Local ecosystem processes modulate ocean acidification and its effect on benthic foundation species

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Abstract

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Ocean acidification poses serious threats to coastal ecosystem services, yet few empirical studies have investigated how feedbacks from local ecological processes may modulate global trends of pH from rising atmospheric CO_2. Just as microclimatic influences cause departures from long-term warming trends in temperature, local processes may decouple local marine environments from the increased anthropogenic CO_2 that dissolves in seawater and reduces pH. Seawater pH has been shown to be an important factor regulating physiological processes of many aquatic organisms, including valuable aquaculture species like Pacific oysters. Understanding 1) whether long-term ocean acidification varies spatially due to local ecological processes, 2) which environmental factors or ecological processes drive variation in seawater pH, and 3) the effects

of this pH variation on marine organisms are critical research needs for climate change adaptation and management of important marine resources. In this dissertation, I found that pH exhibits high variability across spatial and temporal scales in the Salish Sea, exhibiting location-specific long-term changes driven by differences in net ecosystem metabolism (Chapter 1). By mapping pH in important shellfish aquaculture regions of Washington state, I showed that shallow-water environments over tidal flats are more variable in pH than surface waters over deeper channels, associated with bentho-pelagic coupling of organic matter production and decomposition, in addition to characteristic physical changes of temperature and salinity up-estuary (Chapter 2). Using interactions with an autotrophic foundation species (eelgrass *Zostera marina*) along estuarine gradients, I found that growth of two species of oyster were most strongly positively correlated to differences in stable isotope and fatty acid biomarkers of food availability both from river to ocean along the estuarine gradient and in association with eelgrass (Chapter 3). Shell strength, a putative indicator of pH stress, showed a positive response to eelgrass for the native, but negative response for the non-native oyster. Small differences in growth and shell strength were observed in association with eelgrass, but mortality related to predation was much higher in eelgrass. Collectively, these results support the adoption of an ecosystem perspective to ocean acidification as well as other stressors in productive aquatic habitats.

Chapter 1: Patterns of pH variability were quantified as a function of atmospheric CO_2 and local physical and biological processes at 83 sites over 25 years in the Salish Sea and two NE Pacific estuaries. Mean seawater pH decreased significantly at -0.009 ± 0.0005 pH yr^{-1} (0.22 pH over 25 years), with spatially variable rates ranging up to 10 times greater than atmospheric CO_2-driven

ocean acidification. Dissolved oxygen saturation (%DO) decreased by -0.24 ± 0.036% yr^{-1}, with site-specific trends similar to pH. Mean pH shifted from <7.6 in winter to >8.0 in summer concomitant with the seasonal shift from heterotrophy (%DO <100) to autotrophy (%DO>100) and dramatic shifts in aragonite saturation state critical to shell-forming organisms (probability of undersaturation was >80% in winter, but <20% in summer). At multiple scales, %DO overwhelmed the influence of atmospheric CO_2, temperature and salinity on pH, providing strong evidence that local ecosystem processes modulate ocean acidification.

Chapter 2: I mapped surface water pH using a Durafet sensor in seven important aquaculture regions in the Salish Sea, including over intertidal flats that were not generally the focus of sampling efforts collated in Chapter 1. Surveys were repeated in late summer and early spring to investigate seasonal differences in nearshore oceanography. Spatial variation in pH within bays was greater in summer than late spring, and summer surveys also documented greater area experiencing low pH. The areas with low pH tended to be in shallow water. Temperature, salinity, dissolved oxygen and the ratio of chlorophyll to total suspended solids (Chl:TSS) were important factors explaining variation in pH. The strong correlation of pH variability with Chl:TSS provides a mechanistic hypothesis linking food web dynamics and pH stress in coastal areas through the production and decomposition of suspended organic matter. Factors controlling food quality and quantity (primary production, detrital abundance and decomposition) were correlated to pH, indicating pH and food stress to suspension feeders are likely often co-occurring.

Chapter 3: I investigated the effects of an autotrophic foundation species (eelgrass, *Zostera marina*) on intertidal native (*Ostrea lurida*) and non-native oysters (*Crassostrea gigas*) across a range of estuarine conditions in Willapa Bay and Padilla Bay, Washington. I analyzed morphological (growth and shell strength), demographic (survival), and physiological responses (stable isotope and fatty acid signatures) to determine the relative influence of mechanisms by which foundation species affect associated species. Hypothesized mechanisms include top-down and bottom-up food web interactions, and amelioration of pH associated with net photosynthesis (CO_2 uptake) in eelgrass. Eelgrass interactions significantly influenced top-down effects, reducing survival at many sites through provision of habitat for predators. Fatty acid concentration and stable isotope signatures were significant predictors of growth for both species, providing strong evidence of bottom-up effects, including potential fatty acid limitation in native oysters, related to environmental context and modified by eelgrass. Effects of eelgrass on pH stress were inconclusive: Eelgrass had positive effects on growth of both oyster species at down-estuary sites, which is not where pH was likely lowest; also, shell strength in eelgrass increased for native oysters but decreased for non-native oysters. Trade-offs between growth rate and survival were observed at the habitat-scale but to a lesser extent than along the environmental gradient.

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DEDICATION

“Life is a thrill when you’re skill is developed” – Del tha Funky Homosapien

Chapter 1. ECOSYSTEM METABOLISM DRIVES PH VARIABILITY AND MODULATES LONG-TERM OCEAN ACIDIFICATION IN THE NORTHEAST PACIFIC COASTAL OCEAN

1.1 ABSTRACT

Ocean acidification poses serious threats to coastal ecosystem services, yet few empirical studies have investigated how feedbacks from local ecological processes may modulate global trends of pH from rising atmospheric CO_2. We quantified patterns of pH variability as a function of atmospheric CO_2 and local physical and biological processes at 83 sites over 25 years in the Salish Sea and two NE Pacific estuaries. Mean seawater pH decreased significantly at -0.009 ± 0.0005 pH yr^{-1} (0.22 pH over 25 years), with spatially variable rates ranging up to 10 times greater than atmospheric CO_2-driven ocean acidification. Dissolved oxygen saturation (%DO) decreased by -0.24 ± 0.036% yr^{-1}, with site-specific trends similar to pH. Mean pH shifted from <7.6 in winter to >8.0 in summer concomitant to the seasonal shift from heterotrophy (%DO <100) to autotrophy (%DO>100) and dramatic shifts in aragonite saturation state critical to shell-forming organisms (probability of undersaturation was >80% in winter, but <20% in summer). %DO overwhelmed the influence of atmospheric CO_2, temperature and salinity on pH across scales. Collectively, these observations provide evidence that local ecosystem processes modulate ocean acidification, and support the adoption of an ecosystem perspective to ocean acidification and multiple stressors in productive aquatic habitats.

1.2 INTRODUCTION

Nearshore aquatic ecosystems are changing rapidly as a function of altered riverine inputs (Aufdenkampe et al. 2011), eutrophication (Cloern 2001) and loss of foundation species due to human influence (Orth et al. 2006, Zu Ermgassen et al. 2012). Collectively these physical and ecological processes are changing carbon cycling, and interact with atmospheric CO_2 via warming and acidification to influence ecosystem function (Boyd and Hutchins 2012, Bauer et al. 2013). While elevated aqueous CO_2, and the concomitant decrease of pH, has been shown to have negative effects across marine phyla (Kroeker et al. 2010), the influence of long-term anthropogenic CO_2 emissions and the response of biological communities may vary by habitat in relation to local biotic and abiotic conditions (Borges and Gypens 2010, Page et al. 2016, Baumann and Smith 2017). Microbial respiration has long been recognized as a driver of CO_2 exchange with the atmosphere along the aquatic continuum (Gattuso et al. 1998, Cai 2011), particularly in net heterotrophic habitats such as shallow tidal estuaries and fjords that are typically sources of CO_2 to the atmosphere (Cai 2011, Bauer et al. 2013). Conversely, continental shelf and seasonally productive ecosystems can be CO_2 sinks (Martz et al. 2009), suggesting a mechanistic link between local water chemistry and ecological factors such as interactions with organisms that modify the local environment, e.g. ecosystem engineers (Jones et al. 1997), and food web dynamics (Schindler et al. 1997). While studies have shown that biological feedbacks have strong effects on carbonate chemistry on short timescales and small spatial scales (Silbiger and Sorte 2018, Pacella et al. 2018), empirical studies investigating the potential for local ecological modulation of long-term global ocean acidification are lacking.

Currently, organisms are regularly exposed to 'future' levels of lower pH in nearshore ecosystems (Hofmann et al. 2011, Reum et al. 2016). The implications of these periods of low pH may depend on the timing of exposure (Murray et al. 2014), and go beyond the isolated physiological effects of pH when accompanied by multiple stressors like low oxygen or thermal stress (Pörtner 2012). But what controls these pH changes? Seawater pH has been demonstrated to respond to local community composition (Page et al. 2016, Cyronak et al. 2018, Silbiger and Sorte 2018) and changes to water column productivity or suspended particulate matter composition (Bates et al. 2009, Lowe et al. 2016) through the balance of primary productivity and respiration, referred to as net ecosystem metabolism. Few studies have paired quantification of ecosystem processes with pH, yet dissolved oxygen is commonly used as an indicator of ecosystem metabolic state to quantify changes in net ecosystem metabolism (Caffrey 2003). Long-term trends of pH that cannot be explained by increasing atmospheric CO_2 have been observed in coastal systems (Wootton et al. 2008, Duarte et al. 2013) including long-term increases in mean pH (Borges and Gypens 2010, Provoost et al. 2010). Empirical studies that compare trends in metabolic state and pH may fill this knowledge gap. The interaction of biological effects with water mass transport and physical processes could explain local variability of seawater pH on decadal (Baumann and Smith 2017), annual (Dore et al. 2009) and monthly to hourly scales (Hofmann et al. 2011, Ruesink et al. 2015). Addressing ocean acidification from an integrated ecological framework may help define the temporal and spatial structure of exposure to pH stress and the relationship to other energetic and ecological factors at scales relevant to populations and communities (Breitburg et al. 2015).

Previous studies have used long-term monitoring datasets to show that patterns of pH in shallow estuaries are closely linked to dissolved oxygen (Baumann and Smith 2017) and to hypothesize that long-term pH changes in coastal shelf ecosystems are related to ecosystem productivity (Provoost et al. 2010). In the current study, we quantified spatial and temporal seawater pH dynamics within a Northeast Pacific coastal region that includes upwelling-influenced fjord-like estuaries, deep seasonally-stratified embayments, and tideflats. The diversity of habitats sampled in this study is representative of many seasonally productive coastal habitats around the world. We used monthly observations of water properties collected by U.S. state and federal agencies over 25 years to test the relative importance of hypothesized drivers of pH variability at 83 sites across ~7500 km^2 of coastal ecosystems in Washington state. This region supports valuable wild and aquaculture shellfish industries that make up 45% of shellfish aquaculture in the US (Yang et al. 2015) and may be vulnerable to long-term carbonate chemistry changes (Barton et al. 2012). We compared long-term trends of pH to atmospheric CO_2 concentrations, then tested the hypotheses that global ocean acidification is modulated by local changes in net ecosystem metabolism as indicated by dissolved oxygen saturation. We then compared pH to physical and biological variables to test the relative importance of these variables at multiple timescales.

1.3 METHODS

Patterns of seawater pH and the relationship to abiotic and biotic ecological factors were quantified in the coastal waters of Washington State at 83 sites (Supplementary table 1-1) spanning 25 years from 1991-2015 (Fig. 1-1). Sites were distributed throughout five subregions including the Coastal Estuaries (Grays Harbor and Willapa Bay), Hood Canal, South Puget Sound (south of the Tacoma Narrows), Puget Sound (main basin and Whidbey basin) and North Puget Sound (sites north of Admiralty Inlet and in eastern Strait of Juan de Fuca). Environmental

data were collected by the Washington state Department of Ecology (80 sites, WA-DOE) (fortress.wa.gov/ecy/eap/marinewq/mwdataset.asp) and the Padilla Bay National Estuarine Research Reserve (3 sites in North Puget Sound, PB-NERR) (cdmo.baruch.sc.edu/get/export.cfm). Data used in this analysis met rigorous calibration and quality control standards. Data from WA-DOE include monthly water column profiles sampled at 0.5 m depth increments spanning the time period from October 1991 – October 2015 (Bos et al. 2016). Temperature (°C), salinity, dissolved oxygen (DO, mg L^{-1}, % saturation) and pH (NBS scale) data were measured with SBE sensors (Sea-Bird Electronics, Inc.) throughout the study period. WA-DOE monitors permanent 'core' sites and rotating sites; all data were used for seasonal summaries and modeling analyses, whereas trends were assessed with core sites owing to longer periods of observation. PB-NERR data were collected from moored, stationary sensors at 30 min (1995 – 2007) or 15 min (2007 – October 2015) intervals. Temperature (°C), salinity, dissolved oxygen (DO, mg L^{-1}, % saturation) and pH (NBS scale) data were measured using an YSI 6600 multiprobe sonde (YSI, Inc.). These three sites were included in the 'core' sites for long-term analyses.

The methods of data collection differed in that a single time point was associated with multiple depths at WA-DOE sites, but only a single depth at PB-NERR sites. To make observations from these datasets comparable, one observation representing a single time and depth was randomly selected for each month from the 304,989 observations from the WA-DOE and PB-NERR monitoring sites resulting in 5994 observations. For PB-NERR sites, the monthly sample was selected during daytime hours between 0900 and 1700 local time. For WA-DOE sites, one measurement near the surface of each monthly depth profile (0.5 to 4.5m) was selected at random. Observations were filtered to exclude measurements of salinity <15 to restrict error

associated with predicting carbonate chemistry parameters below this level (Gattuso et al. 2015). The subsampling method provided a data set with appropriate weighting (one sample per site and month each year) but used a small fraction of the data. To determine whether the random samples used in analyses gave robust results, multiple subsamples from 'core' sites were used in a bootstrap analysis of long-term trends in pH and dissolved oxygen (250 iterations).

1.3.1 *Multi-decadal pH and %DO trends*

Long-term temporal trends over the 25-year study period were assessed for both pH and %DO saturation with data from the 38 core sites (N = 4961 observations) using a mixed effects model structure with year as explanatory factor and subregion and season as random effects. These smaller-scale factors were considered random effects here, where the focus was on long-term trends, but were explicitly considered for their contributions to pH variability in subsequent analyses (see below). Separate regressions were run for the upper-quartile and lower-quartile of the annual pH distribution. In addition to the region-wide analysis, trends at each core site were assessed separately using linear models. Not all sites were sampled in every month.

1.3.2 *Seasonal and spatial variability of pH and %DO.*

Patterns of variability of mean pH and %DO were investigated using linear models with multiple spatial (site, habitat, subregion) and temporal scales as predictor variables. Habitats included tide flat (22 sites; <~17m depth, sampled over tidal flat or predominantly well-mixed shallow inlets influenced by tidal flats), nearshore (38 sites; water depth was 17 - 60m and close to shore or within a confined embayment) and channel (23 sites; in main channels with water depth >60m). Homogeneity of variance of pH among habitats was tested in each season using Bartlett's test.

Seasons were defined as winter = December, January, February, Spring = March, April, May, Summer = June, July, August, and fall = September, October, and November.

In addition to this statistical modeling, the extent to which surface waters in the region were out of equilibrium with the atmosphere was visualized by developing a metric of the metabolic effect on pH, analogous to %DO. Metabolic effect on pH was calculated by subtracting the predicted 'atmospheric equilibrium' pH based on physical conditions from the observed pH. 'Atmospheric equilibrium' pH was calculated using local temperature, salinity, estimated alkalinity from salinity *sensu* (40) and pCO_2 in the 'seacarb' package in R (Gattuso et al. 2015). We assumed that pCO_2 concentration was in equilibrium with the mean annual atmospheric concentration obtained from NOAA (www.esrl.noaa.gov/gmd/ccgg/trends/). The marine source water that feeds Puget Sound and Hood Canal is characterized by mean pCO_2 concentration near 700 μatm (Murray et al. 2015), but variation of this source is difficult to constrain because of limited observations. We used surface atmospheric CO_2 concentration as the equilibrium point for the calculation of the metabolic effect because a) most ocean acidification model projections consider surface oceans to be in equilibrium with the atmosphere, b) surface waters of this region are in contact with the atmosphere for timescales required to reach equilibration with the atmosphere (Sarmiento and Gruber 2006), c) this assumption simplifies the treatment of different water bodies that vary in their marine and freshwater sources, and d) comparing pCO_2 to atmospheric CO_2 allows for a simple indicator of source-sink dynamics. Using the mean Strait of Juan de Fuca water (~7.8) as a boundary condition would amplify positive anomalies by ~0.2 units without changing seasonal patterns. The predicted pH was subtracted from the observed pH so that positive deviations (CO_2 sink) indicate the influence

of primary productivity while negative deviations (CO_2 source) indicate the influence of respiration.

To investigate potential consequences of pH variation for calcifying organisms, aragonite saturation state was calculated in 'seacarb' with observed pH, temperature, salinity and alkalinity predicted from salinity. The proportion of observations above saturation ($\Omega > 1$) was calculated for each month. The probability of saturation metric is a conservative estimate of stressful carbonate chemistry conditions as organisms have been observed to be sensitive to aragonite saturation states of <1.6 (Waldbusser et al. 2015), and states <1 mark the physical threshold of aragonite dissolution.

1.3.3 *Time of day*

Small-scale (within-day) temporal variability of pH and %DO associated with time of sampling was quantified using linear mixed effects models with random effects of site and season within year (N = 5994).

1.3.4 *Drivers of pH variability*

Analyses and visualizations described in prior sections tested for spatial and temporal variation of pH and %DO at different scales. Here, %DO is incorporated as a potential predictor of pH, along with abiotic and climatic factors considered likely drivers. Subregion and season were identified as random effects following methods in Zuur *et al.* (2009) to constrain spatial and temporal variation. Temperature, salinity and %DO data sampled simultaneous to pH, and monthly atmospheric CO_2 concentration were explanatory factors, excluding interactions. Response and explanatory variables were normalized (mean = 0, SD = 1) to allow direct comparison of variables with disparate ranges of variation. Chlorophyll (Chl) was not sampled at

PB-NERR and many WA-DOE sites and therefore excluded from the full analysis. A supplementary analysis was conducted using the subset of 3123 observations with Chl. All mixed effects models were conducted with 'lmer' in R (Bates et al. 2015), and significance of fixed effects determined with likelihood ratio tests.

1.4 RESULTS

1.4.1 *Multi-decadal pH and %DO trends*

Ecosystem-wide mean seawater pH decreased significantly during the period of sampling at a rate of -0.009 ± 0.0005 pH yr^{-1} (X^2 = 331.5, *df* = 1, p<0.001). Bootstrapped estimates of mean pH trend showed little variation (-0.009 ± 0.0001 pH) associated with the method of subsampling data to unify datasets. Annual lower quartile pH declined significantly over the sampling period (linear regression: $t_{1,23}$ = -2.78, r^2 = 0.22, p = 0.011), whereas annual upper quartile pH did not change over the period (linear regression: $t_{1,23}$ = -0.75, r^2 = 0.023, p = 0.459). pH decreased significantly at 30 of 38 core sites at rates ranging from -0.008 to -0.020 pH yr^{-1} (Fig. 1-2a). The mean rate of pH change varied by subregion, and was greatest in South Puget Sound. %DO decreased in the region by -0.24 ± 0.036% yr^{-1} (X^2 = 42.2, *df* = 1, p<0.001). Significant negative trends were observed at 17 of 38 core sites and ranged from -0.71 to -0.27% yr^{-1}, with one significant positive trend of 0.45% yr^{-1} (Fig. 1-2b). Site-specific trends of pH and %DO were similar to the expected relationship derived from the regression of pH to %DO (Fig. 1-2c; slope = 0.008), but deviations at multiple sites lead to a non-significant relationship between the trends.

1.4.2 *Seasonal and spatial variability of pH and %DO*

Overall, mean daytime pH was 7.92 ± 0.30 in Washington State surface waters during the period from 1991 to 2015 (Table 1-1). Average %DO was 97.8 ± 21.5%. The probability of observing aragonite supersaturation ($\Omega > 1$) shifted from <20% in winter to 88% in July (Fig. 1-3a), concomitant to a system-wide shift from net heterotrophy (%DO <100%) to autotrophy (%DO >100%). The seasonal oscillation of median pH was robust enough to be observed even though mean pH decreased by ~0.22 units over the period of sampling. Metabolic contribution to pH varied considerably among sites and seasons (Fig. 1-1), leading to a range of pH as much as 1.3 units above and below predicted atmospheric equilibrium. These changes were much greater than could be explained by physical factors alone (black boxplots show distribution of 'atmospheric equilibrium' pH; Fig. 1-2a). In total, only 6.6% of observations over 25 years were within one standard deviation of the mean 'atmospheric equilibrium' seawater pH.

