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Research Article

Intertidal Grow-Out Technique, Not Eelgrass (*Zostera marina*), Influences Performance of Pacific Oysters (*Magallana gigas*)

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Colocation of farmed shellfish in seagrass, although not permitted in some regions of the USA, has been proposed as a means to reach cobenefits of habitat and food production. Seagrass could benefit bivalves through protection from predation or abiotic stressors but conversely can reduce water flow and food delivery. At 10 farm sites in Washington state (USA), we tested the performance of Pacific oysters (*Magallana* (*Crassostrea*) *gigas*) grown in ground culture or off-bottom culture across a range of seagrass densities. Oysters averaged 32 m^{-2} and 30% shell cover on ground culture beds. Oysters in off-bottom culture showed a negative relationship between size and density across beds, consistent with growth and mortality through the crop cycle, and the largest oysters were at densities similar to ground culture. Within each farm site, ground and off-bottom beds were selected with each of three categories of seagrass (none, sparse, dense). Dense seagrass on aquaculture beds was half the density of nearby unfarmed seagrass beds. For outplanted oysters over 3–9 months, many aspects of oyster performance improved when oysters were elevated above the sediment. Relative to ground culture, off-bottom oysters had better survival (85% vs. 69%), 7% larger size, and 48% higher condition after 3 months in summer, and the survival advantages persisted over winter. Oyster survival on-bottom was especially impaired in finer sediment. No oyster performance differences were associated with seagrass, except for 9-month results, available for five of 10 farms. After 9 months at these five farms, oyster survival showed a small negative effect of dense seagrass, and shell size showed a small positive effect of sparse seagrass. Consequently, seagrass may not provide a boost to colocated intertidal shellfish, but we found little evidence of trade-offs in which maintaining seagrass would reduce yield of farmed oysters. Moving oysters out of the boundary layer and away from soft sediment improves both survival and tissue growth aspects of yield.

1. Introduction

Intertidal benthic estuarine organisms are exposed to stressors related to the sediment, water column, and air at low tide. Conditions experienced by organisms through the benthic phase of their life cycle often strongly reduce population density relative to what would be expected from settlement alone [1]. Cultivated bivalves can differ dramatically across sites in their growth and survival [2], even when controlling for genetics [3]. Grow-out method is a component of the environment under direct control in the shellfish industry, which markedly affects traits and yield in oysters [2, 4]. Differences in oyster survival and growth across culture practices could be further influenced by sediment conditions or co-occurring seagrass.

Although oysters are a benthic taxon, growing in soft sediment may be challenging for a variety of reasons, and ground-cultured oysters are typically not found on sediments with >4% organic content [5]. Many oyster species naturally grow in reefs and show improved performance on subtidal reefs with greater vertical relief, elevated above low oxygen and protected from sedimentation by water flow [6]. Intertidally, oysters experimentally outplanted on tiles can grow faster when elevated above the sediment relative to those close to the bottom, even if the inundation time is reduced [7]. In aquaculture, species such as *Pinctada imbricata* and *Crassostrea tulipa* have increased yield when grown in suspended culture relative to on-bottom [8, 9], although suspended culture does not always increase yield [2, 10].

Survival could improve away from the benthos because near-sediment conditions can be stressful in terms of low oxygen or burial [11, 12]. In addition to elevating oysters out of stressful abiotic conditions, suspended culture can protect shellfish from benthic predators, which can severely reduce aquaculture yields [13]. Still, predators can recruit to suspended culture [14], and fouling organisms can recruit to shells or gear, impairing growth and survival [15]. Growth could improve with distance from the bottom, as shellfish are in higher flow, receive higher rates of food delivery, and may also experience better food quality with less inorganic component [16].

Species interactions with other engineering species in soft sediment may be facilitative or antagonistic for oysters, even when restricted to a single interacting taxon (seagrass). While prior attention has focused on how to protect seagrass within aquaculture beds [17–19], a new question has recently emerged about whether seagrass could ameliorate low pH conditions for oysters. Empirical data and modeling have addressed how photosynthetic biomass can draw down dissolved inorganic carbon that forms carbonic acid in reaction with water [20–22]. Laboratory experiments have shown improved oyster shell growth in some treatments with seagrass [23, 24]. Field outplants of oysters into seagrass have had more variable results, including reduced survival in seagrass where predator effects were enhanced [25]. In general, multiple pathways exist by which seagrass can influence bivalve performance [26–28]. Seagrass may enhance recruitment and survival of epibenthic bivalves but decrease their growth [29], and distance off-bottom has been shown to alter survival and growth nonlinearly within seagrass [30].

Native eelgrass and other estuarine species have overlapped with oyster aquaculture for more than a century on the USA west coast, including in Willapa Bay, Washington, the single largest production site for cultured oysters in the USA (Pacific oysters, *Magallana gigas*, formerly *Crassostrea gigas* (Thunberg, 1793)) [31]. This region has overcome hatchery failures related to low pH water, which negatively affects larvae of Pacific oysters [32–34]. We used field outplants of Pacific oysters to test two environmental factors that could influence aquaculture yield. Our focal question was how survival, growth, and condition of oysters differed between off-bottom and ground culture intermixed with different seagrass densities. We expected all aspects of performance to improve off-bottom, unless immersion time canceled out this effect. Off-bottom culture could provide a yield advantage particularly in finer sediments. We did not have a strong prediction about how oyster performance would respond to seagrass given the multiple possible pathways of effect [27], but considered the possibility that seagrass would be more facilitative for off-bottom oysters than those growing on ground, in keeping with expectations that seagrass shifts communities from infaunal to epifaunal [35]. The time frame of this study (3–9 months) was relevant to the first year of culture in a multiyear grow-out cycle. Size and density of oysters in each culture method were compared as an index of production (stocking density), independent of the individual-level performance metrics of the outplants.

2. Materials and Methods

2.1. Study Sites. This study of Pacific oyster production and individual outplant performance was carried out at 10 intertidal sites where oysters are farmed in Washington state, USA (Figure 1, Table 1). Half of the 10 sites were sampled in 2020–2021 and the remainder in 2021–2022. Six of the sites were in the coastal estuary of Willapa Bay and four in inland Washington waters (Salish Sea), in keeping with the relative importance of these regions for Pacific oyster production. The coastal estuaries produced nearly 6 million pounds of oysters annually, while the Salish Sea production was around 3 million pounds [36]. Each site covered a spatial extent of <0.5 km, and the two closest sites were 1 km apart, while the longest distance between sites was ~460 km by water (Figure 1).

