NUTRIENT CYCLING BETWEEN CULTIVATED MANILA CLAMS (*V. PHILIPPINARIUM*) AND GREEN MACROALGAE (*ULVA SPP.*) ON THE NORTHERN HOOD CANAL

by

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ABSTRACT

Nutrient Cycling between Cultivated Manila clams (*V. philippinarium*) and Green Macroalgae (*Ulva spp*.) on the Northern Hood Canal

Natalie C. Sahli

Large blooms of green marcoalgae (*Ulva spp*.) occur seasonally throughout the Puget Sound basin; densely covering cultivated manila clams (*Venerupis philippinarum*) and possibly acting as a source of POM to the organisms. Shellfish growers observe a biomass increase of *Ulva spp.* in the presence of *V. philippinarum* monocultures. This observation suggests a fertilization effect between species. Though *V. philippinarium* aquaculture provides millions of dollars of annual revenue to the State of Washington, nutrient cycling dynamics between *Ulva spp*. and *V. philippinarum* remain unquantified for the Northern Hood Canal. To further examine this relationship, fifteen standard commercial *V. philippinarum* mesh bags served as part of an experiment to assess the potential for symbiotic nutrient cycling between the two species on Thorndyke Bay, in Hood Canal, WA from June to September 2015. The randomized bags consisted of treatments containing exclusively *V. philippinarum,* exclusively *Ulva spp.,* and the combination of *Ulva spp*. and *V. philippinarum*. C:N ratios of *Ulva* directly exposed to clams were significantly lower (8.6 ±0.41) relative to the C:N ratios of *Ulva spp*. (12.5 ±0.67) raised without clams, suggesting that ammonium secreted as a metabolic byproduct by the clams provides essential nitrogen to macroalgae tissues. Additionally, unanticipated drought conditions during the experiment yielded $\delta^{13}C$ evidence that *V. philippinarium* (-20.82±0.26‰) feed on sources more isotopically depleted than *Ulva spp*. (12.87±0.67‰) and phyto-POM (15.09± 0.63‰). Evidence for a commensal relationship between these species justifies to shellfish growers that a well-managed integrated system provides means to remove excess nutrients from aquaculture systems via the extraction of nitrogen-rich macroalgae.

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INTRODUCTION

Shellfish monocultures exist within complex functioning shoreline ecosystems. Currently, Washington State shorelines host the most productive shellfish industry in the nation, comprising 31% of the total national market (Washington Sea Grant [WSG], 2015). Washington State's extensive shellfisheries warrant further scientific investigation into potential ecosystem impacts, especially as projections predict substantial industry growth (WSG, 2015; Booth, 2014).

One of the most relevant concerns with shellfish monocultures is their contribution to local nutrient cycling. The Washington coast experiences seasonal upwelling events of nutrient-rich water (Newton et al, 2007). Though nutrients are essential to the functioning of coastal systems, excess nutrients act as a detriment to ecosystems by over stimulating primary production (Newton & Voorhis, 2002). The eventual degradation of primary producers, utilizing these nutrients, depletes local oxygen concentrations and can result in eutrophic conditions. Though upwelling is the main contributor of nutrients to Washington's coastal ecosystems, locally-sourced nutrients can additionally effect water quality conditions (Newton, et al 2007).

The contribution of shellfish monocultures to the marine inorganic nitrogen pool has been clearly documented. Shellfish produce ammonium (NH_4^+) as a metabolic byproduct. This inorganic form of nitrogen is readily taken up by primary producers, such as *Ulva spp*., a seasonal native green macroalgae. The high ammonium concentrations result in increased biomass of *Ulva spp*. near monoculture sites (Saurel et al, 2014; Zertuche-Gonzalez et al, 2008). Though this relationship between shellfish and macroalgae biomass has been demonstrated for certain growing regions, the contribution

of shellfish-produced nitrogen to primary producer tissue in all highly profitable growing regions remains unquantified. If *Ulvoid* tissue acts as a temporary sink for monoculture-derived nitrogen, the species can be managed to mitigate seasonal negative effects of shellfish monocultures.

Ulvoids may also transcend their negative designation as an aquaculture pest species to act as a food source to monocultures. Bivalves have the capacity to incorporate detrital material into their diets when available (Suh & Shin, 2014; Dunton & Schell, 1999). In this sense, shellfish may act to remove labile detritus from the water column, interfering with the harmful decomposition pathway. Evidence for a symbiotic relationship between the two species would support a transition within the industry away from monocultures, towards an integrated macroalgae-shellfish aquaculture system.

Investigating the relationship between seasonal primary producer blooms and shellfish monocultures will lead to a more clear understanding of how the industry can manage aquaculture plots in the face of growth. This thesis attempts to quantify the relationship between seasonal *Ulvoid* blooms and a commercially significant monoculture, as they exist on a productive cultivation site in one of Washington's estuarial bays. The goal of this research is to quantify the contribution of *Ulva spp.* to the diet of a commercially relevant species and to address the contribution of inorganic nitrogen from monocultured species to seasonally occurring macroalgae tissue. These findings will contribute to a larger investigation of how to manage *Ulvoid* blooms in growing regions. Additionally, this research may provide evidence into the sensibility of incorporating primary producers into cultivation sites, as a means of sequestering and removing the nitrogen produced by shellfish during the summer months.

To illustrate the relationship between monocultures and primary producers, the following document will elaborate on the economic and ecological importance of the species, and locale, of interest, ultimately leading to a novel research question. Additionally, a review of relevant research undertaken to this point will be included. Following this will be an elaboration on field and laboratory methods used to quantify the relationship between selected species. The quantitative results of this research and subsequent discussion will follow. Finally, a conclusion, which addresses the economic and ecological implications of these findings, will end this document.

CHAPTER 1: LITERATURE REVIEW

Introduction

Washington State shellfish are globally renowned for their fine quality. As early as the 1860's, the region's abundant native shellfish resources entered into external markets (Pacific Shellfish Institute [PSI]). The high demand for these delicacies brought about a rapid decline in wild native populations, most specifically the native Olympia Oyster (*Ostrea lurida*) (PSI). The solution came with the introduction of the larger Japanese Pacific Oyster (*Crassostrea gigas*) to the region to supplement declining wild stocks (Humphreys et al, 2015). Inevitably, additional non-native species were accidentally introduced through shipments of these Pacific Oysters, including the now commercially significant Manila clam (*Venerupis philippinarum)*(Quayle, 1949 in Humphreys et al, 2015). Both *Crassostrea gigas* and *V. philippinarum* adapted seamlessly to Washington's tidal flats (Humphreys et al, 2015). Today, both populations exist in wild and commercial settings, bringing millions of dollars worth of revenue to the state annually (IEc, 2014).

To maintain a high level of production for global markets, shellfisheries rely on

intensive cultivation of Washington's intertidal flats (PSI). Shellfish are grown often in bags or racks, segregated by species (Toba, Dewey, & King, 2005). These expansive bivalve monocultures require little or no external feed inputs, relying almost exclusively on pre-existing ambient nutrients from the local environment. Shellfish growers and citizens alike herald the nutrient extractive properties of filter-feeding monocultures as an environmental solution to nitrified waters (Shumway et al, 2003). However, an in-depth analysis of the complex nutrient cycling interactions between bivalves and their larger ecosystem reveals a much more complicated story.

Locally concentrated nutrients negatively impact the water quality in certain regions of the Puget Sound (Newton et al, 2007). Nutrients concentrate in certain areas, due to reduced exchange with outside water bodies (USGS). The eutrophic conditions resulting from excess nutrients create harmful conditions for local wild, and cultivated marine, life (Newton, et al 2007). Shellfish accumulate organic and inorganic forms of nutrients into their tissues over their life span, ultimately allowing for the removal of these harmful elements from the system upon harvest (Shumway et al, 2003). However, shellfish also release dissolved inorganic nutrients in the form of ammonium as a metabolic byproduct (Peterson & Heck, 1999). This ammonium fuels the growth of green macroalgae. Although macroalgae temporally sequester nutrients, these nutrients are reintroduced back into the local system upon degradation (Saurel et al, 2014). Evidence exists that macroalgae blooms negatively impact growth of certain shellfish species (though the mechanism by which they do this is unclear).(Lamb, 2015). Before attempting to quantify the potentially complex inter-trophic dynamic between shellfish and macroalgae blooms, understanding the relevant local, and comparable global,

nutrient cycling scenarios is imperative.

One way by which nutrient cycling can be assessed is through using carbon and nitrogen isotopes, in combined with elemental ratios. Stable isotopes of carbon are often used to delineate the dietary composition of a given individual or population. There are two stable isotope forms of carbon: ^{13}C and ^{12}C. Each primary producer has its own unique ^{13}C signature, which carries over into consumers. Hence, in knowing the signatures of all potential food sources and the signature of the primary consumer, the relative contribution of each food source to the total diet can be deduced via a system of equations. Elemental ratios are similar in that primary producer tissues mimic the exact proportions of nitrogen and carbon in the environment (Zertuche-Gonzales et al, 2008; Peterson & Heck, 1999). Hence, lower carbon to nitrogen (C/N) ratios indicate higher ambient nitrogen concentrations. Each of these techniques has been used, respectively, in the study of bivalve diets, and in studying the effect of shellfish on primary producer nutrient assimilation (Suh & Hin, 2014; Zertuche-Gonzales et al, 2008; Dunton & Schell, 1999; Peterson & Heck 1999). Up to this point, a dietary stable isotope analysis for *V. philippinarum* and an elemental ratio analysis of *Ulva spp*. growing near shellfish cultivation plots in the Puget Sound are absent from the current literature.

The following literature review will serve to illuminate the essential pieces of this complex ecological web, using *Venerupis philippinarum* in the productive Northern Hood Canal as the commercially relevant centerpiece. To start, an exploration of this commercially significant bivalve species' dependency on regional baseline conditions, including primary productivity, will be examined. Following, will be an examination of seasonally and spatially relevant primary producers and their specific nutrient exchanges

with aquacultured bivalves. Furthermore, a detailed discussion of regional organic and inorganic nutrient cycling will give specific context to trophic interactions in Washington's estuarial bays. Nutrient cycling at the ecosystem level will provide a transition into the discussion of specific inter-trophic nutrient cycling. Concluding will be a synthesis of all represented scales, presenting an integrated multi trophic systems solution to the environmental stressors emergent from *V. philippinarum* monocultures. This review will demonstrate the need for monoculture diversification in the Washington State shellfish industry, and propose a method of study providing quantitative evidence in favor of this paradigm shift to shellfish growers and marine ecologists, alike.

V. philippinarum Aquaculture in Washington State: ecology of a profitable monoculture

The following section demonstrates the value of *V. philippinarum* aquaculture to the State of Washington and its relevance for intensive study. Research by the industry, into physical applications to monoculture growing sites, has the potential to increase production in the state. Already, certain growing regions yield greater abundances of wild and commercial *V. philippinarum*; notably the Hood Canal and, to a lesser extent, South Puget Sound. These regions are indispensable to *V. philippinarum* aquaculture in Washington. Thus, the commercial and ecological components of these highly productive locales provide context for further research into potential industry innovations. In these regions, the monocultured clams are dependent on larger ecosystem forces and thus cannot be separated from the ecology as a whole. This section will illuminate the connection between the aquaculture industry and the biogeochemical components at growing sites. It will demonstrate a needed shift in the paradigm from monoculture-

intensive research to intensive investigations surrounding the integration of natural ecosystem dynamics into the structure of aquaculture itself.

Economic Importance and Growing Region Characteristics

Washington State boasts the largest grossing shellfish aquaculture industry in the nation (Industrial Economics Incorporated [IEc], 2014), providing 96.9 million dollars of revenue to the state annually (Booth, 2014). *V. philippinarum* aquaculture comprises eleven to sixteen percent of total commercial cultivation, second only to the Pacific Oyster (IEc, 2014). Currently, the industry is exploring new markets for *V. philippinarum*, intending to increase production of the clams without drastically expanding growing sites (IEc, 2014). Innovations in cultivation techniques, which reduce predation by dispensing durable netting, and increase juvenile survivorship, are fueling the growth of the industry (Thompson, 1995). Altering substrates in order to increase the recruitment of spat has been a focus in industry and conservation research. Providing gravely substrates, often enhanced with crushed bivalve shells increases recruitment for hardshell clams (The Nature Conservancy [TNC]). In hatchery settings, individuals are selectively bred to create more robust brood stocks (Johnson, 2008). Historically, industry research in Washington focuses primarily on increasing the production of *V. philippinarum* by selectively breeding populations and/or altering the surrounding environment (TNC; Johnson, 2008; Thompson, 1995). Diversification from the current monoculture paradigm, to more closely mimic natural ecosystem cycling may further improve yields on commercial sites. The following figure exemplifies the increase of manila clam aquaculture in the Puget Sound and Washington Coast.

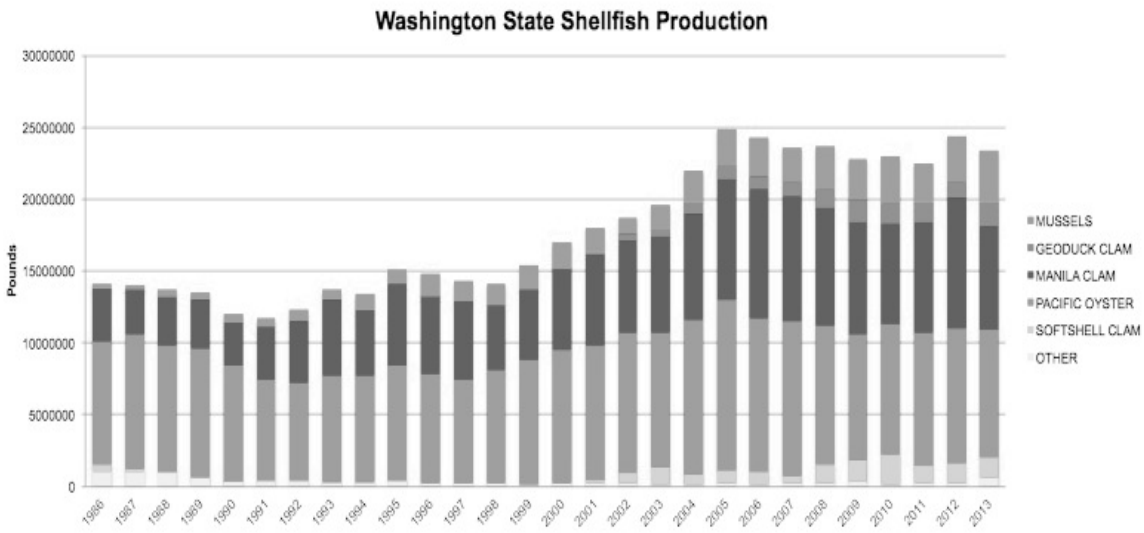

Figure 1. Change in WA state shellfish production from 1986 to 2013 (Washington Sea Grant [WSG] 2015).

Industry projections indicate an increase in *V. philippinarum* production for the all growing regions throughout Washington (Booth, 2014). There are five major growing regions for shellfish in Washington State (Willapa Bay, Grays Harbor, North Puget Sound, South Puget Sound and the Hood Canal) all which support the cultivation of *V. philippinarum.* The regional variability amongst the various growing sites significantly influences the predominance of *V. philippinarum* as compared to other molluscan species. (Booth, 2014). As of 2014, the Hood Canal supports the majority of *V. philippinarum* aquaculture in the state (Booth, 2014). In 2013, manila clam aquaculture accounted for 47% of total aquaculture production in the Hood Canal (WSG, 2015).

The Hood Canal is a primary sub-basin of the greater Puget Sound (Warner & Kawase, 2001). This deep, estuarial fjord is unique in that its mouth is located between two high glacial sills, separating it from the main influx of water from the north. These sills, termed the Admiralty sills, allow water to flow over into the Hood Canal via the Strait of Juan de Fuca. Though the sills do not prevent the influx of water, they greatly

inhibit the outflow of water from the Canal, creating highly stratified conditions in the water column (Warner & Kawase, 2001). These stratified conditions become problematic in the late Summer and Fall, when dissolved oxygen concentrations are low as a result of seasonal upwelling and primary producer degradation (Newton et al, 2007). Riverine inputs into the Hood Canal, additionally remain stratified on the upper surface, further contributing to primary production and eutrophic conditions (Warner & Kawase, 2001).

The unique physical characteristics of the Hood Canal make it well-suited to support *V. philippinarum* cultivation. *V. philippinarum* readily settle in gravel, sand, and mud substrates (Department of Fisheries and Oceans [DFO], 1999). However, the species display higher survivorship in finer-sediment substrates (Thompson, 1995). The upper layers of Hood Canal are comprised predominantly of glacial till and glacial outwash sediments (Washington State Department of Transportation [WSDOT], 2008), providing an ideal habitat type for the clams. Several small streams flow into the Canal, providing a mixture of fine and course sediment to beaches (WSDOT, 2008). The Hood Canal and Puget Sound have experienced significant sediment delivery (Puget Sound Partnership [PSP], 2006). However, the Hood Canal is less populated and has experienced less land change use, which impacts sedimentation in the Puget Sound, reinforcing it as an ideal growing site. Tides in the Hood Canal are less severe than in other growing regions, reducing exposure of intertidal clams to desiccation (Toba, Dewey, & King, 2005). Less drastic tides also reduce the intensity of wave action and beach erosion (WSDOT, 2008). Strong wave action inhibits clam survivorship by washing away particulate substrate. Thus calmer bays, such as those found in the Canal, are preferred for *V. philippinarum* cultivation (Toba, Dewey, & King, 2005).

Inherent physical factors create obstacles to Hood Canal aquaculture. The late summer and early fall is defined by poor water quality in the Canal (Newton et al, 2007). Low dissolved oxygen levels, resulting primarily from the seasonal upwelling of oxygen depleted waters along the Eastern Pacific, present an issue to Hood Canal marine life (Newton et al, 2007). Though shellfish posses a higher tolerance to hypoxic conditions than marine vertebrates, severe oxygen reduction can cause significant stress within organisms (Diaz & Rosenburg, 1995). Harmful algal blooms (HABs) of large green macroalgae similarly stress marine invertebrates. Additionally, certain species of dinoflagellate phytoplankton have been shown to create a starvation response in invertebrates during the late summer and early fall months throughout thr Puget Sound Basin (Jerry Borchert, personal communication, 2015).

The shellfish industry has developed some measures of defense against biotic stressors. Large nets help contain wild juvenile clams and decrease predation risk throughout the two to five year growing period (Booth, 2014). On large scale aquaculture operations, hard plastic 1/2-inch mesh bags protect clams from predators and reduce migration throughout their life cycle (Toba, Dewey, & King, 2005). Aquaculture bags are almost completely submerged in the sediment, allowing clams to burrow to their preferred depth of two to three inches below the surface (Washington Department of Fish an Wildlife [WDFW]).

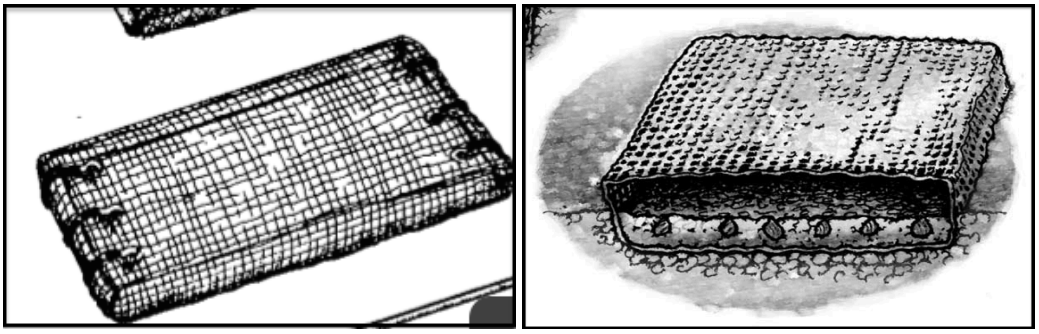

Figure 2. Standard mesh growing bags (Toba, Dewey, & King, 2005).

Often, harvesters remove macro algae from the outsides of the bags during the summer months. Macroalgae reduces the availability of dissolved oxygen within the bags and prevents the circulation of seawater (Toba, Dewey, & King, 2005). Circulating seawater carries phytoplankton-based particulate organic matter, the bivalve's primary food source.

V. philippinarum monocultures: dynamics within regional ecosystems

Cultured and wild *V. philippinarum* derive nutrients from several different sources. *V. philippinarum* primarily consume marine particulate organic matter (POM) (Poulain et al, 2010). The composition of POM in marine waters is composed of several components including " phytoplankton, microphytobenthos, resuspended sediment, terrestrial carbon, [and] marine macro algae detritus" (Poulain et al, 2010). Depending on the availability of POM, filter feeders will consume less preferable organic matter, such as that from sewage runoff (Rensel, Bright & Siegrist, 2011). This is testament to their highly variable and adaptive diets. Cultured bivalves depend most heavily on phytoplankton from the ambient environment, evident from decreased growth rates when phytoplankton are scarce in comparison to alternate nutrient sources (Spillman et al, 2008). In the midst of

seasonally-induced large microalgal blooms in the Puget Sound, bivalve species experience increased growth rates and will selectively feed on phytoplankton over other organic nutrient sources (Rensel, Bright & Siegrist, 2011).

Dissolved inorganic phosphorous increases microalgal productivity (Newton, 2011), which corresponds to higher growth rates in *V. philippinarum.* Phosphorous represents a critical nutrient input affecting the availability of POM. However, in the Hood Canal, nitrogen is considered to be a more significant limiting nutrient than phosphorous (Newton, 2011). Hence, a detailed discussion of phosphorous cycling will be largely omitted from this document in favor of a greater emphasis on nitrogen cycling.

Marine macrophytic detritus can contribute significantly to bivalve diets when abundant (Dunton and Schell 1999). However, detrital material is found less preferable to phytoplankton (Dang et al, 2009 & Rensel, Bright & Siegrist, 2011). In Arachon Bay, France, detritus was consumed only when phytoplankton was less abundant (Dang et al, 2009). Bivalves sort particles by size prior to digestion excreting larger masses of material as pseudofeces (Tucker & Hargreaves, 2009). Hence, the consumption of phytoplankton may be more energetically favorable than the consumption of detritus.

In estuarial food webs, primary consumers rely on local sources of primary producers (Dang et al, 2009). Local sources of organic and inorganic nutrients, and thus the diets of the bivalves utilizing those nutrients, vary regionally. (Rensel, Bright & Siegrist, 2011). Location on the tidal flat (Rensel, Bright & Siegrist, 2011). proximity to freshwater inputs (Kasai, Horie, & Sakamoto, 2004), relative abundance of macroalgae and macrophytes, as well as seasonal productivity of phytoplankton control the type and abundance of nutrients. Assessing these environmental parameters could result in a more

intensive understanding of bivalve-environment interaction to determine the relative local contributors to *V. philippinarum* diets. This understanding could illuminate ways in which aquaculture systems could be manipulated to potentially maximize the availability of preferred food sources. If the preferred food sources of cultivated shellfish have an associated economic value, shellfish growers may be inclined to incorporate a more diverse set of species into cultivation plots.

