Methane emissions along a salinity gradient of a restored salt marsh in Casco Bay, Maine

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Methane emissions along a salinity gradient of a restored salt marsh in Casco Bay, Maine

An Honors Thesis

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

Cailene M. Gunn

Lewiston, Maine
March 28, 2016
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Abstract

This study functions as a pilot project to understand the relationship between salinity and methane emissions on a recently restored salt marsh in Casco Bay, Maine. Long Marsh is a 1.5 mile-long, narrow tidal salt marsh located in Harpswell, Maine that has been tidally restricted by an undersized culvert for over 100 years. Recent restoration efforts on Long Marsh replaced this culvert with a larger one in February, 2014. The salinity gradient has since been restored along much of the marsh, and freshwater vegetation that encroached on the marsh platform has died back. Vegetation and salinity are key indicators and drivers of CH$_4$ emissions on salt marshes. Using static gas chambers, we quantified CH$_4$ fluxes along a salinity gradient at three sites ranging from healthy marsh (salinity of 27 to 31 psu) with Spartina vegetation, to regions invaded by freshwater and Typha vegetation (salinity of 0 to 4 psu). Two transitional sites affected by vegetation changes were also measured. Sampling was executed in the months of July, August and October. CH$_4$ concentrations were determined using a gas chromatograph with a flame-ionization detector. CH$_4$ flux data were determined, and complimented by δ$^{13}$C and % organic carbon values in 50 cm sediment cores at each site.

Lowest CH$_4$ fluxes with least variability were observed at the most saline sites with Spartina vegetation (range of -3.3 to 12.8 μmol CH$_4$/m$^2$ hr). The highest and widest range of CH$_4$ emissions ranged from -0.72 μmol to CH$_4$/m$^2$ hr to 256.3 μmol CH$_4$/m$^2$ hr at the freshest, Typha dominated sites from July to October. The transitional sites exhibited significant variability ranging from -4.6 μmol CH$_4$/m$^2$ hr to 16.9 μmol CH$_4$/m$^2$ hr. For all sites, lowest fluxes were observed in October and highest fluxes in July, suggesting seasonal influence on CH$_4$ emissions. CH$_4$ flux data suggest the reintroduction of healthy tides inhibits methane production and emission. Sediments from transitional sites showed a δ$^{13}$C shift from C4 signal (Spartina; -15 ‰) to C3 signal (Typha; -25 ‰) in the sediment record, which identifies the encroachment of Typha onto the marsh platform due to tidal restriction. Based on average fluxes at freshwater sites for all sampling periods (61.8 μmol CH$_4$/m$^2$ hr) and a calculated 3.11 ha decrease (92%) in Typha area in the first 15 months of restoration, we project a decrease from 75000 gCH$_4$ to 5700 gCH$_4$ ± 3000 gCH$_4$ emitted (per 3 months) since restoration.
1
INTRODUCTION
1.1 Background and Importance of Tidal Salt Marshes

1.1.1 Tidal Salt Marshes

Coastal wetlands, including tidal salt marshes, sea grass beds, and mangrove swamps, exist at the interface between terrestrial and marine systems. They are inundated by tides daily and are extremely dynamic and productive, with their ecological productivity exceeding those of coral reefs and matching those of tropical rainforests (Scott et al., 2014). Of these coastal wetlands, tidal salt marshes are of particular interest due to their widespread distribution and the multitude of ecosystem services they provide.

Overall, it is estimated that tidal salt marshes cover a total area of ~45000 km\(^2\) on earth (Greenberg et al., 2006), about half of which is located in Canada and the United States (Mendelssohn and McKee, 2000). These systems form on low-energy shorelines, predominantly occupying temperate latitudes of 45°N and 45°S, in low topographic areas (Scott et al., 2001). A marsh’s salinity gradient typically ranges from 25-30 psu (practical salinity units) in the healthy marsh regions dominated by highly adapted brackish vegetation, 10-25 psu in the brackish areas leading inland, and 0-10 psu in the marginal and most inland regions (Mitch and Gosselink, 2000).

1.1.2 Vegetation and Zonation

Salt marsh vegetation communities are broadly zoned based on a combination of factors including elevation, hydro-period, soil \(O_2\) availability, and salinity tolerances of individual species (Niering and Warren, 1980; Rabenhorst, 2001). Salinity regimes vary with tidal and groundwater influences, as well as precipitation, runoff, and evapotranspiration (Burdick et al., 1996; Knott et al., 1987; Niering and Warren, 1980). This zonation subdivides the marsh platform into the low marsh areas that are inundated most frequently by tides, and the high marsh areas that are least influenced by the tides (see Figure 1.1). The transition from low marsh to high marsh generally occurs at elevations within ±3 cm of mean high water (Adams, 1963; Niering and Warren, 1980). Low marsh is dominated by tall-form \(S.\ alterniflora\) (\(S.\ alterniflora\)). The high marsh comprises a complex vegetation mosaic predominantly populated by short form \(S.\ alterniflora\), \(S.\ patens\), \(Salicornia europaea\) and \(Distichlis spicata\); \(Distichlis spicata\), \(Juncus gerardii\), among others occupy the higher high marsh (Mitsch and Gosselink, 2000). Freshwater from runoff and groundwater infiltrate the margins and most elevated regions of the marsh, providing salinity conditions (0-8 psu) suitable for stands of freshwater plant species including \(Typha latifolia\), \(Typha angustofolia\), and \(Scirpoides holoschoenus\).

1.1.3 Ecosystem Services

Ecosystem services are defined as the benefits humans derive from ecological systems generated directly from the natural processes and organisms that sustain them (Gedan, 2011). Salt marshes have an abundance of such services. Marshes create sea barriers to prevent shoreline erosion, attenuate waves,
and limit the effects of storm surges and flooding events (King and Lester 1995, Moeller et al. 1996). They also serve as natural filtration systems and nitrogen sinks that filter runoff, decreasing nitrogen input to estuaries (Valiela and Teal, 1979). They provide essential refuge habitat for fish, food for migratory waterfowl, and are the base of complex food webs involving both terrestrial and marine species (Boesch and Turner, 1984). Salt marshes have commercial value for humans as sources of fuel, food, building materials, and shellfishing grounds. Most importantly, salt marshes are extremely effective at sequestering carbon, often in the context of drawing down anthropogenically-influenced atmospheric CO₂.

According to several studies (Bridgham et al., 2006; Choi et al., 2004 and others), estuarine wetlands sequester carbon at a rate about 10-fold higher per unit area than any other wetland ecosystem due to their high sedimentation rates, high soil carbon content, low dissolved oxygen content, and consistent burial rates (Choi et al., 2004). With proper tidal exposure, salt marshes provide the ideal conditions for this carbon sequestration; thus, coastal wetlands could be more valuable carbon sinks than other ecosystems in a warming world (Choi et al., 2004). However, when natural processes are disturbed, the net balance in wetlands can shift from carbon sequestration to emission, in the form of methane (CH₄).

Taking into account its greenhouse effect and rate of drawdown, methane has 25 times the global warming potential of carbon dioxide over a time period of 100 years (Forster et al., 2007), which means small increases in atmospheric concentrations of this gas can have serious climatic implications. An estimated 54-72% of global CH₄ fluxes are from anthropogenic sources including livestock, burning of biomass, and landfills (Bridgham et al., 2013). Freshwater wetlands are the single largest natural source, so there is an increasing concern about potential feedbacks between global climate perturbations and CH₄ emissions from wetlands. Empirical evidence from modeling based on historical datasets by Bridgham et al. (2013) suggest that CH₄ emissions from wetlands are actively and significantly responding to current interannual climate variability, which infers that they will contribute large feedbacks to continued climate change. Subsequently, maintaining the health of marshes and restoring those that have been anthropogenically perturbed is becoming increasingly important in the search for climate change solutions.

Figure 1.1: Vegetation bisect of marsh zones showing type and expected δ¹³C values of major vegetation typical of New England salt marshes. Key to symbols: Sa = Spartina alterniflora; Sp = Spartina patens; Ds = Distichlis spicata; Jg = Juncus gerardii; Ta = Typha angustifolia; Tl = Typha latifolia. Modified from Niering and Warren (1980) and Chmura and Aharon (1995)
1.2 Carbon Biogeochemistry in Salt Marshes

1.2.1 Carbon Sequestration

Blue Carbon

Atmospheric CO$_2$ is assimilated by leaves and shunted, as carbohydrates, to the roots and rhizomes of vegetation during photosynthesis. Much of this organic carbon is buried and stored as belowground organic matter. Because marshes are saturated much of the time, the anaerobic subsurface is conducive to carbon preservation (Stams et al., 2005). The carbon sequestered can be extensive and remain trapped for very long periods of time (centuries to millennia) resulting in substantial carbon stocks (Duarte et al. 2005). This carbon that is sequestered and stored by salt marshes, along with mangroves and seagrass beds, is collectively termed coastal “blue carbon” (Nelleman et al., 2009). Blue carbon describes carbon stored in below and above-ground living biomass, in non-living biomass, and most importantly in the soil. This long-term storage is a function of the anaerobic, saturated state of these systems, allowing for continuous high rates of vertical accretion.

Vertical Accretion

Vertical accretion describes the process by which sediments are deposited over the top of a floodplain during flooding events, resulting in horizontally bedded sediment layers with variable lateral continuity (Hatton, 1983). In a vertically accreting marsh, the sediments become sinks of allochthonous as well as autochthonous organic matter (Schlesinger and Lichter, 2001). In aerobic environments, vertical accretion occurs at slower rates because the high availability of oxygen facilitates aerobic microbial carbon oxidation to release carbon back into the atmosphere (Schlesinger and Lichter, 2001). Conversely, the consistent saturation of coastal wetland soils inhibits this atmospheric exchange resulting in continuous build-up of carbon over time (Chmura et al. 2003).

1.2.2 Carbon Source

Stable Isotope Geochemistry

Isotopes are defined as the variations of a particular element, each having a unique number of neutrons. Stable isotopes are those that do not undergo radioactive decay (Sharp, 2007). Atoms of carbon always possess 6 protons and 6 electrons, but the number of neutrons can change, altering the mass of the atom. $^{12}$C, containing 6 neutrons, is the dominant isotope of C with 98.89% abundance; $^{13}$C is the next most abundant (1.11%) and contains 7 neutrons.

The stable isotopic composition of a sample is presented in delta notation, which expresses the ratio of the heavy stable isotope to the light stable isotope in the sample relative to an international reference standard (VPDB). 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.
Delta notation is identified as follows:

\[ \delta_{\text{sample}} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \]

where \( R = ^{13}\text{C}/^{12}\text{C} \)

Isotopic fractionation refers to the selection and partitioning of isotopes by natural biochemical and kinetic processes as a function of their atomic mass. Biochemical processes will favor particular heavy or light isotopes as a function of available reactants, temperature and other environmental conditions (Sharp, 2007). Kinetic fractionation specifically involves the separation of stable isotopes by mass during unidirectional processes in open systems (Farquhar, et al., 1989). Photosynthesis is a relevant kinetic biological process that fractionates such that the lighter carbon isotope is incorporated in the plant biomass.