Each study site included two grow-out methods: (1) ground culture as single oysters or clusters and (2) off-bottom clusters on longlines or single oysters in flip bags suspended 0.3–0.6 m above the sediment surface (Figure 1). The tidal elevations of these two grow-out methods were similar at the sediment surface ($F_{1,44} = 2.8$, $P = 0.1$); therefore, the off-bottom oysters were slightly higher in tidal elevation than the ground culture oysters. Willapa Bay tidal elevations were extracted from a bathymetry layer constructed from 2002 NOAA LiDAR data and 2005 United States Department of Agriculture (USDA) elevation survey data. Hood Canal and Samish Bay elevations were initially based on publicly available LiDAR data (https://coast.noaa.gov/dataviewer/#/lidar/search/), but these extracted elevations did not align well with our personal experience observing the timing of tides. We instead determined when the water ebbed or flooded by direct observation or by video cameras deployed for other purposes.

In the overall study design, the two aquaculture types were crossed with three levels of colocated eelgrass (*Zostera marina*), resulting in a block of six samples from different "treatments" at each site. Surface sediment samples ($N = 2$–4) were collected from each studied bed within a site, dried, and processed for organic content by loss-on-ignition (500°C for 3 hr), followed by grain size analysis through a sieve series (Wentworth scale, Ro-Tap Sieve Shaker, Mentor, Ohio, USA). Within each site, but outside of actively cultivated aquaculture beds, we sampled areas with high cover of eelgrass to provide an index of local eelgrass density (Table 1), since eelgrass typically occurs at higher density when shoot sizes are smaller [37]. These reference eelgrass beds occurred at an average tidal elevation of 0.08 m (0.09 SE, $N = 15$, all elevations relative to mean lower low water (MLLW)). Aquaculture beds with dense eelgrass (−0.08 m (0.07 SE, $N = 21$)) and sparse eelgrass (0.13 m (0.08 SE, $N = 18$)) were at a similar tidal elevation but slightly higher with no eelgrass (0.2 m (0.06 SE, $N = 21$)).

2.2. Study Design. Each of the 10 sites was considered one block in a completely crossed two-factor design. One factor was culture method (two levels: ground, off-bottom), and the other was eelgrass density (three levels: none, sparse, dense). Off-bottom culture consisted of longlines at five sites and flip bags at six sites. One site (P-S) had both types of off-bottom
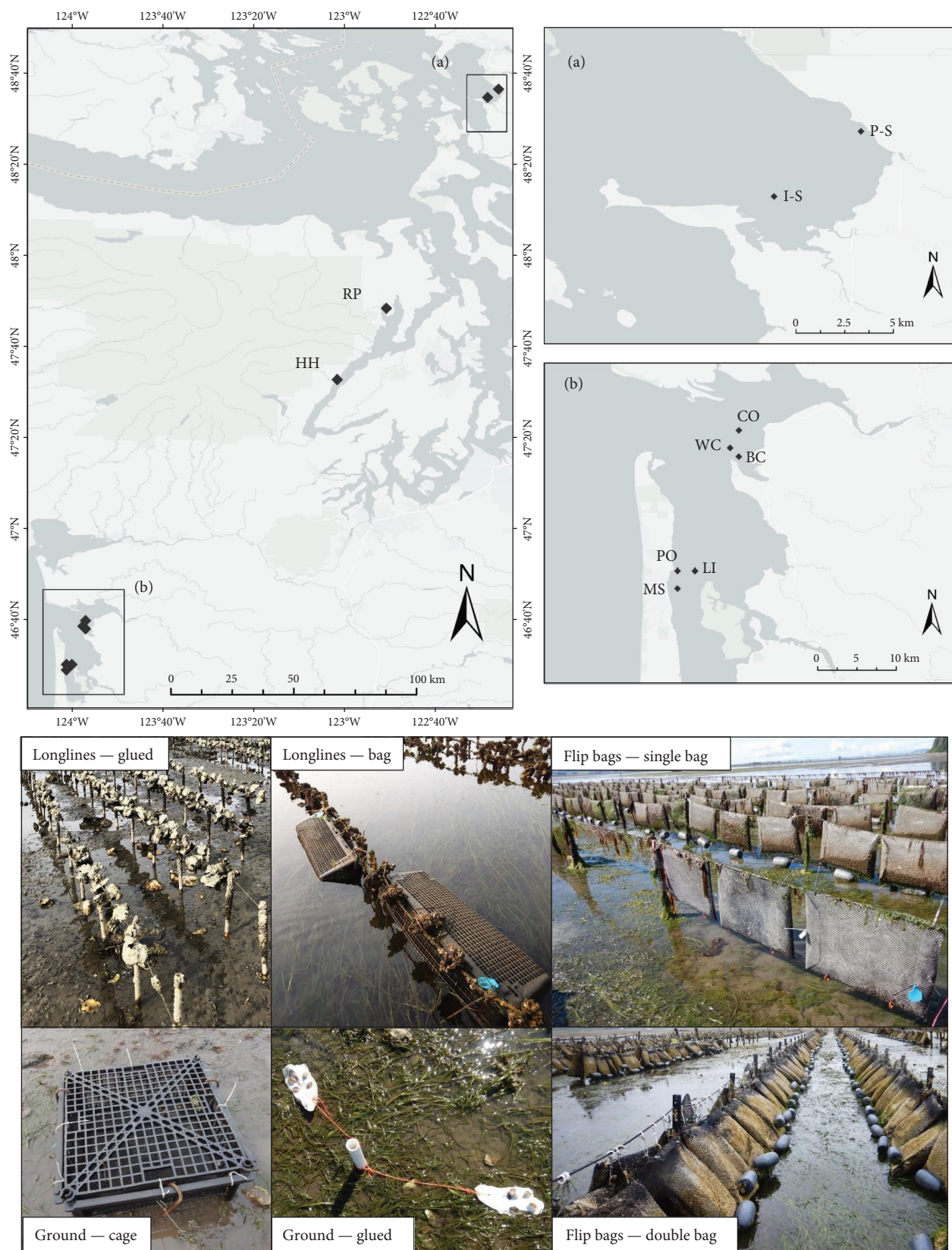
FIGURE 1: Map of study sites in Washington state, USA, including (a) Samish Bay: P-S, I-S; (b) Willapa Bay: CO, WC, BC, PO, LI, MS; and Hood Canal: RP and HH. Grow-out and outplant methods are shown in the images at bottom.

