AN ANALYSIS OF THE EFFECTS OF EELGRASS BEDS ON THE WATER CHEMISTRY OF PORT GAMBLE, PUGET SOUND

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ABSTRACT

An analysis of the effects of eelgrass beds on the water chemistry of Port Gamble, Puget Sound.

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Since the industrial revolution, our oceans have absorbed about 30% of the atmospheric carbon dioxide (CO_2) emissions. Ocean CO_2 uptake has resulted in ocean acidification, which is a progressive decrease of the pH of the world's oceans. Puget Sound has several physical and biogeochemical characteristics that intensify the effects of ocean acidification.

Scientists expect that the continuous acidification of Puget Sound will have detrimental effects on the biodiversity, the function of ecosystems, the local economy, and possibly on human health. Some of these effects have already been observed.

One of the proposed strategies to combat ocean acidification in Puget Sound is the transplantation and restoration of eelgrass (*Zostera marina*) beds in order to utilize them as a carbon sink. However, the carbon capture and sink efficiency of eelgrass beds has not been directly quantified in Puget Sound.

This thesis research, which was conducted during January 2014, examined if eelgrass beds in Port Gamble, WA could significantly increase the pH of the water column directly above them. The experiment measured the rates of change of pH over time as water flowed through two ecosystems: eelgrass beds and a control consisting of mud flats with no eelgrass coverage. Given that eelgrass takes up CO_2 through photosynthesis, we hypothesized that the pH of the water column would increase over time in the eelgrass treatment as a result of photosynthesis rates dominating over respiration rates. Similarly, we expected the pH of the water column in the no eelgrass treatment to decrease over time due to respiration rates dominating over photosynthesis rates.

For this experiment, we attached a water quality monitoring sonde YSI 6600, two garmin gecko GPS instruments, and two video cameras to a floating device, which drifted over the two studied ecosystems. The data obtained from these instruments was used to calculate the rate of change of pH over time for each ecosystem.

The results showed that both treatments (eelgrass and control) exhibited an increase in the rate of change of pH over time. The control treatment showed a more pronounced increase in the rate of change of pH over time (mean=0.00239 pH/minute) than the eelgrass treatment (mean=0.00084 pH/minute). However, a resmpling t-test indicated that there was a no significant difference between the rates of change of pH over time for both treatments (α=0.05, 1000 trials, and p=0.136).

The results from this experiment suggest that eelgrass beds in Port Gamble were not capturing enough carbon during the wintertime to cause a significant increase in the rate of change of pH over time when compared to the control treatment.

This experiment was meant to give scientists a snapshot of the dynamic change of pH that occurs in both ecosystems during the winter; the data presented in this study is not enough to draw conclusions about the carbon sink capacity of eelgrass beds in Port Gamble, Puget Sound. Further research that takes into account variables such as depth, alkalinity, total chlorophyll, irradiance levels, as well as the rates of photosynthesis, respiration, burial, and export, measured during periods of 24 hours or longer, during several months of the year (or at least seasons), are needed to draw definite conclusions about the net carbon sink capacity of eelgrass beds in this region of Puget Sound.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

Ca^{2+}: calcium ion

CA: carbonic anhydrase

$CaCO_3$:calcium carbonate

CO_2: carbon dioxide

$CO_{2(aq)}$: aqueous carbon dioxide also known as carbonic acid

DIC: dissolved inorganic carbon

Dif: difference

gdw/m^2: grams of dry weight per square meter

H^+: proton or hydrogen ion

H_2CO_3: carbonic acid

HCO_3^{2-}: carbonate ion

IPCC: Intergovernmental Panel on Climate Change

LAI: leaf area irradiance, usually measured as photosynthetic leaf area per square meter

NECB: net ecosystem carbon balance

NPP: net primary production

pCO_2: partial pressure of carbon dioxide

ppt: parts per trillion

TA: total alkalinity

tCO_2 eq/ha*yr: metric tons of CO_2 equivalent per hectare per year

WA- DNR: Washington State Department of Natural Resources

[ ]: brackets indicate the "concentration" of the chemical specie placed inside the brackets.

Δ: delta symbol, defined as the change in a particular variable.

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I. INTRODUCTION

As the global combustion of fossil fuels continues to increase exponentially, it is estimated that the world's oceans are absorbing one third of the anthropogenic carbon dioxide emissions (CO_2) or roughly 22 million tons of CO_2 per day (Feely, Sabine, & Fabry, 2006). The intake of massive amounts of CO_2 into the oceans is altering water chemistry, and has resulted in ocean acidification, which is defined as a reduction in the pH of the ocean for an extended period, typically decades of longer (IPCC, 2007). Ocean acidification is having detrimental effects in the biodiversity and function of ecosystems worldwide (Bloom, 2010; Feely, Klinger, Newton, & Chadsey, 2012).

Puget Sound has several biogeochemical characteristics, such as upwelling currents and the input of nutrients through runoff, which intensify the effects of ocean acidification (Feely et al., 2012). Local ocean acidification has caused large-scale larval mortality in commercial oyster hatcheries, negatively affecting the economy of the Pacific Northwest (National Research Council, 2013). Ocean acidification is also expected to reduce biodiversity of local ecosystems as well as the amount and quality of local seafood (Branch, DeJoseph, Ray, & Wagner, 2013). The deterioration in the nutritional quality of local seafood could potentially affect human health (Rossoll et al., 2012).

Because ocean acidification can negatively affect Puget Sound's environment, economy, and human health, scientists have proposed the use of seagrass beds as carbon sinks (Greiner, McGlathery, Gunnell, & McKee, 2013;

Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Seagrasses have the capacity to sequester carbon and bury it in sediments where it can be preserved in the seabed for a period estimated to range from decades to millennia (Dowty et al., 2005; Greiner et al., 2013).

Of the six species present in the Pacific Northwest, *Zostera marina*, also known as eelgrass, is the dominant seagrass in terms of biomass and areal extent, covering about 200 km^2 of the shoreline of Puget Sound (Dowty et al., 2005; Wyllie-Echeverria & Ackerman, 2003). Thus, *Z.marina* is often the proposed specie to be used for ocean-acidification phyto-remediation projects in the Pacific Northwest (Shishido, 2013)

However, the effect that eelgrass beds have on water chemistry, which is reflective of the uptake of CO_2 due to photosynthesis, has not been directly explored or quantified in the Puget Sound region. Estimates of the carbon sequestration capacity remain theoretical and are based on calculations taking into account reported values for density, range, distribution, and net primary production (NPP).

This project represented an effort to explore the carbon capture potential of local eelgrass beds. The objective was to determine if eelgrass beds in Port Gamble could significantly alter the pH of the water over time in order to ameliorate the effects of ocean acidification. The experiment was conducted during the wintertime, when photosynthetic rates were the lowest in the year, to determine if the beds had the capacity to ameliorate the effects of ocean

acidification all year round.

For this experiment, we attached a water quality monitoring sonde YSI 6600, two garmin gecko GPS instruments, and two video cameras to a pair of floating devices labeled “drifters.” One drifter was placed over areas that contained abundant cover of eelgrass and another drifter was placed over areas that contained no visible eelgrass coverage. Each drifter was allowed to drift following the direction of the current, while collecting data of water chemistry parameters. The data from the drifts was used to calculate how the pH of the water was changing over time.

The results from this project showed that during the winter, eelgrass beds in Port Gamble were not capturing enough carbon to significantly increase the pH of the water column over time (ΔpH/min) (α=0.005, 1000 trials, p=0.136).

These results are possibly influenced by variables that were not taken into account in this experiment for simplicity purposes. In fact, we suspect that depth of the water column might have influenced our results as the areas that contained no visible eelgrass coverage had a greater increase in pH over time that the areas that had abundant eelgrass coverage.

Further research that takes into account variables such as depth, alkalinity, and total chlorophyll, as well as the ecosystem’s rates of photosynthesis, respiration, burial, and export, during diel cycles and during different seasons, are needed to determine is eelgrass beds in Port Gamble Bay are a net carbon sink.

This project, which was in collaboration with Washington State

Department of Natural Resources (WA-DNR), served as a pilot project to evaluate the launch of a large-scale multi-location project that will analyze how seagrass beds in Puget Sound modify seawater carbon chemistry to determine if they can help mitigate the local effects of ocean acidification.

II. LITERATURE REVIEW

WHAT IS OCEAN ACIDIFICATION?

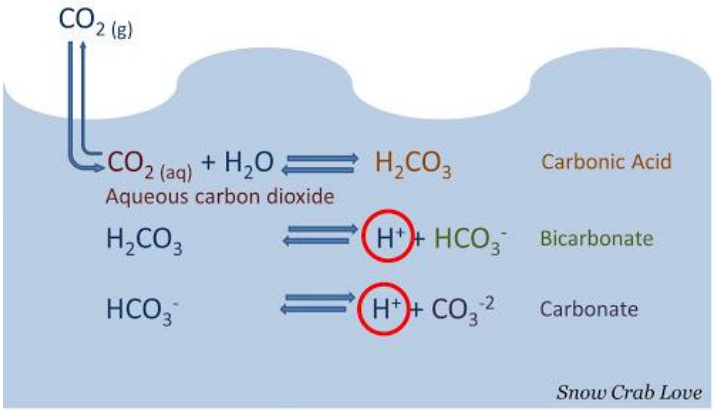
Over the past 250 years, humans have emitted large quantities of carbon dioxide (CO_2) to the atmosphere, increasing the concentrations of atmospheric CO_2. Atmospheric CO_2 levels increased by nearly 45% from preindustrial levels of approximately 270 ppmv (parts per million by volume) to over 400ppmv in 2013 (Bloom, 2010; NOAA, 2013b). This rate of increase, which is driven by anthropogenic activities, is an order of magnitude faster than has occurred in millions of years (Doney, Fabry, Feely, & Kleypas, 2009). In fact, studies of air bubbles trapped in ice cores indicate that current atmospheric CO_2 levels are the highest that have ever been in the past 800,000 years (Doney et al., 2009). The current accumulation of CO_2 in the atmosphere is increasing the natural green house effect and causing global climatic changes (Bloom, 2010).

Atmospheric CO_2 has three fates: it can be absorbed by the terrestrial biosphere, absorbed by the oceans, or it remains in the atmosphere. Since the year 2000, about 30% of the atmospheric CO_2 emitted was absorbed by the terrestrial biosphere, 30% was absorbed into the oceans, and the remaining 40% has persisted in the atmosphere (Gattuso & Hansson, 2011). It is estimated that the oceans are absorbing approximately 22 million metric tons of CO_2 each day, which corresponds to an intake of 8.03 billion metric tons each year (Feely et al., 2006).

In the ocean, carbon dioxide from the atmosphere dissolves in the seawater , following the concentration gradient, achieving equilibrium with the concentration of the atmosphere (The Royal Society, 2005). On land, carbon dioxide is used during photosynthesis and converted into plant tissues (Beer et al., 2010).

By taking in some of the atmospheric CO_2, the biosphere and the oceans mitigate the greenhouse effect. If the oceans and the biosphere did not act as carbon sinks, the current atmospheric CO_2 levels would be far above 450ppmv (parts per million by volume) today, which would translate to a global temperature increase of 2-3°C (Doney et al., 2009). However, the biosphere's capability of absorbing carbon is diminishing, leaving the ocean as the main carbon sink (Doney et al., 2009).

Ocean CO_2 uptake is not benign; it causes a reduction in the ocean's pH and alters the biogeochemical balance of the ocean. Once the molecule of CO_2 dissolves in seawater (H_2O), it forms carbonic acid (H_2CO_3) also known as aqueous carbon dioxide ($CO_{2(aq)}$) (Bloom, 2010). Carbonic acid can dissociate to release a proton (H^+) and a bicarbonate ion (HCO_3^-) (Bloom, 2010). The bicarbonate ion can subsequently dissociate to release a proton (H^+) and become a carbonate ion (CO_3^{-2}) (Equation 1) (Bloom, 2010). Carbonic acid, bicarbonate, and carbonate ions are collectively referred as dissolved inorganic carbon species, and the sum of these chemical species is known as total dissolved inorganic carbon (DIC).



Equation 1. Chemical equations for the dissolution and dissociation of carbon dioxide (CO_2) in ocean water. Released protons shown in circles. Figure reprinted from Snow Crab Love: what is ocean acidification? Retrieved December 15, 2013 from http://snowcrablove.blogspot.com/2012/03/whats-ocean-acidification.html

The seawater reactions for ocean acidification can be reversible; the direction that they follow and how much of the CO_2 dissociates into its subsequent chemical species depends on factors such as salinity, temperature, pH, and water depth (Doney et al., 2009).

The relationship between the DIC species and pH can be modeled by Bjerrum plot, which keeps salinity, temperature, and quantity of dissolved CO_2 at a constant value. The most common representation of a Bjerrum plot assumes DIC=2.1mmol/kg, salinity=35, T=25°C (Figure1) (Zeebe & Wolf-Gladrow, 2001).The plot shows that as the concentration of protons in seawater increases, the protons begin reacting with the carbonate ions, consuming CO_3^{-2} and reforming the bicarbonate molecules. Therefore, as pH decreases the reactions shift toward a higher percentage of bicarbonate ions and a decrease in the percentage of carbonate ions (National Research Council, 2013). For example, at

pH of 8.1, approximately 90% of the inorganic carbon is in the form of bicarbonate ion, 9% is carbonate ion, and only 1% remains as dissolved CO_2, or carbonic acid.

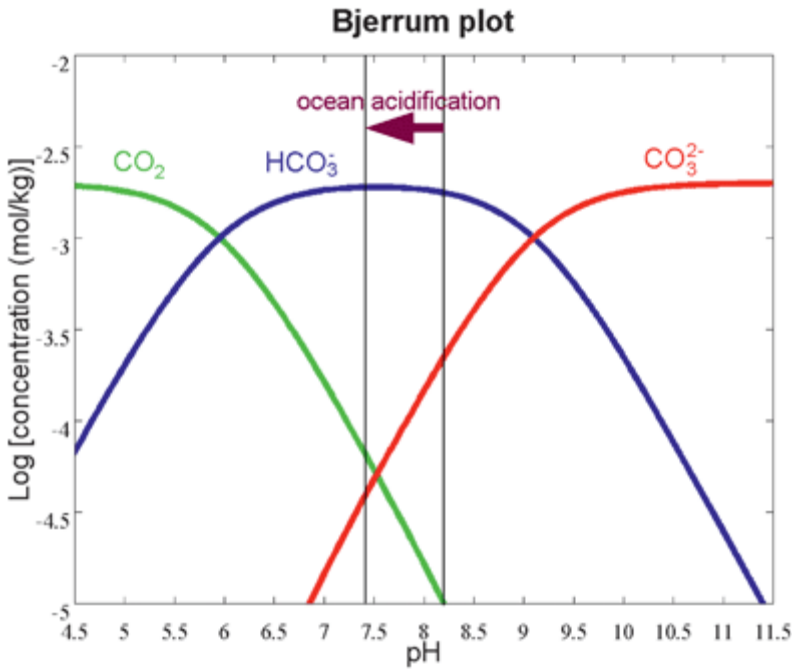


Figure 1. Bjerrum plot illustrating the concentration of DIC species based on pH at DIC=2.1mmol/kg, salinity=35, T=25°C.Reprinted fromOcean acidification: a millennial challenge by M. Hoffman and H.J Shellnhuber, 2010, *Energy and Environmental Science, p1883.*

Acidity is measured as the quantity of protons in the water, thus the increase in protons from CO_2 uptake results in "acidification of the ocean." Acidity is usually measured on the pH scale, which is an inverse, logarithmic scale of the concentration of protons in the water.

$$pH = -\log[H^+]$$

Equation 2. Definition of pH

Therefore, an increase in the concentration of protons, also known as an increase in acidity, is manifested as a decrease in pH. Because the pH scale is logarithmic, a decrease in a unit of pH represents a 10-fold increase in the acidity of the water.

Because the pH of the ocean can change temporarily due to processes like volcanic activity and CO_2 from ocean floor venting, scientists from the Intergovernmental Panel on Climate Change (IPCC), define ocean acidification as "a reduction in the pH of the ocean for an extended period, typically decades of longer, which is primarily caused by the uptake of carbon dioxide from the atmosphere" (National Research Council, 2013). Based on ice cores and boron isotopes, scientist have calculated that since preindustrial times, the average pH in the ocean surface has fallen from 8.21 to 8.10 which corresponds to approximately a 30% increase in the hydrogen ion concentration (NOAA, 2013).

How fast and how much the pH of the ocean can change depends on the alkalinity of the water. Alkalinity can be thought as a measurement of capacity of seawater to resist changes in pH (Shigui Yuan, 2006). The total alkalinity (TA) is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors (weak bases) over proton donors (weak acids) in one kilogram of water (Dickson, Sabine, & Christian, 2007). The following expression represents the major weak acids and weak bases in seawater:

$$TA= [HCO_3^-]+ 2[CO_3^{2-}]+[B(OH)_4^-]+[OH^-]+[HPO_4^2]+2[PO_4^3]+ [SiO(OH)_3-]+[NH_3]+[HS^-]-[H^+]-[HSO_4^-]-[HF]-[H_3PO_4]$$

Equation 3. One of the most used definitions of total alkalinity. This definition was published by Andrew Dickson in 1981. Equation adapted from *CO_2 in Seawater: Equilibrium, Kinetics, Isotopes* (p.28)by E. Zeebe and D. Wolf-Gladrow, 2001, Copyright by Elvesier Oceanography Series.

Since ocean acidification diminishes the amount of dissolved carbonate ions, it also limits the formation of calcium carbonate ($CaCO_3$), which is an important biological component. Many marine organisms build their shells and skeletons from $CaCO_3$ by extracting dissolved calcium (Ca^{2+}), and carbonate (CO_3^{2-}) ions from the water and combining them to form solid crystals of calcium carbonate ($CaCO_3$) (Barton, Hales, Waldbusser, Langdon, & Feely, 2012). While oceanic concentration of Ca^{2+} is relatively abundant, the concentration of CO_3^{2-} ions decreases along with pH (Barton et al., 2012). Therefore, when ocean acidification causes a drop in carbonate ions (CO_3^{2-}), this lowers the potential of calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions to combine and form calcium carbonate ($CaCO_3$).

Calcium carbonate exists in different forms that are categorized by their crystal structure and by the proportion of other elements that are sometimes incorporated in the crystal structure (Fatherree, 2011). The two major forms of calcium carbonate, aragonite and calcite, have different dissolution properties. Elements present in the water can slip in from time to time and take the place of a calcium atom when the crystal is being formed. One of the forms of calcium

carbonate, called calcite forms crystals with a rhombohedral pattern and incorporates magnesium, manganese, and iron (Figure 2) (Fatherree, 2011). Another form of calcium carbonate, called aragonite, forms crystals with an orthorhombic pattern and typically incorporates strontium atoms (Figure 2) (Fatherree, 2011). The structure of aragonite is less stable than that of calcite, so it is more apt to dissolve under similar conditions. In fact, aragonite is about twice as soluble as calcite (Barton et al., 2012). All calcifying invertebrates use one or both of these forms of calcium carbonate to form their skeletons and certain appendages. However, because aragonite is more prompt to dissolution, the decrease in oceanic pH caused by ocean acidification is expected to have a greater impact on calcifying organisms that use aragonite as a building block (Barton et al., 2012).

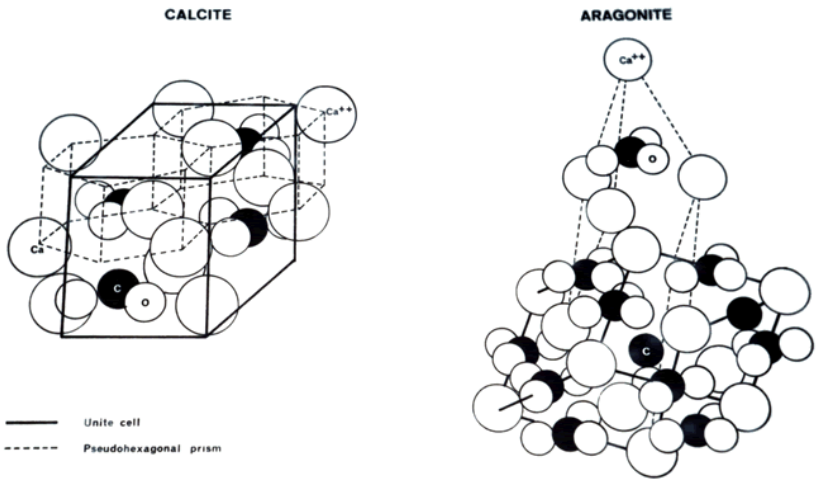


Figure 2. Molecular structures of calcite and aragonite. Reprinted from William Pengelly Cave Studies Trust. Retrived January 4, 2014 fromhttp://www.pengellytrust.org/museum/aragonite.htm.

The “potential” or “energetic favorability” for calcium and carbonate ions

to combine and form calcium carbonate (in the form of calcite or aragonite)is proportional to the saturation state Ω (omega) defined by Equation 3:

$$\Omega_f = \frac{[CO_3^{=}][Ca^{2+}]}{K_{sp,f}}$$

Equation 4. Definition of saturation state. Equation reprinted from the "Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification impacts" by A. Barton, et al., 2012, *Limnology Oceanogr*aphy, 57(3), p698. Copyright 2012 by the Association for the Sciences of Limnology and Oceanography, Inc.

where the subscript f refers to the phase of the mineral being formed, $Ksp,_f$ is the thermodynamic solubility product of that phase and the braquets indicate the concentration of such ions (Barton et al., 2012). Essentially Ω_f is a ratio of the concentration of dissolved ions currently present in a seawater to the concentration of dissolved ions in seawater that is saturated with respect to such ions (Mackie, McGraw, & Hunter, 2011)**.**

When $\Omega_f > 1$, the formation of calcium carbonate structures is favored and excess calcium carbonate precipitates from the water (Barton et al., 2012). The value at which $\Omega_f = 1$ is called that saturation horizon, at this value the concentrations of calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions are in equilibrium with the concentration of their calcium carbonate form ($CaCO_3$). When $\Omega_f = 1$ the rate of dissolution of ($CaCO_3$) into (Ca^{2+}) and (CO_3^{2-}) is the same as the rate precipitation of (Ca^{2+}) and (CO_3^{2-}) into ($CaCO_3$) (Equation5).

$$\text{At } \Omega=1 \; CaCO_{3\,(s)} \leftrightarrow Ca^{+2} + CO_3^{-2}$$

Equation 5. Equilibrium between the soluble and insoluble forms of calcium carbonate at the saturation horizon. Equation adapted from "Future changes in the Baltic Sea acid–base (pH) and oxygen balances" by A. Omsted et al.,2012, *Tellus B: Chemical and Physical Meteorology Vol 64,* Retrieved from http://www.tellusb.net/index.php/tellusb/article/view/19586/htm. Copyright 2012 by Tellus B.

When $\Omega f < 1$ not only organisms have a hard time extracting the carbonate ion from the water, but some of their calcium carbonate structures begin to dissolve as the free floating protons attack the carbonate ions in their shells and skeletons (Feely, Sabine, Hernandez-Ayon, Ianson, & Hales, 2008). Omega (Ω) values are different for calcite and aragonite since these crystal forms have different solubility. Because aragonite is much more soluble than calcite, the aragonite saturation horizon is always nearer to the surface than the calcite saturation horizon (IPCC, 2007). In surface seawater at 25°C and 35 salinity the $Ksp_{(calcite)} \approx 4.3 \times 10^{-7}$ and $Ksp_{(aragonite)} \approx 6.5 \times 10^{-7}$ (Doug Mackie, 2011).

The reduction in oceanic pH coupled with the decrease in the percentage of carbonate ions is changing the structure and productivity of the ocean's biota. Marine organisms evolved to pre-industrial pH and carbonate levels. Studies show that since the Industrial Revolution, oceans have lost approximately 16% of their carbonate ions, which means that calcifying organisms now have less available carbonate to build their skeletons and shells (Barton et al., 2012). In geological time, 250 years is a very short time to adapt to rapidly decreasing pH, carbonate levels, and an increase in dissolved CO_2. As a result, many marine organisms are now exhibiting a decrease in calcification rates, reproduction rates,

abundance, productivity, and range (Portner, 2008). A small number of organisms seem to be benefiting from the decrease in pH and high levels of CO_2 (Portner, 2008). However, the fraction of organisms that are benefiting from these chemical changes is small in comparison with the fraction of organisms that are being negatively affected (Portner, 2008).

RATE OF CHANGE OF OCEAN CHEMISTRY RELATIVE TO PAST EVENTS AND CURRENT CAUSES OF OCEAN ACIDIFICATION

Ocean acidification is a direct consequence of rising atmospheric CO_2 levels (Doney et al., 2009). The chemistry behind ocean acidification is well understood and the causes of ocean acidification have been verified by computer models, hydrographic surveys, and time series data (Doney et al., 2009).

