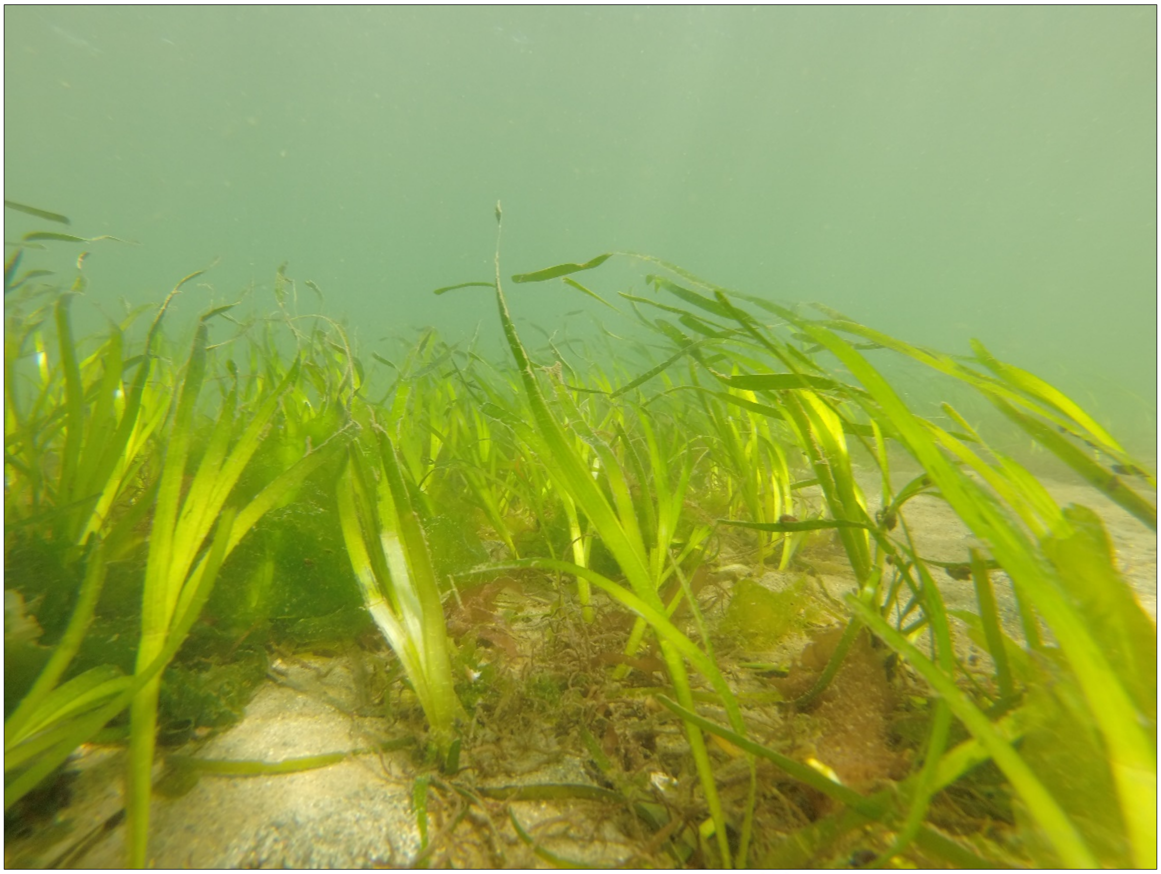
NATURAL RESOURCES

Evaluation of Eelgrass (*Zostera marina* L.) Condition and Environmental Parameters around an Outfall, Orcas Island, WA

September 6, 2016



WASHINGTON STATE DEPT OF
NATURAL RESOURCES
PETER GOLDMARK
COMMISSIONER OF PUBLIC LANDS

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Jeffrey Gaeckle

Nearshore Habitat Program
Aquatic Resources Division

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EXECUTIVE SUMMARY

The Washington State Department of Natural Resources (DNR) is steward of 2.6 million acres of state-owned aquatic land. DNR manages these aquatic lands for the benefit of current and future citizens of Washington State. DNR's stewardship responsibilities include protection of eelgrass (*Zostera marina* L.), an ecologically important nearshore habitat in greater Puget Sound. As monitoring lead for the Puget Sound eelgrass ecosystem indicator, DNR contributes to efforts that aim to achieve the goals set by the Puget Sound Partnership and supports eelgrass conservation and restoration.

The current project was designed to assess the concentration of carbon, nitrogen, metals, and organic contaminants in eelgrass at three sites, two reference and one study site, near the Eastsound Sewer and Water District Outfall Replacement Project study site at North Beach, Orcas Island, WA. Eelgrass area and depth distribution along with carbon, nitrogen, metal and organic contaminant concentrations in eelgrass were measured at three sites on North Beach in 2013. In addition, light and temperature were measured over a period of 30 days in eelgrass at the location of the Eastsound Sewer and Water District Outfall Replacement Project.

There was a significant decline in eelgrass area at the site of the Eastsound Sewer and Water District Outfall Replacement Project between 2013 and 2015. The decline in eelgrass area at the site where the outfall was replaced coincided with a significant decline in the maximum depth of eelgrass. There was also a significant decline in eelgrass area over the three sample years at the western reference site, but the eelgrass depth distribution at this site did not change over the sample period. Eelgrass area at the eastern reference site increased from 2013 to 2014 and the eelgrass depth distribution at remained the same for the entire sample period.

The concentration of carbon, nitrogen and metals in eelgrass were within the range observed in other seagrass, and eelgrass-specific, studies worldwide. The low C:N ratio in the aboveground biomass suggested the eelgrass at North Beach, Orcas Island, grows in an environment with a high nutrient availability. The C:N ratio in the belowground biomass was higher and more similar to results from elsewhere in the world.

The concentration of most metals measured was low compared to a similar study conducted at 15 sites throughout Puget Sound in January 2013 (Gaeckle 2016). There was no pattern in the observed metal concentrations that suggested a loading source at one of the three sites or that eelgrass would be susceptible to stress from metal toxicity.

The concentration of organic contaminants in eelgrass at the three Orcas Island sites was low and often below detectable limits. The low prevalence of organics measured in eelgrass was likely due to the limited amount of these contaminants being discharged into the system during the dry summer months and a limited uptake and accumulation potential by seagrass. Seagrass has low lipid concentrations and therefore has a low affinity and storage capacity for organic contaminants.

Persistent, long-term effects of exposure to metal and organic contaminant concentrations may cause detrimental effects on eelgrass populations over time. In addition, continued monitoring of eelgrass area and depth distribution will indicate whether the outfall modification at Eastsound Sewer and Water District Outfall Replacement Project study will affect eelgrass in the area. Results from three years of sampling show less eelgrass and a shallower deep edge at the Eastsound Sewer and Water District Outfall Replacement Project study site compared to one of the reference sites. The ability to observe changes in eelgrass area and depth distribution and track changes in light availability to seagrass are powerful tools to determine the effects of outfalls that discharge in close proximity to existing eelgrass beds.

1 INTRODUCTION

Outfalls that discharge residential, commercial, and industrial wastewater as well as upland stormwater are abundant throughout Puget Sound (Gaeckle et al. 2015). The construction of the outfall, the chemical composition and volume of the discharge waters, and the properties of the receiving waters affect marine resources in different ways. For seagrasses, the uptake rates and concentrations of nutrients, metals, and contaminants are subject to a high degree of variability due to a number of factors. Research has demonstrated the uptake of these substances and the physiological effects on seagrass (see Gaeckle 2012), but these data tend to be species and location specific. Furthermore, little is known about the concentrations of nutrients, metals, and contaminants in eelgrass (*Zostera marina*) in the Pacific Northwest (Kaldy 2006) and more specifically, in greater Puget Sound. Basic nutrients, nitrogen and phosphorus, are likely abundant in Puget Sound, but whether these substances are at levels that cause toxicity in eelgrass is unknown. The marine nearshore environment where eelgrass grows will experience the greatest effects from metals and contaminants loading due to the proximity to sources (Ecology and King County 2011, Mohamedali et al. 2011). Yet no research has been done to understand the concentration of nutrients, metals, and contaminants in eelgrass and the potential effects these substances have on the health of the dominant marine flora in Puget Sound.

Although tissue nutrient content values for eelgrass are considerably more variable than other seagrasses, it is recommended that an initial baseline assessment of nutrients and δ15N in eelgrass be performed to determine whether plants are exposed to nutrient enrichment (Duarte 1990, Udy and Dennison 1997). In addition, the assessment of metals (e.g., Cd, Cu, Cr, Hg, Ni, Pb, and V) in eelgrass compartments (leaves, rhizomes/roots, epibiota) would characterize metal uptake by eelgrass in Puget Sound and identify whether metal concentrations are at levels that cause physiological effects to seagrass (Lewis and Devereux 2009). Finally, an assessment of PAHs, PCBs, and other organic contaminants (e.g., herbicides, antifouling paints), substances that affect seagrass metabolic processes individually and when combined (Lewis and Devereux 2009), would provide more information on factors that impact eelgrass in Puget Sound. The recommended assessments of nutrient, metal and contaminant concentrations in eelgrass will provide valuable baseline information for decision makers to effectively manage and protect this ecologically significant resource in Puget Sound.

In an effort to assess the effect of an outfall on eelgrass (*Zostera marina* L.) in Puget Sound, Task 3 was to conduct field evaluations of eelgrass and environmental parameters adjacent to an outfall where modifications to the outfall were expected in the near future. A high resolution assessment of eelgrass distribution and abundance at the Eastsound Sewer and Water District Outfall Replacement Project study site at North Beach, Orcas Island, WA, was conducted in Task 3.1 (Gaeckle 2014a, b). Eelgrass plant tissue carbon, nitrogen, metal and organic contaminant concentrations, and environmental parameters at the site provide a 2013 baseline dataset to compare future sampling to and understand the extent and magnitude of the effects from modifications to the Eastsound Sewage Outfall. The work may also provide important baseline information that can be used to evaluate project effectiveness of the outfall modification.

2 METHODS

2.1 Eelgrass area and depth distribution surveys

Eelgrass area and depth distribution at the study and reference sites were collected using underwater videography and Biosonics on August 28, 2013 (Task 3-1, Gaeckle 2014a). Twenty (20) underwater videography transects were surveyed to determine eelgrass area and depth distribution at the Eastsound Sewer and Water District Outfall Replacement Project study site, referred to as outf457-OIC (Orcas Island Center) for this project and at two reference sites, outf455-OIW (Orcas Island West) and outf458-OIE (Orcas Island East) (Figure 1). The surveys were conducted similar to the Submerged Vegetation Monitoring Program (SVMP) methodology (Berry et al. 2003, Gaeckle et al. 2011, Christiaen et al. 2016), except each of the 20 transects was spaced at 10 m intervals across the 200 m width of the median line at each site (Figures 2a-c in Task 3-1, Gaeckle 2014a).

2.2 Eelgrass collection

Eelgrass was collected at the Eastsound Sewer and Water District Outfall Replacement Project study site at North Beach, Orcas Island, WA, on September 26, 2013 (Figure 1, Task 3.1, Gaeckle 2014a). The methods for eelgrass sample collection, processing and storage along with methods that describe the analyses of each sample for carbon, nitrogen, metals, and organic contaminants are described in the Quality Assurance Project Plan and Deliverables 3.1 and 3.2 (Gaeckle 2012a, 2014a, 2014b).

