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

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Abiotic conditions

Sediment properties. Sediment organic content and percent fines were determined in 2-3 surface (2 cm) sediment samples at each position per site. Sediment was initially dried and broken apart mechanically. Samples were ashed at 500°C for 3 hours, and organic content determined by loss-on-ignition. Ashed samples were processed for 2 min in a sieve shaker (Ro-Tap sieve shaker, Mentor, Ohio, USA) to determine grain size fractions (Wentworth scale) and summarized by percent fines (<0.063 mm; Supplemental Fig. 1).

Water temperature. Water temperatures were recorded near study sites and summarized for the year when that site was measured for eelgrass. Water temperatures were downloaded from the surface mooring in closest proximity to the sites in each bay (Supplemental Table 1). Temperatures were downloaded at hourly intervals, except for Hood Canal (approximately 6-hr intervals), for the year in which surveys were carried out at nearby intertidal sites. Temperatures were summarized from 1 Apr through 31 Aug (Supplemental Fig. 2).

Eelgrass measurements

Seasonal patterns occurred in eelgrass (*Zostera marina*) in bays of Washington, Oregon, and California, USA (Supplemental Figure 3). Supplemental Figure 4 provides a schematic diagram of the trait measurements. Measurements included density and maximum length (Supplemental Fig. 5). In situ measurements included density, length, and flowering at 12 sites (Supplemental Fig. 6). Measurements on collected shoots included dry rhizome mass per length, number of recent branches (within the sheath or at the two most recent nodes), and internode length (Supplemental Fig. 7).

Hood Salish Willapa Bay

Canal Sea

Supplemental Figure 1. Elevation and sediment conditions at intertidal positions in and outside oyster aquaculture across 12 sites in Washington and Oregon, USA, distinguishing aquaculture levels (no culture, ground culture, off-bottom culture of longlines or flip bags). Sig = significant difference between ground culture, off-bottom culture, and no culture (none).

HH (47.55°N, -123.03°W),

RP (47.81, -122.85),

IS (48.58, -122.48),

PL (48.61, -122.44),

GH (46.88, -124.08),

CO (46.67, -123.95),

WC (46.65, -123.96),

BC (46.64, -123.95),

LI (46.51, -123.99),

PO (46.50, -124.02),

MS (46.49, -123.02),

TI (45.52, -123.94)



Supplemental Figure 2. Water temperatures across seven bays through spring and summer when sites were sampled for eelgrass (*Zostera marina*). Most records were at hourly intervals, except Hood Canal every 6 hours. See Table 1 for sources. See Supplemental Table 1 for regression variables. Humboldt data at North Spit were much closer to the mouth of the bay than is typical for aquaculture, and coincident data at the CenCOOS station were 0.87°C warmer; CenCOOS data were used through 10 Jul, after which no data were available there, and 0.87°C was added to North Spit records.



Supplemental Figure 3. Eelgrass (*Zostera marina*) across meadows (dense eelgrass outside aquaculture) in the study region, including California bays where eelgrass was measured only outside aquaculture. A) Percent cover in 20 quadrats per site. B) Maximum shoot length of ~50 terminal shoots per site. C) Flowering frequency is number of flowering shoots divided by total shoot count. Bay codes as in Supplemental Table 1. Error bars are SE due to multiple meadows measured in some bays (WBN [6], WBS [6], TO [3], GH [2], SB [2], HC [2]).



Supplemental Figure 4. Schematic diagram of eelgrass (*Zostera marina*) showing trait measurements.



Supplemental Figure 5. Density and maximum length of eelgrass (*Zostera marina*) across meadows (dense eelgrass outside aquaculture) in the study region, including California bays where eelgrass was measured only outside aquaculture. Error bars are standard deviation (20 plots for density, ~50 shoots for maximum length). Site codes as in Supplemental Figure 1.



Supplemental Figure 6. Density and traits of eelgrass (*Zostera marina*) in spring and summer across study sites in Washington and Oregon, USA, distinguishing aquaculture levels (no culture, ground culture, off-bottom culture of longlines or flip bags). These values are from in situ measurements in quadrats at each of 12 sites, showing averages across 20 quadrats (~50 terminal shoots for length). Site codes as in Supplemental Fig. 1.



Supplemental Figure 7. Traits of eelgrass (*Zostera marina*) in spring and summer across study sites in Washington and Oregon, USA, distinguishing aquaculture levels (no culture, ground culture, off-bottom culture of longlines or flip bags). These values are averages across ~50 terminal shoots collected at each of 10 sites. Site codes as in Supplemental Fig. 1.

