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EDITED BY

Rochelle Diane Seitz,
College of William & Mary, United States

REVIEWED BY

Aurélien Boyé,
Institut Français de Recherche pour
l'Exploitation de la Mer (IFREMER), France
Federica Montesanto,
University of Florida, United States

*CORRESPONDENCE

Jennifer L. Ruesink
✉ ruesink@uw.edu

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Intraspecific variation in resilience traits of eelgrass across intertidal stress gradients and oyster aquaculture methods

Jennifer L. Ruesink^{1*}, Katie Houle², Kristy J. Kroeker³,
Brett R. Dumbauld⁴, Fiona C. Boardman¹, Nathaniel S. Lewis⁵,
Brooke A. McIntyre⁴, Andrew D. Suhrbier² and Bobbi Hudson²

¹Department of Biology, University of Washington, Seattle, WA, United States, ²Pacific Shellfish Institute, Olympia, WA, United States, ³Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA, United States, ⁴Agricultural Research Service, United States Department of Agriculture, Newport, OR, United States, ⁵Coastal Oregon Marine Experiment Station, Oregon State University, Newport, OR, United States

Species persist through resistance and recovery traits induced by natural stressors. Whether human activities exacerbate or moderate the effects of natural stressors is an open question. Because eelgrass (*Zostera marina*) creates coastal habitat, its response to natural stressors and human activities is of particular management importance. In this study, traits of intertidal eelgrass were examined across cumulative stressors of emersion and oyster culture, including two culture types: oysters grown directly on sediment (ground culture) or supported by gear above the sediment (off-bottom culture). Summer eelgrass was larger above- and below-ground and branched less than in spring, while density and cover were similar seasonally but declined at higher elevation and in ground culture. Eelgrass traits were divided into those related to resistance (larger above-ground size and below-ground storage) and recovery (increased flowering, branching, and rhizome extension). Resistance traits responded additively to intertidal elevation and oyster culture, with above- and below-ground size reduced in all conditions except that rhizome mass was maintained in ground culture. Smaller above-ground size may confer resistance to emersion stress, as a departure from expectations for other stressors. For resilience traits, flowering increased and internode length declined at higher elevation, whereas these traits did not change in ground culture, and off-bottom culture was associated with shorter internodes (additive cumulative stressor) and tended to moderate the enhanced flowering at higher elevations (non-additive, $P=0.058$). Transitory disturbance in ground culture may reduce eelgrass density with few effects on resilience, whereas off-bottom culture involves longer-term gear placement and trait responses by eelgrass.

KEYWORDS

Zostera marina, stressors, disturbance, perturbation, tidal elevation, eelgrass, resistance, recovery

1 Introduction

Resilience can occur because a system is resistant to adverse conditions or because it recovers rapidly once favorable conditions return (Nimmo et al., 2015). Across seagrass species, resistance and recovery traits generally show tradeoffs (Kilminster et al., 2015; O'Brien et al., 2018), while they collectively contribute to resilience. Similarly, populations within a species can vary in their ability to resist and recover from environmental stress (Tuya et al., 2021). A wide variety of indicators change when seagrasses are exposed to unfavorable environmental conditions (Roca et al., 2016), which is how we use the term “stressor” here. When stressors exceed resistance and recovery capacities of seagrasses, regime shifts can occur to unvegetated states (Unsworth et al., 2015). For intertidal marine organisms, emersion stress varies as a function of elevation, due to drying out at low tide and/or thermal damage in air (Connell, 1972; Peterson, 1991). Coastal human activities may introduce new stressors or modify existing ones associated with elevation gradients. A key question is the extent to which resilience is maintained under human influence, or alternatively, if anthropogenic activities impair resistance and recovery traits (Cabaço and Santos, 2012; Vieira et al., 2020). This study addressed the response of eelgrass (*Zostera marina*) to two types of oyster culture practices across an intertidal stress gradient.

How seagrass responds to multiple stressors has been tested for combinations of temperature, salinity, light, and nutrients (Stockbridge et al., 2020; Ostrowski et al., 2021). However, other factors are at play where intertidal seagrass overlaps with aquaculture. In terms of biological interactions, bivalves can compete for space with seagrass but are increasingly recognized for facilitative roles as bivalves filter water, cycle nutrients, and stabilize sediment (Fales et al., 2020; Gagnon et al., 2020). In terms of physical effects, structures in which bivalves are grown can influence light, water flow, and sediment dynamics, and harvest can cause disturbance (Dumbauld et al., 2009; Forrest et al., 2009; Ferriss et al., 2019; Howarth et al., 2022). In the present study region, ground culture refers to the placement of oysters at low density directly on the sediment surface, often mechanically harvested with a boat-towed dredge, while off-bottom culture occurs when oysters are anchored above the sediment surface on lines strung across vertical pipes. Ground culture places oysters at low densities unlikely to compete with eelgrass (Wagner et al., 2012) but periodically removes eelgrass as oysters are harvested (Tallis et al., 2009), acting as a transitory perturbation (Oliver et al., 2015). Off-bottom culture has more gear present but less intense disturbance at harvest, with gear placement generating a rapid-onset perturbation (Oliver et al., 2015). Ferriss et al. (2019) used a meta-analysis to demonstrate reduced density but enhanced growth and reproduction of eelgrass (*Zostera* spp.) in areas of ground culture, and reduction of density and several traits in off-bottom oyster culture. This result does not address whether bivalve culture effects differ across intertidal stress gradients and whether these two types of aquaculture might differentially affect seagrass traits associated with resilience.

