Measuring the Effects of Eelgrass on pH and Dissolved Oxygen in the Santa Monica Bay

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Introduction

Ocean acidification is a destructive process resulting from increased carbon dioxide content in the Earth's atmosphere. Ocean acidification particularly harms marine organisms relying on calcification to build structures (Kroeker et al., 2013), forcing these organisms to redirect resources from growth and development towards acid-base regulation (Stumpp, Trubenbach, Brennecke, Hu, & Melzner, 2012). Acidification will only continue to increase in the near term as continuing carbon emissions lead to rising partial pressure of CO2 in Earth's atmosphere (Yakushev, 2010). Innovative solutions are needed to mitigate the effects of ocean acidification and protect marine organisms.

One such proposed solution is local biological mitigation of ocean acidification through the use of seagrass meadows. Seagrass meadows have the potential to sequester large amounts of carbon through photosynthesis, drawing comparisons to tropical rainforests in terms of their significant ability to store organic carbon (Fourqurean et al., 2012). In doing so, seagrass raises oceanic pH, with Hendriks et al. (2014) observing a local pH increase of up to .24 pH units during periods of peak photosynthetic activity.

Ample literature is available on the interplay between seagrass and ocean acidification in tropical meadows (Koch, Bowes, Ross & Zhang, 2012; Unsworth, Collier, Henderson & Mackenzie, 2012; Ow, Collier, & Uthicke, 2015). Our study is the first to examine the potential for seagrass meadows in the Santa Monica Bay to act as a localized mitigation strategy against ocean acidification. This is significant because study of seagrass' potential to mitigate ocean acidification is best done on a species or population-specific level. Seagrass species do not exhibit similar ecophysiological responses across the order Alismatales; rather, they are specifically adapted to a wide variety of different environments (Dattolo et al., 2014). Seagrass exhibits a high degree of phenotype plasticity, with different genes within a genotype expressed depending on local conditions (Maxwell et al., 2013), meaning that the response of one meadow to ocean acidification is not necessarily indicative of the response another will have (Garrard, 2014).

Seagrass abundance and productivity increase as carbon dioxide increases. Below-ground biomass increases fivefold, showing great potential for carbon storage and localized ocean acidification mitigation (Russel et al., 2013). Our client, the Santa Monica Bay Foundation, is interested in transplanting seagrass into locations devoid of any seagrass in hopes of locally mitigating ocean acidification and improving resilience to increasing CO2 levels. We hypothesize that as eelgrass (Zostera Pacifica) increases, the pH will increase. To test this hypothesis, we decided to take probe measurements of pH, DO, salinity, temperature, and depth. Analyzing our data for significant correlation between pH and eelgrass density will support whether transplantation can mitigate ocean acidification.

Other parameters we measured are dissolved inorganic carbon, alkalinity, environmental DNA, and nutrients such as nitrite, phosphate, silicate, nitrate, and ammonia. All of these measurements serve to characterize areas and assess the suitability of our control group for eelgrass transplantation. Salinity and temperature have significant influence on eelgrass growth, and have been shown to hinder growth at extreme levels (Nejrup & Pedersen, 2008). Since nutrient uptake by leaf tissues is equal to uptake by root tissues, water column nutrient data is significant for assessment of transplantation viability (Short, 1987). Assessing the health of a potential restoration site increases the success of the operation because it allows for preparatory

measures. Often, efforts are wasted due to inadequate remediation regarding the cause of initial disturbance (Ramesh et al., 2019).

Methods

We used three main pieces of equipment for our data collection: a SmarTROLL pH probe, a five liter Niskin bottle, and a protective cage for the probe that we designed and built ourselves. The SmarTROLL probe was able to continuously measure pH, depth, and dissolved oxygen as we lowered it to the ocean floor. It was calibrated before each boat trip using either a two or three point calibration. The Niskin bottle was used to collect water samples for nutrient concentrations, carbonate chemistry, and environmental DNA (eDNA). The cage for the probe, named *The Charlotte*, was built primarily to protect the probe because it was dropped into the ocean over 100 times over the course of four boat trips. It was built using PVC pipes, PVC cement, rope, zip ties, and adjustable clamps to hold the probe. Attached to the bottom of the cage by ropes is a ½ meter by ½ meter quadrat. A GoPro attached to the bottom of the cage filmed each drop in order to both verify the presence of seagrass as well as to record the density of the seagrass, which was quantified after each trip. A light was also attached to the cage so that the quadrat and seafloor were more clearly visible. Finally, we attached two 1.5 pound weights to *Charlotte* in order to minimize drifting due to the current.

In order to take pH, depth, and dissolved oxygen measurements, we would first go to our study site, Amarillo, and lower the probe and cage once in order to verify there was eelgrass in that area. We selected Amarillo as our study site because it was the site we were able to consistently lower the probe and the cage into seagrass. Collecting data at each drop point took three minutes. One minute was spent lowering the cage and probe to the seafloor, another minute was spent letting the probe collect samples within the eelgrass, and the final minute was spent pulling the rig back up to the boat. This process would be repeated four more times in the same general area because the boat drifted between drop sites. Lowering the probe five times in the same general area allowed us to collect data multiple times at similar depths and also made it more likely for us to lower the rig into eelgrass in that area because the eelgrass was generally patchy and inconsistent. We would then move to a different depth and repeat the entire process over again, starting with lowering the rig once to verify there was eelgrass in that location. We were able to have more successful drops when there were additionally divers that could guide us to areas where the eelgrass was more dense. During each trip, we could collect data using the probe at a maximum of forty drop points before the GoPro would either run out of battery or memory storage.

To obtain a visual density coverage of eelgrass, we estimated total percentage cover for each quadrat that was dropped at different points along our sampling grid. Based on previously published eelgrass sampling methods (Short et al. 2002), GoPro video recordings began for each drop as soon as the quadrat entered the water and were stopped when it resurfaced. We visually inspected screenshots from a segment of the GoPro video that contained the entire quadrat with the best eelgrass coverage for each drop. From this we assigned percentage coverage values. Categories were assigned from sparse to none (0-10%), low density (10-50%), and high density (50-75%) eelgrass quadrat coverage. Dense beds were categorized as >75%.

In addition to data measurements taken by the probe and GoPro, we also collected water samples to examine the carbonate system, environmental DNA, and nutrients at 5 different sites. These sites included Amarillo, Dockweiler, El Pescador, El Matador, and Lechuza, with 3 samples taken at each site. We used a 5-liter Niskin bottle to take a water sample just above the

seafloor and used the same sample for all three tests. Sterile gloves were worn for each test and were changed frequently to avoid contamination, particularly for the eDNA sampling

For the carbonate system sampling, we began by transferring the water from the Niskin into a glass 750mL bottle with four lines of grease applied along the opaque part of the rim. To achieve this, a rubber tube was placed around the opening of the Niskin so the water is forced to flow directly against the bottom of the bottle. We allowed the water to overflow from the bottle, continuously tapping on the side until all of the air bubbles were released. Then, we removed the Niskin tube from the bottle, leaving 1% air space, and added 200 μL of mercuric chloride to the water. Finally, we secured the bottle by putting in a glass stopper and keeping it in place with a plastic clamp and rubber band. Each sample was inverted at least 5 times before it was stored.

The eDNA test was the second sample that was taken. We started by rinsing a sampling pouch 3 times with water from the Niskin bottle and then filling it up to 1.3L. Then we changed to new gloves, hung the sampling pouch so the water could flow downward easily, and attached a sterile tubing set to the opening of the sampling pouch. We then released .3L of water through the sterile tubing set and attached a 0.2 μm sterivex filter to the end after the water had drained. The sample was then allowed to pass through the filter until 1L had been used or it had reached 40 minutes. Once either point was reached, we would use a 3mL syringe to expel the remaining water from the sterivex filter and then seal both ends of it with a blue luer lock cap. It was then labelled and stored on dry ice immediately.