Supplemental Table 1. Sources of water temperature data near all study sites in seven bays (including low- and high-residence time portions of Willapa Bay) along the US west coast. Number of samples spans 1 Apr to 31 Aug in the same year when nearby eelgrass was surveyed.

|  |  |  |  |
| --- | --- | --- | --- |
| Bay | Number of samples | Tide station for elevation estimates by water level | Water temperature source |
| Hood Canal (HC) | 372 | RP: Quilcene, Dabob, Bay, Hood Canal Tidal Station 9445272, NOAA/NOS/CO-OPSHH: Ayock Point Tidal Station 9445388, NOAA/NOS/CO-OPS | ERDDAP ORCA4, 2.75 dbar depth47.803, -122.803 |
| Samish Bay (SB) | 3672 | Bellingham Tidal Station 9449211, NOAA/NOS/NO-OPS | Padilla Bay NERR Ploeg channel48.556, -122.530 |
| Grays Harbor (GH) | 3666 | NANOOS Station 9441102 - Westport, (Data Provider: NOS/CO-OPS) | NOAA Tides & Currents, station 9441102 46.905, -124.105 |
| Willapa Bay North (wk residence time, WBN) | 3626 | (LiDAR data) | NOAA Tides & Currents, station 944091046.708, -123.967 |
| Willapa Bay South (mo residence time, WBS) | 3279 | (LiDAR data) | iButton temperature loggers at Port of Peninsula46.501, -124.028 |
| Tillamook Bay (TI) | 2923 | (LiDAR data) | OR Dept of Environment Quality sonde45.529, -123.937 |
| Humboldt Bay (HB) | 3663 | NA | CenCOOS40.7775,-124.1965NOAA Tides & Currents, station 941876740.767, -124.238 |
| Tomales Bay (TO) | 3270 | NA | NDBC station BDXC1 38.317, -123.071 |

Supplemental Table 2. Results of generalized linear mixed models relating eelgrass amount or traits to factors of season, intertidal elevation, and oyster culture. Values are effect sizes (SE). Elev = Elevation, Gr = Ground culture, Ob = off-bottom culture. Bold shows P<0.05. Italic shows P<0.1.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Response | Cover (arcsine-square root transform) | Density | Max L | Rhizome mass effect size x 100 | Internode L | Branching | Flowering |
| Data distrib | Tweedie | Tweedie | Gaussian | Gaussian | Gaussian | Tweedie | Tweedie |
| Sample size [Sites] | 236 [12] | 236 [12] | 149 [12] | 123 [10] | 123 [10] | 125 (10) | 150 [12] |
| Intercept | -**0.77 (0.19)** | 2.68 (0.28) | **56.04 (5.24)** | **2.70 (0.16)** | **1.40 (0.08)** | **-0.79 (0.20)** | **-4.03 (0.33)** |
| Summer season | 0.17 (0.13) | -0.15 (0.16) | **18.22 (2.65)****Also Su x Ob neg** | **0.37 (0.11)** | -0.023 (0.049) | **-0.70 (0.15)** | 0.41 (0.27) |
| Elevation | **-1.91 (0.38)** | **-2.65 (0.46)** | **-19.58 (8.92)** | **-0.88 (0.32)** | **-0.301 (0.142)** | -0.43 (0.42) | **2.67 (0.76)** |
| Ground culture | **-0.31 (0.15)** | **-0.54 (0.20)** | **-11.33 (3.25)** | -0.07 (0.13) | -0.030 (0.060) | 0.032 (0.173) | 0.35 (0.31) |
| Off-bottom culture | -0.16 (0.17) | *-0.35 (0.21)* | **-13.59 (3.53)** | **-0.35 (0.16)** | **-0.232 (0.073)** | -0.17 (0.21) | -0.34 (0.36) |
| Elev x Gr | -0.16 (0.46) | -0.68 (0.57) | -0.56 (9.72) | -0.17 (0.44) | 0.142 (0.195) | 0.33 (0.56) | -0.68 (1.00) |
| Elev x Ob | -0.13 (0.47) | -0.27 (0.58) | -0.77 (9.77) | 0.66 (0.46) | 0.184 (0.204) | 0.37 (0.60) | *-2.04 (1.08)* |
| McFadden’s pseudo-R2 | 0.11 | 0.04 | 0.05 | 0.03 | 0.31 | 0.29 | 0.18 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Response | Cover | Density | Sqrt (Max L) | Rhizome mass | Internode L | Branching | Flowering |
| Data distrib | NA | NA | Gaussian | Gaussian | Gaussian | Tweedie | Tweedie |
| Sample size [Sites] |  |  | 149 [12] | 123 [10] | 123 [10] | 125 [10] | 150 [12] |
| Intercept |  |  | **7.73 (0.30)** | 2.75 (0.19) | **1.38 (0.82)** | **-0.86 (0.21)** | **-4.1 (0.42)** |
| Summer season |  |  | **1.08 (0.15)** | 0.37 (0.11) | -0.027 (0.049) | **-0.70 (0.15)** | 0.38 (0.28) |
| Sparse eelgrass |  |  | **-0.92 (0.25)** | -0.25 (0.19) | 0.025 (0.082) | 0.19 (0.24) | 0.54 (0.42) |
| Ground culture |  |  | **-0.90 (0.24)** | -0.15 (0.18) | -0.006 (0.078) | 0.036 (0.228) | 0.16 (0.41) |
| Off-bottom culture |  |  | **-0.75 (0.24)** | -0.24 (0.18) | *-0.146 (0.081)* | -0.049 (0.239) | -0.02 (0.43) |
| Sparse x Gr |  |  | **0.77 (0.38)** | 0.22 (0.28) | -0.041 (0.122) | -0.057 (0.354) | -0.14 (0.61) |
| Sparse x Ob |  |  | -0.34 (0.37) | -0.12 (0.28) | -0.193 (0.123) | -0.31 (0.37) | -1.00 (0.67) |
| McFadden’s pseudo-R2 |  |  | 0.16 | 0.02 | 0.29 | 0.28 | 0.08 |