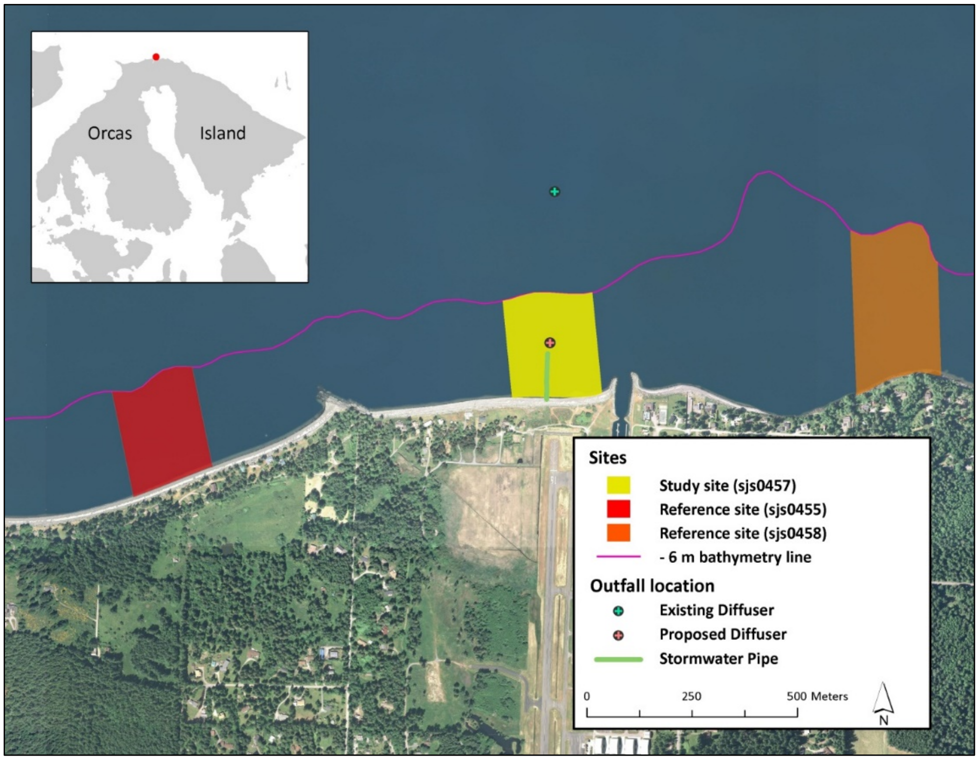

Figure 1. The location of the study site outf457-OIC and reference sites outf455-OIW and outf458-OIE relative to the Eastsound Sewer and Water District Outfall Replacement Project, North Beach, Orcas Island, WA.

Samples were prepared in the DNR Aquatic Botany Laboratory and delivered to the following laboratories for analyses:

- Metals – King County Environmental Laboratory, Seattle, WA
- Carbon and Nitrogen – Stable Isotope Core, Washington State University, Pullman, WA
- Organics – Northwest Fisheries Science Center NOAA-NMFS Laboratory, Seattle, WA

2.3 Light and temperature data collection

Odyssey light sensors (http://odysseydatarecording.com/) and Hobo Tidbit temperature (http://www.onsetcomp.com/) sensors were deployed at three locations (west, mid, and east) at the Orcas Island Center site, outf457-OIC, and one combination light and temperature sensor array was deployed on land. Sensor arrays were deployed September 27, 2013, and were recovered on October 31, 2013. Temperature sensors logged data every 30 minutes from 0100 PST on

September 27, 2013 through 2330 PST on October 30, 2013. Light sensors logged data every 15 minutes from 0100 PST on September 27, 2013 through 2330 PST on October 30, 2013. Sensors at the eastern, mid and western edge of outf457-OIC site were deployed at a depth of -2.5 m relative to Mean Lower Low Water (MLLW). The light and temperature sensors deployed on land were attached, with permission, to the Orcas Island Airport security fence. The in water sensors were cleaned by SCUBA divers on October 15, 2013. All sensors were recovered October 31, 2013. For comparison between sites a subset of the light data collected in a 4-hour period around solar noon were analyzed.

2.4 Seagrass genetics

Three replicate samples and a quality control sample of eelgrass leaf tissue were collected at each site (outf455-OIW, outf457-OIC, and outf458-OIE) and processed according to methods described in Task 3-1, Genetics processing (Gaeckle 2014a). Samples were stored in 50 mL vials with desiccant. Samples will be analyzed when funding becomes available.

3 RESULTS

Two separate field efforts were conducted to collect the underwater videography and BioSonics data and plant samples at the study site, outf457-OIC (Orcas Island Center), and the two reference sites, outf455-OIW (Orcas Island West) and outf458-OIE (Orcas Island East) (Gaeckle 2014a, b). The underwater videography and BioSonics data were collected at the three sites on August 28, 2013, and eelgrass samples for carbon, nitrogen, metals and organic contaminant analysis were collected on September 26, 2013. Additional field efforts were conducted to clean the light sensors on October 15, 2013 at each of the three sites and to recover all the sensors on October 31, 2013.

Subsequent field efforts were conducted in 2014 and 2015 to collect underwater videography and BioSonics data. These data provided additional estimates of eelgrass abundance and depth distribution at the three sites (outf455-OIW, outf457-OIC, and outf458-OIE). The 2014 and 2015 field efforts occurred after the old outfall was decommissioned and the new outfall went online in November 2013.

3.1 Eelgrass area and depth distribution

Eelgrass presence, absence and depth data were collected along 20 transects at each site on August 28, 2013 (Figure 2). Reference site outf455-OIW had the largest amount of eelgrass (4.1 ± 0.1 ha) compared to 1.5± 0.1 ha and 1.3 ± 0.1 ha measured at the study site outf457-OIC and the other reference site outf458-OIE, respectively (Table 1).

Figure 2. Transects to collect underwater videography and BioSonics data to determine eelgrass area and depth at the study site outf457-OIC (Center) and the two reference sites outf455-OIW (West) and outf458-OIE (East) on August 28, 2013.

Identical sample efforts conducted on August 18, 2014 and June 1-3, 2015 provided additional eelgrass area and depth distribution estimates for each site (Table 1 and Table 2). The 2013 data was collected shortly before the old Eastsound Sewage Outfall that extended 518 m (1,700 ft) offshore was replaced with a new outfall. The new outfall was engineered to extend 159 m (520 ft) offshore with the diffuser located in a bare area at -3 m (-10 ft, MLLW) between two eelgrass beds at outf457-OIC (Jen-Jay Inc. 2012).

Table 1. Estimated eelgrass area (ha ± SE) at the study site, outf457-OIC, and the two reference sites, outf455-OIW and outf458-OIE, measured in 2013, 2014, and 2015.

YEAR	outf455-OIW	outf457-OIC	outf458-OIE
	(ha ± SE)	(ha ± SE)	(ha ± SE)
2013 – Aug 28	4.12 ± 0.10	1.41 ± 0.09	1.31 ± 0.19
2014 – Aug 18	3.95 ± 0.14	1.35 ± 0.11	1.48 ± 0.21
2015 – Jun 1-3	3.62 ± 0.13	1.20 ± 0.08	1.34 ± 0.22

The average percent change in eelgrass measured at each site was calculated by comparing the vegetated fractions of each of repeated transect at each site for three different time intervals (Table 2 and Table 3). The average percent change in vegetated fraction varied at each site and over the three measured time intervals (2013-2014, 2014-2015, and 2013-2015). The largest significant decrease in percent change of vegetated fraction was -11.1% observed at outf455-OIW from 2013-2015 (Table 2 and Table 3). While the largest significant increase in percent change of vegetated fraction was observed at outf458-OIE from 2013-2014(Table 2 and Table 3). Eelgrass area at the center site, outf457-OIC, showed a significant 10.5% decrease in vegetated fraction along paired transects between 2013 and 2015 (Table 2 and Table 3). The eelgrass area at the western reference site, outf455-OIW, decreased significantly over each time interval (2013-2014, 2014-2015, and 2013-2015) (Table 2 and Table 3).

Table 2. Percent change in vegetated fraction of the paired transects between each sample year at the study site, outf457–OIC, compared to the two reference sites, outf455-OIW and outf458-OIE.

YEARS	outf455-OIW	outf457-OIC	outf458-OIE
	(% change)	(% change)	(% change)
2013 – 2014	-4.2	-1.7	15.8
2014 – 2015	-6.8	-4.2	2.9
2013 – 2015	-11.1	-10.5	19.7

Table 3. T-test results between the vegetated fraction of paired transects at the study site, outf457–OIC, compared to the two reference sites, outf455-OIW and outf458-OIE, over three time intervals (2013-2014, 2014-2015, and 2013-2015). The t-statistic, degrees of freedom and the p-value are presented for each site and time interval. Significant p-values are bold.

YEARS	outf455-OIW	outf457-OIC	outf458-OIE
	(t-statistic, df, p-value)	(t-statistic, df, p-value)	(t-statistic, df, p-value)
2013 – 2014	-3.072, 19, **0.006**	-0.738, 19, 0.469	2.660, 14, **0.019**
2014 – 2015	-3.050, 19, **0.007**	-1.368, 19, 0.187	-0.337, 14, 0.741
2013 – 2015	-5.035, 19, **0.000**	-2.243, 19, **0.037**	1.625, 14, 0.126

There was no significant difference in the minimum observed eelgrass depth measured at outf455-OIW, outf457-OIC, and outf458-OIE between each of the three sample years, 2013, 2014, and 2015 (Table 4). Statistical results showed p=0.639 at outf455-OIW, p=0.431 at outf457-OIC, and p=0.906 at outf458-OIE.

There was also no significant difference in the maximum observed eelgrass depth at outf455-OIW (p=0.958) and outf458-OIE (p=0.305) between the three sample years, 2013, 2014, and 2015.

However, there was an observed significant difference in the maximum eelgrass depth at outf457-OIC across the three sample years (p<0.001). The results of a Tukey post hoc test showed the maximum depth measured each year at sjs-0457-OIC was significantly shallower than the depth measured the previous year (2013-2014, p=0.028; 2014-2015, p=0.049) and over a two year span (2013-2015, p=0.000).

Table 4. Estimated eelgrass depth distribution (m ± SE) at the study site outf457-OIC and the two reference sites, outf455-OIW and outf458-OIE measured in 2013, 2014, and 2015. There was an observed significant difference in the maximum eelgrass depth at outf457-OIC (p<0.001).

YEAR	outf455-OIW		outf457-OIC		outf458-OIE	
	min	max	min	max	min	max
	(m ± SE)	(m ± SE)	(m ± SE)	(m ± SE)	(m ± SE)	(m ± SE)
2013	-0.80 ± 0.04	-7.66 ± 0.15	-0.64 ± 0.11	-6.04 ± 0.06	-1.18 ± 0.23	-3.45 ± 0.09
2014	-0.74 ± 0.04	-7.60 ± 0.14	-0.89 ± 0.14	-5.81 ± 0.06	-1.19 ± 0.22	-3.28 ± 0.08
2015	-0.79 ± 0.05	-7.62 ± 0.17	-0.70 ± 0.16	-5.61 ± 0.07	-1.18 ± 0.23	-3.35 ± 0.05

3.2 Carbon and Nitrogen

The eelgrass aboveground $\delta^{13}C$ values ranged from -10.7 to -8.0‰ and the belowground $\delta^{13}C$ values ranged between -10.4 and -8.8‰ at outf455-OIW, outf457-OIC, and outf458-OIE. There was no significant difference in the $\delta^{13}C$ in the aboveground and belowground eelgrass compartments at the three sites (Figure 3, Figure 4, Table 5 and Table 6).

