# Habitat effects of macrophytes and shell on carbonate chemistry and juvenile clam recruitment, survival, and growth

Courtney M. Greiner[a,b,*], Terrie Klinger[b], Jennifer L. Ruesink[c], Julie S. Barber[a], Micah Horwith[d]

[a] Fisheries Department, Swinomish Indian Tribal Community, La Conner, WA 98225, USA
[b] School of Marine and Environmental Affairs, University of Washington, Seattle, WA 98105, USA
[c] Biology Department, University of Washington, Seattle, WA 98195, USA
[d] Washington Department of Natural Resources, Olympia, WA 98504, USA

## ARTICLE INFO

## ABSTRACT

Adverse habitat conditions associated with reduced seawater pH often, but not always, negatively affect bivalves in early life history phases. Improving our understanding of how habitat-specific parameters affect clam recruitment, survival, and growth could assist natural resource managers and researchers in developing appropriate adaptation strategies for increasingly acidified nearshore ecosystems. Two proposed adaptation strategies, the presence of macrophytes and addition of shell hash, have the potential to raise local seawater pH and aragonite saturation state and, therefore, to improve conditions for shell-forming organisms. This field study examined the effects of these two substrate treatments on biological and geochemical response variables. Specifically, we measured (1) recruitment, survival, and growth of juvenile clams (*Ruditapes philippinarum*) and (2) local water chemistry at Fidalgo Bay and Skokomish Delta, Washington, USA, in response to experimental manipulations. Results showed no effect of macrophyte or shell hash treatment on recruitment or survival of *R. philippinarum*. Contrary to expectations, clam growth was significantly greater in the absence of macrophytes, regardless of the presence or absence of shell hash. Water column pH was higher outside the macrophyte bed than inside at Skokomish Delta and higher during the day than at night at Fidalgo Bay. Additionally, pore-water pH and aragonite saturation state were higher in the absence of macrophytes and the presence of shell. Based on these results, we propose that with increasingly corrosive conditions shell hash may help provide chemical refugia under future ocean conditions. Thus, we suggest adaptation strategies target the use of shell hash and avoidance of macrophytes to improve carbonate chemistry conditions and promote clam recruitment, survival, and growth.

## 1. Introduction

The absorption of anthropogenic carbon dioxide ($CO_2$) into seawater results in decreased pH, reduced availability of carbonate ions, and lower saturation states of the biominerals organisms use to construct shells and skeletons (Caldeira and Wickett, 2003; Doney et al., 2009; Feely et al., 2004). Studies examining the biological effects of these chemical changes, termed ocean acidification (OA), indicate that bivalves in early developmental stages are most vulnerable to OA (e.g., Gazeau et al., 2013; Kroeker et al., 2013; Parker et al., 2013). Known negative impacts include increased energy demands, impaired neurological functioning, altered behavior, and shell dissolution which can reduce growth and survivorship (Green et al., 2013; Kurihara, 2008; Waldbusser et al., 2015). Although adequate food supply can offset

additional energetic costs caused by environmental stress (Hettinger et al., 2013a; Melzner et al., 2011; Parker et al., 2013), a recent study found that the initiation of feeding is delayed when $pCO_2$ is elevated (Waldbusser et al., 2015). Energetic deficits in early life-history stages may affect bivalve population dynamics either through a reduction in successful recruitment to adult populations (e.g., Melzner et al., 2011; Parker et al., 2013) or through negative carry-over effects that may impair the fitness of adult populations (Hettinger et al., 2013b).

