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A Non-Parametric Statistical Approach to Analyzing Eelgrass Density Data

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ABSTRACT

Golder Associates Inc. was contracted by Olympic Property Group to conduct a diver-based eelgrass survey for the evaluation of eelgrass (*Zostera spp*) density and presence along an existing Wastewater Treatment Plant (WWTP) outfall in Port Gamble. A second location was also surveyed as a control site. Both locations were in the same eelgrass bed, as defined by Virnstein et al (2000). At each location three depth bands were surveyed. Decommission of the outfall pipe is planned for 2017 which will require in-water work for partial or full removal. The control site will be used to determine changes in eelgrass presence and density and if these changes are related to the removal of the pipe or associated with natural changes over time in the larger eelgrass bed. Methods used in the survey were approved by Washington Department of Fish and Wildlife (WDFW) and based on guidelines published by WDFW in 2008. The survey was completed September 23 through 25, 2015. This study compared the use of a parametric t-test with non-parametric shuffling statistical test. Results of both tests compared depths bands at the study and reference sites similarly, but given the variability in bed density it was difficult to obtain power in the t-test and data were not normally distributed, which is an assumption of using a t-test.

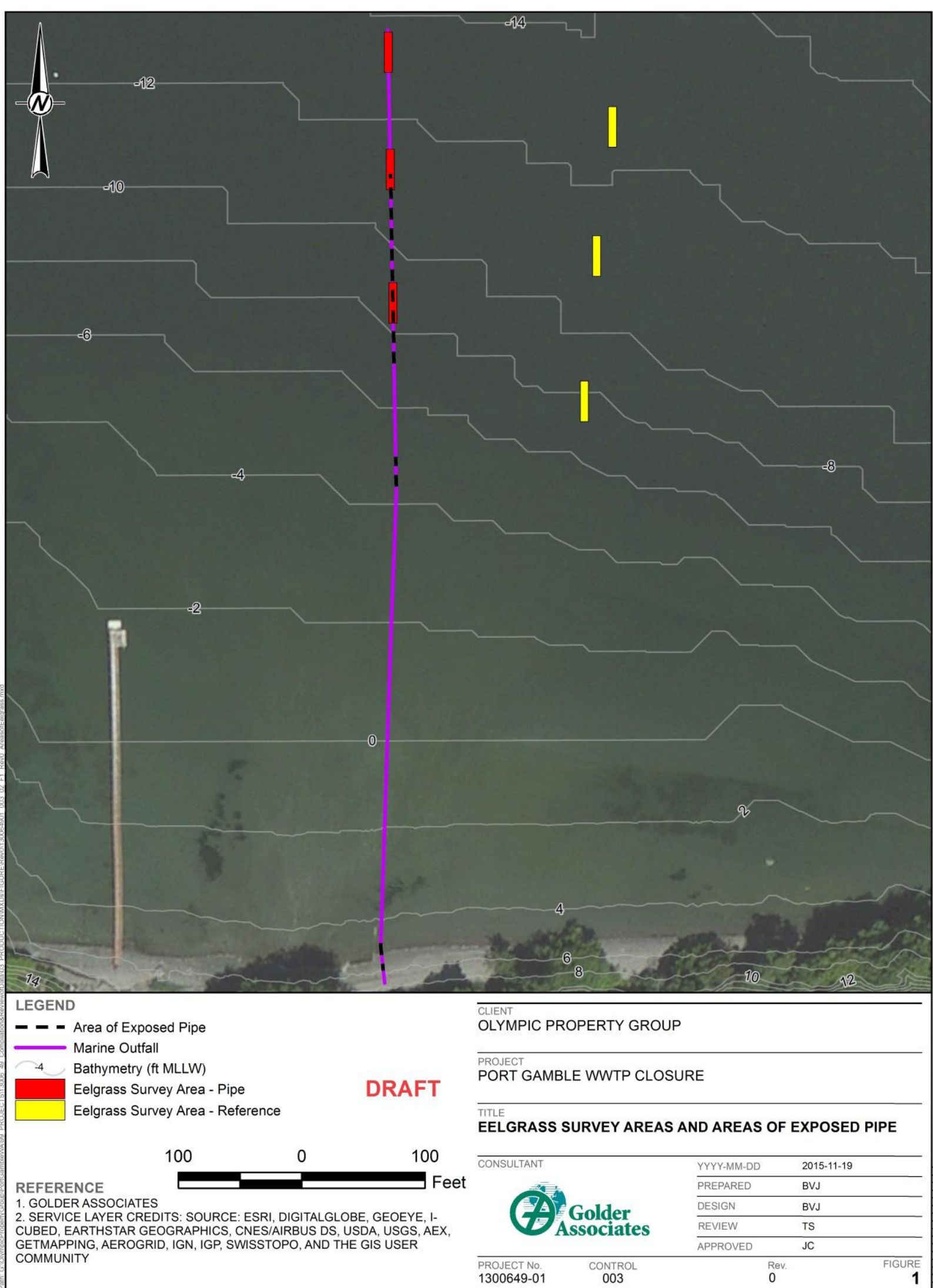


Figure 1. Map of Study Area.

RESULT FIGURES

Site	Mean (count)	Median (count)	Mode (count)	Standard Deviation	Standard Deviation Relative to Mean (%)	Variance	Standard Error
Pipe Shallow	8.3	2	0	9.2	111.3	85.3	1.7
Pipe Mid	11.1	11	13	4.9	43.8	23.5	0.8
Pipe Deep	11.7	11	11	3.4	29.3	11.8	0.6
Reference Shallow	16.7	17	15	4.6	27.4	20.8	0.7
Reference Mid	10.8	10	9	4.9	45.0	23.7	0.8
Reference Deep	6.9	6	6	3.7	53.8	13.9	0.6

Figure 2. Descriptive Statistics for Data at All Sites.