TABLE 1: Characteristics of study sites in Washington state, USA, with outplanted Pacific oysters (*Magallana gigas*).

Site (= block)	Bay	Geolocation N, W	Eelgrass 0.25 m^{-2} (SE)	Outplant method
2020				
LI: Long Island	Willapa	46.51, −124.00	18.0 (1.2)	Longlines: Glued Gr: Cage, glued
PO: Port	Willapa	46.51,−124.02	19.4 (1.9)	Flip bags: Bag Gr: Cage, glued
MS: Middle Sands	Willapa	46.49,−124.02	16.7 (1.5)	Longlines: Glued Gr: Cage, glued
RP: Rock Point	Hood Canal	47.81, −122.85	38.1 (5.9)	Longlines: Bag Gr: Cage
HH: Hama Hama	Hood Canal	47.55,−123.03	184.0 (11.8)	Flip bags: Bag Gr: Cage
2021				
CO: Cutoff	Willapa	46.67,−123.95	19.2 (1.3)	Longlines: Glued Gr: Glued
WC: West channel	Willapa	46.65,−123.96	16.7 (2.8)	Flip bags: Bag Gr: Glued
BC: Bay Center	Willapa	46.64,−123.95	20.0 (1.4)	Flip bags: Bag Gr: Glued
P-S: Plant	Samish	48.61,−122.44	22.7 (2.3)	Flip bags: Bag Longlines: Glued Gr: Glued
I-S: Island	Samish	48.58,−122.48	101.6 (4.2)	Flip bags: Bag Gr: Glued

Note: Two culture methods occurred at each site: off-bottom (longlines or flip bags) and ground (Gr). Single juveniles were deployed in bags or cages or were glued to a larger shell with a hole drilled to attach it in place. Eelgrass density is reported for summer quadrats outside aquaculture ($N = 40$ in Willapa Bay and $N = 20$ otherwise) as an index of site-specific maximum density; sites with denser eelgrass typically have smaller shoots.

culture, which were included as separate replicates. Treatments were selected from the existing mosaic of habitats at each site, but some combinations of culture type and eelgrass were not available within a block, so total sample size was 59 beds across 10 blocks (sites; Table S1). Eelgrass density was measured in spring (April–June) and summer (July–August) in 20 quadrats (0.25 m^2) placed along a 100-m transect at each bed within the 10 sites. Eelgrass was also sampled in meadows outside aquaculture (Table 1). Specifically, two eelgrass beds at Willapa Bay sites and one eelgrass bed at the other four (Table 1). Overall, in summer, dense eelgrass in aquaculture was 52% (SE = 6%, $N = 17$) of counts outside aquaculture, and sparse eelgrass in aquaculture was 18% (SE = 3%, $N = 17$). Eelgrass density on a bed (as a percentage relative to off-aquaculture at the site) was well correlated from spring to summer ($r = 0.72$, $N = 54$).

2.3. Stocking Densities. At each study site, Pacific oysters were sampled on each "treatment" (ground culture and off-bottom culture at three levels of eelgrass) in spring (April–June) and summer (July–August) in order to determine stocking density. For ground culture beds, 20 quadrats (0.25 m^2) were placed along a 100-m transect. Shell cover, number of live oysters, and shell height of up to 10 oysters were recorded in each quadrat. These quadrats were the same in which eelgrass shoots were counted. In longline culture (five sites), some of these quadrats along the 100-m transect fell on the longlines and

some in the space between the lines. Clusters were counted per quadrat, and, if clusters were present, live oysters were counted on one cluster and up to 10 measured for shell height. Standard spacing for commercial longline culture of oysters is one cluster every 20–30 cm along the rope, with ropes at 60–90 cm intervals apart, in some cases leaving out every 4th or 5th rope as an aisle (~5 clusters m^{-2}). This estimate of ~5 clusters m^{-2} was similar to the sampled density of clusters at four sites in summer (clusters m^{-2} (SE) at CO = 4.57 (0.79), LI = 4.51 (0.45), MS = 3.54 (0.14), P-S = 4.73 (1.05)). Due to lack of cluster density data at RP, we assumed 4.5 m^{-2}. Finally, for stocking densities in flip bags (six sites), shellfish farmers grade and split (reduce count per bag) oysters as they grow. A float on the distal end of the bag upends the bag as the water rises, thus tumbling the single oysters and creating a desirable cupped shape without risk of cementing into clusters or onto the bag itself. Flip bags are clipped to lines that are typically spaced at 1.5–2 m, with bag dimensions of 0.7 × 0.5 m (Figure 1). Across sites, bag densities ranged from 0.9 to 1.2 m^{-2}, and each bag yielded about 100 6- or 7-cm oysters after 1–1.5 years (200 per bag at HH) or ~20–30 oysters m^{-2} of growing area. In some cases, these flip bag oysters are transferred to ground culture prior to harvest for sale.

2.4. Oyster Outplants. Single juvenile diploid oysters, for outplant at each bed at the 10 sites, were obtained from a commercial hatchery. In 2020, initial shell heights were 27.6 mm

(SE = 0.8, $N = 30$), and in 2021 were 21.8 mm (SE = 0.6, $N = 30$). In ground culture, oysters were deployed in two ways: plastic cages ($50 \times 50 \times 10$ cm) anchored to the sediment by rebar stakes and containing single juvenile oysters, and shells to which five single oysters were glued, tethered via a 20-cm line that ran from a hole in the shell to a PVC stake (Figure 1). Similarly, on longlines, samples were either mesh bags containing juvenile oysters or shells to which five single oysters were glued (Figure 1). In flip bags, only the flip bag method was used for outplants, and juvenile oysters were contained at a number in keeping with local practice applied to oysters at the outplanted size: 160/bag at Hood Canal sites, 125/bag at Samish sites, and 180/bag in Willapa Bay. These densities were also applied to cages on-bottom or bags on longlines. In all deployments with glued oysters, five were attached to the smooth side of an oyster valve (Gorilla brand caulk), and 10–12 of these shells were attached on each bed, specifically to stakes in ground culture or to the longlines themselves. The deployments spanned two seasonal periods: 3 months of warmest water temperatures from June to September (15–20°C) and the subsequent 6 months of cooler temperatures, including December to March with water temperatures typically <10°C (Figure S1). Oysters were outplanted in June, and up to 10 from cages or bags, or half of the glued shells, were collected in September (3 months) and the remainder in the following March (9 months). Of 59 beds, we recovered outplants from 51 beds after 3 months and 32 beds after 9 months (Table S1). Missing outplants in some cases were removed when beds were harvested.

