

INVESTIGATING THE COLLECTIVE EFFECT OF TWO OCEAN ACIDIFICATION ADAPTATION STRATEGIES ON JUVENILE CLAMS (*VENERUPIS PHILIPPINARUM*)

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A Swinomish Indian Tribal Community Contribution
SWIN-CR-2017-01
September 2017
La Conner, WA 98257

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A thesis
submitted in partial fulfillment of the
requirements for the degree of

Master of Marine Affairs

University of Washington

2017

Committee:

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Program Authorized to Offer Degree:

School of Marine and Environmental Affairs

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Abstract

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Anthropogenic CO₂ emissions have altered Earth's climate system at an unprecedented rate, causing global climate change and ocean acidification. Surface ocean pH has increased by 26% since the industrial era and is predicted to increase another 100% by 2100. Additional stress from abrupt changes in carbonate chemistry in conjunction with other natural and anthropogenic impacts may push populations over critical thresholds. Bivalves are particularly vulnerable to the impacts of acidification during early life-history stages. Two substrate additives, shell hash and macrophytes, have been proposed as potential ocean acidification adaptation strategies for bivalves but there is limited research into their effectiveness. This study uses a split plot design to examine four different combinations of the two substratum treatments on juvenile *Venerupis philippinarum* settlement, survival, and growth and on local water chemistry at Fidalgo Bay and Skokomish Delta, Washington. Results show no macrophyte or shell hash treatment effect on *V. philippinarum* settlement or survival. A significant macrophyte treatment effect was detected on

clam growth, with mean length higher when macrophytes were absent regardless of the presence or absence of shell hash. Additionally, the macrophyte treatment appeared to have an opposite effect on pH than was anticipated, where pH was higher outside of macrophyte beds than inside. Although these results do not support the use of either treatment as an ocean acidification adaptation strategy, the mixed results reported in the literature for both treatments highlight the nascent nature of this research. As atmospheric CO₂ concentrations continue to increase, there is an exigent need for additional studies to determine the specific conditions under which these strategies might help produce conditions conducive to settlement, growth, and survival of bivalves and other calcifying organisms. Such research could help guide local adaptation actions, especially among resource-dependent communities that rely on sustainable fisheries for their health and well-being.

Acknowledgments

First and foremost I would like to thank Swinomish Tribe for providing me with the generous opportunity and support to pursue this degree. This project would not have been possible without the foresight and progressive stance of the Tribal Senate and Fisheries Manager, Lorraine Loomis, to protect the region's natural resources. I would like to thank Terrie Klinger and Jennifer Ruesink for their guidance and support throughout this entire process. I greatly appreciate the knowledge, expertise, and time they have shared with me. It has been an invaluable experience working with them. I would also like to thank Micah Horwith at the Dept. of Natural Resources for allowing me to use the ANENOME project sites and water property data. Thank you to Samish Indian Nation and Skokomish Indian Tribe for permitting me access to their tribal land. I am grateful to Julie Barber and the rest of the Shellfish Department for helping me actualize this project and provide support in and out of the field. I would like to thank my parents and friends for their support and encouragement over the years as well as their countless hours sewing clam bags, smashing shells, and hauling gear into the field. Finally, I would like to thank my spouse. I could never have completed this work without his patience, enthusiasm, and unending support. Funding for this project was provided by the Bureau of Indian Affairs Rights Protection Implementation Climate Change Grant.

Introduction

The atmospheric concentration of carbon dioxide (CO₂) and other greenhouse gases has reached unprecedented levels due to human activity. During the 400,000 years prior to the industrial revolution, atmospheric CO₂ concentrations ranged from 200-280 parts per million (ppm). In 2011, atmospheric CO₂ concentrations reached ~430 ppm with the highest emissions occurring from 2000 to 2010. This sharp change in atmospheric composition has led to an uptake of energy

that has influenced Earth's climate system and resulted in warmer land and ocean surface temperatures, melting sea ice, and rising sea levels (IPCC 2014). While global warming has been moderated due to the absorption of anthropogenic emissions by the ocean, the hydrolysis of CO₂ with seawater has altered the carbonate chemistry of the ocean causing ocean acidification (OA) (IPCC 2014). The impacts of OA include reduced pH, the availability of carbonate ions, and the saturation states of biominerals necessary for calcifying marine organisms to build and maintain shells (Feely et al. 2010; Hofmann et al. 2014; Waldbusser et al. 2015). Since the beginning of the industrial era surface ocean pH has decreased by 0.1, which is equivalent to a 26% increase in acidity (Adelsman and Binder 2012; IPCC 2014). Current projections predict a further 0.3-0.4 decrease in pH by 2100 as atmospheric CO₂ concentrations approach 800ppm (Feely et al. 2004; Caldeira and Wickett 2005; Orr et al. 2005).