Intertidal zones provide several gradients relevant to seagrass resilience traits. Seagrasses can be light-limited at depth and

desiccation-limited at their upper limits, while hydrodynamic energy can act either across or within elevations (Koch, 2001; Yang et al., 2016). These physical factors can require different morphological or demographic strategies at different positions along elevation gradients for seagrass to persist. In terms of size, smaller shoots (=above-ground bundle of leaves) occur with increased water energy and at higher elevations (Keller and Harris, 1966; Boyé et al., 2022). In terms of reproduction, flowering may have a unimodal response to elevation (Olesen et al., 2017). Some responses to environmental gradients, such as larger shoots at depth, may be adaptive for resource (light) acquisition, whereas smaller shoot size may enable closer association with damp sediment and protect from emersion (Shafer et al., 2007). Alternatively, rather than an induction of favorable traits, differential expression of traits across elevational gradients may reflect constraints. Smaller size intertidally could be a result of regular damage (desiccation: (Boese et al., 2005), clipping: (Hernán et al., 2021)), and low light could limit energetic allocation to flowering in subtidal plants (Olesen et al., 2017). Overall, both resistance and recovery traits could involve phenotypic remodeling in ways that generate a morphotype more suited to local conditions, and, particularly for above-ground shoot size, smaller size could improve resistance intertidally. In contrast, across species and in contexts other than intertidal stress, smaller seagrass species are generally considered less stress-resistant than larger species (O'Brien et al., 2018). Because our conclusions are sensitive to this decision to ascribe smaller size as a resistance trait intertidally, we address alternatives in the discussion.

Morphological and reproductive traits that confer resilience (O'Brien et al., 2018) and change with aquaculture (Ferriss et al., 2019) also feature in trait-based frameworks that provide mechanisms for species distributions (Moreira-Saporiti et al., 2023). In part, intraspecific trait variation in *Z. marina* derives from phenotypic plasticity, which is clearly exhibited in seasonal variation in shoot size and branching (Orth and Moore, 1986; Ruesink et al., 2010). Moreover, some aspects of sexual reproductive investment depend on environmental conditions (Backman and Barilotti, 1976; van Katwijk and van Tussenbroek, 2023). Seasonal expression of eelgrass traits often shifts with latitude and temperature; specifically, there is a delay in peak flowering and biomass at higher latitudes and with cooler water temperatures (Clausen et al., 2014; Blok et al., 2018). Warmer sea surface temperatures have been linked to increased (Potouroglou et al., 2014) and decreased flowering (Thom et al., 2003; Qin et al., 2020). Thus, resilience to small-scale stressors such as emersion, flow, and human activities in the intertidal zone could shift due to broader seasonal or interannual change.

In this study, we addressed a critical gap regarding multiple stressors to seagrass by examining traits of *Z. marina* across intertidal stress and oyster culture methods, replicated across 12 sites along the US west coast and Salish Sea. We tested how eelgrass traits responded to natural stressors and were modified in ground culture or off-bottom culture of oysters (Figure 1), near the upper elevation limit of eelgrass. Sampling positions spanned a range of eelgrass cover, while tidal elevation was added for potential explanatory power during analysis. We hypothesized:

1. a decline in the amount of eelgrass (density, percent cover) at higher tidal elevations and with oyster culture.
2. trait variation across intertidal gradients providing resistance to emersion (smaller shoot size, increased rhizome mass) and enhanced recovery (increased sexual and asexual reproduction, and rhizome extension).
3. trait variation specific to each oyster culture method, with recovery traits enhanced by transitory disturbance (ground culture) and resistance traits affected by off-bottom culture. These effects could be particularly pronounced at stressful intertidal elevations, generating non-additive statistical effects of elevation and aquaculture (Figure 1).

2 Materials and methods

2.1 Study design

2.1.1 Environmental conditions at study sites

Eelgrass (*Zostera marina*) has a broad distribution throughout temperate latitudes in the northern hemisphere (Green and Short, 2003). We studied intertidal eelgrass in a mesotidal region of the NE Pacific Ocean, where the upper limits of the distribution emerge regularly on low tides. At 12 sites in five bays (45.5-48° N; Table 1, Figure 2), eelgrass was sampled across an intertidal gradient, in and outside cultivated beds of oysters (*Magallana*

