UNDERSTANDING THE IMPACT OF SEA LETTUCE (*Ulva* spp.) DENSITY ON PACIFIC OYSTER (*Crassostrea gigas*) GROWTH IN PUGET SOUND, WASHINGTON

by
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ABSTRACT

UNDERSTANDING THE IMPACT OF SEA LETTUCE (*Ulva spp.*) DENSITY ON PACIFIC OYSTER (*Crassostrea gigas*) GROWTH IN PUGET SOUND, WASHINGTON

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Biofouling, or the growth of other species besides the cultured species on aquaculture gear is a frequent challenge for shellfish farmers. In areas with dense macroalgae growth, shellfish farmers can frequently spend considerable time managing macroalgae through manual removal from aquaculture gear, particularly during sensitive life stages such as seed cultivation. However, there is little research examining the effects of macroalgae density on oyster growth and survival, and as such, it is unclear if these manual removal techniques are actually needed. This study investigated the relationship between sea lettuce (*Ulva* spp.) density and Pacific oyster (*Crassostrea gigas*) growth and survival on two commercial oyster farms in Puget Sound in hopes of improving the understanding of the necessity for macroalgae removal. Juvenile *C. gigas* were grown in the North Hood Canal and South Puget Sound from April through October 2015 in grow bags with different added wet weights of *Ulva* spp. (3 kg, 1.5 kg, 0 kg). Presence of *Ulva* spp. resulted in significantly smaller shell heights of *C. gigas* at both sites, but did not have a significant effect on mortality. The presence of *Ulva* spp., rather than the amount of *Ulva* spp. in the treatment, had significant negative impacts on oyster shell height. The wet weight of *Ulva* spp. in the treatments decreased in the late summer and fall due to diminished on-site macroalgae presence. However, the shell height of oysters that were exposed to *Ulva* spp. *months earlier* remained significantly smaller on average. In other words, it appeared that early exposure to *Ulva* spp. during growth negatively slows *C. gigas* growth, and it has a lasting effect such that growth is still stunted even when the oysters are no longer exposed to *Ulva* spp. months later. These findings have significant implications for aquaculture practices in that managing *Ulva* spp. on aquaculture gear early in the growing season can lead to more rapid crop turnover and increased production in a growing area over time.

Table of Contents

List of Figures

List of Tables

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I. INTRODUCTION

Washington is the largest producer of farmed oysters in the U.S. A large portion of commercial shellfish production occurs in Puget Sound, an estuarine body of water that extends from British Columbia to western Washington. Along with oysters, other organisms grow in the intertidal zone including macroalgae. Sea lettuce (*Ulva* spp) is a species of macroalgae that is commonly found on oyster farms. Commercial shellfish farms, particularly clam, oyster, and geoduck farms, occur in the intertidal and sub tidal zones, where much of the macro algae is found. As macroalgae accumulates throughout the summer, it drifts over aquaculture gear. When visiting a shellfish farm, it's common to see biofouling algae covering the gear. Algal blooms have been linked to shellfish mortality, although there is not a lot of information about this in the literature (Fisher & Mueller, 2007). There is a perception that macroalgae including sea lettuce could be negatively affecting oyster growth. There are many anecdotal accounts of growers observing increased shellfish mortality in areas covered with large mats of algae (Dewey, 2014). Because of this potential impact to production, some shellfish growers remove macroalgae from aquaculture gear throughout the summer. However, the perceived negative effect of macroalgae on commercial shellfish production hasn't been addressed in the literature.

The modern shellfish industry is a huge economic driver in Washington State, adding hundreds of millions of dollars to the State's economy each year and providing long-term employment in rural areas. Commercial shellfish production is a lucrative industry that contributes approximately $270 million annually to the Pacific Northwest economy (Adelsman & Binder, 2012). Many of the jobs at commercial shellfish farms are living-wage jobs in rural shoreline communities with few opportunities for viable long-term employment. For example, the commercial shellfish industry is the second largest employer in Mason County ("Commercial Shellfish Growers Settlement," 2014). For the shellfish industry to continue to be successful, growers need to ensure that they can produce oysters successfully in Puget Sound.

Puget Sound Basin currently supports a population of 4.4 million people. This population has increased almost 250% since 1960. Approximately 67% of Washington's residents live in the Puget Sound basin ("Threats to Puget Sound," n.d.). With many major roadways right along Puget Sound's shorelines as well as homes, agricultural land, and industrial land, our local pollution drains into the Sound. Nutrients such as nitrogen and phosphorus from fertilizer, leaky septic systems, and runoff enter the water and create an overabundance of nutrients in the water, which is taken up by primary producers such as macro algae. Nutrient pollution is problematic because it causes macroalgae, which occurs naturally in Puget Sound, to bloom and then die, creating areas of hypoxia in Puget Sound.

Puget Sound is not only being impacted by local anthropogenic inputs, it is also experiencing impacts from global anthropogenic activity in the form of ocean acidification. Over the past decade, shellfish growers have seen significant impacts due in part to ocean acidification, including multiple years of oyster hatchery failures along the Pacific Coast (Barton, Hales, Waldbusser, Langdon, & Feely, 2012). Scientists have already documented impacts of ocean acidification in low-flushing areas of the Puget Sound (Feely et al., 2010). In the near future, scientists anticipate that the waters of the

Puget Sound will continue to become more acidic, impacting the ability of shell-building organisms to construct their shells and survive through vulnerable development phases. The combined environmental impacts from both local sources along the water and rising atmospheric carbon dioxide levels driving ocean acidification create water quality issues that impact the entire marine system. With these challenges in mind, shellfish growers have to utilize all available tools to adapt to changing water chemistry.

There is an emerging interest in utilizing the ecosystem services that macroalgae provide to improve water quality in Puget Sound. Phytoremediation in the marine environment, or using plants to improve environmental conditions is a new field of interest to potentially improve water quality locally. Scientists and policy makers are starting to examine the viability of growing and harvesting macroalgae to improve water quality on a local scale. In particular, removing seaweed during its growth period (before decomposition) is a new area of interest for its potential ability to removing CO_2 and nitrogen from the marine system. Altering local water conditions by removing seaweed before it decomposes may potentially have localized benefits including altering pH and aragonite saturation state (Adelsman & Binder, 2012). However, introducing more macroalgae to a shellfish farm may be incompatible with current land use. There is a lack of research that quantifies the local effects of seaweed growth on shellfish, and vice versa. Having a better understanding of the chemical and biological dynamics between seaweed and shellfish growth is critical to shaping more informed management practices to adapt to ocean acidification.

II. LITERATURE REVIEW

Introduction

With 2,800 miles of marine waters and 2,500 miles of shoreline, Puget Sound is the second largest estuary in the United States ("Saving Puget Sound," n.d.). The Sound provides critical habitat connectivity between 10,000 rivers and streams and the Pacific Ocean, through the Strait of Juan de Fuca. The brackish shorelines of Puget Sound also provide a productive area to grow shellfish for food production, a tradition occurring in the region for thousands of years. Shellfish resources including clams, oysters, and geoduck have been an important component of tribal culture and a critical food resource for coastal tribes in Washington for thousands of years ("Traditional Use of Shellfish," 2012). In the 19th century, settlers began commercial harvest of oysters and clams. In the early 20th century, shellfish growers started commercially farming Puget Sound's tidelands by importing Pacific oyster (*Crassostrea gigas*) seed from Japan ("Where We Work: Washington," 2013). Today, the shorelines of Puget Sound continue to provide an important place for shellfish farming for both Tribal and non-tribal communities.

Shellfish farming typically has minimal negative to net positive impacts on the marine environment, and negative impacts are typically short-term. Shellfish farming can often have positive environmental impacts to the marine environment through three dimensional structure and sediment stabilization (Dealteris, Kilpatrick, & Rheault, 2004) (Straus, McDonald, Crosson, & Vadopalas, 2013). Unlike terrestrial forms of farming, shellfish aquaculture requires no inputs (except for the larval grow-out phase in a hatchery). Shellfish consume the nutrients that are overabundant in Puget Sound, cleaning the water to create improved water quality for other species. Shellfish are biological filters that consume phytoplankton, removing these nutrients from the water column. Over the period of an hour, an adult Pacific oyster can filter up 20 gallons of water ("Oyster Restoration Reaches New Depths," 2014). When shellfish are harvested, these nutrients are removed from the system, reducing the nutrient loading in the water.

Ulvoids or sea lettuces (*Ulva spp*) are a genus of seaweeds that grow abundantly throughout the world. While *Ulva* provides important ecosystem functions such as shelter for crustaceans and nutrient cycling, it can be problematic when it blooms and decomposes, creating dead zones in the intertidal zone (Scigliano, 2012). In Puget Sound, ulvoids can form large mats, shading out native sea grasses and creating odor problems. Some shellfish growers view ulvoids as a management issue due to their ability to take over aquaculture beds, causing suffocation for cultured bivalves as well as restricted water flows (Mallet, Carver, & Hardy, 2009).

This literature review will cover the dynamic relationship between Pacific oyster (*Crassostrea gigas*) farming and Sea Lettuce (*Ulva spp.*), a macroalgae that naturally occurs on shellfish farms. The review will discuss the biology of *C. gigas* including the seasonality of growth, growth rates, and triploid oysters and current macroalgae management practices on shellfish farms. The review will then highlight the habitat requirements of ulvoids including biological, chemical, and physical requirements. It will then cover the presence of ulvoids in Puget Sound and factors that affect their growth such as shoreline development, wastewater treatment plants, and agriculture. Finally, the review will provide a brief section on how ulvoids affect *C. gigas*.

Pacific Oyster Biology

C. gigas is the most commonly grown oyster in the world. Originally from Japan, it has been cultivated in Washington since the 1920's. The current value of Pacific oyster culture in Washington is approximately $85 million annually (Harris, 2008). It grows on rocks or soft substrates in the intertidal and subtidal zones. The oyster begins its larval stage as zooplankton for 20-30 days until it sets on a hard surface. The oyster develops as a male for the first year, and then matures either as a male or female in its second year. It is a fast growing oyster, reaching 10-15 cm after 2-4 years depending on the conditions of the bay and growing up to five times faster than other oyster species. *C. gigas* can survive in a larger range of temperature and salinity than other oysters, making it a common oyster to grow in different areas because of its adaptability. It performs well in temperatures between 8-22 degrees Celsius and salinities of 24 to 28 parts per trillion (Harris, 2008).

C. gigas growth rate is highly dependent on the body of water where the oysters are growing. *C. gigas* growth is variable and is determined by water temperature, salinity, turbidity, and food concentration. The growth rates are typically fastest in the spring and summer (Hyun et al., 2001). Pacific oysters require various levels of dissolved oxygen depending on their life stage. Dissolved oxygen levels of 4 mg/L or less can be harmful to larvae, and adults require approximately 100 mg/L ("Crassostrea gigas (Pacific oyster)," 2015). According to a study by Bourgrier et al, oysters that are not in a post-spawning state consume oxygen 80% of the time they are immersed in water (1998). Hypoxia is an on-going issue in Puget Sound, with the most serious hypoxia occurring in South Puget Sound and Hood Canal. The Washington Department of Ecology is working to determine how much these conditions are being influenced by anthropogenic nitrogen inputs and how they affect oysters grown in the region ("Nitrogen in the Puget Sound Ecosystem: Nitrogen Trends in Puget Sound," n.d.).

Triploid Oysters

Recent genetic advances in shellfish farming have allowed farmers to increase their year-long yield. Oysters are typically not as marketable in the summertime because they are using their energy to reproduce. In 1985, shellfish farmers began commercially producing a sterile oyster that is marketable year-round called the triploid oyster. Triploid oysters have three gametes instead of two, and are marketable year-round due to their good flavor and firm texture.

Triploid oysters were historically bred using cytochalasin B (CB) to block the release of the second polar body, rendering the oyster sterile. However, CB is very toxic and most triploids are now bred in a much safer way. The more common way to breed triploids is to cross a tetraploid male with a diploid oyster, producing a sterile oyster with three gametes. In 2000, one third of all oysters produced in Washington and Oregon hatcheries, or 12 billion larvae were triploid oysters grown by breeding a tetraploid with a diploid oyster. Since then, other countries including Australia and France have increased triploid production (Nell, 2002). Triploid oysters are popular in Washington. Not only do they retain their flavor year-round, but they also grow more quickly than diploids at lower latitudes. A study in Hiroshima, Japan found an increase of 81% in whole weight of triploid oysters after 8 months (Nell, 2002).

