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Nitrogen isotopes in Zostera marina: tracking anthropogenic nitrogen in Casco Bay, Maine.

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Nitrogen isotopes in *Zostera marina*: tracking anthropogenic nitrogen in Casco Bay, Maine.

A Senior Thesis

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Abstract

An influx of nutrients into any aquatic system can disturb its ecological balance and cause eutrophication. This is a process that involves a rapid increase in the population of certain primary producers which causes a reduction in available oxygen to be used by other organisms. Previous studies have used stable nitrogen isotope ratios ($\delta^{15}N$) in eelgrass, *Zostera marina*, to better monitor the distribution of anthropogenic nutrients in Casco Bay, Maine. This paper investigates the $\delta^{15}N$ of eelgrass growing at three different sites within Casco Bay (East End Beach, Clapboard Island, Chebeague Island) each at increasing distance from the East End wastewater treatment facility in Portland, Maine, an area of high human activity. There is no statistically significant difference among $\delta^{15}N$ values of eelgrass from all three sites and the average $\delta^{15}N$ values is 6.38 ± 0.40. These values are relatively low and suggest minimal nitrogen input from the East End wastewater treatment plant. Furthermore, there appears to have been a ~1.8‰ decrease in $\delta^{15}N$ values of eelgrass in the south Casco Bay area since 2011 perhaps reflecting that the East End wastewater treatment plant reduced its nitrogen load by ~60-70% in 2017 (A. Brewer; Levin et al., 2019). There is a positive correlation between DIN and chlorophyll concentrations in water from surrounding rivers. Similar research could be used to easily identify areas receiving anthropogenic nitrogen and thus implement changes to prevent the damaging effects of eutrophication and nutrient loading.

1. Introduction

1.1 Summary of the Project

The Casco Bay is an estuary located in the southern coast of Maine. It's biodiversity and ability to sustain nearby civilizations makes the entire watershed one to protect. It supports about 850 species of marine life and contains about 8,200 acres of eelgrass beds (CBEP, 2021). Estuaries act as aquatic boundaries of brackish water that exist between freshwater and saltwater systems (CBEP, 2021).

Nitrogen is an important element that sustains all living organisms. It is the major element in the earth's atmosphere making up about 78.1% of its volume (Stevens, 2019). It also exists in all living cells as nucleic acids DNA and RNA and present in organisms as building blocks for proteins (Smith and Smith, 2001). Because of its importance in living organisms, it is an essential nutrient for agriculture and plant growth and is present in waste products as well as fertilizers (Stevens, 2019).

When increased amounts of nitrogen are found in naturally occurring water bodies, they can interrupt existing ecosystems by causing changes to the distribution of nutrients to inhabitants (McClelland and Valiela, 1998; O'Driscoll et al., 2019). Eutrophication is a possible outcome of rising nitrogen concentrations. This is an anthropogenically influenced occurrence that can cause a dramatic rise in the rate of algal blooms (Lee et al., 2004). Ultimately, the competition for oxygen creates an anaerobic environment that is unable to support aquatic animals and other plant life (Lee et al., 2004).

It is important to monitor these conditions, how they change over time and the specific threshold of marine environments. A way of tracking the source of nitrogen is by analyzing its

stable isotope ratio that is present in eelgrass (McClelland and Valiela, 1998). This is a relatively new method that was used on Casco Bay initially in 2011 to compare nitrogen in the Mackworth Island watershed and Maquoit Bay watershed (Flynn, 2011). This senior thesis project aims to investigate nitrogen isotopes in eelgrass collected from three different sites within Casco Bay. The East End beach, Chebeague Island and Clapboard Island are expected to have varying results with a correlation to their proximity to human activity and the East End wastewater treatment facility. To better understand the source of nitrogen, three freshwater sources will also be monitored. These are the Royal River, Capisic Brook and the Presumpscot River.

1.2 The Casco Bay Watershed

The Casco Bay watershed is an area in which surrounding water bodies are drained into the Casco Bay estuary and it stretches across approximately 200 square miles of land (CBEP, 2021; Figure 1.1).

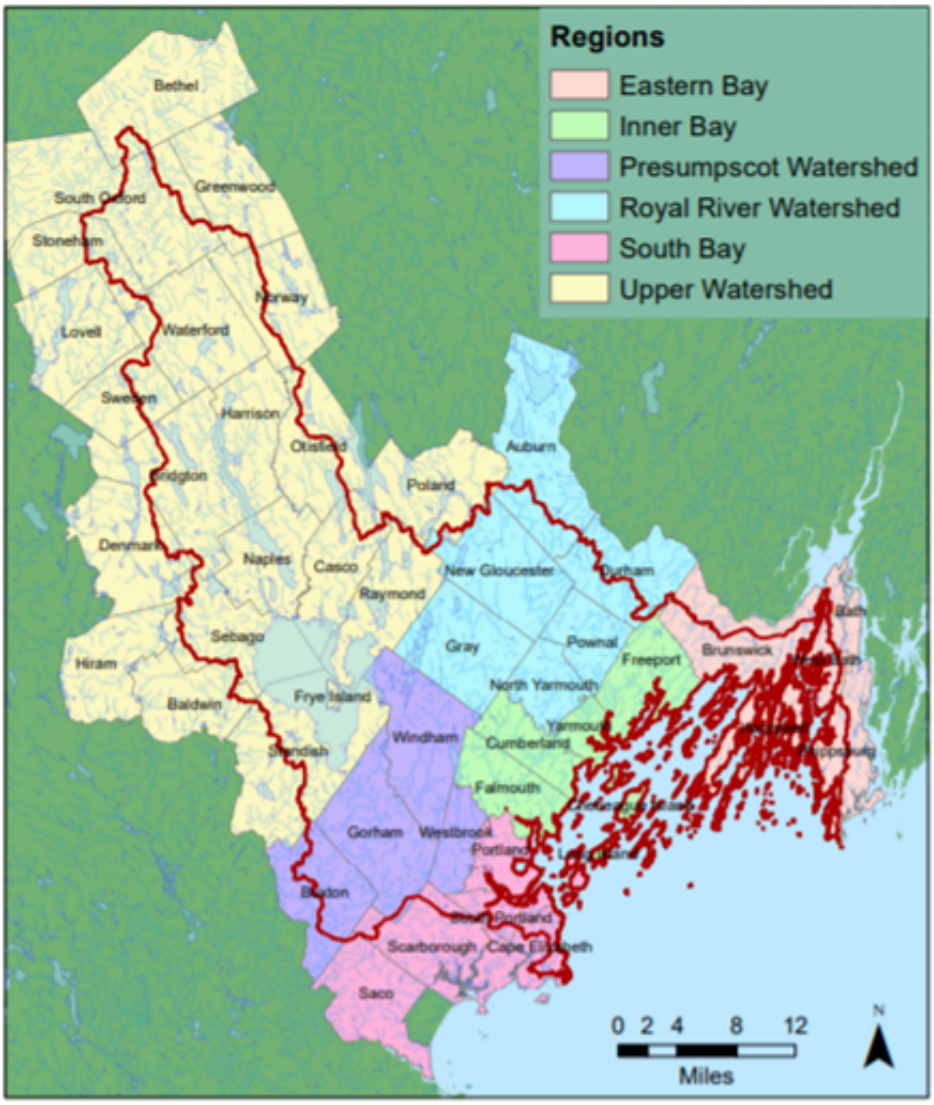

Figure 1.1: Casco Bay Watershed Map. Casco Bay Estuary Partnership. 2010.

The significant networks that drain into the Bay include Sebago Lake, Presumpscot River, Royal River and the Stroudwater Rivers. Most of these hydrological networks support surrounding human settlements physically and economically through different means. From job provision to generation of electricity and waste disposal, these water bodies are a great resource for the development of the Casco Bay area (CBEP, 2021).

In 2010, the Casco Bay Estuary Partnership (CBEP) published a State of the Bay report that monitored water quality, specifically the total nitrogen and dissolved inorganic nitrogen (DIN) within the Casco Bay estuary (Figure 1.2 and 1.3). From this we can observe that there are higher amounts of both dissolved inorganic nitrogen (DIN) and total nitrogen in areas closest to Portland city, an area of high human activity. This is also an area through which the Presumpscot River channels through.

