Blue Carbon Dynamics within the Temperate Subtidal Eelgrass (*Zostera marina*) Meadows of Portage Inlet, British Columbia

by

April Lin

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Declaration of Committee

Name:	**April Lin**
Degree:	**Master of Science**
Title:	**Blue Carbon Dynamics within the Temperate Subtidal Eelgrass (*Zostera marina*) Meadows of Portage Inlet, British Columbia**
Committee:	**Ana Chara-Serna** Supervisor and Chair Faculty, BCIT
	Anayansi Cohen-Fernandez Examiner Faculty, BCIT

Abstract

Seagrass meadows are categorized as blue carbon ecosystems as they are coastal marine environments that contribute immensely to atmospheric carbon dioxide reduction. Despite the importance of their ecosystem services, the carbon stock of many local seagrass meadows and the mechanisms behind their superior sequestration abilities are unknown. This study was the first sediment carbon stock assessment conducted within the subtidal seagrass (*Zostera marina*) meadows of Portage Inlet, Victoria. I projected how the potential loss of the meadows could impact the underlying sediment carbon stock by comparing the seagrass carbon stock to the adjacent unvegetated subtidal mudflat. I modeled the relationship between sediment carbon stock and the productivity measures (in terms of percent coverage, leaf area index and shoot density) of the eelgrass meadows as well as other physical-chemical measures (salinity, temperature, turbidity and wave motion) to determine what factors impacted the sediment carbon stock of the inlet. Data analysis revealed that the loss of the meadows would have little to no impact on the carbon stock of the inlet given that the stock of the meadows (39.4 ± 5.71 Mg C/ha) was statistically comparable to the unvegetated subtidal mudflat (38.9 ± 3.39 Mg C/ha). Model analysis revealed that eelgrass productivity measures could not statistically explain the variation in the carbon stock within the inlet. However, individual models of salinity, temperature and turbidity were more likely to explain the observed variation in sediment carbon stocks. Future studies should continue to analyze the interaction of biological, chemical and physical components in seagrass environments to obtain a more comprehensive understanding of local blue carbon dynamics for restoration and management purposes.

Keywords: Blue carbon; Temperate eelgrass meadows; *Zostera marina*; subtidal; carbon stock; Pacific Coast

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Chapter 1. Introduction

Seagrass meadows are biologically productive coastal environments that contribute immensely to atmospheric carbon dioxide reduction as natural carbon sinks (Macreadie et al., 2021). They are amongst a rising number of other marine and coastal environments, namely salt marshes and mangrove forests, that are being nicknamed blue carbon ecosystems for their ability to both store carbon in the form of vegetative biomass and bury it beneath the underlying soils (Macreadie et al., 2019). Relative to other blue carbon ecosystems, there are 72 species of seagrasses that are distributed across a variety of climatic regions (temperate to tropical) and habitat types (sheltered bays to open ocean) of which they collectively account for an estimated 10% of annual organic carbon burial in the oceans (Duarte et al., 2005; Mazarrasa et al., 2018). Like other blue carbon ecosystems, seagrasses are extremely efficient at sequestering carbon in comparison to terrestrial ecosystems (Prentice et al., 2020). Seagrass meadows can bury an estimated 48-112 Tg of carbon on a global annual basis while occupying only 0.2% of the ocean's surface area (Johannessen, 2022; Mcleod et al., 2011). Terrestrial forests are estimated to bury between 49.3-78.5 Tg of carbon on a global annual basis despite occupying 31% of earth's total land surface area (Mcleod et al., 2011). Therefore, increasing recognition is placed on the pivotal ecosystem service provided by seagrass meadows in the mitigation of atmospheric carbon (Stewart-Sinclair et al., 2021).

A majority of the organic carbon sequestered within seagrass meadows is stored below in the soils rather than in the aboveground vegetation (Serrano et al., 2014). Around 2% of organic carbon sequestered in seagrass meadows can be attributed to vegetative growth in aboveground (leaves, stem, branches) and belowground (rhizomes and roots) biomass (Macreadie et al., 2021; Mcleod et al., 2011; Serrano et al., 2014). 98% of organic carbon sequestration in seagrass meadows is attributed to burial of allochthonous and autochthonous organic matter in the underlying sediment (Duarte et al., 2005; Serrano et al., 2014). The seagrass canopy facilitates capture of particles and provides shelter against tidal currents to allow organic material to settle in the meadow's soils (Mazarrasa et al., 2018). The slow microbial remineralization rate of the marine soils allows for carbon to accrue on a millennial scale given low disturbance conditions (Johannessen, 2022; Mazarrasa et al., 2018; Mcleod et al., 2011). The root system of the meadows can further anchor the soil beds to prevent erosion and resuspension of those particles (Johannessen, 2022).

The capacity and efficiency at which carbon storage is achieved within these seagrass meadows varies depending on the interaction of numerous biological, chemical, and physical properties (Johannessen, 2022; Macreadie et al., 2017; Mazarrasa et al., 2018). Light availability, salinity, ocean temperature, wave intensity, neighbouring landscape features, and turbidity can impact vegetation growth and therefore their capacity for carbon sequestration (Mcleod et al., 2011). Seagrass meadows in sites that are found in areas of lower wave height, lower water depth, lower turbidity, and ample light conditions contain greater sedimentary carbon content (Carr et al., 2016; Samper-Villarreal et al., 2016; Serrano et al., 2014). Studies conducted following marine heat waves found that in sites with substantial seagrass meadow decline, carbon sediment stock was also negatively impacted (Aoki et al., 2021; Arias-Ortiz et al., 2018). Biological interactions such as bioturbation of sediments, interspecific competition, interspecies interactions, disease, and herbivory can draw varying impacts on vegetative health and therefore influence carbon burial (Johannessen, 2022; Mazarrasa et al., 2018; Mcleod et al., 2011). A study conducted in Southeast Australia found that following an overgrazing event by urchins in seagrass meadows, an estimated 57.8 to 104 tons of carbon dioxide were released (Carnell et al., 2020). A laboratory study analyzing the effect of bioturbating shrimp on seagrass meadows found that treatments with bioturbators had a two to five fold increase in the total carbon dioxide release in comparison to control soils (Thomson et al., 2019). Overall, these interactions not only affect the site-to-site variation of carbon storage, but they can generate a spatially heterogenous accrual of carbon within the landscape itself (Macreadie et al., 2019; Prentice et al., 2020). Thus, the carbon stock of any coastal ecosystem should not be generalized through data from neighboring sites and further local investigations are required to capture a more accurate stock assessment. Local assessments can not only highlight the potential benefits of blue carbon environments to decrease greenhouse gas emissions in light of climate change conditions, but can help allocate resources towards the protection and restoration of marine environments.

There is a greater urgence to understand carbon sequestration in seagrass meadows as relative to other blue carbon ecosystems, there is limited data on both regional and local scales to a majority of seagrass species (Mcleod et al., 2011). There are also further pressures to study seagrass ecosystems as they are increasingly impacted by anthropogenic activities and climate change. As of the 1990's, seagrasses have experienced an estimated 7% annual decline because of increasing urbanization, coastal eutrophication, siltation, dredging, aquaculture and invasive species (Waycott et al., 2009). This damage equates to roughly 299 Tg of carbon being released annually on a global scale (Fourqurean et al., 2012). In their degraded state, the natural recovery capacity of these landscapes is not on par with the intensity and scale of human-induced disturbances given the slow accrual of carbon. Moreover, the carbon storing capacity of these ecosystems is more often diminished in their new alternative state. For instance, a study conducted in Rottnest Island, Australia found that of the 4.8 ha of seagrass meadows lost due to mooring activities, an estimated 4.8 kg $/m^2$ of organic carbon was lost as a result when comparing to nearby undisturbed seagrass meadows (Serrano et al., 2014). A similar case study on seagrass beds in Jervis Bay, Australia, reported that a disturbance to the beds 50 years prior had shown that the recovering area contained 35% less carbon stock than the neighboring undisturbed sites (Macreadie et al., 2015). Without management intervention, the mounting combination of stressors will likely convert these carbon sinks into carbon sources.

This study observed the influence of salinity, temperature, turbidity, and wave motion on the underlying carbon sediment stock distribution of the temperate subtidal eelgrass (*Zostera marina*) meadows and subtidal unvegetated mud areas of Portage Inlet, located in the Greater Victoria region of Vancouver Island, British Columbia. Portage Inlet is a shallow basin in which previous carbon stock assessments have not been conducted and thus presents an opportunity to contribute to the growing data set for blue carbon stock assessments. This study attempted to answer the questions of (1) how the potential loss of the eelgrass meadows can impact the underlying sediment carbon stock and (2) what factors impede the eelgrass meadow’s ability to store sediment carbon.