**Identifying Shifts in Carbon Source**

\( \delta^{13}\text{C} \) is a soil property that reflects the signature of the parent vegetation of organic matter at different spatial scales through a sediment record (Guzmán et al., 2013). Plants actively incorporate \( \text{CO}_2 \) through photosynthesis, employing varying degrees of fractionation. Although all plants select for lighter carbon isotopes (\( ^{12}\text{C} \)), plants that adopt a C4 photosynthetic pathway are more efficient at taking up heavier forms of carbon (\( ^{13}\text{C} \)) than C3 plants. C4 plants have adapted this pathway as a response to stressful environmental conditions, to protect from photorespiration when \( \text{CO}_2 \) is limited, in dry environments, and in hypersaline conditions (Lanigan et al., 2008; O’Leary, 1992). C4 plants only fractionate during the diffusion process through stomata, while C3 plants fractionate during both diffusion and carboxylation (O’Leary, 1992). The difference in \( \delta^{13}\text{C} \) values for C3 and C4 plants as a result of fractionation disparities in their photosynthetic pathways is well documented and reflected in \( \delta^{13}\text{C} \) values of their plant tissues. Many marsh plants including *S. alterniflora* and *S. patens*, employ a C4 photosynthetic pathway where average \( \delta^{13}\text{C} \) values approximate -14‰. Plants that employ C3 photosynthesis, including *Typha angustofolia* and *Typha latifolia*, are more isotopically depleted, with average \( \delta^{13}\text{C} \) values of -26‰ (Guzmán et al., 2013). Based on Niering and Warren’s (1980) model of zonation (Figure 1.1) and on these carbon isotope values, \( \delta^{13}\text{C} \) of surface soils should decrease from low to high marsh with more C3 plants occupying the marsh platform with increasing distance from the channel (Johnson et al., 2007). Provided the isotope composition of the surface soils and vegetation does not alter with time, shifts in carbon source and vegetation changes over time can be identified in the geologic record based on \( \delta^{13}\text{C} \) values (Chmura et al., 1995).
1.2.3 Mechanisms Controlling CH$_4$ Production

**Hierarchy of Anaerobic Microbial Metabolism in Coastal Soils**

Methane production is a result of complex microbial processes interacting with belowground carbon that include both syntrophic interactions and competition for substrates. Organic carbon in marsh sediments functions as the electron donor to drive the metabolism of methane-producing heterotrophic microbes called methanogens (Bridgham et al., 2013). This organic carbon must be anaerobically broken down into substrates in order for methanogens to utilize its energy (Figure 1.2). Plant litter inputs are initially broken down by microbial exoenzymes into biopolymers, as soil organic matter and cellulose, and into monomers including simple sugars. Over time and depth, microbial fermentation ensues, further degrading the carbon into lower molecular weight fatty acids and alcohols. The resulting products of fermentation (H$_2$, CO$_2$ and acetate) can then be utilized, by methanogens and other energetically favorable microbial groups, as terminal electron acceptors (TEAs). Based on thermodynamic theory, CH$_4$ production is outcompeted by favorable TEA-reducing processes, which include the following in order of favorability: denitrification (NO$_3^-$), iron-reduction (Fe III), Manganese reduction (Mn III, IV), and sulfate reduction (SO$_4^{2-}$) (Figure 1.2; Bridgham et al., 2013). In a salt marsh, sulfate reduction dominates among anaerobic decomposition processes and has the largest influence on reducing methane production. Furthermore, in fresh water, SO$_4^{2-}$ is not a generally a major ion, thus methanogenesis ensues.

**Salinity, Sulfate Reduction and Methanogenesis**

Due to inundation of seawater by the tides twice daily, nutrients, sediments, and ionic solutes such as K$^+$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$, Cl$^-$, SO$_4^{2-}$, and HCO$_3^-$ are transported into marsh sediments for utilization by plants and microbes (Robenhorst, 2001). These tidal influences contribute a constant supply of SO$_4^{2-}$ ions to marsh sediments to be utilized as a TEA in sulfate reduction, inhibiting CH$_4$ production (Poffenbarger et al., 2011). Both methanogens and sulfate-reducing bacteria use H$_2$ and acetate as substrates when SO$_4^{2-}$ is present. Sulfate-reducers outcompete methanogens for these energy sources due to their more negative Gibbs Free Energy ($\Delta G$) values (-12.6 kcal and -6.1 kcal, respectively; Figure 1.2). The magnitude of $\Delta G$ tells us how far the standard-state is from equilibrium, represented by the equilibrium constant, K. The smaller the value of $\Delta G$, the closer the standard-state is to equilibrium (Stams, 1994). According to Kristjansen and Schonheit (1982), all species of sulfate-reducing bacteria have K <2 uM and all methanogenic bacteria have K > 5uM for H$_2$. This suggests that thermodynamically, sulfate-reducers are more favorable in terms of metabolization and lower K values, yielding a higher affinity for substrates. When tidal marsh soils experience SO$_4^{2-}$ depletion and a transition to freshwater (perhaps due to insufficient tidal flow), competition for TEAs by sulfate reducers decreases and methanogenic metabolism becomes favored (Bartlett et al., 1985; Bridgham et al., 2013; Mitsch et al., 2010).

Poffenbarger et al. (2011) determined the minimum salinity threshold for methanogenesis in salt marshes to be 18 psu based on SO$_4^{2-}$ to salinity ratios (Figure 1.3). At salinities >18 psu, SO$_4^{2-}$ concentrations are high enough such that SO$_4^{2-}$ reduction is energetically favored (Figure 1.2). At salinities <18 psu, variable methane emissions are observed because SO$_4^{2-}$ concentrations are not substantial enough for sulfate reducers to outcompete methanogens (Figure 1.3 b). Marshes have variable salinity levels.
depending on the elevation, geomorphology, hydrologic setting, and vegetation.

The substrates available for these microbial processes that control CH₄ production are dependent on decomposition. Rates of organic matter decomposition in marshes varies with temperature, as found by Keuskamp et al. (2013). Their study measured the mass and carbon loss by decomposition of rooibos and green tea in forest soils at two temperatures (15˚C and 25˚C). They found decomposition rates of tea were higher for the 25˚C plots. Though there is an understanding that temperature drives rates of decomposition (Keuskamp et al., 2013; Davidson and Janssens, 2006), it is unknown how decomposition varies with salinity in marsh settings. These rates of decomposition have potential to illuminate the relationship between salinity and microbial processes and can be used to better understand CH₄ fluxes from marsh sediments.

Figure 1.2: Schematic of CH₄ cycling in wetland ecosystems including hierarchical interactions among microbial processes and their respective free energies; adopted from Bridgham et al. (2013)
Figure 1.3: Relationship between CH$_4$ flux and salinity (a) due to sulfate concentrations (b). This curve suggests increased variability in CH$_4$ flux with decreased salinity (Poffenbarger et al., 2011)

1.2.4 Methane Emissions

CH$_4$ emissions occur when rates of methanogenesis exceed CH$_4$ oxidation, and the net CH$_4$ produced is transported to the atmosphere (Bridgham et al., 2013). While microbial activity regulates methane production, vegetation dynamics function as critical controls over emissions by regulating CH$_4$ transport from sediment to atmosphere. The three modes of methane transference to atmosphere include ebullition, diffusion, and plant-mediated transport through aerenchyma tissues. Ebullition refers to the release of accumulated methane bubbles at the soil surface due to shifts in pressure by external forces or volume. Plant mediated transport is prevalent in anoxic environments where plants utilize their aerenchyma tissue system as a conduit to the atmosphere, (Colmer, 2003; Maricle et al., 2002). These aerenchyma are vessel-like transport structures within the tissues of plants that allow for gas exchange from the atmosphere to the submerged roots of the plant. Plants primarily use aerenchyma for O$_2$ supply to their roots, but they also provide a vehicle for CH$_4$ emission from marsh sediments; this mode of transport is most prolific during periods of high productivity, but continues to slowly function throughout dormancy (Shih and Finkelstein, 2008). Methane emission through aerenchyma varies by plant. Typha
have particularly large aerenchyma compared to *Spartina* species (Shih and Finkelstein, 2008). Root aerenchyma structures in *Spartina* developed to minimize oxygen leakage in response to flooding (Burdick, 1989), while *Typha* adapted to flooding by expanding the cortical air space in the aerenchyma (Chabbi et al., 1999). As a result, gas exchange by *Typha* stands is more effective at CH$_4$ transport to the atmosphere than *Spartina*.

The production and transport of methane varies with season, vegetation, and the conditions of marsh sediments (Bartlett et al., 1987; Emery and Fulweiler, 2014). Emery and Fulweiler (2014) conducted a study on greenhouse gas emissions and net primary productivity on an anthropogenically altered marsh in Massachusetts, largely influenced by the invasive plant species *Phragmites australis*. In comparing greenhouse gas emissions from both unvegetated and vegetated plots of *S. alterniflora* and *P. australis*, they found CH$_4$ fluxes were similar in quantity for both plant species, but varied by season (Figure 1.4). Using multiple regression analysis, it was determined that sediment salinity, temperature, and live biomass were the most significant predictors of CH$_4$ flux for their study. These findings concluded CH$_4$ emissions are positively correlated to live aboveground biomass and negatively correlated to soil salinity.

![Figure 1.4: Monthly CH$_4$ fluxes for *S. alterniflora* and *P. australis* from Emery and Fulweiler (2014). They found flux variation by season where CH$_4$ fluxes are highest during the summer months and lowest in the winter.](image)

### 1.3 Vulnerability and Alteration of Tidal Salt Marshes

#### 1.3.1 Vulnerability and Alteration

*Alteration of Tidal Salt Marshes in Maine*

Coastal wetlands have long been altered by filling, tidal restriction, dredging, ditching, from
contamination by agricultural, urban, and industrial activities, and from the encroachment of invasive species (Taylor, 2008). The vast majority of coastal wetlands in the Northeastern United States have been physically altered by anthropogenic activity, often to the point of completely obliterating the resources by means of dredging or filling. It is estimated that 35% of all tidal salt marshes worldwide have been lost and continue to be destroyed at rapid rates amounting to about 1-2% per year (Pendleton et al. 2012). Salt marshes are extremely prevalent throughout the Gulf of Maine, stretching from Massachusetts to New Brunswick, making Maine an ideal location to study marsh restoration (Taylor, 2008).

**Geology and Marsh Formation in Maine**

Small, narrow salt marshes dominate the coastline of the Gulf of Maine due to its bedrock valleys that were glacially scoured 15,000 years ago. Over a period of 4,000 to 5,000 years these glacially-carved valleys formed into marshes through the accumulation of sediment and colonization of vegetation (Kelley, 1987). In the northeastern coast of Maine, specifically in Casco Bay, the coastline is characterized by a series of indented embayments and north-trending bedrock peninsulas and islands; these features provide a protected environment for salt marsh formation along the major and minor tributaries, yielding back-barrier and fluvial marshes (Kelley et al., 1988). The bedrock of Casco Bay is comprised of Ordovician metamorphic gneiss and schist, and is overlain by the glaciogenic sediments including till and the galciomarine sediments of the Presumpscot Formation (Thompson and Borns, 1985).

**Tidal Restriction**

A significant portion of the salt marshes in Maine have been hydrologically altered by the construction of undersized culverts, which restricts tidal exchange to inland regions. Tidal restriction has been debilitating to salt marshes throughout the state of Maine with the installment of roadways, causeways, and culverts due to coastal population increases. Crain et al. (2009) developed a tidal restriction database using U.S. Geological Survey topographical maps and ground-truthing that identified 283 total restrictions from Kittery to Cape Elizabeth along Maine’s coast, south of Casco Bay. 57 of these identified restrictions were found to be affecting approximately 902 ha of tidal marshes on the upstream side (Crain et al., 2009). The Casco Bay Estuary Partnership (CBEP) identified 128 known or possible tidal restrictions affecting salt marshes and intertidal habitats in Casco Bay alone, these included those by public and private roads, railroads and bridges based on aerial searches (Figure 5; Craig, 2012).

Since tidal energy and the chemistry of seawater play such integral roles in maintaining the delicate balance of marsh ecology, such restrictions have high impact on the zonation of marsh vegetation and biogeochemistry of marsh sediments. The most substantial perturbation in terms of biogeochemical cycling is due to the introduction of freshwater. This disruption frequently results in the conversion of healthy *Spartina* dominated systems to monotypic *Typha* and *Phragmites* stands, along with a salinity decrease in marginal regions of the marsh. In addition to effects on saltwater flooding, tidal restrictions also reduce sediment inputs, prevent marsh migration with rising sea level, and reduce biological exchanges within surrounding estuarine systems (Burdick et al., 1997; Boumans and Day, 1994). Consequently, tidally restricted marshes will continue to degrade habitat and associated biogeochemical
functions. Fortunately, the restoration of these altered systems is feasible by the reintroduction of tidal flow and \( \text{SO}_4^{2-} \) concentrations back into marsh sediments.

