EXAMINATION OF BIVALVE SHELL DEGRADATION FOR ALKALINITY REGENERATION PURPOSES IN HOOD CANAL, WASHINGTON

by

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A Thesis
Submitted in partial fulfillment
of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
June 2014

This Thesis for the Master of Environmental Studies Degree

by

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has been approved for

The Evergreen State College

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ABSTRACT

EXAMINATION OF BIVALVE SHELL DEGRADATION FOR ALKALINITY REGENERATION PURPOSES IN HOOD CANAL, WASHINGTON

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Spreading shell material can buffer corrosive conditions by providing alkalinity regeneration by dissolution of calcium carbonate ($CaCO_3$). This research explored how enhancing the seafloor with a particular size or species of bivalve shell may influence different rates of shell degradation in Hood Canal, Washington. The differences in degradation, measured by changes in mass, was examined over an incubation period of eight weeks among whole and crushed size types for three different species: *Crassostrea gigas*, *Ostrea lurida*, and *Mytilus galloprovincialis.* All shell treatments lost mass, while *M. galloprovincialis* shells degraded the most mass, losing up to 2.78% ± 0.08% of its shell matter. Each species had a significantly different rate of mass loss relative to the other species, whether the shell was crushed ($F_{2,87}$ = 37.39, p<0.0001) or whole ($F_{2,70}$ = 18.74, p<0.0001). For all species, whole shells displayed higher rates of SML than crushed shells for each of the species examined: *M. galloprovincialis* (p=0.02), *O. lurida* (p=0.003), and *C. gigas* (p=0.01). Through $CaCO_3$ dissolution, whole *M. galloprovincialis* and *C. gigas* shells may contribute the most g CO_3^{2-} every year to the seawater (133.3 ± 36.8 and 135.6 ± 18.6 respectively). Both whole and crushed shells of *M. galloprovincialis* contribute the greatest amount of organic matter among all the species through decomposition (11.5 ± 3.5 and 9.5 ± 3.4 respectively). Conversely, whole and crushed shells of *O. lurida* contributed the least amount of CO_3^{2-} and organic matter among all the species (52.7 ± 11.5 and 42.5 ± 25.6; 1.5 ± 0.4 and 1.4 ± 0.4 respectively). Nonetheless, all shell treatments contributed a substantial amount of CO_3^{2-}, relative to organic matter, and are recommended for alkalinity regeneration purposes.

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Acknowledgements

This research was supported by the faculty of The Evergreen State College, notably: Dr. Erin Martin, Dr. Carri LeRoy, Jenna Nelson, Ladd Rutherford, Kaile Adney, and the entire Science Support Center staff. In particular, I would like to thank my advisor, Dr. Erin Martin, for being incredibly supportive and inspiring throughout my time in the Master of Environmental Studies Program.

Thank you to Mitch Redfern, for all the constant support as my field assistant and partner. Thank you to Burke Hales and Joe Jennings at the College of Earth, Ocean, and Atmospheric Sciences at Oregon State University, for providing the water sample analyses, Brady Blake for the input that drove me to understand different perspectives, Brian Allen for guiding and motivating me through the first steps of this process, Andy Suhrbier for taking me on field trips to learn about the instruments that study ocean acidification, Rolin Christopherson for guiding me through the permitting process, Dave DeAndre and Saleh Prohim at Taylor Shellfish for donating fresh shells for this research, the Twanoh State Park rangers, Charlie Korb and Brent, for providing research quarters during the rainy months of February and March, Seattle Shellfish for lending me equipment for this research, Wendi Ruef, Sylvia Musielewicz, and Gretchen Thuesen on

the UW and NOAA teams for sharing their data, knowledge, and boat for the pure sake of science and education.

This research was partially funded by the Evergreen Foundation Grant and was completed under the Right of Entry Permit No. 23-090919 and the Shellfish Transfer Permit No. 14-0144.

CHAPTER ONE: LITERATURE REVIEW

Introduction

Ocean acidification (OA) is the prolonged reduction of seawater *p*H and is threatening marine life globally (Buck & Folger, 2009; Cooley et al., 2009; Doney et al., 2009; Fabry et al., 2008; Orr et al., 2005). OA is primarily caused by the uptake of anthropogenic carbon dioxide (CO_2) from the atmosphere by the ocean (Feely et al., 2004; Orr et al., 2005; Zeebe, 2001), but can also be influenced by local sources such as nutrient runoff, eutrophication, and other natural phenomena (Abril et al., 2003; Borges and Gypens, 2010; Feely et al., 2010). The need for action to prepare for OA is great in the inland and coastal waters of Washington State, a region that is especially vulnerable to synergistic causes of OA (Gazeau et al., 2007).

Probably the largest concern with OA is the magnitude and rapid pace of its effects due to anthropogenic influences. It is estimated that surface ocean *p*H has dropped slightly more than 0.1 *p*H units from 8.25 to 8.14 since the beginning of the Industrial Revolution in 1751 (IPCC, 2013). It is forecasted to decrease another 0.29 *p*H units (near 7.85) by 2100 (Jacobson, 2005). Although these decreases seem miniscule, the *p*H scale is logarithmic, so each *p*H unit is a 10-fold change, where the change from 8.25 to 8.14 corresponds to a 26% increase in the hydrogen ion concentration ([H+]) (IPCC, 2013). These drastic changes in seawater chemistry are reducing the

concentration of the carbonate ion in seawater, and thus the level of calcium carbonate saturation. Calcifying organisms are most vulnerable, as they have difficulty maintaining their exoskeletons (Orr et al., 2005) under these conditions. This is evident, as shellfish hatcheries have experienced losses in oyster larvae since 2008 (Barton et al., 2012). Natural recruitment of other bivalves has also decreased (Place et al., 2008) with ocean acidification being the main culprit.

Shellfish provide many ecosystem services, including provisioning services such as food and income; regulating services such as water quality through the control of eutrophication, algal blooms, and hypoxia; supporting services such as nutrient cycling that maintain ecosystem functions; and cultural services such as spiritual, recreational, and social benefits (Cooley et al., 2009; UNEP and Millenium Ecosystem Assessment Board, 2005). Ocean acidification can disproportionately affect coastal ecosystems and the communities that rely on them by negatively affecting bivalve molluscs and the ecosystem services they provide (Borges and Gypens, 2010; Kelly et al., 2011).

In this literature review, I will discuss how the waters in Washington State are influenced by both global and local causes of OA. Next, I will highlight the implications that OA encompasses, including ecological change (Cooley et al., 2009) and alteration of marine-based resources (Cooley and Doney, 2009). I will then explain why calcifying organisms are most at risk

of dissolution (Gazeau et al., 2007; Miller et al., 2009; Orr et al., 2005) and describe the chemical mechanics behind this phenomenon. Lastly, this literature review will explore how spreading shell material can buffer seawaters from OA and how recent studies have contributed to the understanding of shells' capacity to impact alkalinity. This thesis will explore the notion that the size or species of shell may influence different rates of shell dissolution, which may help restoration organizations manage their approach to shell recycling efforts.

The Causes and Effects of Ocean Acidification in Washington State

In the following sections, both natural and anthropogenic factors that contribute to ocean acidification will be discussed. First, seawater carbonate chemistry will be described to understand the chemical reactions behind ocean acidification. Then, the study region will be discussed, with respect to seawater circulation patterns. Lastly, the global and local contributions to ocean acidification will be discussed further in depth, focusing on why this study region is vulnerable to ocean acidification. These sections will setup the foundations of why this research is needed to further understand the causes and effects of ocean acidification.

Seawater Carbonate Chemistry

The oceanic carbonate system can influence and be influenced by *p*H — the measurement of hydrogen ion concentration, $[H^+]$, in seawater through a series of chemical reactions that occur at equilibrium. The carbonate system comprises three inorganic species of carbon: carbonic acid (H_2CO_3) bicarbonate, (HCO_3^-) and carbonate (CO_3^{2-}). A forth form is carbon dioxide, CO_2 (aq) = aqueous carbon dioxide, which is chemically not separable from H_2CO_3 (Zeebe, 2001). Throughout this literature review, the former H_2CO_3 (Eq. 1) will be used. The sum of the dissolved forms of H_2CO_3, HCO_3^-, and CO_3^{2-} is called the total dissolved inorganic carbon (DIC) (Eq. 2).

$$[H_2CO_3]^* = [CO_2\,(aq)] + [H_2CO_3] \quad (1)$$

$$DIC = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \quad (2)$$

where brackets refer to total stoichiometric concentrations and the asterisk denotes a sum of two compounds.

When CO_2 first dissolves into seawater, it immediately reacts with water, H_2O, and forms H_2CO_3 (Eq. 3). Because H_2CO_3 is a weak acid, it can disassociate into H^+ and HCO_3^-, which can further disassociate into H^+ and CO_3^{2-}. This system is reversible and equilibrates depending on the temperature, salinity, and *p*H of the seawater (Doney et al., 2009). Under

current oceanic conditions, the most abundant form of inorganic carbon is HCO_3^- (91%), followed by CO_3^{2-} (8%), and H_2CO_3 (1%) (Raven et al., 2005). This is illustrated in Fig. 1 by the dotted line that crosses over the concentration curves at *p*H = 8.1. Also indicated in Fig. 1 are the pH values at which $[H_2CO_3] = [HCO_3]$ and $[HCO_3^-] = [CO_3^{2-}]$. The circle and the diamond indicate $pK_1^* = 5.86$ and $pK_2^* = 8.92$ as the equilibrium constants (Zeebe, 2001), or when the stoichiometric concentrations of the products and reactants are equal.

$$H_2O + CO_2 \overset{K_0}{\rightleftharpoons} H_2CO_3 \overset{K_1}{\rightleftharpoons} HCO_3^- + H^+ \overset{K_2}{\rightleftharpoons} CO_3^{2-} + 2H^+ \qquad (3)$$

where K_0, K_1 and K_2 are equilibrium constants.

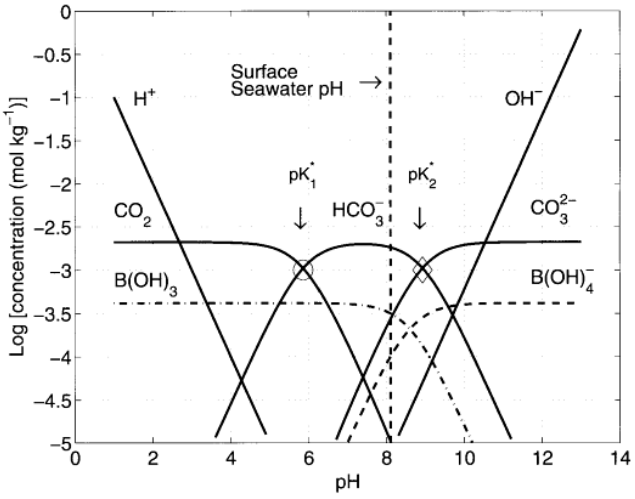

Figure 1. Bjerrum plot of the carbonate system in the ocean graphing the equilibrium relationships (Zeebe, 2001).

Ocean acidification can cause significant shifts in ocean carbonate chemistry by altering carbonate speciation. For instance, if the concentration of H_2CO_3 increases in the water by intrusion of atmospheric CO_2, H_2CO_3 would disassociate into HCO_3^- and H^+ because HCO_3^- is the dominant species of carbon in the seawater. Then, existing CO_3^{2-} reacts with the H^+ by forming more HCO_3^-. The *p*H does not change rapidly because the carbonate system is a natural buffer for seawater, but $[CO_3^{2-}]$ decreases appreciably (Zeebe, 2001), effectively reducing the buffering capacity, also known as alkalinity.

There are many definitions of alkalinity (Andersson et al., 2003; Morse et al., 2007; Rounds, 2006; Wolf-Gladrow et al., Dickson, 2007; Zeebe, 2001), as it is a concept of increasing complexity. Most basically, alkalinity is a measure of the capacity for seawater to resist sudden changes in pH by absorbing hydrogen ions using available bases such as bicarbonate and carbonate. For example, carbonate can absorb two hydrogen ions before turning back into H_2CO_3 because it has a double negative charge. Therefore, having more carbonate ions will increase the seawater's alkalinity. In the ocean, there are more ions than just carbonate and bicarbonate that contribute to the alkalinity. Total alkalinity (TA) is a measure of the alkalinity caused by the presence of both carbonate species and non-carbonate species, including boric acid, and hydroxide. (Dickson et al., 2007) defines TA as:

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^=] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [H_3SiO_4^-] + [NH_3] + [HS^-] - [H^+]_F - [HSO_4^-] - [HF] - [H_3PO_4] \quad (4)$$

where $[H^+]_F$ is the free concentration of hydrogen ions.