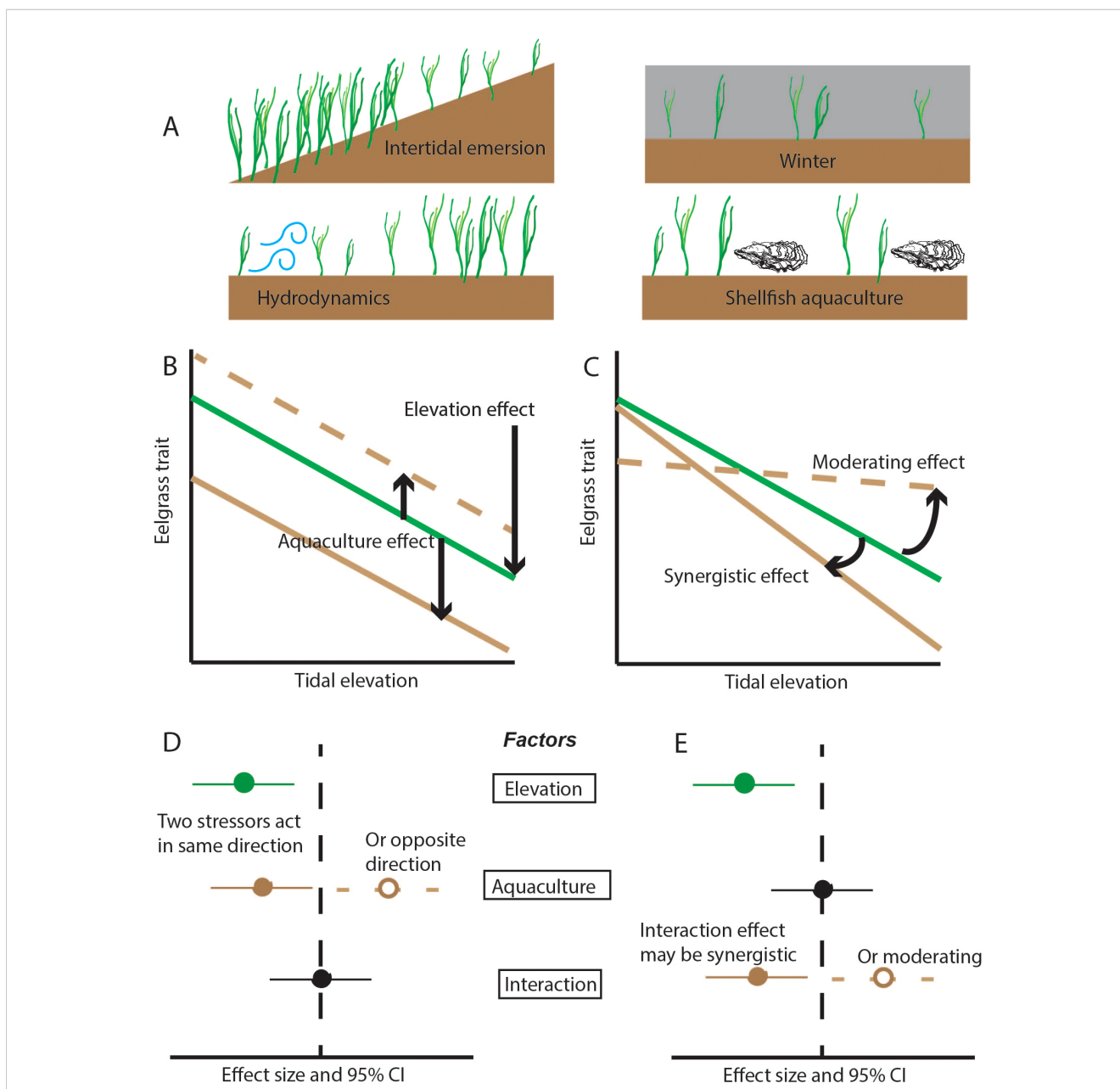


FIGURE 1 Response of eelgrass to multiple stressors. (A) Four stressors that can reduce the amount of eelgrass. While density and percent cover may decline due to environmental stress, remodeling could confer resistance (smaller shoot size, larger rhizome mass), and allocation could promote recovery (increased branching, flowering, and expansion). (B, C) Examples show how a trait such as size can decline at higher elevations, with an additional response to aquaculture that is either a main effect (B) or interactive effect (C). (D, E) Effect sizes consistent with multi-stressor responses in (B, C).

gigas). Two additional bays in California were sampled in eelgrass meadows outside aquaculture (Figure 2). Mean tidal range spans 1.8 to 2.4 m across sites (tidesandcurrents.noaa.gov), and sampling positions were between -0.6 and +0.6 m relative to mean lower low water (Supplementary Figure S1). Sediment was muddy sand, slightly less muddy at higher elevations (Pearson's $r = -0.28$, $t(114) = -3.07$, $P=0.003$), corresponding to a typical pattern of grain size increasing at higher elevations (Peterson, 1991). Organic and mud content were strongly correlated (Pearson's $r = 0.81$, $t(117)=15.08$, $P<0.0001$) (Supplementary Figure S1). Water temperatures were around 10°C in spring at all sites but more variable in summer, ranging from 12.7°C in Tillamook Bay to 19.0°C in Willapa Bay (Supplementary Figure S2).

Tidal elevation was considered a stressor due to emersion time at each study position. Bathymetry in GIS layers was most fully developed for Willapa Bay, where tidal elevations had been surveyed in 2002 by LiDAR (National Oceanic and Atmospheric Administration) and in 2005 by GPS (US Department of Agriculture). In other bays, publicly-available bathymetry did not align well with personal experience of the duration of low tide.

TABLE 1 Bays, sites, and sampling positions included in the study design: three levels of aquaculture (none, ground, off-bottom) crossed with three levels of eelgrass (none, sparse, dense).

Bay (Site) Geoposition	Year sampled	Number of positions at each aquaculture level
Samish, SB (Plant, PL) 48.61, -122.44	2021	FB, No aquaculture: N=3 LL: N=3, <i>in situ</i> eelgrass traits only Ground: N=2 (no sparse)
Samish, SB (Island, IS) 48.58, -122.48	2021	FB, Ground: N=3 No aquaculture: N=2 spring (no sparse)
Hood Canal, HC (Rock Point, RP) 47.81, -122.85	2020	Ground: N=2 (no sparse) LL: N=4 spring No aquaculture: N=3 All: <i>in situ</i> eelgrass traits only
Hood Canal, HC (HamaHama, HH) 47.55, -123.03	2020	FB: N=2 (no sparse) Ground, No aquaculture: N=3 All: <i>in situ</i> eelgrass traits only
Grays Harbor (GH) 46.88, -124.08	2022	No aquaculture: N=3 (2 dense, no sparse) LL, Ground: N=3
Willapa North 46.65, -123.94	2021	No aquaculture: N=5 (2 dense, 1 sparse, 2 unvegetated) FB (at sites BC and WC), LL (at site CO), Ground: N=3
Willapa South 46.50, -124.01	2020	No aquaculture: N=5 (2 dense, 1 sparse, 2 unvegetated) LL (at sites LI and MS), FB (at site PO), Ground: N=3
Tillamook (TI) 45.52, -123.94	2021	LL: N=1 (unvegetated) Ground, No aquaculture: N=3

FB, flip bags; LL, longlines. A "position" is a true replicate, covering approximately 1000 m² with multiple quadrats and eelgrass shoot measurements. Twelve sites included all aquaculture levels and usually three positions differing in eelgrass cover within each aquaculture level (exceptions in final column of this table). See Supplementary Figure S1 for position-specific elevation and sediment properties. Geoposition datum is WGS84.

Instead, the time when water flooded at a sampling position was used to infer tidal elevation, based on where the water level was predicted to be at that time at the closest tide station (tidesandcurrents.noaa.gov; Supplementary Table S1). Video cameras were placed at each position, and the time recorded when the water level reached the camera lens. This time was matched to the corresponding time of observed water level at the nearest buoy station. The height of the camera (0.69 m above sediment) was subtracted from this value as a proxy for elevation. At least three observations from spring and summer videos were used to calculate an average elevation for each position.

2.1.2 Oyster aquaculture at study sites

Ground culture was carried out with clusters of oysters at some sites, but single oysters at others. Off-bottom culture occurred on longlines or in flip bags (Table 1). Photographs of the grow-out methods are provided in Figure 2. These culture "treatments" occurred in similar abiotic environments at each site, with three exceptions: MS off-bottom culture at higher elevation, WC organic content higher in ground culture, and BC less organic and mud outside culture (Figure 2, Supplementary Figure S1). Oyster densities on these beds were reported previously and were generally 1-2 year old Pacific oysters at <70 m⁻² (Ruesink et al., 2023). One site (MS) had longlines harvested and replanted between the spring and summer sampling, but otherwise beds were sampled in the middle of the crop cycle.

