The influence of climate and environment on the growth and survival of Pacific oyster seed in US West Coast estuaries

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Abstract: Pacific oysters *Crassostrea gigas* were introduced to the US west coast in the early 1900's and were initially raised directly from seed (juveniles that had set naturally on cultch shell) imported from Japan. Oysters regularly spawned and became "naturalized" in only several selected West coast locations, such as Willapa Bay, Washington, where conditions allowed for both adult oyster spawning and larval survival, retention and settlement. The shellfish industry relied on "natural or wild" caught seed from these locations or continued seed imports until the advent of hatchery technology in the late 1970's. Recent larval mortality events in hatcheries have been linked directly to changes in seawater chemistry with high pCO$_2$ conditions and acidified water associated with seasonal upwelling along the U.S. West Coast. These conditions may have also resulted in reduced natural oyster sets in Willapa Bay, but estuarine gradients in water chemistry and temperature add complexity making this more difficult to discern. Shellfish hatcheries have adapted to these conditions by measuring seawater carbonate chemistry, buffering incoming water, and adjusting the timing of larval production cycles. While there appear to be larval fitness traits that are genotype-dependent, the potential for breeding programs to improve OA specific traits is only currently receiving attention and remains uncertain. Experiments have rarely been conducted that distinguish success at important physiological transitions in the larval life history and potentially also at the juvenile seed stage when these oysters are out-planted to estuaries with variable conditions.

We review results from recent experiments where survival of oyster larvae raised from crosses of "wild" parental broodstock collected in Willapa Bay was compared to that of larvae raised from controlled crosses with improved lines created by the Molluscan Broodstock Program (MBP) at the Hatfield Marine Science Center (HMSC), Oregon State University. The MBP breeding program was designed to enhance valuable field traits including growth and survival of juvenile and adult oysters, but to date has not explicitly addressed larval traits. Nonetheless, MBP larvae produced more than twice the number of settled spat compared with wild larvae under commercial hatchery conditions (with buffered seawater, pH ~8.3 and Ω_{arag} >2). This advantage also occurred, but to a lesser degree, under unfavorable high pCO$_2$) conditions (pH ~7 and Ω_{arag} <1). Separate experiments were conducted to evaluate survival of juvenile seed along the estuarine gradient and inside and outside eelgrass (*Zostera marina*), a marine plant which has the ability to modify local seawater chemistry. Growth and survival of juvenile oyster seed varied along the estuarine gradient with fastest growth, but lower survival occurring near the estuary mouth and there was no apparent effect of seed source. Results from experiments where MBP seed was planted both inside and outside eelgrass along these same gradients, suggest that this plant can reduce seed growth, especially at locations away from the estuary mouth.

Keywords: Pacific oyster, juvenile, growth, estuary, climate

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Introduction

The Pacific oyster *Crassostrea gigas* was introduced to estuaries along the US West Coast in the early 1900's and largely replaced the native oyster *Ostrea lurida*, which had been extensively fished commercially and mostly overharvested or succumbed to other factors including pollution (Blake and Ermgassen, 2015; Dumbauld *et al.*, 2011; Polson and Zacherl, 2009; Steele, 1964). Pacific oysters were cultured and harvested from leased and privately owned estuarine tidelands and the industry either relied on juvenile seed oysters shipped from Japan or placed cultch shell in several areas where this oyster had naturalized and conditions were conducive for them to spawn (*e.g.* Dabob and Willapa Bay in Washington State, USA and/or Ladysmith Harbor and Pendrell Sound in British Columbia, Canada) (Quayle, 1988). The advent of local hatchery production of oyster larvae and oyster seed in the late 1970's closed the culture life cycle separating the two life history phases and changed the nature of the industry again (Chew, 1984).

The nearshore coastal ocean along the US west coast is part of the California current ecosystem, an eastern boundary upwelling system. Oysters cultured in these systems are thus subject to seasonal events where cold, nutrient rich, high $p\text{CO}_2$ water is transported to the surface and episodic intrusions into estuaries can significantly lower pH and the aragonite saturation state especially near the estuary mouth (Feely *et al.*, 2010; Hauri *et al.*, 2013). Shellfish hatcheries operating in this region have experienced significantly reduced rates of larval growth and survival during these upwelling events (Barton *et al.*, 2012). In response to these challenges, commercial hatcheries now measure seawater carbonate chemistry, strategically time larval production cycles, and chemically buffer incoming seawater in order to maintain optimal carbonate chemistry conditions (Barton *et al.*, 2015).

While there are important exceptions like the Salish Sea in Washington State, most U.S. West coast estuaries are small relative to the spatial extent of the nearby open coastline, so these upwelling events and changes in seawater chemistry can also result in larval oyster mortality and reduced or failed oyster seed sets in estuaries where naturalized oyster populations reproduce. Willapa Bay, which is one of the single largest cultured oyster production sites in the US and the third largest estuary on the US west coast has only a 358 km^2 signature, which roughly equals the size of the James River sub-estuary in Chesapeake Bay, the largest estuary on the US East Coast (11,600 km^2). Estuaries like Willapa Bay also experience less riverine influence during the summer months and have shorter residence times than estuaries where oysters are present on eastern edges of continents (Hickey and Banas, 2003). Gradients in water chemistry and temperature are greatly influenced by short term events that not only influence larvae, but also timing and magnitude of spawning events and retention of larvae in these estuaries making it more difficult to discern reasons for failures in larval seed set (Hales *et al.*, 2017; Ruesink *et al.*, 2018).

