downcore

4.5.1 C/N

C/N ratios can provide a rough estimate of the amount of organic carbon derived from phytoplankton and higher plants contributing to the system. C/N values consistently lie between 10 and 15 in the cores from Maquoit Bay indicating a mix of higher plants and phytoplankton in the sediment. Assuming higher plants have C/N values greater than 15 and phytoplankton has C/N values from 7-10 (Meyers, 1999), the Using a two-end-member mixing model, with phytoplankton on one end and higher plants on the other, it can be determined that C/N ratios range between 11 and 14 in core 6 indicate that approximately 20% of the organic carbon in the entire system at Maquoit Bay is derived from phytoplankton and 80% is derived from higher plants. Care should be taken when interpreting C/N data due to the fact that microbial reworking of organic matter during early diagenesis can potentially modify the C/N ratios of organic matter in sediments (Meyers, 1999). In addition, C/N ratios alone cannot account for bacteria inputs and cannot differentiate between two types of higher plant input (eelgrass and C3 plants). Additionally, there is little accuracy in C/N ratios because higher plant/terrestrial source matter is defined as having a wide range of values (Tyson, 1995), including any ratio greater than 12 (Thorton and McManus, 1994).
4.5.2 δ\textsuperscript{13}C of Bulk Sediments

The δ\textsuperscript{13}C values of the bulk stable isotope data are also only helpful in determining the sources of organic matter to a system, provided that there are two sources of organic matter to an area, each with isotopically distinct values. For example, terrestrial C\textnum{3} plants (i.e., those in the Maquoit Bay watershed) have an average δ\textsuperscript{13}C value of approximately -27‰ and pure eelgrass has a signal of approximately -12‰. The presence of phytoplankton (average δ\textsuperscript{13}C= -25‰) complicates the interpretation of the bulk sediment (Bird, 2005; Kennedy, 2010). The δ\textsuperscript{13}C values for the sediment range between -17‰ and -20‰ in all 3 cores, suggesting that all three sources of organic matter are present in the sediment in varying proportions.

4.6 Organic Matter Characterization

4.6.1 Changes in Compound Specific Data Downcore

Substantial concentrations of chain lengths lower than C\textnum{22} were present in all samples, but may be attributed to the cell-membrane lipid fraction rather than the higher plant leaf wax lipid fraction. Cell membrane lipids are generated by all living organisms and as a result, are not useful biomarkers for higher plants. Furthermore, they are typically more labile and break down easily, and are therefore disregarded in this study as a means of characterizing organic carbon (Eglington and Hamilton, 1967). This trend is visible when comparing carbon chain length to relative percent (Figure 3.12). Thus, the average δ\textsuperscript{13}C from C\textnum{22} and C\textnum{24} chain lengths were calculated and plotted downcore (Figure 4.2).

FAME values of the averaged C\textnum{22} and C\textnum{24} chain lengths range from -21‰ to -25‰, with an average value of -23‰ for core 6 (Figure 4.2). These values show greatest enrichment at the surface and again at 32 cm depth.

Concentrations and relative abundances of individual carbon chain lengths consistently decrease downcore (Figure 3.9), but because higher plant leaf waxes are diagenetically stable (Jaffé, et al., 2006; Meyers, 2007), these gradual decreases in lipid concentration likely represent periods of decreased deposition. Alternatively, it is possible that these compounds are not as
stable as previously thought.

Carbon chain lengths greater than C24 were not present in the eelgrass sample. However, increased concentrations of carbon chain lengths up to C30 were present in the sediment, indicating different sources of HPLW (Figure 4.3). Therefore it can be hypothesized that HPLW concentrations in C22 and C24 are derived from both eelgrass and other C3 plants while HPLW concentrations in C26-C30 are derived from other C3 plants alone.

However, C26 and C28 are slightly enriched in this plot as well. It is possible that the sample of eelgrass analyzed in this study is not completely representative of eelgrass in general and that on average, eelgrass does have substantial concentrations of C26-C30 HPLW. In addition, Figure 4.3 may indicate another source of δ\(^{13}\)C enriched HPLW from the salt marsh just north of Maquoit Bay.

The average δ\(^{13}\)C values for the length of core 6 vary by carbon chain length. The C24 carbon chain length has the most enriched average δ\(^{13}\)C value for the core (-22.7‰ ± .4), while the C30 carbon chain length has the most depleted average δ\(^{13}\)C value for the core (-29.1‰ ± 1.0) (Table 4.2).