Significant spatial and temporal variation of mean pH and %DO was observed at multiple scales (Table 1-2); pH and %DO varied significantly among sites and months (Fig. 1-3), as well as at the subregional and seasonal scale (Table 1-2). Habitat was also a significant predictor of pH and %DO (Table 2; Fig. 1-3). The mean pH at channel and nearshore sites exhibited high variation among seasons (Table 1-1). Tideflats were characterized by significantly greater variation in summer and fall than other habitats (Bartlett's test: Summer, $K^2 = 56.4$, $p < 0.001$; Fall, $K^2 = 18.3$, $p < 0.001$; Fig. 1-2). This variation over the year is largely due to changes in the maximum observed pH, which varied > 1.2 pH from winter to summer, rather than a change in the minimum observed pH which varied ~ 0.3 pH over the year. %DO exhibited a similar pattern (Fig. 1-3b)

1.4.3 *Time of day*

Surface pH increased 0.026 ± 0.001 pH hr^{-1} and %DO increased 1.60 ± 0.010 % hr^{-1} during the daytime sampling period when controlling for interannual and subregional differences ($p < 0.001$).

1.4.4 *Drivers of pH variability*

pH was significantly positively correlated to Dissolved oxygen, temperature and salinity, whereas pH was significantly negatively correlated to atmospheric CO_2 (Table 1-3). The scaled estimate of %DO influence on pH was nearly three times larger than the effect of temperature (0.595 vs. 0.184) and an order of magnitude greater than the effect of salinity (0.030). The effect of atmospheric CO_2 was of similar magnitude but opposite sign as temperature. %DO alone explained 50.9% of the variation in pH, whereas the full model explained 54.8%. Chlorophyll was positively correlated to pH in the subset of data with chlorophyll measurements (Supplementary Table 1-2). The relationship between pH and DO varied slightly by season (Fig. 1-4); the slope of the regression of pH anomaly to %DO was 0.011 in winter ($r^2 = 0.27$, $p < 0.001$), 0.008 during spring ($r^2 = 0.28$, $p < 0.001$), 0.009 in summer ($r^2 = 0.51$, $p < 0.001$) and 0.008 in fall ($r^2 = 0.39$, $p < 0.001$). Both DO and pH were predominately lower than atmospheric equilibrium in fall and winter, with a large shift towards positive pH anomalies and DO supersaturation occurring in spring and summer (Fig. 1-4).

1.5 DISCUSSION

Multi-decadal changes of surface water pH have been observed in marine ecosystems (Bates et al. 2014, Kapsenberg et al. 2017) yet few datasets have quantified parameters necessary to test hypotheses about the relative influence of different processes at multiple timescales (but see(Baumann and Smith 2017). We quantified the effects of atmospheric CO_2, physical and biological factors on seawater pH using a unique dataset spanning 25 years of sampling at 83 sites representing multiple coastal habitats. Surface seawater pH decreased significantly in this NE Pacific coastal ecosystem over the 25-year study period. While pH was negatively correlated to atmospheric CO_2, the rate of pH decrease was ~5 times greater than that predicted from atmospheric CO_2 changes alone, supporting the hypothesis that local processes modulate this global trend. Furthermore, multiple lines of evidence support the hypothesis that local ecosystem metabolism, more than physical oceanographic conditions or atmospheric CO_2, was responsible for the decline. First, the ecosystem-wide trend of -0.009 pH yr^{-1} corresponded to deoxygenation of surface waters at a rate of -0.24% yr^{-1}. Second, the rate of pH and %DO were non-uniform among sites within the same ecosystem, with significant pH decreases ranging from 4-10 times that of open ocean rates of acidification. Third, this system was on average a source of CO_2 to the atmosphere and pH was within one standard deviation of atmospheric equilibrium only ~6% of the time. Fourth, %DO explained a majority of variation in pH across scales, including coherent seasonal variation across the region and observable increases associated with the time of day the samples were collected. Finally, the significant long-term decline of pH was driven by changes to the lower quartile of pH from an intensification of heterotrophic conditions with no corresponding change to the upper quartile range, contrary to model predictions driven by atmospheric changes (Pacella et al. 2018). Collectively, these observations implicate local

ecosystem metabolism as the main driver of variation of pH in NE Pacific coastal habitats. The diversity of habitats represented in this analysis indicates these findings are generalizable to many coastal ecosystems.

This empirical analysis of long-term pH and oxygen changes revealed a primary influence of net ecosystem metabolism on pH dynamics. Biological community composition has been observed to influence carbonate chemistry variation in tide pools and coral reef flats (Page et al. 2016, Silbiger and Sorte 2018); we provide evidence that strong biological influence can extend to an ecosystem-scale. The extremes of pH were far outside the range driven by physical changes alone ($6.5 < pH < 9.3$) and were correlated to extremes of %DO. The widespread prevalence of respiration-driven undersaturation of surface waters in fall and winter means organisms are regularly exposed to stressful carbonate chemistry conditions as a function of the seasonal cycle of biomass production and decomposition. Yet as %DO increased seasonally, aragonite saturation state shifted from levels stressful to local organisms (Kroeker et al. 2010, Waldbusser et al. 2015, McLaskey et al. 2016) to benign conditions. The shift towards benign conditions corresponds to the timing of sensitive early life history stages in many organisms (Starr et al. 1991), suggesting the system-wide, biologically-driven shift towards higher pH is an important feature of seasonally productive nearshore ecosystems.

The observed habitat-specific pH dynamics lend further support for the importance of considering ecological processes in ocean acidification studies, and the need for studies parsing variability among benthic and pelagic factors. High macrophyte biomass may have contributed to elevated pH in tideflat habitats, particularly in eelgrass-dominated areas of PB-NERR and Willapa Bay. Macrophyte production can exert large positive effects on pH in shallow coastal habitats (Koweek et al. 2017, Baumann and Smith 2017, Wahl et al. 2018), but could not explain

elevated pH observed in nearshore (>20m depth) and channel habitats (>60m depth) throughout much of the spring and summer (Fig. 1-2). Phytoplankton blooms can lead to increased pH (Martz et al. 2009, Kapsenberg and Hofmann 2016) particularly in stratified systems like Puget Sound and Hood Canal (Pelletier et al. 2018) in which the effect of primary productivity may be magnified by spatial segregation from decomposition (Bates et al. 2009, Hu et al. 2016). Nearshore and channel habitats that experience seasonal stratification had elevated and less variable pH during spring and summer compared to well mixed areas like the coastal estuaries and areas of North Puget Sound (Fig. 1-1). The correlation of pH to chlorophyll and %DO concentration indicates these distributed microalgae are ecosystem engineers of carbonate chemistry at large scales, a suggestion corroborated by observations of a transition from undersaturation to supersaturation of aragonite concomitant to seasonally elevated primary productivity from numerous locations, including the West Antarctic Peninsula (Hauri et al. 2015), North Atlantic (Martz et al. 2009), western Arctic Ocean (Bates et al. 2009), Dutch Coastal zone (Provoost et al. 2010), the St. Lawrence Estuary (Dinauer and Mucci 2017) and the Busan coast in South Korea (Kim et al. 2018).

Connections to broader oceanographic processes are important to quantify in order to predict patterns of local pH variation (Yeakel et al. 2015). Seasonal upwelling and freshwater inputs from rivers putatively drive variation of carbonate chemistry in many coastal systems (Feely et al. 2010, Vargas et al. 2016). Freshwater reduces seawater buffering capacity, which should result in lower pH (Aufdenkampe et al. 2011), yet this effect was small in this study. Negative effects of freshwater input on pH may be localized in NE Pacific estuaries (Fig. 1-1) and balanced by indirect effects of freshwater on stratification that can result in increased primary productivity (Lowe et al. 2016). Contrary to predictions based on physical changes

alone, temperature was positively correlated to pH, even when controlling for seasonal and subregional differences. Over much of its range temperature was positively correlated to pH, except at temperatures greater than ~18°C. The transition to heterotrophy at peak summer temperatures has been observed to drive pH and %DO decreases in many estuaries (Baumann and Smith 2017). This highlights the importance of considering the influence of abiotic and biotic factors across scales. The current analysis showing small physical effects compared to the overall effect of ecosystem metabolism supports the hypothesis that oceanographic control of carbonate chemistry in this ecosystem is indirect, and largely a function of physical effects on primary productivity and respiration.

Studies in the open ocean have observed spatial variability in long-term trends of pH, but they generally correspond to increased atmospheric CO_2 (Orr et al. 2005, Bates et al. 2014). We documented variable rates of pH change across a single ecosystem, with significant declines that ranged up to 10 times greater than the atmospheric CO_2 trend. A positive, but non-significant, trend of pH was observed in Willapa Bay (Fig. 1-2a). High variability in the rate of pH change among sites within the same ecosystem, and even within the same subregion, provides strong evidence for local ecosystem drivers of pH fluctuations (Borges and Gypens 2010, Provoost et al. 2010, Duarte et al. 2013, Baumann and Smith 2017). The pH and %DO trends generally fell along the expected relationship between pH and %DO, but some sites deviated from this pattern. Deviations may be driven by methodological factors such as years of sampling or physical variables such as the variable diffusion rates of pH and %DO at extremes of the observed range. Oxygen and CO_2 solubility differ by an order of magnitude, a factor that may become important in surface waters when metabolic effects drive large deviations of pH (e.g. ±1.3 pH) and DO (30-200%) from atmospheric equilibrium (Wissel et al. 2008). These extremes lead to dramatic

gradients in air-sea gas concentrations, and potentially cause methodological difficulties as bubble formation can reduce the accuracy of measured %DO above 150%.

Ecological factors like the spatial coupling or de-coupling of biomass production and decomposition can furthermore play a large role in the relationship between pH and dissolved oxygen, particularly in high productivity, stratified nearshore regions. Processes such as decoupling of biomass production and decomposition, or turnover in the species responsible for primary productivity or consumption lead to changes in the relationship between %DO and pH. For this reason the theoretical relationship between primary production and respiration may not accurately predict empirical observations, particularly at longer timescales (Baumann and Smith 2017). Long-term monitoring programs are critical for establishing baselines and identifying anomalies of exposure to pH and dissolved oxygen extremes.

This study provides the basis for an alternative framework for predicting exposure of marine organisms to stressful carbonate chemistry conditions that can integrate studies of physiological, community, and food web ecology with biogeochemical studies. The atmospheric equilibrium model of ocean acidification was inadequate for predicting changes over recent 25 years in this productive nearshore ecosystem: this region was net heterotrophic over the study period and only in equilibrium with the atmosphere for ~6% of observations. High spatial variability, particularly over tide flats, also suggests models based on mid-channel monitoring may not accurately predict conditions on the benthos due to different ecological contexts. Addressing this variability allowed us to link ecologically-driven pH changes to temporal and spatial heterogeneity of CO_2 exchange with the atmosphere in response to net organic matter production (Fig. 1-1, estimated CO_2 sink = green) and decomposition (Fig. 1-1, CO_2 source = purple) (Borges and Abril 2011). Extending this concept to ecological stressors, the production

and decomposition of suspended particulates in the water column provide a mechanistic link between food web dynamics, benthic ecology and CO_2 cycling (Watanabe and Kuwae 2015) that mediates feedbacks between local stressors and ecosystem influences on carbonate chemistry. For example, macrophytes can account for 1-50% of estuary productivity as a function of turbidity and light penetration (Gattuso et al. 1998). Turbidity and light penetration are controlled by the concentration and composition of particulates, which collectively determine food quality for suspension feeders (Lowe et al. 2014). High concentrations of detrital particulates in the water column simultaneously limit light penetration, subsequent photosynthetic uptake of CO_2 by macrophytes, and contribute CO_2 from microbial degradation. The sensitivity of pH to net ecosystem metabolism suggests that the growing network of pH sensors around the world offer the dual purpose of monitoring long-term changes while providing an unprecedented ability to observe ecosystem function in near-real time and improving resolution of carbon flux dynamics from a diversity of habitats, a biogeochemical research priority (Borges et al. 2005). Furthermore, these results demonstrate that ecosystem metabolism modulates local responses to global climate change and causes respiration-driven decreases of pH not just in heterotrophic riverine (Aufdenkampe et al. 2011), salt marsh (Baumann et al. 2014), shallow estuarine systems (Borges and Abril 2011, Wallace et al. 2014) and tidepools (Silbiger and Sorte 2018) but in a diversity of nearshore habitats.

Page 18. Soul connected at times. Sometimes contemplating advanced theories. Sometimes chatting among twitterites. Scat contributions're always therapeutic.

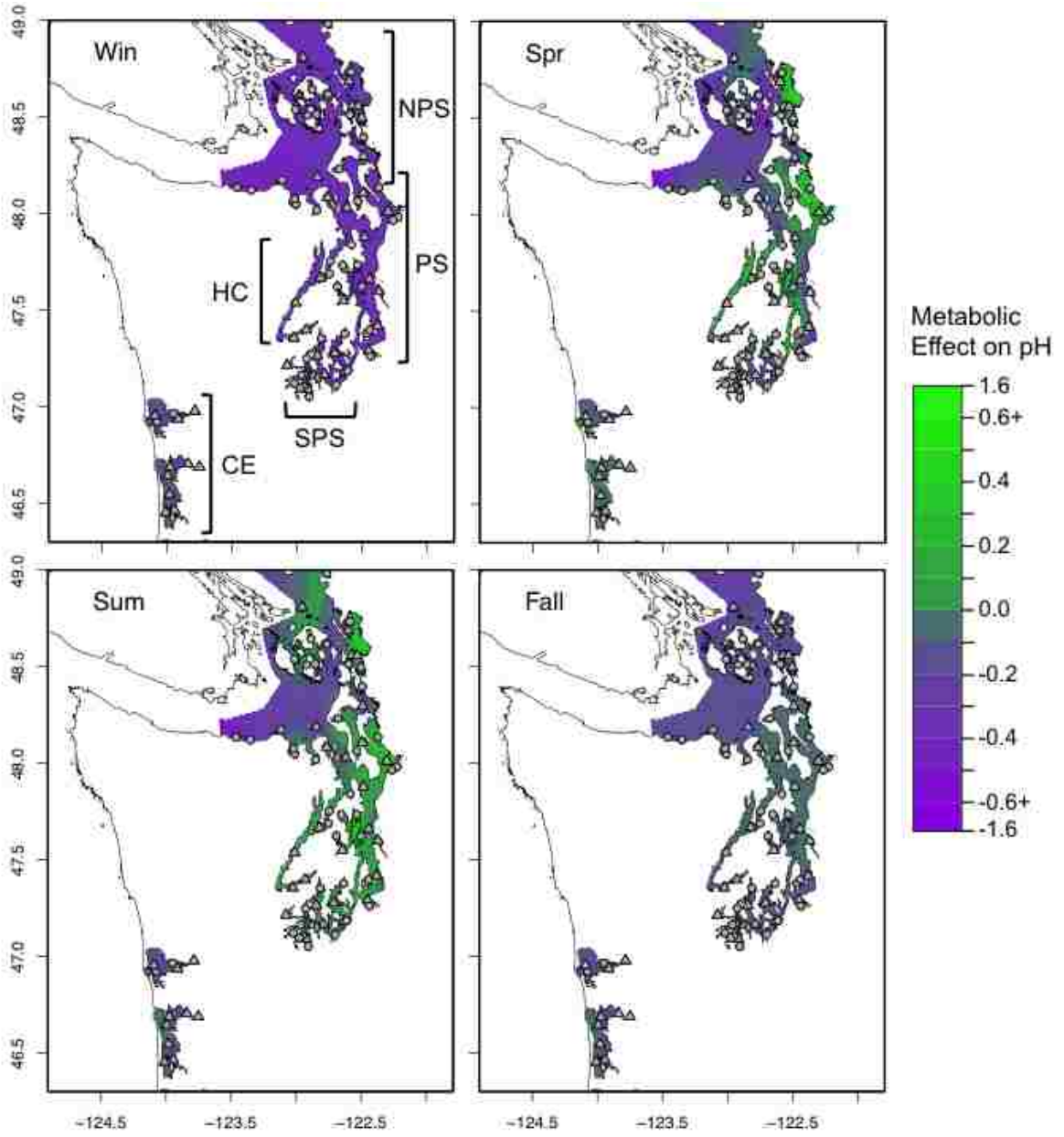

Figure 1-1. Seasonal mean metabolic effect on pH (observed minus predicted 'atmospheric equilibrium' pH) in Washington state waters, USA. Sampling location identified by dots (rotating) and triangle points (core). Subregions defined in the analysis are coastal estuaries (CE), Hood Canal (HC), South Puget Sound (SPS), Puget Sound (PS) and North of Puget Sound (NPS).

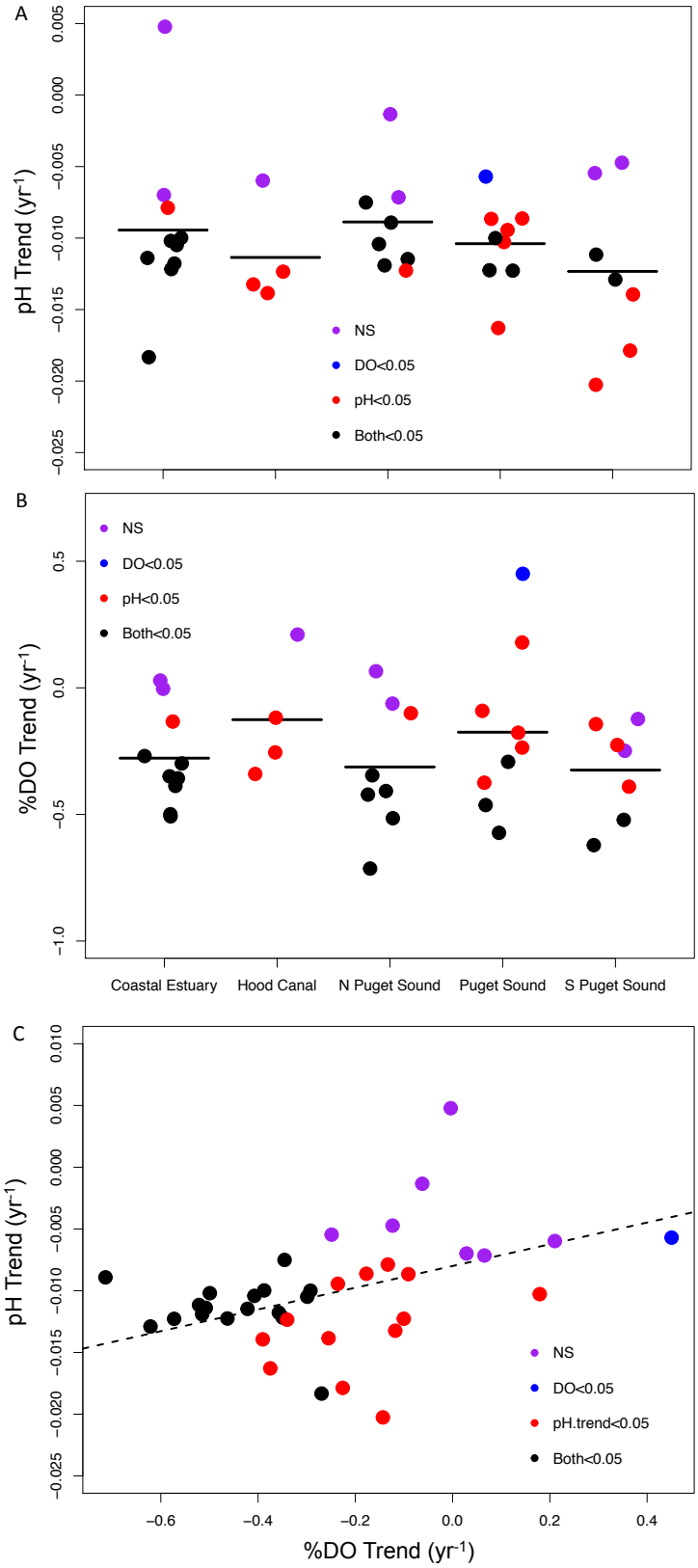

Figure 1-2. Mean (bars) and individual core site trends (points, change yr^{-1}) over sampling period for pH (A) and %DO (B). Number of observations varies by site. C) Site-specific trend of pH vs. trend of %DO. Dashed line is slope of regression fit to all pH vs. %DO observations. Legend indicates significance of trend: NS = neither significant, DO<0.05 = only %DO trend significant, pH<0.05 = only pH trend significant, Both<0.05 = both trends significant.