Improved larval survival and growth under high small 10 day post metamorphosed oyster spat were CO_2 conditions appear to be genotype-dependent fitness traits (Frieder *et al.*, 2017; Pan *et al.*, 2018; Sunday *et al.*, 2011), but the potential for breeding programs to improve these traits has not yet received much attention. A collaborative effort between scientists at Oregon State University and the US West Coast shellfish industry known as the Molluscan Broodstock Program (MBP) was initiated in 1996 to enhance juvenile oyster survival and growth (de Melo *et al.*, 2016; de Melo *et al.*, 2018; Langdon *et al.*, 2003). This selective breeding program uses family-based mating designs and bi-parental crosses to maintain genetic diversity and limit effects of inbreeding. Although seven generations of larval cohorts have been reared in the hatchery, the program is targeted to improve yield of juveniles and adults on farms and there has been no direct attempt to select for larval performance traits. Nonetheless, commercial shellfish hatcheries that partner with MBP reported that larvae spawned from MBP broodstock survived and grew better when compared to wild counterparts, especially during periods of strong upwelling, so experiments were conducted to discern whether unintentional selection for this trait had occurred. Results demonstrated that larvae from MBP

broodstock produced from 37% to 50% more spat than larvae from "wild" broodstock collected from a naturalized population in Willapa Bay (Durland *et al.*, 2019). This advantage occurred under both commercial hatchery conditions (with buffered seawater, pH ~8.3 and Ω_{arag} >2) and to a lesser degree, under unfavorable high pCO$_2$ conditions (pH ~7 and Ω_{arag} <1). This suggests that improvements in larval performance had occurred despite no intentional selection. Further analysis of single nucleotide polymorphisms (SNPs) from pooled larval DNA indicated that larvae from wild stocks had more than twice the number of loci affected by acidified culture conditions, compared to larvae from MBP stocks (Durland, 2019). Functional analysis revealed that the predicted genes associated with changes under OA conditions were linked to the structure and function of cellular membranes (De Wit *et al.*, 2018), but the affected loci were largely exclusive to each parental stock with little overlap, suggesting that development of universal markers and selecting for OA resistance in Pacific oysters will be complicated.

The effect of altered water chemistry on juvenile oysters once they settle naturally or are transplanted from hatcheries and planted on farms for grow-out are less studied. Delayed carry-over effects on juvenile growth due to larval exposure (Gobler and Talmage, 2013; Hettinger *et al.*, 2013) and cross-generational effects when breeding adults are exposed to elevated CO$_2$ have been documented (Parker *et al.*, 2015; Parker *et al.*, 2012), but distinguishing the effects of elevated CO$_2$ and reduced aragonite saturation from other environmental factors in estuaries is difficult. Hollarsmith *et al.* (2020) documented the influence of seasonal processes on both water chemistry and temperature along the estuarine gradient in Tomales Bay, CA. They found that both juvenile Pacific and native oyster growth and survival was more influenced by freshwater runoff during the wet season (though limited in this estuary) than by carbonate chemistry during the summer upwelling season. Growth was highest during the upwelling season and appeared to be related to higher phytoplankton concentrations in the water column especially near mid-bay where other stressors (low

dissolved oxygen, low salinity, high temperature) were less evident.

Finally, there continues to be interest in evaluating the potential role of seagrasses as refugia from the effects of altered water carbonate chemistry in part because these important estuarine plants may also grow more rapidly under elevated CO$_2$ and increase carbon sequestration (Washington State Blue Ribbon Panel on Ocean Acidification, 2012; Cullen-Unsworth and Unsworth, 2016; Kelly *et al.*, 2011). Seagrasses uptake CO$_2$ while photosynthesizing during the day and therefore have the potential to modulate carbonate chemistry at least at very local scales, but they also respire at night and die back during the winter in most temperate estuaries potentially increasing pCO$_2$ (Hendriks *et al.*, 2014). A laboratory evaluation of the interaction between juvenile oysters, the seagrass *Zostera marina* (hereafter eelgrass) and seawater carbon chemistry showed that enhanced daytime seawater pH in the presence of eelgrass did not counteract the negative effects of increased pCO$_2$ when both oysters and eelgrass were present (Groner *et al.*, 2018). However studies conducted in estuaries suggest that growth and survival of juvenile oysters can be enhanced in eelgrass (Lowe *et al.*, 2019; Smith, 2016). This effect was site specific and therefore difficult to attribute to water chemistry and distinguish from effects of other factors like reduced water flow in eelgrass which potentially results in less suspended sediment in the water and enhanced phytoplankton food intake.

Here we present preliminary results from two experiments where juvenile oyster seed was planted inside and outside eelgrass at several locations along the axis of two US West coast estuaries where we expected gradients in water chemistry and environmental conditions to differ.