While the biological effects of high CO₂ in seawater are variable across and within species, studies have found deleterious trends among calcified organisms including decreases in survival, calcification, growth, development, and abundance (Kroeker et al. 2013; Gazeau et al. 2013; Somero et al. 2016). The vulnerability of these shell-forming organisms is greater in early life-history stages. During embryogenesis, bivalves are highly sensitive to changes in seawater because they are most exposed to ambient conditions while forming their initial shell, prodissoconch I, and lack the specialized ion-regulatory epithelia required to maintain an acid-base balance. Decreases in seawater pH and saturation states during this period can increase energy demands in order for larvae to sustain rapid calcification. Because larvae rely almost exclusively on maternal energy reserves for both embryogenesis and metamorphosis, low lipid levels can result in increased failure of settling larvae (Talmage and Gobler 2011; Waldbusser et al. 2013). Moreover, the initial biomineral deposited in the shell forming process is composed

primarily of amorphous calcium carbonate which is particularly susceptible to dissolution when exposed to depressed saturation states (Lannig et al. 2010; Parker et al. 2013; Waldbusser et al. 2015). Under current seawater conditions, shell dissolution has been proposed as a critical factor contributing to high mortality rates observed among newly settled juveniles. Due to the decomposition of organic matter within bottom sediments, the benthic environment juveniles inhabit can be undersaturated with respect to calcite and aragonite (Aller 1982; Green et al. 2009). Studies examining bivalve behavior in porewater with low pH levels have found reduced burrowing activity and higher dispersal rates (Clements et al. 2016). Although adequate food supply can offset additional energetic costs caused by environmental stress (Melzner et al. 2011; Hettinger et al. 2013; Parker et al. 2013), a recent study found that feeding rates are also sensitive to increases in pCO₂ (Waldbusser et al. 2015). Energetic deficits in early life-history stages may affect subsequent population dynamics either through a reduction in successful recruitment into adult populations (Parker et al. 2013) or through negative carry-over effects that may impair the fitness of adult populations (Hettinger et al. 2013).

In coastal environments of the North Pacific, OA is superimposed on seawater conditions that naturally have low carbonate saturation and pH levels due to processes such as upwelling and watershed dynamics (Feely et al. 2010; Somero et al. 2016). Within the Salish Sea, a semi-enclosed estuary in the Pacific Northwest, additional factors, including eutrophication and restricted circulation, further exacerbate the corrosive conditions by creating warm, hypoxic pockets of water (Feely et al. 2010). Interactions between multiple stressors suggest that the occurrence of extreme events is likely to increase in frequency, duration, and magnitude, unlike the gradual shifts that are anticipated in the open ocean (Waldbusser and Salisbury 2014). Even though intertidal species currently experience semidiurnal fluctuations which can produce

seawater conditions that exceed likely projections for 2100, there is evidence that some organisms are approaching their physiological tolerance limits (Somero 2010). Additional stress from abrupt shifts in carbonate chemistry may push populations over critical thresholds and manifest as major changes in ecosystem health (Feely et al. 2010). Laboratory studies suggest modern bivalves may be experiencing selection pressure applied by OA since the industrial revolution (Talmage and Gobler 2011). In the past decade, deleterious impacts in the Pacific Northwest have been observed as high oyster larvae mortalities in hatcheries (Barton et al. 2015) and dissolution in pteropod shells (Bednaršek et al. 2014). A recent analysis of local clam populations has also detected a decline in three native species over the past 29 years, although no specific cause has been identified (Barber et al. in preparation).

These negative trends threaten the livelihoods of communities that depend on healthy coastal ecosystems for marine resources and sustainable fisheries (Cooley et al. 2009; Hale et al. 2009). For example, Coast Salish tribes have relied on the marine environment for physical, cultural, and economic sustenance since time immemorial (Lynn et al. 2013; NWIFC 2016). Clams, in particular, have been an important traditional food and still hold significant meaning in ceremonies (Augustine and Dearden 2014; Deur et al. 2015; Garibaldi and Turner 2004). In addition to providing a reliable food source, activities like harvest and management provide opportunities for cultural values and practices to be passed between generations (Augustine and Dearden 2014; Deur et al. 2015; Poe et al. 2016). The loss of availability and access to culturally important species, such as clams, can directly affect tribal health and well-being (Menezes 2001; Poe et al. 2016; Donatuto et al. 2014). This results in disproportionate challenges for indigenous communities that are already socioeconomically disadvantaged (Lynn et al. 2013; McOliver et al. 2015).

Although the severity and extent of climate change impacts may be minimized by stabilizing current greenhouse gas concentrations, the chemical and biological effects of OA will continue to persist due to the inertia in the climate-carbon system (Archer et al. 2009; IPCC 2014). In terms of ocean chemistry, the time required to return to pre-industrial conditions is on the order of thousands of years (Rau et al. 2012). Therefore, as global and national consensus stymie CO₂ mitigation efforts, action at the local level may provide a faster and more feasible approach for resource-dependent communities to address OA (Adelsman and Binder 2012; Rau et al. 2012; Billé et al. 2013). Identifying appropriate adaptation efforts at the local scale also allows for place-based strategies that address local drivers and socio-ecological vulnerabilities (Ekstrom et al. 2015). However, there are few resources available to guide adaptation efforts (Rau et al. 2012). The majority of scientific literature remains focused on measuring and monitoring climate change-related stressors on specific species and communities. While these investigations are essential to better understand the consequences of global change, the lack of research on proactive strategies limits actions local communities can implement immediately in order to help protect valuable marine resources. Moreover, delay in action may jeopardize the effectiveness of future actions because a continued rise in greenhouse gas emissions will require even greater remediation (Rau et al. 2012; IPCC 2014).