Under moderate carbon emission projections, pH is expected to decline by 0.2–0.3 units by 2100 (IPCC, 2014). Therefore, resource managers are seeking strategies to ameliorate the impacts of OA on culturally, economically, and ecologically-important marine organisms such as bivalves (Rau et al., 2012; WABRPOA, 2012). Because habitat structure in coastal environments can influence survival and growth of
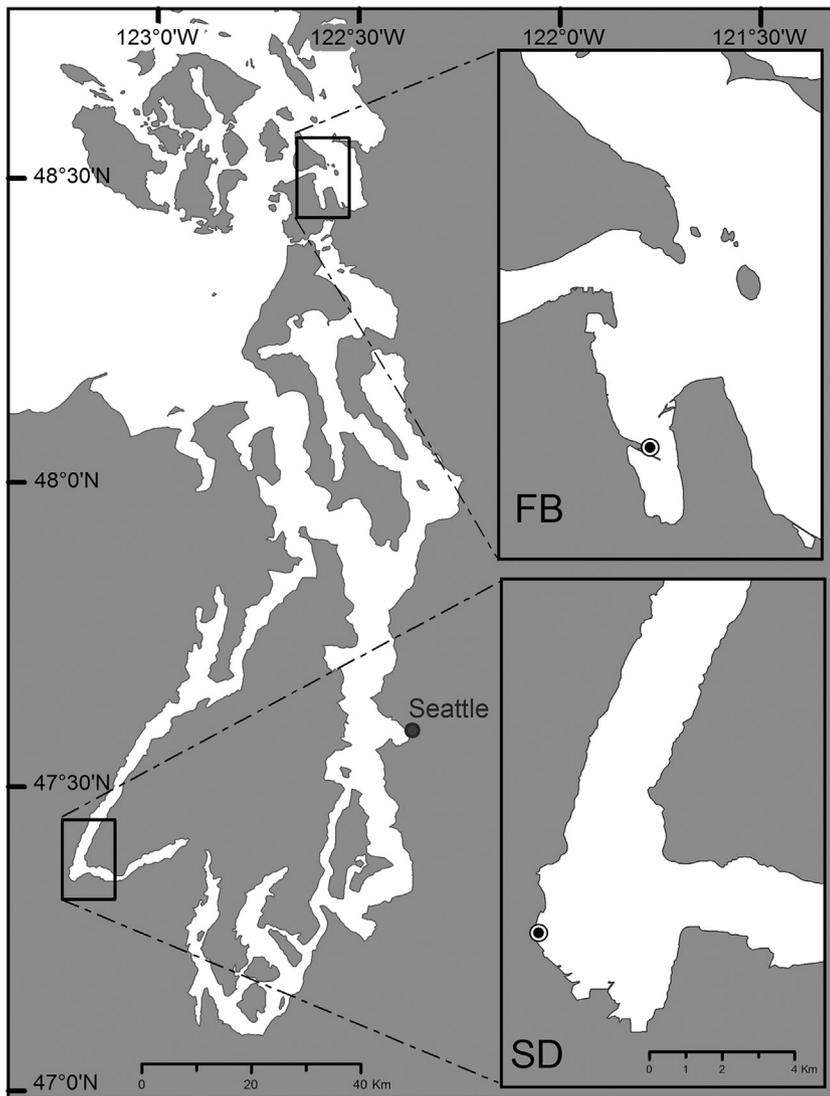
**Fig. 1.** Map of study sites (◉) in Fidalgo Bay (FB) and Skokomish Delta (SD), Washington, USA.

juvenile invertebrates (McDevitt-Irwin et al., 2016; Walters and Wethey, 1996), two habitat modifications have been proposed to mitigate future OA impacts on clams. The first strategy is the addition of shell hash (broken shell) to intertidal clam beds (Billé et al., 2013; Rau et al., 2012; WABRPOA, 2012). Beach coarsening with crushed shell and gravel is known to promote natural recruitment and growth in hardshell clams by increasing substrate stability and interstitial space as well as protecting juvenile clams from predation (Ruesink et al., 2014; Thompson, 1995; Toba et al., 1992). The presence of crushed shell may also increase total alkalinity locally, neutralizing $CO_2$ in the overlying water and increasing biomineral saturation states in sediment porewater (Green et al., 2013, 2009). Although some laboratory and field experiments on juvenile clams have found higher recruitment and survival in sediment with crushed shell (e.g., Clements et al., 2016; Green et al., 2009), other research has found no difference in recruitment or survival between gravel versus shell-enhanced plots (Ruesink et al., 2014; Toba et al., 1992). These differing results suggest that both sediment grain size and sediment pH can influence clam abundance; however, sediment pH may become increasingly important as organisms are exposed to more extreme carbonate chemistry conditions.

A second OA adaptation strategy is the restoration or introduction of photoautotrophs, such as seagrass and macroalgae (collectively termed macrophytes), near shellfish beds (Billé et al., 2013; Hendriks et al., 2015; WABRPOA, 2012). While the structural presence of emergent

macrophytes provides shelter for bivalves from predators and desiccation (Coleman and Williams, 2002; Peterson et al., 1984), recent studies suggest macrophytes may also act as a chemical refuge for calcifying organisms. Via photosynthesis, macrophytes exhibit the ability to draw down $CO_2$ in seawater and increase pH and aragonite saturation state ($\Omega_{aragonite}$), potentially enhancing the calcification processes of co-existing organisms (Buapet et al., 2013; Hendriks et al., 2014; Unsworth et al., 2012). However, the effectiveness of this phytoremediation as an OA adaptation technique for bivalves remains unclear. The magnitude of any buffering effect caused by aerobic photosynthesis within a macrophyte bed is likely to be site specific and would depend on a variety of factors, including structural parameters of the bed, local hydrodynamics, and aerobic respiration, (Cryonak et al., 2018; Hendriks et al., 2014). Moreover, studies investigating the effects of macrophyte presence on clam growth have reported conflicting results (e.g., Everett, 1994; Irlandi and Peterson, 1991; Tsai et al., 2010).

