PHYSICAL FACTORS ASSOCIATED WITH THE LIMITS TO *ZOSTERA MARINA* DISTRIBUTION IN ROSARIO BAY, WA

By

ERICA FRANKLIN

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has been approved by the Department of Biological Sciences
and the Office of Graduate Studies
Walla Walla University

David L. Cowles

Major Professor

Kirt Onthank

Committee Member

Janice McKenzie

Committee Member

Committee Member

Pamela Cress

Dean of Graduate Studies

[illegible] Watkins

Observer of the Process - Graduate Representative

Erica Franklin

Candidate

7-7-2017

Date

ABSTRACT

Eelgrasses and other seagrasses are important habitats along the Pacific Northwest coast for a variety of marine life. They are also widely known to be indicators of coastal ecological health. There are many parameters that affect eelgrass growth, and several of those parameters were investigated in order to determine whether or not those factors were associated with the limits to *Z. marina* growth in Rosario Bay, Washington. Water motion for different locations was measured throughout one summer season using clod cards. The locations chosen were seaward of the eelgrass beds, within the eelgrass beds, and shoreward of the eelgrass beds. Sediment size, the percent organic content, and percent nitrate, phosphate, and sulfate in the sediment was also measured and analyzed for differences among locations. The results showed that water motion did not differ between locations of the bay during the summer measurement season, but sediment size, organic content, and nutrients did vary systematically among locations. The locations on the shoreward side of the eelgrass beds were characterized by a higher amount of gravel, and less organic content than the locations within and seaward of the eelgrass. The locations seaward of the eelgrass beds had a higher amount of nitrate, phosphate, and sulfate than the locations within the eelgrass beds. *Z. marina* may be limited by water action and sediment instability on the shoreward side, and growing where the nutrients within the sediment are better fitted for eelgrass growth. The factors limiting the eelgrass growth in other unoccupied portions of the bay are not obvious.

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Introduction

Background

Zostera marina, more commonly known as eelgrass, is one of twelve species of eelgrass in the genus *Zostera*. There are three species of *Zostera* found in North America, but *Z. marina* is the only species found in Rosario Bay (Fonseca & Uhrin, 2009; Hylarides, 2015). *Z.marina* is a flowering monocot. It is in the pondweed order Potamogetonales and the family Zosteraceae. Due to their thin flattened straplike leaves the family name is derived from the Greek word 'zoster' meaning belt (Fonseca & Uhrin, 2009).

Seagrasses, which include *Zostera marina,* are the only submerged marine plants, and, unlike algae have an underground rhizome and root system. The rhizome is dark brown and in *Z. marina* there are typically two root bundles at each node (Fonseca & Uhrin, 2009). *Z. marina* has terminal shoots located at the end of the rhizome. The branches of the rhizome are alternate and branch irregularly. Each branch becomes an independent shoot with long thin leaves that have a rounded tip (Fonseca & Uhrin, 2009).

Eelgrass generally lives in shallow, protected coastal waters. It also grows in the path of watershed outlets which allows for an influx of nutrients and sediments to the eelgrass from the watersheds (Orth et al., 2006). It can be found in the intertidal and subtidal zones at a shallow enough depth for adequate light penetration (Fonseca &

Uhrin, 2009). It is also susceptible to differences in the sediment size as well as to nitrogen and phosphorus levels (Fonseca & Uhrin, 2009).

Zostera marina is influential on the physical, chemical, and biological environments along coastal waters. It forms an important habitat for many marine creatures, such as many species of fish, decapods, mollusks, birds, and turtles (Fonseca & Uhrin, 2009; Orth et al., 2006; Zimmerman et al., 1991). In addition, eelgrass plants anchor and filter sediments, dampen tidal and wave energy, and contribute to nitrogen and other nutrient cycling (Short, 2014). Seagrass survival can be indicative of the overall ecological health of a coastal ecosystem (Orth et al., 2006).

Around the world, water deterioration has been occurring. As part of this, eelgrass beds have been undergoing large-scale loss throughout the world (Orth et al., 2006). Threats from global climate change, water quality, and localized impacts of sediment contaminants and nutrients have impacts on the eelgrass health (Dennison et al., 1993; Valdemarsen et al., 2010). This deterioration of eelgrass beds has been of some concern in the Puget Sound and has been under much work to determine the cause and how to counteract it (Mumford, 2007).

The viability of eelgrass in many coastal habitats around the world is affected by many environmental parameters. Some of these parameters include substrate nutrients and organic content, light, depth, temperature, water motion, salinity, water nutrients, and competition or predation from other marine organisms (Koch, 2001). Many studies have looked at light and depth as factors that affect eelgrass growth (Fonseca & Uhrin, 2009; Hylarides, 2015; Koch, 2001; Wicks et al., 2009). Besides these, hydrologic factors such as waves, currents, tides and turbulence, geological factors such as sediment size, organic matter content, and nutrients in the sediment also affect their growth (Fonseca & Uhrin, 2009; Koch, 2001; Short, 1987; Wicks et al., 2009).

Water motion and *Z. marina*

Seagrasses are impacted by the velocity of the water around them. *Zostera marina* can tolerate current speeds less than 120-150 cm/sec (Fonseca et al., 1983). Wave action can also be detrimental. The direct impact of waves on submerged aquatic vegetation can be seen when waves and currents erode the edges of a seagrass bed, when the landscape is altered due to wave action, or when plants are broken and removed by storms or boat-generated waves (Koch, 2001). Indirect impacts on the eelgrass beds by water turbulence include sediment suspension, changes in sediment particle size, the extent of water column mixture, and epiphyte growth on the eelgrass (Koch, 2001). Water motion can also directly influence eelgrass growth, damage eelgrass shoots, erode rhizomes from the sediment, scour and redistribute sediment, and cause burial (Thom et al., 2012).

Rosario Bay is in a semi-exposed site and is affected by turbulence generated by waves from the Strait of Juan de Fuca (Hylarides, 2015). Waves pound on the gravel beach and rocky headlands during times of increased wind and during storms. Rosario Bay also experiences high tidal ranges, which induce tidal currents. Further, these large tidal amplitudes influence the depths to which *Z. marina* grows. All the *Z. marina* in Rosario Bay are subtidal (Hylarides, 2015). At high tide the subtidal plants may not have to deal with the turbulence generated by surface wave action as much, but at low tide the plants are closer to the surface and may be influenced by wave action and water drag (Figure 1). This may set an important limit on the depths at which the eelgrass can grow in the bay. The minimum depth of distribution (Z_{min}) of aquatic plants can be determined by the wave mixing depth (Z_{wave}) which extends to a depth equal to half the wavelength (L). Wavelength (L) can be calculated from the wave period (T), where g is the acceleration of gravity (9.805 m/s^2), W stands for the wind velocity (m/s), and F is the fetch (m). The minimum depth of distribution and the wave period (T) can be predicted by the equations

$$Z_{min} = Z_{wave} = \frac{L}{2} \ , \ L = \frac{gT^2}{2\pi} \ , \ T = \left[\frac{0.46W}{g}\right]\left[\frac{gF}{W^2}\right]^{0.28}. \quad \text{(Koch, 2001)}$$

These equations imply that if wave-generated turbulence is important for determining the distribution of eelgrass in Rosario Bay, there should be a minimum subtidal depth Z_{min} within the bay at which the eelgrass is found. Depths shallower than Z_{min} should not support long-term eelgrass growth. Eelgrass depths within the bay can be compared to local weather and wave characteristics to see whether the beds are restricted by $Z_{min,}$

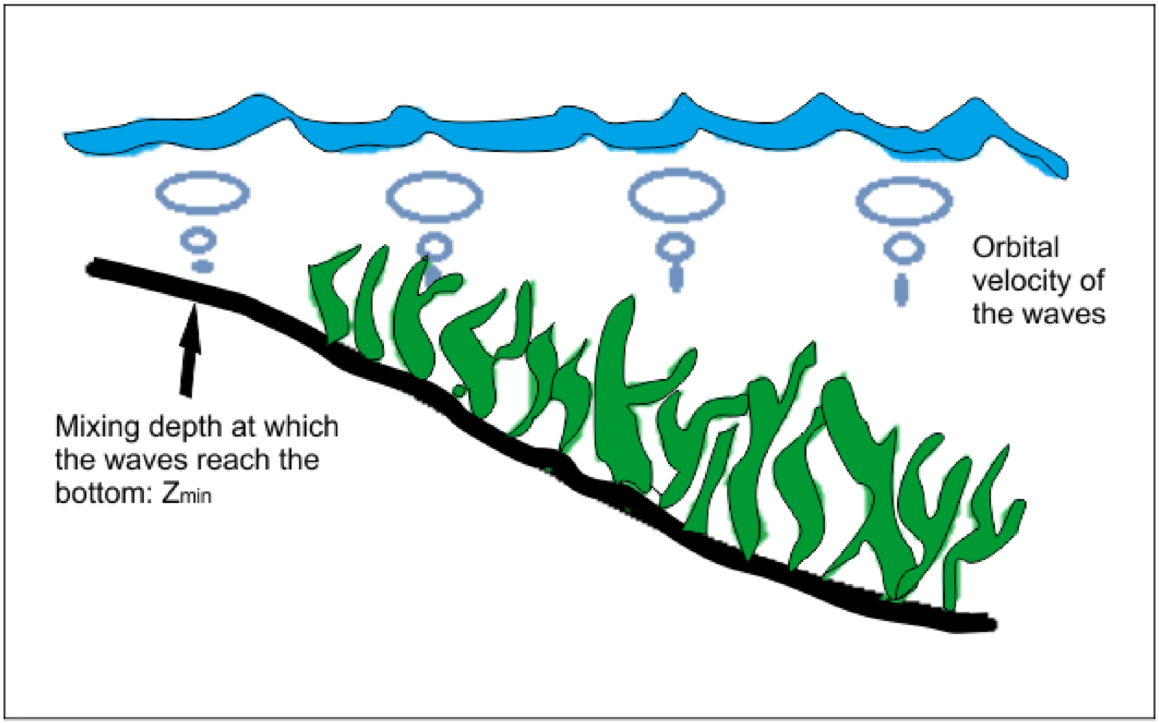


Figure 1. Eelgrass beds can be forced into deeper waters due to exposure to waves. Waves can shift sediment which may affect the ability for the roots to take hold. The zone where waves do not allow eelgrass growth is defined as the depth equivalent to half the wavelength and is the minimum depth of distribution or Z_{min} (Koch, 2001)

and the characteristics of waves which set this limit should be determinable via the equation.

Substrate and *Z. marina*

Sediment characteristics are also important for *Z. marina* growth, morphology, and distribution. Sediment size can affect the availability of nutrients as well as erosion and deposition factors. *Z. marina* grows best in unconsolidated sediment but may also be found in gravel-like substrate along the New England coast (Fonseca & Uhrin, 2009). In healthy *Z. marina* beds the percent silt and clay found by several studies varies from 2.3% to 14% and occasionally to 56% (Koch, 2001). One reason for the large variation is that it can grow in sediment toward the finer end of this range if the sediment is low in hydrogen sulfide, but is excluded when hydrogen sulfide is high (Thom et al., 2012). The distribution of silt-clay in the sediment serves as an indicator of the depositional environment provided by the eelgrass, since this particle size is generally considered more susceptible to erosion than other non-organic sediment sizes (Fonseca et al., 1983).

Nutrients, Organic Content, and *Z. marina*

In order for eelgrass to grow it needs enough nutrients. Nutrient availability is linked to eelgrass growth, abundance, and morphology (Short, 1987). Seagrasses in north temperate climates and in habitats with sediment that comes from land often are limited in nitrogen, but have abundant phosphorus (Short et al., 2014; Short, 1987). In tropical environments or carbonate sediments, however, phosphorus is more limited due to it binding to the sediment (Short, 1987). Eelgrass grows best in areas where the nutrient levels are moderate in sediment, and low to moderate in the water column (Thom et al., 2012). If the water column is high in inorganic nitrogen concentration, however, it can prompt algae blooms which are damaging to the eelgrass growth (Thom et al., 2012).

