



# Influence of seagrass on juvenile Pacific oyster growth in two US west coast estuaries with different environmental gradients

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**ABSTRACT:** Ocean acidification threatens many marine organisms, including oysters. Seagrass habitat has been suggested as a potential refuge for oysters because it may ameliorate stressful carbonate chemistry and augment food availability. We conducted an *in situ* study to investigate whether eelgrass *Zostera marina* habitat affects the growth of juvenile Pacific oysters *Crassostrea gigas* and influences local carbonate chemistry or food quantity at sites where we expected contrasting conditions in two US west coast estuaries. Juvenile oysters were out-planted in typical intertidal on-bottom (just above sediment) and off-bottom (45 cm above sediment) culture positions and in adjacent eelgrass and unvegetated habitats from June to September 2019. Water quality was measured with sondes for 24 h periods each month, and discrete water samples were collected in conjuncture. Results show that eelgrass habitat did not alter average local carbonate chemistry (pH,  $p\text{CO}_2$ ,  $\Omega\text{calcite}$ ), but consistently reduced available food (relative chlorophyll *a*). Eelgrass habitat had little to no effect on the shell or tissue growth of juvenile oysters but may have influenced their energy allocation; oysters displayed a 16% higher ratio of shell to tissue growth in eelgrass compared to unvegetated habitat when cultured on-bottom. At the seascape scale, average site-level pH was negatively correlated with shell to tissue growth but not with shell growth alone. Overall, these findings suggest that juvenile oysters may display a compensatory response and allocate more energy to shell than tissue growth under stressful conditions like acidic water and/or altered food supply due to reduced immersion or eelgrass presence.

**KEY WORDS:** *Crassostrea gigas* · Juvenile · Growth · Eelgrass · *Zostera marina* · Estuary · Climate · Ocean acidification

## 1. INTRODUCTION

Global climate change is causing the oceans to acidify in response to a rapid increase in the concentration of  $\text{CO}_2$  in the atmosphere from human activities and subsequent absorption of  $\text{CO}_2$  in ocean surface waters inducing changes in the carbonate system (reduced pH and carbonate ions; Caldeira & Wickett 2003, Doney et al. 2009, Feely et al. 2009, Jiang et al. 2019). Enhanced atmospheric  $\text{CO}_2$  coupled with riverine discharge, upwelling, and eutrophication are contributing to acidified conditions in

coastal zones (Melzner et al. 2013, Waldbusser & Salisbury 2014, Cai et al. 2017, Lowe et al. 2019a). Ocean acidification threatens many marine organisms, particularly calcifying species such as corals and mollusks (Kroeker et al. 2010, 2013, Chandra Rajan & Vengatesen 2020), and is predicted to have wide-ranging effects on ecological systems and species interactions (Gaylord et al. 2015).

Biotic habitats like macroalgae and seagrasses have been suggested as potential refuge habitats for calcifying organisms including bivalve shellfish against acidified waters due to their ability to uptake

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CO<sub>2</sub> and ameliorate stressful carbonate chemistry conditions (Kapsenberg & Cyronak 2019, Falkenberg et al. 2021). Both shellfish and seagrasses form valuable structured habitats and provide other important ecosystem services in estuaries where shellfish are farmed or fished (Nordlund et al. 2016, Alleway et al. 2019, van der Schatte Olivier et al. 2020, do Amaral Camara Lima et al. 2023), so co-culturing shellfish, like oysters, with seagrass has been suggested as a potentially win-win management strategy for both the aquaculture industry and resource managers (Washington State Blue Ribbon Panel on Ocean Acidification 2012, Clements & Chopin 2017, Nielsen et al. 2018). Nonetheless, the effect of biotic habitats like seagrass on molluscan shellfish performance is complex and difficult to attribute to a single factor like acidification *in situ* due to a suite of other conditions that are also potentially modified by seagrasses and vary spatiotemporally in the estuaries where culture occurs (Kapsenberg & Cyronak 2019, Ducker & Falkenberg 2020, Falkenberg et al. 2021).

Ocean acidification alters seawater carbonate chemistry, creating stressful conditions for bivalve shellfish, such as oysters, that form shells from calcium carbonate (CaCO<sub>3</sub>) minerals. When added to seawater, CO<sub>2</sub> reacts to form bicarbonate (HCO<sub>3</sub><sup>-</sup>) and release hydrogen (H<sup>+</sup>) ions, which increases acidity (lowers pH). Acidified water reduces the availability of carbonate ions (CO<sub>3</sub><sup>2-</sup>) and decreases the saturation state of calcium carbonate (CaCO<sub>3</sub>), making it more difficult for bivalves to form shells, but also causes shell dissolution with subtle dynamics directly linked to organism physiology, energy allocation, the proteome, and molecular traits (Doney et al. 2009, Kroeker et al. 2013, Waldbusser et al. 2015a, Chandra Rajan et al. 2021). Although less investigated, coastal acidification driven by freshwater runoff from the land also affects alkalinity and therefore estuarine carbonate chemistry (Duarte et al. 2013, Fitzner et al. 2018). Early life stages of oysters exhibit the greatest sensitivity to reduced pH with dramatically reduced larval survival and growth during the early post-fertilization period when they are developing shell and later during metamorphosis when they are transforming to a benthic existence (Kurihara et al. 2007, Parker et al. 2013, Waldbusser et al. 2015b, Haley et al. 2018, Dineshram et al. 2021). Sensitivity of older oysters is less studied, but they have also been shown to exhibit reduced growth, reproduction, and survival under reduced pH conditions (Waldbusser et al. 2011, Amaral et al. 2012, Barros et al. 2013, Bednaršek et al. 2022) as well as differing sensitivity and carryover effects from larval

and parental exposure (Hettinger et al. 2012, Parker et al. 2015, Spencer et al. 2020, Lim et al. 2021, Dang et al. 2023).

Commercial shellfish hatcheries operating along the US west coast have already experienced the effects of acidification including significantly reduced rates of larval growth and survival during seasonal periods when upwelling events occur and cold, nutrient rich, high pCO<sub>2</sub> water is drawn into the hatcheries where larvae are cultured (Barton et al. 2012). These hatcheries now measure seawater carbonate chemistry, strategically time larval production cycles, and chemically buffer incoming seawater to maintain optimal carbonate chemistry conditions (Barton et al. 2015). While these strategies have enabled hatcheries to culture larvae and produce viable oyster spat, the effects of acidified water on the success of juvenile oysters once out-planted in these dynamic estuaries are less understood. Reports suggest that ocean acidification has already resulted in a significant reduction in oyster production in the US and Canada, and thus projected losses under future scenarios in these countries and especially those with much larger production like China could be substantial (Narita et al. 2012, Mackenzie et al. 2014, Botta et al. 2020, FAO 2022)

Seagrasses can ameliorate low pH conditions at local scales through the uptake of CO<sub>2</sub> during daytime photosynthesis, but this is potentially offset by respiration at night (Hendriks et al. 2014, Pacella et al. 2018). Seagrass also modifies other site-specific factors such as flow and water residence time (Kowweek et al. 2018, Falkenberg et al. 2021), which in turn can alter seston and food availability by slowing water flow, accumulating fine particles, and altering resuspension of sediment (de Boer 2007, Hasegawa et al. 2008). Therefore, seagrass might limit planktonic food quantity by reducing flow (Bologna & Heck 1999, Allen & Williams 2003), but enhance supply of other potential food like epiphytic algae via deposition, and benthic microalgae and other organic particles via resuspension (Ruesink et al. 2019). The resulting effect of seagrass on oyster performance is therefore complex and difficult to assess *in situ* without measuring and contrasting food availability in concert with pH.

Replicating important *in situ* field conditions like hydrodynamics that cause scale-dependent effects and multiple interactions with other factors like food abundance that influence oyster growth in the laboratory is also difficult (see Noisette et al. 2022 and Vargas et al. 2022 for recent reviews). Nonetheless, results of such recent studies suggest that seagrass

increased pH during the day and had either less influence or even a negative effect at night, which in turn resulted in increased juvenile oyster shell growth in seagrass treatments versus those without seagrass (Ricart et al. 2021a, Garner et al. 2022). Previous studies conducted in the field also suggest that growth of juvenile oysters can be enhanced in seagrass (Smith 2016, Lowe et al. 2019b, Dumbauld et al. 2021), but effects were site-specific, suggesting that multiple factors were involved.

Oyster growers have adopted several culture methods to optimize oyster growth and production on the US west coast, which might also interact with the effects of seagrass habitat to influence oyster performance. Common intertidal culture methods include growing oysters directly on the sediment surface (on-bottom) or suspended above the sediment surface (off-bottom) where they are grown on long-lines, in mesh flip bags or cages suspended from lines or placed on racks generally 0.5 to 1 m above the sediment surface. Off-bottom culture can be more resource intensive for growers but results in desirable characteristics for the restaurant half-shell market (Walton et al. 2013, Thomas et al. 2019). Oysters grown off-bottom have been shown to exhibit faster initial growth, particularly when water inundation time due to tidal elevation is taken into account (Ruesink et al. 2003). Given the effects of seagrass habitat on carbonate chemistry and food availability, interactive effects with culture position might be expected such that oysters grown off-bottom in seagrass habitat would experience ameliorating effects of the seagrass canopy on carbon chemistry and greater access to food while oysters grown on-bottom in seagrass habitat might have less access to planktonic food due to restricted water flow and be more affected by respiration of both seagrass and other organisms at night.