While there is limited research investigating the independent effects of these two potential OA adaptation strategies (i.e., adding shell hash to a beach or restoring macrophyte populations near clam beds), to our knowledge, even fewer studies have integrated the two strategies (but see Ruesink et al., 2014), and no studies have examined their combined effects on both bivalve performance and local seawater chemistry. Therefore, we designed a field experiment that incorporated these two strategies as substrate treatments to investigate their effect on clam

recruitment, survival, and growth (RSG) as well as pH and $\Omega_{aragonite}$ in sediment pore-water and pH in the water column. Because previous studies have demonstrated that each treatment has the capacity to improve OA conditions for calcifying organisms, we hypothesized that 1) the application of both treatments would improve clam RSG, 2) the simultaneous application of both treatments would additively increase pH and $\Omega_{aragonite}$ in pore-water, and 3) the presence of macrophytes would increase pH in the water column relative to unvegetated substrate.

## 2. Methods

### 2.1. Study organism and sites

We used juvenile Manila clams, *Ruditapes philippinarum* (Adams and Reeve, 1850) to evaluate the biological effects of the two substrate treatments (i.e., shell hash and macrophytes). Although Manila clams are non-native in Washington state, USA, they are capable of naturally reproducing in the Salish Sea (Cheney and Mumford, 1986; Ruesink et al., 2014) and have become an economically-important species (Dumbauld et al., 2009). Furthermore, because Manila clams share similar life history and ecological attributes with the native species, *Leukoma staminea*, our results could be generalizable to clam species native to Washington waters (Byers, 2005). Clams used in this study were raised at the Taylor Shellfish hatchery in Hawaii and shipped to a Washington hatchery where they were held in tanks under ambient conditions for one week before being transported to the study sites.

Our study was conducted on two intertidal beaches in Fidalgo Bay (FB) and Skokomish Delta (SD), which are situated within the Washington state portion of the Salish Sea (Fig. 1). Fidalgo Bay is located near the confluence of the Strait of Juan de Fuca and the Strait of Georgia, where well-mixed estuarine waters are strongly influenced by the Fraser River plume. Further south in Hood Canal, SD water conditions are more stratified, with slower circulation and greater variation in temperature and salinity and higher pH in the surface water than in northern Washington waters (Moore et al., 2008). At both study sites, the predominant sediment was a mix of mud and coarse and fine sand with a small percentage (< 10%) of gravel and natural shell hash. The study site at SD was located near a small freshwater stream.

### 2.2. Experimental design

Two factors were tested in our split-plot experimental design. The whole plot factor contained two levels of the macrophyte treatment: macrophytes present (+M) and macrophytes absent (−M). At ~ −0.3 m relative to MLLW an area of vegetated tideflat (+M) adjacent to an unvegetated area (−M) was selected at each study site. Due to differences in the distribution of vegetation between sites, we established plots in an area dominated primarily of macroalgae (*Ulva* spp.) at FB and eelgrass (*Zostera marina*) at SD. Annual net primary productivity rates for seagrass and seaweed are roughly comparable with reported values ranging from 400 to 1900 g C m$^{-2}$ y$^{-1}$ for seaweeds and 300–1000 g C m$^{-2}$ y$^{-1}$ for seagrasses (Mann, 1982).

The sub-plot factor comprised two levels of the shell hash treatment: added shell (+S) and no added shell (-S). Treatment levels were randomly assigned to 16 mesh bags (1 mm mesh, 25 × 25 cm, fully enclosed) nested within each of the macrophyte treatments, resulting in four treatment combinations (+M + S, +M - S, −M + S, −M - S) replicated eight times at each site for a total of 64 samples. Shell hash material consisted of 70% UV-treated Pacific oyster (*Crassostrea gigas*) shell and 30% locally-collected mixed-species clam shell, crushed to ~ < 5 cm particle size. +S treatments consisted of half added shell hash and half local sediment by volume, whereas -S treatments consisted only of local sediment.

In July 2016, we added approximately 100 *R. philippinarum* (mean length = 2.4 mm ± 0.26 SD) to each mesh bag and placed them on the beach at 2 m intervals within each whole plot. The sub-plots were then covered with a 50 × 50 cm piece of vexar mesh to deter predation and help retain added shell. For +S sub-plots, shell hash was also integrated into the sediment under the vexar mesh to increase water exposure to the broken shell. Bags were collected after 55 days and the contents were processed through a sieve series (8, 4, 1 mm). Living clams were preserved in a 10% buffered formalin solution and later identified and enumerated. Lengths to the nearest 0.01 mm were recorded by measuring the longest dimension.