2.1.3 Field data collection at sampling positions

The 12 sites were considered blocked replicates. Sites were selected where three levels of aquaculture occurred within <1 km (none, ground culture, off-bottom culture), and then within each aquaculture level, we specifically selected 3-5 positions with different amounts of eelgrass, ranging from none to the highest cover available (Table 1). A "position" required at least 1000 m² with a similar cover of eelgrass, within a given grow-out method or with no aquaculture present. Although habitat and eelgrass traits were measured in multiple quadrats, position is the true replicate. Where possible, eelgrass was measured along a 100-m transect, but for smaller areas, in two 50-m transects. Along each 100-m length, 20 quadrats (0.25 m²) were spaced at 5-m intervals. Vegetative and flowering shoots were counted in each quadrat. Also, at Hood Canal sites, and for longline culture at PL where no eelgrass was collected, maximum length of five terminal shoots was measured per quadrat. At all other sites, up to 5 terminal shoots were collected with attached rhizome from each of 10 quadrats. Each position was sampled in spring (Apr-Jun) and summer (Jul-Aug). Two seasons were specifically included in the design because of typical changes in traits and density between the beginning and end of the growing season (Clausen et al., 2014; Blok et al., 2018; Ruesink et al., 2022), which also occurred in eelgrass meadows in the study region (Supplementary Figure S3).

2.1.4 Eelgrass trait measurements

Collected shoots were measured for maximum length, sheath length, sheath width, and the length of the most recent fully-extended internode on the rhizome (longest internode length

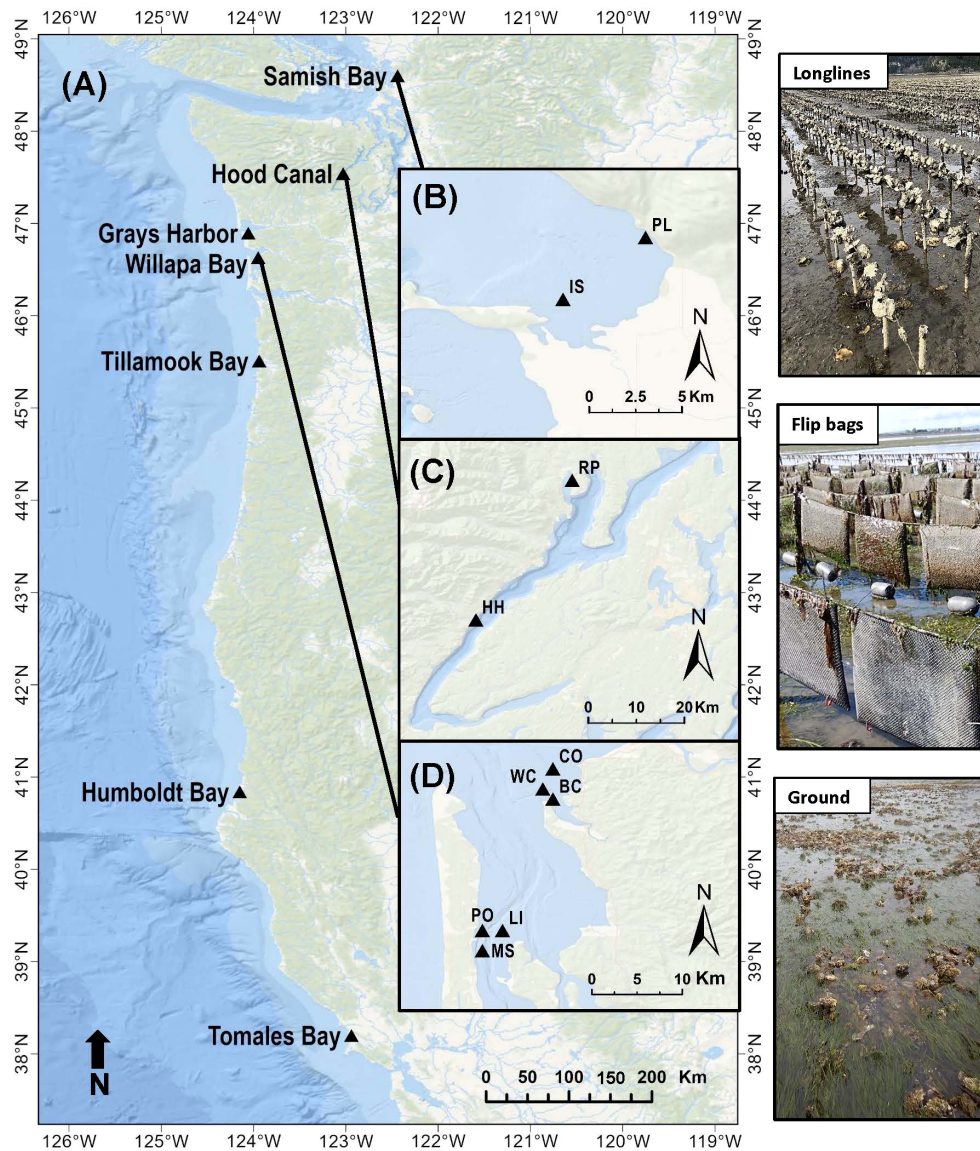


FIGURE 2

Map of study sites in (A) Washington, Oregon and California states, USA. Insets of Washington state sites include (B) Samish Bay: PL (Plant), IS (Island); (C) Hood Canal: RP (Rock Point), HH (Hama Hama); (D) Willapa Bay North: CO (Cut Off), WC (West Channel), BC (Bay Center); Willapa Bay South: PO (Port), LI (Long Island), MS (Middle Sands). Oyster grow-out methods are shown in images to the right. In longline culture, clusters of oysters grow on lines strung across stakes <50 cm above the sediment. In flip bag culture, dozens of single oysters grow in mesh bags attached to lines strung across stakes ~70 cm above the sediment, and the bags upend on higher tides due to the floats on the distal side.

within two nodes; [Supplementary Figure S4](#)). Above-ground material (leaves and sheaths) was dried (60°C for at least 5 days) and weighed. A section of rhizome up to 7 cm in length was dried and weighed separately, then standardized to mass per unit length. Any branches that had formed within the sheath or at the two most recent nodes on the rhizome were counted (and then removed before determining biomass of the terminal shoot).