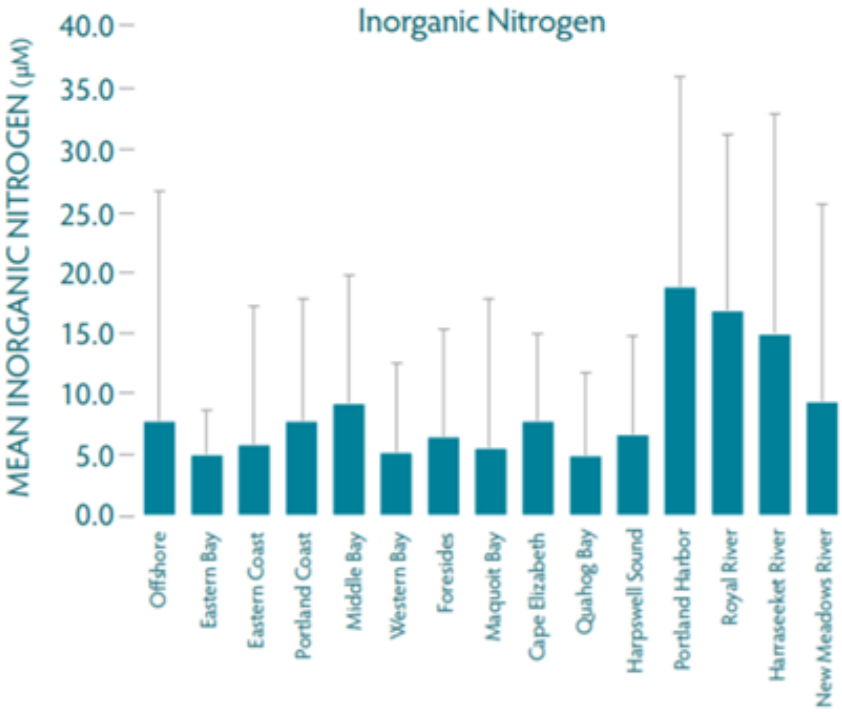

Figure 1.2: Mean Inorganic Nitrogen by region. State of the Bay Report. Casco Bay Estuary Partnership. 2010.

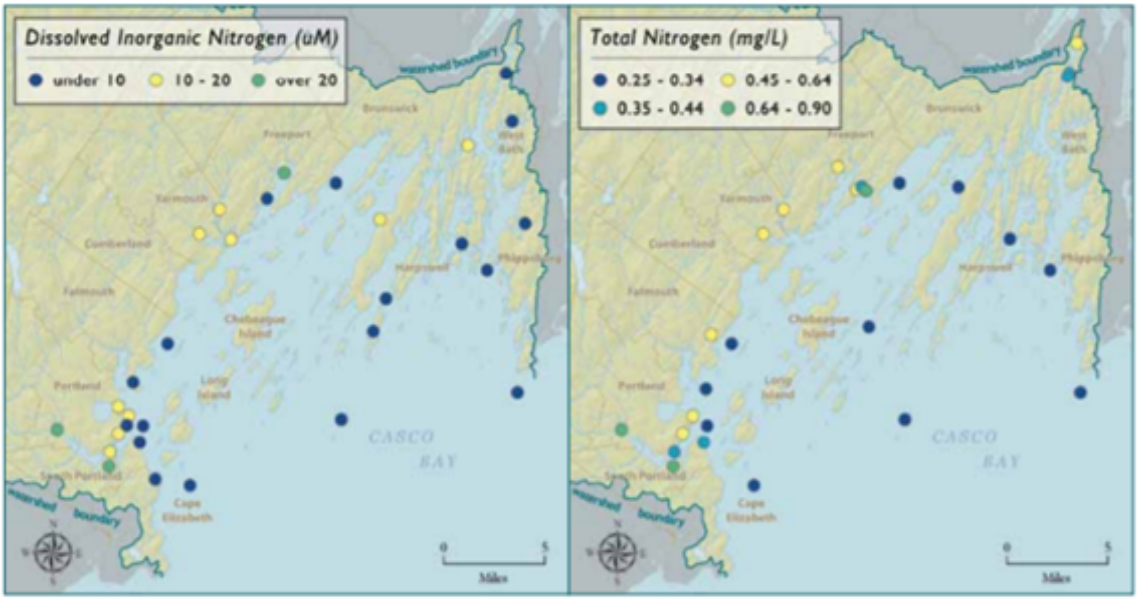

Figure 1.3: Total Nitrogen by region. State of the Bay Report. Casco Bay Estuary Partnership. 2010.

A later report was released in 2015 (Figure 1.4) that showed a similar trend. Although the 2015 report focused solely on total nitrogen concentrations, Portland Harbor, Harraseeket River and New Meadows River had noticeably higher concentrations of total nitrogen, exceeding the remaining 90% of the state that measured below 0.42 mg/l (CBEP, 2015). These are regions of relatively higher human activity and impacts.

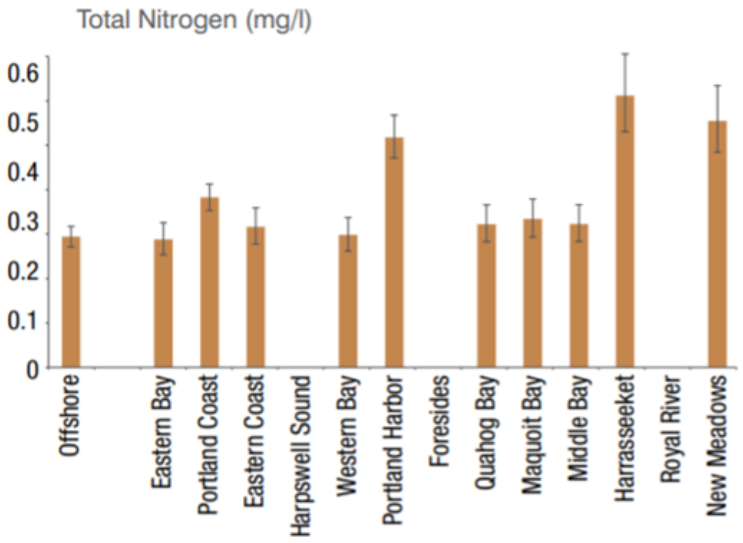

Figure 1.4: Total Nitrogen by region. State of the Bay Report. Casco Bay Estuary Partnership. 2015.

1.3 The Nitrogen Cycle in Estuaries

Although nitrogen makes up for the majority of the earth's atmosphere, it exists in a form, nitrogen gas (N_2), that is not usable by living organisms (Erisman et al., 2013). The nitrogen cycle is a natural process that ensures the conversion of readily available nitrogen within the hydrosphere, biosphere, atmosphere and upper lithosphere (Erisman et al., 2013; Flynn, 2011). There are four main processes in the nitrogen cycle; Nitrogen fixation, Mineralization or Ammonification, Nitrification, and Denitrification (Figure 1.5; Smith and Smith, 2001). Nitrogen fixation is the conversion of gaseous nitrogen into usable forms by nitrogen fixing bacteria such as *Azotobacter*, *Cyanobacteria* and *Rhizobia*. This is one of the hardest conversions as the atoms in gaseous nitrogen are bound together by triple bonds that require high amounts of energy to break (Smith and Smith, 2001; Flynn, 2011).

(i) $N_2 \rightarrow 2N$

$$2N + 3H_2 \rightarrow 2NH_3 \text{ (Smith and Smith, 2001)}$$

Ammonification is the process by which amino acids in organic matter are converted into ammonia.

(ii) $CH_2NH_2COOH + 1.5O_2 \rightarrow 2CO_2 + H_2O + NH_3 + 173$ kcal (Smith and Smith, 2001)

Nitrification involves the oxidation of ammonia into nitrite and nitrate. This is done by some bacteria including *Nitrosomonas* and *Nitrobacter*. Through protonation, ammonia gas, NH_3^-, is converted into ammonium, NH_4 (Flynn, 2011).

(iii) $NH_3 + 1.5O_2 \rightarrow HNO_2 + H_2 + 165$ kcal

$$HNO_2 \rightarrow H^+ + NO_2^-$$

$NO_2^- + 1.5O_2 \rightarrow NO_3^-$ (Smith and Smith, 2001)

Lastly, denitrification, by bacteria *Pseudomonas*, is the reduction of nitrate converting it into nitrogen gas.

(iv) $C_6H_{12}O_6 + 4NO_3^- \rightarrow 6CO_2 + 6H_2O + 2N_2$ (Smith and Smith, 2001)

All of these processes are executed by bacteria that are present in the earth's hydrosphere, biosphere and upper lithosphere (Smith and Smith, 2001). Some of these bacteria live in the root nodules of plants like eelgrass and convert nitrogen in the sediment into nitrogen that can be easily assimilated (NO3- and NH4+) and converted into biomass.