The relationship between eelgrass beds and organic content is complex. Accumulation of organic matter may occur in seagrass beds due to a reduction in water motion (Koch, 2001), but too much organic content may cause issues for the eelgrass growth such as anoxia and hydrogen sulfide buildup in the sediment, depending on the area. In Chincoteague Bay, Maryland, *Z. marina* is usually absent from areas with sediment organic content greater than 4% (Wicks et al., 2009). Plants growing in high organic sediment develop long leaves and short roots which create more drag from the water motion and result in poor anchoring of the plants. These plants are more likely to be dislodged than are plants grown in organic—poor sediment (Wicks et al., 2009). Fine, organic-rich sediments are usually found in relatively calm hydrodynamic conditions, while coarser sediments with lower organic content are characteristic at sites with strong currents or wave turbulence (Koch, 2001). Relatively high sediment organic content, however, may be an indicator for the anoxic conditions that cue seed germination (Koch, 2001).

Eelgrass in Rosario Bay

The eelgrass distribution in Rosario Bay is patchy. The northern and southern limits of the bay, for example, near Rosario and Sares Heads, are characterized by rocks and have little if any eelgrass. Several large patches of eelgrass, however, occur in the sandy subtidal areas near the middle of the bay. Rosario Bay also experiences large tidal amplitudes, which may affect the distribution of *Z. marina*. Large tidal-generated currents occur just outside the bay mouth and may be felt in the bay, and Rosario Bay is also affected by wave action that pounds on the gravel beach and on the kelp beds (Hylarides, 2015). These waves and currents, plus the different types of sediments occurring in the bay, may be important factors setting limits to the extent and location of eelgrass beds in the bay.

Hypothesis:

This study examines the relationship between the distribution of *Zostera marina* eelgrass in Rosario Bay and important physical parameters. I suspect that the distribution and abundance of eelgrass (*Zostera marina*) in Rosario Bay is limited by water turbulence and substrate instability at the shallow end and by nutrient and organic content at other borders. Although that idea cannot be tested directly, tests were performed to test the hypothesis that the distribution and abundance of *Zostera marina* in Rosario Bay is correlated with indicators of an increase in water turbulence and substrate instability at the shallow end and with noticeable changes in sediment type and nutrient and organic content at the other borders of the bed.

Materials and Methods:

Sampling sites and sampling methods

Nine sampling stations were established spanning the existing eelgrass bed within Rosario Bay. The sampling stations were placed at appropriate grid points of an already established eelgrass study grid in Rosario Bay (Cowles, 2011-2013) (Figure 2). The established eelgrass study grid consisted of a 100 m square divided up into 10 x 10 m plots, the corners of which were determined by GPS and referenced also by markers placed on the bottom of the bay at many of the grid corners. The coordinates list the east-west dimension first, then the north-south. I only listed the coordinates as it related to the 100 m grid. To convert the grid coordinates to actual UTM Section 11 coordinates using WGS 84 datum, one must add 0524000 to the east-west dimension and 5362000 to the north-west dimension. In a preliminary diving survey, I noted the 2015 shoreward and seaward limits of the eelgrass bed. I then established sampling stations 1 to 3 shoreward of the eelgrass at coordinates (90,30), (90,40), and (80,50); sampling stations 4 to 6 within the main part of the eelgrass bed at coordinates (50, 60), (50,50), and (40,50); and stations 7 to 9 seaward of the eelgrass at coordinates (20,50), (20,40), and (20,30). Leaded lines were laid down in a path connecting the stations to facilitate rapid location of and direct movement to each station during dives.

Once the stations were established, I conducted a detailed survey of the eelgrass location in the portion of the original 2012 survey surrounding my stations in order to

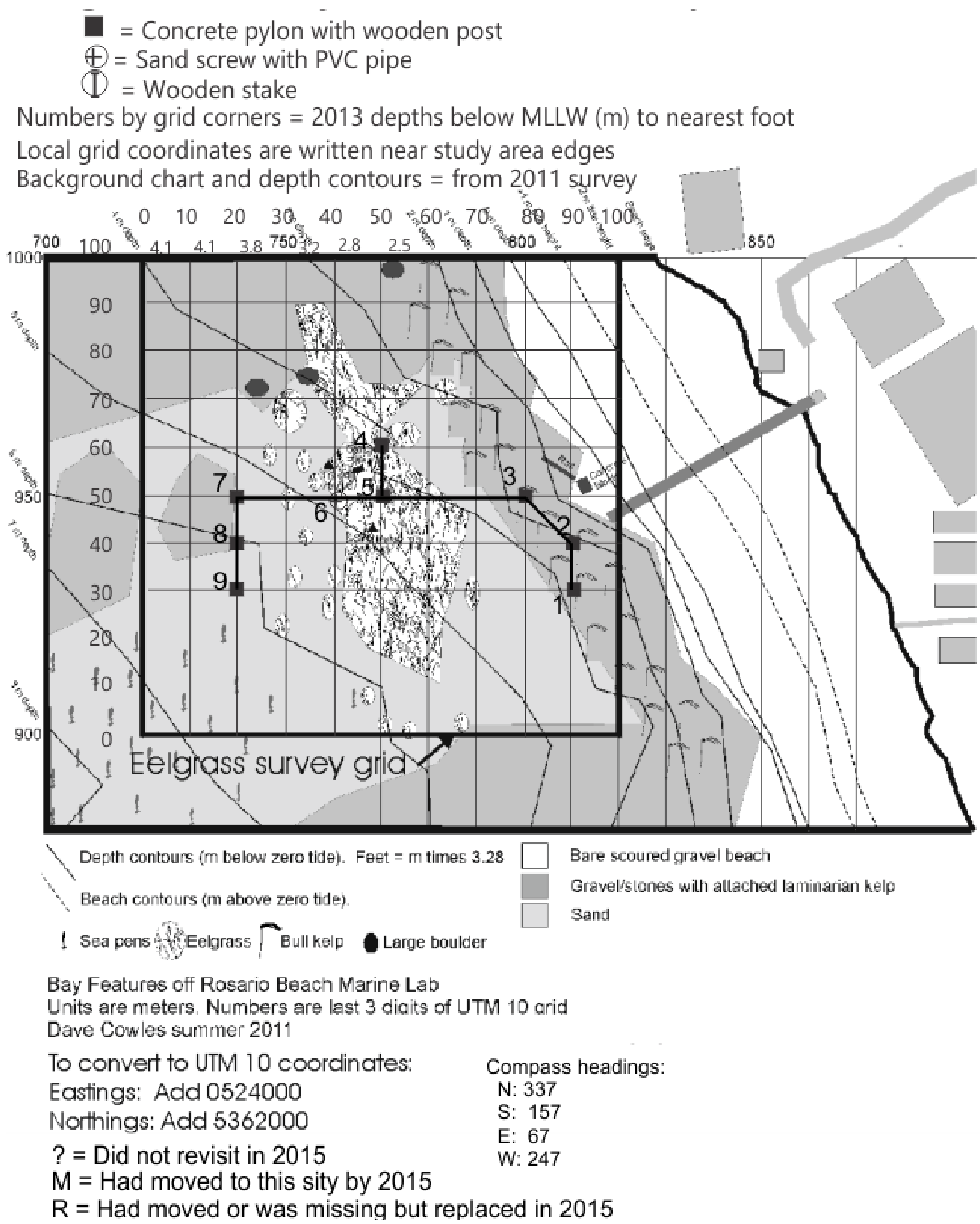


Figure 2. Overlay of Cowles 2011-2013 map showing Rosario Bay, the water depth topography lines, sediment type, the previously (2012-2013 mapped of the eelgrass bed, and sites and leaded line pathways used in this study. (Cowles, 2011-2013).

document any shifts in location of the eelgrass and to provide a higher-resolution map of the eelgrass location. The area surveyed in detail in 2015 was a rectangle stretching from coordinate (20,30) to coordinate (80,70). A leaded line, marked in 1 m increments, was placed east to west between the (20,50) coordinate and the (80-50) coordinate, a second leaded line was laid parallel to the first at 10 m increments to the north and south, and was moved as needed to provide a baseline for measuring each 10x10 m grid. After these lines had established the corners of each grid, divers stretched 10-m lines around the perimeter of the grid and attached to the grid corner markers, and then swam back and forth or around the grid repeatedly within the perimeter lines, mapping eelgrass locations on an underwater slate. The slate had a 10x10 grid on it where divers would write where in the grid eelgrass, algae, or substrate was found. Maps of these grids were then combined into a detailed map of the 60x40 m area, at a resolution of approximately 1-2 meters. Eelgrass did not occur shoreward or seaward of these grid limits.

For the duration of this study the term 'location' for a station will refer to whether the station was seaward, within, or shoreward of the eelgrass bed. Each individual station will be referred to as a site. These sites allowed for comparison of water motion and sediment makeup between where the eelgrass is growing and where it is not growing. The markers placed at each site consisted of either a solid concrete pylon with an attached wooden post or a sandscrew with an attached PVC marker pipe

(Figure 3). At each site, four clod cards were attached to a post sleeve and placed over the site markers to measure water motion on several different occasions during the summer (Figure 4), and six sediment core samples were also taken, once, adjacent to each sampling station and used to analyze sediment size, organic content, and nutrients.

Testing of water motion

To measure the water motion, clod cards were used. A clod card is a plaster of Paris (calcium sulfate) molded object that slowly dissolves when immersed in the water. When the dissolving rate is calibrated against known water motion speeds the cards can be used to determine the overall rate of water motion that occurred where the cards were located in the field during their deployment (Thompson & Glenn, 1994). The clod cards were made using a 1 to 1.6 mass ratio of water to plaster of Paris. They were made in batches of 8 cards at a time. The plaster and water were mixed with a whisk and 400 ml were then placed into each of the eight 18oz disposable plastic cups for each batch. A pre-weighed bolt and numerical tag were embedded into the clod card with the bolt head 2.54 cm from the bottom of the cup by suspending the bolt from a board placed over the cups while the plaster dried (Figure 5). The plaster clod cards were allowed to solidify and then were placed into a dryer at 35˚ C for 24 hours to thoroughly dry the cards. This allowed any excess water to be driven off while not heating the plaster to such an extent that it became brittle. After drying, the initial total weight of

Figure 3. Erica with a sand screw with attached PVC pipe, and a concrete pylon with attached wooden post

Figure 4. The sleeve holding the clod cards before deployment into the bay.