For cover and density data, 4738 quadrats were measured in two seasons across 12 sites, whereas shoot length and flowering data came only from quadrats in positions selected to include eelgrass (3178 quadrats). Other traits were summarized seasonally across all terminal, non-flowering shoots collected from each position across 10 sites (4868 shoots). Prior to statistical analysis, quadrats within

each position were averaged for cover, density, flowering frequency, and shoot length; shoots collected from each position were averaged for other traits. Based on true replicates (positions), sample size for each analysis was 118 for cover and density across 12 sites, 75 for flowering and maximum length across 12 sites, and 63 for traits of shoots collected across 10 sites.

2.2 Data analysis

2.2.1 Amount of eelgrass across stressors

At each site, positions were deliberately selected where no eelgrass was present and in sparse and dense eelgrass ([Table 1](#)),

so the analysis addressed whether small differences in tidal elevation could underlie some of this variability. Also, the design would not be expected to detect differences in the amount of eelgrass across aquaculture “treatments” unless the upper range of densities was altered on aquaculture beds. Nevertheless, we analyzed whether the amount of eelgrass (percent cover in quadrats at low tide, shoot density) responded to the following factors: season, elevation, and oyster culture (none, ground, off-bottom). Initially we included all two- and three-way interactions, but, since statistically-significant interactions of season with other factors did not occur, and we were primarily interested in cumulative stressors, generalized linear mixed models (GLMM) were built with season, elevation, and culture as main effects, plus an elevation x culture interaction. (Zuur et al., 2009) recommend a model comparison approach to determine the appropriate random effect to include (i.e., intercept, intercept and slope, no random effect). Generally, models fit with a random slope failed to converge, and we elected to use site as a random intercept in all models to appropriately account for the study design, which was blocked by site. This random site effect accounts for sites studied in different years as well. Residuals were examined to select an appropriate data distribution (glmmTMB (Brooks et al., 2017), DHARMA packages (Hartig, 2022)) in R (R Core Team, 2023). Datasets of density and cover, which contained many zeros, were fit assuming a Tweedie distribution (log-link). Tweedie falls within the general class of exponential dispersion models and was preferred over zero-inflated models that require interpretation of two outputs: the probability of presence, and the amount when present. Final model results are displayed as effect sizes and 95% confidence intervals for main effects of season, elevation, oyster culture (ground and off-bottom compared to no culture), and the culture x elevation interaction (sjPlot package (Lüdtke, 2023)). Explanatory power of each GLMM was calculated as McFadden’s pseudo- R^2 (Shtatland et al., 2002).

2.2.2 Multivariate trait analysis

Exploratory analyses for eelgrass trait correlations were performed by principal components analysis, normalizing traits by their mean and standard deviation. Specifically, these traits were maximum length, sheath length, sheath width, internode length, above-ground biomass per shoot, below-ground biomass per cm of rhizome, recent branching, and flowering frequency. Each position was a sample, visualized by season and site. Density and cover were not included in this multivariate trait analysis, as these are attributes of the position, not individual-level traits, and additionally positions were deliberately selected to span a large range of amount of eelgrass at each site and in each level of culture.

2.2.3 Traits of eelgrass across stressors

In a multiple-stressors framework, we analyzed two resistance traits (above-ground maximum length, below-ground biomass per cm of rhizome) and three recovery traits (flowering frequency, recent branching, internode length). Because shoots were not collected from two of 12 sites, branching, internode length, and rhizome mass were restricted to samples from 10 sites. GLMMs were generated for each response variable, with season, elevation,

and culture as main effects, the elevation x culture interaction, and site as a random effect. Data distributions for morphological variables were assumed Gaussian, and for flowering and branching with many zeros were tweedie (log-link), following inspection of residuals (Supplementary Table S2). For completeness, we also set up models with season, level of eelgrass (sparse, dense), culture, and the eelgrass x culture interaction as predictor variables for eelgrass traits, which aligned with the *a priori* study design that did not account for tidal elevation. Eelgrass density (sparse, dense) always had poorer relationships with traits than did tidal elevation (Supplementary Table S2).

3 Results

3.1 Amount of eelgrass

Density and cover of eelgrass declined at higher tidal elevations, consistent with intertidal stress reducing the amount of eelgrass. Effect sizes from oyster culture also tended to be negative, significantly so in ground culture (Figure 3). Elevation x culture interactions were not significant: cumulative stressors were additive (Supplementary Table S2). In analyses of both amount and traits of *Z. marina*, McFadden’s pseudo- R^2 values were 0.11-0.31 (corresponding to fixed effects), except lower R^2 values for density and below-ground biomass where site differences were pronounced (site-level variation was 0.30 and 0.31 of total variation in density and below-ground biomass).

3.2 Multivariate trait analysis

The first two principal components explained 68% of total variation in eight eelgrass traits across positions and seasons (Figure 4). Above-ground size variables loaded strongly on PC1. Below-ground variables, specifically internode length and rhizome mass, were less strongly aligned with this size axis. Flowering frequency and recent branching loaded in opposite directions on PC2. Spring samples tended to fall in the lower right-hand quadrant of the component scores, in keeping with smaller shoots that branch frequently in that season. Summer shoots were generally larger with greater flowering frequencies (Figure 4). This multivariate analysis confirmed the importance of sampling in two seasons, while motivating a choice of five traits for univariate analysis: maximum length and below-ground biomass as resistance traits, and flowering, branching, and internode length as recovery traits.

3.3 Resistance traits

Shoot length declined at higher elevations and with both oyster culture methods (Figure 5A). Emersion and culture stressors together were estimated to reduce shoot length by one-third to one-half, based on model estimates (Supplementary Table S2).