At collection, oysters were counted, distinguishing live and dead individuals. We measured shell height and length of all oysters (nearest 0.1 mm) and width for single juveniles. Single juveniles were weighed to determine whole fresh weight and then shucked to dry and weigh the shell and meat separately. Meats were dried for 48 hr at 50°C, followed by 48 hr at 100°C. Shells were dried for 4 days at 100°C.

Survival was calculated based on the number of surviving relative to initial oysters. Some glued juveniles fell off and these were not included in calculations of survival; that is, oyster shell had to remain attached to the glue in order to be scored as dead. Also, any oysters collected at 3 months were subtracted from the initial number used for survival calculations at 9 months. Growth was based on the shell height at collection. Condition index was calculated as dry flesh mass divided by the difference between whole fresh mass and dried shell mass [38]. However, oysters glued to shells were sometimes damaged during removal and, therefore, shell mass was underestimated and not suitable for use in the typical condition index calculations. For single oysters, dry flesh mass per shell height was well correlated with condition index ($r = 0.75$, $N = 1,429$), so we used the former to evaluate condition due to larger sample sizes. Each of the three response variables (fraction surviving, shell height, condition) followed a multilevel structure, since most beds had more than one bag, cage, or shell, within which were several oysters. Oysters within a sample on a bed (i.e., bag, cage, shell) were averaged to generate values that were

independent samples of each bed, and then any independent samples of a bed were averaged for each true replicate, which was one bed with a particular eelgrass density and culture method at a site.

2.5. Data Analysis. Stocking densities were based on calculations from standard practices in flip bag culture (described above) and were determined from spring and summer quadrat data for all longline and ground culture beds. Shell cover, oyster density, and size were summarized for each longline and ground culture bed within the 10 sites in terms of mean and standard error ($N = 20$ quadrats per bed). We tested for correlations between size and density across beds separately in spring and summer. A negative relationship would be expected if beds are at different phases of the crop cycle, planted at a high density of small oysters, which subsequently grow and experience natural mortality before harvest. We also compared likely harvest densities among grow-out methods but did not approach this issue with statistical tests because we did not have direct harvest data from each bed.

Analyses of outplanted oysters were carried out separately for collections at 3 and 9 months. Response variables were oyster survival, shell height, and dry flesh mass per shell height (condition). Fixed effects were two types of aquaculture (ground and off-bottom) and three levels of eelgrass density (no, sparse, and dense), which were organized in a fully-crossed design. Site (block) was included as a random effect, and general linear mixed-effects models were built for each response variable at each time point. Normal distribution of the residuals was used as a diagnostic to assess assumptions about data structure, and Gaussian assumption was suitable for survival, shell height, and condition. Missing data were disproportionately in ground culture treatments, which made us cautious about the ensuing unbalanced design, even with type III sum of squares. We censored sites where more than two of six beds had no data, which reduced sample size to 5 at 9 months, and we imputed other missing data as the average of all extant true replicates; therefore, all missing data were replaced with an identical number that represented the average of those not missing. We note here that this imputation eliminated a significant eelgrass × culture interaction for shell height at 9 months but, otherwise, did not change statistical outcomes. Models were fit by maximum likelihood in order to compare models. As recommended in a study by Zuur et al. [39], the first step was to determine the appropriate random effect structure for each response variable at each collection time, specifically comparing no random effect, intercept only, or random intercept and slope, all in the context of the full model for fixed effects (including culture × eelgrass interaction). Once the appropriate random effect structure was determined, model selection was used to determine fixed effects. Significance of factors was determined by model comparison that adjusts goodness of fit by model complexity, based on chi-square statistics. Models were fit with functions in package lme4 [40], and post hoc tests required lmerTest [41] and emmeans packages [42] in R [43]. We report statistical results, including imputed data (balanced design) and biological differences

without imputed data (percent survival, shell height, and condition); graphs exclude imputed data and include 9-month data that were not analyzed statistically due to >two missing treatments. Parallel models parameterized eelgrass as a continuous rather than categorical factor, where we needed to account for site-level variation in eelgrass density because larger morphotypes occur at lower density (Table 1, unpublished data). Eelgrass density (mean per bed) was, therefore, standardized to density outside aquaculture at each site. Results with continuous eelgrass density were similar to the original design that included eelgrass density as a categorical variable and are reported in the supplemental material (Tables S2–S4).

Oyster survival was tested for a relationship to sediment conditions, specifically the fraction of fine particles (mud). These regression analyses were carried out on 3-month data separately for outplants in ground and off-bottom culture. Four outplants in which mortality was high due to predators were not included in this assessment of sediment impacts. All data visualizations show true replicates (beds) as points, as well as error bars representing the variability of samples within a bed. Data analyses used true replicates, blocked by site.

3. Results

3.1. Stocking Densities. Across the aquaculture beds selected for this study, shell cover averaged 30% (SE 2%, $N = 55$) in ground culture and had a lower total footprint on longlines (9%, SE 4%, $N = 36$, including spring and summer), while densities were generally double on longlines (64 m^{-2} (SE 10, $N = 36$)) relative to on-bottom (32 m^{-2} (SE 6, $N = 55$)). Size and density of oysters were negatively correlated on longlines in spring ($r = -0.92$, $P < 0.0001$, $df = 13$) and summer ($r = -0.66$, $P = 0.007$, $df = 13$), but this relationship did not hold in ground culture in spring ($r = -0.18$, $P = 0.38$, $df = 24$) or summer ($r = -0.35$, $P = 0.07$, $df = 24$) (Figure 2). Accordingly, although overall stocking densities tended to be higher in longlines than ground culture, oysters were at about 20 m^{-2} at a harvestable size in longlines and, therefore, similar to ground culture (Figure 2). Recall from calculations in Methods that, for comparison in terms of production per area, flip bags yield 20–30 oysters m^{-2} at the end of the crop cycle. Therefore, all culture methods provided similar yield per area based on the farm sites included in this study.