***Ulva* spp. growth in Puget Sound**

Ulva spp. is a dynamic macroalgae that responds to both human-caused and natural impacts, with effects locally for marine organisms. There are many different factors that determine the presence and biomass of *Ulva* in a given area including day length, turbidity, weather, bathymetry, nutrient composition of the water, and many others. In general, *Ulva* biomass is highest in the summer and fall and the lowest in the winter and spring. This section will discuss some of these factors to demonstrate how *Ulva* grows in Puget Sound, as well as discuss its role in the marine environment.

Historical Blooms

Ulva spp. is native to Puget Sound and it frequently accumulates in mats over the growing season. Ulvoid mats have historically been a component of Puget Sound's marine ecosystem with the first written historical record of presence in the 1930's. However, scientists and shoreline residents believe the ulvoid blooms have increased since then. Scientists and shoreline residents have observed large biomass of blooms in areas where they do not typically occur on an annual basis (T. A. Nelson, Nelson, & Tjoelker, 2003). There are also anecdotal records of variation of the intensity of blooms in different areas in Puget Sound, particularly in areas that have not typically had ulvoid blooms present (T. A. Nelson et al., 2003) (Frankenstein, 2000).

Anthropogenic factors impacting blooms

Shoreline development

Ulva can outcompete other species in commercial and public areas such as docks and marina. Almost one third of Puget Sound shorelines are armored, meaning they have manmade bulkheads that alter sediment movement (Fresh et al., 2011). Beach armoring is positively correlated with gravelly substrates, which provide structure for ulvoids to attach to. These areas typically have high nutrient inputs (from lawn fertilizer, for example) and obstructions such as jetties and docks, which alter hydrodynamics. The turbidity occurring in these areas from boat traffic also lowers the light intensity in the water column. The lowered light intensity provides an advantage for ulvoids over other seaweeds and seagrasses because it doesn't need as much light intensity as other species grow, and thus outcompetes seagrasses and macroalgae in these areas (Frankenstein, 2000). As more of the shoreline gets developed, *Ulva* has increased habitat where it may not have been able to grow historically.

Wastewater Treatment

The population of Puget Sound basin has increased by almost 250% since 1960 ("Threats to Puget Sound," n.d.). With development comes an increase in nitrogen inputs into groundwater, which eventually reaches the Sound (Frankenstein, 2000). These nitrogen inputs can result in blooms of ulvoids, as nitrogen is a limiting nutrient for *Ulva* spp. in Puget Sound (Frankenstein, 2000). Sewage treatment plants, storm water treatment plants, and storm drains all contribute nutrient additions to the water. Nitrogen is not typically removed from wastewater treatment effluent before its released into Puget Sound. In the summertime, wastewater treatment plants are the largest source of nitrogen into Puget Sound (Mohamedali, Roberts, Sackmann, & Kolossues, 2011). Currently, one of the only wastewater treatment plants that removes nitrogen from their effluent is the LOTT Center in Budd Bay, which removes nitrogen from April to October ("Budd Inlet Treatment Plant," 2015). Failing septic tanks are another significant source of nutrient inputs. Studies have shown that failing septic tanks are one of the largest sources of nutrients entering the groundwater. This water will eventually reach the Sound, providing opportunities for additional ulvoid blooms (Frankenstein, 2000).

Agriculture

Agriculture activities along the shoreline or even further upstream contribute to nutrient inputs in the Puget Sound in the form of non-point nutrient additions and point source additions. Over fertilization of crops as well as animal waste contributes to nutrient run-off that enters the water and increases the amount of available nitrogen and phosphorus. Additional nitrogen inputs into Puget Sound create optimal conditions for increased ulvoid blooms (Frankenstein, 2000).

Impacts of blooms on the nearshore environment

Growth period of Ulva spp.

Ulvoids provide shelter and forage habitat early in the growing season for many marine species. During the growth period, ulvoid blooms are associated with increases in the number and density of grazers and crustaceans that use mats for food and shelter. Seasonality is a significant factor in ulvoid growth. One study from San Quentin Bay, Mexico found a 30 to 40 fold increase in *Ulva spp.* in May and June compared to winter (Zertuche-Gonzalez et al., 2009). Ulvoid mats alter the habitat and food resources for juvenile fish, with resources for seabirds, fish, and mollusks increasing and shelter for perch, greenling increasing as well. Consequently, resources for species such as cod, which feed on crustaceans, are more limited because the algal mats shelter crustaceans (Frankenstein, 2000).

Ecosystem role of macroalgae

Seaweed beds provide community structure roles in the marine environment including substrate stabilization, food and larval deposition, buffering residents from stresses, and providing protection from predators (Bertness, Leonard, Levine, Schmidt, & Ingraham, 1999). One study examined the impact of algal canopy in high and low tidal elevations. The authors found a positive effect on canopy cover effect for understory organisms at stressful high tidal heights. Recruitment, growth, and survival of understory organisms were higher at high tidal levels with canopy cover. At lower tidal heights, the effects were neutral or negative. The authors also noted that consumer pressure was "severe" at low tidal heights. (Bertness et al., 1999).

Decomposition period of Ulva

Large ulvoid blooms can create problematic physical and chemical conditions in the marine environment. Ulvoid blooms can physically block larvae from settling and produce toxic exudates which impact barnacles and herring (Frankenstein, 2000). As ulvoid mats decompose, macro invertebrate use declines. For example, in False Bay on San Juan Island, invertebrates including amphipods, oligochaetes, and polychaetes utilized mats and sediments in the summer. As mats decomposed in the fall, densities of these species decreased (Frankenstein, 2000).

Shading issues

Blooms of ulva can break up and destroy sea grasses such as eelgrass by shading grass out, which reduces sea grass's ability to photosynthesize, and can cause sea grasses to die back. Additionally, ulvoid mats can cover sea grasses completely by growing in large mats. This is problematic because sea grasses create a nursery habitat which is critical to supporting a number of juvenile fish species, such as salmon (T. A. Nelson et al., 2003). Movements from sea grass dominated areas to ulvoid-dominated areas are associated with increased availability of nitrogen (T. A. Nelson et al., 2003).

Ulvoids blooms have impacts to birds as well. Blooms create a physical barrier that blocks access to near shore food resources such as crustaceans for carnivorous birds. Consequently, blooms are associated with an increase in herbivorous birds such as ducks and geese (Frankenstein, 2000).

Ecosystem factors impacting blooms

Growth and decomposition

Earlier research found that *Ulvaria obscura* was a fairly rare species and a spring ephemeral. However, more recent studies have found that *Ulvaria obscura* is more prevalent than previously thought. Nelson and authors (2003) found *Ulvaria obscura* dominating the sub tidal zone throughout the spring and early summer. It can also live in the intertidal zone, but it is not particularly tolerant to desiccation and thus dies off throughout the summer (T. A. Nelson et al., 2003). Blooms typically grow throughout the summer due to solar radiation and decline in the fall as light conditions decrease (Frankenstein, 2000). However, certain parameters can cause bloom declines in summer. Anoxic conditions can cause a decline in blooms. Additionally, high water temperatures can cause bloom declines during the summer (T. A. Nelson et al., 2003).

The growth of ulvoid mats throughout the spring and summer are a large sink for nutrients in the water. Decomposing ulvoid mats input nutrients back into the sediment and the water column. These nutrients are used by algae to support productivity in the summer (Frankenstein, 2000). As ulvoids decompose, particles sink into the sediment where they remain. Studies have found that sediments in ulvoid-rich areas contain more nutrients, particularly nitrogen, than areas without ulvoid accumulations. Eventually, these nutrients are released from sediments into the water column (Frankenstein, 2000). As ulvoid mats decompose, they become a source of nutrients such as particulate organic matter and dissolved organic matter. The absorption and release of organic matter helps distribute nutrients throughout the water column and sediments and contributes to low oxygen concentrations as this material breaks down (Frankenstein, 2000). The decomposition of ulvoids contributes to low oxygen concentrations as this material breaks down (Frankenstein, 2000).

Ulvoid mats can have a negative impact on human usage of beaches and shorelines. The presence of large mats prevents recreation such as swimming, fishing and foraging for clams. The odor of the decomposition mats, particularly the hydrogen sulfide associated with anaerobic decomposition of mats, can be extremely strong and prevent people from recreating on shorelines (Frankenstein, 2000). While the strongest odors typically occur in summer, the odors have been documented in the winter as well.

Nutrient Uptake

The two most important nutrients controlling growth of macro algae are nitrogen and phosphorus. Of the two, nitrogen is the limiting nutrient and ulvoids are thus controlled by the availability of nitrogen in the water. Another important factor controlling ulvoid bloom growth is the supply of nitrogen. If nitrogen availability is low, but it is in constant supply throughout the spring and summer, this could allow ulvoids to grow at a similar pace to higher nitrogen available conditions (Frankenstein, 2000).

Light, temperature, water motion, and CO_2 and O_2 levels all impact nutrient uptake, particularly nitrogen and phosphorus (Gao & McKinley, 1994). Temperature has been associated with an increase in biomass in some studies, and a decrease in biomass in other studies. The study by Nelson found that increasing temperature was positively correlated with an increase in biomass (2003).

Physical factors impacting ulvoid biomass growth

There are many parameters that regulate macro algal biomass growth including nutrient conditions, light conditions, water cycling movement, and day length. These relationships are very complex and researchers are not in agreement about the most significant factors. One study claims that daylength, temperature, and dissolved inorganic nitrogen are the most important factors controlling biomass growth of seaweed (T. A. Nelson et al., 2003). Another study claims that temperature, grazing and stand density were the most important factors (Gao & McKinley, 1994). A third study named shoreline alteration, nutrient inputs, intertidal/subtidal ulvoid beds and hydrodynamics as the most important factors impacting ulvoid blooms (Frankenstein, 2000). Recognizing that all of these parameters may play a different role based on the particular bay, it is important to establish an understanding of what some of the impacts may be.

Light

Irradiance is an important factor controlling the growth of ulvoid blooms. Irradiance, the solar power per unit area, is largely impacted by light conditions, which vary diurnally and seasonally. Along with daylength, irradiance is impacted by local site factors including shading and depth of seaweed. Puget Sound experiences much longer days in summer and fall than spring and winter, allowing for long irradiance periods during the mid and late summer. These changes in light conditions have an impact on photosynthetic rates. In a study of *Ulva rotunda*, researchers found that increasing light conditions in nitrogen-sufficient plants from low conditions to high conditions resulted in a 600% increase in daily surface area growth and a 50% increase in light-saturated photosynthetic capacity (Gao & McKinley, 1994).

Recent research showed a positive correlation between biomass, daylength, and water temperature (Nelson et al. 2003). In this particular study, light was thought to be the primary limiting factor impacting biomass growth. However, light availability for biomass is not completely represented by the daylength variable, as some biomass is growing in sub tidal areas in deeper, more shaded conditions. Photosynthetic rates vary by season and time of day. Furthermore, shading and depth impact the pigmentation of seaweeds (Gao & McKinley, 1994). Water clarity is often lowest in the summer and fall, and highest in the winter, impacting the amount of light that is available to sub tidal macroalgae (T. A. Nelson et al., 2003).

Density

The density of macro algae can impact biomass growth. In areas that have a dense mats of macro algae, light availability, nutrients, and access to inorganic carbon can be limited due to competition (Gao & McKinley, 1994). These conditions can impact aquaculture operations and contribute to growers’ desire to remove ulvoids from their gear. Nelson et al recorded blooms large enough to create hypoxic conditions at night under the ulvoid mat. However, anoxia was not observed in the specific case (T. A. Nelson et al., 2003).

Hydrodynamics

The three topographic features in which ulvoid blooms were most common include enclosed shallow coves with low energy hydrodynamic environments, broad embayments with sloping intertidal flats, and exposed beaches with high wave energy. Additionally, areas with a long-shore trough and soft substrate, a feature that is correlated to beach armoring, accumulate drift ulvoid mats. These areas often have high anaerobic activity, and high odor problems. Substrate is another factor that impacts ulvoid blooms. Ulva grows well on exposed gravelly beaches, because it attaches to the gravelly substrate and grows in the intertidal zone (Frankenstein, 2000).

Turbidity

Turbidity has a large impact on biomass growth. Gao and McKinley found that when the current speed is increased from still to faster currents, macro algae can photosynthesize up to four times as fast (Gao & McKinley, 1994). For areas that experience faster currents, higher photosynthetic rates and thus higher biomass can be expected. Nelson et al found that study sites in isolated bays such as Hood Canal had low ulvoid biomass in comparison to sites with stronger currents and larger water masses, such as Blakely Island (T. A. Nelson et al., 2003).Water motion increases macro algae's ability to uptake nutrients, even in areas that are nutrient limited. This is because macro algae can take in nutrients more easily in flowing water (Gao & McKinley, 1994).