Despite the scarce resources, two working hypotheses regarding bivalve cultivation are garnering attention in the scientific community as potential OA adaptation strategies. The first strategy is the addition of shell hash to clam beds. The general practice of beach coarsening with crushed shell and gravel is a common tactic among shellfish growers and managers to promote natural recruitment in hardshell clams like *Leukoma staminea* and *Venerupis philippinarum*. Studies of substrate additives also suggest that increasing beach grain size enhances post-

settlement survival and growth by altering predator and prey behavior (Thompson et al. 1995; Ruesink et al. 2014). However, the addition of shell may further enhance the benefits of beach coarsening for newly settled clams in increasingly acidic conditions. The presence of crushed shell can increase and stabilize pH and saturation states in porewater which can create a local buffering effect (Green et al. 2009; Clements and Hunt 2014). Laboratory and field experiments on juvenile clams have found higher recruitment and survival in sediment buffered with crushed shell (Green et al. 2009, 2013; but see Ruesink et al. 2014). There is also evidence of shell hash being used as part of an ancient indigenous practice, called clam gardening, to enhance clam bed productivity in British Columbia. The recently rediscovered mariculture technique includes the construction of an intertidal terrace with substratum that is predominantly comprised of shell hash (Harper et al. 1995; Caldwell et al. 2012). Researchers have found significantly higher growth rates of juvenile *L. staminea* and higher post-settlement abundance within the gardens than in non-walled beaches (Grosbeck et al. 2014; Jackley et al. 2016). Although the exact mechanisms at play have yet to be identified, the growing body of research supporting the benefits of shell hash as a substrate additive is building a strong case for implementation as an adaptation strategy (Green et al. 2009; Adelman and Binder 2012; Rau et al. 2012).

A second potential adaptation action that could ameliorate detrimental effects from OA and climate change is the restoration or introduction of marine macrophytes near shellfish beds (Adelman and Binder 2012; Billé et al. 2013). While the structural presence of emergent macrophytes such as seagrasses, kelps, and seaweeds can provide shelter for bivalves from predators and desiccation (Peterson et al. 1984; Coleman and Williams 2002; Mumford 2007), recent studies suggest macrophytes may also act as a chemical refuge for calcifying organisms. Biological activity within productive seagrass, kelp, and algae beds has the potential to increase

pH and aragonite saturation levels (Hendriks et al. 2014; Hendriks et al. 2015). Hendriks et al. (2014) reported a 0.24 unit increase in pH during peak seagrass production, a change large enough to facilitate calcification processes. The authors report a strong correlation between diel pH variability and oxygen concentrations, indicating that metabolic activity is a main driver influencing carbonate chemistry in seagrass canopies. Although additional mechanisms associated with the presence of macrophytes, such as reduced water flow and food delivery within macrophyte beds (e.g. Carroll and Peterson 2013), may dampen the beneficial buffering effect for bivalve communities there could still be great potential in coupling macrophytes with clam beds. The use of macrophytes as a phytoremediation technique would be particularly advantageous because photosynthesizing organisms may be less affected by increased CO₂ concentrations (Adelsman and Binder 2012; Harvey et al. 2013; Kroeker et al. 2013).

The efficacy of both macrophytes and shell hash as adaptation strategies is yet to be determined. Due to the complexities of the coastal environment and dynamic interactions between stressors, robust results are lacking regarding the degree and magnitude of influence of either strategy on local seawater chemistry and bivalve survival. Additional research is necessary to better understand their potential as effective adaptation tools and determine appropriate implementation practices to maximize ecological benefits. For instance, it is possible that combining these two strategies may be more effective than using either strategy in isolation, especially under future ocean conditions. Previous research conducted by Ruesink et al. (2014) assessed the effect of crushed shell as part of a substrate treatment with crushed rock and the removal of eelgrass on *V. philippinarum* density. Compared to sandy sediments, the authors found higher clam densities when the substrate was amended regardless of the presence or absence of eelgrass. To expand upon this finding as a prospective OA adaptation strategy, I

investigated the potential benefits of applying four different combinations of shell hash and macrophytes as a substrate treatment. I hypothesized that adding both shell hash and macrophytes would yield higher clam settlement, survival, and growth than adding either amendment in isolation. I further hypothesized that adding both shell hash and macrophytes would result in higher mean pH than adding either amendment alone.

Methods

Study organism and sites

Juvenile *V. philippinarum* were used to evaluate the biological effects of the treatment combinations. Commonly referred to as manila clams, *V. philippinarum* were introduced to the eastern Pacific in the 1930s (Magoon and Vining 1981) and have become a commercially important species for resource-dependent communities throughout the Salish Sea (Dethier 2006; Dumbauld et al. 2009). Optimum conditions for growth are temperatures between 13 and 21°C (Dethier 2006) and salinities between 16 and 33ppt (Numaguchi 1998). Although manila clams can tolerate a wider range of temperatures and salinities than the confamilial native species *L. staminea* (Family Veneridae) (Dethier 2006), they share similar life history and ecological attributes (Byers 2005). Therefore, studies of *V. philippinarum* may elucidate potential responses in other tribally-important clam species. The clams for this study were raised at the Taylor Shellfish hatchery in Kona, HI and shipped to the Quilcene, WA hatchery where they were held in ambient conditions until they were transported to the study sites. The mean length at the start of the study was 2.4mm (± 0.26 SD).

The study was conducted at two intertidal beaches, Fidalgo Bay (FB) and Skokomish Delta (SD), situated within the southern portion of the Salish Sea in Washington State (Figure 1).

These two locations were selected from among the study sites included in the Washington Department of Natural Resources (WA DNR) ANENOME program.