A plot δ\(^{13}\)C for different carbon chain lengths from C22 to C30 downcore shows that C22 through C26 generally follow the same general trend downcore (Figure 4.3). The C26 chain length does not follow the trend perfectly, while the C30 chain length is quite depleted relative to the smaller chain lengths. This supports that chain lengths from C22 to C24 include the HPLW lipids derived from eelgrass, while chain lengths greater than C24 represent the HPLW lipids from C3 \(^{13}\)C-depleted terrestrial sources. Additional studies are required to determine the concentrations of other HPLW that are present in eelgrass.

4.6.2 Weighted Mean Isotopic Value of Higher Plant Leaf Waxes

The weighted mean isotopic value of HPLW’s takes into consideration the concentration and isotopic composition of several higher plant leaf waxes rather than just one (Figure 3.13; Makou et al., 2007). The equation for calculating the weighted mean isotopic value of higher plant leaf waxes is as follows (where [ ] = concentration):
\[ \frac{(\delta^{13}C_{C22} \times [C22] + \delta^{13}C_{C24} \times [C24])}{([C22] + [C24])} \]

The C22 and C24 carbon chain lengths were used in this equation because they are present in both C3 plants and eelgrass. Values are significantly more enriched at the surface of the core than the following 5 cm interval. From 13 cm to 32 cm depth, the sediment becomes gradually more enriched in HPLW lipids followed by a decrease at the bottom of the core. Throughout, values have a small range of 3‰. In addition, the eelgrass sample exhibits the most enriched value, indicating that the modern plant tissue is more enriched in HPLW lipids than the sediment below. These shifts in isotopic values throughout the core might be indicative of changes in eelgrass growth and organic matter deposition of higher plants.

### 4.6.3 Organic Carbon Cycling at Maquoit Bay- Terrestrial, Bacterial and Phytoplankton Contributions

A normalized plot of terrestrial plant fatty acids, phytoplankton fatty acids and bacterial fatty acids was constructed using a method described by Gong and Hollander (1997). In this plot, terrestrial plant fatty acids include carbon chain lengths greater than 21; phytoplankton fatty acids include carbon chain lengths that are even-numbered from 12 to 20; and bacterial fatty acids include carbon chain lengths that are odd-numbered from 13 to 19 (Figure 4.4).

The bacterial biomarkers generally have the smallest contribution throughout the core and represent a consistent percentage of the organic matter inputs in Maquoit Bay. Bacterial fatty acids lie around 14% for the entire core, ranging between 11% and 16%. Such stable values might suggest the presence of bacterial processes with depth. Active bacteria have been found at depths over 50 cm (Fisher et al., 2003), however, the depth of the active bacterial zone is perhaps a topic for future study (Fisher et al., 2003).

Terrestrial and phytoplankton biomarkers have similar percent contributions to the system downcore. Terrestrial biomarkers range from 41% to 46% while phytoplankton biomarkers range from 38% to 43%. This analysis gives us more accurate information than C/N ratios, but requires far more intensive methods. The sediment sampled at the top and at the bottom of the core contains equal percentages of both terrestrial and phytoplankton biomarkers.
Throughout the middle of the core, there is a slightly stronger signal of terrestrial biomarkers than phytoplankton biomarkers (6% more terrestrial on average). Since terrestrial biomarkers generally contribute the most fatty acids to the entire sediment core (45% on average), there is a clear and stable presence of HPLW fatty acids downcore. This indicates that there has been higher plant contribution to the mudflat at Maquoit Bay for an extended period of time, which is represented by the entire length of the core.

4.6.4 Two-End-Member Mixing Modeling

By targeting higher plant leaf waxes, the individual amounts of organic carbon from allochthonous terrestrial input (C3 plants) and autochthonous terrestrial input (eelgrass) can be determined using a 2-end-member mixing model. However, it is important to note that this comprehensive model of organic carbon assumes that there are no salt marsh plants within the system at Maquoit Bay.

