INTERACTIVE EFFECTS OF OCEAN ACIDIFICATION AND FOOD AVAILIABILTY ON PREDATOR-PREY DYNAMICS IN THE ROCKY INTERTIDAL ZONE

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ABSTRACT

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Climate change induced ocean acidification will cause the average ocean pH to decrease over the 21st century. Many studies have investigated effects of exposure to low pH on marine organisms in the lab, however, only a very few have done so in the field. During bouts of strong upwelling, nearshore and intertidal areas in northern California can experience extended periods of acidified waters. I investigated the potential effects of such low pH conditions on the California mussel (*Mytilus californianus*), because of the important ecological role it plays in the rocky intertidal zone. I found that mussels living under low pH generally had thinner and weaker shells, making them more vulnerable to predation by crabs. However, when mussels had access to higher levels of food, the negative effects of low pH can apparently be mitigated. I will test these ideas under controlled conditions in the lab in the near future.

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS I

LIST OF FIGURES III

CHAPTER

1. INTRODUCTION 1

The California Mussel 2
Low pH in Northern California 3
Sampling Sites 4
Mussel Shell 4

2. METHODS AND MATERIALS 5

3. RESULTS 7

4. DISCUSSION 13

REFERENCES 15

LIST OF FIGURES

FIGURE

1. pH Data over Time………………………… 9

2. pH Data by site…………………………….. 9

3. Shell Length………………………………...10

4. Shell Thickness……………………………..10

5. Mean Breaking Force……………………….11

6. Byssal Thread Strength……………………..11

7. Chlorophyll-a……………………………….12

CHAPTER 1

INTRODUCTION

Since the beginning of the industrial revolution around the turn of the 19th century, there has been a need to burn carbon based fuels to keep the gears of industry spinning. The burning of hydrocarbon fuels releases carbon dioxide into the atmosphere, and for centuries was never given a second thought. Now, two hundred years later we are now starting to realize that this activity is not without consequence. As the amount of anthropogenically released carbon dioxide in the atmosphere has increased, there have been significant associated changes to the climate. These changes include increased storm frequency and elevated sea water temperatures (Lesser, 2016). However, the effect of anthropogenically released carbon dioxide is not limited to the alteration of the climate. In recent decades, there is evidence to show that the increased volumes of carbon dioxide in the atmosphere is also lowering the pH levels of surface seawater. (Kroeker *et al.* 2014) This is because Earth's oceans operate as a sink that absorbs carbon dioxide directly from the atmosphere. If the oceans are absorbing large quantities of carbon dioxide this can affect the carbonate buffer system. Carbonate dissolution found in marine sediments is the eventual buffer to neutralizing the anthropogenically released carbon dioxide (Emerson, 1990). When the carbon dioxide levels are increased the buffer is less effective and the pH will decrease. The lower pH may cause developmental and other structural problems in a variety of different organisms that inhabit the rocky intertidal zone.

Although lowered pH will have an effect on many different species I will study the morphological effects on the California mussel (*Mytilus californianus)*. There are a variety of species that live in the rocky intertidal region and form calcium carbonate shells. I chose the

California mussel because of its ecological role it plays in the rocky intertidal zone as a foundation species. Ocean acidification will happen throughout the ocean but I will focus on the rocky intertidal region found around Humboldt County, CA. Since the California mussel was chosen for study the field data would be collected in the rocky intertidal zone. The rocky intertidal zone is considered by some to be a natural laboratory because it is easily accessible and has a high diversity of species to study. My study involved the analysis of data on seawater pH, mussel morphology, and local chlorophyll-a levels collected at four different sites in collaboration with Dr. Paul Bourdeau and his team at Humboldt State University. I then use the results of this exploratory data analysis to design a controlled laboratory experiment that I am currently working on here at CSULB. My controlled laboratory experiment will be different than other ocean acidification experiments because it looks at how mussels are affected by ocean acidification in a broader ecological context. Specifically, by placing mussels in the proximity of predatory crabs, and manipulating food availability in a set pH level environment. This is to make the comparison of field to lab data to get an idea of what exactly is happening and will happen in the future to the California mussel under ocean acidification conditions.