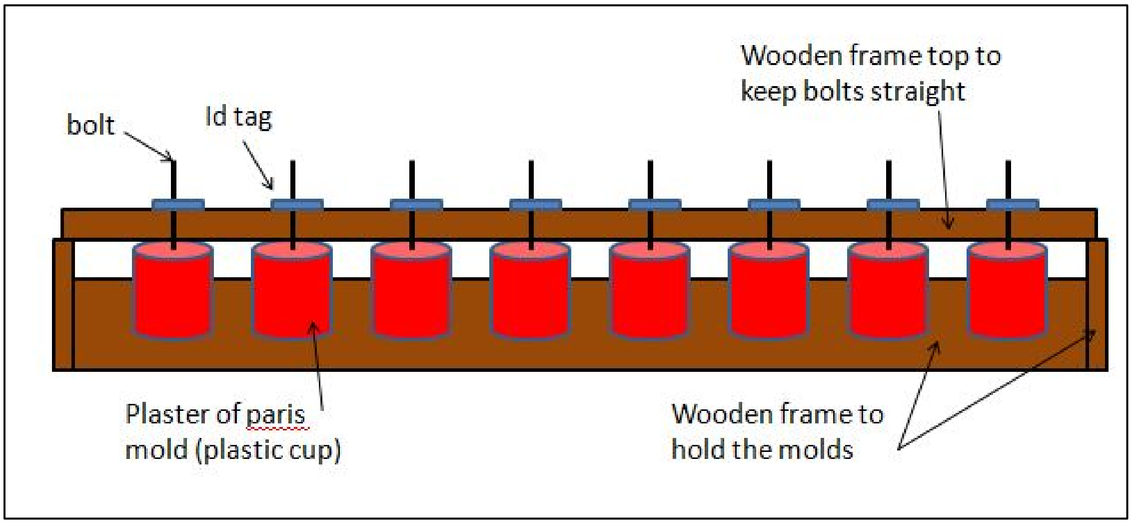


Figure 5. Wooden frame used to make the clod cards. The base had slots to hold plastic cups used as a mold for the plaster of Paris, at the top another wooden board kept the bolts and id tags straight and in the proper position as the plaster of Paris hardened.

each clod card assembly including the bolt, nut, id tag, and clod card was measured and recorded. The cards were then attached to a post sleeve stand in a 4 clod card array that would suspend the cards about ½ m from the sea bottom when placed over the pylon or sandscrew plot corner markers. The arrays were then transported out to directly over each of the sampling sites by rowboat to avoid any effects of water movement over the clod cards during placement, then carried directly down to the site by divers and deployed with the 4 clod cards facing directly north, south, east, and west. The clod cards on each array had already been recorded as to which clod card number was facing which direction. The SCUBA team would then swim to the next station using the underwater path, ascend, pick up the next clod card array from the boat, and repeat the process for all the stations. The clod cards were retrieved after approximately 48 hours of deployment in the same way. After retrieval, the clod cards were dried for 24 hours at 35˚ C and weighed again to calculate plaster mass loss from the cards during deployment. These clod card deployments occurred on July 26-28, August 2-4, August 9-13, and August 16-18.

In order to calibrate the amount of water action affecting the cards, a water motion calibration device was created using a plastic wading pool and a rotational arm attached to a motor suspended over the water in the pool (Figure 6). The clod cards were screwed into the arm and suspended low enough that the entire plaster component was underwater. The arm was set to rotate at pre-determined speeds using a stepper motor so that the speed was precisely controlled. A baffle with slotted

Figure 6. Water Motion Simulator setup in the marine lab used to make the calibration curve for the clod cards. The green buckets held the control clod cards with no water motion. The yellow arm above the pool rotated at a given speed. Six clod cards were attached at a time, giving 3 different speeds due to the circumference they traveled. A stepper motor was located in the black covering above the yellow metal arm and turned the arm at a very stable, repeatable speed. The clod cards were screwed into the yellow rotational arm. The hose brought new sea water into the pool.

divisions for the clod cards to pass through was placed in the water to prevent water currents from forming as the rotational arm turned. Clean water was slowly added to the pool and an equal amount was allowed to overflow from the pool during the calibration process to prevent oversaturation of water by the plaster material which was slowly dissolving from the clod cards as they moved through the water. Two clod cards were circulated at each calibration speed, and two control clod cards were also suspended in still water of the same temperature (+/- 1 ˚ C) for comparison of dissolution rate at zero speed. Calibration curves of clod card mass loss at these known speeds were generated to calculate average water motion around the clod cards deployed in the bay based on their mass loss (Figure 7).

To measure the wave depth interaction, data was used from the Naval Air Station, Whidbey Island (NASWI) that gave wind speed and direction every half an hour for a year. The fetch was calculated by measuring the distance from the far shore (m) on Google Earth© from the given wind directions at 10 degree increments. The wave depth interaction was then calculated using the equation from Koch et al. (2001).

Sediment tests

Six cores of sediment were collected at each of the sampling stations using SCUBA. In order to collect sediment cores, a hole was punctured into the bottom of a 50 ml falcon tube (opposite of the cap). The cap was removed and the tube was pushed

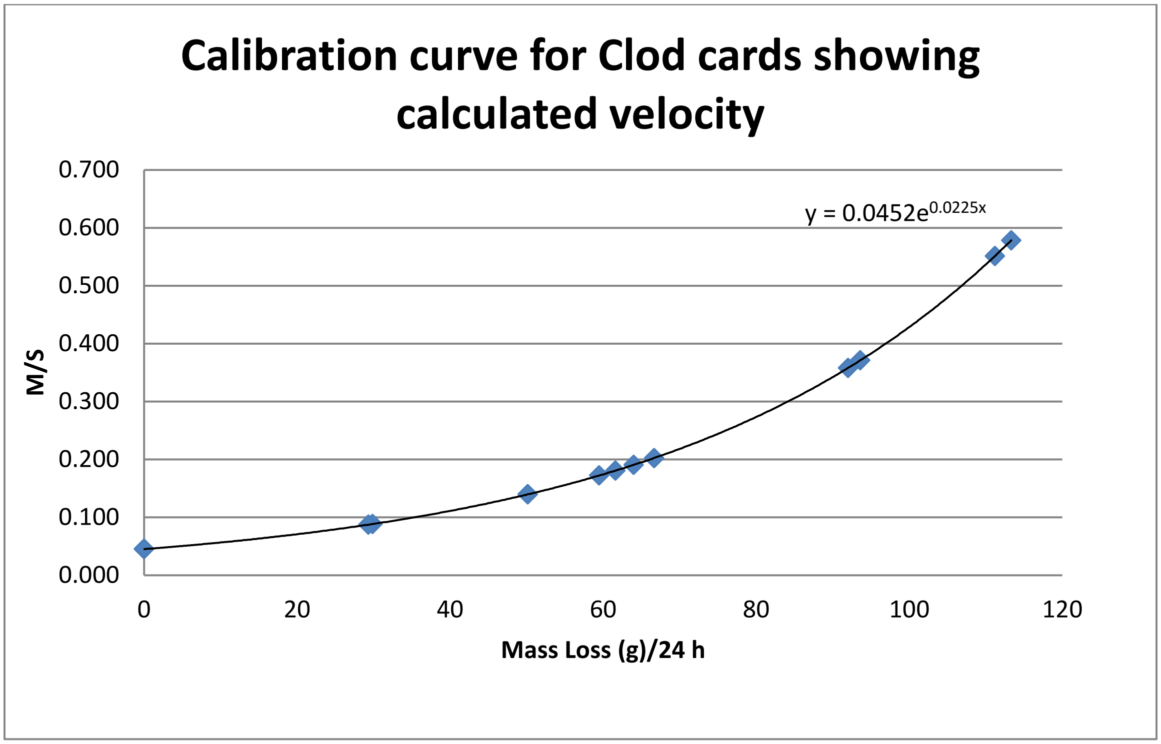


Figure 7. Calibration curve used to find the velocity of the water (m/s) from the mass lost in 24 hours (g/24 h). This calibration curve was determined by taking the amount of mass lost at a given velocity and a given time.

upside-down 8-9 cm into the sediment, which allowed the sediment to form a core inside of the tube. In order to collect the now full falcon tube, the sediment beside it was dug into and the lid was screwed back on and the tube transported upside down back to the laboratory so that all the sediment remained in the tube (Figure 8).

All of the cores were initially dried in the 35 ˚ C dryer and then later dried at 105˚ C before measuring the initial dry weight. After drying, three of the six cores from each site were shaken for 10 minutes through a series of sieves, first of 2 mm gap and then 0.05 mm gap to determine the particle sizes that the sediment was composed of. Sediment particles greater than 2 mm diameters were classed as gravel according to the Wentworth size scale (Wentworth, 1922). Particles less than 2 mm but greater than 0.05 mm were classed as sand. Particles less than 0.05 mm diameter were classed as silt and clay. The dry mass of each size class of sediment after passing through the sieves was again measured and used to determine the percentage of each sediment size class in each sample (Figure 9).

To test for organic content, I used the ash-free dry weight (AFDW) method. This analysis was performed only on the combined sand and silt/clay portion of the sediment since the gravel portion would not be expected to have any substantial organic content. The sand and silt portions from these same cores were combined, placed in crucibles, and then heated to 440˚ C using a muffle furnace and left for 16 hours. At this temperature the organic material was burned off. After the furnace cooled enough that

Figure 8. Sediment cores were taken with falcon tubes with a hole drilled in the small end to allow water to exit as the tubes were pushed mouth-first into the sediment. Once the sample was taken the lid was screwed back on and then they were stored and transported like this until analysis could be done

Figure 9. Sediment went through 2 different sized sieves (2mm, and 0.5 mm) and was separated by size to determine the percent gravel (right), percent sand (center), and percent silt/clay (left) for each site. This was done for three samples taken at each site.

the crucibles could be safely handled with gloves and tongs they were removed from the muffle furnace. Ten ml of distilled water was added to the sample. The crucibles with the sediment samples and water were then placed back in the 105˚ C drier and dried for an additional 24 hours and then reweighed. This step of re-wetting and re-drying the samples after the muffle furnace was used because clay has a high affinity towards water and may have retained some water during the initial weighing even after drying at 105˚ C, so adding water back to the sample and then re-drying the samples at 105˚ C allowed for a more precise measurement of how much organic content was present. The mass difference between the initial dry weight and the weight after the muffle furnace and drier is the mass of the organic material that burned off in the muffle furnace (Figure 10). In addition, any dried organic material such as twigs or leaves that were found in the gravel fractions were weighed and their mass was added to that of the organic material from the sand/silt/clay fraction as determined above to give the total organic content of each core sample.

The remaining three sediment cores from each site were used to test for sediment nutrient content. The cores were first dried at 105˚ C and the gravel portion was removed using a 2 mm sieve. 2.3 g of the dried silt/sand portion of each core was then combined with 7 ml of universal solution (3% acetic acid, 10% sodium acetate, in distilled water) and shaken for 1 minute according to the LaMotte Soil Nutrient Test Kit Instructions (LaMOTTE Company, 2013). The sand and silt were then filtered out of the

Figure 10. Sediment samples from an individual site. The top three trays contain samples that haven't gone through the muffle furnace. The bottom three trays contain samples that went through the muffle furnace. There was a color change in the sediment as the organic material was burned off.

solution by filter paper (LaMotte 0465-H) and standard nitrate, phosphate, and sulfate tests (Nitrate-N 3649-SC, Phosphate-Low 3653-SC, Sulfate 3665-SC) were performed on the remaining liquid using the LaMotte SMART 3 colorimeter (1996, 26617 RMN). (Figure 11).

Statistical Analysis

Analysis of the data was done on SPSS version 24 and compared the stations shoreward of the eelgrass beds, inside the eelgrass beds, and seaward of the eelgrass beds with each other in terms of water motion, sediment nutrient content, sediment organic content, and sediment particle size. Since different sediment cores had different masses, the sediment size fractions and organic content cores were compared in terms of percent of the core's total mass instead of actual mass of each constituent. To normalize these percentage data an arcsine transform was used before analysis. A MANOVA was used on the sediment size using R version 3.2. This was done because the proportions may have a direct effect on one another since they were from the same samples. A one-way ANOVA was used for more of the data sets, with a Tukey or a Dunnett T3 and Games-Howell non-parametric post-hoc test depending on if transformations made the data variances equal or not. If the tests indicated the data was not parametric appropriate transformations were done to make them parametric before analysis, or a Kruskal-Wallis test was used.

Figure 11. Sediment nutrient tests for sulfate, nitrate, and phosphate. A blank is on the left. These tests were done on the sand/silt portions of three of the sediment samples taken from each site.