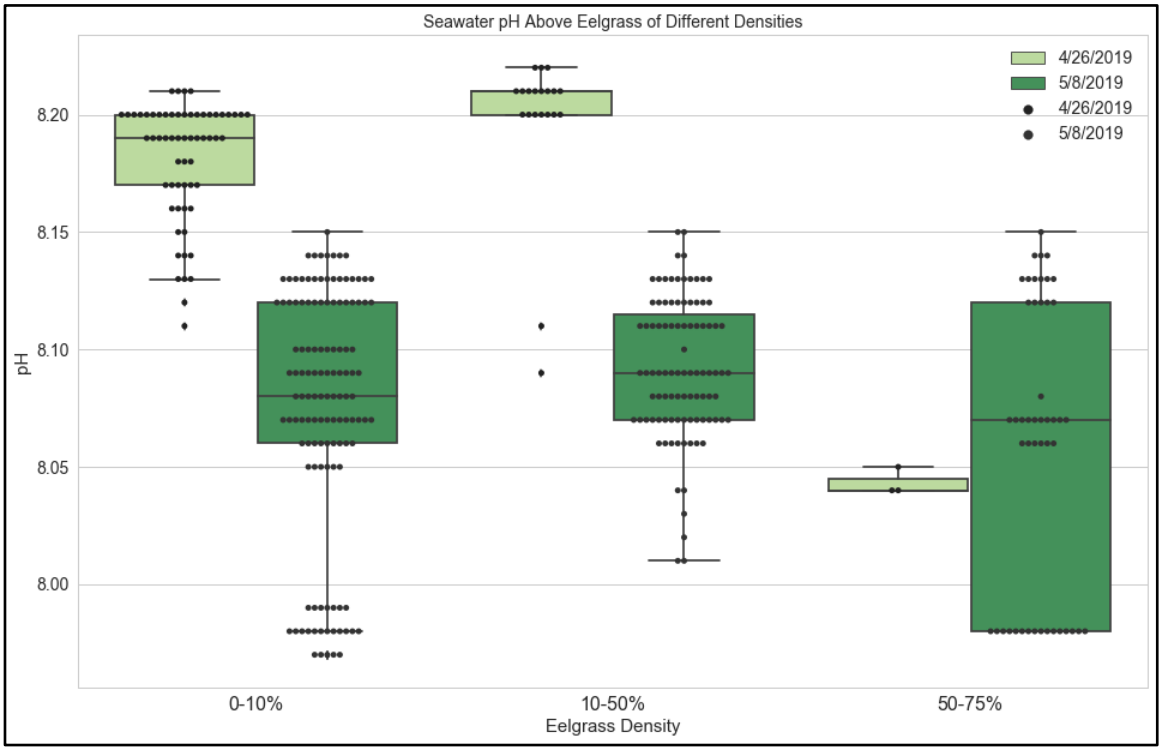
The final test was the nutrient sampling. We began by taking 2-50mL tubes and rinsing them 3 times in the Niskin water. The bottles were then filled up to the 45mL mark and placed on dry ice.

Results

pH Observations

The results of our project are presented in the following three figures from the Amarillo sampling trip on May 8, 2019, with further analysis in the discussion section below. Weather conditions on May 8th were cloudy and overcast and sampling period ranged from from 10 am to 3 pm.

Figure 1: Seawater pH Above Eelgrass Beds of Different Densities at Amarillo (5/8/19)



This figure shows boxplots of pH vs eelgrass density at Amarillo on two separate sampling dates, 4/26/19 in light green and 5/8/19 in dark green. Each black dot represents a drop of the quadrat that was then visually analyzed for eelgrass coverage, ranging from sparse to none (0-10%), low density (10-50%), and high density (50-75%). On the April 26th sampling trip, we were not able to locate sufficient patches of eelgrass and thus returned to Amarillo with help from The Bay Foundation divers to find higher density patches on May 5th. The similar distributions for each density classification from the May 5th trip suggests that increasing eelgrass density does not influence pH.

Table 1: Mean pH for Each Density Category of Eelgrass at Amarillo (5/8/19)

Eelgrass Density	Mean pH
0-10% Coverage	8.0770
10-50% Coverage	8.0885
50-75% Coverage	8.0530

Table 2: T-test Results for Mean pH Samples Above Different Eelgrass Densities at Amarillo (5/8/19)

05/08/2019 Amarillo Trip - Welch's T-test (Assumes unequal Population variances)	T-statistic	P-Value

(0-10% coverage) vs. (50-75% coverage)	-2.0645	0.0441
(0-10% coverage) vs. (10-50% coverage)	2.0737	0.0392
(10-50% coverage) vs. (50-75% coverage)	-3.0962	0.0033

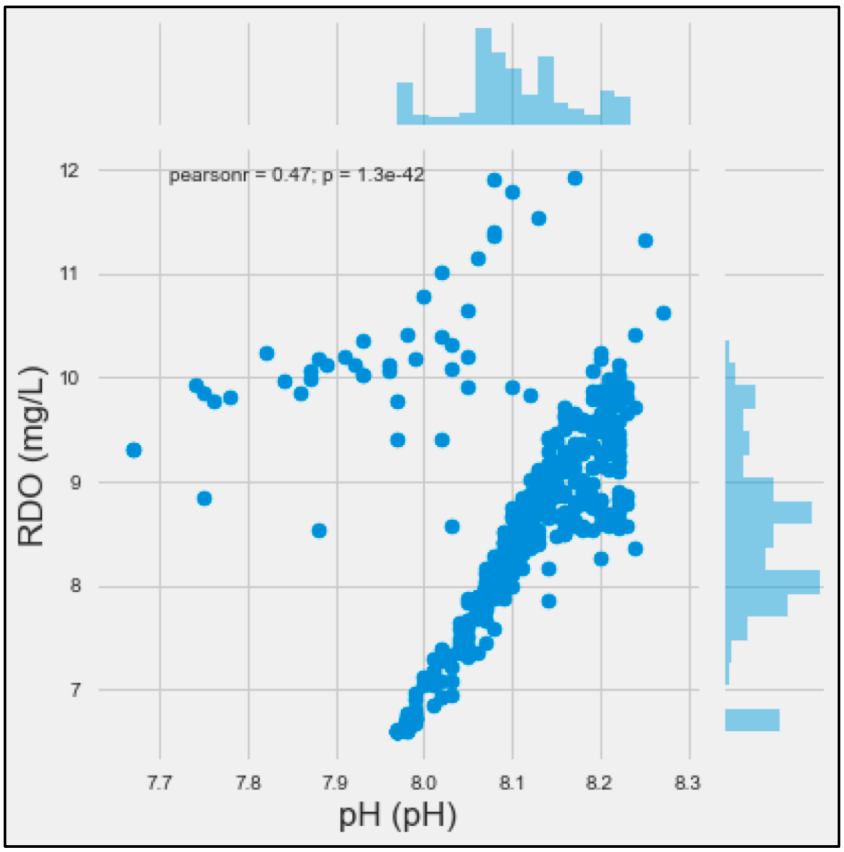
Table 3: Standard Deviation and number of pH Samples in Each Eelgrass Density Category at Amarillo (5/8/19)

Density category	Standard Deviation of pH	# of Data Points
Sparse Eelgrass (0-10% coverage)	0.0473	132
10-50% Coverage Eelgrass	0.0365	96
50-75% Coverage	0.0678	40

T-tests were performed to test the differences in means between each eelgrass density category, the results of which are displayed in Table 2. Mean pH's across all different densities differed by $\leq .05$ (Table 1). The pH of the sparse to none eelgrass category (0-10% coverage) was 0.024 pH units higher than the mid-density category (50-75% coverage) which translates to a -2.0645 t-statistic with a 0.0441 P-value. This is statistically significant at a confidence level of 95%. The result is inconsistent with our hypothesis that pH would be greater above eelgrass beds of higher density than those above lower density eelgrass. Indeed, the low density eelgrass category (10-50% coverage) had a higher mean pH than both the sparse to none and mid-density groups.