Several stations around the word have been recording how the decrease in pH corresponds to an increase in dissolved CO_2. The Hawaii Ocean Time-Series (HOT) station ALOHA has been recording the increase in atmospheric CO_2 and the increase of oceanic dissolved CO_2 since 1988 (Figure 3). Other time series studies have confirmed this trend, studies such as the Bermuda Atlantic Station Time-Series Study, and European Station Time-Series, have documented the progressive decrease in oceanic pH since the 1980's (Doney et al., 2009). All of these time series studies indicate that the oceanic pH has been decreasing at a rate of 0.02 units per decade (Doney et al., 2009).

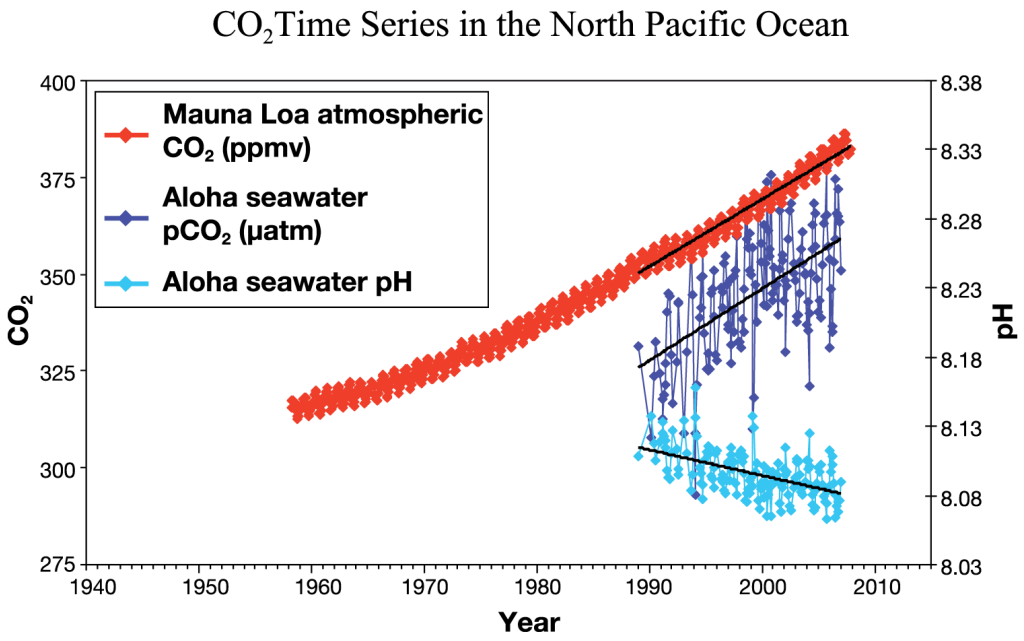


Figure 3. The Time Series for Station ALOHA in Mauna Kea Hawaii shows that the increase in atmospheric carbon dioxide is correlated to an increase in the dissolved CO_2 in seawater (pCO_2) and a decrease in the pH of the seawater (Doney et al., 2009).

Additionally, studies have confirmed that the increase in atmospheric CO_2 is due to anthropogenic CO_2 emissions and not due to natural causes such as an increase in respiration rates. Fossil fuel combustion and the production of cement, followed by deforestation, are the main causes of today's ocean acidification (IPCC, 2013). It has been estimated that between 1800 (the beginning of the Industrial Revolution) and 1994 the oceans have absorbed about 48% of the total CO_2 emitted by human activities (The Royal Society, 2005).

Furthermore, computerized models that simulate the Earth's physical properties (ocean currents, climatic patterns, ocean depth, etc) have predicted steep decreases in pH if we continue with the current rates of human CO_2 emissions onto the future (Doney et al., 2009; National Research Council, 2013). Computerized models predict a further decrease of 0.3–0.4 pH units, which is

equivalent to a 100-150% raise in acidity, by the end of this century (Doney et al., 2009). The models also indicate that by the year 2100 the ocean pH will reach between 7.6 and 7.9 pH units if we continue with "business as usual" (The Royal Society, 2005).

Although fluctuations in atmospheric CO_2 levels have been common throughout Earth's history, past increases in CO_2 occurred over millions of years and thus the rate of increase of CO_2 differs greatly from the current rapid increase driven by human activities (Figure 4) (National Research Council, 2013). In the past, when atmospheric CO_2 raised slowly, because of increased respiration rates and volcanic activity, ocean pH and carbonate levels remained relatively stable. This was because the slow raise in CO_2 levels was balanced by the rate of dissolution of existing calcium carbonate deposits in the ocean (thousands of years), the weathering of terrestrial rock (hundred thousand years or more) and release of minerals and gases from tectonic processes (millions of years) (National Research Council, 2013). However, the current rate of dissolution of CO_2 into the ocean water is faster than the time required for natural processes to buffer the changes in the pH of the ocean.

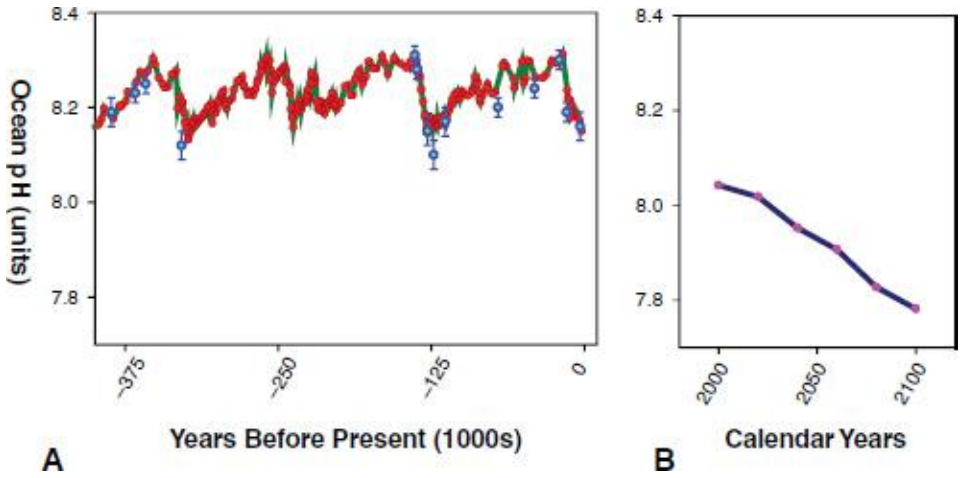


Figure 4. Estimated past, present, and future average oceanic pH. The pH in Panel A was calculated from boron isotopes, planktonic foraminifera shells and from ice core records of pCO_2, where alkalinity, salinity, and nutrients were assumed to remain constant. In panel B, the scale of the x-axis has been expanded to illustrate the pH trend projected over the next century. Future pH values (average for ocean surface waters) were calculated by assuming equilibrium with atmospheric pCO_2 levels and constant alkalinity. Future atmospheric pCO_2 levels were assumed to follow the business-as-usual CO_2 emissions scenario. Reprinted from "Ocean Acidification: A National Strategy to Meet the Challenges of a Changing Ocean" by National Research Council, 2010. Copyright 2010 National Academies Press.

OCEAN ACIDIFICATION AND PUGET SOUND

a) Puget Sound description

Puget Sound is an inlet of the Pacific Ocean in western Washington State. Puget Sound is composed of a complex estuarine system of interconnected fjords and basins comprising 2329 km^2 including 168 km^2 of water. There are four major divisions in the Sound that are categorized by presence of sills, or submarine ridges that constrict the flow of water from one subdivision of the Puget Sound Basin to the next (Nelson, 1999). These divisions are the Main Basin, Whidbey Basin, Southern Basin, and Hood Canal Basin. The Main Basin is comprised by Admiralty Inlet and the Central Basin (Figure 5) (Nelson, 1999).

Figure 5. Map of Puget Sound with its respective basins. Reprinted from Wikimedia Commons: Map of Puget Sound, n.d. Retrieved Octber 28, 2014 from http://commons.wikimedia.org/wiki/File:Map-pugetsound-vector.svg

The relative volume and area, in each basin is illustrated in the following table (Table 1) adapted froms Julie Nelson's *Physical and biological oceanography of the Puget Sound* (n.d).

	Main Basin	**Main Basin**	**Whidbey Basin**	**Southern Basin**	**Hood Canal Basin**
	Admiralty Inlet	Central Basin			
Area	16%	30%	23%	16%	15%
Volume	13%	46%	17%	9%	16%

Table 1: Subdivisions of Puget Sound and their relative water volume of the 168 cubic kilometers of total water volume of Puget Sound (Nelson, 1999).

Barring water input from precipitation, water enters Puget Sound from river and stream runoff at the surface, and from ocean upwelling at the bottom (Lincoln, 2000). Water mostly exits on the surface, through the seaward end via Admiralty Inlet and the Strait of Juan de Fuca (Lincoln, 2000). Puget Sound extends approximately 160 kilometers (100 miles) from Deception Pass in the north to Olympia in the south (Lincoln, 2000). Its average depth is 62 meters (205 feet) and its maximum depth, off Point Jefferson between Indianola and Kingston, is 280 meters (930 feet) (Lincoln, 2000). The depth of the Main Basin, between the southern tip of Whidbey Island and Tacoma, Washington, is approximately 180 meters (600 feet) (Lincoln, 2000).

In Washington, the effects of ocean acidification are intensified by the ocean circulation patterns and by anthropogenic influences. The relative importance of these local drivers varies by location and by season (Washington

State Blue Ribbon Panel on Ocean Acidification, 2012). For example, acidification along the outer coast of Washington and Puget Sound is strongly influenced by coastal upwelling while acidification in shallow estuaries, including those in Puget Sound, may be particularly influenced by eutrophication (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

In Puget Sound, observations show that during the winter the waters are well-mixed and less acidic, while summer and fall are characterized by poorly-mixed, layered waters that confine corrosive waters to deeper subsurface areas (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Many parts of Puget Sound are corrosive to aragonite in the deeper waters. The following section introduces the local drivers that intensify the effects of ocean acidification in Puget Sound.

b) Upwelling

In the Pacific Northwest, upwelling currents bring highly acidified deep waters to the ocean's surface (Welch, 2013). In all oceans, only the surface layer of the ocean (down to about 100 m on average) is well mixed and in contact with the atmosphere (The Royal Society, 2005). The $CO_{2(gas)}$ dissolves unto the surface waters following the concentration gradient between the atmosphere (more CO_2) and the ocean (less CO_2) (Archer, 2010). The carbonic acid formed gets transported into the deep ocean by downwelling currents, which occur when currents converge or when the wind drives the surface waters against the coastline, and by the biological pump (Archer, 2010; NOAA, 2014).

The biological pump is the mechanism by which marine organisms cycle oceanic carbon (Alley, 2002) Plankton and other photosynthetic organisms take up CO_2 during photosynthesis and convert it to biomass. A portion of this biomass gets eaten by heterotrophs, which convert the carbon in the biomass into fecal pellets that sink easily; another part of the biomass dies and sinks to the bottom of the ocean. The fecal pellets and the dead biomass that sinks into the deep waters decomposes thanks to the action of detritivores, releasing CO_2 back into the water (Alley, 2002; Archer, 2010). As more CO_2 is transferred into deeper colder waters, these waters become saturated with CO_2 and become "acidified" (Welch, 2013). Thermohaline circulation pushes deep cold waters from the North Pacific Ocean unto the Pacific West Coast (Hickery & Banas, 2003). Once the acidified deep waters reach the West Coast, they resurface due to a process called "upwelling." In the Pacific Northwest, upwelling happens when strong northerly winds push the surface water away from the coast (Figure 6) (NOAA, n.d.-a). These winds transport offshore surface water southward (orange arrow in Figure 6), with a component transported away from the coastline due to the Earth's rotation (light green arrow) (NOAA, n.d.-a). This makes room for the deeper colder waters to travel along the continental shelf and replace the wind-blown waters (dark blue arrow) (Welch, 2013). Thus, upwelling currents bring acidified waters to the surface. The water that is resurfacing right now in the Pacific Northwest was last exposed to the atmosphere a half-century ago, when CO_2 levels were much lower (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). This means that the water that will be upwelled in the future

will be increasingly be more corrosive.

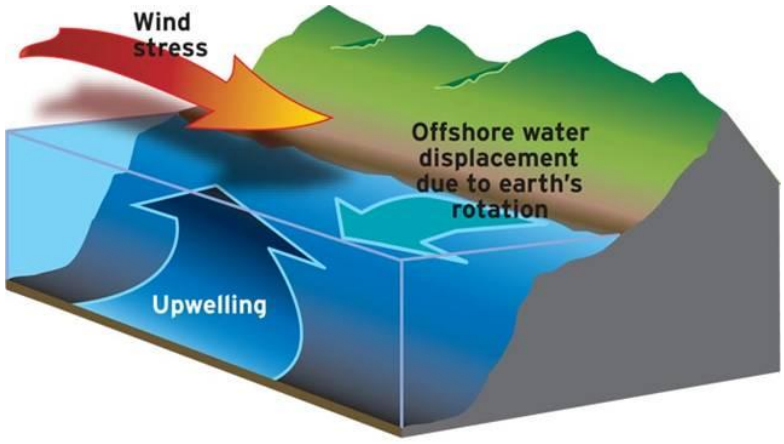


Figure 6. Diagram of and upwelling current along the coast of Washington State. Reprinted from Coastal Upwelling by NOAA, n.d. Retrieved from http://www.nwfsc.noaa.gov/research/divisions/fe/estuarine/oeip/db-coastal-upwelling-index.cfm

In the Pacific Northwest, upwelling winds are prevalent in the late summer and early fall, usually from April to November, off the Washington and Oregon coast (Feely et al., 2012). As these winds push the surface water west and away from the coast, water upwells into the Strait of Juan de Fuca, flowing over into Puget Sound (Nelson, 1999; Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Historically, upwelling currents were considered beneficial for the economy and for the ecosystems because they brought nutrients back to the surface. As marine organisms die, most of the carbon is consumed by other organisms in the surface waters or released back to the atmosphere by the decomposition process (The Royal Society, 2005). However, some of the organic material falls as particle sediments to deep waters. Upwelling currents bring large

amount of this organic material, which is nutrient rich, back to the surface. This organic material "fertilizes" the surface waters. Thus, areas with upwelling currents have high biological productivity and are considered good fishing grounds (Thomson, 1981). However, as human CO_2 emissions increase, the beneficial effects of upwelling currents are being overrun by the effects of ocean acidification (Feely et al., 2002).

c) Shallow carbonate saturation horizons

The North Pacific Ocean has a shallower saturation horizon for both calcite and aragonite than other regions in the world. Two things account for this difference in the calcite and aragonite horizons: the latitude, and the deep ventilation and deep-water currents (Doney et al., 2009; Feely et al., 2002). First, since calcium carbonate ($CaCO_3$) solubility increases with decreasing temperature and pressure, carbonate saturation states are lowest in cold high-latitude regions, such as in the Pacific Northwest and at depth (Doney et al., 2009). Second, the deep ventilation and deep-water circulation in the Pacific North permit the accumulation of CO_2 from both anthropogenic and natural sources, and as waters become acidified, the saturation horizon lowers (Feely et al., 2002). The difference in saturation horizons depths between the North Pacific Ocean and other regions has been documented. For example, Feely et al (2002) determined that the aragonite saturation horizon ranges from 120-580 meters in the North Pacific compared to 200-1320m in the South Pacific.

Human CO_2 emissions have resulted in the shoaling of the calcite and

aragonite horizons in all oceans; however, in the Pacific Northwest this shoaling has been more dramatic than in other regions (Feely et al., 2008; IPCC, 2007). In the Pacific Ocean, there is a pronounced shoaling of the aragonite and calcite saturation states from south to north and from west to east because of the higher total dissolved inorganic carbon (DIC) concentrations in northern and eastern regions relative to the alkalinity concentrations (Figure 7). This means that in the North Pacific Ocean waters are already naturally more acidic (Feely et al., 2002). When studies compared the preindustrial saturation horizons to the present-day saturation horizons, a steep reduction in the saturation horizon depth was detected in the Pacific North Ocean where the horizon was reduced by 30-100 meters as compared to 30-80 meters in the South Pacific (Feely et al., 2002).

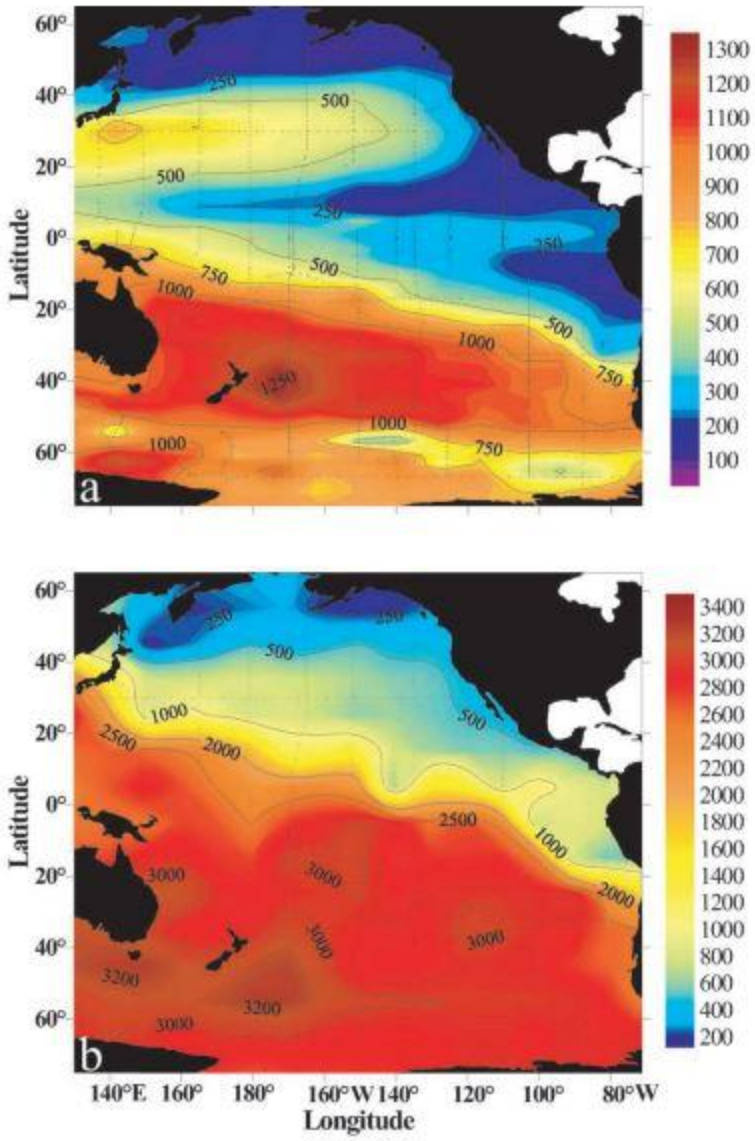


Figure 7: Estimated aragonite (top) calcite (bottom) saturation horizon depths, in meters, for the Pacific Ocean for the year 2002. Figure reprinted from "In situ calcium carbonate dissolution in the Pacific Ocean" by Feely et al.,2002, Global Biogeochemical Cycles, Vol 16, No 14 p91-6.

In Puget Sound, the subsurface waters from the Juan de Fuca Straight to the Main Basin are usually undersaturated with respect to aragonite in the winter and summer (Feely et al., 2010). In the summer, upwelling waters enter the Juan the Fuca Straight and mix with the inland waters of Puget Sound thanks to tidal

currents and vertical mixing, thus decreasing $\Omega_{aragonite}$ values. In the winter, decreased photosynthesis and runoff lead to hypoxic conditions and thus to the undersaturation of aragonite (Feely et al., 2010). The combination of ocean acidification and the complex pH patterns that exist in Puget Sound, have already caused a decrease of 0.05–0.15 pH units in surface waters and a decrease of 0.09–0.33 points in the aragonite saturation state (Busch, Harvey, & McElhany, 2013).

d) Long residence times

An important physical characteristic of an estuary is its ability to exchange water with the open ocean. Exchange helps cleanse the deep basins of the sound and prevent them from becoming naturally stagnant from organic decay. Exchange has also played a role in the transport of pollutants from Puget Sound into the open ocean. When an estuary, like Puget Sound, has a slow "rate of exchange" with the open ocean, acidified waters get trapped in the estuary for long periods of time (Andutta, Ridd, Deleersnijder, & Prandle, 2013).

Residence time, is a measure of how long it takes to completely flush out an estuary (Andutta et al., 2013). This measurement gives scientists an idea of how long it takes for an estuary to flush out its water and replace it with new water. In order to calculate the residence time, scientists usually use computer models that place tracers on "virtual particles" and then run simulations to determine of how much time it takes to flush those particles out of the system (Andutta et al., 2013).

The residence time of the different basins in Puget Sound varies according to the seasonal winds, the freshwater inputs from rivers and melted snow, the tidal currents inside of Puget Sound, and the upwelling currents in the ocean. In general, Puget Sound is considered to have slow residence times because most of the water ends up recirculating multiple times inside the sound before exiting to the ocean (Table 2) (Washington State Department of Ecology, 1986). For example, fresh water on the surface of the Main Sub-basin takes about a week to get from the mouth of the Dunamish River to the Admiralty sill (Entranco Engineers, Inc, 1988). Then, due to the local current, this water spends about 10 days going back to its starting point; the surface water must make the trip twice, on the average, before reaching the Strait of Juan de Fuca and exiting to the ocean (Entranco Engineers, Inc, 1988).

	Replacement Time (days)				Net Seaward Transport ($10^3 m^3/s$)			
	Whidbey Basin	Southern Puget Sound	Hood Canal	Entire Puget Sound	Whidbey Basin	Southern Puget Sound	Hood Canal	Entire Puget Sound Area
January-February	46	33	272	113	7.3	5.5	1.1	17.2
February-March	32	45	85	62	10.5	4.1	3.4	31.4
March-April	54	41	202	582	6.2	4.5	1.4	3.3
April-May	30	28	97	166	11.2	6.5	3.0	11.7
May-June	44	114	672	184	7.7	1.6	0.4	10.6
June-July	25	54	328	99	13.5	3.4	0.9	19.7
July-August	30	80	152	124	11.2	2.3	1.9	15.7
August-September	47	70	191	132	7.2	2.6	1.5	14.8
September-October	93	80	149	407	3.6	2.3	1.9	4.8
October-November	57	51	215	120	5.9	3.6	1.3	16.2
November-December	76	174	183	480	4.4	1.1	1.6	4.1
December-January	18	35	101	146	18.7	5.2	2.9	13.3
MEAN	40	56	177	152	8.4	3.3	1.6	12.8

Table 2. Calculated residence times (replacement times) for the major subdivisions of Puget Sound during different months. Reprinted from "State of the Sound, 1988" report by Entranco Engineers, Inc, 1988, Puget Sound Water Quality Authority.

e) Eutrophication

The near surface waters of the Puget Sound are highly productive due to nutrients delivered from upwelled waters and rivers that flow into the estuary (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Human activities often increase the flow of nutrients from land to marine waters resulting in eutrophication, or the over-abundance of nutrients in the water. Eutrophication can substantially acidify the water by causing algae blooms (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). When the bloom ends, the algae die and sink to the bottom where they are broken down by decomposing bacteria that consume oxygen and release large amounts of carbon dioxide (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). If this happens, the water becomes supersaturated with carbon dioxide which leads to a higher concentration of protons and thus a considerable decrease in pH (Abril et al., 2003)

In Puget Sound, agricultural runoff, pollutants, and soil erosion can acidify coastal waters at substantially higher rates than atmospheric carbon dioxide alone (Abril et al., 2003; Feely et al., 2010; Kelly et al., 2011). Municipal and industrial wastewater discharges can significantly reduce the pH of the water near the discharge point, especially in poorly flushed areas (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Anthropogenic eutrophication has caused several “dead zones” in Puget Sound throughout the years. A dead zone is an area that does not have enough

oxygen to support marine life (NOAA, 2014b). As the oxygen decreases, many organisms die or leave the area and the zone becomes a biological dessert (NOAA, 2014b). An area that has had dead zones repetitively through the years is Hood Canal (Moriarty, 2011). Hood Canal is a popular waterway with a booming year-round population on Puget Sound's west side. The dead zones in Hood Canal have been caused by overloaded and failing septic systems and by oil spills. Extensive dead zones caused massive fish kills in Hood Canal in 2003, 2006, and 2010 (Moriarty, 2011). Other dead zones have appeared in other Puget Sound locations such as: West Point in Seattle, Budd Inlet in Olympia, Penn Cove on Whidbey Island and Bellingham Bay (Moriarty, 2011). In 2008, Washington Department of Ecology developed a computer model and water-sampling program to identify the anthropogenic nutrient inputs and points of low dissolved oxygen in Puget Sound (Mohamedali, Roberts, Sackmann, & Kolosseus, 2011). This program identified more than 100 locations where the water quality was impaired due to low oxygen concentrations and/or high levels of pollutants (Figure 8) (Mohamedali et al., 2011).