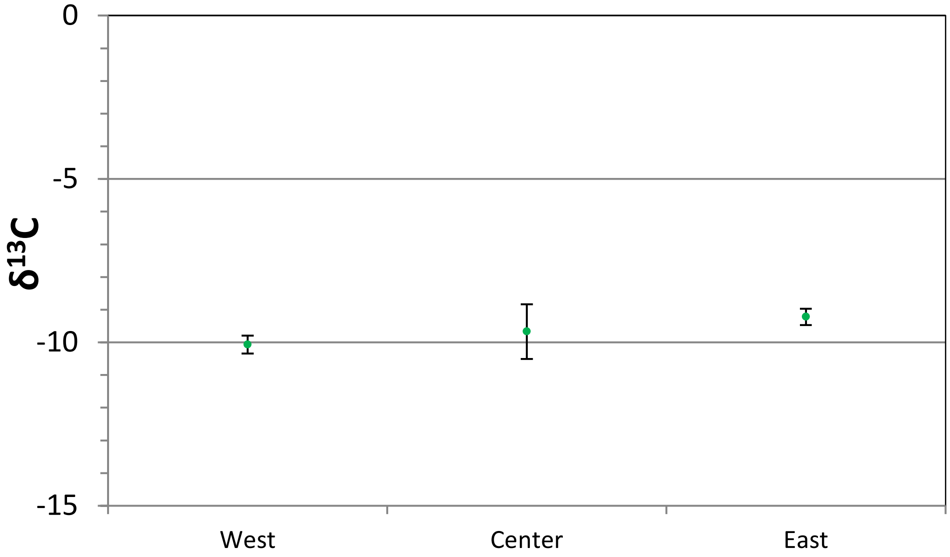

Figure 3. The concentration (± SE, n=3) of $\delta^{13}C$ in aboveground eelgrass (*Zostera marina*) at outf455-OIW, outf457-OIC and outf458-OIE sampled in September 2013.

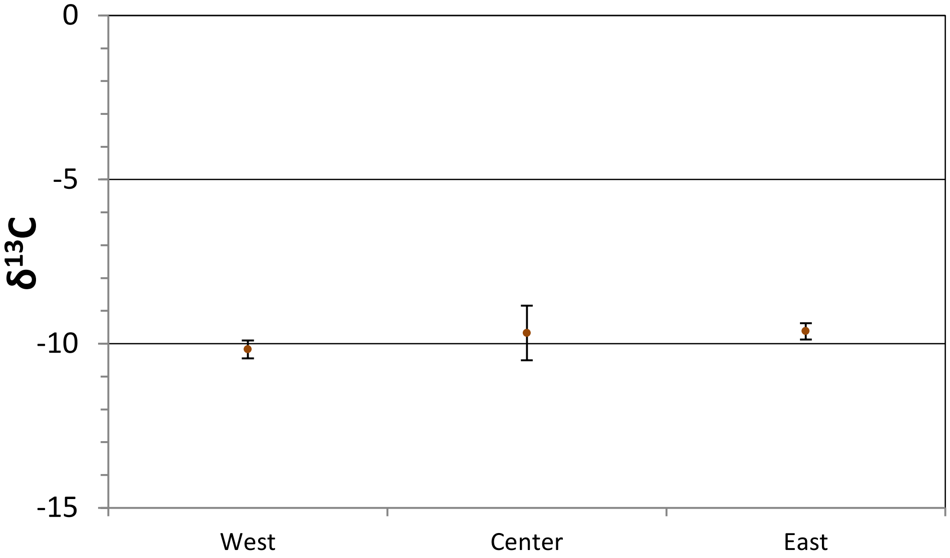

Figure 4. Average concentration (± SE, n=3) of $\delta^{13}C$ in belowground eelgrass (*Zostera marina*) at outf455-OIW, outf457-OIC and outf458-OIE sampled in September 2013.

Table 5. Average (± SE, n=3) $\delta^{13}C$ values for above- and belowground eelgrass (*Zostera marina*) compartments collected at outf455-OIW, outf457-OIC and outf458-OIE in September 2013.

SITES	**ABOVEGROUND**				**BELOWGROUND**			
	%C	**(SE)**	**$\delta^{13}C$**	**(SE)**	**%C**	**(SE)**	**$\delta^{13}C$**	**(SE)**
outf455-OIW	32.6	4.5	-10.1	0.3	35.1	0.4	-10.2	0.2
outf457-OIC	36.5	0.5	-9.7	0.8	34.5	0.0	-9.7	0.5
outf458-OIE	36.2	0.4	-9.2	0.2	34.9	0.3	-9.6	0.4

The eelgrass aboveground $\delta^{15}N$ values ranged from 4.7 to 7.0‰ and the belowground $\delta^{15}N$ values ranged between 3.2 to 5.4‰. There average aboveground $\delta^{15}N$ values were significantly different between the three Orcas Island sites (p=0.002; Figure 5, Table 5). A Tukey's post hoc test found the measured $\delta^{15}N$ values at outf458-OIE were significantly higher than the values observed at outf455-OIW and outf457-OIC (p=. There was no significant difference in the measured aboveground eelgrass $\delta^{15}N$ values at outf455-OIW and outf457-OIC.

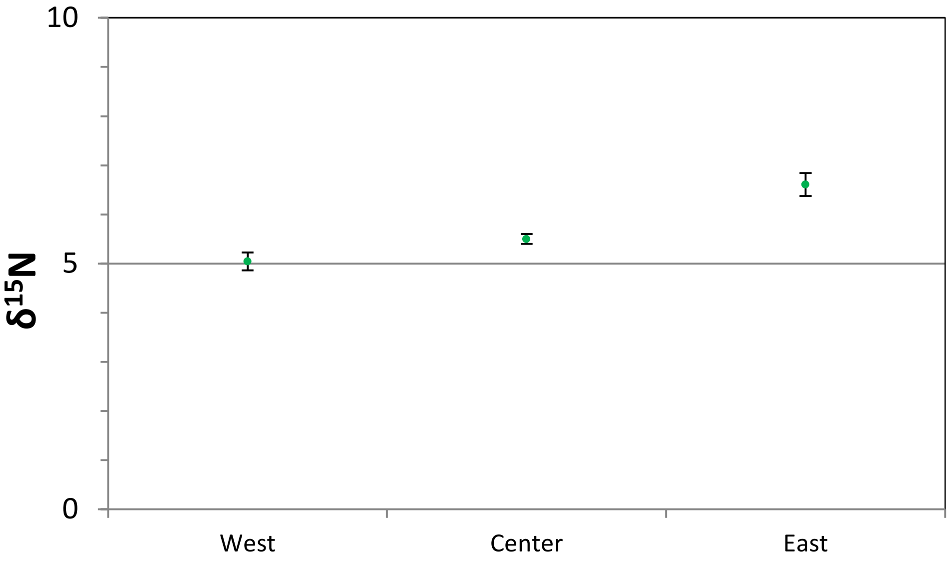

Figure 5. The concentration (± SE) of $\delta^{15}N$ in aboveground eelgrass (*Zostera marina*) at outf455-OIW, outf457-OIC and outf458-OIE sampled in September 2013.

The belowground $\delta^{15}N$ values were also significantly different between the three Orcas Island sites ($p<0.001$; Figure 6, Table 6). A Tukey's post hoc test found the measured belowground eelgrass $\delta^{15}N$ values at outf458-OIE were significantly higher than the values observed at outf455-OIW and outf457-OIC. There was no difference in the mean belowground eelgrass $\delta^{15}N$ values between outf455-OIW (p=<0.001) and outf457-OIC (p=<0.001) (Figure 6).

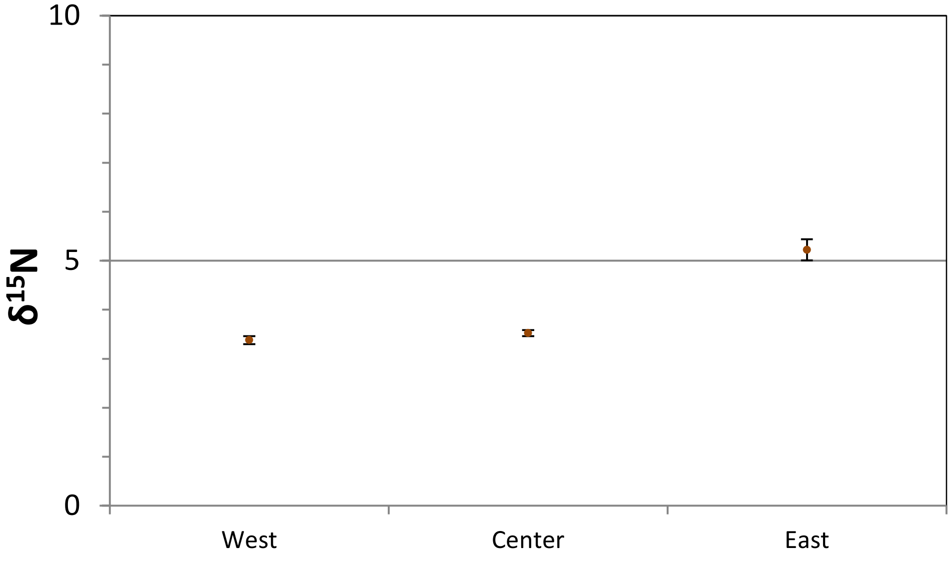

Figure 6. The concentration (± SE) of $\delta^{15}N$ in belowground eelgrass (*Zostera marina*) at outf455-OIW, outf457-OIC and outf458-OIE sampled in September 2013.

Table 6. Average (± SE, n=3) $\delta^{15}N$ values for above- and belowground eelgrass (*Zostera marina*) compartments collected at outf455-OIW, outf457-OIC and outf458-OIE in September 2013.

SITES	**ABOVEGROUND**				**BELOWGROUND**			
	%N	**(SE)**	**$\delta^{15}N$**	**(SE)**	**%N**	**(SE)**	**$\delta^{15}N$**	**(SE)**
outf455-OIW	2.2	0.2	-10.1	0.2	1.4	0.0	-10.2	0.1
outf457-OIC	3.1	0.1	-9.7	0.1	1.4	0.0	-9.7	0.1
outf458-OIE	2.9	0.0	-9.2	0.2	1.5	0.1	-9.6	0.2

The aboveground eelgrass biomass C:N ratios ranged from 9.4 at outf457-OIC to 13.2 at outf455-OIW and the belowground biomass C:N ratio ranged from 17.3 at outf458-OIE to 22.6 at outf455-OIW. The C:N ratio was significantly different in the aboveground biomass measured between the three sites; outf455-OIW, outf457-OIC, and outf458-OIE (p=0.009, Figure 7). A Tukey's post hoc analysis found the C:N ratio was significantly lower at outf457-OIC, compared to outf455-OIW (p=0.010) and outf458-OIE (p=0.024) where the ratios measured were not significantly different between these two sites (Figure 7).