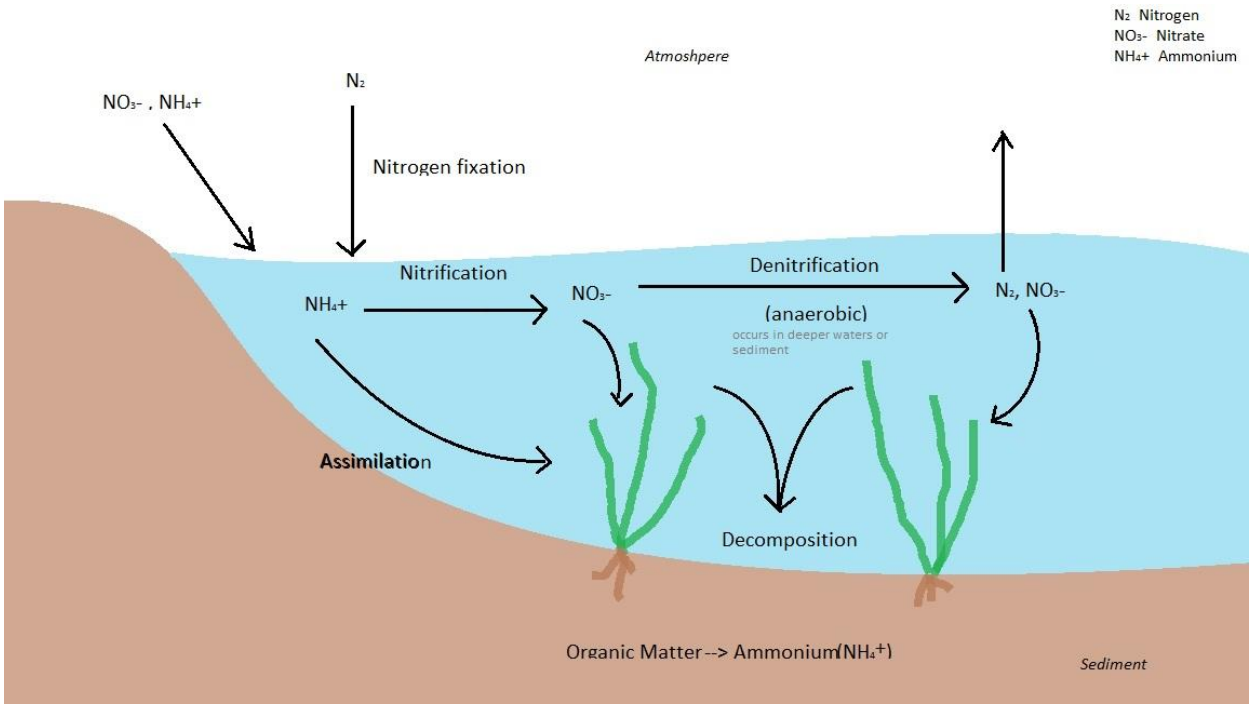

Figure 1.5: Diagrammatic representation of the nitrogen cycle in marine environments.

1.4 Nitrogen Isotopes

Human influences in anthropogenic nitrogen loading can be reflected in the isotopic ratios of nitrogen (McClelland and Valiela, 1998). Delta (δ) values are the ratio of the heavy to light isotopes in a sample measured relative to the ratio of heavy to light isotopes of a standard multiplied by 1,000 and is given in units of permil (0/00). For nitrogen, the heavy to light isotopes of interest are $^{15}N/^{14}N$).

$$\text{Delta } (0/00) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

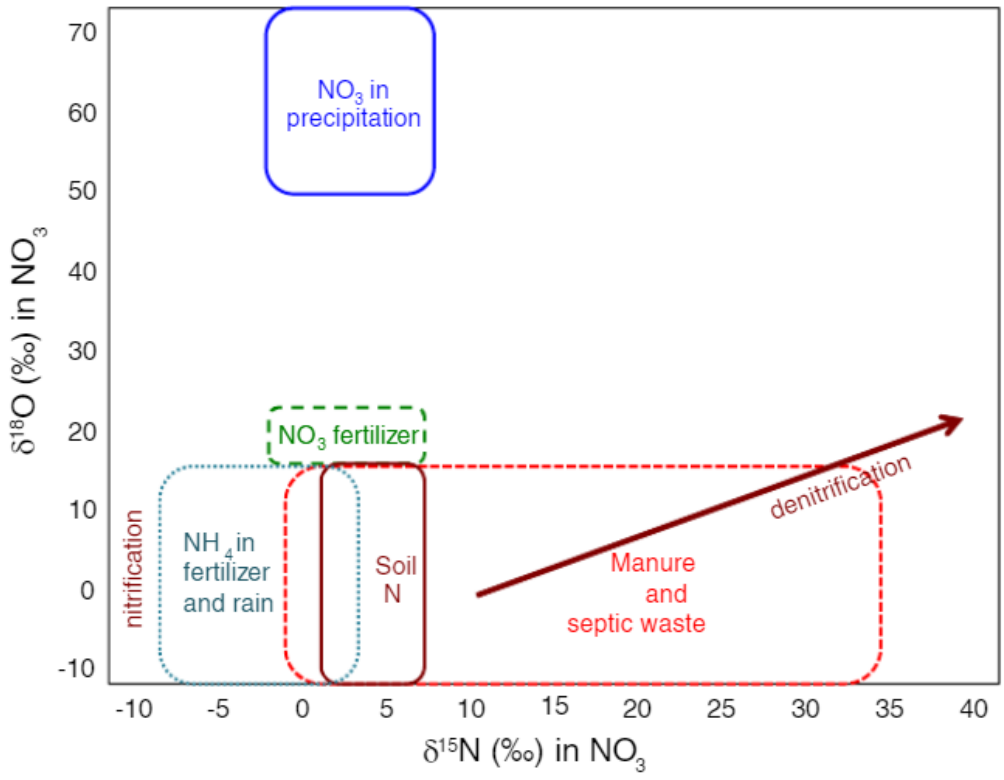

Figure 1.6: Typical δ^{18}O-NO_3 and δ^{15}N-NO3 ranges for nitrate sources and the processes that alter these values. Modified and redrawn from Kendall (1998) and Kendall et al. (2008) (Glibert et al., 2019)

Figure 1.6 shows that NO_3^- with higher δ^{15}N values is mostly derived from manure and septic waste. If poorly disposed of, these materials can end up polluting natural bodies of water. Stable isotopes can be used, and have been used, to track anthropogenic nitrogen in coastal areas (Glibert et al., 2019). Using Isotope Ratio Mass Spectrometry, we can detect isotopic ratios of nitrogen within eelgrass, *Zostera marina*.

1.5 Research Questions

The combination of this research brought about the questions for this project: How have the nitrogen inputs changed over the last ten years? What is the source of nitrogen? How can we identify nitrogen from anthropogenic sources?

2. Methods

2.1 Field Methods

2.1.1 Sampling in Casco Bay Estuary

Three sampling sites were selected within the Casco Bay estuary; Chebeague island, Clapboard Island and East End Beach (Figure 2.1). At these locations, eelgrass, water and surface sediment samples were collected. Eelgrass was analyzed for size and $\delta^{15}N$ and the waters were analyzed for DIN, chlorophyll and phaeophytin.

On July 1st 2021, Angela Brewer from the Maine Department of Environmental Protection (DEP) led a sampling survey. Here members of the team went diving at depths of 1.4m at Chebeague Island, 1.9m at Clapboard Island and 1.3m at East End. At each site there were four sampling stations located along an intermediate-depth permanent transect at 3m, 10m, 18m and 19m along the transect. At each sampling station, 1 surface sediment core and 3 eelgrass plants were collected. At each site, 1 liter of water was collected.

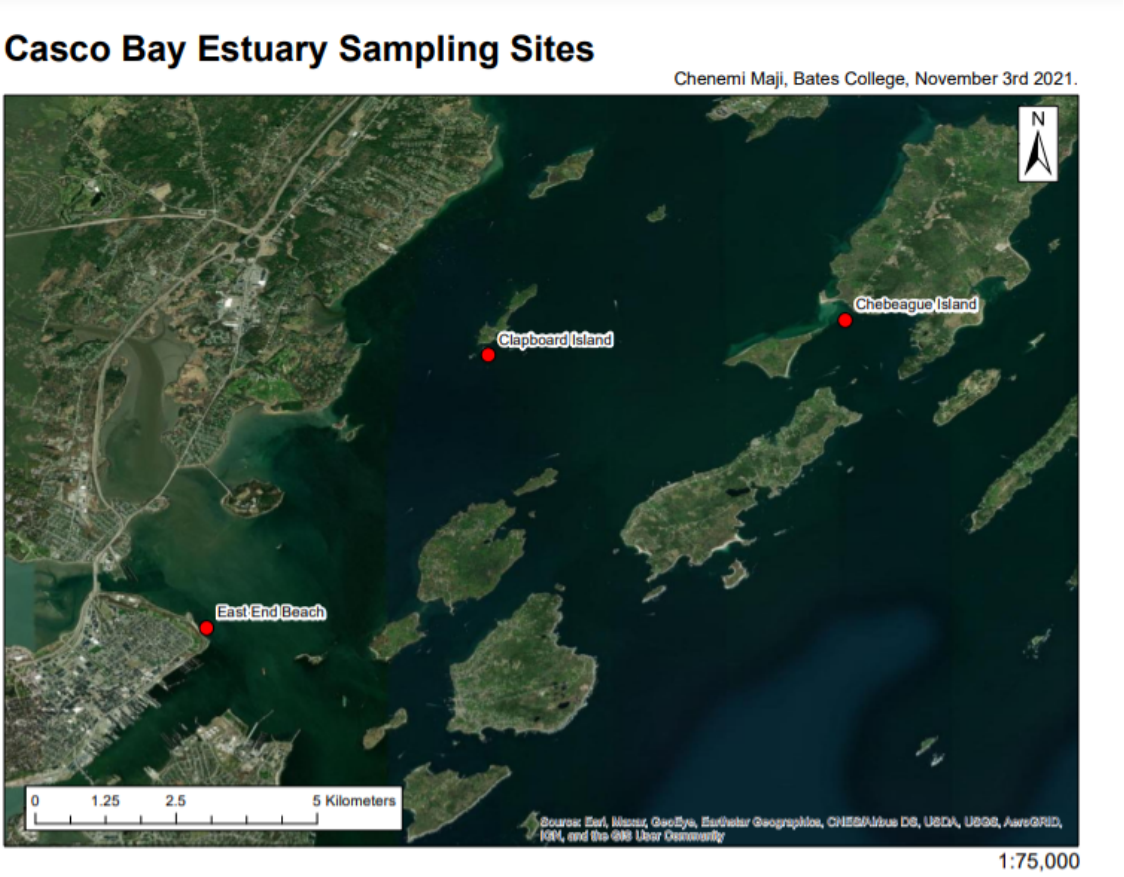

Figure 2.1: Map of the Casco Bay estuary sampling sites

The surface cores were collected using a ~9 cm diameter pvc pipe that was pushed 5-10cm into the sediment. The top of the pvc pipe was then capped with the palm of a hand to create suction, lifted out of the sediment bed and transferred into a whirl pack. The whirl pack was then labeled and placed in an ice chest for preservation.