Tidal currents

The primary forces that control the surface currents in Puget Sound are tidal range, tidal height, and wind. The large majority of sites with ulvoid blooms have eddies off shore during most of the tide. These eddies capture and concentrate drifting ulvoids.

Ulvoids are also impacted by littoral drift due to drift cells carrying ulvoids towards an obstruction such as jetties, where ulvoids tend to accumulate (Frankenstein, 2000). The area of Puget Sound that experiences the most eddy activity is central Puget Sound. Central Puget Sound is also where most of the ulvoid blooms are reported (Frankenstein, 2000). Eddies also impact the concentration of nutrients in the water, with higher nutrient concentrations in areas that have eddies. Eddies and currents are a determining factor in the amount of nutrients that remain in near shore areas (Frankenstein, 2000).

Ulva and grazing

Bivalves are opportunistic feeders, utilizing resources in the water column including ulvoids (Frankenstein, 2000). Invertebrates also graze on *Ulva* including the mud snail (*Ilyanassa obsoleta*) in the Eastern US (Giannotti & McGlathery, 2001). However, ulvoid mats have been documented to be problematic for bivalves and other invertebrates. Studies have shown that mats have reduced numbers and densities of benthic macro invertebrates such as bivalves and some polychaetes (Frankenstein, 2000). A 1999 study of clam resources in Dungeness Bay found a positive correlation between clam densities and lack of ulvoid mats (Frankenstein, 2000). In areas where ulvoids were present, there was a positive correlation between clam size and presence of ulvoids. There was also an increase in dead clams on beaches where ulvoids were present compared to beaches where ulvoids were absent. The correlation between dead clams and ulvoid presence is consistent with the literature's previous findings (Frankenstein, 2000).

Macroalgae management practices on oyster farms

On oyster farms, ulvoid biofouling is sometimes a constant management issue. Biofouling, or the growth of other species besides the cultured species on aquaculture gear is a frequent challenge for shellfish farmers. Aquaculture gear provides three-dimensional habitat in the intertidal area that can increase habitat for macroalgae. Macroalgae will either latch on to the three-dimensional habitat or drift macroalgae will get stuck on the gear. The accumulation of macroalgae on shellfish farms can be an issue for growers when the macroalgae gets extremely dense. Removing algae from aquaculture gear by scraping it off grow bags and other equipment is time-consuming and costly for shellfish farmers (Mallet et al., 2009). Up to 15% of annual operating costs can be spent on controlling biofouling issues including macroalgae (Adams, Shumway, & Whitlatch, 2011). Growers often pile removed algae on the beach adjacent to the shellfish harvest area. These piles, which initially removed the algal biomass from the water, create a subsequent problem later in the summer. As the seaweed decomposes, the nutrients from the algae reenter the water. Hypothetically, these nutrient additions alter water chemistry, potentially increasing nutrient loads of harmful CO_2 back in the water column as the nutrients disperse locally. Shellfish, already severely impacted by the unavailability of $CaCO_3$ due to anthropogenic CO_2 emissions (Feely et al., 2010), may then experience local impacts from CO_2 entering the water column from algal decomposition.

Impact of shellfish aquaculture activities on ulvoid presence

Multiple studies have noted that shellfish aquaculture practices often increase algal biomass by providing structure for algae to attach to in the intertidal environment. A study of clam farming in coastal BC found that *Ulva spp*. increased as a result of farming practices. The study found that when predator nets were installed, *Ulva sp.* increased. As compared to the reference sites, the farm site had noticeably more *Ulva* spp. One study found that biofouling increased the weight of a predator net by 200 fold, causing increased dragging (Swain & Shinjo, 2014). A study by Bendell (2014) found that there was as significant difference in macroalgae biomass per bottom unit between a sandy beach and aquaculture mesh (Bendell, 2014). Another study by Van Alstyne et al found an increase in macroalgae with shellfish farming activities (K.L. Van Alstyne, Flanagan, & Gifford, 2011). One explanation for the increase in macroalgae is the intertidal pools left by recreational harvesters that were enriched with nutrients including ammonia that supported macroalgal growth (Bendell, 2014).

Oyster Recruitment

Thomsen et al. researched the effects of drift algae and sediment on *Crassostrea virginica* reefs and associated sessile organisms. They found that accumulation of drift algae as well as sediments reduce richness and abundance of sessile organisms associated with oyster reefs. They also found that oyster recruitment was reduced due to drift algae, which they attributed to reduced light penetration, smothering, and interference with feeding apparatus (Thomsen & McGlathery, 2006).

Allelopathic properties

Nelson tested the effects of *Ulva fenestra* and *Ulvaria obscura* extracts on larval development for Pacific oyster as well as *Ulva, Ulvaria,* and *Fucus gardneri* growth in Puget Sound. They found that *Ulva* and *Ulvaria* slowed oyster larvae development (2003). The authors found that *Ulva* had a larger effect than *Ulvaria.* Based on their results they concluded that *Ulva and Ulvaria* likely have allelopathic properties. Researchers estimated that the maximum concentration of algae that Pacific oysters experience in the field is a layer of seaweed 5 cm deep, or “six layers” of algae thick

(Timothy A. Nelson, Lee, & Smith, 2003). However, *Ulvaria* is the only macroalgae with documented allelopathic properties (Kathryn L. Van Alstyne, Harvey, & Cataldo, 2014). *Ulva* and *Ulvaria* extracts significantly affect the growth of *Ulva* and *Ulvaria*, meaning that *Ulva* and *Ulvaria* don't grow as well in areas where dense ulvoids are present.

Not only did *Ulva* and *Ulvaria* affect the growth of macroalgae, but they also affected oyster growth and development. Oysters did not develop normally under tested concentrations. In fact, no oyster larvae developed normally to the D-hinge stage while 92.8% of control larvae developed successfully. One researcher found that *Ulva lactuca* extracts inhibited development of crab larvae and crab larvae had zero larvae success. The combination of Nelson's research and Johnson's research point to a general toxicity of ulvoids for multiple species. The extract was more toxic for *Fucus* zygotes than oyster larvae, suggesting that there is a difference in sensitivities to the toxicity of the extract between animals and plants. For *Ulvaria*, the plant did not need to die for the toxic compounds to be released. This phenomenon is very rare among plants: there are few cases of macrophytes producing toxics that slow growth of other macrophytes (Timothy A. Nelson et al., 2003).

The properties of the extracts are still not completely understood. One explanation for *Ulvaria* is a polymerization of oxidized dopamine that blocks membranes and direct effects of dopamine. Another explanation is an antiherbivore activated defense system. A third explanation is bacteria creating toxins or anaerobic conditions in the extracts (Timothy A. Nelson et al., 2003).

Impact on primary productivity (food sources)

Large algal blooms and subsequent desiccation could result in decreased primary productivity due to hypoxic conditions and resulting reduced colonization success by other organisms. There is a potential that large blooms would prevent food from reaching oysters, thus slowing their growth (Timothy A. Nelson et al., 2003, p. 200)

Water flows with seaweed

Multiple studies have discussed how fouled aquaculture equipment slows water flows. Mallet et al summarizes three studies, which have hypothesized that reduced water flows from fouled gear slows rate of food supply as well as waste removal (2007). However, other studies have addressed benefits of fouled gear to culture species. Sea scallops and pearl oysters have experienced better performance due to fouling (Mallet et al., 2009). Research into the impact of algal canopies on the subtidal zone has found that canopies "can increase the re-suspension and deposition of sediment, enhance larval settlement and increase food supply to filter feeders" (Bertness et al., 1999). However, more studies lean towards the idea that algal mats slow water flows, impacting bivalves negatively. One study found that water circulation in aquaculture gear is reduced (Swain & Shinjo, 2014).

Although there was no direct link to water flows, one study found that sea scallops increased mass by 68% with regular changing of nets that removed fouling organisms (Mallet et al., 2009). The same study found that regular bag turning can provide effective control of fouling for Eastern oysters in bag on bottom culture.

Future scenarios of *Ulva* biomass levels in Puget Sound

Changes in the water conditions in Puget Sound have already been attributed to ocean acidification, and the conditions will continue to change in the future (Feely et al., 2010). For example, Dr. Feely's research attributes 24-49% of the pH decrease in Hood Canal's deep waters since pre-industrial levels to ocean acidification (Feely et al., 2010). Scientists are currently conducting extensive research to better predict how the marine ecosystem will be impacted by these drastic changes in water quality, and a lot is still unknown. Scientists are predicting that 49-82% of the pH decrease in Hood Canal will be attributed to ocean acidification in the future (Feely et al., 2010). These acidic conditions will make it increasingly difficult for vulnerable calcifiers to build their shells.

Ulva is not CO_2 limited and the saturation state is currently much higher than current atmospheric levels of CO_2. In order to create saturation conditions for macro algal photosynthesis based on CO_2, conditions of at least five times ambient air concentration of CO_2 were needed (Gao & McKinley, 1994). Although *Ulva* is not carbon limited, the increase in carbon dioxide may result in an increase of algal biomass. The increase in atmospheric CO_2 is predicted to increase primary productivity by phytoplankton, resulting in altered conditions for macro algae as well (Gao & McKinley, 1994). The effect that this added biomass will have on shellfish is not yet known. However, experiments have found that *Ulva lactuca* utilizes bicarbonate and possibility utilizes CO_2 for cell processes (Gao & McKinley, 2000). This study also found that macroalgal use of CO_2 most often results in elevated pH levels. They found this to be true even with elevated concentrations of CO_2. It is questionable whether the amount of increased photosynthesis will result in a net gain or a net loss of pH conditions. This would probably vary on a bay-to-bay basis, depending on the algal biomass, rates of decomposition and photosynthesis, and flushing of nutrients in that particular area.

Conclusion

Ulvoids, ubiquitous in Puget Sound, have impacts on the nearshore environment through photosynthesis, respiration, and decomposition. Ulvoids interacts with *C. gigas* in multiple ways including physically, biologically, and chemically. Ulvoids uptake large amounts of nutrients including carbon, nitrogen, and phosphorus from the water column, and transport these nutrients through tidal movement. As nutrient inputs into Puget Sound continue to grow, ulvoid blooms become a more frequent occurrence with potentially widespread effects on the marine ecosystem. Biofouling of shellfish aquaculture gear with ulvoids is well documented by shellfish growers (Adams et al., 2011). However, this interaction has not been studied closely in Puget Sound. Understanding the interaction between ulvoids and oysters can help shape management practices for commercial oyster farms in Puget Sound.

My thesis research explored the impact of a particular genus of seaweed, *Ulva* spp. on Pacific oyster (*Crassotrea gigas*) production. I examined different levels of *Ulva* spp. densities in close proximity to planted oysters in grow bags in order to get a better understanding of the dynamics occurring between *Ulva* spp. densities, growth of the seaweed, and growth and survival of Pacific oysters in different density scenarios of *Ulva* spp. The goal of my research was to inform commercial shellfish aquaculture management practices surrounding seaweed issues in aquaculture in Puget Sound. Getting a better understanding of ulvoid growth throughout the growing season and the impact of different ulvoid biomass levels on the growth of shellfish is important to establish better management practices surrounding ulvoid management in the shellfish aquaculture industry.

III. METHODS

Study design

This field experiment took place on two commercial oyster farms in Puget Sound (Washington) to test the effect of sea lettuce (*Ulva* spp.) density on Pacific oyster *(Crassostrea gigas)* growth and survival in aquaculture settings in order to understand how to better manage *Ulva* spp. biofouling on commercial oyster operations. The experiment was designed to determine if *Ulva* spp. management is necessary on commercial oyster farms.

Study site

The study was conducted at two different sites in Puget Sound that varied in their topography, tidal patterns, and flow rates. These sites were selected to provide a basis for comparison between two areas of the Puget Sound with different site-specific dynamics in order to better control potential confounding variables. One of the sites was Baywater Shellfish Farm on Thorndyke Bay in North Hood Canal and the other site was Gosser Farm in Peale Passage in South Puget Sound (see Figure 1). These sites differed in their onsite dynamics because the residence time of water in North Hood Canal is typically shorter than South Puget Sound (Harrington, 2005) (Babson, Kawase, & MacCready, 2006). Hood Canal is a deep fjord with a sill at the entrance that restricts water circulation from Admiralty Inlet (Roberts, Newton, & Hannafious, 2005). Residence time of water in Hood Canal can be a year or more. However, Thorndyke Bay is close in proximity to Admiralty Inlet and has a shorter residence time of water than other areas of Hood Canal. On average, the residence time of water in Northern Hood Canal is approximately 23 days, half as long as the residence time of water in South Puget Sound

(Babson et al., 2006). Other major differences include the steepness of the beach; Thorndyke Bay is a wide, shallow bay while Peale Passage has a steeper gradient.