In order to understand how CO_2 emissions are affecting the ocean, we must understand the sea–air CO_2 exchange. Due to thermodynamics, equilibrium at the sea-air interface can be characterized as an equality of partial pressures. For example, when the partial pressure of carbon dioxide in the air is greater than that of seawater's, the seawater draws in the atmospheric CO_2. The partial pressure of CO_2 (pCO_2) refers to the gas phase that is in equilibrium with that of seawater (Zeebe, 2001).

DIC and pCO_2, alkalinity, and pH constitute four measureable factors in the carbonate system that can be determined analytically (Wolf-Gladrow et al., 2007). The knowledge of any two of them allows us to calculate the carbonate chemistry of a seawater sample. For this thesis, the DIC and pCO_2 values were measured and entered into a computer-modeling program to calculate pH, alkalinity, and the calcium carbonate saturation values.

Water Circulation of Puget Sound and Hood Canal

Puget Sound is a semi-closed estuary in Washington State with many freshwater inputs and an oceanic passage at its northern end through the Strait of Juan de Fuca (Fig. 2). It consists of interconnected basins separated by sills. A shallow sill at Admiralty Inlet limits the exchange of seawater to

and from the Pacific Ocean (Ebbesmeyer and Barnes, 1980). Although tidal currents and vertical mixing are strongest at Admiralty Inlet, there is little water movement and strong vertical stratification below Admiralty Inlet (Feely et al., 2010). This contributes to the sluggish circulation in the inlets that branch from Admiralty Inlet, including Hood Canal.

Hood Canal is a deep, natural fjord forming one of the major basins of Puget Sound in Washington State. In Hood Canal, the seawater circulates slowly and has a residence time that varies from 64 to 121 days (Babson et al., 2006; Warner et al., 2001). This residence time is long, compared to the northern Whidbey Basin, whose residence time varies from 33 to 44 days (Babson et al., 2006).

Circulation is driven by new water inputs, wind, and upwelling currents. During winter months, circulation is gradual, due to the intrusion of newer, denser ocean water (Warner et al., 2001). During summer months, circulation is more rapid due to northerly winds that push the surface layer in the main stem of Hood Canal northwards, resulting in upwelling of deeper waters (Feely et al., 2010).

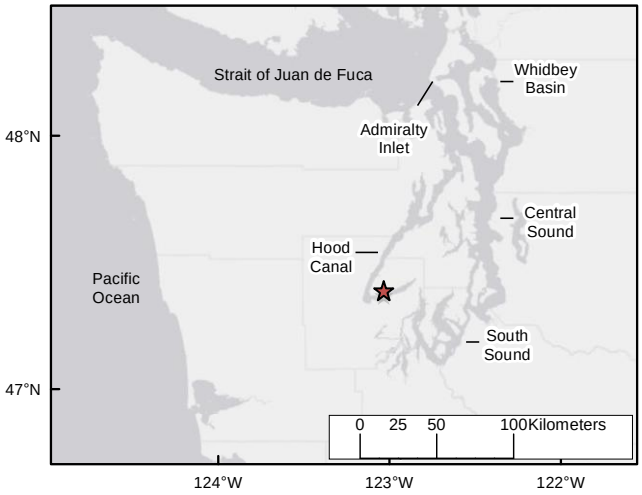

Figure 2. Map of the main bodies of water surrounding Washington State, including Admiralty Inlet and the main Puget Sound Basins. The star represents the study site in Southern Hood Canal.

Upwelling exposes deep water rich with CO_2 and nutrients to the surface. Because of the slow water circulation in Hood Canal during winter conditions, the water stratifies vertically with cold, salty water in the depths. Compared to the main basin of the Puget Sound, Hood Canal has a stronger stratification that separates the upper and lower layers of the water column (Warner et al., 2001). The lack of flushing in Hood Canal during winter causes confinement of acidic waters, which makes it an excellent site for studying the effect of ocean acidification on shell dissolution on the seafloor.

Global Contributions to Ocean Acidification

Rates of atmospheric CO_2 emissions have increased exponentially since the Industrial Revolution (Orr et al., 2005). Human activities cause a net CO_2 flux to the atmosphere from burning fossil fuels, cement production, and

land use change such as deforestation (Chapin et al., 2011; IPCC, 2013). From 1750 to 2011, anthropogenic CO_2 emissions have released 545 gigatonnes of carbon (Gt C) to the atmosphere. From these cumulative anthropogenic CO_2 emissions, 240 Gt C (~44%) have been stored in the atmosphere, 150 Gt C (~27%) have been accumulated in natural terrestrial ecosystems, and 155 Gt C (~28%) have been absorbed by the ocean (IPCC, 2013).

The total ocean uptake flux, including the anthropogenic CO_2, is estimated to be 2.7 ± 0.5 Gt C in 2011 (Quéré et al., 2013). This rate differs among spatial and temporal values, though the general trend in the literature confirms that seawater and atmospheric pCO_2 correlate (Doney et al., 2009) indicating that human emissions of CO_2 have caused and will further cause CO_2 absorption by seawater due to air-sea CO_2 exchange (Cooley et al., 2009; Doney et al., 2009; Orr et al., 2005).

In Washington State, the other mechanism for the invasion of CO_2 into coastal waters is attributed to upwelling events. The change in seasonal wind directions dictate the upwelling or downwelling currents that occur over the oceanic continental shelf and the sills of Puget Sound (Feely et al., 2010). Upwelling exposes water rich with CO_2 from the deep water to the coast. Globally upwelling water contains CO_2 that has accumulated from past anthropogenic additions, biological respiration, and physical-chemical processes due to the ocean's thermohaline circulation patterns (Chapin et al.,

2011; Orr et al., 2005).

Global contributions of CO_2 may have a profound impact on pH and CO_3^{2-} availability. The increases of atmospheric CO_2 — and consequently the H_2CO_3 in the seawater —have a relatively small effect in waters with a high *p*H and alkalinity. However, as more atmospheric CO_2 is absorbed and $[CO_3^{2-}]$ declines, the change in the H_2CO_3 and $[H^+]$ gets stronger as CO_2 is added, effectively lowering *p*H and CO_3^{2-} levels (Orr et al., 2005; Zeebe, 2001).

Local Contributions to Ocean Acidification

In Puget Sound, global influences can account for 24-49% of the *p*H decrease in the deep of waters of Hood Canal relative to pre-industrial levels (Feely et al., 2010). However, recent studies demonstrate that local sources of nutrients delivered by freshwater inputs, pollutants, soil erosion, water circulation, biological processes can acidify coastal waters at substantially higher rates than atmospheric carbon dioxide alone (Abril et al., 2003; Borges and Gypens, 2010; Feely et al., 2010; Kelly et al., 2011). These impacts are likely to be intensified when combined with other stressors in coastal ecosystems, such as overfishing, habitat destruction, and temperature increases (Kelly et al., 2011).

Estuaries within Puget Sound often experience eutrophication through the over-abundance of nutrients in the water (Mackas and Harrison, 1997; Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Eutrophication can be natural or human-caused, though the leading cause in Puget Sound is attributed to agricultural nutrient runoff and insufficient wastewater treatment (Feely et al., 2010; Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Eutrophication can cause disproportionate plant and algae growth that can lead to hypoxic zones after aerobic bacteria break down the organic material and deplete dissolved oxygen levels. Waters then becomes supersaturated with respect to CO_2 due to aerobic respiration, which can lead to an increase in $[H^+]$ and decrease in $[CO_3^{2-}]$ (Abril et al., 2003).

In estuaries, low salinity and high productivity due to riverine inputs create conditions that can be corrosive to shells due to inputs of organic matter that can fuel net respiration due to natural or anthropogenic stimulated respiration processes in the estuary (Abril et al., 2003; Feely et al., 2010; Kelly et al., 2011). Further, estuarine carbonate chemistry can be variable and complex due to many biogeochemical processes driven by production and respiration cycles, freshwater input, and atmospheric CO_2 (Waldbusser et al., 2011) and it is unclear to what magnitude these local impacts contribute to acidification.

Calcium Carbonate Mechanics

Calcium Carbonate Saturation State

The calcium carbonate ($CaCO_3$) saturation state is the equilibrium between the solid state and solution. One well-known and profound effect of OA is the lowering of $CaCO_3$ saturation states (Zeebe, 2001), which can impact calcifying organisms negatively (Feely et al., 2010; Green et al., 2013, 2009). Because Ca^{2+} is such an abundant ion in seawater, the $CaCO_3$ saturation state (Ω) in seawater is controlled by the amount of CO_3^{2-} available (Eq. 5). As mentioned before, when $[H_2CO_3]$ increases in seawater, $[CO_3^{2-}]$ is reduced because H^+ binds to it, leading to decreased CO_3^{2-} availability and lower $CaCO_3$ saturation states. As ocean *p*H falls, decreasing levels of CO_3^{2-} limits calcifying organisms to form their shells or skeletons because of lower aragonite and calcite saturation states (Feely et al., 2010).

$$\Omega_{\text{calcium carbonate}} = [CO_3^{2-}]\,[Ca^{2+}] / K^*_{sp} \qquad (5)$$

where K^*_{sp} is the solubility product, or equilibrium constant, of $CaCO_3$.

The saturation horizon is the depth in the ocean that identifies the boundary between super-saturation and under-saturation of $CaCO_3$ (Orr et al., 2005). As particles fall through the water column, respiration occurs, and CO_2 accumulates with depth, making it more acidic. Above the saturation horizon, the saturation state (Ω) is greater than 1 and $CaCO_3$ does not readily

dissolve into Ca^{2+} and CO_3^{2-}. However, below the saturation horizon, the saturation state (Ω) is less than 1, and $CaCO_3$ will dissolve. When the saturation state (Ω) equals 1, the reaction is in equilibrium (Eq. 6) and $CaCO_3$ is dissolving at the same rate that it is precipitating.

$$CO_3^{2-} + Ca^{2+} \rightleftharpoons CaCO_3 \quad (6)$$

The carbonate compensation depth occurs at a depth in the ocean where production is exceeded by dissolution. Surface seawater is supersaturated with respect to calcite and aragonite (Morse et al., 2007). However, $CaCO_3$ can dissolve in waters supersaturated with $CaCO_3$ (Waldbusser et al., 2011), demonstrating that the carbonate compensation depth can be correlated — but not directly dependent upon — certain values of the saturation horizon.

Both dissolution and precipitation of $CaCO_3$ can occur depending on carbonate saturation levels. Precipitation and dissolution are quantified by their rates. Solubility quantifies the dynamic equilibrium state achieved when the rate of dissolution equals the rate of precipitation. The typical unit for dissolution is mol/s, compared to the unit for solubility as mol/kg (Chang, 2013).

Biogenic Calcification

Biogenic calcification is the biological ability to precipitate $CaCO_3$ from seawater (Eq. 6), most often in the phase of calcite or aragonite (Doney et al., 2009; Morse et al., 2007). This thesis will be examining the shells from bivalve molluscs, which are made up of mostly mineral calcium carbonate, $CaCO_3$, an important component of marine sediments and biological organisms.

$$CO_3^{2-} + Ca^{2+} \rightleftharpoons CaCO_3 \quad (6)$$

Different organisms from all three domains of life are able to produce minerals that serve a variety of functions. In fact, living organisms utilize more than sixty different minerals, including amorphous minerals, inorganic crystals, and organic crystals (Addadi and Weiner, 1992). Calcium minerals represent about 50% of all known biogenic mineralization, reflecting calcium's abundance in the ocean as well as its versatile use in cells (Addadi and Weiner, 1992).

Aragonite and calcite are both mineral forms of $CaCO_3$, though they differ in the positions of their CO_3^{2-} (Addadi and Weiner, 1992), which influences the crystalline structure and solubility constant (Morse et al., 2007). Aragonite is also both denser and more soluble than calcite (Morse et al., 2007). This chemical and anatomical difference between aragonite and

calcite may have powerful biological implications, as changes in seawater chemistry may affect aragonite-calcifiers more negatively than calcite-calcifiers (Morse et al., 2007).

Aragonite plays an important role in the calcifying life stages of pteropods, corals, and larval oysters (Barton et al., 2012). Larval oysters precipitate aragonite to form their initial larval shell. Following settlement, they then form their shell out of calcite. They seem to be particularly susceptible to changes in seawater chemistry when the larval shell is formed from the more-soluble aragonite (Barton et al., 2012; Beniash et al., 2010; Kurihara et al., 2007).