Shellfish aquaculture exists within the context of a larger ecosystem. The ecosystem services necessary for shellfish survival represent only one aspect of a larger interaction. The byproducts of these monocultures similarly feed back into the surrounding environment. Environmental impacts from shellfish monocultures can be both positive and negative. Shellfish aquaculture increases biodeposition of undigested or excreted POM into the sediment, fueling denitrifying bacteria, theoretically removing nitrogen from the system (Shumway et al, 2003). This sedimentary organic matter (SOM) fuels benthic primary consumers at the base of the food chain (Shumway et al, 2003). Additionally, shellfish filter both particulate organic and inorganic forms of nitrogen, resulting in N assimilation in their tissue, helping mitigate potentially harmful eutrophic conditions (Shumway et al, 2003). The structure of aquaculture additionally provides refuge for native species, and supports wild shellfish recruitment (Saurel et al, 2014).

Unfortunately, extensive shellfish monocultures have the potential to negatively impact the environment. Dense monocultures adversely affect benthic biodiversity (Sequeira et al, 2008). In addition, cultivated *V. philippinarum* compete with native clams and oysters for desirable POM, reducing growth rates in wild species (Sequeira et al, 2008) and changing phytoplankton species composition (Saurel et al, 2014). Evidence in

the literature, which conflicts with industry publications, suggests shellfish farms contribute further to eutrophication through biodeposition (De Casbaianca, Laugier, & Marinho-Soriano, 1997). Ammonium excretion by *V. philippinarum* increases with summer temperatures (Mann & Glomb, 1978), fueling the growth of primary producers (Saurel et al, 2014). Shellfish monocultures can act as significant nutrient sources to local primary producers. Zertuche-Gonzalez, 2008, concluded that ammonium concentrations were high enough to significantly increase the proliferation of the green seaweed, *Ulva spp.,* sharing the same bay as oyster monocultures in Baja California. Local evidence from the Puget Sound suggests that manila clam monocultures similarly increase the abundance of seaweed species in a harvest area (Saurel et al, 2014). Dense *Ulva spp*. blooms outcompete and shade other macrophyte communities, reducing marine plant diversity. Overall, alternative aquaculture systems warrant investigation as a means to reduce any potential negative impacts of monocultures on local ecosystems.

Primary Producers in the Puget Sound in Hood Canal: spatial and seasonal dynamics of *Ulva spp.* and Phytoplankton

The previous section demonstrates the importance of regional ecology to productive *V. philippinarum* aquaculture in the state of Washington. This section will further expand on the dynamics of primary producers, specifically phytoplankton and *Ulva spp*. While phytoplankon is the established preferred primary food source of cultivated bivalves, detrital macroalgae can contribute to bivalve diets when present. Distribution of both primary producers is variable amongst growing sites in the Puget Sound. Similar to *V. philippinarum*, primary producer populations respond to the biogeochemical forcing in a given region. The abundance and composition of primary producers in an area can have significant impacts on growing shellfish. Additionally, shellfish can influence the

abundance and physiology of certain primary producers. Understanding the ecology of these primary producers will illuminate the key components of seasonal and spatial algal nutrient inputs into productive aquaculture settings. Additionally, this information will contribute further insight into the feasibility and possible benefits of incorporating native macroalgaes into integrated mariculture systems.

Macroalgae Blooms: dynamics of a nutrient source and sink

Ulva spp. is a genus of green sea lettuce native to the Puget Sound and Hood Canal. During the growing months of June-September, *Ulva spp*. is the dominant macroalgal species in the intertidal zone (Western Washington University [WWU]). *Ulva spp*. attaches to substrates, yet the majority exists as floating in large masses in shallow, sandy protected bays (WWU). *Ulva spp*. generally prefers areas of softer substrates over rocky intertidal zones (Nelson, 2008). Reduced nitrogen input into marine ecosystems in the late summer months, due to decreased river export, limits *Ulva spp.* growth (Nelson, 2008). Decay typically happens during the late summer/early fall (Nelson, 2008). Low salinity environments and low light levels have a negative effect on the macro algae blooms (Nelson, 2008). This combination of factors make sandy, well-exposed *V. philippinarum* growing sites excellent habitats for Ulva spp..

Often *Ulva* survives better in these near shore environments than other macrophytes. Evidence in the last decade suggests that Ulva spp. outcompetes marine macrophytes in their traditional habitat, as it more readily adapts to high intensity light levels, partial desiccation and eutrophic conditions (Nelson, 2008). Economically feasible macroalgae show declining population levels throughout the certain regions of the Sound

due to increasing sedimentation from anthropogenic sources (Mumford, 2007). *Ulva spp*. thrive in highly sedimented zones, and they can serve as an experimental proxy by which to assess detrital contributions, and nutrient uptake of macroalgae as a whole to altered near shore ecosystems.

Seaweeds are generally perceived to be the least represented primary producer contributors to global marine food webs (Nelson &Tjoelker, 2003). However, *Ulva spp*. is a significant contributor of nutrients in marine near shore ecosystems. In the case of eutrophic near shore coastal ecosystems, seaweeds represent a main component of primary production (Zertuche-Gonzalez et al, 2008). *Ulva spp*. decays rapidly, with a half life of 8 days (Zertuche-Gonzalez et al, 2008). Some nutrients are remineralized into the environment; however, labile organic components are reintroduced into the food web during decomposition (Zertuche-Gonzalez et al, 2008).

Other macroalgaes, such as kelp, contribute significantly to benthic food webs (Dunton & Schell, 2003). Though detrital incorporation is variable among benthic invertebrates, significant evidence exists supporting the importance of detrital macro algae throughout near shore trophic levels (Dunton &Schell, 2003). Juvenile *V. philippinarium* readily incorporate marine detritus, which can compose over 50% of their diet (Suh & Shin, 2013). In comparable environments, adult clams incorporate 20-30% floating or settled detritus (Suh and Shin, 2013). Detrital incorporation in benthic communities varies based on locale (Suh and Shin, 2013). Incorporation rates of macroalgae detritus remain unknown for adult *V. philippinarium* in the Hood Canal growing region.

The relative abundance of *Ulva spp. in* the intertidal zone allows it to be a

significant sink for nutrients (Hanisak 1993 in Zertuche-Gonzalez et al, 2008). Nitrogen, phosphorous and carbon comprise three ambient nutrients able to be stored in *Ulva* tissue (Zertuche-Gonzalez et al, 2008). *Ulva spp*. abundance increases significantly near oyster and manila clam beds due to higher ammonium concentrations (Saurel et al, 2014; Zertuche-Gonzalez et al 2008). The tissue of *Ulva spp*. near oyster beds in Bahia San Quintin lagoon in Mexico reach maximum values for Ulva nitrogen saturation (around ~2.3%) for most of the growing season (Zertuche-Gonzalez et al, 2008). In certain high light intensity, low-nutrient environments, maximum nitrogen absorption reflects maximum growth rate (Zertuche-Gonzalez et al, 2008).

If the *Ulvoids* are left to naturally decay, which is likely, unless physically removed, the absorbed nutrients will return to the environment during decomposition in the Fall. If the area experiences reduced water circulation over the winter, these nutrients may possibly linger to exacerbate the following Spring's bloom, with the onset of warmer temperatures and increased light. This phenomenon as can be seen in the Bahia San Quintin lagoon (Zertuche-Gonzalez et al, 2008). Removal of *Ulva spp.* tissue prior to degradation can reduce eutrophic conditions (Zertuche-Gonzalez et al, 2008). A well-managed integrated aquaculture system could result in the removal of this short-term nutrient sink from the ecosystem. Thus, the resulting ecosystem would be less susceptible to eutrophic conditions resulting from shellfish monocultures and natural seaweed degradation.

Seasonal ecology of phytoplankton and response to nutrient variability

Phytoplankton blooms occur in the Puget Sound and Hood Canal primarily between

the months of April to September (Nakata & Newton, 2001). Generally blooms will occur at temperatures greater than thirteen degrees Celsius (Greengrove et al, 2014), and thus are not dependent exclusively on season. Blooms are uncommon during the winter months due to limitations in temperature and light (Greengrove et al, 2014). A variety of physical environmental factors influence bloom severity "…including vertical advection and turbulence, modulation of underwater light intensity by self-shading and inorganic particles, sinking of algal cells, and occasional rapid horizontal advection of population from [a given region] by sustained winds." (Winter et al, 1975).

Microalgae blooms comprise the largest component of total marine primary production. Marine phytoplankton blooms are fueled primarily by available nitrogen in marine ecosystems (Winter, 1975). Phytoplankton assimilate dissolved organic nitrogen (DON), ammonium, nitrate, nitrite, and, to a lesser degree, atmospheric nitrogen gas (Voss et al, 2011). Ammonium is the most readily absorbed form of N by phytoplankton, especially in low light intensity and nutrient-limited conditions (Dortch, 1990). Though the rate of ammonium uptake in phytoplankton exceeds that of nitrate, conditions of high nitrate saturation produces more productive blooms for a majority of species (Dortch, 1990). Recent increases in nutrient introduction by anthropogenic sources has resulted in increasing localized bloom size throughout the Puget Sound region (Kangaonkar et al, 2012).

In the Puget Sound, seasonal nutrient upwelling during the summer months and anthropogenic nutrient inputs control the proliferation of phytoplankton (Kangaonkar et al, 2011). These large seasonal blooms act to effectively feed cultured bivalves throughout the region (Rensel, Bright & Siegrist, 2011). However, the byproduct of

microalgae degradation can significantly contribute to eutrophic conditions (Voss et al, 2011). The decomposition of phytoplankton reduces dissolved oxygen concentrations below the surface, especially in the summer months when water layers are highly stratified by temperature (Newton et al, 2007, 2005; Voss et al, 2011). Thus, the degradation of these large blooms negatively impacts water quality and habitat conditions for native species.

Inter-trophic Nutrient Cycling: tracing fundamental elements through complex ecosystems

The previous two sections demonstrate 1) the dependence of a commercially important species on local physical and ecological dynamics 2) the seasonal and spatial ecology of primary producers, the food sources of *V. philippinarium*, in relation to aquaculture sites. This section will attempt to further synthesize the fundamental relationship amongst environmental nutrient availability, primary consumers (i.e., shellfish), and primary producers in aquaculture settings. Elemental carbon and nitrogen comprise the core transferable units upon which to quantify the story of dynamic inter-trophic exchange. The physical forcings of the local environment come into play as a baseline for available nutrients. Additionally, the differential characteristics of available carbon and nitrogen species can be used to assess the relative movement of the elements from their ecological baseline through trophic levels. Similarly, the relative abundance of specific elemental components in primary producer tissue serves to illuminate the effect of external mechanisms on the ready available forms of these elements. Synthesizing components of environmental nutrient determination and elemental transfer between trophic levels can act to quantify a symbiotic relationship within a given locale. Symbiotic multi-trophic relationships in aquaculture settings represent solutions to effect

nutrient determinations in eutrophically stressed coastal environments.

Organic and Inorganic Nutrient Sources and Fates in Puget Sound Estuaries

Organic nutrients enter the Puget Sound through primarily allochthonous pathways. The majority of Dissolved Organic Nitrogen (DON) enters the Puget Sound by riverine transport (Mackas & Harrison, 1997). Evidence suggests that DON and particulate organic N, exported by wetland-dominated Hood Canal tributaries, can exceed inorganic N export in other tributaries (Steinburg et al, 2010). Particulate and dissolved organic nutrients contribute significantly to eutrophic conditions in estuaries by fueling primary productivity and increasing local sedimentation (Mackas & Harrison, 1997). The local degradation of marine primary producer tissue acts as an autochthonous source of particulate organic nitrogen (PON) and dissolved organic nitrogen (DON) (Mackas & Harrison, 1997).

Average inputs of particulate organic nitrogen (PON) and dissolved organic nitrogen (DON) into Puget Sound estuaries were assessed in 1997 to be 1400-1500 tonnes/day (Mackas & Harrison, 1997); a value that likely has increased with land use change and increasing population in the Puget Sound basin. Of this organic input, ~75% terminates in primary producer tissue (Mackas & Harrison, 1997). Organic nitrogen leaves estuarial systems through advective export of incorporated, particulate, and dissolved organic nitrogen, marine-life harvest, predation, and to a lesser extent, denitrification (Harrison et al, 1997). In poorly flushed basins, DON and PON compound localized eutrophic conditions (Mackas & Harrison, 1997).

Nutrient cycling in marine near shore ecosystems represents a complex process

inclusive of terrestrial and open ocean influences (Khangaonkar et al, 2012; Voss et al, 2011). Terrestrial nutrient inputs dominated by "rivers, non-point source runoff and nearly 100 wastewater discharges" threaten the health of the Puget Sound, primarily poorly flushed basins (Khangaonkar et al, 2012). In basins such as the South Puget Sound and Hood Canal, these stagnant inorganic and organic terrestrial nutrients exacerbate eutrophic and harmful hypoxic conditions (Khangaonkar et al, 2012; Newton et al, 2007).

Seasonal nutrient upwelling events from the open oceans (which contribute the majority of nutrients into the Puget Sound basin) occur from November to Februrary (Khangaonkar et al, 2012). Upwelled nutrients from the Pacific Ocean enter the Puget Sound basin by way of the Admiralty Inlet (Khangaonkar et al, 2012). In the Hood Canal, inorganic N inputs from upwelling comprise about 98% of the total N delivered to the surface (Steinnburg et al, 2010). Certain sub-basins receive significant N inputs via watershed export (Steinnburg et al, 2010). However, the majority of the Hood Canal cycles according to seasonal upwelling influence. Despite the dominance of upwelling as a source of nutrient availability in the Canal, seasonal autochthonous and allochthonous sources represent important factors in localized nutrient cycling.

Inorganic nutrient loading significantly affects local marine chemistry. Dissolved inorganic nutrients (DIN) exceed organic forms of N in the Puget Sound (Steinnburg et al, 2010; Mackas & Harrison, 1997). Streams and rivers provide additional contributions of DIN to marine systems (Newton et al, 2011). Freshwater inorganic nutrient inputs account for 421 ± 162 metric tons of inorganic nitrogen per year (Paulson et al, 2004 in Newton et al, 2011). Bacterially mediated atmospheric fixation of N contributes additional inorganic nutrients into the Hood Canal at 30 ± 11 metric tons per year

(Paulson et al, 2004 in Newton et al, 2011). In contrast, the primary mechanisms by which nitrogen is naturally lost from the Washington coastal shelf are through bacterially-mediated denitrification and burial in sediment (Christiansen, Smethie, & Devol, 1987). Inorganic nutrient cycling represents an incredibly complex process involving both abiotic and biotic components. Due to the limited scope of this project, the discussion of inorganic nutrient cycling will be constrained primarily to the inorganic N exchange between macrobiota.

The balance of nitrogen in a system is dependent on a variety of environmental factors. These variable concentrations of nutrients represent the fundamental environmental parameters that shape nutrient assimilation and movement in an ecosystem. Understanding nutrient exchange on a local level can illuminate forcings which may have a profound impact on species within a regional ecosystem.

Stable isotope methodology: tracing source elements through trophic levels

Stable isotopes behave in a predictable manner from individual to ecosystem. Stable nitrogen and stable carbon isotopes are most commonly utilized to study inter-trophic dynamics and nutrient cycling in complex systems (Ryabenko, 2013). Stable nitrogen isotopes are found in two forms: ^{15}N and ^{14}N (Ryabenko, 2013), whereas stable carbon isotopes take the form of ^{12}C and ^{13}C (Farquhar, Ehleringer, & Hubick,1989). The forms differ in their neutron counts; the "heavier isotope" having the higher amount of neutrons of the two forms (Ryabenko, 2013). The heavier form of each isotope is significantly less abundant than the lighter form (Ryabenko, 2013). The exact environmental abundance varies with local biogeochemical cycling and can experience

wide temporal variation. However, species will assimilate both forms present in the environment in a specific and predictable manner. Isotope relative abundances allow for the delineation and comparison of trophic levels. Measure of relative abundance is expressed in comparison to a standard and are defined by the following equations for carbon and nitrogen (Ryabenko, 2013)

1a. $$\delta C^{13}(\text{vs. V} - \text{PDB}) = [(\frac{C^{13}}{C^{12}})_{sample} \div (\frac{C^{13}}{C^{12}})_{V-PDB} - 1]\times 1000$$

*****_where V-PDB represents the Vienna PeeDee Belemnite International Standard_

b. $$\delta N^{15}(vs.\,air) = [(\frac{N^{15}}{N^{14}})_{sample} \div (\frac{N^{15}}{N^{14}})_{air} - 1]\times 1000$$

Primary producers differ in their rates of assimilation of heavy and light N isotopes (Ryabenko, 2013). Nitrogen isotope abundance in primary producers is a reflection of both the relative occurrence of species-specific discriminatory molecular reactions and environmental $^{15}N/^{14}N$ ratio (i.e. the $^{15}N/^{14}N$ of nutrients the primary producers consume). The tissue of local primary producers will proportionally reflect environmental abundance of 15N /^{14}N (Cloern, 2002). The ratio of heavy to light isotope in tissues is referred to as the "relative abundance" (Ryabenko, 2013). Relative abundance can differ amongst individuals within the same trophic level (Cloern, 2002). However, the change in relative abundances of N isotopes from one trophic level to the next is large enough to delineate food webs in a given ecosystem (Ryabenko, 2013).

Fractionation of available inorganic N species within a given ecosystem depends on a variety of environmental parameters. Some of the determining factors in available N species isotope relative abundance baseline values include microbial species composition (and rate of metabolization), dissolved oxygen concentration, the availability of N in a system, the presence of N-assimilating biota, and the biogeochemical source of the N-species (Hoefs, 2009; Hein et al, 1995). Macroalgae more readily assimilate the heavier

form of nitrogen into their tissues, especially if their N source is ammonium (Altabet, 1988). High ambient ammonium concentrations correspond to a higher environmental relative abundance of ^{15}N (Cloern, 2002). Ammonium's enrichment, relative to other species of inorganic N, is attributed to significant fractionation during the nitrification process (NH_4^+ to NO_2) (Brandes & Devol, 1997). However, this enrichment does not occur in anoxic environments, which may be observed in the Hood Canal during late summer (Brandes & Devol, 1997). In N limited systems, fractionation is relatively zero between forms of DIN, as N-consumers do not exhibit an isotope preference during limitation (Hoefs, 2009). In anoxic systems, the $\delta^{15}N$ value for nitrate is triple the value of nitrate in oxic systems due to the dominance of denitrification (Hoefs, 2009). Field observations aiming to average isotope fractionation between anoxic and oxic marine environments yield an average value of 4.5‰ for NH_4^+, and a value -10‰ for NO_3^- (Hoefs, 2009). These values exemplify the tendency of ammonium to have a higher enrichment value compared to more oxidized nitrate species.

Micro and macroalgae differ in their capacity to assimilate ammonium. Ammonium assimilation occurs at a much faster rate than nitrate assimilation for both macro and microalgae (Hein et al, 1995). However, microalgae assimilate ammonium at a more rapid rate than do macroalgae (Hein et al, 1995). Additionally, microalgae simultaneously display a preference for less enriched ammonium species (Altabet,1988). Microscopic primary producers have a much higher turnover rate and therefore disproportionately utilize the lighter, more energetically favorable, isotope forms (Altabet,1988). Incorporation of heavy isotopes is positively correlated with the residence time of an organism in a given environment (referred to as incubation period) (Ryabenko, 2013). Incubation period is inversely related to turnover rate. Hence, this discrimination against isotopically enriched ammonium results in higher concentrations of ambient enriched

NH_4^+.When ammonium, in any form, is present in high concentrations, the enzyme which facilitates nitrate uptake is severely limited in microalage, resulting in disproportionate ammonium uptake (Hein et al, 1995). Hence, the fractionation values for ammonium in nutrient dense, productive systems far exceeds that of nitrate.

Carbon isotope signatures are the primary metric used to quantify the dietary contributions to consumers (whereas nitrogen isotopes, because of their high fractionation rates through trophic positions, delineate trophic level). Moving up trophic positions in an ecosystem will result in an isotopic enrichment proportional to trophic level for C and N isotopes. Both macro and microalgae synthesize carbohydrate structures from carbon dioxide through the photosynthetic process (Farquhar, Ehleringer, & Hubick,1989). The photosynthetic process selectively discriminates between the heavy and light isotope, preferring the light isotope (Farquhar, Ehleringer, & Hubick,1989). Green macroaglae is more enriched relative to microalgae (Dunton & Schell, 2003). In the Puget Sound *Ulva spp.* has a relative abundance of $\delta^{13}C$ -13.0 to -6.7‰ and phytoplankton is more depleted with $\delta^{13}C$ values ranging from -20.0 to -18.1 ‰ (Howe, Simenstad,& Ogsto, 2012). *V. philippinarum* incorporate both detrital macroalgae and phytoplankton into their diet. The relative abundance of C isotope contributed from each component will be proportionately reflected in the tissue of *V. philippinarum,* as represented by the following equation (barring trophic fractionation values for *V. philippinarum*) (Duedero et al, 2009):

2. $$\delta\,^{13}C_{\text{phytoplankton}} \times F_{\text{phytoplankton}} + \delta\,^{13}C_{\text{ulva}} \times F_{\text{ulva}} = \delta\,^{13}C_{\text{clam}}$$

where $\delta\,^{13}C$ represents the relative abundance of ^{13}C; and F represents the fraction of each dietary component

As elements move through a trophic system, each higher trophic level will metabolically incorporate the heavier isotope at a species-specific rate (Zanden & Rasmussen, 2001). This processes is referred to as fractionation and leaves the body

tissues relatively enriched with the heavier isotope (Suh & Shin, 2013). *V. philippinarum* fractionate nitrogen by an average amount of 2.9‰ (Duedero et al, 2009) and carbon by 0.6 ‰ (Suh & Shin, 2013). Often a standard trophic fractionation rate of 3.4 ‰ and 0.8 ‰ for nitrogen and carbon, respectively, can be applied to most trophic studies (Suh & Shin, 2013). Not all tissues in a consumer will assimilate isotopes equally (Kidd et al, 1995). Muscle tissues retain the average signatures of diet over longer periods of time (Kidd et al, 1995). In bivalves, the stomach gland tissue best reflects short term diet (Raikow & Hamilton, 2001). Hence, the stomach gland of *V. philippinarum* will most accurately reflect diet based on environmental composition of primary producers at a given point in time after applying the 0.6 ‰ fractionation rate (Suh & Shin 2014).

Nutrient exchange and quantification in integrated aquaculture systems

Integrated multitrophic aquaculture (IMTA) systems are a sustainable alternative to traditional monocultures. These systems can operate in temperate marine waters and incorporate commonly cultured regional species (Barrington et al., 2009). The most commonly utilized model comprises of fed finfish, filter feeding shellfish, and extractive macroalgae (Barrington et al., 2009). However, systems can be simplified from this model to a primary producer/primary consumer system based on fundamental principles of nutrient cycling. In this context, macroalgae acts as an extractive component for inorganic nutrients produced by shellfish (Barrington et al., 2009). Filter feeding bivalves incorporate detrital macroalgae into their diets in traditional monoculture settings (Ruesnick et al, 2014). Thus it is reasonable to assume that in an integrated aquaculture system, shellfish will similarly ingest a portion of ambient detrital cultivated macroalgae. IMTA systems help mediate negative aquaculture contributions to eutrophication by

confining nutrient cycling within the system. Despite extensive literature supporting the benefits of IMTA, only a 16% increase in the practice has been observed in temperate marine environments for 1999-2009 (Barrington et al., 2009).