Thorndyke Bay (latitude, 47.8042; longitude, –122.7344), is a shallow bay (approximately 750 m long) along the North Hood Canal in Jefferson County. Thorndyke Bay is bordered to the north by North Thorndyke Reach and to the south by the southeast side of the Toandos Peninsula. Thorndyke Creek runs through timberland into the estuary. The drift cell runs left to right, so water circulation patterns presumably transport freshwater from the creek toward the field site ("Coastal Atlas," 2015). This experiment took place just east of the delta of Thorndyke Creek (approximately 500 meters to the E how far N). Thorndyke Bay is an area of ecological significance as one of the best examples of an intact creek estuary in Jefferson County. The bay is an important regional site for overwintering waterfowl.

Taylor Shellfish's Gosser Farm is located in Peale Passage on the southwest side of Harstine Island directly east of Squaxin Island. The Gosse farm is approximately 450 meters wide and is a commercial farm for oysters, clams, and geoduck. Peale Passage (latitude, 47.2645; longitude, –122.9231) is a small channel fed by Pickering Passage to the north and has a right to left drift cell. Water circulation in Peale Passage is limited by long residence times and slow flushing rates. On average, the residence time of water in South Puget Sound is approximately 46 days (Babson et al., 2006). The uplands directly above the site are a mix of residential and timberland.

Aquaculture methodology

The experiment was conducted from April 2015 through October 2015. Hard plastic mesh oyster grow-out bags (9 mm mesh size formed in a box style) were deployed at both sites in late April with a set amount of oysters (n=150) and various levels of *Ulva* spp. based on treatment. The experiment was conducted using a randomized design where 5 replicates of 4 treatments, totaling 20 grow bags, were attached to a line along a tidal elevation of 0. The grow bag configuration and tidal elevation was identical at both sites (see Appendix D). Commercial oyster production was manipulated at two sites by controlling the amount of *Ulva* spp. in each bag. The four treatments included:

"Control" (no *Ulva* spp. accumulation, 150 oysters)

"1.5 kg" (1.5 kg *Ulva* spp. added, 150 oysters)

"3 kg" (3 kg *Ulva* spp. added, 150 oysters)

"Ulva" (1.5 kg *Ulva* spp added, no oysters)

Oysters were randomly distributed into grow bags filled with equal amounts of oysters (n=150) of similar size (29.1 ± 1.0 mm at Thorndyke Bay on April 9 and 29.9 ± 1.1 mm at Peale Passage on April 21). This size distribution was chosen because it is consistent with the industry standard for these bags (Davis, 2015). All oysters used in the experiment were triploid Pacific oysters from the same family that were produced at the Taylor Shellfish Hatchery in Quilcene and were grown at the Taylor Shellfish Floating Upwelling Nursery System (FLUPSY) in Oakland Bay, which is located in Shelton, WA. They were obtained from the FLUPSY as ½ inch juvenile oysters (see Appendix B). The experiment was conducted during the summer and fall in order to capture the growth of the oysters during the primary growing season of triploid Pacific oysters (Mvungi, Lyimo, & Bjork, 2012). During their first year of cultivation, oysters are the most sensitive to impacts(Pogoda, Buck, & Hagen, 2011). Therefore, juvenile oysters were selected for this experiment for their potential response to the *Ulva* spp.

Oyster methodology

(*a) Sampling*

Oysters were sampled on a bi-weekly basis during accessible tides. All grow bags were sampled during low tide. Oysters were removed from grow bags to measure growth and assess mortality. All oysters sampled were immediately returned to grow bags.

(b) Growth

Shell height, defined as "the longest measurement from the umbo to the edge of the shell" (Cotter et al., 2010) was used as the primary measurement for oyster growth rate. A sub-sample of 10 oysters per bag were measured by sampling every 10th oyster counted. Shell growth was measured using a digital caliper (Electronic Digital Caliper 0-150mm). Initial shell length ranged from 29.1 ± 1.0 mm to 29.9 ± 1.1 mm.

(c) Mortality

Oyster mortality was measured by counting dead oysters, which were identified by oysters with shells gaping open and unable to close their shell when handled. Dead oysters were kept in the bag, consistent with commercial aquaculture practices. All oysters sampled were immediately returned to grow bags.

Ulva methodology

(a) Sampling

Commercial oyster production was manipulated at each site by controlling the amount of *Ulva* spp. in each bag. The placement of *Ulva* spp. inside the bags was to mimic natural accumulation of *Ulva* spp. in the grow bags. *Ulva* spp. weights were adjusted on a bi-weekly basis to maintain a target weight for each treatment. *Ulva* spp. was initially added to the oyster grow bags at Thorndyke Bay on April 20 and Peale Passage on April 21. From April through October, *Ulva* spp. was weighed and adjusted on a bi-weekly basis (every other week) except for September and October due to the tide. *Ulva* spp. was gathered on site from nearby sources, drained of water in a salad spinner, and weighed with a kitchen scale. It was measured into the predetermined amounts for each treatment (1.5 kg, and 3 kg increments), and placed inside the grow bags on top of the oysters.

(b) Growth

Ulva spp. growth was assessed two weeks following the placement of a predetermined treatment amount of *Ulva* spp. in each bag. While effort was made to maintain consistency in treatments, this was not always the case due to the dynamic nature of the marine environment. After two weeks, all *Ulva* spp. was removed from grow bags using gloves. *Ulva* spp. was then spun with a salad spinner. The wet weight of the *Ulva* spp. was recorded. *Ulva* spp. quantities were adjusted to maintain target treatment weights (3 kg, 1.5 kg, 0 kg). Most often, this required supplementing the current *Ulva* spp. in the bag with additional *Ulva* spp. on site. *Ulva* spp. was identified to genus to use for the treatments.

Data analysis

Oyster growth data were normally distributed and were analyzed using a repeated measures MANOVA (JMP Pro 11). Oyster growth was assessed over time as well as within the treatment variation. Both site and treatment effects were analyzed in the MANOVA. Oyster mortality data were also analyzed using an MANOVA. The following variables were not normally distributed: mortality. Non-parametric analysis was used to analyze mortality.

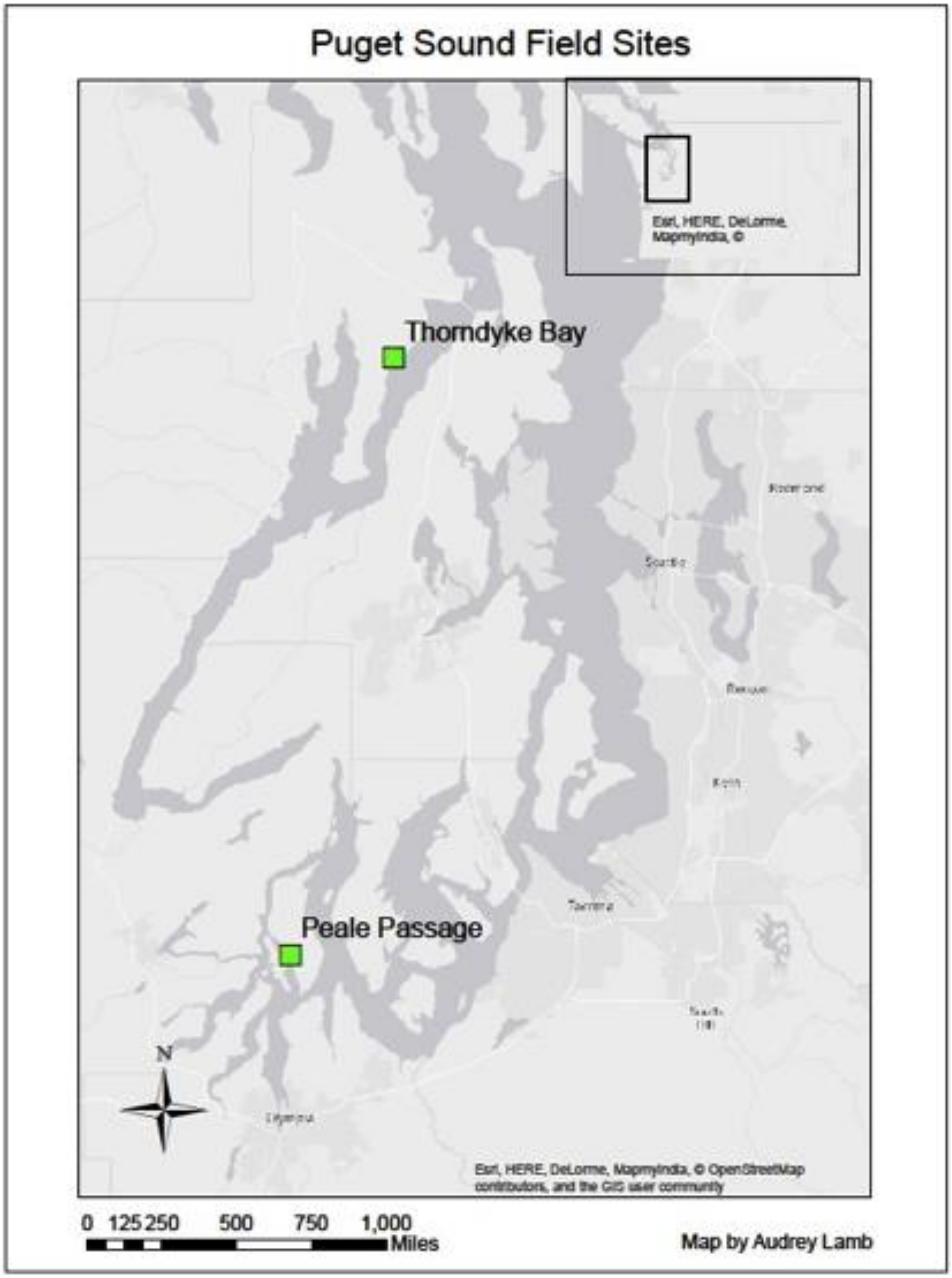

Figure 1: Map of field sites in Puget Sound. Thorndyke Bay is located in North Hood Canal and Peale Passage is located in South Puget Sound on Harstine Island.

IV. RESULTS

Ulva spp. weights

Fluctuations in *Ulva* spp. levels were recorded at each site and the amount of *Ulva* spp. that remained in the bags at the next sampling event was used to determine the mass of *Ulva* spp. that the oysters had been exposed to for the preceding two weeks. Both site and time had a significant effect on *Ulva* spp. weight. The amount of *Ulva* spp. in each treatment was significantly larger at Thorndyke Bay than Peale Passage ($F_{(5,45)}$=1.864, p<0.0001). Time also had a significant effect on *Ulva* spp. weight. There was a significant difference in the amount of *Ulva* spp. in each treatment between months (F($_{1.45)}$=13.260, p<0.0001).

Thorndyke Bay

At Thorndyke Bay, *Ulva* spp. weights varied within each treatment. For the 3 kg treatment, values ranged from 1.0 kg ± 0.1 kg to 2.5 ± 0.0 kg with the lowest weight occurring in May and the peak being two months later in July. The mean value for the 3 kg treatment was 2.1 ± 0 kg. Looking at the graph (see Figure 3), values for the 3 kg target treatment were always less than 3 kg. For the 1.5 kg treatment, values ranged from 0.4 ± 0.0 kg to 1.6 ± 0.1 kg with the lowest value occurring in May and the highest value occurring in June. The 1.5 kg treatment without oysters (control) had a similar trend, with values ranging from a low of 0.9 ± 0.1 kg in June to a high of 1.6 ± 0.1 kg in July. The control (oysters, no, *Ulva* spp.) only had *Ulva* spp. detected in late July with a mean value of 0.5 ± 0.5 kg. *Ulva* spp. weights were collected in October for half of the bags but due to an incoming tide not all *Ulva* spp. weights were recorded. For the bags that were recorded, *Ulva* spp. weights were the lowest of any weights recorded during the experiment with mean values for the 3 kg treatments of 0.4 ± 0, 1.5 kg treatments of 0.2 ± 0.1 kg, and 0 kg *Ulva* spp. for the control and *Ulva* spp. only treatments (see Table 2).