![Tidal Restrictions](image)

**Figure 1.5:** The 128 known or possible tidal restrictions identified in Casco Bay, ME by the CBEP (Craig, 2012)

Quantification and assessment of marsh responses to restoration is critical for motivating and implementing restoration projects. Burdick et al. (1996) contributed to the information available for marsh response in a study that measured the effect of long-term tidal restriction and subsequent restoration at Drakes Islands Marsh in Maine, and Mill Brook Marsh in New Hampshire. They monitored before and after tidal restoration (8 years post-restoration) conditions for surface elevation, water levels, salinity, plant cover, and fish use on these marshes. Based on their findings, they hypothesized time scales of restoration presented in Figure 1.6. They observed restoration of hydrology and fish use within days to weeks, and observed vegetation and soil responses on week to year timescales. They found that \textit{S. alterniflora} began to re-dominate the marsh platform after several months of restoration and vegetation shifts were observed for eight years after. They hypothesize that fish community development, plant succession and elevation response would take decades to be fully reestablished. Although Burdick et al. (1996), among others, provide a comprehensive assessment of tidal reintroduction on restricted marshes in Northern New England, there is still a major deficit of information available on the effects of tidal restoration on methane emissions and carbon sequestration. The quantification of methane emissions and subsequent carbon stock data are needed to properly advise and motivate restoration efforts on tidally restricted marshes in terms of their carbon budget (Buchsbaum, 2001; Burdick et al., 1996; Silliman et al., 2009).
1.3.2 Purpose

Tidal restrictions have the capacity to influence vegetation type, microbial activity, and carbon cycling; the interplay among these factors will be explored on a recently restored salt marsh in Casco Bay, Maine. This study addresses two main objectives: (1) to understand the relationships between salinity, vegetation, and CH$_4$ emissions along a salinity gradient at Long Marsh, and (2) to compare soil salinities, decomposition rates, carbon isotope data, and carbon stocks based on bulk density to further understand the effects of tidal restriction on marsh health. Finally, annual methane emission projections on Long Marsh will be made based on vegetation mapping by the CBEP of *Typha* area before and after restoration, assuming *Typha* as a proxy for methane emissions.

1.3.3 Study Area

The site for this study is located on Long Marsh in Casco Bay, Maine in the town of Harpswell (Figure 1.7). It is a 1.5-mile long, linear marsh that occupies a glacially carved valley. Over 100 years ago a 3-foot wide culvert was built on the Northern, open-ocean end of the marsh, restricting tidal flow to its most inland regions (Coffin, 1938). This marsh has been subject to decades of limited tidal exchange resulting in freshwater pooling, groundwater intrusion and the expansion of brackish vegetation onto the marsh platform. Over the course of tidal restriction, the site experienced substantial changes in marsh vegetation including the replacement of healthy *Spartina*-dominated regions with freshwater *Typha* along the margins. In February 2014, the CBEP was funded by the Maine Department of Transportation to
widen the 3-foot culvert on Long Marsh to 14 feet in diameter as one of their many restoration projects in Casco Bay (Figure 1.8). Since restoration, tidal exchange has drastically increased and subsequent dieback of freshwater vegetation has occurred (Figure 1.8).

Long Marsh provides ideal conditions to study CH$_4$ fluxes along a salinity gradient of a restored marsh due to its pristine, highly saline site North of the culvert that was unaffected by the tidal restriction. This region is able to simulate a reference marsh representing tidally-exposed “unaltered” marsh conditions. Additionally, a gradual salinity gradient exists from the culvert to the inland, freshwater region of the marsh. Dieback and vegetation shifts on the margins of several sites provide experimental sites for restoration response. The CBEP of Portland, Maine has monitored vegetation changes over 12 transects for the past 4 years, providing valuable information about shifts in plant populations. However, no CH$_4$ flux data was obtained before restoration so the CH$_4$ flux component of the study takes a space-for-time

Figure 1.7: Location map of Long Marsh located in the town of Harpswell, in Casco Bay within the Gulf of Maine
Figure 1.8: Image of the culvert restricting Long Marsh before restoration (3 feet wide; 2013) and after restoration (14 feet wide; 2015). Photos by the CBEP.
2

METHODS
Five sites for CH$_4$ sampling and sediment core collection were chosen based on their unique salinities, dominant marsh vegetation, and their responses to tidal restriction and subsequent restoration (Figure 2.1). At each site CH$_4$ flux samples were collected using a closed static gas chamber method adopted from Emery and Fulweiler (2014). Additionally, average soil salinities were determined, and sediment cores were obtained for isotope analyses and carbon density at each site. A tea bag index experiment by Keuskamp et al. (2013) was employed at three of the sites to determine rates of decomposition.

2.1 Field Sites

All field sampling occurred at sites along two transects; one along a normal salinity gradient, and the other across a transitional region of the marsh. The sites of the normal transect include a highly saline site (SAL; Figure 2.1 a) located approximately 160 meters north of the culvert. The SAL region is dominated by healthy *Spartina alterniflora* and *Spartina patens* vegetation with soil salinity levels ranging from 26 to 30 psu. This site represents the region of the marsh that was unaffected by the installation of the culvert and ensuing tidal restrictions. The intermediate site along the normal salinity gradient transect is a brackish region (BRACK; Figure 2.1b) located approximately 780 meters inland from the culvert. This site remains highly saline with levels ranging from 24 to 30 psu. The vegetation in this region is predominantly *S. patens* with some *Juncus girardii*. Pools and small channels are present throughout the BRACK location. The freshwater site (FRESH Figure 2.1c) is located about 1200 meters from the culvert and is the most inland site. Soil salinity levels range from 0-6 psu and the area is dominated by *Typha angustifolia*.

The transitional transect comprises two sites that experienced expansion of *Typha* onto the marsh platform likely due to the emplacement of the undersized culvert. The transitional site on the eastern side of the channel (TE; Figure 2.1d) approximately 300 meters south of the culvert, has a combination of live *S. patens* and dead *Typha*, which experienced dieback post tidal restoration with evidence of some patches of new *Typha* growth. Soil salinity at this site currently ranges from 15 to 25 psu. Evidence of pooling and the impoundment of water is observed at this site. The transitional site on the western side of the marsh (TW: Figure 2.1e) is located around 600 meters inland from the culvert, with salinity levels ranging widely from 22 to 30 psu. The vegetation experienced 100% dieback of *Typha* and pre-existing woody vegetation with some new growth of *Schoenoplectus acutus* and *Salicornia europaea*.

2.2 Salinity Measurements

At each site soil salinities were determined at 20 cm depths using a soil sipper and portable refractometer. In the instances where soil waters obtained by the sipper were too clouded from sediment to measure, the water samples were filtered on-site using Kimwipes. At several sites the sediment was too compact and impermeable to extract soil water samples, in which case we dug a small, 20 cm deep core and extracted the soil waters that pooled in the hole. Salinity measurements were taken in July and October.
Figure 2.1: Five sampling sites with three collars as triplicates at each site were chosen based on vegetation and salinity. (a) Saline, SAL; (b) Eastern transitional, TE; (c) Western transitional, TW; (d) Brackish, BRACK; and (e) Fresh, FRESH. The transect along the normal salinity gradient comprises sites a, d and e; the transitional transect includes sites b and c. Photographs by Kückens, 2015.
2.3 CH$_4$ Field Sampling

2.3.1 Static Gas Chamber Design

Static gas chambers were modeled after Emery and Fulweiler (2014). Plexiglas cylinders 20.2 cm in diameter were used for the body of each chamber, cylinder heights varied, including 0.465 meters, 0.61 meters, and 1.5 meters, to account for varying heights of vegetation. Cylinders were designed to fit on top of the sampling collars to create a closed chamber. The chamber was capped with a Plexiglas cap rimmed with adhesive foam for an airtight seal. An airtight septum was installed in the side of each chamber 30.5 cm from its base for syringe sampling. Affixed inside each chamber was a battery powered fan to homogenize the gases captured, as well as a temperature and humidity gauge to monitor interior conditions (Figure 2.2)

Figure 2.2: Static gas chamber design and field set-up for sampling CH$_4$ fluxes, method adopted from Emery and Fulweiler (2014)
2.3.2 Exetainer and Sampling Preparation

Prior to sampling, 12 mL glass Labco exetainer vials were purged with helium, an inert gas, to remove sources of contamination and trace CH$_4$ introduced by air. Each exetainer was then evacuated with a 60 mL syringe to create a negative pressure vessel, and capped with an airtight septum cap. All sampled gases were collected and stored in these exetainer vials at room temperature.

2.3.3 Field Sampling

Three collar locations at each site were spaced 3 to 7 meters apart as triplicates (collars 1, 2 and 3 = C1, C2 and C3) to properly represent each site for gas sample collection. These steel collars were 20.2 cm in diameter and 8 cm deep and were inserted into the marsh soils to isolate the selected representative area of marsh vegetation and sediment for static chamber sampling. These collars were allowed 48 hours to one week to equilibrate before sampling. Sampling at each collar site was conducted on a large bench to avoid ebullition and stress to marsh sediments. At the time of sampling a chamber of appropriate height was placed atop the collar and capped, enclosing all vegetation within the chamber. Samples were taken immediately after sealing the chamber (t = 0 min), then at 1 min, 5 min, 10 min, and every 10 minutes following until 40 minutes elapsed (n = 7 per collar site). At each sampling interval a 25 mL sample was extracted through the chamber septum using a 30 mL syringe with a stopcock. Each 25 mL sample was immediately stored in a prepared exetainer vial. This process was repeated for each collar at all five sites. Additionally, three atmospheric samples per sampling round were obtained for baseline CH$_4$ concentration comparison. Three full rounds of sampling were executed for repetition and seasonal shifts. These sampling rounds occurred in late July to early August (Round 1), late August (Round 2), and late October (Round 3; Table 2.1). For all intents and purposes Rounds 1, 2, and 3 will be referred to as July, August and October sampling periods, respectively.

Table 2.1: Dates of CH$_4$ flux sampling

<table>
<thead>
<tr>
<th>Site</th>
<th>Sampling Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Round 1 (July)</td>
</tr>
<tr>
<td></td>
<td>7/29/15</td>
</tr>
<tr>
<td>SAL</td>
<td>x</td>
</tr>
<tr>
<td>BRACK</td>
<td>x</td>
</tr>
<tr>
<td>FRESH</td>
<td>x</td>
</tr>
<tr>
<td>TE</td>
<td>x</td>
</tr>
<tr>
<td>TW</td>
<td>x</td>
</tr>
</tbody>
</table>
2.4 GC-FID Analysis

2.4.1 Determining CH$_4$ Concentrations

Analysis of CH$_4$ was conducted using a gas chromatograph with a flame ionization detector (GC-FID), model Agilent 6890N. This GC-FID is equipped with a 30m column (Carboxen 1006 PLOT Capillary GC, I.D. 0.53 mm, 0.3 μm; Sigma Aldrich, # P0040523) and FID conditions: 300°C; H$_2$, 30.0 mL/min, 2.2 psi; airflow, 400.0 mL/min, 2.2 psi. Helium was used as the carrier gas with pressure control (25 mL/min). Samples were injected isothermally at an inlet temperature of 200 °C. The method used was titled CARBOXEN.M. The oven temperature program was as follows: initial 35°C hold for 14 minutes. Ramp 1: 25°C/min to 55°C hold 15 minutes, totaling 29.8 minutes for each injection. Septa for the GC-FID were replaced every three site runs (n=21).