The California Mussel

The California mussel (*Mytilus californianus*) is a member of the Phylum Mollusca in the Class Bivalvia. It has a range from Mexico to Alaska and is found primarily in the rocky intertidal zone. The California mussel is a suspension feeder which means that it will actively take in food from filtering seawater. It has a soft interior that is made up of the ctenidia (gills) and other digestive organs. These organs are covered by a calcium carbonate shell which is formed by accretionary growth. This means that the shell has layers and layers of calcium carbonate that over time forms a rigid exterior. The California mussel reproduces by broadcasting spawning its gametes into the water column, where it will develop into a trochophore larvae. The trochophore larvae is a free-swimming larva that feeds in the water column. After spending some time as the trochophore the larvae will settle on a hard substrate and undergo metamorphosis. After metamorphosis it will begin development into the juvenile California mussel. The mussel is able to survive on the coastal rocks because it secretes byssal threads (Lesser, 2016). Byssal threads are a filamentous attachment structure that keeps the mussel attached to the substrate and will not degrade with water exposure. The mussel is uniquely adapted to live under the variable abiotic and biotic factors. The California mussel is commercially harvested for food consumption in northern California.

Low pH in Northern California

California mussels are distributed throughout most of the west coast of North America. Humboldt California is the area that we decided to take pH samples and mussel morphology from (Peacor *et al.* 2012). This is due to a seasonally occurring decrease in pH that is caused by upwelling of deeper waters off of the Humboldt coast. That means that the organisms that live in the rocky intertidal zone there are exposed to consistently lower pH levels than other populations found along the west coast (Barth *et al.* 2007). The intertidal organisms can then be studied to see what types of morphological changes have been made in response to living in this area. These lower pH levels are approximately the pH levels predicted to be found oceanwide in the next fifty to one hundred years from now. This makes for an ideal location to be able to study the effect that ocean acidification will have on the California mussel. From studying that region, predictions can be made about how climate change may affect the mussel populations living in other rocky intertidal regions along the west coast of North America.

Sampling Sites

There were four total sampling sites that run from south to north. The southernmost sampling site was Belinda Point, located near Fort Bragg, CA. North of Belinda Point is Devil's Gate, found just south of Cape Mendocino, CA. North of Devil's Gate is Bakers Beach, located near Humboldt State University in Trinidad, CA. The northernmost point is Point St. George, which lies just south of the Oregon border. The reason that these sites were chosen is because the two southern sites are not generally exposed to strong upwelling events (and associated low pH conditions), whereas the two northern sites are routinely exposed to upwelling (Feely *et al.* 2008). pH levels are generally predicted to get lower the higher north the sampling site.

Mussel Shell

The mussel shell provides a barrier from the harsh variables of the outside world. Without its shell, the mussel can be easily preyed upon or weakened to such a state that the slightest wave is able to knock it from its attachment. Ocean acidification causes the calcium carbonate shell that the mussel has to not be able to form properly, causing deformations. This is not the only species of invertebrate that is facing negative effects from ocean acidification (Lesser, 2016). Any species that forms a calcium carbonate shell is at potential risk to having shell deformations. If the mussel is diverting its energy to reinforce its shell, then it may be diverting energy from other vital systems (anything from reproduction to feeding ability). The shell deformations also make predation by brown rock crabs (*Romaleon antennarium*) much more likely (Kroeker *et al.* 2016). This in turn could lead to population declines in the California mussel. For this reason, I focused on investigating the potential effects of low pH conditions on mussel shell parameters.

CHAPTER 2

MATERIALS AND METHODS

The pH data came from three different sources: the Bakers Beach data came from the CENCOOS online data repository that takes pH data from the Trinidad pier which is close to Bakers Beach (collected from 2009 to 2017); the Belinda Point and Devil's Gate pH data came from the OMEGAs online data repository (collected from 2012-2013); and the Point St. George pH data was collected by Dr. Bourdeau and his team (collected from 2017). These data were then analyzed to determine the averages and standard errors from each data set to see what type of trends in the pH levels there were, based on each of the four sites. Concentrations of chlorophyll a (a proxy for mussel food availability) were calculated from seawater samples collected at each site. Bulk water samples were filtered, pigments extracted in 90% acetone, and chlorophyll-a concentrations estimated from measurements of absorbance on a spectrophotometer.