Our primary research objective in this study was to determine if the presence of seagrass affects the growth of juvenile oysters out-planted using on- and off-bottom culture positions during their first summer growing season at locations in US west coast estuaries where previous studies had shown that both food and water chemistry

varied. To investigate the underlying mechanisms of how seagrass might influence juvenile oyster growth, we specifically examined whether seagrass habitat altered local carbonate chemistry ( $p\text{CO}_2$ , pH, calcite saturation) and food quantity (chlorophyll *a* concentration [chl *a*]). Finally, we explored the relative influence of water conditions (temperature, pH, [chl *a*]) on several measures of oyster growth to further understand key drivers of *in situ* juvenile oyster performance.

## 2. MATERIALS AND METHODS

### 2.1. Study sites

We conducted this study in 2 estuaries along the US west coast with active commercial shellfish aquaculture operations: Netarts Bay, Oregon (45.4025, -123.9444, Fig. 1) and Willapa Bay, Washington (46.6622, -124.0106). We chose these estuaries based on expected unique gradients and established study

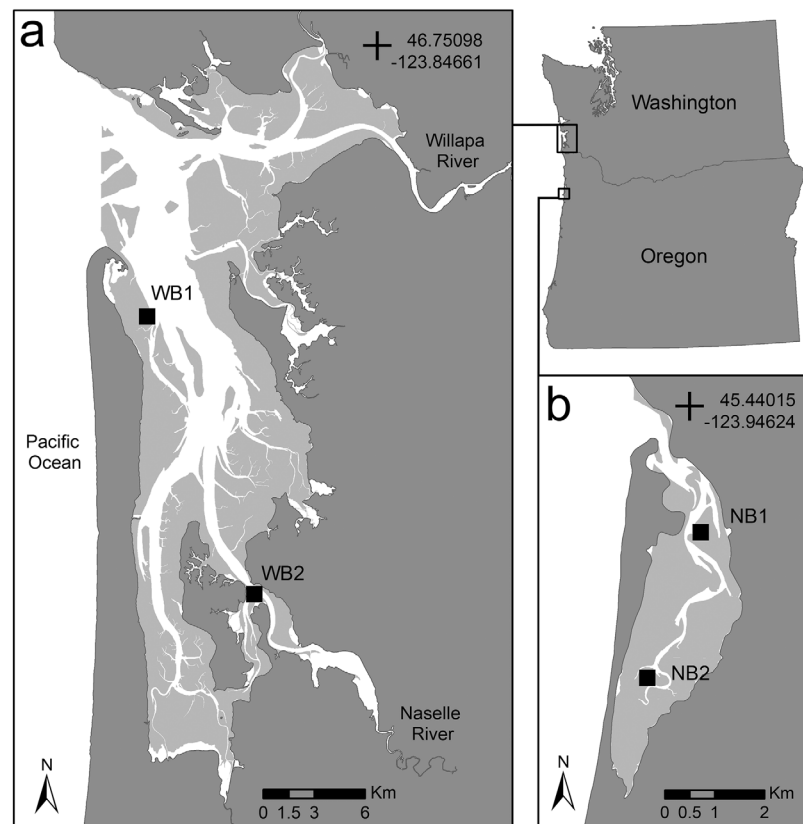


Fig. 1. Locations (black squares) of experimental sites in (a) Willapa Bay, Washington (WB1, WB2), and (b) Netarts Bay, Oregon (NB1, NB2), USA. At each experimental site, juvenile oysters were out-planted in eelgrass and adjacent unvegetated habitats, and positioned to grow on-bottom (at the sediment surface) and off-bottom (45 cm above the sediment)

sites to represent variable conditions at each endpoint of those gradients (referred to hereafter as up-estuary and down-estuary and representing a location near the freshwater end and the ocean or estuary mouth, respectively). We also chose intertidal sites where seagrass habitat, unvegetated mudflat habitat, and nearby Pacific oyster *Crassostrea gigas* (recently reclassified as *Magellana gigas*, but see Bayne et al. 2019) aquaculture co-occurred at approximately the same tidal elevation in each estuary. Seagrass habitat in these estuaries predominantly consists of the endemic eelgrass *Zostera marina* with some mixed presence of the introduced eelgrass *Z. japonica* especially at higher tidal elevations (Ruesink et al. 2010, Shafer et al. 2014, Dumbauld et al. 2022).

Netarts Bay is a relatively small and tidally flushed estuary, with over ~75% of its total volume exchanged on every tidal cycle (6.22 h). It has a relatively small watershed (36.3 km<sup>2</sup>) fed by several small streams whose summertime freshwater contribution to flushing is negligible (Glanzman et al. 1971, McCallum 1977, Davis & McIntire 1983). Therefore, patterns in carbonate chemistry (Fairchild & Hales 2021) and microalgal food availability were expected to be similar across the estuarine gradient and driven by ocean conditions during this summertime study. Pacific oyster culture operations occur in this estuary, but production is limited due to its small size. Netarts Bay is important to the shellfish industry because it is home to the Whiskey Creek Shellfish Hatchery, the largest independent producer of oyster larvae in the US Pacific Northwest (Barton et al. 2012), where impacts of acidic water entering the bay were first documented and linked to this strong ocean influence (Barton et al. 2015).

Willapa Bay is the third largest estuary and the single largest producer of oysters on the US west coast, and often leads the country in farmed shellfish production (Northern Economics 2013, USDA 2019). Commercial aquaculture beds occupy approximately 4888 ha, or roughly 23% of the intertidal area of this estuary (Dumbauld & McCoy 2015). Although culture methods have shifted with market demand, and the majority of actively farmed beds currently consist of on-bottom oyster culture where oysters are planted directly on the sediment surface, growers still practice off-bottom culture where oysters are raised on long-lines or other structures above the sediment surface (Dumbauld & McCoy 2015). Willapa Bay has a much larger watershed (2857 km<sup>2</sup>) than Netarts Bay, but is still strongly tidally influenced with a characteristically weak estuarine gradient in

the summer months when rivers have little discharge. Though an estimated half of the bay's water volume is exchanged and replaced by ocean water on every tidal cycle, the water has a 3 to 5 wk residence period in the upper estuary compared to 1 wk in the lower estuary (Banas et al. 2007). Upwelling also occurs in the nearshore coastal ocean off Willapa Bay and unfavorable carbon chemistry conditions have been previously correlated with poor oyster larval survival in this estuary (Hales et al. 2017). Nonetheless we expected more favorable carbonate chemistry (Ruesink et al. 2015, 2018) and higher food availability (Ruesink et al. 2003) for juvenile oyster growth to occur at the down-estuary site near the mouth of the estuary compared to the up-estuary site near the Naselle River where coastal acidification and freshwater alkalinity had previously been shown to cause lower pH values (Ruesink et al. 2015).

## 2.2. Oyster deployments

Juvenile oysters were out-planted within eelgrass habitat and adjacent unvegetated habitat along the same tidal elevation at 2 experimental sites located at opposite ends of the gradient in each estuary (Fig. 1). We utilized juvenile Pacific oysters *C. gigas*, sourced from the Oregon State University's Molluscan Broodstock Program (MBP), for outplants because the commercial industry primarily uses this species for production in the region. The MBP hatchery does not buffer incoming seawater, but water is only drawn during periods when conditions are favorable. While unintentional selection for fast larval growth has been documented in this hatchery (Durland et al. 2021), we utilized a single family for all outplants to avoid any differential carryover effects. Juvenile oysters were individually glued to experimental ceramic tiles using a non-toxic aquarium glue (4 individuals per tile; 11 × 11 cm tiles) with umbos oriented toward the center to encourage growth away from each other. Tiles were kept in hatchery conditions to allow juvenile oysters to deposit shell onto tiles for 2 wk and then out-planted to each experimental site in mid-June. At each outplant site, we placed 16 tiles of oysters: 8 tiles per habitat type (seagrass and unvegetated), with 4 tiles within each habitat type per culture position ('on-bottom' and 'off-bottom'). Tiles were attached to PVC poles at 5 and 45 cm heights above the sediment surface to represent on- and off-bottom culture positions. Tiles were oriented vertically and parallel with the current.

### 2.3. Growth metrics

We took photographs of oysters pre-outplant and post-outplant to measure shell growth, and we processed oysters at the end of the season to measure tissue mass growth. Oysters were  $16.3 \pm 3.2$  mm (mean  $\pm$  SD) in height (measured from umbo to longest axis of growth) when out-planted. Each month we used a small brush to remove biofouling from around each oyster. Shell height and width (widest part parallel to umbo) were measured for each oyster from pre- and post-outplant photos using ImageJ software to calculate an estimated shell area ( $\text{mm}^2$ ). Shell area was traced for a subset of oysters and found to be highly correlated with shell height  $\times$  width (Pearson's;  $t_{102} = 24.79$ ,  $\text{cor} = 0.98$ ,  $p < 0.0001$ ), so shell height  $\times$  width was used for estimating shell growth. The following equation was used to calculate daily shell growth for each oyster to be used in analyses:

$$\text{Shell growth} = \frac{\text{Final shell area (mm}^2\text{)} - \text{Initial shell area (mm}^2\text{)}}{\text{Total outplant days}} \quad (1)$$

At the end of summer, we retrieved the tiles and shucked each oyster to separate shell and somatic tissue. The bottom shell of each oyster could not be cleanly separated from the tiles, so we only dried ( $45^\circ\text{C}$ ) and weighed the tissue to obtain final tissue mass. The following equation was used to calculate daily tissue mass growth for each oyster to be used in analyses:

$$\text{Tissue growth} = \frac{\text{Final dried tissue mass (g)}}{\text{Total outplant days}} \quad (2)$$

In addition, the ratio of shell to tissue growth for each oyster was calculated and normalized to be between 0 and 1 as a proxy of energy allocation toward shell and somatic tissue for analysis. Oyster survival was not calculated or utilized for analyses, because we could not confidently determine the cause of mortality for lost oysters. Lost oysters could have been predated, have died as a result of stressful conditions, or have been simply knocked off the tiles by drifting debris.