Methods

We conducted experiments in two estuaries along the US West Coast. Netarts Bay, Oregon, USA (45°25' N, 123°56' W) is a small tidally flushed estuary, with over ~75% of its total volume turning over every tidal cycle (6.22 hours) (**Fig. 1**). It has a relatively small (36.3 km) watershed whose summertime

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freshwater contribution to flushing is negligible (Glanzmann *et al.*, 1971; Kentula and McIntire, 1986). Patterns in carbonate chemistry were therefore expected to be almost entirely driven by conditions on the coastal shelf, perhaps at both the marine end near the estuary mouth and at the riverine end during the summer when this experiment was conducted. Netarts Bay is also home to the Whiskey Creek Shellfish Hatchery, the largest independent producer of oyster seed in the Pacific Northwest

(Barton *et al.*, 2012) where a continuously-monitoring carbonate chemistry analyzer has been in place since 2010 (Barton *et al.*, 2015). Though still dwarfed in size by estuaries along the US East coast as noted above, Willapa Bay, Washington USA (46°32' N, 123° 59' W) is the third largest estuary along this coast. It has a much larger watershed than Netarts Bay (2,857 km^2) (**Fig. 2**), but is still strongly tidally-influenced, especially in the summer when river flow is virtually non-existent. Though roughly half the water volume
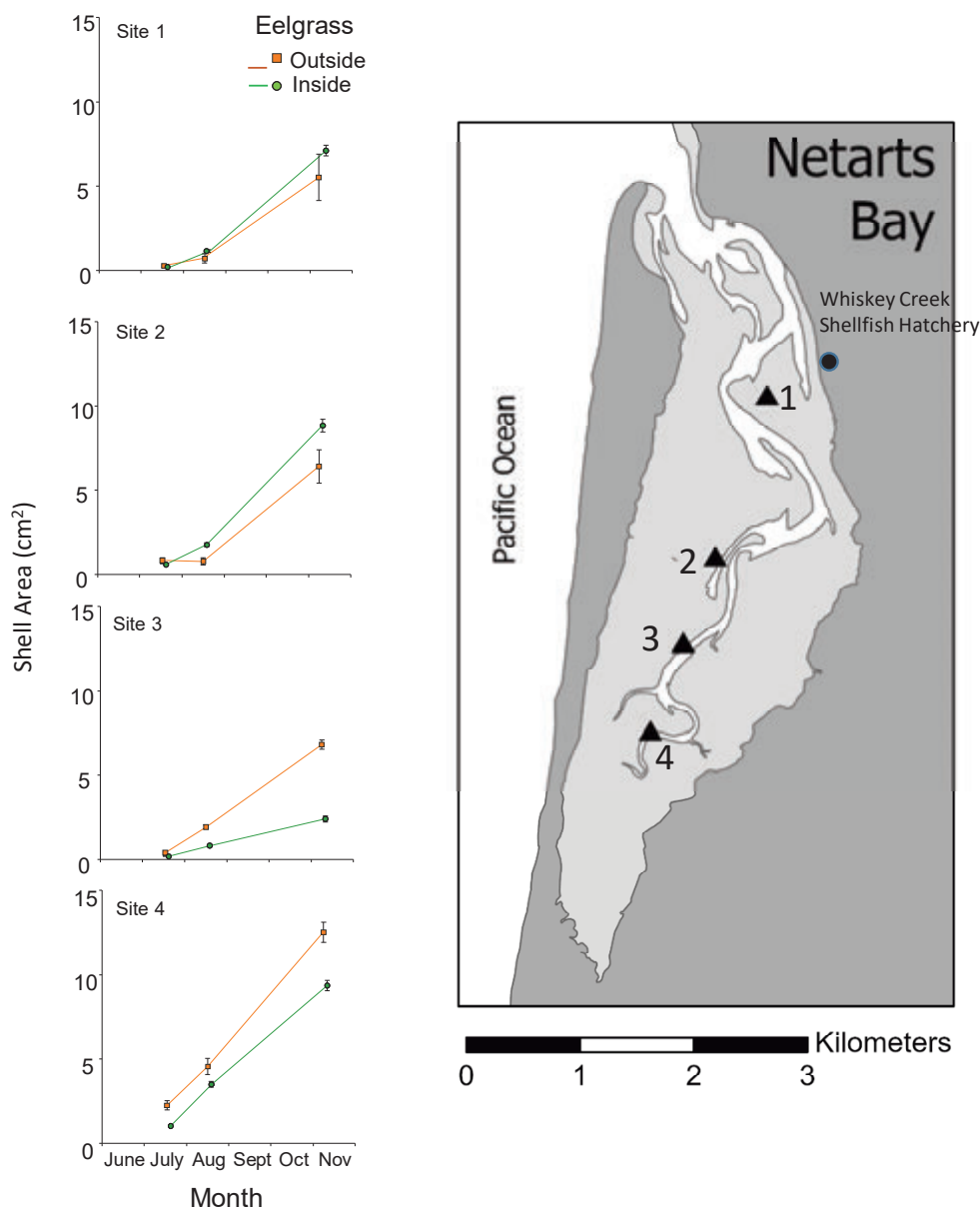

Fig. 1. Results of the 2015 experiment in Netarts Bay, Oregon, USA. Oysters were deployed in May at 4 sites shown in map (right). Average shell area measurements of oysters (cm^2±SE) in eelgrass habitat (green) and outside open habitat (orange) is shown for three time points in July August and November (left).

is exchanged on every tide and replaced with new ocean water, the water at the southern end of the estuary has a 3 to 5 week residence period (Banas *et al.*, 2004; Banas *et al.*, 2007). It is the single largest producer of oysters on the US West coast and often in the US. Carbonate chemistry has been shown to be influenced by upwelling near the ocean endpoint at the estuary mouth, but also by alkalinity

and other factors near the Naselle River (Ruesink *et al.*, 2018; Ruesink *et al.*, 2015), so we expected a potentially different gradient and influence of carbonate water chemistry on oyster survival and growth in this estuary than Netarts Bay.