In one study, early development in juvenile oysters slowed when exposed to acidified conditions (*p*H = 7.4) (Kurihara et al., 2007). *In situ* experiments in surface waters validate previous lab-based experiments that highlighted decreased calcification rates in juvenile Pacific oysters (*C. gigas*) (Barton et al., 2012). In another study, shells of the larval Mediterranean mussel *(Mytilus galloprovincialis*) dissolved due to the expenditure of more energy needed to maintain calcifying functions in very acidic waters (Kurihara, 2008). These studies have alluded that lower calcium carbonate saturation states are due to decreasing pH and $[CO_3^{2-}]$.

Shell Composition

Although bivalve shells are mostly made of $CaCO_3$, there are additional components within it that need to be considered when examining shell degradation, including organic matter and other inorganic elements. The inorganic and organic matter in shells was examined throughout the literature to understand if shell mass loss can be attributed mineral dissolution or organic decomposition.

According to a study on the chemical-mechanical characteristics of oyster shell, $CaCO_3$ accounts for 96% of the inorganic material in its mineral phase of calcite (Yoon et al., 2003). The remaining mass was composed of seven other minerals, including silica, magnesia, and sodium oxide in trivial amounts (Yoon et al., 2003). Therefore, mineral dissolution can be principally attributed to dissolution of $CaCO_3$.

Depending on the proportion of organic matter of the shell, it can slow mineral dissolution, thus potentially making shell degradation less favorable for alkalinity regeneration purposes. The organic matter found in bivalve shells can be described as an organic protein matrix that is enclosed around the $CaCO_3$ crystals (Simkiss, 1965; Weiner & Hood, 1975). The proportion of organic matter proportions in bivalve shells can depend on species and life stage (Barros et al., 2013; Barton et al., 2012; Glover and Kidwell, 1993; Goulletquer and Wolowicz, 1989; Kvenvolden et al., 1980; Waldbusser et al., 2011; Weiner and Hood, 1975). As such, the protein proportion in shell can

vary from 0.1 to 10% of shell weight between different species of bivalves (Almeida et al., 1998). (Goulletquer and Wolowicz, 1989) measured the organic material in clam shells and found that the average percentage of shell organic matter in the shell varied from 2.38% for *Cardium edule* and 2.34% for *Cardium glaugum* to 2.80% for *Ruditapes phillippinarum*. The proportion of organic matter in bivalve shells is understudied and little is known on this actual figure.

Within the organic matrix of the bivalve shell, there is a mixture of glycoproteins, mucopolysaccharides, lipids and amino acids, with differences that depend on the species (Weiner and Hood, 1975). (Simkiss, 1965) compared organic matter of the California mussel (*Mytilus californius*), the Eastern oyster (*Crossostrea gigas*), and a snail (*Australorbis globratus*) and found that the protein in molluscan shells resembled each other closely compared to the snail shell, indicating that bivalve shells have a generally similar organic matrix compared to other taxa. More research is needed to analyze the proportions of organic matter in different species of shells to examine if organic decomposition may be the mechanism for shell degradation rather than mineral dissolution.

Responding to Ocean Acidification: Shell Recycling

Utilizing Shells as an Alkalinity Buffer

Calcium carbonate plays an important role in regulating carbon sequestration by the oceans. However, the rate of anthropogenic CO_2 additions is outpacing the ocean's ability to restore oceanic pH and carbonate chemistry. By putting shells back into targeted areas, local waters may be able adjust to the abnormal amounts of CO_2 that is invading the waters.

Recognizing the risks of ocean acidification to Washington, Governor Christine Gregoire created the Washington State Blue Ribbon Panel on Ocean Acidification to chart a course for addressing the causes and consequences of acidification. In a collaborative effort to strategically respond to the effects of OA, the Panel created both short and long-term goals. One possible short-term strategy to combat locally intensified acidification is to return shell material to coastal habitats where shellfish are present. Spreading shell material ($CaCO_3$) can buffer corrosive conditions by increasing seawater alkalinity (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). The dissolution of $CaCO_3$ provides alkalinity regeneration by buffering weak acids, such as H_2CO_3 (Abril et al., 2003; Morse et al., 2007; Waldbusser et al., 2013). Conversely, the physical removal of shell from the system also would result in the loss of a substrate that can contribute to alkalinity through dissolution.

In addition to alkalinity regeneration, shells can also be used as important substrate for oyster reef restoration (Brumbaugh and Coen, 2009). Physical habitat restoration for shellfish most often involves placing fresh, weathered, or fossilized dredged shell directly on the bottom of the seafloor. Shells act as a suitable habitat for shellfish larvae to settle, grow, and die (Brumbaugh and Coen, 2009). Enhancing an area with shells may increase shellfish populations as well as restoring the shell resource.

Shell recycling can also provide ecological benefits. Shells form complex structures that provide refuge or hard substrate for other species of marine plants and animals to inhabit, enhancing biodiversity (Dumbauld et al., 2009; Gutiérrez et al., 2003). On the Pacific Coast, shells of the Pacific oyster placed at high densities in the intertidal zone provide excellent habitat for juvenile Dungeness crabs (Ruesink et al., 2006).

Alkalinity Regeneration Performance in Shells

Studies in the primary literature have tested if shell recycling can indeed provide alkalinity regeneration by shell dissolution. Most studies took place in the Chesapeake Bay, an area that has been overharvested and polluted heavily. One study added crushed shells of the hard shell clam (*Mercenaria mercenaria)* to a mudflat before seeding it with *M. mercenaria* juveniles. The addition of shell material caused the $CaCO_3$ saturation state to increase from $\Omega = 0.25$ to $\Omega = 0.53$ (Green et al., 2009), which is a small,

yet effective change which increased the number of live clams almost three-fold in two weeks, suggesting that settling clam larvae respond greatly to increased $CaCO_3$ saturation (Green et al., 2009). In a similar and more recent study, Green et al. (2013) tested their experiment again to examine if clam larvae respond positively to increased $CaCO_3$ saturation in both a lab observation and field manipulation study. They found that aragonite saturation state rose from $\Omega = 0.68$ to $\Omega = 1.30$ in sediments that were buffered with shell material. Further, *M. mercenaria* increased their burrowing recruitment in the buffered sediments, suggesting that shell recycling could indeed provide alkalinity regeneration to sediments and positively influence shellfish that depend on an elevated saturation state.

(Waldbusser et al., 2011) examined different types of intact oyster *(Crassostrea virginica)* shell at different levels of *p*H to determine if fresh, weathered, or dredged shells would have differing dissolution rates in a lab setting. Fresh shells were collected from a local oyster house and had their tissue removed 24 h before the experiment; weathered shells had been placed in a land-based area for 2 y before the experiment; and dredged shells were collected from a marina, where they had been on the seafloor for up to several hundred years (Waldbusser et al., 2011). Fresh and weathered shells had the highest in shell mass losses, followed by the fossilized, dredged shell. Fresh shells dissolved slightly faster than weathered shells, though not significantly. This could be because fresh shells can lose up to 10-25% of its

weight almost immediately after death (Waldbusser et al., 2011). One possible explanation for the higher dissolution found in fresh shells may be attributed to remineralization of organic matter by microbes on the fresh shell surface, and the consequent event of metabolic CO_2 production (Waldbusser et al., 2011). Remineralization of the shells' organic material would contribute to a mass loss without an increase (and potentially a decrease) in alkalinity (Waldbusser et al., 2011). Further research is needed to address if shell mass losses can be attributed to $CaCO_3$ dissolution or organic decomposition.

Shell size as a factor for dissolution has not been formally studied. From a first-order perspective, more surface area accelerates thermodynamic shell degradation (Waldbusser et al., 2011), promoting higher alkalinity levels. This thesis examined how different shell sizes could impact dissolution.

Shell Budget

Altering the shell balance via shellfish harvest or shell recycling in an estuary may have significant geochemical (Waldbusser et al., 2011) and biological implications (Kidwell and Jablonski, 1983). Increasing inputs into the shell budget encourages settlement of calcifying organisms due to the higher carbonate availability and suitable substrate. According to the taphonomic hypothesis (Kidwell and Jablonski, 1983), increasing shell

content may provide a positive feedback process that provides cultch, or hard substrate for larval calcifying organisms to grow on. These calcifying organisms will eventually die and add a buffer to the waters as their shells dissolve. As they grow, they are forming their shells out of $CaCO_3$ by using the surrounding available CO_3^{2-}. When they eventually die, their shells dissolve, adding Ca^{2+} and CO_3^{2-} back into the water while providing further substrate for future calcifying organisms, repeating the process. The taphonomic hypothesis can work with a caveat. Organisms that thrive from shell additions must remain in the water indefinitely to ensure the positive feedback process between shell formation and dissolution continues. This taphonomic feedback hypothesis may have powerful implications for shellfish aquaculture that disrupts the $CaCO_3$ budget. If shells are taken out of the system or cannot be achieved by natural populations of molluscs, waters may need assistance via shell additions in areas with heavy shellfish aquaculture harvests (Brumbaugh and Coen, 2009; Waldbusser et al., 2011).

In a study on the decline of the Eastern oyster *(Crassostrea virginica)*, the population decline is often associated with a decline in the shell bed, and ultimately a decline in the shell resource, which lowers the available CO_3^{2-} in the seawater (Powell and Klinck, 2007). Oyster reefs need a balance among oyster settlement, growth, and mortality to sustain themselves (Waldbusser et al., 2011). When the shell budget decreases, a negative feedback occurs where the current shellfish populations utilize the sparse amounts of CO_3^{2-}

from the seawater to form $CaCO_3$, which is not being supplemented by shell dissolution. When there is less carbonate in the ocean, there is less opportunity for carbonate to buffer acids, such as H_2CO_3. Further, any shell formation by living shellfish will contribute to a decrease in alkalinity production and an increase in $[H^+]$ (Waldbusser et al., 2013). Because oyster larvae have lower survival rates in low-alkaline, high-acidity environments (Barros et al., 2013; Barton et al., 2012), oyster populations tend to decline in areas where there is not a cycle of $CaCO_3$ replacement by shellfish mortality or shell additions.

By pulling carbonate from marine waters, calcifying organisms may play a role in sequestering carbon in the $CaCO_3$ of shells, reducing concentrations of carbonate that may have once been CO_2 as a greenhouse gas and dissolved into seawater as H_2CO_3 and reacted into HCO_3^- and consequently CO_3^{2-}. It has been suggested that one oyster can sequester 8.36 grams of carbon every 2 years (Hickey, 2008). However, $CaCO_3$ formation alone will not be strong enough to curb for the increasing amounts of CO_2 absorbed into the ocean via fossil fuel combustion, based a study that modeled the $CaCO_3$ saturation state for the 21st century, especially considering dissolution occurs as a result of increasing CO_2 absorption (Andersson et al., 2003). Further, the formation of $CaCO_3$ decreases both DIC and TA. As a result, the system shifts to higher H_2CO_3 levels and lower

pH levels (Zeebe, 2001). In other words, the current rate of acidification is outpacing the ocean's capacity to restore oceanic *p*H and [CO_3^{2-}].

The feedback system between shellfish and shells has powerful implications for Washington State, as it is one of the largest shellfish aquaculture industries in the nation. Little to no research has been done on the current fluxes of shell material to the shellfish consumers and back to the estuary. In commercial shellfish aquaculture, shells return to the waters if shellfish growers harvest only the meat and return the shells to their respective bays, usually as a planting material for juvenile oysters. Further research is needed to establish if shell material can be a renewable, infinite resource (Powell and Klinck, 2007).

Shell-Recycling Programs

No shell-recycling programs currently exist in Washington State. Shell enhancement programs do exist for the purposes of native oyster restoration. As of 2013, the species of shell used for shell additions is locally grown Pacific oyster *(Crassostrea gigas),* as it is the most farmed oyster and shell is readily available from known sources. Shells must sit out for two years in piles before they can be placed in the water to prevent the spread of the oyster drill (Cohen and Zabin, 2009; Washington Department of Fish and Wildlife, 2013). The main reason for the lack of a shell-recycling program in Washington State can be attributed to the precautionary concern for disease

introductions via shell transfers. The transfer of shell material currently requires a permitting process through Washington Department of Fish and Wildlife, which also manages invasive species. The Shellfish Transfer Permit was established to reduce the risk of transferring marine invasive species, such as oyster drills *(Ocicebrellus ornatus)*, from one water body to another (WDFW, 2013). Various approaches have been used or recommended to reduce the risk of transporting oyster drills and harmful shellfish pathogens from local and non-local sources (Cohen and Zabin, 2009).

On the East Coast and Gulf, shell-recycling programs encourage local oyster consumers to recycle their oyster shells rather than to send them to the landfill as trash or employing them for landscaping purposes (Bushek et al., 2004). With the appropriate protocol, a shell collection and deposition program could be implemented to help protect local waters against OA, while also supplementing current native oyster restoration projects and engaging citizens and businesses with the local impacts of acidification.