Shellfish monocultures produce high concentrations of ammonium, which compound with environmentally sourced nutrients to exacerbate harmful local conditions. C:N ratios indicate the relative nitrogen assimilation of a primary producer in relation to a nitrogen source (Peterson & Heck, 1999). The carbon nitrogen ratio within the tissue of a primary producer is a direct reflection of available environmental nitrogen (Peterson & Heck, 1999). Thus, increased amounts of available ammonium accessible to *Ulva spp*. tissue will presumably result in a lower C:N ratio within that tissue. Assessing the C:N ratio of *Ulva spp*. near *V. philippinarum* would quantify the relative assimilation of bivalve-produced DIN for applications to a bioremediative IMTA setting.

Quantifying detrital contribution from primary producer to primary consumer is possible using stable isotope methodologies (Dunton & Schell, 2003). A case for the co-cultivation of seaweed and bivalves could result from the quantification of detrital material into bivalve diets. Evidence from Pacific Oysters (*Crassostrea gigas*) suggest that in the presence of abundant detrital material, dietary incorporation reaches a maximum of ~9% detrital material (Ruesnick et al, 2014). However, inter and intra species variability as well as regional environmental conditions may allow *V. philippinarum* in the Hood Canal to incorporate higher levels of detrital macroalge into their diet. Additionally, smaller *V. philippinarum* have an increased capacity for detritus uptake (Suh & Shin, 2013). If stable carbon isotope analysis illuminates *V. philippinarum* as a significant sink for detrital material, it would represent in piece of an

environmentally beneficial symbiosis in an aquaculture context.

Examining Case Studies and Identifying Existing Questions: a novel project proposal

Marine ecological literature warrants further quantitative investigation into the interaction between *V. philippinarum* and *Ulva spp*. in the Northern Hood Canal. The following section extensively examines case studies relevant to nutrient exchange between these species in attempts to diversify the literature applied to a commercially relevant scenario in the Northern Hood Canal. Additionally, this section will pose a novel research question surrounding this distinct set of species, and unique locale. This question will ultimately serve to address the quantitative component of a larger purpose: the possibility of supporting innovation within the shellfish industry.

The impact of shellfish monoculture on macroalgae proliferation

The ability for dense bivalve monocultures to positively impact macrophyte growth is well-documented in the literature. Peterson and Heck use a simple experiment to quantify the effect of ammonium, introduced by suspension feeding bivalves, specifically *Modiolis americanus,* on the growth of the seagrass *Thalassia testudinum*. The study controls for confounding factors by conducting the experiment within laboratory settings. This controlled environment allows for the researchers to determine exact species densities and proximity to one another. The results demonstrate two fundamental concepts. 1) Ambient ammonium concentration increases with increasing bivalve density; 2) C:N ratios in macrophyte leaf tissue are negatively correlated to shellfish density. In treatments where shellfish density is at 0, *Thalassia testudinum* tissue displays a C:N ratio of 16.31 ±0.38. Whereas treatments containing 500 and 1500 mussels display C:N

ratios of 14.85±0.67 and 13.37±0.38 respectively (Peterson & Heck, 1999). As C:N ratio in *Thalassia testudinum* tissue is inversely correlated with shellfish density, it suggests that the presence of bivalves increases nitrogen assimilation in primary producers, lowering C:N ratios.

More recent studies attempt to quantify the effect of pre-existing monocultures on macroalgae growth in the field. Similar to the Perterson and Heck study, a study undertaken by Zertuche-González et al in 2008, focuses on species of *Ulva* found in a sub-tropical bay near intensive shellfish monocultures. The study relies primarily on *Ulva* dry weights obtained from plots, and quantitative results from a C,H,N analysis. Researchers additionally monitored total dissolved inorganic nitrogen (TDIN) over the two year study. An increase in TDIN, specifically ammonium, resulted in greater %N in *Ulva* tissue. Increases in %N also corresponded to greater biomass. The study concluded that *Ulva* responds to environmental fluxes in TDIN and can act as a temporary nutrient sink.

A Puget Sound study, undertaken by Saurel et al, 2014 utilized a combination Farm Aquaculture Resource Management (FARM) model, combined with mid-year *Ulva* biomass measurements. Results from the simulations and field support the prediction that ammonium contributes significantly to macroalgal production. Each *V. philippinarum* individual produces 0.3 g of ammonium over a growing cycle of three years. In the first year of the study, the average difference between *Ulvoid* biomass growing on predator exclusion nets was 1 gdw (gram dry weight) m^{-3} higher for nets exposed to*V. Philippinarum* relative to controls that contained no V. philippinarum. The subsequent two years showed an increasing difference between nets associated with *V. philippinarum*

and nets unassociated with *V. philippinarum* (differences were ~2.5 and ~4.0 gdw m^{-3} respectively).This indicates accumulation of nutrients over the study period, despite the seasonality of *Ulvoid* blooms. The Saurel et al study, proposes the crucial consideration of utilizing the harvestable *Ulva* biomass to prevent nitrogen from reentering the system via tissue degradation.

The above studies serve to support further quantitative assessment of nutrient cycling on a *V. philippinarum* cultivation plot in the northern Hood Canal. Both the Peterson and Heck study and the Zertuche-González et al 2008 study quantify the amount of N assimilated into macrophyte tissue in the presence of shellfish. However, neither study uses *V. philippinarum* as a species of interest. Additionally, both of these studies focus on subtropic shellfish cultivation scenarios, leaving space to quantify interactions in temperate regions. The Saurel et al. (2014) study examines the temperate Puget Sound, and utilizes *V. philippinarum* as a species of interest. However, this study investigates biomass gains apart from nutrient composition within *Ulva* tissue. Though these studies reflect and support the conception that shellfish cultivation leads to increased nitrogen assimilation/biomass increase in primary producers, further specific investigation is warranted.

Extrapolating research from *V. phili*ppinarum cultivation sites in the North Puget Sound can help illuminate the biomass proliferation scenario taking place on aquaculture sites in the northern Hood Canal. The extent of N assimilation into *Ulva* tissue as a result of *V. philippinarum* cultivation remains undefined for this region. The following question poses to address this unknown:

Do V. philippinarium monocultures result in increased nitrogen assimilation, as measured by C:N ratios, in Ulva spp. tissue?

If successfully answered, the results from this investigation will better inform the management of seasonal *Ulvoid* blooms on aquaculture sites in the region, and have positive environmental implications.

Potential V. philippinarium dietary shift during seasonal macroalgae blooms

Shellfish are well-known to have variable diets, largely dependent on the nutrient sources available in their local environments. Research abroad of *V. philippinarum,* and local studies focusing primarily on the Pacific Oyster *C. gigas,* give insight to the environmental and physiological factors that dictate dietary preferences in cultivated bivalves. However, the literature alludes to circumstances which remain largely un-investigated. The capacity of *V. philippinarum* to alter their diet to consume detrital material during summer algal blooms in the northern Hood Canal is a circumstance which calls for further scientific exploration.

Case studies from outside the temperate Pacific Northwest provide evidence of *V. philippinarum*'s ability to incorporate detrital material as a dietary supplement. Suh and Shin 2013 quantify the intra-specific differences in dietary preferences of manila clam age classes in the Korean Yellow Sea. The study investigates the ability of size class to determine the dietary incorporation of detrital vs. microalgal material. Results indicate that larger clams more readily incorporate microalgae into their diets as opposed to detrital material. However, the incorporation of detrital material is variable based on seasonal availability, and in some cases comprises 20-30% of the larger clam diet. Smaller juvenile clams incorporate much higher proportions of detritus into their diets

(47.1 to 51.2%) (Suh & Shin 2013).

A separate study from the W. Pacific, specifically the Miya Estuary of Japan, investigates the potential of *V. philippinarum* to incorporate terrestrial material into their diets. The paper by Kasai, Horie, & Sakamoto 2004, concludes that marine POM (MPOM) was highly preferred over terrestrial POM (TPOM) because of the high lignin content of TPOM, and the high concentration of nitrogen in MPOM. However, during heavy rain events, when high concentrations of TPOM were present, the isotopic signatures of the clams shifted to more closely reflect the TPOM signature due to the availability of this material.

Many of the case studies involving delineating diet via isotopic signature in the Pacific Northwest focus primarily on *C. gigas*. In one study, conducted by Ruesink et al in 2013, researchers assessed growth rates in *C. gigas* as correlated with environmental parameters such as temperature, salinity, sediment type, dissolved oxygen concentration and resource availability, throughout the Puget Sound. This study takes into account the variability in primary producer composition, such as the abundance of microalgae, microphytobenthos, and macroalgae, over the study area. Organism growth was strongly positively correlated with temperature. However, no significant conclusions could be drawn in terms of broad dietary preference comparing within seasons. A relative isotopic depletion occurred in clam tissues in the winter when terrestrial inputs were presumably higher. Otherwise, the isotopic signature of the organisms similarly reflected that of the primary producers in the local environment, which was not a significant factor in determining growth. Hence, this comparative study over wide geographic range yielded results indicating a certain dietary specificity for each test region.

A more recent study conducted by Conway-Cranos et al in 2015 focuses on a wider range of the Puget Sound basin, including the Hood Canal. The study examines the primary dietary inputs to *C. gigas* for each specific locale. Isotope data indicate that macrophytes, including *Ulvoid* species, upland vegetation, sea marsh, and marine grasses, comprise a large portion (over 40%) of *C. gigas* diets in the Hood Canal. A stable isotope study, conducted by NOAA in 2011, reports the capacity of *C. gigas* to consume particulate effluent when present nearby, despite an indicated preference for microalgae (Rensel, Bright, and Seigrist, 2011). Overall, the literature from within the Puget Sound region suggests the tendency of *C. gigas* to uptake the POM characteristic to a given region.

The aforementioned studies indicate that bivalves have the capacity to shift their diets as a reflection of available food sources. Literature focusing on *V. philippinarum* diets in the W. Pacific gives insight into the environmental and physiological parameters affecting food preference. However, these studies do not take into account the high *Ulvoid* bloom density, which occurs in N. Hood Canal growing areas. Studies from the Puget Sound region often focus on quantifying the readiness of commercially significant C. *gigas* to incorporate available organic matter. Additionally, this research does not focus specifically on seasons, or subregions, most affected by dense *Ulvoid* blooms. Hence, there remains a need to assess the assimilation of available environmental components into *V. philippinarum* diets during the prolific summer macroalgae blooms. The following question poses to address this unknown:

Does V. philippinarium's microalgae-based diet shift to include proportions of detrital Ulva spp. tissue during seasonal macroalgae blooms?

Answering this question will inform the aquaculture industry of the seasonal nutrient

cycling dynamics which take place on *V. philippinarum* plots during the summer Ulvoid blooms.

Potential symbiosis between Ulva spp. and V. philippinarum

Based on the above conclusions, the possibility of symbiotic nutrient cycling between *V. philippinarum* and *Ulva spp.* seems highly likely. A vast body of literature exists, which addresses nutrient exchange in controlled integrated multitrophic aquaculture (IMTA) contexts. Integrated Multitrophic Aquaculture is the co-cultivation of two or more species in order to mimic the nutrient cycling scenarios present in natural ecosystems. One of the more comprehensive studies of IMTA, undertaken by Barrigngton et al in 2009, remarks on the effectiveness of co-cultivating shellfish and macroalgae. The role of bivalves and macroalgae in IMTA systems remain consistent with the species' ability to cycle nutrients in their native ecosystems.

Macroalgae acts as an inorganic nutrient extractive agent for the inorganic effluent produced by other species in the system (Barrington et al, 2009). In 2007, Buschmann conducted a study focusing on the Pacific Coast of S. America. In Chile, macroalgae is commercially farmed in conjunction with salmon, abalone, and mussels (Buschmann, 2007). The study attempts to optimize the depth and structure of macroalgae cultures to maximize inorganic N extraction to prevent the proliferation of eutrophying algal blooms. Studies of IMTA off the coast of South Africa report that using macroalgae with abalone aquaculture reduces inputs of nitrogen into surrounding ecosystems by an average of 4.4 tons per year, once harvested (Nobre, et al, 2010). The international success of macroalgae in reducing large quantities of inorganic off puts from shellfish aquaculture

farms is promising for areas of intensive shellfish monocultures, such as the Puget Sound.

Bivalves act to incorporate particulate organic matter as a result of effluent or tissue degradation within IMTA systems. A study by Bolton et al in 2008 assesses the strengths of co-cultivating *Ulva spp.* and abalone. As abalone readily consume particulate *Ulva spp.*, co-cultivating the species would reduce the required feed input into the abalone. The inorganic nitrogen produced by the abalone would, in turn, fertilize the *Ulva spp.* Additionally, the harvested *Ulva spp.* would be processed for meal on shore to be fed back to the abalone, supplying the majority of their dietary needs. The ability of particulate *Ulvoid*s to contribute to abalone diets may closely mimic the dietary intake of *V. philippinarum* during summer *Ulvoid* blooms on the Hood Canal.

Integrated Multitrophic aquaculture systems are not standard practice in Washington. However, strong local interest exists in researching IMTA systems. NOAA conducted a study in 2011 which placed Pacific Oysters (*C. gigas*) and Blue Mussels (*M edulitus*) near working finfish aquaculture pens in an open-exchange system in the Puget Sound. This system allowed for both effluent and phytoplankton-based POM to flow through to the shellfish. The study uses stable isotope signature from the pen effluent to quantify the uptake of the effluent in adjacent shellfish plots. The results indicate that growth near effluent increases, though shellfish species selectively feed on phytoplankton. These findings support the benefits of IMTA systems (Rensel, Bright, Seigrist, 2011). Currently there is strong interest in understating the benefits of shellfish-macroalgae open exchange systems. Research utilizing pre-existing species on potential sites in the Hood Canal and Puget Sound may give insight into the interaction of cultivated macroalgae and pre-existig bivalve infrastructure, barring the finfish

component often common to IMTA systems.

As a vast body of literature hails the positive environmental impacts of IMTA systems, Washington's willingness to participate in IMTA-related research could greatly benefit local aquaculture and ecosystems. IMTA has a strong foothold in S. Africa and the Pacific coast of South America. However, the ecosystem dynamics and commercially relevant species of these locales do not directly translate to Washington aquaculture systems. The above studies provide strong support for the integration of macroalgae and shellfish aquaculture based on quantitative principles of nutrient cycling. Studies in temperate regions, such as the Bay of Fundy (Barrington et al, 2009) and Puget Sound, demonstrate the ability for shellfish to act as an organic nutrient extractor from farm effluent. The missing piece is quantifying nutrient exchange in the field using commercially relevant *V. philippinarum* and native *Ulva spp.*. Fortunately, the dense summer *Ulvoid* blooms offer the perfect naturally occurring opportunity to study nutrient exchange between these species prior to investing in additional infrastructure.

CHAPTER 2: MANUSCRIPT

Introduction

The Hood Canal is a deep fjord comprising part of the main Puget Sound basin in Washington State. This canal provides vital habitat to a wealth of native and established marine species. Several molluscan species endemic to the Canal serve to support Washington State's flourishing seafood economy. The most profitable of these commercial species, the Pacific Oyster (*Crassostrea gigas)* and the Manila clam (*Venerupis philippinarum)*, were introduced into the region from the Western Pacific in

the early 20th century (Humphreys et al, 2015). Over time, these species established themselves into Washington's tidal flats, providing the foundation for the modern multi-million dollar shellfish industry. Today, Hood Canal growing regions lead the State in manila clam production (Booth, 2014). As the Canal is vital to the health and continued success of the shellfish industry, understanding the interactions of the local environmental fluxes and clam monocultures is of utmost importance.

Though the Hood Canal provides ideal physical habitat for the introduced *V. philippinarum*, biogeochemical cycling in the Canal can result in undesirable seasonal growing conditions within the region. Evidence suggests that the seasonal blooms of macroalgae, including *Ulva spp.*, negatively impact marine life, including certain species of cultivated shellfish (Newton et al, 2007). *Ulvoid* bloom intensity has increased in recent years for aquaculture sites on the Northern Hood Canal, presumably as a result of increased anthropogenic nutrient loading. When large blooms of primary producer degrade, dissolved oxygen is rapidly depleted. For the Hood Canal, eutrophication is especially severe, as the exchange of water from the Canal basin into the Strait of Juan de Fuca is limited by geographic barriers (United States Geological Survey [USGS]).

Negative impacts from the deterioration of large macroalgae blooms on *V. philippinarum* growth remain unquantified for the northern Hood Canal growing regions. Existing evidence suggests, that when present in high densities, *Ulvoids* decrease the overall growth of Pacific Oysters (Lamb, 2015). However, there is some question as to whether the *Ulvoid* densities of 0, 1.5, and 3.0 kg in the *C. gigas* experiment accurately reflected true densities present on the bags (Lamb, 2015). Additionally, the mechanism inhibiting successful growth in this species remains unknown. Discussions among

growers hypothesize that *Ulva spp*. draws down vital DO within the bags, or that the *Ulvoids* reduce the flow of more labile food sources through the bags. Uncertainty exists as to whether the dense blooms have a similar impact on commercially significant cultivated species, such as *V. philippinarum.* Field and industry observations suggest that mortality in *V. philippinarum* populations does not increase during the summer harmful bloom events (Joth Davis, personal communication, 2015). *Ulva* may provide a food source to shellfish grown for aquaculture purposes, negating some of the negative impacts described previously. Hence, a more complex, and possibly synergistic, mechanism may underlie the seasonal coexistence of these two species.

It is unknown as to what degree the *Ulva spp*. blooms, which cover the *V. philippinarum* cultivation site, provide an alternate food source to the local clam population. Growers speculate that dense blooms inhibit to the flow of phytoplankton-based seston to the clams. *V. philippinarum* depend heavily on phytoplankton-based seston as a primary food source (Conway-Cranos et al, 2015; Suh & Shin, 2014; Resnsel, Bright, Seigrist, 2011; Kasai, Horie, & Sakamoto, 2004). However, *Ulva spp*. degrades rapidly (Zertuche-González et al, 2008). Hence, the detrital material could be supplementing *V. philippinarum* diets. The negative impacts of *Ulva spp.* on *C. gigas* growth may not directly translate to *V. philippinarum* due to the intraspecies differences in feeding behavior and/or cultivation method. Hence, the effect of *Ulva spp.* on *V philippinarum* may represent a relationship that exceeds the initial negative stigma.

In addition, recent evidence suggests that macroalgae *Ulva spp*. benefits from cultivated shellfish (Saurel et al, 2014; Zertuche-Gonzalaz et al, 2008). Monocultures contribute to the annual increase in prolific blooms in monoculture-supporting bays

(Saurel et al, 2008; Zertuche-Gonzalaz et al, 2008). The capacity for shellfish cultivation to significantly increase the nutrient assimilation and proliferation of primary producers is true for the Puget Sound (Saurel et al, 2014). However, within the Canal, specifically the Northern Hood Canal, the transfer of inorganic nutrients from *V. philippinarum* monocultures to *Ulva spp*. remains unexamined. Question remains as to whether shellfish monocultures exacerbate local blooms, further contributing to regional poor water quality conditions post degradation. If *Ulva spp*. proves to act as a significant temporary sink for inorganic nutrients released by shellfish cultivation, industry could effectively manage the blooms so as to reduce negative environmental impacts. Management (ie. removal) could additionally decrease the proliferation of *Ulva spp*. in subsequent years by removing excess nutrients from the poorly flushed system.

Ecological and economic and value exists in investigating the relationship between seasonal macroalgae blooms and *V. philippinarum* monocultures. The question which most adeptly addresses the concerns of ecologists and industry follows:

Are commercially farmed manila clams (Venerupis philippinarium) and seasonally occurring green macroalgae (Ulva spp.) involved in symbiotic nutrient cycling in the context of bag aquaculture on the Northern Hood Canal?

Do V. philippinarium monocultures result in increased nitrogen assimilation, as measured by decreased C:N ratios, in Ulva spp. tissue?

Does V. philippinarium's microalgae-based diet shift to include higher proportions of detrital Ulva spp. tissue during seasonal macroalgae blooms?

To answer this question, an experiment will take place allowing for the quantification of nitrogen assimilation in primary producer tissue via elemental ratios. Stable isotope analysis will allow for the quantification of *Ulvoid* contribution to *V. philippinarum* diets. Tissue will be examined from a treatment, which mimics the status

quo summer conditions, as well as control treatments, which isolate *V. philippinarum* and *Ulva spp*. tissue from one another.

Quantifying the extent of nutrient cycling between the two species will illuminate the role of *V. philippinarium* cultivation in enhancing seasonal harmful macroalgae blooms. Additionally, the contribution of *Ulva spp*. to summer *V. philippinarium* diets, and the subsequent effect on growth rate will be illuminated. Findings could help promote sustainable management practices, and encourage the diversification of industry towards the co-cultivation of shellfish and macroalgae. Ideally, a more thorough understanding of this multitrophic interaction will result in a net positive ecosystem benefit surrounding *V. philippinarium* cultivation.

Materials and Methods

Study Location and Background

This study took place on the Northern Hood Canal. As a whole, the Hood Canal is a deep estuarial fjord comprising part of the main Puget Sound basin (Warner & Kawase, 2001). It is separated from external water bodies via the Admiralty Sill, which restrict water from circulating out of the Canal in the north (Warner & Kawase, 2001). Thorndyke Bay, which comprises the location of this study, is an estuarial bay located in the Northern Hood Canal, at the Admiralty Sill. The Thorndyke Bay ecosystem represents a rare, pristine mid-sized creek estuary, identified as a "priority conservation area." (Harrington, 2005). The beach provides excellent habitat for wintering waterfowl (WDFW, 2004 in Harrington, 2005) and shellfish. Due to the lack of development or alteration in and around the Thorndyke Creek estuary, the area functions as high value

native species habitat (Harrington, 2005).

Figure 3. Location of the study site, Thorndyke Bay, situated in the Northern Hood Canal. All field measurements were collected at this site.

The Thorndyke Bay estuary is composed primarily of sandy glacial sediment, deposited via the erosion of exposed bluffs in the north (Harrington, 2005). The absence of shoreline development in the area allows for continued natural deposition of sediments to the beaches. Vegetation along the shoreline is comprised primarily of salt marsh commonly associated with small stream mouths. The stream running into the area,

Thorndyke Creek, supports a 32 acre marsh above the high tide line (Harrington, 2005). This marsh is classified as a low, silty marsh, and forms at the mouth of a mid-sized creek. Thordyke Bay itself is a shallow body of water with relatively weak tides (Cannon, 2005). As the bay is shallow, the low tide line is relatively far from shore.