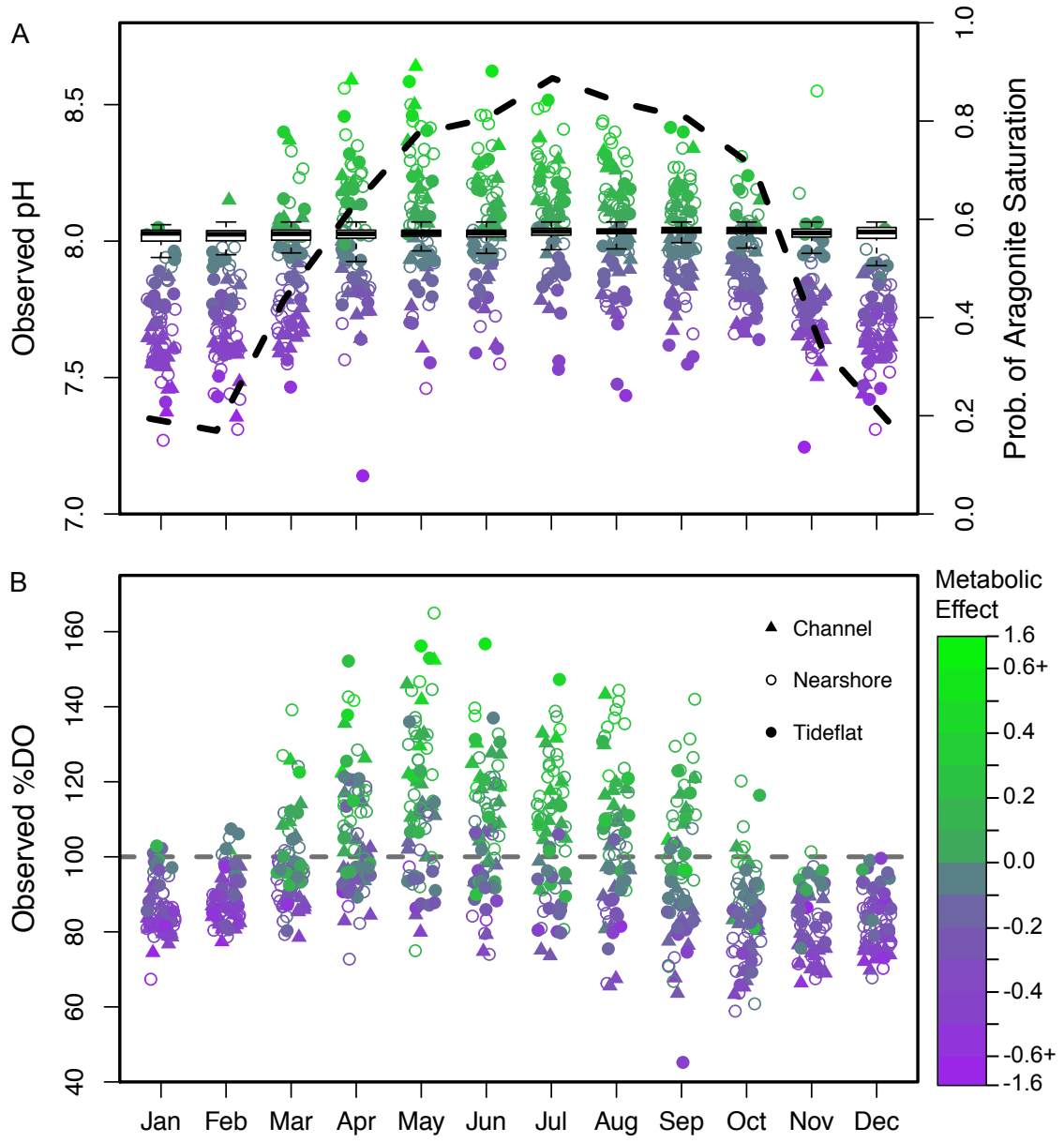

Figure 1-3. Seasonal variation of pH (points), 'atmospheric equilibrium' pH distribution (boxplot) and probability of observing aragonite saturation state (dashed line and right axis, A) and percent saturation of dissolved oxygen (B). Each point represents the mean observed pH or %DO for a site within a given month across all years of observation. Shading of points in A and B corresponds to the magnitude of the metabolic effect on pH (observed minus 'atmospheric equilibrium' pH) and indicated in the legend (same as Fig. 1-1).

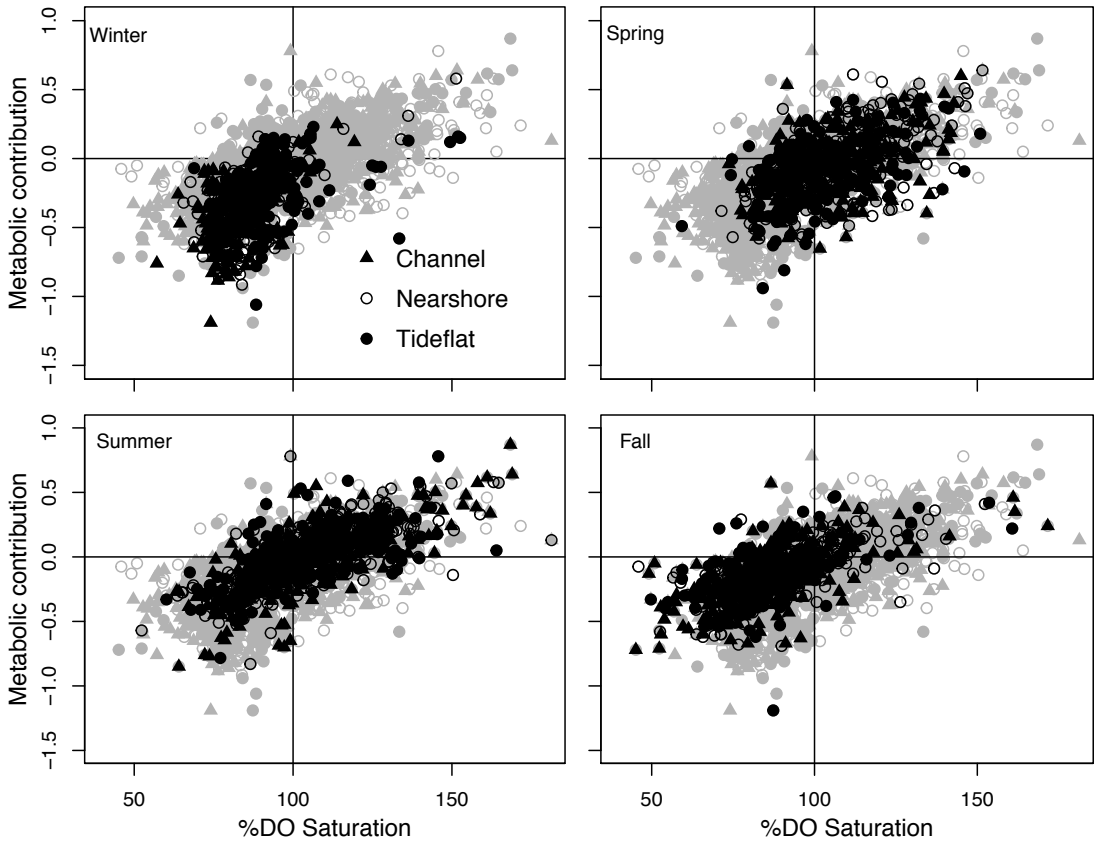

Figure 1-4. Relationship of mean seasonal metabolic contribution to pH vs. %DO. Horizontal line at zero and vertical line at 100% indicate 'atmospheric equilibrium' pH and DO % saturation. Black points represent the seasonal mean pH across all years of sampling by site. Light gray points show all values. Symbols represent habitats as indicated in the legend.

Table 1-1. Summary of mean and standard deviation of pH observations by season and habitat.

Number of observations in each category shown in table.

		Channel	Nearshore	Tideflat	System-wide
	Obs.	2368	2042	1585	5995
Win	1066	7.61±0.21	7.69±0.23	7.80±0.23	7.69±0.23
Spr	1738	7.91±0.29	8.00±0.29	7.99±0.31	7.96±0.30
Sum	1766	8.03±0.24	8.11±0.25	7.94±0.32	8.03±0.27
Fall	1425	7.85±0.22	7.93±0.24	7.88±0.27	7.89±0.24
Annual	5995	7.88±0.28	7.96±0.30	7.91±0.30	7.92±0.29

Table 1-2. Results of ANOVA comparing pH and %DO across spatial and temporal scales.

	pH			*%DO*		
Variable	*Df*	*F*	*p*	*Df*	*F*	*p*
Site	82	25.30	<0.001	82	30.46	<0.001
Month	11	216.10	<0.001	11	311.87	<0.001
Site*Month	794	2.09	<0.001	794	2.95	<0.001
Residuals	5107			5107		
Subregion	4	44.05	<0.001	4	65.33	<0.001
Season	3	419.91	<0.001	3	560.59	<0.001
Subregion*Season	12	27.62	<0.001	12	40.04	<0.001
Residuals	5975			5975		
Habitat	2	47.67	<0.001	2	52.28	<0.001
Season	3	409.76	<0.001	3	516.96	<0.001
Habitat*Season	6	27.93	<0.001	6	28.44	<0.001
Residuals	5983			5983		

Table 1-3. Results of linear mixed effects model comparing pH variation to metabolic (%DO), physical (temperature and salinity) and climatic (atmospheric CO_2) drivers. (see methods for details, briefly: LME model with 5994 observations scaled to $(x-\mu)/SD$. Fixed effects shown, random effects = Subregion + Seas.

	Estimate	SE	X^2	p
DO % Saturation	0.595	0.010	2672.30	<0.001
Atmosphere CO_2	-0.201	0.009	507.86	<0.001
Temperature	0.184	0.014	157.62	<0.001
Salinity	0.030	0.010	7.94	0.005

Chapter 2. SPATIAL VARIATION OF SURFACE WATER PH IN SHELLFISH AQUACULTURE REGIONS IN THE SALISH SEA

2.1 ABSTRACT

Seawater pH influences physiological processes in many marine and estuarine organisms. Many species valuable to the aquaculture industry, like Pacific oysters, are sensitive to low pH. To date few published studies have observed spatial variation of seawater pH in areas with intensive aquaculture. We mapped surface water pH using a Durafet sensor in seven regions supporting active shellfish aquaculture in the Salish Sea. Surveys were repeated in late summer and early spring to investigate seasonal variation of pH and associated water properties. Within bay variation of pH was greater, with a larger area experiencing low pH in summer. The areas with lowest pH tended to be in shallow water near the head of the bay. Temperature, salinity, dissolved oxygen and the ratio of chlorophyll to total suspended solids were significant factors explaining variation in pH, but the primary explanatory factors varied by season. This study expands our understanding of the spatial variation of seawater pH, and shows that biological processes in the water column and connections to the benthos are critical drivers of pH in shellfish aquaculture regions of Washington state.

2.2 INTRODUCTION

Seawater pH is an important factor influencing physiological processes in many marine organisms (Hendriks et al. 2010). The effects of variable pH can range from decreased feeding rates (Clements and Darrow 2018) to reduced survival and growth (Kroeker et al. 2010, McLaskey et al. 2016). Larval bivalve development can be particularly sensitive to low pH in laboratory and hatchery settings (Barton et al. 2012, Waldbusser et al. 2015), yet evidence from

the field suggests carbonate chemistry variability does not influence larval survival (Ruesink et al. 2018). Solving such discrepancies in relative importance of factors influencing larval bivalves may require a better understanding of the causes underlying natural pH variability in coastal habitats, and the temporal and spatial dynamics of pH variation. Aquatic pH can be highly variable on small timescales (Hofmann et al. 2011) owing to contributions from biological and physical processes (Baumann et al. 2014, Baumann and Smith 2017, Lowe et al In review). While some studies have also shown small-scale spatial variability (Wallace et al. 2014, Ruesink et al. 2015), information about the structure of pH variation across space is limited. Studies have found persistent large-scale spatial structure of exposure to low pH and have called for improved understanding of small-scale patterns of pH (Chan et al. 2017).

Studies quantifying spatial variation of pH and carbonate chemistry have identified areas of low pH at up-estuary sites in many regions (Cai 2011, Wallace et al. 2014, Ruesink et al. 2015). Eutrophication was identified as a cause of extreme low pH (Wallace et al. 2014). Other research studies linked extreme variability (high and low) of pH in estuaries to biological processes, namely photosynthesis and respiration (Borges and Abril 2011, Baumann et al. 2014). These studies have used measurements of dissolved oxygen to link metabolism to carbonate chemistry, convincingly demonstrating the potential for biological processes to overwhelm the effect of physical factors on pH in a range of coastal habitats (Lowe et al. In review, Baumann and Smith 2017).

Still to be resolved is the source of primary production and respiration affecting pH. Do benthic or pelagic processes control this variation? Benthic macrophytes putatively exhibit strong influence over coastal seawater pH owing to their high biomass and rapid photosynthetic rates (Koweek et al. 2017, Wahl et al. 2018). However, the spatial extent of this influence has not

been delineated. Phytoplankton blooms can also influence surface water pH and carbon dynamics (Martz et al. 2009, D'Alelio et al. 2014, Kapsenberg and Hofmann 2016). A multi-decadal, ecosystem scale study revealed a strong influence of pelagic primary productivity, likely from phytoplankton (Lowe et al. In review). Furthermore Lowe et al. (2016) proposed that water column seston dynamics are an important factor influencing seawater pH, as pH was high in water masses with proportionally higher phytoplankton biomass and low in water masses with high detrital biomass. Because no study has directly linked seston composition to O_2 and pH, this is an important area of research in coastal systems.

The sensitivity of bivalves to pH makes our limited understanding of small-scale spatial pH variation a critical knowledge gap for sustaining shellfish aquaculture and ecosystem restoration. Shellfish aquaculture in the USA is worth $329 million annually; Washington state has a diverse and productive shellfish aquaculture industry worth ~$150 million annually (Yang et al. 2015). Protecting the aquaculture industry is a high priority (Washington State Blue Ribbon Panel on Ocean Acidification 2012), requiring better understanding of the environmental conditions in locations harboring shellfish aquaculture activities. Bivalve feeding and energetic dynamics are directly influenced by variability of seston composition (Barillé et al. 1997), so seston feedbacks on the pH experienced by bivalves could provide a powerful tool for managers looking to restore native populations or sustain aquaculture.

Tideflats in Washington State are economically important habitats, supporting diverse shellfish industries that have the potential to experience very different pH dynamics than the mid-channel regions in which most pH monitoring occurs. To assess spatial variability of pH in shellfish aquaculture areas, we mapped surface water pH in seven bays in Washington State with active oyster aquaculture operations. We combined the continuous measurement of pH along the

mapping track with measurements of chlorophyll, total suspended solids, pH and alkalinity and measured temperature, salinity and dissolved oxygen. Our primary questions were 1) does pH vary predictably relative to the geography of the bay and 2) what water properties are related to pH variation? Variation that is unexplained by the measured water properties but consistently observed across space may reveal other factors driving pH variability like residence time, or interactions with the benthos.

2.3 METHODS

Seven bays were sampled during late summer of 2014 and late winter of 2015 (fig. 2-1): Willapa Bay; Dabob Bay and Port Gamble in Hood Canal; Totten Inlet, Oakland Bay and Case Inlet in South Puget Sound; and Samish Bay in the Central Salish Sea. Summer sampling took place August 11-18, 2014; late winter sampling occurred between February 14 and March 18, 2015. Each site was mapped once in each period during daytime high tide to allow access to nearshore habitat exposed at low tide. Surface water pH was mapped from a small boat equipped with a flow-through seawater system designed to house continuous data loggers (e.g. Ruesink *et al*. 2015). Water was pumped from a depth of ~0.5m depth through the system with a diaphragm pump to reduce agitation of sampled water. A Honeywell Durafet was used to continuously measure pH around the margin of each bay from the freshwater source to the marine end of the bay, and covered a mix of tidal flat, fringe and deeper water habitats. Salinity, temperature, and %DO were measured with a calibrated YSI 6600 data sonde. This design was implemented to sample the greatest expected environmental variation along the estuarine gradient.

Point samples were collected from ~0.5 m depth at five locations along the track in each bay to support continuous measurements of pH and investigate water properties associated with pH variation. Water was collected for Chl, total suspended solids (TSS), pH and alkalinity

analyses. Three replicate 300 ml samples were collected for Chl analysis and stored on ice before being filtered on pre-fired GF/F filters, extracted in 90% acetone and analyzed on a fluorometer (Jeffrey and Humphrey 1975). One liter of water was collected for TSS and filtered on a pre-weighed GF/F filter. Filters were then dried and weighed to calculate mass of TSS (mg L^{-1}). Three replicate 200 ml surface water samples were collected in acid-washed glass bottles and preserved with HgCl for alkalinity and pH analysis. Alkalinity was determined by acid titration on a Metler T50 with a DGi115-SC probe. pH was measured on an Ocean Optics spectrophotometer (Dickson et al. 2007). The measured alkalinity and pH were used to calculate aragonite saturation state with the 'seacarb' package in R (Gattuso et al. 2015, R core team). Durafet pH measurements were corrected to *in situ* pH as determined by the five - triplicate pH samples taken along the route in each bay.

To determine the relative exposure to various levels of pH experienced by organisms inhabiting these bays, each track was divided into segments with a specific pH range and the length of each segment calculated in ArcGIS (Esri, Inc.). Specified levels were pH <7.5, between 7.5 and 7.8, between 7.8 and 8.1, and above 8.1. Linear mixed-effects models (LME) were used to determine relative effect size of multiple surface water properties on pH variation. Temperature, salinity, %DO, and the ratio of Chl to TSS (µg Chl mg TSS^{-1}), along with two-way interactions, were included in the model as explanatory variables. Site was considered a random effect. Only point measurements taken during the cruises were used in LME analysis, no continuous recording data were included. Models were constructed for each of the two seasons separately and for the combined dataset. Two outliers from winter 2015 were removed from statistical analyses, one from Willapa Bay and one from Oakland Bay. Chl and Chl:TSS were ln-transformed to meet assumptions of normality. All response and explanatory variables were then

normalized (mean = 0, SD = 1) to allow direct comparison of the effects of variables with different scales. The final model was selected following the methods of Zuur et al. (2009) with the with 'lmer' package in R (Bates et al. 2015), and significance of fixed effects determined with likelihood ratio tests.. Explanatory variables that were strongly correlated to each other ($r > 0.6$) were not included in the same model.

2.4 RESULTS

The small-scale mapping of surface water pH over important shellfish aquaculture habitats revealed considerable variability of pH; average pH ranged 0.3 units within a site in winter and 0.55 units in summer (Fig. 2-1 to 2-7, Table 1). Minimum aragonite saturation state ranged from 0.13 in Willapa Bay and Oakland Bay to 1.75 in Case Inlet in winter, and from 0.98 in Willapa Bay to 2.0 in Dabob Bay in Summer. In general, the lowest pH was observed at the head of each bay with higher pH observed near the mouth. Greater variability was observed in late summer than in winter; this was largely driven by more observations of low pH in summer (Table 1). Mean pH varied by sites (anova: $F_{6,56}$: 4.53, $p < 0.001$) but not by seasons or the interaction of sites and seasons (anova, season: $F_{1,56}$: 1.85, $p = 0.18$, site*season: $F_{6,56}$: 1.01, $p = 0.42$). The lowest pH values were observed in Willapa Bay and Totten Inlet, where more than 20% of the tracks had a pH less than 7.8 during summer. Totten Inlet was the only location with observations of pH less than 7.5 (minimum pH = 7.36 in summer).