One interesting shell-recycling project occurred from 1960 until 2006 in the state of Maryland to restore previously harvested oyster reefs. Approximately 196 million bushels of dredged oyster shell were replaced in Chesapeake Bay during a 46-year period from the program's inception to termination (Waldbusser et al., 2011). This is likely the largest coordinated shell-recycling/reef-restoration effort to-date, and also perhaps the largest alkalinity buffering experiment conducted. Unfortunately, the shell-recycling

program has been discontinued because of the lack of accessible shell material (Southworth et al., 2010).

A large percentage of the oysters used for East Coast shell-recycling efforts come from Gulf of Mexico populations (Brumbaugh and Coen, 2009). A conscious effort is being made to quarantine the non-local shell, based on concerns that the shells may harbor nonnative pathogens or exotic hitchhikers. Using the results from Bushek et al. (2004), recycled shells are quarantined in a designated land-based area for a minimum of one to three months before being used for shell recycling. East Coast and Gulf shell-recycling programs values differ, that is, they are willing to risk the spread of invasive species in exchange for the benefits of recycling shell material. In Washington, however, precautionary values prevent shell-recycling programs from existing on the basis that land-based weathering or quarantine may be inadequate to neutralize all pathogens for disease transfers (Blake, 2013). Very little research has been done regarding shell material as a vector for diseases and invasive species. In a review of shell storage, (Cohen and Zabin, 2009) identified concerns regarding invasive species, but did not address controlling pathogens. More research is needed to address the concerns of invasive species management in shell-recycling programs.

CHAPTER TWO: MANUSCRIPT

Formatted and prepared for: Journal of Shellfish Research

This manuscript is a preliminary draft submitted to fulfill graduation requirements for The Evergreen State College Master of Environmental Studies program. The following document has not been edited, reviewed, or otherwise endorsed by any of the listed co-authors and serves only to exemplify the potential final journal submission.

EXAMINATION OF BIVALVE SHELL DEGRADATION FOR ALKALINITY REGENERATION PURPOSES IN HOOD CANAL, WASHINGTON

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ABSTRACT: Spreading shell material can buffer corrosive conditions by providing alkalinity regeneration by dissolution of calcium carbonate ($CaCO_3$). This research explored how enhancing the seafloor with a particular size or species of bivalve shell may influence different rates of shell degradation in Hood Canal, Washington. The differences in degradation, measured by changes in mass, was examined over an incubation period of eight weeks among whole and crushed size types for three different species: *Crassostrea gigas*, *Ostrea lurida*, and *Mytilus galloprovincialis.* All shell treatments lost mass, while *M. galloprovincialis* shells degraded the most mass, losing up to 2.78% ± 0.08% of its shell matter. Each species had a significantly different rate of mass loss relative to the other species, whether the shell was crushed ($F_{2,87}$ = 37.39, p<0.0001) or whole ($F_{2,70}$ = 18.74, p<0.0001). For all species, whole shells displayed higher rates of SML than crushed shells for each of the species examined: *M. galloprovincialis* (p=0.02), *O. lurida* (p=0.003), and *C. gigas* (p=0.01). Through $CaCO_3$ dissolution, whole *M. galloprovincialis* and *C. gigas* shells may contribute the most g CO_3^{2-} every year to the seawater (133.3 ± 36.8 and 135.6 ± 18.6 respectively). Both whole and crushed shells of *M. galloprovincialis* contribute the greatest amount of organic matter among all the species through decomposition (11.5 ± 3.5 and 9.5 ± 3.4 respectively). Conversely, whole and crushed shells of *O. lurida* contributed the least amount of CO_3^{2-} and organic matter among all the species (52.7 ± 11.5 and 42.5 ± 25.6; 1.5 ± 0.4 and 1.4 ± 0.4 respectively). Nonetheless, all shell treatments contributed a substantial amount of CO_3^{2-}, relative to organic matter, and are recommended for alkalinity regeneration purposes.

KEY WORDS: *ocean* acidification, alkalinity regeneration, calcium carbonate, *p*H, shell, dissolution, Hood Canal

SHORT RUNNING TITLE: Shell Degradation for Alkalinity Regeneration

ACKNOWLEDGEMENTS: Thank you to the College of Earth, Ocean, and Atmospheric Sciences at Oregon State University, Carri LeRoy, Jenna

Nelson, Ladd Rutherford, Kaile Adney, Brady Blake, Brian Allen, Andy Suhrbier, Rolin Christopherson, Dave DeAndre, Saleh Prohim, Charlie Korb, Wendi Ruef, Sylvia Musielewicz, Gretchen Thuesen, and Mitch Redfern. This research was partia**lly funded** by the Evergreen Foundation Grant and was completed under the Right of Entry Permit No. 23-090919 and the Shellfish Transfer Permit No. 14-0144.

Introduction

Ocean acidification (OA) is the prolonged reduction of seawater *p*H that can cause significant shifts in ocean carbonate chemistry by altering carbonate speciation. These drastic changes are altering a wide range of marine organisms and the food webs that depend on them (Buck & Folger, 2009; Cooley et al., 2009; Doney et al., 2009; Fabry et al., 2008; Orr et al., 2005). Changes in seawater chemistry are reducing ocean carbonate (CO_3^{2-}) concentrations, and thus calcium carbonate ($CaCO_3$) saturation. Because Ca^{2+} is such an abundant ion in seawater, the $CaCO_3$ saturation state (Ω) in seawater is controlled by the amount of CO_3^{2-} available. One well-known and profound effect of OA is the lowering of $CaCO_3$ saturation states (Zeebe, 2001), which can impact calcifying organisms negatively (Feely et al., 2010; Green et al., 2013; Green et al., 2009). Calcifying organisms are most vulnerable to OA, as they have difficulty forming and maintaining their calcareous exoskeletons in conditions with low $CaCO_3$ saturation (Orr et al., 2005). This is evident, as shellfish hatcheries have experienced losses in oyster larvae since 2008 (Barton et al., 2012). Natural recruitment of other bivalves has also decreased (Place et al., 2008), with ocean acidification as the main culprit.

Probably the largest concern with OA is the magnitude and rapid pace of its effects due to increasing anthropogenic CO_2 in the seawater. Rates of atmospheric CO_2 emissions have increased exponentially since the Industrial

Revolution circa 1751 (Orr et al., 2005). From these cumulative anthropogenic CO_2 emissions, 240 Gt C (~44%) have been stored in the atmosphere, 150 Gt C (~27%) have been accumulated in natural terrestrial ecosystems, and 155 Gt C (~28%) have been absorbed by the ocean (IPCC, 2013). It is estimated that surface ocean *p*H has dropped slightly more than 0.1 *p*H units from 8.25 to 8.14 since the beginning of the Industrial Revolution (IPCC, 2013). It is forecasted to decrease another 0.29 *p*H units (near 7.85) by 2100 (Jacobson, 2005) as CO_2 levels increase in the atmosphere and ocean.

Washington State is a region that is especially vulnerable to the synergistic causes of OA. The main mechanism for the invasion of CO_2 into coastal waters is through upwelling events, which bring seawater rich with CO_2 from the deep ocean to the coast. Water that upwells onto Washington's coast contains CO_2 that has accumulated from past anthropogenic additions, biological respiration, and physical-chemical processes due to the ocean's thermohaline circulation patterns (Chapin et al., 2011; Orr et al., 2005).

In Puget Sound, upwelling events can account for 24-49% of the *p*H decrease relative to pre-Industrial Revolution levels (Feely et al., 2010). Recent studies demonstrate that local sources can also contribute to ocean acidification in marine waters of OA, such as, nitrogen and sulfur oxide gases, nutrients and organic carbon from wastewater discharges, and polluted runoff from land-based activities (Abril et al., 2003; Borges and

Gypens, 2010; Feely et al., 2010; Kelly et al., 2011; Washington State Blue Ribbon Panel on Ocean Acidification, 2012). These local sources of OA can disproportionally affect coastal ecosystems, economies, and cultures that rely on vulnerable calcifying organisms (Cooley and Doney, 2009; Cooley et al., 2009).

In a collaborative effort to strategically respond to the effects of OA in Washington State, Governor Christine Gregoire created the Washington State Blue Ribbon Panel to address the causes and consequences of acidification. One possible short-term strategy to combat locally intensified acidification is to return shell material to coastal habitats where shellfish are present. Spreading shell material ($CaCO_3$) can buffer corrosive conditions by increasing seawater alkalinity (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). The dissolution of $CaCO_3$ provides alkalinity regeneration by buffering weak acids, such as H_2CO_3, the byproduct of CO_2 (Abril et al., 2003; Morse et al., 2007; Waldbusser et al., 2013).

Although few studies have examined shell dissolution for alkalinity regeneration purposes, those that exist have demonstrated significant results that increased the $CaCO_3$ saturation in seawater. Green et al. (2009) added crushed shells of the hard shell clam (*Mercenaria mercenaria)* to a mudflat in Southern Maine before seeding it with *M. mercenaria* juveniles. The addition of shell material caused the $CaCO_3$ saturation state (Ω) to increase from $\Omega = 0.25$ to $\Omega = 0.53$ (Green et al., 2009), which is a small, yet significant, change

(Green et al., 2009). In a similar and more recent study, Green et al. (2013) tested their experiment again to examine if clam larvae respond positively to increased $CaCO_3$ saturation in both a lab observation and field manipulation study. Aragonite saturation state rose from $\Omega = 0.68$ to $\Omega = 1.30$ in sediments that were buffered with shell material. Further, *M. mercenaria* increased their burrowing recruitment in the buffered sediments, suggesting that $CaCO_3$ additions could indeed provide alkalinity regeneration to sediments and positively influence shellfish that depend on an elevated $CaCO_3$ saturation state.

Different species of bivalve shells may contain different proportions of inorganic and organic matter, and thus degrade at different rates. Mineral dissolution can be principally attributed to dissolution of $CaCO_3$, as 96% of the inorganic matter in bivalve shell is $CaCO_3$ (Yoon et al., 2003). The remaining mass was composed of seven other minerals, including silica, magnesia, and sodium oxide in trivial amounts (Yoon et al., 2003). Conversely, the organic matter in shell can vary from 0.1 to 10% in bivalve, based on different species of bivalves (Almeida et al., 1998). The proportions of inorganic and organic matter in bivalve shells may have implications for shell degradation, though it is poorly understood.

This study examined how bivalve shell degradation occurs during winter and early spring conditions in Hood Canal, Washington to determine if bivalve shells contribute to alkalinity regeneration through $CaCO_3$

dissolution. This study also aimed to determine if the degradation rates of bivalve shells differ among different species of bivalve shells. The species of shells examined included the Pacific oyster *(Crassostrea gigas)*, Olympia oyster *(Ostrea lurida)*, and Mediterranean mussel *(Mytilus galloprovicialis).* These species were chosen because of their existing influence in shell-recycling projects *(C. gigas)*, their potential to understand native oyster bed mechanics *(O. lurida),* and the surplus of shell material from commercial processing *(M. galloprovicialis)*, as well as a need for general understanding of shell characteristics among different species.

This study also examined if shell degradation may differ between a whole and crushed size. From a first-order perspective, crushed shell has more potential to dissolve than whole shells, as there is more surface area, and surface area is a first-order control on $CaCO_3$ dissolution (Green et al., 2013, 2009; Morse et al., 2007), however, shell size as a factor for degradation has not been formally studied.

Materials and Methods

Study Site

In order to examine shell degradation in a location where it would naturally occur, research took place in Southern Hood Canal, which forms one of the major basins of Puget Sound in Washington State. Research was conducted in February and March of 2014 due to Hood Canal's low $CaCO_3$

saturation state and pH values during winter conditions (Feely et al., 2010). The lack of flushing in Southern Hood Canal during winter causes confinement of acidic waters, which makes it an ideal site for studying the effect of ocean acidification on shell dissolution on the seafloor. The sample site was ~200 m from the shore in the sublittoral zone of Southern Hood Canal (47°22'49"N, 122°58'09"W) (Fig. 3). An Oceanic Remote Chemical Analysis (ORCA) buoy was located ~3 km from the sample site (47°22'30"N, 123° 0'30"W) (Fig. 3).