The Baywater Shellfish Company operates in the intertidal zone just north of Thorndyke Creek. Baywater Inc harvests four types of shellfish for local commercial distribution, including the manila clam, *Venerupis philippinarum.* Every year, from May through August, green macroalgae blooms of *Ulva spp*. completely cover *V. philippinarum* plots (Joth Davis, personal communication, 2015). Hence, this site provides the essential components for studying the extent of nutrient cycling between *Ulva spp*. and *V. philippinarum.* To quantify nutrient cycling at peak *Ulva spp*. density and maximum *V. philippinarum* metabolic rate, data collection took place from June 2015 through September, 2015. The site's rare position amidst and unfettered estuarial ecosystem, and unique susceptibility to intensive *Ulvoid* blooms, designated it as the ideal site for this research project.

Duration of study

This study began on June 27th, 2015 and ended on September 5th 2015. This time frame was chosen in attempts to capture the majority of the *Ulvoid* growing season. All data was collected on an outgoing tide of < 0.0 ft.

Field Design

All samples were collected from a working *V. philippinarum* aquaculture plot. In this plot, *V. philippinarum* were segregated into in-ground aquaculture bags containing

approximately 100-300 live individuals each. The bags were laid in several dozen rows perpendicular to the shoreline, each row containing individuals of a similar age. All bags were industry standard of 1/2'' hard plastic mesh, with the dimensions of 18" x 32" x 4."

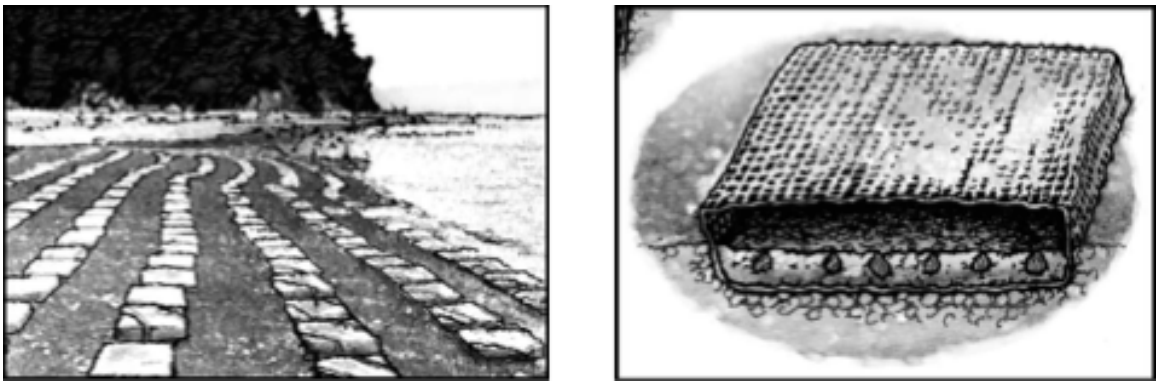

Figure 4. The image on the left portrays the standard layout of in-ground aquaculture bags along the intertidal zone. Bags are distributed in a uniform grid pattern. Rows of similarly aged clams run perpendicular to the waterline. The Right-hand image is a cross section of an in-ground aquaculture bag. Clams remain submerged in the sediment, while the top of the bag remains exposed. Toba, Dewey, & King, 2005.

One row containing 15 total bags, running perpendicular to the waterline (~20 m from the high tide line) was used for this experiment. The 15 bags were segregated into three separate treatments, 5 replicates of each, to assess the extent of nutrient cycling between the bag-cultivated clams and seasonally occurring green macroalgae. Replicates were randomized. The first treatment acted as a control and contained exclusively *V. philippinarum,* in order to assess the dietary intake of *V. philippinarum* in the absence of a dense *Ulva spp*. covering. The second treatment, also a control, contained no clams within the bag, but maintained a dense *Ulva spp*. covering on top of the bag. The purpose of this treatment was to assess the extent of nitrogen uptake in *Ulva spp*. in the direct absence of *V. philippinarum.* The third treatment mimicked the status quo during the

summer growing season, containing both *V. philippinarum* and *Ulva spp*.. The goal of this treatment was to assess dietary composition in *V. philippinarum* individuals directly contacting *Ulva spp*.. Additionally, this treatment addressed nitrogen uptake in *Ulva spp*. directly in contact with *V. philippinarum.* The three treatments contained within the experimental row are summarized below:

Control 1: Exclusively V. philippinarum (no Ulva spp.)

Control 2: Exclusively *Ulva spp*. (no *V. philippinarum*)

Treatment: *V. philippinarum* and *Ulva spp*

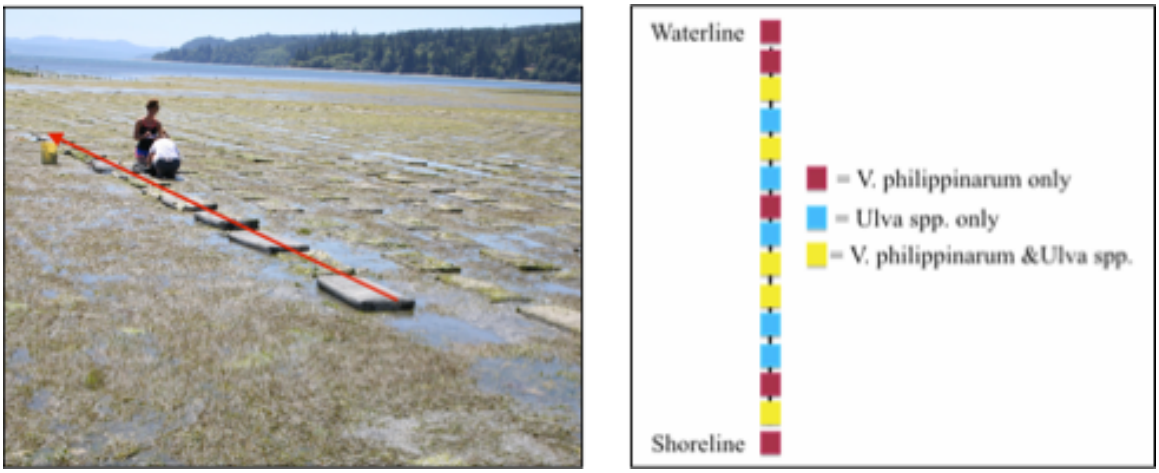

Figure 5. The lefthand picture is the experimental row in the field. The righthand image is a digram of the three treatments as they were randomly distributed throughout the experimental row.

Treatments containing *V. philippinarum,* were standardized to include 150 clams, two years of age, for each bag prior to the experiment. The quantity 150 was chosen to reflect the average number of live clams per bag as observed in the surrounding aquaculture plot. It was essential to ensure that all clams be of similar size and age, as ammonium excretion (Mann & Glom, 1978) and preference for detritus (Suh and Shin, 2014) are size/age-dependent factors. In choosing clams all of the same age and broodstock, it was assumed that size be consistent amongst individuals.

Initially, each bag within the experimental row was covered in thick attachments of *Ulva spp*.. These attachments had formed in May, and as such, were well established by the time the experiment began. The *Ulva spp*. only control bags, as well as the combination treatment bags, were left with the pre-existing attachments. These attachments were left in tact to most accurately mimic the status-quo conditions on the bags during summer bloom events. No additional *Ulva spp*. was introduced into the treatments. This experiment relied exclusively on the regeneration of *Ulva spp*. attachments on the in-ground bags to capture the fluctuations in natural density throughout the growing season. *Ulva spp*. also existed in a free-floating form. However, this unattached *Ulva spp.* is randomly deposited and removed via daily tidal action. Hence, free-floating *Ulva spp*. was not considered as a variable in this experiment. Pre-existing attachments on *V. philippinarum* only treatments were severed at the onset of the experiment, and scraped weekly to prevent further growth and potential reduction in treatment effect.

Sample Collection and Processing

Data Collection in the Field

V. philippinarum:

To detect any potential treatment effect unaccounted for by quantitative nutrient analysis, clam growth was measured in each of the 10 total clam bags every other week from June 27th 2015 to August 15th, 2015. Bags were dumped into large plastic sorting trays and the height and width of every tenth clam measured with calipers. Any mortalities in the bags were recorded, and the dead individuals were removed from the

total population. Weekly, 3 randomly chosen individuals were collected from each bag and put on ice for transportation to the laboratory for isotopic content and elemental ratio analyses preparation.

Ulvoid abundance fluctuated in response to ambient conditions, which was unanticipated. Hence, qualitative designations were utilized to capture these changes in *Ulvoid* abundance for July 31, 2015 through September 5, 2015. In order to estimate the amount of *Ulva spp.* attached to the *Ulva spp.* control and combination treatment bags, qualitative proxy measurements were taken twice during the experiment. To not disturb the tissue on the experimental row, proxy high, med, and low samples were collected from working *V. philippinarum* bags in the surrounding cultivation plot to obtain dry weight measurements. All ten *Ulva spp.*-containing bags in the experimental row were visually assessed and designated high, med, and low in relation to their density of *Ulva spp.* attachments. A general guideline designated an *Ulvoid* covering from 0-33% of the surface of the exposed bag as low, 33-66% as medium, and 66-100% as high. Depending on daily conditions, bags ranged from three-quarters of the bags covered in "low" density *Ulva spp.* to four fifths of the bags covered in "high" densities of *Ulva* spp. These samples were transported in ziplock bags on ice to the laboratory to obtain dry weight.

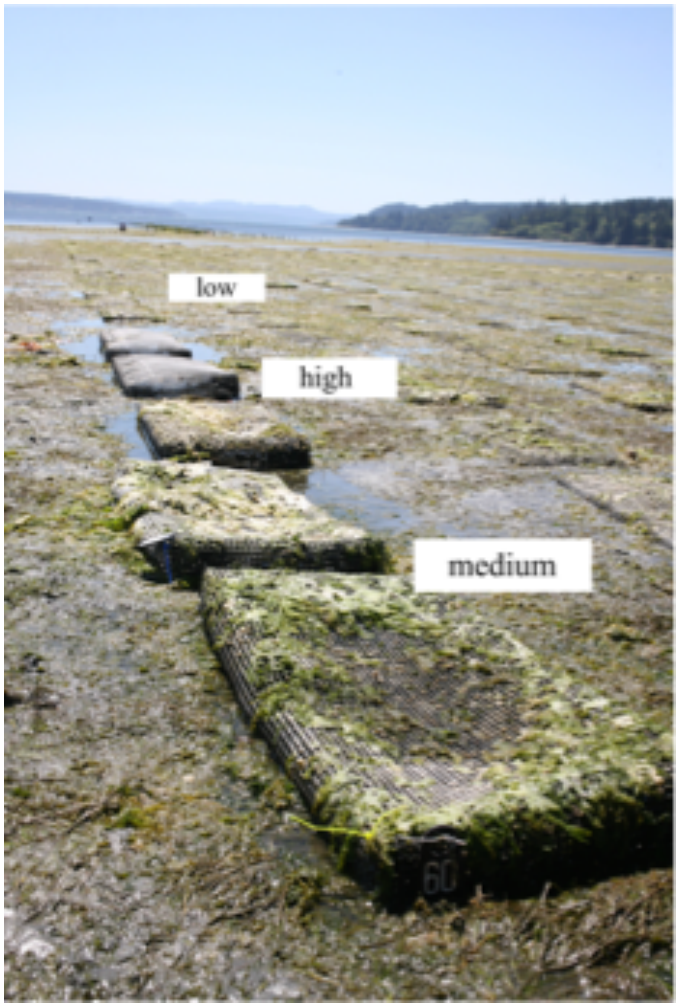

Figure 6. Examples of bags receiving a low, medium, and high designation. This photo was taken during the mid-July *Ulvoid* die-off. Hence, bags in this picture represent the lower end of *Ulva spp.* coverage.

Weekly, approximately 5 g of *Ulva spp* tissue was collected from each of the ten bags in the experimental row containing *Ulva spp.,* and transported on ice in small ziplock bags to the laboratory to be prepared for isotope and C/N ratio analysis.

Phytoplnkton-based Seston

In order to assess the relative contribution of phytoplankton-based seston to *V. philippinarium* diets in the various treatments, phytoplankton was collected weekly starting July 13, 2015 and running through the end of the experiment. Samples were

collected on an incoming tide using a phytoplankton net into an acid washed (HCl) amber bottle. All excess seston on phytoplankton net was rinsed into bottle with DI water in the field. Bottles were then transported indirectly on ice to the laboratory for isotope analysis preparation.

Laboratory Sample Preparation

V. philippinarum:

In the laboratory, samples from each of the ten bags were prepared for isotopic analyses of $\delta^{13}C$ and ^{15}N following the procedures of Levin and Currin (2012). To do so, clams were separated into 24 oz plastic containers and covered with ~20 oz of seawater collected on site. The clams were then allowed to purge their stomach contents for 24 hours to prevent any potential cross-contamination into the stomach gland during dissection. Dissection tools and surface were sterilized with 70% ethanol prior to the dissection of each individual. Abductor mussels on the clam were cut using dissecting scissors and the clam was internally rinsed with DI water. The stomach gland was removed, rinsed with DI water, and stored in a small plastic ziplock. The stomach gland was chosen as it most accurately reflects short-term dietary preferences in marine invertebrates (Levin and Currin, 2012). Clam stomach glands from individuals in the same bag were combined to form a composite sample to reflect the average diets of the clams within each individual aquaculture bag. Stomach glands were then frozen at -20 °C for 5-8 months prior to analysis preparation.

Three composite samples, belonging to each of the treatment categories, from each week of data collection were removed from the freezer in February 2016 and allowed to

thaw. Samples were then placed in combusted petri dishes and dried at 60 °C for 24 hrs (Levin and Currin, 2012). Samples were then placed into combusted ceramic mortar and pestle and pulverized to a fine powder prior to preparation for isotopic analysis, described below.

Ulva spp.:

Once in the laboratory, the 5 g samples were squeezed to remove DI water and frozen at -20 °C (Levin and Currin, 2012) for 5-8 months prior to analysis preparation.

Samples were removed from the freezer in February 2016 and allowed to thaw. Samples were then placed in combusted 60 x15 mm petri dishes via sterilized forceps and dried at 60 °C for 24 hrs (Levin and Currin, 2012). Samples were then placed into combusted ceramic mortar and pestles and pulverized to a fine powder prior to encapsulation.

The three qualitative *Ulva* samples were rinsed 3x with DI water. Qualitative samples were spun in separate batches according to classification in a plastic salad spinner for 10 seconds to remove excess water. The samples were then transferred to foil to be dried in the oven at 60°C for 24 hrs. Dry weights were and recorded.

Phytoplankton-based Seston:

Once in the laboratory, samples were filtered through a combusted glass vacuum filtration apparatus using a GF/F filter. Filters were dried in the oven at 60 °C for 24 hrs (Levin and Currin, 2012). Dried filters were wrapped in combusted foil and stored in a cool, dry place for 5-8 months prior to encapsulation.

Stable Isotope Analysis

Pulverized composite samples of both *Ulva spp*. and *V. philippinarium* were later transferred into pre-combusted 5x8 mm tin capsules for isotope analyses. Approximately 2.0 mg of Ulva *spp*. sample, and 1.25 mg of *V. philippinarium* sample were weighed into separate capsules using the Perkin Elmer AD 6000 Ultra Microbalance, folded and shipped to the UC Davis Stable Isotope Facility for C/N ratio determination and $\delta^{13}C$ and ^{15}N analysis via a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (stableisotopefacility.ucdavis.edu, 2/22/2016).

In February 2016, filters were removed from foil and prepared on a surface sterilized with 70% ethanol. A sterilized 5 mm diameter cork borer was used to remove 8 small sections of the filter. Measurements were made of the diameter of seston on the full filter in order to calculate the proportion of material removed and sent for analysis. The 8 sections were placed in a pre-combusted 9x10 mm tin capsule. The capsule was then folded into a cube <8 mm and placed in a 48-well tray. The tray was sent for C/N ratio determination and ^{13}C and ^{15}N analysis at UC Davis as described previously.

Statistical Analysis

Statistical analysis took place in JMP pro version 12. To visualize growth trends, *V. philippinarium* measurement values were averaged from every replicate, for each collection date. Growth rate was calculated for each replicate by taking the difference between the final and initial measurements and dividing by the time elapsed. For growth rates, variance homogeneity was tested using Levene's test, normality was assessed using

Shapiro-Wilke's test. Data was normally distributed and displayed the similar variance. Treatment effect on *V. philippinarium* growth was analyzed using one-way ANOVA, followed by a Tukey's HSD test. Carbon and nitrogen ratio data was transformed from micrograms to moles, which were used to find the atomic ratio. Data was normally distributed and displayed equal variance. Hence, means between treatments were compared using one way ANOVA. Analysis via Tukey's HSD test verified significant differences between treatment means.

Isotope relative abundance values for phytoplankton and *Ulva spp*. were compared using a t-test and assessed for normality via a Shapiro-Wilke's test. The two groups were shown to be normally distributed. Carbon isotope relative. abundance values for the *V. philippinarium* stomach glands were adjusted to reflect a fractionation of +0.6‰. This fractionation value reflected that used in Suh & Shin 2013 dietary isotope analysis of *V. philippinarium.* Nitrogen relative abundance values were adjusted to reflect a +2.9‰ fractionation rate. Values for both isotopes were normally distributed via Shapiro-Wilke's test.

Results

V. philippinarum growth

Mean length measurements of *V. philippinarum,* taken at intervals throughout the summer, are displayed in Tables 1 and 2. Initial average measurements ranged from 3.76-4.12 cm for individuals in *V. philippinarum* only (control) bags. For individuals in *V. philippinarum* and *Ulva spp.* (treatment) bags, initial average values ranged from 3.85-4.10 cm. Final measurements for individuals in control bags ranged from 4.12-4.27 cm, and for individuals in treatment bags from 4.18-4.29.

Replicate/Date	6/27/15	7/18/15	8/1/15	8/15/15
Control 1	4.12 ± 0.13	4.01±0.09	3.92±0.09	4.24±0.08
Control 2	4.12±0.08	4.15±0.06	4.11±0.05	4.27±0.05
Control 3	4.11±0.06	4.11±0.06	4.14+/0.09	4.27±0.06
Control 4	4.11±0.06	4.11±0.09	4.30±0.08	4.27±0.02
Control 5	3.76±0.06	3.82±0.09	3.97±0.11	4.12±0.06
Mean	**4.04±0.07**	**4.04±0.06**	**4.09±0.07**	**4.26±0.05**
Treatment 1	3.92±0.07	4.00±0.03	4.00±0.09	4.18±0.06
Treatment 2	3.92±0.07	3.96±0.03	3.90±0.08	4.24±0.06
Treatment 3	3.85±0.09	3.91±0.03	4.05±0.08	4.24±0.09
Treatment 4	3.87±0.06	3.96±0.02	3.96±0.07	4.27±0.05
Treatment 5	4.10±0.07	4.02±0.02	4.00±0.07	4.29±0.05
Mean	3.91±0.04	4.00±0.02	3.98±0.02	4.24±0.01

Table 1. Average length measurements in cm coupled with standard error for each of the five *V. philippinarum* only control and treatment replicates. Measurements were taken from late June- Mid August.

Control values show a universal increase in average length over the measurement period. Wide variation exists between the means of the initial measurements (0.36 cm), as well as the means of the final measurements (0.14 cm). Additionally, negative growth trends between initial and final measurements were recorded. This may be due to small sample size and natural wide variability in shell lengths as the same individuals were not repeatedly sampled.

All length values show an increase over the measurement period. Lesser variation (0.18 cm) exists in the initial measurements of the treatment bags, as compared to that of the control bags. Means of the final measurements also exhibit a lesser difference from initial values than those of the control replicates (0.11 cm). Additionally, negative growth trends between initial and final measurements were recorded. This may be due to small sample size, as clams were only measured four times throughout the experiment, and natural wide variability in shell lengths. To account for measurement inconsistencies throughout the measurement period, only final and initial values were taken into

consideration for the growth rate (Fig. 7)

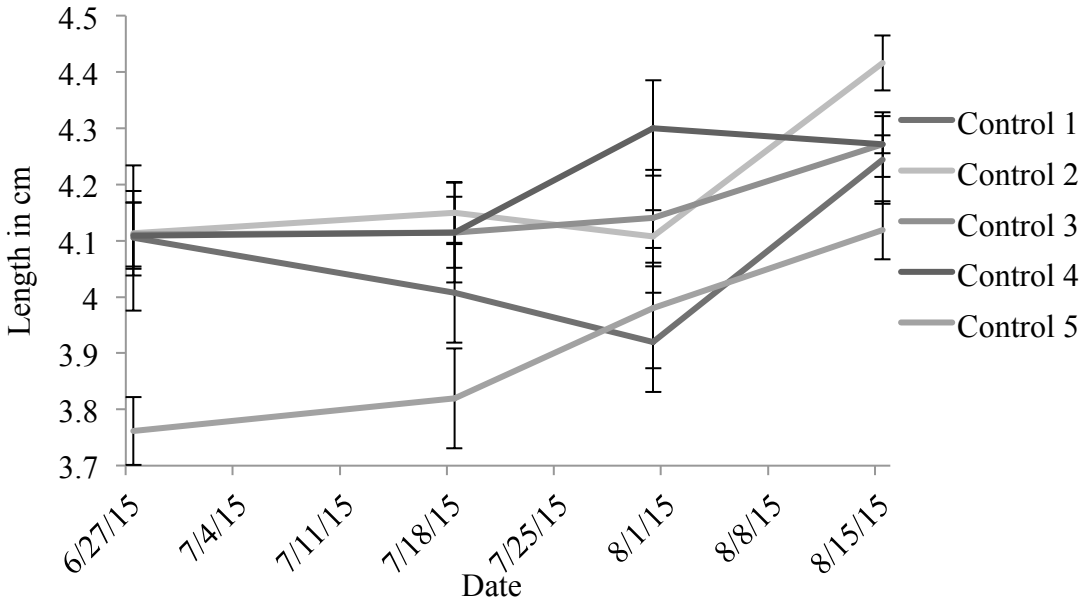

Figure 7. Length in centimeters of *V. philippinarum* control group over time. Each line represents a different replicate within the control group.

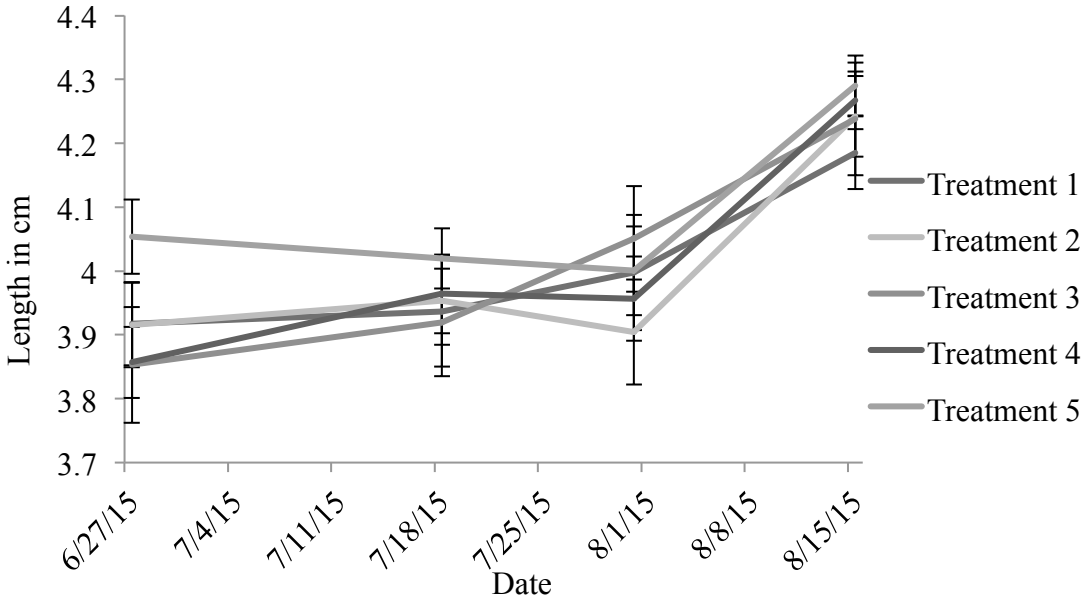

Figure 8. Length in centimeters of *V. philippinarum* grown with ulva spp. (treatment group) over time. Each line represents a different replicate within the treatment group.