3.2. Oyster Outplants. Oysters performed better in off-bottom than ground culture in a manner that was generally unrelated to local eelgrass density. Survival over 3 months improved off-bottom (85% (SE 5%)) relative to on-bottom conditions (69% (SE 6%); nine sites, Figure 3, Tables S2 and S3). This survival difference by culture method persisted after winter (84% (SE 5%) off-bottom vs. 72% (SE 6%) ground culture), with an additional main effect of eelgrass given lowest survival in dense eelgrass (five sites, Tables S2 and S3). The magnitude of difference was 66% survival in dense eelgrass and 84% with sparse or no eelgrass, but

pairwise contrasts were not strong statistically ($P = 0.05$). For oysters in ground culture, survival declined in finer sediments (regression $r^2 = 0.25$, $t_{1,15} = -2.23$, $P = 0.04$; Figure 4). No relationship between survival and sediment conditions was evident for off-bottom oysters (regression $r^2 = 0.01$, $t_{1,26} = -0.56$, $P = 0.6$; Figure 4). The pattern, as shown in Figure 4, has proportion of fine sediment ($< 63 \mu$ grain size) as the independent variable, ranging from 0.2% to 39% by mass. Fines were highly correlated with organic content, which ranged from 1.2% to 7.1% ($r = 0.78$, $N = 59$). Oyster drills (*Ocinebrellus inornatus*) caused mortality on-bottom at I-S and in longlines at MS, based on drill holes in upper valves. Newly recruited crabs that were able to get through the plastic grid of cages were likely responsible for mortality when mostly shell fragments remained (vs. whole shells).

Shell height was 7% greater (51.33 mm (SE 1.64) vs. 47.85 mm (SE 1.74)) and condition was 48% greater (0.00879 gDW mm^{-1} (SE 0.00079) vs. 0.00592 gDW mm^{-1} (SE 0.00125)) off-bottom relative to ground culture after 3 months (Figure 5). Although mean condition remained 45% greater off- than on-bottom at 9 months, this difference was no longer statistically significant (0.016 gDW mm^{-1} (SE 0.002) vs. 0.011 gDW mm^{-1} (SE 0.003); Tables S2 and S3). For shell height at 9 months, oysters were 5%–8% larger in sparse than in dense or no eelgrass (Tables S2 and S3). Shell height doubled over the 3 summer months (~25 mm linear extension), but growth overwinter slowed substantially (14 mm linear extension in 6 months). Condition initially was 0.0029 gDW mm^{-1} ($N = 60$) and increased from 3 (0.0077 gDW mm^{-1}) to 9 months (0.014 gDW mm^{-1}). This temporal change is expected for the condition metric used in this study, since volume increases as a cubic function of length. In comparison to models with eelgrass as a categorical variable, eelgrass incorporated as a continuous linear variable was never a significant predictor of oyster performance (Table S4). These models with continuous eelgrass were consistent with categorical eelgrass in identifying oyster performance differences by culture method, and, in addition, better oyster condition off- than on-bottom persisted to 9 months (Table S4).

4. Discussion

A key element of successful intertidal shellfish farming is recognizing which environmental factors affect yield and can be controlled. On a bed-specific scale, culture practices determine whether oysters are on or above the sediment, which further determine structural complexity and disturbance that may alter grain size [44] and seagrass co-occurrence [18]. Despite their adult life history as benthic organisms, oysters may not have an optimal habitat on-bottom due to near-bed properties involving high concentrations of fine inorganic particles. Ground culture growth and survival can be constrained, especially among dispersed single oysters or clusters, which are unlike natural patterns of gregarious settlement and reef formation. Off-bottom and suspended culture methods are particularly advantageous in fine sediments of low bulk density [9, 45].
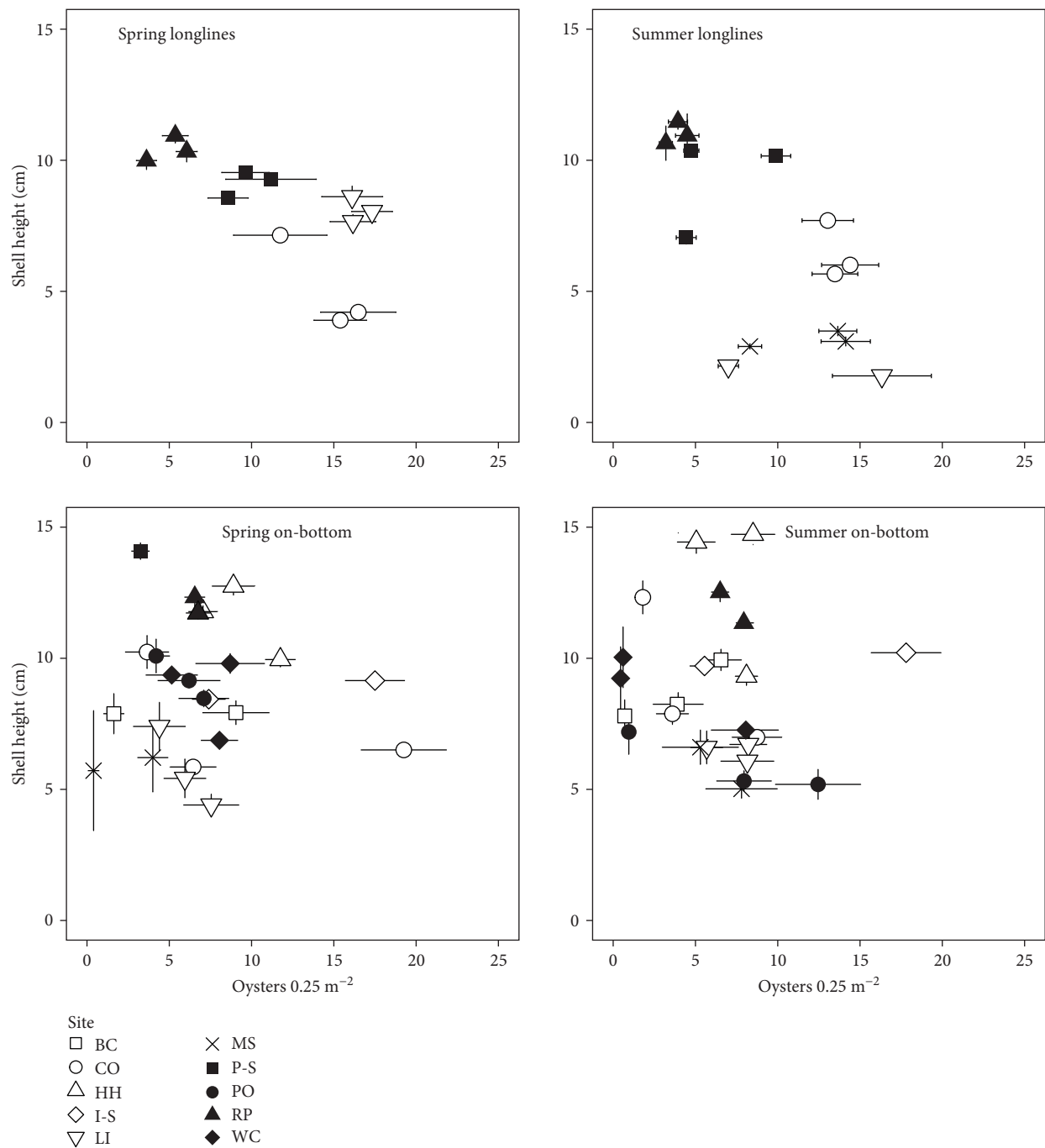
FIGURE 2: Stocking attributes of commercial oyster beds (*Magallana gigas*) across sites in Washington state, USA. Error bars show standard error of 20 quadrats (density) and sometimes fewer for shell height if oysters were not present in all quadrats. *x*- and *y*-axes scales are equivalent for all panels, which cutoff some points: dense, newly-strung cultch at three MS longline beds in spring; dense, newly-strung cultch at one LI longline bed in summer; dense, moderately-sized oysters at one I-S ground culture bed in spring and summer; and dense, moderately-sized oysters at one MS ground culture bed in summer.