Results

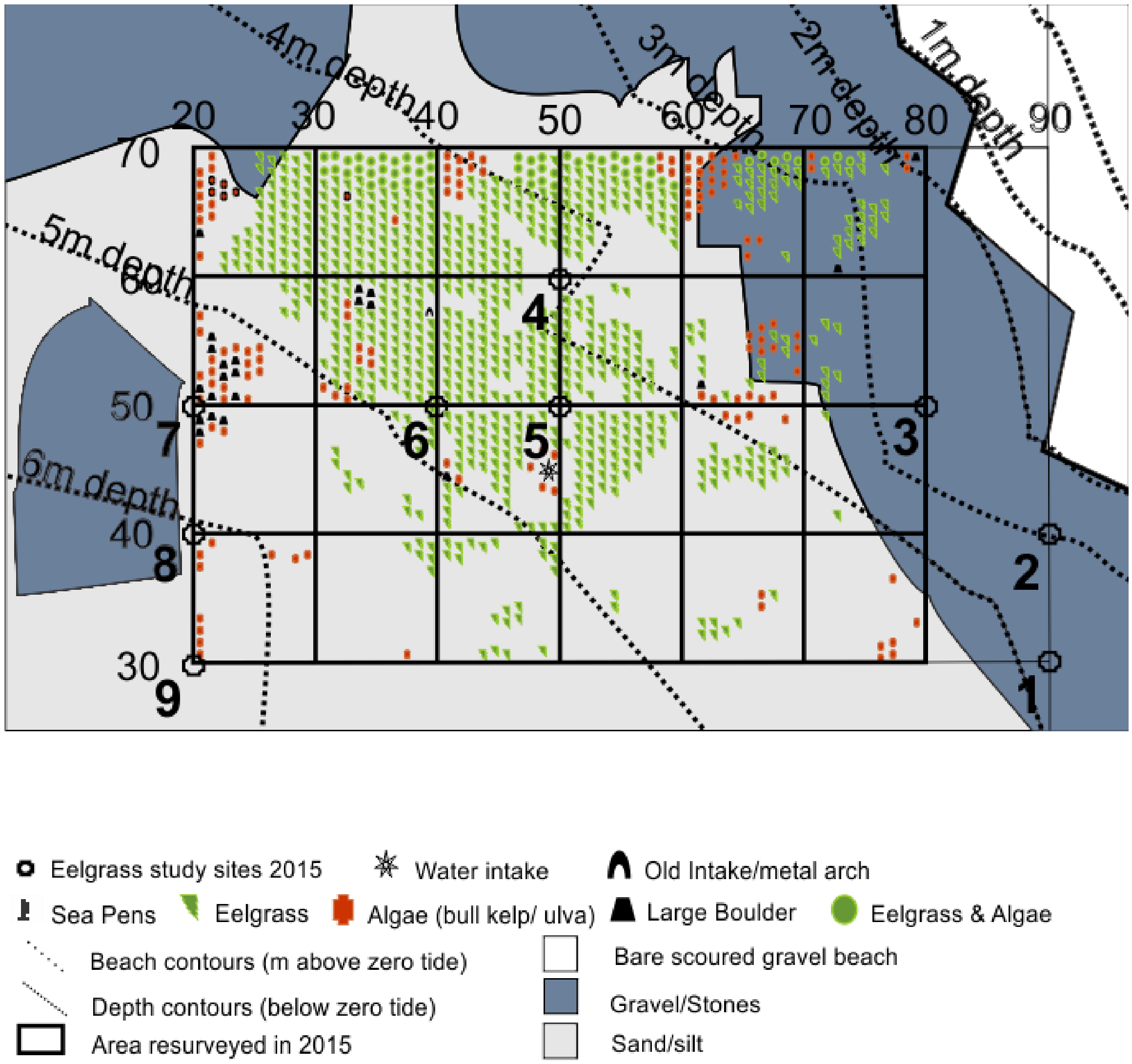
Mapping of the Rosario Bay Eelgrass Bed

My detailed eelgrass mapping grid (Figure 12, Figure 13) covered the majority of the eelgrass bed within Rosario Bay. A mix of eelgrass and algae extended a short distance north of the grid, and then turned to algae in the rock and gravel substrate there. A few scattered clusters of eelgrass extended out into the sand to the south of the grid. No eelgrass occurred either shoreward or seaward of the grid. The region shoreward of the grid was covered with gravel and larger scattered stones.

On the shoreward side, neither eelgrass nor algae occurred at depths shallower than 2 m below the zero tide line, an area dominated by clean, round gravel and smaller stones. Depths from 2-3 m below zero tide had abundant *Ulva* algae attached to the gravel and stones, along with scattered brown and red algae attached to the larger stones. A few small patches of eelgrass extended into the lower parts of this area. Almost all the eelgrass occurred at depths below 3m, and abundant eelgrass did not occur until depths below 4 m. By 5 m depth the eelgrass bed was beginning to dissipate, and I did not find eelgrass at depths below 7 m.

The substrate shoreward of the eelgrass was mostly gravel with scattered larger stones. Within the eelgrass bed was silty sand. Southwest of the eelgrass bed the sloping bay bottom was covered with silty sand, with a patch of large algae-covered boulders not far directly west of the bed.

Figure 12. Map of Rosario Bay in 2015 showing the water depth, substrate type, eelgrass location (in detailed 60x40 m grid within the original 100x100 m grid by Cowles, 2011-2013), and station locations. In the area outside of the (20,30) to (80,70) rectangle the grid was not surveyed for eelgrass growth during the 2015 summer. In the areas seaward and shoreward of the survey grid there was no eelgrass growing.



Grid coordinates are written near the study area edges
Background chart, depth contours, and sea pen location from 2011/2013 survey
Bay features off Rosario Beach Marine Lab
Units are meters. Numbers are last 3 digits of UTM 10 grid
To convert to UTM 10 coordinates
Eastings: Add 0524000
Northings: Add 5362000

Figure 13. Detailed map of eelgrass growth within the (20,30) to (80,70) rectangle surveyed in 2015. Along the northern edge the eelgrass was intermixed with algae. Along the southern side the eelgrass was patchy and sparse. The eastern side was characterized by gravel and large smooth stones, whereas the western side had large rough boulders and algae.

Water motion

None of the sampled periods during this study included the high waves and water motion that periodically take place in Rosario Bay. However a comparison of mass loss from clod cards between trial periods showed that there was a significant difference between trial periods with the greatest wear per 24 hours during the July 26-28 deployment (2-Way ANOVA, $P<0.005$). The least wear was the last trial period, August 16-18. There was no significant difference between the 3rd and 4th trial periods (2-Way ANOVA, $P=0.992$) but there was a difference between the first two trial periods and all the subsequent trials (Table 1, Figure 14). Occasionally divers observed crabs climbing on the clod cards, potentially causing excess wear.

Comparison among locations seaward, within, and shoreward of the eelgrass bed showed no significant difference in wear (2-Way ANOVA $P=0.231$) (Table 1, Figure 15) Additional comparisons were made between whether the cards were facing north/south or east/west. It was noticed that three clod cards showed sharply increased wear, more than twice the average wear for the other cards at their site. These cards were removed from the analysis as outliers, with the presumption that the excess wear was caused by crab gymnastics. After removal of these outliers, the cards facing northing or south at each site (approximately parallel to the beach) had 7.2% more wear than those facing east or west (perpendicular to the beach), a significant difference ($F=4.764$, $P=0.031$) (Figure 16).

Table 1. Mean calculated water motion (m/s) for the four different trial periods run during July and August 2015. The coordinates list the east-west dimension first, then the north-south. To convert the coordinates to actual UTM Section 11 coordinates using WGS 84 datum, add 0524000 to the east-west dimension and 5362000 to the north-west dimension.

Site #	Coordinates	Location	Mean Calculated Water m/s Trial 1 (7/26-7/28)	Mean Calculated Water m/s Trial 2 (8/2-8/4)	Mean Calculated Water m/s Trial 3 (8/9-8/13)	Mean Calculated Water m/s Trial 4 (8/16-8/18)
1	90,30	Shoreward	0.29	0.14	0.11	0.37
2	90,40	Shoreward	0.74	0.17	0.14	0.10
3	80,50	Shoreward	0.38	0.15	0.11	0.05
4	50,60	Within Eelgrass	0.59	0.13	0.10	0.06
5	50,50	Within Eelgrass	0.20	0.30	0.09	0.32
6	40,50	Within Eelgrass	0.59	0.11	0.09	0.08
7	20,50	Seaward	0.27	0.21	0.10	0.05
8	20,40	Seaward	0.27	0.13	0.10	0.25
9	20,30	Seaward	--	0.13	0.13	0.07

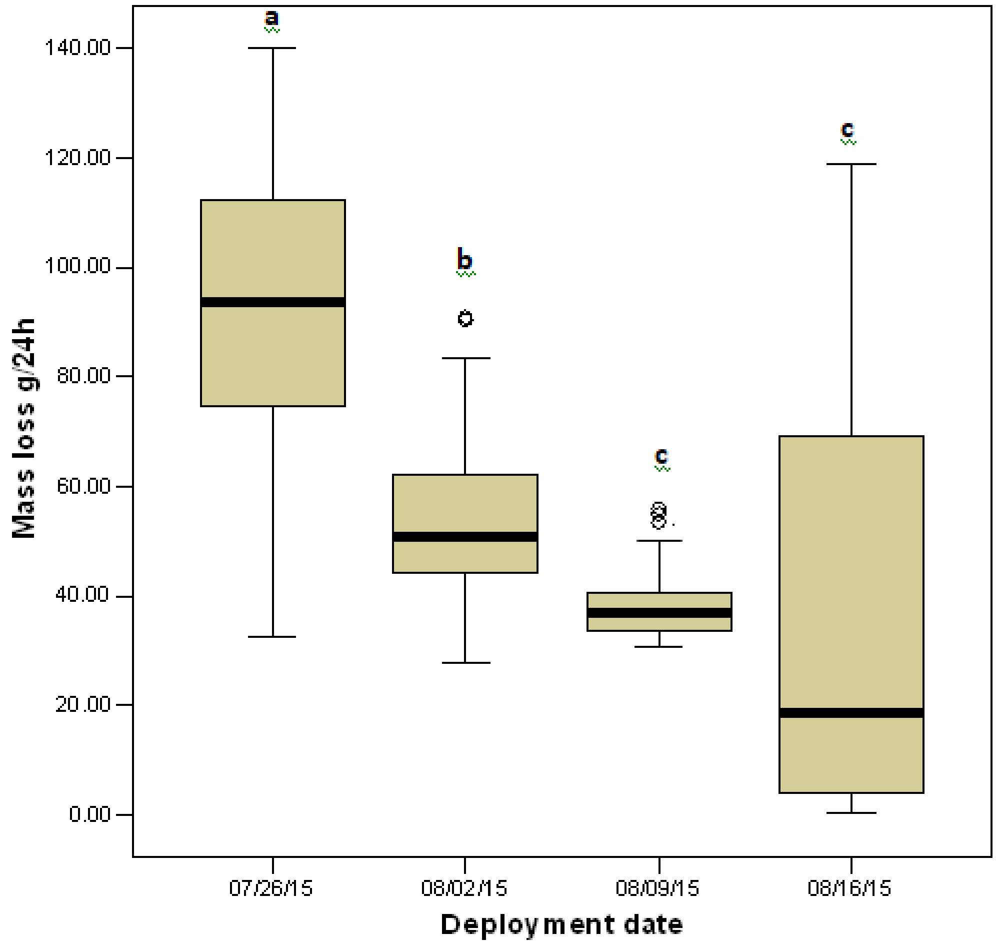


Figure 14. Box plot comparison of the mean mass loss on the clod cards per 24 hours of the different trial periods. The first trial period had more wear than any of the succeeding trial periods, The second trial period had more wear than the last two trial periods but less than the first trial period, The last two trial periods showed the least amount of wear and were not different from each other.