Site	Estimated Sample Size for 0.9 Power	Estimated Sample Size for 0.7 Power	Actual Samples Collected
Pipe Shallow	411	206	30
Pipe Shallow - West	4425	2214	16
Pipe Shallow - East	46	23	14
Pipe Mid	64	32	38
Pipe Deep	29	14	35
Reference Shallow	25	13	40
Reference Mid	67	30	39
Reference Deep	96	48	40
All Shallow	127	64	70
All Mid	66	33	77
All Deep	74	37	75

Figure 2. Results of WDFW Sample Size Calculator.



Photo 1. Photo of study site.

METHODOLOGY

Divers surveyed three sites at different water depths in the eelgrass bed along the pipe and in the same bed at the reference location. The reference location was assumed to be far enough away from the pipe to not be affected by pipe removal, but close enough to the pipe location that any other environmental factors affecting the bed as a whole will impact the density similarly in both locations. The surveyed sites are shown in Figure 1. Both the pipe and reference locations each have a shallow, mid, and deep site. Depths were estimated based on depth readings using a handheld tape measure dropped overboard from the diver support vessel at the time of the survey and corrected to Mean Lower Low Water (MLLW) based on predicted tides for Port Gamble. Estimated depths for shallow sites were -1 to -2 meter (m) relative to MLLW, mid sites -2 to -3 m MLLW, and deep sites -3 to -4 m MLLW.

Each site was 10 m long by 4 m wide and surveyed using a Generalized Random Tessellation Stratified (GRTS) method where a grid was imposed over the site and points were randomly selected to identify the position of the top right corner of a 0.25 m quadrat.

For the purposes of this survey the null hypothesis is that there is no difference in the density of eelgrass in the bed area surrounding the pipe as compared to the reference area when comparing the same depth band. In future surveys data would primarily be comparing data over time at the same location and depth band, i.e. pipe shallow changes over time. If the trend is towards increasing density at the reference location this may signify that there was negative impact on the eelgrass bed from pipe removal.