Chapter 2. Goals and Objectives

The goal of this study was to understand what factors impact the eelgrass meadow's ability to store carbon through modeling and projecting the potential loss of sediment carbon stock given the degradation of the eelgrass meadows within Portage Inlet.

Goal 1: Determine how the potential loss of the eelgrass meadows can impact the sediment carbon stock of the inlet.

> **Objective 1.1:** Determine the current sediment carbon stock through the collection of sediment cores.
>
> **Objective 1.2:** Project the potential loss of carbon by comparing the sediment carbon stock to the adjacent unvegetated subtidal mudflat (mud arm) of the inlet

Goal 2: Identify factors that may interfere with the ability of the eelgrass meadow to sequester and store sediment carbon

> **Objective 2.1:** Assess the relationship between the productivity of the eelgrass meadows (in terms of percent cover, shoot density, and leaf area index) and the carbon sediment stock
>
> **Objective 2.2:** Assess the relationship between the physical-chemical factors (salinity, temperature, turbidity, and wave motion) and the carbon sediment stock

Chapter 3. Methods

3.1. Site Area: Portage Inlet

Portage Inlet is located in the Greater Victoria region of Vancouver Island, British Columbia (48°27'41.5"N, 123°25'09.3"W). It forms the head of a saltwater estuary arm where connections of freshwater from the Colquitz, Hospital, and Craigflower Creek flow through to the Gorge Waterway into Victoria Harbour. The Inlet is characterized as a 70 ha shallow tidal lagoon with a seabed that is predominately mud sediment and whose harbour depth reaches less than 2 m (Capital Regional District, 2000). The marine vegetation is dominated by 50 ha of eelgrass meadows of the common eelgrass species *Zostera marina* (Capital Regional District, 2000). The inlet's eelgrass meadows provide a refuge for juvenile salmonids, marine invertebrates and feeding grounds for migrating waterfowl (Capital Regional District, 2000).

It is important to note that due to accessibility and safety concerns, the study was restricted to the western portion of Portage Inlet. The extent of which the inlet was surveyed towards its most eastern coast was denoted by the municipal boundaries (Fig.1). Portage Inlet is categorized under the district municipalities of View Royal and Saanich. This study was conducted within the boundaries of the View Royal township.

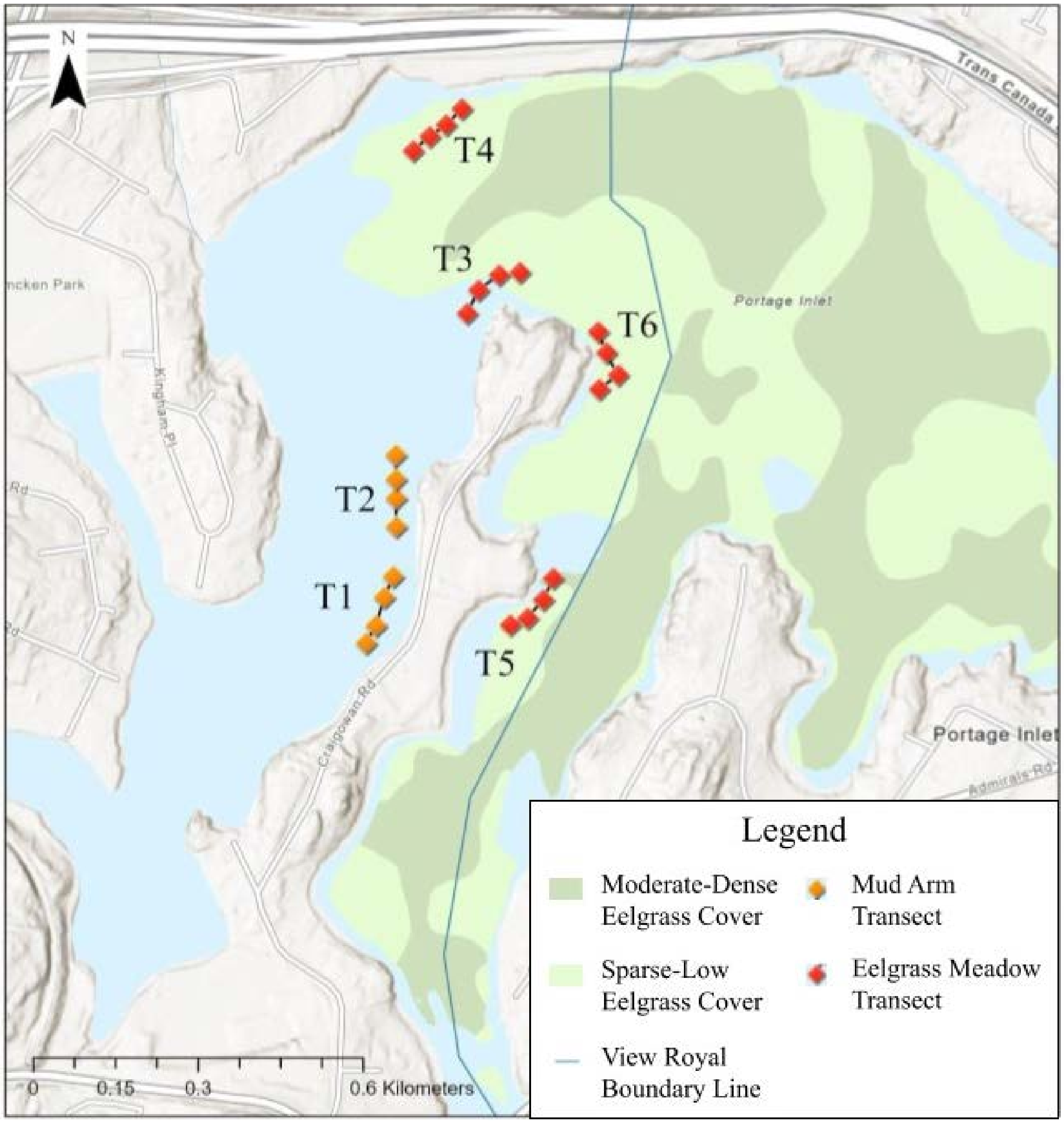

Figure 1. Map of blue carbon study site at Portage Inlet, Victoria, BC. Study was conducted in 2023. Location of 6 transects and 24 monitoring plots are pictured above. Transects 1-2 (T1-T2, red diamonds) represent monitoring plots conducted in the unvegetated subtidal mudflat (mud arm) and transects 3-6 (T3-T6, orange diamonds) represent monitoring plots conducted in the subtidal *Zostera marina* meadows. Transects were restricted within the township of View Royal and the city boundary line marks the extent of which the inlet was surveyed towards its most eastern coast. Basemaps of the eelgrass cover were taken from a subtidal survey conducted by the regional government in 2000 to estimate initial starting locations for transects (Capital Regional District, 2019a). Eelgrass cover was broken into two categories for mapping: sparse-low eelgrass cover (represents <25% coverage) and moderate-dense eelgrass cover (represents ≥ 25% coverage)(Capital Regional District, 2000). Additional basemaps layers were taken from ArcGIS Online and the Capital Regional District (boundary line)(Capital Regional District, 2019b; Esri, 2024).

3.2. Site Set-up

Six subtidal transects were systematically stratified across Portage Inlet to capture a representative sample of the carbon stock. Two transects were conducted along the unvegetated subtidal mudflat (mud arm) of the inlet and four transects were spread out across the subtidal eelgrass meadows. Transects were located approximately 20 meters away from the vegetated shoreline. Each transect was 100 meters in length and contained 4 x 1.0 m^2 monitoring plots spaced at a distance of approximately 25 meters apart. Monitoring plots were marked by driving 4 x 18 in length garden stakes in the sediment. A PVC float was attached to one of the stakes within each monitoring plot so the plot location was easily visible from the surface of the water. GPS coordinates of the monitoring plots were recorded (Appendix 1, Fig.1).

3.3. Monitoring of Eelgrass Productivity

To monitor the productivity of the eelgrass meadows, measurements of: shoot density, leaf area index (LAI) and percent cover were taken three times during the study for each monitoring plot. Data collection was limited to only three of the four eelgrass meadow transects as tidal interactions had rendered one transect inaccessible for these particular measurements in the later season. Percent cover was defined as the percent coverage of eelgrass shoots within the 1.0 m^2 monitoring plot. Shoot density was defined as the number of eelgrass shoots within the 1.0 m^2 monitoring plot. LAI was calculated as the shoot density multipled by the average width (mm) and length (mm) of five eelgrass shoots within the 1.0 m^2 monitoring plot (Durance, 2002). The five eelgrasses were chosen by measuring the individual closest to each corner of the monitoring plot and one random individual within the plot.

3.4. Monitoring of Physical-chemical Factors Influencing Productivity

To identify other potential factors that may influence the sediment carbon stock of the eelgrass meadows, measures of salinity, temperature, turbidity, and wave motion were taken at each monitoring plot.