### 2.3. Juvenile recruitment, survival, and growth

Because individual clams within the bags did not constitute independent subsamples, clam RSG were analyzed using a single mean value for each variable from each sub-plot. *R. philippinarum* were sorted by size to distinguish outplanted individuals from new recruits. Individuals ≤ 2.4 mm were assumed to have recruited naturally based on typical seasonal recruitment patterns, while clams > 2.4 mm were assumed to have been outplanted for the study (Williams, 1980). The number of clams ≤ 2.4 mm was used to calculate an index of recruitment. The number of clams > 2.4 mm was used to generate an index of survival and the lengths of clams > 2.4 mm were used to estimate growth. At FB, the −M + S treatment combination was removed from the growth analysis because five of eight sub-plots contained no *R. philippinarum* larger than 2.4 mm. Bags with fewer than 25 individuals larger than 2.4 mm were also eliminated from the growth analysis to strengthen statistical power. Consequently, there was an unbalanced number of replicates in the remaining treatment combinations.

### 2.4. Environmental parameters

In early August, pore-water samples were collected at the sub-plot level at SD to evaluate habitat treatment effects on pH and $\Omega_{aragonite}$ in the hyporheic (sub-surface) water. Methods, modified from Tsai et al. (2010), consisted of one 100 mL sample of pore-water per sub-plot collected from three banjo filters buried approximately 4 cm deep under the vexar mesh that covered each clam bag. Filters were installed at low tide and pore-water samples were collected 24 h later. Samples were placed in 0.12 L acid washed glass bottles and temperature readings were taken using a handheld pH10A pen (YSI). The samples were then fixed with 0.3 mL saturated solution of HgCl$_2$ and sealed with a Phenolic PolyCone cap. Samples were analyzed for salinity with a Haach conductivity meter, pH using an Ocean Optics CCD spectrophotometer following SOP 6 protocol, and total alkalinity using an open cell titrator per SOP 3b protocol (Dickson et al., 2007). These measurements were then used to calculate dissolved inorganic carbon and aragonite saturation state using CO$_2$ calc (Robbins et al., 2010).

To assess whether macrophytes had an effect on water column pH, custom-built pH sensors were deployed in unvegetated (−M) and eelgrass-dominated areas at FB and SD. Because the sensors were part of the Washington Department of Natural Resources' Acidification Nearshore Monitoring Network (ANeMoNe), which specifically targeted eelgrass beds, the pH sensor at FB was placed in a *Z. marina* bed that was ~0.3 m deeper than the *Ulva*-dominated area where the clam bags and pore-water filters were placed. At the Skokomish Delta site there was a small freshwater source < 4.5 m from the *Z. marina* bed. The sensors were placed ~ −0.61 to −0.76 m and ~ −0.46 to −0.61 m relative to MLLW at FB and SD, respectively. pH (total scale) measurements were collected every 10 min from 23 June - 20 July 2016. Because hours of sunlight were used as a proxy for photosynthetic activity, data were divided into daytime (0600–2100) and nighttime (2100–0600) intervals based on the hours of sunrise and sunset over the monitoring period. Median pH from each daytime and nighttime interval was extracted for quantitative analyses, yielding 27 measurements in each of the whole plots by time interval (+M day, +M night, −M day, and –M night).

The custom-built pH sensors use Honeywell Durafet© technology to autonomously measure pH with minimal drift over time. Identical sensors have been used to measure pH in California (Hofmann et al., 2014), and the commercially available Sea-Bird SeaFET pH sensor uses the same underlying technology. Before and after deployment, ANe-MoNe sensors were calibrated in the laboratory against Tris-buffered synthetic seawater (following SOP 6a in Dickson et al., 2007) across five temperatures, to calculate the instrument-specific electrode standard potential, $E^*$. Once the instrument-specific $E^*$ is known, pH can be calculated from the recorded temperature and voltage output of the durafet in a deployed sensor (Bresnahan et al., 2014).

### 2.5. Statistical analyses

Linear mixed-effects models (LMM) were applied to clam RSG to test for differences between the treatment combinations in a manner that accounted for the sample imbalance as well as the crossed and nested structure of the experimental design. Based on Akaike Information Criterion, the best fitting models for clam RSG included macrophyte and shell treatments, plus their interaction, as fixed effects and study site as a random effect (Zuur et al., 2009). To analyze pH and $\Omega_{aragonite}$ in our pore-water samples, two-way ANOVAs were performed with the substrate treatments as factors. We also used two-way ANOVAs on water column pH at the two study sites with macrophyte treatment and daytime/nighttime interval as factors. Assumptions of normality and homogeneity of variance were met for all tests, except at FB where the water column pH variance was heterogeneous. Because of the FB variance, a significance factor of $\alpha = 0.01$ was used to minimize the likelihood of making a Type I error (Keppel, 1991). A significance factor of $\alpha = 0.05$ was used for all other tests. Statistical analyses were performed in R version 3.4.2 (R Core Team, 2017) using lme4 and lmerTest packages (Bates et al., 2015; Kuznetsova et al., 2016).