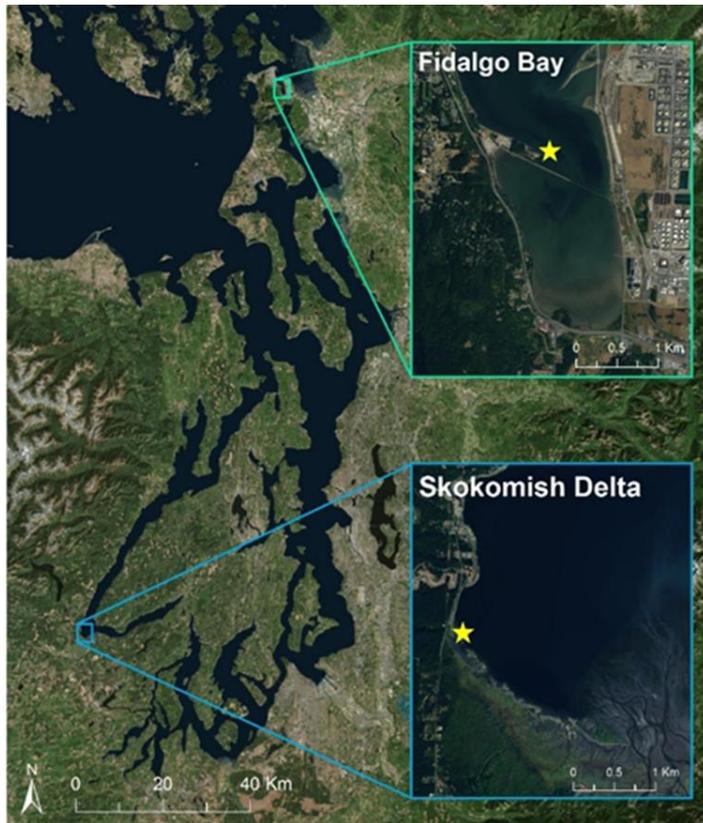


Figure 1. Map of Puget Sound study sites in WA. FB=Fidalgo Bay, SD=Skokomish Delta. Stars indicate location of field experiment.

Experimental design

To test whether clam settlement, survival, and growth varied with the different treatment combinations, a split plot design was employed. Sub-plots with the shell hash treatment were crossed within whole plots of the macrophyte treatment that were nested within a study site (Figure 2). The macrophyte treatment consisted of two levels, bare (B) and present (M). The treatment level was determined using similar criteria as WA DNR's ANENOME program. At each study site, one *bare* whole plot was delineated where no marine vegetation was on the

beach. A paired *present* whole plot was located where macrophytes (e.g., *Zostera spp.*, *Ulva spp.*, and *Lactuca spp.*) existed at least every 20cm throughout the entire plot. There were also two levels tested for the shell hash treatment in the sub-plots: shell (S) where broken shell was added and no shell (N) where no broken shell was added to local sediment. The shell hash was 70% *Crassostrea gigas* (Pacific oyster) and 30% native clam shell collected from the study location. All shell was crushed manually into pieces ranging from 5cm in length to a coarse powder. While oyster and clam shell are constructed from different mineral species, calcite and aragonite respectively, both have been used in prior studies examining the effects of shell hash (see Green et al. 2009; Ruesink et al. 2014).

In June 2016, a transect was placed normal to shore at -0.3 to -0.46m relative to MLLW within each whole plot at both study sites. The sub-plots consisted of sixteen 25cm x 25cm bags made of 1mm mesh screen that were placed along the transect every 2 meters and filled with local sediment. Each bag was randomly assigned a shell hash treatment level, eight with shell (S) and eight without added shell (N). The shell bags were filled with enough of the pre-crushed shell hash mix to create a 50:50 mix of shell hash to sediment. Approximately 100 of the 2.4mm *V. philippinarum* clams were added to each of the 16 bags. Then the bags were closed with zip ties to retain the clams and shell hash as well as reduce predation from smaller organisms such as *Hemigrapsus* spp. and set flush with the surface of the sediment. In order to deter predation from fish and seabirds, a 50cm x 50cm piece of anti-predator netting was staked into the substrate over each bag. To maximize the effect of the shell hash treatment, sub-plots with added shell also had shell hash mixed into the sediment surrounding the mesh bags under the predator net.