Biweekly measurements of barometric pressure, conductivity and temperature were taken using a YSI at each respective plot during the midpoint of high and low tide. Similar protocols were followed for measurements of turbidity using a turbidimeter. Barometric pressure, temperature and conductivity were used to generate practical salinity through RStudio using the "gsw" package. Under the "gsw" package, the gsw_SP_from_C function uses formulations from the 1978 Practical Salinity Scale (Fofonoff & Millard Jr, 1983; Hill et al., 1986). This function required the use of sea pressure, however barometric pressure was substituted in its place. Measurements of water quality were taken within a meter from the surface water level, thus the differences of the two pressures were negligible.

Wave motion was monitored indirectly through clod cards using the procedures of Githaiga et al. (2019). Clod cards were mixed using 100 mL of water to 80 g of Plaster of Paris and molded using ice cube trays (4.3 L x 3.0 W x 2.5H cm per cube). Each cube was left to dry for a minimum of 3 days and then it was sanded to a weight of 9.5g $\pm$ 1.5g. The plaster cubes were glued onto a plastic sheet (7.6 x 5.2 cm) using marine epoxy glue (Amazing Goop ®) and the combined weight was recorded. Clod cards were strapped to each monitoring plot around the level of the sediment surface for 24 hours until removed for drying. Each clod card was air dried for a minimum of 48 hours until a constant weight and the final weight was recorded. The percentage weight loss of the clod card acted as a measure to describe the current speed that the eelgrass would be subjected to. Clod cards were conducted on a biweekly basis.

$$percent\ weight\ loss = \frac{final\ weight\ after\ 24h\ exposure}{initial\ weight} * 100$$

3.5. Sampling of Sediment Cores

The procedures for sediment core sampling in loose saturated soils were based on methods proposed by Fourqurean et al. (2012). One sediment core was taken at each of the 24 monitoring plots to assess the carbon stock of the eelgrass meadows and the mud arm. The sediment corer was a predrilled PVC pipe that was 5.08 cm in diameter and outfitted with 4 sampling ports running along its length. Each sampling port was 2.5 cm in diameter and was spaced at 5 cm intervals down the length of the pipe. Each sampling port was thus positioned at 2.5 cm, 7.5 cm, 12.5 cm and 17.5 cm to represent the respective soil depth intervals of 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm. Post extraction of the soils, a sub-corer was used to sample each of the four sampling ports and the volume of the subsample was noted. The sub-corer was made using a cut-off 25 mL polyethylene syringe (2.0 cm in diameter). Each sub-core sample was stored on ice in transit within a sterile bag and then refrigerated at 4ºC until laboratory analyses could occur.

3.6. Soil Carbon Stock Quantification

Procedures for the quantification of carbon stock measurements were based on methods outlined by Howard et al. (2014). All 24 core samples were calculated by employing the following equations in the preceding order.

1. $SDBD\left(\frac{g\,C}{cm^3}\right) = \frac{mass\ of\ dried\ soil\ (g)}{original\ wetted\ volume\ (cm^3)}$
2. $\%LOI = \frac{dry\ mass\ before\ combustion\ (mg) - dry\ mass\ after\ combustion\ (mg)}{dry\ mass\ before\ combustion} * 100$
3. $\%Corg = EA\ value\ slope\ (\%LOI) + EAvalue\ intercept$
4. $SCD\left(\frac{g\,C}{cm^3}\right) = \left(\frac{\%Corg}{100}\right) * SDBD\left(\frac{g\,C}{cm^3}\right)$
5. $Carbon\ Stock\ Core\left(\frac{g\,C}{cm^3}\right) = \sum_{i=0}^{n} SCD_i * 5cm$

Following equation (1), the soil dry bulk density (SDBD) was determined through the weighing and volumetric measurements of the sub-core samples. Each sub-core sample was placed in a pre-weighed container and oven-dried at 60 ºC for 72 hours until a constant dry weight was achieved (Howard et al., 2014). To check for dry weight, samples were taken out from the oven at the 24-hour mark and were left to cool in a silica desiccator cabinet for one hour prior to weighing (Postlethwaite et al., 2018). A less than 4% change in the dry weight was defined as a constant weight and thus the final 72-hour dry weight was taken for carbon stock calculations.

In equation (2), the percent loss-on-ignition (%LOI) was determined by homogenizing the dried sub-core samples using a mortar and pestle, and then placing them in a muffle furnace for 4 hours at 550 ºC (Postlethwaite et al., 2018). During homogenization of samples, larger allochthonous and autochthonous debris (e.g., stones, twigs, shells, plant material) were removed (Howard et al., 2014). Equipment used for homogenization was also cleaned between samples using 70% ethanol to prevent cross contamination. When removed from the muffle furnace, samples were placed in a silica desiccator for one hour prior to the weighing.

In equation (3), the percent organic carbon (%Corg) was determined through two methods due to the cost constraint of lab processing. The first method involved processing the direct measure of %Corg by selecting a subset of samples to undergo elemental analysis and processing via CO2 coulometer. One sediment core for every transect, along with all its sub-core samples, were taken for analysis. The resulting analyses gave the total carbon (%TC) and inorganic carbon (%IC). The %Corg was taken by subtracting the %TC from the %IC. The second method took the %LOI to convert into %Corg using a regression line against the subsamples taken for elemental analysis and processing via CO2 coulometer (Howard et al., 2014). A total of 6 slope equations were generated to convert the %LOI into %Corg (Fig. 2).

By using equations (4) and (5) the total carbon stock for one core sample was taken by summing the product of the soil carbon density (SCD) at each 5cm depth interval. With respect to equation (5), n is equal to the number of sub-cores, i is the depth of each core in 5 cm interval and $SCDi$ is the SCD of each 5cm interval of soil. The core carbon stock was then converted to common units and averaged to determine the carbon stock in megagrams carbon per hectare (Mg C/ha) for the eelgrass meadows and mud arm respectively.