The WDFW Sample Size Calculator was used to estimate the amount of samples (N) required to obtain a statistical power of 0.9 and 0.7 (Figure 3). Power is the likelihood of correctly rejecting the null hypothesis. With lower statistical power it is more likely to obtain a Type 2 error, in which one may say there is no difference in the density of eelgrass when in reality there is a difference, which would mean accepting the null hypothesis when in reality it should be rejected. A Type 2 error would make it more likely to conclude that there is no effect from the project when in reality there is an effect. In practice collecting the required sampling size was found to be difficult. This was due to the variability in shoot density; higher variability requires larger sample sizes.

This high variability invalidates the use standard parametric statistics for analysis, and the power calculations used as part of the WDFW Calculator only apply when using parametric statistics. There are two basic assumptions when using parametric statistics, the first is that data are collected from random, independent samples. The second is that data are from a normal distribution. The first assumption is considered the most important because of the data is properly collected through a randomized study design, alternative appropriate models can be used to analyze the data and should be considered in future revisions of sampling methodology by WDFW.

Statistics were completed on the data using MatLab ®. Initially descriptive statistics were calculated on raw data and a Kolmogorov-Smirnov goodness-of-fit test was completed to see if distributions at each site were normally distributed. For this survey no data were normally distributed, a square-root transformation was tested on all data, but data were still not normally distributed thus non-parametric statistical methods were used for analysis. Although data not normally distributed should not be analyzed with parametric tests, a t-test p-value is also presented Figure 2-1. These p-values represent the likelihood that the null hypothesis is correct (<0.05) or to reject the hypothesis (=>0.05). It is likely that these results are valid as data was collected from a random independent distribution, which likely represents the mean value of shoot density accurately. The low standard error of the mean also suggests that the mean values are reasonable (Figure 2). Parametric statistics, such as the t-test are suggested for use in WDFW survey guidelines, but based on this analysis there may be alternatives that are more appropriate and easier to obtain an acceptable amount of data to use.

A resampling method was used to compare means between the pipe and reference locations at the shallow, mid, and deep sites. This method shuffled the count values at a given depth band and redistributed the values into two new groups; the difference between means of these resampled groups was then calculated 1000 times. A p-value was calculated by taking the number of times the absolute difference of the means in the resampled data was greater than the absolute difference of the means and dividing that by the number of times shuffled (1000). The p-values for this test indicate how likely it is by random chance that the difference of the means between the two sites would differ from what was observed during this survey. It does not represent the ability to reject or not reject the null hypothesis.



Photo 1. Photo of kelp crab utilizing eelgrass on at the study site.