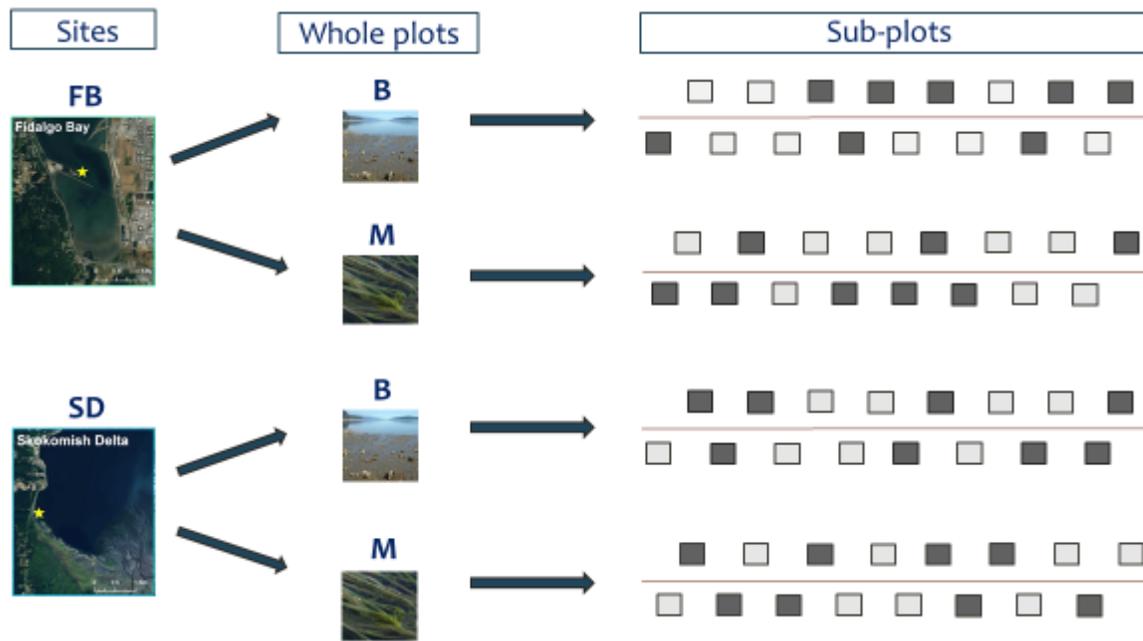


Figure 2. Split plot experimental design. Site: FB=Fidalgo Bay, SD=Skokomish Delta; Macrophyte treatment: B=bare, M=macrophytes present; Shell hash treatment: N=no shell hash added (dark square), S=shell hash present (light square).

After 55 days, the bags were retrieved and the contents were sieved using a series of 1mm, 4mm, and 8mm sieves. All clams determined to be alive at the time of retrieval were preserved in a 10% buffered formalin solution and later identified and enumerated. Lengths to the nearest 0.01mm were also recorded by measuring along the longest axis parallel to the shell hinge using digital calipers.

Juvenile settlement, survival, and growth

Because the individual clams within the bags were not independent subsamples, clam abundance and growth were analyzed using a single mean value from each sub-plot to assess treatment effects. *V. philippinarum* collected from each bag were divided into two categories relative to the initial size of outplanted clams. Individuals smaller than 2.4mm were assumed to have recruited naturally into the bags based on the typical seasonal recruitment patterns, while clams larger than

2.4mm were assumed to have been outplanted. Therefore, counts of clams <2.4mm were used to calculate an index of recruitment, counts of clams >2.4mm were used to generate an index of survival, and the lengths of clams >2.4mm were used to estimate growth. At Fidalgo Bay, the FBBS (bare of macrophytes, shell hash added) treatment combination had to be removed from the clam growth analysis because five of the eight sub-plots contained no *V. philippinarum* larger than 2.4mm, resulting in a sample size of less than five. Additionally, bags with fewer than 25 individuals larger than 2.4mm were eliminated from the growth analysis for statistical reasons, resulting in an unbalanced number of replicates in the remaining treatment combinations. A linear mixed effects model was applied to each biological factor to test for differences between treatment combinations in a manner that accounted for the imbalance as well as the crossed and nested structure of the experimental design. For all analyses, shell and macrophyte treatments and their interaction were considered fixed factors, with study site and whole plot (macrophytes present or bare) as random factors. Statistical analyses were performed in R version 3.3.1 (R Core Development Team) using lme, vegan, and ggplot packages.

Environmental parameters

At each site, WA DNR placed sensor packages in bare and eelgrass-dominated areas. The packages consisted of Durafet pH, CTD and DO sensors. The sensors measured water property data every ten minutes over the course of the study. At the end of field season WA DNR staff retrieved the sensors and downloaded the data for pH, temperature, and salinity data. Readings taken between June 22 and August 15 were extracted and used for qualitative analysis to assess general site characteristics over the duration of the study. Measurements collected while the sensors were exposed during extreme low tides were excluded from the analysis.

Results

Juvenile settlement, survival, and growth

No significant effect of macrophyte additions or shell hash additions was detected on clam settlement or survival (Table 1). Clam growth (as measured by shell length) was highest in treatments without added shell hash or macrophyte biomass (Figure 2). This effect was significant for macrophyte treatments but not for shell hash (Table 1). The effect of macrophyte additions on clam growth was negative: clams in plots where macrophytes were absent were larger than those in plots where vegetation was present regardless of shell treatment (Figure 2).

Table 1. Statistical results testing all four substrate treatment combinations at FB and SD on abundance of juvenile clams < 2.4mm, clams > 2.4mm, and mean clam length > 2.4mm. Linear mixed-effects models included beach (FB and SD) and macrophyte treatment as random effects.

Effect	Index of Settlement		Index of Survival		Mean Length	
	DF	F-value[P-value]	DF	F-value[P-value]	DF	F-value[P-value]
Macrophyte Treatment	1,40	3.02 [0.08]	1,50	1.50 [0.22]	1,50	55.44 [P<0.05]
Shell Hash Treatment	1,40	2.51 [0.11]	1,50	0.10 [0.76]	1,50	0.07 [0.80]
Macrophyte x Shell	1,40	0.61 [0.43]	1,50	0.62 [0.43]	1,50	0.28 [0.60]