Biomass per rhizome length was reduced at higher elevation and in off-bottom culture, but not in ground culture (Figure 5B). No significant interaction effects were found between the two stressors. Eelgrass shoots in summer were larger both above- and below-ground than in spring (Supplementary Table S2).

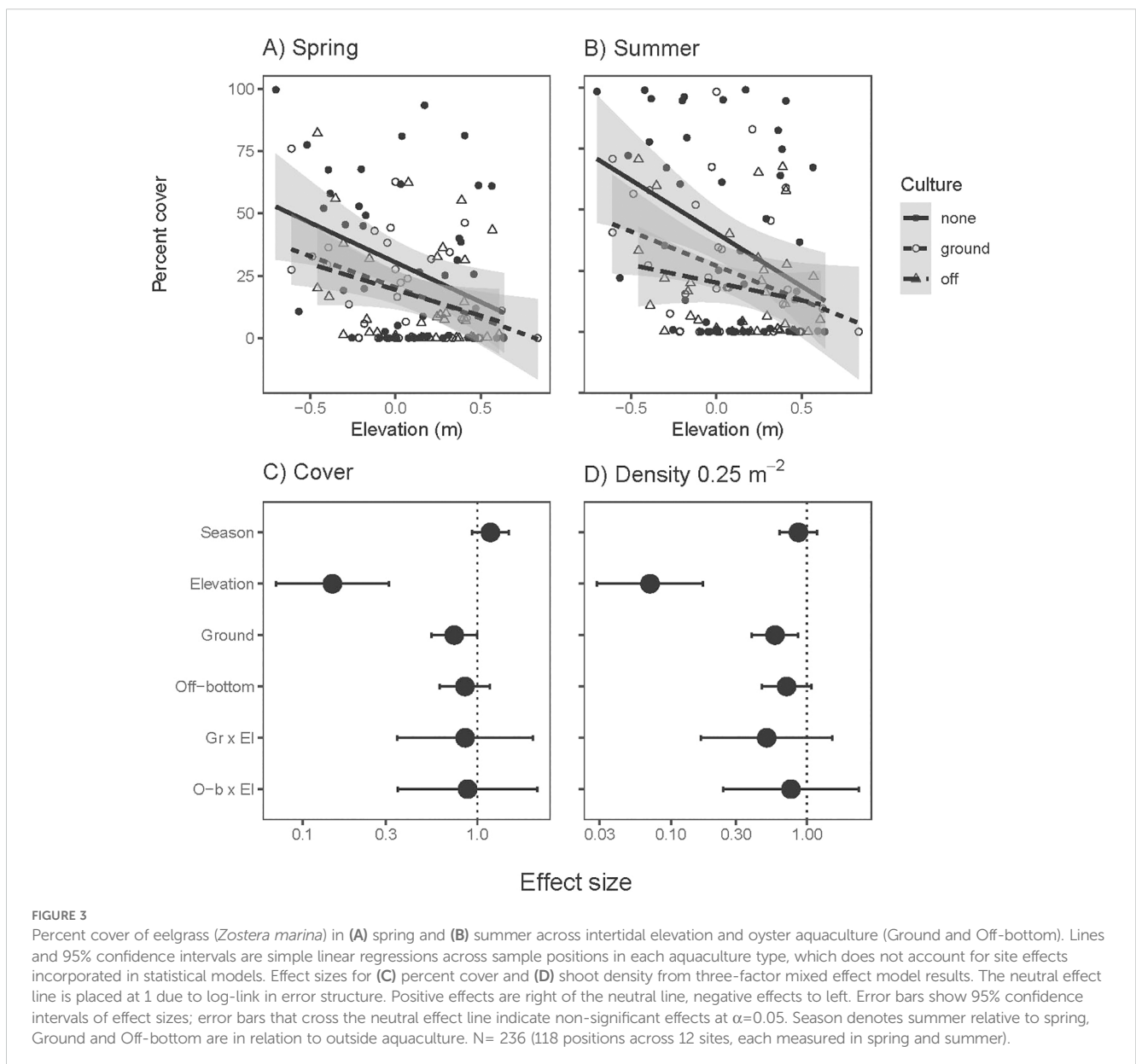
Internode length was shorter at higher elevations and in off-bottom culture (Figure 6C, Supplementary Table S2).

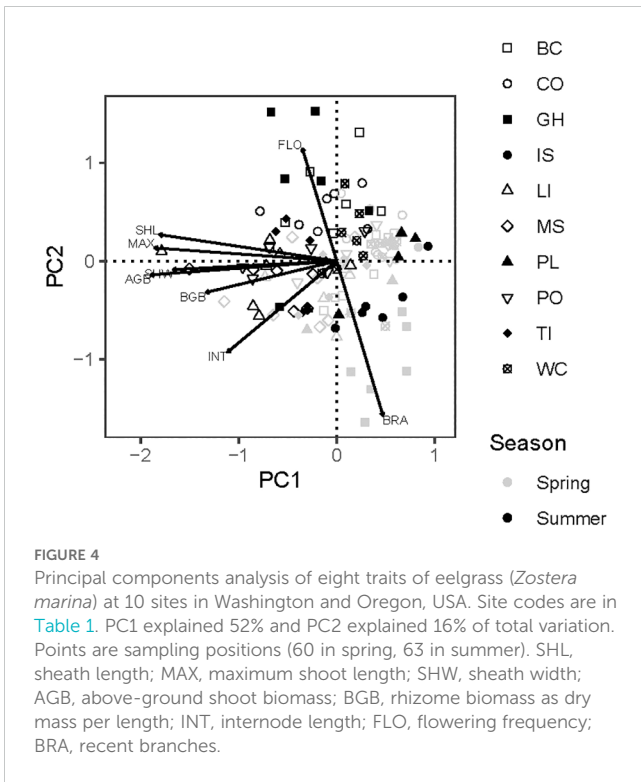
3.4 Recovery traits

Flowering frequency increased at higher elevations, but the increase at higher elevation tended to be moderated in off-bottom culture (negative interaction effect, $P=0.058$; Supplementary Table S2). Flowering had a positive effect size for ground culture, although not significant (Figure 6A). Branching frequency averaged 0.16/node in spring and 0.08/node in summer, and season was the only significant predictor (Figure 6B, Supplementary Table S2).