The eelgrass replicates were collected at random from each sampling point. This random collection of shoots and leaves were snipped from three separate plants and combined into one labeled collection bag. Similar to the collected cores, the samples were chilled for preservation while being transported to the lab.


Figure 2.2: Transect identification buoy (left). Diving team from DEP on sampling survey (right).

2.1.2 River Sampling

Sampling of freshwater inputs was done on the Presumpscot River, Capisic Brook and Royal River within the Casco Bay watershed (Figure 2.3). The river sampling was done in two separate events; on the 22nd of July and the 29th of September. On each day, 1 liter water samples were collected in dark media bottles for chlorophyll testing. There was also on site filtration done using a 25mm, 0.45 um polypropylene syringe filter into 250 ml chemically preserved bottles for DIN analyses. These water samples were sent out to be analyzed for NOx and Chlorophyll content by the Department of Environmental Protection.

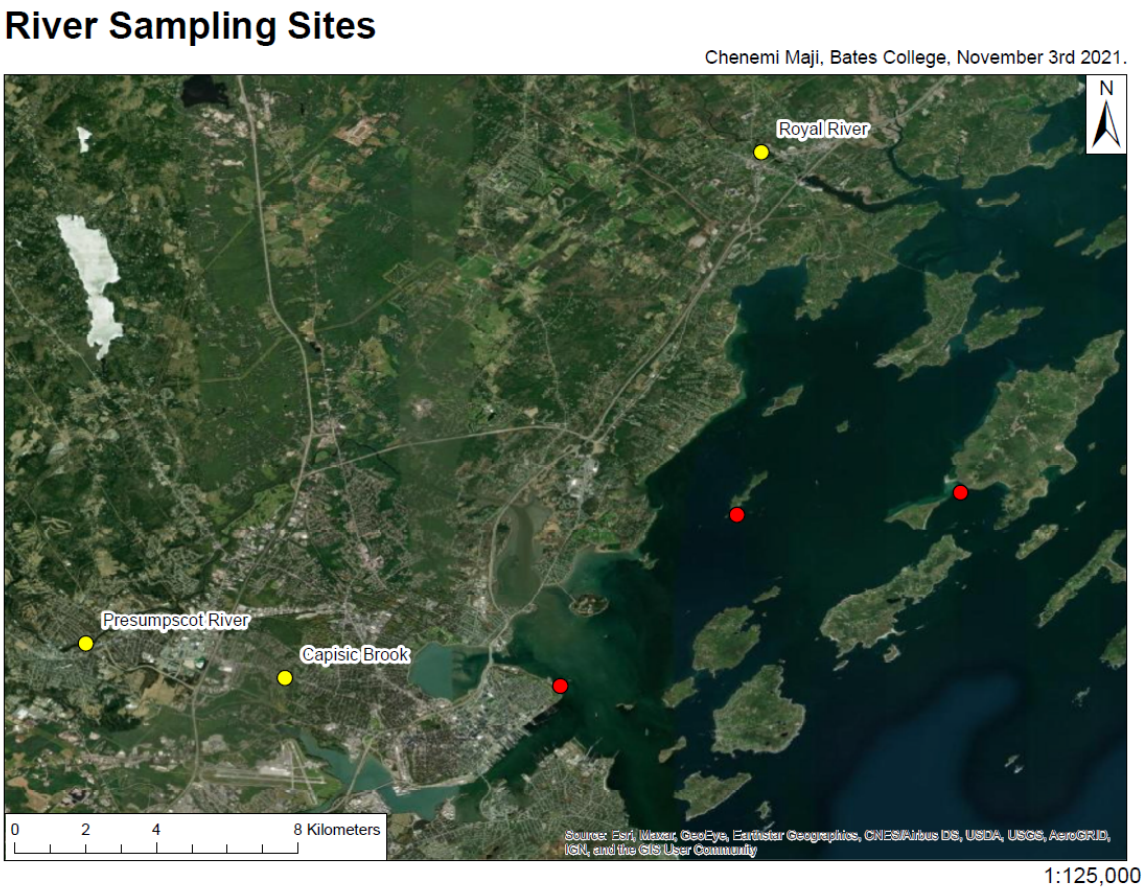

Figure 2.3: Map of the river sampling sites.

2.2 Laboratory Methods

To prepare the eelgrass for analysis, they were scraped within 24 hours to remove epiphytes and measured to record length. The samples were then divided and labeled as individual plant units and then frozen right after to prevent decomposition. The eelgrass samples were then freeze dried and ground into a fine powder using the shatterbox grinder to homogenize for analysis. About 2-4 milligrams of each sample was weighed out and packed into tin cups to be run in the EA-IRMS (Flynn, 2011).

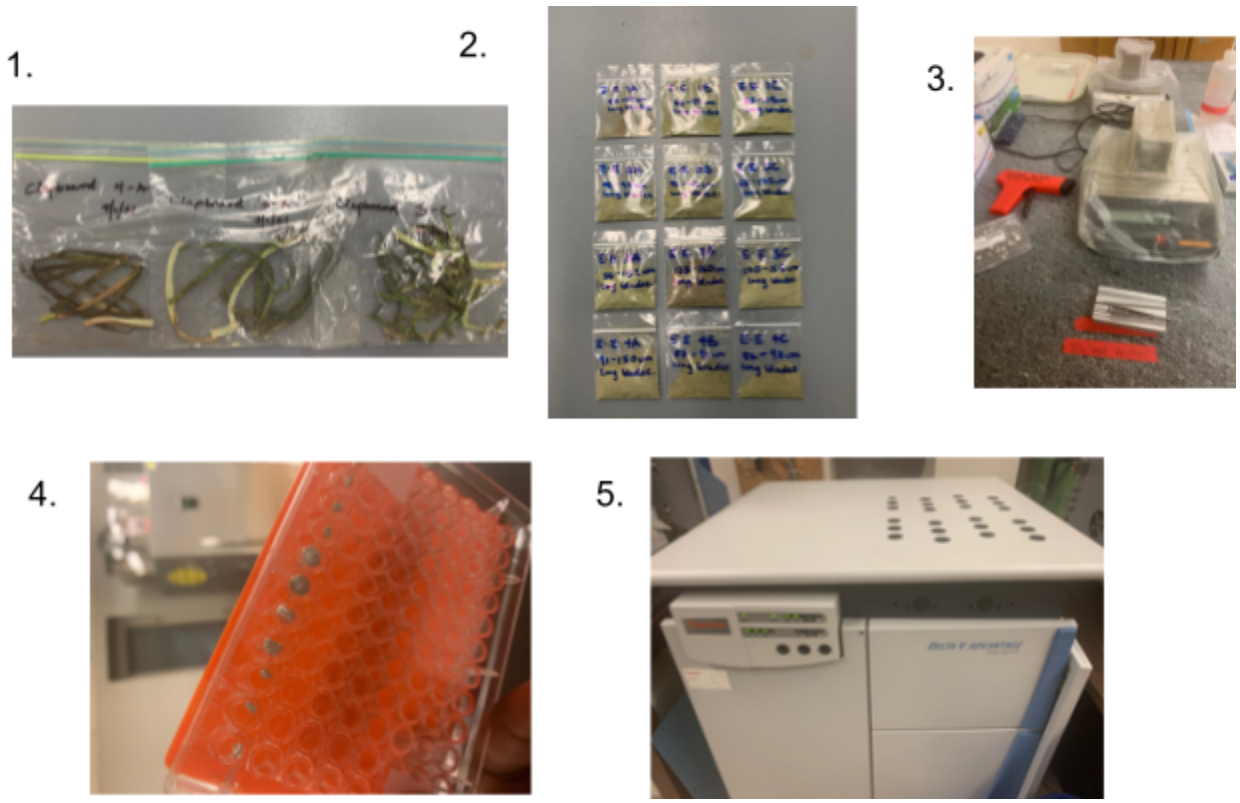

Figure 2.4: Stages of eelgrass prep. 1. Sub sampled individual eelgrass shoots. 2. Homogenized eelgrass. 3. Equipment used to weigh out and package eelgrass samples. 4. Samples to be run in EA-IRMS. 5. The Spectrometer main body.