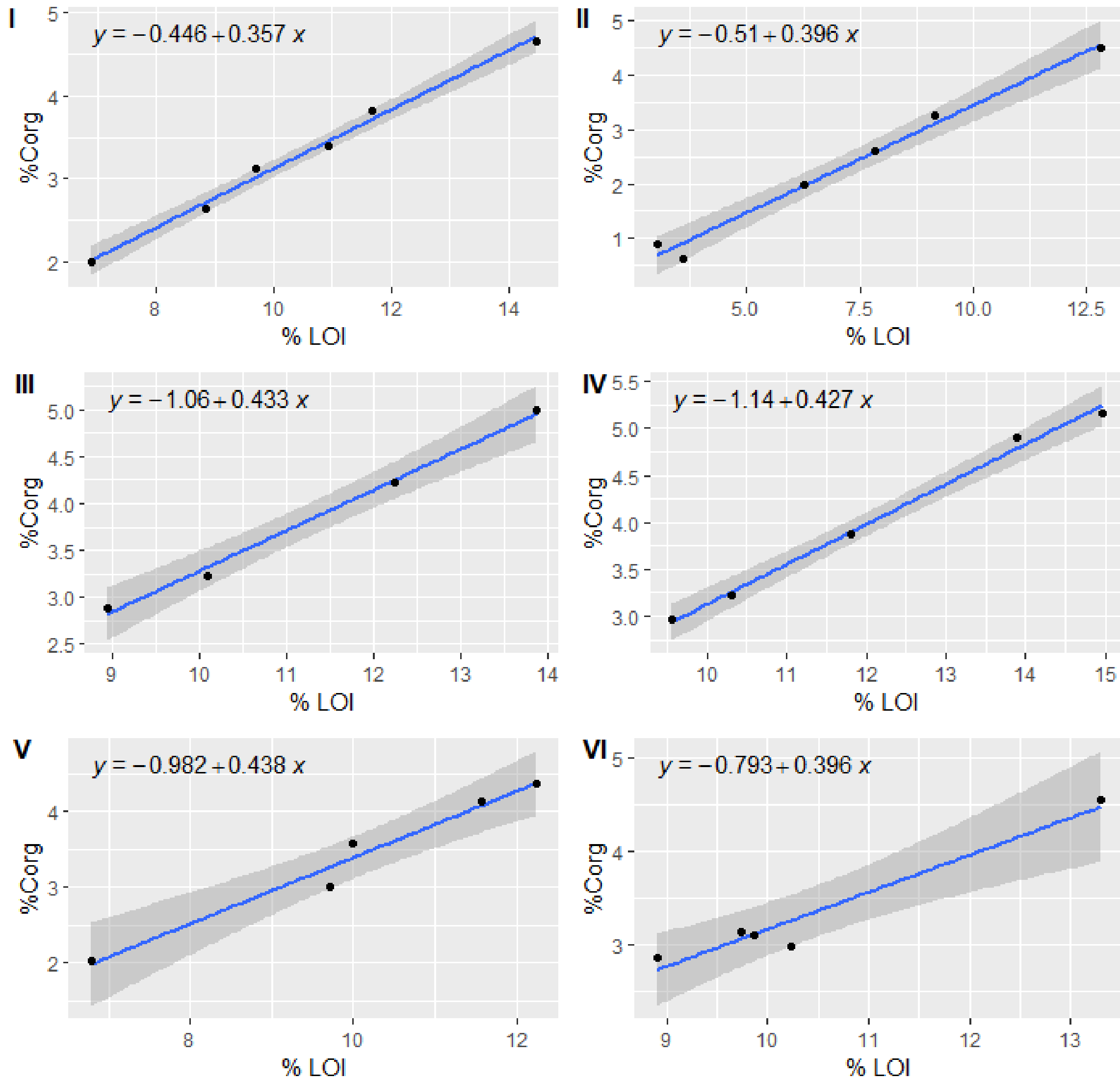

Figure 2. Percent organic carbon (%Corg) plotted against percent loss on ignition (%LOI) for sediment samples collected in Portage Inlet, Victoria, BC. I-II represent the sediment cores taken from the subtidal unvegetated mud arm and III-VI represent sediment cores taken from the subtidal eelgrass meadows (*Zostera marina*). Trendlines from the equations were applied to each sediment core subsample to convert the %LOI to %Corg as part of the carbon stock estimation.

3.7. Statistical Analysis

All analyses were conducted in R studio and the significance level was set to $\alpha = 0.05$ (RStudio Team, 2023). A linear mixed effects model was used to compare carbon stock differences between and within the transects and monitoring plots of the respective mud arm and eelgrass meadow. Multivariate linear mixed effect models were used to compare the carbon stock to the selected physical-chemical measures (salinity, temperature, turbidity and wave motion) as well as eelgrass productivity measures (percent cover, shoot density, and LAI). Data sets were averaged by monitoring plot and R package scale was used to standardize measures across all models. Variability in response effects from differences between and within transects and monitoring plots were accounted for through blocking measures. Model selection was assessed by using a stepwise forward selection approach. Variance inflation factors (VIF) for each model were also calculated and candidate predictors were tested in combination to generate VIFs less than 3. The degree of correlation was also further tested between predictors using Pearson's correlational test.

Chapter 4. Results

4.1. Carbon Stock

The average estimated carbon stock between the two sites were comparable to one another, with the eelgrass meadow's carbon stock at 39.4 $\pm$ 5.71 Mg C/ha and the mud arm's carbon stock at 38.9 $\pm$ 3.39 Mg C/ha (Table 1). The eelgrass meadow's range in estimated carbon stock averages per monitoring plot were more widespread than those of the mud arm's as it contained both the highest and lowest estimates (Table 1). Visualization of the average carbon stock per transect can be found in figure 4. Futher inspection into the %Corg revealed that both sites exhibit a decreasing trend in %Corg at further depth levels; the greatest amount of %Corg is found within the surface level depth (0-5cm). At every depth level, the eelgrass meadows also contained a greater %Corg than the mud arm (Fig. 5).

When accounting for the estimated hectare occupancy of the mud arm versus the eelgrass meadows, the eelgrass meadow's carbon stock was roughly 1970.53 Mg C (50ha) and the mud arm's carbon stock was roughly 778.39 Mg C (20ha). Though the current stock of the eelgrass meadow was larger based on its respective area of occupancy, the linear mixed effects model done on the average carbon stock for each site yielded insignificant differences between the eelgrass meadows and mud arm ($p=1.000$, $t=0.001$, $df=4, 20$). There was no difference in the average carbon stock within the eelgrass meadows and the mud arm. Visualization of the model can be found in figures 3-4.

Table 1. Summary of the carbon stock calculations (Mg C/ha) for the unvegetated subtidal mudflat (mud arm, 20 ha) and the eelgrass meadows (50 ha) residing in Portage Inlet, Victoria, BC. One sediment core was taken to the depth of 20 cm at every monitoring plot. There were six transects of which each contained four monitoring plots. Transect 1-2 represented cores taken from the unvegetated subtidal mudflat and transects 3-6 represent cores taken from the eelgrass meadows.

Core Identification	Monitoring Plot Average Stock (Mg C /ha)			Total Stock Estimate (Mg C)
	Mean ± SE	Lower Range	Upper Range	
Mud Arm	38.9 ± 3.39	35.6	45.8	778.39 (20ha)
Transect 1	37.6 ± 2.26	35.6	40.7	
Plot 1	40.7 ± 0.99			
Plot 2	36.2 ± 0.20			
Plot 3	37.8 ± 2.16			
Plot 4	35.6 ± 0.51			
Transect 2	40.3 ± 4.09	36.2	45.8	
Plot 1	40.6 ± 0.83			
Plot 2	36.2 ± 0.43			
Plot 3	38.5 ± 5.84			
Plot 4	45.8 ± 4.04			
Eelgrass Meadows	39.4 ± 5.71	34.3	53.7	1970.53 (50ha)
Transect 3	36.7 ± 3.95	34.3	42.6	
Plot 1	42.6 ± 1.02			
Plot 2	35.0 ± 1.01			
Plot 3	35.0 ± 1.09			
Plot 4	34.3 ± 0.93			
Transect 4	34.3 ± 0.50	33.8	34.8	
Plot 1	33.8 ± 0.66			
Plot 2	34.0 ± 0.63			
Plot 3	34.7 ± 0.81			
Plot 4	34.8 ± 0.54			
Transect 5	44.3 ± 6.84	37.9	53.7	
Plot 1	44.3 ± 0.88			
Plot 2	37.9 ± 1.97			
Plot 3	41.1 ± 2.30			
Plot 4	53.7 ± 3.15			
Transect 6	42.3 ± 3.68	38.1	46.6	
Plot 1	38.1 ± 1.61			
Plot 2	46.6 ± 4.20			
Plot 3	43.7 ± 1.05			
Plot 4	40.9 ± 0.60			

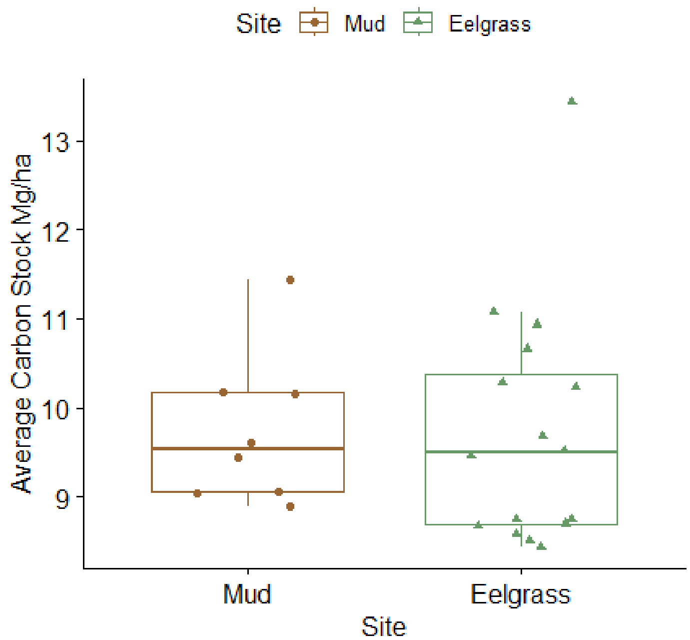

Figure 3. A comparison of the average carbon stock (Mg C/ha) of sediment cores taken in *Zostera marina* eelgrass meadows (n=16) and an unvegetated subtidal mudflat (n=8) within Portage Inlet, Victoria, BC.