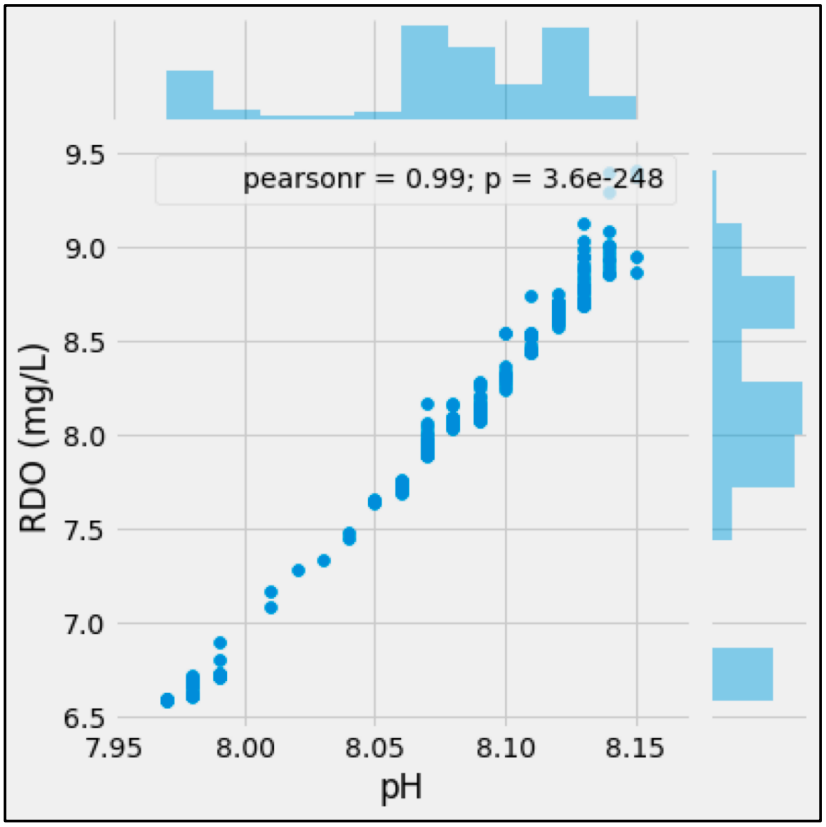
Dissolved Oxygen Observations

Figure 2: pH vs. DO in Full Water Column at Amarillo on 5/8/19



This figure shows the pH data points from eelgrass sites in Amarillo on 5/8/19. Each dot represents a pH value compared to the rugged dissolved oxygen concentration for the entire water column.

Figure 3: pH vs. DO at the Seafloor at Amarillo on 5/8/19



This figure shows the pH and RDO of samples from Amarillo on 5/8/19. Each dot represents a pH value and its rugged dissolved oxygen concentration.

Throughout the water column, we found that the correlation between pH and rugged dissolved oxygen (RDO) was moderate, with a pearson's R coefficient of 0.47. However, when calculating the correlation coefficient for samples within a foot of the seafloor, we resolved an r-value of 0.99. This is a distinct signature of biological influence on the seawater chemistry. We can't conclude that this relationship is due solely to photosynthetic activity of eelgrass since there was little correlation between eelgrass density and pH (or RDO). A few possible explanations for the differing pH vs. DO correlation coefficients throughout the water column and at the seafloor are the presence of photosynthetic microorganisms, other macrophytes on the seafloor,and thermochemical relationships. This includes air/sea exchange of carbon dioxide into DIC, optimal temperatures for growth/photosynthesis, and pressure gradients that affect the autotrophic rates of aquatic vegetation.. Notably, the pH vs. DO correlation at the seafloor at Amarillo on April 26th was 0.86 (Figure 12, Appendix).