### 2.4. Water properties

To directly compare water conditions experienced by oysters grown in eelgrass versus unvegetated habitat, we deployed 6600 series YSI<sup>®</sup> sondes alongside oysters in each habitat at each site for separate 24 h periods once a month from June to September 2019. Each YSI sonde measured temperature, salinity, relative [chl *a*], and pH every 15 min. Relative [chl *a*]

values were not true concentrations of chl *a* in the water column, but rather relative measures based on sensor calibrations using rhodamine dye solution. Sensors were secured in a PVC cup with holes drilled in the sides to allow for water movement while keeping the sensors wet during periods of low tide. Additional measurements of water quality and carbonate chemistry were made by collecting discrete water samples with a Niskin bottle during high slack tide on one afternoon each month at both sites and habitat types in each estuary. Water samples were collected 25 cm above the seafloor to match the average depth of growing oysters, transferred without bubbles to acid-washed amber glass bottles (355 ml), poisoned with 0.3 ml saturated solution of  $\text{HgCl}_2$ , and crimp-sealed with gas-tight metal caps. Concurrently, temperature, salinity, and dissolved oxygen concentration ([DO]) were measured with a handheld ProSolo YSI<sup>®</sup> unit. Water samples were analyzed for dissolved inorganic carbon ( $C_T$ ) and partial pressure of carbon dioxide ( $p\text{CO}_2$ ) on a Burke-o-Lator analyzer (Hales et al. 2004, Bandstra et al. 2006) at the US EPA Pacific Coastal Ecology Branch. Estimated analytical accuracy was 0.06–0.16% for  $C_T$  values and 0.79% for  $p\text{CO}_2$  values. The full carbonate chemistry system was calculated with CO2SYS (Lewis & Wallace 1998, Orr et al. 2018) using  $\text{CO}_2$  system constants from Millero (2010),  $K_{\text{HSO}_4}$  constants of Dickson (1990), and  $K_{\text{HF}}$  constants of Perez & Fraga (1987). In this study, we focused on the carbonate saturation state of calcite ( $\Omega_{\text{calcite}}$ ) rather than aragonite because, although less soluble than aragonite, it is thought to be the primary material utilized for shell building in post-settlement juvenile oysters (Gazeau et al. 2007, Ries 2011, Dodd et al. 2021). Estimated standard sampling errors for metrics of interest were calculated based on a subset of Niskin sampling when 3 crimp-sealed bottle samples from each of 8 Niskins were collected and processed.

### 2.5. Statistical analyses

#### 2.5.1. Oyster growth

We examined whether the presence of eelgrass, referred to as habitat (eelgrass, unvegetated) for analysis, affected the shell and tissue growth of juvenile oysters out-planted in 2 culture positions (on-bottom, off-bottom) using generalized linear models in R version 4.2.2 (R Core Team 2022). Individual oyster growth measures were averaged for each tile, which was used as the experimental unit within each model ( $n = 4$ ). All tiles were recovered from experi-

mental treatments except for 1 lost tile each in treatment NB1 off-bottom in eelgrass, NB1 off-bottom in unvegetated, and NB2 off-bottom in unvegetated. Variation in shell growth ( $\text{mm}^2 \text{d}^{-1}$ ), tissue growth ( $\text{g d}^{-1}$ ), and the ratio of shell to tissue growth as response variables were related to effects of site, habitat, and culture position using weighted generalized least square models assuming a Gaussian error distribution and allowing for unique variance structures by site and culture position using the 'nlme' package in R (Pinheiro et al. 2021). Tissue growth was square-root transformed to meet assumptions of normality. Significance of effects was assessed using likelihood ratio tests to determine the most parsimonious final models following Zuur et al. (2009). Following the principle of marginality, models with significant interaction terms were subset to test effects of interest using one-way ANOVAs and pairwise Tukey HSD tests.

### 2.5.2. Water properties

To investigate whether habitat type altered carbonate chemistry or food availability at each site, we assessed time-series plots of monthly 24 h sonde deployments and compared monthly discrete measures. First, we evaluated whether paired YSI sondes deployed in adjacent habitat types were concurrently measuring the same water mass, and therefore able to measure a habitat effect if one existed. To do so, we compared temperature and salinity values across each time series because we did not expect habitat to significantly affect these parameters. We then compared metrics of interest, i.e. pH and relative [chl *a*], for periods of the tide when the same water mass covered both habitats. YSI-specified sensor accuracies were included for each plotted measure to enable visual assessment of the magnitude of differences between sensor readings on paired instruments. Sensor accuracies were as follows: temperature was  $\pm 0.15^\circ\text{C}$ , salinity was  $\pm 0.1$  ppt, pH was  $\pm 0.2$  units, and relative [chl *a*] had none as a comparative sensor measure. Paired measurements with a magnitude greater than sensor accuracies were considered significantly different. Summary statistics and Wilcoxon tests were

used to compare discretely collected carbonate chemistry metrics ( $p\text{CO}_2$ , pH,  $\Omega\text{calcite}$ ) by habitat within each site. To evaluate site-level differences, summary statistics of monthly salinity and temperature sonde measures were compared.

### 2.5.3. Water properties and oyster growth

To further understand the underlying drivers of oyster growth among sites, we cautiously assessed the relative influence of monthly water conditions on growth. Monthly measures of sonde-collected mean relative [chl *a*], mean temperature ( $^\circ\text{C}$ ), and discretely sampled pH were utilized as orthogonal predictors of tile-averaged shell growth ( $\text{mm d}^{-1}$ ) and the ratio of shell to tissue growth in separate linear regressions (i.e. each tile-averaged growth response corresponded to monthly water measures for each site and habitat treatment).

## 3. RESULTS

### 3.1. Oyster growth

The effect of habitat on shell growth varied by site (site  $\times$  habitat interaction: Table 1) and only margin-

Table 1. Results of generalized least squares models for Pacific oyster shell and tissue growth

Response	Effect	df	<i>F</i>	<i>p</i>
Shell growth	Site	3,49	1005.33	<0.0001
	Habitat	1,49	0.003	0.96
	Position	1,49	70.77	<0.0001
	Site $\times$ Habitat	3,49	8.50	0.0001
	Site $\times$ Position	3,49	11.29	<0.0001
	Habitat $\times$ Position		Removed <sup>a</sup>	
	Site $\times$ Habitat $\times$ Position		Removed <sup>a</sup>	
Tissue mass	Site	3,51	303.98	<0.0001
	Habitat	1,51	2.05	0.12
	Position	1,51	77.59	<0.0001
	Site $\times$ Habitat		Removed <sup>a</sup>	
	Site $\times$ Position	3,51	8.08	0.0002
	Habitat $\times$ Position	3,51	7.42	0.0088
	Site $\times$ Habitat $\times$ Position		Removed <sup>a</sup>	
Shell to tissue	Site	3,49	19.96	<0.0001
	Habitat	1,49	1.09	0.30
	Position	1,49	19.09	0.0001
	Site $\times$ Habitat		Removed <sup>a</sup>	

<sup>a</sup>Effects were removed from full models using log likelihood ratio testing at the 95 % confidence level ( $p \geq 0.05$ )

ally by culture position (habitat × position interaction: log likelihood ratio  $\chi^2 = 3.48$ ,  $df = 20$ ,  $p = 0.06$ ). Eelgrass had a weak negative effect on shell growth at Willapa Bay sites and a weak positive effect on shell growth at Netarts Bay sites (Fig. 2a). Oysters had 12 and 4% lower shell growth in eelgrass than unvegetated habitat at WB1 and WB2, respectively (Tukey tests:  $p = 0.11$  and  $0.71$ , respectively, Fig. 2a). In comparison, oysters had 46 and 31% higher shell growth in eelgrass than unvegetated habitat at NB1 and NB2, respectively (Tukey tests:  $p = 0.21$  and  $0.63$ , respectively, Fig. 2a). Follow-up testing showed that culture position did not greatly mediate the effect of habitat; on-bottom cultured oysters grew just

$2.1 \text{ mm}^2 \text{ d}^{-1}$  on average more in eelgrass than at unvegetated sites (Tukey:  $p = 0.43$ , Fig. 3a), while off-bottom cultured oysters grew just  $1.6 \text{ mm}^2 \text{ d}^{-1}$  less in eelgrass than in unvegetated habitat (Tukey:  $p = 0.72$ , Fig. 3a).