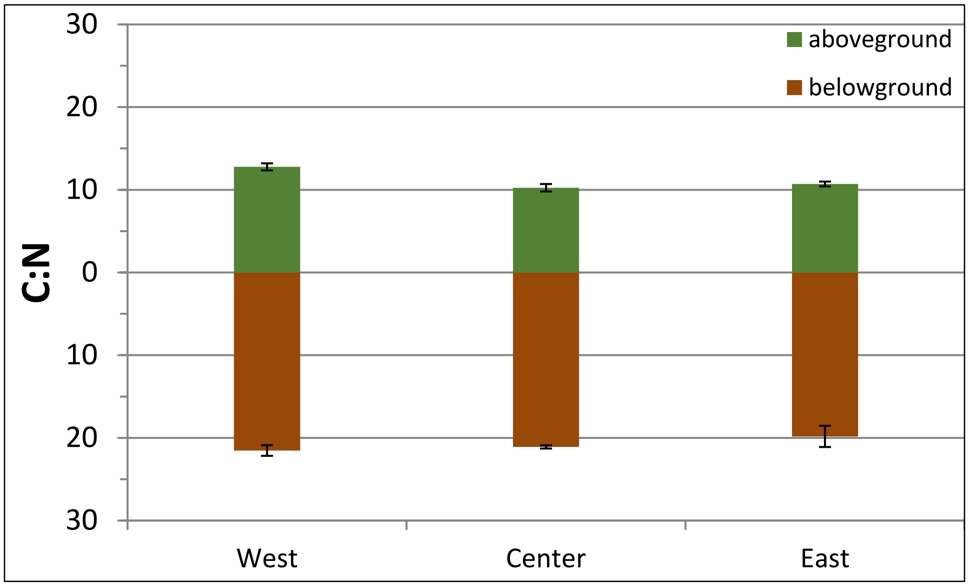

Figure 7. Average (± SE, n = 3) above- and belowground eelgrass (*Zostera marina*) C:N ratio collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

3.3 Metals

The average concentration (μg gdw^{-1}, ± SE) of metals in the aboveground compartment of eelgrass at outf455-OIW, outf457-OIC, and outf458-OIE was significantly different for mercury, iron and zinc but not for the other metals analyzed. Similar results were observed for the concentration of metals in belowground compartments of eelgrass. In addition a significant difference was observed in concentrations of iron, vanadium and zinc (μg gdw^{-1}, ± SE) measured in the belowground compartments of eelgrass sampled at the three sites sampled.

Mercury (Hg)

There was a significant difference in the average mercury concentration measured in the aboveground eelgrass compartment between samples collected at Orcas Island West, Center and East (p=0.002, Figure 8). A Tukey's post hoc test showed mercury concentration in the aboveground eelgrass biomass was significantly lower at outf458-OIE compared to outf455-OIW (p=0.013) and outf457-OIC (p=0.002); while the post hoc showed no significant difference in Hg concentration between outf455-OIW and outf457-OIC (Figure 6). There was no significant difference in the mercury concentration measured in the belowground eelgrass biomass between the three sites (p=0.519).

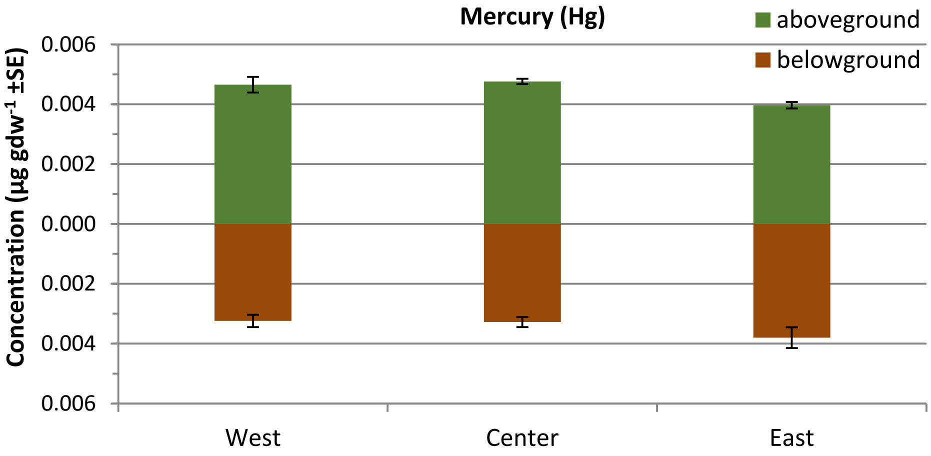

Figure 8. The average concentration (µg gdw^{-1} ±SE) of mercury (Hg) in above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Arsenic (As)

There was no significant difference observed in the concentration of arsenic measured in above- and belowground eelgrass compartments from samples collected at outf455-OIW, outf457-OIC, and outf458-OIE (aboveground p=0.754, belowground p=0.062; Figure 9).

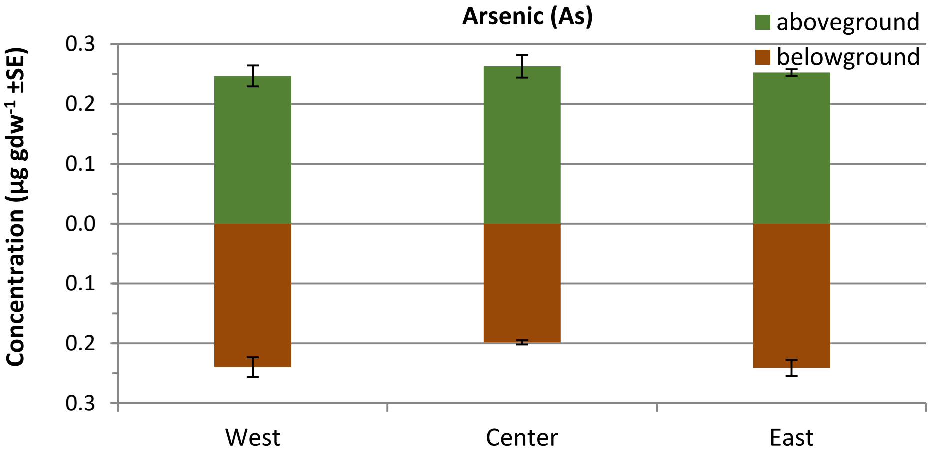

Figure 9. The average concentration (µg gdw^{-1} ±SE) of arsenic (As) in above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Cadmium (Cd)

There was no significant difference observed in the concentration of cadmium measured in the above- and belowground eelgrass compartments between Orcas Island West, Center and East (aboveground p=0.929, belowground p=0.509; Figure 10).

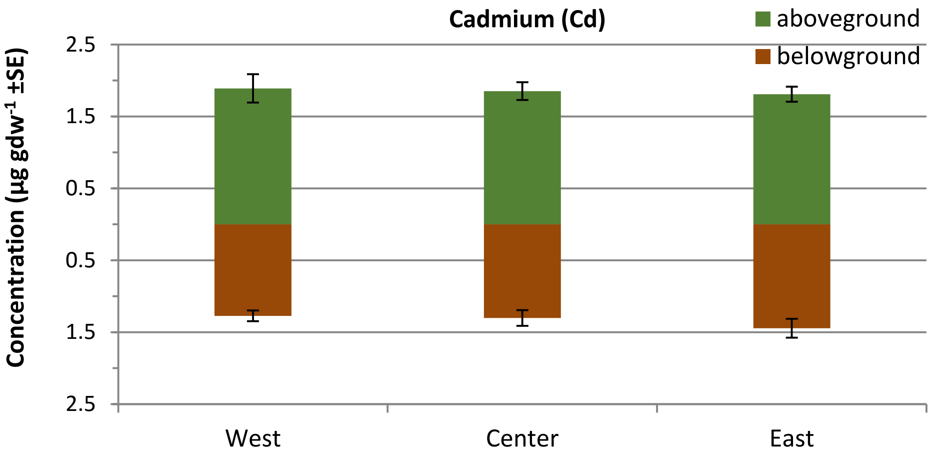

Figure 10. The average concentration (µg gdw^{-1} ±SE) of cadmium (Cd) in above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Chromium (Cr)

There was no significant difference observed in the concentration of chromium measured in the above- and belowground eelgrass compartments between Orcas Island West, Center and East (aboveground p=0.087, belowground p=0.694; Figure 11).

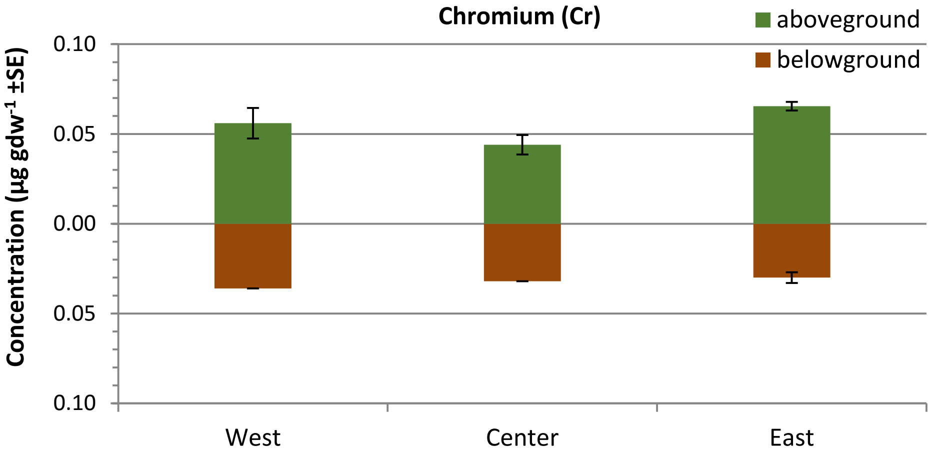

Figure 11. The average concentration (µg gdw^{-1} ±SE) of chromium (Cr) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Copper (Cu)

There was no significant difference observed in the concentration of copper measured in the above- and belowground eelgrass compartments between Orcas Island West, Center and East (aboveground p=0.596, belowground p=0.194; Figure 12).

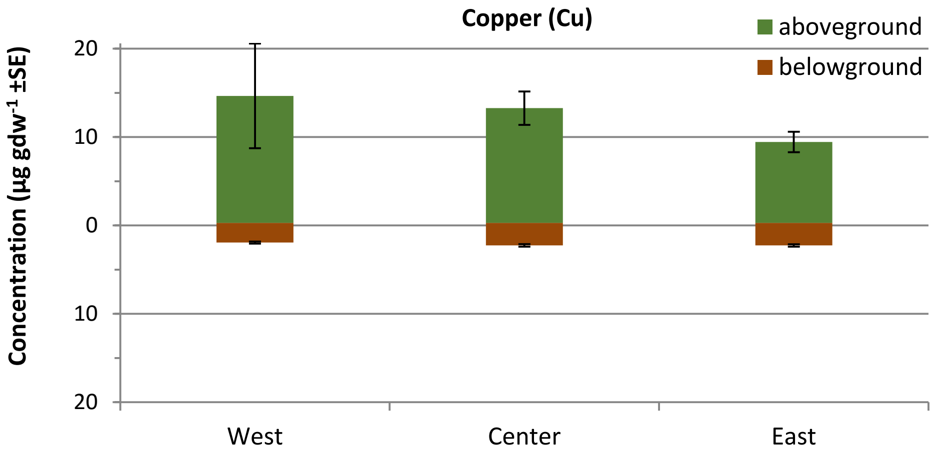

Figure 12. The average concentration (µg gdw^{-1} ±SE) of copper (Cu) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Iron (Fe)

There was a significant difference in the average iron concentration measured in the aboveground eelgrass compartment between samples from outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (p=0.046, Figure 13). A Tukey's post hoc test showed iron concentration in the aboveground eelgrass biomass was significantly lower at outf455-OIW (West) compared to outf457-OIE (East), and there was no significant difference between the iron concentration between samples collected at outf458-OIE (East) and outf457-OIC (Center) and outf455-OIW (West) and outf457-OIC (Center) (Figure 11).