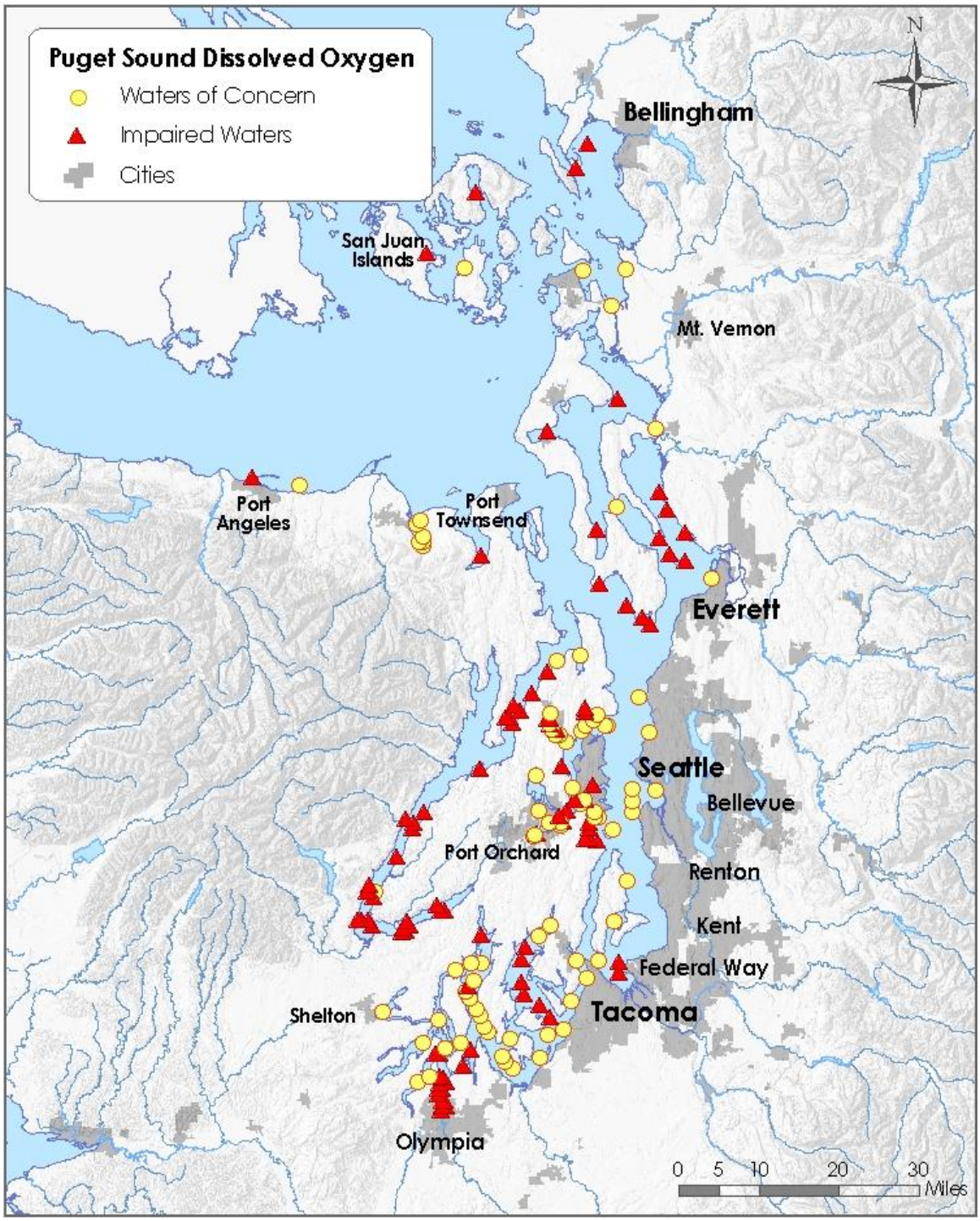


Figure 8. Locations of impaired water quality areas in Puget Sound in 2008. Reprinted from Puget Sound Dissolved Oxygen Model Nutrient Load Summary for 1999-2008. Washington State Department of Ecology, p1.

f) Freshwater inputs

Freshwater inputs from rivers can contribute to ocean acidification by delivering large quantities of freshwater and dissolved organic carbon. Freshwater usually has a lower pH than saltwater; the pH of freshwater is dependent on the dissolved minerals and organic materials that the water carries (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). pH values for freshwater range from 6.5 to 8.5 in Puget Sound with values usually averaging around 7 pH units (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Since freshwater is typically more acidic than saltwater, the areas where freshwater and seawater meet can sometimes be corrosive to calcifying organisms (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

BIOLOGICAL AND ECOLOGICAL IMPLICATIONS OF OCEAN ACIDIFICATION IN PUGET SOUND

a) Phytoplankton

Phytoplankton, also known as microalgae, are microscopic, free-floating, unicellular photosynthetic organisms (NOAA, n.d.-b). Like land plants, phytoplankton have chlorophyll to capture sunlight and turn it into chemical energy. Phytoplankton consumes carbon dioxide, and release oxygen. All phytoplankton photosynthesize, but some get additional energy by consuming other organisms (NOAA, n.d.-b). Phytoplankton are either naked, cells surrounded by only a cell membrane, or surrounded by calcified structures in the

form of scales of shells (Feely et al., 2012).

Ocean acidification is expected to result in substantial alterations the distribution, composition of phytoplankton populations (Blue Ribbon Panel on Ocean Acidification, 2012). These effects will influence the composition and productivity of marine ecosystems, and possibly the global cycling of carbon (Feely et al., 2012).

Phytoplankton species have shown diverse responses to elevated values of carbon dioxide partial pressures (pCO_2) under laboratory conditions. The partial pressure of carbon dioxide(pCO_2) is defined as the pressure that would be exerted by the molecules of carbon dioxide if all the other gases were removed from the air (Jacob & Mickley, 2014). As pCO_2 increases more CO_2 dissolves in the surface waters following the concentration gradient. Studies show that an increase in pCO_2 (and correspondingly and increase in dissolved CO_2) result in increases and decreases in growth rate (depending on the specie), change in calcification rates, decreased size, changes in their nutritive value, and changes in the production of toxic compounds (Feely et al., 2012). The vast taxonomic diversity encompassed by phytoplankton contributes to the differences in responses; genetic variability within the same specie has also been reported to influence phytoplankton's response to ocean acidification (Feely et al., 2012).

Phytoplankton's possible production of toxic compounds in response to ocean acidification is of concern because such compounds are toxic to humans and fish. The effects of these toxins will be explored in the following section

titled “Socioeconomic Impacts of Ocean Acidification in Puget Sound.”

b) Animal Calcifiers

In Puget Sound, 30 percent of marine life — some 600 species — draw upon carbonate ions to grow (Welch, 2013). Puget Sound calcifiers include calcifying plankton, oysters, clams, scallops, mussels, abalone, crabs, geoducks, barnacles, sea urchins, sand dollars, sea stars, and sea cucumbers, and many other organisms. Even some seaweeds produce calcium carbonate structures (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Ocean acidification is affecting shell formation rates, energy usage, and survival of shellfish larvae (Talmage & Gobler, 2010; Waldbusser et al., 2013). The larvae of Pacific oyster *Crassostrea gigas* (Waldbusser et al., 2013), northern quahog clam *Mercenaria mercenaria*, and the bay scallop, *Argopecten irradians* (Talmage & Gobler, 2010) have all shown increased mortality at current and future seawater pH levels. As Waldbusser (2013), explains young shellfish larvae do not have developed feeding organs; thus, they rely on the energy they extracted from the egg to build their shell. For example, Pacific oyster larvae only have about 48 hours to precipitate roughly 90 percent of their body weight. Since the carbonate ion concentrations are very low in acidified waters, calcifiers have to spend more energy trying to extract these ions from the water. Adult oysters and other bivalves grow slower because of this increased energy expenditure; however, shellfish larvae cannot delay their growth, they must build a shell before they run out of energy. Unfortunately, many larvae do end up running out of

energy before they can develop a protective shell and a feeding organ (Waldbusser et al., 2013).

Ocean acidification can limit the growth of calcifiers. Studies have shown that planktonic calcifiers such as copepods (small crustaceans) and pterapods (small snails) grow more slowly in acidified waters (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). The growth rates of several species of mussels (Gaylord et al., 2011), oysters (Waldbusser et al., 2013) are also decreased under acidified conditions.

Laboratory experiments show that bivalves exposed to current levels of acidity develop shells that are brittle and more easily crushed and bivalves exposed to future levels of acidity show malformations in their shells. Increased levels of dissolved CO_2 have been correlated to decrease in shell strength and thickness in many species of bivalves (Gaylord et al., 2011; Talmage & Gobler, 2010). California mussels *Mytilus californianus* (Gaylord et al., 2011), northern quahog clam, *Mercenaria mercenaria*, and the bay scallop, *Argopecten irradians* (Talmage & Gobler, 2010) have shown that the structural integrity and strength of their shell is compromised at concentrations lower than the present levels of carbon dioxide (390 ppm of pCO_2). For example, in 2010 Tamage and Gobler discovered that scallops grown at pre-industrial levels (250ppm CO_2) had shells with ridges while scallops grown at current pH levels had very few ridges. Additionally, scallops grown under future CO_2 levels (750 ppm) had shells that were riddled with holes, pockmarks, and crevices (Figure 9)

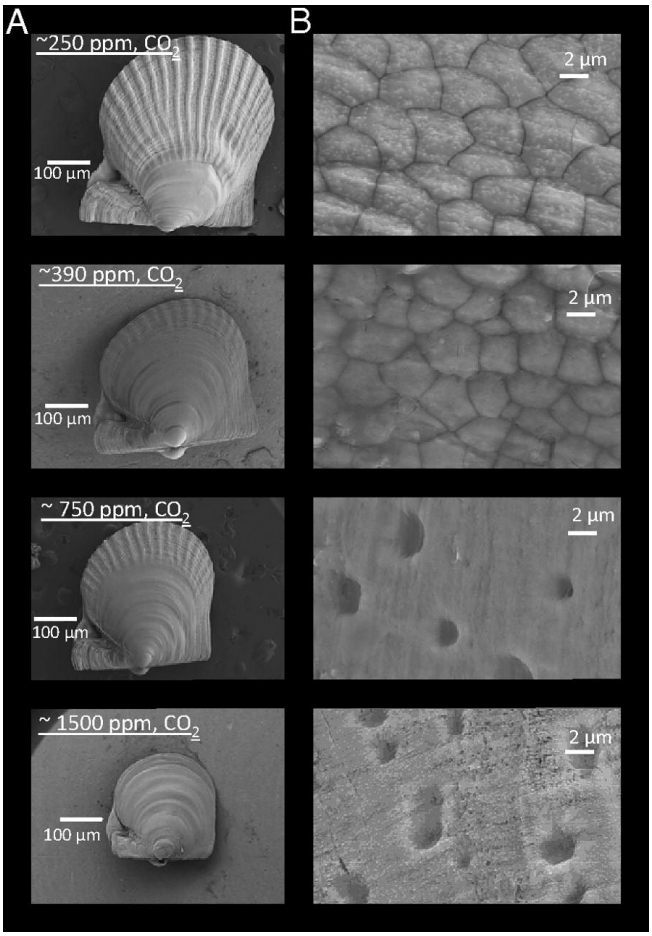


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Figure 9. Pictures and electron micrographs of scallops grown under pre-industrial, current, and future partial carbon dioxide (pCO_2) levels. Under pre-industrial levels of pCO_2 (250 ppm) scallops have ridges. Under current pCO_2 levels (390 ppm) scallops begin losing their ridges. At future pCO_2 levels scallops shells are comparatively small and smooth; shells also show microscopic holes and ridges. Reprinted from "Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish" by S. Talmage and C. Gobler, 2010 *Proceedings of the National Academy of Sciences* Vol 107(40) p 17250

At high levels of ocean acidification, the exoskeleton of calcifiers begins to dissolve. Under the pCO_2 levels predicted for 2100, the shells of pterapods completely dissolve in 45 days (Figure 10). Since pterapods are an important food source for salmon, seabirds, and whales, the increased dissolution of pterapods shells is expected to disturb the food web of Puget Sound (Washington

State Blue Ribbon Panel on Ocean Acidification, 2012)

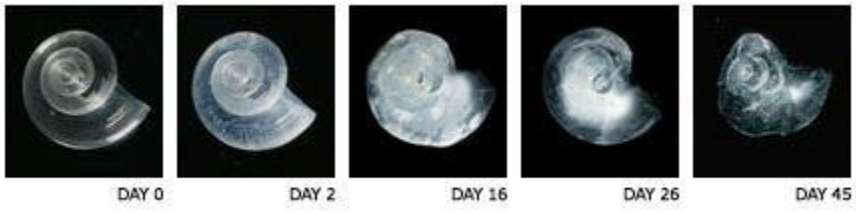


Figure 10. Dissolution of pterapods shells under pCO_2 levels predicted for 2100. Reprinted from *Acid Threat* by J.S. Holland, 2007. Retrieved from http://ngm.nationalgeographic.com/2007/11/marine-miniatures/acid-threat-text. Copyright by National Geographic.

These changes in organisms shell's morphology and functionality result in decrease survival rates. The thinner, frailer shells make individuals more subject to predation and environmental stressors. Mollusks with thinner shells are more prone to predation by crustaceans and carnivorous snails (Gaylord et al., 2011). Additionally, the decrease in shell thickness and strength decreases the odds of organisms surviving the crushing wave action of storms and desiccation caused by tides (Gaylord et al., 2011).

Ocean acidification also has ecological implications in Puget Sound. Calcifiers provide habitat, shelter, and/or food to other organism in the food web; a decline of calcifiers has rippling effects through the ecosystem. For example, rockfish and sharks rely on the habitats created by the deep-water corals of the Olympic Coast (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Yet, such corals are at the frontier of ocean acidification because cold waters have lower pH values than the water that bathes shallow reefs (Guinotte et

al., 2006). In fact, cold-water corals in the North Pacific are thought to be surviving at the marginal levels of the aragonite horizon; any further decrease in pH and these cold water corals will begin dissolving (Guinotte et al., 2006).

c) Macroalagae and seagrasses

Marine macroalgae (seaweeds) and seagrasses are benthic multicellular photosynthetic organisms (Feely et al., 2012). As phytoplankton, macroalgae and seagrasses use the energy from the sun to convert carbon into biomass, releasing oxygen in the process (Feely et al., 2012). Macroalgae belong to the algae family and are structurally much simpler than plants; they lack specialized organelles and cells found in plants. Most species of macroalagae are uncalcified but a few species are calcifiers. Seagrasses are aquatic flowering plants that have long and narrow leaves and grow on meadows that resemble as grassland; thus these marine plants were named "seagrasses" because they superficially resemble the terrestrial grasses (Larkum, Orth, & Duarte, 2006).

Macroalagae are a very diverse group so their response to ocean acidification is expected to vary from specie to specie (Feely et al., 2012). Predictions of their future response to ocean acidification are based on current observed trends and on their physiological requirements (Feely et al., 2012).

Studies show that the relative abundance of non-calcifying versus calcifying macroalgae will change with increasing acidification. Porzio et al (2011) studied the responses of 101 species of macroalgae from around the world to a natural decrease in seawater pH from 8.1 to 7.8 units. This study reported that

there was an overall 5% decrease on macroalgae species richness as the pH decreased to 7.8. They also found that as the pH dwindled, the abundance of calcifying macroalgae decreased while the abundance of non-calcifying algae increased. When the pH reached 6.7, where carbonate saturation levels $\Omega < 1$, calcareous species were absent and there was a 72% reduction in species richness. Under these high CO_2 conditions, Porzio et al. observed an overall decrease in the reproduction rates of most species, with a few exceptions that showed enhanced reproduction rates (Porzio, Buia, & Hall-Spencer, 2011).

Studies show that non-calcifying macroalagae and seagrasses have the potential to increase photosynthesis under acidifying conditions (Feely et al., 2012). According to Washington's Blue Ribbon Panel on Ocean Acidification (2012), most seagrasses and macroalgae are able to use bicarbonate (HCO_3) in addition to CO_2 to fuel photosynthesis. Studies have shown that the non-calcifying algae, unaffected by reductions in carbonate ions, have the potential to increase their growth and photosynthesis under high HCO_3 conditions. Non-calcifying macroalgae and seagrasses also appear to be robust enough to withstand the reduction in seawater pH (Feely et al., 2012).

However, a study by Arnold et al. (2012) indicates than under elevated pCO_2, some seagrasses lose the ability to produce phenolic compounds that protect these plants against herbivores, pathogens and, damage by UV radiation. These effects and implications of ocean acidification on seagrasses will be discussed under the section titled **"**Eelgrass (*Zostera marina*) Biology, Ecology and socioeconimical Importance in Puget Sound", which appears later in this

chapter (Arnold et al., 2012).

d) Ecosystems

The ecological implications of ocean acidification are critical. Scientist predict that at the pH forecasted for the year 2100, ecosystems will have a significant reduction in biodiversity (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Such predictions are based on laboratory experiments that simulate future pH levels, current observations in places with high carbon dioxide levels, and on paleontological research. Scientists have discovered a few places where carbon dioxide levels are naturally high, thanks to under-sea volcano vents. By studying these "natural laboratories" scientist have gained insight on what ecosystems will look like under future carbon dioxide levels (Riebesell, 2008; Washington State Blue Ribbon Panel on Ocean Acidification, 2012). As predicted, these studies confirm that at future ocean acidification conditions (pH < 7.5) there is a pronounced loss of biodiversity no indication of adaptation or replacement of sensitive species by others capable of filling the same ecological niche (Figure 11) (Riebesell, 2008). Paleontological studies confirm this conclusion, research on fossils and chemicals in ancient rocks indicates that past ocean acidification events (due to natural causes such as volcanic eruptions) have been accompanied by major marine extinctions (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Figure 11. Low and high carbon dioxide communities. The figure on the left shows a diverse marine ecosystem in normal (low) carbon dioxide conditions (mean pH 8.2). The photo on the right shows a high carbon dioxide ecosystem (pH 7.8) in the volcanic under-sea vents of Ischia Island in Italy. The high carbon dioxide ecosystem has less biodiversity than the low carbon dioxide ecosystem. Reprinted from Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response by Washington State Blue Ribbon Panel on Ocean Acidification, 2012, Washington Department of Ecology. Copyright by David Littswager (left) and Luca Tiberti (right).

A study done by Busch et al. (2013) showed that ocean acidification will result in drastic changes to the structure of Puget Sound's food web. This study was based on a computer simulation model of Puget Sound's food web. In this simulation, scientists removed or decreased the abundance of various functional groups of organisms, based on the current and predicted effects of ocean acidification (Busch et al., 2013). The computer analysis showed that ocean acidification resulted in increases in the biomass of some groups and decreases in other groups. For example, a decrease in the population of copepods (predator) resulted in an increase in the population of microzooplankton (prey) and a decrease in the population of herring (feeds on copepods) (Busch et al., 2013). A combined scenario, considering multiple decreases in the population of functional groups affected by ocean acidification, resulted in an overall decrease in the

amount of seafood that the food web can produce. For example, a moderate scenario assuming a 25% decrease in the productivity of Puget Sound's ecosystems, showed that the following populations will decrease: shrimp (3% decrease), cancer crab (12%), bivalves (2%) ,salmon (3%), herring (3%), salmon, rockfish (2%) , sea urchin (1%), among other species (Busch et al., 2013).

Ocean acidification is expected to cause a shift from calcifying benthic communities to plant-based communities. Since reproductive success of so many calcifying species will be impaired, there will be a community shift from calcifier-based ecosystems, such as coral reefs, to non-calcifying based ecosystems like seaweeds and seagrass beds (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

However, non-calcifying autotroph based food webs are not immune to the effects of ocean acidification. Several studies have shown that ocean acidification reduces the nutrient content of calcifying and non-calcifying photosynthetic organisms, inducing food quality deterioration through the food web (Bellerby et al., 2008; Rossoll et al., 2012; Urabe, Togari, & Elser, 2003). Increased CO_2 stimulates carbon fixation in photosynthetic organisms thereby reducing the nutrient content relative to carbon (Bellerby et al., 2008; Urabe et al., 2003). Other studies, point towards an impact in the synthesis of fatty acids by phytoplankton (Rossoll et al., 2012). Fatty acids play an important role in the development, growth and reproduction of heterotrophs, who can only acquire the nutrient through their diet (Rossoll et al., 2012). A decrease in extracellular pH can affect the intracellular processes of phytoplankton, which control the

enzymatic activity for the production of fatty acids (Rossoll et al., 2012). Thus, a decrease in the phytoplankton's production of fatty acids is expected to constrain the development, growth and reproduction rates of heterotrophic organisms throughout the food web (Rossoll et al., 2012).

SOCIECONOMIC IMPACTS OF OCEAN ACIDIFICATION IN PUGET SOUND

a) Economy

Washington State is the largest producer of farmed shellfish in the U.S, with more than 300 farms accounting for 25% of the total domestic production by weight, and an annual revenue of about $107 million (Pacific Shellfish Institute, 2013). Overall, Washington's seafood industry generates over 42,000 jobs in Washington and contributes at least $1.7 billion to gross state product through profits and employment at neighborhood seafood restaurants (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

The Pacific Northwest shellfish industry has experienced major failures in its oyster hatcheries because the decrease in pH has increased oyster larvae mortality (National Research Council, 2013). Around 2005, Puget Sound oyster farmers began noticing elevated larvae mortality rates during certain parts of the year, namely shortly after the prevailing wind switched and caused seasonal upwellings along the Washington coast. Initially, the farmers thought that the deaths were due to a pathogenic bacterium, *Vibrio tubiashii*, so they invested in expensive filtration systems that would remove the pathogen from the water.

However, the oyster larvae continued dying at an alarming rate. Closer examination of the dead larvae revealed that they were unable to form the required shell studies confirmed that the water being pumped into the hatcheries was acidified (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). In order to utilize water that is suitable for growing oysters, oyster farmers have begun "buffering" the water in the hatcheries by adding antacid substances or more calcium carbonate (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Antiacid substances and calcium carbonate represent an extra cost for hatcheries and therefore a increased cost for the oyster consumer.

In order to guard against ocean acidification effects, some Washington Oyster farmers have relocated their hatchery sites away from Puget Sound. Goose Point Oysters and Taylor Shellfish have established hatcheries in Hawaii where they raise their younglings and then ship them back to Washington for the remainder of their growth cycle. Relocating hatcheries to Hawaii means less jobs in Washington State; if more hatcheries follow this relocation pattern, the state economy could suffer.

b) Tribes

Ocean acidification has also cultural implications. Among Puget Sound's population the Native American community has been affected the hardest by the effects of ocean acidification. Tribes harvest shellfish for subsistence and ceremonial purposes. In fact, almost all of the commercial wild clam fisheries in Puget Sound are tribal (Washington State Blue Ribbon Panel on Ocean

Acidification, 2012).

The predicted decrease in the abundance of pterapods is expected to result to have a negative impact on the abundance of fish species that feed on them, such as pink salmon, mackerel, and herring. These fish species are an important food source for Washington's tribes (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

The possible decline of salmon is of special importance because in addition of being a food source, it also has spiritual and cultural significance for the Pacific Northwest Tribes. For example, many tribes celebrate the annual salmon return as this event signifies renewal and continuation of life. Salmon is also offered as one of the traditional fish foods during religious ceremonies and rituals. One creation legend teaches that salmon is one of the "First Foods":

> *When the Creator was preparing to bring humans onto the earth, He called a grand council of all the animal people, plant people, and everything else. In those days, the animals and plants were more like people because they could talk. He asked each one to give a gift to the humans—a gift to help them survive, since humans were pitiful and would die without help. The first to come forward was Salmon. He gave the humans his body for food. The second to give a gift was Water. She promised to be the home to the salmon. After that, everyone else gave the humans a gift, but it was special that the first to give their gifts were Salmon and Water. When the humans finally arrived, the Creator took away the animals' power of speech and gave it to the humans. He told the humans that since the animals could no longer speak for themselves, it was a human responsibility to speak for the animals. To this day, Salmon and Water are always served first at tribal feasts to remember the story and honor the First Foods.*(The Columbia River Inter-tribal Fish Comission, 2014)

Salmon is also tied to the tribes' sense of place and history. They believe that the creator placed them in the locations where the salmon would return and that they are obliged to remain and to protect these places. Additionally, Northwest Tribes were able to flourish thanks to trade economies based on salmon. Many of the ancient Indian trade routes connected salmon fisheries to towns. Thus, since ancient times salmon has shaped the lives of the Northwest tribes and a decline in salmon population would impacts the tribes sense of place and identity (Columbia River Intertribal Fish Comission, n.d.)

> *"Without salmon returning to our rivers and streams, we would cease to be Indian people"*
>
> -*Indian proverb* (Columbia River Intertribal Fish Comission, n.d.)

> *"My strength is from the fish; my blood is from the fish, from the roots and berries. The fish and game are the essence of my life. I was not brought from a foreign country and did not come here. I was put here by the Creator"*
>
> -*Chief Weninock, Yakama, 1915* (Columbia River Intertribal Fish Comission, n.d.)