RESULTS and CONCLUSIONS

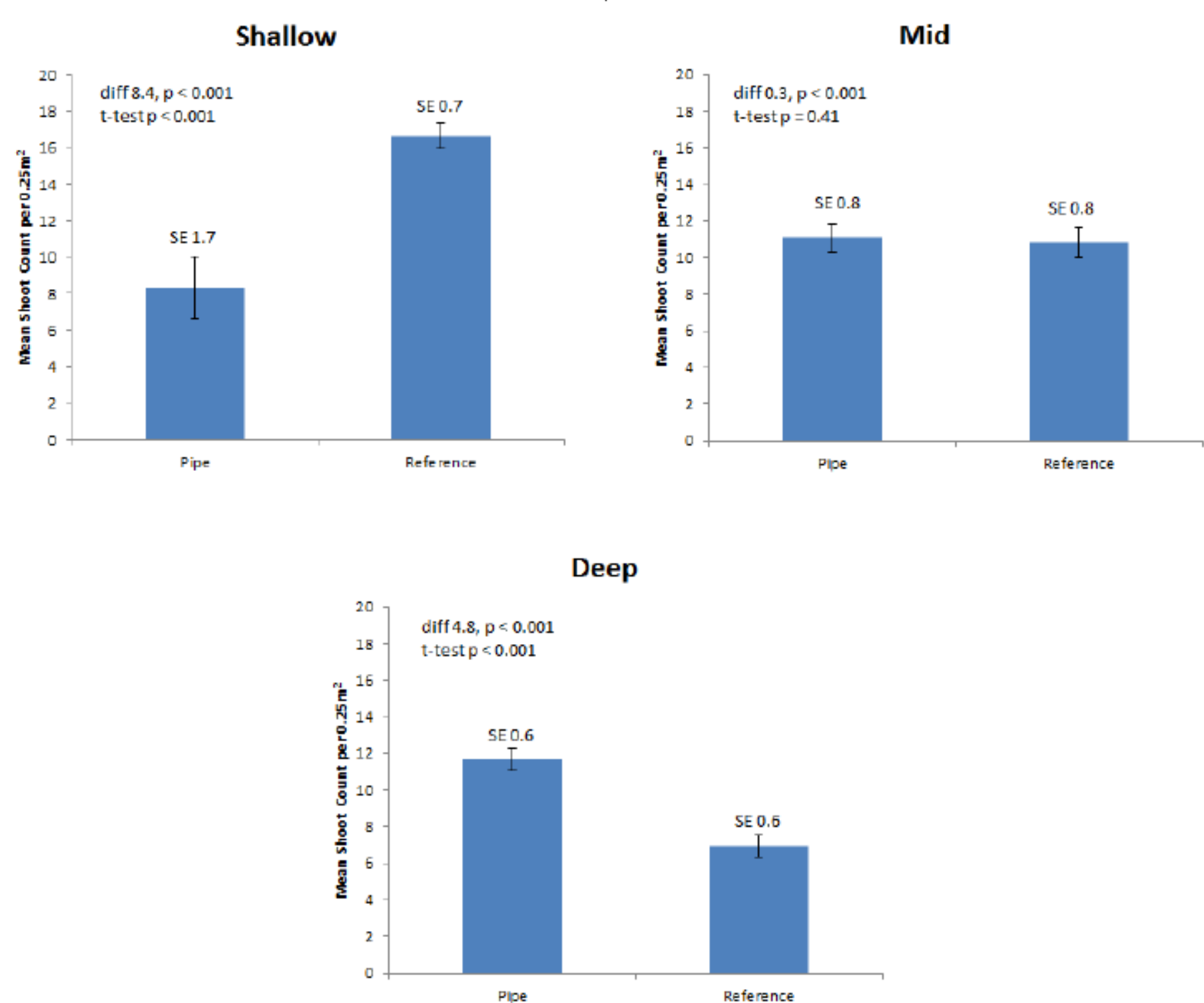


Figure 2. Bar graphs showing the mean shoot density per quadrat surveyed at each depth band, contrasting between the pipe and reference locations. The diff value is the difference between the mean shoot density values. The p-value was calculated using the methodology described, with the first p-value being from the resampling method and the second from the t-test. Error bars are presented as standard error values.

This initial survey did not always meet the target for a 0.9 statistical power. At many of the sites, collecting a sufficient sample size to meet the statistical goals would have been infeasible in a reasonable amount of time. The in-water time for this survey was 8 hours. WDFW also acknowledges that there is inherent variability in eelgrass beds and that power may be lowered from 0.9 if needed, but this reduces confidence in the results. If there is less confidence in the statistical results (low statistical power) WDFW indicates they may require more mitigation.

One possible alternative to standard parametric statistics with data that is highly variable would be to use non-parametric statistics. Non-parametric statistics do not require a normal distribution of input data. The resampling method used as an alternative in the study was simple to employ and results are easier to understand by non-scientists. In this study MatLab was used to perform analysis, but there is also versions of this in R, as well as an add-in for Excel.



Photos (Clockwise). 1. Photo of exposed pipe high in the intertidal zone. 2. Sunstar. 3. Epiphytes on eelgrass. 4. Diver at the study site.

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