There was significant interaction between site and culture position (site × position: Table 1) driven by a large difference in shell growth of on- and off-bottom oysters at sites in Netarts Bay (Fig. 4a). Oysters grew  $12.7 \text{ mm}^2 \text{ d}^{-1}$  more shell area on- than off-bottom at sites in Netarts Bay (Tukey:  $p < 0.001$ ) compared to just  $1.4 \text{ mm}^2 \text{ d}^{-1}$  more shell area on- than off-bottom at sites in Willapa Bay (Tukey:  $p = 0.45$ ). When comparing oysters grown on-bottom, site had a signifi-

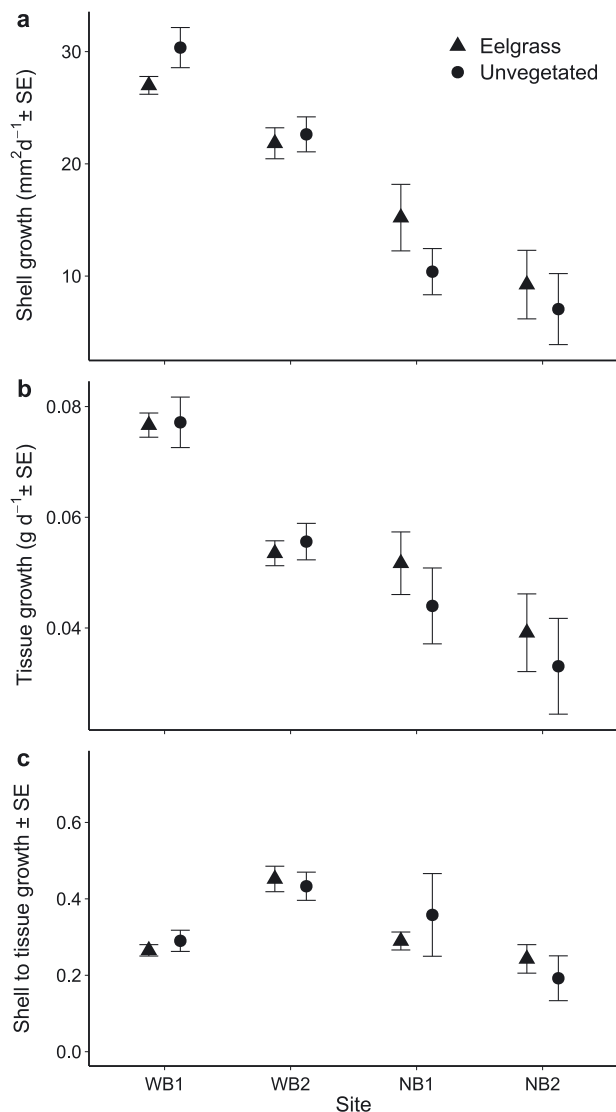


Fig. 2. Growth of oysters averaged by tile ( $n = 61$ ) for each site and habitat measured as (a) shell area ( $\text{mm}^2 \text{ d}^{-1} \pm \text{SE}$ ), (b) shell tissue ( $\text{g d}^{-1} \pm \text{SE}$ ), and (c) shell to tissue (ratio normalized between 0 and 1  $\pm \text{SE}$ )

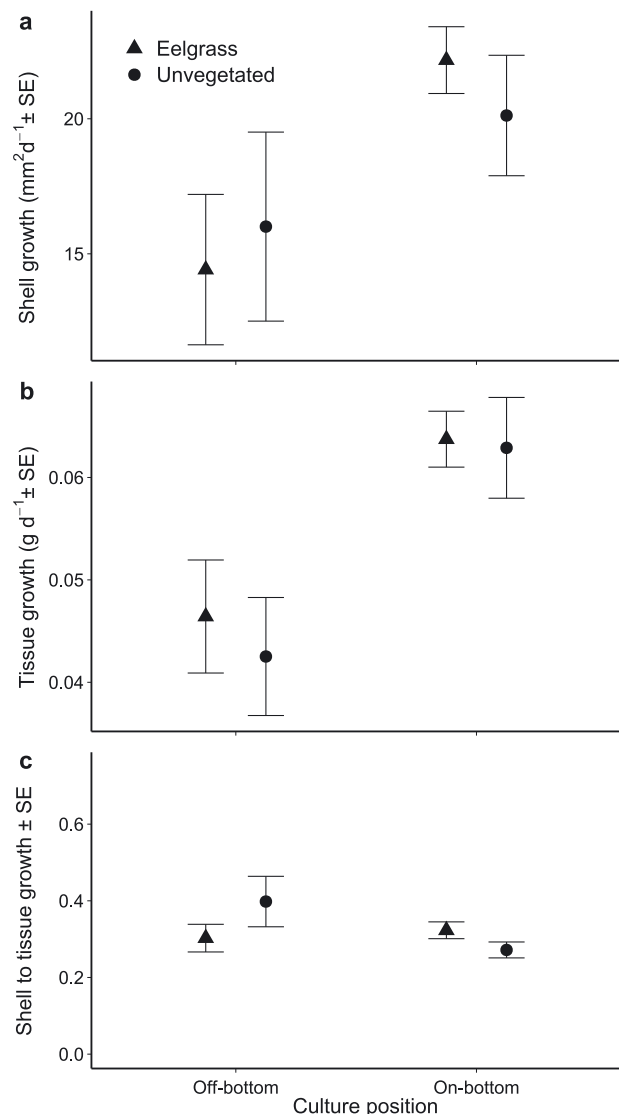


Fig. 3. Growth of oysters averaged by tile ( $n = 61$ ) for each culture position and habitat measured in (a) shell area ( $\text{mm}^2 \text{ d}^{-1} \pm \text{SE}$ ), (b) tissue ( $\text{g day}^{-1} \pm \text{SE}$ ), and (c) shell to tissue (ratio normalized between 0 and 1  $\pm \text{SE}$ )

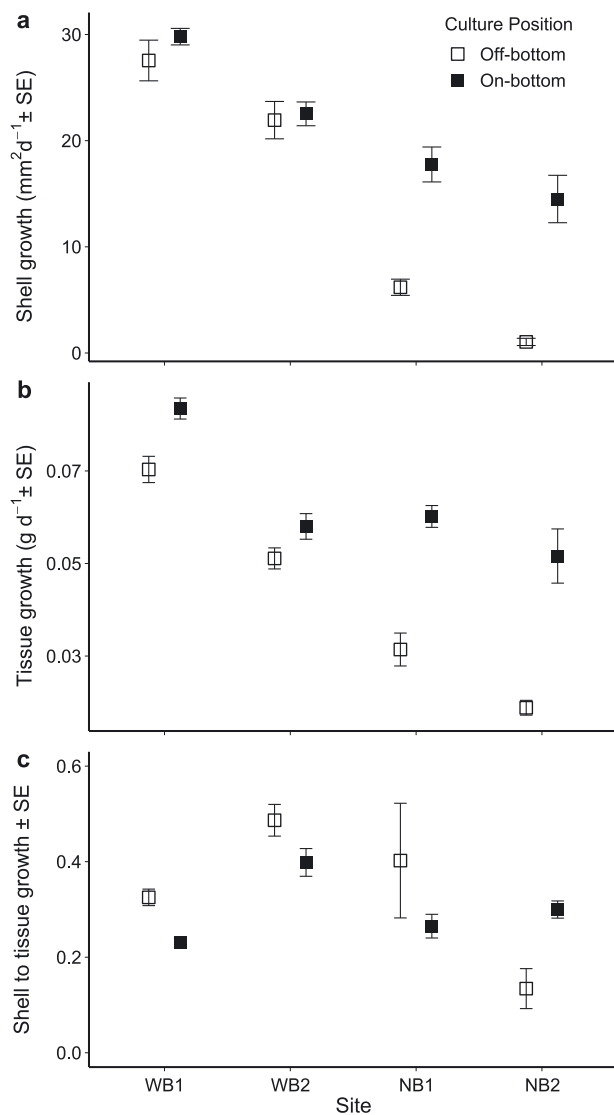


Fig. 4. Growth of oysters averaged by tile ( $n = 61$ ) for each site and culture position measured in (a) shell area ( $\text{mm}^2 \text{d}^{-1} \pm \text{SE}$ ), (b) tissue ( $\text{g d}^{-1} \pm \text{SE}$ ), and (c) shell to tissue (ratio normalized between 0 and 1  $\pm \text{SE}$ )

cant effect on shell growth ( $F_{3,28} = 18.57$ ,  $p < 0.001$ , Fig. 4a). Specifically, oysters had higher shell growth at sites in Willapa Bay than Netarts Bay (Tukey;  $p < 0.001$ ), and shell growth decreased from ocean to river in each estuary as follows: 29.8, 22.5, 17.8, and 14.5  $\text{mm}^2 \text{d}^{-1}$  at WB1, WB2, NB1, and NB2, respectively (Fig. 4a).

Similar to results for shell growth, habitat had little overall effect on tissue growth. However, differing model results indicate that the effect of habitat on tissue growth did not vary much by site (site  $\times$  habitat interaction: log likelihood ratio  $\chi^2 = 7.04$ ,  $\text{df} = 18$ ,  $p = 0.07$ ) but did vary by culture position (habitat  $\times$  posi-

tion interaction: Table 1). Follow-up tests revealed that habitat had little to no effect on tissue growth at any site (Tukey;  $p > 0.39$  for all within-site comparisons, Fig. 2b). The slight positive effect of eelgrass observed on tissue growth at NB1 was minimal (0.001  $\text{g d}^{-1}$  difference, Tukey;  $p = 0.40$ , Fig. 2b). The interactive effect between habitat and culture position was also minor, with just a slight positive effect of eelgrass for off-bottom cultured oysters (9% difference in tissue growth, Tukey;  $p = 0.63$ , Fig. 3b) and an even smaller effect of eelgrass for on-bottom oysters (1% difference in tissue growth, Tukey;  $p = 0.88$ , Fig. 3b).

Like shell growth, tissue growth also varied by site for on-bottom cultured oysters ( $F_{3,28} = 14.74$ ,  $p < 0.001$ , Fig. 4b). However, the effect of site was mostly driven by higher tissue growth at WB1 compared to the other sites (48% higher than other sites, Tukey tests:  $p < 0.001$  for each comparison), which all had similar tissue growth (within 17%, Tukey tests:  $p > 0.35$  for each comparison). Tissue growth decreased from ocean to river in each estuary, but this effect was less apparent than that for shell growth.