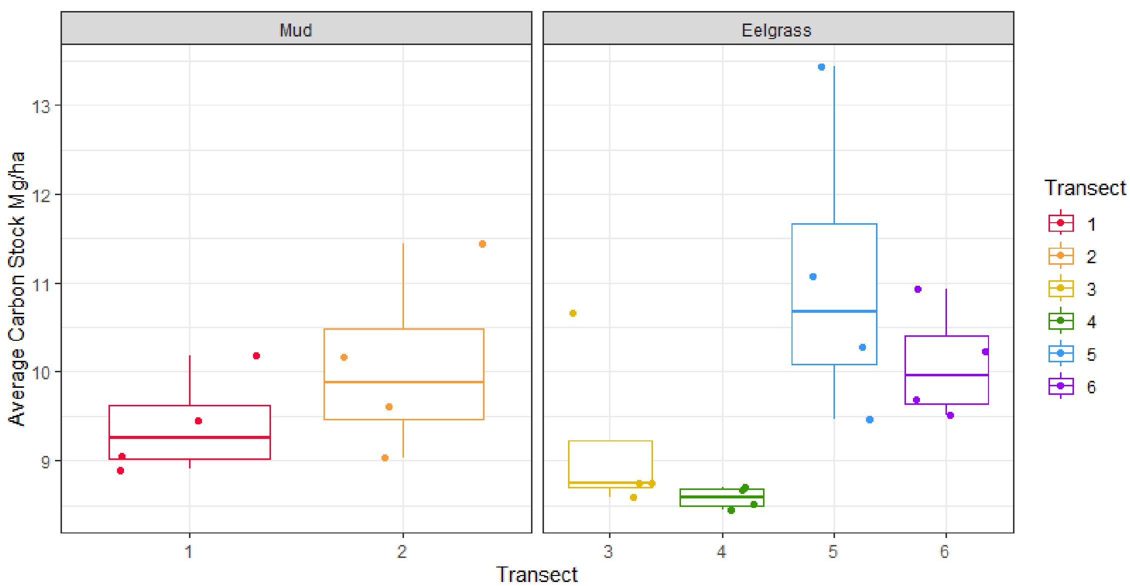

Figure 4. Boxplot of the average carbon stock (Mg C/ha) amongst 6 different transects taken within Portage Inlet, Victoria, BC. Sediment cores from transects 1-2 were collected from an unvegetated subtidal mudflat (n=8). Sediment cores from transects 4-6 were collected from a subtidal eelgrass meadow of the species *Zostera marina* (n=16).

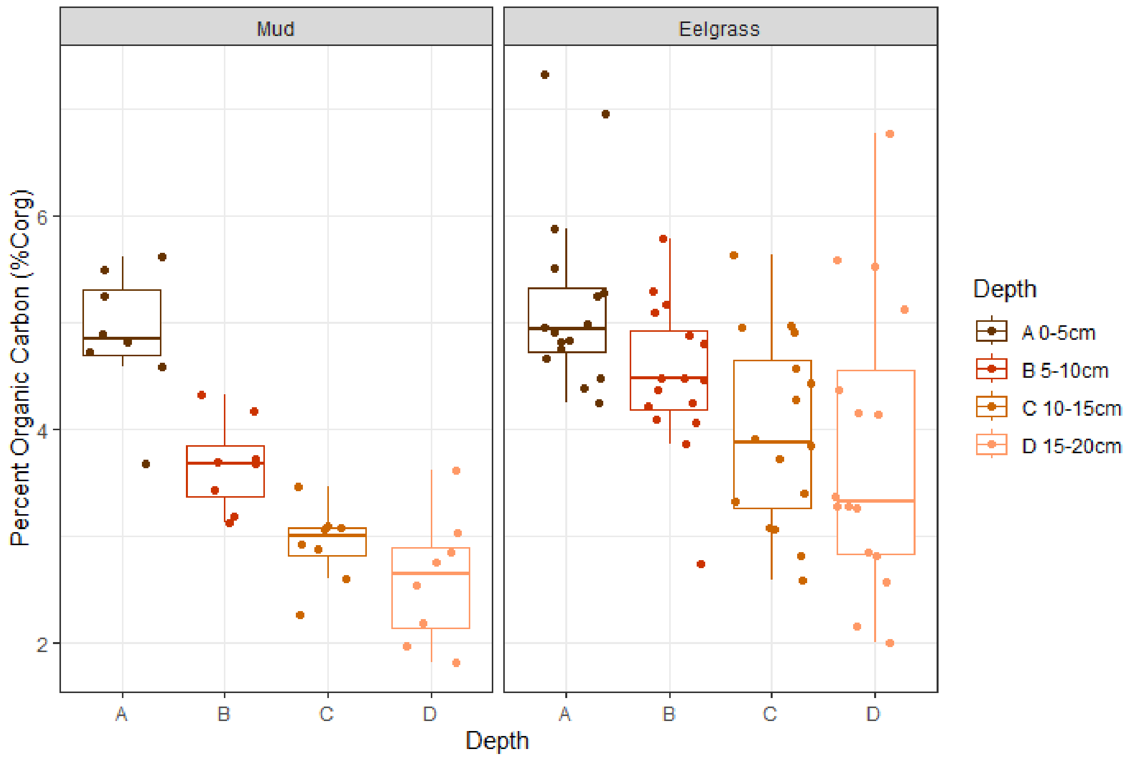

Figure 5. A Boxplot comparison of the percent organic carbon (%Corg) amongst four different depth levels and two different site categories within Portage Inlet, Victoria, BC. 8 cores were collected from an unvegetated subtidal mudflat and 16 cores were collected from a subtidal eelgrass meadows of the species *Zostera Marina*. Each lettered core depth corresponds to the depth interval at which the sample was taken at 5 cm increments below the sediment surface level. Level A represents the shallowest depth from 0-5 cm and level D represents the deepest depth cored at 15-20 cm (n=24). (Level B: 5-10 cm, Level C: 10-15 cm, Level D: 15-20 cm).

4.2. Physical-chemical Measures

Average water quality measures between the mud arm and eelgrass meadows were found to be similar for salinity, temperature, turbidity and wave motion. Over the progression of the study period: the average salinity was 0.2 PSU greater in the eelgrass meadows, the average temperature was the same in both sites at 24.6°C, the average turbidity was greater in the mud arm by 0.07 NTU, and the average wave motion was 1.8% greater in the eelgrass meadows (Table 2). Though all physical-chemical measures were similar between the two sites, the range in turbidity for the eelgrass meadows was nearly three times as large as the mud arm (Table 2).

Multivariate linear mixed effects models done on all combinations of standardized physical-chemical measures (temperature, turbidity, salinity and wave motion) and their respective interactions yielded signficant explanatory power for individual models of temperature (p=1.76e-

4, x^2=14.06, df=4, 20), turbidity (p=5.87e-3, x^2=7.59, df=4, 20), and salinity (p=2.27e-4, x^2=13.60, df=4, 20) (Table 3). Salinity exhibited a positive effect on the average carbon stock, whereas the temperature and turbditiy exhibited a negative effect. The salinity model had the highest explanatory power, with marginal R^2 value of 0.426 and a conditional R^2 value of 0.487. That is 42.6% of the variation in average carbon stock can be explained by the salinity model when accounting for only fixed effects and a futher 6.1% can be added when accounting for both fixed and random effects. The temperature model had a marginal R^2 value of 0.410 and a conditional R^2 value of 0.437. That is 41% of the variation in average carbon stock can be explained by the temperature model when accounting for only fixed effects and a futher 2.7% can be added when accounting for both fixed and random effects. The turbidity model had a marginal R^2 value of 0.266 and a conditional R^2 value of 0.303. That is 26.6% of the variation in average carbon stock can be explained by the turbidity model when accounting for only fixed effects and a futher 3.7% can be added when accounting for both fixed and random effects. Summary results of the mixed effects models can be found in table 3.

Out of all the statistically signficant model combinations, the temperature model was considered as the best fit model. Though the salinity model had the highest explanatory power it was negatively correlated to the temperature (p=3.79e-12, t=-13.54, df=22). This was to be expected as the values taken to form the PSU were based on the same values of temperature taken from the temperature model. A further examination of VIF between temperature and salinity confirmed values greater than 7 when both were featured as fixed effects in the same model. Conversely, the turbidity model has the lowest explanatory power in comparison to both salinity and temperature models. It is noted that the turbidity is positively correlated to the temperature (p=1.02e-7, t=7.74, df=22). An examination of VIF between temperature and turbidity revealed values just over 3 when both were featured as fixed effects in the same model. Temperature was a more independent measure than salinity alone and had the higher explanatory power in comparison to the turbidity variable. Therefore, temperature differences best explained the variation in the average carbon stock across Portage Inlet.

Table 2. Summary statistics for physical-chemical measures of salinity (PSU), temperature ($°C$), turbidity (NTU), and wave motion (% current speed) in Portage Inlet, Victoria, BC. Measures were averaged across 8 monitoring plots located in the unvegetated subtidal mudflat (mud arm) and 16 monitoring plots located in the subtidal eelgrass meadow (*Zostera marina*).

Site	Mud Arm		Eelgrass Meadow	
	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range
Salinity (PSU)	32.3 $\pm$ 2.68	27.4 – 37.1	32.5 $\pm$ 1.85	29.2 – 36.3
Temperature(°C)	24.6 $\pm$ 0.41	20.9 – 27.2	24.6 $\pm$ 1.60	21.7 – 27.7
Turbidity (NTU)	1.31 $\pm$ 0.33	0.79 – 2.20	1.24 $\pm$ 0.84	0.26 – 4.26
Wave Motion* (%Current Speed)	85.8 $\pm$ 7.00	74.4 – 102	87.6 $\pm$ 8.81	58.5 – 103

* Wave motion was monitored indirectly through clod cards using the procedures of Githaiga et al. (2019).The percentage weight loss of the clod card acted as a measure to describe the current speed that the eelgrass would be subjected to.