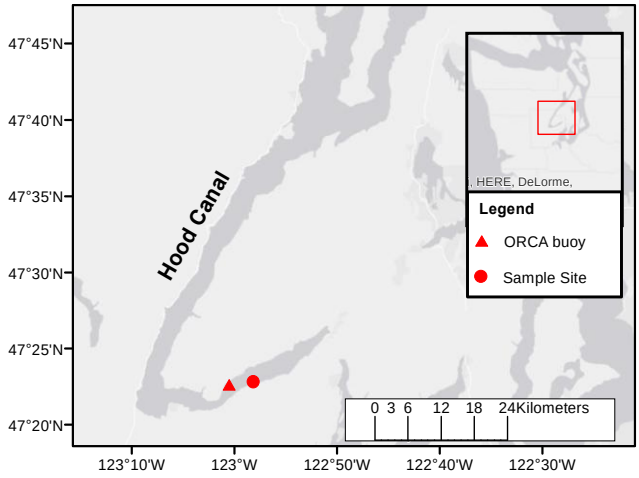

Figure 3. Map of the sites of interest in Southern Hood Canal include the ORCA buoy and the sample site. The ORCA buoy took pCO_2 measurements every three hours for the duration of this project. The sample site is the location of shell incubations and bi-weekly water sample collections.

Shell Collection and Preparation

Adult shellfish were collected in December of 2013 from Totten Inlet, Washington by Taylor Shellfish Farms. The animals had all their meat removed prior to receipt and were eliminated of all bio-foul by rinsing with

deionized water and lightly brushing them. Baking the shells for one hour at 100°C in a drying oven (Yamato Scientific Co., Ltd., Tokyo, Japan) provided consistent dry mass measurements, but was also required for the shell transfer permit through Washington Department of Fish and Wildlife (WDFW). Heat treatment, such as baking, ensures that invasive biota, such as oyster drills and their eggs, would be killed before returning the shells back to the water ((Washington Department of Fish and Wildlife, 2013)

Two size types, whole and crushed, were examined for the purposes of this study. Shells were crushed with a hammer after the baking treatment, and then processed through a series of sieves to achieve the desired particle size range (0.3 cm to 1.0 cm diameter) for each species. Whole shells ranged in size among species, but were consistent within each species.

Shells in 0.028-mm-mesh bags were deployed on long lines anchored to the seafloor in the sublittoral zone, where water depth varied (range = 6-14 m) depending on tidal cycles. The mesh bags were used to permit water to flow through without allowing the crushed fragments of shell to escape. A total of 192 bags were deployed, which included a crushed or whole shell treatment for each species (Fig. 4). Shells were deployed for a maximum of eight weeks, with four retrieval dates throughout.

Every other week, shells were collected to measure mass changes over time. Upon collection, shells from the mesh bags were given the same treatment as before they were deployed: rinsed and lightly brushed to remove

any sediment and bio-foul, then dried for 1 hour at 100°C before taking final shell mass measurements. A control group that was not deployed was also rinsed, dried, and measured in two-week increments to assess whether the experimental treatment caused significant changes in mass.

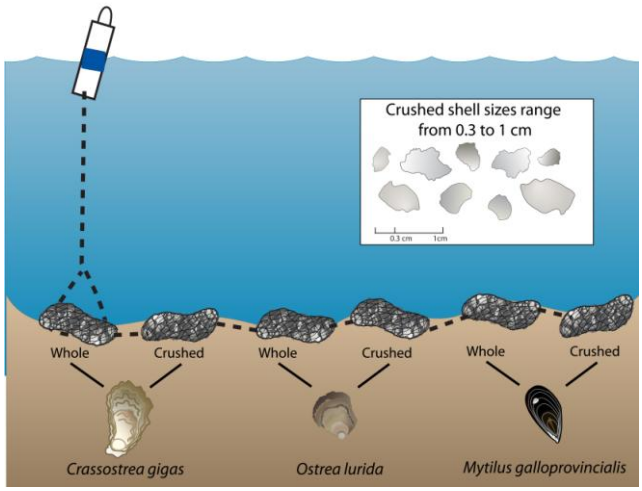

Figure 4. The shell incubation design. The collection of bags included three species, two size types, four collections, with eight replicate bags for each treatment type.

Seawater Collection and Analysis

Seawater carbonate chemistry was analyzed to characterize chemical changes in Southern Hood Canal for the duration of this study. DIC, pCO_2, alkalinity, and *p*H constitute four measureable factors in the carbonate system that can be determined analytically (Wolf-Gladrow et al., 2007). The knowledge of any two of them allows us to calculate the carbonate chemistry of a seawater sample. According to Bandstra (2006), alkalinity and pH measurements suffer when high accuracy is required, so this study focused on measuring *p*CO2 and DIC instead.

During biweekly shell retrievals, water samples were collected at multiple slack tides within a daily cycle to examine the within-day variation of seawater chemistry. Water samples were collected in a Van Dorn bottle ~0.1 m above the seafloor where the shells rested and water depth varied (range = 6-14 m) depending on tidal cycles. Salinity and temperature were measured at the same depth of water collections using a YSI Model 85 Handheld meter (YSI, Incorporated, Yellow Springs, Ohio).

Water samples for DIC and pCO_2 analysis were carefully transferred from the Van Dorn bottle into 355 mL amber-glass bottles with polyurethane-lined metal crimp-sealed caps, filled to within 1.5 cm of the top, preserved with 30 μL of a saturated HgCl2 solution to stop biological activity from altering the carbon distributions in the sample container before analysis (Dickson, et al., 2007).

DIC and pCO_2 samples were analyzed at the College of Earth, Ocean, and Atmospheric Sciences at Oregon State University. The two analyses are performed sequentially on the same sample, generally the DIC first followed by the pCO_2. The laboratory system is based on a gas-permeable membrane contactor, through which a flowing gas stream continuously and quantitatively strips CO_2 from acidified seawater. The CO_2 content of this strip-gas stream, which is proportional to the DIC of the seawater stream via a simple mass balance, is then analyzed using a non-dispersive infra-red (NDIR) gas analyzer (Model 840A, LI-COR, Inc., Lincoln, NE) (Bandstra et

al., 2006). The *p*CO2 is also determined using the NDIR. A continuously re-circulated air stream equilibrates with the sample and passes through the NDIR. After inputting the *in situ* temperature and salinity of the water samples, a modeling program, created by Dr. Burke Hales, computes the unmeasured terms in the carbonate chemistry system, including alkalinity, *p*H, and calcium carbonate saturation values (Bandstra et al., 2006).

For supplemental pCO_2 data, an Oceanic Remote Chemical Analysis (ORCA) buoy automatically took air and surface seawater pCO_2 measurements every three hours. The seawater measurements were taken at the surface of the seawater, while the air measurements were taken 1-2 m above the surface. Averaged pCO_2 measurements are post-calibrated using a simple linear regression between original averaged measurements and span coefficients, a method similar to the post-calibration established by (Feely et al., 1998). All data from the ORCA buoy are preliminary and have not gone through the quality control process (Mathis et al., 2014).

Organic Matter in Shells

The proportions of organic matter in shells before and after the experiment were examined. Fresh shells that were not placed in seawater were compared to shell treatments that had been incubated in the seawater for different durations of time. Shells were combusted in a muffle furnace at 475°C for 2 hours, which ignited the organic material, leaving only the

inorganic material (Goulletquer and Wolowicz, 1989; Rodhouse, 1990). The proportions of organic and inorganic matter were determined by measuring shell mass before and after the combustion process.

Statistical Analyses

All statistical analyses were run in JMP Pro (version 10.0.1.1; SAS Institute, Cary, North Carolina). Shell degradation values were reported as % mass remaining, so they were arcsine square root transformed before statistical analysis. Assumptions of normality and homogeneity of variances were tested using a Shapiro-Wilke's test and Levene's test. Because variation increased through time, data did not meet the assumptions initially. However, following natural logarithmic transformations, the data were normal and homoscedastic. Results were reported as mean with standard error (S.E.) and an alpha (α) of 0.05.

To examine the relationships between shell degradation for each species and size of shell, a one-way analysis of variance (ANOVA) and post-hoc comparisons (Tukey's honest significant difference, HSD) were used. To examine patterns in both treatments and percentage of mass loss (g) through time, an analysis of covariance (ANCOVA) model was used to determine the effects of species, size, and collection date (covariate). For each shell treatment, correlation analysis was used to evaluate whether the shell mass through time was significant (Pearson's correlation coefficient, r at $\alpha = 0.05$).

Mass loss rates (% wk^{-1}) were calculated by dividing the percentage of mass loss value by the number of weeks the shells were incubated.

Results

Seawater Chemistry

The carbonate chemistry values of DIC, pCO_2, alkalinity, pH, $\Omega_{calcite}$, and $\Omega_{aragonite}$ from the sample site are shown in Table 1. There were no correlations or apparent patterns found over time (among or within days) for any of the carbonate chemistry parameters, so the values were averaged with a standard error and used to characterize the general seawater chemistry at the sample site. Water samples collected at the sample site (n = 16) were consistently undersaturated with respect to aragonite (Ω = 0.59 – 0.91). Calcite saturation values were sometimes undersaturated, though often supersaturated (Ω = 0.92 – 1.41). The pH values ranged from 7.60 – 7.85. Further, pCO_2 values were consistently supersaturated (551.11 - 1134.01, relative to atmospheric CO_2 levels (~400 ppm) (Etheridge et al., 1996).

Table 1. Carbonate chemistry and calcium carbonate saturation values in Southern Hood Canal during February-April 2014.

Sample	DIC ($\mu mol\ kg^{-1}$)	pCO_2 (μatm)	alkalinity ($\mu mol\ kg^{-1}$)	pH	$\Omega_{calcite}$	$\Omega_{aragonite}$
2/3/14 8:20	1011	2073	2080	7.64	0.96	0.62
2/3/14 13:45	898	2039	2063	7.69	1.12	0.73
2/3/14 17:30	982	2088	2096	7.66	0.98	0.63
2/3/14 23:30	900	2072	2089	7.69	1.06	0.68
2/18/14 12:30	1048	2093	2110	7.64	1.07	0.70
2/18/14 17:30	551	1843	1905	7.85	1.41	0.91
2/19/14 7:45	900	2065	2080	7.70	1.07	0.69
3/4/14 13:00	1066	2106	2121	7.63	1.06	0.68
3/4/14 19:15	1134	2100	2094	7.62	0.92	0.60
3/5/14 8:30	1004	2090	2111	7.65	1.11	0.72
3/18/14 7:30	904	1943	1963	7.67	1.03	0.67
3/18/14 13:00	636	1845	1894	7.80	1.32	0.85
3/18/14 18:40	696	1850	1890	7.76	1.21	0.78
4/3/14 14:30	749	1929	1964	7.75	1.19	0.77
4/3/14 21:00	1028	2022	2033	7.63	0.99	0.64
4/4/14 7:00	1115	2039	2041	7.60	0.93	0.60

Data retrieved from the ORCA buoy were used to characterize air and surface seawater pCO_2 levels in Southern Hood Canal (Fig. 5). Values for air pCO_2 were relatively consistent (range = 400.3 – 441.3 μmol/mol). Values for surface seawater pCO_2 were variable (range = 62.6 – 756.9) with a temporal trend that decreased as time moved forward from winter to early spring (Fig. 5) (R^2=0.75, p<0.0001).

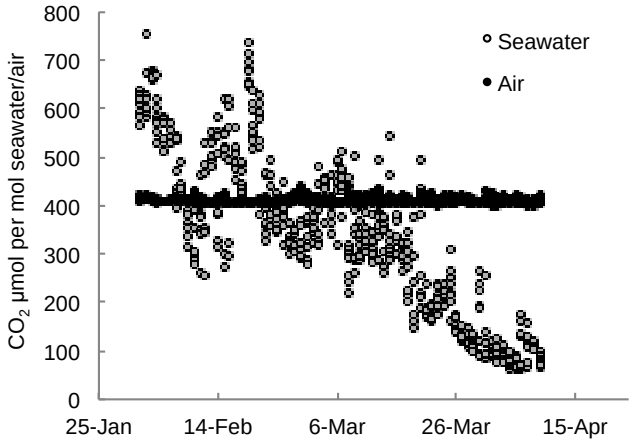

Figure 5. The temporal variation of air and surface seawater pCO_2 in Southern Hood Canal for the duration of this study.

Shell Mass and Degradation Rate

Shell degradation was calculated every week by calculating the percentage of shell mass loss (SML). All treatments showed decreasing SML over the eight-week study period (Table 2). Shells of *M. galloprovincialis* showed the greatest SML, losing up to 2.61% ± 0.06% as crushed shell and 2.78% ± 0.08% as whole shell after the incubation period of eight weeks. The next largest change in SML was observed in *C. gigas*, whose shells lost up to

1.23% ± 0.04% as crushed shell and 2.23% ± 0.38% as whole shell. Finally the smallest change in SML occurred for *O. lurida*, whose shells lost up to 0.78% ± 0.02% as crushed shell and 0.87% ± 0.05% as whole shell.

For each species and size treatment, SML increased with time (Fig. 6), except for whole *C. gigas* shells (Fig. 6a), which had no significant relationships with time (Table 3) due to the extraordinary variation within each week's measurements. Nonetheless, *C. gigas* shells lost mass and showed an insignificant pattern over time.