We saw the most notable habitat effect on the ratio of shell to tissue growth. Although there was little to no habitat effect at each site (site  $\times$  habitat interaction: log likelihood ratio  $\chi^2 = 2.40$ ,  $\text{df} = 15$ ,  $p = 0.49$ , Fig. 2c), there was a notable habitat effect that varied by culture position (habitat  $\times$  position: Table 1). Oysters cultured on-bottom had 16% higher shell to tissue growth in eelgrass compared to unvegetated habitat (Tukey;  $p = 0.10$ , Fig. 3c), and oysters cultured off-bottom had 28% lower shell to tissue growth in eelgrass compared to unvegetated habitat (Tukey;  $p = 0.20$ , Fig. 3c). There was also an interactive effect between site and culture position (site  $\times$  position; Table 1), as oysters had higher ratios of shell to tissue cultured off-bottom compared to on-bottom at all sites except NB2, where the opposite was observed (Fig. 4c, Tukey tests:  $p < 0.01$  for WB1,  $p = 0.07$  for WB2,  $p = 0.22$  for NB1, and  $p < 0.01$  for NB2).

When comparing oysters grown on-bottom, site had a significant effect on the ratio of shell to tissue growth ( $F_{3,28} = 11.33$ ,  $p < 0.001$ , Fig. 4c). In contrast with individual metrics of shell and tissue growth, the ratio of shell to tissue growth for on-bottom cultured oysters was highest at WB2, intermediate at NB1 and NB2, and lowest at WB1 (Fig. 4c). There was a pattern of increasing shell to tissue ratio from ocean to river in Willapa Bay (61% higher at WB2 than WB1, Tukey's:  $p < 0.001$ ) and only slightly in Netarts Bay (11% higher at NB2 than NB1, Tukey's:  $p = 0.69$ ).



### 3.2. Water properties

Paired YSI sonde measures revealed that the same water mass flowed over adjacent eelgrass and unvegetated habitats for the majority of each 24 h deployment, as shown by minimal detectable differences (within sensor accuracies) in temperature and salinity between habitats at each site, with some notable differences to consider (Figs. 5 & 6; Figs. S1–S5 in the Supplement at [www.int-res.com/articles/suppl/q015p287\\_supp.pdf](http://www.int-res.com/articles/suppl/q015p287_supp.pdf)). Specifically, at WB1, sig-

nificant differences in temperature and salinity measures (greater than sensor accuracies) occurred at variable and inconsistent times of the tidal cycle (up to 1.22°C and 0.79 ppt different, Fig. 5; Figs. S1 & S2). At WB2, significantly higher temperatures and salinities were measured in eelgrass habitat during several daytime low slack tides (up to 1.22°C and 0.79 ppt different, Fig. 5; Figs. S1 & S2). Most apparent, at NB1, eelgrass habitat was consistently significantly warmer and less saline than the adjacent unvegetated habitat on flood tides (up to 7.36°C and

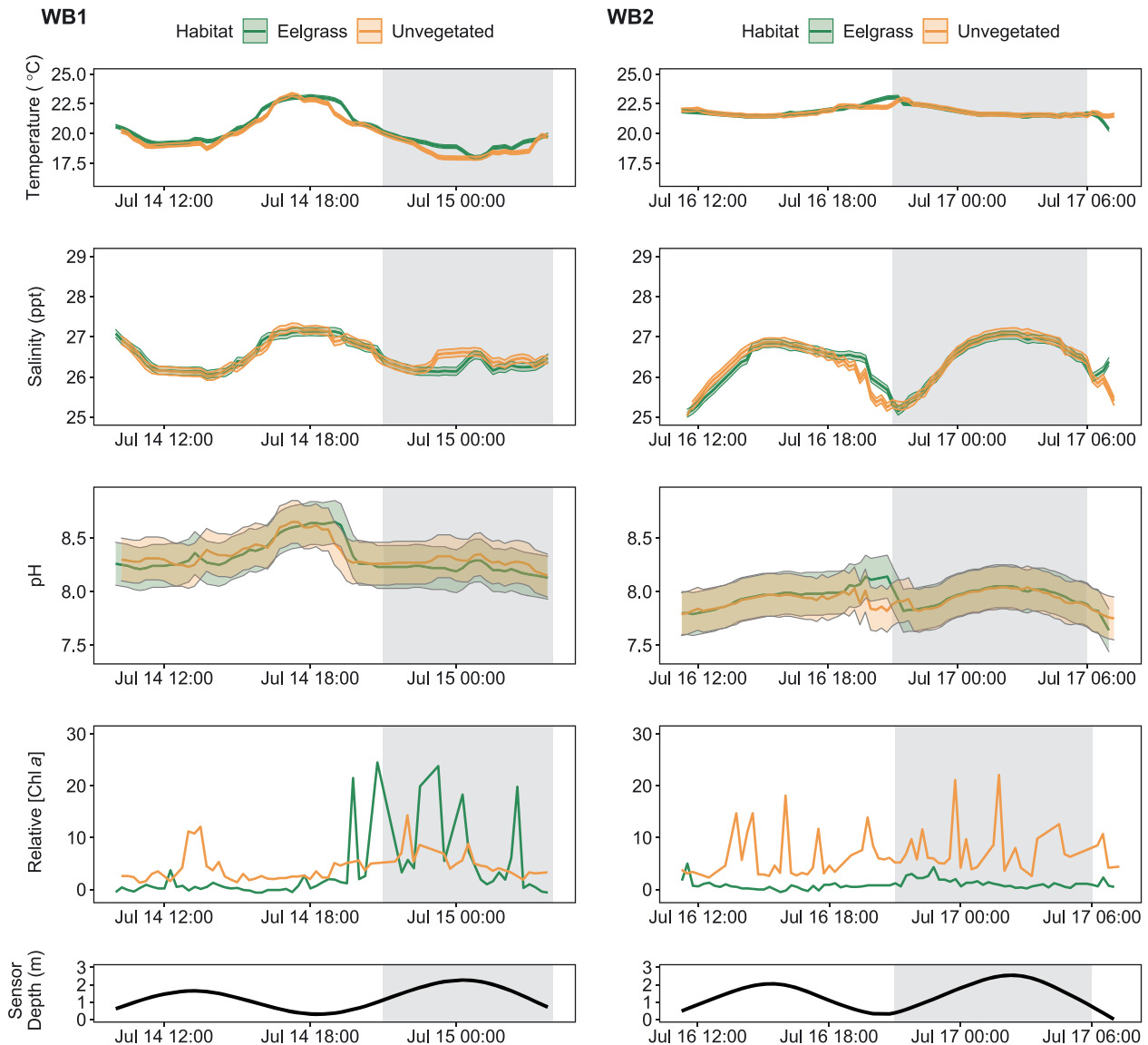


Fig. 5. Temperature (°C; sensor measure  $\pm$  accuracy), salinity (ppt; sensor measure  $\pm$  accuracy), pH (sensor measure  $\pm$  accuracy), relative chlorophyll *a* concentration ([chl *a*]), and sensor depths (m) measured every 15 min over 24 h deployments in July at each oyster outplant site in Willapa Bay (WB1, WB2) in adjacent eelgrass (green) and unvegetated habitat (yellow). Each metric shows raw sensor measures  $\pm$  sensor accuracy across each time series. Gray zones show hours of darkness based on first and last light. Note consecutive deployment dates for each site