Table 3. Summary of linear mixed-effects models evaluating differences in physical-chemical measures of salinity (PSU), temperature ($°C$), turbidity (NTU), and wave motion (% current speed) against the average carbon stock (Mg C/ha) found in Portage Inlet, Victoria, BC. Physical-chemical measures were averaged across 24 monitoring plots, 8 of which were located in the unvegetated subtidal mudflat and 16 of which were located in the subtidal eelgrass meadow (*Zostera marina*).

	Model evaluation				
Fixed Effects	R_m^2	R_C^2	df	x^2	p
Salinity (PSU)	0.426	0.487	4, 20	13.60	2.27e-4
Temperature (°C)	0.410	0.437	4, 20	14.06	1.76e-4
Turbidity (NTU)	0.266	0.303	4, 20	7.59	5.87e-3
Wave Motion* (%Current Speed)	0.001	0.335	4, 20	0.016	8.99e-1

Note: The marginal $R^2(R_m^2)$ explains the amount of variance due to fixed effects within the model. The conditional $R^2(R_C^2)$ explains the amount of variance due to fixed and random effects.
* Wave motion was monitored indirectly through clod cards using the procedures of Githaiga et al. (2019).The percentage weight loss of the clod card acted as a measure to describe the current speed that the eelgrass would be subjected to.

4.3. Eelgrass Productivity

Percent cover, shoot density and LAI decreased over the progression of the study period for all transects monitored (Fig. 6). The average percent cover, shoot density and LAI was greatest in transect 3. For the duration of the monitoring period: the average percent cover of transect 3 was 2.5 times greater than that of transect 6 and 1.5 times greater than transect 4, the average shoot density of transect 3 was similar to transect 4 and greater than transect 6 by 1.6 times, and the average LAI of transect 3 was 1.3 times greater than that of transect 4 and 1.5 times greater than transect 6. Further summary statistics can be found in table 4.

Multivariate linear mixed effects models done on all combinations of standardized productivity measures (percent cover, shoot density and LAI) and their respective interactions yielded insignficant explanatory power for all model combinations. Neither individual models of percent cover (p=0.133, x^2=0.100, df=2, 8), shoot density (p=0.221, x^2=0.187, df=2, 8), or LAI (p=0.164, x^2=0.127, df=2, 8), could explain the variation in the average carbon stock found within Portage Inlet (Table 5). Percent cover as the only fixed effect variable had the highest explanatory power, with marginal R^2 value of 0.149 and a conditional R^2 value of 0.543. That is 14.9% of the variation in average carbon stock can be explained by this model when accounting for only fixed effects and a futher 39.4% can be added when accounting for both fixed and random effects. Shoot density as the only fixed effect variable had a marginal R^2 value of 0.094 and a conditional R^2 value of 0.474. LAI as the only fixed effect variable had a marginal R^2 value of 0.100 and a conditional R^2 value of 0.562. All 3 models were found to exhibit negative effects on the average carbon stock.

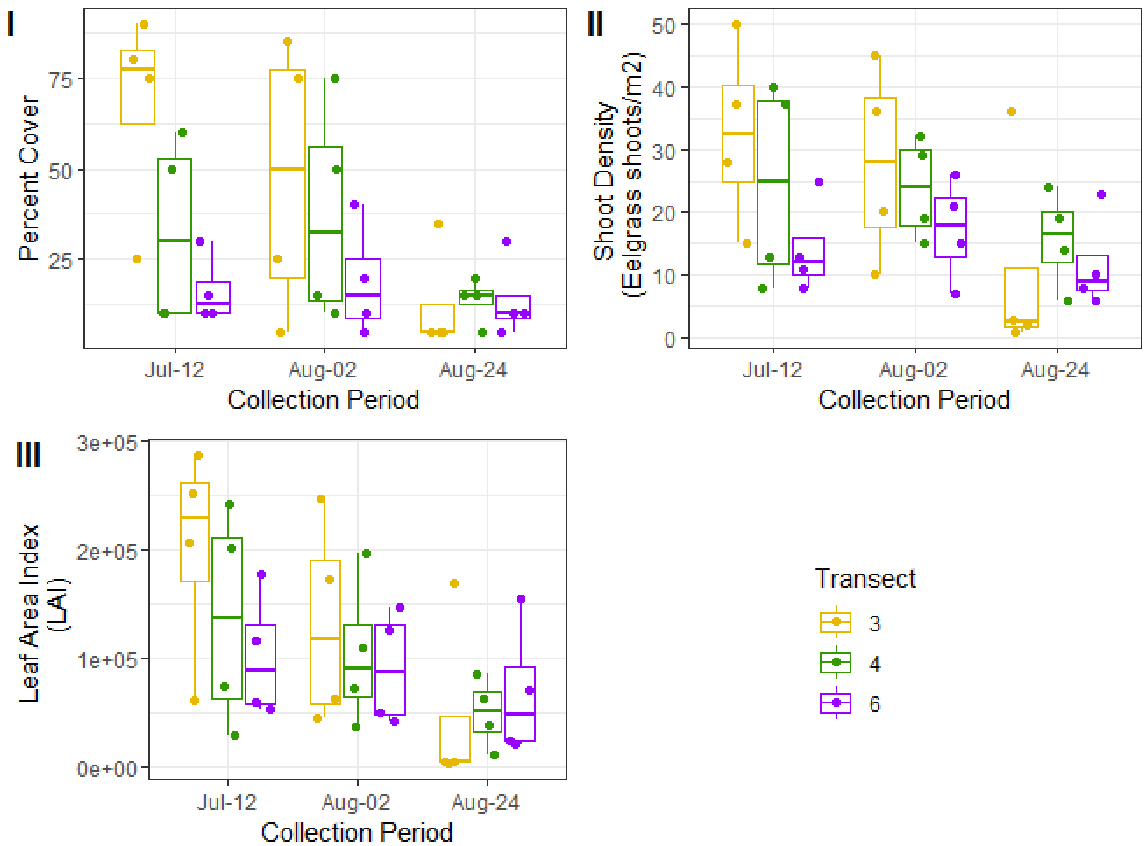

Figure 6. Boxplots of productivity measures for three different transects of eelgrass meadows (Zostera Marina) plotted against time. Study data was collected in Portage Inlet, Victoria, BC, over the course of three data collection periods. Amongst four transects conducted in the eelgrass meadows, three of the four transects were chosen for assessment. Each transect contained four monitoring plots in which were included in the data above. Plot I, percent cover was defined as the percent coverage of eelgrass shoots within the 1.0 m^2 monitoring plot. Plot II, shoot density was defined as the number of eelgrass shoots within the 1.0 m^2 monitoring plot. Plot III, LAI was calculated as the shoot density multipled by the average width (mm) and length (mm) of five eelgrass shoots within the 1.0 m^2 monitoring plot. The five eelgrasses were chosen by measuring the individual closest to each corner of the monitoring plot and one random individual within the plot.

Table 4. Summary statistics concerning the productivity measures of the eelgrass meadows (*Zostera Marina*) within Portage Inlet, Victoria, BC. Percent cover, shoot density, and lead area index (LAI) were monitored in three transects conducted in the eelgrass meadows. Each transect contained four monitoring plots and were averaged for summary assessment. Percent cover was defined as the percent coverage of eelgrass shoots within a 1.0 m^2 monitoring plot. Shoot density was defined as the number of eelgrass shoots within a 1.0 m^2 monitoring plot. LAI was calculated as the shoot density multiplied by the average width (mm) and length (mm) of five eelgrass shoots within a 1.0 m^2 monitoring plot. The five eelgrasses were chosen by measuring the individual closest to each corner of the monitoring plot and one random individual within the plot.

Transect	3		4		6	
	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range
Percent Cover	42.5 $\pm$ 35.51	5 – 90	27.9 $\pm$ 23.8	5 – 75	16.3 $\pm$ 11.3	5 – 40
Shoot Density	23.6 $\pm$ 17.4	1 – 50	21.3 $\pm$ 11.1	6 – 40	14.4 $\pm$ 7.42	6 – 26
LAI	126841.5 $\pm$ 107024.2	3346.77 – 287346.20	97356.06$\pm$ 75601.76	12096.75 – 242101.45	87256.31 $\pm$ 53999.96	21649.84 – 177257.70

Table 5. Summary of linear mixed-effects models evaluating differences in the productivity measures of eelgrass meadows (percent cover, shoot density, and leaf area index (LAI)) against the average carbon stock (Mg C/ha) found in Portage Inlet, Victoria, BC. Measures were average across 12 monitoring plots located in the subtidal eelgrass meadows (*Zostera marina*).