Samples were injected with a glass, gas-tight 10 mL syringe, inserting 5 mL into the GC. Upon injection, compounds were isothermally separated; the routine retention time for CH$_4$ was 7.8 minutes. While moving through the column, some compounds within the gas adsorb onto the stationary phase in the column, while others separate out and continue through to the end of the column. The separated gases pass through a hydrogen flame, which ionizes the organic compounds present in the sample. The resulting ion stream then enters the detector, which records each compound that passes through. The concentration of CH$_4$ is proportional to the ion current detected (Fessenden and Fessenden, 1993). GC-FID were processed using OpenLab software in a computer. Area under each CH$_4$ curve was integrated for each run (pA), and then converted into a concentration using a standard calibration curve.

2.4.2 Standard Curve

The standard calibration curve used to determine CH$_4$ concentrations was based on the following equation: $y = 32.496 - 1.4848$, with an $r^2$ value equal to 0.9994 (Figure 2.3). Standards for the curve were mixed using 5 ppm CH$_4$ and 5 ppm He for dilution at concentrations of 5 ppm, 2.5 ppm, 1.5 ppm and 0 ppm.

2.4.3 Quality Assurance and Instrumental Error

Standards of various concentrations (5 ppm, 2.5 ppm, 1.5 ppm and 0 ppm) were run through the GC every three to five site runs (n=21-35) for quality assurance to determine instrumental reproducibility and stability over time. The 5ppm CH$_4$ control standards indicated no temporal changes over time, with average area of 159.3 pA and a 3.1% coefficient of variation; 2.5 ppm standards averaged 80.2 pA with a 3% coefficient of variation (Appendix C). These values imply a 3% GC-FID instrumental error. Sample deterioration over storage time was accounted for by running standards made 9/30/2015 once per month for the duration of the GC-FID analyses (September through December). No statistically significant temporal degradation was observed.

Random error in curve integration was determined to be ± 2% based on comparing curve area
integration by two different scientists (Gunn and Dostie). Thus, the total instrumental and random error associated with determining CH$_4$ concentrations using the GC-FID is estimated to be ±5% (See Appendix C).

Figure 2.3: Standard curve based on known standards used to calculate all CH$_4$ concentrations and fluxes; $R^2 = 0.9994$

2.4.4 Outliers and Discarded Values

Outliers due to due to systematic or documented field error were eliminated. Systematic error includes improper sample volume injection, sample contamination by improper extainer evacuation or leaks, unidentified broken seal during sampling etc. Prior to calculating CH$_4$ fluxes, the Q-test was applied to suspected CH$_4$ concentration outliers based on the equation:

$$Q_{exp} = \frac{|\text{outlier} - \text{closest value}|}{\text{range}}$$

$Q_{exp}$ was compared to a 99% confidence interval $Q_{crit}$=0.068 for 7 samples. All values where $Q_{exp} > Q_{crit}$ were discarded.
Furthermore, all known field error was documented and accounted for preceding calculations (Appendix A). Values affected by field error were discarded; these sources of error include known burping of sediment, disruption of static chamber setup, breaking of the chamber seal, improper sampling time, etc. All outliers were removed prior to calculating average fluxes for each site. Values affected by field error notes and discarded outliers (5 total) from systematic error are displayed in Appendices A and B respectively.

2.4.5 Flux Calculations

First, CH$_4$ concentrations over time (40 minutes) were plotted to determine the rate at which CH$_4$ concentrations (ppm converted to μmol /mol$_{gas}$/min) were changing in the chamber. The volume of gas in the chamber was determined using the ideal gas law. Based on this quantity, fluxes of CH$_4$ were calculated taking chamber volume, average atmospheric temperature, average chamber temperature and chamber footprint for each sampling site into account. A sample calculation in Table 2.2 below describes how to determine moles of gas molecules within a single static chamber where P (assumed constant at 1 atm) and R are constants.

Table 2.2: Sample calculation of how to determine moles of gas within a static chamber

<table>
<thead>
<tr>
<th>Pressure (atm)</th>
<th>Volume of Chamber (L)</th>
<th>Gas Constant (L<em>atm/K</em>mol)</th>
<th>Temperature (K)</th>
<th>Gas Molecules in Chamber (moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>V</td>
<td>R</td>
<td>T</td>
<td>n = PV/RT</td>
</tr>
<tr>
<td>1</td>
<td>V</td>
<td>0.0820</td>
<td>273 + (T in °C)</td>
<td>mol</td>
</tr>
</tbody>
</table>

\[ \text{slope} \times n = \text{flux CH}_4 \]

Fluxes are presented in μmol CH$_4$/m$^2$/hr. CH$_4$ concentrations determined through comparison to the standard curve are in parts per million per minute (μmol /mol$_{gas}$/min), thus, moles of gas must be converted to μmol e (n*1000). This is then converted to μmol CH$_4$/min:

\[ \frac{\mu \text{mol CH}_4}{\text{mol-min}} \times \text{moles gas} = \frac{\mu \text{mol CH}_4}{\text{min}} \]

This is then converted to μmol CH$_4$/m$^2$/hr by dividing by the area of the chamber footprint (m$^2$) and converting minutes to hours:

\[ \frac{\mu \text{mol CH}_4}{\text{min}} \times \frac{60 \text{ min}}{\text{hr}} \times \frac{1}{0.0324 \text{ m}^2} = \frac{\mu \text{mol CH}_4}{\text{m}^2 \cdot \text{hr}} \]

This is then converted to mass (g) to compare against Poffenbarger et al. (2011) salinity versus flux curve, as follows:

\[ \frac{\mu \text{mol CH}_4}{\text{m}^2 \cdot \text{hr}} \times \frac{1 \text{ mol CH}_4}{1 \times 10^8 \mu \text{mol CH}_4} \times \frac{16.04 \text{ g}}{1 \text{ mol CH}_4} = \frac{g \text{ CH}_4}{\text{m}^2 \cdot \text{hr}} \]
2.5 Sediment Core Analysis

2.5.1. Core Retrieval

Sediment cores were used to determine the nature of the substrate including $\delta^{13}C$ and carbon density based on % carbon (%C) and dry bulk density at each flux site. The upper 50cm of marsh sediment at each collar site was sampled using a Dutch peat corer (diameter=2.54 cm). Each 50 cm core was divided into 10 cm subdivisions and frozen the day of retrieval for 30-60 days.

2.5.2 Preparation

All sediment core samples were freeze-dried using a LabConco freeze drier at -40°C with a vacuum at $100 \times 10^{-3}$ Mbar. Each freeze-dried sample was weighed for dry bulk density. The sample was then homogenized in a shatterbox for 2 minutes. Between 0.5 and 2.0 mg of sediment was weighed out and packaged in a tin capsule for analysis by Isotope Ratio Mass Spectrometry (IRMS). Bulk geochemical parameters (i.e., %C, C/N, and $\delta^{13}C$) were obtained.

Internal lab standards for the IRMS including Acetanilide, Caffeine, and Cod Muscle tissue were run through IRMS. These standards were selected because they have consistent $\delta^{15}N$ and $\delta^{13}C$ ratios covering a range of values. All other sample values are determined based on the IRMS output of these values. Additionally, the standards were analyzed every 15 samples (beginning, middle, end) during the run for quality control to ensure instrumental consistency for the duration of each run.

2.5.3 Isotope Ratio Mass Spectrometry

Carbon isotope measurements of bulk organic matter were made in the EGL at Bates College using a ThermoFinnigan Delta V Advantage stable isotope ratio mass spectrometer (IRMS) fixed to a Costech elemental analyzer (EA) via a Conflo III combustion interface. All stable carbon isotope values are reported in delta ($\delta$) notation units of per mil (‰) based on VPDB standard (Craig, 1957).

During an IRMS-EA run, the freeze-dried and homogenized sample contained in a tin capsule is dropped into the EA and oxidized under high heat, transforming organic matter into $\text{CO}_2$, $\text{N}_2$ and $\text{H}_2\text{O}$. The tin capsule facilitates flash combustion to 1300°C, leaving $\text{NO}_x$, $\text{CO}_2$, He and $\text{H}_2\text{O}$ flowing through the first tube with $\text{CrO}_3$ and $\text{CO}$. $\text{NO}_x$ acts as the reagent at 1050°C. These gases are reduced as they flow through the second tube (at 600°C) with elemental copper reagents converting $\text{NO}_x \rightarrow \text{N}_2$. Remaining gases flow through a water trap with magnesium perchlorate, then through a GC that separates the gases by atomic mass ($\text{He}, \text{N}_2$ then $\text{CO}_2$). Once separated, they are sent to the IRMS through a combustion interface, where the gases are reduced. The gases generated ($\text{CO}_2$) are introduced to the evacuated IRMS chamber where they are bombarded by electrons emitted by a tungsten filament. The charged ions then pass through a voltage gradient (low to high) that accelerates and focuses them into a beam. The ions then run through a magnetic field and are deflected into Faraday cups based on their charge-to-mass ratio, where they are detected. These signals are then generated into an ISODAT reading where ratios are determined relative to a standard. This EA-IRMS analysis provided values for % C and $\delta^{13}C$. 
2.5.4 Carbon Density

Dry bulk density was determined by obtaining a dry mass for each 10 cm subsample and dividing by the volume of each sample using the equation:

\[
\text{Dry Bulk Density (g/cm}^3) = \frac{\text{Dry mass (g)}}{50.7 \text{ cm}^3}
\]

Carbon density was then calculated based on the average %C determined for the upper 50 cm of the cores.

\[
\text{Carbon Density (gC/cm}^3) = \frac{g_{\text{carbon from %C}}}{100 \ g_{\text{sediment}}} \times \text{Bulk Density (g sediment/cm}^3)
\]

2.6 Tea Bag Index

The TBI experiment by Keuskamp et al. (2013) adopted a simplified litterbag experiment using tetrahedron-shaped Lipton 5 cm tea bags with synthetic 0.25 mm mesh. The mesh size allowed microorganisms to enter the bags, excluding all macrofauna. The experiment used green and rooibos tea. Each tea bag was weighed dry before deployment. Following the TBI protocol by Keuskamp et al. (2013), green and rooibos tea bags were buried pairwise at 8 cm depths for 90 days (July 23 - Oct 21). Five pairs were installed at each of the sites along the normal salinity gradient transect, SAL, BRACK and FRESH. After the 90 day duration, the tea bags were removed and soil temperature data were collected at each site. These bags were then scraped of excess sediment on mesh of the bags and dried in a Fisher Scientific Isotemp drying oven at 70°C for 48 hours. The bag labels were removed and the dried bags were weighed, then the contents of each tea bag were removed and weighed. Percent mass and carbon loss of each tea bag was calculated by averaging the contents of bags of each type of tea prior to burial, then determining percent change equation with post-burial content masses:

\[
\frac{\text{Pre – burial dry mass of tea contents} - \text{post burial dry mass of contents (g)}}{\text{pre – burial dry mass of tea bag contents}} \times 100 = \text{Percent loss}
\]
3

RESULTS
3.1 Salinity

Salinity measurements taken in July and October show slightly elevated average salinities in October than July for each site except TW (Table 3.1; Figure 3.1). Largest increases in salinity from July to October occurred at the BRACK site, increasing by 4.3 psu. The normal salinity gradient is evident, gradually decreasing in salinities from SAL to BRACK to FRESH, observed in both months. Transitional sites show variable salinities.