There was also a significant difference in the average iron concentration measured in the belowground eelgrass compartment between samples from outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) (p=0.036, Figure 11). A Tukey's post hoc test showed iron concentration in the aboveground eelgrass biomass was significantly lower at outf457-OIC (Center) compared to outf458-OIE (East), and there was no significant difference between the iron concentration between samples collected at outf455-OIW (West) and outf457-OIC (Center) and outf455-OIW (West) and outf458-OIE (East) (Figure 13).

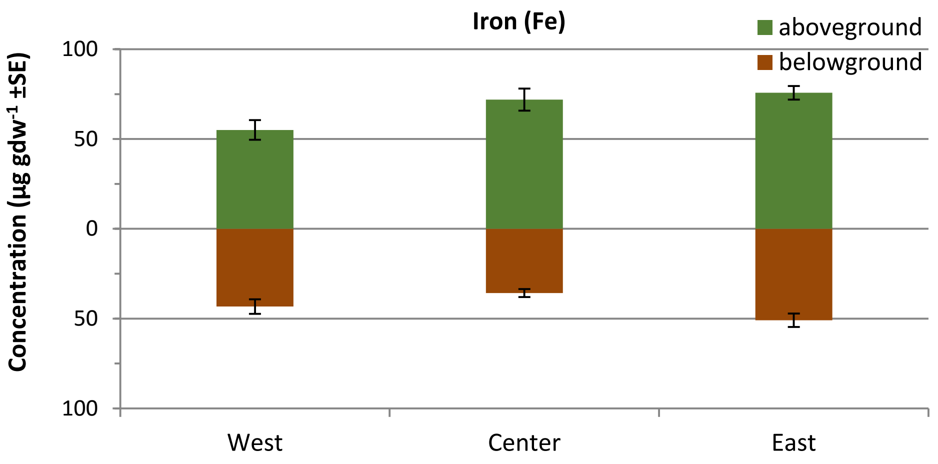

Figure 13. The average concentration (µg gdw^{-1} ±SE) of iron (Fe) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Lead (Pb)

There was no significant difference observed in the concentration of lead measured in the above- and belowground eelgrass compartments between outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (aboveground p=0.357, belowground p=0.966; Figure 14).

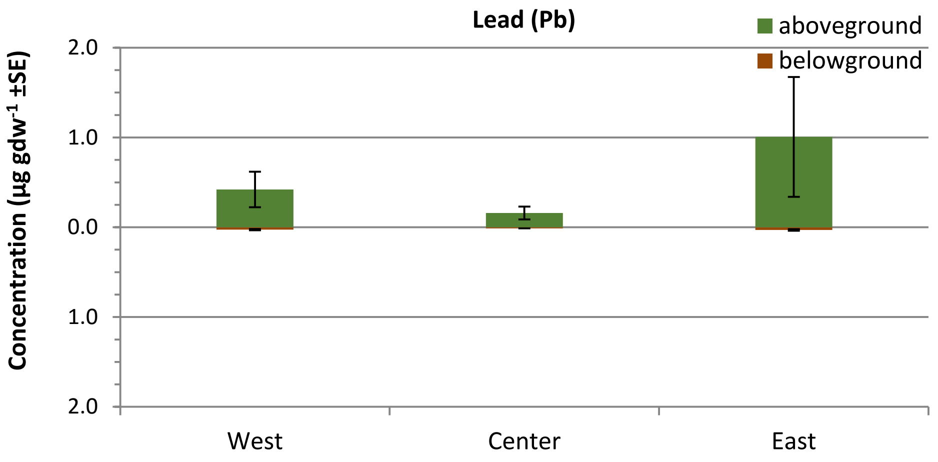

Figure 14. The average concentration (µg gdw^{-1} ±SE) of lead (Pb) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Nickel (Ni)

There was no significant difference observed in the concentration of nickel measured in the above- and belowground eelgrass compartments between outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (aboveground p=0.412, belowground p=0.108; Figure 15).

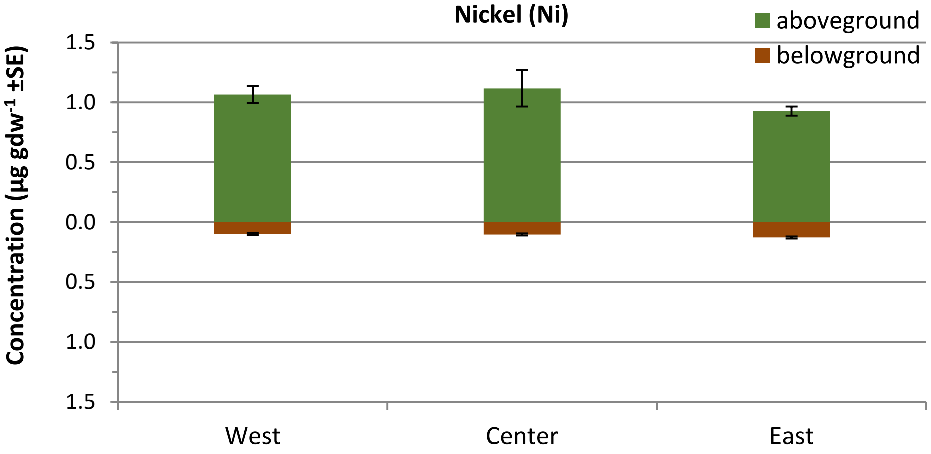

Figure 15. The average concentration (µg gdw^{-1} ±SE) of nickel (Ni) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Vanadium (V)

There was no significant difference observed in the concentration of vanadium measured in the aboveground eelgrass compartments between outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (p=0.562; Figure 16). There was a significant difference in the vanadium concentration measured in the belowground eelgrass compartment between outf455-OIW, outf457-OIC, and outf458-OIE (p=0.039, Figure 16). The concentration of vanadium measured in the belowground eelgrass biomass was significantly higher at Orcas Island East compared to Orcas Island Center. However, the vanadium concentration in the belowground eelgrass biomass was not different between samples collected at outf455-OIW (West) and outf457-OIC (Center), and outf455-OIW (West), outf458-OIE (East) (Figure 16).

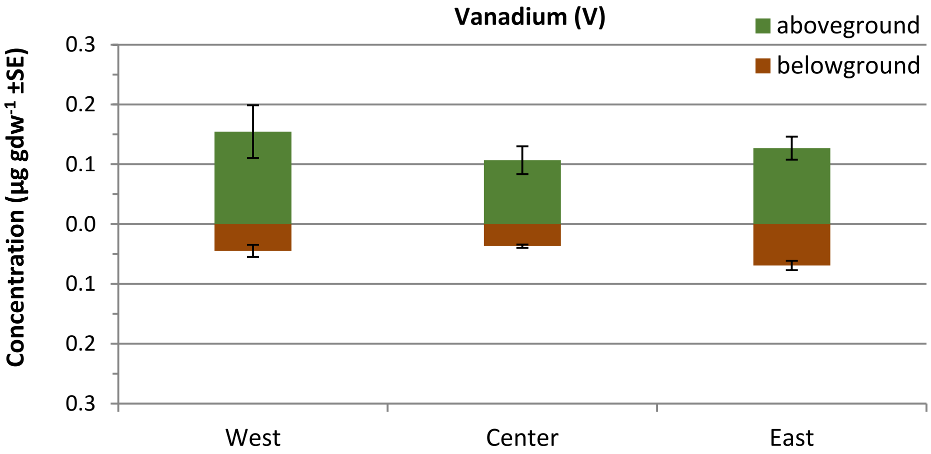

Figure 16. The average concentration (µg gdw^{-1} ±SE) of vanadium (V) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

Zinc (Zn)

There was a significant difference in the average zinc concentration measured in the aboveground eelgrass compartment between samples from outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (p=0.002; Figure 17). A Tukey's post hoc test showed zinc concentration in the aboveground eelgrass biomass was significantly lower at outf455-OIW (West) compared to outf458-OIE (East), and there was no significant difference between the zinc concentration between samples collected at outf458-OIE (East) and outf457-OIC (Center) (Figure 17).

There was also a significant difference in the average iron concentration measured in the belowground eelgrass compartment between samples from outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (p=0.039, Figure 17). A Tukey's post hoc test showed iron concentration in the belowground eelgrass biomass was significantly lower at outf457-OIC (Center) compared to outf458-OIE (East), but there was no significant difference between the iron concentration between samples collected at outf455-OIW (West) and outf457-OIC (Center), and outf455-OIW (West) and outf458-OIE (East) (Figure 17).

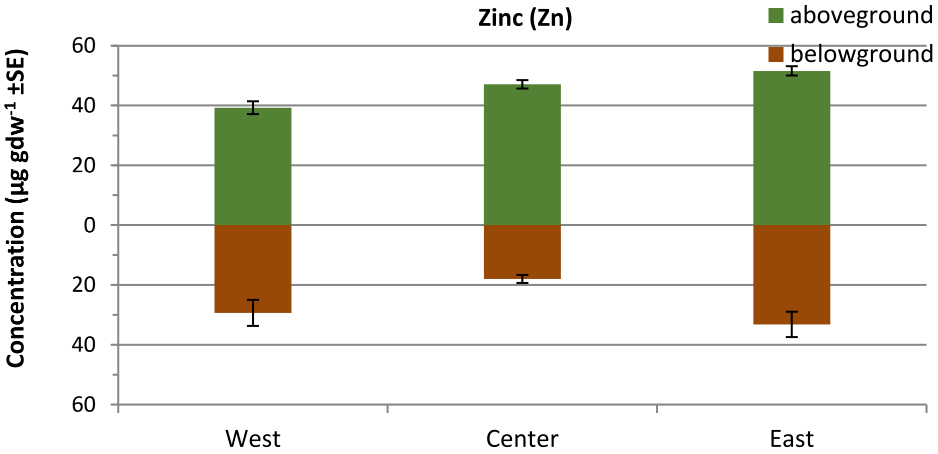

Figure 17. The average concentration (µg gdw^{-1} ±SE) of zinc (Zn) in the above- and belowground eelgrass compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

3.4 Organics

3.4.1 Polycyclic Aromatic Hydrocarbons

Low molecular weight PAHs

The above- and belowground low molecular weight (LMW) polycyclic aromatic hydrocarbons (PAHs) consisted of the sum of 22 analytes (Appendix A, Table A-2). There was a significant difference in the average LMW PAH concentration for the aboveground biomass between samples from outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (p=0.019; Figure 18). A Tukey's post hoc test showed LMW PAH concentration in the aboveground eelgrass biomass was significantly lower at outf458-OIE (East) compared to outf457-OIC (Center) (p=0.017), and there was no significant difference between the LMW PAH concentration between samples collected at outf455-OIW (West) and outf457-OIC (Center) and between outf455-OIW (West) and outf458-OIE (East) (Figure 18).

There was no significant difference observed in the concentration of LMW PAHs measured in the belowground eelgrass compartments between outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) (p=0.698; Figure 18).