## 3. Results

### 3.1. Juvenile recruitment, survival, and growth

At the end of 55 days, 55% of the mesh bags contained at least 80 live clams > 2.4 mm in length, with median sizes ranging from 5 to 8 mm among treatments. The number of clams ≤ 2.4 mm, considered to be new recruits, ranged up to 100 per bag but was generally fewer, with a median count of 11.5 recruits among treatments and median lengths ranging from 1.94 to 2.22 mm. Neither recruitment nor survival showed a statistically significant response to shell hash or macrophyte treatments (Table 1). However, regardless of the shell treatment, clams grew faster in areas without macrophytes (Table 1, Fig. 2).

### 3.2. Environmental parameters

In SD pore-water, pH and $\Omega_{aragonite}$ values ranged from 7.05 to 7.86 and 0.36 to 1.65, respectively (Table 2, Fig. 3). The two-way ANOVAs revealed a significant macrophyte treatment effect on pH ($F_{1,19} = 20.48$, $P = 0.00023$) and $\Omega_{aragonite}$ ($F_{1,19} = 19.20$, $P = 0.00032$), as well as a significant shell hash treatment effect on pH ($F_{1,19} = 6.85$, $P = 0.017$) and $\Omega_{aragonite}$ ($F_{1,19} = 11.70$, $P = 00029$). Measurements for both carbonate chemistry parameters were higher when macrophytes were absent and shell hash was present (Fig. 3).

From 23 June to 20 July, median pH in the water column at FB and SD ranged from 7.34 to 8.09 and 7.11 to 8.30, respectively. At FB, pH was higher during the day than at night, but no macrophyte treatment effect was detected. Conversely, the macrophyte treatment had a significant effect on pH at SD, with higher pH outside of the eelgrass bed than inside the bed. No daytime/nighttime interval effect was observed at SD (Table 3, Fig. 4).

**Table 1**

Statistical results testing substrate treatment combinations at Fidalgo Bay and Skokomish Delta on abundance of juvenile clams ≤ 2.4 mm (index of recruitment), clams > 2.4 mm (index of survival), and mean clam length as an estimate of growth of *Ruditapes philippinarum* > 2.4 mm. Linear mixed-effects models included macrophyte treatment, shell hash treatment and their interaction as fixed effects and study site (Fidalgo Bay and Skokomish Delta) as a random effect. SD = standard deviation.

| Effect | Index of recruitment | | Index of survival | | Mean length | |
|---|---|---|---|---|---|---|
| Fixed | DF | F-value [$P$] | DF | F-value [$P$] | DF | F-value [$P$] |
| Macrophyte treatment | 1, 38 | 2.64 [0.11] | 1, 50 | 1.64 [0.21] | 1, 48 | 55.77 [< 0.05] |
| Shell hash treatment | 1, 39 | 1.14 [0.29] | 1, 50 | 0.02 [0.89] | 1, 48 | 0.15 [0.70] |
| Macrophyte x Shell | 1, 39 | 0.61 [0.44] | 1, 50 | 0.65 [0.43] | 1, 48 | 0.28 [0.60] |

| Random | Variance | SD | Variance | SD | Variance | SD |
|---|---|---|---|---|---|---|
| Study site | 146.3 | 12.09 | 0.0 | 0.00 | 0.2 | 0.45 |

## 4. Discussion

Contrary to our hypotheses, the combined presence of macrophytes and shell hash did not improve carbonate chemistry or juvenile clam RSG. In fact, at both sites, clams grown in the presence of macrophytes were smaller than those grown in the absence of macrophytes. Moreover, water column pH inside the eelgrass bed at SD was unexpectedly lower than outside the eelgrass bed and macrophyte presence had no effect on water column pH at FB despite the detection of a diurnal signal. However, we did observe the highest median pH and $\Omega_{aragonite}$ in sediment pore-water where macrophytes were absent and shell hash was added. Thus, while we did not find an effect of shell hash addition on clam RSG, our results do suggest that the addition of shell hash could provide chemical refugia for clams under future ocean conditions.

It is possible our confounding macrophyte treatment results could reflect a number of environmental factors that were not controlled for in our field experiment. Structured habitat, including coarse grain size and vegetation, can alter predator-prey interactions, sediment stability, and recruitment cues, which in turn can influence clam behavior and survival (e.g., Clements and Hunt, 2014; Green et al., 2013; Irlandi and Peterson, 1991). We attempted to minimize variability by positioning plots on similar substrates and by adding anti-predator netting which appeared to reduce predation from *Hemigrapsus* sp. as there were few indications of crushed clam shells. Therefore, the observed negative effect of macrophytes on clam growth suggests that unidentified factors associated with aquatic vegetation influenced growth. For example, the baffling effect created by seagrass and macroalgae blades are known to reduce flow velocities around and within macrophyte beds and influence particle settlement (Ginsburg and Lowenstam, 1958). These changes to local habitat conditions have been reported to cause both increases (Peterson et al., 1984) and decreases (Allen and Williams, 2003; Carroll and Peterson, 2013) in food delivery to bivalves. At both of our study sites, we quantified an observed growth response that is consistent with food limitation, perhaps related to the baffling effect.