	% Growth length		% Growth length
Treatment 1	6.8	**Control 1**	3.4
Treatment 2	8.3	**Control 2**	7.3
Treatment 3	10.0	**Control 3**	3.9
Treatment 4	10.6	**Control 4**	6.3
Treatment 5	5.8	**Control 5**	9.5
Mean	**8.3±1.0**	*Mean*	**6.1±2.7**

Table 2. Mean length growth rates for each of the treatment and control replicates between June 27th and August 15th.

In this case, the experimental treatment has a is 2.2% higher growth rate than the *V. philippinarum* control group. This difference, however, is not significant (t=1.53, df=4, p=0.16).

Mean height measurements of *V. philippinarum,* taken at intervals throughout the summer, are displayed in Table 3. Initial average measurements ranged from 1.83-2.11 cm for individuals in *V. philippinarum* only (control) bags. For individuals in *V. philippinarum* and *Ulva spp.* (treatment) bags, initial average values ranged from 1.90 to 2.02 cm. Final measurements for individuals in control bags ranged from 1.98 to 2.02 cm, and for individuals in treatment bags from 1.92 to 2.09 cm.

Replicate/Date	6/27/15	7/18/15	7/31/15	8/15/15
Control 1	1.96±0.05	1.96±0.04	1.99±0.04	2.10±0.03
Control 2	2.03±0.05	2.14±0.05	2.02±0.03	1.98±0.04
Control 3	2.11±0.03	2.05±0.04	1.93±0.03	2.01±0.01
Control 4	2.05±0.01	2.02±0.01	1.96±0.01	2.02±0.01
Control 5	1.83±0.03	1.97±0.05	1.97±0.05	1.76±0.04
Mean	**2.00±0.05**	**2.01±0.04**	**1.98±0.02**	**1.98±0.06**
Treatment 1	1.98±0.04	1.98±0.04	2.04±0.04	1.92±0.05
Treatment 2	1.97±0.05	1.88±0.03	1.96±0.04	2.09±0.04
Treatment 3	1.96±0.05	1.92±0.04	1.85±0.06	1.95±0.05
Treatment 4	1.90±0.03	1.91±0.03	1.94±0.03	1.86±0.03
Treatment 5	2.02±0.03	2.02±0.04	1.93±0.06	1.88±0.03
Mean	**1.96±0.02**	**1.94±0.03**	**1.95±0.03**	**1.94±0.04**

Table 3. Average height measurements in cm coupled with standard error for each of the five *V. philippinarum* only control and treatment replicates. Measurements were taken from late June- Mid August.

The measurements for the control group indicate that only one of the five replicates showed positive growth. Of the remainder of the control group, one showed no growth and three showed negative growth. Of the replicates displaying no, or negative, growth between initial and final values, intermediate values indicate evidence of positive growth rates in two. Additionally, initial value measurements vary widely (0.38 cm). Final measurement values range by 0.24 cm. The lowest observed mean of 1.76 cm was taken on the final measurement day. Whereas the highest mean value was recorded on the second day of measuring.

The treatment group mirrored the control in that one bag had an increase in growth, one did not change, and three decreased from initial to final measurement. Again, two of the four replicates, which either did not grow, or remained the same between initial and final measurements, showed an increased mean size in intermediate measurements. Additionally, the mean initial measurement valued varied by 0.12 cm. The final values varied more widely (0.21 cm). Both the lowest and highest means were recorded on the

final measurement date. Measured shell heights between treatments and over time displayed stochastic tendencies.

Using the means displayed in the above tables, % growth over time was calculated for each replicate between the treatment and control groups. The results are displayed in the Table 6:

	% Growth height		% Growth height
Treatment	-2.7	**Control**	7.0
Treatment	6.0	**Control**	-2.4
Treatment	0.0	**Control**	-4.6
Treatment	-1.6	**Control**	-1.3
Treatment	-7.3	**Control**	-3.9
Mean	**-1.1+/1.9**	*Mean*	**-1.1±2.1**

Table 4. Mean growth rates for each of the treatment and control replicates between June 27th and August 15th. The means of all replicates in each treatment and control were taken for both length and height.

The values demonstrate the difference between the mean of % growth between the treatment and control groups using the parameter of height. The mean % growth for height between both groups is equal at -1.1%. Taking into account the standard error of ±2.0 %, it is unlikely the shell heights actually experienced negative growth.

Figure 9 demonstrates the difference between treatments on using the parameters of length and height.

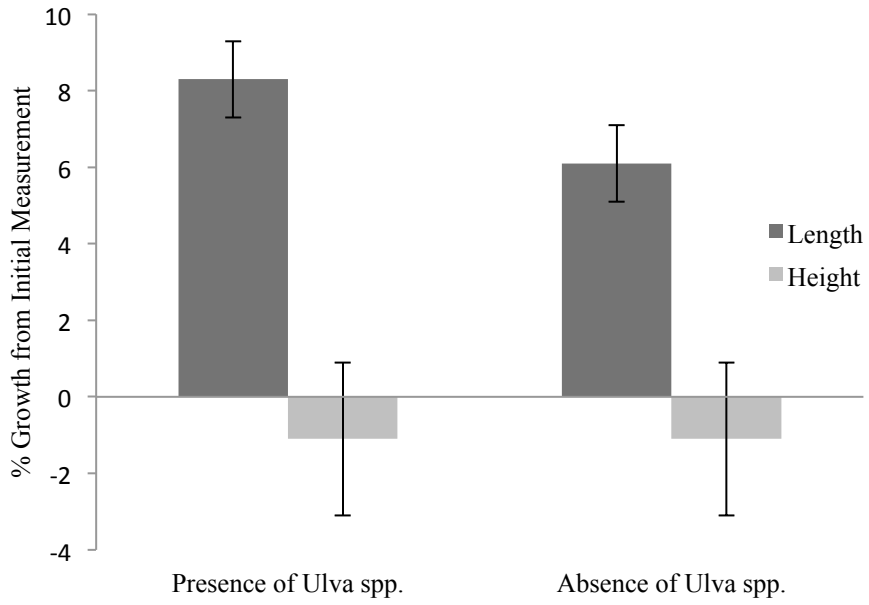

Figure 9. The mean % growth values for height and length between *V. philippinarium* treatment and control groups. Means do not appear to be significantly different in either case.

The above figure demonstrates the relative equality of the % growth means between the control and treatment groups for each parameter.

Statistical analysis of the mean percent growth for shell length between control and treatment groups does not demonstrate a significant difference between groups ($t=1.53$, $df=4$, $p=0.16$). Additionally, analysis shows there is no significant difference between mean% height growth between control and treatment groups ($t=0.33$, $df=4$, $p=0.98$). This analysis concludes that the presence of *Ulva spp*. does not have a significant effect on the growth of *V. philippinarum* for this experiment.

Ulva spp. Abundance

Ulva spp. abundance varied between sampling dates. As a large storm took place 8/20/2015 which deposited deeper water *Ulvoid*s onto the entire cultivation plot,

abundances were only recorded for 7/31/15 and 9/5/15.

Qualitative estimates, as achieved by drying and weighing proxy *Ulvoid* masses, indicate 7/31/15 had an overall lower *Ulvoid* density covering (2.4-8.1 grams dry weight [gdw]). The second collection date, 9/5/15 had slightly more dense *Ulvoid* attachments (3.4-10.2 gdw). For both dates, all high designations were concentrated in the treatment groups, whereas low and medium designations were present throughout. Figure 10 shows the differences in frequency distribution for high, medium, and low values for the both collection dates combined

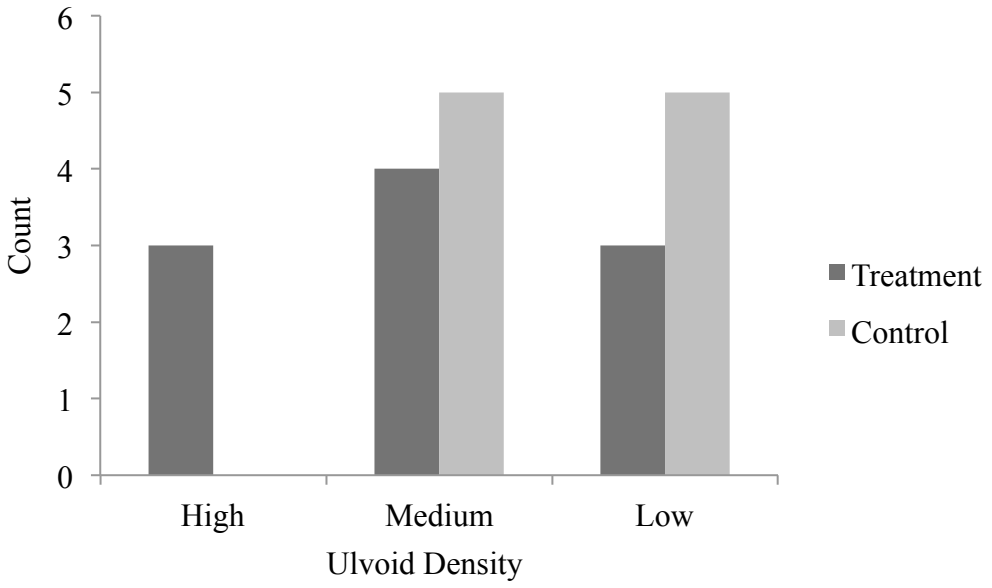

Figure 10. Frequency distribution of high, medium, and low *Ulvoid* density designations between control and treatment groups. N=10.

From figure 10, it is clear that the treatment group displays a greater amount of high *Ulvoid* density counts, and relatively reduced medium and low *Ulvoid* density counts.

Substituting the proxy dry weight values for the high, medium, and low designations, yields no significant difference for the means of the control (ulva only) (5.0 ±0.8 gdw) and treatment (5.4 ± 0.8 gdw) groups for the 7/31/15 date (t=0.17, df=9,

p=0.56). The control (3.4 ±1.0 gdw) and treatment (7.3 ±0.9 gdw) means are additionally not significantly different for the 9/5/15 date (t=1.47, df=9, p=0.18).

Despite the lack of significance, it is clear that a pattern exists between control and treatment bags. Bags containing *V. philippinaurium* have an observably higher *Ulvoid* density than bags not containing *V. philippinaurium.* A larger sampling size, and longer sampling period is necessary to determine if there is a true statistical difference between the treatments.

Carbon to Nitrogen Ratios

Carbon to nitrogen ratios for *Ulva* clustered around lower values for the treatment group containing *V. philippinarum* and *Ulva spp.*(X=8.6±0.41)*,* and higher values for the control group containing *Ulva spp*. exclusively (X=12.5±0.67). Values for the treatment group ranged from 6.6 to 11.6, whereas values for the control group ranged from 9.6 to 16.3. The highest value for the control group was collected on September 5th (16.3), the lowest on July 19th (9.6). For the treatment group, the highest value was collected August 15th (11.6), the lowest on July 13th (6.6).

A majority of treatment and control C/N ratios showed significant differences when partitioned by collection date. Only two of the three dates, 7/19/15 (p=0.84) and 7/31/15 (p=0.07) did not display a significant difference between the treatment groups. The following figure displays the differences between mean values by date.

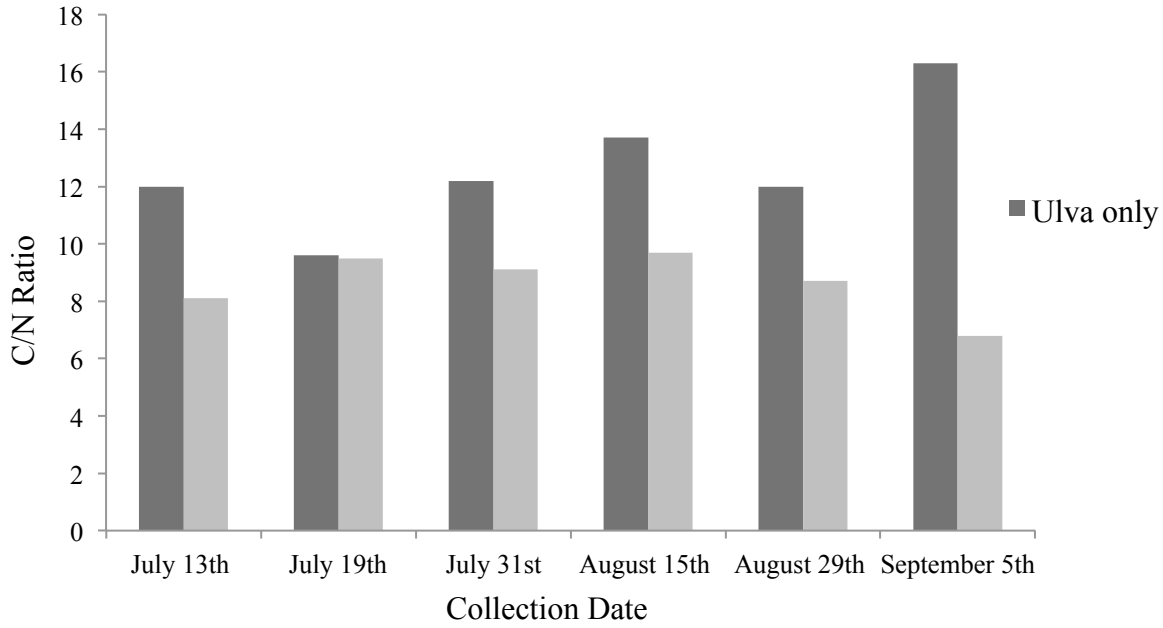

Figure 11. A graphical depiction of C/N ratios for treatment (C/U) and control (U) groups by date. Treatment groups represent an average of 3 measurements, whereas 3 control group averages were taken July 13th. All other control values were based off of one measurement per date.

This visualization represents the clear tendency of C/N ratios for replicates in the treatment group to be lower than those of the control group.

The mean values are significantly different from one another. The mean $C:N_{ulva}$ for all replicates in the treatment group was 8.6 ±0.41. The mean value for the *Ulva spp*. treatment was 12.5 ±0.67. The results of the t-test indicate that the two values are significantly different from each other $p<0.001$. These results indicate that the *V. philippinarium* in the treatment group had a significant effect on the nitrogen assimilation into the *Ulva spp.* tissue.

The C/N ratios for the phyto-POM ranged from 4.11 to 5.10, and did not overlap with the C/N ratio range of *Ulvoid* tissues on the control or treatment bags. There was a significant difference between the C/N ratio of *Ulvoid* tissue and phyto-POM ($p<0.0001$).

The C/N ratios for *V. philippinarium* ranged from 4.07 to 5.86 for the group grown without *Ulva* and from 3.82 to 5.19 for the treatment group grown with the *Ulva.* Neither control nor treatment *V. philippinarium* ratios overlapped with ratios of control or treatment *Ulva* tissue. There was a significant difference between control and treatment groups for the *V. philippinarium* ratios (p=0.038). There was a significant difference between *V. phillippinarium* control group and the phyto-POM (p=0.27) and *V. phillippinarium* control group and the phyto-POM (p=0.71). The difference between the control and treatment groups does not reflect the expectation that *V. phillippinarium* exposed to *Ulva* would have a higher C/N ratio (see Appendix 1).

Dietary Isotope Analysis

δ ^{13}C analysis

For *V. philippinarium* control and treatment stomach glands, δ ^{13}C values were similar in range (control= -19.1 to -22.6 ‰; treatment=-18.7 to -22.0‰). Though the range for the treatment values was slightly lower than that of the control, mean δ ^{13}C values between treatment and control *V. philippinarium* stomach glands did not show a significant difference between groups (p=0.16).

For δ ^{13}C values *Ulva spp*. overall exhibited the widest range of all test groups (-9.0 to -17.1‰). *Ulva spp*. tissue from the *Ulva* only control (-13.0 to-17.1‰, X=-14.30 ± 0.67) and the *Ulva* tissue from the *Ulva* and *V. philippinarium* treatment (-9.0 to -16.5‰, X=-12.15± 0.58) showed a significant difference between control and treatment groups in terms of mean δ ^{13}C signature (t=-2.4, df=25, p=0.03). This finding suggests that the presence of *V. philippinarium* had an unexpected enrichment effect on the δ ^{13}C signature

of the associated *Ulva spp.*

δ ^{13}C values for the stomach glands in the *V. philippinarium* only group (-19.1 to -22.6 ‰, X= -20.82±0.26) and for the stomach glands in the combination treatment group (-18.7 to -22.0 ‰, X=-20.26±0.27) did not intersect with the range of values from any of the primary producer groups, including phytoplankton-based particulate organic matter (POM). δ ^{13}C values for phytoplankton ranged from -14.9 to -17.4 ‰

Figure 12 summarizes the ranges for control and treatment *Ulva spp.*, control and treatment *V. philippinarium* groups, and the range of phytoplankton-based POM.

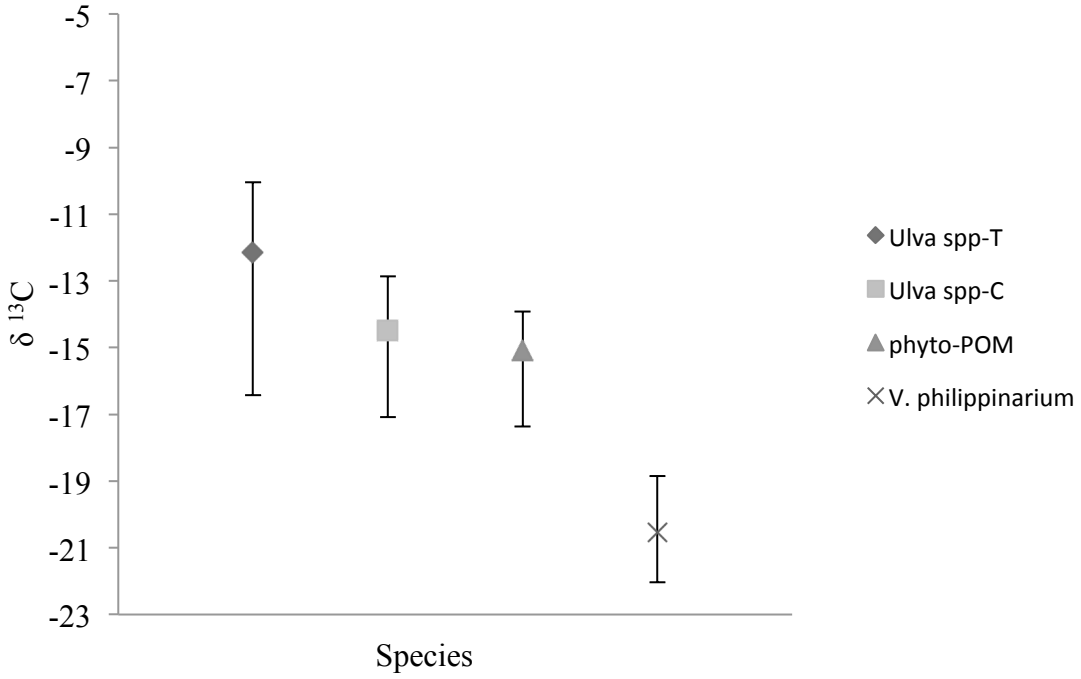

Figure 12. This figure exhibits the mean isotopic signature with ranges for *Ulva spp*. control and treatment groups and the *V.philippinarium* control and treatment stomach gland (after taking into account 0.6‰ fractionation factor for the stomach gland). The mean δ^{13}C value for phyto-POM is also displayed. *Ulva spp*. groups have significantly different δ^{13}C signatures from one another. *V.philippinarium* control and treatment do not show a significant difference. However, *V.philippinarium* combined control and treatment group lies outside of the range for all primary producers.

$\delta^{13}C$ mean values for *V.philippinarium* were statistically indistinguishable (t=-9.66, df=37, p=0.16). Hence, *Ulva spp*. application had no detectable effect on *V.philippinarium* dietary preference as indicated from the stable isotope analysis. However, *V. philippinarium* presence had an unexpected enrichment effect on $\delta^{13}C$ signature of *Ulva spp*.. As such, *Ulva spp*. tissue must be divided into two classes (treatment and control) for accurate comparison with other primary producers.

The average mean $\delta^{13}C$ for all *Ulva spp*. in the experiment (12.87±0.67‰) and phytoplankton-based seston (15.09± 0.63‰) were found to be significantly different from one another (t=-9.41, df=28,p=0.03). There was a significant difference between the *Ulva spp*. treatment group and phytoplankton (t=2.52, df= 21, p<0.0001). However, there was no significant difference between *Ulva spp*. control (X=-14.30 ±0.67‰) and phytoplankton (t=0.62, df= 11, p=0.75). Figure 13 represents the $\delta^{13}C$ values for treatment and control *Ulva spp*. groups, as well as the $\delta^{13}C$ value for phytoplankton.

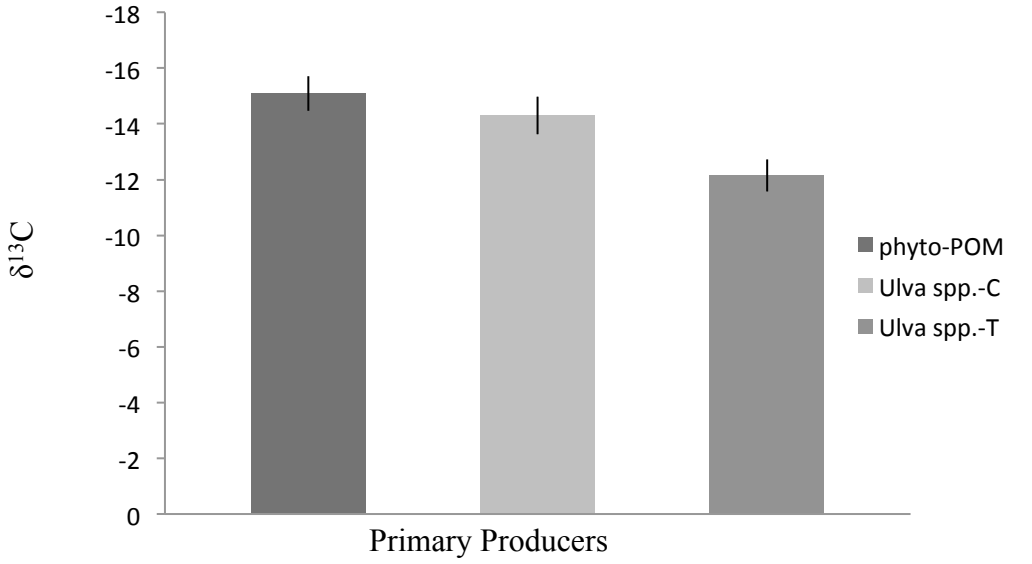

Figure 13. The differences in mean $\delta^{13}C$ values for treatment (X_T= -12.15± 0.58‰) and control (X_C=-14.30 ±0.67‰) Ulva spp. groups, and phytoplankton-based POM (X_{Phyto}=-12.87±0.67‰). There is no significant difference between X_C and X_{Phyto} values. All other groups are significantly different.