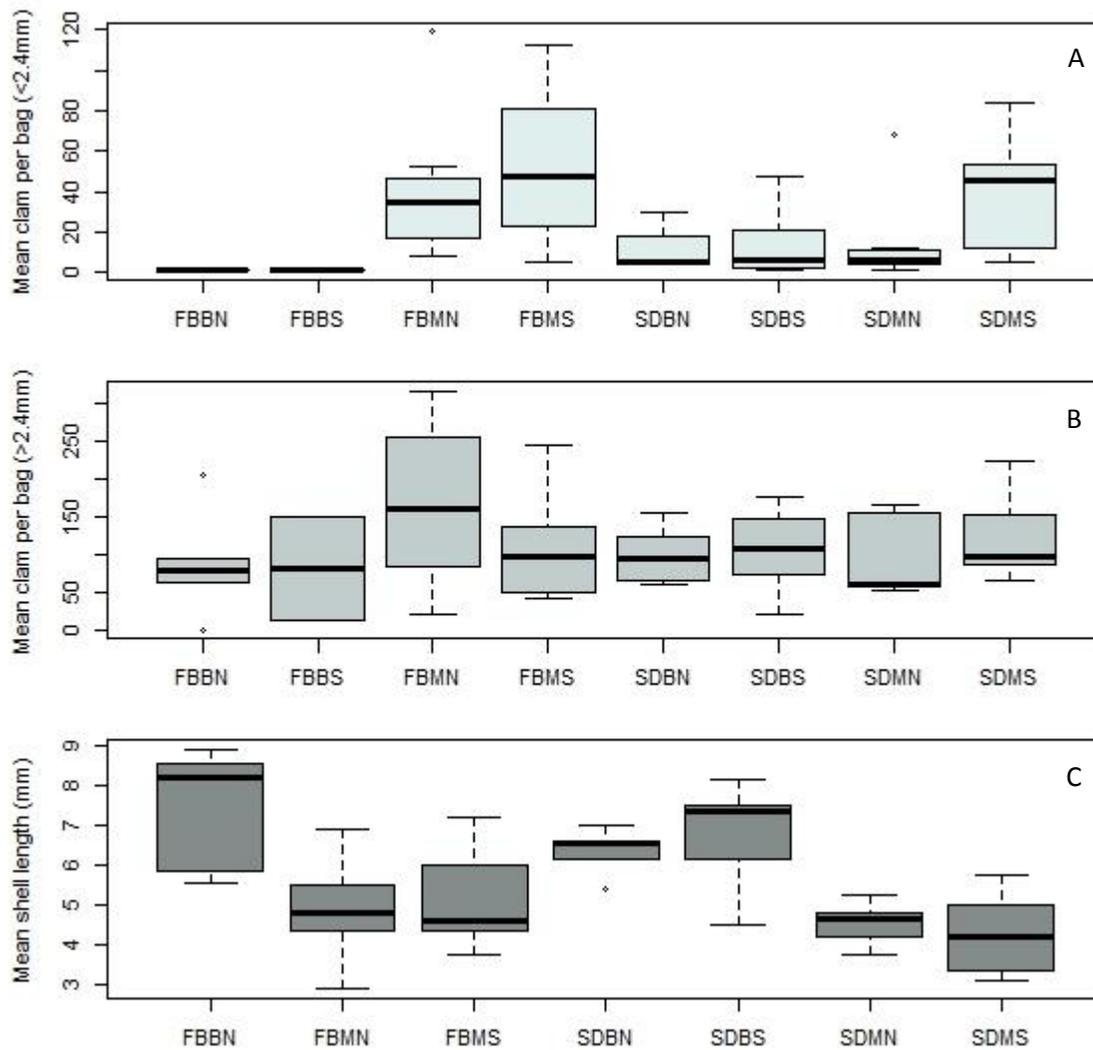


Figure 2. Box plots of the A) index of settlement, B) index of survival, and C) clam growth by substrate treatment combination. FB=Fidalgo Bay, SD=Skokomish Delta; B=bare, M=macrophytes present; N=no shell hash added, S=shell hash added.

Environmental parameters

pH varied between treatments within sites and between sites (Figure 3). Visual inspection of the pH data over the course of the experiment suggests that at both sites the median pH was lower in treatments where macrophytes were present compared with bare treatments. The range of variation in pH was greatest at FBM and the only site where acidic seawater was observed.

Seawater temperature over the experimental period was similar between treatments and sites (Figure 3). The range of temperature variation was greatest at SDB.

Salinities at FB were between 28 and 30 ppm. At SDB, salinity was anomalously low (<15ppm).

Salinity at SDM was higher, approaching values observed in FB, and had the greatest variation measurements.