No aspects of meat or shell growth or survival improved consistently with eelgrass in our study. These sorts of tests need additional attention, since facilitation is possible between seagrass and bivalves [27, 46]. In laboratory experiments, seagrass may improve oyster shell extension [24] or reduce shell growth under elevated pCO_2 [23]. In several field trials, surrounding eelgrass often has no effect or reduces the numbers or sizes of bivalves [25, 47]. In the present case, the design was not set up to tell if eelgrass facilitated oyster yield in some sites and impaired it in others, which could lead to overall no effect. That said, for some response variables, incorporating a random slope in addition to random site intercept was warranted statistically (Tables S2 and S3), and these random slopes can be interpreted to
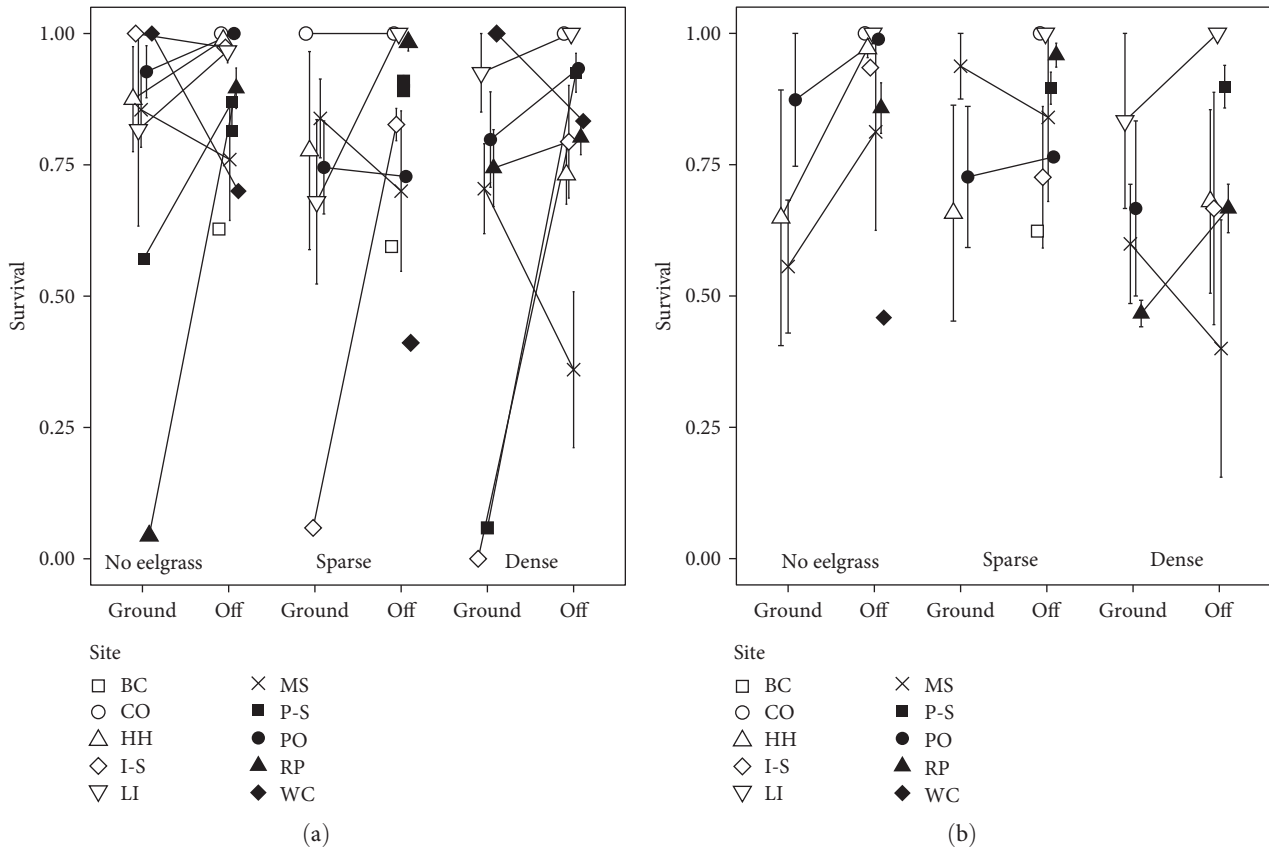
(a)

(b)