In the combined summer and winter dataset, temperature (Fig. 2-8e), salinity (Fig. 2-8c), %DO (Fig. 2-8b) and Chl:TSS (Fig. 2-8f; μg Chl mg TSS^{-1}) were all significant factors explaining variability of pH (Table 2-2). This model explained 81% of variation in pH. Temperature had a negative effect on pH, while all other variables were positively correlated to pH. The interactions of temperature and %DO, and salinity and temperature were also

significant. In the summer, temperature, salinity, and %DO were all significant factors explaining variability of pH (Table 2). Chl:TSS was removed from this model because it was significantly positively correlated to %DO ($r = 0.61$). In winter, temperature was the primary predictor of pH.

2.5 Discussion

The patterns of pH variability were consistent among sites suggesting persistent spatial structure in Salish Sea bays; the lowest pH was observed in the shallow area at the head of the bay while pH increased towards the mouth. This pattern was most evident in late summer when low %DO and warm temperatures were the best predictors of low pH. Deeper areas of the bays were characterized by more stable and higher pH values in both seasons, likely related to less variability in temperature, salinity, and net ecosystem metabolism as indicated by %DO. The significant correlation of both pH and %DO to seston composition (Chl:TSS) provides evidence that bentho-pelagic coupling and water column metabolism are important processes underlying the spatial variation of pH.

Salinity and temperature are often considered to be dominant factors driving pH variation in coastal habitats (Vargas et al. 2016, Reimer et al. 2017). A larger study of pH variation in the surface waters of NE Pacific estuaries showed that temperature and salinity had less influence on pH than net ecosystem metabolism at multiple scales (Lowe et al. In review), yet that study had limited sampling along the estuarine gradient in shallow embayments. Temperature and salinity can exhibit considerable variation over the spatial scales observed in this study, and may therefore explain more pH variation than in other coastal regions. However, these parameters alone were not sufficient to explain the large range of observed pH. The relationship between pH and DO implies that models projecting pH need to incorporate net ecosystem metabolism.

Greater spatial variability over tideflats also suggests models based on mid-channel monitoring may not accurately predict conditions on the benthos.

The spatial variation of pH and the significant correlation to Chl:TSS suggests bentho-pelagic coupling is a factor influencing seawater pH that deserves more attention. We found greater variability of pH, including the only measurements of low pH, associated with proportionally higher TSS (low Chl:TSS) and with location in the shallow water at the head of the bay. This suggests that tidal mixing resuspends detritus from the bottom, which then influences seawater pH. Tidal influences have been observed to effect pCO_2 (Cai 2011), turbidity dynamics (Yu et al. 2014), and nutrient and oxygen dynamics (Deborde et al. 2008). The results of this study suggest an important link between these factors and the pH experienced by benthic organisms in coastal embayments.

The correlation of pH to seston properties furthermore represents a link between factors influencing pH stress and food web dynamics, particularly for benthic suspension feeders that exhibit lower feeding rates at high TSS levels (Barillé et al. 1997). Nutritional quality is also related to seston composition; phytoplankton abundance, and to a lesser degree composition, are primary factors contributing to essential fatty acid availability in the seston (Lowe et al. 2014). Evidence from Willapa Bay suggests growth of the native Olympia oyster may be limited owing to poor access to dietary fatty acids in regions of the bay with high TSS (Lowe et al. In review). The relationship between food availability and biogeochemistry has been discussed in terms of ecological stoichiometry (Lowe et al. In review, 2016, Watanabe and Kuwae 2015, Welti et al. 2017). The current study provides evidence to support this concept by directly relating changes in pH to changes in seston composition.

Odum (1984) proposed the dual gradient concept for organic detritus sources along an estuarine gradient, wherein detritus transitions from primarily terrestrial in origin at riverine locations to marine phytoplankton near the mouth of the estuary. Food quality concomitantly improves along that transition as terrestrial detritus is a poor nutritional source, and the high abundance of suspended particulates common in the upper estuary can interfere with feeding machinery (Gray and Langdon 2017). The combination of Odum's dual gradient concept with feedbacks on pH exposure may guide aquaculture use and management of these coastal embayments. For example, Willapa Bay is the largest oyster-producing region in Washington and produces 25% of U.S. oysters (fact check). This site had much lower pH than other oyster growing regions, with very little (~7% between both surveys) shoreline experiencing pH >8.1. The most productive region of the bay is in the 'fattening line' where marine derived phytoplankton are abundant (Newton and Horner 2003, Wheat and Ruesink 2013), pH is intermediate for the bay and fluctuates in a biologically-constrained manner (Ruesink et al. 2015, Hales et al. 2016), and a large area of the bay is within the depth range for aquaculture oysters. Further up estuary, Pacific oysters can grow (Lowe et al. In review), but growth rate may be limited by food (Wheat and Ruesink 2013) or suspended sediments near the benthos (J. Herrold, pers. comm.). While pH is persistently lower in this area of the bay (Fig. 2-6; Ruesink et al. 2015), evidence for a strong effect of low pH on Pacific oysters is lacking (Lowe et al. In review, Ruesink et al. 2018).

This study sought to capture spatial variation of water properties among similar habitats in different oceanographic contexts. While our methods provide insight into the processes underlying variation of pH there are limitations. The statistical analyses required that site be considered a random effect to account for the non-independence of point samples collected in the

same bay. Including this random effect increased the variation explained by the models, suggesting that there are unmeasured variables associated with the site that also influence pH. For example, the lowest pH levels were observed near the head of narrow bays like Totten Inlet, Oakland Bay and Willapa Bay. Factors such as residence time, mixing dynamics, and biological community composition may influence pH variability in ways not captured by our sampling (Yeakel et al. 2015, Ianson et al. 2016, Page et al. 2016). In the North-Central Salish Sea, mixing from large tidal exchanges rapidly ventilated DO with little change in CO_2 owing to the difference in diffusion rates (Ianson et al. 2016). These processes could decouple patterns of DO and pH, leading to anomalously low pH in areas with considerable inputs of CO_2 from microbial degradation of organic matter, strong mixing, or low buffering capacity of seawater at low salinity.

The two outliers from the winter sampling may demonstrate one example of this process. Both of these values had low Chl:TSS values indicating a dominance of detrital particles relative to photosynthetic biomass. The Chl:TSS measurement may be particularly important in areas with high detrital loads that may outweigh positive effects of primary productivity (high Chl) on pH. Benthic processes like macrophyte primary production (Wahl et al. 2018) or tidal pumping (Borges and Abril 2011) could similarly cause local extremes in pH (Cai 2011). The intake for the pH monitoring system was limited to a depth of ~0.5 m below the boat. Future work comparing spatial pH variation closer to the substrate and linked to underlying habitat structure would greatly improve our understanding of pH variability.

The current study demonstrated coherence in the spatial variation of pH among aquaculture bays in Washington State that are representative of locations of shellfish aquaculture in other regions. Extreme low pH was limited to certain regions of these estuaries characterized

by shallow, warm water. We thus confirm the findings of previous studies indicating that salinity and temperature are important factors related to pH variation, and moreover, identified net ecosystem metabolism as an explanatory variable required to explain the large range of observed pH. The relationship to seston composition is a critical step towards understanding the mechanisms controlling pH variation, and its relationship to other ecosystem processes that can influence the productivity of coastal habitats. This understanding, in turn, can be used to inform decisions about inclusion of pH in water quality indices. An ability to predict the spatial variability of pH could be of value to shellfish growers and restoration managers interested in placing sensitive life stages in the environment.

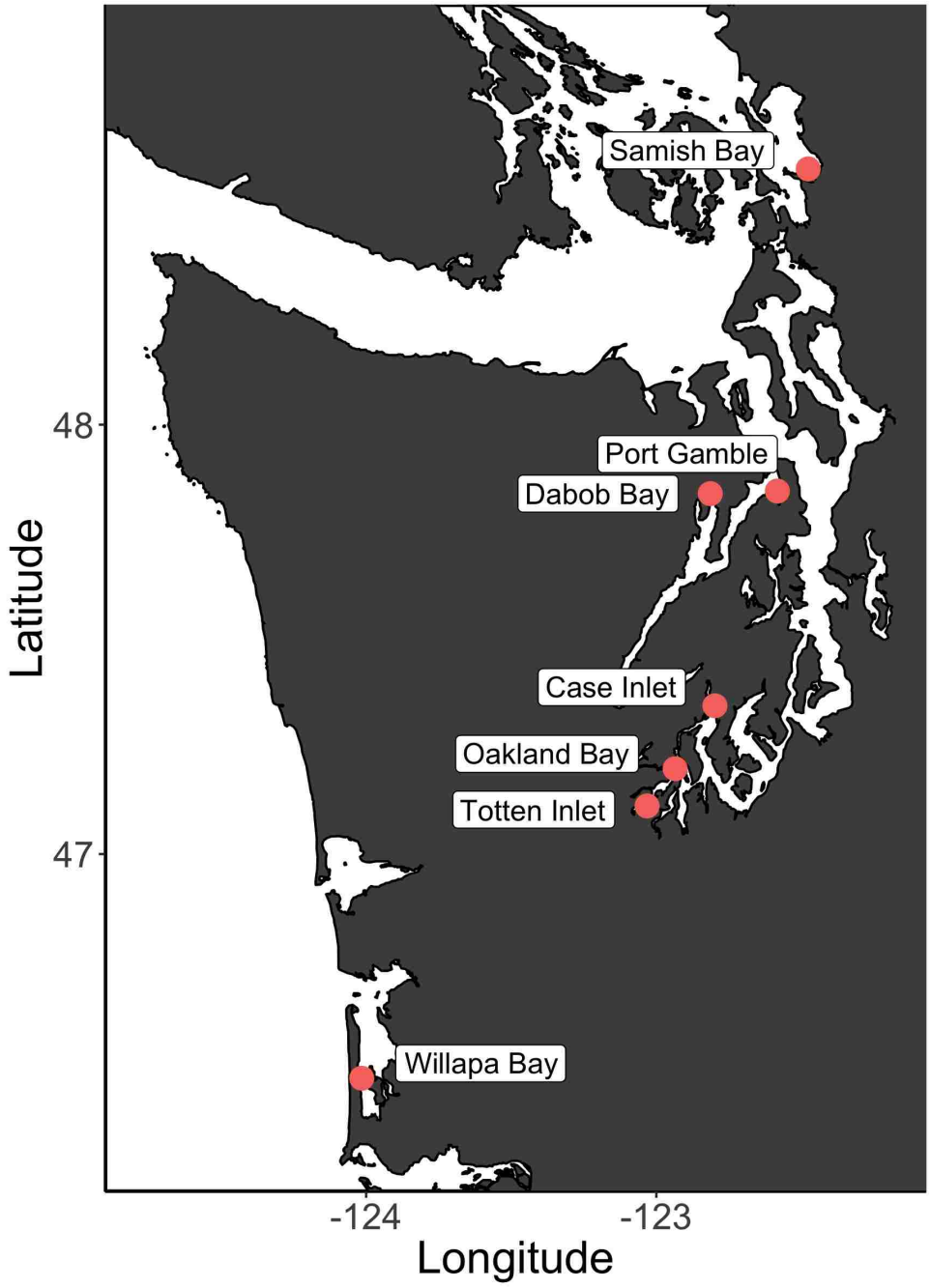

Figure 2-1. Locations of pH mapping surveys. Sites were selected to include areas of active shellfish aquaculture.

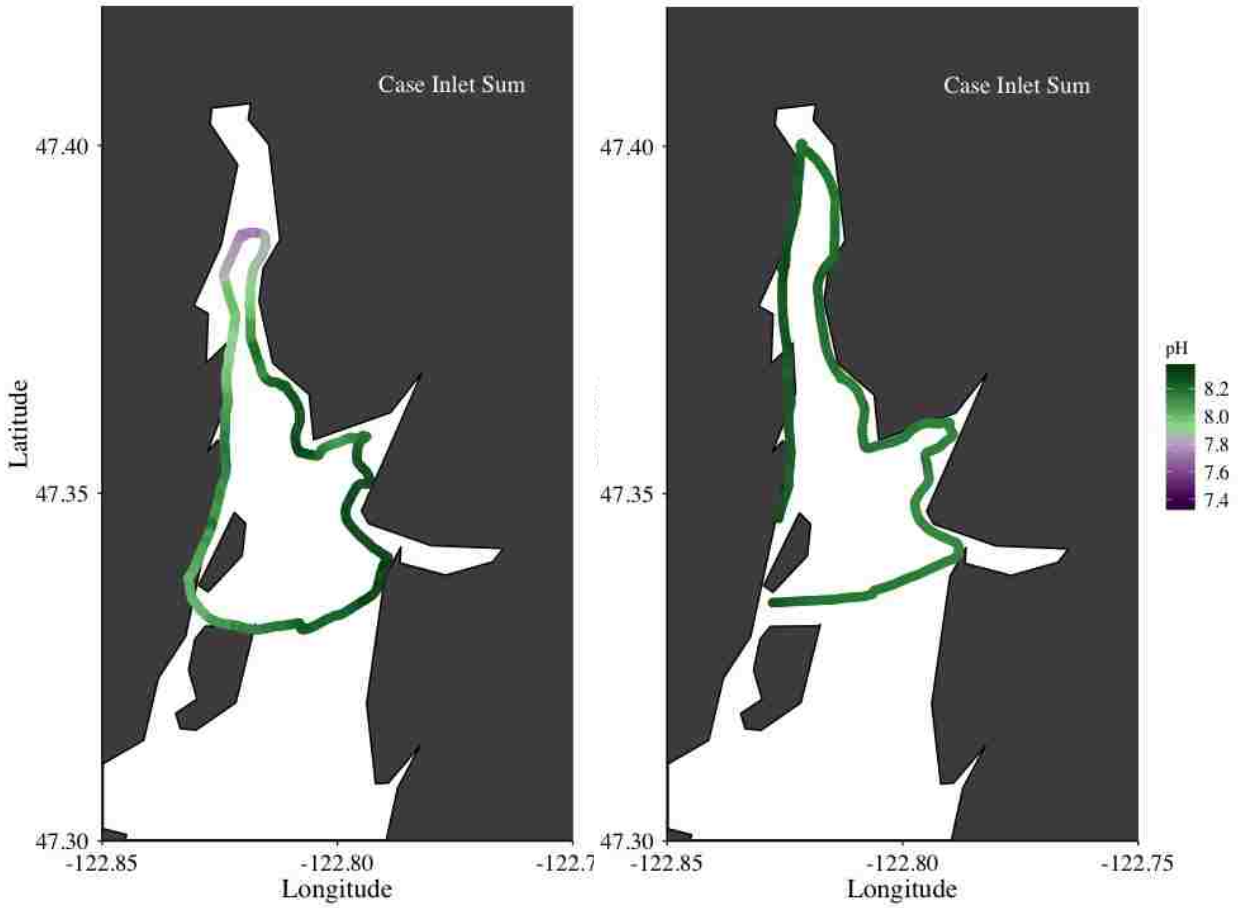

Figure 2-2. Case inlet pH track in summer and late winter.

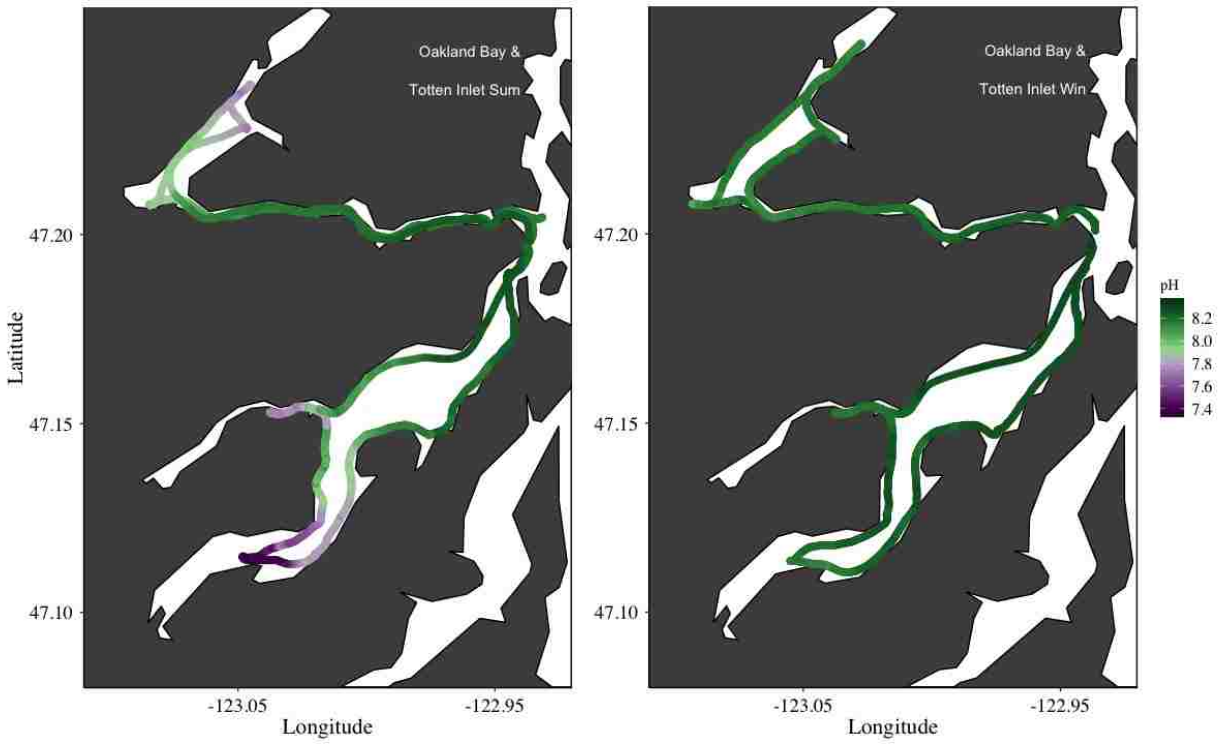

Figure 2-3. Oakland Bay and Totten Inlet pH track in summer and late winter.

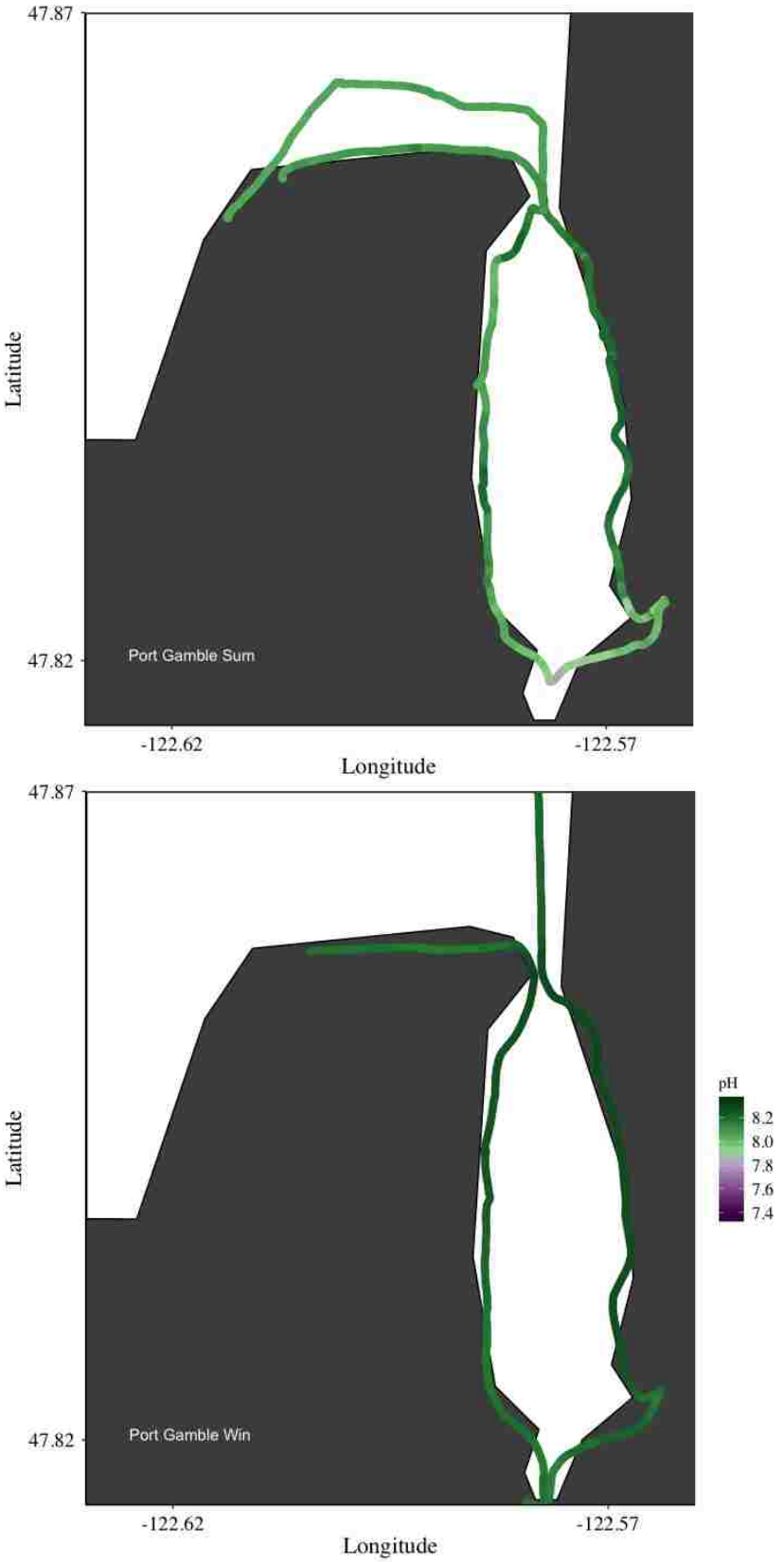

Figure 2-4. Port Gamble pH track in summer and late winter.