Experimental plantings of juvenile oyster seed (spat) were made in two separate years to contrast growing conditions inside and outside of eelgrass at
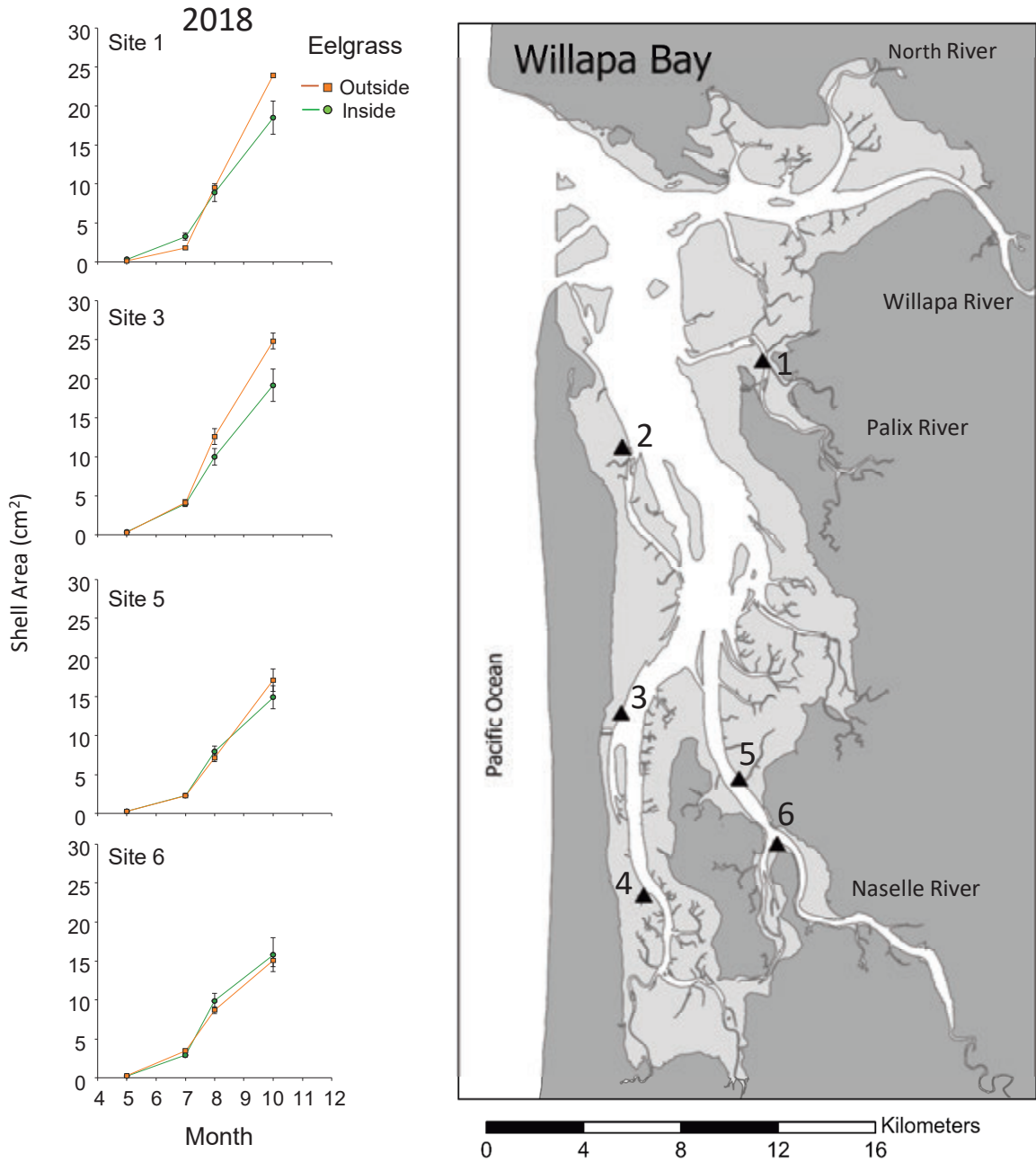

Fig. 2. Results of the 2018 experiment in Willapa Bay, Washington, USA. Oysters were deployed in May at six sites (map on right). Average shell area measurements of oysters (cm^2 ± SE) in eelgrass habitat (green) and outside open habitat (orange) is shown for three time points in July August and October.

several sites along the estuarine gradient in these two estuaries. Our methods differed slightly and were briefly as follows:

Netarts Bay 2015

Aged Pacific oyster shell was used as cultch for this experiment. Five shells were labeled with an individual plastic identification tag and placed in each of 32 small mesh bags (2.5 cm mesh) and these placed in a setting tank at the Whiskey Creek Shellfish hatchery on 5/11/2015. Eyed oyster larvae (MBP Pod, cohort 26) were placed in the tank and allowed to set on these shells following standard hatchery practices. Bags were removed from the tanks and deployed at 4 sites along the estuarine gradient in Netarts Bay (**Fig. 1**) on 5/20/2015. Four shell bags were deployed within eelgrass and four outside eelgrass in bare habitat at the same tidal elevation. Shell bags were attached to PVC pipes so they were suspended approximately 20 cm above the sediment. Poles with bags were placed approximately 5 m apart and each pole had copper tape wrapped around it at the sediment surface in order to prevent access by oyster drill predators. Oyster shells were removed from the bags and photographed at roughly monthly intervals thereafter and returned to the lab for final measurements on 9/30/2015. Temperature was recorded continuously at 15 min intervals using Hobo® data loggers. Photographs from each time point were examined and oyster measurements (length and width) made using Image J (v1.49®) on size calibrated images. Oyster cultch shell labels and shape enabled each individual oyster to be labeled and recorded so that they could be identified and counted in subsequent photos. An assessment of fouling which became an obvious potential factor (% cover, mostly barnacles) was also made. At the conclusion of the experiment oysters were counted, measured (length, width in cm) with calipers, removed from the cultch shell and both dry and wet meat and shell weight measurements made.

Willapa Bay 2018

After being set as singles in the MBP hatchery at HMSC, five oyster spat were cemented (cyanoacrylate glue) to the rough side of small ceramic tiles (5cm×5cm) in this experiment. Tiles were labeled with an individual plastic identification tag and then held for 24 hours in tanks before being transported and deployed at 6 sites along the estuarine gradient in Willapa Bay, Wa (**Fig. 2**) on 5/20/2018. Tiles were attached to PVC stakes at two positions: 20 cm above the sediment and just above the sediment surface. Two tiles were attached back to back at each position: one with spat from an MBP cohort and one with spat from broodstock collected from a "wild" population in Willapa Bay. Three stakes with tiles were deployed within eelgrass and three outside eelgrass in bare habitat at the same tidal elevation at each site. Poles with tiles were placed approximately 10 m apart and each pole had copper tape wrapped around it at the sediment surface in order to prevent access by oyster drills. Tiles were visited and photographed at roughly monthly intervals thereafter and returned to the lab for final measurements on 10/30/2018. Fouling organisms were removed from areas surrounding each oyster using a toothbrush before photographs were taken at each time point. Photographs from each time point were examined and oyster measurements (length and width) made using Image J (v1.49®). Temperature was recorded continuously at 15 min intervals using Hobo® data loggers deployed in two habitat treatments at each site and YSI® sondes were deployed at two locations (Nahcotta and Palix) for 24 hours in August. At the conclusion of the experiment oysters were counted, measured (length and width in cm) with calipers, scraped from the tile and both dry and wet meat and shell weight measured.

Data Analysis

Data from both experiments were analyzed using general linear and linear mixed effect models in R (R Core Team, 2019). Here we present data for shell size (length×width) examined over the summer deployment period. Because we were able to track individual oysters in both experiments, we then evaluated oyster growth for discrete time intervals for which we had the most complete data: 1) an initial interval fairly close to deployment when oysters first experienced estuarine conditions and 2) over the entire period or at the end of the period

when we collected the oysters and made additional measurements (*e.g.* tissue and shell mass). For the 2015 Netarts Bay experiment there were two categorical factors of interest represented in this model: eelgrass (present or absent) and location (4 sites). We also examined and added the proportion of each cultch shell covered with fouling organisms (mostly barnacles) as a factor in this model for the initial period where we collected this information. Models for the second 2018 Willapa Bay experiment included the same primary categorical treatments: eelgrass (present or absent) and location (6 sites), but also included height (20cm above sediment, on bottom) and seed source (MBP, Wild). Growth was evaluated as a normalized daily average = final size (area in mm^2) – initial size (area in mm^2) / days in interval / initial size. Complete models with interaction terms were initially examined, the most important random component determined if needed, and then the most parsimonius model selected by comparing full and nested models with log-likelihood tests and comparing Akaike information criterion (AIC).

Results and Discussion

Oyster growth differed along the estuarine gradient with oysters reaching a larger size outside eelgrass than within eelgrass at the two southernmost locations in Netarts Bay in 2015 and reaching the largest size at site 4 located furthest from the estuary mouth (**Fig. 1**). The opposite pattern was observed at the two sites closer to the estuary entrance, where oysters grew to a larger size within eelgrass resulting in significant interaction between location and eelgrass factors in models for final shell size, but not for normalized growth from August to November (**Table 1**). Oyster mortality across the whole season was highest at locations away from the ocean, but there was no discernable trend attributable to eelgrass presence. Fouling on the cultch shells, mostly by barnacles, was apparent at the first sampling effort in July, highest at sites closest to mouth and higher outside eelgrass than inside eelgrass (**Fig. 3**). Fouling was not a significant factor however when included in the models for size or growth at this time point (**Table 1**). Smith (2016) conducted experiments at site 1 in Netarts Bay during the same year, but repeatedly deployed small 10 day post metamorphosed oyster spat monthly from March – October and monitored for short term growth and mortality. Smith (2016) documented a significant effect of eelgrass on in- situ carbonate chemistry, but only observed significant growth and survival advantages for oyster spat placed in eelgrass during May. Survival of these