Peale Passage

When *Ulva* spp. was detected at Peale Passage, the mean for the 3 kg treatment was 1.1 ± 0.1 kg and the means for the 1.5 kg treatments with and without oysters were both 0.5 ± 0.1 kg. By August, there wasn't sufficient *Ulva* spp. on site to meet the treatment levels for the bags. Interestingly, *Ulva* spp. weight in the 3 kg treatment peaked one month earlier than Thorndyke Bay in June. Values for the 3 kg treatment ranged from 2.6 ± 0.1 kg in June to 0.1 ± 0.0 kg in August. Oysters in the 1.5 kg treatment were exposed to less than half of the targeted *Ulva* spp. weight in most months (see Figure 2). The maximum value for the 1.5 kg treatment occurred in June with 0.8 ± 0.0 kg and the minimum occurred in August with 0.1 ± 0.1 kg. The 1.5 kg treatment without oysters (control) had maximum values of 0.7 ± 0.1 kg and 0.7 ± 0.0 kg in June and July respectively. The lowest value for the 1.5 kg treatment without oysters was 0.1 ± 0.0 kg in August. For much of the experiment, *Ulva* spp. was not detected in the control treatment (oysters, no *Ulva* spp.), but there were slight increases detected in June and July. There was no *Ulva* spp. detected in any of the treatments in October (see Table 1).

Oyster growth rate

Ulva spp. weight had a significant effect on oyster growth at each site and across both sites. Shell height in the control treatment (150 oysters, no *Ulva* spp.) was significantly larger than both the 3 kg and 1.5 kg treatments at both sites (see Figure 4). As discussed below, significant differences in oyster height were not detected between the medium and high *Ulva* spp. treatments until October.

Thorndyke Bay

The significant difference between shell height of the control (oysters, no *Ulva* spp.) as compared to the high and medium treatments was not apparent immediately. The initial mean shell height for oysters at Thorndyke Bay was 29.1 ± 1.0 mm. The treatments weren't put into place until a month after the oysters were put into their bags. Shell height growth rates always increased for the 3 kg and control treatments from April with a mean of 29.1 ± 1.06 mm to October with means of 61.0 ± 3.2 mm for the 3 kg treatment and 83.8 ± 3.9 mm for the control. Shell height always increased for the 1.5 kg treatment from a mean of 29.1 ± 1.06 mm to 59.4 ± 3.7 in August. However, mean shell height for the 1.5 kg treatment decreased from 59.4 ± 3.7 in August to 51.9 ± 3.4 mm in October. The oysters did not get smaller, so it is likely that smaller oysters were sampled in October than August on average. Post ad-hoc tests showed that by July, the mean shell height for oysters in the control treatment was significantly larger than the 3 kg and 1.5 kg treatments at Thorndyke Bay ($F_{(2,12)}$=5.099, p<.0001). This trend continued throughout the rest of the experiment. Even as *Ulva* spp. weights decreased to almost 0 kg in the treatments in October, there continued to be a significant difference between the control treatment and the 3 kg and 1.5 kg treatments with a difference of approximately 20 mm on average (see Figure 3). Conversely, there was not a significant difference in size between the oysters in the 3 kg and 1.5 kg treatments $F_{(2,12)}$=5.099, p<.0001). At the final sampling date in October the mean shell height of oysters in the control treatment was 22.8 ± 0.7 mm larger than the 3 kg treatment (see Table 3).

Peale Passage

The oysters at Peale Passage exhibited a similar trend throughout the summer with slight variations. The initial mean shell height when the oysters were planted in April was 29.3 ± 1.1 mm. The treatments were put into place two weeks after the oysters were put in their bags. Shell height growth rates always increased from April with a mean of 29.3 ± 1.1 mm to October with means of 78.0 ± 5.9 mm for the 3 kg treatment, 81.5 ± 3.7 for the 1.5 kg treatment, and 97.6 ± 3.2 mm for the control (150 oysters, no *Ulva* spp.) (see Table 4). Post ad-hoc tests showed that by June, the mean shell height for oysters in the control treatment was significantly larger than the 3 kg and 1.5 kg treatments at Peale Passage ($F_{(2,12)}$=7.309, p<.0001). This trend continued through July. In August, there was a significant difference between the shell height for oysters in the 3 kg treatment and control treatment. A post ad-hoc test showed that shell heights for the 1.5 kg treatment were not significantly different from the 3 kg or control treatments in September. Shell height increased for all treatments in October, with shell height in the control significantly larger than the 3 kg and 1.5 kg treatments ($F(_{2,12})$=6.03, p<.0001) (see Figure 2).

Although the site dynamics were different in both sites, shell height response was consistent at both sites for the summer (see Appendix B). Through September, site did not have a significant effect on shell height, although it was approaching significance (see Figure 4) ($F_{(1,25)}$=0.18, p<.0439). In October, oysters in all treatments at Peale Passage grew considerably and at that point site did have a significant effect ($F_{(2, 24)}$=6.10, p<.0001).

Oyster survival

Oyster survival was noted in each bag and dead oysters were counted on a bi-weekly basis. The 3 kg, 1.5 kg, and control treatments started with 150 live oysters in each bag. Survival values ranged from 150 ± 1 to 147 ± 4 at Thorndyke Bay (see Table 5) and 150 ± 1 to 147 ± 3 at Peale Passage (see Table 6) over the entire experiment. Oyster mortality declined slightly (between 150-147) depending on the treatment, over the course of the duration of the study. Survival was non-normally distributed and was analyzed using non-parametric Monte Carlo simulation. Neither site nor treatment had a significant effect on oyster survival (DIF=1.4, p=0.297).

Tables

Table 1

Mean *Ulva* spp. weights of treatments at Peale Passage, Washington. “3 kg” signifies treatment with a targeted weight of 3 kg *Ulva* spp. and 150 oysters. “1.5 kg” signifies treatment with a targeted weight of 1.5 kg *Ulva* spp. and 150 oysters. “0 kg” signifies treatment of 0 kg *Ulva* spp., 150 oysters. “1.5 kg, no oysters” represents treatment with 1.5 kg targeted *Ulva* weight and no oysters.

Date	**3 kg**		**SE**	**1.5 kg**		**SE**	**0 kg**		**SE**	**1.5 kg, no oysters**		**SE**
5/17/15	1.2	±	0.1	0.4	±	0.1	0.0	±	0.0	N/A	±	N/A
6/16/15	2.6	±	0.1	0.8	±	0.0	0.3	±	0.2	0.7	±	0.1
7/1/15	1.0	±	0.1	0.7	±	0.0	0.1	±	0.0	0.6	±	0.1
7/13/15	1.0	±	0.3	0.5	±	0.2	0.2	±	0.2	0.7	±	0.0
7/28/15	0.5	±	0.0	0.4	±	0.1	0.0	±	0.0	0.4	±	0.0
8/30/15	0.1	±	0.0	0.1	±	0.1	0.0	±	0.0	0.1	±	0.0
10/29/15	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0
Total Mean	0.9	±	0.1	0.4	±	0.1	0.1	±	0.1	0.4	±	0.0

Table 2

Mean *Ulva* spp. weights of treatments at Thorndyke Bay, Washington. Abbreviations are as defined in Table 1.

Date	**3 kg**		**SE**	**1.5 kg**		**SE**	**0 kg**		**SE**	**1.5 kg, no oysters**		**SE**
5/4/15	1.0	±	0.1	0.4	±	0.0	0.0	±	0.0	N/A	±	N/A
5/20/15	2.5	±	0.4	1.4	±	0.1	0.0	±	0.0	N/A	±	N/A
6/4/15	2.5	±	0.0	1.6	±	0.1	0.0	±	0.0	1.3	±	0.1
6/15/15	2.1	±	0.1	0.6	±	0.3	0.0	±	0.0	0.9	±	0.1
7/3/15	2.2	±	0.1	1.3	±	0.1	0.0	±	0.0	1.6	±	0.1
7/14/15	2.2	±	0.2	1.3	±	0.1	0.0	±	0.0	1.5	±	0.1
7/31/15	2.0	±	0.2	1.5	±	0.2	0.5	±	0.5	1.5	±	0.1
8/28/15	1.9	±	0.1	1.1	±	0.1	0.0	±	0.0	1.4	±	0.1
10/27/15	0.4	±	0.0	0.2	±	0.1	0.0	±	0.0	0.0	±	0.0
Total Mean	1.9	±	0.1	1.0	±	0.1	0.1	±	0.1	1.2	±	0.1

Table 3

Mean shell height (mm) of oysters in treatments at Thorndyke Bay. Abbreviations are as defined in Table 1. Mean shell height was calculated using a weighted mean.

Date	3.0 kg		SE	1.5 kg		SE	0 kg		SE
9-Apr	29.1	±	1.0	29.1	±	1.0	29.1	±	1.0
20-Apr	30.2	±	0.9	30.6	±	0.9	33.5	±	0.8
4-May	32.3	±	0.7	37.4	±	1.0	37.1	±	1.0
20-May	40.7	±	1.3	38.0	±	1.3	38.1	±	1.4
4-Jun	40.4	±	1.5	37.4	±	1.3	42.2	±	1.3
15-Jun	42.2	±	1.4	42.9	±	1.3	44.9	±	1.7
3-Jul	50.5	±	1.8	47.2	±	2.2	51.7	±	1.7
14-Jul	53.6	±	2.0	49.2	±	2.7	54.1	±	2.6
31-Jul	59.4	±	2.6	51.1	±	2.9	61.6	±	2.0
28-Aug	60.7	±	3.3	59.4	±	3.7	71.9	±	2.7
27-Oct	61.0	±	3.2	51.9	±	3.4	83.8	±	3.9

Table 4

Mean shell height (mm) of oysters in treatments at Peale Passage, Washington. Abbreviations are as defined in Table 1. Mean shell height was calculated using a weighted mean.

Date	3.0 kg		SE	1.5 kg		SE	0 kg		SE
21-Apr	29.3	±	1.1	29.3	±	1.1	29.3	±	1.1
17-May	34.9	±	0.9	33.7	±	1.2	35.9	±	1.0
16-Jun	41.2	±	1.1	40.5	±	1.8	41.9	±	1.6
1-Jul	42.4	±	1.7	45.8	±	1.7	48.5	±	1.7
13-Jul	42.2	±	1.7	45.1	±	2.1	54.8	±	1.9
28-Jul	46.5	±	1.8	48.2	±	2.8	59.7	±	1.9
30-Aug	61.1	±	3.7	60.6	±	3.0	75.1	±	2.9
29-Oct	78.0	±	5.9	81.5	±	3.7	97.6	±	3.2

Table 5

Oyster survival in treatments at Thorndyke Bay, Washington. Abbreviations are as defined in Table 1.

	3.0 kg		SE	1.5 kg		SE	0 kg		SE
April	150	±	0	149	±	1	150	±	1
May	149	±	1	149	±	1	148	±	3
June	149	±	1	149	±	1	147	±	4
July	149	±	0	149	±	1	147	±	4
August	148	±	1	148	±	1	147	±	4
October	148	±	1	148	±	1	147	±	4

Table 6

Oyster survival in treatments at Peale Passage, Washington. Abbreviations are as defined in Table 1.

	3.0 kg		SE	1.5 kg		SE	0 kg		SE
May	150	±	1	150	±	1	150	±	0
June	N/A	±	N/A	N/A	±	N/A	N/A	±	N/A
July	147	±	3	148	±	2	149	±	1
August	147	±	3	148	±	1	149	±	1
October	147	±	3	148	±	1	149	±	1

Figures

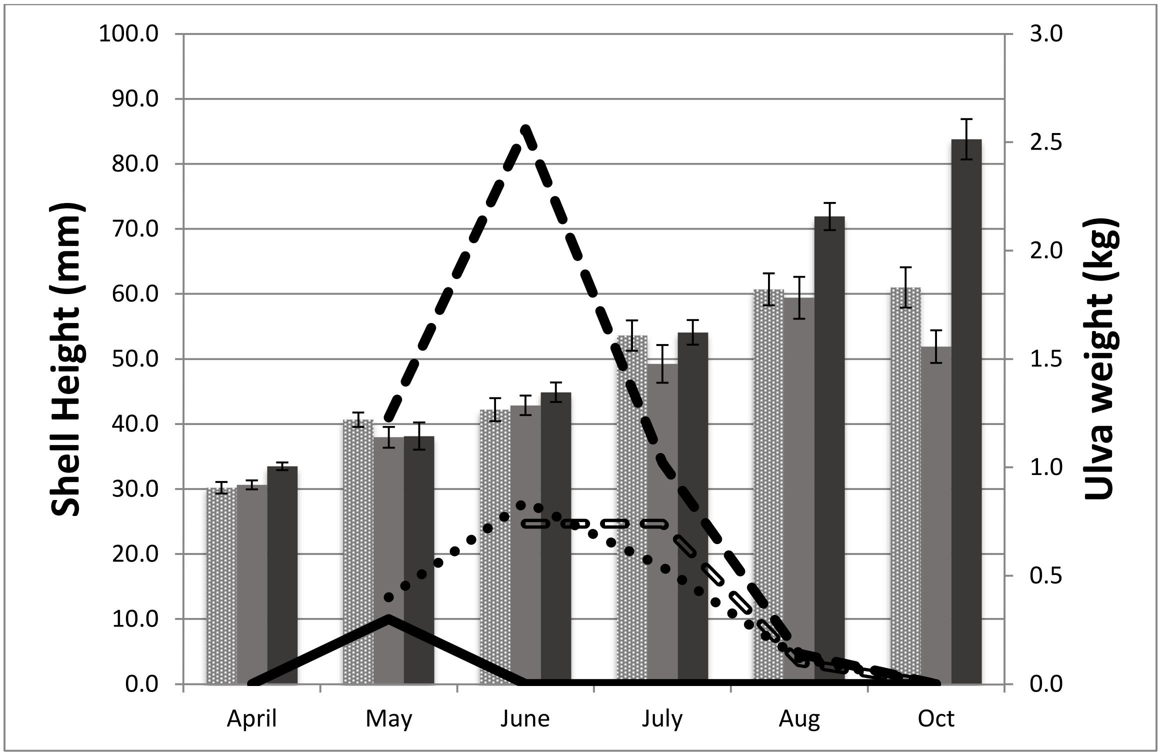

Figure 2: Mean *C. gigas* shell height (mm) at Peale Passage for length of the experiment (primary y-axis). Patterned bar signifies treatment with 3 kg target weight *Ulva* spp. and 150 oysters. Grey bar signifies treatment with 1.5 kg target weight, 150 oysters. Dark grey bar signifies "control" treatment with 0 kg *Ulva* spp. target weight, 150 oysters. Secondary y-axis represents actual mean *Ulva* spp. weight in bags (kg) after two weeks. Dashed line represents 3 kg *Ulva* spp. target weight with oysters. Dotted line represents 1.5 kg target weight *Ulva* spp.*,* no oysters. Outlined dashed line represents 1.5 kg target weight *Ulva* spp. with oysters. Black line represents 0 kg target weight *Ulva* spp. with oysters.