The mussel morphological data was broken up into several different categories. The first was shell length measured in millimeters (mm) to the nearest 0.1 mm using digital calipers. This was measured precisely determine how long the mussel is from the anterior to posterior margins. The mean shell thickness was also measured in millimeters. Shell thickness can be a difficult to measure, so the average of three measurements was used to be precise as possible. The shell breaking force was measured with an Instron Materials Testing System that crushes the shell until it breaks. The shell breaking force is measured in Newtons (N). Finally, the byssal attachment strength was measured by ripping the mussels directly off the rock with a hand-held spring scale. The force needed to rip each mussel off the rock was measured in Newtons (N).

The mussel morphology data was organized by site and the average and standard deviation calculated for each of the different mussel measurements. Analyses of variance (ANOVAs) were done on each data set to compare site means; visual inspection of residuals was done before each test to check for equal variances and normality. All statistical analyses were done in Minitab 17.

CHAPTER 3

RESULTS

pH Data over Time

At Trinidad Pier, near Humboldt State University in northern California, there are regular fluctuations of seawater pH throughout the year. The highest pH values are found in the winter months, whereas the lowest pH values are found in spring and summer (Fig. 1; CENCOOS data).

pH Data by Site

Across collection sites that encompassed different latitudes, the farther north the sampling site, the lower the seawater pH. pH values at Belinda Point and Devil's Gate to the south were not significantly different from one another and were both significantly higher than at Bakers Beach and Point St. George to the north (Fig. 2; ANOVA, ($F_{3,\,684} = 62.1$, $P < 0.001$).

Shell Length

Mussel shell length varied across sites, but there was no obvious trend towards a decrease in shell length with increasing latitude (Fig. 3; ANOVA, $F_{3,\,256} = 20.3$, $P < 0.001$).

Shell Thickness

Mussel shell thickness (corrected for shell length) also varied across sites. Belinda Point and Devil's Gate were not significantly different from one another, but shell thickness decreased at Bakers Beach and then increased at Point St. George, the northernmost site (Fig. 4; ANOVA, $F_{3,\,261} = 16.6$, $P < 0.001$).

Mean Breaking Force

The amount of force needed to break a mussel shell (corrected for shell thickness), a measure of shell strength, exhibited a pattern similar to that of shell thickness. Mussels from the two southern sites, Belinda Point and Devil's Gate, required higher amounts of force to break the shell and were not significantly different from one another. At Bakers Beach there was a decrease in the amount of force needed to break the shell, whereas the force values for Point St. George were higher than the Bakers Beach value (Fig. 5; ANOVA, $F_{3, 224} = 4.0$, $P = 0.008$)

Byssal Thread Strength

The force needed to rip the mussel's byssal threads from the substrate (corrected for planform area) exhibited a different pattern. Belinda Point had the lowest thread strength and was significantly different from Devil's Point, which was less than at Bakers Beach and Point St. George (Fig. 6; ANOVA, $F_{3, 148} = 25.1$, $P < 0.001$).

Chlorophyll-a Levels

Chlorophyll-a levels were relatively low at Belinda Point and Devil's Gate and not significantly different from one another. In contrast, at Bakers Beach the chlorophyll-a values were higher than Belinda Point and Devil's Gate and even more so at Point St. George. The levels at Point St. George were significantly higher than the other three sites (Fig. 7; ANOVA, $F_{3, 44} = 42.8$, $P < 0.001$)