δ ^{15}N analysis

V.philippinarium $\delta^{15}N$ were enriched comparatively to all primary producer values when adjusted with a 2.9 ‰trophic fractionation factor. Control $\delta^{15}N$ values for *V.philippinarium* ranged from 10.0 to 10.8‰. Treatment $\delta^{15}N$ values ranged from 10.1 to 10.8. Control and treatment *V.philippinarium* groups did not display a significant difference.

Primary producer values were relatively similar in terms of $\delta^{15}N$ values. The range of values for the *Ulva spp*. control group was 7.2 to 9.2‰. The treatment group ranged from 7.6 to 9.6‰. Though the treatment group had a slightly higher range of values, *Ulva spp*. $\delta^{15}N$ values did not display any significant difference between control and treatment groups (t=1.65, df=25, p=0.51). The same was true for means δ ^{15}N values between treatment (X=10.4 ±0.12‰.) and control (10.4±0.05‰.) groups of stomach glands (p=0.78). Phytoplankton $\delta^{15}N$ values ranged from 7.9 to 8.8‰. Mean $\delta^{15}N$ phytoplankton values (8.3±0.88‰) were not significantly different from mean *Ulva spp*. $\delta^{15}N$ values (t=,0.29, df=35, df=28, p=0.66).

Mixing Model

As the mean ^{13}C signatures of both primary producer groups were significantly different from one another, the following system of linear equations was used to deduce the proportion of dietary source contributions to the control and treatment *V. philippinarium* diets.

3a. $\delta^{13}C_{phyto\text{-}POM} \times F_{phyto\text{-}POM} + \delta^{13}C_{Ulva} \times F_{Ulva} = \delta^{13}C_{V.\ philippinarium}$

b. $F_{phyto\text{-}POM} + F_{Ulva} = 1$

*where $\delta^{13}C_{V.\ philippinarium\ only}$ ranged from -19.1 to -22.6 ‰; $\delta^{13}C_{Ulva\ only}$ ranged from -13.0 to-17.1‰,; $\delta^{13}C_{phyto\text{-}POM}$ranged from 14.9 to -17.4 ‰ and $\delta^{13}C_{V.\ philippinarium\ combination}$= -18.7 to -22.0

‰; $\delta\ ^{13}C_{Ulva\ combination}$=--9.0 to -16.5‰; $\delta\ ^{13}C_{phyto\text{-}POM}$=-14.9 to -17.4 ‰ and $F_{phyto\text{-}POM}$=the fraction of phyto-POM in the diet; F_{Ulva}=the fraction of *Ulva spp*. in the diet**.**

In attempting the mixing model for the *V. philippinarium* only control group, I used the range of *V. philippinarium* control signatures, the range of *Ulva spp.* control signatures and the range of POM signatures. For the *V. philippinarium* combination treatment, I used the range of *V. philippinarium* combination signatures, the range of *Ulva spp.* combination signatures, and the range of POM signatures. However, the signatures of both source contributors were enriched compared to the signature of the stomach gland. Hence, any combination of both test sources will not account for the signature found in the stomach glands of the control and treatment groups.

Discussion

V. philippinarum growth

The results from the growth data do not reveal any significant effect of the presence of Ulva spp. on *V. philippinarium* shell growth. Shell length exhibits an average increase that is slightly higher in the presence of *Ulva* relative to the absence of *Ulva spp.*(control). For shell height, both the treatment and control groups did not differ in growth rate. The growth rate increased over the summer most likely due to increased metabolic activity in the warmer months. This data was collected over a much shorter time period than studies in the literature focusing primarily on growth rates, which typically last from 6 months to 2 to two years (Lamb unpublished data, 2015; Suh & Shin, 2012). Results suggest that a longer period of data collection is needed to yield a more meaningful result.

The effect of *Ulva spp* on the growth rates of *V. philippinarium* does not compare

to the effect of *Ulva spp.* on *C. gigas* at the same site. When *C. gigas* was exposed to dense concentrations of *Ulva spp.* at the Thorndyke Bay site during the summer of 2015, growth rate was negatively impacted (Lamb, 2015). However, *C. gigas* growth rate did not become negatively impacted until mid-July, and the trend was more pronounced later in the study (August-October) (Lamb, 2015). This reinforces the necessity of extending the measurements of *V. philippinarium* further into the season for a more accurate cross-species comparison.

Despite the discrepancy between the response of *C. gigas* and *V. philippinarium* in the field, it is possible that the summer *Ulvoid* blooms do not have a significant impact on *V. philippinarium* growth. The study by conducted by Lamb in 2015 examines the effect of 0, 1.5, and 3.0 kg of *Ulva spp*. biomass on *C. gigas* growth. In contrast, this study, using *V. philippinarium,* depends exclusively on natural density of *Ulva spp*. on site to mimic the natural conditions. This approach did not allow for the control of *Ulva spp*. densities for the duration of the project. The summer of 2015 was especially warm and dry; resulting in drastically decreased natural *Ulvoid* density (Joth Davis, personal communication). Hence, the discrepancies in *Ulvoid* density may account for the differences in treatment effect observed between the Lamb 2015 results and these results.

Carbon to Nitrogen Ratios

Carbon/nitrogen (C:N) ratios show a marked decrease for *Ulva spp*. tissue with *V. philippinarium;* Values ranged from 8.6±0.41 in the presence of *V. philippinarium*, and 12.5±0.67 in the absence, being significantly lower in the presence of clams. One reason for the lower C:N ratios in the presence of *V. philippinarium* could be that there is

increased nitrogen assimilation in the tissue of *Ulva spp*. when grown in proximity to the clam. *V. philippinarium* actively produces ammonium as a metabolic byproduct (Saurel et al, 2014), which is the most readily utilized form of nitrogen by *Ulva spp*. (Saurel et al, 2014; Zertuche-Gonzalez et al, 2008). Hence, it is assumed that the ammonium produced by *V. philippinarium* has a fertilization effect for *Ulva spp*. tissue. Additionally, the production of ammonium is positively correlated to higher temperatures due to heightened metabolic activity (Mann & Glomb, 1978). Hence, the seasonality of the *Ulvoid* blooms coincides with an assumed increase in local ammonium concentrations near *V. philippinarium* beds in the warm summer months.

To test if daily mean temperature had an effect on nitrogen assimilation in *Ulva spp*. tissue (hypothetically by increasing *V. philippinarium* metabolic activity) a regression was run using weekly average temperature data from the National Weather Service (NWS) for Seattle. Figure 14 demonstrates the relationship between daily average temperature and C/N ratio for *Ulvoid* tissue exposed to *V. philippinarium.*

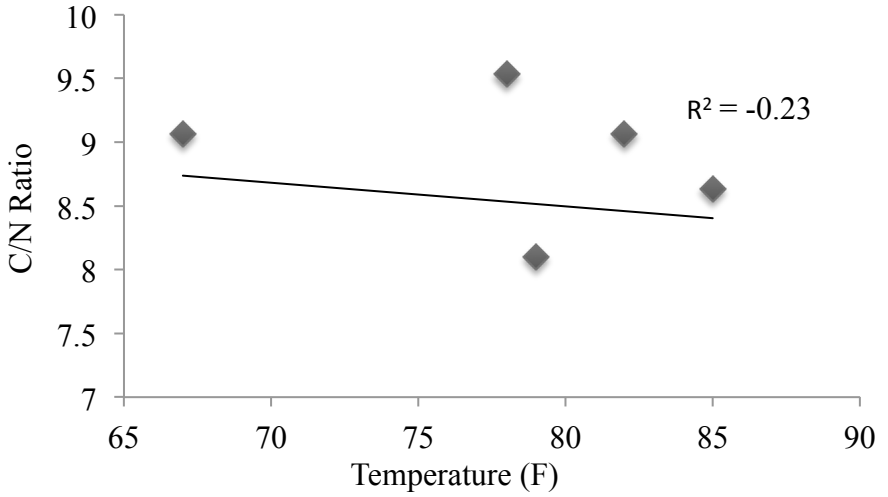

Figure 14. Linear regression using temperature as a predictive variable of C/N ratio. The relationship between daily temperature and C/N is not significant (R^2=-0.23, p=0.18).

This analysis indicates that weekly temperature and C/N ratio are not significantly correlated (R^2=0.-0.23, p=0.18). However, the C:N ratio decreased with increasing temperature, as expected. Despite the weekly temperature not acting as a strong force on nitrogen assimilation, the continued presence of *V. philippinarium* did have a significant effect on overall nitrogen assimilation.

As the presence of *V. philippinarium* appears to have a fertilization effect for *Ulva spp*., it can be assumed that *V. philippinarium* monocultures exacerbate local bloom severity. In Samish Bay, *V. philippinarium* monocultures have been shown to significantly increase the biomass of local *Ulva spp.*(Saurel et al, 2014). Though no equivalent biomass measurements exist for Thorndyke Bay, elevated nitrogen levels in *Ulvoid* tissue imply increased growth biomass production due to heightened nutrient acquisition. This biomass is of concern for the aquaculture industry, as it makes shellfish harvest more difficult, and has been shown to negatively impact the growth of commercially significant *C. gigas* when present in high densities (Lamb, 2015).

The capacity for *Ulvoid* blooms to act a significant sink for nitrogen in Thorndyke Bay has significant implications for the surrounding ecosystem. The tissue effectively stores excess nitrogen released by *V. philippinarium* monocultures. However, the degradation of this tissue releases the stored nitrogen back into the local ecosystem. Additionally, the process of primary producer degradation consumes dissolved oxygen (DO) in the water column, increasing harmful eutrophic conditions. The Hood Canal experiences seasonal decrease in DO due to oxygen-poor upwelling and limited flushing of the Hood Canal Basin (Newton et al, 2007). In the Southern reaches of the Canal, this poor water quality can result in lethal conditions for local sea life (Newton et al, 2007).

Though Thorndyke Bay is not highly susceptible to low DO of this capacity, *V. philippinarium* monocultures and *Ulvoid* blooms co-occur in the South Hood Canal. Hence, the nitrogen from degrading *Ulvoid* blooms may be locally exacerbating pre-existing DO issues on aquaculture farms throughout the Hood Canal. Ultimately, research into biomass proliferation and nitrogen assimilation in areas more strongly impacted by poor water quality is necessary to determine the extent of the problem in these areas.

Isotope Analysis

$\delta^{13}C$ stable isotope values for phytoplankton reflected the typical range of values for the Hood Canal (Conway-Cranos et al, 2015), and for *Ulva* in the N. Puget Sound (Howe, Simenstad,& Ogsto, 2012). As expected, the overall signature of the *Ulvoid* tissue was slightly enriched compared to that of the phytoplankton-based POM. This is due to the increased utilization of the heavier isotope of CO_2 in macroscopic primary producers (Altabet,1988).

Interestingly, *Ulvoid* tissue collected from bags containing *V. phillipinarium* were significantly enriched when compared to *Ulvoid* tissue grown independently of *V. phillipinarium*. Analyzed separately, tissue not exposed to *V. phillipinarium* was statistically indistinguishable from pytoplankton-based seston. Though phytoplankton and both control and treatment *Ulva spp*. isotopic ranges fall within values supplied by the literature, phytoplankton-based seston values fall toward the bottom end of the range. Conway–Cranos et al provides a range of -14.9 to -25.3 ‰ for phytoplankton-POM in nearby Dosewallips. Phytoplankton-POM values, collected from July 13th through September 5th, lie from -14.9 to -17.4 ‰ . It is possible that phytoplankton is naturally relatively enriched at the Thorndyke Bay site from July to September. Alternatively, the

decomposition of high abundances of *Ulvoid* species may have resulted in enriched POM. As *Ulvoid* species degrade more rapidly than other macrophytes (Zertuche-Gonzalez et al, 2008), it is possible that larger proportions of the enriched tissue were present in the POM samples, further enriching the signature.

The significant difference between control and treatment *Ulvoid* tissue indicates that the presence of *V. phillipinarium* has an enrichment effect on *Ulva spp*.. An investigation into this mechanism reveals that *Ulva spp*. growing on *V. phillipinarium* bags may be disproportionately integrating bicarbonate (HCO_3^-) relative to CO_2. Of the species of dissolved inorganic carbon (DIC), HCO_3^- is relatively enriched (Boutton, 1991). The rate of HCO_3^- uptake in *Ulvoids* positively correlates with temperature and desiccation (Axelsson, Larsson, & Ryburg, 1999). Assimilation of HCO_3^- also occurs with lower levels of dissolved oxygen (DO) (Axelsson, Larsson, & Ryburg, 1999). This is due to an adaptive mechanism within the *Ulvoid* tissue, which adjusts for the reduced concentrations of all DIC species in the absence of oxygen (Axelsson, Larsson, & Ryburg, 1999). HCO_3^- forms faster than CO_2 in marine environments. As the treatment and control bags were relatively similar in terms of temperature and desiccation status, reduced DO, caused by *V. phillipinarium* respiration, may partially account for the tissue enrichment difference between treatments.

To determine if *V. philipinnarium* respiration has an effect on bicarbonate uptake, it is important to demonstrate that *Ulvoid* net photosynthesis is greater than the *V. philipinnarium* respiration rate. In the North Adriatic clams respire at a rate of 0.014 ± 0.009% grams dry weight per day (gdw d^{-1}) at 20 degrees C (Solidoro et al, 2000). At 18 degrees C, *Ulva fenestrata* experiences a max gross primary production rate of 22.63 ±

2.7 gdw h^{-1}, when completely submerged in seawater and a reparation rate of 7.54±1.60 gdw h^{-1}. In air, the gross photosynthetic rate is 15.46 ± 2.33, with a respiration rate of 8.06±0.53 gdw h^{-1} (Quadir, Harrison, & DeWreede, 1979). Normalizing units, it becomes clear that *Ulva fenestrata* photosynthesizes at a rate 8 to 25 times the respiration rate of *V. philipinnarium.* Though there is a difference of 2 degrees C between these measurements, *V. philipinnarium* respires slightly slower at 18 degrees C (Solidoro et al, 2000), implying that the photosynthetic rate of *Ulva fenestrata* would continue to outpace *V. philipinnarium* respiration at this temperature.

It is hypothesized that *Ulva spp*. in the Northern Hood Canal uptakes nitrogen produced by *V. philipinnarium* respiration, perhaps resulting in an initial increase in growth rate. However, as biomass continues to increase, *V. philipinnarium* CO_2 may not be able to meet the DIC demands of the rapidly photosynthesizing *Ulvoid*s. Where fertilized *Ulva* spp. has rapidly consumed CO_2, the DIC composition would shift in favor of HCO_3^-. In these circumstances, the *Ulvoid*s could employ their HCO_3^- uptake mechanism. Relying primarily on HCO_3^- would account for a tissue enrichment in the treatment setting. The control *Ulva spp*. bags may also experience a shift from CO_2 to HCO_3^- due to their own photosynthetic needs. However, it can be assumed that their growth rate, and therefore, photosynthetic capacity is not as great, due to lack of initial fertilization effect from *V. philipinnarium* nitrogen.

Results from this study do not show a significant difference between *Ulvoid* density between bags cointaining *V. philipinnarium* and empty bags. However, density data available in this study is minimal, and the trend implies that biomasses are higher in *Ulvoid*s exposed to *V. philippinarium* as compared to *Ulvoid*s not directly exposed.

Ultimately, a more thorough and long-term study into relative biomasses of *Ulvoid*s on *V. philipinnarium* bags should be done in conjunction with an *Ulvoid* tissue relative abundance study.

For *V. phillipinarium,* $\delta^{13}C$ values were indistinguishable between the presence or absence of *Ulva spp.*. This indicates that the presence of *Ulva spp.* in this particular experiment did not have a significant effect on the dietary preference of *V. phillipinarium.* These results, however, may not directly translate to years displaying more average bloom densities. This experiment was designed to mimic the status-quo conditions of *Ulvoid* attachment to *V. phillipinarium* bags by not controlling for *Ulvoid* densities on treatment bags. However, the summer drought shifted the bloom season earlier and visibly reduced the amount of *Ulva spp*. attached to all *V. phillipinarium* growing bags on the site. It is unclear if non-drought bloom conditions would result in a dietary shift, due to their usual capacity to completely smother the bags, hypothetically restricting the inflow of POM to *V. phillipinarium*. Preferably, this experiment would be repeated during a non-drought year to test the effect of status quo *Ulva spp*. covering on *V. phillipinarium* diet.

Though the effect of *Ulvoid* presence on *V. philippinarium* could not be determined, the results illuminated interesting information about *V. philippinarium* diets during seasonal drought. There was no significant difference between $\delta^{13}C$ signatures of *V. philippinarium* between treatments. Hence, it can be assumed that the stomach gland $\delta^{13}C$ signatures were an accurate representation of diet in the absence of obstruction. Analyzing the data under this assumption revealed that the combination of detrital *Ulva spp*. and phytoplankton-POM did not account for the entirety of *V. philippinarium* diet.

The $\delta^{13}C$ isotopic signature for *V. philippinarium* fell far outside of the ranges of both primary producers, being significantly depleted.

To account for the depleted isotopic values of the stomach glands, values of salt marsh grass, specifically *Glaux maritima* and *Salicornia virginica,* from nearby Dosewallips and Hamma Hamma were substituted into a source proportion estimator. Marsh grasses and upland vegetation are depleted in regards to *Ulva* and phytoplankton signatures. The signatures found in the 2015 Conway-Cranos et al study showed averages signatures of $\delta^{13}C = -28.0 \pm 0.6‰$ and $\delta^{13}C = -27.6 \pm 0.9‰$ for each of the two Hood Canal sites. Sea marsh detritus was found to comprise 35-45% of *C. gigas* diet at these sites. Upland vegetation was not found to be a significant dietary contributor to *C. gigas* (~2%) (Conway-Cranos et al, 2015). As Thorndyke Bay is surrounded on both sides by sea marsh, it can be assumed that there is a supply of marsh detritus to bivalve diets in this region (Harrington, 2005).

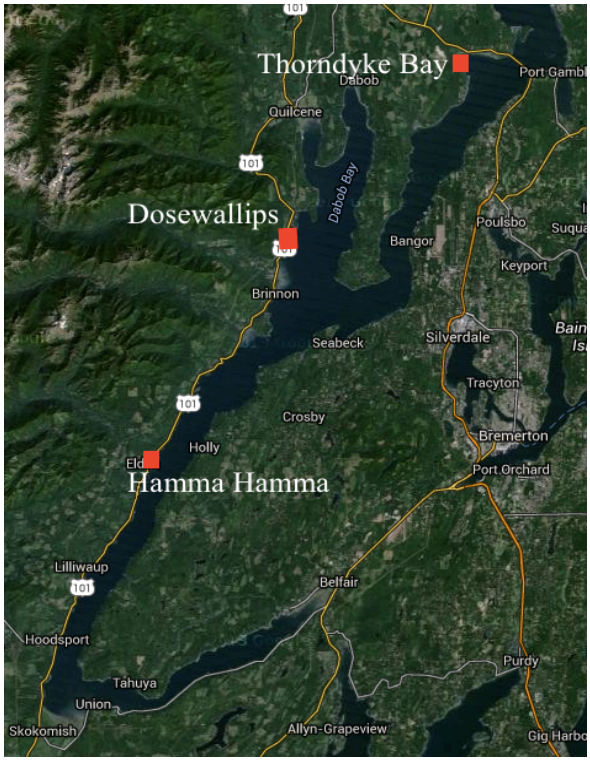

Figure 15. Map of Thorndyke Bay, Dosewallips, and Hamma Hamma site location along the Hood Canal. The Hamma Hamma site showed an average Salt Marsh signature of δ ^{13}C -28.0 ±0.6‰ and the Dosewallips site, an average signature of δ ^{13}C -27.6±0.9‰ (Conway-Cranos et al, 2015).

As there was no significant difference between treatment and control groups for *V. philippinarium* stomach gland signatures, the δ ^{13}C signature ranges were taken from the combined group (-22.1 to -19.1 ‰) to assess the possibility of a third dietary contributor. For *Ulva spp*., the experimentally measured total δ ^{13}C values of -15.7 to -9.9 ‰ were input into the mixing model. Phytoplankton δ ^{13}C measured range values of -16.44 to -14.07 ‰ were used. For sea grasses, the entirety of the reported values were used (-28.0 to -27.0 ‰) (Conway-Cranos et al, 2015). Table 5 shows the results from the analysis performed by ISOSOURCE addressing the mean value of probable % contribution of primary producer to overall dietary composition. ISOSOURCE computes a frequency histogram of all possible solutions to the three-end member mixing model

using δ ^{13}C data.

Primary Producer	*Ulva spp.*	Phyto-POM	*G. maritima* and *S. virginica*
Mean Estimated Contribution	23-24%	26-32%	44-50%

Table 5. The mean range of estimated source contributions in percentages to *V. philippinarium* diet using δ ^{13}C values for *Ulva spp,* Phyto-POM, and *G. maritima* and *S. virginica*. Estimates indicate a high contribution from *G. maritima* and *S. virginica*, followed by, Phyto-POM and *Ulva spp*. respectively.

The lower end of the salt marsh and phyto-POM values found in the ISOSOURCE model are consistent with the values presented in the Conway-Cranos 2015 *C. gigas* study (35-45%). The values in table 5 may over represent actual contribution of the three primary producer categories to *V. phillippinarium* diet, as the full range of potential source contributions was not included in this post-hoc analysis. Additionally, values for *Ulva spp.* could not be directly compared to this study, as *Ulvoid*s were included amongst nine species as intertidal macrophytes (Conway-Cranos et al, 2015). Figure 16 displays the value ranges for all separately considered primary producers and the combined primary consumer values.