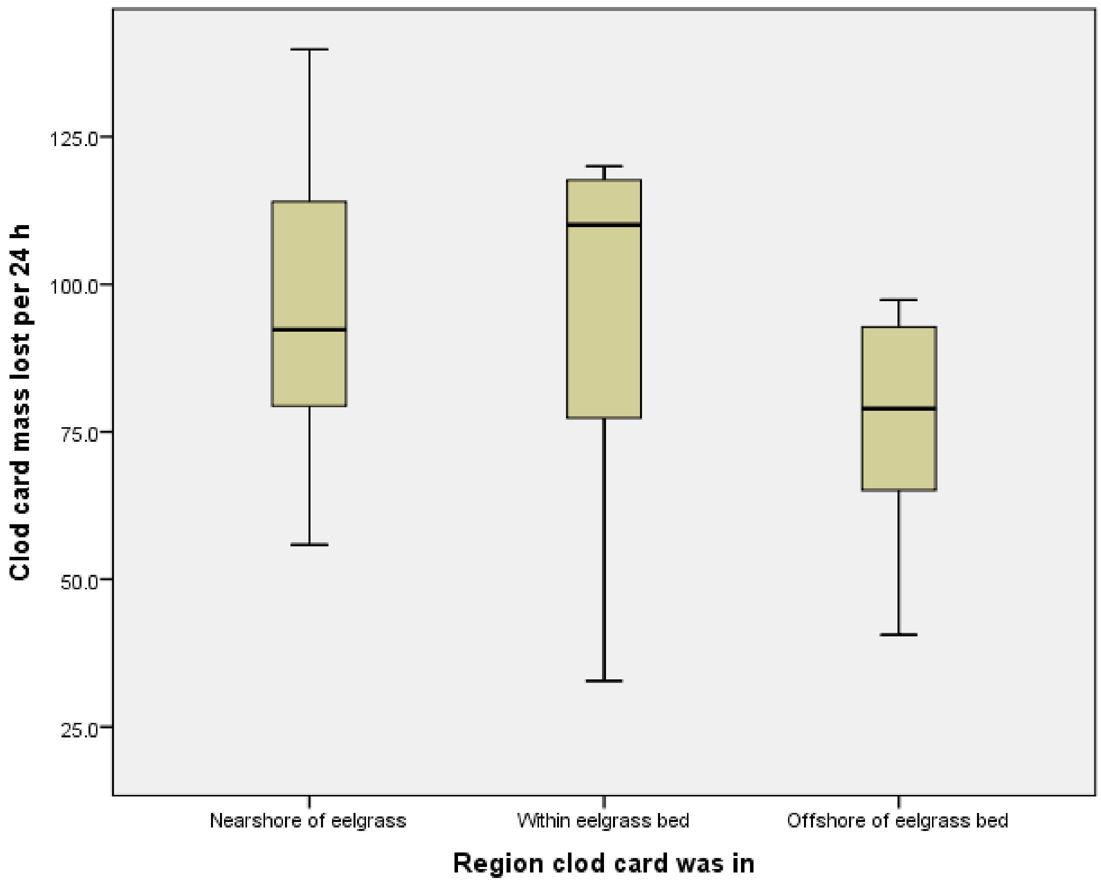


Figure 15. Comparison among locations during the July 26-28 2015 trial period, showing no significant difference in wear between locations. ANOVA (F=1.540, P=0.231)

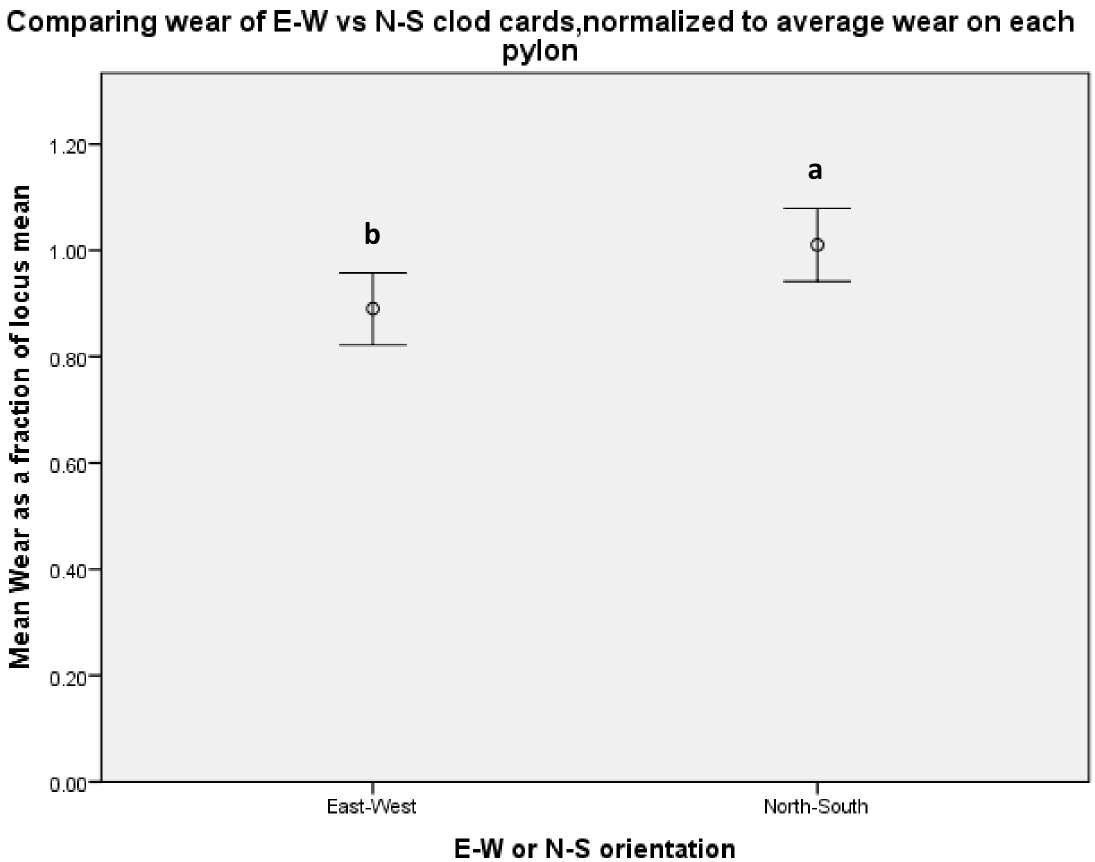


Figure 16. Comparison of clod card wear between those facing east-west and those facing north-south in Rosario Bay, after 3 outliers were removed. The cards facing north-south had a significantly higher wear than those facing east-west. ANOVA ($F=4.764$, $P=0.031$). Locus mean is the mean wear for all the clod cards at a particular station.

Using the data set from Hylarides thesis (2015) and the equation for calculating the depth of wave interaction from Koch et al (2001) it was determined that when the wind is >0 m/s and from the ocean, the wave depth interactions are deep enough to affect the entire bay including the eelgrass beds. While most of the wave depth interaction was <2 m there was still a substantial number that interacted at >10 m depth (Figure 17).

Sediment Grain Size

A comparison of the dry mass of all sediment samples taken shows that although there was variation in the sample dry masses at each station there was no significant difference among stations. Therefore the sediment cores from the different stations are comparable to one another and can be validly compared. (Table 2, Figure 18).

After Arcsine transformation, the MANOVA comparing the percent of each core sample in the different size classes (gravel, sand, or silt/clay) showed that the locations seaward, within, and shoreward of the eelgrass regions differed significantly from one another in the proportion of these size classes ($F=18.631$, $P<0.0005$). Since the MANOVA showed that there was a difference, an ANOVA with Tukey post hoc tests was performed comparing the proportion of each size class by region. Gravel percentage was not homoscedastic when considered alone so the arcsine of the inverse of gravel percent was taken and that was used for this analysis. ANOVA with Tukey post-tests

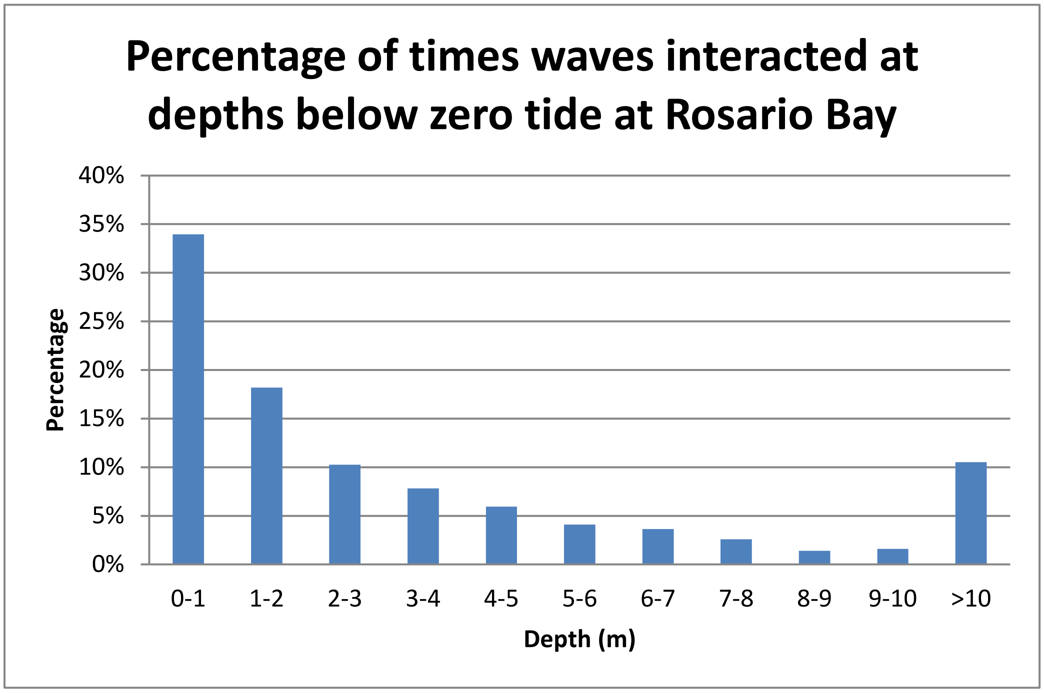


Figure 17. Frequency of wave interaction based on the equation in Koch (2001) which calculates depth of wave interaction with the bottom based on wave height. Wave height was calculated from weather data recorded at half-hour intervals by the Whidbey Island Naval Air Station for Hylarides (2015).

Table 2. Station coordinates and regions with mean average sediment types, mean average percent organic content, and mean average mg nutrients per gram sediment of nitrate, phosphate, and sulfate. Nutrient content is as a proportion of the fine sediment (excluding gravel). The coordinates list the east-west dimension first, then the north-south. To convert the coordinates to actual UTM Section 11 coordinates using WGS 84 datum, add 0524000 to the east-west dimension and 5362000 to the north-west dimension.