Salinity and Temperature Observations

Figure 4: pH profile vs Salinity in Full Depth at Amarillo 5/8/19

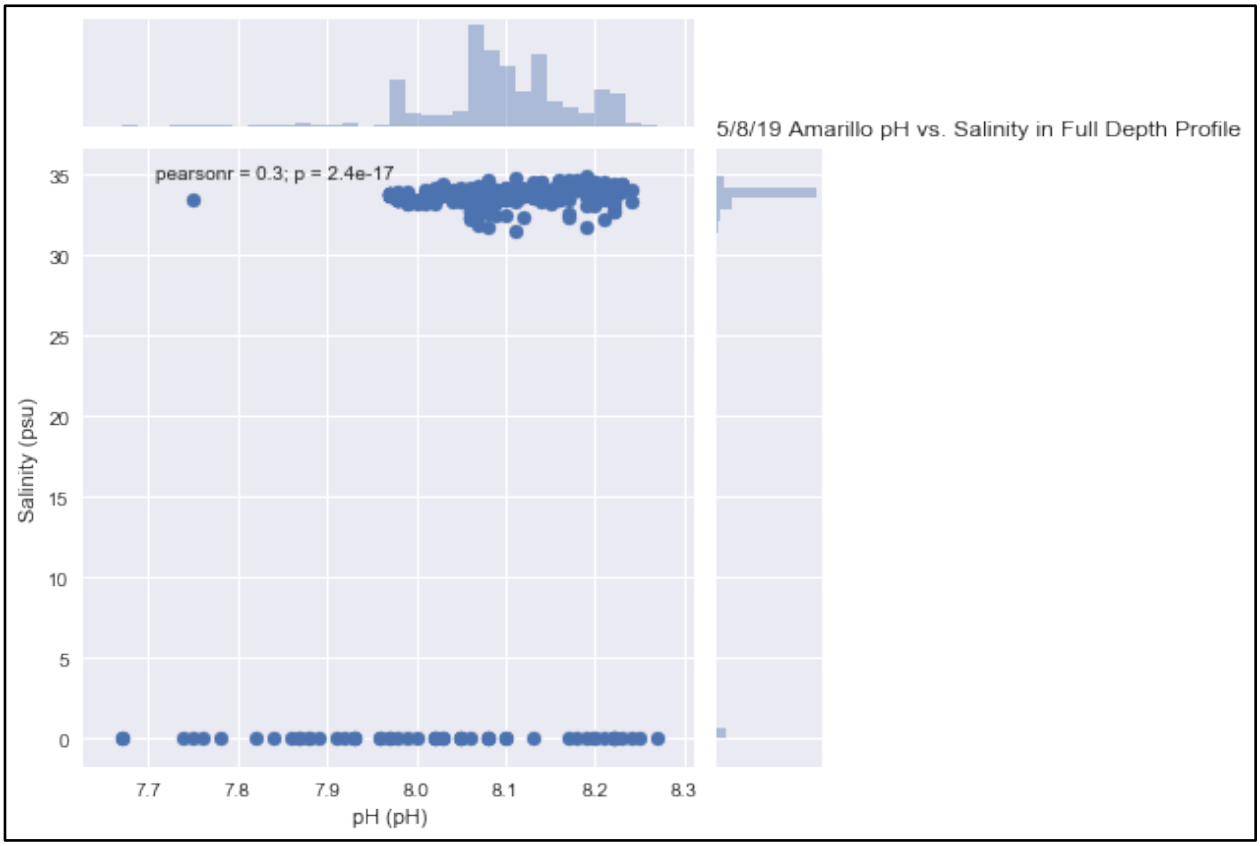


Figure 5 [illegible] t Amarillo on 5/8/19

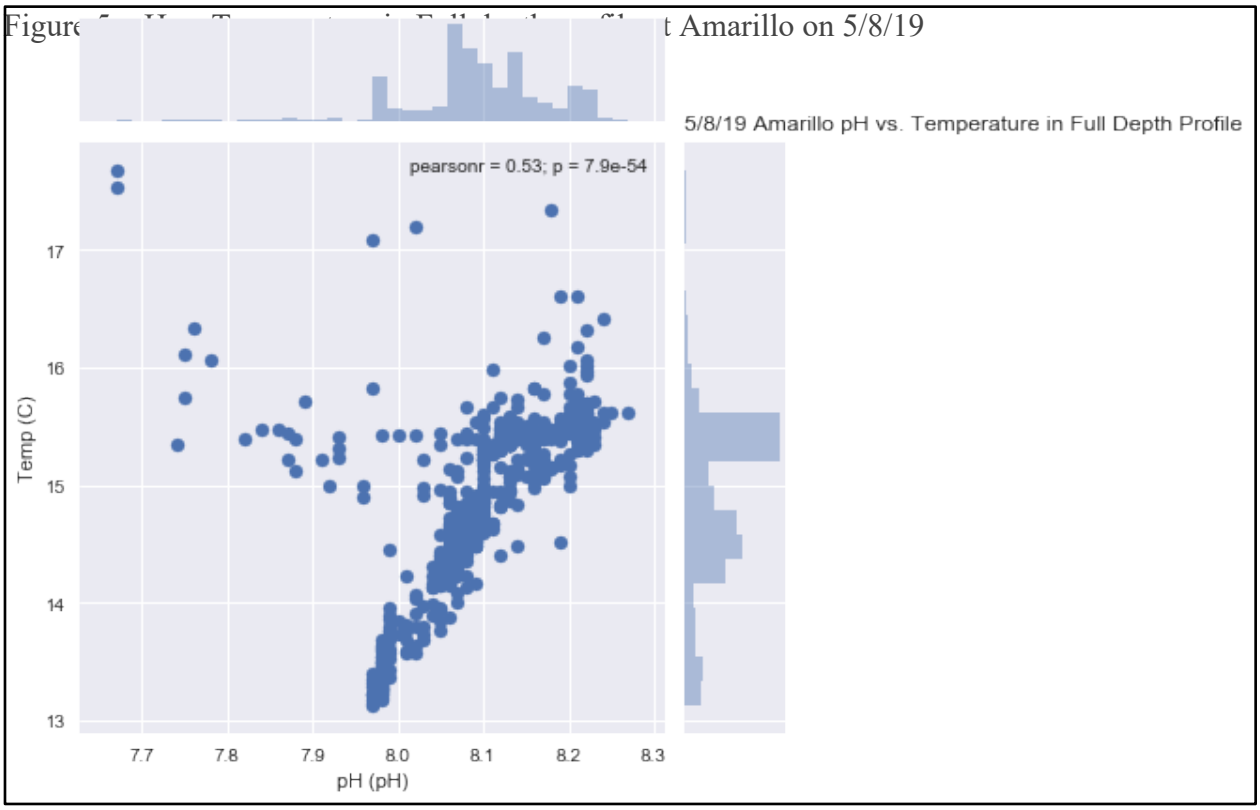
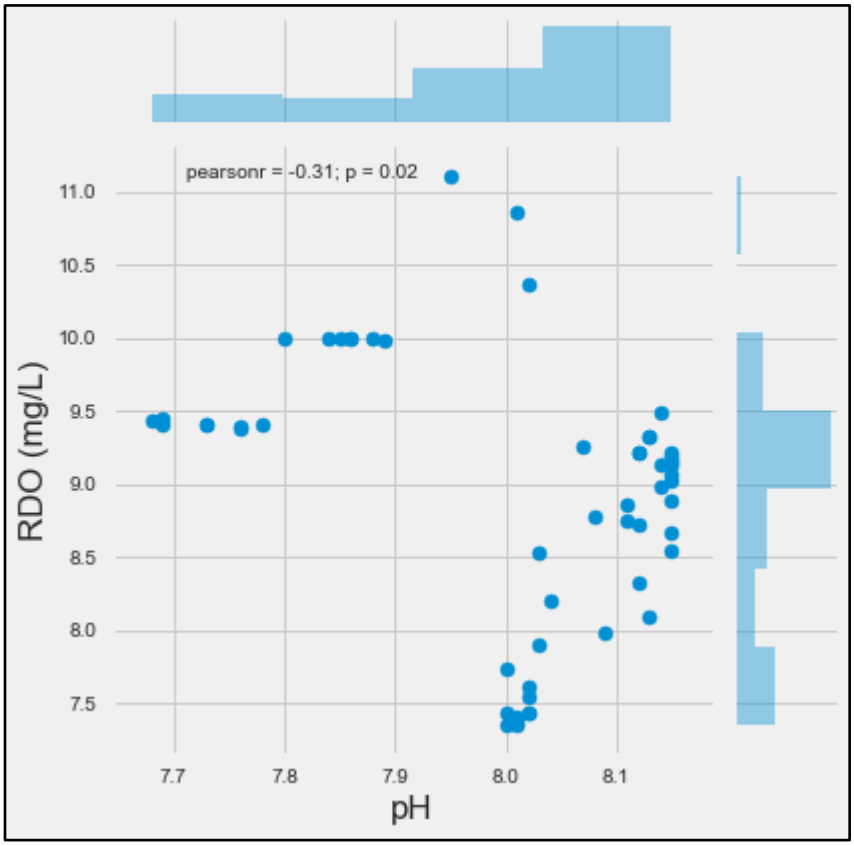
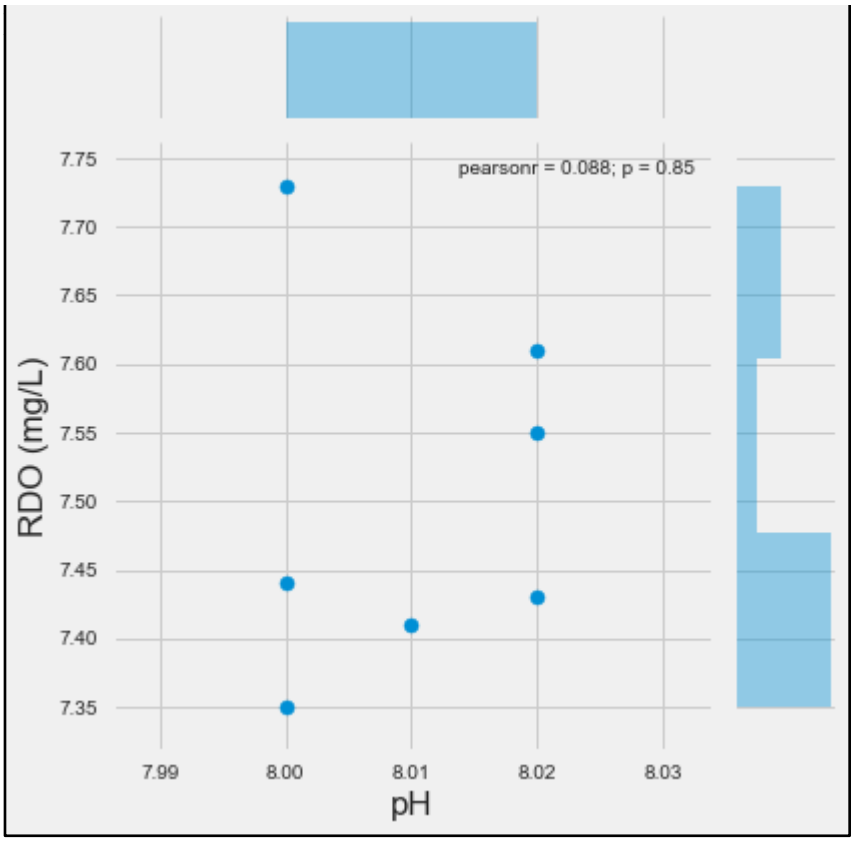


Figure 4 above shows a low correlation coefficient of r=.3 between salinity and pH in the entire water column, indicating that the two are not correlated. Dissolved oxygen and salinity showed a similar low level of correlation (Figure 7). Figure 5 shows a moderate correlation of r= .53 between pH and temperature throughout the water column. This correlation is likely due to temperature having some effect on seagrass metabolism which in turn can influence photosynthesis rates and pH. Figure 8 shows a strong positive correlation of r= .81 for DO and temperature. As temperature increases, solubility of oxygen decreases. This plot contradicts this trend. This could be attributed to uncertainties in water column temperatures due to local upwelling off the Santa Monica coast or seasonal variations. Outside temperature conditions were 19.4 degrees celsius on sampling date.
The temperature and salinity depth profiles for our sampling on April 26th and May 5th are displayed in Figures 9 and 10 in the appendix.