A major concern for the tribes is that ocean acidification can be exacerbated by other climate change variables such as an increase in ocean water temperatures, eutrophication and changes in rainfall patterns. Certain tribal areas

are prone to low levels of oxygen which has historically caused fish and shellfish mortalities. Now these tribes are facing the double threat of dealing with hypoxia and ocean acidification at the same time. For example, since 2006, Quinault have documented the mortality of thousands fish and crab during the late summer months due to low oxygen conditions (Handsen Terri, 2014). The threat of ocean acidification combining with low oxygen zones is a concern for the Quinault who are currently working with University of Washington and NOAA scientists determined these hypoxia events were also related to ocean acidification (Handsen Terri, 2014).

Because ocean acidification along with other climate change factors will affect the tribes' natural and cultural resources, several Northwest tribes have already devised climate adaptation plans that include measures to mitigate the effects of ocean acidification. For example, the Jamestown Sk'lallam Tribe created a climate change working group that devised strategies to combat the decline in salmon and shellfish, their strategies include habitat restoration, employing monitoring programs to ensure sustainable harvesting, restoring the natural habitats, monitoring water quality, rebuilding stocks and transplanting shellfish to areas that are more suitable for reproductive success (Jamestown Sk'lallam Tribe, 2013). In a way tribes are at the forefront of the research and mitigation strategies for ocean acidification because tribes practice the seventh generation sustainability principle, which implies that they consider how the next seven generation will be affected by the decisions they make today. Thus, many of their mitigation plans consider how the environment will change during the

next seven generations (Columbia River Intertribal Fish Comission, n.d.)

In 2012 the Hoh, Makah and Quileute tribes and the Quinault Indian Nation organized the inaugural First Stewards symposium, a national event that examined the impact of climate change on indigenous coastal cultures. This event, hosted in Washington D.C, consisted or a dialogue between native leaders, climate scientists, policy-makers and non-government organizations, with the objective of devising adaptations and mitigation strategies to cope with climate change and ocean acidification. This dialogue explored solutions based on scientific research as well as in traditional ecological knowledge.

The First Stewards symposiums continue to be organized every year and the northwest tribes continue to be at the forefront of the mitigation strategies required to combat ocean acidification.

c) Human Health

Recent research has demonstrated that the toxicity of some toxin forming phytoplankton increases under conditions of high CO_2 in seawater. These phytoplankton can produce harmful algae blooms that are characterized by rapid growth and entrainment of toxins. Such toxins are noxious to humans and fish. When shellfish consume this phytoplankton, they too become toxic to humans (Brown, 2012). In the past, harmful algae blooms have resulted in the closure of recreational and commercial fisheries in Puget Sound. Three species of phytoplankton (two species of *Pseudonitzchia* and one of *Karlodinium*) were shown to produce more toxins when grown in seawater with high CO_2

concentrations. These findings suggest that harmful algae blooms inside Puget Sound could become more toxic under conditions of ocean acidification, with consequent impacts on food webs, human health, and economy (Brown, 2012; Feely et al., 2012)

Additionally, the predicted deterioration of nutrient content thorough food webs and the direct impacts on commercially harvested species caused by ocean acidification, is expected to reduce the nutritional quality and quantity of seafood (Branch et al., 2013; Rossoll et al., 2012). As explained in previous sections, ocean acidification might impair seafood production by changing the biochemical composition of algae and its transfer to higher trophic levels and by impacting the metabolism of seafood species (Rossoll et al., 2012). In the future, human population growth will translate to an increased demand for protein, yet fish and mollusk protein quantities are expected to decrease under ocean acidification (Branch et al., 2013). Thus, human diets will be affected and humans will either be forced to find other sources of protein or their health will bear the effects of an improper nutrition.

EELGRASS (*Zostera marina*) BIOLOGY, ECOLOGY, AND SOCIOECONIMICAL IMPORTANCE IN PUGET SOUND

a) Eelgrass as a carbon sink

Seagrasses are flowering plants (angiosperms) adapted to the marine environment that have a grass-like appearance (Larkum et al., 2006; Mumford, 2007). They comprise four marine angiosperm families, 12 genera, and 58 known

species (Hartog & Kuo, 2006). All seagrass species evolved from land plants that returned to the sea approximately 100 million years ago (Touchette & Burkholder, 2000)

Seagrass beds, are one of the most productive ecosystems on Earth and have capacity to sequester sizable amounts of carbon and store it in their sediments (Duarte, Kennedy, Marbà, & Hendriks, 2013). It is estimated that even though seagrass meadows occupy less than 0.1 percent of the world's oceans, they are responsible for 10-20 percent of all carbon buried annually in the sea (Duarte et al., 2011; Greiner et al., 2013).

Due to the noticeable carbon sink capacity of seagrass beds, researchers have proposed that strategies based on the conservation and reforestation of seagrass beds, along with salt-marshes and mangrove forests, could be used to mitigate the effects of climate change and ocean acidification by storing carbon in their sediments (Duarte et al., 2011).

In the Pacific Northwest, the most abundant seagrass specie is *Zostera marina*, a native species also known as eelgrass (S. Beer & Rehnberg, 1997). While only six species of seagrass are present in the Pacific Northwest, *Z. marina* is the dominant seagrass in terms of biomass and areal extent, stretching from southeastern Alaska to Baja California, Mexico (Wyllie-Echeverria & Ackerman, 2003). In Washington State, *Z. marina* beds represent 37% of the shoreline vegetation (Dowty et al., 2005); while in Puget Sound, eelgrass beds are widely distributed covering about 200 km^2 of the shoreline .

Due to its due to its abundance and prevalence in the inland waters of Puget Sound, Z. *marina*, has the potential to serve as a local carbon sink, thus contributing to ameliorate the local effects of ocean acidification (Shishido, 2013). In 2012, the Washington State Blue Ribbon Panel on Ocean Acidification, recognizing the importance of eelgrass, stated the need to "preserve Washington's existing native seagrass and kelp populations and, where possible, restore these populations " in an attempt mitigate and adapt to the effects of ocean acidification (Washington State Blue Ribbon Panel on Ocean Acidification, 2012a, p. 30, Action 6.3.1). Additionally, the panel also recommended the development of "vegetation-based systems of remediation for use in upland habitats and in shellfish areas" (Washington State Blue Ribbon Panel on Ocean Acidification, 2012a, p. 30, Action 6.1.1).

These phyto-remediation strategies have been hindered by the gaps in our knowledge of the mechanisms and rates of carbon sequestration and carbon burial of local seagrasses, particularly of eelgrass (Duarte et al., 2013). Consequently, this thesis project represents an attempt to quantify the effectiveness of local eelgrass beds in mitigating ocean acidification.

The following section represents a summary of the published research on the habitat requirements of eelgrass, and on the presumed mechanisms of carbon sequestration and burial.

b) Eelgrass description

Zostera marina plants are easily recognizable. They have long, narrow, ligulate leaves about 31 to 53 centimeters long (12 to 20 inches) with parallel edges and three veins running along their length (Figure 12 and 13) (Larkum et al., 2006). The leaves are usually green, but when cast up on the shore they turn black, and eventually grayish-white when bleached by the sun (Larkum et al., 2006). Blade width varies with depth. The blades from deeper plants are one to two centimeters wide, while intertidal plants are two to five millimeters wide (Mumford, 2007). Leaves emerge from a perennial creeping rhizome (Larkum et al., 2006). Roots from the rhizome serve as the main means of nutrient uptake (Mumford, 2007).

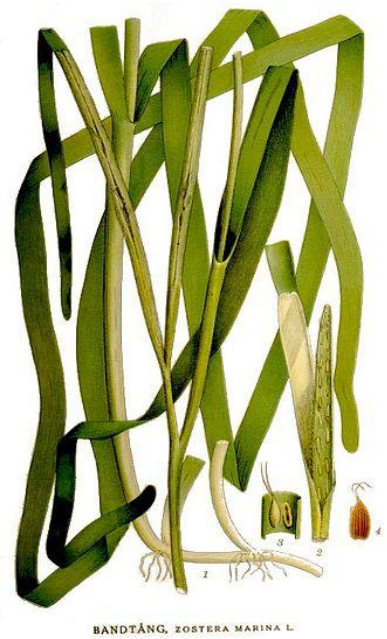


Figure 12. Illustration of *Zostera marina*. Reprinted from *Bilder Ur Nordens Flora* by C.A.M Lindaman 1905. Kessinger Publishing, LLC. Copyright 2010 by Kessinger Publishing, LL

Figure 13. Eelgrass bed on Bainbridge Island, WA. Reprinted from USGS Multimedia Gallery by David Ayers, 2012. Retrieved from http://gallery.usgs.gov/photos/11_07_2012_lPGs3WU321_11_07_2012_1#.VF11sPmor0t

c) Carbon sequestration in seagrass beds

Seagrasses rank amongst the most productive populations on the biosphere (Duarte and Chiscano, 1999). Seagrasses only occupy 0.15% of the ocean surface, yet they contribute to an estimated 1% of the primary net production of the global oceans (Duarte & Chiscano, 1999). On average, net primary production for a square meter area covered by seagrass is about 461 gdw/ m^2 (grams of dry weight per meter square) .This means that seagrasses are about 11 times more productive, in terms of biomass, than macroalgae whose biomass is about 40.7 gdw/ m^2 (Table 3) (Duarte and Chiscano, 1999). The productivity of *Z. marina* is dependent upon the environmental conditions of each area, but estimates of its net primary productivity (NPP) range from about 200 to 341.82 g C/m^2 *yr (Mariko-Shishido, 2013)

Average biomass and net primary production of different plant communities

Community	Biomass (g DW m^{-2})	Production (g DW m^{-2} per day)	Reference
Forests			
Tropical	45000	5.2	Whittaker (1975)
Temperate	35000	3.4	Whittaker (1975)
Boreal	20000	2.2	Whittaker (1975)
Grasslands			
Savanna	4000	2.4	Whittaker (1975)
Temperate	1600	1.6	Whittaker (1975)
Tundra and alpine	600	0.4	Whittaker (1975)
Swamp and marshes	15000	5.5	Whittaker (1975)
Cultivated land	1000	1.8	Whittaker (1975)
Phytoplankton	9.2	0.35	Cebrián and Duarte (1994)
Microphytobenthos		0.13	Charpy-Roubaud and Sournia (1990)
Coral reefs	2000	0.8	B = Whittaker (1975) P = Crossland et al. (1991)
Macroalgae	40.7	1.0	B = Cebrián and Duarte (1994) P = Charpy-Roubaud and and Sournia (1990)
Marsh plants	767	3.0	B = Cebrián and Duarte (1994) P = Woodwell et al. (1973)
Mangroves		2.7	P = Lugo et al. (1988)
Seagrasses	461	2.7	This study

Table 3. Average biomass per meter square of different autotrophic populations. Adapted from "Seagrass biomass and production: a reassessment" By C.M Duarte & C.L Chiscano, 1999, *Aquatic Botany*, 65(4).

In addition to the high productivity of seagrass plants, these plants and sediment beds usually host a variety of associated microalgae and phytoplankton that also contribute significantly to total ecosystem production (Sybill Jaschinski, Daniela C. Brepohl, & Ulrich Sommer, 2008). Thus, even though primary production is dominated by seagrasses, other organisms such as epiphytes, red algae, sand microflora, and phytoplankton inhabiting the same ecosystem can also act as carbons sinks (Sybill Jaschinski et al., 2008) . Seagrass themselves contribute to a modest 1% of the net primary production, yet their ecosystem production is estimated to be 12% of that of the global ocean (Duarte & Chiscano, 1999).

Most seagrasses, including *Z. marina*, and other marine macroalgae can obtain energy from two forms of DIC: aqueous carbon dioxide $CO_{2(aq)}$ and bicarbonate (HCO_3^-) (Koch, Bowes, Ross, & Zhang, 2013; Palacios & Zimmerman, 2007). $CO_{2(aq)}$ is absorbed through passive diffusion, while (HCO_3^-) is dehydrated (either internally or externally) and converted back into $CO_{2(aq)}$ for assimilation (S. Beer & Rehnberg, 1997; Palacios & Zimmerman, 2007). Eelgrass, as most marine macro-autotrophs, actively secrete hydrogen ions (H^+) into localized regions of the surface of its leaves, to lower the pH and promote the dehydration of HCO_3^- (Carr & Axelsson, 2008; Koch et al., 2013). *Z. marina*, and other seagrass species also secrete carbonic anhydrase (CA) from their leaves. CA is an enzyme that catalyzes the interconversion of HCO_3^- to $CO_{2(aq)}$; this enzyme is pH dependent and most effective at low pH values (Koch et al., 2013). It is worth to note that the reaction catalyzed by CA is reversible and can change direction under high pH values (Koch et al., 2013). The $CO_{2(aq)}$ formed in these acid regions of the leaf might be taken up actively, but more likely, it just diffuses through the plasma membrane (Carr & Axelsson, 2008). $CO_{2(aq)}$ is largely absorbed by the leaves of seagrasses with a small uptake happening in the roots and rhizomes (S. Beer, 1989).

The ability of seagrasses and other marine macroalgae to utilize HCO_3^- is advantageous as [HCO_3^-] currently represents about 88% of the total DIC content of seawater (Palacios & Zimmerman, 2007). Furthermore, because seagrasses and macroalgae have a higher affinity for $CO_{2(aq)}$ than HCO_3^-, ocean acidification is expected to enhance their competitive advantage (Koch et al., 2013). Since

seagrasses and macro algae absorb $CO_{2(aq)}$ through passive diffusion and this process requires less energy than the dehydration or active transport of HCO_3^-, these organisms have a higher photosynthetic affinity for $CO_{2(aq)}$ than for HCO_3^- (Koch et al., 2013). It is estimated that marine macroalgae fulfill 80-90% of their carbon requirements from the dehydration of HCO_3^-, while only 10% of their carbon requirements are achieved through the absorption of $CO_{2(aq)}$ (S. Beer & Koch, 1996; Palacios & Zimmerman, 2007). In contrast, seagrasses fulfill 50% of their carbon requirements by dehydrating HCO_3^-, while utilizing $CO_{2(aq)}$ to carry out the remaining 50% of their carbon requirements (Palacios & Zimmerman, 2007). Consequently, the predicted increase of $CO_{2(aq)}$ due to ocean acidification, will likely result in a greater competitive advantage for seagrasses than for macroalgae (S. Beer & Koch, 1996). As the atmospheric $[CO_2]$ continues to rise, by the year 2100, $[CO_{2(aq)}]$ in the ocean will increase by more than 250%, while $[HCO_3^-]$ will only increase by 24%, and $[CO_3^{2-}]$ will decrease by more than 50% (Koch et al., 2013). Even though in absolute terms (mol/kg) HCO_3^- will still be the most abundant specie of DIC, this increase in $[CO_{2(aq)}]$ will reduce the photosynthetic energy expenditure for seagrasses and macroalgae, which will probably result in an increase of biomass (Koch et al., 2013). Evidence of this increase in biomass will be presented later in this chapter.

Once $CO_{2(aq)}$ enters the eelgrass plant, it is photosynthesized using the C3 pathway. Most seagrasses and macroalgae utilize this pathway; however, a few seagrasses, such as *Cimodocea nodosa* utilize the C4 pathway (Koch et al., 2013). High $[CO_2]$ benefits C3 photosynthesis because high levels of CO_2 minimize

photorespiration in C3 plants, thus increasing their photosynthetic efficiency (Koch et al., 2013). In contrast, C4 photosynthesis is saturated at current atmospheric [CO_2], thus C4 plants are operating at their maximum efficiency, and further increases in [CO_2] will not result in higher rates of photosynthesis (Koch et al., 2013). Additionally, C4 photosynthesis is more energy intensive than C3 photosynthesis, hence C4 plants show less productivity at high [CO_2] than C3 plants.

d) Carbon burial in seagrass beds

The high rates of carbon burial in seagrass beds are due to their high primary production, their capacity to capture particles from the water column and deposit them in soils, and their low rate of herbivory (Fourqurean et al., 2012).

Seagrasses develop lush canopies that slow the water flow and trap sediments and organic matter suspended in the water (Fourqurean et al., 2012). Depending on shoot density and seagrass species, the flow reduction due to current deflection by the canopy ranges from 2- to more than10-fold compared to water flow outside the seagrass bed (Duarte et al., 2013). Seagrass canopies can also dampen the waves by creating friction against incoming water (Duarte et al., 2013; van Katwijk, Bos, Hermus, & Suykerbuyk, 2010). This friction reduces the wave size and leads to a wave induced transport of particles known as Stokes Drift, which further contributes to the deposition of sediments and organic matter in seagrass beds (Duarte et al., 2013). Filtering organisms living on the leaves of seagrasses also contribute to trapping and depositing particles in the bed (Marba,

2009). Seagrass meadows retain the particles and sediments they trap because the canopy prevents their re-suspension, and because the sediments are anchored by a dense network of clonal rhizomes and roots that can extend several meters below the ground (Fourqurean et al., 2012; Marba, 2009). In fact, two thirds of the biomass of seagrasses is buried in soil in the form of rhizomes and roots (Fourqurean et al., 2012).

Due to the typically high sedimentation rates in seagrass beds, some of the belowground biomass and the dying annual tissues are progressively buried (Duarte et al., 2010; Mateo, Romero, Pérez, Littler, & Littler, 1997). The amount of biomass that is buried can be quite large thanks to low herbivory rates in seagrasses (Duarte et al., 2010). Duarte and Cebrian (1996) report that only about 18% of the seagrass biomass is consumed by herbivores; in contrast, about 33% of the biomass of macroalgae is consumed by herbivores.

The low herbivory rates are due to nutrient limited tissues, that are hard to digest or/and contain unpalatable and, in some cases, toxic compounds (Duarte et al., 2010; Thayer, Bjorndal, Ogden, Williams, & Zieman, 1984). Seagrasses have high C:N:P (carbon, nitrogen, phosphorous) ratios, which means that these plants have a low nutrient content relative to their fiber content. Gattuso, Frankignoulle, & Wollast, (1998) report that these ratios range from 204:4:1 to 3550:61:1, with an average of 474:24:1. One study by Duarte (1990) reports a C:N:P ratio of 255:15:1 for *Z. marina* (Kaldy, 2006). The content of nitrogen (protein) tends to decline with the age of the leaf, making mature leaves less nutritious (Thayer et al., 1984). Hence, the relatively low content of nitrogen and phosphorous, coupled

with the presence of relatively high amounts of structural carbohydrates, such as lignin, which have classically been viewed as indigestible to many herbivores, limit the amount of predation on seagrasses (Thayer et al., 1984).

Many species of seagrass also contain other types of phenols, sulphated phenols, and sulphated flavones that are toxic, unpalatable, prevent herbivore settlement, and/or bind to proteins and carbohydrates making them impossible to digest (Thayer et al., 1984). *Zostera marina* contains zosteric acid and caffeic acid, which prevent the settlement of marine bacteria, algae, barnacles, and tube worms that can pray on its tissues or disrupt their clonal network (Buchsbaum, 1990; Grignon-Dubois, 2010). *Z. marina* also contains rosmarinic acid, which has antibacterial, antifungal and antiviral properties (Grignon-Dubois, 2010). Additionally, seagrasses have relatively high ash (mineral) content (varying according to specie and location) which is acerbic to herbivores (Thayer et al., 1984). Thus in addition to having a high amount of fiber compared to a low concentration of nutrients, seagrasses contain a wide variety of chemical substances that deter predation.

Due to their high sedimentation rates, abundant underground biomass, and low rates of herbivory, seagrass beds build thick deposits of carbon (both autochthonous and allochthonous) that grow at a rate of 1mm per year (Duarte et al., 2011). The estimated concentration of organic carbon for these deposits is around 4.1% (Fourqurean et al., 2012) .

However, high organic carbon burial rates do not guarantee a high carbon

sink capacity. In order for seagrass beds to be considered effective carbon sinks, the organic carbon must remain trapped in their sediments for a period ranging from centuries to millennia (Duarte et al., 2013). As Duarte et al. (2011) explain, seagrasses have several mechanisms that allow for the millenary preservation of carbon. First, the low nitrogen and phosphorous content in seagrass tissues makes them a poor substrate to support microbial growth and therefore the tissues are recalcitrant to decomposition. Second, seagrass sediments are often anoxic, which leads to inefficient microbial metabolism, thus favoring the preservation of buried seagrass tissues. The anoxic conditions are a consequence of the constant deposition of sediments and particulates in seagrass beds. Third, the rhizomes and the wave dissipation action of the canopy prevent the resuspension of buried carbon. Finally, an obvious reason for the preservation of tissues is that underwater sediments are free of fires, which are responsible for high proportion of the CO_2 that is released from land carbon sinks (Duarte et al., 2013). As Duarte states, "*The combination of all these factors leads to high carbon preservation in seagrass sediments, which together with high metabolic inputs and particle trapping rates explain the role of seagrass meadows as intense carbon sinks in the biosphere"*(Duarte et al., 2013).

Coastal vegetated ecosystems store more carbon in their sediments than tropical forest do in their soils. Estimations point that seagrasses are responsible 10% of the annual carbon burial that occurs in the oceans, or 27.4 Tg C/yr (Fourqurean et al., 2012). Seagrasses bury more than twice the amount of carbon in their sediments ($500tCO_2$/ha) than tropical forest do in their soils ($200tCO_2$/ha)

(Figure 14) (Murray, Pendleton, Jenkins, & Sifleet, 2011).

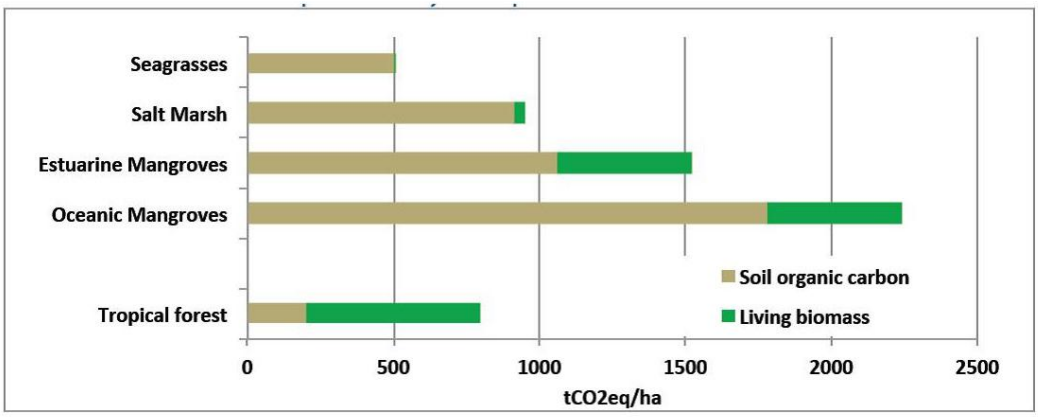


Figure 14. Global averages for carbon pools (soil organic carbon and living biomass) of focal coastal habitats (in tones of CO_2 equivalent per hectare per year). Tropical forests are included for comparison. Only the top meter of soil is included in the soil carbon estimates. Reprinted from "Green Payments for Blue Carbon: Economic Incentives for Protecting Threatened Coastal Habitats" by B. Murray et al., 2011. Report by Duke University Nicholas Institute for Environmnetal Policy Solutions.

The rate of carbon burial in specific seagrass ecosystems is dependent on many variables including seagrass specie, temperature, irradiance, nutrient load, sediment characteristics, depth, range, and age of the seagrass meadows (Greiner et al., 2013). Currently, there are only a few studies that have measured the carbon sequestration and/or burial rates in mono-specific meadows (Greiner et al., 2013). For *Z. marina* reported estimates of carbon burial rage from 36.68 ±2.79 g C m^2 /yr (Greiner et al., 2013) to 181 ± 18 g C m^2 /yr (Kaldy, 2006). This wide range might partially be attributed to the inconsistencies among different sampling and analysis methods (table 4) (Greiner et al., 2013).

Literature values for *Zostera marina* production rates

Location	Units	Leaves	Rhizome	References
Denmark	g C m^{-2} d^{-1}	1.72	0.45	Sand-Jensen (1975)
Japan	g dw m^{-2} d^{-1}	2.3	1.5	Mukai et al. (1979)
France	g C m^{-2} y^{-1}	389	183	Jacobs (1979)
Netherlands	g dw m^{-2} d^{-1}	0.0–3.5		Nienhuis and DeBree (1980)
Alaska	g C m^{-2} y^{-1}	3.3–8		Zieman and Wetzel (1980)
Denmark	g C m^{-2} y^{-1}	805		Borum et al. (1984)
Oregon	g dw m^{-2} d^{-1}	1–14		Kentula and McIntire (1986)
	g C m^{-2} y^{-1}	175–537		Kentula and McIntire (1986)
	g dw m^{-2} d^{-1}	0.5–3.5	0.25–0.5	This study
Virginia	g C m^{-2} y^{-1}	452		Murray and Wetzel (1987)
Mass	g dw m^{-2} y^{-1}	385–626		Roman and Able (1988)
Washington	g C m^{-2} y^{-1}	199.7		Thom (1990)
Washington	g C m^{-2} d^{-1}	1.5–9.8		Nelson and Waaland (1997)
	g dw m^{-2} y^{-1}	1767		Nelson and Waaland (1997)
Virginia	mg dw sht^{-1} d^{-1}	0.2–2.2	0.06–2.0	Moore et al. (1996)
World wide	g dw m^{-2} d^{-1}	5.2	1.7	Duarte and Chiscano (1999)
N. Carolina	g C m^{-2} y^{-1}		55–102	Kenworthy and Thayer (1984)

Note that the units vary between studies.