Using HPLW fatty acid data from the C22 and C24 chain lengths and the following mixing model equation,

\[
(\delta^{13}C_{\text{eelgrass}_{\text{C22-C24}}})(X) + (\delta^{13}C_{\text{C3}_{\text{C22-C24}}})(1-X) = \delta^{13}C_{\text{sediment}_{\text{C22-C24}}}
\]

\[
(-19.0)(X) + (-36)(1-X) = -23.5
\]

where \(X\) = the fraction of carbon from eelgrass and

\((X-1)\) = the fraction of carbon from C3 plants.

the fraction of organic carbon derived from eelgrass can be determined. Typical C3 plants have \(\delta^{13}C\) values ranging between -38‰ and -34‰ (Reddy, et al., 2000). A value of -19.0‰ was used for the \(\delta^{13}C_{\text{eelgrass}_{\text{C22-C24}}}\) and an average value of -36‰ was chosen for this mixing model, based on published values (Reddy, et al., 2000). It was determined that 73.5% of the higher plant input is derived from autochthonous eelgrass. Therefore, the remaining 26.5% of the higher plant input is derived from allochthonous C3 plants. Thus, the percent of organic carbon derived from eelgrass, has an uncertainty of 15%. This value represents the error associated with the calculation used to determine the final percentages of organic carbon derived from each individual source (Appendix A).
The percentage of higher plant input from eelgrass was plotted against depth for core 6 (Figure 4.2) and varies between 50-75% through time with peaks at the surface and at 35 cm depth. Variations are likely due to eelgrass productivity relative to other source contributions.

### 4.6.5 Organic Carbon Characterization

The results from the mixing model are then normalized to the total organic carbon inputs from phytoplankton, bacteria, eelgrass and C3 plants (Figure 4.4). According to the lipid biomarker and HPLW isotope data, phytoplankton accounts for ~41% and bacteria accounts for ~14% of the organic carbon in the sediment. The remaining ~45% is then derived from C3 plants and eelgrass.

In the previous mixing model, ~74% of the higher plant input is derived from eelgrass, which is ~33% of the total organic carbon signal. Therefore, the remaining ~27% of the higher plant input is derived from C3 plants, which is ~12% of the entire organic carbon signal. The percent of organic carbon derived directly from eelgrass ranges from ~29-38% and is relatively stable with depth (Figure 4.2).

To summarize, phytoplankton accounts for ~41% of the organic carbon, eelgrass accounts for ~33% of the organic carbon, bacteria accounts for ~14% of the organic carbon and C3 plants account for the final ~12% of the organic carbon in the system at Maquoit Bay. These values were relatively consistent throughout the core, indicating that processes of organic carbon deposition and decomposition have remained relatively stable throughout the time period studied. Finally, the complete organic carbon signal from the sediment in Maquoit Bay has been successfully characterized.

### 4.6.6 Implications

Another study of seagrasses found that 50% of the organic carbon in the surface sediment in seagrass beds was derived from seagrass (Kennedy et al., 2010). A 3-end-member-mixing model was used to compare two isotopic ratios. However, Kennedy does not account for microbial processes at the surface of the sediment in the study. The use of two separate models allows for 4 source contributions to be accounted for, thereby increasing the accuracy of the data.
Figure 4.1 shows a map of Maquoit Bay shows the stratigraphies of the cores that were taken, as well as their relative depths.
Figure 4.2 plots the δ¹³C FAMEs show the average of C22 and C24 chain lengths downcore for core 6, Weighted Mean Isotopic value of HPLW of chain lengths from C22-C24, % of higher plant carbon derived from eelgrass according to the 2-end-member mixing model and the % organic carbon derived from eelgrass according to the 2-end-member mixing model used in conjunction with the source contribution plot.
Figure 4.3 shows a plot $\delta^{13}C$ for different carbon chain lengths from C22 to C30 downcore.
Figure 4.4 is a normalized plot of terrestrial plant fatty acid, phytoplankton fatty acid and bacterial fatty acid contributions in Maquoit Bay. Terrestrial plant fatty acids include carbon chain lengths greater than 21; phytoplankton fatty acids include carbon chain lengths that are even-numbered from 12 to 20; and bacterial fatty acids include carbon chain lengths that are odd-numbered from 13 to 19.
### Table 4.1

<table>
<thead>
<tr>
<th>Eelgrass</th>
<th>Bulk isotopes from sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>%OC</td>
<td>36.1</td>
</tr>
<tr>
<td>%N</td>
<td>1.7</td>
</tr>
<tr>
<td>$\delta ^{13}$C</td>
<td>-9.7‰</td>
</tr>
<tr>
<td>$\delta ^{15}$N</td>
<td>6.1‰</td>
</tr>
<tr>
<td>C/N</td>
<td>24.9</td>
</tr>
</tbody>
</table>

Table 4.1 compares the bulk isotope data from the EA-IRMS for a pure eelgrass sample and the average values from the sediment core in Maquoit Bay.