Table 1. Results of linear mixed effect models ("nlme" package, Pinheiro *et al.* 2019) used to examine the effect of habitat and site on shell area and growth of oysters in Netarts Bay in 2015. Individual cultch shells were used as random intercept terms in each model

Response	factor	Full Model			Final Model		
		df	F	*p*	df	F	*p*
Shell area (Nov)	site	3,92	27.41	<0.001			
	eelgrass	1,92	19.56	<0.001			
	site*eel	1,92	11.30	<0.001			
Normalized growth (Aug – Nov)	site	3,94	14.50	<0.001	3,94	13.34	<0.001
	eelgrass	1,94	9.40	0.003	1,94	9.0	0.004
	site*eel	1,94	0.26	0.856			
Shell area (July)	site	3,84	43.45	<0.001	3,89	42.31	<0.001
	eelgrass	1,84	14.64	0.002	1,89	3.76	<0.001
	barnacles	1,84	3.68	0.058			
	site*eel	3,84	0.41	0.744			
	barn*eel	1,84	0.31	0.576			

small spat planted in subsequent months appeared to correlate with an overall declining trend in daily pCO_2 minima. These findings agree with other research and suggest that additional variables like water flow and tidal exposure must be taken into account (Koweek *et al.*, 2018).

Though experimental design and deployments were slightly different than those in the 2015 Netarts experiment, oyster growth also differed along the estuarine gradient in Willapa Bay in 2018. Oysters consistently reached a larger size at locations closest to the estuary mouth in this estuary (Sites 1–3) **(Fig. 2)**, but few oysters survived at Sites 1 and 2 due at least in part to experimental problems (early losses of glued oysters from tiles and lost stakes). Oysters obtained a larger size outside of eelgrass than within eelgrass at these locations, but there was significant interaction between location and eelgrass in models for both shell area and normalized growth **(Table 2)**, because no eelgrass effect was observed at locations away from the mouth (Sites 4–6) **(Fig. 2)**. We also deployed oysters from two parental sources (selected MBP family and "wild") at two heights above the sediment in this experiment. While there was no significant effect of oyster source, there was significant interaction between height above sediment and location **(Table 2)**. Oysters grew larger 20 cm above the bottom at locations near the estuary mouth and the opposite effect was observed at sites 5 and 6. Previous authors also found enhanced
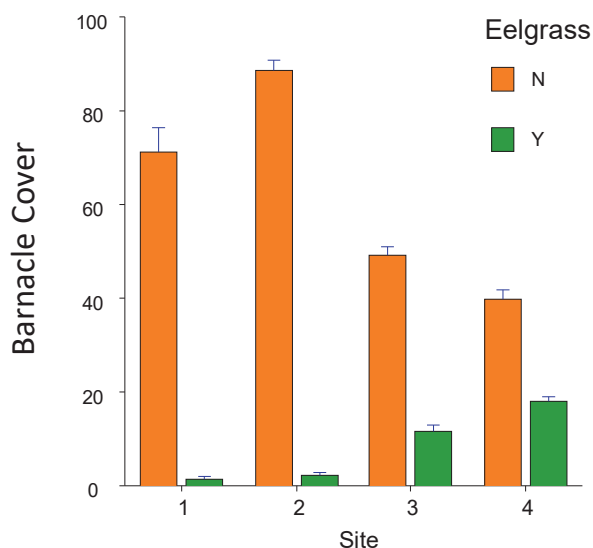

Fig. 3. Comparison of average percent barnacle cover measured on shells deployed in eelgrass (green bars) and outside eelgrass (brown bars) at each site in Netarts Bay during July 2015.

Table 2. Results of general linear models used to examine the effect of habitat (eelgrass, open), seed source (MBP, Wild), position (off bottom, on bottom), and site on shell area and growth of oysters in Willapa Bay in 2018. Only sites 3–6 used in these models due to lack of sufficient data at sites 1–2

Response	factor	Full Model			Final Model		
		df	F	*p*	df	F	*p*
Shell area (August)	site	3	9.31	<0.001	3	9.47	<0.001
	eelgrass	1	3.59	0.061	1	3.66	0.058
	position	1	2.48	0.118	1	2.52	0.115
	source	1	3.57	0.061	1	3.63	0.059
	site*eel	3	3.97	0.010	3	4.04	0.009
	site*pos	3	3.41	0.020	3	3.47	0.018
	site*source	3	0.35	0.789			
Normalized growth (July – Aug)	eelgrass	1	0.001	0.970	1	0.001	0.970
	site	3	3.19	0.026	3	3.15	0.026
	position	1	0.49	0.487	1	0.49	0.487
	source	1	1.24	0.267			
	site*eel	3	2.45	0.067	3	2.53	0.060
	site*pos	3	3.02	0.033	3	3.02	0.044
	site*source	3	1.25	0.295			