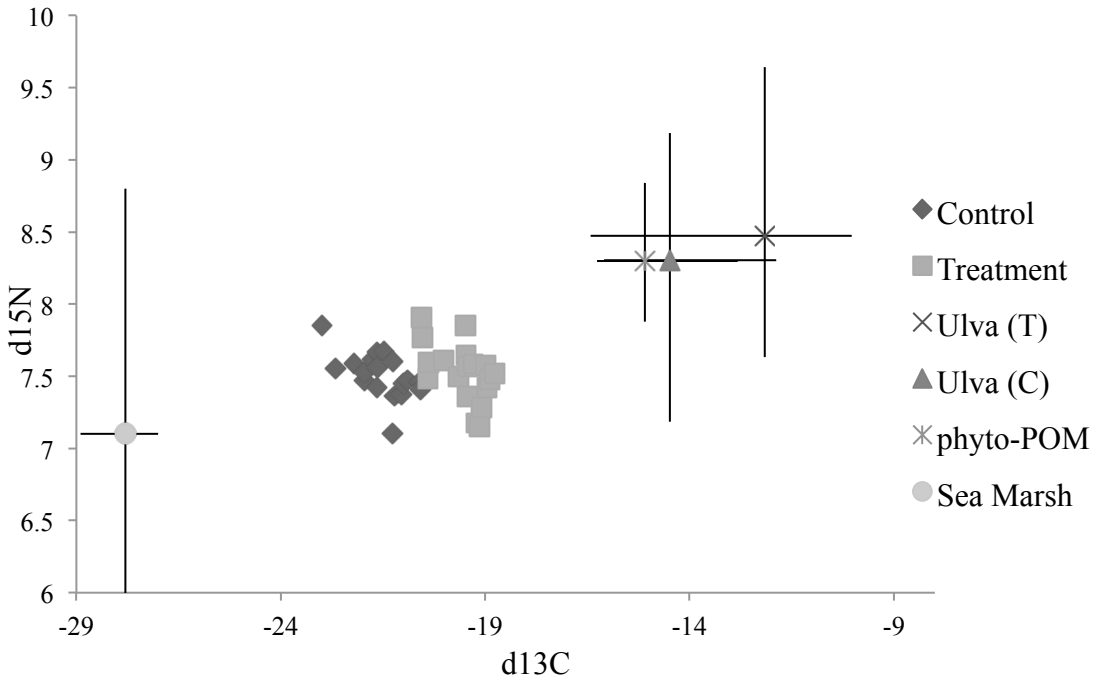

Figure 16. A mixing model comparing δ ^{13}C and δ ^{15}N ratio values of primary producers and *V. philipinarium* (y-range extends to 5). Raw values for treatment and control groups for *V. philipinarium* were included, though the groups did not show any significant difference for δ ^{13}C between treatments (p=0.16). *Ulva* control (*Ulva* C) and treatment (*Ulva* T) group means, along with the range of values were included. *Ulva* treatment and control groups showed significant differences between groups and hence were treated as separate dietary contributors. The phytoplankton-based POM mean and range was also included. The combined isotopic means and ranges of*G. maritima* and *S. virginica* were also included.

Figure 16 demonstrates that with the added combined signatures of *G. maritima* and *S. virginica, V. philippinarium* values from this study fall between the ranges of food sources. Hence, incorporating depleted sources into a model or future study is crucial for this site.

Overall, this study was able to illuminate that *Ulva spp*. has no observable impact on *V. philippinarium* dietary preference during years of severe drought. Additionally, this study uncovered the need to include more dietary source components, especially local sea marsh grasses, in subsequent analysis of bivalve diets at this site. Thus, more isotope-

based studies are needed to 1) determine the effect of normal density *Ulvoid* blooms on *V. philippinarium* diets, and 2) illuminate the baseline source contributors to *V. philippinarium* diet in the Thorndyke Bay region of the Northern Hood Canal.

Conclusion

Evidence for a Mutualistic Interaction

The original intent of this study was to explore whether *V. philippinarium* and *Ulva spp.* are involved in a symbiotic relationship during the seasonal proliferation of *Ulvoid* blooms. This study was part of an effort to explore the relationship between cultivated bivalves and macroalgae on a future macroalgae cultivation site in the Northern Hood Canal. Though it remains undetermined as to whether a symbiotic relationship exists, it is evident that at the very least, a commensal relationship exists between *V. philippinarium* and *Ulva spp.*.

This study confirmed the capacity for *V. philippinarium* to increase nitrogen assimilation in *Ulva spp.* tissue. This contribution of nitrogen remained previously unquantified for these species in the Northern Hood Canal. However, increased inorganic nutrient assimilation into macroalgae tissue in the presence of bivalves is one of the fundamental principles behind integrated (IMTA) multitrophic aquaculture systems (Barrington, 2009). It may not be desirable, from an ecosystem perspective, to increase nutrient flow to *Ulvoid* tissue (for reasons discussed later). However, if the capacity for *V. philippinarium* to contribute nitrogen to macroalgae tissue translates to a cultivatable species, IMTA could be successful in the Northern Hood Canal. Whole bay studies in the Northern Puget Sound show that *Ulvoid* tissue abundance increases near whole plots of *V. philippinarium*. Hence, it is reasonable to assume that the fertilization effect is not

limited strictly to tissue attached to growing bags.

Various IMTA systems count detrital algae from the system as a significant contributor to bivalve diets. These systems are often closed systems with reduced variety of feed input (Barrington, 2009). Hence, bivalve diets in these circumstances would mirror the availability of food sources. However, in open ecosystem settings, such as in Thorndyke Bay, bivalves can be more selective of their food sources. *V. philippinarium* does not always feed proportionally based on food availability (Suh & Shin, 2014). As seen in this study, *V. philippinarium,* uninhibited by barriers, incorporates isotopically depleted food sources. It is uncertain whether dense *Ulvoid* barriers would cause a significant shift in this behavior. Macroalage cultivation systems may not cause a large disruption of POM. Hence, their application to the *V. philippinarium* growing site may not force an unfavorable dietary shift. The response of *V. philippinarium* to these new source components would be a more relevant study in terms of the site-specific IMTA configuration.

Overall, the commensal relationship illuminated by this study is evidence for the potential success of macroalgae cultivation in Thorndyke Bay. Further investigation is necessary to determine how various macroalgae species and densities would impact *V. philippinarium* diet, and most importantly, growth. However, findings from this study indicate that low-interference macroalgae growth does not have a significant negative impact on *V. philippinarium* diet or growth. Meanwhile, cultivated *V. philippinarium* significantly increase nitrogen assimilation in *Ulvoid* tissues. These findings support the inclusion of macroalgae cultivation on the Northern Hood Canal.

Ecosystems Implications

During a time of increasing anthropogenic impacts on Washington's coastal ecosystems, understanding the sources and fates of nutrients is essential. Shellfish monocultures serve to sequester particulate organic nitrogen (PON), integrating it into their tissues, and allowing it to be removed from the local ecosystem upon harvest (Shumway et al, 2003). However, during the summer months, when shellfish are most metabolically active, this organic PON is more rapidly transformed into ammonium (Mann & Glomb, 1978). Ammonium is the most readily uptaken form of nitrogen by *Ulvoid*s and phytoplankton (Saurel et al, 2014; Zertuche-Gonzalez et al, 2008; Dortch, 1990). This study provides evidence of increased nitrogen assimilation in *Ulvoid* tissue associated with *V. philippinarium*. Hence, the transformative capacity of *V. philippinarium* provides an easily accessable fertilizer to macroalgae blooms.

Though the fertilization effect of *V. philippinarium* on primary producer tissue can be harnessed to promote macroalgae cultivation, its current relationship with *Ulva spp*. has presumably negative ecosystem impacts. As demonstrated by this study, *Ulvoid* tissue assimilates higher nitrogen concentrations near *V. philippinarium* monocultures. This increases overall *Ulvoid* biomass (Saurel et al, 2014; Zertuche-Gonzalez et al, 2008) and locally concentrates nutrients. Upon degradation of *Ulva spp*. tissue, these nutreints are released back into the ecosystem as particulate organic matter. The aerobic digestion of this POM consumes dissolved oxygen and leaves areas more susceptible to seasonal eutrophic conditions.

Shoreline systems, which experience dense *Ulvoid* blooms, high volumes of riverine nitrogen inputs, and low rates of flushing, may be most affected by nitrogen

outputs from *V. philippinarium* monocultures. These more eutrophically susceptible areas occur widely in the Southern Hood Canal (Newton et al, 2007). Though the primary driver of eutrophication in the Hood Canal are geochemical forcing from the open ocean, anthropogenic effects such as industry and nutrient runoff have compounding, localized effects. In order to help negate any negative contributions of the shellfish industry to seasonally concentrating nutrients, *Ulvoid* blooms should be removed from cultivation sites. In doing so, shellfish will have a higher positive net benefit on surrounding shoreline ecosystems.

Confounding Factors

This research project took place in the midst of a historic drought in Washington State. The warmer and drier than average conditions abnormally impacted the growing season of *Ulva spp*.. Industry observations and Puget Sound literature indicate the normal *Ulvoid* growing season lasts from June through September (Joth Davis, personal communication, 2015; Nelson et al, 2000). However large quantities, *Ulvoid* species were observed in Thordyke Bay as early as May during the study year (Joth Davis, personal communication, May 2015). Hence, the proposed study timeline was unable to capture the beginning of the true *Ulvoid* growing season. Additionally, the extreme warm, dry temperatures, in combination with the large tide runs of July, caused severe desiccation in *Ulva spp.* covering the *V. philippinarum* cultivation plots. The original experimental design was based on the capacity for *Ulva spp*. to form strong attachments to the mesh growing bags. However, these attachments were weakened and severed with the mass degradation of *Ulva spp*. tissue from July 18th- July 31st, 2015. Though the nearshore

Ulva spp. tissue was significantly affected, deeper free-floating *Ulva spp*. continued to proliferate observably less affected.

Figure 18. This photo was taken of the entire *V. philippinarium* cultivation site on July 18th, 2015. The severity of *Ulvoid* die off is evident by the amount of mesh growing bag exposed. In normal conditions, bag surfaces would be entirely covered in *Ulvoid* attachments.

Free-floating *Ulvoid* and terrestrial and marine macrophyte species posed an obstacle to the integrity of the experimental design throughout the field data collection process. Established *Ulva spp*. attachments persisted on their respective bags throughout the experiment (barring the July 18th- July 31st, 2015 window). However, tidal deposits of free-floating *Ulvoid*s and macrophytes species served to contaminate the non-*Ulva spp*. control bags. In attempts to prevent the reduction of treatment affect by free-floating tidal vegetation, the control bags were thoroughly scraped each week throughout the

experiment. However, abundance and residence time of free-floating masses remains unknown for the periods between visits.

Additionally, an uncharacteristically severe storm hit the Washington coast on the data collection day of August 31, 2015. This storm redistributed large quantities of free floating *Ulvoid*s into the upper intertidal zone, completely covering the experimental row. Additionally, sediment and particulate marcrophytes were redistributed into the water column. This temporary shift in seston composition did not mirror the week proceeding or following the storm.

Figure 19. This photo was taken August 31st following the large late-summer storm. The photo is of the high shoreline, usually free of *Ulvoid* species. Clearly, large masses of Ulva spp. were redistributed much higher onto the intertidal zone and shoreline than normal. Photo credit: Joth Davis

To better buffer the experimental row from confounding environmental factors, and

increase treatment effect, a shift in the experimental structure is recommended. This buffer could be achieved by implementing a secondary containing structure on the outside of the *V. philippinarum* mesh bags. This structure would act as a barrier to prevent the influx of free-floating *Ulvoid* masses on incoming tides. Additionally, the barrier would help contain the degrading *Ulva spp*. within the surface area of the bag. This method would also allow for the direct quantification of mass algal mass, and allow the researcher to maintain *Ulva spp*. biomass at consistent levels throughout the experiment. This barrier, however, would not allow for the true quantification of particulate *Ulva spp*. in clam diets given environmental fluxes present in the natural environment.

Recommendations for Future Research

This study warrants further investigation into the seasonal dynamics taking place in Thorndyke Bay. The results from this study confirmed increased nitrogen assimilation in *Ulvoid* tissue associated with *V. philippinarum.* Hence, it would be interesting to quantify the associated response in *Ulva spp*. biomass at this site. Quantifying the abundance of *Ulvoid* biomass over several seasons would give insight into how locally concentrated nutrients, from shellfish cultivation and from degrading *Ulvoids*, are effecting subsequent year's blooms. Additionally, expanding this study to include the eutrophically-stressed Southern Hood Canal could illuminate the seasonal contribution of shellfish cultivation to increasing primary production, and subsequent decomposition.

After a preliminary IMTA system is introduced in the Northern Hood Canal, it would be beneficial to quantify the effect of bivalve species on nitrogen assimilation and

biomass accumulation in cultivated macroalgal species. Findings from this type of study would help the industry make a case for the expansion of IMTA, especially if macroalgae proves an economically viable product. Additionally, this type of study would allow for ecologists to quantify the removal of nitrogen from these ecosystems through macroalgae harvest. Increased removal of nutrients from these systems may also aid in bolstering the argument for IMTA systems throughout the state. Additionally, it would be interesting to investigate the effect of macroalgae cultivation on seasonal *Ulvoid* blooms. It is possible that through the repeated harvest of seasonal nutrients from the system, *Ulvoid* blooms may become less severe. This would have positive implications for growers, and for the surrounding ecosystem.

Many questions about *V. philippinarum* diet were posed by this study. This research took place under the assumption that *V. philippinarum* were eating primarily *Ulva spp*. and phyto-POM. However, it was illuminated that their diet is likely to be far more expansive, consisting of much more depleted sources. An investigation into source contributors to *V. philippinarum* diets is imperative to understanding how these preferences respond to seasonal influences. Additionally, it would be interesting to investigate the dietary preferences of *V. philippinarum* in non-drought years. Traditioanlly, dense *Ulvoid* mats completely cover *V. philippinarum* plots. It is possible they are pressured to switch their diets to incorporate more *Ulvoid* detritus during normal years. It would also be interesting to see how these normal summer conditions impact the growth of *V. philippinarum* in the covered bags.

After the introduction of the IMTA system, it would be interesting to note any change in *V. philippinarum* feeding preferences. It is possible that a different species of

macroalgae may be more preferable to *V. philippinarum*. If this is the case, IMTA systems have a higher chance at industry acceptance. Investigating the interaction of cultivated macroalgae with *C. gigas* is another important study. As *C. gigas* is the most profitable bivalve in Washington state (Booth, 2014), it is important to monitor its success in regards to new industry innovations.

Integrating Findings into industry Practices

The results from this study have both short-term and long-term implications for the shellfish industry. As *Ulva spp*. acts as a temporary nutrient sink, and significantly reduces *C. gigas* growth when present in dense quantities (Lamb, unpublished data, 2015), there is rationale for better management practices. For these reasons, the removal of *Ulvoid* blooms from shellfish cultivation sites would have positive impacts ecologically and economically. Currently, the removal of *Ulvoid* species is seen by the industry to be expensive and time consuming (Joth Davis, personal communication, 2015). A cost-benefit analysis would be necessary to determine the amount of resources optimal to devote to the removal of *Ulvoid* species. It is reasonable to assume that over time, removing *Ulva spp*. from growing sites will result in reduced need for removal in subsequent years. Hence, a larger upfront investment in removing the blooms could have lasting benefits to a given harvest sites.

The integration of macroalgae aquaculture to shellfish growing beds would, too, have positive benefits for growers. Cultivated macroalgae would sequester nutrients in a similar manner to *Ulvoid* species, without experiencing rapid decomposition rates (Zertuche-Gonzalez, 2008). This would allow for the longer-term sequestration of nutrients prior to harvest. Additionally, cultivating macroalgae for developing local

markets would yield a direct economic benefit to growers.

Overall, removing inorganic nitrogen from shellfish growing regions during the summer months is of utmost importance to Hood Canal growers. Macroalage has the capacity to sequester nitrogen during the summer growing season. Removing the currently dense *Ulvoid* blooms from growing areas is a practice growers can adopt to reduce the negative indirect effects that shellfish cultivation has on surrounding estuarial ecosystems. For a longer-term solution, macroalgae cultivation can be introduced to growing areas to more reliably sequester nitrogen throughout the entire growing season. The harvest of these species would result in increased profits to growers, and allow for the active removal of shellfish-based inorganic nitrogen. Regardless of tactic, growers must look to utilizing the benefits of integrated ecosystem functions for an environmentally conscious approach shellfish cultivation in the Hood Canal.

Bibliography

Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries, 25(4)704-726.

Altabet, M. A. (1988). Variations in nitrogen isotopic composition between sinking and suspended particles: Implications for nitrogen cycling and particle transformation in the open ocean. Deep Sea Research Part A. Oceanographic Research Papers, 35(4),535-554.

Barrington, K., Chopin, T., & Robinson, S. (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper, 529, 7-46.

Bode, A., Alvarez-Ossorio, M. T., & Varela, M. (2006). Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. Marine Ecology Progress Series, 318(8).

Bolton, J. J., Robertson-Andersson, D. V., Shuuluka, D., & Kandjengo, L. (2009). Growing Ulva (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. Journal of Applied Phycology, 21(5), 575-583.

Booth, S.R. (2014).Crop Profile for Bivalve (Oysters, Clams, Geoduck Clams and Mussels) Aquaculture in Washington. Retrieved from http://www.ipmcenters.org/cropprofiles/docs/WAbivalve.pdf

Boutton, T. W. (1991). Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. *Carbon isotope techniques*, *1*, 173.

Buschmann, A. H., Varela, D. A., Hernández-González, M. C., & Huovinen, P. (2008). Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of Macrocystis and Gracilaria as biofilters. Journal of Applied Phycology, *20*(5), 571-577.

Cloern, J. E., Canuel, E. A., & Harris, D. (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnology and oceanography, 47(3), 713-729.

Christensen, J. P., Smethie, W. M., & Devol, A. H. (1987). Benthic nutrient regeneration and denitrification on the Washington continental shelf. Deep Sea Research Part A. Oceanographic Research Papers, 34(5), 1027-1047.

Conway-Cranos, L., Kiffney, P., Banas, N., Plummer, M., Naman, S., MacCready, P., & Ruckelshaus, M. (2015). Stable isotopes and oceanographic modeling reveal spatial and

trophic connectivity among terrestrial, estuarine, and marine environments. *Marine Ecology Progress Series*, *533*, 15.

Dang, C., Sauriau, P. G., Savoye, N., Caill-Milly, N., Martinez, P., Millaret, C., & De Montaudouin, X. (2009). Determination of diet in Manila clams by spatial analysis of table isotopes. Marine Ecology Progress Series, 387, 167-177.

De Casabianca, M. L., Laugier, T., & Marinho-Soriano, E. (1997). Seasonal changes of nutrients in water and sediment in a Mediterranean lagoon with shellfish farming activity (Thau Lagoon, France). ICES Journal of Marine Science: Journal du Conseil, 54(5), 905-916.

Department of fisheries and Oceans (1999). Stock Assessment Report. Retrieved from:http://www.dfo-mpo.gc.ca/CSAS/CSAS/status/1999/c6-03e.pdf

Deudero, S., Cabanellas, M., Blanco, A., & Tejada, S. (2009). Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel Mytilus galloprovincialis. Journal of Experimental Marine Biology and Ecology, 368(2), 181-188.

Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and marine biology. An annual review, 33, 245-03.

Dortch, Q. (1990). The interaction between ammonium and nitrate uptake in phytoplankton. Marine ecology progress series. Oldendorf, 61(1), 183-201.

Dunton, K. H., & Schell, D. M. (1987). Dependence of consumers on macroalgal (Laminaria solidungula) carbon in an arctic kelp community: δ13C evidencee. Marine Biology, 93(4), 615-625.

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. Annual review of plant biology, 40(1), 503-537.

Feely RA, Scott, DC, Cooley SA. (2009). Ocean acidification: present conditions and future changes in a high CO2 world. Oceanography. 22(4), 36-47.

Feely RA, Sabin CL, Hernandez-Ayon JM, Ianson D, Hales B. (2008). Evidence for upwelling of corrosive “acidified” water onto the continental shelf. Science. 320, 1490-1492.

Greengrove, C., Masura, J., Moore, S., Bill, B., Hay, L., Eldred, K., ... & Stein, J. Alexandrium bloom ecology in Puget Sound: cyst distribution, growth and viability.

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. Phycologia, 32(2), 79-99.

Hein, M., Pedersen, M. F., & Sand-Jensen, K. (1995). Size-dependent nitrogen uptake in micro and macroalgae. Marine ecology progress series. Oldendorf, 118(1), 247-253.

Hoefs, J. (1997). Stable isotope geochemistry (Vol. 201). Berlin: Springer.

Howe, E. R., & Simenstad, C. A. (2015). Using stable isotopes to discern mechanisms of connectivity in estuarine detritus-based food webs. Marine Ecology Progress Series, 518, 13-29.

Humphreys, J., Harris, M. R., Herbert, R. J., Farrell, P., Jensen, A., & Cragg, S. M. (2015). Introduction, dispersal and naturalization of the clam Ruditapes philippinarum in British estuaries, 1980–2010. Journal of the Marine Biological Association of the United Kingdom, 1-10.

Industrial Economics, Incorporated (2014). Marine Sector analysis Report. Retrieved from:http://msp.wa.gov/wp-content/uploads/2014/03/AquacultureSectorAnalysis.pdf

Kasai, A., Horie, H., & Sakamoto, W. (2004). Selection of food sources by Ruditapes philippinarum and Mactra veneriformis (Bivalva: Mollusca) determined from stable isotope analysis. Fisheries science, 70(1), 11-20.

Kidd, K. A., Schindler, D. W., Hesslein, R. H., & Muir, D. C. G. (1995). Correlation between stable nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food web. Science of the Total Environment, 160, 381-390.

Khangaonkar, T., Sackmann, B., Long, W., Mohamedali, T., & Roberts, M. (2012). Simulation of annual biogeochemical cycles of nutrient balance, phytoplankton bloom (s), and DO in Puget Sound using an unstructured grid model. Ocean Dynamics, 62(9), 1353-1379.

Mackas, D. L., & Harrison, P. J. (1997). Nitrogenous nutrient sources and sinks in the Juan de Fuca Strait/Strait of Georgia/Puget Sound estuarine system: assessing the potential for eutrophication. Estuarine, Coastal and Shelf Science, 44(1), 1-21.

Miller WA, Reynolds AC, Sobrino C, Reidel GF (2009). Shellfish face uncertain future in high CO2 world: influence of acidification on oyster larvae calcification and growth in estuaries. PLOS1.4(5), 5661.

Mumford, T.F. 2007. Kelp and Eelgrass in Puget Sound. Puget Sound Nearshore Partnership Report No. 2007-05. Published by Seattle District, U.S. Army Corps of Engineers, Seattle, Washington.Available at www.pugetsoundnearshore.org

Mann, R., & Glomb, S. J. (1978). The effect of temperature on growth and ammonia excretion of the Manila clam Tapes japonica. *Estuarine and Coastal Marine Science*, *6*(3), 335-339.

Nakata, K., & Newton, J. A. (2000). Seasonal patterns and controlling factors of primary production in Puget Sound's Central Basin and Possession Sound. In Puget Sound Water Quality Action Team, 2002. Proceedings of the 2001 Puget Sound Research

Conference. T. Droscher, editor. Puget Sound Water Quality Action Team. Olympia, Washington.

Needoba, J. A., Sigman, D. M., & Harrison, P. J. (2004). The Mechanism of Isotope Fractionation During Algal Nitrate Assimilation as Illuminated by the 15N/14N of Intracellular Nitrate. *Journal of Phycology*, *40*(3), 517-522.