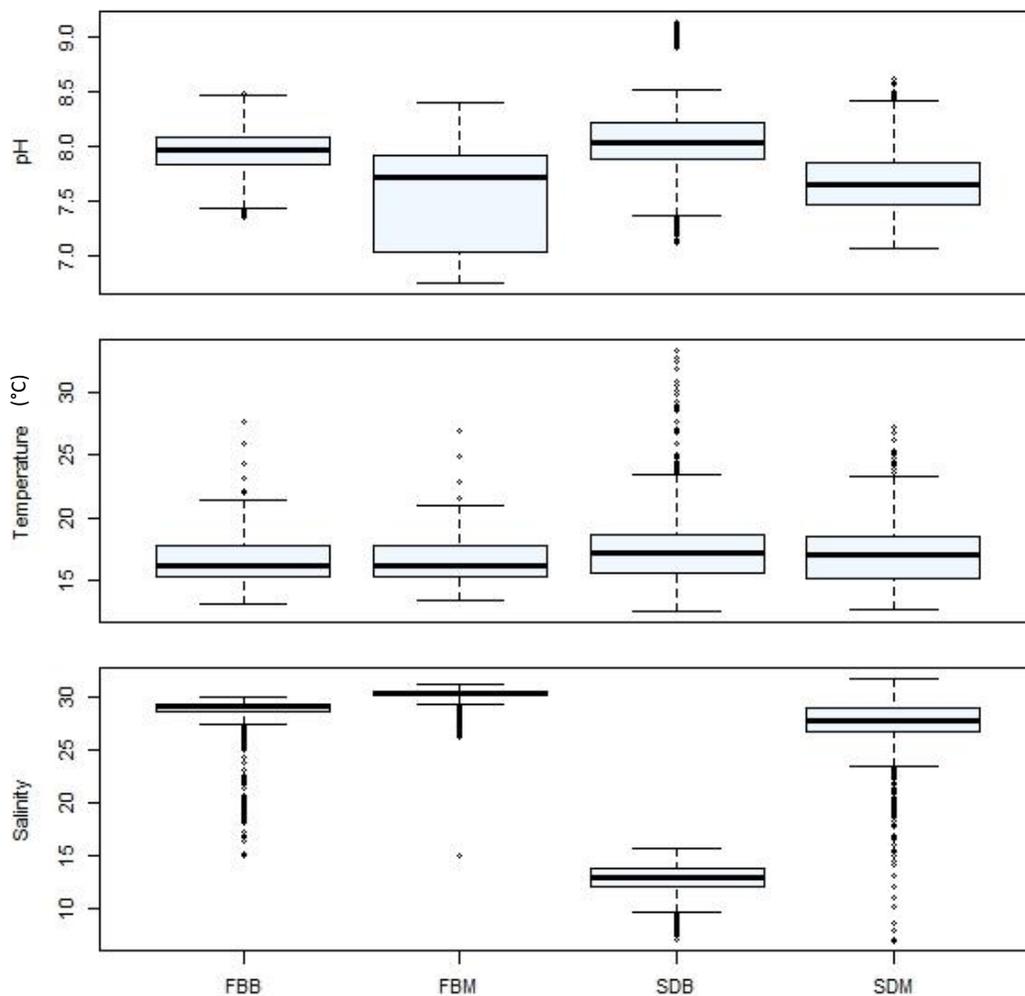


Figure 3. Box plots based on water property monitoring results from Fidalgo Bay and Skokomish Delta at the macrophyte treatment level (B=bare, M=macrophytes present) from June 22 – August 15, 2016.

Table 2. Water property monitoring results from Fidalgo Bay and Skokomish Delta at the macrophyte treatment level (B=bare, M=macrophytes present) from June 22 – August 15, 2016.

Site	Seawater temperature (°C)		Salinity		pH	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Fidalgo Bay - B	13.13-27.62	16.52 ± 1.8	15.01-29.98	28.16 ± 2.3	7.35-8.48	7.95 ± 0.2
Fidalgo Bay - M	13.43-26.87	16.56 ± 1.7	15.01-31.05	30.06 ± 0.8	6.75-8.41	7.56 ± 0.4
Skokomish Delta - B	12.46-33.33	17.16 ± 2.4	7.10-15.69	13.00 ± 1.2	7.12-9.14	8.14 ± 0.5
Skokomish Delta - M	12.64-27.22	16.94 ± 2.2	7.02-31.59	27.61 ± 1.9	7.07-8.61	7.66 ± 0.3

To examine the influence of photosynthesis and respiration on seawater pH, daytime measurements made between 4:00 p.m. and 7:00 p.m. were plotted separately from nighttime measurements made between 2:00 a.m. and 5:00 a.m. (Figure 4). Visual inspection of these data show higher pH during the day than at night. Additionally, daytime and nighttime pH at the bare sites where macrophytes were absent were higher than the respective measurements at the sites where macrophytes were present.

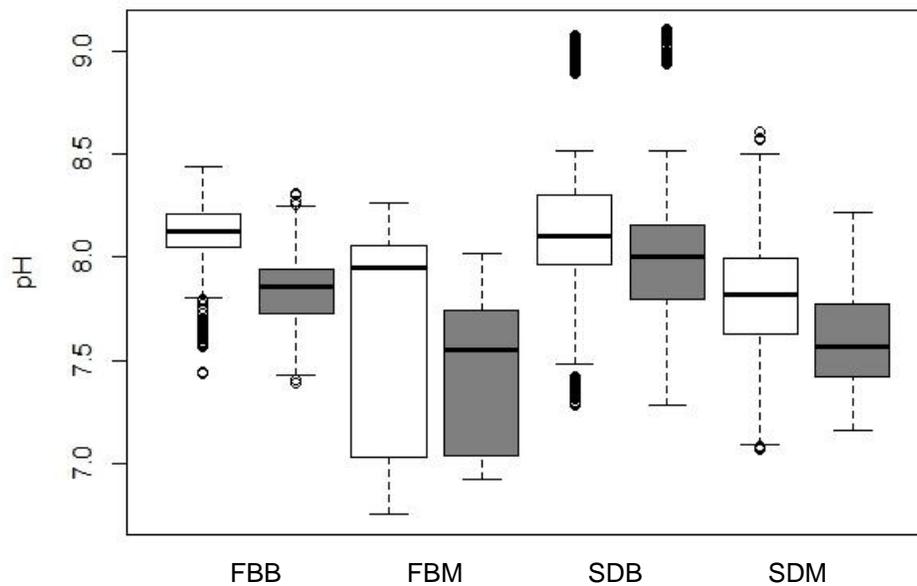


Figure 4. Box plots based on daytime (white) and nighttime (grey) pH levels at macrophyte treatment sites (B=bare, M=macrophytes present) in Fidalgo Bay (FB) and Skokomish Delta (SD).