The sediments were rinsed in epure to remove salts prior to freeze drying and isotopic analysis. Epure was added to the sample container and decanted three times. The sediments were then frozen and further sub-sampled to create a more efficient freeze drying process. The Elemental Analyzer-Isotopic Ratio Mass Spectrometer (EA-IRMS) was then used to determine the nitrogen and carbon isotope ratios of all samples.

Three individual plants from different sampling stations at each site were selected. They were subsampled along shoot length and measured for $\delta^{15}N$ to gauge the isotopic variability within a plant. The result of this isotopic data analysis showed no systematic variability through the shoot of the plant (Table 2.1; Figure 2.5). Thus homogenization was chosen as an alternative method.

2.3 Data to show Isotopic Variability along Eelgrass shoots.

Below are the data generated to show isotopic variability along the plant shoots. East End has the longest leaves and Clapboard Island, the shortest. The absence of a trend in any form can be observed in Figure 2.5. A close range of values can be observed, however it is not systematic, making it necessary to homogenize the entire sample of leaves.

Sample ID	Distance from tip (cm)	Mass of sub-sample (g)	$\delta^{15}N$ (‰)
East End - sampling station 1, Plant 1	0	1.782	6.35
East End - sampling station 1, Plant 1	10	3.447	5.55
East End - sampling station 1, Plant 1	20	3.388	5.58
East End - sampling station 1, Plant 1	30	3.127	5.62
East End - sampling station 1, Plant 1	40	3.474	5.49
East End - sampling station 1, Plant 1	50	3.632	4.8
East End - sampling station 1, Plant 1	60	3.091	4.49
East End - sampling station 1, Plant 1	70	3.714	5.07
Chebeague - sampling station 3, Plant 1	0	2.238	5.53
Chebeague - sampling station 3, Plant 1	10	4.763	6.14

Chebeague - sampling station 3, Plant 1	20	5.177	6.88
Chebeague - sampling station 3, Plant 1	30	5.206	7.16
Chebeague - sampling station 3, Plant 1	40	3.532	7.54
Clapboard - sampling station 4, Plant1	0	3.787	5.38
Clapboard - sampling station 4, Plant1	10	6.465	5.14
Clapboard - sampling station 4, Plant1	20	4.523	5.71
Clapboard - sampling station 4, Plant1	30	3.902	4.57

Table 2.1: δ^{15}N of sub-sampled 2 cm long eelgrass shoots from each site.

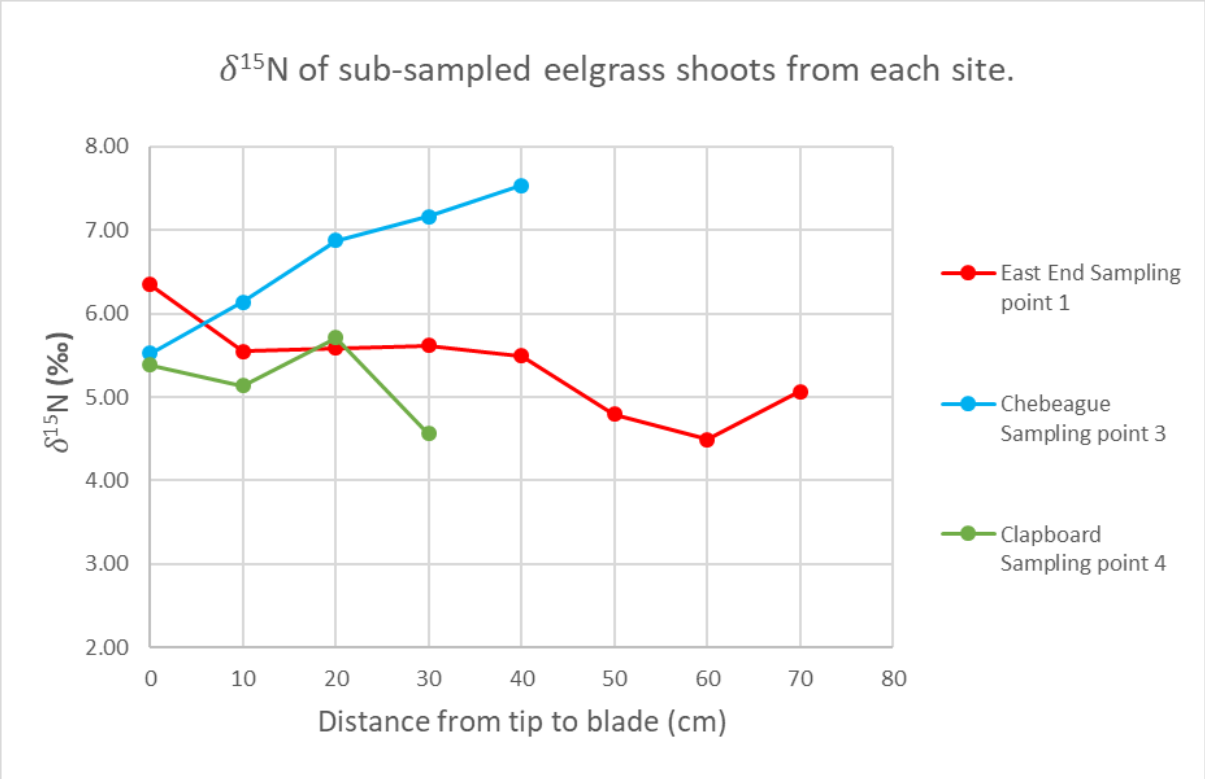

Figure 2.5: δ^{15}N of sub-sampled eelgrass shoots at increasing distance from the tip from each site.

2.4 Data Analysis

The IRMS gives organized data on the isotopic ratio of elements like Nitrogen and Carbon. This includes percentage amounts of Carbon and Nitrogen present, Carbon to Nitrogen molar ratio, ratios of $\delta^{15}N$ present in each sample. The d15N values from each site were compared using statistical tools such as the T-test and generated p-values.

3. Results

3.1 Data from EA-IRMS on Eelgrass Samples

All of the eelgrass data were obtained by the EA-IRMS in the Environmental Geochemistry Laboratory, Department of Earth and Climate Sciences (formerly Geology) at Bates College.

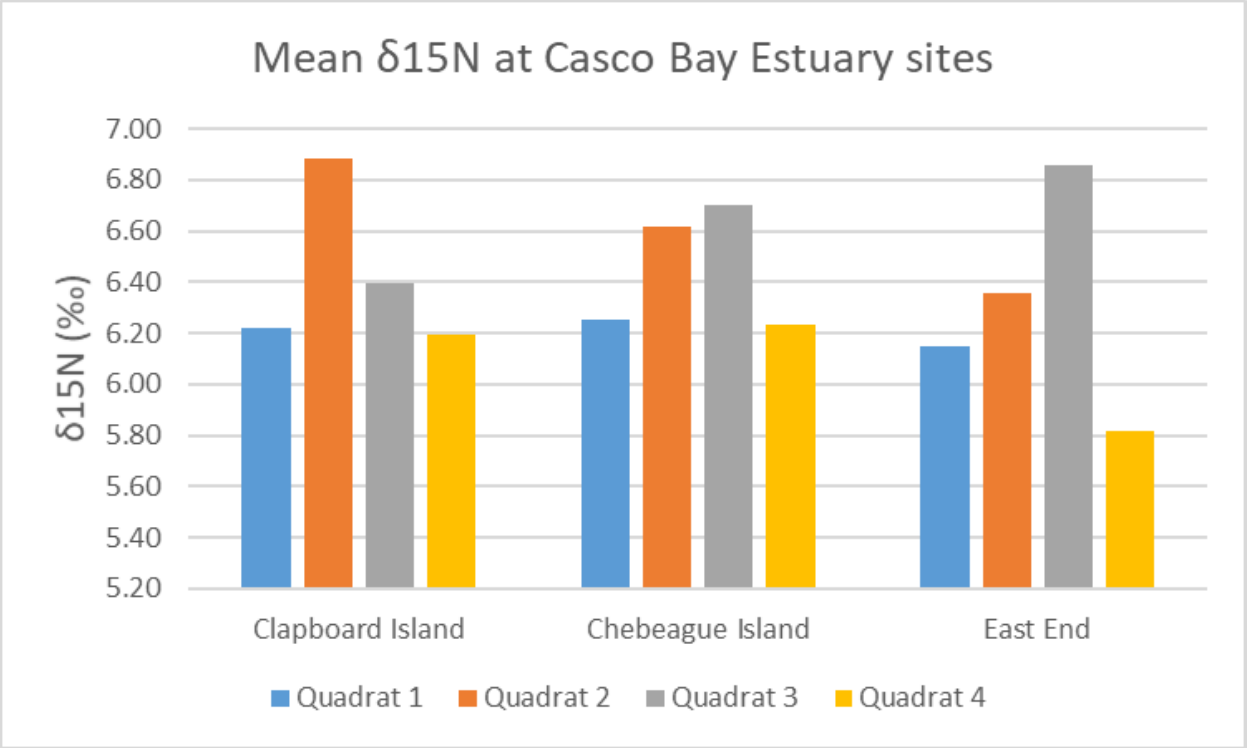

Figure 3.1: Average δ^{15}N values in eelgrass at each of the four (4) sampling stations at each sampled site, Clapboard Island, Chebeague Island and East End Beach, within Casco Bay.