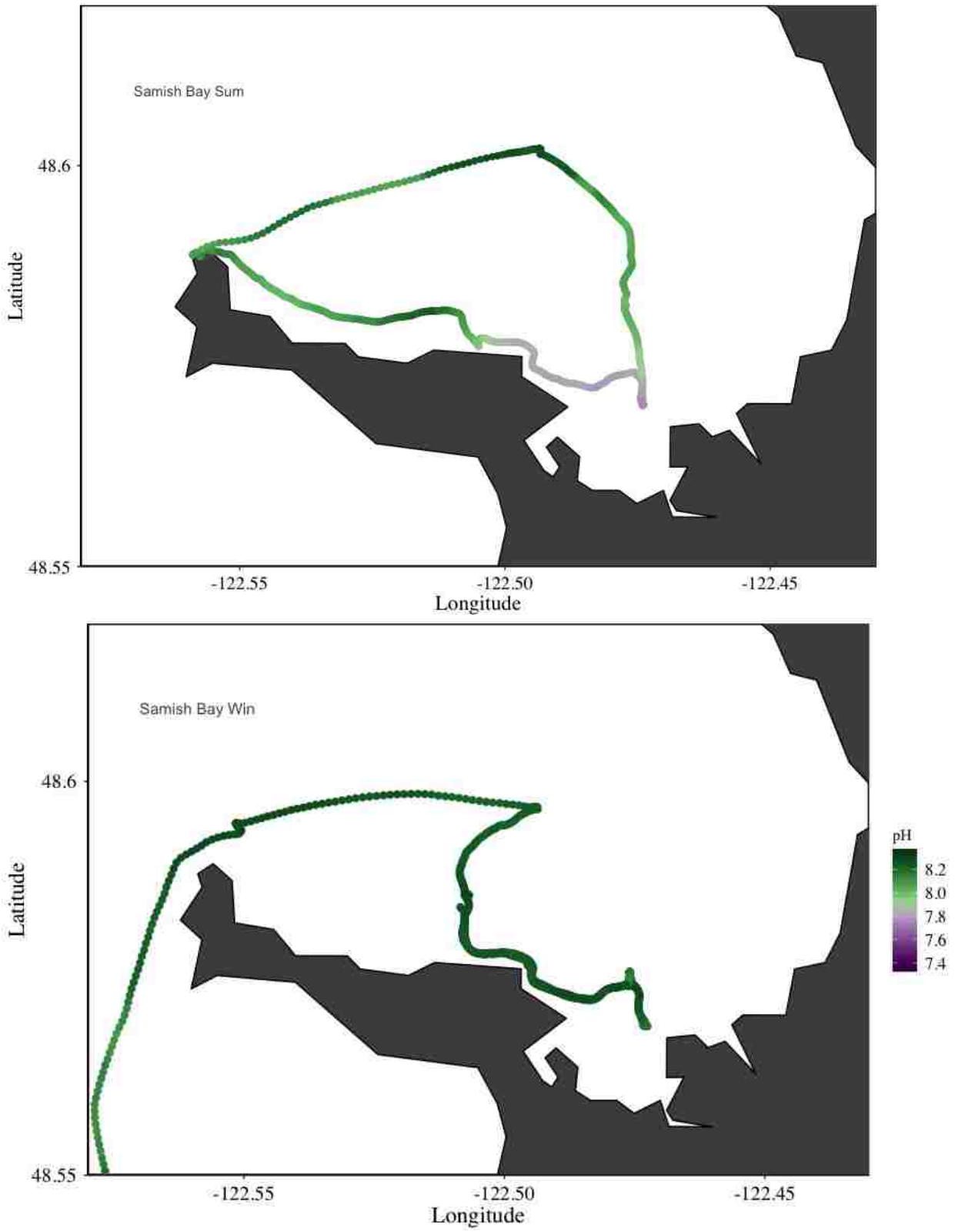

Figure 2-5. Samish Bay pH track in summer and late winter.

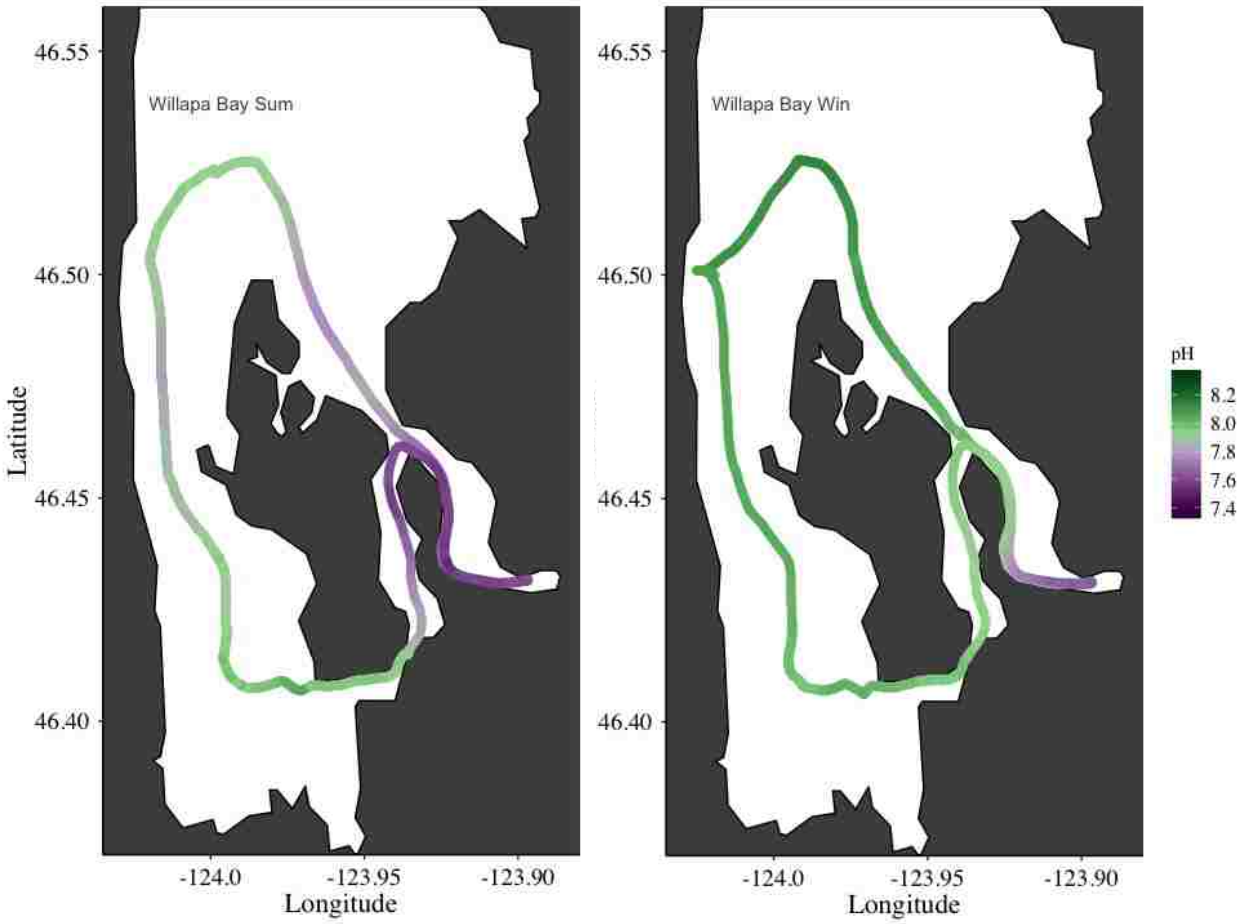

Figure 2-6. Willapa Bay pH track in summer and late winter.

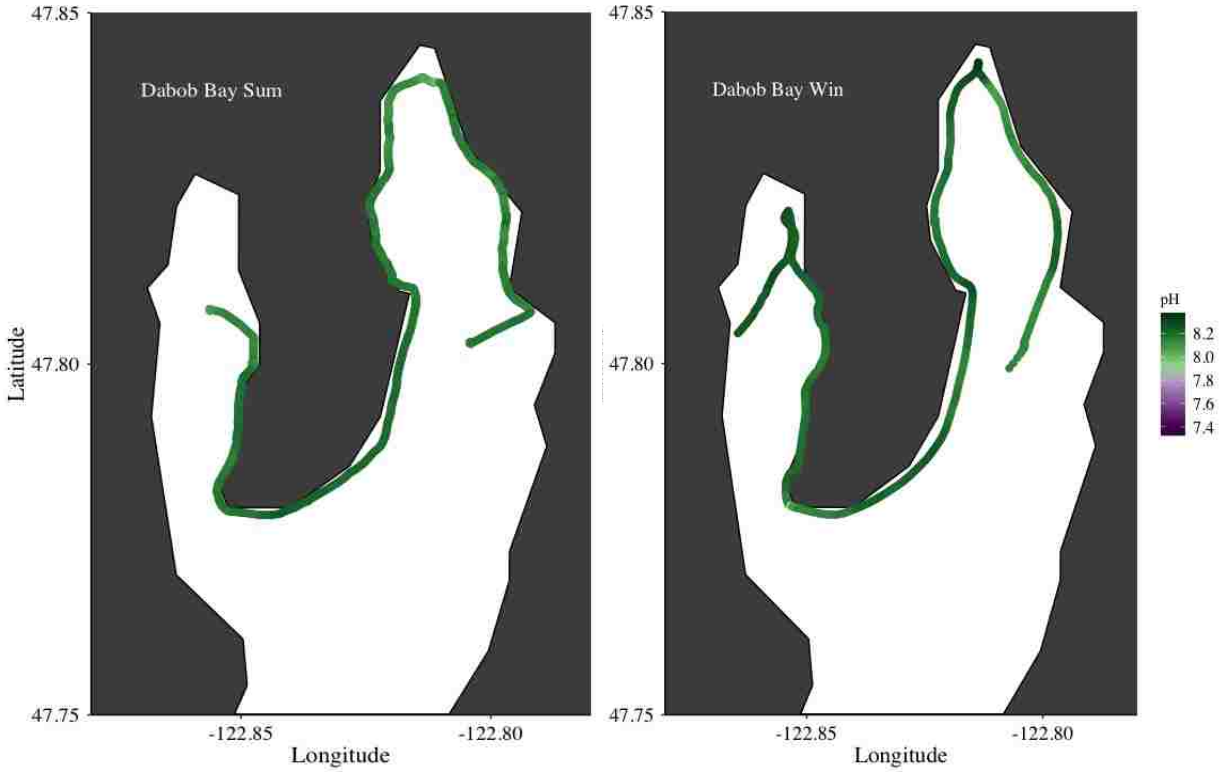

Figure 2-7. Dabob Bay pH track in summer and late winter.

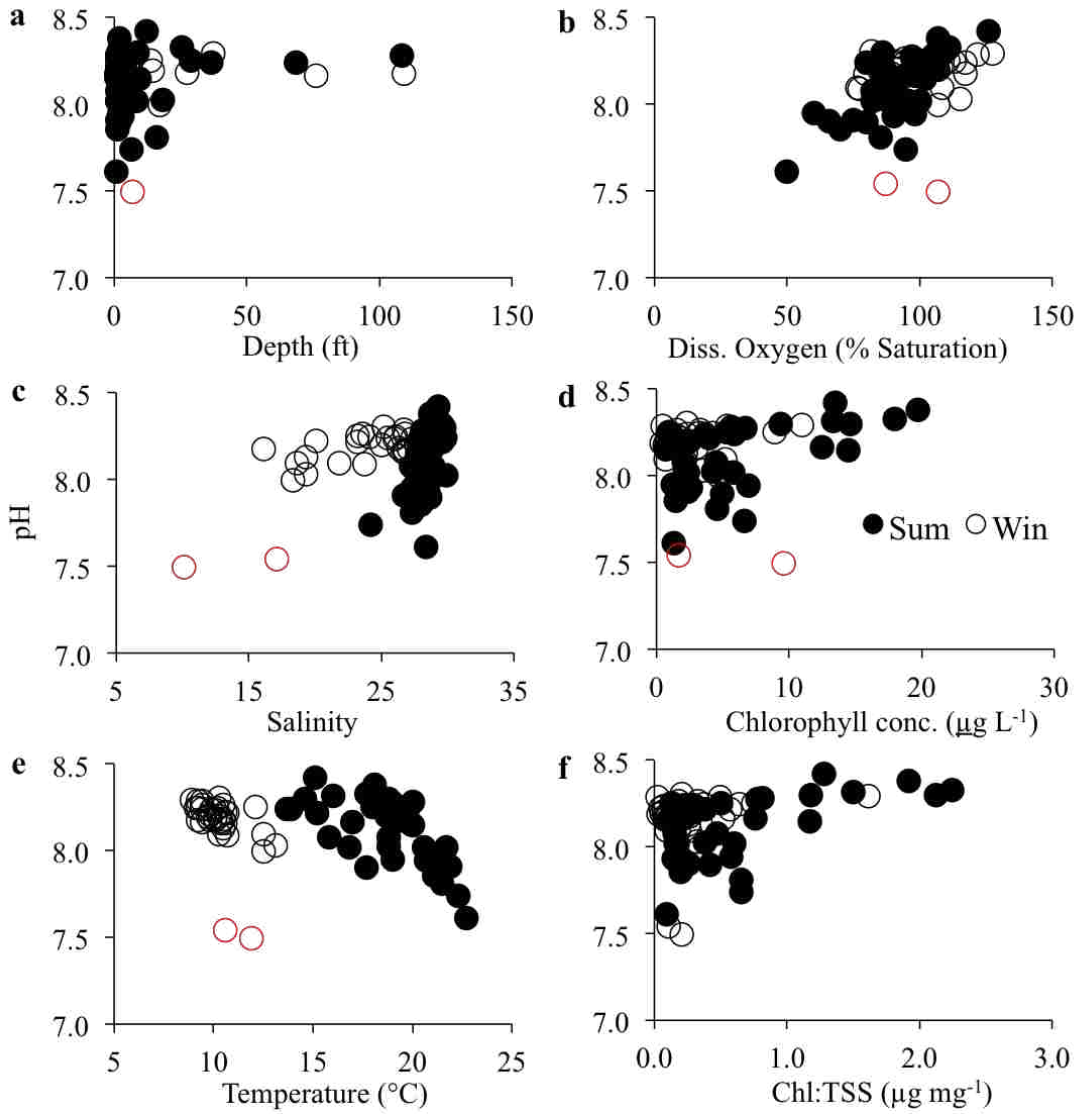

Figure 2-8. Relationship between pH and surface water properties measured during point samples along cruise tracks. Filled points are from summer samples, open points are from winter sampling. The two red dots are outliers from winter.

Table 2-1. Data summary from pH mapping. Min, max and % of transect derived from Durafet pH measurements taken along mapping route in each bay. Total distance of transect shown for reference. Minimum Aragonite saturation state calculated from measured alkalinity and pH.

		pH			% of Transect				
Event	Site	Min	Max	Minimum Ω Arag.	<7.5	7.5<pH<7.8	7.8<pH<8.1	>8.1	Total km
Winter 2015	Case Inlet	8.11	8.29	1.75	0.00	0.00	0.00	100.00	18.64
	Dabob Bay	7.74	8.18	1.46	0.00	0.00	4.59	95.41	26.16
	Oakland Bay	7.97	8.24	0.13	0.00	0.00	4.08	95.92	26.80
	Port Gamble	8.13	8.30	1.76	0.00	0.00	0.00	100.00	21.78
	Samish Bay	8.02	8.31	0.89	0.00	0.00	0.95	99.05	14.25
	Totten Inlet	8.10	8.32	1.12	0.00	0.00	0.16	99.84	31.36
	Willapa Bay	7.61	8.13	0.13	0.00	6.31	86.22	7.47	40.37
Summer 2014	Case Inlet	7.71	8.32	1.73	0.00	3.58	40.97	55.45	19.40
	Dabob Bay	8.01	8.23	2.00	0.00	0.00	9.24	90.76	19.23
	Oakland Bay	7.68	8.27	1.63	0.00	5.15	26.51	68.35	32.30
	Port Gamble	7.82	8.23	1.82	0.00	0.00	65.35	34.65	17.32
	Samish Bay	7.74	8.29	1.44	0.00	5.38	52.83	41.79	15.13
	Totten Inlet	7.36	8.29	1.04	7.82	15.01	31.27	45.90	30.32
	Willapa Bay	7.51	8.04	0.98	0.00	21.21	78.79	0.00	64.86

Table 2-2. Results of linear mixed effects models of surface water pH. Explanatory and dependent variables were scaled prior to analysis to make estimates of effect of each variable comparable.

Dataset	Model r^2	Variable	Estimate	SE	*t*	*p*
Combined	0.81	Intercept	0.135	0.151	0.89	0.386
		Salinity	0.500	0.096	5.21	<0.001
		Temperature	-0.619	0.088	-7.03	<0.001
		%DO	0.372	0.074	5.07	<0.001
		ln(Chl:TSS)	0.171	0.083	2.06	0.045
		T * %DO	0.313	0.056	5.63	<0.001
		S * T	0.421	0.1058	3.98	<0.001
Summer	0.93	Intercept	0.000	0.147	0.00	1.000
		Salinity	0.211	0.064	3.33	0.003
		Temperature	-0.488	0.104	-4.71	<0.001
		%DO	0.522	0.051	10.24	<0.001
Winter	0.43	Intercept	0.000	0.133	0.00	1
		Temperature	-0.662	0.135	-4.92	<0.001

Chapter 3. EFFECTS OF SEAGRASS ON THE SURVIVAL AND GROWTH OF NATIVE AND NON-NATIVE OYSTERS ALONG AN ESTUARINE STRESS GRADIENT

3.1 ABSTRACT

Foundation species play important roles in marine ecosystems by provisioning habitat, mediating energy flow, altering species interactions and other pathways, but the extent to which these effects depend on the local ecological context is still poorly known. We investigated the effects of an autotrophic foundation species (eelgrass, *Zostera marina*) on intertidal native (*Ostrea lurida*) and non-native oysters (*Crassostrea gigas*) across a range of estuarine conditions in Willapa Bay and Padilla Bay, Washington. We analyzed morphological (growth and shell strength), demographic (survival), and physiological responses (stable isotope and fatty acid signatures) to determine the relative influence of mechanisms by which foundation species affect associated species. Hypothesized mechanisms include top-down and bottom up food web interactions and amelioration of pH associated with eelgrass. Eelgrass interactions significantly influenced top-down effects, reducing survival at many sites. Fatty acid concentration and stable isotope signatures were significant predictors of growth of both species providing evidence of strong bottom-up effects, including potential fatty acid limitation in native oysters, related to environmental context and modified by eelgrass. Effects of eelgrass on pH stress were inconclusive: Eelgrass had positive effects on growth of both oyster species at down-estuary sites, whereas shell strength in eelgrass increased for native oysters but decreased for non-native oysters. Trade-offs between growth rate and survival were observed at the habitat-scale but to a lesser extent than along the environmental gradient. Understanding the feedbacks among

foundation species, associated species, and local environmental conditions will improve ecosystem-based management strategies.