growth of Pacific oysters near or close to the mouth of this estuary (Ruesink *et al.,* 2003). Lowe *et al.* (2019) also documented little difference in oyster growth between eelgrass and open habitat treatments at the same riverine endpoint (site 6). They found significantly larger oysters in eelgrass than outside this habitat at their site closest to the ocean. While we observed the opposite trend, this agrees with our observations for Netarts Bay above. These authors and others (Hollarsmith *et al.,* 2020) mostly attribute trends in oyster growth to the concentration of phytoplankton as food and found that trends in

survival might also reflect predator abundance or even abundance of bacteria and disease as stressors in these two habitats rather than water chemistry. In single 24 hour records, we observed higher levels of food (measured as relative chlorophyll a) within eelgrass at two of the stations along this gradient in Willapa Bay and more dramatic fluctuation at the location closest to the estuary mouth where we also observed colder average temperatures over the entire experimental period and greater fluctuations and differences in pH (**Fig. 4**). This location (site 1) is however potentially also more affected by freshwater
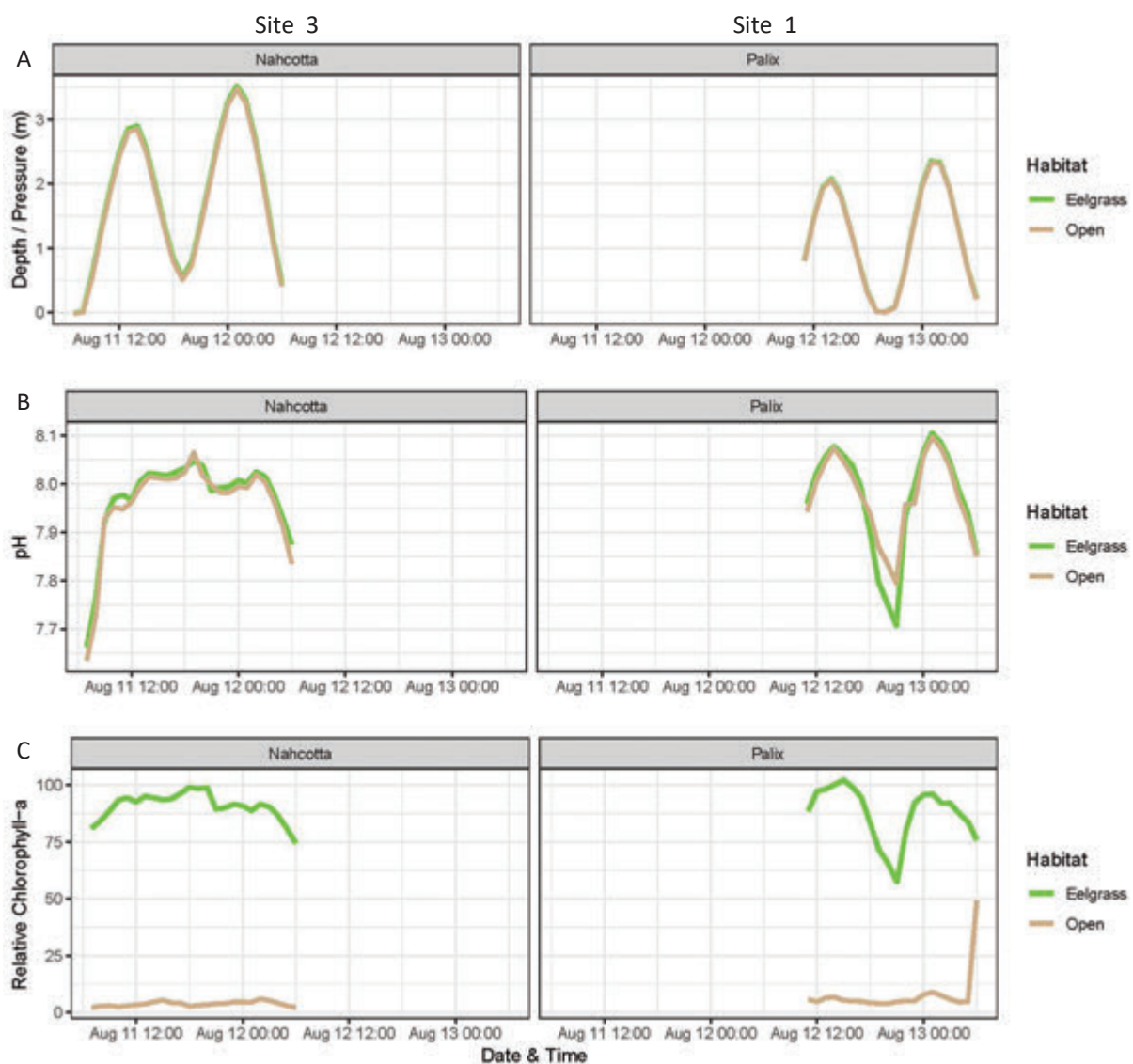

Fig. 4. Twenty-four hour records for A) water depth – following the tide, B) pH , and c) relative chlorophyll a, at intertidal sites 1 (Palix River) and 3 (Nahcotta) where oysters were deployed in Willapa Bay. Note the fluctuating values of both parameters at site 1, but differences in pH in eelgrass at low water at night at this site and consistently higher chlorophyll a in eelgrass at both locations.

from the Palix River as potentially evidenced by a drop in pH and chlorophyll a at low tide. We have not yet directly related this environmental data directly to oyster growth and survival, in part because we recognize that the density and spatial configuration of eelgrass will also affect water flow and food supply so static measurements and simple site/treatment characterizations and point in time estimates of these water chemistry parameters need to be refined.