Table 4. Estimated production rates for Z. marina found in published literature. Reprinted from "Carbon, nitrogen, phosphorus and heavy metal budgets: How large is the eelgrass (Zostera marina L.) sink in a temperate estuary?" by J.E Kaldy, 2006, *Marine Pollution Bulletin*, 52(3).

In Puget Sound, the carbon sink capacity of eelgrass has never been directly measured. In 2013, a study by Caitlin Mariko-Shisido calculated the amount of carbon that could be removed from seawater by eelgrass via photosynthetic assimilation. Mariko Sishido utilized estimates of eelgrass abundance, distribution, and regional net primary productivity in order to calculate the rate of carbon assimilation. This study calculated the carbon uptake of eelgrass to be 10 billion g C /yr (grams of carbon per year) for the Puget Sound Basin, and a range from 100 million to 10 billion g C/yr for individual sub-basins (Table 5) (Shishido, 2013). These estimates show that the maximum daily increase in pH ranged from 0.02 to 0.05 pH units to a positive daily change in pH from 0.01 units to 0.05 pH units, which is considered insufficient to offset daily increases in pH due to anthropogenic carbon (Table 6) (Shishido, 2013). Thus according to estimates, *Z marina* might not cause a pronounced shift in the carbonate chemistry, necessary for mitigating the effects of ocean acidification (Shishido, 2013).

The lack of direct measurements for the carbon draw-down potential in different areas of Puget Sound, justifies the methodology and objectives for this thesis, which attempts to directly measure the carbon sequestration potential of eelgrass in Port Gamble, Puget Sound.

	Source	NPP ($gC\ m^{-2}\ y^{-1}$)	Areal Extent (minimum estimate) (m^2)	C Draw-down Rate (minimum estimate) $gC\ y^{-1}$	Areal Extent (maximum estimate) (m^2)	C Draw-down Rate (maximum estimate) $gC\ y^{-1}$
Entire Basin	Minimum NPP	199.7	9.69×10^7	1.49×10^{10}	1.17×10^8	2.34×10^{10}
	Maximum NPP	255.5	9.69×10^7	3.31×10^{10}	1.17×10^8	4.01×10^{10}
Central Puget Sound	Minimum NPP	199.7	2.86×10^6	5.71×10^8	3.47×10^6	6.93×10^8
	Maximum NPP	255.5	2.86×10^6	9.77×10^8	3.47×10^6	1.19×10^9
Hood Canal	Minimum NPP	199.7	5.36×10^6	1.07×10^9	7.89×10^6	1.58×10^9
	Maximum NPP	255.5	5.36×10^6	1.83×10^9	7.89×10^6	2.70×10^9
North Puget Sound	Minimum NPP	199.7	5.90×10^7	1.18×10^{10}	7.04×10^7	1.41×10^{10}
	Maximum NPP	255.5	5.90×10^7	2.02×10^{10}	7.04×10^7	2.41×10^{10}
San Juan-Strait of Juan de Fuca	Minimum NPP	199.7	1.25×10^7	2.49×10^9	1.55×10^7	3.09×10^9
	Maximum NPP	255.5	1.25×10^7	4.36×10^9	1.55×10^7	5.29×10^9
Saratoga-Whidbey	Minimum NPP	199.7	1.72×10^7	3.44×10^9	2.01×10^7	4.02×10^9
	Maximum NPP	255.5	1.72×10^7	5.90×10^9	2.01×10^7	6.88×10^9

Table 5. Minimum and maximum estimates of the metabolic carbon sink capacity of *Z. marina*, in g C m^2/yr, using upper and lower bounds of net primary production (NPP) and minimum and maximum estimates of area occupied by Z. marina in different Basins of Puget Sound. Reprinted from *Carbon draw-down potential by the native eelgrass Zostera marina in Puget Sound and implications for ocean acidification management* by C. Mariko Shishido, 2013. University of Washington School of Marine and Environmental Affairs

Selected Site	NPP	+ %Δ in DIC for selected site	+ Δ in pH
Dabob Bay	Low	0.72	0.03
	High	1.23	0.05
Padilla Bay	Low	1.12	0.03
	High	1.91	0.05
Picnic Cove	Low	0.23	0.02
	High	0.40	0.03
Jamestown	Low	0.20	0.01
	High	0.34	0.02

Table 6. Positive change in dissolved inorganic carbon (DIC) and pH projected for several sites in Puget Sound based on estimates of abundance, distribution, and regional net primary productivity (NPP). Reprinted from *Carbon draw-down potential by the native eelgrass Zostera marina in Puget Sound and implications for ocean acidification management* by C. Mariko Shishido, 2013. University of Washington School of Marine and Environmental Affairs

e) The effects of ocean acidification on seagrasses

As mentioned earlier, ocean acidification is expected to increase the productivity of seagrasses, such as eelgrass, because high [$CO_{2(aq)}$] benefits their carbon sequestration and photosynthetic metabolism. Most experiments studying how ocean acidification affects seagrasses have been performed under controlled laboratory conditions and have focused on examining the short-term effects of [$CO_{2(aq)}$] enrichment. These show that under elevated [$CO_{2(aq)}$] seagrasses exhibit increase photosynthetic rates, reproduction, and underground biomass (Koch et al., 2013)

Beer and Koch (1996) demonstrated that at elevated [$CO_{2(aq)}$] both seagrasses and macroalgae ramp up their photosynthetic rates. Beer and Koch measured the photosynthetic rates of two seagrasses including *Z. marina*, and three macroalgae species at increased concentrations of DIC. The concentrations of DIC were gradually adjusted by injecting small amounts of 100 mM $NaHCO_3$

until the seawater reached a pH of 6; the authors recorded how the photosynthetic rates of these autotrophs changed in response to each injection. Their experiment showed that the photosynthetic rates of all autotrophs studied increased due to the increased availability of $CO_{2(aq)}$ (Figure 15) (S. Beer & Koch, 1996). It is worth mentioning that although macroalgae also increase their photosynthetic rates under high [$CO_{2(aq)}$] conditions, and are sometimes more efficient than seagrasses in their assimilation of organic carbon, macroalgae do not possess the carbon burial capacity that seagrasses have because they lack roots and rhizomes.

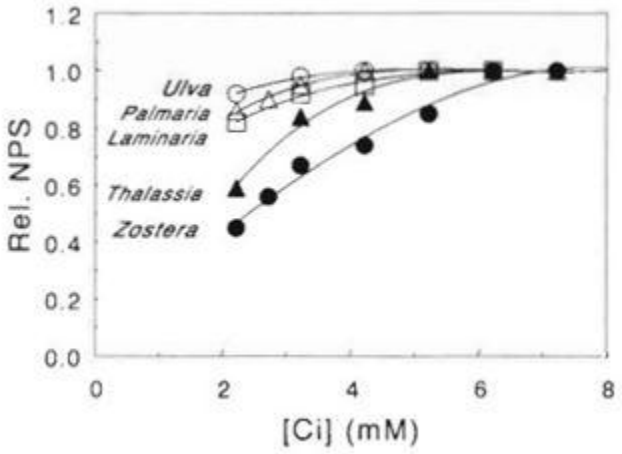


Figure 15. Net photosynthetic rates (NPS) of two seagrass species: *Zostera marina* and *Thalassia testidinum* and three marine macroalgae: *Ulva lactuca, Palmata palmate,* and *Laminaria saccharina* in natural seawater (2. 2mM DIC) and following additions of DIC (here labeled as Ci). The additions of DIC consisted of injections of μliter amounts of a100 mM NaHC03 solution. Figure shows that under high CO_2 conditions macroalgae are more efficient at sequestering dissolved inorganic carbon. Reprinted from "Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments" by S.Beer and E. Koch, Marine Ecology Progress Series,141(1), p201.

Jiang et al. (2011) reported a statistically significant increase in leaf growth rates and belowground nonstructural carbohydrates of [$CO_{2(aq)}$] enriched

seagrass *Thalassia hemprichii* compared to the un-enriched treatment. The authors studied the response of these plants under four different concentrations of [$CO_{2(aq)}$], which are equivalent to pH values of 8.10 (un-enriched treatment), 7.75 (the projected value for 2100), 7.50 (the projected value for 2200) and 6.2 (an extreme value)(Jiang, Huang, & Zhang, 2011) .

Hendricks et al. (2013) reported that photosynthetic activity in shallow seagrass meadows (5-12 feet) of *Pocedonia oceanica* buffered the local effects of ocean acidification along the coast of Mallorca, Spain. Hendricks et al. correlated the diurnal change in seawater pH with the metabolic activity of *P. oceanica* meadows *(*photosynthetic leaf area per m^2 (LAI), dissolved oxygen, shoot density, biomass) while taking into account environmental parameters (temperature, irradiance, salinity, residence time, etc). The magnitude of diurnal pH variation was strongly related to seagrass productivity, with the largest ranges coinciding with the peak in seagrass productivity, which happens in June. The range of pH measurements was also influenced by oxygen concentrations (max and mean; $F = 61.86$, $p < 0.0001$nd $F = 18.29$, $p < 0.01$, respectively). The main factor affecting oxygen concentrations was determined to be LAI which affected both max ($r^2 = 0.60$, $F = 13.71$, $p < 0.01$), and mean ($r^2 = 0.60$, $F = 13.51$, $p < 0.01$) oxygen concentrations. The authors concluded "that metabolically intense seagrass meadows actively control the carbonate system in their canopies" (p. 12325) and that the carbon draw down happening in the seagrass beds minimizes the effects of ocean acidification and offers a refuge to calcifiers and organisms that are sensitive to pronounced pH changes (Hendriks et al., 2013).

Just as experiments studying $CO_{2(aq)}$ enrichment show an increase in photosynthetic rates and biomass of seagrasses, an experiment studying a decrease in $[CO_{2(aq)}]$ showed a decrease in photosynthetic activity. This experiment, performed by Hellblom and Björk (1999), analyzed the result of diluting the DIC content while keeping all other variables constant in aquaria containing *Z. marina* specimens. Their experiments showed that photosynthesis was significantly inhibited when the DIC concentration decreased from 2mM to 1mM; however, respiration was not inhibited (Hellblom & Björk, 1999).

Unsworth et al. (2012) developed a computer model that analyzed the pH buffering capacity of seagrass meadows near scleratinian coral reefs in the Indo-Pacific Ocean. This study suggested that increases up to 0.38 pH units, and Ω aragonite increases of up to 2.9 in seagrass meadows (with a 24 h water residence time and 1 m depth seagrass meadow) could potentially enhance calcification of scleratinian corals downstream of seagrasses by 18% (Unsworth, Collier, Henderson, & McKenzie, 2012).

Studies examining the long-term effects of ocean acidification on seagrasses are scarce, but the results from these few studies are consistent with the increase in underground biomass observed in short-term studies. For example, a study by Palacios and Zimmerman (2007) examined the effects of long-term enrichment of $CO_{2(aq)}$ (over the period of two years) on the performance of *Z.marina* growing under 33% surface irradiance. Palacios and Zimmerman simulated the current $[CO_{2(aq)}]$ and the $[CO_{2(aq)}]$ predicted for the year 2100 (36 μmol CO_2) and 2200 (85 μmol CO_2) by injecting flue glass into aquaria. They

discovered that even though the enrichment did not alter leaf size or leaf sugar content, the $CO_{2(aq)}$ enrichment led to significantly higher underground production of rhizomes and an increase in vegetative proliferation. Shoots growing under [$CO_{2(aq)}$] predicted for 2100 were 25% larger than those from current [$CO_{2(aq)}$], and shoots grown under [$CO_{2(aq)}$] predicted for 2200 were 50% larger than those from current [$CO_{2(aq)}$] (Palacios & Zimmerman, 2007).

Even though studies show that seagrasses have the potential to reduce the effects of ocean acidification, experiments also show that under high [$CO_{2(aq)}$] the nutritional quality of seagrasses decreases and they lose their ability to produce anti-herbivory compounds. A study by Arnold et al (2012) indicates than under elevated pCO_2, some seagrasses lose the ability to produce phenolic compounds that protect these plants against herbivores, pathogens, and damage by UV radiation. Arnold et al. (2012) reported that the reduction of these defense compounds resulted in higher rates of herbivory and lower overall productivity. Since some phenolic compounds are antimicrobial or protect the plants against UV radiation, the authors of this study expect that the reduction of phenols will result in increased pathogen infections and increased tissue damage (Arnold et al., 2012).

f) Eelgrass habitat requirements

Nutrient requirements: Eelgrass typically inhabits areas that are naturally phosphorus or nitrogen limited (Touchette & Burkholder, 2007). Seagrasses may be limited by nitrogen in nutrient poor waters with sandy or shallow organic

horizon sediments or limited by phosphorous in carbonate sediments (Touchette & Burkholder, 2000). Due to this limitation, *Z. marina*, as most seagrasses, developed active uptake systems for NO_3^- and PO_4^{-3}) and NH_4^+ (Touchette & Burkholder, 2000). Excessive enrichment of nitrogen and phosphorus is correlated to eelgrass decline. Excess nitrogen in the in the water column can inhibit seagrass growth by stimulating epiphyte overgrowth and by consuming the plant's energy reserves (Touchette & Burkholder, 2000, 2007). Scientists hypothesize that eelgrass must have evolved in nitrogen-limited waters, where nitrogen peaks (sudden rises in concentration) were infrequent. Supposedly, during these peaks eelgrass plants would use energy to assimilate as much nitrogen as they could and then convert it to amino acids (Touchette & Burkholder, 2007). Some scientists think that eelgrass plants never developed a mechanism that stops the uptake of nitrogen; in nitrogen rich waters eelgrass plants are thought to consume all their energy reserves uptaking more nitrogen than they need to survive (Touchette & Burkholder, 2007). It is important to note that external variables, such as irradiance and temperature, affect eelgrass' assimilation capacity of these nutrients (Peralta, Bouma, van Soelen, Pérez-Lloréns, & Hernández, 2003).

However, due to abundant runoff and freshwater inputs, which act as nutrient sources, *Z. marina* in Puget Sound is not limited by the concentration of nitrogen or phosphorus (Mumford, 2007). In fact, these inputs are believed to be beneficial as *Z. marina* has been shown to respond favorably to low or moderate N and/or P enrichment (Touchette & Burkholder, 2000).

Temperature: The ideal temperature for maximum eelgrass growth is around 20°C, but *Zostera marina* can survive in temperatures that range from 5°C to 30°C (Touchette & Burkholder, 2007). Natural populations of eelgrass exist under temperatures ranging from arctic waters to temperate estuaries (Nejrup & Pedersen, 2008). Seasonal growth is closely associated with temperature. Low water temperatures (<5 °C) reduce photosynthesis and growth, and impede sexual reproduction, but do not affect mortality (Nejrup & Pedersen, 2008). High temperatures (25–30 °C), on the other hand, increase mortality and decrease the photosynthesis and growth rates (Nejrup & Pedersen, 2008).

Light and depth: Eelgrass needs high levels of light to grow and reproduce; because of this, it is typically only found in shallow waters that are less than 10 meters (Mumford, 2007). Eelgrass habitat is constrained to a depth gradient that represents at its upper boundary the likelihood of exposure to desiccation at low tide, and at its lower boundary light attenuation in the water column (Dowty et al., 2005; Mumford, 2007). A survey by the WA-DNR ongoing Puget Sound's Submerged Vegetation Monitoring Project (SVMP) found that eelgrass depth range varies throughout the sound, with the San Juan Straits having the widest depth range and Seratoga-Whimbey region having the narrowest depth range (Figure 16) (Dowty et al., 2005).

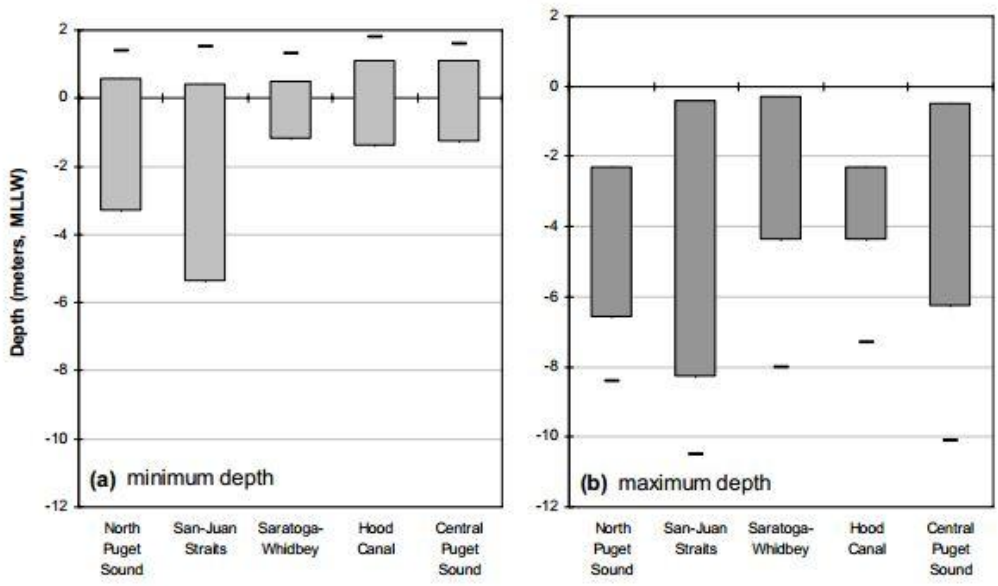


Figure 16. Site-level minimum and maximum *Zostera marina* depth results summarized by Puget Sound regions. Reprinted from Puget Sound "Submerged Vegetation Monitoring Project 2003-2004 Monitoring Report" by P. Dowty et al., 2005. Published by Washington State Department of Natural Resources. Retrived from http://www.dnr.wa.gov/Publications/aqr_nrsh_03_04_svmp_rpt.pdf

Salinity: The optimum salinity for eelgrass is between 10% and 25%, although eelgrass can be found in waters ranging from 2% to 40% of salt concentration (Nejrup & Pedersen, 2008). Eelgrass is well adapted to tolerate changes in salinity, as estuaries often experience variations in freshwater inputs that cause rapid changes in salinity (Nejrup & Pedersen, 2008). Even though eelgrass can maintain a positive carbon balance at extreme salinities, studies show that growth, reproduction, and germination are affected when salinity falls below or shoots above the optimal parameters (Nejrup & Pedersen, 2008).

Substrate: Eelgrass tends to grow in unconsolidated substrates ranging from gravelly sand to fine muds and silts, with a general preference towards finer particle sizes (Kenworthy & Fonseca, 1977). In Puget Sound, *Z. marina* is

primarily found in the subtidal zone, rooted in sand or mud in shallow waters, where the currents are not too strong (Mumford, 2007). However, in some moderately high-energy environments, such as Salmon Bank, eelgrass can be found growing in finer substrates trapped between cobbles and boulders (Mumford, 2007).

g) Distribution and density of eelgrass beds in Puget Sound

Eelgrass in broadly distributed through Puget Sound. Thus, eelgrass is found in areas from +1.8 to -8.8 meters (relative to mean lower low water) in Puget Sound, with beds being more abundant around 0.0 meters. (Mumford, 2007). In general, eelgrass beds are found throughout Puget Sound except for south of Anderson Island and Carr Inlet in southern Puget Sound, possibly due to the extreme tidal range or seasonal lack of nutrients (Figure 17) (Mumford, 2007).

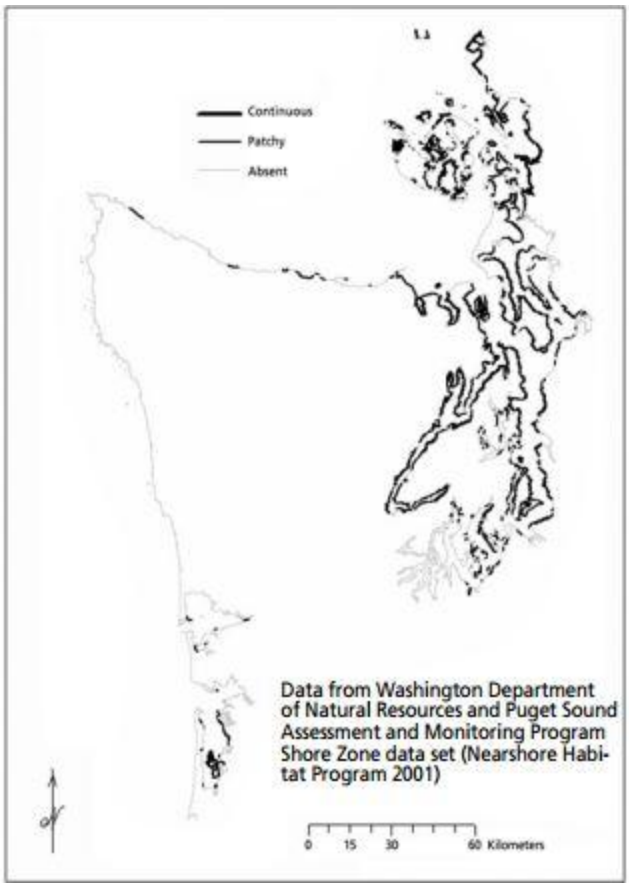


Figure 17. Distribution of eelgrass *(Z. marina)* in Puget Sound. Reprinted from *Kelp and Eelgrass in Puget Sound* by T. Mumford, 2001, Washington Department of Natural Resources, Aquatics Division.

Eelgrass beds in Washington State have been mapped by the WDNR and published in the ShoreZone database (Nearshore Habitat Program 2001). According to the database, eelgrass beds represent 37% of the shoreline vegetation of Washington State (table 7). Additionally, the Puget Sound Assessment and Monitoring Program, led by WDNR has been monitoring five regions within Puget Sound since the year 2000 and has estimated that there are 200 km^2 of *Z. marina* in the shorelines of Puget Sound.

County Name	Total Miles	Percent of Shoreline with Aquatic Vegetation			
		Eelgrass	Floating Kelp	Non-floating kelp	Sargas-sum
Clallam	254	20%	40%	80%	1%
Grays Harbor	187	5%	> 1%	6%	> 1%
Island	214	63%	10%	18%	8%
Jefferson	254	58%	7%	33%	18%
King	123	62%	13%	27%	25%
Kitsap	254	48%	> 1%	21%	21%
Mason	232	28%	> 1%	24%	33%
Pacific	276	22%	> 1%	1%	> 1%
Pierce	239	26%	7%	44%	19%
San Juan	408	41%	31%	63%	47%
Skagit	229	51%	12%	26%	15%
Snohomish	133	22%	1%	1%	3%
Thurston	118	4%	> 1%	24%	4%
Whatcom	147	55%	7%	18%	34%
Total	3067	37%	11%	31%	18%

Table 7. Length of shoreline with eelgrass, floating and non-floating kelp by Puget Sound counties (Mumford, 2007). Reprinted from *Kelp and Eelgrass in Puget Sound* by T. Mumford, 2001, Washington Department of Natural Resources, Aquatics Division.

The density of the eelgrass beds depends on the conditions of the habitat. In areas where conditions are thought to be most suitable, beds are dense and continuous, while other less suitable areas have patchy beds (Mumford, 2007). Continuous beds are usually found in extensive tideflats, and more fragmented beds in areas are found raveled edge shorelines (Mumford, 2007). Little is known about the interannual variation of the density of beds, but the variation is expected to be less than 10 percent (Mumford, 2007).