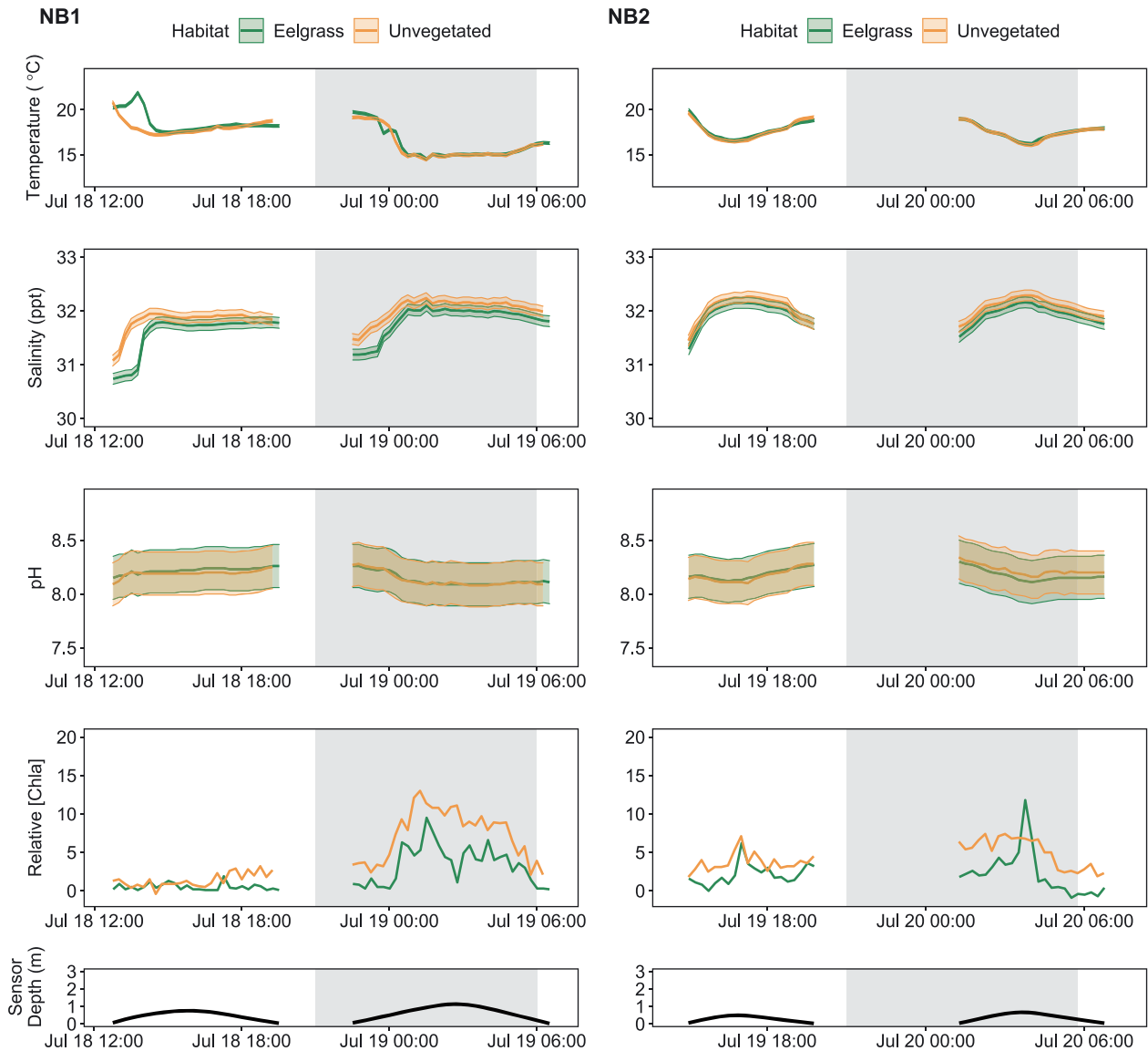


Fig. 6. Temperature ( $^{\circ}\text{C}$ ; sensor measure  $\pm$  accuracy), salinity (ppt; sensor measure  $\pm$  accuracy), pH (sensor measure  $\pm$  accuracy), relative chlorophyll *a* concentration ([chl *a*]), and sensor depths (m) measured every 10 min over 24 h deployments in July at each oyster outplant site in Netarts Bay (NB1, NB2) in adjacent eelgrass (green) and unvegetated habitat (yellow). Gray zones show hours of darkness based on first and last light. Note consecutive deployment dates for each site

1.82 ppt different, Fig. 6; Figs. S3–S5). This observation suggests that different water masses might have flowed over adjacent habitats during each flood tide and then mixed as the site covered. Given these noted differences, we compared habitat conditions outside these periods and overall.

Habitat had no detectable influence on carbonate chemistry conditions. Only considering periods of the tide when the same water mass likely covered adjacent habitats, pH was not detectably different. Further, this held true over the entire tide cycle of each deployment; pH conditions were similar even during the

most noted periods of different temperature and salinities. Discrete sampling also showed little to no difference in carbonate chemistry measures between habitats at each site (Table 2; Wilcoxon for all within-site habitat tests:  $p > 0.48$ ,  $n = 4$ ), pH (Wilcoxon for all within-site habitat tests:  $p > 0.33$ ,  $n = 4$ ), and  $\Omega_{\text{calcite}}$  (Wilcoxon for all within-site habitat tests:  $p > 0.48$ ,  $n = 4$ ).

In contrast, habitat did have a detectable influence on food quantity; unvegetated habitat consistently had higher relative [chl *a*] values than adjacent eelgrass habitat. Relative [chl *a*] measures were highly variable, but more frequently greater in unvegetated com-

Table 2. Summary statistics (mean ± SD, min, max) of water parameters by site and habitat type measured from monthly YSI sonde deployments and discretely collected Niskin samples. Discrete sample standard sampling errors are given in parentheses associated with each metric

	WB1		WB2		NB1		NB2	
	Eelgrass	Unvegetated	Eelgrass	Unvegetated	Eelgrass	Unvegetated	Eelgrass	Unvegetated
<b>YSI sonde</b>								
Tidal height (m)	0.5	0.0			1.0	1.5		
Salinity (ppt)	28.6 ± 1.48, 26.0, 32.1	27.9 ± 1.42, 22.0, 30.1			32.6 ± 0.7, 30.7, 33.8	32.5 ± 0.7, 30.3, 33.6		
Temperature (°C)	19.1 ± 1.5, 12.2, 23.3	20.8 ± 1.07, 16.6, 23.1			15.4 ± 2.9, 8.9, 17.4	16.9 ± 2.1, 10.4, 24.0		
Relative [Chl a]	3.3 ± 3.6, 0.0, 24.5	2.7 ± 2.0, 0.0, 13.9			4.3 ± 3.6, 0.1, 16.4	3.2 ± 3.4, 0.0, 21.0		
pH (total scale)	8.24 ± 0.12, 7.94, 8.65	7.92 ± 0.10, 7.63, 8.14			8.10 ± 0.19, 7.66, 8.41	8.13 ± 0.12, 7.83, 8.50		
n (# of sensor measures)	257	253	253	253	288	274	288	274
<b>Discrete samples</b>								
Alk. (µeq kg <sup>-1</sup> , ± 7.04 standard sampling error)	2078.3 ± 49.8, 1955, 2191	2077.9 ± 50.1, 1955, 2188			2244.7 ± 16.8, 2213, 228	2230.2 ± 24.9, 2170, 2287		
pCO <sub>2</sub> (µatm, ± 10.25 standard sampling error)	361 ± 89, 245, 433	345 ± 78, 262, 413			398 ± 107, 269, 517	347 ± 111, 212, 482		
pH (total scale, ± 0.01 standard sampling error)	8.07 ± 0.09, 7.99, 8.19	8.08 ± 0.08, 8.01, 8.16			8.05 ± 0.10, 7.95, 8.16	8.10 ± 0.12, 7.97, 8.26		
Ωcalcite (± 0.09 standard sampling error)	3.9 ± 0.6, 3.1, 4.5	4.0 ± 0.6, 3.2, 4.5			4.0 ± 0.9, 3.1, 5.1	4.5 ± 1.1, 3.3, 5.9		
n (# of samples)	4	4	4	4	4	4	4	4

pared to adjacent eelgrass habitat during each 24 h deployment (Figs. 5 & 6; Figs. S1–S5) and overall, in average and cumulative measures (Table 2). Unvegetated habitats had 34, 48, 39, and 57 % higher relative [chl a] present than adjacent eelgrass habitats at WB1, WB2, NB1, and NB2, respectively.

Sites varied characteristically by salinity, temperature, and carbonate chemistry from expected ocean and freshwater influence. Willapa Bay sites exhibited a stronger freshwater influence than Netarts Bay sites; WB1 and WB2 were less saline (3.4 ppt lower) and warmer (3.0°C higher) than NB1 and NB2 (Table 2). Within each estuary, sites showed little difference in salinity or temperature. WB1 was only slightly more saline and cooler than WB2 (1.25 ppt and 1.63°C different), and NB1 was essentially the same in salinity and only minimally cooler than NB2 (0.09 ppt and 1.55°C different). WB2 contrasted all other sites with the lowest range of salinity and most acidic conditions (Table 2). WB2 was on average 163 µatm higher in pCO<sub>2</sub>, 0.17 units lower in pH, and 1.3 units lower in Ωcalcite than the other sites.

### 3.3. Water properties and oyster growth

Only on-bottom cultured oyster growth measures were utilized to examine relative influence of water properties on growth to exclude the high variability in growth observed by culture position (Table 1); very little growth was observed at sites in Netarts Bay (Fig. 4a). Regressions suggest that shell growth (mm d<sup>-1</sup>) was positively correlated with temperature (linear regression: slope = 0.65, adjusted r<sup>2</sup> = 0.06, df = 116, p < 0.001), but not with pH (slope = -2.7, adjusted r<sup>2</sup> = -0.008, df = 116, p = 0.60) or relative [chl a] (slope = 0.44, adjusted r<sup>2</sup> = -0.008, df = 116, p = 0.10). In contrast, the ratio of shell to tissue growth was most strongly negatively correlated

with pH (slope =  $-0.54$ , adjusted  $r^2 = 0.13$ ,  $df = 116$ ,  $p < 0.001$ ), weakly positively correlated with temperature (slope =  $0.01$ , adjusted  $r^2 = 0.04$ ,  $df = 116$ ,  $p = 0.06$ ), and not correlated with relative [chl *a*] (slope =  $-0.01$ , adjusted  $r^2 = 0.04$ ,  $df = 116$ ,  $p = 0.19$ ).

## 4. DISCUSSION

### 4.1. Seagrass habitat

Our primary objective in this study was to contrast juvenile Pacific oyster growth in eelgrass versus unvegetated habitat and evaluate whether any differences observed were due to known effects of this plant on carbonate chemistry and the quantity of food available to these filter feeders. Results showed that oysters grew larger when planted closer to the estuary mouth and positioned on-bottom, but eelgrass habitat had little to no effect on growth as measured by shell size or tissue mass alone across outplant sites. The only notable effect of eelgrass habitat was for the ratio of shell to tissue growth where oysters planted on-bottom devoted more resources to shell relative to tissue when planted in eelgrass. Oysters planted off-bottom generally displayed lower shell relative to tissue growth in eelgrass habitat, but this effect was not consistent across sites and was likely due to differences in tidal elevation. Expected gradients were present in both estuaries with slightly warmer temperatures and notably more acidic conditions observed at the up-estuary site in Willapa Bay. While there was consistently less food available (measured as relative [chl *a*]) in eelgrass compared to unvegetated habitat at all sites, there were no observable differences in average carbonate chemistry conditions (measured as  $p\text{CO}_2$ , pH, and  $\Omega_{\text{calcite}}$ ) between habitats. Despite the lack of a habitat effect on carbonate chemistry conditions, these measures of carbonate chemistry at the site level appeared to be correlated with a higher ratio of shell to tissue growth for juvenile oysters.