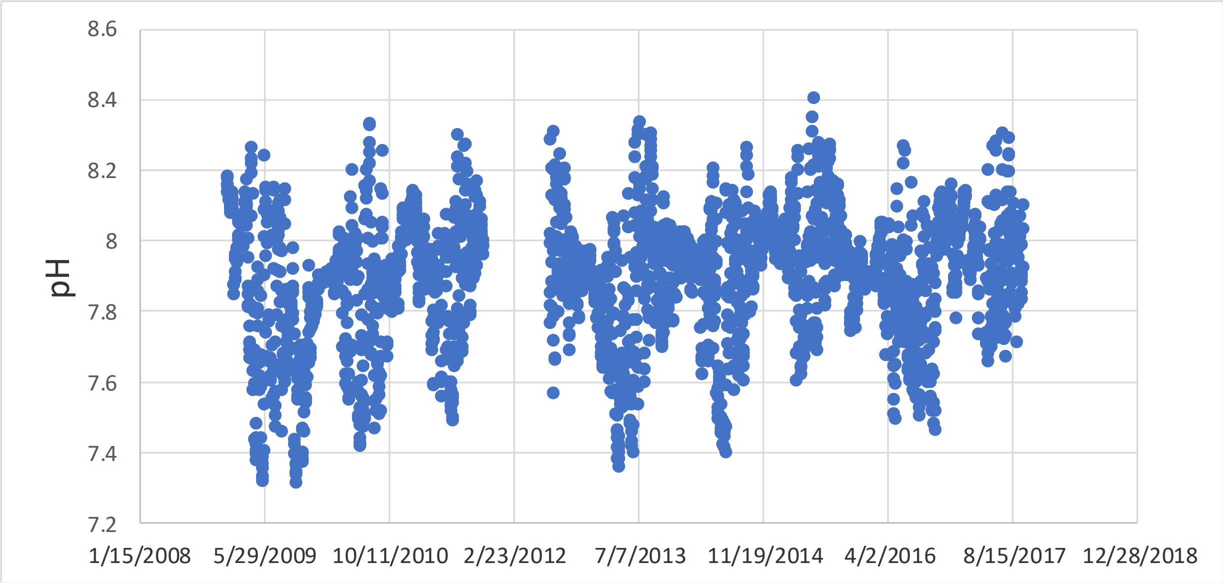

Figure 1:
CENCOOS pH data collected at Trinidad Pier, CA. Graphed out against date/year.

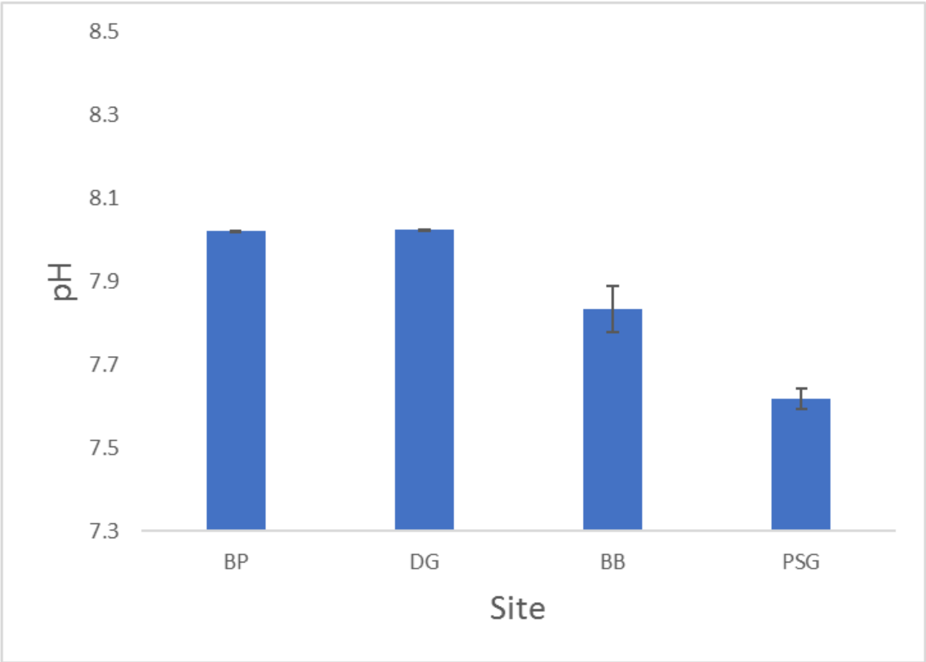

Figure 2:

Combination of CENCOOS and OMEGAS pH data graphed against site. BP= Belinda Point, DG = Devil's Gate, BB = Bakers Beach, PSG = Point St. George. Error bars are +/- 1 SE.