Other factors that limit density and distribution of *Z. marina* include competitors and water quality degradation. *Z. marina* has only few competitors;

these include the introduced brown *seaweed Sargassum muticum*, the sand dollar *Dendraster excentricus* and possibly the newly discovered kelp species in Hood Canal, *Chorda filum* (Mumford, 2007). If the environment has excessive nutrients, algal species might overgrow and limit photosynthesis for eelgrass. Sea lettuce (*Ulva sp*) has been known to grown on the water surface and block sunlight; similarly, epiphytes can flourish the blades of eelgrass, blocking light, and gas exchange (Mumford, 2007). Due to their relatively high light requirements, eelgrass beds thrive in shallow waters, where they are vulnerable to damage by human activities that reduce the water quality (Fonseca, Kenworthy, & Thayer, 1998). Increased runoff from nutrient loading activities, such as logging and agriculture, have resulted in a decrease of eelgrass growth (Wolf, 2007). Exposure to toxic substances, such as petroleum products and metals like cadmium, impairs photosynthesis and respiration, and limits eelgrass growth and distribution (Mumford, 2007). Physical disturbances such as oyster culture, high-energy boat wakes, the dredging and filling required to maintain shipping lanes, and construction of under and over water , also impact eelgrass habitat (Mumford, 2007; Wolf, 2007)

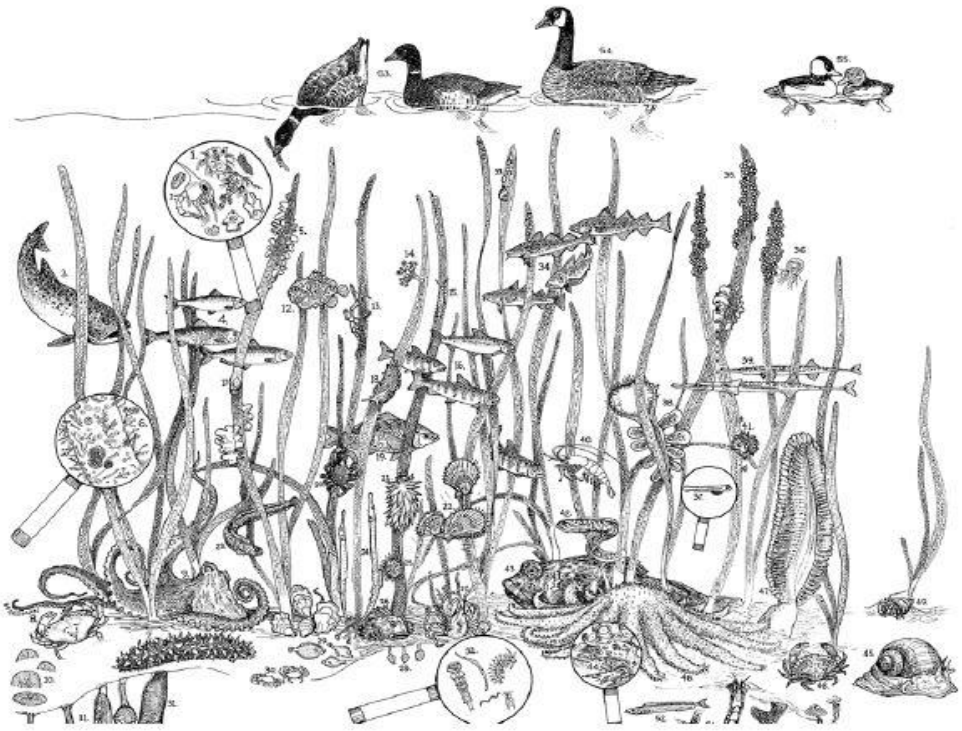
h) Ecological function and socio-economical importance of eelgrass

Eelgrass serves a wide variety of ecological functions in Puget Sound ecosystems, including fueling the food web, stabilizing sediments, and providing habitat, nursery, and protection to many species (Mumford, 2007). Eelgrass is highly productive, annually producing large amounts of biomass that fuels

nearshore food webs directly through detritus pathways and consumption by several species of birds and indirectly by feeding many species of invertebrates (Figure 18) (Larkum et al., 2006; Mumford, 2007).

Eelgrass fuels the food web by providing food to crustacean and bird species. Ducks, swans and other species of goose, are known to stop in eelgrass beds during their migrations (Mumford, 2007). For example, the Pacific black brant migration is closely linked to the distribution of *Z. marina* beds from Mexico to Alaska (Larkum et al., 2006). Crustacean species such as members of the isopod genera *Synidotea* also feed on eelgrass (Mumford, 2007).

Zostera marina beds provide structure for habitat and nursery of many species. Many organisms, including microalgae and macroalgae, copepods, amphipods and snails, inhabit the eelgrass blades and rhizomes (Mumford, 2007). Marine birds such as the Great Blue Heron feed extensibly on the small invertebrates found in eelgrass beds (Mumford, 2007). Fishes such as juvenile salmonids utilize eelgrass beds as migratory corridors, as they pass through Puget Sound; the beds provide protection from predators and abundant food, such as small crustaceans (Mumford, 2007). Several species of flounder, weakfish, blue crab, bay scallops, lobsters and striped bass, require eelgrass habitats at a point of life history (Mumford, 2007). Additionally, many fish and crustaceans species lay their eggs on eelgrass, including species of commercial interest such as Dungeness crab *(Metacarcinus magister)* and Pacific herring *(Clupea pallasii)* (Mumford, 2007).



1. Zooplankton
2. Larval crab
3. Salmon
4. Herring
5. Epiphytic macroalgae
6. Epiphytic microalgae, Hydozoa, and bryozoa
7. Sea cucumber
8. Dungeness crab
9. Octopus
10. Sand dollars
11. Clams and cockles
12. Pacific spiny Lumpsucker
13. Caprellid amphipod
14. Stalked jellyfish
15. Eelgrass isopod
16. Juvenile salmon
17. Bubble shell
18. Opalescent nudibranch
19. Perch
20. Juvenile kelp crab
21. Alabaster nudibranch
22. Scallop
23. Gunnel
24. Bay pipefish
25. Sea urchin
26. Juvenile sculpin
27. Decorator crab
28. Juvenile clams
29. Juvenile flounder And sole
30. Juvenile crab
31. Geoduck
32. Sediment microfauna
33. Snail and snail eggs
34. Juvenile cod, tomcod And wall-eyed pollock
35. Herring eggs
36. Jellyfish
37. Larval fish
38. Mellbae-hooded nudibranch
39. Tubesnout
40. Shrimp
41. Brooding anemone
42. Prickleback
43. Sculpin
44. Bacteria on detritus
45. Moonsnail
46. Sunflower seastar
47. Sea pen
48. Red rock crab
49. Hermit crab
50. Worms
51. Ghost shrimp
52. Sand lance
53. Black Brant
54. Canada Goose
55. Bufflehead

Figure 18. The eelgrass meadow: A world of microhabitats. . Reprinted from *Kelp and Eelgrass in Puget Sound* by T. Mumford, 2001, Washington Department of Natural Resources, Aquatics Division. (Mumford, 2007)

Eelgrass also provides sediment stabilization. Their dense interlocking rhizomes effectively grab and anchor the sediments, protecting the bottom from erosion, while the blades slow water currents, dampen waves, and trap sediments, increasing the rate of deposition (Larkum et al., 2006; Mumford, 2007)

The socieconomical importance of eelgrass can be related to its connection to commercial seafood species and with its cultural significance to Native American Tribes. In addition to housing many commercial seafood species, eelgrass is used as ceremonial material in Native American Rituals (Mumford, 2007).

Several studies have attempted to associate a monetary values to eelgrass habitats, based on their economical contribution. Costanza et al. (1997) calculated that eelgrass was worth $19,004 per hectare per year, while McArthur and Boland (2006) found that the loss of 16% of eelgrass on an area corresponding approximately to 1° latitude and longitude, resulted in an economic loss of $235,000 per year, due to a reduction of seafood catch.

The ecological and socioeconomical importance of *Z. marina* was highlighted in the 1930's when 90% of the North Atlantic *Z. marina* died off, due to "wasting disease" (Larkum et al., 2006). This massive die-off led to reductions in estuarine and costal food web productivity, which resulted in the disappearance of the commercially harvested scallop *Argopecten irraiance* and in a drastic reduction of brant geese populations (Larkum et al., 2006).

Seagrass plants can absorb heavy metals and incorporate them in their tissues, thus they have been proposed as a bio-remediation mechanism for dealing with metal contaminated waters. Similarly, seagrasses can be used as ecological indicators to assess the water quality and level of metal contamination of an ecosystem (Thangaradjou, Raja, Subhashini, Nobi, & Dilipan, 2013). Kaldy

(2006) determined that *Z. marina* plants incorporated 73–90% of metals in the water into new leaf tissue. Nickel and zinc are incorporated into eelgrass tissues faster than arsenic, cadmium, chromium or copper (Table 8)(Kaldy, 2006). Because of their high productivity and low herbivory rates, eelgrass plants could potentially be used to accumulate heavy metals that are dissolved in the water; the metals could then be extracted from the plants and be disposed in an environmentally safe way.

Date	C[a]	N[a]	P[b]	As[c]	Cd[c]	Zn[b]	Cr[c]	Ni[b]	Cu[c]
Leaves	137 (17)	11 (1.4)	1668 (150)	1491 (206)	756 (95)	13 (1.2)	3078 (966)	11 (9)	3856 (626)
Rhizome	37 (4)	1 (0)	199 (33)	212 (12)	59 (12)	1.8 (0.3)	564 (227)	3.5 (5.8)	461 (90)
Root	7.3 (0.8)	0.4 (0.0)	21 (2)	132 (14)	2 (1)	0.7 (0.1)	570 (277)	0.4 (0.17)	313 (67)
Total	181 (18)	13 (1.4)	1888 (154)	1836 (206)	817 (96)	15 (1.3)	4213 (1030)	15 (11)	4629 (636)
Potential export[d]	1.8×10^5	1.3×10^4	1.9×10^3	1.8	0.82	15	4.2	15	4.6

Values represent means ± (SD). Note that units vary between the different elements and are denoted with a superscript. Elements are denoted using traditional chemical symbols. Potential export ($kg\ y^{-1}$) calculated assuming 1 km^2 of *Z. marina* habitat in Yaquina Bay, Oregon (Wyllie-Echeverria and Ackerman, 2003).

[a] Units $g\ m^{-2}\ y^{-1}$.
[b] Units $mg\ m^{-2}\ y^{-1}$.
[c] Units $\mu g\ m^{-2}\ y^{-1}$.
[d] Units $kg\ y^{-1}$.

Table 8. Budget calculations for the incorporation C, N, P and metals into new *Zostera marina* leaf, rhizome, and root tissues. Reprinted from "Carbon, nitrogen, phosphorus and heavy metal budgets: How large is the eelgrass (*Zostera marina*) sink in a temperate estuary?" by J. Kaldy, 2006, Marine Pollution Bulletin, 52(3)

i) Protective status of eelgrass in Washington State

Because of the ecological importance of eelgrass and its susceptibility to human disturbances, eelgrass has been given regulatory protection under a variety of federal, state and local laws (Mumford, 2007). The EPA is concerned with the protection of eelgrass under the Clean Water Act; EPA must guarantee that the water quality does not affect the physical and biological integrity of the nation's

waters (Nelson Walter, 2009). In Washington State, eelgrass has been designated as critical habitat under the Critical Areas Ordinance by the Department of Ecology, while WA- DNR has designated areas of *Z. marina* as habitats of special concern and has a no-net-loss policy for shoreline development (Dowty et al., 2005). Additionally, the Puget Sound Partnership designated eelgrass one of the top five indicator species to estimate the health of the Puget Sound and has developed a set a target of increasing eelgrass habitat in Puget Sound by 20 percent by the year 2020 (Puget Sound Partnership, 2012).

j) Suggestions for future research on eelgrass

After reviewing the scholarly literature, it is clear that there are some robust estimates of the rate of carbon assimilation and the rate of carbon burial of seagrasses (as a group) at a global scale. There are also estimates of net primary production (NPP) rates, and amount of carbon stored in sediments and biomass for tropical and subtropical seagrass species. However, very limited information was found regarding the rate of carbon uptake and the amount of carbon stored in biomass and sediments of seagrass beds located in temperate latitudes. Even through the irradiance levels, and thus NPP rates are lower in temperate regions, it is important to calculate this rate in order to have a more complete estimate of how much carbon is stored in seagrass ecosystems at a global and regional scale.

Similarly, there are only a handful of field studies that have directly measured whether or not seagrass species can increase the pH of seawater to significantly ameliorate ocean acidification. Most estimates on the change of pH

or the change of DIC caused by seagrasses were obtained by ether doing theoretical calculations of by conducting laboratory experiments that carefully control most variables that affect photosynthesis. More field studies are needed in order to have more realistic estimate of the contribution of seagrasses to the mitigation of ocean acidification.

Only one estimate for the carbon draw-down potential of eelgrass in Washington State was found. This estimate was theoretical and was calculated with statistics gathered by past studies on the characteristics of different areas in Puget Sound. No studies regarding the carbon burial capacity of eelgrass in Washington State or in the Pacific Northwest were found. More studies, whether they are in a laboratory or in the field, are needed to examine the carbon uptake of eelgrass and it subsequent effect on pH in Washington state marine waters. This thesis represents the first field study that attempted to estimate the change in pH over time and the change of DIC over time over eelgrass beds located within an area of Puget Sound.

III. METHODS

PURPOSE OF EXPERIMENT

The purpose of this experiment was to compare the rates of change of pH over time between two ecosystems, *Zostera marina* beds and bare mud flats, located in Port Gamble, WA to determine if the carbon sequestration capacity of *Z. marina* beds would be capable of locally buffering the impacts of additional acidity caused by the oceanic uptake of anthropogenic CO_2. Our experiment was conducted during daylight hours of January 19 and January 20, 2014 and thus our results only represent a snapshot of what the rates of change of pH over time are like for these two ecosystems during wintertime in Port Gamble. It important to keep in mind that during the winter, solar irradiance is low which leads to reduced photosynthesis and consequently reduced carbon sequestration rates, which may suggest our results represent a minimum (or conservative) uptake. In spite of the reduced irradiance, we expected to see a positive rate of change of pH over time for the *Z.marina* beds as a result of net photosynthesis in this ecosystem. For the bare mud flats, we expected to see a decline in pH over time, resulting from net respiration in the ecosystem.

STUDY AREA

The experiment took place in Port Gamble, Kitstap County, Washington from 9am to 3pm of January 19 and January 20, 2014 (Figure 19). Port Gamble, also known as Gamble Bay, is an inlet located in the northwestern shore of the

Kitsap Peninsula in Kitsap County, Washington, United States. Port Gamble lies within the Upper Hood Canal Watershed and the mouth of the inlet is located on the north, where it opens up to Hood Canal.

Figure 19. Location of Port Gamble (denoted by the red square) within Puget Sound, Washington.

Several factors influenced the selection of this site for the study. The main factor was that Port Gamble reportedly contained significant intertidal eelgrass

beds along the shoreline. Newfields Northwest, and environmental consulting group, had previously conducted scuba transect surveys in this area and they had determined the locations that contained *Z. marina* beds (NewFields Northwest, 2007). Another factor was the ease of access to Port Gamble and the fact that WA-DNR had already obtained authorization from the S'klallam Tribe to conduct experimental work within their reservation. A decisive factor was that Port Gamble is considered to have "excellent" quality marine waters and thus this decreased the risk of having to account for contamination or other variables that might have influenced the results of the experiment.

According to the 2013 Water Quality Monitoring Report from the Kitsap Public Health District, the overall marine water quality for Port Gamble is classified as "excellent." The majority of the waters of Gamble Bay are approved for shellfish harvesting except for the northeast area where there is a permanent closure zone around the outfall of a sewage treatment plant. All water quality stations in Port Gamble met the stare bacterial standards during the 2012-2013 year. However, there were temperature exceedances at three of the four stations during the 2013 summer months (Kitsap Public Health District, 2013). Additionally, there is concern among the S'klallam Tribe, Washington Department of Natural Resources (DNR), and Washington's Department of Ecology that historical operations of the former sawmill released pollutants to the water including wood waste, cadmium, mercury, petroleum hydrocarbons, carcinogenic polycyclic aromatic hydrocarbons (cPAHs), dioxins/furans, sulfide and ammonia (Port Gamble S'Klallam Tribe, 2014; Washington State Department

of Ecology, n.d.). Some of these contaminants have been found on soil surrounding the mill site and in shellfish tissues, although the these contaminants are absent in the marine waters of the bay or in concentrations below those that would risk human health (Kitsap Public Health District, 2013; Washington State Department of Ecology, n.d.)

Several communities live along the edges of Gamble Bay. Along the east side of the bay, lies the S'klallam Tribe Indian Reservation and the Little Boston community. The right side of the Bay contains the town of Port Gamble along with the remnants of mill that operated under the Puget Sound Mill Company, later known as Pope & Talbot, Inc, from 1853 to 1995. The mill was removed in 1997 and the fill area has since been leased for log sorting, wood chipping, and other light industrial activities (Washington State Department of Ecology, n.d.). The community of Gamblewood is located in the south side of the bay. Unincorporated residential housing can be found on both sides of the bay. The communities of Kingston, Poulsbo, and Hansville are located in the vicinity of the bay (Port Gamble S'Klallam Tribe, 2014).

The surrounding communities harvest shellfish and fish from Gamble Bay. Gamble Bay is the last bay in Kitsap County open for commercial shellfish harvesting of geoduck, clams, and oysters (Port Gamble S'Klallam Tribe, 2014). The Bay also contains Dungeness Crab and shrimp (NewFields Northwest, 2007). The S'klallam Tribe has a salmon hatchery located in the north west side of the bay (Port Gamble S'Klallam Foundation, 2014). Salmonoid fish such as Chum, Coho, Chinook, Pink Salmon, and Cutthrow Trout frequent the waters of Gamble

Bay (NewFields Northwest, 2007). Forage fish such as herring, surf smelt and sand lance inhabit and spawn in the waters of Gamble Bay (NewFields Northwest, 2007).

Since several communities depend of the marine resources of Gamble Bay, it is essential that these resources are protected from the effects of ocean acidification. Eelgrass beds are considered critical habitat for a number of fish and invertebrate species, and thus are considered protected habitat. In addition to providing special habitat, eelgrass beds might mitigate the impacts of ocean acidification. The Olympic Property Group (a real estate subsidiary of Pope Resources, the former owner of the Port Gamble mill) and DNR have considered developing eelgrass mitigation and restoration projects in Gamble Bay (NewFields Northwest, 2007).

EXPERIMENTAL DESIGN

a) Construction of drifting devices

Four circular floating devices of about 1meter in diameter were constructed using ½ inch PVC pipes and 1 inch industrial tubing. Each circular device had console in the center for the attachment of instrumentation; the console was constructed in a way that allowed the instrumentation to be submerged a couple of inches from the surface of the water. Two pairs of circular devices were tied to each other and were referred to as one "drifter" (Figure 20).

Figure 20. Image of one of the drifters

b) Preparation for Drifts

A water quality monitoring sonde YSI 6600 was attached using zip ties to one console in each drifter in order to measure temperature, salinity, and depth. The YSI measured these parameters every minute for the duration of the experiment.

Two GPS Gamin gecko devices were also attached to each drifter (one in each console). The GPS devices would provide information on the spatial location of the drifters for every minute of the experiment. Two devices were used to ensure that if one device failed we would still have spatial data from each drift.

To measure pH, two custom-made voltmeters crafted using two Honeywell Durafet II electrodes were attached to each drifter, to record voltage every minute. These pH sensors where built according to specifications in the

publication titled *Testing the Honeywell Durafet® for seawater pH applications* by Martz, Connery, and Johnson (2010). According to Martz, Connery and Johnson (2010) the theoretical accuracy of the sensors is ± 0.0005 pH units; however, based on our experimental test the accuracy of the sensors was ±0.001 pH units. Our test consisted of submerging the sensors in a seawater reference solution of pH 7.95 at room temperature for a period of one hour and then calculating the pH based on the measured voltage. (Andrew Dickson's Laboratory seawater reference solution batch 134, bottled September 27, 2013). Deviations between the measured pH and the pH of the standardized solution were noted and later used as correction factors when calculating the measured pH for each drift

Two Go-Pro waterproof cameras were attached to each circular frame using zip ties in order to record video footage of the underwater ecosystem and calculate percent cover of eelgrass.

Twenty clean brown beer bottles where disinfected and prepared for the collection of water samples immediately before commencing the drifts. The beer bottles were prepared by washing them with 5ml of 38% HCl, followed by a 5ml wash with commercial bleach.

We also had two kayaks and a small 15-foot aluminum boat. These vehicles where used to scout the area and in the case of the boat to get information on depth and presence of eelgrass by utilizing the sonar and depth meter.

c) Site selection

Sites were selected based on the presence of significant subtidal *Z. marina* beds that where in proximity of areas that showed no apparent vegetation coverage and were classified as mud flats. Thus, each was comprised of two ecosystems: a subtidal *Z. marina* bed ecosystem, which was referred to as the "eelgrass treatment," and a mud flat ecosystem containing little to no observable vegetation, referred to as the "no eelgrass treatment". Five areas, each containing a pair of ecosystems were selected for this study, for a total of five "eelgrass" treatment replicates and five "no eelgrass" treatment replicates.

For the site selection process, we considered the distance of each site from the shoreline, the estimated density of eelgrass, and the strength of the current in each area. The boat sonar was used to confirm the presence and absence of eelgrass in each of the paired ecosystems. The sonar was also used to estimate the density of eelgrass in each bed, only beds that resulted in an acoustic signature characteristic of dense eelgrass beds were selected. Dr. Alan Trimble and Dr. Jennifer Ruesnik from the University of Washington's Biology Department evaluated the sonar signal. After using the sonar, the presence or absence of eelgrass was confirmed visually by drifting on top of each ecosystem in two kayaks. Paired ecosystems that had the same composition covering an area of at least 30x15m, with the longest side of the area parallel to the direction of the current were chosen. Only areas within five meters from the shoreline where considered. The areas chosen where then narrowed down by only considering

areas that showed they had slow currents (those moving at a speed of less than 3 meters per minute). The intensity of the currents was tested by deploying the drifters and observing how fast the drifters moved with the current.

The criteria explained above, resulted in a total of five paired ecosystems contained within two areas, which are delineated by the rectangles in the map (Figure 21). The relative closeness of the paired ecosystems ensured that environmental conditions, such as wind and current, were the same for both ecosystems and therefore the same for both drifters.

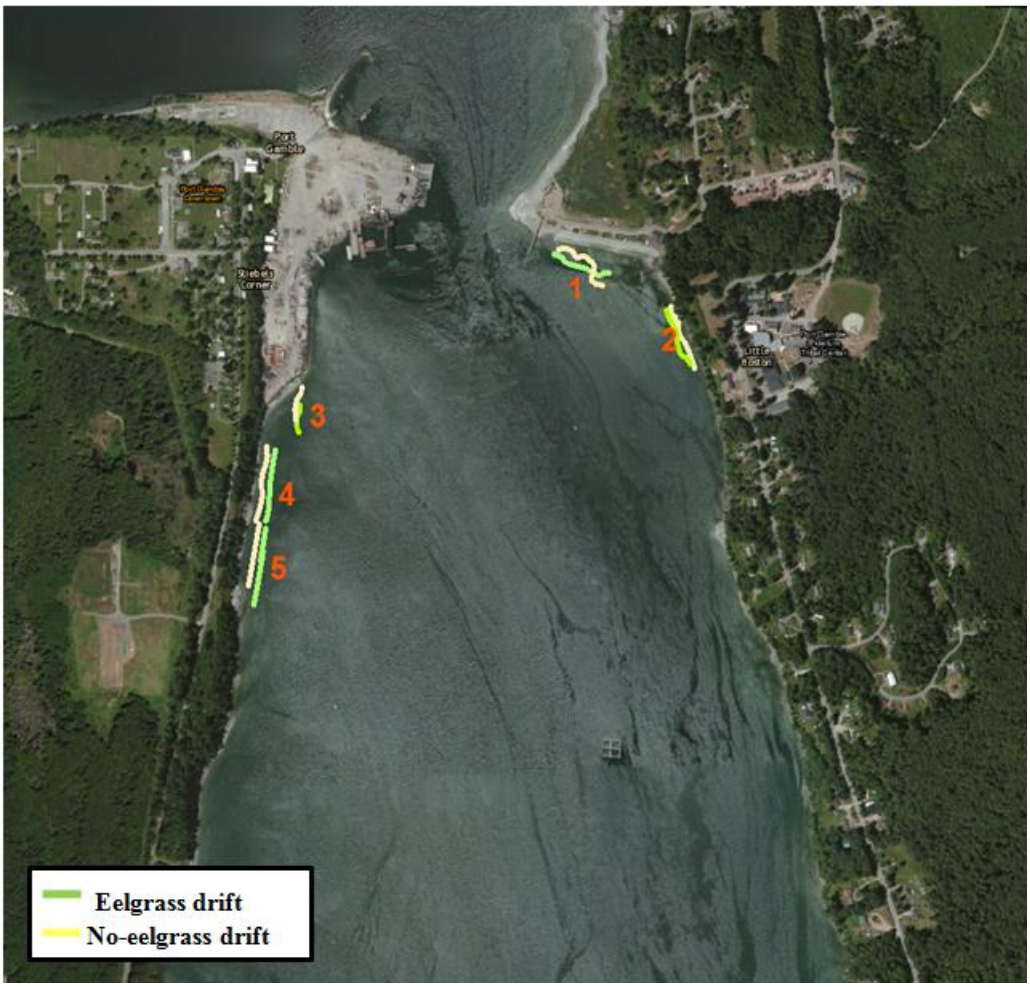


Figure 21. Location of each of the ten drifts (five drifts per treatment) within Port Gamble.

We found some of the introduced specie *Zostera japonica* growing in close proximity to *Zostera marina* beds. However, we did not attempt to avoid *Z. japonica* in this experiment, as the *Z. japonica* density was very low (less than 15 shoots per square meter) and the plants were not observed directly on *Z. marina beds*. Because the density of *Z. japonica* was so low, for experimental purposes, it was assumed that *Z. marina* was contributing to 99% of the carbon sequestration of that area, ad that any contribution to carbon sequestration by Z. japonica was negligible.

d) Drifts

Each drifter was allowed to drift over the assigned ecosystem in the direction of the current. The drifters where allowed to drift with the current for a period that ranged from 35 minutes to one hour. Each drift was ended after the researchers observed that the drifters where drifting onto a different ecosystem or onto deeper waters, or after the one-hour period was over.