Because ocean pH dynamics show daily and seasonal variations partially driven by biological activity (Hofmann et al., 2011; Wootton et al., 2008), we hypothesized that water column pH would show a detectable response consistent with the metabolic influence of macrophytes. Yet, during hours of photosynthetic activity we observed higher pH levels at FB regardless of the presence of eelgrass (Fig. 4). This finding suggests that other metabolic activities or physical factors drive pH patterns at FB, for instance metabolic contributions from benthic
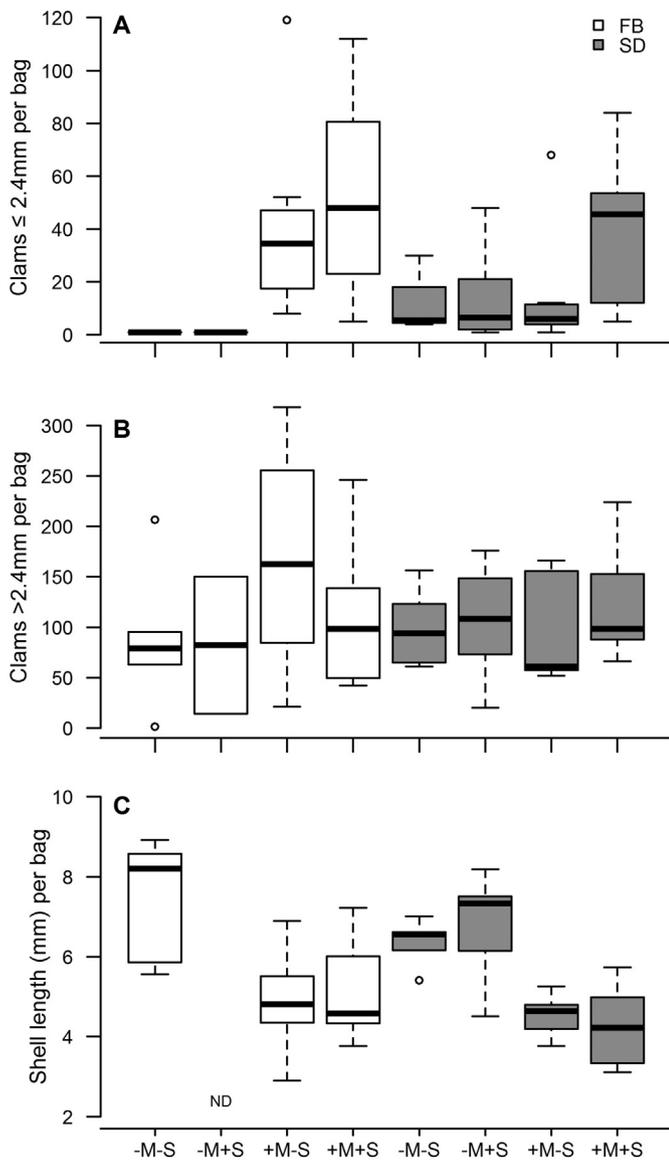
**Fig. 2.** Box plots of A) index of recruitment, B) index of survival, and C) estimated clam growth by substrate treatments (recruits excluded). Bold lines in boxes are median values. FB = Fidalgo Bay, SD = Skokomish Delta, −M = macrophytes absent, +M = macrophytes present, -S = no shell hash added, +S = shell hash added, ND = no data.
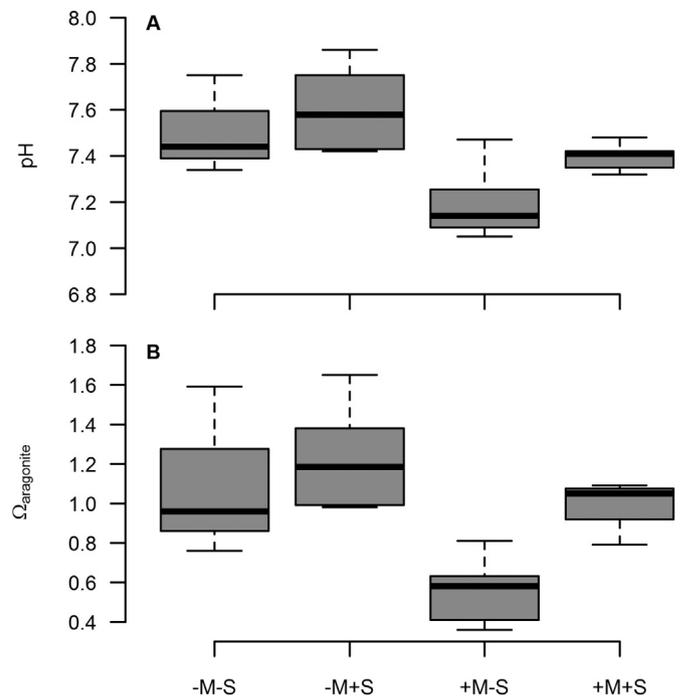