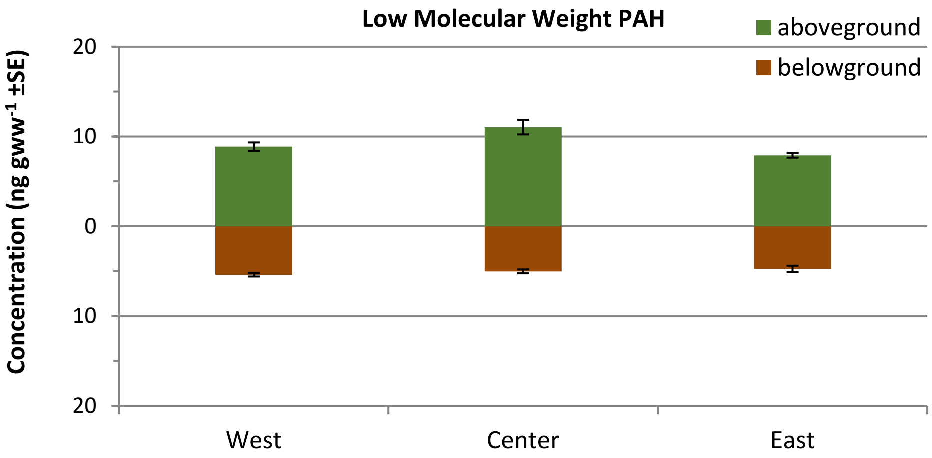

Figure 18. Average concentration (ng gww^{-1} ±SE) of low molecular weight PAHs in the above- and belowground compartments from samples collected at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013.

High molecular weight PAHs

The above- and belowground high molecular weight (HMW) polycyclic aromatic hydrocarbons (PAHs) consisted of the sum of 20 analytes (Appendix A, Table A-2). The concentration of HMW PAHs in the above- and belowground eelgrass biomass collected from outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) were considered no detects or below the limit of quantification (LOQ). Therefore, there was inadequate HMW PAH data to analyze.

3.4.2 Persistent Organic Pollutants

The concentration of persistent organic pollutants (POPs; polychlorinated byphenyls, PCBs; polybrominated diphenyl ethers, PBDEs; and dichlorodiphenyltrichloroethane, DDTs) in the above- and belowground eelgrass biomass collected from outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East) were considered non-detects or below the limit of quantification (LOQ) for most of the analyzed eelgrass samples. The study relied on a minimum of three eelgrass biomass samples for a baseline assessment of POPs at each of the three sites. However, the analysis only produced a maximum number of two samples for any POP-Site-plant compartment combination. The majority of the samples were considered non-detects or below the limit of quantification (Table 7).

Table 7. Number of eelgrass biomass samples analyzed for Persistent Organic Pollutants (POPs) in the above- (AG) and belowground (BG) eelgrass biomass at outf455-OIW (West), outf457-OIC (Center) and outf458-OIE (East) in September 2013. An analysis of POPs was not possible as only a few samples provided valid data (Valid Sample) and most were considered below the Limit of Quantification (LOQ Samples). The POPs analyzed included: polychlorinated byphenyls, PCBs; polybrominated diphenyl ethers, PBDEs; and dichlorodiphenyltrichloroethane, DDTs.

Persistent Organic Pollutant		**Valid Samples**	**Valid Samples**	**LOQ Samples**	**LOQ Samples**	**TOTAL**
		AG	BG	AG	BG	
PCBs	outf455-OIW	2	1	1	2	6
	outf457-OIC	1	1	2	2	6
	outf458-OIE	2	2	1	1	6
PBDEs	outf455-OIW	0	0	3	3	6
	outf457-OIC	0	0	3	3	6
	outf458-OIE	0	0	3	3	6
DDTs	outf455-OIW	1	0	2	3	6
	outf457-OIC	0	0	3	3	6
	outf458-OIE	0	0	3	3	6

3.5 Environmental Data

3.5.1 Temperature

Three temperature sensors were deployed in water at -2.5 m (MLLW) on the eastern, mid, and western edge of outf457-OIC (Center) site. The average temperature measured over the approximate 30 day period at the three locations (west, mid, and east) within outf457-OIC was 10.5˚C, with a minimum temperature of 10.2˚C and a maximum of 11.5˚C (Figure 19, Table 8). In contrast, the average temperature on land was 9.8˚C with a minimum temperature of 7.3˚C and a maximum of 13.2˚C (Figure 19, Table 8).

Table 8. Minimum, maximum and average temperature measured at the eastern, mid, and western edge of outf457-OIC (Center) and on land between September 27, 2013 and October 30, 2013. Temperature was recorded every 30 minutes with a Hobo Tidbit temperature logger.

TEMPERATURE	**WEST**	**MID**	**EAST**	**LAND**
Sep 27 – Oct 30, 2013	**(water, ˚C)**	**(water, ˚C)**	**(water, ˚C)**	**(air, ˚C)**
Minimum	10.1	10.2	10.2	7.3
Maximum	11.5	11.5	11.5	13.2
Average	10.5	10.5	10.6	9.8

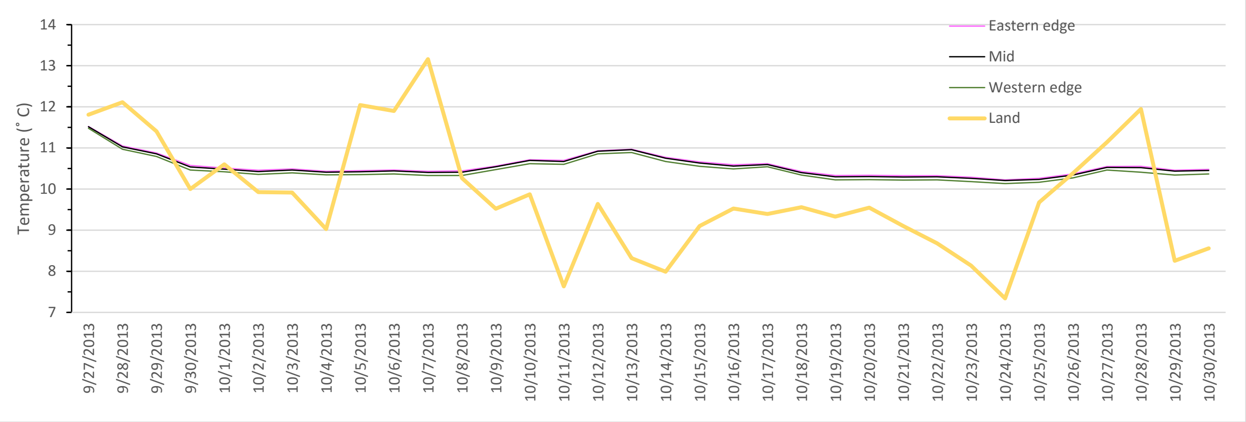

Figure 19. Daily average temperature measured at the eastern, mid, and western edge of outf457-OIC (Center) and on land between September 27, 2013 and October 30, 2013. Temperature was recorded every 30 minutes with a Hobo Tidbit temperature logger.

3.5.2 Light

Three light sensor arrays were deployed in water (-2.5 m, MLLW) at the eastern, mid and western edge of outf457-OIC (Center) from September 27, 2013 through October 30, 2013. Each array included a light sensor at the bottom and a second sensor 1 m higher to determine light available to eelgrass at the site and light attenuation due to the water column (Figure 20a). The land sensor was attached in an unobstructed location on the Orcas Island Airport security fence (Figure 20b).

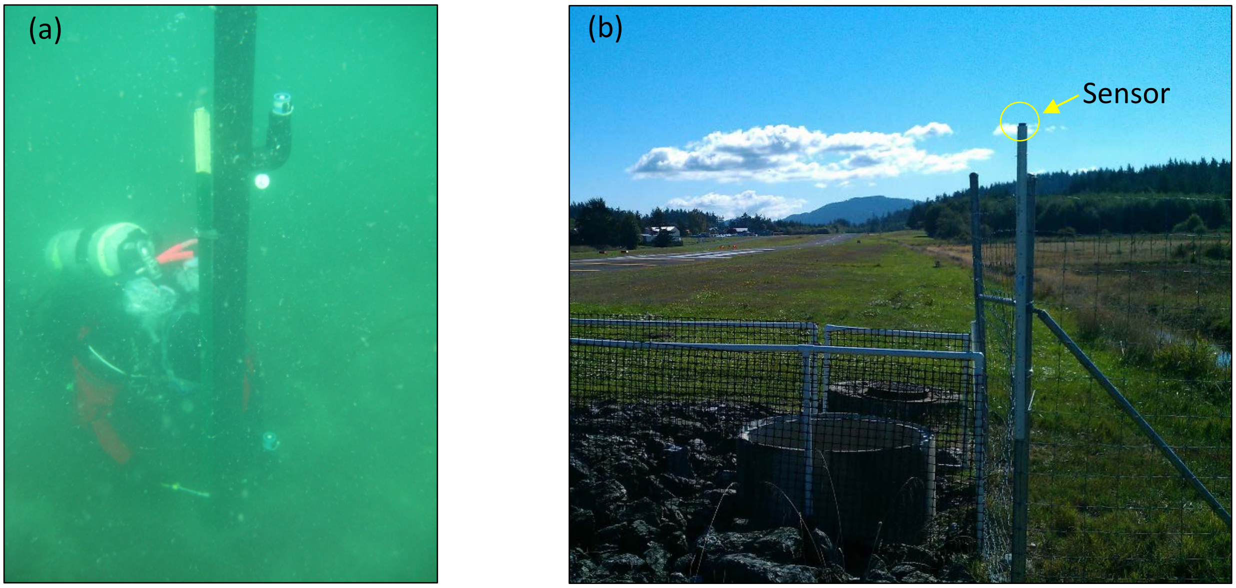

Figure 20. (a) In water light sensor array with one sensor at the bottom and the other sensor 1 m higher. Each light sensor array was install at -2.5 m (MLLW) at the eastern, mid and western edge of outf457-OIC (Center). Note: the white object just below the upper sensor is a temperature sensor. (b) On land light sensor attached to the Orcas Island Airport security fence. All sensors were deployed from 0100 PST, September 27, 2013, through 2330 PST, October 30, 2013.

Table 9. Location (latitude – longitude) of the light and temperature sensor arrays deployed at -2.5 m (MLLW) at the eastern, mid and western edge of outf457-OIC (Center) and at an unobstructed location on land at the Orcas Island Airport.

SITE	LOCATION	LATITUDE	LONGITUDE
Outf457-OIC (Center)	West	48˚42.870	-122 ˚54.716
Outf457-OIC (Center)	Mid	48˚42.900	-122 ˚54.605
Outf457-OIC (Center)	East	48˚42.887	-122 ˚54.644
LAND	Orcas Island Airport	48˚42.788	-122 ˚54.654

The in-water sensors were prone to natural fouling and were cleaned by SCUBA on October 15, 2013 (Figure 21). Preliminary review of the light data showed only six (6) days of data were useful for analysis following initial sensor deployment and six (6) additional days after the sensors were cleaned for a total of 12 days of valid data.