Our preliminary conclusions are:

- Oyster larvae have clearly been shown to be sensitive to water chemistry including low aragonite saturation state, but other conditions are important and genotype/broodstock may play an important role in initial spat settlement and size.

- Location or planting site for juvenile spat (seed) within the estuary is important. Juvenile oyster seed generally grows faster near the mouth of US West Coast estuaries, but there are important finer scale, within estuary spatial and temporal gradients.

- The effect of eelgrass on oyster seed also depends on planting site. We suspect this is due to several interacting factors including water flow, food supply, and perhaps settlement and competition with other invertebrates, and not water temperature or modifications to water chemistry. However, this data and relationship remains to be collected and further examined at appropriate spatial and temporal resolution.

- Juvenile oyster growth is generally faster above the sediment surface, but this varies along the estuarine gradient as well. This is also likely due to food supply and perhaps other factors influencing oyster feeding behavior including sediment load but needs to be further explored in these estuaries.

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(1) Barton A., Waldbusser G. G., Feely R.A., Weisberg S. B., Newton J. A., Hales B., Cudd S., Eudeline B., Langdon C. J., Jefferds I., King T., Suhrbier A., and McLaughlin K., 2015: Impacts of Coastal Acidification on the Pacific Northwest Shellfish Industry and Adaptation Strategies Implemented in Response. *Oceanography*, **28(2)**, 146–159.

This is a review of the history and science underpinning the effects of changing seawater chemistry on bivalve shellfish larvae and the impacts that have already taken place to the commercial shellfish aquaculture industry on the US West Coast. Multiple authors contributed to this review which addresses a broad audience but covers the leading research on direct effects to bivalve larvae as well as monitoring seawater conditions and adapting to these changes.

(2) Durland E., Waldbusser G., and Langdon C., 2019: Comparison of larval development in domesticated and naturalized stocks of the Pacific oyster *Crassostrea gigas* exposed to high *p*CO$_2$ conditions. *Mar. Ecol. Prog. Ser.*, **621**, 107–125.

Two replicated experiments were conducted where Pacific oyster larvae from "wild" parent crosses and those from selected stocks (OSU Molluscan Broodstock Program) were raised and set under favorable hatchery conditions (buffered seawater, pH ~7.8 and Ω_{arag} ~2) and unfavorable (pH ~7.4 and Ω_{arag} ~1) conditions. Early larval development was inhibited by acidified seawater and it affected the timing, but not magnitude of larval mortality. The effect on metamorphosis (setting) was variable, but MBP larvae produced more and larger spat in ambient and high pCO$_2$ seawater, respectively.

(3) Hales B., Suhrbier A., Waldbusser, G. G., Feely R. A., and Newton J. A., 2017: The carbonate chemistry of the "Fattening Line," Willapa Bay, 2011–2014. *Estuaries Coasts,* **40**, 173–186.

The authors present detailed data on seawater chemistry (especially pCO$_2$ and aragonite saturation state) for Willapa Bay, Washington where Pacific oysters have been the mainstay of the oyster aquaculture industry for almost 100 years and there is a long term record of spawning and setting. They reconstruct this record for a longer historical period and their data suggest that recent conditions provide a smaller window of optimal conditions (low aragonite saturation state and warm enough temperatures for oyster spawning) than occurred historically. While they did not sample larvae and therefore can't confirm effects, they substantiate the complexity of measuring these effects and attributing them to a single cause in an estuary where conditions are variable.

(4) Lowe A. T., Kobelt J., Horwith M., and Ruesink J., 2019: Ability of eelgrass to alter oyster growth and physiology is spatially limited and offset by increasing predation risk. *Estuaries Coasts*, **42**, 743–754.

The authors planted juvenile seed oysters and measured water chemistry inside and outside of eelgrass (*Zostera marina*) at several locations in Willapa Bay and in the Salish Sea in Washington state. They demostrate that while eelgrass modified carbonate chemistry, it did not appear to be the primary variable influencing juvenile oyster growth and survival and, instead, predation was higher and thus survival lower inside eelgrass beds where surviving oysters also grew slower.

(5) Ruesink J. L., Roegner G. C., Dumbauld B. R., Newton J. A., and Armstrong D. A., 2003: Contributions of coastal and watershed energy sources to secondary production in a Northeastern Pacific estuary. *Estuaries*, **26(4B)**, 1079–1093.

These authors measured growth of juvenile Pacific oyster seed deployed at numerous locations throughout Willapa Bay, and used stable isotopes to distinguish marine versus terrestrial energy sources. Results demonstrated a very distinct along estuary gradient with oysters growing faster near the mouth and slower at greater distance from the mouth and away from the mouth. Oysters also displayed distinct stable isotope ratios that reflected a strong marine signal near the mouth (−18 δ^{13}C) and a stronger terrestrial signature at distance −22 δ^{13}C). Oysters grown just off the bottom grew slower than those off-bottom at any given tidal elevation.