Nelson, T. A., Haberlin, K., Nelson, A. V., Ribarich, H., Hotchkiss, R., Alstyne, K. L. V., & Fredrickson, K. (2008). Ecological and physiological controls of species composition in green macroalgal blooms. Ecology, 89(5), 1287-1298.

Nelson, T. A., Nelson, A. V., & Tjoelker, M. (2003). Seasonal and spatial patterns of" green tides"(ulvoid algal blooms) and related water quality parameters in the coastal waters of Washington State, USA. Botanica Marina, 46(3), 263-275.

Newton, J., Bassin, C., Devol, A., Kawase, M., Ruef, W., Warner, M., & Rose, R. (2007, March). Hypoxia in Hood Canal: An overview of status and contributing factors. In Proceedings of the 2007 Georgia Basin Puget Sound Research Conference. Puget Sound Action Team, Olympia, Washington.

Newton, J. A., Bassin, C., Devol, A., Richey, J., Kawase, M., & Warner, M. (2011). Hood Canal dissolved oxygen program integrated assessment and modeling report: I. Overview and results synthesis.

Newton, J. and Van Voorhis, K., 2002, Seasonal Patterns and Controlling Factors of Primary Production in Puget Sound's Central Basin and Possession Sound, Washington State Department of Ecology, Environmental Assessment Program, Publication #02-03-059, Olympia, WA.

Nobre, A. M., Robertson-Andersson, D., Neori, A., & Sankar, K. (2010). Ecological–economic assessment of aquaculture options: comparison between abalone monoculture

and integrated multi-trophic aquaculture of abalone and seaweeds. *Aquaculture*, *306*(1), 116-126.

Peterson, B. J., & Heck, K. L. (1999). The potential for suspension feeding bivalves to increase seagrass productivity. Journal of Experimental Marine Biology and Ecology, 240(1), 37-52.

Poulain, C., Lorrain, A., Mas, R., Gillikin, D. P., Dehairs, F., Robert, R., & Paulet, Y. M. (2010). Experimental shift of diet and DIC stable carbon isotopes: Influence on shell δ 13 C values in the Manila clam Ruditapes philippinarum. Chemical Geology, 272(1), 75-82.

Quadir, A., P. J. Harrison, and R. E. DeWreede. "The effects of emergence and submergence on the photosynthesis and respiration of marine macrophytes." *Phycologia* 18, no. 1 (1979): 83-88.

Raikow, D. F., & Hamilton, S. K. (2001). Bivalve diets in a midwestern US stream: a stable isotope enrichment study. Limnology and Oceanography, 46(3), 514-522.

Resnsel,J., Bright,K., and Segreist,Z. (2011). Intergrated Shellfish Mariculture in the Puget Sound. Retrieved from http://www.aquamodel.net/Downloads/Puget%20Sound%20IMTA%20final%20report%2031May2011.pdf

Robinson, S. M. C., Martin, J. D., Cooper, J. A., Lander, T. R., Reid, G. K., Powell, F., & Griffin, R. (2011). The role of three dimensional habitats in the establishment of integrated multi-trophic aquaculture (IMTA) systems. Bulletin of the Aquaculture Association of Canada, 109(2), 23-29.

Ruesink, J. L., Trimble, A. C., Berry, H., Sprenger, A. G., & Dethier, M. N. (2014). Environmental Correlates of Growth and Stable Isotopes in Intertidal Species Along an Estuarine Fjord. Estuaries and coasts, 37(1), 149-159.

Ryabenko, E. (2013). Stable isotope methods for the study of the nitrogen cycle. Topics in Oceanogr, 1-40.

Saurel, C., Ferreira, J. G., Cheney, D., Suhrbier, A., Dewey, B., Davis, J., & Cordell, J. (2014). Ecosystem goods and services from Manila clam culture in Puget Sound: a modelling analysis. Aquaculture Environment Interactions, 5, 255-270.

Sequeira, A., Ferreira, J. G., Hawkins, A. J. S., Nobre, A., Lourenço, P., Zhang, X. L., & Nickell, T. (2008). Trade-offs between shellfish aquaculture and benthic biodiversity: a modelling approach for sustainable management. Aquaculture, 274(2), 313-328.

Shumway, S. E., Davis, C., Downey, R., Karney, R., Kraeuter, J., Parsons, J., & Wikfors, G. (2003). Shellfish aquaculture–in praise of sustainable economies and environments. World Aquaculture, 34(4), 8-10.

Solidoro, C., Pastres, R., Melaku Canu, D., Pellizzato, M., & Rossi, R. (2000). Modelling the growth of Tapes philippinarum in Northern Adriatic lagoons. *Marine Ecology Progress Series*, *199*(137-148).

Spillman, C. M., Hamilton, D. P., Hipsey, M. R., & Imberger, J. (2008). A spatially resolved model of seasonal variations in phytoplankton and clam (Tapes philippinarum) biomass in Barbamarco Lagoon, Italy. Estuarine, Coastal and Shelf Science, 79(2), 187-203.

Suh, Y. J., & Shin, K. H. (2013). Size-related and seasonal diet of the clam (Ruditapes pinarum), as determined using dual stable isotopes. Estuarine, Coastal and Shelf Science, 135, 94-105.

The Pacific Shellfish Institute (2013). Where we Work Washington. Retrieved from:http://www.pacshell.org/washington.asp

Thompson, D. S.. (1995). Substrate Additive Studies for the Development of Hardshell Clam Habitat in Waters of Puget Sound in Washington State: An Analysis of Effects on Recruitment, Growth, and Survival of the Manila Clam, Tapes philippinarum, and on the Species Diversity and Abundance of Existing Benthic Organisms. Estuaries, 18(1), 91–107. Retrieved from http://www.jstor.org/stable/1352285

Toba, D., Dewey,B., & King, T. (2005). Small Scale Clam Farming for Pleasure and Profit in Washington State. Retrieved from: http://articles.extension.org/sites/default/files/Small-scale%20clam%20farming%20for%20pleasure%20and%20profit%20in%20Washington.pdf

Tucker, C. S., & Hargreaves, J. A. (Eds.). (2009). Environmental best management practices for aquaculture. John Wiley & Sons.

United States Geological Survey. An Introduction to Hood Canal. Retrieved from http://wa.water.usgs.gov/projects/hoodcanal/data/HC.pdf on 2/23/16.

Voss, M., Baker, A., Hermann, W. B., Conley, D. J., Deutsch, B., Engel, A., ... & Grizzetti, B. (2011). Nitrogen processes in coastal and marine ecosystems. The European Nitrogen Assessment: Sources, Effects and Policy Perspectives, 1, 147-176.

Warner, M. J., Kawase, M., & Newton, J. A. (2001, February). Recent studies of the overturning circulation in Hood Canal. In Proceedings of the 2001 Puget Sound Research Conference, Puget Sound Action Team, Olympia, WA.

Washington Department of Fish and Wildlife (2015). Clams: manila clams. Retrieved from:http://wdfw.wa.gov/fishing/shellfish/clams/manila_clams.html

Western Washington University. Ecology of Harmful Macroalgal Blooms. Retrieved from: http://www.wwu.edu/algae/eblooms.htm

Winter, D. F., Banse, K., & Anderson, G. C. (1975). The dynamics of phytoplankton blooms in puget sound a fjord in the northwestern united states. Marine Biology, 29(2), 139-176.

Zanden, M., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. Limnology and oceanography, 46(8), 2061-2066.

Zertuche-González, J. A., Camacho-Ibar, V. F., Pacheco-Ruíz, I., Cabello-Pasini, A., Galindo-Bect, L. A., Guzmán-Calderón, J. M., ... & Espinoza-Avalos, J. (2009). The role of Ulva spp. as a temporary nutrient sink in a coastal lagoon with oyster cultivation and upwelling influence. Journal of Applied Phycology, 21(6), 729-736.

Appendices

APPENDIX A: Carbon and Nitrogen raw data and atomic ratios

C Amount (ug)	N Amount (ug)	C/N mass ratio	C/N atomic ratio	ID number
Ulva spp.				
443.77	57.9	7.66	6.57	1-CU-270
461.22	54.71	8.44	7.23	1-CU-05
468.47	38.39	12.2	10.46	1-CU-32
610.92	53.65	11.39	9.76	2-CU-32
555.17	48.24	11.51	9.86	2-CU-270
257.63	24.8	10.39	8.9	2-CU-02
324.52	24.14	13.44	11.52	3-CU-32
319.53	39.45	8.1	8.53	3-CU-270
489.41	49.17	9.95	8.31	3-CU-60
365.03	37.64	9.7	9.1	4-CU-05
550.33	51.84	10.61	11.58	4-CU-02
546.62	40.49	13.5	7.98	4-CU-32
340.22	36.51	9.32	7.71	5-CU-60
397.43	44.19	8.99	10.36	5-CU-270
421.13	34.83	12.09	6.92	5-CU-02
297.28	36.82	8.07	6.75	6-CU-60
812.12	103.09	7.88	6.69	6-CU-05
528.27	67.77	7.8	11.82	6-CU-32
507.28	36.8	13.78	11.85	1-U-36
422.16	30.5	13.84	12.05	1-U-28
592.63	42.13	14.07	12.2	1-U-04
716.63	63.927	11.21	9.61	2-U-04
226.03	15.88	14.23	12.2	3-U-28
374.07	23.45	15.95	13.67	4-U-36
550.5	39.45	13.95	11.96	5-U-88
655.97	34.4	19.07	16.35	6-U-227
V. philippinarium				
808.27	124.65	6.48	5.56	1-C-228
794.50	131.96	6.02	5.16	1-C-213
689.42	106.11	6.49	5.57	1-C-402
805.05	133.64	6.02	5.16	1-CCU-60
562.21	100.41	5.6	4.8	1-CCU-05
719.17	118.94	6.05	5.19	1-CCU-32
697.96	115.13	6.06	5.2	2-C-228
885.58	129.47	6.84	5.86	2-C-92
537.46	98.64	5.45	4.67	2-C-402
290.70	56.57	5.14	4.41	2-CCU-32

568.95	104.33	5.45	4.67	2-CCU-60
702.19	120.34	5.84	5	2-CCU-02
573.02	105.04	5.46	4.68	3-C-99
607.93	106.56	5.7	4.89	3-C-213
720.66	118.71	6.07	5.2	3-C-92
703.23	131.40	5.35	4.59	3-CCU-270
490.04	93.77	5.23	4.48	3-CCU-60
569.25	103.85	5.48	4.7	3-CCU-02
565.37	104.10	5.43	4.66	4-C-228
614.81	121.18	5.07	4.35	4-C-270
524.94	107.53	4.88	4.18	4-C-99
593.35	119.72	4.95	4.25	4-CCU-02
566.94	102.16	5.55	4.76	4-CCU-32
550.99	123.37	4.47	3.82	4-CCU-05
596.60	99.61	5.99	5.13	5-C-228
666.73	130.00	5.13	4.4	5-C-99
698.21	124.54	5.61	4.81	5-C-92
630.16	127.55	4.94	4.24	5-CCU-05
613.15	120.67	5.08	4.36	5-CCU-32
469.47	93.35	5.03	4.31	5-CCU-60
751.06	117.64	6.38	5.47	6-C-402
549.36	115.67	4.75	4.07	6-C-213
692.86	144.11	4.81	4.12	6-C-99
626.12	115.14	5.44	4.66	6-CCU-02
700.07	136.27	5.14	4.4	6-CCU-60
546.77	109.88	4.98	4.27	6-CCU-05
Phyto-POM				
668.87	122.25	5.47	4.69	P-1
1916.68	373.25	5.14	4.4	P-2
569.88	118.75	4.80	4.11	P-3
2568.18	452.16	5.68	4.87	P-4
375.23	63.05	5.95	5.1	P-5

APPENDIX B. Stable isotope raw data

ID number	δ13C	δ15N
Ulva spp.		
1-CU-270	-16.42	7.63
1-CU-05	-16.52	8.16
1-CU-32	-16.62	7.88
2-CU-32	-14.60	7.95
2-CU-270	-10.94	8.28
2-CU-02	-14.33	8.05
3-CU-32	-10.90	8.86
3-CU-270	-9.90	8.67
3-CU-60	-10.85	8.23
4-CU-05	-9.79	8.72
4-CU-02	-9.22	8.51
4-CU-32	-9.03	8.57
5-CU-60	-12.70	9.23
5-CU-270	-12.19	9.64
5-CU-02	-11.58	9.34
6-CU-60	-10.69	8.10
6-CU-05	-10.85	8.23
6-CU-32	-11.90	8.41
1-U-36	-17.08	8.15
1-U-28	-16.28	7.18
1-U-04	-12.29	8.90
2-U-04	-16.32	7.65
3-U-28	-14.57	8.21
4-U-36	-12.40	8.90
5-U-88	-13.95	9.18
6-U-227	-12.95	8.26
V. philippinarium		
1-C-228	-19.50	7.67
1-C-213	-19.15	7.85
1-C-402	-19.67	7.56
1-CCU-60	-19.10	7.59
1-CCU-05	-18.79	7.91
1-CCU-32	-19.21	7.77
2-C-228	-19.49	7.48
2-C-92	-20.41	7.47
2-C-402	-19.32	7.53
2-CCU-32	-18.91	7.61
2-CCU-60	-19.00	7.60
2-CCU-02	-19.43	7.61

3-C-99	-20.54	7.50
3-C-213	-20.62	7.67
3-C-92	-21.06	7.42
3-CCU-270	-19.43	7.56
3-CCU-60	-18.96	7.85
3-CCU-02	-20.56	7.65
4-C-228	-21.01	7.57
4-C-270	-20.40	7.67
4-C-99	-21.78	7.10
4-CCU-02	-20.60	7.60
4-CCU-32	-21.66	7.36
4-CCU-05	-20.03	7.58
5-C-228	-22.69	7.18
5-C-99	-21.64	7.36
5-C-92	-21.97	7.37
5-CCU-05	-20.90	7.45
5-CCU-32	-22.23	7.15
5-CCU-60	-21.28	7.28
6-C-402	-23.01	7.57
6-C-213	-21.27	7.47
6-C-99	-21.49	7.46
6-CCU-02	-21.97	7.40
6-CCU-60	-21.23	7.42
6-CCU-05	-21.67	7.48
phyto-POM		
P-1	-15.52	8.36
P-2	-14.22	8.84
P-3	-13.92	8.20
P-4	-14.44	7.88
P-5	-17.36	8.22

APPENDIX C: Detailed materials and methods

Field Data Collection

*All data to be collected on tide run of <0.0 ft

Materials:

- *15x Manila clam bags covered in Ulva: bags must be of 1/2 in mesh (industry standard) and partially submerged in intertidal sediment*
- *1,500 Manila clams*: clams of intermediate age/size (clams=1.5 yr)
- *2x 200 mL dark plastic bottles*: *Bottles must be rinse thoroughly 3x with DI water. They must then be submerged in a 1.2M acid bath for 24 hours prior to sample collection. Bottles must be re-rinsed 3x with DI water after acid bath.*
- *2x 2L bottles: bottles must be rinsed 3x with DI water and 3x with seawater*

before sample collection

- *Phytoplankton net: rinse net with 3x with DI water before sample collection to clean and after sample collection to wash remnant phytoplankton into collection bottle*
- *2x Hand Calipers: 1x electronic, 1x manual*
- *3x gallon ziplock bags: bags must be thoroughly rinsed clean in standard water prior to sample collection*
- *1 large plastic tub*: *able to hold ~150 manila clams*
- *20 small ziplock bags*

Site Preparation:

1) Designate one row of 15 clam bags containing 1.5 year old clams
2) Remove bags from sediment and standardize 10 bags to hold 150 manila clams
3) Remove all clams from 5 bags, replace with several heavy rocks for weight (designate bags "Ulva only" and mark with blue zip-tie)
4) Randomize bags and return to original location in the sediment
5) Remove *Ulva* from 5 randomized clam bags (designate bags "clam only" and mark with pink zip-tie)
6) Remaining 5 bags will be designated "both" and marked with a yellow zip-tie
7) Attach a numerical marker to each of the 15 bags
8) Leave experiment for one week before collecting the first round of data

Data collection:

Clams:

1) Every other week measure clam growth in each of the 10 clam bags
2) Dump each bag into plastic tray to count
3) Count off 10 clams, measuring the height and width of every tenth clam with calipers until all clams are counted
4) Record measurements and total number of clams in bag. Also record mortalities. Remove dead clams from the bag.
5) Collect three random individuals from from each bag to put in ziplock container
6) Store individuals on ice, avoiding direct contact with ice

Ulva:

1) Collect Ulva samples (>5g) from the 10 bags weekly
2) Samples will be placed in small ziplock bags and transported on ice, avoiding direct contact with ice
3) Weekly, note Ulva abundance on bags (Low, med, high)
4) Qualitatively match "low, med, high" coverings on 10 treatments to "low, med, high" coverings on rows outside of experiment
5) Scrape a representative sample of each of the three categories from external bags
6) Put each sample in large ziplock bag
7) Transport on ice

ISOTOPE METHODOLOGY

UC Davis Stable Isotope Facility Protocol

Materials:

- *2 x plastic leader bottles: Bottles must be rinse thoroughly 3x with DI water. They must then be submerged in a 1.2 M acid bath for 24 hours prior to sample collection. Bottles must be re-rinsed 3x with DI water after acid bath.*
- *20 x GF/F 47 micrometer filters: Filters must be stored in an aluminum foil pouch and combusted at 450 degrees C in a muffler furnace for 4.5 hours. After combustion, pouch must be stored in a dry, isolated place.*
- *20 x (10x10) squares of aluminum foil: foil must be stored in a larger foiled pouch and combusted with the GF/F filters*
- *Vacuum stopper & vacuum column: The top of the stopper, as well as both openings on the column must be wrapped in tin foil. The wrapped pieces must be put in a 500 degree C oven for 4 hours.*
- *Dissection tools: All dissection tools and tray must be sterilized (rinsed with 70% ethanol) before touching the organisms.*
- *54 x small glass petri dishes: Cover glass the openings of petri dishes in foil. Combust in muffle furnace at 500 degrees C for 4 hours.*
- *Metal scupula: Wash with lab soap between samples. Follow with DI water rinse. Rinse with 70% ethanol. Repeat between samples*
- *Small cork border: Wash with lab soap between samples. Follow with DI water rinse. Rinse with 70% ethanol. Repeat between samples*
- *2x forceps: Wash with lab soap between samples. Follow with DI water rinse. Rinse with 70% ethanol*
- *Mortar and pestle: Wash with lab soap between samples. Follow with DI water rinse. Rinse with 70% ethanol Repeat between samples.*
- *150x 5x8 mm Tin Capsules: Combust at 500 degrees C for 4 hours inside a 200 mL glass beaker covered in foil.*
- *2x 96-well tray: Assign unique name to trays. Group samples of similar material together.*
- *1x 48 well tray*
- *crushing rod*
- *cup holder*

V. Phillippinarium

Freezer Prep:

1) In the lab, put samples from each treatment into separate labeled 20 oz tupperware
2) Samples must be completely submerged in filtered seawater for at least 24 hours to allow for the cleansing of gut contents
3) The 10 treatments will require 4 L of seawater which will be filtered through a course mesh
4) Using nitrile gloves, organisms must be moved to a sterilized dissecting tray
5) A sterilized dissecting tool must be inserted into the partially open clam under water and the abductor muscles carefully cut with sterilized dissecting scalpel
6) The inside of the organism must then be rinsed with DI water
7) After the gills are moved aside by a sterilized dissecting pin, the visceral mass must be located
8) Using sterilized dissection scissors, the membrane of the visceral mass must be carefully cut to expose the stomach gland

9) The stomach gland then must be properly removed using sterilized tweezers
10) The 3 stomach glands associated with a given treatment must then be transferred in to a properly labeled plastic bag to be frozen in a -20 degree C freezer until further processing
11) The dissecting tray must be rinsed with DI water and 70% ethanol between each separate dissection

Shipping Prep:
1) Remove samples from freezer and allow to de-thaw until sample separates from bag
2) Dry each sample in separate, labeled glass petri dish at 60 degrees C for 24 hrs
3) Pulverize each sample separately in mortar and pestle
4) Use scupula to weigh 1.25 mg of dry sample from mortar into Sn capsule
5) Secure material inside of capsule
6) Organize capsules into 96-well tray leaving no empty wells between samples
8) Secure small samples by placing an index card (cut to size) over wells before securing lid

Ulva spp.
Freezer Prep:
Approximately >5 grams of ulva should be collected randomly from the tops of the ten treatments and put in fresh, labeled plastic bags
Additionally, high, medium, and low coverage clam bags must be identified to qualitatively reflect the amount of ulva on treatment bags in the same growing area
All the ulva must be removed and stored in separately labeled plastic bags from these clam bags once every two weeks
All ulva samples contained in the plastic bags must be transported back to the lab on ice
Samples for isotopic analysis must be removed using nitrile gloves and rinsed with DI water
Isotope samples must then be placed into fresh, properly labeled miniature plastic bags to be frozen in a -20 degree C freezer until further processing
Qualitative Ulva abundance samples must be removed from bags and rinsed 3x with water
After the final rinse, all excess water must be removed from samples by manually compressing tissue until no runoff is observed
These 3 samples must then be placed on foil and dried at 60 degrees C for 24 hours before weighing.

Shipping Prep:
1) Remove samples from freezer and allow to de-thaw until sample separates from bag
2) Dry each sample in separate, labeled glass petri dish at 60 degrees C for 24 hrs
3) ulverize each sample separately in mortar and pestle
4) Use scupula to weigh 2.0 mg of dry sample from mortar into Sn capsule
5) Secure material inside of capsule
6) Organize capsules into 96-well tray leaving no empty wells between samples
8) Secure small samples by placing an index card over wells before securing lid

Phytoplankton

Freezer Prep:

1) Sample must be collected using a 20 micrometer plankton net to capture the water.
2) The water must be drained from the bottom of the net to fill 2 dark plastic liter bottles
3) The bottle must be stored on ice during transport to the lab
4) To prep for vacuum filtration of the water sample, all pre-combusted glassware must be thoroughly cleaned with ethanol
5)The apparatus will include a cylinder clamped on top of GF/F filter which rests on a stopper platform.
6) The stopper will seal a 1000 mL flask
7) The first flask will be connected to a second waste trap 1000 mL flask by a rubber hose. The rubber hose will be attached atop a second dual valve stopper. This stopper will also be connected to a vacuum by a hose
8) The vacuum must be turned on slowly before pouring the contents of the liter bottle through the filer
9) After the liquid has passed through the filter, the column must be unclamped, and the G/F filter removed by sterilized tweezers and placed into a pre-combusted 10x10 cm sheet of aluminum foil.
10) Fold the aluminum foil into a pouch around the filter using sterilized forceps
11) Place pouch in a 60 degree C drying oven for 24 hours
12) After contents have dried, fold filter into quarters using sterilized forceps
13) Re-wrap pouch and place in medium-sized desiccator until analysis

Shipping Prep:

1) Using sterilized forceps remove filter from foil pouch
2) Use sterilized hole punch to remove a piece of filter
3) Place circular piece into tin capsule
4) Secure material inside of capsule
5) Organize capsules into 96-well tray leaving no empty wells between samples
6) Secure small samples by placing an index card over wells before securing lid