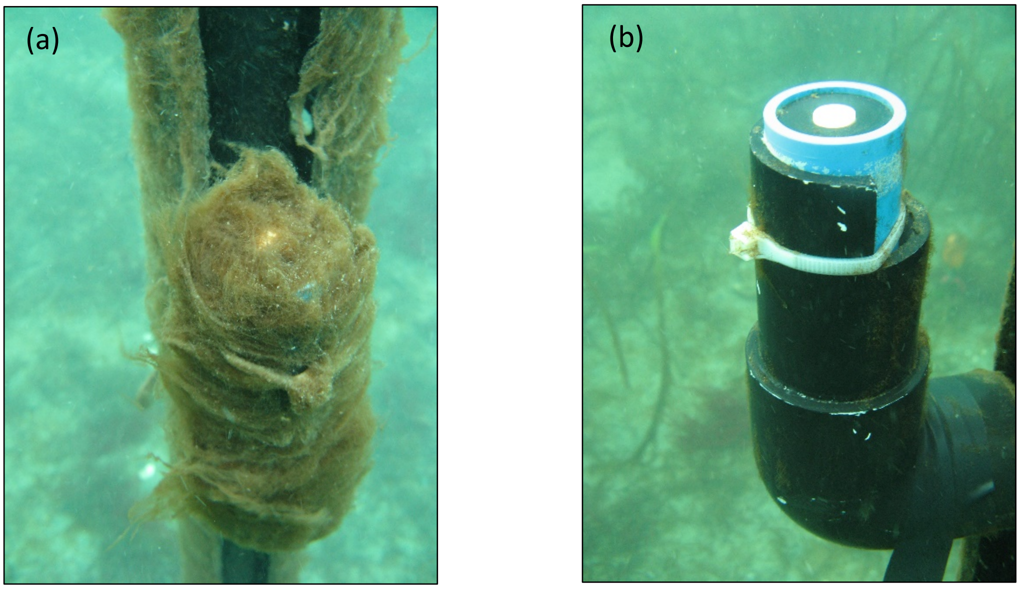

Figure 21. Photos of the bottom light sensor at the western edge of outf457-OIC (Center) on October 15, 2013, prior to cleaning (a) and after cleaning (b).

The daily average available light eelgrass received relative to ambient land conditions at the western, mid and eastern edge of outf457-OIC (Center) over the course of the 12 valid days varied between 7.6% measured at the bottom sensor located on the eastern edge of outf457-OIC (Center) up to 22.5% measured at the top sensor on the western edge of outf457-OIC (Center) (Figure 22).

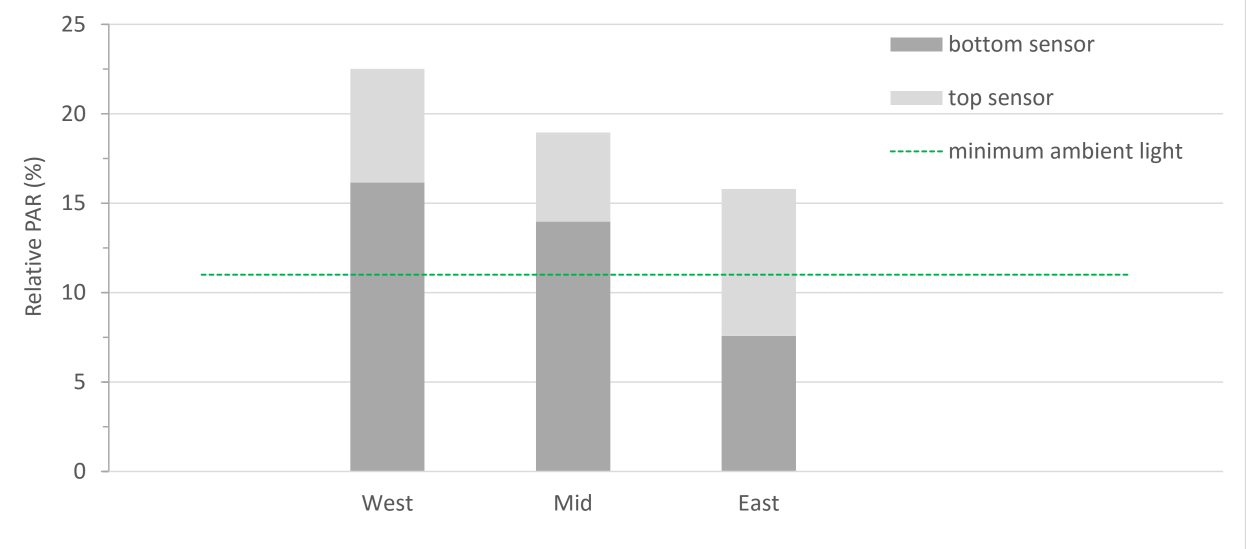

Figure 22. Photosynthetically available radiation (PAR) measured at the bottom and top sensors deployed in -2.5 m (MLLW) at the eastern, mid and western edge of outf457-OIC (Center) relative to PAR measured at the sensor on land (Orcas Island Airport) over 12 days from September 27, 2013 through October 21, 2013. Seagrass requires a minimum of 11% ambient light to survive (Duarte 1991) and as much as 30% (Dennison et al. 1993).

It is apparent that water column properties at the eastern edge of outf457-OIC (Center) inhibit more light than the mid and western edge of the site (Figure 22, Figure 23).

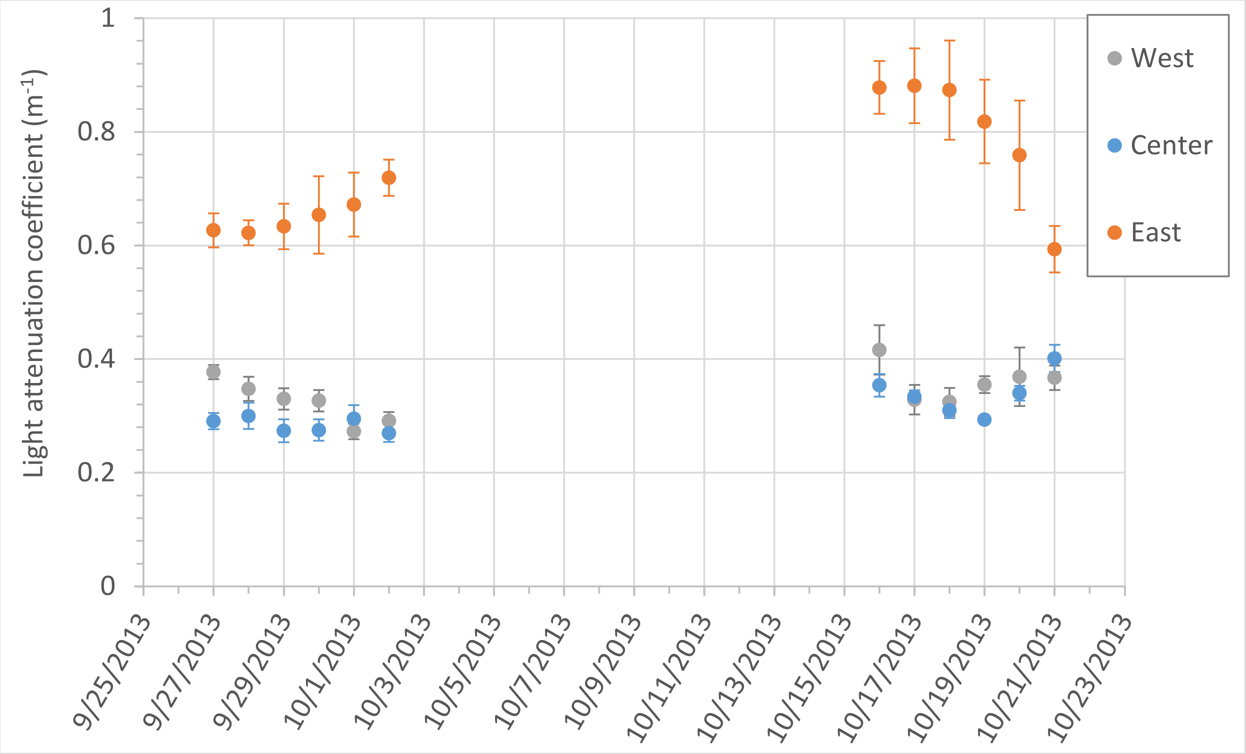

Figure 23. Light attenuation coefficient (K_d, m^{-1}) for the western, mid and eastern edge of outf457-OIC (Center). Light sensors were deployed in -2.5 m (MLLW) at each location from September 27, 2013, through October 30, 2013.

4 DISCUSSION

4.1 Eelgrass Area and Depth Distribution

The eelgrass area and depth distribution varied between the three sites along the north shore of Orcas Island. Although there were observed significant changes in eelgrass area at each site over the three sample years, the change in eelgrass area at the center site, outf457-OIC – the location with the replaced outfall – coincided with the only significant change in the deep edge of the bed (Table 4). The measured changes in the deep edge of the bed at outf457-OIC could be related to a reduction in water clarity due to the outfall effluent. Eelgrass at outf458-OIE did not grow as deep as compared to the other two sites. This could be due to differences in water clarity between the sites, as outf458-OIE had the lowest amount of surface light remaining at the bottom during the short light sensor deployment at these sites. Continued monitoring of eelgrass area and depth distribution at these sites will indicate whether these changes were year-to-year natural variability or a response to the persistent stressor on seagrasses caused by wastewater effluent.

4.2 Carbon and Nitrogen

The nutrient content, %C, %N, $\delta^{13}C$ and $\delta^{15}N$, in the above- and belowground eelgrass compartments varied between the three sites on the north shore of Orcas Island. There was no obvious pattern indicating a gradient towards higher or lower areas of nutrient enrichment or loading between the three sites. The average %C values measured at outf455-OIW, outf457-OIC and outf458-OIE in September 2013 was 34.9 and similar to values measured in eelgrass throughout Puget Sound in January 2013 (Gaeckle 2016). The %N measured in eelgrass in August 2013 was lower (2.1%) compared to nitrogen concentrations (3.4%) measured throughout Puget Sound in January (Gaeckle 2016). The percent carbon and nitrogen measured in eelgrass in January and September were within the range of results from global research (28 – 43% carbon and 1.2 – 5.6% nitrogen) (Duarte 1990). The slightly lower values of nitrogen in eelgrass during the September 2013 sampling could be a result of seasonal nitrogen availability and eelgrass growth rates (Duarte 1990, Fourqurean et al. 1997). Nitrogen is typically limited in the nearshore during summer growing seasons and growth rates are often elevated increasing plant biomass while reducing total nutrient concentrations. The low C:N ratio in the aboveground biomass is indicative of high growth rates and nutrient availability. Interestingly, the C:N ratio in the aboveground biomass (11.2 ± 0.44) was nearly half the C:N ratio in the belowground biomass (20.8 ± 0.5). Although eelgrass is producing individual rhizome segments at a rate equal to the production of a new leaf, the concentration of nitrogen in the leaves (2.7 ± 0.2) seems to be nearly twice that measured in the rhizomes (1.4 ± 0.0).

4.3 Metals and Contaminants

The majority of the metal concentrations measured in eelgrass at the three Orcas Island sites in September 2013 were low compared to concentrations measured in eelgrass at 15 sites throughout Puget Sound in January 2013 (Gaeckle 2016). Only the concentration of lead (Pb) in the aboveground biomass was nearly 3 times higher at Orcas Island than the combined average measured throughout Puget Sound (Table 10). The concentration of seven metals (Hg, As, Cd, Cr, Cu, Fe, and V) in the aboveground biomass were over two times greater at the 15 sites sampled in January 2013, than observed at Orcas Island. Only two metals (Ni and Zn) had similar concentrations (Table 10).

In the belowground biomass, the concentration of only two metals (Hg and Cd) measured in eelgrass at Orcas Island was comparable to the concentration measured at the 15 sites throughout Puget Sound (Table 10) (Gaeckle 2016). The concentration of the other eight metals were 2 to nearly 12 times higher in the eelgrass samples from Puget Sound, compared to samples from Orcas Island (Table 10).