Site #	Coordinates	Location	Mean % Gravel	Mean % Sand	Mean % Silt/ Clay	Mean % Organic	Mean Nitrate (mg/g sed)	Mean Phosphate (mg/g sed)	Mean Sulfate (mg/g sed)
1	90,30	Shore-ward	82.77	17.09	0.14	1.10	0.007	0.002	0.139
2	90,40	Shore-ward	89.90	10.04	0.06	1.46	0.008	0.004	0.074
3	80,50	Shore-ward	77.11	22.76	0.13	0.93	0.010	0.005	0.029
4	50,60	Within Eelgrass	0.17	99.01	0.82	1.07	0.009	0.008	0.048
5	50,50	Within Eelgrass	0.00	98.91	1.09	0.93	0.009	0.008	0.112
6	40,50	Within Eelgrass	16.33	82.82	0.85	0.81	0.008	0.010	0.128
7	20,50	Seaward	21.86	76.93	1.21	0.97	0.011	0.008	0.153
8	20,40	Seaward	9.55	89.69	0.77	0.85	0.009	0.008	0.124
9	20,30	Seaward	17.79	81.59	0.62	0.85	0.006	0.014	0.151

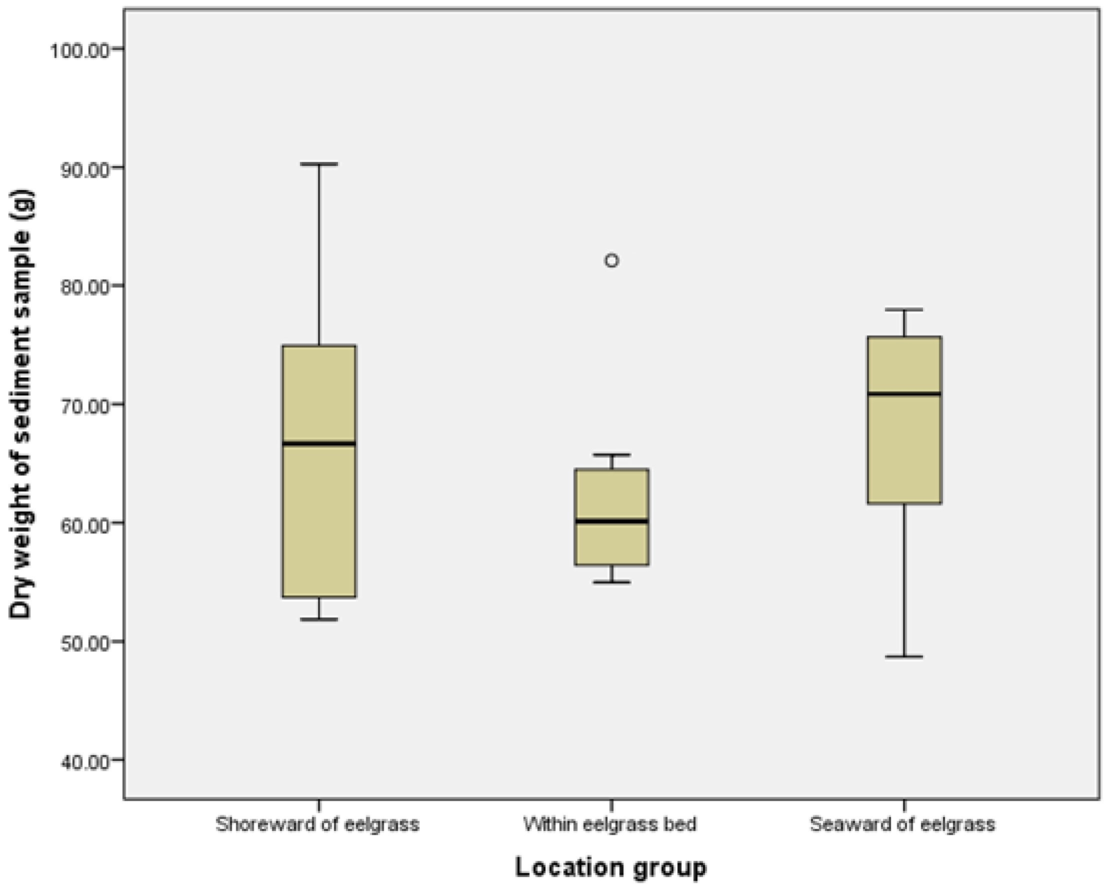


Figure 18. Comparison of dry weight between different locations to make sure they were comparable to each other, showing no significant difference between sites.

showed that all groups were different from each other in gravel content ($F = 106.432$, $P<0.0005$). The highest percentage of gravel was found in the sediment shoreward (east), and the lowest within the eelgrass beds (Table 2, Figure 19). The sediment seaward of the eelgrass beds had an intermediate level of gravel that was different from either shoreward or within.

A comparison of the percent of sand in the sediment also showed that there was a significant difference among all three locations, with the least percent being shoreward, the most within the eelgrass, and an intermediate level seaward ($F = 120.823$, $P<0.0005$) (Figure 20) These data were homoscedastic so didn't need to be further transformed before analysis. The data for the percent silt and clay in the sediment were not homoscedastic and further transformations didn't fix the problem. An ANOVA with post-hoc tests that are not sensitive to homoscedasticity; Tamhane, Dunnett T3, and Games-Howell, was therefore performed on these data. The ANOVA was significant ($F=21.341$ $P<0.0005$), and all three post-hoc tests showed the same results: shoreward had significantly less silt than either within or seaward sediment samples, but within and seaward had no difference in percentages (Figure 21).

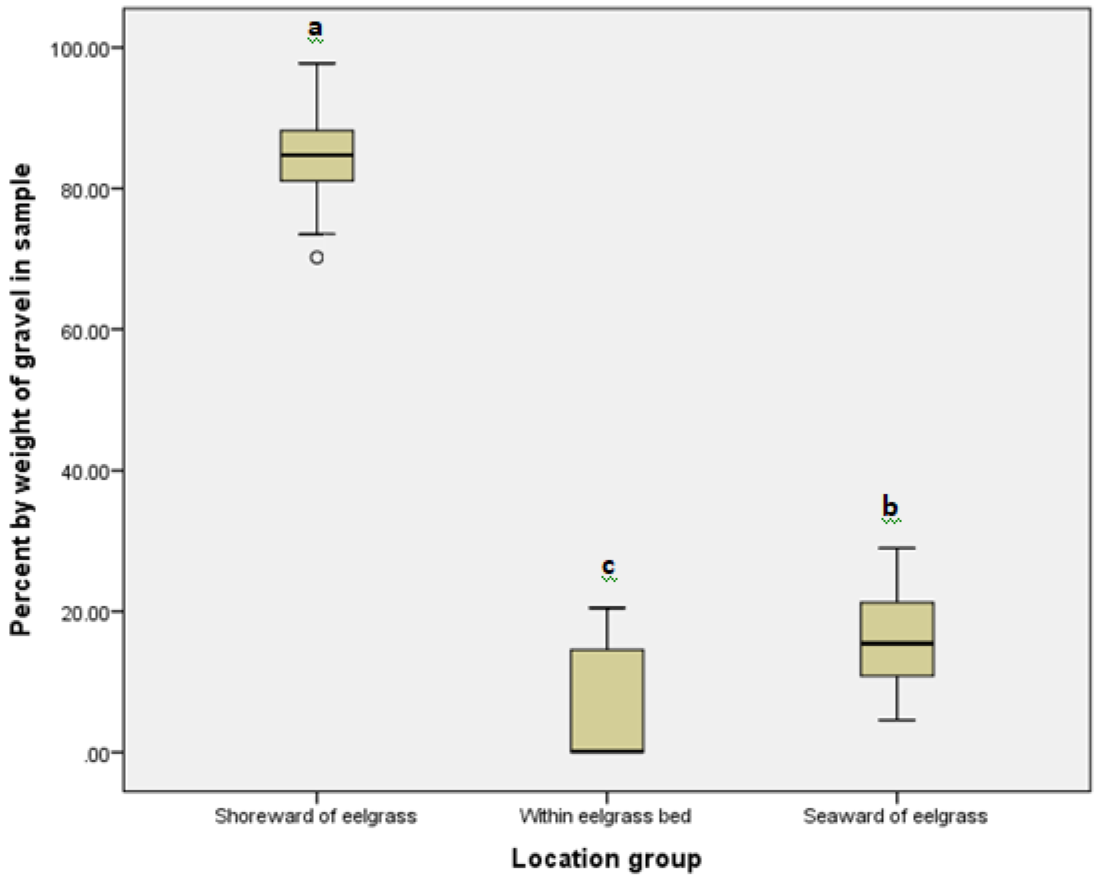


Figure 19. Comparison of percent gravel between locations. Letters indicate groups with significant differences from each other. The highest amount of gravel was shoreward while the least amount was within the eelgrass beds. (F=106.432, P<0.0005)

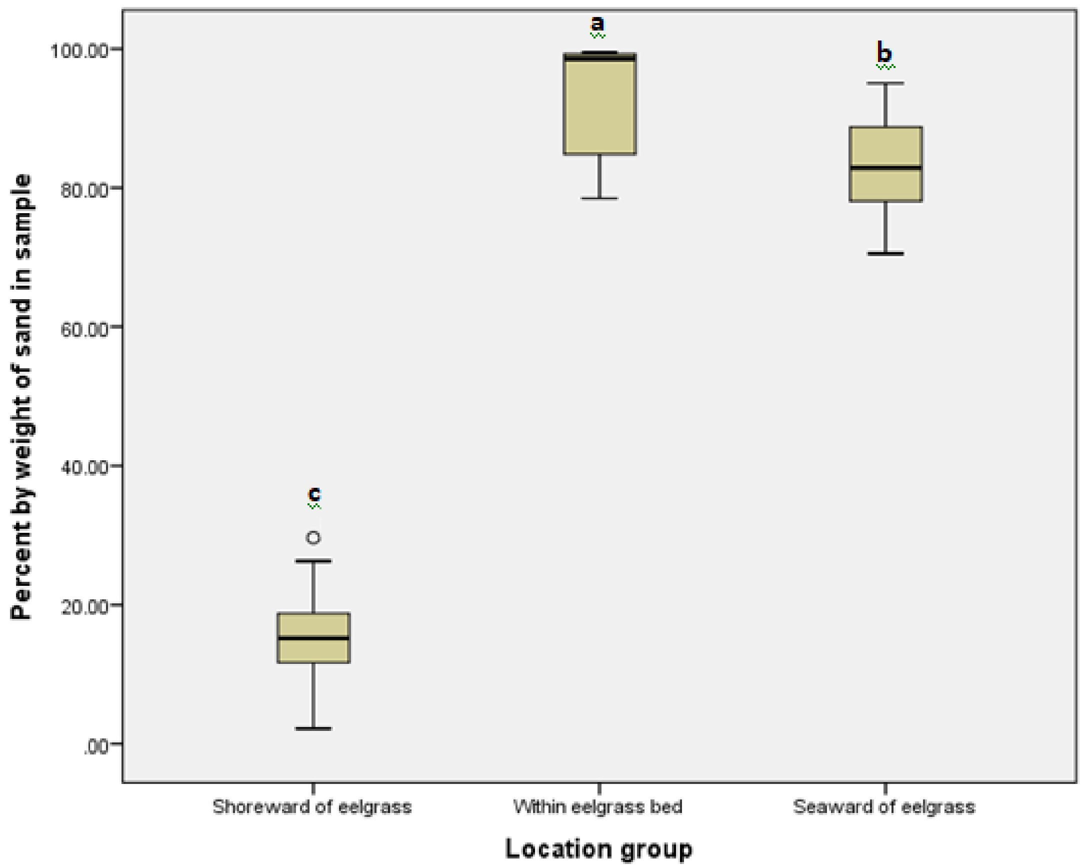


Figure 20. Percent sand in different locations. Letters indicated significant difference from each other. The percentage of sand was significantly lower in the shoreward group than either of the other two locations. (F=120.823, P<0.0005)