3.2 INTRODUCTION

Foundation species provide habitat and alter interactions between sympatric organisms (MacArthur 1964, McDevitt-Irwin et al. 2016) and thus affect community structure and ecosystem resilience (Dayton 1972, Ellison et al. 2005, Altieri et al. 2007). The effects of foundation species are often the result of altered abiotic conditions (Jones et al. 1997, He and Bertness 2014), therefore the magnitude and direction of foundation species effects are likely to depend on ambient abiotic conditions and ecological context. Naturally occurring environmental gradients offer opportunities to explore when and how foundation species effects manifest. For example, competitive interactions can shift to facilitative interactions along environmental stress gradients in montane (Callaway et al. 2002), salt marsh (Altieri et al. 2007) and other environments (He et al. 2013). These studies have used environmental gradients to shape our understanding of the context-dependency of species interactions (Bruno et al. 2003).

Macrotidal estuaries are an ideal location to study interactions among foundation species due to conspicuous habitat mosaics formed on tidal flats and dramatic environmental gradients that characterize the surrounding water bodies. Foundation species form habitat mosaics that provide natural settings for replicated species-interactions studies. The environmental gradients allow comparison of species interactions across a variety of environmental contexts within the same system (He et al. 2013). Seagrasses, such as *Zostera marina*, herein eelgrass, are important autotrophic foundation species in temperate estuaries (Orth et al. 2006) that influence many sympatric species including bivalves (McDevitt-Irwin et al. 2016). Seagrass can influence

bivalve performance through multiple pathways, including modification of habitat availability, species interactions, food quality and environmental stressors (Orth et al. 1984, Ruesink et al. 2006). Experiments that rely on natural environmental gradients provide realistic tests of ecological hypotheses (Chamberlain et al. 2014), but are vulnerable to confounding variables. Parsing these mechanisms in natural systems therefore requires measuring multiple response variables, as well as environmental indicators of each pathway. The expected responses of three aspects of bivalve performance (survival, growth and shell strength) in response to independent ecological factors are shown in Fig. 3-1, along with the expected effect of seagrass on performance. In the current study we employed multiple techniques to apply evaluate these factors in a natural setting for two species of economically and ecologically important bivalves.

Bivalves within seagrass beds typically have improved survival rates but slower growth (Orth et al. 1984). Top-down processes can explain improved survival rates and slower growth for bivalves in seagrass beds (Fig. 3-1a,d.). Reduced predator abundance in seagrass is expected to result in higher bivalve survival than in unvegetated habitat where predators have easier access to prey (Carroll and Peterson 2013). Predator abundance affects bivalve performance through direct consumption or indirect changes to behavior or energetic allocation (Nakaoka 2000). Survival and growth of bivalves are likely inversely correlated to predator exposure (Richards et al. 1999, Bible et al. 2017), while shell strength is expected to be positively correlated to predator abundance owing to morphological plasticity and increased energetic allocation to shell building in response to predators (Fig. 3-1g). Patterns of predation intensity along the estuarine gradient are predator specific, but osmotic stress generally reduces crab and snail abundance from ocean to river such that predation should decrease as salinity decreases with distance up-estuary (Attrill 2002).

Bottom-up processes that modify the quantity and quality of available food sources, collectively referred to as food availability, influence survival and growth (Reusch and Williams 1999). Bivalve growth is predicted to increase with food availability (Fig. 3-1e), whereas survival is likely to be constant above a threshold of food limitation (Fig. 3-1b) and shell strength weakly related to food availability owing to allometric increases with growth (Wilkie and Bishop 2012; Fig. 3-1h). Flow is often reduced within a seagrass bed (Reusch 1998, Bologna and Heck 1999, Allen and Williams 2003), which is expected to lower food availability and therefore bivalve growth (Fig. 3-1e). Changes in the sources and production of suspended particulates along the estuarine gradient result in a general pattern of predominantly low-quality detrital sources up-estuary and progressively higher food quality as particulates become dominated by phytoplankton towards the ocean (Odum 1984, Newton and Horner 2003). Stable isotope ($\delta^{13}C$) and fatty acid (FA) biomarkers vary in relation to seston composition; $\delta^{13}C$ becomes enriched, FA composition changes and FA concentration (mg g^{-1}) increases as seston shifts from terrestrial or riverine detritus to phytoplankton (Budge et al. 2014, Lowe et al. 2014). Food availability also depends on the concentration of total suspended solids as high concentrations can reduce feeding efficiency (Barillé et al. 1997).

In addition to altering food web interactions, seagrasses modify the abiotic environment experienced by associated organisms. Photosynthetic foundation species may improve carbonate chemistry for calcifiers (e.g. Wahl et al. 2017), a pathway likely to gain importance with ocean acidification but already amenable to test in the variable pH conditions of estuaries (Murray et al. 2015, Ruesink et al. 2015). Ruesink et al. (2015) described a persistent pH gradient decreasing from the mouth towards the head of the estuary in Willapa Bay, Washington,. This pattern of pH change along estuarine gradients is driven by interactions of physical changes like salinity with

respiration of organic carbon (Borges and Abril 2011) or conversely, localized primary productivity driving pH increases (Baumann and Smith 2017). Extrapolating from these studies, habitat-specific amelioration of poor carbonate chemistry conditions will putatively lead to increased growth and shell strength in seagrass (Fig. 3-1f,i), while survival would likely only be reduced at the up-estuary reach where pH is lowest (Fig. 3-1c). Shell growth and shell strength are expected to decrease from ocean to up-estuary sites as carbonate chemistry stress reduces energy available for shell growth (e.g. Waldbusser et al. 2015; Fig. 3-1f,i).

Native and non-native species often exhibit disparate responses to local environmental conditions (Sakai et al. 2001, Lenz et al. 2011). Two species of oyster currently reproduce in Pacific Northwest estuaries and present an opportunity to study the effect of foundation species on bivalves with different evolutionary histories. *Ostrea lurida*, herein 'native' oysters, are slow-growing specialists with a shared evolutionary history with *Z. marina* in Pacific Northwest estuaries. Native oysters were harvested to near extinction in the early 1900s and are currently the focus of restoration efforts throughout the Pacific Northwest region (Trimble et al. 2009, Wasson et al. 2014). Their lack of recovery has been attributed to sensitivity to many factors including competition with non-native species, habitat loss, and changing abiotic conditions (Trimble et al. 2009, Pritchard et al. 2015). *Crassostrea gigas*, herein 'non-native' oysters, are fast-growing generalists that have naturalized in many of the countries in which they are cultivated (Ruesink et al. 2005). *C. gigas* were introduced to Washington estuaries from Japan subsequent to the collapse of native oyster populations and today support a valuable commercial fishery in the state (Washington Sea Grant 2015). Previous studies have demonstrated differences in physiological processes like filtration rates (Gray and Langdon 2017), sensitivity to seawater pH (Waldbusser et al. 2016), as well as vulnerability to predation (Buhle and

Ruesink 2009). Understanding the responses of these bivalves to foundation species interactions and environmental variability will inform a valuable framework for improving restoration and management efforts.

In the current study, an *in situ* common-garden transplant experiment was used to test the influence of foundation species on the primary ecological factors driving bivalve performance across an environmental gradient. Native and non-native oysters were deployed to eelgrass and unvegetated habitat in distinct environmental contexts to compare their responses to oceanographic variability (site), foundation species interactions (eelgrass vs. unvegetated habitat), and the influence of environmental context on interspecific interactions (habitat by site interaction). Following deployment, we measured survival, growth, biochemical indicators of food availability, and shell strength. We tested the hypothesis that foundation species mediate bivalve responses to predation pressure (survival), food availability (growth, biochemical indicators) and carbonate chemistry (shell strength). Context-dependent species interactions would manifest as significant habitat by site interactions if present, whereas consistent effects of associating with eelgrass would result in significant habitat effects and no interaction with site.

3.3 METHODS

3.3.1 *Experiment deployment*

The experiment took advantage of the natural mosaic of eelgrass habitat in the lower intertidal zone of Washington estuaries. Cohorts of native and non-native oyster species were sourced from Taylor Shellfish Farms, and outplanted to eelgrass (minimum 6 m diameter) and unvegetated habitat patches. Initially, native oysters were 18.4 ± 2.5 mm and triploid non-native oysters were 20.9 ± 2.9 mm. Sites were selected to span the large range of environmental

conditions inhabited by eelgrass and oysters in Washington State. Three sites were selected in Willapa Bay and Padilla Bay. These sites vary in temperature, salinity, and pH (Table 3-1) and also in expected food quality as it relates to position along the estuarine/salinity gradient and oceanographic influence (e.g. Newton and Horner 2003). Sites in Willapa Bay, listed from river to ocean, were: WB1, WB2, WB3. Sites in Padilla Bay were: PB1, PB2, PB3. Three replicate pairs of eelgrass and unvegetated habitat patches were established within each site.

Oysters were deployed in each replicate patch as a set of 10, glued as individuals to a ceramic tile (15 x 15 cm) and oriented with the oysters facing down just above the sediment surface. Tiles were deployed during the week of 12-Jul-2015. One tile of each native, non-native or mixed assemblage was deployed to each replicate habitat (3 tiles x 2 habitats x 3 patches, resulting in 18 tiles per site) at approximately -0.5 feet below mean lower low water. Oysters were retrieved 26-Oct-2016, after 3.5 months, at which point the surviving oysters were counted (for survival analyses, out of 15 per species per habitat patch), measured (shell height from umbo to edge along the longest axis of growth) and the tiles were individually bagged and frozen until dissections.

3.3.2 *Sample preparation*

During dissection, somatic tissue was separated from the shell then freeze-dried and weighed for somatic tissue mass. One oyster was selected from each recovered tile for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid biomarker analyses. Samples were sent to the Washington State University Isotope Core Lab for carbon and nitrogen isotope analysis following standard protocols. Fatty acid methyl esters were extracted using a modified-Folch method on 10 mg of dry tissue (Taipale et al. 2011), and analyzed using Gas chromatography with flame ionization detection. Fatty acids were identified by comparison to a sample library of known peaks

generated by GC-Mass spectrometry analysis of a Nu-Chek Prep 68D fatty acid standard and a subsample of oyster tissue samples. FA concentrations (mg FA g tissue^{-1}) were calculated by multiplying the FA peak area by the slope of the best-fit regression of peak area to the concentration of the FA standard at five dilutions and divided by the mass of sample extracted.

Shell strength (N mm^{-1}) was calculated as the maximum load (N) sustained by the shell prior to cracking divided by the shell thickness (mm). Shell thickness at this point within the margin of new growth was measured using calipers prior to crushing from above with a custom 0.3 mm ball attached to a vertically mounted digital force gauge (Omega model DFG51-50) lowered at approximately 1/3 mm second^{-1}. Maximum load, the highest point on the load-time curve, was extracted with LabVIEW software (National Instruments, Inc.).

3.3.3 *Statistical analysis*

Oyster survival, final shell height, and tissue mass were response variables examined across the main factors of site, habitat, species identity and all two- and three-way interactions. Within a site, the paired habitat replicates were set as a random effect, requiring a mixed-effect model structure. Survival (out of 15 initial outplants) was evaluated assuming binomial error distribution (logit-link) in the lme4 package in R (Bates et al. 2015, R Development Core Team, 2016). Shell height and tissue mass (log-transformed) were evaluated assuming Gaussian error distribution in the nlme package in R (Pinheiro et al 2016). Significance of fixed effects was determined by likelihood ratio tests. In accordance with the principle of marginality, models with significant interaction terms were subset to test fixed effects of interest. Two Padilla Bay sites (P2, P3) were excluded from statistical analyses due to very low oyster survival.

Proportional composition of 22 fatty acids was analyzed using permutational multivariate ANOVA. The three-factor PERMANOVA model of proportional fatty acid composition was

conducted with the 'adonis' function in the 'vegan' package in R (Oksanen et al 2016). Species, site and habitat were main effects in this analysis. Only fatty acids representing >0.05% of the total were included in the analysis and all fatty acid proportions were arc-sine square-root transformed prior to analysis.

Tissue chemistry (FA concentration) was used as a response variable to test hypotheses relating to food availability, and shell strength was used a response variable to test hypotheses relating to carbonate chemistry. Comparisons between tissue mass and FA (mg g^{-1}), and shell height and shell strength were done with untransformed data using ANCOVA with species as a categorical covariate.

3.4 RESULTS

3.4.1 *Survival*

Oyster survival was generally lower in eelgrass than unvegetated habitat, and down-estuary compared to up-estuary (Fig. 3-2). There was a significant habitat by site interaction for native oysters, but not non-native oysters. Both species experienced lower survival in eelgrass at WB2, WB3 and PB1, but not at WB1 (Table 3-2). On average, native and non-native oyster survival was 13.3% and 18.4% lower in eelgrass than in unvegetated habitat, respectively. Across the estuarine gradient, native oyster survival was much higher (>90%) at WB1 than at WB2 and WB3 (<35%). Non-native oysters exhibited a similar pattern with >90% survival at WB1, <65% survival at WB2 and <55% at WB3. Small holes in the shells of oysters at PB2 and PB3 indicated high predation by invasive oyster drills that resulted in near zero survival for both species (Fig. 3-2).

3.4.2 *Growth*

Association with eelgrass did not affect native or non-native oyster growth in Willapa Bay, but at PB1, native oysters grew 20% faster in eelgrass (Fig. 3-3A), and non-native oysters grew 15.5% faster in eelgrass (Fig. 3-4A). Across the estuarine gradient, native oysters grew slowly at WB1 (~0.3 mm month^{-1}), quickly at WB2 (~3.1 mm month^{-1}) and at intermediate rates at WB3; native oysters at PB1 grew at ~2.3 mm month^{-1} (Fig. 3-3A). Non-native oysters grew slowest at WB1 (~7.6 mm month^{-1}), at intermediate rates at WB2, and most quickly at WB3 (~11.2 mm month^{-1}); Non-native oysters at PB1 grew at 9.7 mm month^{-1}.

Patterns of somatic tissue growth were similar to shell height (Fig. 3-3, 3-4). For both species, tissue mass was significantly greater in eelgrass at PB1, but not at sites in Willapa Bay (Fig. 3-3B, 4B; Table 3-3). Across the estuarine gradient, Native oyster tissue mass increased from river to ocean, with no growth at WB1, intermediate growth at WB2 and max growth at WB3; PB1 showed intermediate growth. Non-native oysters followed a similar relative pattern from river to mouth, but maintained positive growth rates at WB1.

3.4.3 *Shell strength*

Association with eelgrass significantly increased shell strength for native oysters ($F_{1,180} = 8.18$, $p = 0.005$), and significantly decreased shell strength for non-native oysters ($F_{1,214} = 6.19$, $p = 0.014$). There was no habitat by site interaction for native (Site*Habitat: $F_{3,180} = 1.26$, $p = 0.290$) or non-native oysters (Site*Habitat: $F_{3,214} = 0.701$, $p = 0.552$). Across the estuarine gradient, shell strength did not vary by site for native (Site: $F_{3,7} = 3.016$, $p = 0.104$; Fig. 3-3C) or non-native oysters (Site: $F_{3,8} = 2.69$, $p = 0.117$; Fig. 3-4C).

3.4.4 *Stable isotope and fatty acid biomarkers*

Association with eelgrass did not affect $\delta^{15}N$ of oyster tissue (Table 3-3), and there was no interaction between habitat and site (Fig. 3-3D, 3-4D; habitat*site: $F_{3,22} = 0.29$, $p = 0.83$ and $F_{3,21} = 1.57$, $p = 0.22$ for native and non-native oysters, respectively). $\delta^{15}N$ of native oyster tissue varied significantly among sites (Table 3-4), ranging from 8.18‰ at PB1 to 9.46‰ at WB3 with no trend across the Willapa Bay estuarine gradient. $\delta^{15}N$ of non-native oyster tissue increased along the estuarine gradient, from 8.51‰ at WB1 to 9.46‰ at WB3; $\delta^{15}N$ of non-native oyster tissue at PB1 was 8.2‰. The two species had similar $\delta^{15}N$ values at all sites except WB1, where native oysters were more enriched than non-native oysters (9.24‰ vs. 8.51‰).

The influence of eelgrass on oyster tissue $\delta^{13}C$ was site dependent (habitat*site: $F_{3,22} = 2.85$, $p = 0.06$ and $F_{3,21} = 9.80$, $p < 0.001$ for native and non-native oysters, respectively). $\delta^{13}C$ of both species was significantly enriched in eelgrass at PB1, but not at sites in Willapa Bay (Fig. 3-4C). Across the estuarine gradient, mean $\delta^{13}C$ of native oysters increased linearly from WB1 to WB3; Native oyster $\delta^{13}C$ at PB1 was similar to WB3 (Fig. 3-3E; Table 3-4). Mean $\delta^{13}C$ of non-native oysters followed a similar pattern of enrichment from up-estuary to lower estuary sites (Fig. 3-4E).

Association with eelgrass did not affect total fatty acid (FA) concentration (mg g tissue^{-1}), and there was no interaction between habitat and site for either species ($p > 0.20$ in all cases). FA concentrations varied significantly across the estuarine gradient for both native and non-native oysters (Fig. 3-3F, 3-4F; $F_{3,8} = 58.75$, $p < 0.001$ and $F_{3,7} = 8.21$, $p = 0.011$ for native and non-native oysters, respectively) and were significantly correlated to $\delta^{13}C$ (Native: slope = 0.21, $r^2 = 0.75$, $df = 31$, $p < 0.001$; Non-native: slope = 0.44, $r^2 = 0.36$, $df = 31$, $p < 0.001$). FA concentrations in native oysters were lowest at WB1 and WB2 and increased to a maximum of

27.5 mg g tissue^{-1} at WB3. Native oysters at PB1 had FA concentrations similar to WB3. FA concentrations in non-native oysters increased from ~9.6 mg g tissue^{-1} at WB1 to ~14.5 mg g tissue^{-1} at WB3, and FA concentrations at PB1 were similar to WB3. Proportional FA composition in native oysters was significantly different in and out of eelgrass at WB1 ($F_{1,9}$ = 9.09, P = 0.015) and PB1 ($F_{1,10}$ = 3.12, p = 0.043), but not at WB3 or WB2. Association with eelgrass had no effect on FA composition in non-native oysters, and there was no interaction between habitat and site. FA composition varied significantly across the estuarine gradient for native and non-native oysters (Table 3-5).

Somatic tissue mass was positively correlated to FA concentration for both native oysters (Linear regression: slope = 0.004, r^2 = 0.55, df = 31, p <0.001; Fig. 3-5) and non-native oysters (Linear regression: slope = 0.03, r^2 = 0.13, df =31, p = 0.020), with significantly different slopes for each species (ANCOVA interaction: $t_{3,62}$ = 2.92, p = 0.005). Tissue mass was also positively correlated to δ^{13}C for both native oysters (Linear regression: slope = 0.02, r^2 = 0.47, df = 36, p <0.001) and non-native oysters (Linear regression: slope = 0.06, r^2 = 0.28, df =34, p <0.001), with significantly different slopes for each species (ANCOVA interaction: $t_{3,70}$ = 2.87, p = 0.005). Shell height was not related to shell strength for native oysters (Linear regression: slope = 2.21, r^2 = 0.008, df =193, p = 0.117, Fig. 3-5) or non-native oysters (Linear regression: slope = 0.33, r^2 = 0.004, df = 228, p = 0.879), and the slopes were not significantly different (ANCOVA interaction: $t_{3,421}$ = -0.66, p = 0.509).

3.5 DISCUSSION

The foundation species, *Zostera marina*, significantly affected survival, growth, shell strength and tissue chemistry of native and non-native oysters. Large, negative population-level effects of eelgrass were observed on the survival of both species and contrasted weak physiological-level

benefits of associating with eelgrass, particularly for native oysters that exhibited greater sensitivity to environmental changes along the gradient and associated with eelgrass habitat. The magnitude of these effects was contingent upon local environmental context, resulting in an observed trade-off between growth and survival along the gradient, and to a lesser extent in eelgrass. There are three primary mechanisms that could explain this pattern (Fig. 3-1): differences in predation and predator-induced morphological changes, alteration of food availability, or amelioration of environmental stress.