**Fig. 3.** Box plots of Skokomish Delta porewater sample A) pH and B) $\Omega_{aragonite}$ by substrate treatments. Bold lines are median values. There was a significant macrophyte treatment effect on pH and $\Omega_{aragonite}$, as well as a significant shell hash treatment effect on pH and $\Omega_{aragonite}$. −M = macrophytes absent, +M = macrophytes present; -S = no shell hash added, +S = shell hash added.

**Table 3**
Results from two-way ANOVAs assessing the main effects of macrophyte treatment and daytime/nighttime interval on median pH measurements collected at Fidalgo Bay and Skokomish Delta.

| Site | Effect | DF | F-value [P] |
|---|---|---|---|
| Fidalgo Bay | Macrophyte treatment | 1, 104 | 1.24 [0.27] |
| | Time (day, night) | 1, 104 | 23.25 [< 0.001] |
| | Macrophyte x Time | 1, 104 | 0.75 [0.39] |
| Skokomish Delta | Macrophyte treatment | 1, 104 | 26.74 [< 0.001] |
| | Time (day, night) | 1, 104 | 0.015 [0.90] |
| | Macrophyte x Time | 1, 104 | 0.57 [0.45] |

and pelagic microbial primary producers (e.g., Hendriks et al., 2014; Thom and Albright, 1990). Photosynthetic microbes are capable of increasing pH by drawing down $pCO_2$ through photosynthesis. Although microbes typically contribute proportionally more to primary

productivity in winter when macrophyte biomass is low (Buapet et al., 2013; Thom and Albright, 1990), in the central Salish Sea phytoplankton may play a larger role in nearshore metabolism than previously expected (Lowe et al., 2016). This could potentially explain the strong diurnal signal at FB.

Furthermore, even though macrophyte-dominated ecosystems are typically net autotrophic, factors such as climatic conditions, nutrient availability, and hydrodynamics can influence site-specific productivity
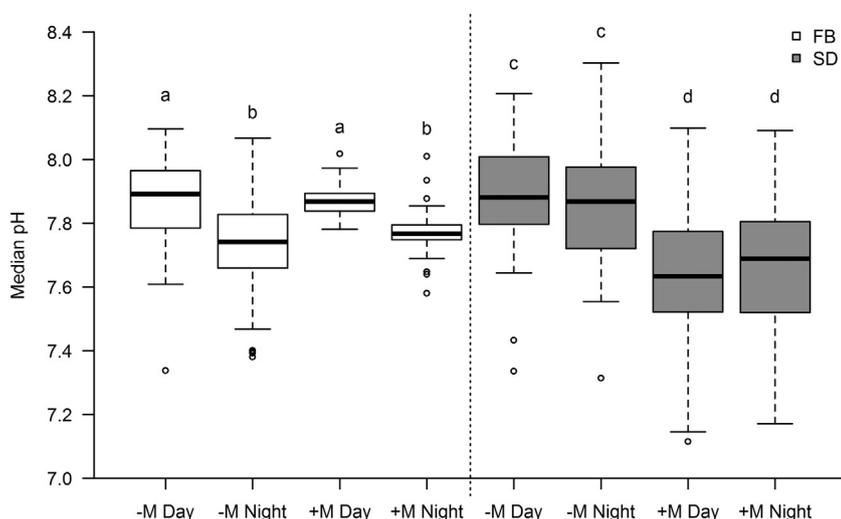
**Table 2**
Geochemical data from pore-water samples collected at Skokomish Delta for different substrate treatment combinations. pH units are total scale. −M −S, n = 3; −M + S, n = 6; +M -S, n = 7; +M + S, n = 7. −M = macrophytes absent, +M = macrophytes present, -S = no shell hash added, +S = shell hash added.