4 Discussion

Numerical and cover reductions reliably indicate stress for seagrass (Roca et al., 2016). In this study, the amount of eelgrass consistently declined at higher elevations, and oyster culture added stress, even though we deliberately selected positions in and out of culture across a full range of eelgrass densities. Intraspecific trait variation revealed a more complex set of responses to stressors, with some cases showing eroded resilience while others maintained resistance or recovery traits in oyster aquaculture. Eelgrass in ground culture was smaller above- but not below-ground. In off-bottom culture, eelgrass size decreased both above- and below-



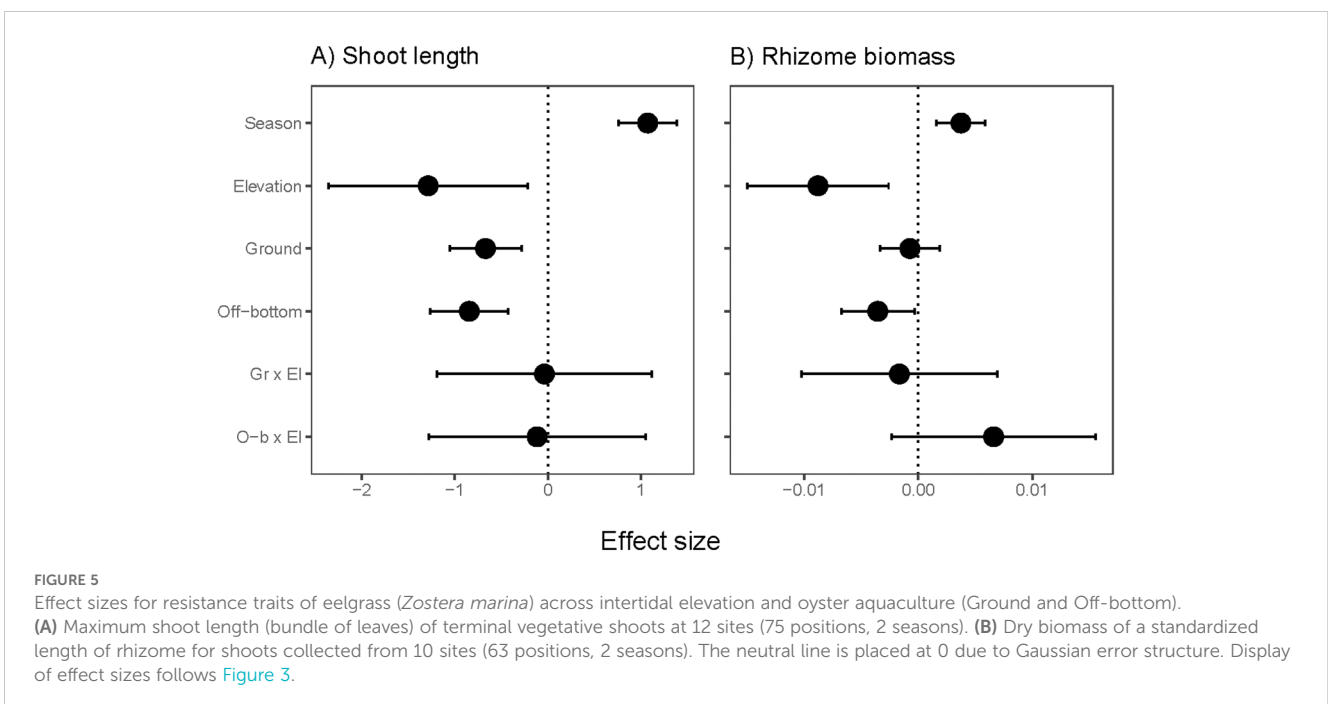


ground, and while branching did not change, recovery traits of flowering and rhizome extension diminished in some parts of the tidal elevation gradient.

Our field study design, where stressor treatments occurred at each site (blocked design), highlighted environmentally-modulated trait changes. However, due to low replication of treatments within sites, we could not explore how tidal elevation responses might

differ under varying culture methods at each site (including e.g., singles vs. clusters, or longlines vs. flip bags). Overall, the impact of aquaculture on resilience traits of eelgrass depended on the oyster grow-out method (Figures 1, 5, 6). This general result aligns with a meta-analysis showing that both ground culture and off-bottom culture reduce the amount of eelgrass, with only off-bottom culture affecting reproduction (Ferriss et al., 2019). Multiple stressors often act additively on seagrasses (Stockbridge et al., 2020), and this observational study was no exception. Statistically, only flowering showed an elevation x culture interaction, with large but not significant effect size ($P=0.058$), in which the enhanced flowering typical of higher elevations was induced less in off-bottom culture (Figure 6A).

Multivariate trait analyses in seagrasses commonly visualize correlated traits (e.g. (Nomme and Harrison, 1991)). When density and traits are both included, smaller shoots typically occur at higher density, with a second axis corresponding to the amount of eelgrass (i.e., cover, mass per area, or leaf area index; (Olesen and Sand Jensen, 1994; Vieira et al., 2018; Boyé et al., 2022; Duffy et al., 2022)). In our analysis (Figure 4), we focused on individual traits rather than population-level characteristics (density, cover). Both multivariate and individual trait analyses showed a seasonal shift from small, rapidly-branching shoots in spring to larger shoots in summer, when flowering became more evident (Figure 4, 5, 6, Supplementary Figure S3). Our results confirm that longer shoots share covarying traits like wider and longer sheaths, faster leaf and rhizome extension, and larger rhizomes (Ruesink et al., 2018; Boyé et al., 2022). This covariation makes it notable that shoot length but not rhizome mass differed in ground culture (Figure 5), a functionally-important decoupling of above- and below-ground responses. Smaller size of shoots above-ground may protect from further damage, but smaller storage below ground could undermine eelgrass’s ability to survive