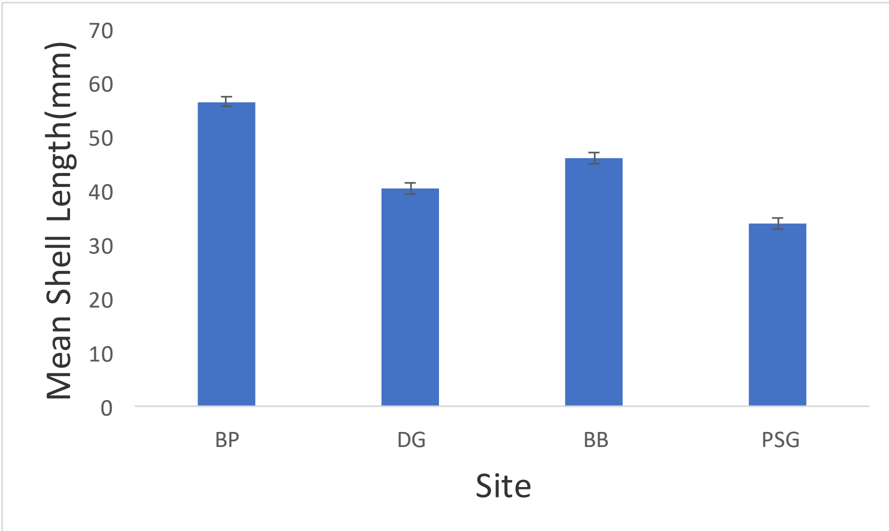

Figure 3:

Mean Shell Length is represented in millimeters and graphed against collection site. BP= Belinda Point, DG = Devil's Gate, BB = Bakers Beach, PSG = Point St. George. Error bars are +/- 1 SE.

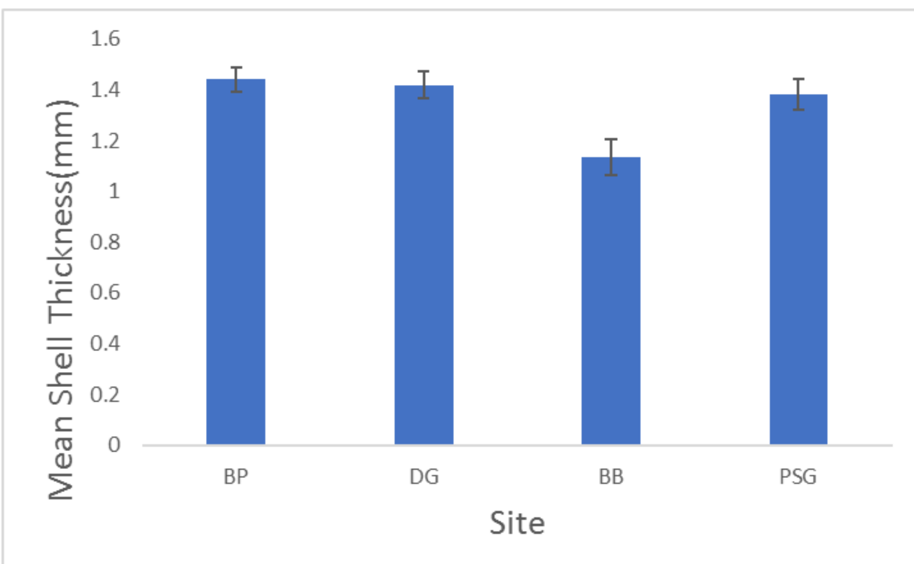

Figure 4:

Mean shell thickness in millimeters(mm) is graphed against collection site. BP= Belinda Point, DG = Devil's Gate, BB = Bakers Beach, PSG = Point St. George. Error bars are +/- 1 SE.