Table 10. Average concentration (µg gdw^{-1} ±SE) of 10 metals in the above- and belowground biomass of eelgrass measured at Orcas Island in September 2013 and at 15 sites throughout Puget Sound from January 2013 (Gaeckle 2016).

METAL	ABOVEGROUND		BELOWGROUND	
	Orcas Island	**Puget Sound**	**Orcas Island**	**Puget Sound**
	µg gdw^{-1}	µg gdw^{-1}	µg gdw^{-1}	µg gdw^{-1}
	mean (± SE)	mean (± SE)	mean (± SE)	mean (± SE)
Hg	0.004 (0.000)	0.009 (0.002)	0.003 (0.000)	0.004 (0.001)
As	0.262 (0.009)	0.892 (0.230)	0.218 (0.007)	0.485 (0.125)
Cd	1.890 (0.101)	4.429 (1.144)	1.264 (0.060)	1.451 (0.375)
Cr	0.060 (0.003)	0.124 (0.032)	0.032 (0.002)	0.140 (0.036)
Cu	13.506 (2.559)	29.818 (7.699)	2.414 (0.108)	5.274 (1.362)
Fe	71.411 (4.100)	183.838 (47.467)	41.967 (2.744)	162.945 (42.072)
Pb	0.651 (0.307)	0.228 (0.059)	0.028 (0.007)	0.082 (0.021)
Ni	1.068 (0.071)	1.524 (0.393)	0.108 (0.008)	0.400 (0.103)
Va	0.144 (0.020)	1.277 (0.330)	0.045 (0.005)	0.528 (0.136)
Zn	46.044 (2.329)	79.578 (20.547)	24.367 (2.329)	52.962 (13.675)

The higher average metal concentrations measured in January at 15 sites throughout the Sound could be due to the higher availability of these metals in the environment and the difference in eelgrass growth rate between January and September. It is likely that metal and other contaminant concentrations are more available during the winter, a period of increased discharge into Puget Sound. Eelgrass plant growth rates will also affect concentrations of metals and contaminants.

During winter months, times of slow growth and high contaminant availability, eelgrass will uptake metals and increase concentrations of these substances in their above and belowground biomass. In contrast, metal concentrations will decrease during the summer as eelgrass growth rates spike and dilute the concentration of metals that are already less abundant because of reduced inputs and availability in Puget Sound.

There were few observed differences in concentration of metals in eelgrass between the Orcas Island sites. Out of the ten metals, only concentrations of iron (Fe), vanadium (V) and zinc (Zn) had significant differences in above- or belowground concentrations between the three sites. However, it is not clear what is driving differences in iron, vanadium and zinc concentrations between the three sites. There is no clear source of iron, vanadium or zinc in the area and the metal concentration pattern between the sites does not align with the predominant longshore drift.

4.4 Organics

Organic contaminants measured at the three Orcas Island sites include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and persistent organic pollutants (POPs). The majority of these substances enter the nearshore environment through stormwater runoff with a small amount entering the system through atmospheric deposition. Seagrass is typically protected from these contaminants through an antibacterial biofilm on the leaves of the plant (Gunnarsson et al. 1999). Seagrass is also less prone to absorb and store organic contaminants because of their low lipid concentration. The percent lipids in Puget Sound eelgrass sampled in January 2013 ranged from 0.07 – 0.17% (Gaeckle 2016). Therefore, the storage capacity of organic contaminants in eelgrass is limited by naturally low lipid concentrations in the plants.

The concentration of organics in eelgrass at the three Orcas Island sites was low, and for PCBs, PBDEs and DDTs, below the limit of quantification. The only valid data to compare between the three sites on Orcas Island was the concentration of low molecular weight (LMW) PAHs in eelgrass. The difference between the concentration of LMW PAHs measured in January 2013 and September 2013 were negligible in the belowground biomass and only 1.4 times different in the aboveground biomass (Table 11).

Table 11. Average concentration (ng gdw^{-1} ±SE) of Low Molecular Weight (LMW) polycyclic aromatic hydrocarbons (PAHs) in the above- and belowground biomass of eelgrass measured at Orcas Island in September 2013 and at 15 sites throughout Puget Sound from January 2013 (Gaeckle 2016).

	ABOVEGROUND		BELOWGROUND	
	Orcas Island	Puget Sound	Orcas Island	Puget Sound
	ng gdw^{-1}	ng gdw^{-1}	ng gdw^{-1}	ng gdw^{-1}
	mean (± SE)	mean (± SE)	mean (± SE)	mean (± SE)
LMW PAH	9.3 (0.5)	13.1 (0.8)	5.1 (0.2)	5.7 (0.2)

Similar to the assessment of metals, the higher concentration of LMW PAHs in eelgrass measured during the winter was likely a response to higher availability of these organic contaminants in the system and slower eelgrass growth rates (Gaeckle 2016). Outfall effluent could be a source of relatively higher LMW PAHs in eelgrass at outf457-OIC site, but at the time eelgrass was sampled at this site, the Eastsound Sewage Outfall was still discharging 518 m (1,700 ft) offshore. The concentration of the other organic contaminants measured for this study were too low to quantify and resulted no additional analyses (Table 7).

4.5 Environmental Parameters

The temperature and light data provided a short assessment of environmental parameters at outf457-OIC, the location of the outfall modification. The observed water temperatures were typical of conditions in the San Juan Island, Puget Sound, and within the suitable range for eelgrass.

The light conditions were more informative on water quality parameters and possible factors that may cause eelgrass to grow to different depths. The relative light was lower on the eastern edge of outf457-OIC compared to the central and western edge of the site (Figure 22). A similar result was observed in the higher light attenuation at the eastern edge of outf457-OIC compared to the western and central locations within outf457-OIC. Although, only 100 m apart, if the pattern of reduced light continued east, then it would support the reason for the shallower deep edge depth of eelgrass at the eastern reference site, out458-OIE. The deep edge of the eelgrass at outf458-OIE was between 2.5 to 4.3 m shallower than the deep edge of eelgrass at outf457-OIC and outf455-OIW, respectively (Table 4).

Although temperature and light data was only collected at the west, center and east edge of outf457-OIC between September 27 and October 30, 2013, it provides a baseline for future comparison, particularly if eelgrass evaluations show declines in area and depth distribution. A long-term assessment of light could indicate changes in the light availability attributed to the new outfall location.

5 CONCLUSION

Eelgrass is an important habitat in Puget Sound and supports numerous ecosystem functions. It is considered a significant indicator of ecosystem health (Dennison et al. 1993, Krause-Jensen et al. 2005, Orth et al. 2006), and has been identified as an indicator to track the recovery of Puget Sound (PSP 2010). To further assess stressors that cause eelgrass decline in the Sound (Thom et al. 2011), it is critical to understand the effects of outfall construction and effluent on eelgrass. Outfalls that discharge residential, commercial, and industrial wastewater along with upland stormwater are abundant throughout developed coastal areas, particularly in Puget Sound (Gaeckle et al. 2015). Furthermore, outfall construction and discharge can affect marine organisms and processes, specifically eelgrass (Gaeckle 2012b). The potential impacts of outfalls to eelgrass range from physical effects on the environment where it grows to effects on the plants from increased turbidity, changes in flow patterns and an excess of chemicals that could affect seagrass physiology.

The current study assessed the concentration of carbon, nitrogen, 10 metals, and a suite of organic contaminants in the above- and belowground compartments of eelgrass at three sites along North Beach, Orcas Island. The importance of this location was that the center site, outf457-OIC, had an existing sewage outfall pipe relocated to a bare area between two seagrass beds. The preliminary eelgrass bed monitoring was completed one month before installation of the new, shallow outfall pipe installed between two seagrass beds on North Beach. Therefore, data from the initial monitoring could be used as a baseline for subsequent seagrass bed evaluations. Two additional years of eelgrass area and depth distribution data collection suggested some changes were in effect at the deep eelgrass edge of the center site; the outfall replacement site. The deep eelgrass edge at the center site moved shallower suggesting conditions (e.g., water clarity, light) became less suitable for eelgrass at the deeper depth. There was also evidence of a decrease in eelgrass area over the three sample years at this site.

As with the larger soundwide assessment of carbon, nitrogen, metals and organic contaminants in eelgrass (Gaeckle 2016), the concentrations measured at the three sites on Orcas Island were within the range observed in other seagrass, and eelgrass specific, studies worldwide. Organic contaminants concentrations were low, but the low levels observed were likely due to limited uptake and accumulation potential by seagrass because of generally low lipids in seagrass biomass. The C:N ratio in the aboveground biomass at Orcas Island was also low and suggests a naturally high nutrient environment, however, the pattern was not consistent in the belowground biomass. The carbon, nitrogen, metal and organic contaminant concentration data in eelgrass acquired for this study was collected prior to the installation of the new outfall. These data are baseline data to compare future assessments now that the new outfall is discharging effluent in shallow water between two eelgrass beds.

There is a paucity of studies, local and global, that have evaluated the effects of metal and organic contaminants on eelgrass physiology (Lewis and Devereux 2009), therefore it is uncertain whether the concentrations measured in eelgrass along North Beach will affect eelgrass in this area. Continued assessment of contaminants in eelgrass along with tracking changes in eelgrass area and depth distribution will provide an opportunity to assess the effects of outfalls on this critical habitat in Puget Sound.

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7 APPENDIX

Table A-1. Name and abbreviation of low (LMW) and high molecular weight (HMW) polycyclic aromatic hydrocarbon (PAH) analytes measured in eelgrass (*Z. marina*) compartments (leaves, rhizomes, roots) at three sites, outf455-OIW (West), outf457-OIC (Center), and outf458-OIE (East), on the north shore of Orcas Island, WA, USA.

LMW PAH NAME	ABBREVIATION	HMW PAH NAME	ABBREVIATION
naphthalene	NPH	fluoranthene	FLA
C1-naphthalenes	C1NPH	pyrene	PYR
C2-naphthalenes	C2NPH	C1-fluoranthenes/pyrenes	C1FLA
C3-naphthalenes	C3NPH	C2-fluoranthenes/pyrenes	C2FLA
C4-naphthalenes	C4NPH	C3-fluoranthenes/pyrenes	C3FLA
acenaphthylene	ACY	C4-fluoranthenes/pyrenes	C4FLA
acenaphthene	ACE	benz[a]anthracene	BAA
fluorene	FLU	chrysene	CHR
C1-fluorenes	C1FLU	C1-benzanthracenes/chrysenes	C1CHR
C2-fluorenes	C2FLU	C2-benzanthracenes/chrysenes	C2CHR
C3-fluorenes	C3FLU	C3-benzanthracenes/chrysenes	C3CHR
dibenzothiophene	DBT	C4-benzanthracenes/chrysenes	C4CHR
C1-dibenzothiophenes	C1DBT	benzo[b]fluoranthene	BFF
C2-dibenzothiophenes	C2DBT	benzo[k]fluoranthene	BKF
C3-dibenzothiophenes	C3DBT	benzo[e]pyrene	BEP
C4-dibenzothiophenes	C4DBT	benzo[a]pyrene	BAP
phenanthrene	PHN	perylene	PER
anthracene	ANT	indeno[1,2,3-cd]pyrene	IDP
C1-phenanthrenes/anthracenes	C1PHN	dibenz[a,h]anthracene	DBA
C2-phenanthrenes/anthracenes	C2PHN	benzo[ghi]perylene	BZP
C3-phenanthrenes/anthracenes	C3PHN		
C4-phenanthrenes/anthracenes	C4PHN		