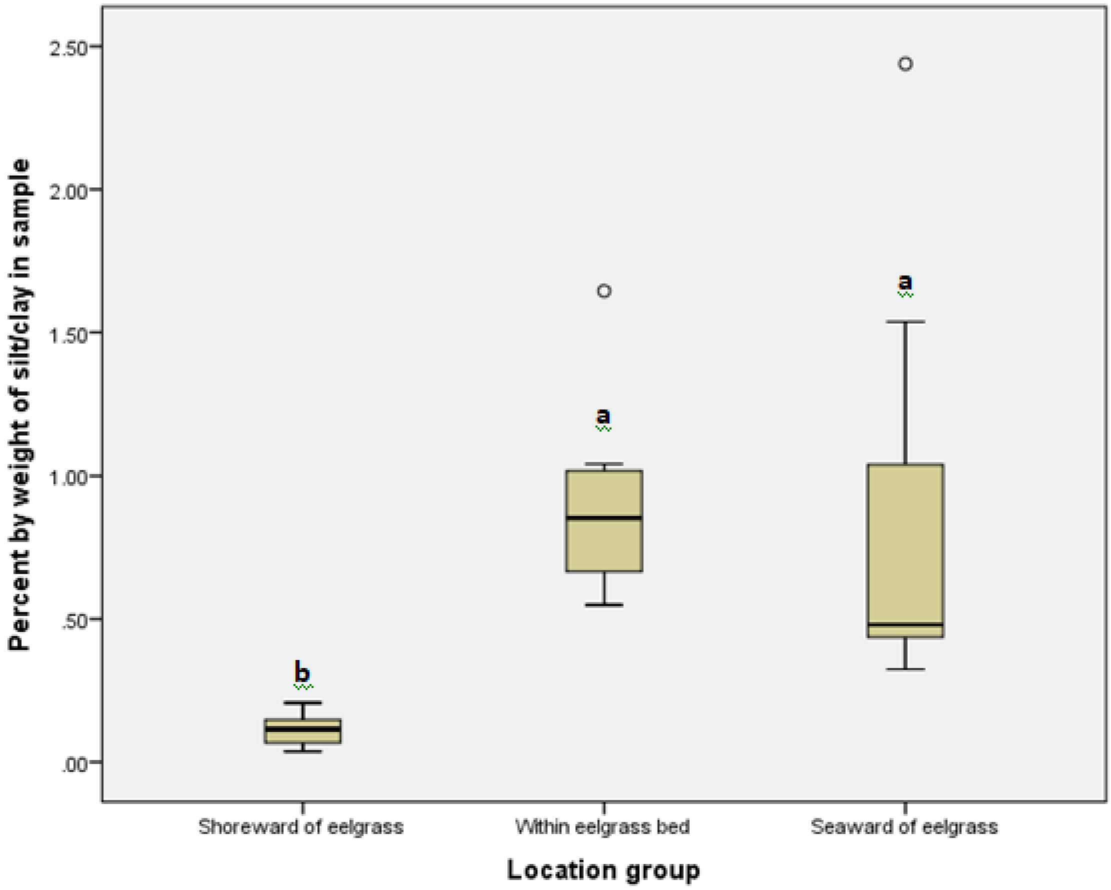


Figure 21. Percent silt/clay in different locations. Letters indicate significant difference between the different locations. (F=21.341, P<0.0005).

Sediment Organic Content

One-way ANOVA analysis with Tukey post-hoc test of arcsine-transformed percentage data showed that there was significantly less organic content in the shoreward sediment than within or seaward of the eelgrass bed ($F=31.223$, $P<0.0005$). There was no difference in organic content between within-eelgrass or seaward sites ($P=0.658$) (Figure 22). Note that organic content is the organic proportion of the fine (non-gravel) portion of the sediment only.

Sediment Nutrient Contents

Nutrient content of the sediment was also measured as a proportion of the fine portion of sediment only. Nitrate levels in the sediment from the three different locations were compared by one-way ANOVA ($F=3.799$, $P=0.038$). A comparison of the nitrate levels showed that the sediment seaward of the eelgrass had higher nitrate content than did the sediment within the eelgrass bed ($P=0.046$), but it was not different in nitrate content from the sediment shoreward of the bed($P=0.961$)(Table 2, Figure 23). The shoreward sediment was not different in nitrate level from sediments either within or seaward of the eelgrass ($P=0.109$, 0.961). Sediment within the eelgrass had nitrate levels that were only lower than seaward sediments but not different from shoreward sediments ($P=0.046$, $P=0.109$). After phosphate levels were square root transformed to normalize the data, an ANOVA with Tukey post-test showed that

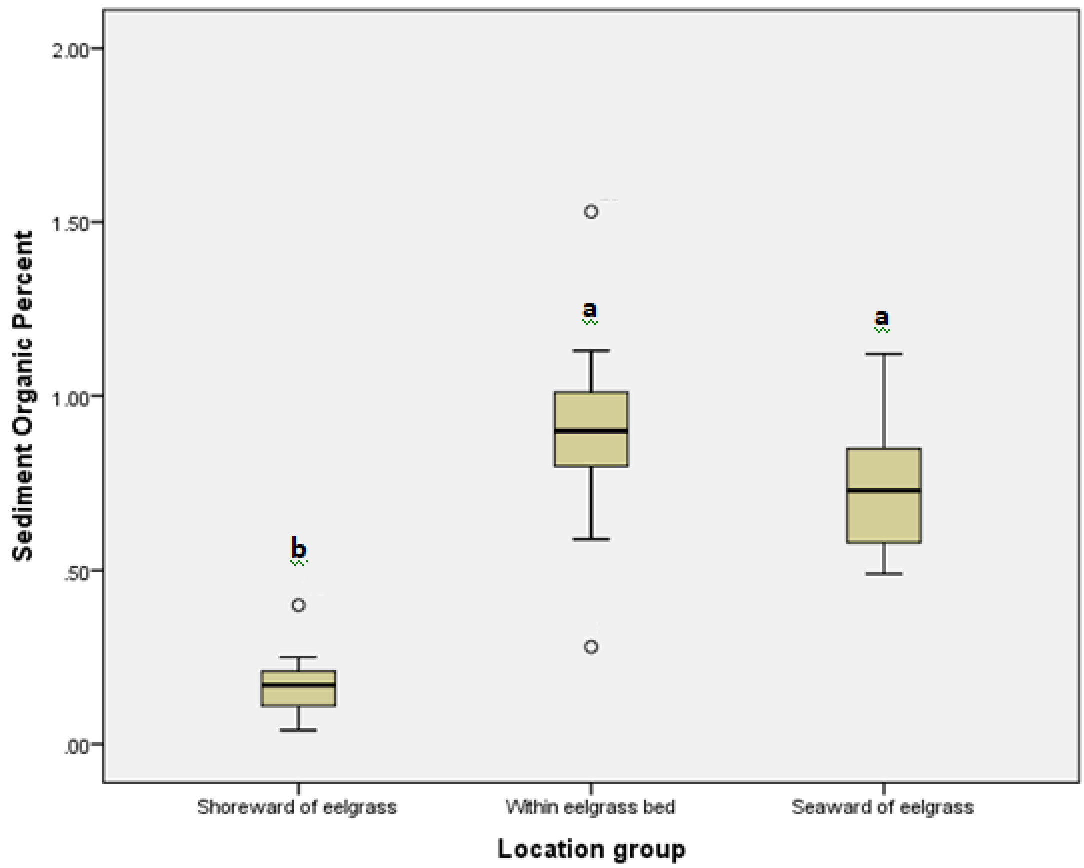


Figure 22. Percent organic sediment, showed that there was significantly less organic content in the shoreward sediment than within or seaward (F=31.223, P<0.0005). Letters indicate significance.

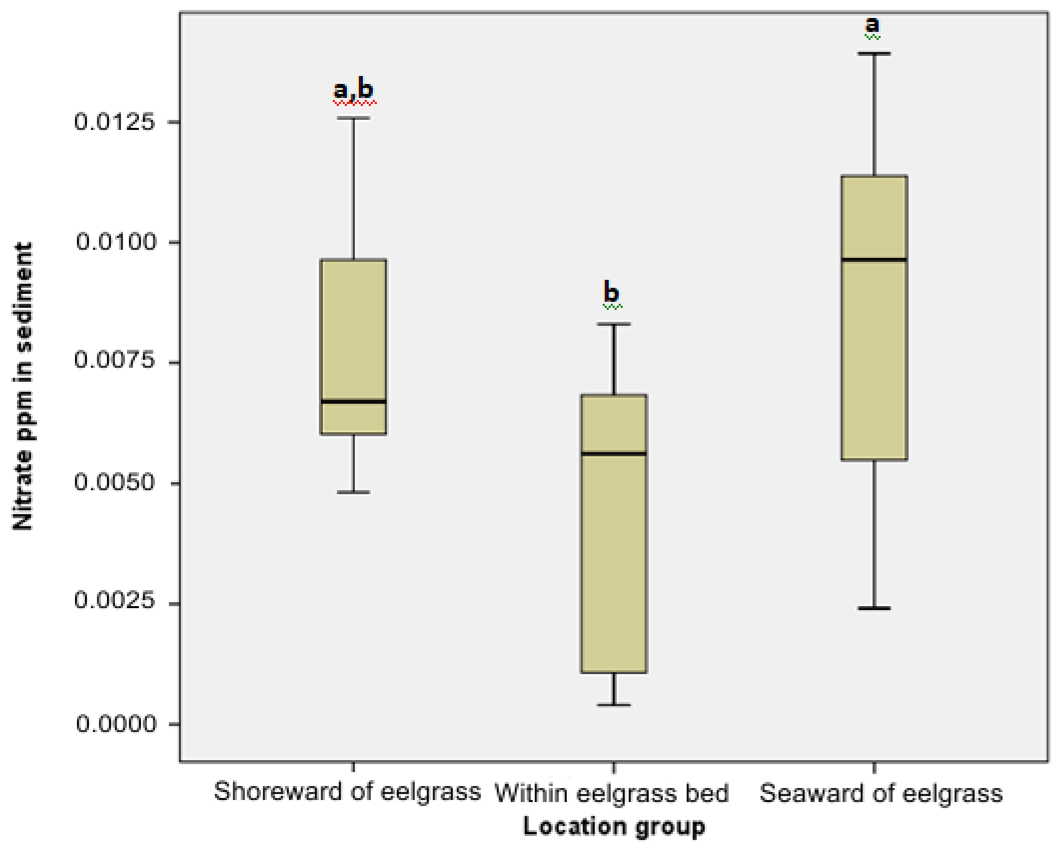


Figure 23. Nitrate levels between locations, letters show significance. ($F=3.799$, $P=0.038$)

seaward sediments were significantly higher in phosphate than sediments in either shoreward or within the eelgrass beds ($F = 8.738$, $P=0.011$, 0.002), but shoreward sediments and sediments within the eelgrass beds were not different from each other ($P=0.920$) (Table 2, Figure 24). Sulfate levels were unable to be transformed to make them normal, since the skewness for the shoreward sediment was 1.238. After a non-parametric Kruskal-Wallis test showing there was a difference, an ANOVA and Tukey showed that the sediment within the eelgrass was higher in sulfate levels than either seaward or shoreward ($F=7.403$, $P=0.011$, 0.007). Sulfate levels in locations on either side of the eelgrass beds, shoreward or seaward, were not statistically different from each other ($P=0.998$) (Table 2, Figure 25).