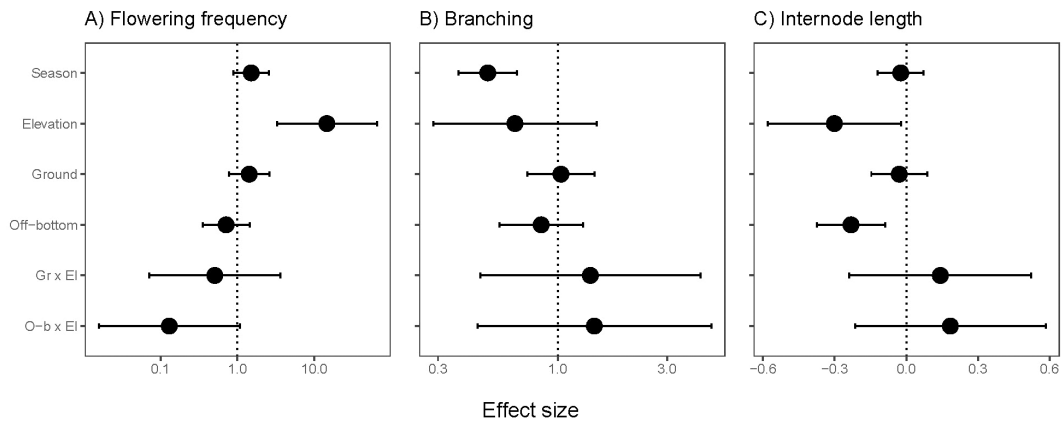


FIGURE 6

Effect sizes for recovery traits of eelgrass (*Zostera marina*) across intertidal elevation and oyster aquaculture (Ground and Off-bottom). (A) Flowering frequency based on quadrats at 12 sites (75 positions, 2 seasons). (B) Branching and (C) internode length of shoots collected from 10 sites (63 positions in 2 seasons). The neutral line is placed at 0 for Gaussian error structure and at 1 for flowering and branching with log-link error structure. Display of effect sizes follows Figure 3.

stressful conditions. Leaf damage may drive phenotypic plasticity in above-ground size, as subsequent leaves emerge smaller and less exposed to desiccation (Ruesink et al., 2012).

Tidal elevation strongly influenced both the amount and traits of eelgrass. Cover and density declined at higher elevations, consistent with sampling near the upper limit of eelgrass (Figure 3). Both above- and below-ground size declined at higher elevation, generally reducing resistance traits. Recovery traits were either induced (flowering) or reduced (internode length) at higher elevation, and branching also had a negative mean response to elevation. Although flowering is a resilience trait, recovery depends on successful seed set and germination, which we did not investigate. Shoot size has been shown repeatedly to decline at higher intertidal elevations (Keller and Harris, 1966; Boese and Robbins, 2008; Ruesink et al., 2012). Increased flowering at higher intertidal elevations has been reported in Willapa Bay and other coastal estuaries (Boese and Robbins, 2008; Ruesink et al., 2012). These responses, while common intertidally, are not universal. Flowering frequency may be unresponsive to depth (Yang et al., 2013; von Staats et al., 2021; Lekammudiyanse et al., 2024). Subtidal eelgrass studies report varying trait patterns with depth, such as taller shoots but unimodal relationships for amount of eelgrass and investment in flowering (Olesen et al., 2017). In *Z. marina*, long-term shading can reduce but sediment nutrients may increase flowering frequency (Backman and Barilotti, 1976; Johnson et al., 2017). Since our study occurred in the low-intertidal zone, we did not expect light limitations, unlike in subtidal eelgrass or under floating oyster bags where growth and flowering decline (Dennison and Alberte, 1985; Skinner et al., 2013), although wide gear spacing allows persistent seagrass (Ferretto et al., 2022). The timescale of our study cannot address interannual flowering variation, which can be substantial. We found <1% flowering in Hood Canal and Samish Bay in this study, but these bays have previously been

reported to have up to 7% and 10% flowering, respectively (Yang et al., 2013).

While elevation had strong statistical effects, emersion is not definitively the most important abiotic stressor for eelgrass, especially since we did not stratify samples across tidal elevations. Sediment properties, which reflect the overlying hydrodynamic regime, could also influence eelgrass traits through nutrient availability, phytotoxins, or anchoring (Koch, 2001). Lower-elevation sediments were generally muddier, which could drive trait responses if nutrient-rich muddy sediments promote growth or sandy sediments indicative of higher energy induce smaller shoots with low drag (Baden and Boström, 2001; Yang et al., 2013). Internode length might respond to local resource conditions as a foraging strategy to remain in high-resource patches (short internodes) or access new resources (long internodes) (De Kroon and Hutchings, 1995). We did not capture a range of sediment conditions that appeared stressful, so it was also not possible to address whether oyster culture could moderate this stressor.

Oyster aquaculture along the US west coast occurs on broad tidal flats overlapping with eelgrass at low intertidal elevations (Dumbauld and McCoy, 2015) where eelgrass already experiences strong environmental gradients of stress and seasonal light and thermal constraints. Our findings suggest that oyster aquaculture acts additively with natural stressors in most cases, with non-additive (marginal significance) effects observed in higher-elevation off-bottom culture where flowering decreased (Figure 1). Resistance and recovery, especially if induced by stress, have been essential for eelgrass to persist in the variable intertidal environment. Declining resilience traits, particularly when eelgrass amounts are low, raise concerns. Future work can therefore address not only aquaculture practices that maintain co-located seagrass (e.g. (Ferretto et al., 2022)), but also how they may enhance eelgrass resistance and recovery traits.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Mendeley Data DOI: [10.17632/d8vgk47fnz](https://doi.org/10.17632/d8vgk47fnz); <https://data.mendeley.com/datasets/d8vgk47fnz>.

Author contributions

JR: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. KH: Writing – review & editing, Investigation, Data curation. KK: Writing – review & editing, Validation, Supervision, Investigation. BD: Writing – review & editing, Resources, Investigation, Data curation. FB: Writing – review & editing, Investigation, Data curation. NL: Writing – review & editing, Investigation. BM: Writing – review & editing, Investigation. AS: Writing – review & editing, Investigation. BH: Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1427595/full#supplementary-material>

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