Table 2. The percentages of shell mass losses over time. These values are shown in this table as averages with standard errors.

Shell Treatment	Week 2	Week 4	Week 6	Week 8
Whole *C. gigas*		1.40 ± 0.28	1.69 ± 0.10	2.23 ± 0.38
Crushed *C. gigas*	0.20 ± 0.05	0.78 ± 0.02	1.10 ± 0.06	1.23 ± 0.04
Whole *M. galloprovincialis*	0.57 ± 0.03	1.44 ± 0.04	2.40 ± 0.06	2.78 ± 0.08
Crushed *M. galloprovincialis*	0.26 ± 0.03	1.49 ± 0.10	2.10 ± 0.04	2.61 ± 0.06
Whole *O. lurida*	0.24 ± 0.02	0.78 ± 0.10	0.86 ± 0.04	0.87 ± 0.05
Crushed *O. lurida*	0.11 ± 0.01	0.55 ± 0.08	0.79 ± 0.05	0.78 ± 0.02

SML occurred very linearly over time for *M. galloprovincialis* shells, decreasing between each week ($p<0.003$) and explained powerfully by time (R^2=0.92, $F_{1,29}$ = 335.49, $p<0.0001$). This relationship is the strongest among all sizes and species of shells (Table 3) (Fig. 6c). Comparatively, crushed *C. gigas* shells had consistent SML that decreased significantly between weeks

2 and 4 ($p<0.0001$) and week 4 and 6 ($p=0.017$), but not between week 6 and week 8 ($p=0.58$), demonstrating a steady SML for the first 6 weeks that slows for the last 2 weeks (Fig. 6a). Changes in SML of crushed *O. lurida* shells resemble the changes in crushed *C. gigas* shells, where SML slows with time (Fig. 6a and 6b) between weeks 6 and 8 ($p=0.99$). For the whole *O. lurida* shells, the SML slowed more promptly, that is, shells were different between weeks 2 and 4 ($p<0.05$), but not significantly different between weeks 4 and 6, nor weeks 6 and 8 ($p=0.67$, $p=0.99$).

Table 3. The relationships between time (covariate) and the percentage of shell mass loss for each shell treatment using Pearson's correlation coefficient, r and the R^2 and F-value results from the ANCOVA test. Asterisks denote p-values that are statistically insignificant ($\alpha > 0.05$).

Shell Treatment	r	R^2	F-value
Whole *C. gigas*	0.51*	0.25*	$F_{1,9} = 3.06$*
Crushed *C. gigas*	0.89	0.79	$F_{1,28} = 105.86$
Whole *M. galloprovincialis*	0.94	0.92	$F_{1,29} = 335.49$
Crushed *M. galloprovincialis*	0.92	0.84	$F_{1,29} = 157.26$
Whole *O. lurida*	0.72	0.58	$F_{1,29} = 39.99$
Crushed *O. lurida*	0.86	0.73	$F_{1,27} = 72.60$

The rates of shell degradation were calculated by dividing SML by the amount of time (weeks) the shells had been incubated to yield values in units of percentage of mass change per week. *M. galloprovincialis* shells showed

significantly higher rates of shell mass loss compared to *C. gigas* and *O. lurida* shells for both size treatments ($p=0.006$, $p<0.001$) (Fig. 7). Shells from *C. gigas* and *O. lurida* had similar rates of loss (Fig. 7). For all species, whole shells displayed higher rates of SML than crushed shells for each of the species examined (Fig. 8): *M. galloprovincialis* ($p=0.02$), *O. lurida* ($p=0.003$), and *C. gigas* ($p=0.01$) (Fig. 8).

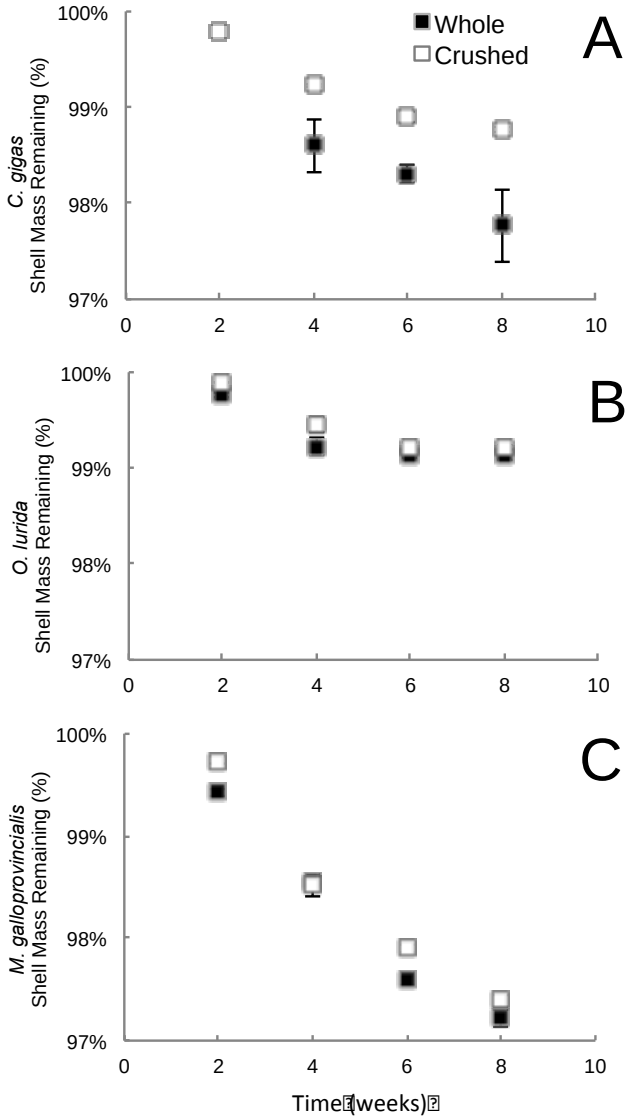

Figure 6. The shell mass loss throughout the eight-week study for the crushed and whole size varieties of *C. gigas* (A), *O. lurida* (B), and *M. galloprovincialis* (C) species.

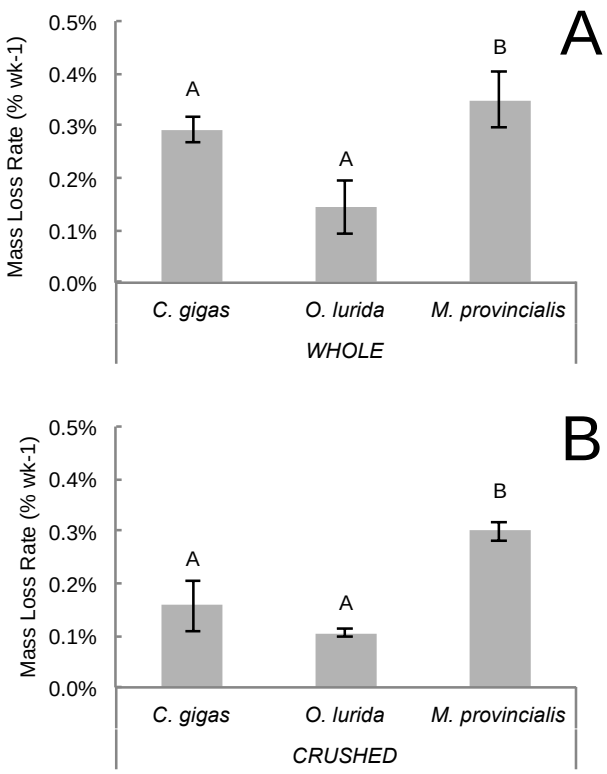

Figure 7. The differences of mass loss rates (% wk^{-1}) among the species for each whole (A) and crushed (B) shell treatment. Letters above each bar signify statistical differences between species from Tukey's HSD test, so that letters connected by the same letter are not significantly different.

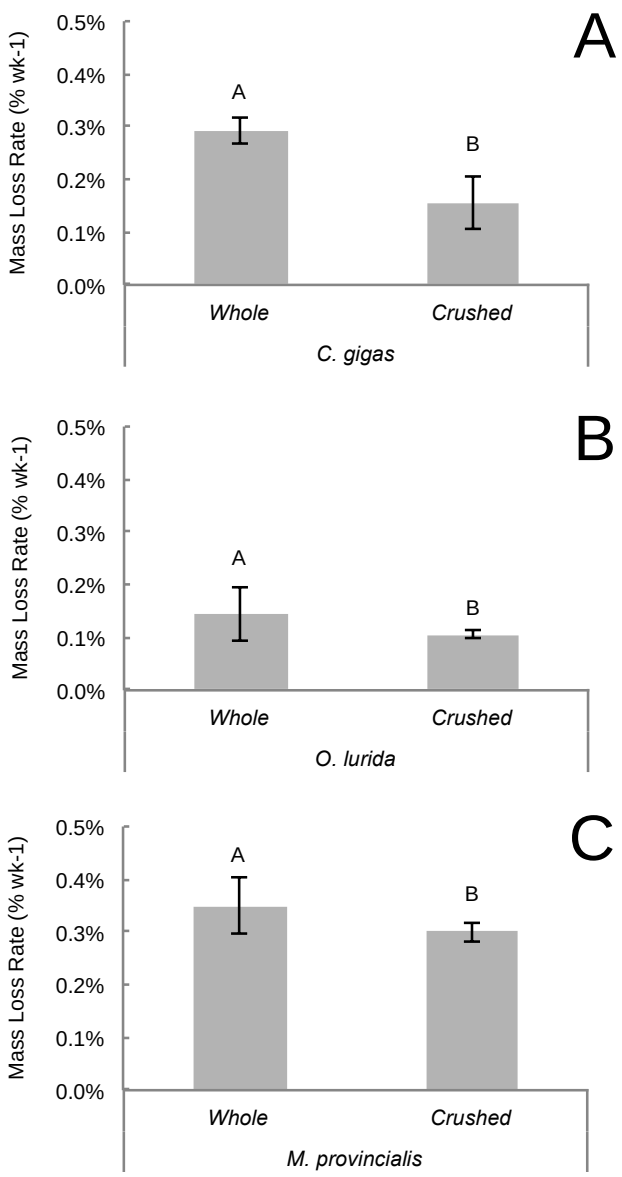

Figure 8. The differences of shell mass loss rates (% wk^{-1}) between the size treatments of *C. gigas* (A), *O. lurida* (B), and *M. galloprovincialis* (C) shells.

Organic Matter in Shells

Based on organic matter analysis before and after the experiment, all shell treatments experienced organic matter decomposition during incubation in seawater (Table 4) (Fig. 9). Whole *M. galloprovincialis* shells had the highest amount of organic matter among all shell treatments, before and after the experiment (5.47% ± 0.11%, 4.77% ± 0.14% respectively) and also exhibited the highest loss of organic matter during incubation (0.70% ± 0.12%). Shells of *C. gigas* and *O. lurida* had relatively similar proportions of organic matter in their shells. For all species, whole shells contained a higher proportion of organic matter than their respective crushed shell types.

Crushed *C. gigas* and whole *M. galloprovincialis* shells experienced significant correlations over time, demonstrating that organic matter decomposition slowed with time as $CaCO_3$ dissolution quickened (Fig. 10) ($p=0.0246$, $p<0.01$). All other shell treatments show generally consistent pattern, suggesting that organic decomposition occurred very linearly alongside $CaCO_3$ dissolution.

Table 4. The differences in proportions of organic matter (%) for each shell treatment before and after placing shells in the seawater.

Shell Treatment	Before	After	Difference
Whole *C. gigas*	2.35 ± 0.08	1.95 ± 0.17	0.41 ± 0.12
Crushed *C. gigas*	1.79 ± 0.14	1.44 ± 0.02	0.35 ± 0.08
Whole *O. lurida*	2.08 ± 0.18	1.44 ± 0.08	0.64 ± 0.13
Crushed *O. lurida*	1.99 ± 0.03	1.98 ± 0.10	0.01 ± 0.07
Whole *M. galloprovincialis*	5.47 ± 0.11	4.77 ± 0.14	0.70 ± 0.12
Crushed *M. galloprovincialis*	4.67 ± 0.41	4.51 ± 0.04	0.15 ± 0.22

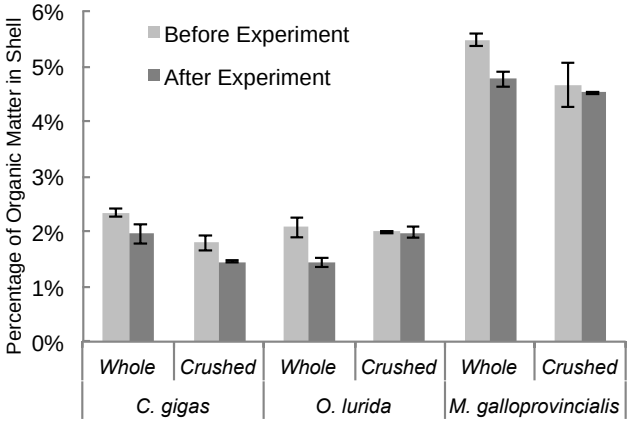

Figure 9. The percentage of organic matter in each shell treatment before and after the experiment.