Figure 3.1: Salinity measurements taken at all three collars at each of the five sites in (a) July and (b) October
Table 3.1: Average salinities at each site in July and October

<table>
<thead>
<tr>
<th>Site</th>
<th>July Average Salinity (psu)</th>
<th>July StDev</th>
<th>October Average Salinity (psu)</th>
<th>October StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>27.3</td>
<td>1.2</td>
<td>28.3</td>
<td>1.5</td>
</tr>
<tr>
<td>BRACK</td>
<td>23.7</td>
<td>0.6</td>
<td>28.0</td>
<td>2.6</td>
</tr>
<tr>
<td>FRESH</td>
<td>1.7</td>
<td>2.1</td>
<td>4.0</td>
<td>2.6</td>
</tr>
<tr>
<td>TE</td>
<td>20.7</td>
<td>5.1</td>
<td>22.3</td>
<td>5.5</td>
</tr>
<tr>
<td>TW</td>
<td>28.3</td>
<td>1.5</td>
<td>25.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

3.2 CH$_4$ Fluxes

3.2.1 Uncertainty

The error associated with determination of CH$_4$ fluxes using the GC-FID and integration methods amounted to ±5%. As explained in methods, this includes a ±3% coefficient of variation of standards over time (instrumental error) and a ±2% uncertainty for area integration (random error). We propagated the instrumental error through a full flux calculation for FRESH C1 August sampling, determining this total possible uncertainty based on maximum and minimum slopes (Figure 3.2). The calculated uncertainty for this site amounts to 67.4 ± 12.5 μmol CH$_4$/m$^2$ hr. Then a ±2% error was applied to the calculate flux, as this uncertainty affects full flux calculations rather than individual CH$_4$ concentrations, as integration techniques are applied by site rather than sample. This total error equals 67.4 ± 14 μmol CH$_4$/m$^2$ hr at FRESH C1 (August). This instrumental and random error is less than uncertainty associated with variability among triplicates at the FRESH field site in August (± 30.6 μmol CH$_4$/m$^2$ hr); for all sites and all sampling periods, error as a result of variability in the field exceeded instrumental and random error. This suggests field error to be more significant than the instrumental and random error associated with CH$_4$ flux calculations. The datum that lie outside the margin of error in Figure 3.2 can be attributed to field error and cannot be quantified, thus it is accounted for by assigning uncertainty based on variability among triplicates at each site (Table 3.2; Figures 3.3 and 3.4).

Figure 3.2: Maximum and minimum slopes based on ±3% to calculate uncertainty extremes from instrumental error in CH$_4$ flux determination for FRESH C1 August (= ± 12.5 μmol CH$_4$/m$^2$ hr) with a ±2% random error applied, totaling 67.4 ± 14 μmol CH$_4$/m$^2$ hr
3.2.2 CH₄ Fluxes in July, August and October

Average CH₄ flux data from static gas chamber sampling are compiled in Table 3.2 and Figures 3.3 a-c for all collars at all sites SAL, BRACK, FRESH, TE and TW, over three sampling periods in July, August and October.

Table 3.2: Average CH₄ Fluxes from C1, C2 and C3 at each site in July, August and October

<table>
<thead>
<tr>
<th>SITE</th>
<th>JULY</th>
<th>AUGUST</th>
<th>OCTOBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₄ Flux</td>
<td>StDev</td>
<td>Range</td>
<td>CH₄ Flux</td>
</tr>
<tr>
<td>SAL</td>
<td>5.3</td>
<td>6.5</td>
<td>1.5-12.8</td>
</tr>
<tr>
<td>FRESH</td>
<td>76.6</td>
<td>16.3</td>
<td>47.4-256.3</td>
</tr>
<tr>
<td>BRACK</td>
<td>4.8</td>
<td>2.3</td>
<td>2.6-7.2</td>
</tr>
<tr>
<td>TE</td>
<td>7.5</td>
<td>2.5</td>
<td>5.3-10.2</td>
</tr>
<tr>
<td>TW</td>
<td>3.2</td>
<td>7.2</td>
<td>-2.8-11.1</td>
</tr>
</tbody>
</table>

In July, the FRESH site experienced the largest and most variable fluxes of CH₄ ranging from 47.4 to 256.3 μmol CH₄/m² hr and averaging 76.6 ± 16.3 μmol CH₄/m² hr. (Table 3.1; Figure 3.3 a). Lowest emissions were observed at the TW site, the region that experienced most considerable dieback of freshwater vegetation as measured by the CBEP. TW fluxes averaged 3.2 ± 7.2 μmol CH₄/m² hr (-2.8 to 11.1 μmol CH₄/m² hr). Emissions from the BRACK and SAL were 4.8 ± 2.3 μmol CH₄/m² hr and 5.3 ± 6.5 μmol CH₄/m² hr, respectively.

Overall, average CH₄ fluxes were higher during the August sampling (Table 3.2, Figure 3.3b) than July for all sites except FRESH. Largest and most variable fluxes were again observed at the FRESH site (average 47.9 ± 30.6 μmol CH₄/m² hr), but displayed a smaller range than in July (12.6 to 67.4 μmol CH₄/m² hr). The next highest fluxes came from the TE site where evidence of some new Typha growth was observed. These fluxes averaged 12.7 ± 5.5 μmol CH₄/m² hr with a maximum output of 16.9 μmol CH₄/m² hr, falling in the range of the FRESH site. SAL emitted the least amount of CH₄ with smallest variability (3.2 ± 2.9 μmol CH₄/m² hr). Both July and August sampling rounds demonstrate an inverse relationship between CH₄ flux and salinity along the normal salinity gradient, with variability in trends along the transitional transect.

In October, negative fluxes were detected at 40% of collar locations, and at all sites except TW (Figure 3.3 c). Average flux values at BRACK and SAL were as low as -0.6 ± 4.5 μmol CH₄/m² hr and -0.1 ± 3.0 μmol CH₄/m² hr, respectively. The largest negative flux measured -5.2 μmol CH₄/m² hr at C1 located furthest from the channel. These negative fluxes may indicate the presence of uptake by CH₄ oxidation. Overall, fluxes measured during October revealed the lowest emissions of the three rounds of sampling, with average fluxes less than 3 μmol CH₄/m² hr for all sites except FRESH.
Figure 3.3: Fluxes at each collar for all five sites during sampling periods in (a) July; (b) August; and (c) October. These data depict the variability among collars within each site.
3.2.3 Average Monthly Fluxes

A compilation of averages per site (n=3) for all three monthly rounds summarizes magnitude of fluxes for the three months, as well as average fluxes for all sites relative to one-another (Figure 3.4). Fluxes in July and August were high compared to October for all sites. This indicates an influence of season on the emission of methane from marsh sediment and vegetation. July showed the highest variability among sites ranging from 3.2 to 76.6 μmol CH₄/m² hr, while October showed marginal variability among its sites (-0.6 to 10.3 μmol CH₄/m² hr). Variability among collars at each site is apparent (Figure 3.4 error bars; Figure 3.3), alluding that multiple factors independent of season impact methane flux. Measured variability exceeds instrumental uncertainty for fluxes (section 3.1; Figure 3.1), indicating variability due to spatial heterogeneity among collars.

Relative to one-another, sites along the normal salinity gradient transect (SAL, BRACK, FRESH) displayed a negative correlation between methane flux and salinity. For all sampling rounds the FRESH and SAL sites showed highest and lowest CH₄ fluxes, respectively. Overall, the TE site had higher CH₄ fluxes than the TW and BRACK sites.

Figure 3.4: Compiled average fluxes CH₄ from July, August and October sampling periods. Overall, average CH₄ fluxes were lowest at the SAL site and highest at the FRESH site, with variability at the BRACK and transitional sites. CH₄ fluxes at all sites were lower in October than July and August.
3.3 Sediment Core Analyses

3.3.1 Core Descriptions

General stratigraphy descriptions for all sediment cores for the upper 50 cm were noted. Overall the thickness of the observed peat units for each core decrease with site distance from the open ocean.

**SAL**

The SAL C1 core was obtained in a *S. patens*-dominated area. The upper 13 cm comprised dark brown peat with approximately 40% decomposed roots and rhizomes. Dense, grey clay was observed from 13-28 cm. The sediment from 33-50 cm was dark brown and contained larger roots and debris, with small clumps of grey clay.

The SAL C2 core was taken in an area dominated by *S. alterniflora* on the *S. patens* margin. The upper 25 cm of this core comprised brownish-grey peat with fibrous roots and rhizomes. The sediment transitioned to a clay-rich grey sediment with larger roots and sticks from 25-50 cm.

SAL C3 was located in a region dominated by both *S. alterniflora* and *S. patens*. The upper 8 cm comprised a brown, organic rich peat with highly fibrous root networks. From 8-30 cm the peat sediment was grey in color with larger roots and rhizomes, and transitioned to a dense grey clay.

**BRACK**

The BRACK C1 core was retrieved in a region inhabited by *S. patens* and *J. gerardii*. The upper 10 cm comprised organic–rich, dark brown sediments with large amounts of fibrous root material. Lenses of decomposing *S. patens* fragments were observed in dark brown peat from 10-30 cm, and the remaining 20 cm included homogenous, dark brown, dense, clay sediment.

The BRACK C2 core, obtained in an area with both *S. patens* and *J. gerardii*, comprised light brown sediment with large roots in the upper 5 cm, a dark brown peat with small roots from the middle unit. From 12 to 50 cm the core comprised thick, greyish brown, clay-rich sediment.

Lastly, the BRACK C3 core, taken in a region dominated by healthy *S. patens* and consisted of brown peat with decomposing *S. patens* in the upper 5 cm, transitioning to thick, clay-rich sediment s from 5-15 cm. From 15-50 cm sediments were dense, grey and clay-rich with some fibrous root material.

**FRESH**

All three FRESH cores were obtained in regions dominated by *Typha* vegetation. The core at FRESH C1 included loose, silty, brown peat in the upper 8 cm. From 8 to 25 cm the sediments observed were denser with fibrous root material. From 20-50 cm the sediments were grey, extremely dense and clay rich, with some root material.

The FRESH C2 core comprised grey, loose peat in the upper 5 cm, with large amounts of fibrous root material in silt from 5 to 20 cm. The bottom 30 cm was made up of a dense, grey, clay-rich sediment with a lot of fibrous plant material.

The core obtained at FRESH C3 comprised organic-rich peat in the upper 10 cm that was grey in
color and included decomposing plant matter and larger root and rhizomes fragments. From 10 to 50 cm depths the sediment was a dense, greyish-brown, impermeable clay.

**TE**

Cores obtained at the TE site were taken from regions with variable vegetation and marsh sediment. The core obtained at TE C1 was from a primarily unvegetated area with some living *Salicornia* and dead *Typha*. The upper 3 cm of the core was highly organic peat and decomposing plant material, from 3-20 cm the sediments were dark brown, peat with fibrous root material and rhizomes. From 20-25 cm the sediments transitioned from silt to greyish-brown clay. The lower 25 cm comprised brownish-grey clay with fibrous root material.

The TE C2 was obtained in an area dominated by dead *Typha* stands with some living *S. alterniflora* and *Salicornia*. The upper 10 cm of the core was composed of highly decomposed, dark-brown peat, with lenses of silt and thick roots. From 10-22 cm the sediments were silty and dark brown, transitioning to grey, fibrous clay from 22-40 cm. The lower 10 cm of the core was predominantly thick, grey clay with large amounts of roots and rhizomes.

Lastly, the TE C3 core was obtained in a region with 50% living *Typha* and 50% dead *Typha*. The upper 10 cm was silty, organic-rich, dark brown sediment with decomposing *Typha* stalk fragments. From 10-40 cm the sediments were dark-brown, organic rich peat with some clay. The lower 10 cm was predominantly brownish-grey clay with large roots.

**TW**

Cores obtained at collars in the TW site were also retrieved from diverse collar sites. The TW C1 core was obtained at a region dominated by dead *Typha* vegetation. The upper 5 cm of the core was organic-rich, dark brown peat with decomposing *Typha* fragments. From 5 to 12 cm the sediments transitioned to denser, greyish-brown sediment with fibrous root material and rhizomes. From 12 to 25 cm the core comprised extremely dense, light brown, impermeable clay. The clay was so dense the remaining 25 cm of the core could not be obtained (note n=2 for TW C1).