Figure 3.1 shows the lowest δ^{15}N value in sampling station 4 at East End and the highest in sampling station 2 at Clapboard Island. The least amount of variation can be seen across the sampling stations in Chebeague Island and the most variable data are at East End.

The average $\delta^{15}N$ values of eelgrass from Clapboard Island ranged between 6.19‰ and 6.88‰, from Chebeague Island ranged between 6.23‰ and 6.70‰ and from East End ranged between 5.82‰ and 6.86‰ (Figure 3.1). There were no systemic changes in isotope values along the transects sampled.

To summarize this data further, we see that the average $\delta^{15}N$ at each site range from 6.29‰ to 6.45‰ at East End beach and Chebeague Island respectively (Figure 3.2). A p-test was used to compare the average $\delta^{15}N$ values from East End and Chebeague Island and no significant difference was found. This gave a p-value of ~0.18. The standard deviation for all site averages was also less than 1 which shows all the data from each site are tightly clustered.

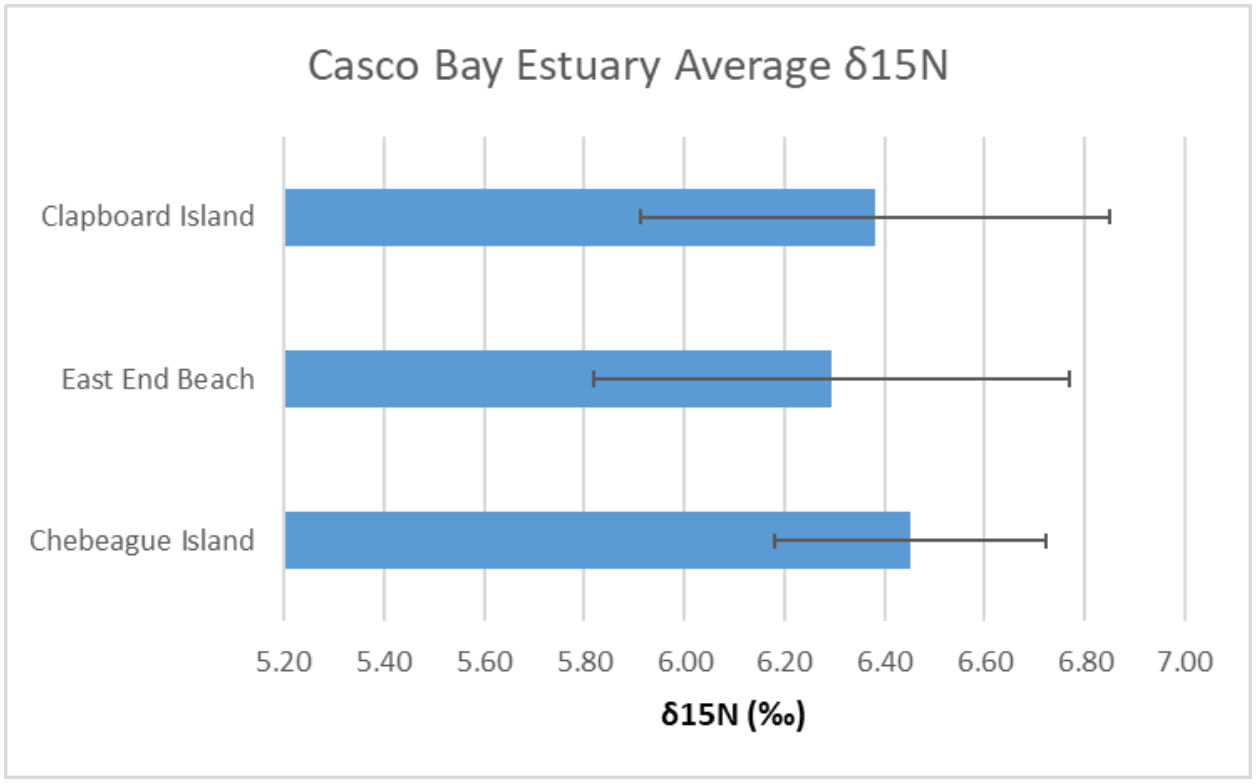

Figure 3.2: Average $\delta^{15}N$ in eelgrass at each sampled site, Clapboard Island, Chebeague Island and East End Beach, within Casco Bay.

In comparison to a thesis written in 2011 (Flynn, 2011), we can see that there is definitely more isotopic homogeneity in eelgrass from the Casco Bay area now relative to 2011 (Figure 3). However, that research focused on Mackworth Island and Maquoit Bay, which are not the sites investigated in this project. Mackworth Island is in very close proximity to East End beach and is at the mouth of Presumpscot river. Maquoit Bay is further from the remaining four sites and comparatively farther from a densely populated area.

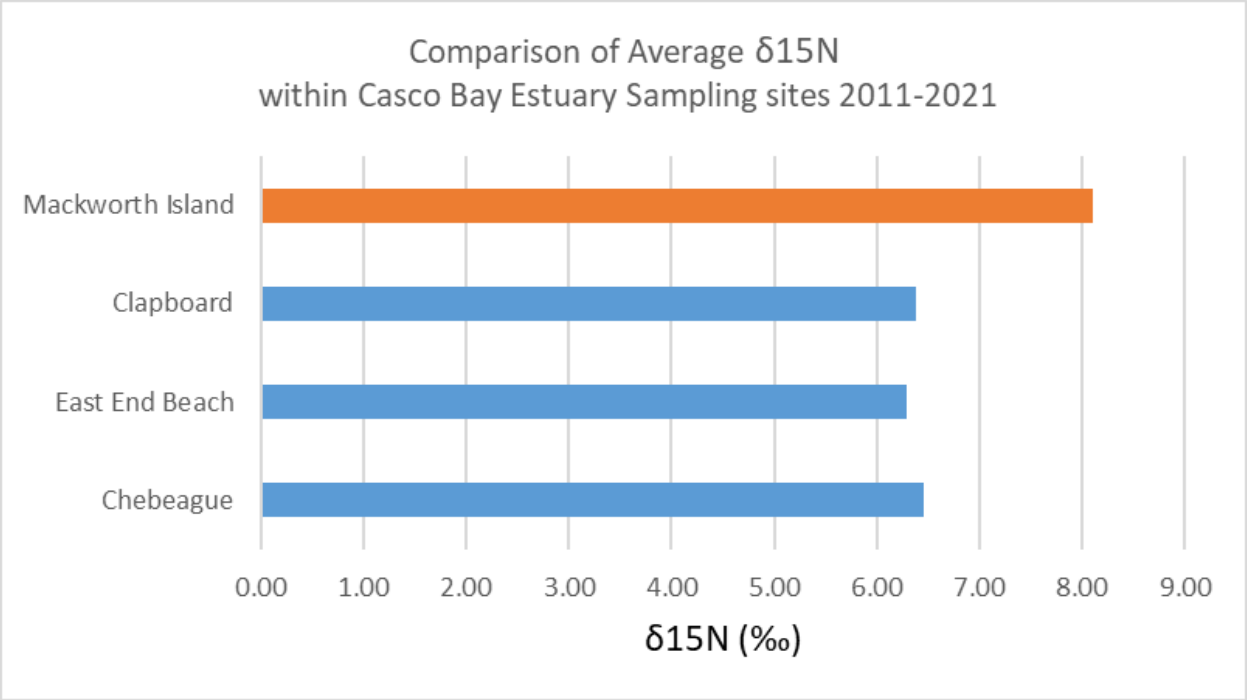

Figure 3.3: Comparison of the average $\delta^{15}N$ in eelgrass from 2021 in Blue, to sites sampled in 2011, in Orange, within Casco Bay. The East End Beach and Mackworth Island are the most directly comparable in terms of location.