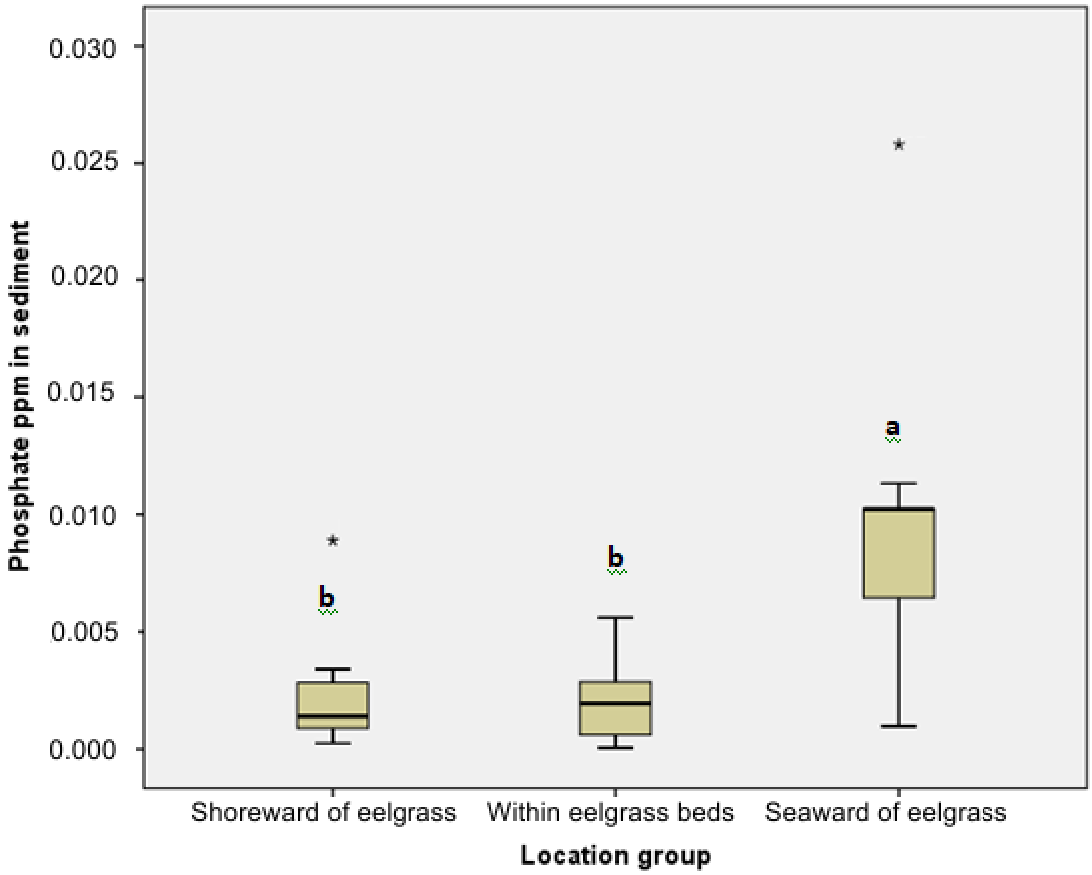


Figure 24. Phosphate levels in sediment locations, letters indicate significance (F=8.738, P=O.011,

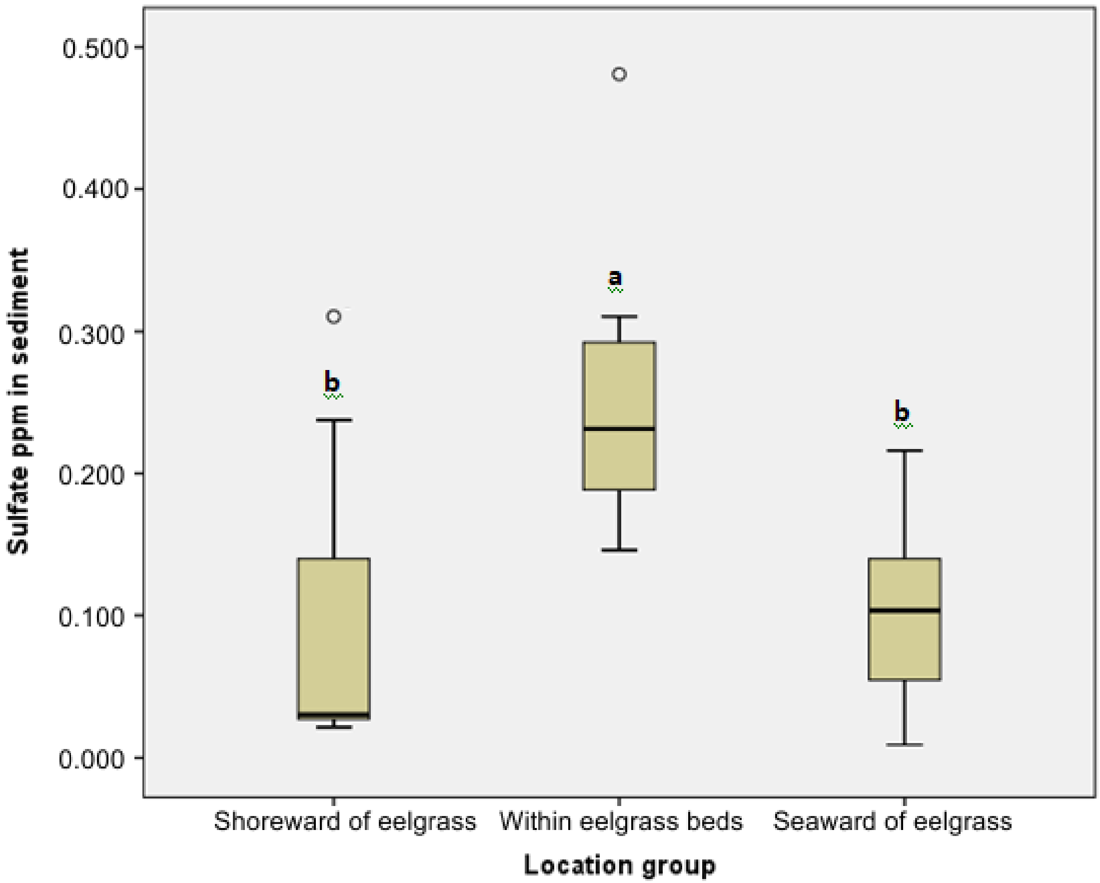


Figure 25. Sulfate levels between locations. Letters indicate significance. Data was not normal, with a skewness of 1.238. (F=7.403, P=0.003)

Discussion

A *Z. marina* population has been established in Rosario Bay for many years and it appears to flourish in nearby bays (Padilla Bay and Bowman's Bay) as well. Previous studies have determined that the population in Rosario Bay is receiving enough light (Hylarides, 2015). Therefore in this study I looked at other parameters as possible reasons that the eelgrass patch in Rosario Bay is small and not found in all portions of the bay. The main portion of the eelgrass bed appears to be quite stable, occurring at virtually the same position in 2015 as it did in 2012 (Figures 2, 12, 13). Comparison of the 2012 and 2015 maps suggest possible shifts in the position of some of the smaller, peripheral patches, but it is not clear whether these shifts are real or simply due to the higher spatial resolution of the 2015 survey. The 2012 survey was made by swimming a series of transects from edge to edge of the 100-m grid by compass heading, counting kicks and recording the eelgrass locations along each transect and had less spatial accuracy than did this survey (Cowles, personal communication).

During this study it was found that the amount of water motion did not vary during the duration of my study between the different locations. This may be due to being able to test water motion only during the summer, and furthermore only being able to dive when it was relatively calm so the clod cards didn't show as much wear as may be expected to occur during periods of greater water motion. It is known that especially during the winter months, storms bring stronger water motion, which changes the beach profile and even moves driftwood that has been lodged on the beach. Although the eelgrass bed is entirely subtidal and thus not directly exposed to the surface waves, we observed while diving that winter 2015 wave action had been great enough to dislodge and tumble 25 kg concrete pylons which David Cowles had placed near my inshore sites. Some of the pylons were moved up to at least 10 meters from their original site. All the pylons placed at depths of less than 2 m below zero tide level were moved, as were several pylons at depths between 2 and 4 meters (Cowles, personal communication). While this specific study didn't show any substantial difference in water motion between the different locations, such may not be the case in other times of the year. Eelgrass growth could also clearly be affected by waves coming from outside the bay. Using wave depth data collected from June 2013-June 2014 at different times of the day the waves were seen to be frequently large enough to substantially interact with the bottom of the bay. Using the equation of Koch et al. (2001) to calculate the minimum depth that *Z. marina* could grow, it is clear that wave depth interactions down to depth of 10 m or more are very common (Figure 17). Therefore the entire bay, including where the eelgrass grows, is impacted by at least some wave action. During storms the wave action can be major (Figure 26). The depth that the eelgrass in Rosario Bay grows is within the range of minimum and maximum depth means for eelgrass bed sites around the San Juan Islands/Strait of Juan de Fuca ((Mumford, 2007).

The reason why the clod cards indicated greater water motion during the July 26-28 deployment than during later deployments is not clear. The most obvious drivers

Figure 26. Winter storm Feb 1998 showing waves almost to the beach cabins. Storms like this could impact the sediment and eelgrass growth. Photo taken by David Habenicht.

of water motion are tidal exchange and wave action, which is driven by wind speed. Maximum tidal range during that deployment was 2.3 m compared to 2.0 to 2.8 m in the later deployments. Maximum steady wind speeds at NASWI during the July deployment dates were 4.9 m/s gusting to 8.0 m/s. For the later deployments maximum wind speeds ranged from 4.5 to 7.6 m/s and gusts ranged up to 10.3 m/s so both tidal ranges and wind speeds were comparable and moderate in all deployments. It is possible that wave action from an offshore storm increased water motion during the July deployment beyond that expected from local wind conditions.

The differences between sediment particle sizes showed that the sediment shoreward of the eelgrass was heavily dominated by gravel, which made up only a small proportion of the sediment within and seaward of the eelgrass (Figure 19). In contrast, the sand and silt/clay content of the eelgrass bed and seaward was high, but was very low in the shoreward sediment (Figures 20, 21). This difference mirrored in the organic content, which was also significantly higher in and seaward of the eelgrass beds, and tended to be highest within the eelgrass bed (although this difference was not significant) (Figure 22). This shows that between the eelgrass bed and the shore was a substantial change in substrate to sand and gravel, which may play a role in why the eelgrass was not growing there. Eelgrass has an underground rhizome and root system that may be impacted by the sediment size. In more gravelly sediment the rhizome may have trouble growing through the sediment or receiving nutrients from the sediment. This could also be an indicator that the current flow in the eelgrass bed is less intense allowing for silt/clay particles to settle in those areas (Fonseca et al., 1983). In the areas of the bay where larger stones were located there was also an increase in the amount of algae seen, there may be some competition for nutrients and space seen in areas where both algae and eelgrass are trying to grow together. With less sand/silt there is less sediment that is able to trap nutrients that are vital for plant life. Furthermore, the sand and gravel, which changes to clean gravel and stones as the beach slopes up closer to shore, are doubtless indications of unstable substrate which is periodically disturbed by wave action. Such turbulence and instability likely would prevent establishment of eelgrass at those depths, even if enough sediment existed there to support its growth.

Sediment nutrient levels varied between the different locations as well. The levels of nitrate between the different locations was slightly higher seaward than within the eelgrass bed but was not significantly different from shoreward of the bed while within and shoreward showed no difference (Figure 23). Phosphate was the highest seaward of the eelgrass bed, while sulfate was the highest within the bed (Figures 24,25). However, these nutrient levels apply only to the fine (non-gravel portion of the sediment and fail to take into consideration that there was less sand/silt in the shoreward locations than elsewhere in the bay. Since the sediment shoreward of the beds had dramatically higher gravel content and less fine sediments, the total availability of nutrients would be much higher within and seaward of the eelgrass beds. The higher sulfate levels within the bed probably reflect the higher organic content there, which leads to higher sulfur levels within the fine sediment. The slightly lower level of nitrate could be reflective of the nitrate uptake through the roots of the eelgrass.

Conclusion

In conclusion, the eelgrass beds in Rosario Bay seem stable. The beds are likely limited on the shoreward side by substrate instability due to water motion, lack of fine sediment, and nutrient limitation. The limitation on the northern side could possibly be caused by competition with brown and red algae. The limitations on the seaward side and the southern side are less obvious.

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