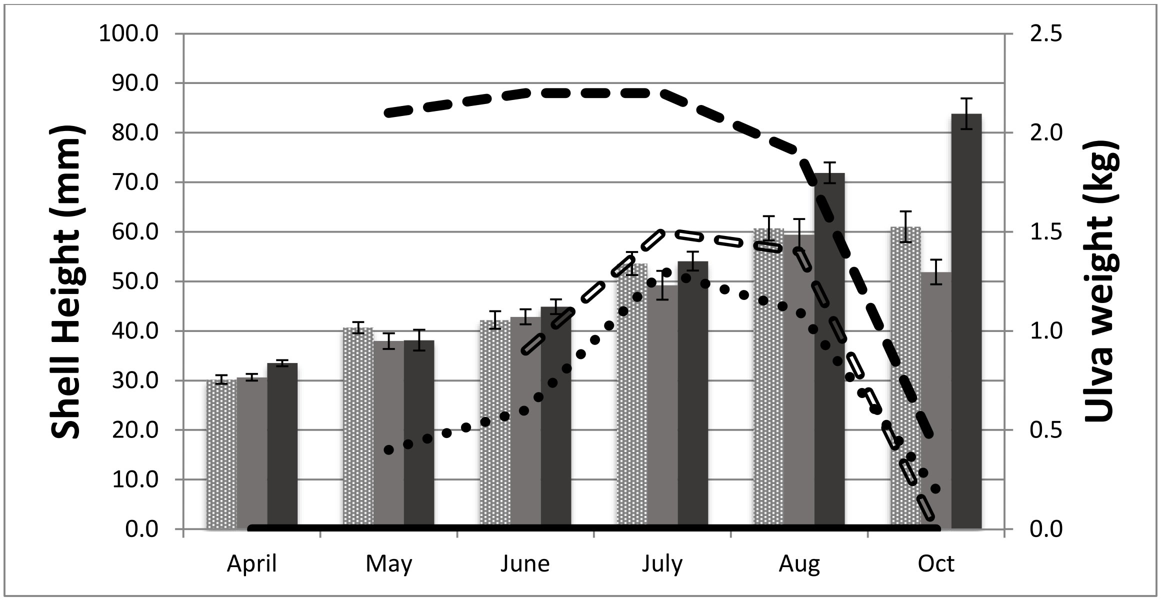

Figure 3: Mean *C. gigas* shell height (mm) at Thorndyke Bay for length of the experiment (primary y-axis). Patterned bar signifies treatment with 3 kg target weight *Ulva* spp. and 150 oysters. Grey bar signifies treatment with 1.5 kg target weight, 150 oysters. Dark grey bar signifies "control" treatment with 0 kg *Ulva* spp. target weight, 150 oysters. Secondary y-axis represents actual mean *Ulva* spp. weight in bags (kg) after two weeks. Dashed line represents 3 kg *Ulva* spp. target weight with oysters. Dotted line represents 1.5 kg target weight *Ulva* spp., no oysters. Outlined dashed line represents 1.5 kg target weight *Ulva* spp. with oysters. Black line represents 0 kg target weight *Ulva* spp. with oysters.

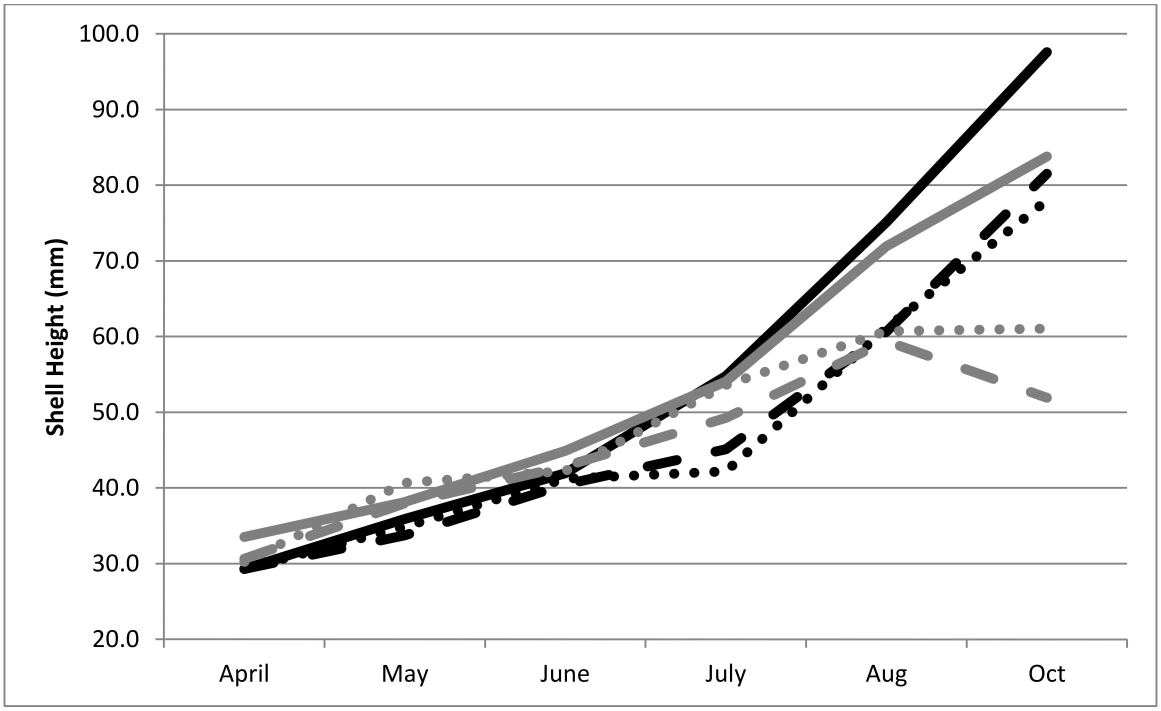

Figure 4: Mean *C. gigas* shell height (mm) at Thorndyke Bay and Peale Passage for the length of the experiment. Black lines signify Peale Passage treatments with solid line representing control

treatment (oysters, no *Ulva* spp.), dashed line representing 1.5 kg *Ulva* spp. treatment, and dotted line representing 3 kg *Ulva* spp. treatment. Grey lines signify Thorndyke Bay with solid line representing control treatment (oysters, no *Ulva* spp.), dashed line representing 1.5 kg *Ulva* spp. treatment, and dotted line representing 3 kg *Ulva* spp. treatment.

V. DISCUSSION

Introduction

These results are one of the first studies to provide evidence for the negative impact that *Ulva* spp. can have on juvenile *C. gigas* growth. The results demonstrated that when *Ulva* spp. was present, it significantly decreased the shell height of oysters. The presence of *Ulva* spp.*,* rather than the amount of *Ulva* spp. added, had significant negative impacts. The amount of *Ulva* spp. in the treatments decreased in the late summer and fall due to diminished on-site *Ulva* spp. presence. However, the shell height of oysters that were exposed to *Ulva* spp. *months earlier* remained significantly smaller on average. In other words, it appeared that early exposure to *Ulva* spp. during growth negatively slows *C. gigas* growth, and it has a lasting effect such that growth is still stunted even when the oysters are no longer exposed to *Ulva* spp. months later. These findings have significant implications for aquaculture practices in that managing *Ulva* spp. on aquaculture gear early in the growing season can lead to more rapid crop turnover and increased production in a growing area over time.

***Ulva* spp. dynamics**

Ulva spp. accumulation and residence time affects whether it interacts with aquaculture on-site. *Ulva* spp. accumulation varies on a site-by-site basis depending on currents, freshwater inputs, and nitrogen and light availability, among other factors (Frankenstein, 2000). *Ulva* spp. production is driven by time periods when nitrogen is abundant and light is available. The annual cycle for *ulva* spp. in Puget Sound begins in springtime when daylength increases, peak growth occurs mid-summer due to increased primary productivity in the water as well as increased irradiance, and then *ulva* spp. decomposes in late summer and fall after it blooms. *Ulva* spp. occurs year round in low levels and it has the ability to overwinter in some areas. However, it is absent from other sites (or present at very low levels) starting in late summer through early spring. *Ulva* spp. growth is very site-specific, and thus its interactions with aquaculture are site specific as well.

This study saw a higher amount of *ulva* spp. on site at Thorndyke Bay relative to Peale Passage both in quantity as well as residence time. Peak *ulva* spp. growth occurred in July in Thorndyke Bay where *ulva* spp. weight was 2.5 times larger in the 3 kg treatment as compared to the control. *Ulva* spp. at Peale Passage peaked in June with 2.2 times more *Ulva* spp. in the 3 kg treatment as compared to the control. *Ulva* spp. resources decreased in August to almost 0 at Peale Passage and less than one third of the treatment target weights at Thorndyke Bay. *Ulva* spp. weight continued to decrease at Thorndyke Bay through the remainder of the experiment.

Differences in on-site topology, water quality dynamics, and biological communities could potentially explain some of the variability in *Ulva* spp. presence between the two sites. Thorndyke Bay is a very shallow bay as compared to Peale Passage, which has a steeper gradient. The shallow depth at Thorndyke Bay allows increased light penetration during more of the tidal cycle and larger surface area allows for increased light penetration deeper in the water column as well as further into the bay, which could provide favorable conditions for *Ulva* spp. to grow in relative to Peale Passage. There is also a freshwater stream, Thorndyke Creek that empties into the bay, providing a possible source of nitrogen throughout the year. North Hood Canal also has a shorter residence time for water than South Puget Sound, which could increase nutrient exchange and thus influence availability of nutrients for *Ulva* spp. (Harrington, 2005). *Ulva* spp. is typically present at Thorndyke Bay year-round and can be observed overwintering in oyster and clam bags. Shellfish farmers at Peale Passage have observed *Ulva* spp. seasonally, with some *Ulva* spp. occurring in the summer and hardly any growing on-site for the rest of the year (Prohim, 2015). *Ulva* spp. does not typically accumulate in bags at Peale Passage during most of the year. These site-specific dynamics influenced the presence of *Ulva* spp. at both sites throughout the experiment.

Oyster response

Oyster growth rates varied between the two sites, although not significantly. It is common to see differences in oyster growth rates between sites. Growth performance can be affected by a number of variables including food availability (phytoplankton quantity and quality), turbidity, seston concentration, temperature, salinity, and pollution (Kochmann & Crowe, 2014) (Cassis, Pearce, & Maldonado, 2011). Specific parameters for these two sites are currently unknown. It is important to note that Washington produces some of the fastest-growing oysters in the world and the growth rates in Washington are higher than most studies from the East Coast of the US as well as Europe. As a means of comparison, Diedrich et al conducted a study in the Wadden Sea in North Germany examining growth rates of oysters in different substrates (2006). The experiment started with juvenile oysters planted in June 2002 that had a mean shell height of 27 ± 1.0 mm, and oysters grew to a mean length of 45.9 ± 1.0 mm by November 2002 (Diedrich, 2006).