Association with eelgrass significantly reduced survival of both species of oyster at sites with intermediate survival, but had no effect at the up-estuary site in WB and the two sites in PB with high oyster drill prevalence. These differences highlight the importance of ecological context when considering species interactions; low and variable salinity at WB1 can exclude many oyster predators, while behavior of snail predators in PB was evidently not influenced by eelgrass. Sites with low survival also showed rapid growth and high FA concentrations, suggesting that environmental stress and food availability did not contribute to survival rates. Increased habitat complexity associated with eelgrass ought to provide refuge from predators and result in higher bivalve survival (Irlandi and Peterson 1991, Carroll and Peterson 2013, McDevitt-Irwin et al. 2016), yet this was not observed at our sites. The relationship between eelgrass morphology and its role as a foundation species may contribute to this disparity. The large, sparse eelgrass in WB and PB1 (20-136 shoots m^{-2}) would not impede mobile predators such as crabs to the same degree as the dense seagrass found in other locations (e.g. 14,000 shoots m^{-2}; Irlandi and Peterson 1991), and may instead provide shelter for these predators. Many red rock crabs (*Cancer productus*) were observed hiding under tiles in eelgrass during the study, especially at WB3 and WB2, where they have been observed at intermediate densities

relative to oyster and unvegetated habitat (Holsman et al. 2006). Oyster drill predation is observed in many habitats in Washington and may be less influenced by eelgrass-interactions as evidenced by the high mortality in both habitats in PB2 and PB3 (Grason and Buhle 2016, Valdez et al. 2017).

Top-down interactions were expected to contribute to a significant effect of eelgrass on oyster shell strength (Bible et al. 2017). Native and non-native oysters exhibited species-specific, and opposite, responses to eelgrass interactions with no habitat by site statistical interaction. The increased shell strength of native oysters in eelgrass did not match the predicted response to eelgrass based on predation (Fig. 3-1g: habitat). Nevertheless, it was consistent with a morphological response to increased predation in this habitat at some sites. Higher native oyster shell strength was also observed at WB1 where no predation was evident, suggesting an alternative driver. Non-native oysters had lower shell strength in eelgrass, including patches with lower survival, which is inconsistent with a morphological response to increased predation.

Bottom-up processes are major factors driving organism performance; the availability of limiting nutrients such as essential fatty acids affects growth, survival and reproductive output (Winder et al. 2017). Seston composition changes considerably along estuarine gradients (Odum 1984, Newton and Horner 2003, Cloern et al. 2017) and is locally-modified by the presence of foundation species (de Boer 2007, Hasegawa et al. 2008). Seagrasses can influence food availability for bivalves via modulated flow, increased sedimentation, or modified resuspension of sediment or benthic or epiphytic algae (Orth et al. 1984, McDevitt-Irwin et al. 2016). The depletion of carbon isotopes and altered fatty acid composition along the estuarine gradient provided evidence of variable food availability among sites. Effects of eelgrass interactions on growth were limited to positive effects at the most oceanic sites, especially at PB1 where

increased growth was correlated to significant changes in Native oyster tissue chemistry ($\delta^{13}C$ and fatty acids). While this effect of eelgrass was counter to predictions (Fig. 3-1e), increased growth of benthic suspension feeders within eelgrass is not unprecedented (Judge et al. 1993), and may be driven by increased habitat-specific organic matter retention (Duarte et al. 1999, Granata et al. 2001, González-Ortiz et al. 2014). Eelgrass is unlikely to be contributing directly to oyster diet, but may instead increase resuspension (Ruesink et al. in review) or support productive epiphytes that contribute to oyster diet (Nelson and Waaland 1997, Ruesink 2016).

Growth of both species was positively correlated to FA concentration as predicted (Fig. 3-1e), and food availability differences across site had a much greater effect on growth of both species than did habitat. Similar patterns of $\delta^{13}C$ and fatty acid variation among sites show the two species consumed similar components of the seston, but morphological differences indicate differences in assimilation or energy allocation. Native oysters exhibited no growth and poor condition up-estuary, that when combined with enriched $\delta^{15}N$ at this site, indicates starvation (Doi et al. 2017). In contrast, non-native oysters exhibited rapid growth throughout the study region with smaller differences of FA concentration and growth among sites. High suspended sediment loads interfere with oyster filtration (Barillé et al. 1997). Native oysters reduce clearance rates faster than the generalist non-native oyster in response to increased turbidity (Gray and Langdon 2017), suggesting the high turbidity at WB1 as likely a factor contributing to FA limitation in the native oyster. While we found no evidence of food availability effects on survival during the 3.5-month study, the differences in FA concentration at the end of the study provide vastly different energy reserves to oysters entering the low-food winter period. Native oysters were more sensitive to changes in food availability than the introduced species, as evidenced by the significant site-specific effects of eelgrass interactions and FA limitation of

growth at the up-estuary site. Overall, the positive correlation of growth to $\delta^{13}C$ and total FA concentration supports previous conclusions that access to marine phytoplankton or isotopically-enriched benthic microalgae drives the large-scale and habitat-scale differences in growth of oysters (Ruesink et al. 2003, Lebreton et al. 2011).

Foundation species can dramatically reduce the effects of environmental stressors, thereby facilitating improved performance in associated organisms (Altieri et al. 2007, He et al. 2013). In aquatic ecosystems, autotrophic foundation species have the potential to mediate local pH through photosynthetic uptake of carbon dioxide (Koweek et al. 2017), that may provide a refuge from low pH for calcifying species (Ruesink et al. 2015, Nielsen et al. 2018, Wahl et al. 2018). Native oysters exhibit reduced growth in experiments covering the pH range observed along the estuarine gradient in Willapa Bay (pH ~7.8 - 8.0, Table 3-1; Hettinger et al. 2012); post-settlement non-native oysters have shown negative, positive, or no difference in growth at reduced pH (Wright et al. 2014; Ko et al. 2014; Timmins-Schiffman et al. 2014), suggesting effects depend on interactions with other environmental conditions or on local adaptation (Wright et al. 2014). The growth patterns of both species, and native oyster shell strength, observed in this study were consistent with expectations in response to eelgrass-mediated and site-specific pH increases along the estuarine gradient (Fig. 3-1f, i). Non-native oyster shell strength, however, exhibited the opposite response to eelgrass and was stronger in unvegetated habitat. Furthermore, shell height did not vary significantly with shell strength as expected assuming a high energetic cost to shell construction under stressful carbonate chemistry conditions, nor did the magnitude of the eelgrass effect change predictably along the gradient with large-scale changes in pH. The slight habitat-specific enrichment of $\delta^{13}C$ may reflect a physiological response to the environment stress rather than food availability, as physiological

responses related to temperature (Pernet et al. 2007) and season (Richoux and Ndhlovu 2015) also influence biomarker composition. But this is weakly supported by data and altered biomarker signatures generally reflect changes in diet (Peterson and Fry 1987, Galloway et al. 2014). These results should motivate future studies that simultaneously observe *in-situ* habitat-specific growth, food availability and environmental conditions to partition the relative influence of carbonate chemistry on bivalves in coastal ecosystems.

Ecosystem-based management strategies require an understanding of the feedbacks among foundation species, associated species, and the local environment in order to improve efficacy of management efforts. Many studies in other regions observed a trade-off between food-mediated growth and predation intensity associated with foundation species, but have been limited in space and identification of contrasting mechanisms. We were able to identify multiple mechanisms of foundation species' influence by combining ecological techniques and using environmental variation to test effects of species interactions in a natural context. These results provide context for this trade-off, and suggest that eelgrass restoration efforts at estuarine sites with greater oceanic influence may result in a greater physiological benefit to oysters, primarily for the native species in this region, at the risk of greater predation. Moreover, restoration efforts focused on benthic suspension-feeding bivalves may benefit more from monitoring local food availability and predation pressure, as these factors were the primary drivers of oyster growth and survival, respectively, identified in this study.

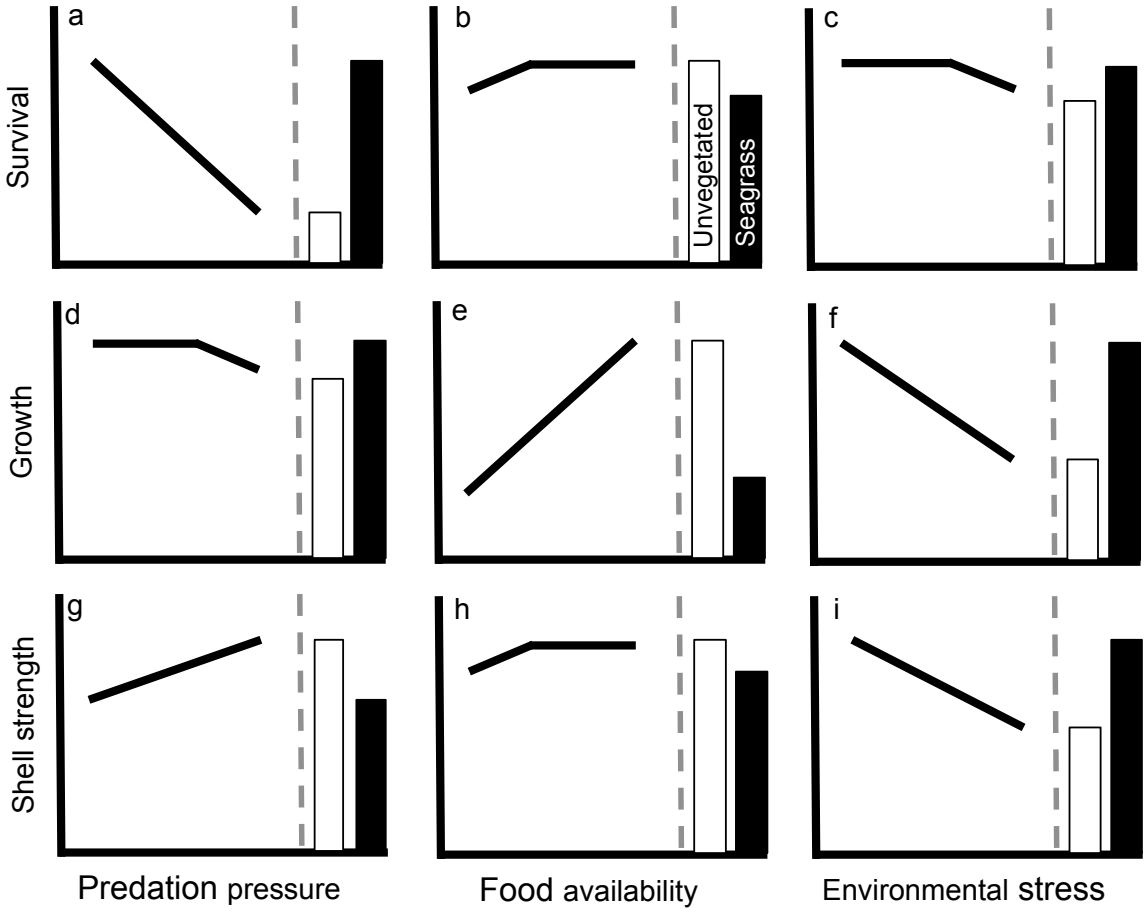

Figure 3-1. Predicted effects of associating with foundation species on survival (a-c), growth (d-f), and morphology (g-i) to predation (a,d,g), food availability (b,e,h) or environmental stress (c,f,i). Predictions assume that the response varies with intensity of the explanatory factor (solid line); nonlinearities occur when a response is expected only under extreme conditions. Predicted responses of bivalves associated with seagrass (filled bar) or in unvegetated habitat (hollow bar) are shown to the right of the vertical dashed line in each panel. These predications are based on modification of the intensity of the explanatory factor by seagrass. For example, in a) survival is predicted to decrease as predation pressure increases; eelgrass putatively decreases predation pressure on sessile benthic invertebrates resulting in higher survival of bivalves in seagrass than in unvegetated habitat

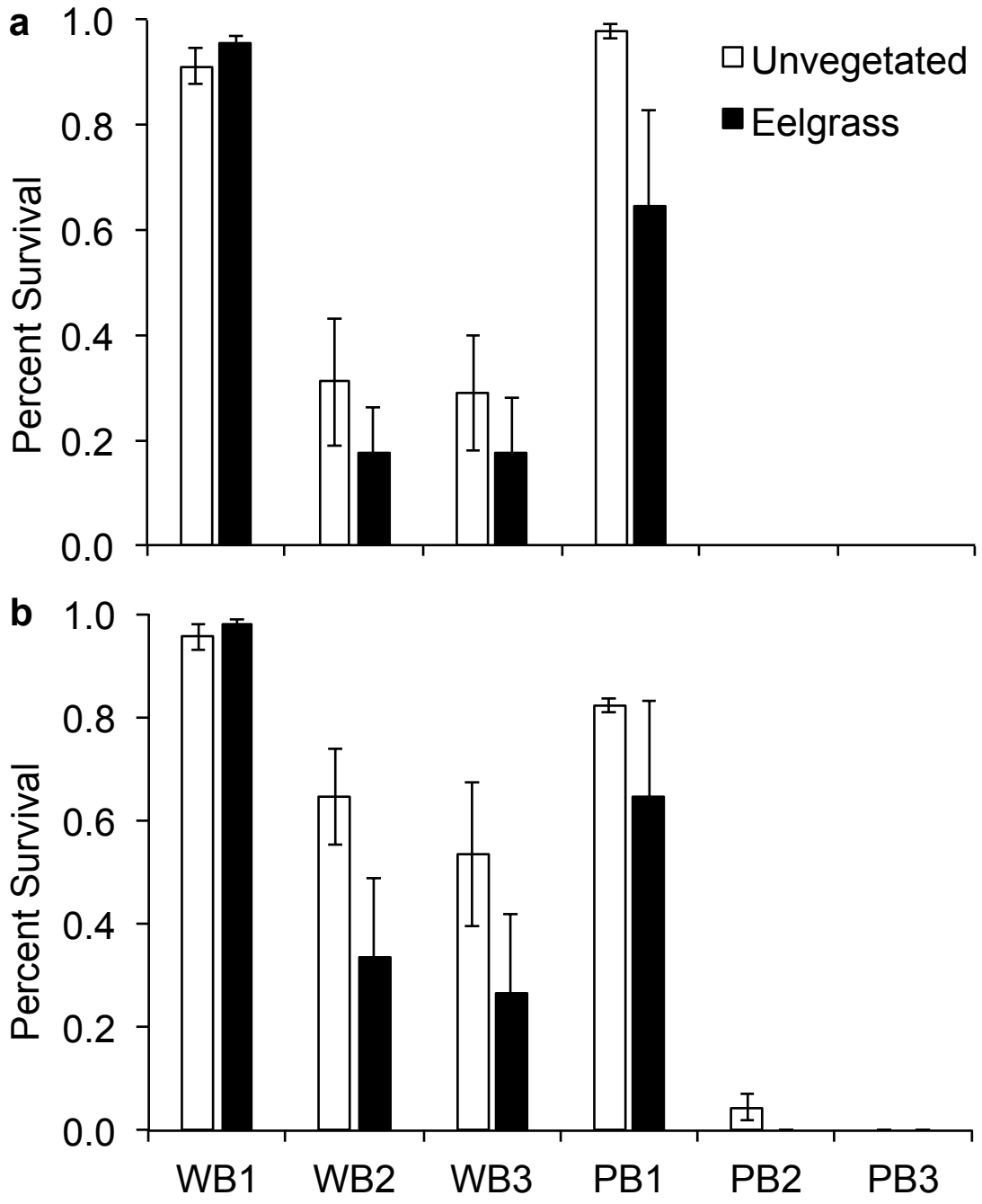

Figure 3-2. Survival of native (A) and non-native (B) oysters after 3.5-month deployment in eelgrass or unvegetated habitat across two estuarine gradients in Willapa Bay (WB) and Padilla Bay (PB). Site abbreviations are: WB, 1 – River, 2 – Midbay, 3 – Ocean; PB, 1 – South, 2 – Midbay, 3 – North. PB2 and PB3 were removed from statistical comparisons due to zero survival.

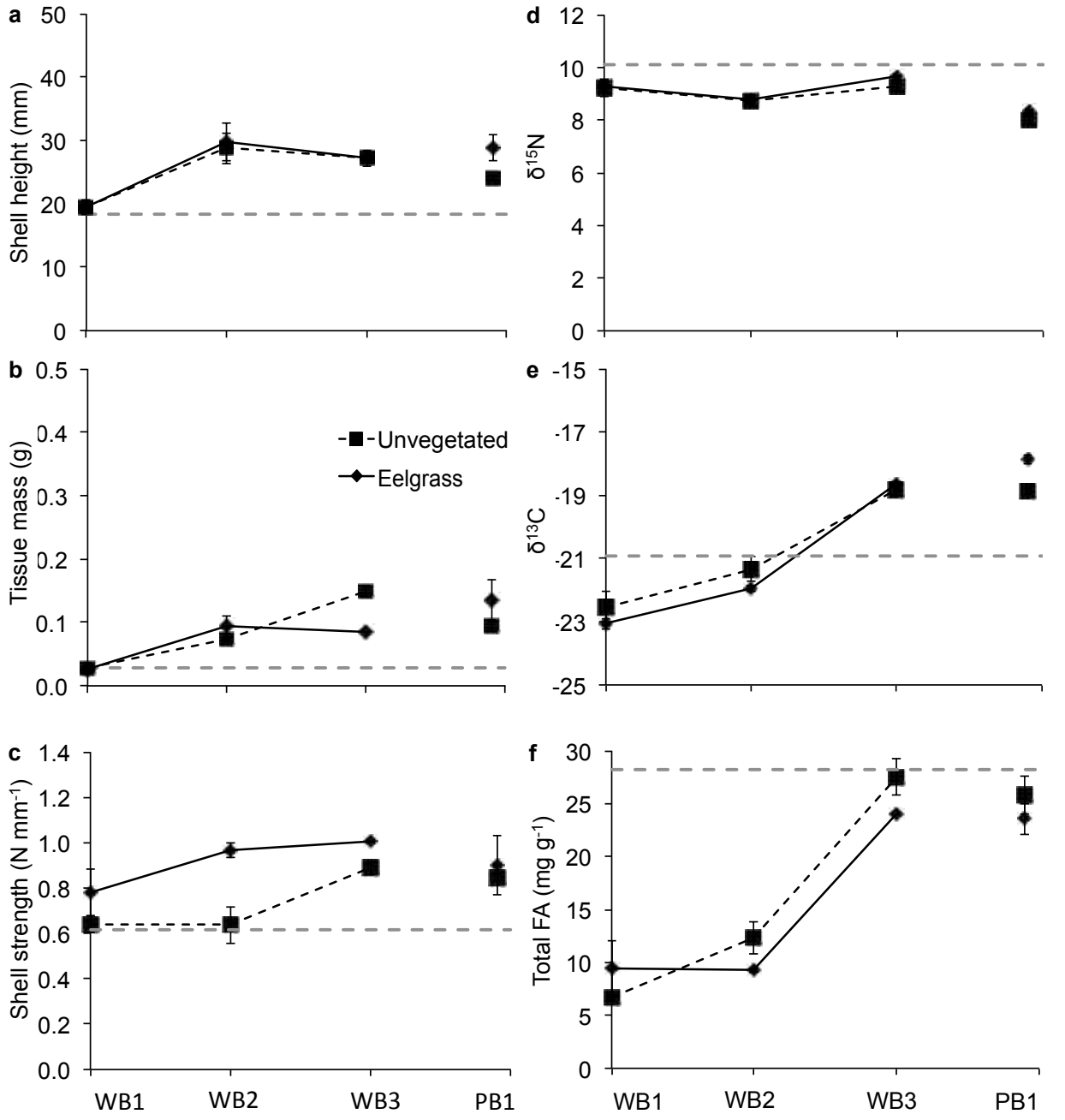

Figure 3-3. Native oyster *Ostrea lurida*. Shell height (A), tissue mass (B), shell strength (C), $\delta^{15}N$ (D), $\delta^{13}C$ (E), and total FA (mg g^{-1}) (F) in eelgrass (diamond, solid line) and unvegetated (square, dashed line) at the end of the experiment. Dashed gray line indicates mean value at the beginning of the experiment