A kayaker was assigned to each drifter; the kayaker's job was to ensure that the drifter stayed in the designated ecosystem, to record information pertinent to the experiment, and to collect water samples. The kayakers followed the drifters within a distance of 5 meters to confirm that the drifter was within the designated ecosystem (eelgrass bed or mud flat) and to keep the drifters from being run over by boats. The kayaker was instructed to end the drift if the drifter was driven by wind or currents into a different ecosystem. During the experiment, there was no reported turbulence created by nearby boats that could have affected

the measured parameters. The kayakers also recorded information pertinent to the experiment, such as the start and end time of each drift, as well as any observable flora and fauna. The kayakers collected two replicate water samples at the beginning and end of the drift; these water samples were collected directly next to the drifter and were intended to provide information on the alkalinity, spectrophotometric pH, and salinity of the water. The purpose of collecting the water samples was to determine how the water chemistry changed as the water moved through each ecosystem. Because we worked with paired ecosystems that were close to each other, we assumed that any water chemistry changes in each ecosystem could be attributed to photosynthesis and respiration within each ecosystem. Immediately after collecting the water samples, the kayakers transferred them into a the research boat, where the samples were poisoned with 30μL saturated mercuric chloride, capped and stored in a cooler for laboratory analysis.

The procedure of deployment of the drifters and collection of water samples was repeated ten times, one for each replicate, during the course of two days. At the end of each day, information from the YSI sensors, GPS equipment, GoPro cameras, and electrodes was downloaded and transferred into a computer for future analysis. The water samples were transported to the laboratory at the end of day, where they were stored at room temperature until analysis.

e) Video footage analysis

The video footage was used in order estimate the percent cover of eelgrass and confirm that the drifters were placed on top of the desired ecosystem. The footage for all of the replicates represented more than eight hours of film. Static frames corresponding to 30 second intervals were studied and percent cover of eelgrass, algae, and bivalves was estimated. The estimations of percent cover where done by dividing each frame into four quadrants of equal size and estimating what percentage of each quadrant was covered by each one of these organisms. The individual estimates for percent area covered by the organisms in each quadrant were then added in order to calculate the total percent. Observations on the species of flora and fauna observed, estimated depth, and visibility were also noted.

f) Water chemistry analysis

The replicate water samples collected at the beginning and end of each ecosystem were analyzed in WA-DNR Water Chemistry Lab located in Olympia, WA. Samples were analyzed for spetrophotometric pH, total alkalinity (TA) and salinity. For pH analysis, the Hoffman Lab protocol for determination of the pH of seawater using indicator dye m-cresol purple was followed (appendix A) using an Ocean Optics Ocean View Spectrometer. For determination of total alakalinity, a Mettler-Toledo T-50 automatic titrator was used and the Hoffman Lab Protocol for total alkalinity titration of seawater was followed (appendix B). For

determination of salinity a Milwaukee MA887 digital refractometer was used.

Unfortunately, the results for TA and spectrophotometric pH varied widely between the replicates and showed large standard errors and coefficients of variance. Based on the large variation found within replicates and on the fact both analytical techniques gave accurate values for the reference solutions used, it was concluded that the bottles were probably contaminated with the residue from the acid-bleach wash and that this residue was affecting the test results. Because of the contamination of the water samples, total alkalinity and spectrophotometric pH could not be accurately determined. The analysis of salinity, on the other hand, was not affected by the sample contamination. In order to obtain pH values necessary to calculate the change in DIC for each ecosystem, the pH data measured by the YSI instruments on the drifters was used. In order to obtain total alkalinity (TA) values, which are also necessary to calculate the change in DIC, the lowest and highest values of a range of TA measurements that previously collected by DNR during March 2014 in Port Gamble were used. The reasoning behind using the highest and lowest TA values available for Port Gamble was so that we could capture as much of the natural variability of the real TA values in Gamble Bay. Also, photosynthesis and respiration do not affect the alkalinity of the sample, and as such, the eelgrass should not affect these values considerably.

g) Data organization and statistical analysis

Since the custom-made voltmeter used only measured the change in the electric potential of the water, these values had to be converted to pH. This

conversion was performed using the equation suggested in *Testing the Honeywell Durafet® for seawater pH applications* by Martz, Connery, & Johnson (2010) (Equation 6).

$$pH = \frac{E - E^*}{S}$$

Where

E= electrode potential (i.e voltage) of the second half-cell forming the circuit in the custom made pH meter.

E*= electrode potential (i.e voltage) of the first half-cell forming the circuit in the custom made pH meter.

$S = R \times T \times \ln(10)/F$ (R is the gas constant 8.3145 J/ K*mol, T is temperature in Kelvin; F is the Faraday constant 96485 C/mol)

Equation 6. Conversion of electric voltage to pH. Adapted from "Testing the Honeywell Durafet for seawater pH applications" by Martz et al., 2010, *Limnology and Oceanography: Methods, 8*

All data corresponding to pH, temperature, salinity, percent cover of eelgrass, bivalves and macro-algae, and other observations regarding the ecosystem, were compiled into an excel spreadsheet (Appendix C). Since the YSI and Durafet electrode only measured the parameters each minute, the values from the previous minute were assumed to be the same for the subsequent 30 second interval.

The video footage revealed that there was some eelgrass present in the mud flats treatment, which was assumed to contain little to no vegetation; the video also showed that the coverage of eelgrass in the eelgrass treatment was, in some cases, sporadic with bare patches. Thus, for the five eelgrass beds replicates

the average eelgrass coverage was 18% with a median of 15%. For the five "no eelgrass" replicates the average eelgrass cover treatment was 7% a median of 0%.

In an effort to amplify the difference between the two treatments, each eelgrass replicate was broken down into ten-minute intervals consisting of 20 data points (one every 30 seconds) and only the ten-minute intervals that showed average eelgrass coverage of 20% or more were included on the "eelgrass treatment." Similarly, only the ten-minute intervals that showed an average eelgrass cover of 5% or less were included under the "no eelgrass treatment." The 10 minute intervals were classified according to the percent cover of eelgrass that they showed and not according to the initial ecosystem classification. The re-classification of treatments resulted in nine replicates for the eelgrass treatment and eleven replicates for the no eelgrass treatment (Figure 22 and 23). A resampling- t test confirmed that the difference between the average eelgrass cover for the newly classified replicates for eelgrass treatment (mean = 25.15%) and the no eelgrass treatment (mean=0.60%) was significant (α=0.05, p=0.000).

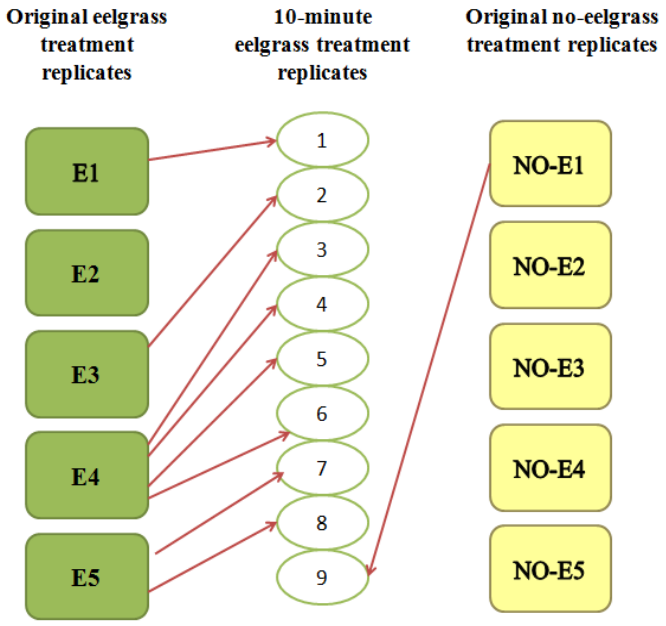


Figure 22. Diagram of the reclassification of eelgrass treatment replicates. Only 10 minute intervals that had an average cover of ≥ 20% eelgrass were used to create the nine 10-minute no-eelgrass treatment replicates.

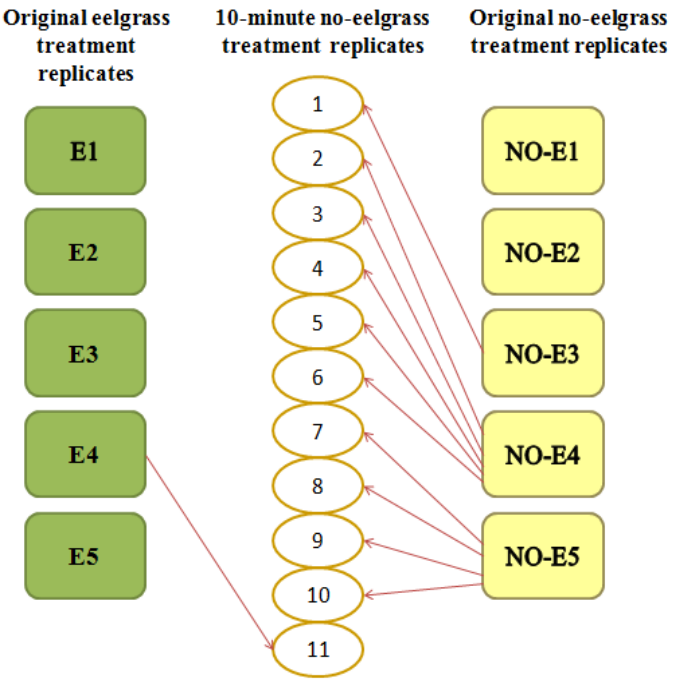


Figure 23. Diagram of the reclassification of no-eelgrass treatment replicates. Only 10 minute intervals that had an average cover of ≤ 5% eelgrass were used to create the eleven 10-minute eelgrass treatment replicates.

Before beginning with the statistical analysis the first 30-second data point from the reclassified replicate number seven of the eelgrass treatment was labeled an outlier, because the video footage revealed that the drifter was being placed in the water at that moment and this affected the pH value of that first data point. Similarly, the first four 30-second data points for reclassified replicate seven for the no eelgrass treatment were labeled as outliers for the exact same reasons. Thus, none of these data points were taken into account for the statistical analyses.

The change in pH over time (ΔpH/s) was calculated for each of the re-classified replicates by obtaining the slope of pH plotted against time. A Shapiro Wilks' test and a Levene's test was performed in order to determine if the rates of change of pH over time met the assumptions of normality and homogeneous variances required to perform a parametric t-test or a parametric one-way ANOVA. Since the data did not pass the assumptions required for a parametric test, a resampling t-test was performed in order to evaluate if there was a significant difference in ΔpH/s between the two treatments. After performing the resampling t-tests, ΔpH/s was converted into rates of change of pH over each minute (ΔpH/m) for the rates to be more applicable to field studies.

Lastly, because the water samples were contaminated, the upper and lower limits of a range of alkalinity values from unpublished data collected by WA-DNR in Port Gamble during March 2014, were used, along with the pH values from the custom made pH sensors, to obtain estimates of the DIC values for each

replicate using CO2SYS software. DIC measurements where calculated for every 30 second intervals in each one of the 10 minute replicates. The estimates of DIC were computed by inputting the measured temperature, salinity, pH (from field voltmeters), pressure (from YSIs estimated by using the conversion that 1m of depth =1 decibar of pressure) and the upper and lower values for the range of total alkalinity in Port Gamble. Because we had two different values for alkalinity (1963.10 mmol/kg = lowest and 2069.1 mmol/kg = highest) we had two different estimates for DIC for every 30 seconds of the 10-minute replicates.

From the resulting DIC values for each replicate, the rate of change of total DIC (micromoles per kilogram of seawater) over time (seconds) (Δμmol of DIC/kg *s) was calculated by plotting the DIC values over time and calculating the slope.

Four separate resampling t-tests were then performed in order to determine if there was a significant difference between the ΔDIC/s values for the following groups: eelgrass using high TA value vs. eelgrass using low TA value, no eelgrass using high TA value vs. no eelgrass using low TA value, eelgrass using low TA value vs. no eelgrass using low TA value, eelgrass using high TA value vs. no eelgrass using high TA value. After performing each test the results were converted into rates of change of DIC over each minute (ΔDIC/m units= μmol/kg* m) and rates of change of DIC per hour (ΔDIC/m units= μmol/kg* h) t make the results more applicable.

IV. RESULTS

For the eelgrass treatment, all nine 10 minute replicates showed that pH increased over time (Figure 24). For this treatment, replicate seven shows the highest rate of increase in pH over time (Figure 25). The average initial pH for the eelgrass treatment was 8.022 ± 0.012 (standard error) and the average final pH was 8.025 ±0.011 (standard error); a one-way parametric ANOVA revealed that values were not statistically different from each other (α=0.05, p=0.736). The maximum absolute difference for this treatment (final pH minus initial pH was 0.018 pH units, while the minimum difference was 0.001 pH units.

For the no eelgrass treatment, nine of the eleven replicates showed that pH increased over time (Figure 26). Two replicates, replicates 5 and 7, showed a decrease in pH over time (Figure 27). The average initial pH for the no eelgrass treatment was 8.109±0.026 (standard error), while the average final pH was 8.158±0.033(standard error). A one-way parametric ANOVA indicated that these values were not statistically different from each other (α=0.05, p=0.247). The maximum absolute difference for this treatment (final pH minus initial pH) was 0.147 pH units (indicating a decrease of pH over time), while the minimum difference was 0.007 pH units.

The no-eelgrass treatment had a significantly higher average pH, average temperature, and average salinity than the eelgrass treatment (Table 9). The results indicated that the no eelgrass treatment had a significantly higher temperature than the eelgrass treatment (difference=0.35°C, α=0.05, number of

trials=1000 and p=0.012). Salinity was also significantly higher in the no eelgrass treatment than in the eelgrass treatment (dif=0.33 ppt, α=0.05, number of trials=1000, and p=0.001). Similarly, pH was also significantly higher in the no eelgrass treatment than in the eelgrass treatment (dif=0.12 pH units, α=0.05, 1000 trials and p=0.002).

A resampling t-test was used to asses if there was a significant difference in the rates of change of pH over time (ΔpH/min) between the eelgrass treatment (mean=0.000843 pH/minute) and the no eelgrass treatment (mean=0.0239 pH/minute). The results showed that the rates of ΔpH/minute were not significantly different between treatments (α=0.05, 1000 trials and p<0.136) (Table 9).

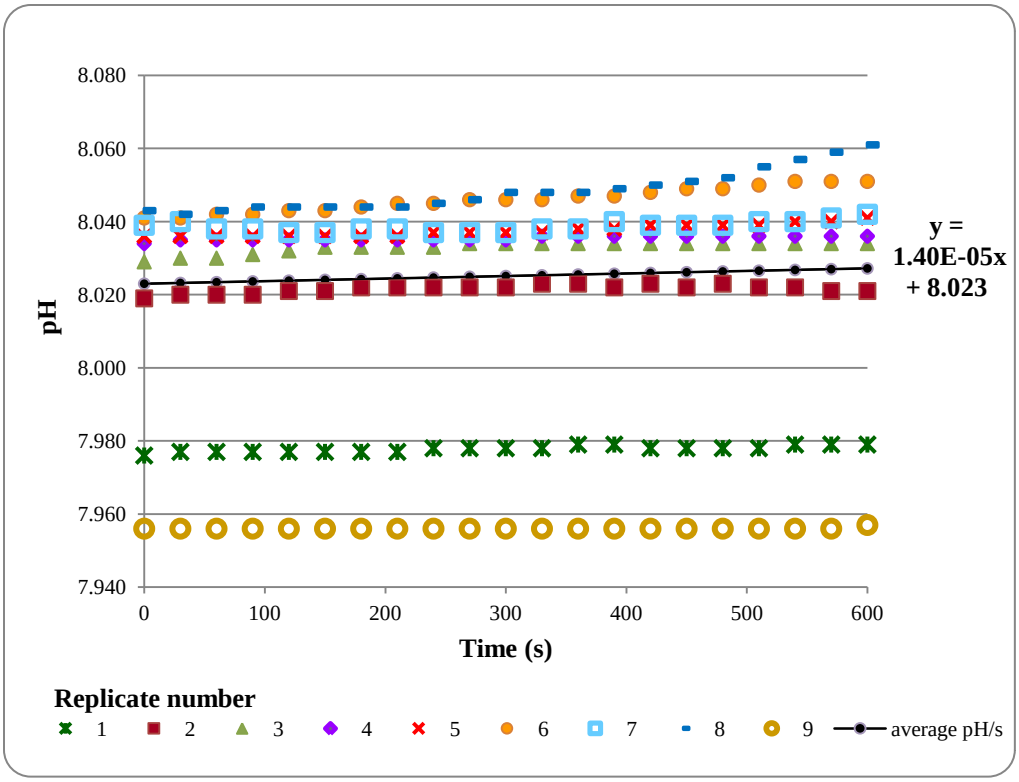


Figure 24. Change in pH over time for all of the nine replicates in the eelgrass treatment. The continuous black line represents the average change pH/s for all the replicates within this treatment, the equation on the right describes this line. The average rate of pH/s was then converted to pH/min for statistical analysis.

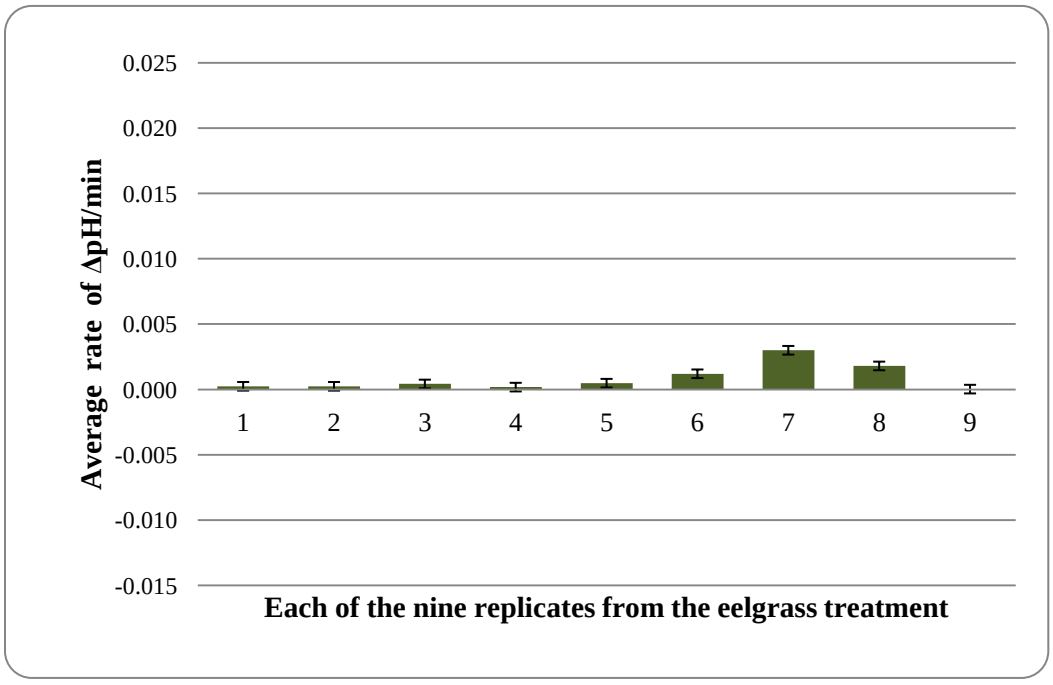


Figure 25. Average rate of change in pH/min for all the replicates in the eelgrass treatment. Error bars denote the standard error of the mean.

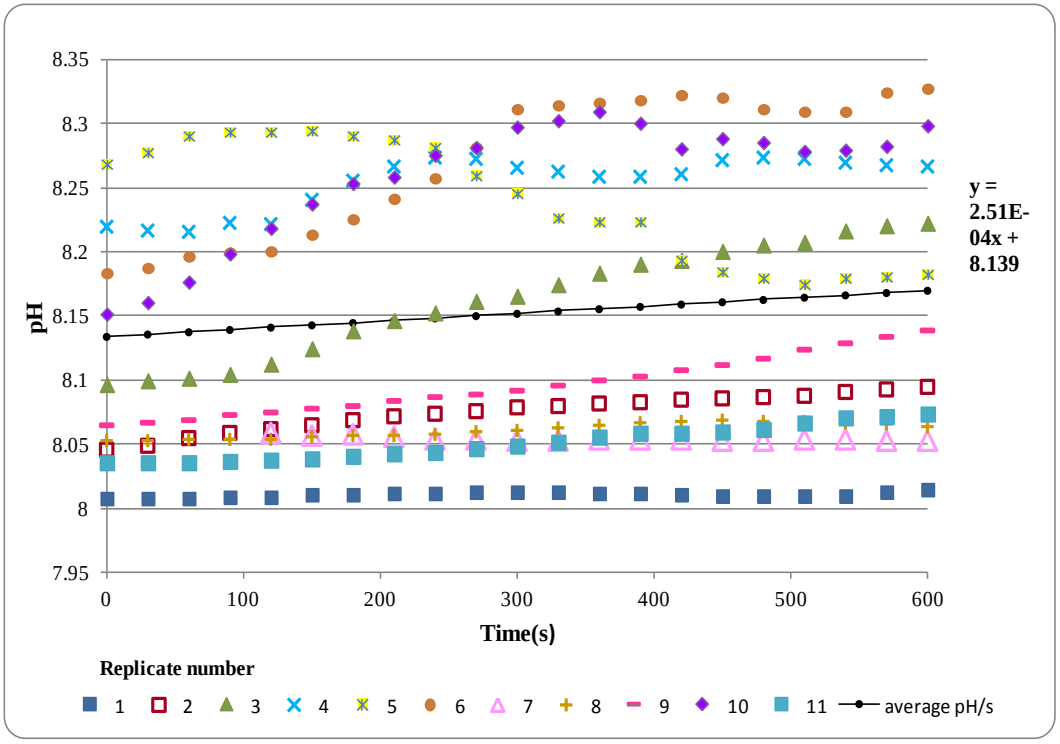


Figure 26. Change in pH over time for all of the eleven replicates in the no eelgrass treatment. The continuous black line represents the average change pH/s for all the replicates within this treatment, the equation on the right describes this line. The average rate of pH/s was then converted to pH/min for statistical analysis

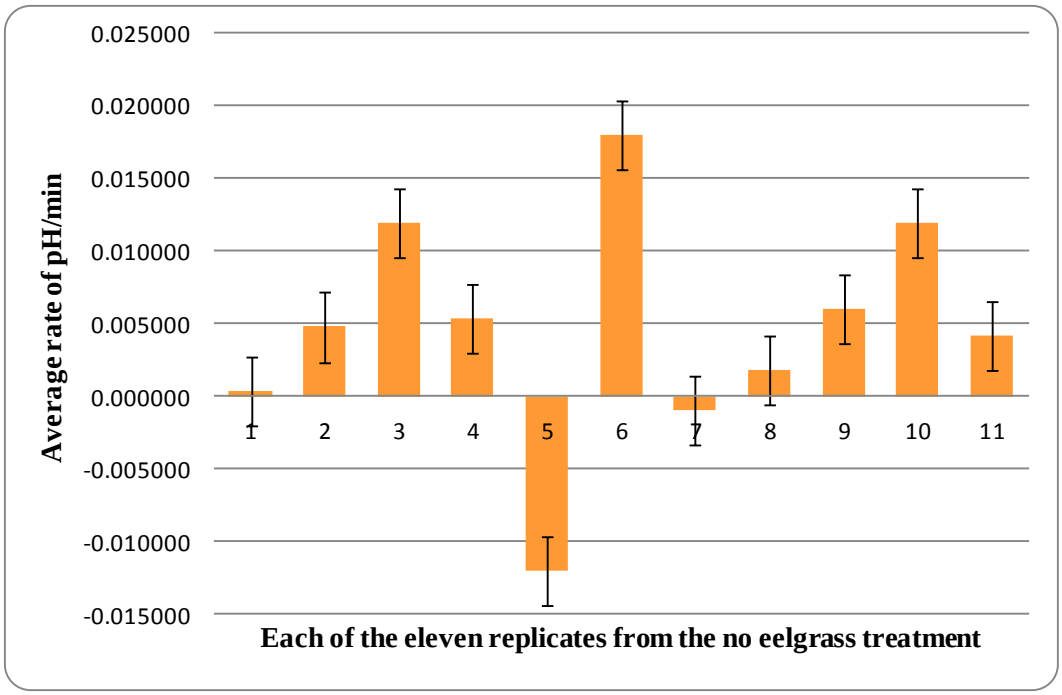


Figure 27. Average rate of change in pH/min for all the replicates in the no eelgrass treatment. Error bars denote the standard error of the mean.