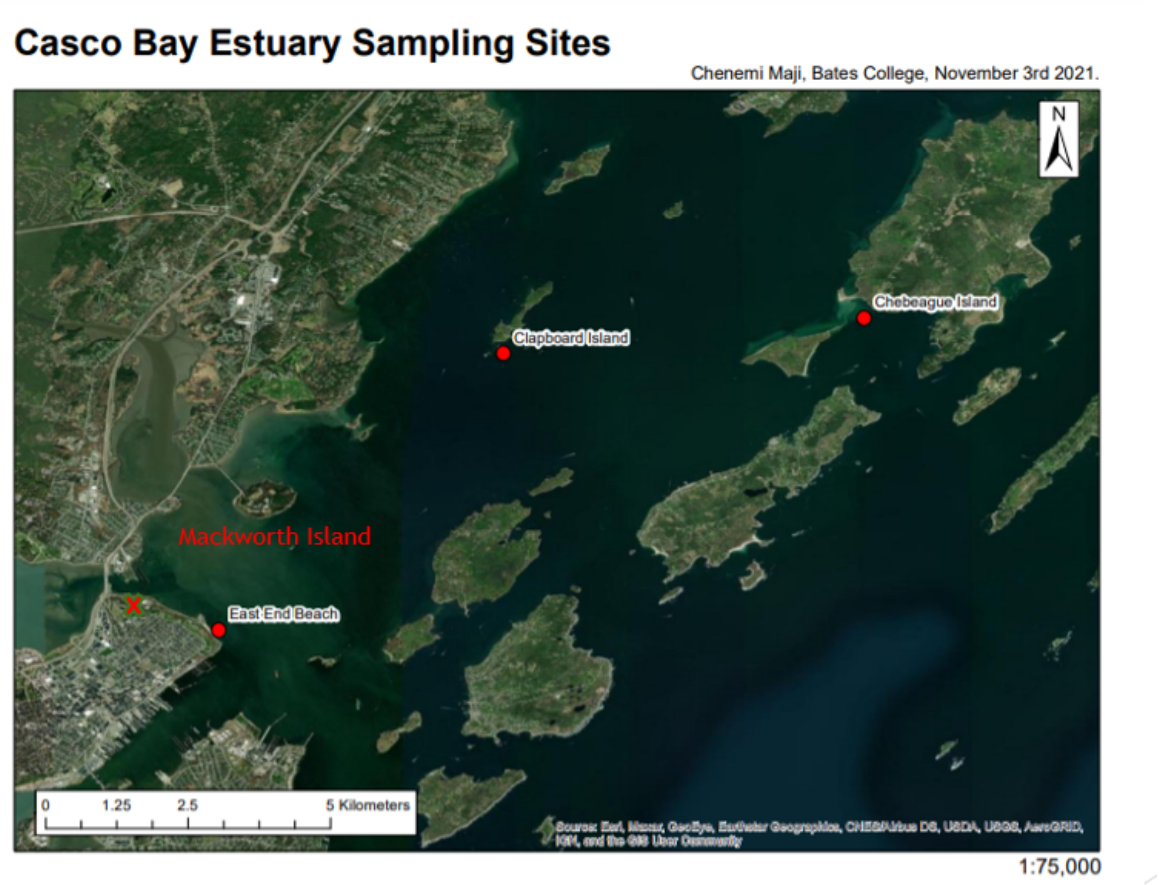

Figure 3.4: Casco bay estuary sampling sites and Mackworth Island from Greg Flynn 2011 research. Red 'X' marks East End wastewater treatment facility.

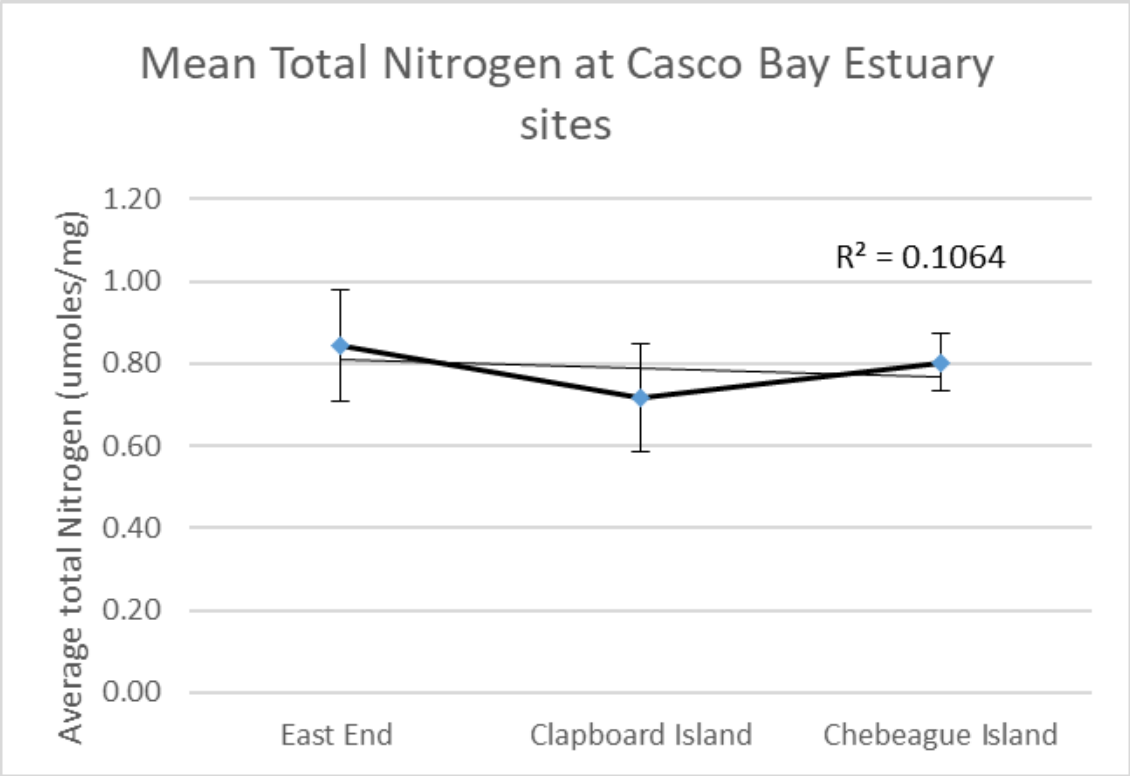

Figure 3.5: Comparison of the mean total nitrogen present in eelgrass at each sampled site within Casco Bay at increasing distance from the wastewater treatment facility.

East End eelgrass has the highest mean total nitrogen content, 0.85 umoles/mg which can be compared to the lowest value of 0.72 umoles/mg at Clapboard Island (Figure 4). The highest carbon to nitrogen ratios of eelgrass samples can be seen in the Clapboard sampling stations and The lowest ratios can be found in the East End sampling stations (Table 3.1).

Site name	Eelgrass Leaf Average C/N Ratio
Chebeague sampling station 1	39.57
Chebeague sampling station 2	38.43
Chebeague sampling station 3	38.21

Chebeague sampling station 4	32.98
East End sampling station 1	30.95
East End sampling station 2	32.28
East End sampling station 3	37.76
East End sampling station 4	33.77
Clapboard Island sampling station 1	44.76
Clapboard Island sampling station 2	53.73
Clapboard Island sampling station 3	39.61
Clapboard Island sampling station 4	39.34

Table 3.1: Mean carbon to nitrogen ratio of eelgrass samples in each sample sampling station.

3.2 Data from DEP on Water Samples

Below are two tables that summarize the data collected from water samples that were sent out to the Department of Environment Protection for analysis. This includes tests for chlorophyll and nitrate, nitrite and ammonia as nitrogen. These tests were done for both river water samples collected in August and September 2021 and estuary water samples collected in the month of July.

SAMPLE POINT NAME	PARAMETER NAME	CONCENTRATION	REPORTING LIMIT	PARAMETER UNITS	ANALYSIS DATE
CAPISIC BROOK	CHLOROPHYLL A - PHAEOPHYTIN	1.080	0.100	UG/L	8/2/2021
PRESUMPSCOT RIVER	CHLOROPHYLL A - PHAEOPHYTIN	1.060	0.067	UG/L	8/2/2021
ROYAL RIVER	CHLOROPHYLL A - PHAEOPHYTIN	0.750	0.100	UG/L	8/2/2021
CAPISIC BROOK	CHLOROPHYLL A - PHAEOPHYTIN	3.280	0.180	UG/L	9/22/2021
PRESUMPSCOT RIVER	CHLOROPHYLL A - PHAEOPHYTIN	0.675	0.041	UG/L	9/22/2021
ROYAL RIVER	CHLOROPHYLL A - PHAEOPHYTIN	1.720	0.042	UG/L	9/22/2021
CAPISIC BROOK	NITRATE + NITRITE AS NITROGEN	0.472	0.002	MG/L	8/2/2021
PRESUMPSCOT RIVER	NITRATE + NITRITE AS NITROGEN	0.0754	0.002	MG/L	8/2/2021
ROYAL RIVER	NITRATE + NITRITE AS NITROGEN	0.150	0.002	MG/L	8/2/2021
CAPISIC BROOK	NITRATE + NITRITE AS NITROGEN	0.629	0.050	MG/L	9/17/2021
PRESUMPSCOT RIVER	NITRATE + NITRITE AS NITROGEN	0.026	0.050	MG/L	9/17/2021
ROYAL RIVER	NITRATE + NITRITE AS NITROGEN	0.260	0.050	MG/L	9/17/2021

CAPISIC BROOK	PHAEOPHYTIN	0.620	0.100	UG/L	8/2/2021
PRESUMPSCOT RIVER	PHAEOPHYTIN	0.979	0.067	UG/L	8/2/2021
ROYAL RIVER	PHAEOPHYTIN	1.070	0.100	UG/L	8/2/2021
CAPISIC BROOK	PHAEOPHYTIN	1.020	0.180	UG/L	9/22/2021
PRESUMPSCOT RIVER	PHAEOPHYTIN	0.554	0.041	UG/L	9/22/2021
ROYAL RIVER	PHAEOPHYTIN	0.920	0.042	UG/L	9/22/2021

Table 3.2: Chlorophyll, Nitrate, Nitrite and Phaeophytin concentrations of river water samples in August and September.