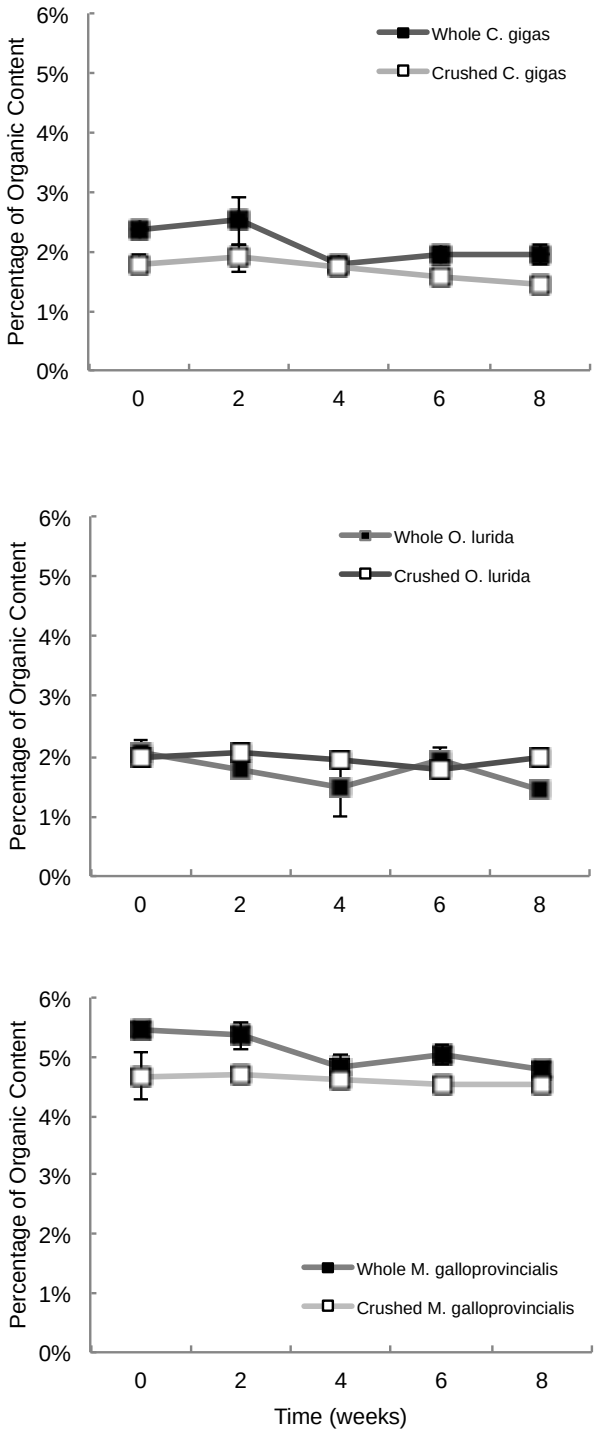

Figure 10. The percentage of organic matter in each shell treatment over time.

Contribution of Carbonate and Organic Matter to the Seawater

All shell treatments have the ability to contribute a substantial amount of CO_3^{2-}, relative to organic matter, and could be used for alkalinity regeneration purposes (Table 5). These values were calculated using the SML and organic matter values, so they are highly variable given the extrapolation of these data. This calculation assumes that all mineral dissolution is $CaCO_3$ dissolution. Through $CaCO_3$ dissolution, whole *M. galloprovincialis* and *C. gigas* shells may contribute the most g CO_3^{2-} every year to the seawater (133.3 ± 36.8 and 135.6 ± 18.6 respectively). Both whole and crushed shells of *M. galloprovincialis* contribute the greatest amount of organic matter among all the species through decomposition (11.5 ± 3.5 and 9.5 ± 3.4 respectively). Conversely, whole and crushed shells of *O. lurida* contributed the least amount of CO_3^{2-} and organic matter among all the species (52.7 ± 11.5 and 42.5 ± 25.6; 1.5 ± 0.4 and 1.4 ± 0.4 respectively).

Table 5. The calculated annual contribution of carbonate and organic matter (g) per kg of shell material.

Shell Treatment	**g CO_3^{2-} per kg shell yr^{-1}**	**g organic matter per kg shell yr^{-1}**
Whole *C. gigas*	135.6 ± 18.6	4.4 ± 0.7
Crushed *C. gigas*	63.3 ± 17.7	1.7 ± 0.5
Whole *O. lurida*	52.7 ± 11.5	1.5 ± 0.4
Crushed *O. lurida*	42.5 ± 25.6	1.4 ± 0.4
Whole *M. galloprovincialis*	133.3 ± 36.8	11.5 ± 3.5
Crushed *M. galloprovincialis*	119.9 ± 37.6	9.5 ± 3.4

Discussion

Seawater Chemistry

Values of DIC, pCO_2, alkalinity, pH, $\Omega_{calcite}$, and $\Omega_{aragonite}$ from the sample site showed low variation with no temporal trends that correlated with changes in shell degradation over time. However, carbonate chemistry values correspond similarly to previous research that took place in Hood Canal in February 2008 (Feely et al., 2010). The low variability and lack of trends in the data may be attributed to measurements being taken near the seafloor, where water is often confined with less physical activity (Warner et al., 2001).

Values from the ORCA buoy showed high variability compared to the sample site. Because measurements were taken at the surface of the seawater near the ORCA buoy, more variability was expected, as the surface water pCO_2 can vary drastically from seawater depths (Feely et al., 2010) due to changes in biological and physical conditions (Takahashi et al., 2009).

Data from the ORCA buoy demonstrated a decrease in pCO_2 over time (Fig. 5), potentially caused by an increase in phytoplankton photosynthesis in early spring. The data from the ORCA buoy may be more informative than the data from the sample site due to the higher sample size and temporal resolution. If this is the case, the observed slowing of shell dissolution (Fig. 6) is expected due to the decrease in pCO_2 over time (Fig. 5). However, coastal waters are subject to highly variable carbonate chemistry (Green et

al., 2009), so more research over different temporal and spatial scales is needed to investigate how changes in carbonate chemistry may affect coastal ecosystem functions, such as shell degradation.

Areas with high pCO_2 and low $CaCO_3$ saturation states are likely to be adversely affected by ocean acidification and may result in substantial ecological, economic, and cultural effects (Green et al., 2009). Since there have been no high-quality, long-term measurements of all carbonate chemistry factors in Southern Hood Canal, it is not possible to determine the extent of how ocean acidification is affecting the region. Nonetheless, aragonite saturation was consistently undersaturated ($\Omega = 0.59 – 0.91$) and calcite saturation was sometimes undersaturated ($\Omega = 0.92 – 1.41$), demonstrating that Southern Hood Canal would be an ideal site to use shell recycling for alkalinity regeneration purposes because of its consistently low $CaCO_3$ saturation state values and high pCO_2 levels during winter conditions.

Shell Degradation: $CaCO_3$ Dissolution and Organic Decomposition

Shells from *M. galloprovincialis* degraded the fastest and most among all bivalve species. This can be partly explained by differences in shell mineralogy, as the shells of oyster species, such as *C. gigas* and *O. lurida,* are mainly composed of calcite (Stenzel, 1963 in (Gazeau et al., 2007), while the shells of mussel species, such as *M. galloprovincialis,* can contain up to 83%

of aragonite (Hubbard et al., 1981(Gazeau et al., 2007). Aragonite and calcite are both mineral forms of $CaCO_3$, though aragonite is also both denser and more soluble than calcite (Morse et al., 2007). During the experiment, seawater was sometimes undersaturated with respect to calcite, but it was consistently undersaturated with respect to aragonite. These data suggest that *M. galloprovincialis* shells dissolve the most $CaCO_3$ in the form of aragonite, contributing more CO_3^{2-} to the seawater.

Another explanation for the higher degradation rates in *M. galloprovincialis* shells can be explained by the organic matter decomposition that is taking place during the shell degradation process. Shells of *M. galloprovincialis* contained a higher proportion of organic matter and contributed the most organic matter to seawater (Table 5), which does not contribute to alkalinity regeneration. However, *M. galloprovincialis* shells also released the most $CaCO_3$ from dissolution.

The decomposition of organic matter in shells has been discussed by (Waldbusser et al., 2011), who showed that fresh *Crassostrea virginica* shells degraded slightly faster than weathered shells (stored at an upland location for ~2 y). Fresh shells, as were used for this study, have more organic matter to decompose. Similarly, *M. galloprovincialis* shells contained the highest proportions of organic matter and exhibited the greatest degradation. Although organic matter decomposition does not contribute to alkalinity regeneration, the consequent event of microbes respiring CO_2 may create

conditions that are more favorable for alkalinity regeneration via mineral dissolution (Waldbusser et al., 2011).

All shell treatments generally experienced consistent proportions of organic matter decomposition and $CaCO_3$ dissolution. For shells of the oyster species, *C. gigas* and *O. lurida*, there was a fast rate of SML followed by a slower rate of loss after 4-6 weeks, demonstrating that these species of shells experience a slower rate in both decomposition and dissolution with time. SML for *M. galloprovincialis* did not slow over time, indicating that this species of shells experience a constant rate of both decomposition and dissolution. These short-term measurements only provide a glimpse into shell dissolution mechanics, though these data are within the range of other previous measurements of shell degradation (1.05 – 7 %(Hales and Emerson, 1997; Hecht, 1933; Waldbusser et al., 2011). More research is needed to examine if shell degradation would continue to occur at similar rates over a longer study period.

For all species, whole shells had a higher rate of shell degradation than crushed shells. From a first-order perspective, crushed shells have more potential to dissolve, as there is more surface area, which is a first-order control on dissolution (Morse et al., 2007), but this was not observed. One possible explanation for the consistently higher degradation rates in whole shells may be attributed to the method of deployment, in which the crushed shells may have repositioned in heaps during incubation so that diffusion

between the shells and seawater decreased. Additionally, too much crushed shell can result in sulfide generation due to decreased diffusion between sediment in water paired with hypoxic conditions (Green et al., 2013, 2009). Another possible reason for the higher degradation in whole shells could be attributed to the higher proportion of organic matter in whole shells. The organic matter in shells is found within them as an organic matrix and as a protective outer layer as a sheath (Simkiss, 1965; Weiner and Hood, 1975). During the crushing process, if organic matter crushed into smaller pieces more easily, it is possible that small pieces of the organic matter found in shells were crushed too small (<0.3 cm) to be used for this study. This hypothesis is supported by the fact that crushed shells contained less organic matter than whole shells even before shells were placed in the water for the experiment (Fig. 9).

Future Considerations of Shell-Recycling Programs

In Washington State, shell is sometimes introduced back into the seawater as a settling surface to encourage the establishment of juvenile bivalve populations. The use of whole shells is the traditional technique for shell enhancement as a substrate to seed native and cultured oyster beds. Whole shells would be more suitable due to their higher rates of dissolution and existing influence in shell enhancement projects. When shells are over-planted in soft muds, burial can occur, which slows dissolution enormously

and results in $CaCO_3$ preservation (Morse et al., 2007). A thin base layer to the seafloor is recommended so that burial is minimal and so that there is space for dissolution and juvenile oyster larvae settlement.

The main reason for the lack of a shell-recycling program in Washington State can be attributed to the precautionary concern for disease and invasive species introductions by shell transfers. The Shellfish Transfer Permit was established to reduce the risk of transferring marine invasive species, such as oyster drills *(Ocicebrellus ornatus)* from one water body to another (WDFW, 2013). Shells must weather for two years in piles before they can be placed in the water to prevent the spread of the oyster drill (Cohen and Zabin, 2009; Washington Department of Fish and Wildlife, 2013).

The use of fresh shells may not be appropriate for shell-recycling purposes because of possible disease transfers (Washington Department of Fish and Wildlife, 2013) and their high proportions of organic matter content. The process of land-based weathering is highly recommended for all shells, as it allows the organic matter to break down before returning shells to the water. Weathered shells can still contribute to alkalinity (Waldbusser et al., 2011), so a land-based weathering treatment can help minimize organic decomposition. Weathering has other positive effects, such as minimizing the transfer of the invasive oyster drill by storing it in a land-based area for ~3 months (Cohen and Zabin, 2009).