FIGURE 3: Survival of Pacific oysters (*Magallana gigas*) across sites in Washington state, USA. (a) Fraction surviving after 3 months (September) and (b) after 9 months (March). Error bars show SE of multiple samples (cages, bags, or glued cultch) within shellfish aquaculture beds across three levels of eelgrass (no, sparse, dense), with lines connecting on and off-bottom in each site.
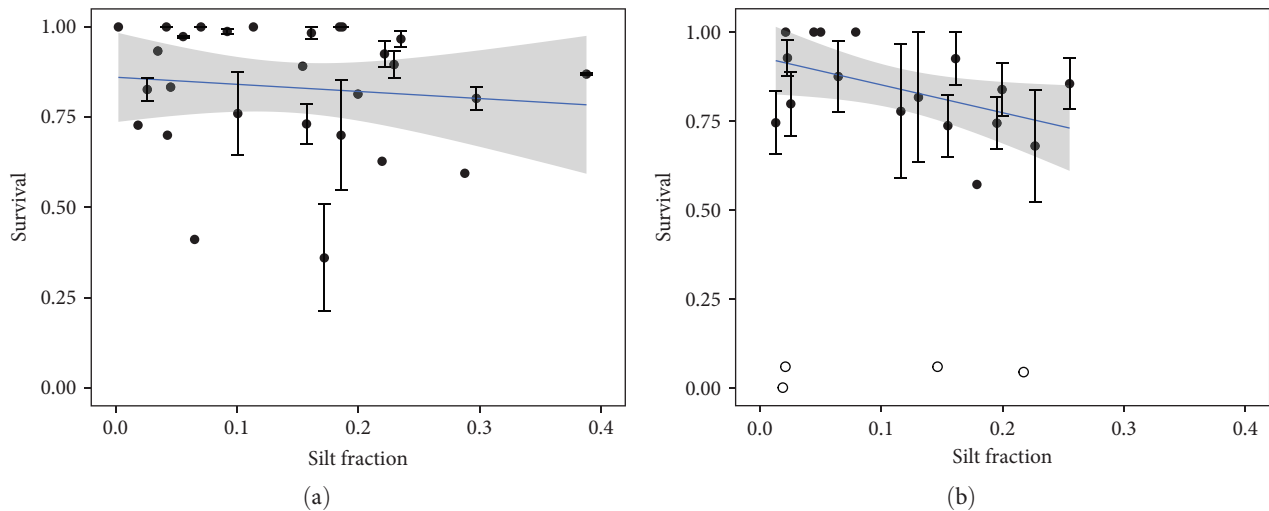

(a)

(b)

FIGURE 4: Survival of Pacific oysters (*Magallana gigas*) across sediment conditions (silt fraction $<63\,\mu$ grain size) after 3 months in Washington state, USA. (a) Fraction surviving in off-bottom culture and (b) ground culture. Open circles represent cases where outplants had very low survival from predation, and these were not included in regression analyses. Error bars show SE of multiple samples (cages, bags, or glued cultch) within shellfish aquaculture beds, but only mean values were used in regression analysis.

mean that the magnitude of any benefit of culturing oysters off-bottom differed by site, which is also evident in the site-specific slopes, as shown in Figures 3 and 5. Statistically, fine sediments reduced on-bottom oyster survival, and we also observed several cases of high mortality due to predators (Figure 4). Eelgrass can cause accumulation of organics and fines [48] and modify trophic interaction strength [49]. Accordingly, an important result of our study is that
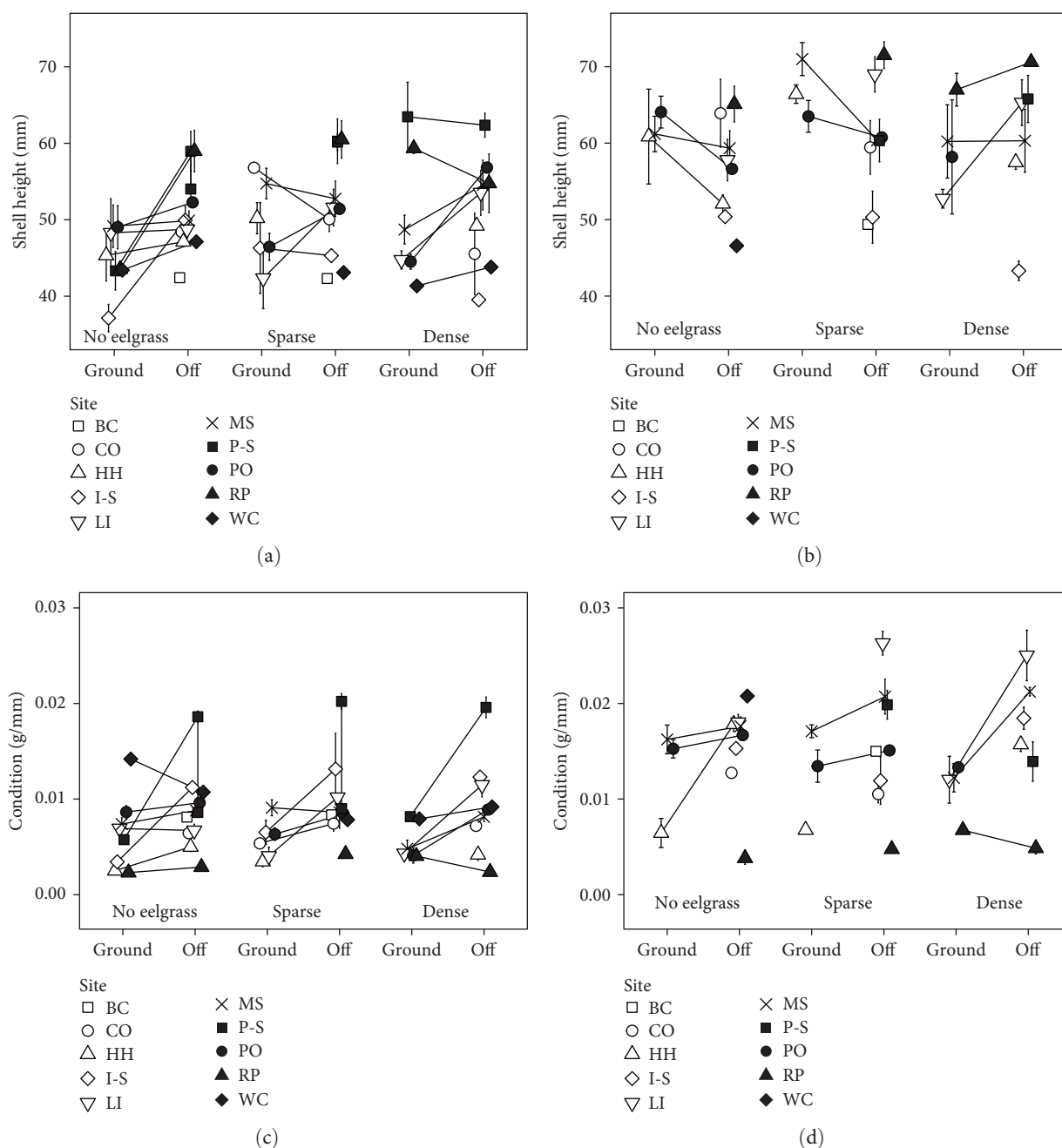
FIGURE 5: Shell size and condition of Pacific oysters (*Magallana gigas*) across sites in Washington state, USA. (a) Shell height after 3 months (September) and (b) after 9 months (March). (c) Condition as dry flesh mass per shell height after 3 months (September) and (d) after 9 months (March). Error bars show SE of multiple samples (cages, bags, or glued cultch) within shellfish aquaculture beds across three levels of eelgrass (no, sparse, dense), with lines connecting on and off-bottom in each site. P-S site contained separate beds of flip bags and longlines as off-bottom culture. Visualizations include all sites, although statistical analysis incorporated nine sites at 3 months and five sites at 9 months.

eelgrass did not impair oyster performance on shellfish beds. In other words, practices that maintain some eelgrass—through relatively low shell cover and disturbances that are not too frequent or intense—do not correspond with a yield loss in individual oyster performance. We did not explore stocking density as a predictor of individual performance since farmers typically do not exceed stocking densities that stunt their product, and carrying capacity was not a focus of this study.