SAMPLE POINT NAME	**PARAMETER NAME**	**CONCENTRATION**	**REPORTING LIMIT**	**PARAMETER UNITS**
CHEBEAGUE ISLAND	AMMONIA AS NITROGEN	0.012	0.01	MG/L
CLAPBOARD ISLAND	AMMONIA AS NITROGEN		0.01	MG/L
EAST END	AMMONIA AS NITROGEN	0.067	0.01	MG/L
CHEBEAGUE ISLAND	CHLOROPHYLL A - PHAEOPHYTIN	1.95	0.08	UG/L
CLAPBOARD ISLAND	CHLOROPHYLL A - PHAEOPHYTIN	2.85	0.08	UG/L
EAST END	CHLOROPHYLL A - PHAEOPHYTIN	2.56	0.08	UG/L

CHEBEAGUE ISLAND	NITRATE + NITRITE AS NITROGEN		0.002	MG/L
CLAPBOARD ISLAND	NITRATE + NITRITE AS NITROGEN		0.002	MG/L
EAST END	NITRATE + NITRITE AS NITROGEN	0.0119	0.002	MG/L
CHEBEAGUE ISLAND	PHAEOPHYTIN	0.521	0.08	UG/L
CLAPBOARD ISLAND	PHAEOPHYTIN	0.584	0.08	UG/L
EAST END	PHAEOPHYTIN	1.01	0.08	UG/L

Table 3.3: Chlorophyll, Nitrate, Nitrite, Ammonia and Phaeophytin concentrations of estuary water samples in July.

Nitrate (NO_3^-) and Nitrite (NO_2^-) concentrations are harder to detect in samples from the estuary. The only site where there were detectable concentrations of nitrate and nitrite is at East End at 0.0119 mg/L (Table 3.3). As Figure 3.6 below reflects, the highest concentration of chlorophyll and $NO_3^- + NO_2^-$ was seen at Capisic Brook in September. The lowest concentration of these was at Presumpscot River in September.

Table 3.2 and 3.3 above show that Capisic Brook is the only fresh water source with similar chlorophyll content as the sites in the estuary which are all ≧ 2ug/l.

To further understand how nutrients affect primary productivity, measured chlorophyll concentrations can be compared with river sample nitrate and nitrite concentrations. The data collected in August and September can further be compared. There is a clear positive correlation between the variables in September (Figure 3.6). The same positive correlation is present in data from August, however, there is an outlier in Presumpscot River. In September, Capsic and Royal Rivers have higher values in both variables than in August. Again, the Presumpscot River does not follow this apparent trend. The Royal River and Capisic Brook both had increased chlorophyll and NO_3^- + NO_2^- concentration from August to September while Presumpscot River saw a slight decrease (Figure 3.6).

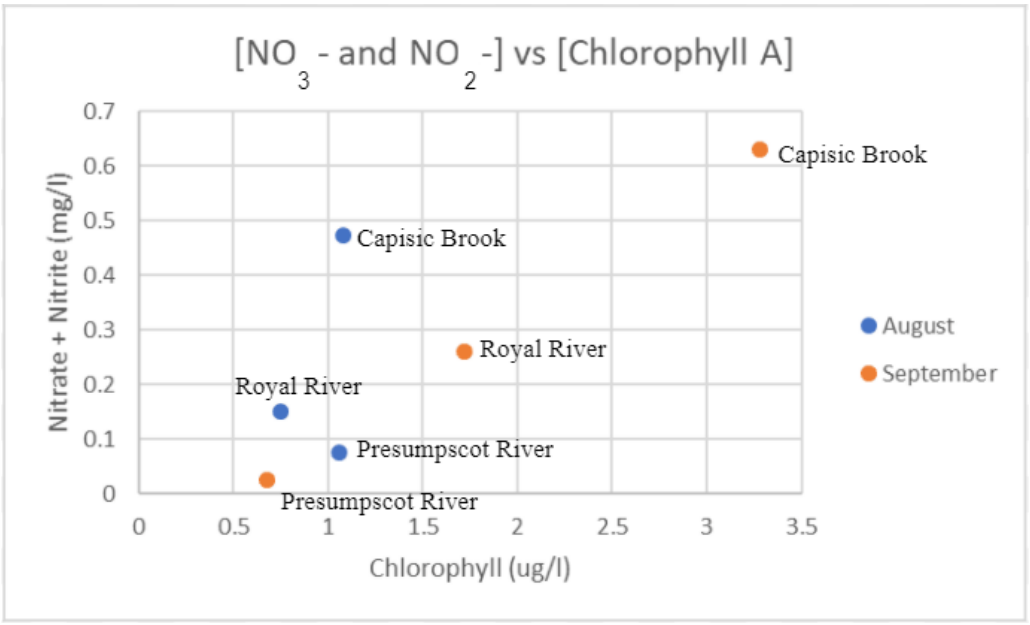

Figure 3.6: Nitrate + Nitrite vs Chlorophyll concentrations of river water samples in August and September.

4. Discussion

4.1 Marine Results

The general trend shows no statistically significant difference in the $\delta^{15}N$ values of eelgrass at Chebeague Island, Clapboard Island and East End beach. Flynn, 2011, reports that Mackworth Island, a site across the way from East End (Figure 3.4), had a nitrogen isotopic ratio of 8.1‰ which signifies a greater presence of ^{15}N in the eelgrass samples. Comparing this value to that of East End, the results also show a 1.81‰ decrease in the $\delta^{15}N$ values in the south Casco Bay area closest to the wastewater treatment facility since 2011. In 2017 the East End Wastewater Treatment Facility made internal changes, one of which was to upgrade the aeration system (Levin et al., 2019). In the first season after implementation, May 2018, this led to a drop in the average sludge volume index (SVI) from 250 mL/g to 120 mL/g. A positive secondary effect of this was a reduction in the effluent total nitrogen by 72% exceeding the goal of 20-40% (Levin et al., 2019).

It can also be observed that East End water samples have the most DIN and ammonia concentrations (Table 3.3). Eelgrass samples at the East End site also have the highest total nitrogen composition. This corresponds to the reports from the CBEP in 2010 and 2015 where Portland Harbor had higher concentrations of total nitrogen and DIN than most investigated sites in Casco Bay.

Research has been done that made use of eelgrass as a nutrient pollution indicator. This study focused on three sites in New England, the Great Bay Estuary, Narragansett Bay and Waquoit Bay (Lee et al., 2004). The competition for nutrients in coastal ecosystems is carried out by primary producers (Fong et al., 2008). In cases of nutrient loading, less adaptive primary

producers are exposed to nutrient deficiency while more adaptive ones, like certain microalgae can increase in population (McClelland and Valiela, 2003). Seagrasses are less adaptive and over time can experience nutrient deficiency and population decrease which has been repeatedly reported in Waquoit Bay, Massachusetts (Valiela et al., 1992). The carbon to nitrogen ratios of these unhealthy seagrasses in this paper range from ~18-28 (Lee et al., 2004). These are much lower than the carbon to nitrogen ratios of eelgrass in this experiment which range from ~31-54. However, noting that the lowest C/N ratio was observed in eelgrass from the East End, this may signify the occurrence of nutrient loading at a slow rate over time.

4.2 Freshwater Results

Capisic Brook had the highest NO_3^- + NO_2^- concentrations as well as chlorophyll concentrations of all the freshwater sources. This could be as a result of its observed smaller size and lower volume of water compared to the other sites. The area was also surrounded by foliage and deer tracks which might indicate the deposition of more organic matter rich in nutrients.

Presumpscot River had the lowest concentrations (Figure 3.6) and the only negative change from August to September. Presumpscot river seemed to visually be the largest of all three sites. This could mean greater dilution of input materials. Heavy storm events around the sampling day in August and the poor visibility of the water could mean there was a disturbance in settled sediment. This may have influenced the values obtained from the collected samples.

4.3 Limitations to Study

It is important to note that these are preliminary interpretations from a relatively small dataset. Future studies should be focused on sites dispersed through the entire Casco Bay estuary and data could be collected for longer periods of time. Another modification could be to

investigate benthic nitrogen because denitrification occurs deep in the soil and that accounts for nutrients that aren't necessarily in circulation. Flux and other water discharge patterns could also be monitored as it may give insight on the movement of nutrients in freshwater systems.

5. Conclusions

There is no statistically significant difference among $\delta^{15}N$ values of eelgrass from all three sites in the Casco Bay Estuary. The average $\delta^{15}N$ values for these is 6.38 ± 0.40. There also appears to have been a ~1.8‰ decrease in $\delta^{15}N$ values of eelgrass in the south Casco Bay area since 2011. This could reflect that the East End wastewater treatment plant reduced its nitrogen load by ~70% in 2017 (A. Brewer; Levin et al., 2019).

Eutrophication and nutrient pollution are modern environmental problems that should be monitored and prevented. The results of this experiment show that this is very impactful research. It provided supporting evidence of improving conditions within Casco Bay. However, there is a lot that is unknown and greater trends that may not be observed as this is a simple snapshot in time. More extensive research needs to be done on this topic as it can help prevent irreversible damage to this great ecosystem.

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