The TW C2 core was obtained in a location dominated by *Scirpoides holoschoenus* and *Salicornia* vegetation. The upper unit of the core was primarily dark brown, silty peat with some woody fragments and small roots. From 14 to 48 cm the sediment was dark-brown, organic matter with large roots and partially-decomposed woody fragments, and no evidence of clay. The deepest 2 cm of the core indicated a transition to grey, clay-rich sediment.

The core obtained at TW C3 was taken in a primarily unvegetated region with some live *Scirpoides holoschoenus* and dead *Typha*. The upper 10 cm was dark-brown peat, transitioning to dark-brown, organic-rich sediment with large amounts of roots and rhizomes. The lower unit was dominated by decomposing roots and brown organic material.
3.3.2 δ¹³C

For sites along the normal salinity gradient δ¹³C signatures reflect overlying vegetation to a large degree and suggest consistency in plant carbon source with depth. SAL δ¹³C values hovered around -15 ± 1.1‰ throughout core depth with little variability among collar sites (Figure 3.5a, Table 2). The SAL δ¹³C values are dominated by C4 signatures reflecting the overlying Spartina species. There was only slight variability among FRESH cores (± 4.5‰) ranging from -27.4‰ in the upper 10cm, gradually increasing to -24‰ at depth (Figure 3.5 c). FRESH δ¹³C signals reflect the predominance of C3 plants such as Typha, at the site. More variability was observed among cores at the BRACK site, which revealed δ¹³C signature ranges for both C3 and C4 plants (range of 25.2‰ to -15.6‰; average -20‰; Figure 3.5b). Cores at BRACK C1 and C2 exhibited ranges of -25.2‰ to -20.7‰, a combination of C3 and C4 signals, reflecting overlying J. gerardii and S.patens. The δ¹³C values of the BRACK C3 core were dominated by C4 plant signatures (range -19.6‰ to -15‰), reflecting overlying S.patens. The disparity in δ¹³C signals among collar sites at BRACK suggests mixed plant source throughout the site.

The δ¹³C values for cores at the transitional sites indicate notable shifts in plant carbon source through the sediment record, increasing in δ¹³C with depth. Average δ¹³C values at TW and TE both show an overall increase with depth, with largest shifts occurring between 20 and 30 cm (Figure 3.5 d and e). δ¹³C values of the TE cores averaged -24.0‰ in the upper 10 cm, increasing to -18.6‰ by 50 cm. All three TE cores show a similar trend, but vary ±1.6 to 2.9‰. Much higher variability was observed among TW collar sites amounting to ± 4.5‰ at 25 cm. This variability is largely influenced by the anomalous core at TW C3 due to its dense, clay-rich impermeable sediment. δ¹³C values were only possible to obtain in the upper 30 cm of this core, revealing a δ¹³C range from -25.2 to -24.8‰, which is depleted relative to TW C2 and C3 (range -24.25 to -16.8‰ in the upper 30 cm; Figure 3.5e). This inconsistency is likely attributable to differences in source plant and matrix characteristics. Generally, cores C2 and C3 display similar trends, showing most enriched δ¹³C signals at 25 cm depth (-9.1‰ and -16.8‰ at C2 and C3, respectively; Figure 3.5 d and e), with a slight depletion to 50 cm to -22.7‰ and -21.3‰ at C2 and C3 respectively. Shifts are more apparent at cores from C2 and C3 than C1. We hypothesize that the shifts in δ¹³C signals are indicative of vegetation response to perturbation of the marsh ecosystem.

Table 3.3: Average δ¹³C from 0-50 cm from cores at each site, in triplicate

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>SAL δ¹³C</th>
<th>StDev</th>
<th>BRACK δ¹³C</th>
<th>StDev</th>
<th>FRESH δ¹³C</th>
<th>StDev</th>
<th>TE δ¹³C</th>
<th>StDev</th>
<th>TW δ¹³C</th>
<th>StDev</th>
</tr>
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<tbody>
<tr>
<td>0-10</td>
<td>-15.9</td>
<td>1.1</td>
<td>-22.1</td>
<td>2.2</td>
<td>-27.4</td>
<td>0.8</td>
<td>-24.0</td>
<td>1.6</td>
<td>-24.8</td>
<td>0.4</td>
</tr>
<tr>
<td>10-20</td>
<td>-15.5</td>
<td>0.4</td>
<td>-21.9</td>
<td>3.8</td>
<td>-25.5</td>
<td>0.7</td>
<td>-23.4</td>
<td>1.3</td>
<td>-24.2</td>
<td>0.7</td>
</tr>
<tr>
<td>20-30</td>
<td>-15.6</td>
<td>0.2</td>
<td>-21.9</td>
<td>4.5</td>
<td>-25.9</td>
<td>0.8</td>
<td>-21.0</td>
<td>2.6</td>
<td>-20.3</td>
<td>4.3</td>
</tr>
<tr>
<td>30-40</td>
<td>-16.2</td>
<td>0.6</td>
<td>-21.0</td>
<td>4.7</td>
<td>-23.3</td>
<td>0.7</td>
<td>-18.8</td>
<td>1.9</td>
<td>-20.0</td>
<td>0.1</td>
</tr>
<tr>
<td>40-50</td>
<td>-15.3</td>
<td>0.3</td>
<td>-19.6</td>
<td>2.5</td>
<td>-24.3</td>
<td>1.6</td>
<td>-18.6</td>
<td>2.9</td>
<td>-22.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Figure 3.5: δ¹³C for upper 50 cm of sediment cores taken at all three collars for all five sites. Data show consistent signal with depth along the normal salinity gradient (a, b, and c), and shift in signal with depth in cores from transitional sites (d and e).
3.3.3 % Carbon

Total %C of the core sediments was found to be low at the sites along the normal salinity gradient (range 5.1 to 10.5% for SAL, BRACK and FRESH; Figure 3.6 a-c) compared to core sediments from the transitional sites (range 11.4 to 36.2% for TE and TW; Figure 3.6 a and b; Table 3.3; Appendix D). Narrow variability for sites along the normal salinity gradient was observed; ±4.1%, 3.9% and 3.3% for SAL, BRACK and FRESH respectively. A slight decrease in average %C with depth was observed for all normal transect sites, with largest %C decreases in the upper 10cm (Figure 3.6 a-c). Decreases in %C with depth occurs due to decomposition and respiration, as well as inputs from inorganic sources.

The % carbon of cores within each transitional site were higher than the normal salinity gradient transect, and highly variable (±10.6% and 16.3% for TE and TW, respectively). Average %C at TE showed an initial increase in %C in the upper 15 cm, with a rapid decrease between 15 and 25 cm depths. TE C3, however, showed exact opposite trends, with a rapid decrease to 15 cm, then a gradual increase in %C. Much like $\delta^{13}C$ values for the TW site, C1 increased the variability among collar sites, while C2 and C3 followed similar trends, increasing in %C with depth (Figure 3.6e).

Average profiles for $\delta^{13}C$ (Figure 3.5) and %C (Figure 3.6) at all five sites show opposing trends, suggesting an inverse between the two parameters. These trends are most apparent in sites and TE and TW, suggesting decreased %C with enrichment in $\delta^{13}C$ values. If this apparent correlation is significant, it may suggest that C4 plants are associated with lower %C, thus lower decomposition rates.

Table 3.4: Average %C from 0-50 cm from cores at each site

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>SAL %C</th>
<th>StDev</th>
<th>BRACK %C</th>
<th>StDev</th>
<th>FRESH %C</th>
<th>StDev</th>
<th>TE %C</th>
<th>StDev</th>
<th>TW %C</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>9.6</td>
<td>4.1</td>
<td>10.5</td>
<td>3.9</td>
<td>6.6</td>
<td>2.3</td>
<td>18.9</td>
<td>6.1</td>
<td>20.0</td>
<td>6.1</td>
</tr>
<tr>
<td>10-20</td>
<td>6.4</td>
<td>2.7</td>
<td>6.3</td>
<td>2.6</td>
<td>5.3</td>
<td>2.0</td>
<td>19.4</td>
<td>1.0</td>
<td>16.4</td>
<td>9.6</td>
</tr>
<tr>
<td>20-30</td>
<td>6.1</td>
<td>1.3</td>
<td>5.6</td>
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<td>1.8</td>
<td>5.4</td>
<td>2.1</td>
<td>2.7</td>
<td>0.8</td>
<td>12.3</td>
<td>8.6</td>
<td>35.1</td>
<td>7.7</td>
</tr>
<tr>
<td>40-50</td>
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<td>2.4</td>
<td>5.6</td>
<td>1.6</td>
<td>3.7</td>
<td>3.3</td>
<td>13.3</td>
<td>10.2</td>
<td>36.2</td>
<td>9.9</td>
</tr>
</tbody>
</table>

All raw $\delta^{13}C$ and %C data are presented in Appendix D.
Figure 3.6: %C for upper 50 cm of sediment cores taken at all three collars for all five sites. There is a slight decrease with depth at sites along the normal salinity gradient (a, b, and c), and variability with depth in cores from transitional sites (d and e). Overall, transitional sites show higher %C than those along the normal salinity gradient.
3.4 Tea Bag Index

The Tea Bag experiment was conducted during a 90 day period where soil salinity temperatures ranged from 22.7 °C at burial to 10.1°C at retrieval, with mean monthly temperatures presented in Figure 4.1. The data show that % mass loss for green tea over a 90 day period was lowest at the most saline site (49.7%) and higher at the BRACK and FRESH sites, with mass loss of 54.5% and 53.1 % respectively (Table 5; Appendix E). Significant variability in %C loss was observed at all sites and was largest at the FRESH site (± 11.1). Largest loss in carbon for green tea was observed at the FRESH site averaging a 20.1% decrease.

The % mass loss was significantly lower for rooibos tea compared to green tea for all samples. Largest losses in mass were observed at the BRACK site (18.5%) followed by the FRESH site (16.8%). Highest decreases in %C were observed at the FRESH site, followed by the SAL site (10.4% and 10.1%, respectively). For both green and rooibos tea, the FRESH site seemingly holds mechanisms that contribute the largest breakdown of carbon relative to BRACK and SAL sites.

<table>
<thead>
<tr>
<th>SITE</th>
<th>GREEN</th>
<th>ROOIBOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Mass Loss</td>
<td>% Carbon Loss</td>
</tr>
<tr>
<td>SAL</td>
<td>49.7</td>
<td>15.2</td>
</tr>
<tr>
<td>BRACK</td>
<td>54.5</td>
<td>9.6</td>
</tr>
<tr>
<td>FRESH</td>
<td>53.1</td>
<td>20.1</td>
</tr>
</tbody>
</table>
4
DISCUSSION
4.1 CH₄ Emissions

4.1.1 Influence of Season and Temperature

Over the course of the three-month sampling period we found season and related soil temperature to be the primary drivers of methane emission observed at Long Marsh. Largest fluxes at each site occurred during the months of July and August compared to October (Figure 3.4). Atmospheric temperatures from the Augusta, ME weather station in July, August and October averaged 20.3°C, 16.7°C, and 8.9°C, respectively (Figure 4.1; National Weather Service, 2015). Measured soil temperatures at Long Marsh for July, August and October were 20.3 °C, 22.7 °C and 10.1 °C, respectively (Figure 4.1). Both atmospheric and soil temperatures correlate to general flux trends for each sampling month. According to Zeikus and Winfrey (1975), in the presence of proper nutritional requirements, methanogens are metabolically active between 4°C and 45 °C (Le Mer and Roger, 2001).

Additionally, the physical condition of plants changes with season. Plant biomass is higher and more prolific in July and August relative to October, when plants begin to transition to dormancy. Aerenchyma structures require upstanding macrophytes to maximize gas transport from the root system. During dormancy, aerenchyma lose some of their structure and are less erect, decreasing CH₄ transport (Shih and Finkelstein, 2008). Several Typha plants in sampling collars transitioned to dormancy by the October sampling period, which likely slowed this transport mechanism.