During our April 5th sampling trip, we collected 4 samples off the coast of Dockweiler beach, the transplant site proposed by The Bay Foundation. The following figures display data from Dockweiler.

Figure 6: pH vs. DO in Full Depth Profile at Dockweiler from 4/5/19





There is a moderate negative correlation between the pH and DO throughout the water column at Dockweiler with a pearson's R coefficient of -0.31. There is a lack of correlation at the seafloor with an R value of 0.088. This could be due to the lack of macrophytes on the seafloor at Dockweiler. It is unclear how this chemical ratio will affect the transplanting prospects of eelgrass at the site.

Discussion

Outcome

To evaluate the ability of eelgrass to buffer ocean acidification in its surrounding waters, we sampled chemical parameters in locations with different densities of eelgrass. Through this sampling approach, we aimed to compare the pH and DO values of seawater above different densities of eelgrass beds to evaluate whether denser patches of eelgrass contribute to larger increases in pH and DO. We also chose to sample in locations with a variety of depths to evaluate whether bathymetry had an influence on the ability of eelgrass to buffer ocean acidification. The collected data was inconsistent with our hypothesis that eelgrass increases the pH and DO of the water in its surroundings.

The April 26th and May 8th data were collected in the same geographic area during the daytime. In each sample, we analyzed the temperature and salinity profiles to evaluate prevailing

oceanic conditions, and calculated pearson's correlation coefficient between pH, DO, temperature, and salinity to assess whether pH and DO differences were attributable to biotic or physical factors.

Reasons for Lack of Evidence of Eelgrass pH Modulation

Low Density

There are several possible reasons for why our data did not evince any influence eelgrass had on the chemistry of its surrounding water.

Two explanations seem plausible. First, the densities of eelgrass present in the Amarillo study location could be too low to have a measurable impact on seawater chemistry. Indeed, other studies exploring the capacity of seagrass to buffer ocean acidification have measured shoot densities of 0 to 778 shoots per meter squared of *Posidonia oceanica (I. E. Hendriks et al., 2013)* and of 0 to 1900 shoots per meter squared of *Thalassia testudinum (Barry et al. 2013).* Both of these studies explored macrophyte environments in shallow coastal habitats with depths ranging from 5-12 m, a similar depth range to that of our study location. These studies analyzed the pH of water near different species of seagrass similar to *Zostera marina* and observed statistically significant increases in daytime pH due to photosynthesis. Hendriks et al. measured daytime fluctuations of 0.24 pH units in their higher density *Posidonia oceanica* quadrats during the month of June. Barry et al. observed daily pH fluctuations of 0.47 pH units and significant increases in DO which were attributed to photosynthesis by the seagrass, *T. testudinum*. To the contrary, our *Zostera Marina* observations included a much lower range of densities between 0 and 160 shoots per meter squared. The low densities of eelgrass likely did not achieve a rate of photosynthesis high enough to alter the pH and DO of the surrounding waters.

Although we could not attribute differences in pH to eelgrass photosynthesis, we did observe a high degree of correlation (r=0.99) between pH and dissolved oxygen at maximum depths, while neither pH nor DO were correlated with temperature or salinity. This suggests that the pH and DO values are driven by biotic factors including the diurnal cycles of photosynthesis and respiration.

Macrophyte/ microorganism presence

There are several biotic organisms in the Amarillo study site that could explain the high degree of correlation between the pH and DO. The main organism is Giant Kelp (*Macrocystis pyrifera*) which is present in large quantities at Amarillo. As presented in the 2018 Practicum report "An Analysis of the Potential of Giant Kelp and Eelgrass to Lower the Acidity of the Santa Monica Bay", Kelp forests contribute to significant daytime increases in pH throughout the water column. The presence of Giant Kelp and other macrophytes likely explains the correlation between pH and DO. (Hoshijima et al. 2019) also reported that areas inside kelp forests had higher diurnal DO cycles than areas not located in kelp forests, specifically near the top at the kelp canopy where maximum photosynthesis occurs. Their main conclusions were that kelp

forests consistently produced higher pH's and DO environments throughout the entire water column, which is why we saw a large degree of correlation. A study by Yafeng Zhang et al. showed that as phytoplankton increased, pH increased and DIC decreased at upper levels of the water column. (Zhang et al., 2019)**.** A study similar to our own was conducted on macrophytes. Fluctuations of pH and DO in unvegetated sites were largely attributed to photosynthetic activity of phytoplankton. (Carter, Rybicki, & Hammerschlag, 1991). Though further analysis of our nutrient, eDNA, and carbonate chemistry data is necessary to make any definite conclusions, a likely explanation for lack of visible pH influence by eelgrass is significant influence from phytoplankton and macrophyte photosynthesis.

Weather

Second, the cloudy weather conditions on our sampling days might have lowered the photosynthetic output of the eelgrass, contributing to negligible fluctuations in pH and DO. During both the April 26th and May 8th sampling trips, the weather was overcast and cloudy. This might have limited the rate of eelgrass photosynthesis and obfuscated their influence on the pH and DO of the surrounding waters. During the April 5th trip, we collected light samples throughout the day. Earlier in the day, it was sunny out with low cloud coverage and the light values recorded were 820 umol and 1309.5 umol. Later in the day when it was overcast, the light values dropped to 282.2 umol and 182.3 umol. In a study by Luis G Egea et al., the researchers grew *Zostera noltei*, a species of eelgrass, in a controlled environment under light conditions of 1250 umol. This value was set to mimic typical sunny conditions. Under darker, overcast weather conditions, the eelgrass located on the seafloor might have been much less productive than it would have been under sunny conditions. This is a significant factor in our experiment that could have contributed to the lack of trends attributable to eelgrass photosynthesis.

Future Direction

Future studies on eelgrass at depths of 5-12 meters in the Santa Monica Bay can improve upon our study design with several distinct approaches.

Diurnal Trends

One such method would be to focus on discerning the photosynthesis-driven diurnal flux in pH and dissolved oxygen in seawater above eelgrass meadows by collecting time series data. Given the fragmented, short term (seconds to minutes) scale of our sampling method, we did not have the resolving power to piece out diurnal trends in pH and DO. In their 2014 study, Hendrix et al. used a moored sensor located 0.1 m above a dense eelgrass bed to record pH and DO every 15 minutes for two weeks. Using a fixed sensor to record time series data enables researchers to assess the changes in pH and DO with respect to parameters that influence the photosynthetic output of macrophytes. Comparing trends in pH and DO with time of day, luminosity, and other

parameters gives researchers more power to distinguish the ability of eelgrass beds to modify the chemical nature of the seawater around them.

Future practicum teams can use the custom-built PVC rig and IN-Situ probe to replicate a moored sensor by anchoring it in one spot for an extended period of time. The rig should be weighed down and modified to anchor it at a specified height above the seafloor. Additionally, the Go Pro video camera we used could not share video feed in real time, which is necessary to ensure the probe is situated above eelgrass while sampling. Therefore, a different camcorder with live video feed will be needed to design a moored sensor. Once properly modified, the probe can be lowered in the water, and anchored over a spot of eelgrass for several hours, to resolve subtle changes in chemistry reflecting the diel changes in photosynthesis and respiration.

Nutrient, Carbonate Chemistry, and eDNA Analysis

In order to better characterize and understand the biological taxa present in distinct locations, future teams can use the 5L niskin bottle we purchased to collect water samples. After being treated with mercuric chloride to kill living organisms, the sample can be tested for dissolved inorganic carbon (DIC), alkalinity, and nutrient content. Quantifying dissolved inorganic carbon and alkalinity can help us get a more complete picture of the carbonate system. Nutrient analysis tests for quantity of phosphate, nitrate, nitrite, ammonia, and silicate. The water can also be filtered and later used in environmental DNA analysis. Using environmental DNA, we can determine the relative diversity of species present in the area two hours before the water sample was obtained. Both these analyses give us insight into the biological diversity and seawater chemistry in the location of interest. This also tells us how seagrass affects local ecosystems beyond altering pH and dissolved oxygen. Some species have linear relationships with pH and growth rate, other species are more closely linked to aragonite saturation levels and other factors.