Scaling up from individual performance to whole-bed production reinforced the distinctions of culture method. Relative to ground culture, off-bottom culture had a reduced direct footprint of shell cover but more oysters per area, a feature of the three-dimensional positioning of oysters. This apparent benefit of off-bottom culture (lower footprint, more production) is of course mitigated by the labor costs of placing, maintaining, and collecting oysters by hand, as well as material costs of posts, lines, and bags. Optimizing production

for profitability would require consideration of existing capital investments (i.e., boats, land area). Any restrictions on the spacing of gear would also reduce the financial benefits of off-bottom culture. For instance, in their Biological Programmatic Opinion related to species listed under the US Endangered Species Act, the National Marine Fisheries Service (NMFS) noted "only oyster longlines (with flip bags ok) spaced laterally at 10 feet intervals shall be used in fallow areas that have been colonized by eelgrass" [50] p. 98. Thus, line spacing in the current study was tighter than NMFS allows in fallow areas. One other aspect of off-bottom culture is evident from the size–density relationships across beds, as shown in Figure 2. The clear negative relationship for longlines implies that farmers start the crop cycle with a consistent oyster density (oysters per seeded cultch and spacing of clusters), which declines due to natural attrition as the oysters grow. From spring to summer, mean size increased on all longline beds except for LI where oysters were harvested and restrung in between sampling. In contrast, absence of a clear trade-off between size and density in ground culture may reflect that we selected beds used for different purposes (growing vs. fattening vs. holding), including some with low oyster density that may have been harvested without replanting. The shell cover from benthic surveys (30%) is typical for ground culture (as reported in a study by Wagner et al. [19]), but the oyster densities were rather low as compared to $50\,\mathrm{m}^{-2}$ market-sized oysters reported for fattening beds [51].

Yield is heavily influenced by survival, particularly acute events such as low-salinity stress [52, 53], burial by sediment [11], and seasonal disease outbreaks [54, 55]. Additionally, summer mortality events are globally observed in Pacific oysters but remain multifaceted in terms of drivers [56–58]. One year of outplants overlapped with the June 2021 heat dome (Figure S1) that caused high mortality in intertidal bivalves regionally [59], but we recorded 20% mortality of outplants in 2020 ($N = 27$) and 26% in 2021 ($N = 24$), suggesting that this heat wave did not markedly interfere with aquaculture yield at these sites. Our ability to draw conclusions about factors influencing overwinter performance was restricted by loss of replicates, and for those that remained, cumulative survival was estimated at 84% for the first 3 months and 79% by 9 months ($N = 32$), suggesting low additional mortality overwinter and/or as oysters grew. Such high overwinter survival has been reported previously, e.g., for *Crassostrea virginica* in Delaware Bay [60]. The statistical results for survival and shell height were more complex after 9 than 3 months, including a slight height advantage in sparse eelgrass rather than the more straightforward difference between culture methods. We interpret these winter outcomes cautiously, since eelgrass is typically at its lowest biomass overwinter [61] and would be expected to have least effect during that time.

Shell growth provides an index of body size increase coincident with the mantle secreting shell, whereas condition accounts for storage of energy resources [62]. Because off-bottom oysters occurred over tidal flats of similar tidal elevation to ground culture, their immersion time was shorter; however, the intertidal range of 0 to $+0.5\,\mathrm{m}$ relative to mean lower low water does not typically alter shell extension rates [7]. The small improvements in shell growth off-bottom were consistent with past work [7] but dwarfed by improvements in meat weight and, therefore, condition of off-bottom oysters. Similarly, oysters in floating culture also have shown high condition relative to those growing on-bottom [63]. Further, suspended culture can result in slower shell growth than off-bottom racks, while the off- versus on-bottom benefit appeared especially where sediments consisted of relatively high fractions of fine particles [2].

5. Conclusions

Grow-out techniques strongly influenced survival and condition of individual Pacific oysters and the shell cover footprint in aquaculture. Ground culture became more challenging as the mud content of the sediment increased, whereas off-bottom culture was robust to this sediment gradient. Co-occurring eelgrass had little effect on oysters in this study. We, therefore, conclude that grow-out technique, not eelgrass, is a better predictor of Pacific oyster performance. Interspersion of ecosystem engineers, here eelgrass and oysters, could result in trade-offs in relative abundance if a primary component of their interaction involves space competition or reduction in environmental quality for the other species. Practices associated with shellfish aquaculture may also constitute disturbances that reduce abundance or size of seagrass [64]. In the present study, beds without eelgrass occurred at higher tidal elevations where eelgrass may be limited from desiccation [65, 66]. Long-term colocation of farmed shellfish and seagrass appears possible, even if the interactions are not directly facilitative, on low intertidal flats of Washington state where environmental conditions are suitable for both taxa at low densities: for oysters due to standard growing practices, and for eelgrass due to typical large widely-spaced morphotype (Table 1). These biological responses can help farmers make decisions about practices that optimize production, capital and labor inputs, and environmental impacts, including colocation with eelgrass.

Data Availability

Disclosure

The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or the U.S. Government.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

Supplementary Materials

Figure S1: daily water temperatures at two stations in Willapa Bay, Washington, USA from May 2020 to April 2022, spanning the study period. Table S1: persistence of Pacific oyster (*Magallana gigas* = *Crassostrea gigas*) outplants in each treatment at the 10 outplant sites in Washington State, USA. Tables S2–S4: statistical results testing survival, growth, and condition of Pacific oysters at 3 and 9 months after outplant in a crossed design (culture type × eelgrass levels). Separate tables are provided for results, including imputed data for missing values (Table S2), excluding missing values (Table S3), and parameterizing eelgrass as a continuous variable based on site-standardized density (Table S4). (*Supplementary Materials*)

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