Variable	Eelgrass treatment	SEM	No eelgrass treatment	SEM	p-value α=0.05
% of estimated eelgrass coverage	25	1	1	0	<0.001
Average temperature (°C)	8.49	0.02	8.84	0.08	0.012
Average salinity from water samples (ppt)	28.91	0.12	29.24	0.03	0.336
Overall average pH	8.02	0.01	8.14	0.03	0.002
Average ΔpH/s	1.40E-05	5.50E-06	3.98E-04	2.51E-04	0.136
Average ΔpH/minute	8.43E-04	3.30E-04	2.39E-02	1.51E-02	0.136
Average ΔpH/hour	5.06E-02	1.98E-02	1.43E+00	9.05E-01	0.136

Table 9. Comparison between variables for eelgrass and no eelgrass treatments and their respective standard error of the mean (SEM).

For the eelgrass treatment, using the lowest alkalinity value (1963.1 μmol/kg) the rate of change of DIC over each minute (ΔDIC/min) for each replicate ranged from -0.003 to -0.594 μmol of DIC/kg * min (indicating an increase in pH). Using the highest alkalinity value (2069 μmol/kg) the ΔDIC/min for each replicate in the eelgrass treatment ranged from -0.003 to -0.62 μmol of DIC/kg*min (indicating an increase in pH). The average ΔDIC/min for all replicates in the eelgrass treatment was -0.18 ± 0.06 μmol of DIC/kg (calculated using low TA) or 0.19 ± 0.06 μmol of DIC/kg* min (calculated using high TA). This rate is equivalent to an uptake of 10.8 ± 3.7 μmol of DIC/kg * hour

(calculated using low TA value) or 11.2 ± 3.9 μmol of DIC/kg* hour (calculated using high TA value) (Table 10).

For the no eelgrass treatment, using the lowest alkalinity value (1963 μmol/kg) the average rate of DIC over each minute (ΔDIC/min) ranged from 6.3 μmol of DIC/kg*min (indicating a decrease in pH) to -8.0 μmol of DIC/kg*min (indicating and increase in pH). For the same treatment, using the highest alkalinity value (2069.1 μmol/kg) the rate of ΔDIC/min ranged from 6.6 μmol of DIC/kg*min (indicating a decrease in pH) to -7.9 μmol of DIC/kg*min (indicating an increase in pH). The average (ΔDIC/min) for all replicates in this treatment was -2.0 ± 1.2 μmol of DIC/kg*min (calculated using low TA value) and -2.0 ±1.2 μmol of DIC/kg*min (calculated using high TA value). This rate of change is equivalent to an uptake of 117.8 ± 68.7 μmol of DIC/kg* hour (calculated using low TA value) and 121.5 ± 70.2 μmol of DIC/kg* hour (calculated using high TA value) (Table 10).

	EELGRASS				NO EELGRASS			
	Average ΔDIC µmol/kg* min	SEM for average ΔDIC µmol/kg * min	Average ΔDIC µmol/kg * hour	SEM for average ΔDIC µmol/kg *hour	Average ΔDIC µmol/kg* min	SEM for average ΔDIC µmol/kg * min	Average ΔDIC µmol/kg * hour	SEM for average ΔDIC µmol/kg *hour
Calculated using lowest alkalinity value (1963.1 mmol/kgSW)	-0.18	0.06	-10.8	3.7	-2.0	1.2	-117.8	68.7
Calculated using highest alkalinity value (2069.1 mmol/kgSW)	-0.19	0.06	-11.2	3.9	-2.0	1.2	-121.5	70.2

Table 10. Average rate of change of dissolved inorganic carbon (DIC) over time and its respective standard error of the mean (SEM) for both treatments.

The two resampling t-tests performed to determine if the TA value used had a significant effect on the average ΔDIC/min within each treatment were not significant. Hence, the average ΔDIC/min for eelgrass treatment calculated using the low TA value (1963.1 µmol/kg) was not significantly different from the average ΔDIC/min for the eelgrass treatment calculated using the high TA value (2063.1 µmol/kg), the same result applies for the no eelgrass treatment.

The two resampling t-tests performed to determine if the average ΔDIC/min between the eelgrass treatment and the no eelgrass treatment was significantly different when using the same TA value. The results showed that there was no significant difference between the average ΔDIC/min calculated using the low TA value for eelgrass (-0.2±0.1 µmol of DIC/kg*min) and no

eelgrass treatment (-2.0±1.2 µmol of DIC/kg*min) (α=0.05,1000 trials, p=0.166). Likewise, there was no significant difference between average ΔDIC/min calculated using the high TA value for eelgrass (-2.0±1.2 µmol of DIC/kg*min) and the no eelgrass treatment (2.0±1.2 µmol of DIC/kg*min)((α=0.05,1000 trials, p=0.142).

In general, the calculations for the carbonate chemistry for both treatments (calculated using the same TA values) showed that the no-eelgrass treatment resulted in lower average DIC and lower average pCO_2 values than the eelgrass treatment. Similarly, the no eelgrass treatment contained a higher proportion of the DIC in the form of carbonate ion (CO_3^{-2}) than the eelgrass treatment (Table 11 and Table 12).

Experimental variable	Eelgrass	SEM	No eelgrass	SEM
TA value used	1963.1	N/A	1963.1	N/A
Average pH	8.02	0.01	8.14	0.03
Average temperature (C°)	8.49	0.02	8.84	0.08
Average pCO_2 (µatm)	369.7	10.7	278.39	21.01
Total DIC (µmol/kg)	1840.7	3.6	1643.4	12.4
Total H_2CO_3 (µmol/kg)	17.6	0.5	13.1	1.0
Total HCO_3^- (µmol/kg)	1730.8	5.1	1658.9	18.9
Total CO_3^{-2} (µmol/kg)	92.26	2.01	120.8	7.5
Ω calcite	2.27	0.05	2.97	0.19
Ω aragonite	1.4	0.03	1.9	0.1

Table 11. The average values of carbonate chemistry variables and their respective standard error of the mean (SEM) for each treatment calculated using the lowest total alkalinity (TA) value available for Port Gamble.

Experimental variable	Eelgrass	SEM	No eelgrass	SEM
TA value used	2069.10	N/A	2069.10	N/A
Average pH	8.02	0.01	8.14	0.03
Average temperature (C°)	8.49	0.02	8.84	0.08
Average pCO_2 (µatm)	390.2	11.2	293.9	22.2
Total DIC (µmol/kg)	1942.5	3.7	1892.8	12.9
Total H_2CO_3 (µmol/kg)	18.6	0.6	13.9	1.1
Total HCO_3^- (µmol/kg)	1826.6	5.3	1751.4	19.8
Total CO_3^{-2} (µmol/kg)	97.4	2.1	129.33	8.2
Ω calcite	2.4	0.05	3.1	0.2
Ω aragonite	1.5	0.03	2.0	0.1

Table 12. The average values of carbonate chemistry variables and their respective standard error of the mean (SEM) for each treatment calculated using the highest total alkalinity (TA) value available for Port Gamble.

V. DISCUSION OF RESULTS

The results showed that the pH increased over time in all the replicates of the eelgrass treatment, which suggests that this ecosystem was acting as a net autotrophic ecosystem during the period when the experiment took place. These findings are consistent with previous studies (Hendriks et al., 2013; Shishido, 2013; Unsworth et al., 2012) which have shown that the carbon uptake of seagrasses can lead to an increase in the pH of seawater. Unsworth et al. (2012) reported an increase between 0.01 and 0.06 pH units for the water column directly above eelgrass beds located in the Indo Pacific region during winter (with a depth of 1 meter, 6 hr residence time and 25°C) (Unsworth et al. supplemental data). Shishido (2013) estimated a maximum increase of 0.05 pH units in the water column above *Z. marina* beds in Puget Sound (with a 6 h residence time, temperature and season not specified). If we were to calculate the ΔpH/hr from the maximum increase in pH reported by these studies, we would obtain a rate of change of 0.008 to 0.01pH units/hr, which seems very low compared to our reported rate of 0.05 pH units/hr for the eelgrass treatment. Both Unsworth et al. (2012) and Shishido (2013) assume a static system, in which the water remains on top of the seagrass beds for 6 hours as it is being diluted. Thus, it was expected that the change in pH reported by both studies would be higher than the change in pH reported in our study, since we witnessed movement of the water column over time. It is important to emphasize that direct comparisons between the results of our study and those of Unsworth et al.(2012) or Shishido (2013) are impossible

because unlike these studies, we did not take into account net primary production rates (NPP), shoot density, light intensity, area occupied by the seagrass bed, or the hydrodynamics of the place.

The results showed that on average, the pH also increased over time in the no eelgrass treatment; this is contrary to what was expected. We expected that the no eelgrass treatment would show a decrease in pH over time resulting from respiration being greater than photosynthesis in this ecosystem. The video footage revealed that the no eelgrass treatment had a considerable amount of mollusks (oysters and clams) and echinoderms (sea stars and sand dollars); thus, the expectation was that the respiration and calcification rates of these organisms would result in a decrease in pH. Respiration decreases the pH by adding more carbon dioxide (CO_2) to the water and calcification decreases the pH by taking up carbonate (CO_3^{-2}) from the water, thus reducing alkalinity and by releasing CO_2 as a by-product of the calcification reaction. However, our results suggest that for the no eelgrass treatment, other processes that decreased CO_2 dominated over the processes that increased CO_2 during the duration of our experiment.

In spite that the no eelgrass treatment had positive average rate of change in pH/time, there was no significant difference between the ΔpH/ time for both treatments. The most logical explanation for this result is that photosynthesis rates were constrained due to winter conditions. It is well known that low temperatures and low irradiance levels of photosythetically active radiation (PAR) slow photosynthesis in *Z. marina* (Larkum et al., 2006; Mumford, 2007; Nejrup & Pedersen, 2008; Touchette & Burkholder, 2007). Our data shows that during our

experiment, the average temperature for the water column above eelgrass beds was 8.49°C. This temperature is quite low considering that the lowest temperature that *Z. marina* can survive in is 5°C and that the ideal temperature for maximum eelgrass growth is around 20°C (Touchette & Burkholder, 2007). Similarly, eelgrass is known for needing high levels of light to grow and reproduce. During the summer, when most of *Z. marina* growth happens, Puget Sound gets approximately 5 to 6 hours of peak solar irradiance, while during winter, Puget Sound only gets approximately 0.8 to 1.6 peak solar irradiance hours (Honsberg & Bowden, n.d.; National Renewable Enegy Laboratory, n.d.). Thus, it is most likely that low temperatures and low irradiance levels significantly limited photosynthesis in eelgrass beds during our experiment.

In addition to photosynthesis being constrained by low irradiance and low temperatures, it is feasible that other variables, such as the differences in photosynthesis rates of other species and differences in depth affected the ΔpH/time for the no eelgrass treatment, resulting in an overall increase of pH/time (as further explained below). This overall increase in ΔpH/time for the no eelgrass treatment probably thwarted any significant carbon uptake that occurred in the eelgrass treatment, which resulted in the rates change of pH/time being statistically equal between both treatments.

The overall increase in pH/time for the no eelgrass treatment could also be attributed to shallower water column depth. The video footage showed that the no eelgrass areas were consistently shallower (approximately 0.5 meters in depth)

than the eelgrass areas (approximately 1-1.5 meters in depth). Assuming that alkalinity was the same for both treatment areas, shallower areas contain less water and thus are more sensitive to changes in the concentration of hydrogen ions or DIC for a given amount of biological activity. For example, a decrease in the concentration of hydrogen ions ($[H^+]$) in a shallower water column would result in a greater decrease in pH, than if the water column was deeper because there is proportionately more water to dilute the concentration of H^+ in the latter case. The sensitivity of shallower waters to changes in $[H^+]$ is supported by the data, which shows that the no eelgrass treatment exhibited greater variability, having a larger standard error than the non eelgrass group. In fact, two of the 11 replicates for the no eelgrass treatment showed a decrease in pH over time, while none of the replicates for the eelgrass treatment showed a decrease in pH over time.

A less plausible explanation for the no eelgrass treatment having a higher (although not significant) ΔpH/time than the eelgrass treatment is that the no eelgrass treatment had a higher net photosynthesis rate than the eelgrass treatment. This could be due to the no eelgrass treatment having a higher amount of non-seagrass photosynthetic organisms and/or due to the eelgrass treatment having a higher ecosystem respiration rate. The first point is supported by the video footage, which showed that although the no eelgrass treatment did not contain a significant amount of seagrass, there were other photosynthetic species present in this ecosystem. Abundant red and green algae were observed in the no eelgrass treatment; predominant species were the green algae *Ulva sp*. and the red

algae *Hildenbrandia sp and Gracilaria sp*. It was not possible to quantify the percent cover of algae because some video frames where blurry and in many cases it was impossible to distinguish crustose algae from shadows in the sediment and rocky structures. Because we did not quantify photosynthetic activity, the contribution that other photosynthetic organisms (such as algae and phytoplankton) had on the ΔpH/time for each treatment is unknown. However, a study by Ziegler & Benner (1999) indicates that the gross primary production of benthic algae can be about half that of seagrass beds and that occasionally, the NPP of benthic algae communities can be higher than that of seagrass beds. The second point, which assumes that the eelgrass treatment had a higher respiration rate than the no eelgrass treatment, can be attributed to eelgrass beds being a highly productive ecosystem. Eelgrass beds produce a large amount of plant biomass that harbors many species of fish and invertebrates and that fuels detritivorous pathways. Hence, it is reasonable to assume that the eelgrass treatment might have a higher rate of ecosystem respiration, due to higher heterotrophic consumption and decomposition, than the no eelgrass treatment.

In conclusion, our experiment showed that eelgrass beds in Port Gamble did not have a significant effect in the change of pH over time, when compared to the control treatment. Thus, eelgrass beds in Port Gamble were not capturing enough carbon to cause a significant change in ΔpH/time during the winter days. These results are possibly influenced by variables, such as depth of the water column and difference in respiration and photosynthesis rates between treatments, which were not taken into account in this experiment for simplicity purposes.

The results of this experiment are only applicable to diurnal low tide winter conditions in Port Gamble. Even though inferences on the carbon assimilation capacity of eelgrass beds in Port Gamble can be drawn based on this experiment, further experiments are necessary in order to determine if eelgrass beds in Port Gamble are net carbon sinks. A carbon sink is an ecosystem that has a positive net ecosystem carbon balance (Archer, 2010). A net ecosystem carbon balance (NECB) is defined as the net rate of organic carbon accumulation in (or loss from) ecosystems. Thus, a NECB implies that the ecosystem absorbs more carbon than it releases thought a defined period. In order to determine if eelgrass beds in Port Gamble Bay are carbon sinks, one would need to study how much carbon is captured and released through a year. Seagrasses, like all photosynthetic organisms, release carbon dioxide at night when respiration is dominating due to the lack of photosynthesis. This change in their metabolic cycle is highly dependent on the amount of irradiance and temperature. It is common knowledge that some seagrass ecosystems switch from being carbon sinks during the summer and spring, to being carbon sources during wintertime (Beer & Waisel, 1979). This happens because as irradiance decreases during the winter, their photosynthetic rates decrease, sometimes falling below their respiration rates. Additionally, in certain regions of the world, seagrasses are annual plants with their leaves dying during the winter (but their rhizomes and roots surviving underground). The fate of the dead biomass depends on the decomposition and burial rates of each site. Therefore, in order to determine if eelgrass beds in Port Gamble Bay are net carbon sinks, one would need to study the ecosystem's

photosynthesis and respiration rates, as well as the burial and export rates, during night and day and during different seasons of the year. Determining whether an ecosystem is a net carbon sink is a massive undertaking. Thus, even through inferences can be made about how eelgrass beds in Port Gamble Bay act during other months of the year, and at different irradiance levels based on the results of this experiment, the data presented in this study is not enough to draw definite conclusions about the net carbon sink capacity of eelgrass beds in this region of Puget Sound.

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VII. APPENDICES

APPENDIX A

Determination of the pH of seawater using the indicator dye m-cresol purple (Hofmann lab protocol)

We follow SOP 6b (Guide to Best Practices, Dickson et al., edited 1/28/2009) for the spec pH method. Major alterations we have had to make for our lab are:

-We collect water samples for pH analysis in either 125mL glass stoppered bottles with no headspace. (SOP 6b indicates that the water samples should be collected in the optical cells directly. This is not practical for our lab since we have no good way of storing or warming all of those cells at once).

-We use a BioSpec 1600 spectrophotometer which holds cells with a 1 cm path-length (rather than the 10 cm one specified in SOP6b). Therefore, we add **3mL** of seawater sample to each cuvette and **50uL** of dye.

Summary of relevant variables:

A_1 (absorbance 1) = absorbance at 578nm
A_2 (absorbance 2) = absorbance at 434nm
A_1/A_2=ratio of the two absorbances
A_1/A_{2corr}=ratio corrected for the addition of dye.
*730 is a non-absorbing wavelength. This value is used to correct for background noise associated with the spec

Preparation of dye and determination of dye correction factor

- Prepare a 2mmol solution of m-cresol purple in MilliQ. Adjust the pH to 7.9 (i.e. the approximate pH of seawater) using HCl.

- For each batch of dye prepared, a correction factor for the addition of dye must be determined. A full explanation of this is in section 8.3 of the Guide to Best Practices (Dickson *et al*., edited 1/2009). Briefly, you should:

  -Prepare seawater samples with three distinct pH values (e.g. 7.8, 8.0, 8.2)

For each sample:

-Measure and record absorbance of sample at 730, 578, and 434nm.

-Add 50uL of m-cresol purple and measure and record absorbance at the three wavelengths.

-Add a second 50uL of m-cresol purple and measure and record absorbance at the three wavelengths.

-Determine A_1/A_2 for each addition of dye (see SOP 6b and pH calculation worksheet).

-Perform a linear regression A_1/A_2 vs. $\Delta A_1/A_2$

A_1/A_2corr = (A_1/A_2)-V[a+b(A_1/A_2)] (see 8.3, SOP 6b)

Sampling and storage of sample before analysis

- Collect seawater sample in 125 mL glass stoppered bottle OR scintillation vial using silicon tubing[1]. There should be no headspace in either collection vessel.
- pH analysis should be performed immediately after collection. Place capped or stoppered samples in 25ºC water bath to begin warming prior to analysis.

Measurement procedure

- Clean and dry a quartz cuvette.
- Pipet 3mL seawater sample into quartz cuvette. Cap the sample and carefully clean the exterior of the cell with a Kimwipe.
- Place in warming chamber. Warm sample to exactly 25ºC.
- Measure and record the absorbances at the three wavelengths (730nm, 578nm, 434nm).
- Pipet 50uL dye into the cuvette, replace the cap, and invert the cell to mix the sea water and dye.
- Return the cell to the spectrophotometer and again measure the absorbances at the three wavelengths.

[1] If doing both pH and TA analysis, we collect sample in 125mL stoppered bottle. pH analysis only requires 3mL of sample, which we remove from the bottle before performing TA analysis. If only measuring pH, we use scintillation vials to limit the water being removed from treatment buckets.

Calculation and expression of results

See section 8 of SOP6b and annotated pH calculation worksheet

Note: you need salinity to calculate pk2. We use a YSI 3100 conductivity/salinity meter and generally take one salinity measurement for each bucket once a day and then use that value for calculation of the pH throughout the day.

APPENDIX B

Hofmann Lab – Total Alkalinity Titration Protocol

Rivest, E.B., Bitter, M.B., Hancock, J.R., (2013)

1. Turn on Mettler Toledo T50 titrator
2. Open LabX titration on desktop
3. Press Purge option on tablet
    a. Leave pH probe and bubbler out
    b. Attach purge acid cup
    c. Gently dislodge air bubbles from lines between titrant and burette, and burette and cup, during purge
    d. Press back twice to return to home (post purge)
4. Remove Purge acid cup and dispose liquid
5. Rinse all probes with DI water and dry with kimwipe
6. Collect 98-101 grams of filtered sea water (FSW) for titration
7. Collect approximately 75 grams of FSW and measure salinity using bench-top meter
8. Attach FSW cup to titrator
9. Make sure bubbler is turned on
10. Insert pH probe into sample cup (make sure probe is filled with solution)
11. Open plug on pH probe
12. In Lab X titration, click 'Analysis tab'. Make sure titrator is in "idle" mode
13. Right click 'EQP_Rivest 2012-020', click "run"
    a. Enter sample id and mass
    b. Click "start"
14. Once titration begins (propeller begins to spin), insert bubbler into sample cup
15. Observe first sample trajectory carefully for any abnormal spikes in graph
16. After completion of titration, open R on desktop.
    a. Within R program, open 'TA_Emily (1).R'
17. On desktop, open 'R data files' folder
    a. Open 'Result_TA.csv'
18. On LabX, click the 'Reports' tab. Find the sample run using the time stamp. Expand the menus until you select option 'EQP titration'

    a. Copy and paste the data below the graph into ‘Result_TA.csv’
    b. Make sure that there no rows that contain data from a previous titration. If there are, delete them
    c. Save the spreadsheet
19. On R-Script, change the weight, salinity and name of sample.
    a. Copy and paste the entire code onto R-console.
    b. Actual results will appear in grey
    c. For non-poisoned samples, use TA x 1,000,000
    d. For poisoned samples, use TA corrected (TA x 1,000,000 x 1.002)
    e. Record TA to two decimal places
20. After each titration, rinse and dry all probes
21. Dispose samples into proper waste containers
22. Repeat for second FSW sample and reference both samples for similarity with each other and with previous dates’ FSW samples
23. Make sure to always record results in general lab notebook as well as your own.
24. If necessary, continually repeat new FSW samples until results become consistent.
25. Next, repeat steps 6-21 with CRM from Dickson lab. Use salinity from CRM certification. Make sure your calculated TA is within +/- 10umol/kg of certified value.
26. If results are accurate, move on to your samples. For each sample, repeat steps 6-21.
27. Re-run a CRM sample after every 10 experimental samples and at the end of your day of titrations. This confirms that the pH probe did not drift over time.
28. To shut down, rinse and dry all probes. Put the plug back on the pH probe and store it in its specific storage solution. Place a cup of DI on the titrator for the other probes. Turn off the bubbler. Shut off the titrator and close Lab X.

APENDIX C First segment showing how data was organized in a spreadsheet

				SEDIMENT TYPE						VEGETATION											BIVALVES					
date	real time	elapsed time	mixed?	mud	sand-granule	peeble	cobble	boulder	% rock	eelgrass % cover	percent cover algae				total cover algae	ulva	gracilaria	Hildenbrandia		% cover diatoms	shell %	type of shell				
1/19/14											unk	re	brow	gree					genus/sp observed			frag	clam	oyster	barn	muss
Drift 2	13:55:00	0:00:00	n	x					0	30		10			10						2	x				
No	13:55:30	0:00:30	n	x					0	35		15			15						1	x				
eelgrass	13:56:00	0:01:00	n	x					0	25		1			1						2	x				
	13:56:30	0:01:30	n	x					0	30		1			1						1	x				
	13:57:00	0:02:00	n	x					0	25		10			10						1	x				
	13:57:30	0:02:30	n	x					0	25		15			15					nv	2	x				
	13:58:00	0:03:00	n	x					0	20		15			15			x		nv	1	x				
	13:58:30	0:03:30	n	x					0	50		35			35					nv	1	x				
	13:59:00	0:04:00	n	x					0	35		30			30			x		nv	1	x				
	13:59:30	0:04:30	n	x					0	30		40			40			x		nv	3	x				
	14:00:00	0:05:00	n	x					0	25		10			10			x		0	5	x				
	14:00:30	0:05:30	n	x					0	15		25			25			x		0	2	x				
	14:01:00	0:06:00	n	x					0	5		50			50					0	1	x				
	14:01:30	0:06:30	n	x					0	0		65		1	66					0	<1	x				
	14:02:00	0:07:00	n	x					0	0		80			80						<1	x				
	14:02:30	0:07:30	n	x					0	0		80			80						<1	x				
	14:03:00	0:08:00	n	x					0	0		80			80						<1	x				

Second segment showing how was organized in a spreadsheet

BIVALVES						FAUNA		NOTES						
shell %	type of shell						% fauna		Time	Temp	SpCond	Sal	Depth	Battery
	frag	clam	oyster	barn	muss				hh:mm:ss	C	mS/cm	ppt	meters	volts
2	x								13:55:00	7.61	44.3	28.3	0.054	12.7
1	x					fish		bottom within 3 feet						
2	x					tiny see-through fish			13:56:00	8.04	43.79	27.97	0.07	12.7
1	x					seastar								
1	x								13:57:00	8.21	43.62	27.86	0.075	12.7
2	x					sun star		Bottom 2-3 feet						
1	x								13:58:00	8.27	43.56	27.82	0.077	12.7
1	x							white crustose stuff on the bottom. Not sure what it is.						
1	x								13:59:00	8.29	43.55	27.81	0.077	12.7
3	x													
5	x								14:00:00	8.3	43.55	27.82	0.077	12.7
2	x													
1	x								14:01:00	8.3	43.55	27.82	0.077	12.7
<1	x													
<1	x								14:02:00	8.31	43.56	27.82	0.077	12.7
<1	x													
<1	x					anemone			14:03:00	8.31	43.56	27.83	0.078	12.7