A site-dependent effect of eelgrass habitat on juvenile oyster growth has been previously reported at some of the same sites in both of these estuaries. Results were similar to those we report for growth measured as shell size. Highest values were reported at sites near the estuary mouth, but effects of eelgrass were inconsistent at sites along the estuarine gradient in both estuaries and the largest habitat differences were observed at sites in Netarts Bay (Smith 2016, Lowe et al. 2019b, Dumbauld et al. 2021). These authors and others who have examined the potential

effects of carbonate chemistry alone on juvenile oyster growth in field studies conducted in US west coast estuaries (Venkataraman et al. 2019, Hollarsmith et al. 2020) mostly evaluated oyster growth measured as shell size. They either did not concurrently measure both food and water chemistry, or they measured these variables, but not at intertidal sites where most oysters are cultured in these estuaries. Since the only habitat effect we observed was for a ratio of shell growth to tissue growth, our results suggest that measuring growth as shell size alone may not capture any subtle effects of eelgrass habitat on oyster performance and that effects of eelgrass on water chemistry, though difficult to quantify at appropriate scales *in situ*, may not be significant.

Results of several recent laboratory mesocosm studies where both water chemistry and seagrass habitat could be controlled suggest that effects on oyster growth depend on experimental treatments (both seagrass and water chemistry), but oyster physiology and how growth is measured are also important. Garner et al. (2022) found that the presence of eelgrass *Zostera muelleri* enhanced juvenile oyster *Saccostrea glomerata* shell growth at ambient pH ( $8.1 \pm 0.02$ ) but not at reduced pH treatment levels ( $7.85 \pm 0.15$ ) where the opposite effect occurred. Habitat and pH treatments were crossed, but treatment levels were static, and because food was held constant, the authors attributed this effect to pH. Ricart et al. (2021a) demonstrated that *Z. marina* density was important and positively affected pH during daylight hours but not at night in mesocosm experiments. Both shell and tissue growth of juvenile Pacific oysters deployed in these mesocosms for 30 d were affected, but shell growth increased linearly while tissue weight and therefore peak oyster condition (tissue weight/total weight) values occurred at intermediate eelgrass shoot density (200–300 shoots  $\text{m}^{-2}$ ). They attributed the effect of eelgrass density on shell growth to ameliorated pH and suggested that shell and tissue growth were potentially uncoupled such that tissue weight might be more sensitive to food concentration and reduced at high eelgrass density. We did not evaluate the effects of eelgrass density, but eelgrass presence consistently reduced food quantity available to oysters at all sites; while tissue weight was not consistently reduced, the ratio of shell to tissue growth was enhanced for oysters grown on bottom in eelgrass at all sites, which is consistent with such a decoupled effect (enhanced tissue growth relative to shell growth when food is limited).

In other experiments designed to examine the physiological effects of acidified seawater alone, re-

duced shell growth when observed was linked to shell thinning and dissolution (Timmins-Schiffman et al. 2012, Wright et al. 2014) and not always reflected in measurements of shell size. Similarly in multifactorial experiments with a crossed design, Ko et al. (2014) found that shell growth increased in low pH treatments, but decreased at high temperatures and low salinity, such that overall decreases in growth occurred only when all 3 of these parameters were at stressful levels. Bednaršek et al. (2022) examined oyster growth and physiology under more realistic scenarios where treatments included fluctuating pH based on values measured over multiple tidal cycles during the summer season in US west coast estuaries and found that the level of shell dissolution depended more on frequency of exposure to low pH rather than the level itself in static exposures. These authors observed either no effect or a much weaker effect of pH on tissue mass in mesocosm experiments, but food was either not measured or held constant. A meta-analysis of the few published papers that included experimental results for mollusks and other invertebrates with crossed factors including food indicated that enhanced food had positive effects on both growth and calcification, and that the negative effects of reduced pH, at least on calcification, were mitigated when food was enhanced (Ramajo et al. 2016, 2018, Brown et al. 2018). Calcification is an energy-intensive process, and elevated  $p\text{CO}_2$  has also recently been shown to decrease clearance rates and ingestion, increasing oxygen consumption and ammonia excretion, and thereby decreasing overall scope for growth (Jiang et al. 2021). When measured, calcification as a trait has not been shown to be significantly affected, but it is not clear whether shell dissolution is also a stressor decreasing overall scope for growth. The metabolic tradeoffs and biomineralization processes for shell differ amongst species, and the mechanisms remain under study (Chandra Rajan et al. 2021, Lee et al. 2021, Dang et al. 2023, Mele et al. 2023). These laboratory mesocosm studies do not adequately replicate the physicochemical characteristics of dynamic estuarine systems where levels of stressors vary in space and time and where exposure to multiple stressors have been shown to have interactive effects on marine organisms like oysters (Gunderson et al. 2016). Nonetheless, the results of these experimental studies conducted to date provide context for the site-specific effects we observed and the lack of a consistent effect of eelgrass habitat on juvenile oyster growth across sites. Highest juvenile oyster growth (measured as either shell size or tissue mass) was ob-

served at the down-estuary location in Willapa Bay where both food and water chemistry were favorable, and lowest growth was observed at the up-estuary site in Netarts Bay where water chemistry was favorable, but tidal elevation resulted in much less immersion time and therefore lower food availability. Because eelgrass reduced food availability (measured as relative [chl *a*]) across all sites, but did not consistently affect carbonate chemistry, we would expect reduced oyster growth especially for on-bottom oysters located in eelgrass. Instead, we observed interaction between site and position, but also site and habitat for shell growth, and the only consistent habitat effect was for a ratio of shell to tissue growth which was higher for oysters grown on-bottom in eelgrass. Although counterintuitive, this suggests that food was important, as we would expect a reduction in food to increase the amount of energy devoted to shell relative to tissue, despite values for each of these individual measures. Similarly, we might expect the opposite effect if carbonate chemistry was influenced by habitat (e.g. eelgrass would increase pH and thereby decrease the amount of energy oysters devoted to shell versus tissue). Several alternatives are also possible, including, for example, that relative [chl *a*] values measured did not adequately represent food assimilated by oysters. We therefore discuss this result in the context of differences in food and water chemistry that we expected along estuarine gradients and with hydrodynamics based on previous studies in the sections below.

## 4.2. Estuarine gradient

Distinguishing the effect of seagrass on pH from its effect on food availability at intertidal estuarine sites *in situ* has proven difficult because these variables not only co-vary, but are hard to quantify simultaneously over appropriate temporal scales (e.g. daily semidiurnal tidal cycle, day/night cycle, and weekly, monthly, and seasonal cycles affecting both ocean and riverine source water) and spatial scales (e.g. location along the estuarine gradient, tidal height, distance from channel) that are relevant to the organism (Waldbusser & Salisbury 2014). In this study, data collected using 24 h sonde deployments and discrete water samples during daytime flood tides at each intertidal site directly alongside the oysters at monthly intervals reflected this relatively high temporal and spatial variability, but generally followed expected large-scale patterns in hydrodynamics and

the correlated changes in temperature, salinity, and pH across each tidal cycle at sites in each estuary.

The ocean influence was strongest at both sites in Netarts Bay where the highest mean salinities ( $\bar{x} = 32.5$  and 32.6 ppt at down- and up-estuary endpoints, respectively) and lowest mean temperatures ( $\bar{x} = 16.0$  and 16.9°C at down- and up-estuary endpoints, respectively) were observed. Less fluctuation occurred over the tidal cycle in Netarts Bay, and pH and relative [chl *a*] values at both sites also reflected the dominant ocean influence. pH generally decreased on flood tides and increased on ebb tides, while relative [chl *a*] increased on flood tides. There were no obvious diel patterns, but pH declined to its lowest values on high slack tides especially at night, likely indicating that upwelled water had entered the bay and then been exposed to benthic metabolism (Fairchild & Hales 2021). Calcite saturation values varied more across months than by site or habitat on individual sampling dates, but discrete sample results corroborated values measured with sensors and suggested that pH was lowest at the ocean endpoint in Netarts Bay.

As expected, a stronger estuarine gradient was observed in Willapa Bay, but lower salinities ( $\bar{x} = 28.8$  and 27.6 ppt) and warmer temperatures (18.3 and 19.9°C) were evident at both endpoints compared to those observed in Netarts Bay. Slightly greater fluctuations were observed over tidal cycles in Willapa Bay with an atypical pattern of declining salinity observed on some flood tides at the down-estuary endpoint, potentially due to the general influence of the Columbia River plume during relaxation events (Banas et al. 2009, Giddings & MacCready 2017). While both pH and relative [chl *a*] values reflected the ocean influence at the down-estuary site with pH generally decreasing on flood tides and increasing on ebb tides, the presence of more saline water on ebb tides at this site suggests potentially reversed flow at some time points. pH values at the up-estuary site were the lowest observed in this study (generally <8), and the ocean influence at this site was muted and delayed. Relative [chl *a*] values were also low at this site and increased on the ebb tide instead of the flood tide, reflecting the contribution of terrestrial or riverine sources. There were no clear diel patterns nor habitat differences in pH, but relative [chl *a*] values were generally higher outside eelgrass and at night. Discrete bottle samples taken at high tide during the day again corroborated the sensor measurements and suggested that both pH and calcite saturation were lowest at the riverine endpoint in Willapa Bay as had been previously documented (Ruesink et al. 2015, 2018).

The magnitude of fluctuations in both pH and relative [chl *a*] in both estuaries varied by deployment date and were less dramatic in September during a period of low upwelling in the ocean adjacent to Willapa Bay (coastal upwelling transport indices [CUTI] at 46° N = 0.16 to 0.20) and relaxation/downwelling adjacent to Netarts Bay (CUTI indices at 45° N = -0.2 to 0) than during earlier months. pH values appeared to drop but remained slightly higher in eelgrass compared to unvegetated habitat at low slack tides during the day, but these differences were measured within sensor error. Consistent differences in salinity and temperature during these low slack tides suggest that eelgrass habitat might retain water during some periods (e.g. neap tide series and/or the high–low semidiurnal tide) when it affects more of the water column and water is moving slowly.