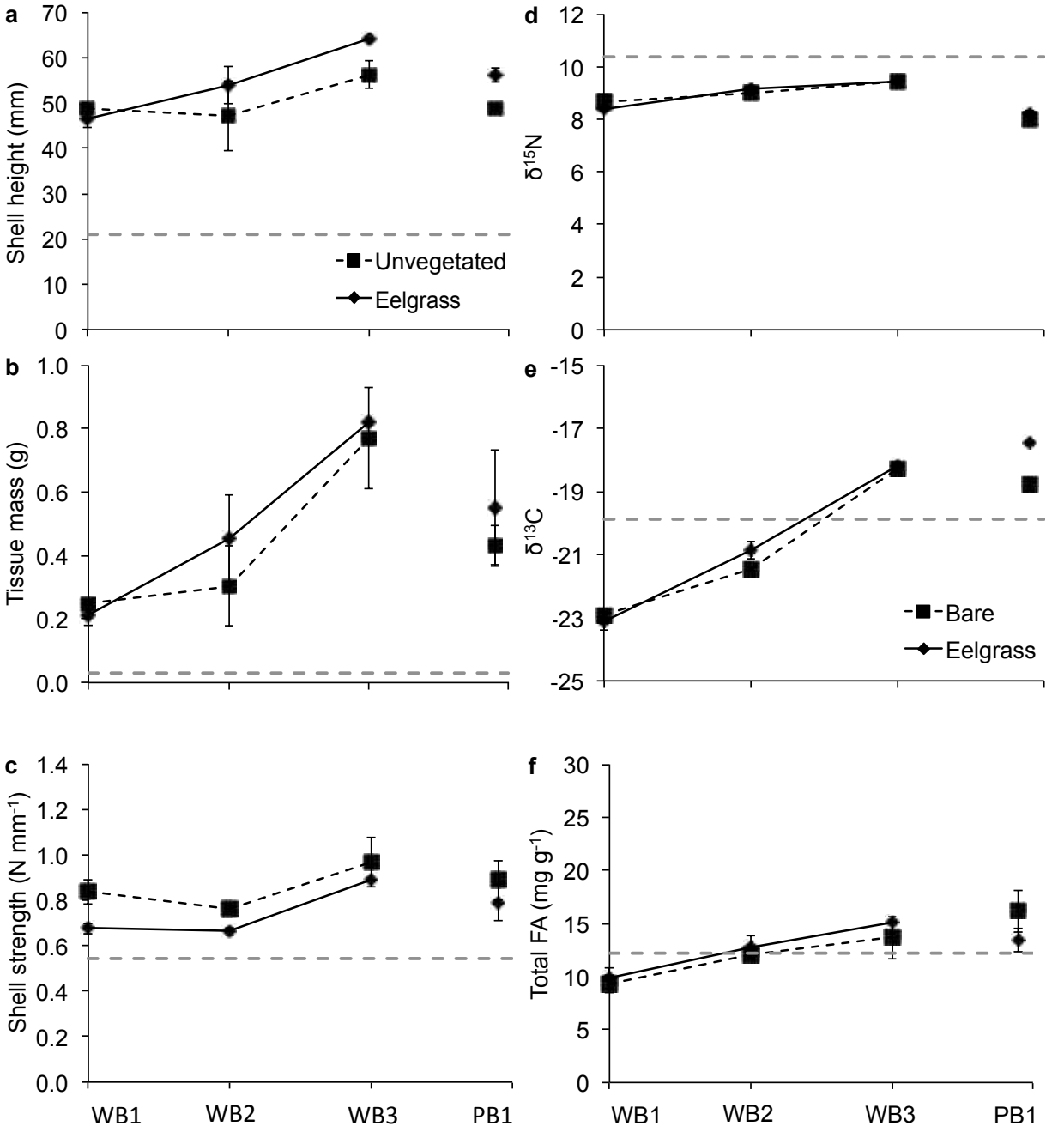

Figure 3-4. Non-native oyster *Crassostrea gigas*. Shell height (A), tissue mass (B), shell strength (C), $\delta^{15}N$ (D), $\delta^{13}C$ (E), and total FA (mg g^{-1}; F) in eelgrass (diamond, solid line) and unvegetated (square, dashed line) at the end of the experiment. Dashed gray line indicates mean value at the beginning of the experiment

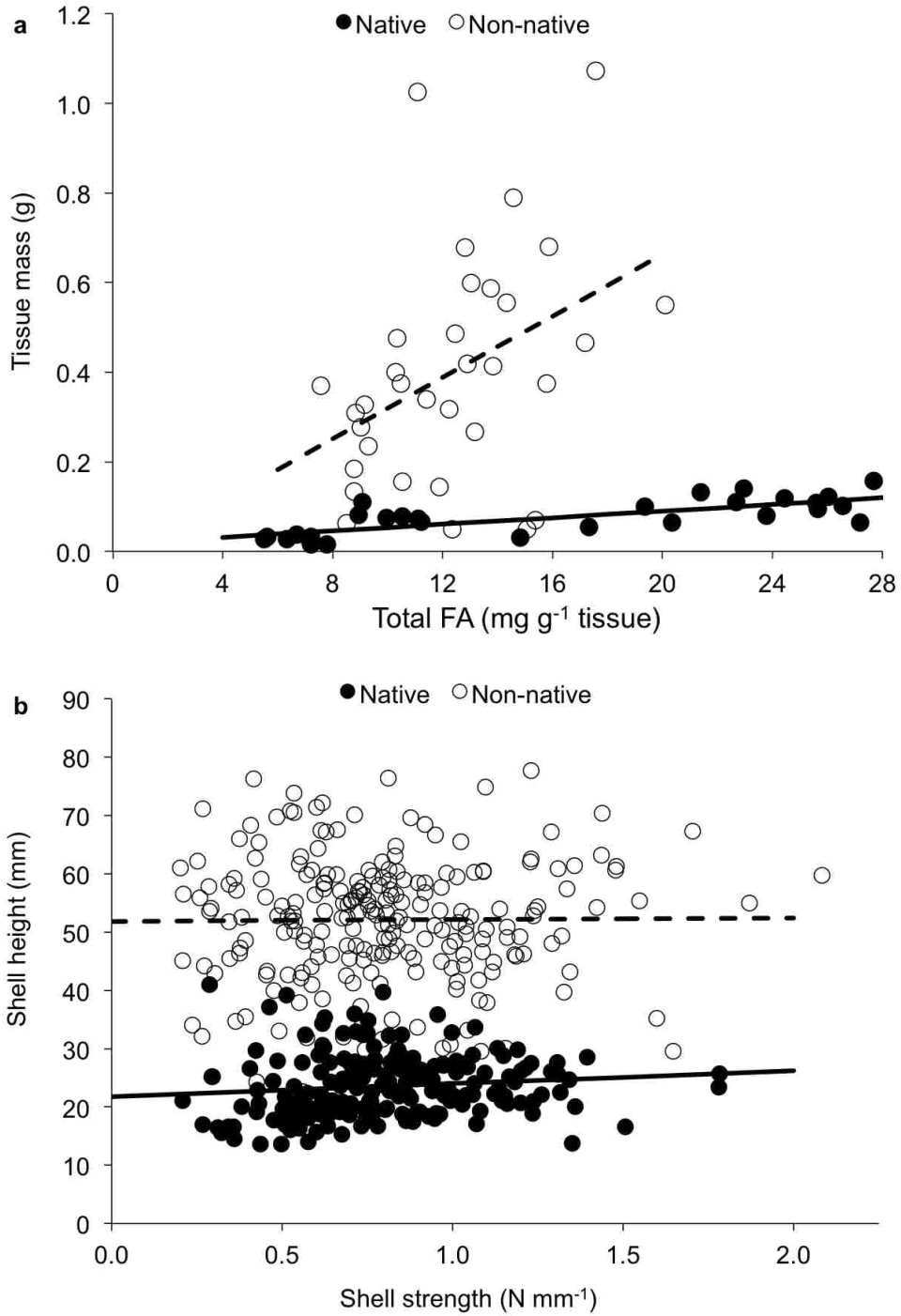

Figure 3-5. Native and non-native oysters. Test of hypothesized mechanisms relating to food quality (dry tissue mass vs. FA concentration) and carbonate chemistry variation (shell height vs. shell strength). Raw data is shown

Table 3-1. Mean monthly conditions near each location during July – October. Data sourced from Washington Department of Ecology monthly sampling events and the Padilla Bay National Estuarine Research Reserve monitoring.

Region	Site	Latitude	Longitude	pH	Salinity	Temperature (°C)
Willapa Bay	WB1	46.4539	123.9213	7.8 ± 0.35	25.9 ± 2.68	17.5 ± 1.17
Willapa Bay	WB2	46.4325	124.9965	7.9 ± 0.29	27.9 ± 1.80	17.3 ± 1.07
Willapa Bay	WB3	46.6283	124.0335	8.1 ± 0.42	29.3 ± 1.81	14.5 ± 1.51
Padilla Bay	PB1	48.4809	122.5277	8.0 ± 0.20	29.1 ± 0.95	13.4 ± 1.70

Table 3-2. Results of linear mixed-effects analysis of survival of native and non-native oysters in Willapa and Padilla Bay.

Species	Effect	X^2	*df*	*p*
Native	Site	34.04	6	<0.001
	Habitat	37.87	4	<0.001
	Site:Habitat	21.40	3	**<0.001**
Non-native	Site	14.70	6	**0.023**
	Habitat	30.94	4	**<0.001**
	Site:Habitat	5.13	3	0.162

Native oysters	Site	X^2	*df*	*p*
Habitat	WB3	2.41	1	0.120
	WB2	3.20	1	0.074
	WB1	0.74	1	0.390
	PB1	34.51	1	**<0.001**

Table 3-3. Results of linear mixed effects models testing effect of habitat within site on morphological and biomarker response variables with significant statistical interactions in the full model.

		Native			Non-native		
Response	Site	df	*F*	*p*	df	*F*	*p*
Shell height	WB1	1,80	0.00	0.962	1,83	2.07	0.154
	WB2	1,17	0.23	0.636	1,38	0.03	0.872
	WB3	1,17	0.02	0.904	1,32	1.82	0.187
	PB1	1,69	30.38	**<0.001**	1,62	9.20	**0.004**
Tissue mass	WB1	1,80	0.01	0.938	1,83	4.48	**0.037**
	WB2	1,17	1.45	0.245	1,38	0.45	0.507
	WB3	1,17	10.94	**0.004**	1,32	0.74	0.397
	PB1	1,69	12.29	**<0.001**	1,62	5.94	**0.018**
δ13C	WB1	1,9	1.22	0.299	1,8	2.07	0.188
	WB2	1,3	1.46	0.314	1,5	6.08	0.057
	WB3	1,2	0.09	0.795	1,2	14.25	0.064
	PB1	1,8	63.35	**<0.001**	1,6	21.99	**0.003**
δ15N	WB1	1,9	0.0189	0.8938	1,8	1.971	0.198
	WB2	1,3	0.026	0.8829	1,5	4.48	0.088
	WB3	1,2	0.827	0.4592	1,2	0.013	0.921
	PB1	1,8	4.742	0.0611	1,6	1.177	0.320

Table 3-4. Results of linear mixed effects models testing effect of site along the gradient by habitat on morphological and biomarker response variables by species with significant statistical interactions in the full model.

		Native			Non-native		
Response	Habitat	df	*F*	*p*	df	*F*	*p*
Shell height	Bare	3,8	27.79	**<0.001**	3,8	6.52	**0.015**
	Eelgrass	3,4	18.39	**0.008**	3,4	19.92	**0.007**
Tissue mass	Bare	3,8	24.96	**<0.001**	3,8	8.53	**0.007**
	Eelgrass	3,5	17.61	**0.004**	3,4	8.99	**0.030**
$\delta^{13}C$	Bare	3,8	110.39	**<0.001**	3,7	219.33	**<0.001**
	Eelgrass	3,5	301.91	**<0.001**	3,4	155.03	**<0.001**
$\delta^{15}N$	Bare	3,8	17.86	**<0.001**	3,7	12.94	**0.003**
	Eelgrass	3,5	8.49	**0.021**	3,4	56.10	**0.001**

Table 3-5. Species-specific results of PERMANOVA on proportional fatty acid composition of native and non-native oysters.

	Effect	df	SSE	MSE	*F*	*R2*	*P*
Native	Site	3	0.18	0.06	15.77	0.65	0.001
	Habitat	1	0.02	0.02	5.33	0.07	0.011
	Site:Habitat	3	0.03	0.01	2.63	0.11	**0.038**
	Residuals	12	0.04	0.00		0.17	
	Total	19	0.27			1.00	
Non-native	Site	3	0.09	0.03	3.10	0.42	**0.028**
	Habitat	1	0.01	0.01	0.56	0.03	0.565
	Site:Habitat	3	0.01	0.00	0.43	0.06	0.860
	Residuals	11	0.11	0.01		0.50	
	Total	18	0.22			1.00	

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SUPPLEMENTARY TABLES

Supplementary table 1-1. Locations and descriptions of sampling sites.

Site	Subregion	Habitat	Sampling	Max Depth (m)	Latitude	Longitude
GYS004	Coastal Estuary	Tideflat	Core	24	46.9779	-123.7846
GYS008	Coastal Estuary	Tideflat	Core	7	46.9373	-123.9132
GYS009	Coastal Estuary	Tideflat	Rotating	16.5	46.9645	-123.9496
GYS015	Coastal Estuary	Tideflat	Rotating	16.5	46.9229	-124.0768
GYS016	Coastal Estuary	Tideflat	Core	13	46.9534	-124.093
WPA001	Coastal Estuary	Tideflat	Core	12	46.6873	-123.7499
WPA003	Coastal Estuary	Tideflat	Core	10.5	46.704	-123.8374
WPA004	Coastal Estuary	Tideflat	Core	18.5	46.6868	-123.9735
WPA006	Coastal Estuary	Nearshore	Core	21.5	46.5454	-123.9802
WPA007	Coastal Estuary	Tideflat	Core	18	46.4532	-124.0096
WPA008	Coastal Estuary	Tideflat	Core	19.5	46.4632	-123.9413
WPA113	Coastal Estuary	Tideflat	Core	12	46.644	-123.993
HCB003	Hood Canal	Channel	Core	162	47.5379	-123.0096
HCB004	Hood Canal	Nearshore	Core	55.5	47.3562	-123.0249
HCB007	Hood Canal	Nearshore	Core	37	47.3981	-122.9296
HCB008	Hood Canal	Channel	Rotating	112	47.7533	-122.745
HCB009	Hood Canal	Channel	Rotating	97	47.6883	-122.75
HCB010	Hood Canal	Channel	Core	103	47.67	-122.82
PGA001	Hood Canal	Nearshore	Rotating	22.5	47.8398	-122.5813
ADM001	Central Salish Sea	Channel	Core	153	48.0298	-122.6179
ADM002	Central Salish Sea	Channel	Core	97	48.1873	-122.843
Bayview	Central Salish Sea	Tideflat	Core	4.5	48.4961	-122.5021
BLL009	Central Salish Sea	Nearshore	Core	31	48.6859	-122.5996
BLL011	Central Salish Sea	Nearshore	Rotating	24	48.7332	-122.5846
DIS001	Central Salish Sea	Nearshore	Rotating	42.5	48.0181	-122.8479
DRA002	Central Salish Sea	Nearshore	Rotating	23	48.9832	-122.763
DUN001	Central Salish Sea	Nearshore	Rotating	19.5	48.1731	-123.1146
EAS001	Central Salish Sea	Nearshore	Rotating	34	48.6429	-122.8835
FID001	Central Salish Sea	Tideflat	Rotating	16.5	48.5126	-122.5952
FRI001	Central Salish Sea	Nearshore	Rotating	19.5	48.5382	-123.013
FSH001	Central Salish Sea	Tideflat	Rotating	5.5	48.5098	-122.918
Gong	Central Salish Sea	Nearshore	Core	20	48.5583	-122.5725
GRG002	Central Salish Sea	Channel	Core	205.5	48.8082	-122.9541
JDF005	Central Salish Sea	Nearshore	Rotating	39.5	48.0609	-123.031
JDF007	Central Salish Sea	Nearshore	Rotating	29	48.0484	-123.0096
LOP001	Central Salish Sea	Nearshore	Rotating	16	48.5132	-122.8513
PAH003	Central Salish Sea	Nearshore	Rotating	22.5	48.1348	-123.4607
PAH008	Central Salish Sea	Nearshore	Rotating	19	48.1215	-123.3513

Ploeg	Central Salish Sea	Tideflat	Core	4.5	48.5563	-122.5308
PTH005	Central Salish Sea	Nearshore	Core	36.5	48.0831	-122.7646
RSR837	Central Salish Sea	Channel	Rotating	60	48.6165	-122.763
SEQ002	Central Salish Sea	Nearshore	Rotating	31.5	48.0765	-123.018
ADM003	Puget Sound	Channel	Core	214	47.879	-122.4832
CMB003	Puget Sound	Channel	Core	158.5	47.2904	-122.4501
CMB006	Puget Sound	Nearshore	Rotating	53.5	47.2615	-122.4373
DYE004	Puget Sound	Nearshore	Rotating	28.5	47.6223	-122.6896
EAG001	Puget Sound	Nearshore	Rotating	20.5	47.6217	-122.5017
EAP001	Puget Sound	Channel	Core	212.5	47.417	-122.3804
ELB015	Puget Sound	Channel	Core	131.5	47.5965	-122.3696
HLM001	Puget Sound	Nearshore	Rotating	53.5	48.0637	-122.5332
PMA001	Puget Sound	Nearshore	Rotating	51.5	47.7348	-122.5346
PNN001	Puget Sound	Nearshore	Rotating	31	48.2309	-122.6757
POD006	Puget Sound	Tideflat	Rotating	16	47.7148	-122.6346
POD007	Puget Sound	Tideflat	Rotating	6	47.7332	-122.6513
PSB003	Puget Sound	Channel	Core	110	47.6598	-122.4429
PSS008	Puget Sound	Nearshore	Rotating	37.5	47.9815	-122.2235
PSS010	Puget Sound	Channel	Rotating	109.5	47.965	-122.2633
PSS019	Puget Sound	Channel	Core	107	48.0109	-122.3013
QMH001	Puget Sound	Nearshore	Rotating	21.5	47.3798	-122.4662
QMH002	Puget Sound	Tideflat	Rotating	13	47.3965	-122.4429
SAR003	Puget Sound	Channel	Core	150	48.1076	-122.4915
SIN001	Puget Sound	Nearshore	Core	18	47.5493	-122.6435
SKG001	Puget Sound	Nearshore	Rotating	29.5	48.3957	-122.4896
SKG003	Puget Sound	Nearshore	Core	25	48.2965	-122.4896
SUZ001	Puget Sound	Channel	Rotating	107	48.1351	-122.3707
BML001	S Puget Sound	Tideflat	Rotating	14	47.3776	-122.6337
BUD002	S Puget Sound	Tideflat	Rotating	14	47.0515	-122.9063
BUD005	S Puget Sound	Nearshore	Core	20	47.092	-122.9182
CRR001	S Puget Sound	Channel	Core	110	47.2765	-122.7096
CSE001	S Puget Sound	Channel	Core	66.5	47.2645	-122.8443
CSE002	S Puget Sound	Nearshore	Rotating	24.5	47.3532	-122.8146
DNA001	S Puget Sound	Channel	Core	51.5	47.1615	-122.8718
ELD001	S Puget Sound	Nearshore	Rotating	23	47.1062	-122.9499
ELD002	S Puget Sound	Tideflat	Rotating	15	47.0962	-122.9754
GOR001	S Puget Sound	Channel	Core	171	47.1832	-122.6346
HND001	S Puget Sound	Nearshore	Rotating	25.5	47.1512	-122.8343
NSQ001	S Puget Sound	Nearshore	Rotating	33	47.1123	-122.6985
NSQ002	S Puget Sound	Channel	Core	111	47.1673	-122.7882
OAK004	S Puget Sound	Nearshore	Core	28	47.2134	-123.0777
PCK001	S Puget Sound	Nearshore	Rotating	22	47.2484	-122.9249
STL001	S Puget Sound	Channel	Rotating	122.5	47.1848	-122.6112

TOT001	S Puget Sound	Nearshore	Rotating	31.5	47.1643	-122.9646
TOT002	S Puget Sound	Tideflat	Rotating	17	47.1215	-123.0213

Supplementary table 1-2. Results of linear mixed effects model comparing pH variation to metabolic (%DO), biological (chlorophyll), physical (temperature and salinity) and climatic (atmospheric CO_2) drivers. (see methods for details, briefly: LME model with 3123 observations scaled to $(x-\mu)/SD$. Fixed effects shown, random effects = Subregion + Seas.

Variable	Estimate	SE	X^2	p
DO % Saturation	0.598	0.016	1141.00	<0.001
Atmosphere CO_2	-0.163	0.012	165.76	<0.001
Temperature	0.127	0.020	40.58	<0.001
Salinity	0.047	0.014	11.04	<0.001
Chlorophyll	0.042	0.014	8.81	0.003

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