Discussion

Experimental results give no indication of a positive influence of shell hash or macrophytes on growth or survival of juvenile clams. Moreover, such additions do not appear to increase local seawater pH. These results were consistent across the two study sites.

Even though the lack of shell effect on settlement supports the findings of Ruesink et al. (2014), the absence of a substrate effect on survival is inconsistent with the results of both

Ruesink et al. (2014) and Thompson (1995). Notably, all treatments in this study were covered with anti-predator netting, which reduces the role of structural protection from predators achieved by adding material of large grain size; this differed from the experimental configuration used by both Ruesink et al. (2014) and Thompson (1995). Furthermore, neither Ruesink et al. (2014) nor Thompson (1995) tested a treatment consisting only of shell hash. Crushed shell may not have the same effect as gravel due to different physical characteristics. Including a treatment that has comparable amounts of those found in traditional clam gardens may raise any shell hash effect to a detectable level and help elucidate mechanisms responsible.

These findings may reflect site-specific factors or changes in processes that were not controlled for in the study. For example, the experimental manipulations could have altered hydrodynamics, predator-prey interactions, sediment stability, and recruitment cues, which in turn could have influenced clam behavior and survival (Irlandi and Peterson 1991; Green et al. 2013; Clements and Hunt 2014). The high settlement index observed in the combined presence of macrophyte and shell hash could have resulted from the low flow characteristic typical of marine plant communities (Ginsburg and Lowenstam 1958) coupled with a recruitment cue from shell hash. However, the decline in the index of survival and mean growth in the combined macrophyte and shell hash treatments suggest that any initial recruitment benefits are quickly lost. This could potentially be due to burial of newly settled clams via deposition of fine-grained particles, which tends to be higher in seagrass beds than in areas lacking vegetation. The lower flow velocities characteristic of seagrass beds could also reduce food supply, although Irlandi and Peterson (1991) reported similar chl *a* concentrations inside and outside of seagrass beds, suggesting no effect of seagrass on planktonic food availability. Peterson et al. (1984) attributed high growth rates in *Mercenaria mercenaria* to the higher concentration of food particles

deposited in seagrass meadows due to the baffling effect created by *Z. marina* blades. Based on the biological findings from this experiment and the mixed results in the literature, there is a clear need for additional research to assess interactions between clams and macrophytes. Due to the variable responses observed across different life history stages, it may also be informative to monitor biological responses over a longer duration. Some species prioritize calcification over other biological processes and are capable of growing shell under low pH conditions at a cost to other metabolic functions (Wood et al. 2008). Incorporating later life stages into such experiments may allow better consideration of energetic consequences.

The treatment effects on water properties were predominantly inconsistent with expectations. The larger range in pH at sites where macrophytes were present as well as the measurements of higher pH in the daytime than at nighttime within each macrophyte site can be expected due to photosynthetic activity. However, the observation that median pH levels were higher at sites without macrophytes is perplexing. Photosynthesis affects pH by removing CO₂ from seawater during the day, thereby raising seawater pH (Hendriks et al. 2015). Results from this study show higher pH levels at sites *without* macrophytes than at sites with macrophytes during hours of active photosynthesis (Fig. 4). While this may partially be explained at the Skokomish Delta beach because of a freshwater input next to the macrophyte site, there was no such freshwater source at the Fidalgo Bay beach.

One important factor that affects the magnitude of metabolic influence on pH is the residence time of the water parcel exposed to the vegetation (Hendriks et al. 2014). The majority of low tides during the summer occur during mid-day, limiting the immersion time during daylight hours. Even though the three-hour period from 2:00-5:00 p.m. was least influenced by low tides, the exposure time may have been too limited to produce any detectable impact on

local water chemistry. Repeating the experiment within a more enclosed system such as a tidal lagoon could provide a better opportunity to test the effects of vegetation on seawater pH. Notably, large differences between the sites used in this study and those used by Hendriks et al. (2014) discourages direct comparisons between the two studies. The seagrass meadows studied by Hendriks et al. (2014) were intentionally selected to minimize the influence of watershed dynamics on pH, whereas watershed effects were substantial at the SD site used in this study. Moreover, the size and density of the vegetated areas in this study may not have been sufficient to produce a detectable signal. Collecting data on additional metrics such as dissolved oxygen and chl a in addition to pH may improve resolution in future studies.

Experimental results from this study do not support the use of shell hash or macrophytes as an adaptive strategy to promote growth or survival of juvenile clams. However, the findings do not suggest research on either strategy should be halted. Instead, the mixed results on shell hash and macrophytes highlight the nascent nature of this research. Additional studies are necessary to resolve the conflicting findings and determine appropriate applications and locations at which these strategies may effectively promote amiable conditions for bivalve populations. Subtle differences may have been obscured by the dynamic interactions characteristic of nearshore systems in Puget Sound. Such complex interactions and feedbacks between abiotic and biotic factors increase the difficulties inherent in identifying effective OA adaptation strategies. Nonetheless, identifying effective actions that can help ecologically important species acclimatize or adapt to these unprecedented changes is essential in order to sustain marine ecosystems as they now exist. Furthermore, it is imperative to the health and well-being of coastal communities dependent on a healthy marine environment for physical,

economic, and cultural sustenance. As human activity continues to release greenhouse gasses, the need to take action especially at the local level is ever more exigent.

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