Effect of *Ulva* spp. presence on shell height

Shell height for all three treatments almost doubled between July and October, which is consistent with seasonality for oyster shell height development (Hyun et al., 2001) (see Figure 4). Oyster growth at Peale Passage in the late summer and fall was greater than growth at Thorndyke Bay, suggesting that on-site variability between the two sites was a potential factor. One potential explanation is the absence of *Ulva* spp. at Peale Passage during that time period. At both sites, there was a similar trend between the treatment weight (3 kg vs. 1.5 kg) and the shell height response, where oysters exposed to 1.5 kg of *Ulva* spp. had larger shell heights than oysters in the 3 kg bags (except for the 1.5 kg treatment at Thorndyke Bay in October). However, this response was not significant and the presence of *Ulva* spp. was more of a determining factor for shell height than amount of *Ulva* spp.

The presence of *Ulva* spp. had a strongly significant effect on shell height at both sites in July through the rest of the experiment. At the final sampling date in October, *C. gigas* mean shell height for the control were approximately 25% larger than the 3 kg treatment and 20% larger than the 1.5 kg treatment at Peale Passage. This difference was more pronounced at Thorndyke Bay, with mean shell height of the control almost 40% larger than the 3 kg treatment and 60% larger than the 1.5 kg treatment. These differences are not only significantly different statistically, but could also be significant in the marketplace. Oysters that are consistently smaller by 20 mm or more could mean the difference between an oyster ready for market in October and an oyster that wouldn't be ready for market until the following year. This size difference could result in thousands of dollar of lost profit as well as additional costs of continued management of the current crop and a delayed outplanting of new crops. For these biological and commercial reasons, it is important to identify variables that could explain the causes for these differences in shell height.

Physical interactions

Shading

Several observations of *Ulva* spp. negatively impacting oysters are consistent with observations of the same phenomenon in other studies. Multiple studies have noted an effect of macroalgae on oyster recruitment and oyster larval development. Thomsen and McGlathery (2006) researched the effects of drift algae and sediment on *Crassostrea virginica* reefs and associated sessile organisms. They found that oyster recruitment was reduced due to the presence of drift algae, which they attributed to smothering, interference with feeding apparatus, and the indirect effects of light penetration limiting food productivity (Peckol & Rivers, 1996). *Ulva intestinalis* has been shown to decrease light penetration by decreasing surface irradiance by 50% with one layer and over 80% with three layers (Mvungi et al., 2012).

Water flow

One explanation for this trend is that water circulation in bags with *Ulva* spp. was slower than water circulation in the control bags. When the grow bag is submerged, the macroalgae in the bag slows down the water flow over the oysters (Mallet et al., 2009). Water flow has a strong effect on passive filter feeders (Sanford, Bermudez, Bertness, & Gaines, 1994). Oysters access their food, phytoplankton, through the water column and current spend affects food availability. Thus, with slower water flow rates, the oysters have less opportunity for consumption and growth (Cassis et al., 2011). Water flow rate was not studied specifically in this experiment, but is a potentially explanatory factor that should be studied further. This potential finding has repercussions for other types of aquaculture where flow is inhibited by biofouling of bivalves on other organisms. For example, multiple studies have seen an effect of algal biofouling on flow rates in aquaculture. Claereboudt et al studied the effect of biofouling of mollusks on juvenile sea scallops (*Placopecten magellanicus*) and found that changing the nets regularly resulted in a 68% increase in muscle mass and a 4.8% increase in shell height (Claereboudt, Bureau, Cote, & Himmelman, 1994).

Sedimentation

Macroalgae can alter water currents or act as a physical barrier to oyster settlement. Macroalgal canopies have physical impacts that have cascading effects, including concentrating pelagic larvae and decreasing water flow leading to sedimentation (Kathryn L. Van Alstyne et al., 2014). Kochmann and Crowe examined the effect of biotic interactions on the establishment of *C. gigas* including the effect of macroalgae cover of *Fucus serratus* on *C. gigas* juvenile development and survival. The authors found that macroalgae cover did not have a significant effect on oyster growth or survival, and there was a non-significant trend toward greater oyster growth in cages with macroalgae. The authors suggested that these results may be due site-specific dynamics including communities of other organisms that oysters compete with (Kochmann & Crowe, 2014).

Chemical interactions

Water quality

There are a number of factors that may explain why oysters with *Ulva* spp. present in the bag had a decreased growth rate as compared with the control. The presence of *Ulva* spp. blooms can influence local water quality dynamics in a bag through the growing cycle of the macroalgae. For example, a study by Mvungi et al examined the impact of *Ulva intentinalis* layers on *Zostera marina* productivity (2012). The authors observed increases in pH due to *Ulva* spp. photosynthesis up to 10.6 (Mvungi et al., 2012). Increased growth rates in the bags with 3 kg of *Ulva* spp. as compared to the other two treatments were observed at Peale Passage in May and Thorndyke Bay in June, although these differences were not significant. This could potentially be explained by the growth of *Ulva* spp. during this period and subsequent water quality improvements, but more research would be needed to confirm this finding. Lomstein et al found an increase in dissolved oxygen (DO) concentrations when *Ulva lactuca* was added to a lab experiment from 11 to 23 mmol m−2 d−1 that remained through the incubation period (Lomstein, Guldberg, Neubauer, & Finster, 2006). Although pH may increase locally during growth, it could also decrease during decomposition (Felix & Pradeepa, 2012). *Ulva* spp. can decrease DO concentrations during decomposition (Rinehart, Guidone, Ziegler, Schollmeier, & Thorner, 2014). Decomposition was observed at Peale Passage on July 13th (see Appendix C) and was not observed at Thorndyke Bay during sampling. The impact of decomposition could be another explanatory factor in the significant difference between treatments with *Ulva* spp. present and without *Ulva* spp. Decay was observed at Peale Passage in June and

Thorndyke in August. According to Brush, decay occurs when oxygen levels are low and respiration is reduced (2010). Decay of macroalgae is directly related to temperature and indirectly related to C:N and C:P ratios of the macroalgae (Brush & Nixon, 2010). More research is needed to quantify the impacts of the water quality dynamics.

Allelopathy

Chemical interactions could also play a role in the impact of *Ulva* on oyster growth. *Ulva* has been observed to inhibit the growth and development of other plants and animal species (Timothy A. Nelson et al., 2003), suggesting a potential for general toxicity, or allelopathic properties. Nelson also noted another study that found *Ulva lactuca* extract inhibited development of crab larvae and the larvae had zero success, or 100% mortality (Timothy A. Nelson et al., 2003). Currently, *Ulvaria obscura* is the only known algae to produce dopamine. *Ulvaria* releases dopamine during desiccation and rehydration process, which occurs frequently during tidal cycles (Kathryn L. Van Alstyne et al., 2014). Van Alstyne et al noted that *Ulvaria* released 7-100% of dopamine in their tissues when desiccated on the beach for 75 minutes and then re-immersed in seawater (2014). The authors also observed a rapid 0.7 drop in pH in the surrounding seawater. The authors did not see the same drop in pH when *Ulva lactuca* was exposed to similar conditions (Kathryn L. Van Alstyne et al., 2014). However, Nelson's research suggests that *Ulva* exhibits slowed growth when exposed to its own extract and could be a potential explanatory factor for the stark difference in size between *C. gigas* exposed to *Ulva* and the control.

Biological interactions

Ulva spp. concentrations varied between sites and decreased over time in the course of the experiment. There are a number of factors that influence macroalgal loss including decay, respiration, grazing, and decay through drift (Brush & Nixon, 2010). One study hypothesized that *Ulva* biomass can be lost due to marginal tissue becoming reproductive and producing spores as it is integrated into the water column (Zertuche-Gonzalez et al., 2009). There is no data available for Peale Passage or Thorndyke Bay that addresses the strongest influences at either of those sites. However, the increased consistent loss of *Ulva* spp. at Peale Passage as compared to Thorndyke Bay suggests that the *Ulva* spp. at Peale Passage may have experienced a more robust grazing regime. Invertebrates including snails and crabs were found inside the bags at both sites.

Additional observations

Another interesting finding was that actual *Ulva* spp. weights for the targeted 1.5 kg treatment differed in the bags that had oysters and bags without oysters, which both had 1.5 kg of *Ulva* spp. added to it. At Thorndyke Bay, where there was more *Ulva* spp. on site, bags with a treatment weight 1.5 kg of *Ulva* spp. and 150 oysters had less *Ulva* spp. remaining in the bags after two weeks than bags with a treatment weight of 1.5 kg of *Ulva* spp. and no oysters by 15-50%. At Peale Passage, a similar trend was observed in July when the bags with a treatment weight 1.5 kg of *Ulva* spp. and no oysters had approximately 37% more *Ulva* spp. than bags with a treatment weight of 1.5 kg and oysters. This trend was not significant but it did occur throughout multiple sampling periods and at both sites. This suggests that there may be a feedback of oysters on *Ulva.*

This observation differs from findings of the literature by Ale et al, who found that *Ulva lactuca* showed a favorable growth response to ammonium, which oysters secrete, as a nitrogen source (Ale, Mikkelsen, & Meyer, 2011). In their experiment, Ale et al observed that *Ulva lactuca* prefers ammonium over nitrate as a source of nitrogen (Ale et al., 2011). Their findings suggest that oysters may provide beneficial conditions for *Ulva* to grow in and thus may support more *Ulva* growth on site. Lomstein et al found that in a controlled lab experiment, ammonium efflux accounted for 83% of the total nitrogen efflux after *Ulva lactuca* was added to the flow-through mescosm (2006). However, this experiment suggests that other negative impacts may dominate this positive impact.

Shell height was selected as the metric or proxy for oyster growth in this study due to time constraints of collecting data during a low tide. Shell height is one parameter to measure growth, but there are other important parameters that help tell a complete story. Many studies examine meat weight (dry weight) as a parameter of growth. Others measure shell height as well as width. Shell height provides data on the length of the shell growth but it does not always reflect characteristics that are important qualities in a commercial oyster. Commercial oysters are marketed based on the shell size, meat weight, meat quality and color, and shell appearance. For example, the oysters at Peale Passage that made up growth in the late summer and fall were what’s known in the industry as “skinny” (Davis, 2015). Skinny oysters have a long narrow shell and thus the internal meats are also long and skinny. Skinny oysters can occur when oysters experience rapid growth or when bags are not turned on a regular basis

This type of oyster is less desirable in the marketplace because of its shape and smaller meat size (Davis, 2015). Oysters under the “control” treatment at Peale Passage were not observed to be skinny, while oysters under both *Ulva* treatments were skinny by late October. This trend was not observed at Thorndyke Bay where growth rates in the late summer and early fall were slower for the oysters in the two *Ulva* treatments. While the shell height metric portrayed a lot of growth in the late summer and fall particularly for oysters at Peale Passage, this may not have reflected the true size of the oyster.

There were also noticeable differences in shell color at both sites between the different treatments. The difference in shell color was more apparent at Thorndyke Bay, where oysters in the *Ulva* treatments often had light-colored shells and oysters in the control treatment had external shells with more purple coloring to them. One explanation for this is that shell color differed based on the level of stress that the oyster was experiencing. Changes in shell color under stress is a phenomenon that other researchers have observed with bivalves include conversion of purple internal shell to white in *Corbicula fluminea*.

Recommendations

Ulva presence had a persistent effect on diminished oyster height throughout the experiment. Even though *Ulva* weights in treatment bags diminished considerably at both sites by August, a discrepancy in growth rates between treatments exposed to *Ulva* and the control remained through the production period. This preliminary research suggests that to maximize growth, farmers should begin to think about ways to move *Ulva* from seed growing areas. For tidelands where *Ulva* growth accumulates in bags, efforts should be focused on removal early in the growing season (May-July). Oysters that were exposed to treatment levels during this period did not recover in their growth rates in this experiment. This is also typically the peak growing season for *Ulva*. Removing *Ulva* early in the season increases the likelihood that oysters will be ready for market earlier and growers can introduce another crop earlier in the growing season. Other suggestions include regular bag turning to help control fouling on top of bags (Mallet et al., 2009).

Ulva growth is very site specific and growers need to account for site-specific differences when determining how to manage their beds. For example, *Ulva* naturally accumulates in grow-out bags at Thorndyke Bay but not at Peale Passage. Managing *Ulva* in bags is not necessarily a critical issue for growing areas that have more ephemeral *Ulva* populations.

This experiment was conducted at two field sites with different site dynamics and produced consistent results at both sites. Studies examining the interaction between oysters and macroalgae are rare and more research needs to be done before general conclusions about the interactions between *Ulva* spp. and *C. gigas* in Puget Sound can be concluded. However, the benefit of having two field sites illustrates that this phenomenon is not site-specific. Although site-specific dynamics were very different at both sites, shell height growth rates were strongly significant and had similar trends at both sites in the 1.5 and 3 kg treatments. This experiment demonstrates that the interaction between these two species is worth examining further.