The use of *M. galloprovincialis* and *C. gigas* shells is highly recommended for alkalinity regeneration purposes because of the high rates of $CaCO_3$ dissolution that may occur with these species of shells. However, all bivalve species and sizes of shells from this study can contribute to alkalinity regeneration. This research may guide restoration organizations manage their approach to shell-recycling by understanding the potential to use different types of available shell material for alkalinity regeneration purposes.

CHAPTER THREE: General Conclusions and Discussion

Introduction

This study presents the first of its kind investigating shell degradation for alkalinity regeneration purposes in Washington State. Southern Hood Canal is an ideal site to use shell recycling for alkalinity regeneration purposes because of its consistently low $CaCO_3$ saturation state values. The use of *M. galloprovincialis* shells is highly recommended for alkalinity regeneration purposes because of the high rates of $CaCO_3$ dissolution that may occur with these species of shell. However, all bivalve shell species from this study can contribute to alkalinity regeneration. More research is needed to examine if shell degradation would continue to occur at similar rates over a longer study period. This research may guide restoration organizations manage their approach to shell-recycling by understanding the potential to use different types of available shell material for alkalinity regeneration purposes. The current limitations regarding shell recycling include, but are not limited to: disease and invasive species management, alkalinity regeneration performance, lack of infrastructure, and ethical considerations. These potential consequences should be addressed before any operations take place.

Alkalinity Regeneration Performance

This study demonstrated that shells have the potential to offer alkalinity regeneration to marine waters. Preliminary results also show how much these amounts are. However, these results may change as seawater chemistry naturally changes on a seasonal basis. Further, these results may be perceived as idealistic, in that shells did not have any organic tissue remaining on them and contributed the minimum amount of anticipated organic matter to marine waters. Realistically, more organic matter would be contributed to the seawater, due to processing and handling of shells. Additionally, eight weeks is a short period of time and this study only provides a glimpse into shell dissolution mechanics. Some possible mechanisms that may deter shell dissolution from occurring over time include shell burial, algae and bio-foul growth on shell substrate, and piling shells too high. More research is needed to examine if shell degradation would continue to occur at similar rates over a longer study period.

Disease and Invasive Species Management

The main reason for the lack of a shell-recycling program in Washington State can be attributed to the precautionary concern for disease and invasive species introductions by shell transfers, as shells can be a vector for disease (Cohen and Zabin, 2009). Shells from restaurants, oyster bars, and processing operations from outside Washington State may have

potentially infected locations that may enter the markets without testing for invasive species or pathogens. Various approaches have been used or recommended to reduce the risk of transporting and introducing undesirable organisms with shells (Cohen and Zabin, 2009), but more research is needed based on the lack of knowledge in this field.

A large percentage of the oysters used for East Coast shell-recycling efforts come from non-local populations (Brumbaugh and Coen, 2009). A conscious effort is being made to quarantine the non-local shell, based on concerns that the shells may harbor pathogens or invasive species. Recycled shells are weathered in a designated area for a minimum of one to three months before being used for shell enhancement (Bushek et al. 2004), though it is unknown if this time frame is sufficient for pathogen removal. East Coast and Gulf shell-recycling programs values differ from the West Coast by their willingness to risk the spread of invasive species in exchange for the benefits of restored oyster beds and improved water quality through bivalve filtration (Brumbaugh and Coen, 2009; Cohen and Zabin, 2009).

Currently, land-based weathering is the treatment established in Washington State to reduce the spread of oyster drills *(Ocicebrellus ornatus)* from one water body to another (Cohen & Zabin, 2009, WDFW, 2013), but it is not known to be effective strategy to neutralize all biota and disease. In a review of land-based weathering, (Cohen and Zabin, 2009) identified concerns regarding invasive species, but did not address controlling pathogens. The

research by (Bushek et al., 2004) investigated if land-based weathering would be a sufficient treatment for the protozoan oyster disease, *Perkinsus marinus.* While a storage period of 90 days eliminated most of *P. marinus,* cells with unknown viability was observed from shell material that had been stored for 6 months. One disease of immediate concern is the oyster herpes virus 1 (OsHV-1), which can cause up to 100%, mortalities in young *Crassostrea gigas* (Segarra et al., 2010). Currently this disease has been observed as virulent in *C. gigas* stocks cultivated in France, Australia, New Zealand and present in Japan and the United Kingdom (Friedman et al., 2005; Le Deuff et al., 1996; Renault et al., 1995, 1994). An outbreak of this disease puts at risk the natural and commercial populations of shellfish in Washington State, which provide many important ecosystem services and economic provisioning. No study has examined if land-based weathering would be an effective approach to prevent pathogenic outbreaks during shell transfer, demonstrating a need for treatment and monitoring strategies.

The conditions that are needed to permit non-local shell transfers should include a treatment strategy, either by a heat, weathering, or chemical treatment, so that no viable viral, bacterial, or protozoan agents remain viable. The effectiveness of land-based weathering is based on time, climate, pile location, maintenance, and the tolerations of the organism of concern. More research is needed to address these factors, especially to identify the ideal amount of time for land-based weathering to neutralize

pathogens and minimize the organic matter in different species of shells. Heat is another method that could be used to treat shell. The Coast Seafoods plant in Willapa Bay used a propane blast heater on an external conveyor system to prevent the spread of an invasive crab (*Carcinus maenas)*, which had become established in Humboldt Bay a few years earlier (Cohen and Zabin, 2009). However, the stainless steel conveyor belt paired with the saltwater environment did not hold up well (T. Morris, pers. comm.) and they eventually switched to land-based weathering as a form of treatment. A monitoring program would be necessary to ensure the effectiveness of any treatment. The shell-recycling program in Washington State must be prepared to both treat and monitor all shells to ensure no disease outbreak in marine waters.

Shell-recycling programs encourage local oyster consumers to recycle their oyster shells rather than to send them to the landfill as trash or employing them for landscaping purposes (Bushek et al., 2004). To ensure that the public does not put shells back in the water themselves, an outreach component to the shell-recycling program is essential to ensure the success of disease and invasive species management. With the appropriate protocol, a shell collection and deposition program could be implemented to help protect local waters against OA, while also supplementing current native oyster restoration projects and engaging citizens and businesses with the local benefits of recycling shell.

Ethical Considerations

Further research is needed to address the ethics of adding shells to marine waters. Shell addition manipulates ocean chemistry, which may have the potential to damage or alter marine ecosystems in unforeseen ways (Buck and Folger, 2009). Shell additions can also alter natural habitats, if added in vast quantities in areas where native oysters would not naturally live and deposit their shells. These potential consequences should be addressed before any massive operations take place. Management agencies must ask if the costs of doing nothing are more harmful than modifying the existing infrastructure.

Adding shells into the water may be a form of restoration, rather than a form of new alteration. Historically, reefs and beds formed by oysters such as the Olympia oyster *(Ostrea lurida)* were dominant features in many estuaries throughout their native ranges (Brumbaugh and Coen, 2009). Recycling shells can restore ecosystem function by providing a positive feedback process that provides $CaCO_3$ back into the system (Kidwell and Jablonski, 1983). Shells act as a suitable habitat for shellfish larvae to settle, grow, and die (Brumbaugh and Coen, 2009). These calcifying organisms will eventually die and add a buffer to the waters as their shells dissolve. As they grow, they are forming their shells out of $CaCO_3$ by using the surrounding available CO_3^{2-}. When they eventually die, their shells dissolve, adding Ca^{2+} and CO_3^{2-} back into the water while providing further substrate for future

calcifying organisms, repeating the process. Enhancing an area with shells may increase shellfish populations as well as restoring the shell resource. Enhancing an area with shells may increase shellfish populations as well as restoring the shell resource. If shells are taken out of the system without being recycled, a negative feedback will occur that make it difficult for sustainable shellfish population (Brumbaugh and Coen, 2009; Waldbusser et al., 2011).

Responding to Ocean Acidification in Washington State

The need for action to prepare for ocean acidification (OA) is great in the inland and coastal waters of Washington State, a region that is especially vulnerable to synergistic causes and effects of OA (Gazeau et al., 2007). The absorption of anthropogenic carbon dioxide (CO_2) by the ocean and the localized impacts that exacerbate levels of CO_2 in Washington State are causing pH to decrease and along with it the saturation state of $CaCO_3$ in seawater, which threatens calcifiers who depend on $CaCO_3$ to survive. Many of Washington State's marine species are calcifiers including oysters, clams, scallops, mussels, abalone, crabs, geoducks, barnacles, sea urchins, sand dollars, sea stars, sea cucumbers, and some seaweeds (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). In Washington's marine waters, as with the global marine ecosystem, ocean acidification is expected to significantly impact food web structures and functions, as well as

individual species.

This research has strong implications for different fields of study, including marine biology, ecology, wildlife management, commercial aquaculture, and environmental policy. Collaboration between these disciplines is required to tackle ocean acidification at different scales. Various collaborative organizations among shellfish farmers, commercial harvesters, the Tribe, state, local, and federal agencies, and other stakeholders, such as the Blue Ribbon Panel on Ocean Acidification, are identifying and pursuing research, policy, and education goals that will best prepare communities in Washington State to adapt to changes in the marine ecosystem. Continued research and education on ocean acidification will allow those who depend on the ocean for its ecosystem services to respond to OA in a sustainable approach.

Economic and Cultural Impacts of Ocean Acidification

Although OA is a global issue, Washington State is particularly susceptible to its effects, which impact the state's environment, economy, and culture. OA can disproportionately affect coastal ecosystems and the communities that rely on them by negatively affecting shellfish and the ecosystem services they provide (Borges and Gypens, 2010; Kelly et al., 2011). Shellfish provide many ecosystem services, including provisioning services such as food and income; regulating services such as water quality through

the control of eutrophication, algal blooms, and hypoxia; supporting services such as nutrient cycling that maintain ecosystem functions; and cultural services such as spiritual, recreational, and social benefits (Cooley et al., 2009; UNEP and Millenium Ecosystem Assessment Board, 2005).

Washington is the top producer of farmed clams, oysters and mussels in the nation, with an annual value of over $107 million ("Washington State Shellfish Initiative," 2011). People have been farming shellfish in Washington since the mid-1800s (Toba and Nosho, 2004). Today, Washington State's shellfish industry directly and indirectly employs over 3200 people and annually contributes an estimated $270 million to the state's economy ("Washington State Shellfish Initiative," 2011). Shellfish farmers are significant private employers in rural coastal areas of Washington. In Pacific and Mason counties alone, the industry generates over $27 million annually in payroll ("Washington State Shellfish Initiative," 2011).

Washington's recreational shellfish activities are also economically and culturally significant. Over 300,000 licenses are purchased annually to harvest shellfish, providing over $3.3 million of revenue to the state ("Washington State Shellfish Initiative," 2011). On average 244,000 digger trips are made per season for recreational razor clam harvest on Washington's coast bringing an estimated $22 million to coastal economies ("Washington State Shellfish Initiative," 2011).

Shellfish have also played a significant role in the diets and economies of western Washington Native American tribes for thousands of years (Northwest Indian Fisheries Commission, 2013). Currently, Washington tribes engage in subsistence harvest of shellfish (Northwest Indian Fisheries Commission, 2013), therefore tribes are vulnerable to ocean acidification. Therefore, tribal leaders are needed as important stakeholders in ocean acidification policy and research.

Key Policy Changes to Address Ocean Acidification

Because ocean acidification is primarily caused by the increase of global carbon dioxide emissions, policies that advocate for carbon dioxide emission reductions are needed to protect marine waters from OA. This is a comprehensive and challenging task, as it requires collaboration between regional, national, and international entities. Washington State is leading the way in OA science and policy. Recognizing the risks of OA to Washington, former Governor Christine Gregoire created the Washington State Blue Ribbon Panel on Ocean Acidification (The Panel) to chart a course for addressing the causes and consequences of acidification ("Washington State Shellfish Initiative," 2011). The Panel is comprised of different stakeholders, including scientists and policymakers, who studied ocean acidification in depth from different perspectives. In doing so, it recognized that global CO_2 emissions are the leading cause of OA, and that necessary

reductions in emissions must be met (Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Conclusion

The rapid pace of OA does not give humans, ecosystems, or organisms much time to adapt. Ultimately, policy and human behaviors need to change to minimize OA, however, those factors take time. Both short- and long-term strategies are required to successfully offset the magnitude of OA. Shell-recycling programs can improve water chemistry in Washington State as a short-term restorative approach to combat OA. Given that shell-recycling programs encompass several purposes, including mitigation against OA, providing restoration material for native oysters, and engaging citizens and businesses in combatting OA, the benefits may very well outweigh the potential costs.

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