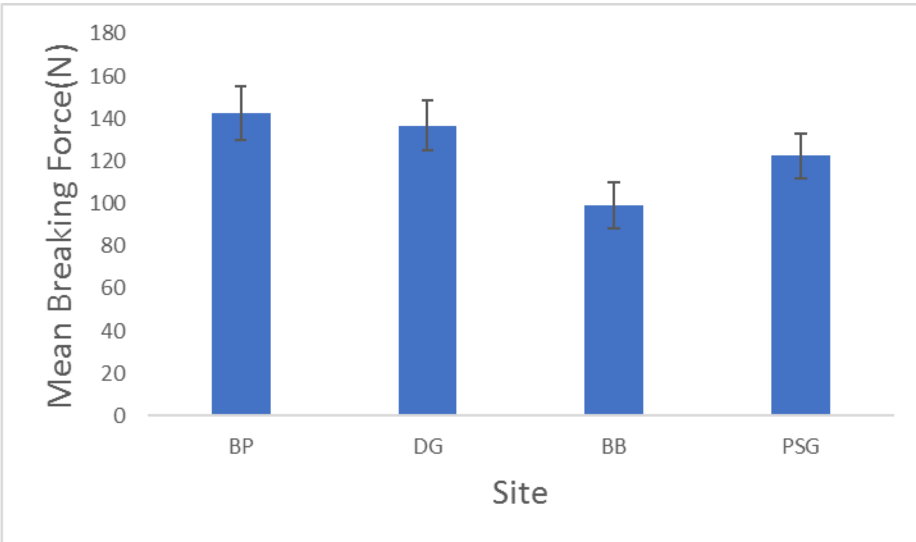

Figure 5:
Mean breaking force in Newtons(N) is graphed against collection site. BP= Belinda Point, DG = Devil's Gate, BB = Bakers Beach, PSG = Point St. George. Error bars are +/- 1 SE.

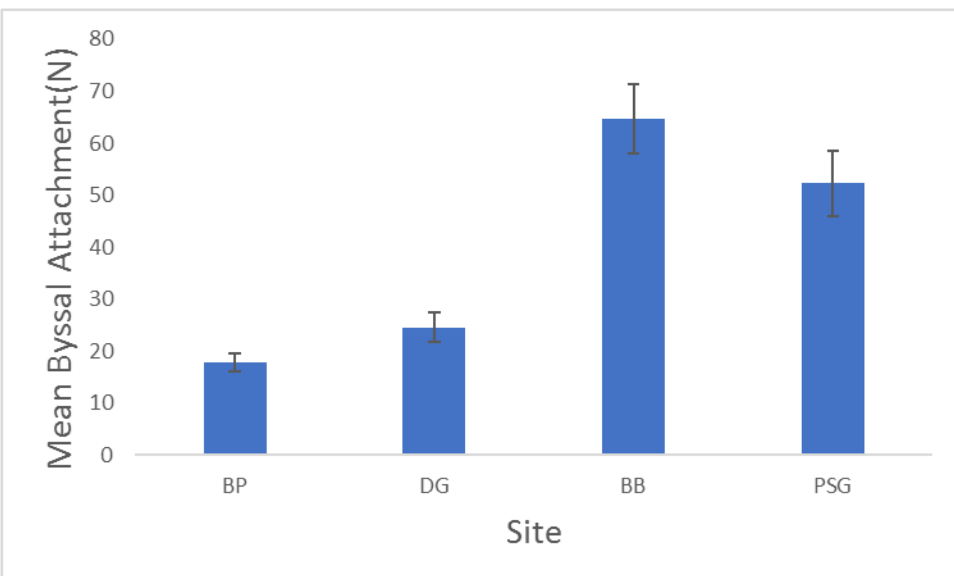

Figure 6:
Mean Byssal Attachment measured in Newtons(N) against collection site. BP= Belinda Point, DG = Devil's Gate, BB = Bakers Beach, PSG = Point St. George. Error bars are +/- 1 SE.