Future experiments on this issue should also consider examining water quality parameters as a key component of the study. Water quality measurements including pH and DO were included in the study design. However, due to the limited scope of the project, these measurements were not taken on a regular basis. It would be useful to use equipment that could take frequent measurements in the bag throughout the day (pulse) instead of obtaining measurements only during field collection in order to get a better understanding of the water quality dynamics. The toxicity of *Ulva* to itself as well as other organisms is another emerging field that needs more research in order to better understanding the interactions occurring.

Design considerations

The study design was effective and allowed researchers to observe an ecosystem effect. However, in a future follow up study, there are some factors that should be considered. Utilizing a power analysis in the study design in order to determine the amount of samples may have helped increase the F-value for shell height and provided more informative results for this measurement. The amount of replicates (5) was quite large and could probably be reduced while samples per bag (10) could be increased. These values would provide more information about the growth dynamics occurring in each bag. Now that a significant effect has been observed for treatments of 3 kg and 1.5 kg, it would be informative to see if there is still an effect with less *Ulva*. Treatments of 0.5 and 1 kg should be considered for a follow-up experiment. Measuring the amount of macroalgae that naturally accumulates in grow bags at different locations could help to inform this parameter. Additionally, including water quality measurements in the study design would be informative to identify site-specific factors that may help to explain the differences in shell height. Water flow, pH, temperature and DO should all be considered for future follow up studies.

Conclusion

There are many factors that potentially affect the growth of *C. gigas* in commercial operations. In this experiment, there was a significant size difference between *C. gigas* exposed to *Ulva* spp. in the bag and without *Ulva* spp. in the bag. This indicates a relationship between *Ulva* spp. and oyster growth. With the introduction of *Ulva* spp in a bag comes many potential changes in the local environment including physical, chemical, and biological changes. This study established that there was an effect of *Ulva* spp. on *C. gigas* growth. Additional research is needed to explain the particular components of *Ulva* spp. presence that had the strongest effect on *C. gigas* growth. Understanding the key components of *Ulva* spp. presence that affect oyster growth is very important to developing successful growing practices on oyster farms.

VI. INTERDISCIPLINARY CHAPTER

Introduction

Washington State has been proactive on addressing ocean acidification through the creation of the Governor's Blue Ribbon Panel (2012) and Marine Resources Advisory Committee (2013-present), and the Washington Shellfish Initiative (2012) by providing funding and resources to support research and collaborative efforts between growers, scientists, and policymakers. Through the Marine Resources Advisory Committee, policymakers and scientists have set priorities for Washington to focus on in order to better equip the state to adapt to ocean acidification. One of the key early action items in the 2012 Blue Ribbon report is to "develop vegetation-based systems of remediation for use in upland habitats and in shellfish areas" (Adelsman & Binder, 2012). This action item recognizes that seaweeds are a prolific resource and have the ability to potentially improve water quality for calcifying organisms.

The abundance of algae in Puget Sound presents an opportunity to both improve environmental conditions and create a product from seaweed that could be important in the marketplace. The total of all products made from seaweed worldwide is $5.6 billion annually, with many value-added products made from seaweed ("A guide to the seaweed industry," 2015). Currently, the vast majority of seaweed production occurs in Asia. Commercial harvesting of seaweed in the U.S. is restricted by permit regulations. Currently, Maine is the only state that has a commercial seaweed harvesting industry. Commercial harvesting is not permitted at this time in Washington.

Phytoremediation

There has been a lot of interest around harnessing the potential of seaweed here in Washington. The Puget Sound Restoration Foundation (PSRF) was awarded a $1.5 million Ocean Challenge grant through the Paul Allen Foundation in 2015. The challenge was targeted toward the scientific community to develop science-based solutions to the social and environmental effects of ocean acidification (Hickey, 2015). PSRF is currently developing a seaweed demonstration farm in Hood Head, North Hood Canal to look at the impact of growing sugar kelp on the marine environment. They hope to have a better understanding of the impact of seaweed farming on surrounding water chemistry. PSRF is partnering with other research institutions including University of Washington to look at the impact of seaweed farming for sensitive calcifiers such as pteropods.

Food

Ulva is an edible seaweed that can be harvested for human consumption. It is one of many types of seaweed in the Pacific coastal region that are edible. Seaweed has been harvested as a food product in coastal communities for thousands of years, particularly Asian countries. It is now being consumed in many other countries as well. Seaweed is extremely nutritious and contains high levels of iodine and iron. It also contains calcium, magnesium, potassium, sodium, iron, chromium, copper, and trace minerals which are all important for bodily functions ("Nutritional Information," 2015). *Ulva* in large quantities is a good source of fiber, protein, plant pigments, and plant sterols (Moll, 2013). Seaweed is harvested commercially in 35 countries and the top three producing countries for edible seaweeds are China, Korea, and Japan. There has not historically been as much demand for edible seaweed in the United States, but this is starting to change. Chefs in high-end restaurants in New York are featuring seaweed on their menus as a sustainable local food.

Developing a regional market for seaweed is critical to establishing seaweed harvesting on a larger scale.

Biofuel

Seaweed represents an untapped biofuel market. Seaweed is extremely prevalent worldwide and utilizing this resource could significantly reduce our dependence on fossil fuel. Gao and McKinley estimate that substituting seaweed for fossil fuels to offset carbon dioxide emissions could be a net cost of zero or potentially less than zero (Gao & McKinley, 1994). Compared to first-generation biofuel sources such as bioethanol, seaweed has much lower growing and processing costs (Dibenedetto, 2010). In addition, replacing first-generation biofuels such as bioethanol with seaweed would free up terrestrial land that could be used for growing food crops. Researchers in Norway are looking at the viability of extracting bio-oil from sugar kelp (*Laminaria saccharina*) to be used as biofuel. Their preliminary results suggest that it is possible to get bio-yields of 79% with very high heating mechanisms. Using seaweed for bioenergy (ethanol, etc.) can also free up terrestrial land for farming food products or other land uses. Of course, there are technical challenges that prevent seaweed biomass from being utilized for bioenergy on a global scale on the magnitude of replacing carbon. Understanding the role that algal blooms play in the ecosystem is of primary importance before any type of implementation efforts can be explored.

Fertilizer

Seaweed has been used as a fertilizer for thousands of years. Salish tribes in the San Juan Islands used to fertilize their camas gardens with seaweed (Wagner, 2012). Seaweed contains concentrated amounts of nutrients such as phosphorus and nitrogen that have high demand in agriculture. For example, phosphorus is a finite material that is currently mined with significant environmental impacts. Seaweed contains phosphorus that can be utilized in much less impactful way. Seaweed can be used to make excellent local fertilizer for the Pacific Northwest. It is processed commercially for liquid seaweed extracts, a rapidly growing market for organic fertilizer.

Other uses

The seaweed industry extends beyond edible seaweeds for consumption. Seaweed is also used as a food additive and an anti-bacterial agent. It is harvested for its hydrocolloids that are used a thickening agent in cosmetics. There is also potential to use seaweed for wastewater treatment through absorption of heavy metal ions in polluted water ("A guide to the seaweed industry," 2015).

Ulva spp. has potentially beneficial chemical effects that could promote human health and safety. The extract of *Ulva lactuca* has been found to slow the growth of harmful algal blooms (HABs) in lab experiments. Authors Tang and Gobler found that environmentally realistic levels of *Ulva lactuca* strongly slowed the growth of seven common HABs due to heat-stable allelochemicals (Tang & Gobler, 2011). This research demonstrates that there are many other potential uses for seaweed, particularly *Ulva* that could be explored through additional research.

Conclusion

The Puget Sound region has the ability to contribute to a profitable worldwide industry of seaweed harvesting and products. There is a lot of emerging interest in harvesting seaweed from Puget Sound as well as growing it commercially. The seaweed industry is very profitable; total worldwide value of all industrial products made from seaweed is $590 million (“Prospects for seaweed production in developing countries,” 2015). There are also implications of harvesting macroalgae from Puget Sound for commercial use that must be addressed before seaweed is harvested on a large scale. Removing seaweed, full of micronutrients out of the water will likely have impacts on the marine ecosystem. It is unknown whether these impacts would be significant or not. This issue is further complicated by the fact anthropogenic nitrogen plays a role in seaweed growth in Puget Sound. Before seaweed harvesting is explored on a commercial scale, it’s important to understand the potential implications of this practice to the marine ecosystem.

VI. CONCLUSION

Washington is one of the largest producers of farmed shellfish in the United States, producing approximately 85% of the farmed shellfish on the West Coast (Adelsman & Binder, 2012). A major portion of this production occurs in Puget Sound. Shellfish farming is an important economic driver in rural coastal towns along Puget Sound, and is also a source of local, sustainably grown food. Unlike terrestrial farming, which can often have harmful environmental impacts including problematic waste streams and point-source pollution, shellfish farming often has a net positive effect on the local ecosystem (Fisher & Mueller, 2007). Shellfish, as filter feeders, consume nutrients from the water that are then removed when the shellfish are harvested. Additionally, by consuming phytoplankton, shellfish improve the clarity of the water and allow sunlight to penetrate deeper into the water, supporting the growth of native species including eelgrass (*Zostera marina*) (Fisher & Mueller, 2007). In addition, shellfish farming provides an impetus for political action to improve clean water in Washington State. This political pressure has far-extending impacts for the local population and the environment far beyond shellfish farming.

The future health of Washington's marine waters is uncertain. Scientists are observing the beginning of what could be enormous changes in the water chemistry and thus species composition of our marine waters. The local shellfish industry, dependent on highly sensitive shellfish larvae, is the canary in the coalmine for this problem. Shellfish growers are adapting to this "new normal" proactively by using adaptive techniques including controlling water chemistry conditions in hatcheries for shellfish larvae and utilizing hatcheries to grow out shellfish until they are less vulnerable instead of relying on natural sets. However, continuing to produce shellfish in increasingly acidic conditions will require more adaptation and creativity.

Local impacts on Puget Sound can alter nutrient availability for macroalgae, impacting the frequency and amount of algal blooms. These impacts contribute to nutrient pollution, which has detrimental effects for water quality in some areas of Puget Sound (Mohamedali et al., 2011). Macroalgae exist naturally and are an important component of marine systems. They provide shelter, transport, and food for many organisms low on the food chain including shelter for crustaceans from predators (Frankenstein, 2000) and uptake of anthropogenic CO_2 during photosynthesis. They can also be harmful to water quality, shading out native sea grasses and creating oxygen deficient environments, killing marine species and potentially leading to local dead zones. Researchers have linked the increase in nutrient run-off into the Sound to an increase in algal blooms (Frankenstein, 2000).

Understanding the relationship between seaweed and shellfish growth is key because macroalgae occurs on many shellfish farms during certain times of the year, and growers can spend a lot of time managing biofouling on their gear. Quantifying the impact of *Ulva* spp. weight on commercially grown *C. gigas* oysters is critical to making informed management decisions. When developing plans to introduce macroalgae culture or harvest to a shellfish farm, the impacts to the growth of the crop must be taken into account along with macroalgae's ability to alter local water chemistry. Providing shellfish growers with the best available tools to adapt to more acidic marine waters is critical to ensuring the survival of the shellfish industry in Washington State.

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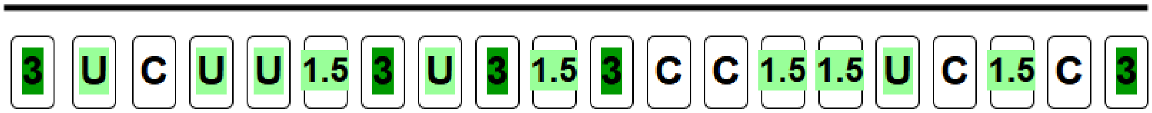
Appendices

Appendix A. Field Sites in spring and then during peak *Ulva* spp. bloom at a) Thorndyke Bay and b) Peale Passage

Appendix B. *C. gigas* growth comparison at completion of experiment (October 2015) at a) Thorndyke Bay and b) Peale Passage. “3 kg” indicates 3 kg *Ulva* spp. added to treatment and “control” indicates 0 kg *Ulva* spp. added.

Appendix C. Study Design

Graphic representation of study design at each site.



C= Control, 0 kg *Ulva* spp., 150 oysters

U= *Ulva* spp., 1.5 kg *Ulva* spp., 150 oysters

1.5= 1.5 kg *Ulva* spp., no oysters

3= 3 kg *Ulva* spp., 150 oysters