Precipitation is an additional factor that often fluctuates with season and can influence CH₄ production; increased precipitation can flush soils and sediments, and dilute concentrations of available substrates for methanogens (Kelley et al., 1988). Based on monthly precipitation values from the Augusta, ME weather station, rainfall for July, August and October of 2015 was recorded to be 7.0 cm, 11.5 cm and 15.8 cm, respectively (Figure 4.1). We observed overall CH₄ fluxes to be higher in July for all sites excluding FRESH, even though average monthly precipitation was 4.5-8.8 cm less. If precipitation was a driving variable, we would expect to see lowest CH₄ fluxes in July, and higher fluxes in August and October. Precipitation values do not corroborate our dilution hypothesis for this particular study, but may contribute to flux changes in scenarios with substantial monthly precipitation variability.

4.1.2 Relationship between Salinity and CH₄ Fluxes

Observed CH₄ fluxes along the normal salinity gradient transect confirm the negative correlation between CH₄ and salinity proposed by Poffenbarger et al. (2011; Figure 4.2). Salinity levels lower than 18-20 ppt (also psu, as measured on Long Marsh) show methane fluxes, with increasing emissions and variability as salinity decreases. Based on Long Marsh data, the threshold for methane emissions is 18 ppt, showing CH₄ fluxes < 0.54 g CH₄/m²/yr with porewater salinities >18 ppt (Figure 4.2). This is in strong agreement with Poffenbarger et al.’s (2011) findings. This relationship is likely driven by concentrations of SO₄²⁻ present, which is positively related to salinity in seawater (Bridgham et al., 2000; Poffenbarger et al., 2011; Robenhorst, 2001). Sulfate brought in by tides catalyzes sulfate reduction in
marsh sediments inhibiting methanogenesis (Poffenbarger et al., 2011). Therefore, the absence of sulfate in FRESH and BRACK regions (below 18 psu) results in methanogenesis due to lack of competition by other microbes (Bartlett et al., 1985; Mitsch et al. 2010). This relationship could further be verified by measuring SO$_4^{2-}$ concentrations during CH$_4$ flux sampling.

Figure 4.1: 2015 daily temperature (orange) and precipitation (blue) from the Augusta, ME weather station (July-October) show seasonal trends; data obtained from the National Weather Service Forecast Climate Report, 2015. Measured soil temperatures from Long Marsh in Jul, Aug, Oct (green) show slightly higher soil temperature compared to atmosphere.
4.1.3 CH₄ Uptake

Negative fluxes were observed during the October sampling period, indicating additional microbial processes steering CH₄ emissions. Methanotrophy, a form of methane oxidation, is currently best understood in aerobic environments, but Chowdhury and Dick (2013) and others report evidence of partial CH₄ oxidation in marine anoxic sediments and submerged soils (Caldwell et al., 2008; Zehnder and Brock, 1980). The mechanism for these processes involves upward diffusion of methane produced in deep sediment that is then consumed by methane-oxidizing prokaryote populations called methanotrophs. Sulfate-driven anaerobic oxidation of methane is mediated by a syntrophic consortium of methanotrophs and sulfate reducing bacteria, utilizing sulfate as a terminal oxidant.

According to Valentine and Reeburgh (2000), this process is estimated to consume 5-10% of the net atmospheric CH₄ flux, which would result in negative fluxes in our sites. Like methanogenesis, methanotrophy varies with season as a function of temperature and shifts with plant communities. Working hypotheses by Kevbrina et al. (2000) suggest that methanotrophs are less sensitive to temperature than methanogens, and thrive in cooler environments than methanogens, with relatively stable rates of methanotrophy between -1°C and 30°C (La Mer and Roger, 2001). In October, marsh sediment temperatures ranged from 10°C -10.6 °C and 40% of fluxes determined were negative,
suggesting methanotrophic processes dominate methanogenesis in colder months. Further studies need to be conducted to understand hierarchical interactions of microbial processes at play in order to quantify effects of methanotrophy on overall CH$_4$ production.

4.1.4 Expected Annual Trends

Emery and Fulweiler (2014) found similar seasonal CH$_4$ flux trends to ours on Long Marsh during the months of July, August and October (Figure 1.4; Figure 3.4). Large emissions were observed in July, followed by August, and substantially lower fluxes were observed in October. This study found a mean flux range of -11 to 54 μmolCH$_4$/m$^2$/hr for all sampling periods, except May, which appears to be an outlier. Emery and Fulweiler (2014) determined fluxes from S. alterniflora and P. australis vegetation (freshwater). We compared findings, assuming conditions and response from S. alterniflora are similar in both studies, and that P. australis and T. latifolia have comparable salinity tolerances and methane transport mechanisms. However, numerical fluxes are not similar. Contrary to our study’s findings, S. alterniflora was associated with higher fluxes than P.australis, a freshwater plant. However, our monthly relationships of large fluxes during July and August, and low or negative fluxes in October shadow Emery and Fulweiler’s (2014) findings. Based on these similarities, we deduce that if projected over a 12 month period, similar general monthly trends (relative to one-another) would be found at Long Marsh, excluding May.

Emery and Fulweiler (2014) found over half of the measured fluxes to be zero or below detection limits, which we would expect on Long Marsh if sampling spanned a 12-month period. They found negative fluxes during months of October, January and March; they attribute negative fluxes to the methanotrophic activity. Emery and Fulweiler (2014) focused solely on vegetated and unvegetated plots of S. alterniflora and P. australis, while Long Marsh has much more diverse and variable vegetation, which may contribute to the observed differences in CH$_4$ production. Thus, large assumptions are made in comparing studies; in order to determine representative annual fluxes on Long Marsh, indicating data for all 12 months must be obtained.

4.1.5 Organic Matter Decomposition

Keuskamp et al. (2013) found that temperature is a driver of TBI, concluding higher temperatures result in higher rates of decomposition. They measured TBI at two temperatures, 15°C and 25°C using forests soils in a controlled setting. We related our TBI findings by plotting them on the curve obtained by Keuskamp et al.’s (2013) study, assuming a mean soil temperature of ~15.8°C based on average daily temperatures in July through October from the Augusta, ME weather station and measured soil temperatures on Long Marsh (Figure 4.3; Figure 4.1). We observed slightly lower average mass loss compared to Keuskamp et al. (2013), possibly due to frequent, minute temperature fluctuations daily and monthly, as well as differences in soil biogeochemistry in marsh soils versus forest soils. Keuskamp et al. (2013) did not analyze salinity as a variable, however, assessing TBI for each site relative to one-another indicates lower rates decomposition over a 90 day period at the SAL site compared to BRACK and FRESH (Figure 4.3). This then suggests lower microbial activity in higher salinity environments. These
findings are loosely supported by %C data where we found lower %C values in the upper 10cm for the FRESH site (%6.6) compared to SAL and BRACK sites (average 9.6% and 10.5%, respectively; Table 3.2). %C loss from tea bags at these sights follows similar trends (Table 3.5). These data suggest higher decomposition in lower salinity environments. Since decomposition processes produce the substrates utilized by methanogens in methanogenesis, these data provide further evidence that low salinity (high decomposition) is associated with higher methane production.

No data were collected for TBI at the transitional sites on Long Marsh, but based on the sediment characteristics, we expect to see fairly low rates of decomposition at these sites. The %C of the upper 10cm of cores for TE and TW were 18.9% and 20.0%, respectively. These values are high compared to the normal salinity gradient sites, suggesting less decomposition present to break down buried organic matter.

![Figure 4.3: Mass loss of tea bags at SAL, BRACK and FRESH displayed as average relative mass remaining for green and rooibos tea bags at each site (n= 5 for each tea type). Lowest mass loss at SAL for both types of tea. Long Marsh data plotted against Keuskamp et al.’s (2013) findings for green and rooibos tea mass loss at 15°C and 25°C.](image-url)
4.2 Sources of Buried Organic Matter

4.2.1 Shifts in Vegetation Based on $\delta^{13}$C

Though carbon isotope analyses of sediment cores, we confirm that surficial plant communities dominate the carbon source of sediments at sites along the normal salinity gradient. The $\delta^{13}$C values observed for C4 and C3 plants at SAL and FRESH, respectively, indicate *Spartina* and *Typha* communities are long established in these corresponding regions. The presence of both C3 and C4 signals that comprised the BRACK sediment cores suggest that both *Spartina* and *J. gerardii* have extensively dominated this region. Consistency of $\delta^{13}$C throughout the sediment record for all three of these sites, suggests firmly established overlying vegetation communities.

Transitional sites reveal shifts in carbon source within the sediment record, likely due to the encroachment of *Typha* onto the marsh platform. The upper 10 cm surface sediments from TE cores are dominated by C3 plant signatures (average -24.0 ‰) reflecting the overlying *Typha* debris; these values become more enriched at depth to reflect $\delta^{13}$C values closer to C4 plant signals (average -18.6 ‰ at 50 cm; Figure 3.4d). TW cores follow a similar trend with enriched $\delta^{13}$C values at depth relative to the surface (Figure 3.4 e). At both transitional sites, the most significant shift in $\delta^{13}$C occurs between 20 and 30 cm depths.

Interpretations based on these preliminary data find that this $\delta^{13}$C enrichment at approximately 25 cm depth represents the period of time when C3 plants inundated the marsh platform as tidal restriction and freshwater influence initiated. A thorough chronology of the sediments would facilitate a more comprehensive understanding of the organic matter replacement and turnover on Long Marsh. Additionally, further data including time of infrastructure construction, rates of accretion and the rooting depth of *Typha* are needed to substantiate this hypothesis.

4.2.2 Relative Marsh Age

Based on sediment core descriptions, we deduce that Long Marsh is experiencing transgression of sea level and has migrated inland over time. For all cores, we observed an abrupt shift from clay to peat, indicating the point in the sediment record where marsh sedimentation and accretion originated (see section 3.3.1). In comparing thickness of the peat units observed for the various sites, the northernmost sites are thicker than the most inland regions. Listed from northernmost to southernmost sites, peat horizon ranged from 13-30cm at SAL, 10-40cm at TE, 5-14cm at TW, 5-30 cm at BRACK, and 5-10 cm at FRESH. Varying rates of decomposition, the mobilization of carbon, and variable degrees of mineral deposition contribute to this apparent trend in peat thickness. However, thicker peat generally indicates a longer-lived marsh platform. Basal $^{14}$C ages on the peat, sedimentation rates, and accretion models are needed to corroborate this notion.
4.3 Restoration

4.3.1 CH₄ Projections from Vegetation Mapping

Typha-dominated regions exhibited largest CH₄ fluxes with the most variability, which we surmise is primarily due to depleted SO₄²⁻ concentrations facilitating methanogenesis, and to its transport through aerenchyma and ebullition. Since Typha is highly adaptive and quickly invades the marsh platform in areas of low salinity, the migration of Typha stands is an indicator of increased CH₄ emissions from the marsh. Based on these associations, marsh response to restoration in the context of CH₄ emissions can theoretically be estimated using Typha vegetation mapping. We deduce that our calculated average fluxes projected over Typha-dominated (FRESH) regions on Long Marsh pre and post restoration will provide an estimate of changes in methane emissions in response to altered tidal flow. Such estimates are founded on the assumption that Typha is the dominant marker for CH₄ emissions. Since this assumption does not incorporate CH₄ emissions from other types of marsh vegetation, these Typha-based projections are more successfully used to quantify relative changes over time, rather than total fluxes.

The CBEP runs a monitoring program for vegetation change on Long Marsh and provides a map of the area of Typha stands 6 months before the restoration (July 2013) and 15 months after (July 2015; Figure 4.4). Before restoration, 3.37 ha of Typha was present on the marsh platform as a result of over 100 years of tidal restriction. This area decreased to 0.26 ha by July of 2105- a 92% dieback of Typha in just 15 months. They also observed salinity increases from 8 psu to 26 psu at site TE. Based on these responses to a simple tidal reintroduction project, Long Marsh can be considered a case study of successful restoration.