Conclusion & Recommendations for The Bay Foundation

Our data demonstrates that under the conditions in which we sampled, eelgrass does not have a significant effect on pH values. We can attribute our inconclusive data to overall low sample size, zero samples with high densities, and low densities across all four sampling trips. Additionally, the weather on our third and fourth trips was overcast, which might have lowered the rates of photosynthesis during our sampling. Eelgrass beds do have the potential to mitigate acidification (Unsworth et. al, 2012, Hendriks et. al, 2014), but future research in the Santa Monica Bay will need to be done in higher density meadows with more favorable weather conditions for photosynthesis.

Appendix

Figure 8: Seawater pH Above Eelgrass of Different Densities at Different Depths

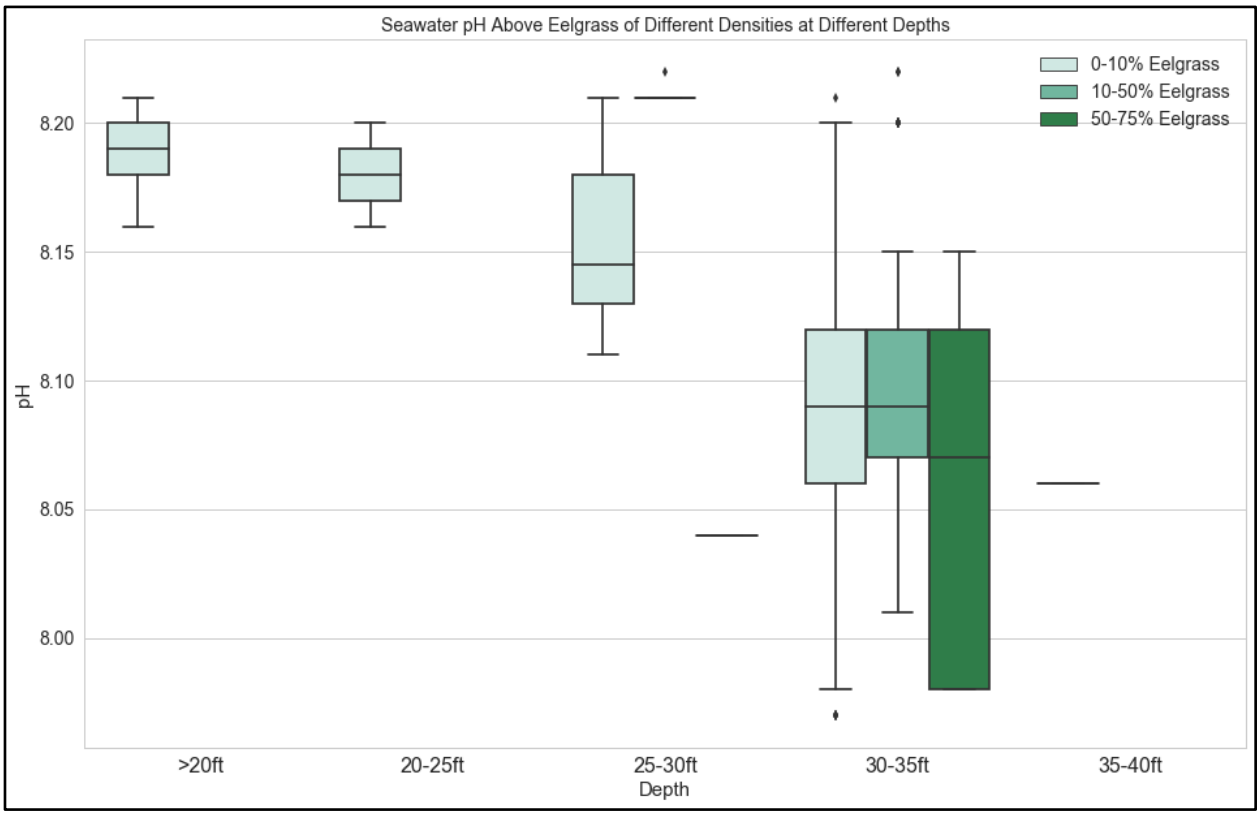


Figure 9: DO vs Salinity in Full depth profile at Amarillo on 5/8/19

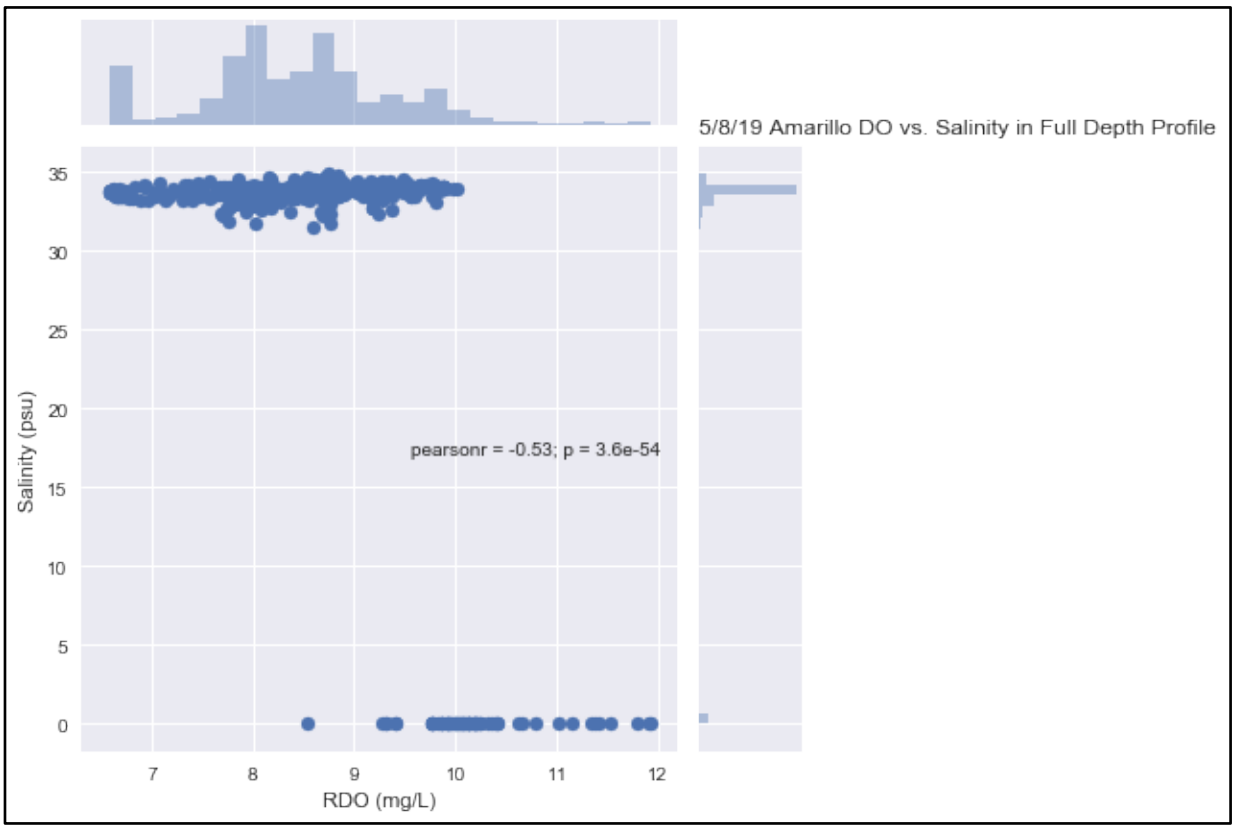
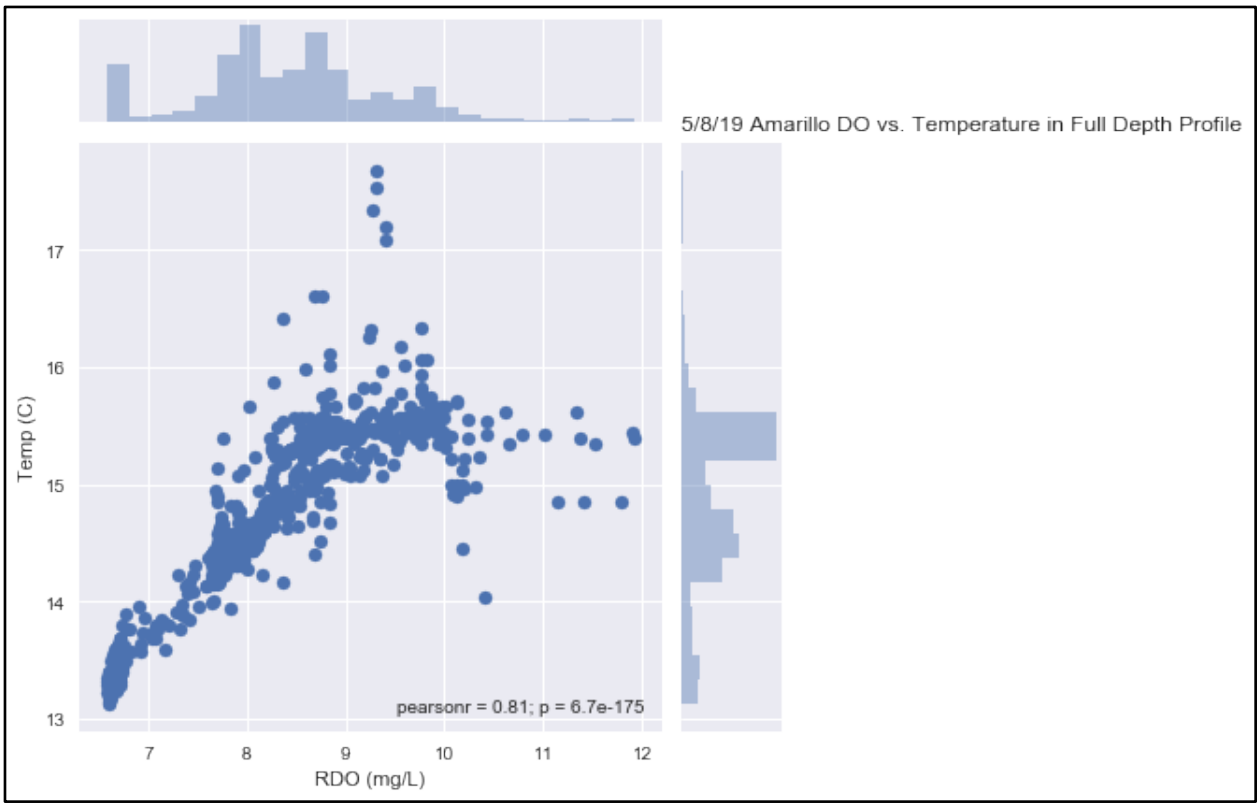
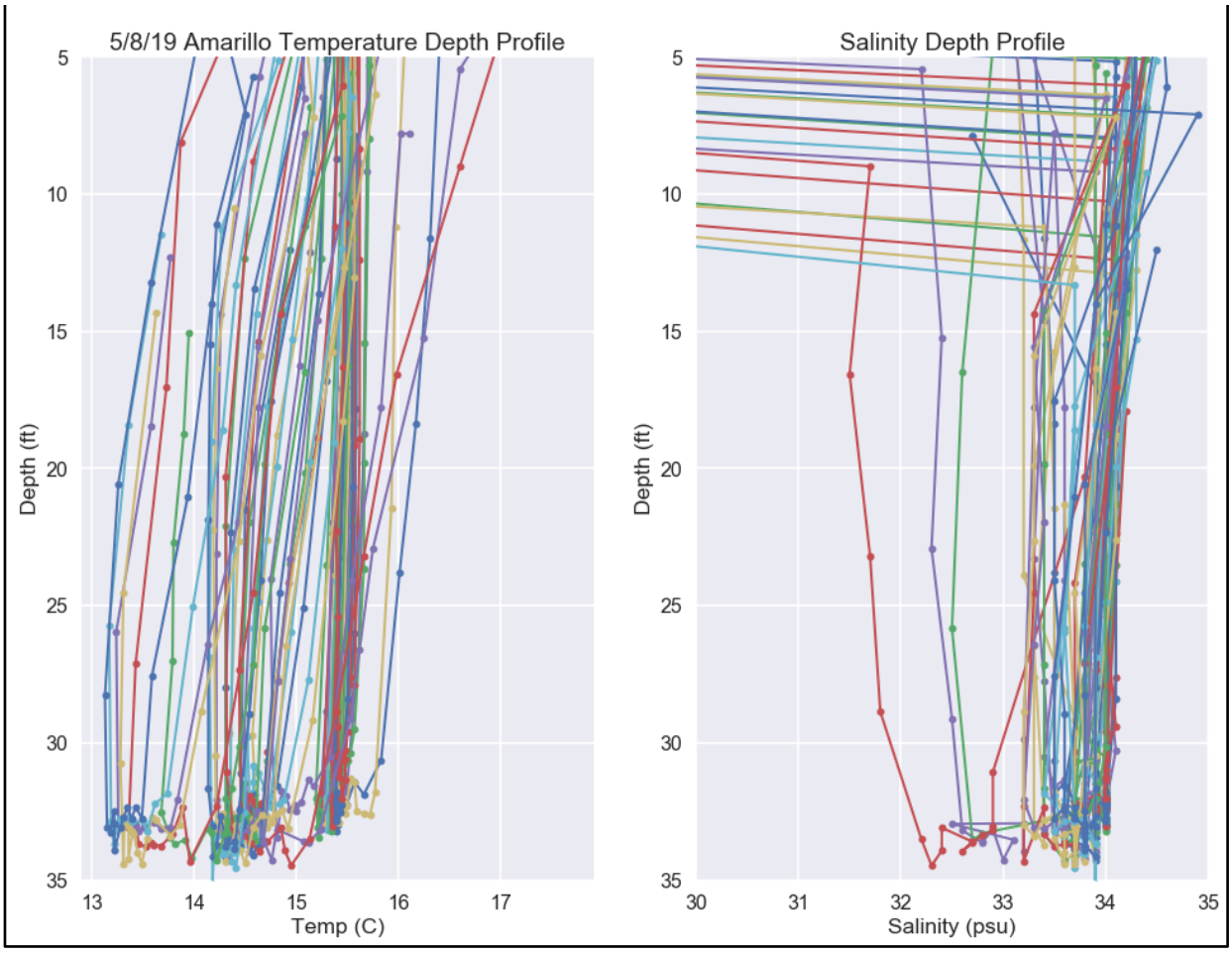


Figure 10: DO vs Temperature in Full depth profile at Amarillo on 5/8/19




5/8/19 Amarillo Temperature Depth Profile
Depth (ft)
Temp (C)
5
10
15
20
25
30
35
13
14
15
16
17
Salinity Depth Profile
Depth (ft)
Salinity (psu)
30
31
32
33
34
35