	Model evaluation				
Fixed Effects	$\boldsymbol{R_m^2}$	$\boldsymbol{R_C^2}$	df	$\boldsymbol{x^2}$	p
Percent Cover	0.149	0.543	2, 8	0.100	0.133
Shoot density	0.094	0.474	2, 8	0.187	0.221
Leaf Area Index (LAI)	0.100	0.562	2, 8	0.127	0.164

Note: The marginal $R^2(R_m^2)$ explains the amount of variance due to fixed effects within the model. The conditional $R^2(R_C^2)$ explains the amount of variance due to fixed and random effects.

Chapter 5. Discussion

5.1. Carbon Stock Variation

The primary objective of this study sought to determine how the loss of the eelgrass meadows within Portage Inlet could impact the underlying sediment carbon stock by projecting carbon loss using the carbon stock of the adjacent unvegetated mud arm in Portage Inlet. The data concluded that there were no statistically significant differences between the average carbon stock found in the eelgrass meadows (39.4 $\pm$ 5.71 Mg C/ha) and the adjacent mud arm (38.9 $\pm$ 3.39 Mg C/ha). Core samples taken from both sites had comparable carbon stocks and showed similar trends of decreasing %Corg with increasing depth levels. This projection implied a scenario in which a loss of the eelgrass meadows would have little to no impact on the carbon stock of the inlet. This would indicate that the *Zostera marina* meadows within the inlet stored carbon on a comparable scale to the unvegetated subtidal mudflat.

When conducting comparisons of sediment carbon stock, the general assumption is that vegetated coastal ecosystems are often regarded to have higher sediment carbon content compared to their unvegetated counterparts; this trend is not always definitive along the Northern Pacific Coastline (Prentice et al., 2020). Some studies within this region have reported *Z.marina* meadows to have a greater carbon stock over neighbouring unvegetated sediments (Oreska et al., 2017; Postlethwaite et al., 2018), whereas others have reported the stock assessments to be equal or vice versa (Douglas et al., 2022; Prentice et al., 2019). This observed variation in carbon stock can be attributed to the meadow's inherently low capacity for sequestering carbon (Postlethwaite et al., 2018; Rohr et al., 2018) and high capacity to dampen waves in adjacent seascapes (Huxham et al., 2018; Reidenbach & Thomas, 2018; Twomey et al., 2020). In comparison to other seagrass species, *Z.marina* has a smaller canopy, shallower root system, patchy nature and persists in soils of higher oxygen content and low anoxic sediment (Murphy et al., 2021; Rohr et al., 2018). Given unfavorable environmental conditions, these attributes would prove disadvantageous as they can foster higher microbial remineralization rates of sediment carbon (Howard, 2018) and an overall lower sediment carbon stock. Moreover, seagrass meadows have a dampening effect on wave energy which allows for vertical accrual of sediment further up in the shoreline (Huxham et al., 2018; Risandi et al., 2023). This effect can be increasingly significant along long continuous patches of seagrasses; Fonseca and Cahalan's (1992) study has shown that *Z.marina* is amongst one of four other seagrass species found to reduce wave energy by as much as 40% per meter of seagrass given instances where seagrass length was similar to water depth. This would therefore increase the sediment carbon stock for adjacent seascapes that are not vegetated.

When applying these principles to Portage Inlet, it can be theorized that the eelgrass meadows within the inlet were providing a service of wave relief to the mud arm. The mud arm is well enclosed by a barrier of eelgrass and is higher inshore from the force of open coastal waters. This in combination with high bouts of inwelling from detrital seagrass wrack and allochthonous inputs from two different creek systems could have increased the sediment carbon stock of the mud arm. While consequently, other stressors to the seagrass meadows may have created adverse conditions that would foster higher remineralization rates of carbon and decrease the inherent sediment carbon stock of the meadows. When observing the %Corg at each depth level for Portage Inlet, the remineralization rate of the eelgrass meadows appeared similar to that of the mud arm as both seascapes had a comparable decrease in the %Corg with increasing depth. Seagrass depth profiles with this inherent shape are implied to have a high turnover and remineralization of carbon while the organic matter is decomposed (Kindeberg et al., 2019). Despite the advantage of having a vegetated canopy and rhizome mat to facilitate particulate carbon capture, the eelgrass meadows were still unable to bury more organic carbon in comparison to the adjacent unvegetated mud arm. These factors in combination could be plausible explanations for why the two seascapes share similar carbon stock estimates. Therefore, it is not advisable to suggest that a total loss of eelgrass within Portage Inlet would have no impact on the underlying sediment carbon stock.

Further variations in the observed carbon stock within Portage Inlet may have also been influenced by elements of the study methodology. The core sediment samples were primarily collected towards the western portion of the Inlet due to accessibility issues and safety concerns. This prevented sampling in areas where the eelgrass meadows were perhaps more continuous, less patchy and further away from the meadow’s edge. It has been established that the carbon stock of seagrass meadows can be impacted by spatial components where greater amounts of carbon can be found in the middle of the meadow rather than the edges (Oreska et al., 2017). Moreover, core sediment samples were only taken to the depth of 20 cm and were further subsampled at each 5cm increment. This was done to decrease the time constraints associated with processing samples and to reduce the difficulties associated with sampling in the subtidal zone. It is recommended that studies quantifying carbon stock estimates take sediment samples to the depth of refusal (depth at which the sediment core can no longer be pushed through) or at least 1m below the surface level to create a more accurate calculation (Howard et al., 2014).

5.2. Variation from Physical-chemical and Productivity Measures

The secondary objective of this study sought to determine what site-specific factors may have interfered with the ability of the eelgrass meadows to store sediment organic carbon. Out of the four assessed physical-chemical factors (temperature, turbidity, salinity and wave motion), individual models of temperature, turbidity and salinity were significant factors attributed to the carbon stock variation. However, the temperature model was ultimately chosen as the best fit model as it had the highest explanatory power and better independence from other variables. Out of the three productivity measures (percent cover, shoot density and LAI), neither models were statistically significant.

The temperature of the eelgrass meadows within Portage Inlet was on average 24°C with the highest temperature peaking around 28°C. The optimum temperature range for temperate eelgrass to sufficiently conduct photosynthesis would be around 10°C to 25°C (Lee et al., 2007). Anything above this threshold is found to induce significant stress to the eelgrass as higher temperatures increase respiration and decrease photosynthetic activities (Gao et al., 2019; Staehr & Borum, 2011). This effectively hinders their metabolic function and decreases the eelgrass's carbon storage capabilities from low biomass production (Staehr & Borum, 2011). This relationship is highlighted in my study as the temperature model showed a significant negative effect to the carbon stock; an increase in temperature would imply a decrease in carbon stock.

The Salinity of the eelgrass meadows within Portage Inlet was on average 32.5 PSU with the highest salinity peaking around 36.3 PSU. The optimal salinity range for temperate eelgrass growth would fall between 10 to 30 PSU (Phillips, 1974). Higher salinity stress is not often examined for eelgrass species, so although the meadows within Portage Inlet are slightly above the optimal growth range there exists site-to-site acclimatization mechanisms that would grant some leniency in range tolerances (Murphy et al., 2021). Low salinity values are more often a threat to eelgrass in the Pacific Coast as climate models project higher precipitation levels and therefore an increase in freshwater input to marine environments (Cummins & Masson, 2014; Greene & Pershing, 2007). This would be especially damaging to more enclosed coastal areas like Portage Inlet. Low salinity values below 20 PSU are associated with slower growth, higher mortality and lower seedling establishment in temperate eelgrass species (Murphy et al., 2021). This relationship is similar to the findings within my study as the salinity model showed a positive effect to carbon storage; that is an increase in salinity would imply an increase in carbon stock.

The turbidity of the eelgrass meadows within Portage Inlet was on average 1.24 NTU with the highest turbidity measurement around 4.26 PSU. Eelgrass meadows are greatly affected by light limitation from turbid waters and a decline can decrease their abilities to photosynthesize, which limits the growth of biomass available for carbon sequestration (Short et al., 2011; Waycott et al., 2009). This relationship is similar to the findings within my study as the turbidity model showed a negative effect to carbon storage; that is an increase in turbidity would imply a decrease in carbon stock. The explanatory power of the turbidity model was perhaps not as strong in comparison to the salinity or temperature models because of an additional confounding factor. During the monitoring period, it was noted that the inlet became eutrophic towards the end of the study and most eelgrass shoots were covered in epiphytes. The progression of the eutrophication in certain plots can be pictured in figure 7. Epiphytes can reduce photosynthesis in the eelgrass leaves by reducing available light intensity and carbon uptake (Sand-Jensen, 1977). The excess epiphytes and floating algae that came about with the eutrophication, most likely impacted the turbidity readings for the eelgrass meadows. This is perhaps why the range of turbidity was twice as great in the eelgrass meadows in comparison to the mud arm.