| Carbonate chemistry parameter (Mean ± SE) | Substrate treatment | | | |
|---|---|---|---|---|
| | -M -S | -M + S | + M -S | + M + S |
| Temperature (°C, at collection) | 23.23 ± 0.24 | 22.67 ± 0.06 | 22.80 ± 0.23 | 22.86 ± 0.24 |
| Temperature (°C, at measurement) | 25.11 ± 0.05 | 24.73 ± 0.08 | 24.91 ± 0.04 | 24.83 ± 0.06 |
| $A_T$ (µmol kg$^{-1}$) | 2546.25 ± 139.13 | 2561.43 ± 153.40 | 2616.10 ± 129.15 | 3078.15 ± 102.83 |
| $C_T$ (µmol kg$^{-1}$) | 2519.31 ± 179.50 | 2514.82 ± 170.30 | 2707.16 ± 140.35 | 3094.98 ± 106.18 |
| Salinity | 27.43 ± 0.09 | 25.60 ± 1.31 | 27.21 ± 0.12 | 27.53 ± 0.09 |
| pH | 7.51 ± 0.12 | 7.60 ± 0.07 | 7.19 ± 0.06 | 7.39 ± 0.02 |
| $\Omega_{aragonite}$ | 1.10 ± 0.25 | 1.23 ± 0.10 | 0.55 ± 0.06 | 0.99 ± 0.05 |

**Fig. 4.** Box plots of water property monitoring results from Fidalgo Bay and Skokomish Delta inside and outside of eelgrass beds (−M = outside, +M = inside) during the day (0600–2100) and night (2100–0600) from 23 June – 20 July 2016. Letters above treatments indicate statistical difference determined by two-way ANOVAs. Bold lines are median values and the dashed line separates the two statistical tests.

and the magnitude of a buffering effect in nearshore environments (Gattuso et al., 1998; Hendriks et al., 2014; Thom and Albright, 1990). The lack of an observable diurnal signal in pH at SD suggests that processes other than aerobic metabolism dominate at this site. For example, the freshwater source adjacent to the eelgrass bed may have obscured the metabolic signal and contributed to the low pH readings. At FB, the strong and consistent flushing typical of this location (Moore et al., 2008) may have reduced residence times and limited our ability to detect an influence of eelgrass on water chemistry (Unsworth et al., 2012). Moreover, the sensors inside and outside of the eelgrass bed may have been too close together to detect a difference between the two treatment levels (sensors were ~30 m apart at FB and about ~125 m apart at SD).

We were not surprised that pore-water pH and $\Omega_{aragonite}$ were higher in the shell hash sub-plots, but we did not find the expected additive effect of macrophytes plus shell hash on carbonate chemistry parameters. This may be due to the split-plot design of the experiment in which the macrophyte effect was tested over a large spatial area and allowed for other factors to confound any difference detected between the vegetated and un-vegetated plots. However, at the sediment-water interface, macrophytes can provide a source of detrital organic matter and may be enhancing the deposition of other organic matter via the baffling effect which in turn promotes respiration and sediment acidification. Seagrass roots can further enhance carbonate dissolution by directly adding $O_2$ to the sediment (Burdige et al., 2008; Widdicombe et al., 2011). Assessing local pore-water hydrodynamics and the capacity of root structures or blades to influence carbonate chemistry would provide a more comprehensive understanding of macrophyte effects on clam habitat.

Unlike seagrass and macroalgae, the addition of shell hash increased the average pore-water $\Omega_{aragonite}$ by 0.39 units and pH by 0.15 units. Most interestingly, no treatment effect was detected on clam RSG despite these increases. While this result is at odds with the contention that shell dissolution contributes to high post-settlement mortality rates observed among juvenile clams (Green et al., 2009), the range of pore-water pH observed in our treatments was higher than pH levels tested in similar studies that found significant decreases in *R. philippinarum* survival (Basallote et al., 2015; Rodríguez-Romero et al., 2014). It is also possible that our outplanted Manila clams were already too large to be affected by the undersaturated conditions they were exposed to in this study. Even though bivalves in early life-history stages are especially vulnerable to OA (Gazeau et al., 2013; Kroeker et al., 2013; Parker et al., 2013), there may be a size-dependent effect even amongst smaller clams where individuals are more resistant to changes in carbonate chemistry as their size increases (Green et al., 2009; Waldbusser

et al., 2010). Increasing the magnitude of the buffering effect may also increase the likelihood of observing an effect on clam RSG. A larger buffering effect may be achieved by reducing the size of the shell hash from fragments < 5 mm to a uniform 1 mm fragment, thereby increasing the dissolution rate (Green et al., 2013; Morse, 1983). Repeating our study under more corrosive conditions and addressing factors of shell and clam size in future studies could provide greater insight into effective shell hash application techniques that elicit both physical and biological responses. Because settlement and early post-settlement events often govern overall population dynamics of bivalves and other marine invertebrates (Gosselin and Qian, 1997; Hunt and Scheibling, 1997; Olafsson et al., 1994), creating chemical refugia that benefit larvae and juveniles could help promote successful recruitment to adult populations under increasingly corrosive conditions. Although more testing of shell hash effects on pore-water would be ideal, our results suggest that the addition of shell hash does have potential for use as an adaptation strategy.

## Declarations of interest

None.

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