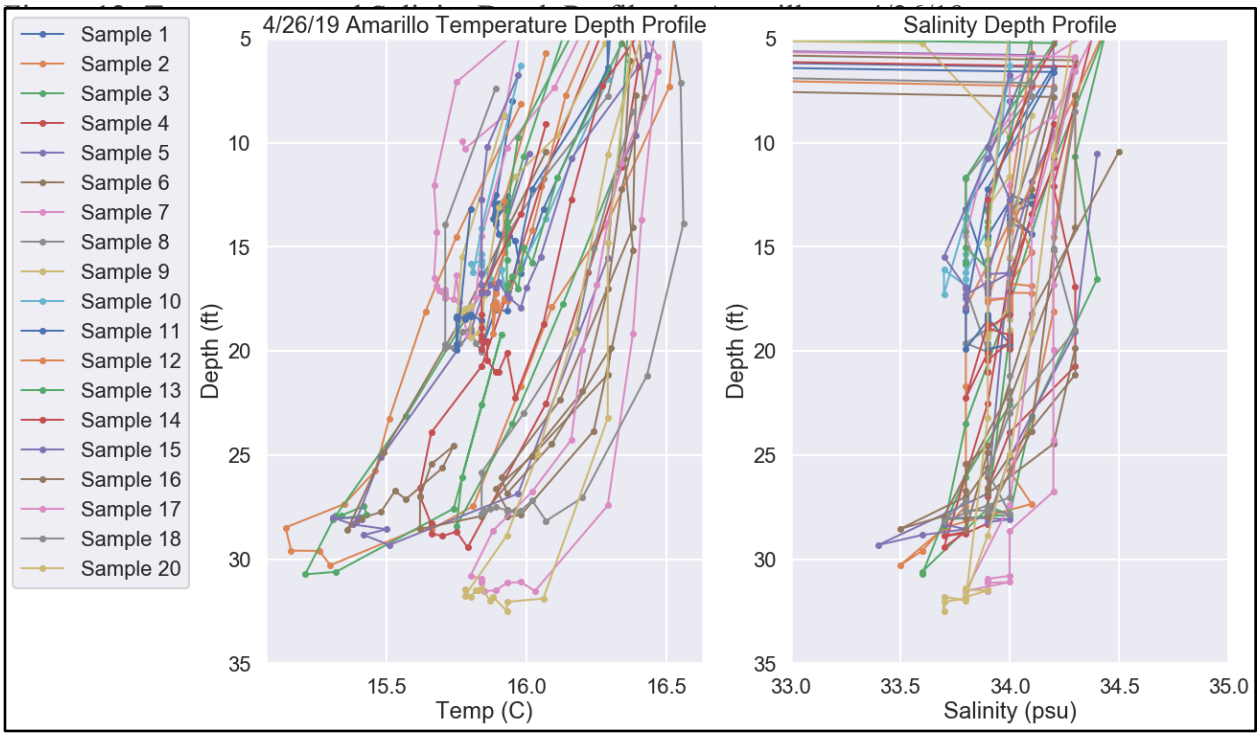


Figure 13: Seafloor pH vs. DO at Amarillo on 4/26/19

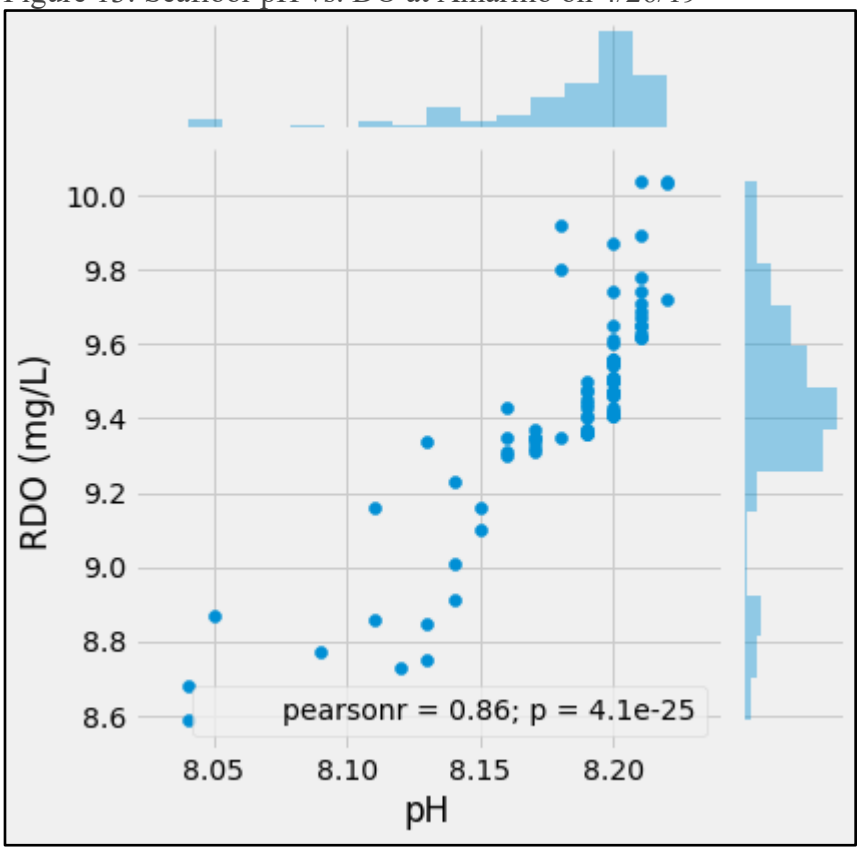


Figure 14: Map of pH Recordings at the Seafloor at Amarillo on 5/8/19

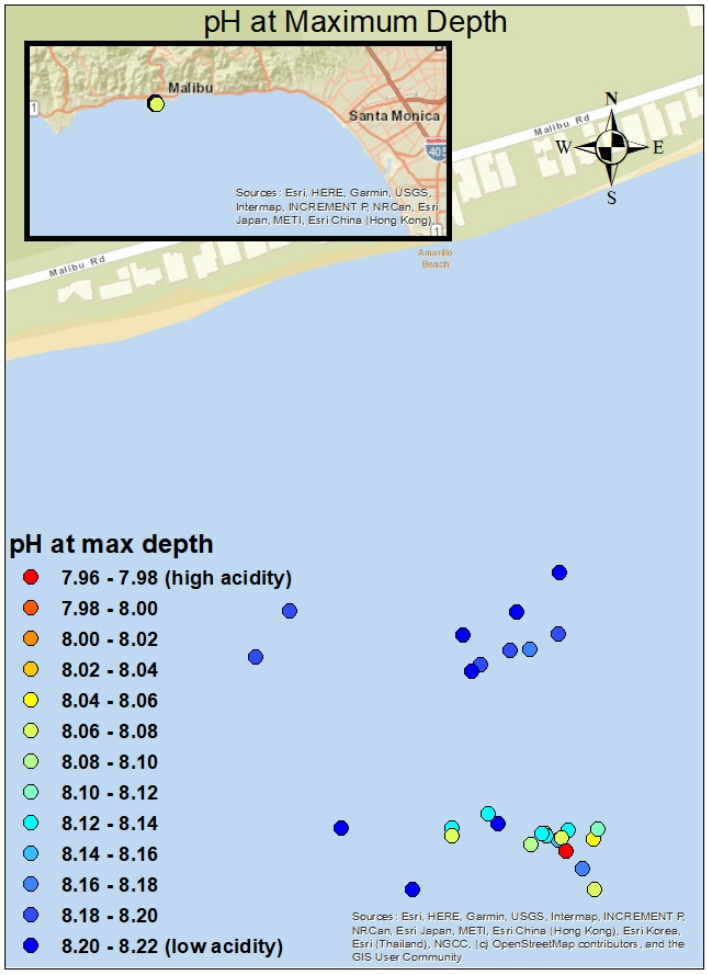
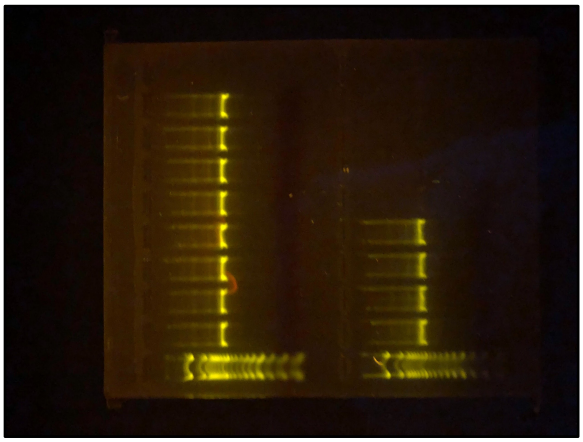


Figure 15: eDNA Results



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