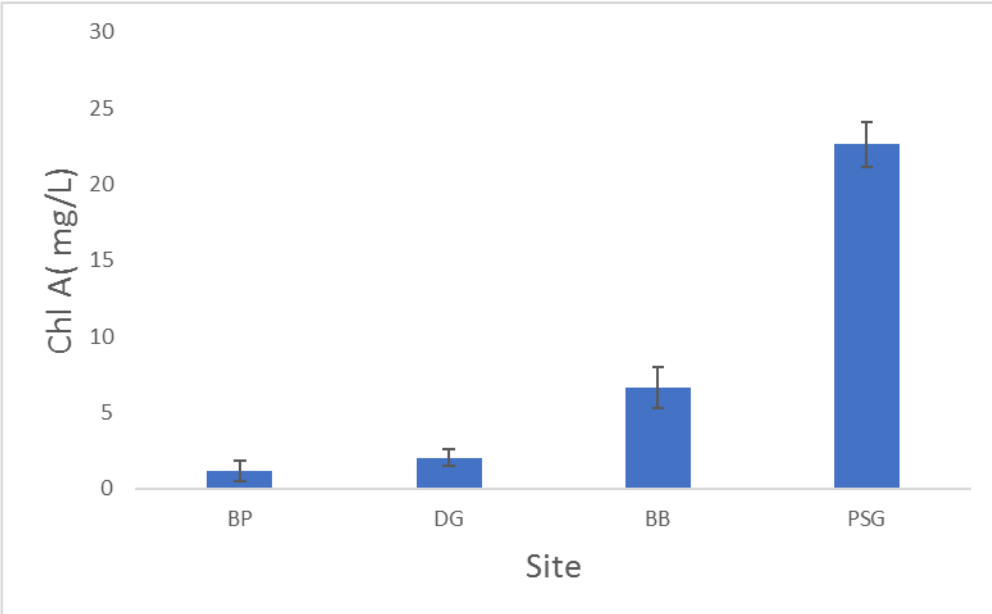

Figure 7:

Chlorophyll A concentration (mg/L) plotted against collection site. BP= Belinda Point, DG = Devil's Gate, BB = Bakers Beach, PSG = Point St. George. Error bars are +/- 1 SE.

CHAPTER 4

DISCUSSION

Nine years of daily pH data shows that there are consistent fluctuations seawater pH at Trinidad pier in Humboldt County, CA. This means that year after year, a variety of organisms in the nearby rocky intertidal zone are routinely exposed to potentially stressful low pH conditions. Daily average pH values also differ spatially, with mean pH levels decreasing from south to north, presumably due to more upwelling in the northern areas.

Mussels appear to be sensitive to localized reductions in pH that have been observed in these locations. Although mussel shell length was variable, across sites, there was not a significant trend in a decrease in the shell length with increasing latitude. This could mean that the mussels sampled at each site were not representative of the entire population, or that the distribution of energy under these stressful conditions is not being allocated to shell length. In contrast, mussels from Belinda Point and Devil's Gate, the two southernmost sites, had the thickest shells and required the highest values of force needed to crush the shells. Although mussels from the two northern sites had thinner, weaker shells, those from Point St. George were not nearly as negatively affected by low pH when compared to individuals from Bakers Beach. One possible reason for this unexpected observation is the high concentration of chlorophyll-a at this site, indicative of an increase in local phytoplankton concentration (food available to the mussels). That increase in available energy could allow mussels at Point St. George to counteract the otherwise negative effects of low pH conditions.

These northern California areas are often pummeled by large storms and byssal threads are the mussels' most important asset for remaining attached to the substrate. Mussels from the two southern sites required lower amounts of force needed to remove them from the rock surface when compared to those from the northern sites. One possible theory to explain why the mussels are secreting more byssal threads in these sites is that the wave forces are more intense in the north. Another possibility is that the mussels are stressed by the decreased pH levels (as evidenced by the thinner shells), and it is more energetically favorable to form a byssal thread proteins. These results are a snapshot of how the pH and mussel morphology are found in the field. These results will then be compared to a lab experiment here at CSULB. The lab experiment will involve manipulating seawater pH levels and food availability to mussels. Similar results from the lab experiment that would mean that mussels are negatively impacted by low pH levels, but only when food is limiting.

The observation that mussels exposed to low pH conditions have thinner, weaker shells raises an interesting question about predator-prey dynamics in this system. Such mussels could be potentially more susceptible to predation. In northern California, brown rock crabs will feed heavily on the California mussel when compared to other prey options (Bourdeau, unpublished data), using their claws to either crush or peel open the shell of their prey. By taking the mussels from the lab experiment and feeding them to crabs to determine the rate at which they are eaten, I will get a better sense of the role that the effects of low pH on mussels play in the predator-prey interactions. Understanding what ecological effects will impact the mussels under ocean acidification conditions can lead to more accurate predictions in the future, and will allow my results to be placed in a broader ecological context.

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