Wave motion was not a statistically significant variable to explain the carbon stock variation and this could be due to the set-up of the clod cards. In Githaiga et al.'s (2019) study, they employed the same use of clod cards as an indirect measure for water velocity and they found that a higher placement of the clod cards in water column equated to exposure of eelgrass to higher speeds of water current. Given that the clod cards in this study were placed at the level of the sediment surface, the current velocity experienced between vegetated and unvegetated sites were most likely similar. Future studies should take note to test for multiple height variations or seek alternative methodologies for a more direct measure of current speed.

Overall variations in explanatory power between the temperature, salinity and turbidity exist because of limitations associated with project methodologies. Temperature and salinity were highly correlated because the values to form the PSU were based on the same values of temperature taken from the temperature model. If this study had used a seperate instrument for salinity measurements, the high collinearity could have potentially been avoided. Moreover, all physical-chemical measurements were taken at discrete sampling times rather than in continuous succession. Due to budget constraints data loggers were unavailable for every monitoring plot, but had this been employed it could have provided more insight as to daily fluctuations. In addition, long-term monitoring would have been beneficial as my study results cannot comment as to the variation in the sediment carbon stock on a greater temporal scale.

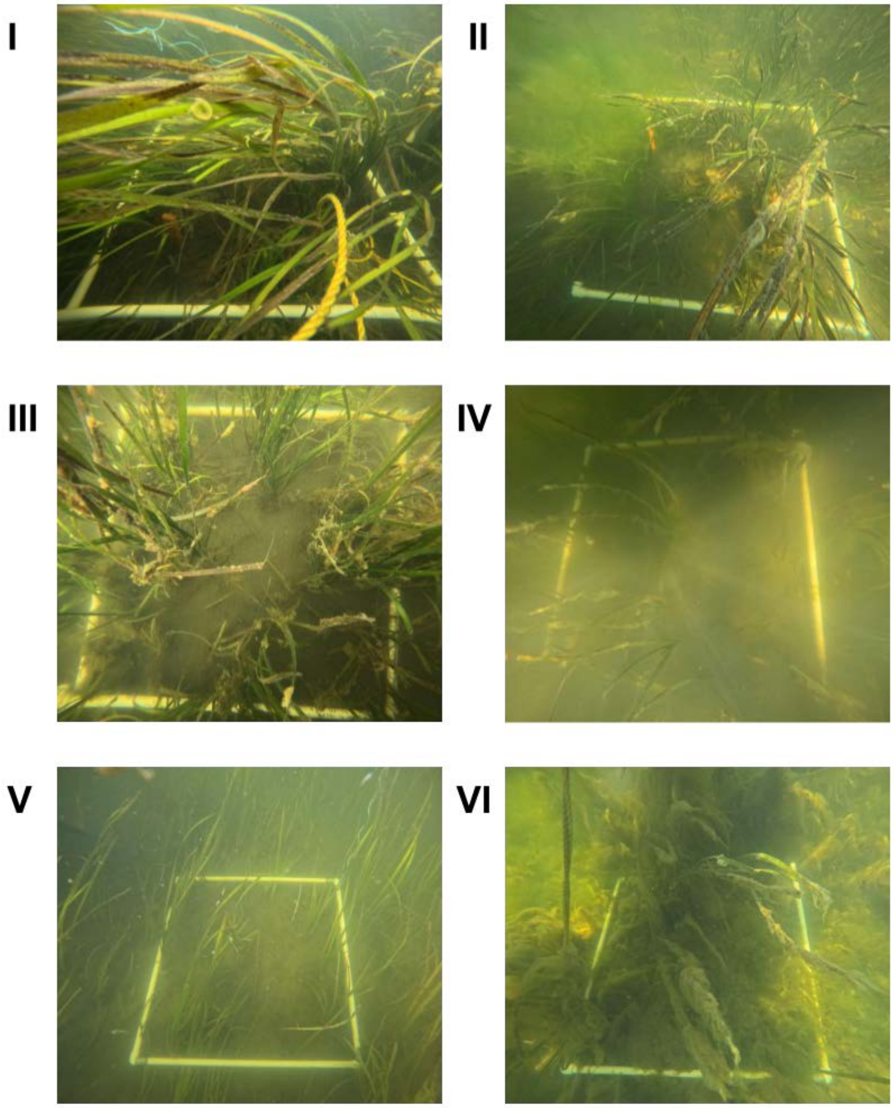

Figure 7. Before and after snapshots of eelgrass (*Zostera* marina) monitoring plots taken over a progression of 43 days (July 12 to August 24) at Portage Inlet, Victoria, BC. The pairings of Plot I-II, Plot III-IV, and Plot V-VI, each represent the progression of one respective monitoring plot.

The overall productivity measures (percent cover, shoot density and LAI) were unable to explain the variation in the sediment carbon stock. All productivity measures had negative effects on the carbon stock model, where an increase in productivity implied a decrease in carbon stock for the eelgrass meadows. This is contrary to the findings of most studies concerning high productivity and carbon storage within eelgrass meadows. Seagrass canopies with more structural complexity are found to better reduce hydronamic energy as they can filter and bury more organic particulate matter (Mazarrasa et al., 2018). Seagrass meadows with higher biomass (shoot density and LAI) are also found to have greater carbon stocks (Kim et al., 2022; Samper-Villarreal et al., 2016).

Instances where higher biomass may be associated with decreased carbon storage would arise if intense canopy density began to facillitate self-shading and decreased light accessibility (Collier et al., 2012; Murphy et al., 2021). In these cases, it would appropriate for a decrease in biomass to signal an increase in carbon storage. Though the productivity measures declined over the study period, it is unclear whether this was indicative of similar coping mechanisms for self-shading from earlier in the season. Future studies should explore the implications of sediment carbon stock given self-shading events in eelgrass meadows. Studies should also monitor producitivity measures on an annual timeline to capture the extent of variability in association with the carbon stock.

Chapter 6. Conclusion

This study conducted the first sediment carbon stock assessment within the subtidal eelgrass meadows of Portage Inlet, Victoria. Study findings suggest that the sediment carbon stock within the eelgrass meadows were statistically comparable to the adjacent unvegetated subtidal mudflat (39.4 ± 5.71 Mg C/ha, 38.9 ± 3.39 Mg C/ha). Model analysis revealed that all productvitiy measures examined could not statistically explain for the variation in the carbon stock within the inlet, however individual models of salinity, temperature and turbidity were more likely attributed to the variation seen. Future studies should continue to analyze the interaction of biological, chemical and physical components in seagrass environments to obtain a more comprehensive understanding of blue carbon dynamics. Futhermore, these studies should be carried out with a greater consideration to temporal changes in the interaction of site attributes for restoration and management purposes.

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Appendix A. Sample Site Information

Table A.1. Summary of sediment core locations taken in Portage Inlet, Victoria, BC. A total of 24 sediment cores were taken across 24 monitoring plots divised into 6 transects. Transect 1-2 represent cores taken from the unvegetated subtidal mudflat (mud arm) and transects 3-6 represent cores taken from the eelgrass meadows. Core ID represents the particular transect (T) and monitoring plot (MP) in which the sediment core was taken. Additional information on location (latitude and longitude) and average water depth are listed. The average water depth was taken by measuring the distance between the sediment surface and the water at surface level during the midpoint of low and high tide across 3 consecutive days.

Core ID	Latitude	Longitude	Average Water Depth (m)
Mud Arm			
T1MP1	48.457429	-123.425409	0.60
T1MP2	48.457619	-123.425248	0.648
T1MP3	48.457925	-123.425115	0.661
T1MP4	48.45815	-123.424968	0.676
T2MP1	48.45869	-123.424919	0.695
T2MP2	48.458996	-123.424921	0.729
T2MP3	48.459203	-123.424923	0.757
T2MP4	48.459464	-123.424925	0.759
Eelgrass Meadows			
T3MP1	48.460998	-123.423761	0.895
T3MP2	48.46125	-123.423574	0.987
T3MP3	48.461414	-123.423237	0.994
T3MP4	48.461442	-123.422899	1.06
T4MP1	48.463211	-123.423847	0.995
T4MP2	48.463039	-123.424103	1.07
T4MP3	48.462912	-123.424372	1.14
T4MP4	48.462758	-123.424642	1.11
T5MP1	48.457627	-123.423057	1.26
T5MP2	48.4577	-123.422773	1.33
T5MP3	48.457899	-123.422518	1.46
T5MP4	48.458133	-123.422358	1.66
T6MP1	48.460178	-123.421604	1.11
T6MP2	48.460332	-123.421294	1.08
T6MP3	48.460574	-123.421485	1.21
T6MP4	48.460799	-123.421622	1.12