#### 4.3. Culture position and tidal elevation

Tidal elevation affects juvenile oyster growth by altering immersion time, but also by altering other factors like fouling by competitors (Bishop & Peterson 2006) and position above the substrate (on- and off-bottom; Ruesink et al. 2003) that cause interactive effects. When oysters are emersed at low tide, they close their shells and cannot feed, but gas exchange is also restricted and they experience greater respiratory and metabolic acidosis which can impact energy available for growth (Pörtner 2008, Eilers et al. 2009, Scanes et al. 2017). We attempted to avoid some of these effects by deploying oysters at the same tidal elevation and removing fouling organisms each month from the area surrounding individual oysters measured in this study. Unfortunately, after deployment, we discovered that tidal height, estimated from cumulative immersion time, differed at each of the sites. Thus, immersion time is incorporated in the site effect and is likely at least partially responsible for significant interaction effects that we observed between position (oysters planted 0.45 m off-bottom and directly on the sediment surface) and site in model results for all 3 measures of oyster growth. This effect was most notable for shell growth, with oysters deployed on-bottom in Netarts Bay growing 12.7 mm<sup>2</sup> d<sup>-1</sup> more shell area than those grown off-bottom. The effect was less apparent for tissue growth, but translated into a similar effect for the ratio of shell to tissue growth with higher shell to tissue growth for off-bottom oysters at all sites except NB2, where tidal elevation was highest (+1.5 m mean lower low water, MLLW) and where the oposite

effect occurred. These results contrast sharply with results of a previous study conducted in Willapa Bay where oysters deployed on bottom grew slower than those deployed 60 cm above the sediment surface, by an amount roughly proportional to tidal height or immersion time (Ruesink et al. 2003). These authors only presented data on shell growth and found that this effect diminished in the mid- to high intertidal zone ( $> +1.8$  m MLLW) where shell growth was reduced for oysters deployed both on and off-bottom. The authors examined shell growth along a transect within sites where elevation and thus immersion time could be evaluated and distinguished from other on/off-bottom effects, whereas we could not easily determine whether the position–site interaction was due solely to elevation. Seasonal food supply and potentially other seston in the water column versus that in the benthic boundary layer could also be important factors (Lee et al. 2017, Noisette et al. 2022).

#### 4.4. Hydrodynamics and food quality

The effect of seagrass on water flow and water residence time and secondary effects on both food and water chemistry likely also influenced our results. While the local hydrodynamic effects of seagrass beds have been well documented (Fonseca & Koehl 2006, Koch et al. 2007, Hasegawa et al. 2008), fewer studies have explicitly evaluated these secondary effects especially over shallow intertidal areas where resuspension often governs water properties, especially during large tidal exchanges or during storm and wind events (Widdows et al. 2008). One such study conducted in several estuaries, including Willapa Bay, suggests that while water flow was reduced in eelgrass and total suspended solids and chl *a* concentrations were greater in surface water flowing over eelgrass, only total suspended solids decreased as water depth increased (Ruesink et al. 2019). These researchers used Lagrangian surface drifters to monitor water as the tide flooded over these habitats and thus measured these properties at increasing distances above the sediment surface where bottom cultured oysters reside over time. Because our instruments were deployed just above the sediment surface for the entire tidal cycle, they show the increase in [chl *a*] as the tide floods, especially at the sites near the estuary mouth but generally lower values within eelgrass than outside eelgrass. We suspect that large differences in amplitude of [chl *a*] peaks between individual dates and especially between sites may be due to differences in flow,

e.g. strength of the tidal currents and perhaps also wind events. Nonetheless, when averaged across dates, higher relative [chl *a*] values were found outside eelgrass than inside eelgrass at all sites in our study. No relationships were observed between relative [chl *a*] values and oyster shell or tissue growth across sites, but the ratio of shell to tissue growth was higher for oysters grown on-bottom in eelgrass. As noted above, this suggests that food was important because we would expect a reduction in food to increase the amount of energy devoted to shell relative to tissue especially when other stressors like carbonate chemistry which could produce the opposite effect did not occur. Lowe & Ruesink (2021) conducted a manipulative field experiment and deployed oysters at 2 sites close to where we deployed oysters in Willapa Bay. While these authors found that oysters at the up-estuary site (close to WB2) grew faster than those at the down-estuary site (close to WB1), they also showed that artificially augmented food levels had no effect on juvenile oyster shell growth at either site. This suggested that suspended food was not limiting shell growth at either location, and despite consistently lower pH values at the up-estuary site similar to those we measured, they attributed this to effects of fouling that occurred at the down-estuary site. They based this argument on a second experiment where reduced oyster shell and tissue growth were observed at the up-estuary site when water chemistry was altered by buffering pH and food was added. Had they contrasted these 2 values, the most reduced shell to tissue ratio in their mesocosms would have been for the buffer treatment, which would mirror our results and would be related to enhanced pH at this location.

Relative values of [chl *a*] may reflect food quantity, but they do not necessarily reflect food quality or the ability of oysters to feed when sediment and other non-organic particles are present in the seston. While outside the scope of our research, the reduced [chl *a*] levels that we observed in eelgrass habitat at all sites could be due to other effects on food quality such as shading effects on benthic diatoms or reduced presence of dinoflagellates due to production of allelopathic chemicals by eelgrass itself (Jacobs-Palmer et al. 2020). Even fewer studies have examined the effects of seagrass and flow on water chemistry (reviewed with other coastal engineering species by Noisette et al. 2022), but a recent *in situ* experiment conducted in a seagrass bed on the coast of Saint Martin demonstrated that seagrass increased water residence time and therefore enhanced the day–night effects on pH, especially at a location where

ambient flow was weaker (James et al. 2020). We did not assess flow at our sites, but a previous study in Willapa Bay suggests that water residence time is significantly influenced on a local scale with significant cross-shore gradients independent of tidal height and along the estuarine gradient (Wheat et al. 2019).

#### 4.5. Larger spatial and temporal scales

Although models and direct measurements have demonstrated that seagrass can buffer local pH in temperate estuaries, this effect varies over daily tidal cycles and across broader annual temporal scales due to seagrass metabolism (Koweek et al. 2018, Ricart et al. 2021b). Its ability to serve as a refuge for oysters and thereby benefit oyster aquaculture at these scales is therefore less certain. Instrument resolution prevented us from discerning differences in pH measured inside and outside eelgrass at individual points in time over 24 h sampling periods, and we were not able to deploy them for extended periods in these intertidal areas, but our results suggest that when integrated across the four 24 h time series, there was no consistent effect of eelgrass habitat on juvenile oyster growth over the summer at our sites. Nonetheless, our results confirm that ambient levels of pH can vary widely and are likely modulated by local ecosystem metabolism (Cheng et al. 2015, Lowe et al. 2019a) and therefore could affect oyster growth if conditions change. Under laboratory conditions, Garner et al. (2022) found a positive effect of eelgrass on oyster shell growth at ambient  $p\text{CO}_2$  but not at elevated  $p\text{CO}_2$  levels representing these future scenarios. Even if we were unable to detect the relatively small and short-term reductions in pH that eelgrass has previously been shown to provide under current conditions, this benefit seems even less likely to occur over longer temporal scales with higher predicted atmospheric  $\text{CO}_2$  levels and the decreased ability of this seagrass to buffer more asymmetrical extremes in carbonate chemistry under future conditions (Pacella et al. 2018).

#### 4.6. Conclusions and application

Pacific oyster aquaculture continues to be an important industry around the world. The US west coast industry has already experienced the effects of altered water chemistry on survival of larval oysters in hatcheries. Lower oyster production has been attributed to acidification in other countries as well,

with potentially dire consequences for large producers like China especially under future climate change projections. Our results suggest that eelgrass habitat did not alter average local carbonate chemistry (pH,  $p\text{CO}_2$ ,  $\Omega_{\text{calcite}}$ ) conditions, but consistently reduced potential food availability for juvenile oysters (relative chl *a*) and thus might not mitigate acidified conditions. Juvenile oyster growth measured as shell size was not consistently influenced in eelgrass habitat, but a ratio of shell to tissue growth was enhanced for oysters grown on-bottom in this habitat. Average site-level pH across estuaries was also negatively correlated with shell to tissue growth, suggesting that juvenile oysters may display a compensatory response and allocate more energy to shell than tissue growth under stressful conditions (low pH and/or altered food supply due to reduced immersion or eelgrass presence).

Ocean and coastal acidification are just one result of climate change, and the physiological response of both juvenile and adult oysters to reduced pH combined with other stressors should continue to be evaluated, especially considering availability of advanced genomic tools suggesting that observed phenotypic responses like those we measured could be plastic (Dang et al. 2023, Wang et al. 2023). Evidence also suggests that these responses are non-linear with tolerance thresholds or tipping points (pH values around 7.3–6.9) below which they will be more dramatic in the future (Somero et al. 2016, Lutier et al. 2022). Both seagrass and oyster habitats have other attributes and provide numerous ecosystem services at the seascape scale, including carbon sequestration, sediment/shoreline stabilization, and habitat provision, that will also change under future 2011, Plummer et al. 2013, Lemasson et al. 2017, Unsworth et al. 2018, Ricart et al. 2020, Barrett et al. 2022). Finally, co-culture has been shown to benefit seagrass as well (Groner et al. 2021, Agnew et al. 2022), and this interaction should continue to be evaluated.

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