![Figure 4.4: Area of Typha stands pre (2013) and post (restoration) mapped by the Casco Bay Estuary Partnership. Map shows a 92% decrease in Typha area from 3.37 ha to 0.26 ha due to restoration.](image)

We use a calculated average flux of $61.8 \pm 33.2 \, \mu\text{molCH}_4/\text{m}^2\text{hr}$ (from all 3 months, n=9) from the Typha dominated FRESH collar sites to project CH₄ fluxes over a three month period. Using dieback data from the CBEP presented in Figure 4.4, we calculate that with the 92% loss in Typha 15 months post-restoration, there was a decrease from ~75000 gCH₄ to ~5700 gCH₄ ± 3000gCH₄.
Calculations were based on the following:

Convert $\mu$mol CH$_4$/m$^2$/hr to g CH$_4$:

$$
\frac{61.8 \ \mu\text{mol CH}_4}{m^2 hr} \times \frac{1 \ \text{mol}}{10^6 \ \mu\text{mol}} \times \frac{16.04 \ g}{1 \ \text{mol CH}_4} = \frac{9.9 \times 10^{-4} \ g \text{CH}_4}{m^2 \cdot hr}
$$

Project over the three months,

$$
\frac{9.9 \times 10^{-4} \ g \text{CH}_4}{m^2 hr} \times 2232 \ \text{hr (in 3 months)} = \frac{2.2 \ g \text{CH}_4}{m^2}
$$

Project over area (before restoration, 2013):

$$
\frac{2.2 \ g \text{CH}_4}{m^2} \times \frac{10^4 \ m^2}{1 \ \text{ha}} \times \frac{3.37 \ ha}{7.5 \times 10^4 \ \pm \ 4.0 \times 10^4 \ g \text{CH}_4 \ \text{before}}
$$

$g$CH$_4$ before - $g$CH$_4$ after = 75000 gCH$_4$ - 5700 gCH$_4$ = 69000 gCH$_4$, no longer emitted

The calculated uncertainty amounts to 53%, and is applied to the resultant gCH$_4$ emitted, thus the CH$_4$ flux decrease is substantial enough to significantly impact net carbon sequestration regardless of the uncertainty applied.

Based on Long Marsh’s response to tidal reintroduction as a result of a simple restoration project, it is clear that vegetation on marshes responds positively to restoration. Simple steps such as culvert replacement can be made to mitigate current greenhouse gas contributions on tidally restricted marshes. Findings by Burdick et al. (1996) found salinity increases of 20 ppt and dramatic vegetation changes (~50% dieback of freshwater vegetation in 9 months). Burdick et al. (1996) observed that the reduced flood frequency, salt and sediment exchange, decreased elevation, and decline in fish populations caused by tidal restriction were alleviated within 8 years post-restoration. Based on similar vegetation and salinity responses on Long Marsh in the first 15 months, we expect reclamation progression as projected by Burdick et al. (1996) in Figure 1.6 to occur on Long Marsh.

### 4.3.2 Carbon Density and Preliminary Carbon Stocks

Understanding the carbon density of marsh sediments provides information as to how a marsh might respond to various other perturbations, including gradual rise in sea level. Carbon density can be used to project carbon stocks for a given area of marsh. Also, quantifying total carbon stocks of wetlands can be used to place value on wetlands as carbon sinks, which may have applications in environmental economics. Average carbon density was calculated based on average dry bulk density over the upper 50 cm (averaged from all sites) and %C for cores at each (Table 4.1).
For carbon stock calculations we used averages from the sites along the normal salinity gradient (SAL, BRACK, FRESH) as they are more heavily represented on the marsh and less variable than transitional locations. Note that this results in a modest carbon stock estimate because the most carbon-dense sites are unrepresented. Carbon density from the three sites averaged 0.034 gC/cm$^3$ ± 0.004 gC/cm$^3$ (Table 4.1), which translates to a carbon stock of 170 ± 20 Mg C/ha for the upper 50 cm. This projected for the entire study area of ~11 ha (CBEP) totals 1870 ± 220 MgC stored in the upper 50 cm for all of Long Marsh based on the calculation:

$$\frac{61.8 \mu\text{mol CH}_4}{m^2 \text{hr}} \times \frac{1 \text{ mol}}{10^6 \mu\text{mol}} \times \frac{16.04 g}{1 \text{ mol CH}_4} \times \frac{9.9 \times 10^{-4} g\text{CH}_4}{m^2 \times \text{hr}} = 170 \frac{Mg C}{ha} \times 11 \text{ ha} \approx 1870 \pm 220 MgC$$

The mean carbon stock for tidal salt marshes determined by the IPCC (2013) is 255 Mg C/ha for one meter of depth. Although our calculations provide only a rough estimate of carbon stocks on Long Marsh, we obtained values only slightly lower than IPCC published data. Note that our values are calculated for the upper 50 cm rather than 1 meter, so the carbon stocks are not directly comparable. These estimates do confirm that Long Marsh stores carbon in its sediments, and with a carbon density similar to other marshes in Maine (0.030-0.070 gC/cm$^3$ at Sprague Marsh, Phippsburg, ME; Pickoff, 2013). However, our projections can only provide preliminary values for the upper 50 cm of sediment on Long Marsh. These measurements are based on a small sample size and assume consistent vegetation, carbon density and burial rates. These assumed conditions are rarely observed on salt marshes, including Long Marsh. Further studies must be conducted in order to obtain a more representative carbon stock for Long Marsh.

<table>
<thead>
<tr>
<th>SITE</th>
<th>Avg C Density (gC/cm$^3$)</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>0.031</td>
<td>0.005</td>
</tr>
<tr>
<td>BRACK</td>
<td>0.038</td>
<td>0.01</td>
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<td>FRESH</td>
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<td>0.05</td>
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<tr>
<td>TW</td>
<td>0.060</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### 4.4 Future Work

The purpose of this study was to determine CH$_4$ emissions along a salinity gradient on a transitional salt marsh in order to begin quantifying marsh response to restoration. CH$_4$ fluxes, TBI and carbon isotope analyses are consistent with the conclusion that tidal reintroduction inhibits the emission of CH$_4$, and that vegetation is an environmental indicator of these changes. However, this study is a pilot project that only spans 3 months of field sampling, and minor alterations to the methods and future work can help inform and fortify the current relationships identified. Further analyses throughout growth
season are needed to understand the relationships between plant productivity and methane transport via aerenchyma and diffusion.

Regarding field sampling using static gas chambers, we would like to assess the impact of isolating marsh areas using metal collars during sampling. The insertion of the collar irrefutably cut through roots and rhizomes, killed some plants, and perturbed the marsh sediment and surrounding biology. Some of the vegetation at the collar rim was seriously affected and may have altered methane emission. To test this, we employed a preliminary comparison during the October sampling period where we measured CH$_4$ fluxes at the FRESH C2 site side by side, one chamber using the collar and another without. Data suggest that CH$_4$ emissions were slightly higher at the chamber without the collar (Appendix B) This may be due to more active plant populations in the collar, and less perturbation to the subsurface. Additionally, a tight seal on the collar was a source of uncertainty at all sites, which was found negligible for the noncollar chamber sampling. Although the collars were given at least 48 hours to equilibrate, we conclude that not using collars may result in more accurate, representative gas samples. The challenge in this alternative method would be determining a technique that would ensure consistent site location for replication. Further work comparing methods must be done to validate this hypothesis.

In assessing the effect of chamber height and volume variation on CH$_4$ concentrations, no apparent impact was observed between 0.45 m$^2$ and 0.61 m$^2$ chambers. Further work would need to be done to study the impact of the 1.542 m$^2$ chambers, as these were only used for sampling FRESH sites. Sampling time is also a factor that should be assessed. CH$_4$ concentration readings over 40 minutes provided adequate flux readings, however, increased sampling interval over a longer sampling time may provide more accurate flux data. Additionally, abrupt changes in shade, humidity and temperature over the course of a sampling period should be evaluated for impact on CH$_4$ concentrations.

In order to determine more representative annual CH$_4$ fluxes, sampling must be executed the same time each month for all 12 months of the year, allowing for proper analysis of seasonal influence as well as a more representative average flux for Typha-dominated regions for annual projections. Accordingly, additional replication at each site and an increased number of sites along the salinity gradient would decrease uncertainty.

The determination of sedimentation and accretion rates, and CO$_2$ fluxes in conjunction with CH$_4$ fluxes, would also allow for a better understanding of carbon cycling in this marsh. Quantifying such stocks and ecosystems services in the context of economic benefit or loss would serve to inform and motivate further restoration projects.

In order to properly assess the complete marsh response to restoration, the study could have been modeled after Burdick et al. (1996) where sediment analyses, fish population data, elevation data, and salinity levels were obtained before restoration. Our study did not have access to these pre-restoration conditions, however, based on comparisons, we come to similar conclusions as Burdick et al. (1996) regarding reintroduction of tidal flows in using CBEP and SAL sites as pre-restoration references.
5

CONCLUSION
CH$_4$ fluxes determined at sites along a normal salinity gradient, and one across a transitional region of Long Marsh suggest the reintroduction of healthy tides inhibits methane production and emission. Highest and most variable CH$_4$ fluxes were observed at the least saline site with *Typha* vegetation, while lowest CH$_4$ fluxes with least variability were observed at the most saline sites with *Spartina* vegetation. These data suggest an inverse relationship between CH$_4$ flux and salinity due to SO$_4^{2-}$ concentrations with a <18 ppt salinity threshold for methane emissions, as proposed by Poffenbarger et al. (2011). This relationship is attributed to interactions between sulfate reducing bacteria and methanogens in marsh sediments, where sulfate reduction outcompetes methanogenesis with sufficient availability of SO$_4^{2-}$. Additionally, we conclude that season has a large influence on methane emissions, likely due to the interplay of temperature, plant dormancy and methanotrophy. Based on the determination of carbon source based on $\delta^{13}$C, we conclude that surface vegetation is an indicator of salinity, and thus methane emissions on site. %C and decomposition further add to these findings by indicating an inverse relationship between decomposition and salinity. *Typha* was found to be a direct marker for methane emissions and can be used as a proxy to project annual CH$_4$ emissions from a marsh area. Based on the 92% dieback of *Typha* after 15 months of tidal reintroduction mapped by the CBEP, we conclude that Long Marsh has experienced a substantial decrease in the amount of CH$_4$ emitted since restoration, equating to ~36000 gCH$_4$ per 3 months. Centered on Long Marsh’s response to restoration, we conclude that tidal reintroduction is highly effective at re-establishing healthy salinity gradients and brackish marsh vegetation, thus inhibiting methane production.

As a pilot project, this study was effective in its aims to quantify CH$_4$ emissions using a static gas chamber sampling method. We found that this method was successful in capturing CH$_4$ samples, however uncertainty could be minimized by small alterations in the static chamber design, particularly regarding the use of collars. Sediment core analyses corroborated findings relating methane fluxes to both salinity and vegetation, and provided belowground carbon source signatures that confirmed hypotheses regarding vegetation shift from *Spartina* (C4) to *Typha* (C3) as a result of tidal restriction. In addition to modified sampling methods and larger sampling sizes, this study would benefit from a better understanding of accretion rates and carbon sequestration on the marsh. Additionally, pre-restoration monitoring of CH$_4$ fluxes and rates of decomposition is necessary in order to fully understand the scope of restoration.

As highly productive, dynamic and responsive ecosystems that provide a plethora of ecosystem services, the restoration and management of salt marshes is critical. This study contributes to a pool of scientific evidence that suggests tidal restriction of salt marshes contributes to the natural emission of CH$_4$. It is important to maintain the balance of marsh ecosystems and reclaim those that have been altered because their carbon sequestration capabilities may be critical in the search for climate change solutions. Tidal restriction is rampant throughout Casco Bay and the Gulf of Maine, and may be alleviated through simple and effective culvert removal projects, as observed on Long Marsh.
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