### Table 4.2

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>$\delta ^{13}$C Core Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>C22 avg</td>
<td>-24.2‰</td>
</tr>
<tr>
<td>C22 std</td>
<td>1.1</td>
</tr>
<tr>
<td>C24 avg</td>
<td>-22.7‰</td>
</tr>
<tr>
<td>C24 std</td>
<td>0.4</td>
</tr>
<tr>
<td>C26 avg</td>
<td>-23.1‰</td>
</tr>
<tr>
<td>C26 std</td>
<td>0.6</td>
</tr>
<tr>
<td>C28 avg</td>
<td>-24.9‰</td>
</tr>
<tr>
<td>C28 std</td>
<td>0.9</td>
</tr>
<tr>
<td>C30 avg</td>
<td>-29.1‰</td>
</tr>
<tr>
<td>C30 std</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 4.2- The average $\delta ^{13}$C values with standard deviations for the length of core 6 by carbon chain length.
Conclusion
In this study, the organic carbon from sediment cores taken from an eelgrass bed was characterized by targeting Higher Plant Leaf Wax fatty acid biomarkers. The eelgrass bed in Maquoit Bay exhibits a complex system of deposition and decomposition. The sources of organic matter deposition and diagenesis include phytoplankton, autochthonous eelgrass, bacteria and other allochthonous C3 plants. The degree to which this organic matter is produced and deposited depends on tidal fluctuations, river inputs, wind and runoff. A 2-end-member mixing model was used in conjunction with a source contribution plot to characterize the organic carbon recovered from sediment cores.

Once the organic carbon was completely characterized according to 4 separate sources, it was determined that less of the organic carbon was derived from eelgrass than was originally expected, based on a previous study (Kennedy et al., 2010). In general, of the total organic carbon within the sediment at Maquoit Bay, 33% is derived from eelgrass.

Further studies might target the n-alkane lipid biomarkers, for which there is a more accurate analysis of organic matter characterization that has been applied successfully in several studies (Hernandez, et al., 2001; Jaffé, et al., 2006; Xu, et al., 2007). Once a set of methods that can detect eelgrass organic matter through time is established, the methods can then be utilized in other areas, such as the Gulf of Maine. With the data compiled from this thesis, there is potential to characterize organic matter in sediment cores from Turner Farms in Penobscot Bay. Organic matter in fish bones from Turner Farms has been analyzed to determine baseline conditions in primary production and food web interactions in the system prior to and through colonization by western Europeans. Using the methods laid out in this thesis, the characterization of organic matter from a sediment core from Turner Farms could act as a supplement to current data and as a historical record of eelgrass recovery through time.

An eelgrass profile through time will be helpful in advocating for the preservation of this primary producer. According to the results of this study, eelgrass can act as a significant carbon sink for the local ecosystem, and actions should be made to preserve it.

The purpose is to archive eelgrass recovery through time by characterizing the organic
matter present in eelgrass beds to determine the amount of organic carbon derived directly from eelgrass. It is necessary to study these important marine habitats and protect them from further human-induced decline. Since European settlement, these habitats have been in a state of decline that may be associated with anthropogenic activities.
References


Ouellette, N., 2003, Lipid biomarkers and paleovegetation determinations for the last 30,000 years at Elikchan Lake, Northeast Siberia. Bates College Department of Geology, Lewiston, Maine.


Rodgers, K., 2005, Stable carbon isotope analysis of lake core sediments and lipid biomarkers as a proxy for Late Pleistocene carbon cycling at Elikchan Lake, NE Siberia. Bates College Department of Geology, Lewiston, Maine.


Mixing Model:

Best:

\[(\delta^{13}C \text{ eelgrass})(X) + (\delta^{13}C \text{ C3 plants})(1-X) = \delta^{13}C \text{ sediment}\]

\[-19.0(X) + (-36)(1-X) = -23.5\]

\[X = .735 = 73.5\%\]

Max:

\[(\delta^{13}C \text{ eelgrass})(X) + (\delta^{13}C \text{ C3 plants})(1-X) = \delta^{13}C \text{ sediment}\]

\[-19.0(X) + (-38)(1-X) = -21.8\]

\[X = .853 = 85.3\%\]

Min:

\[(\delta^{13}C \text{ eelgrass})(X) + (\delta^{13}C \text{ C3 plants})(1-X) = \delta^{13}C \text{ sediment}\]

\[-19.0(X) + (-34)(1-X) = -25.6\]

\[X = .560 = 56.0\%\]

Best Value +/- Uncertainty: 73.5% + 14.7%