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Flowering effort, phenology, and morphology in intertidal seagrass (Zostera marina) vary along a temperature gradient within a Northeast Pacific estuary

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Research Article

Keywords: Seagrass flowering, phenology, morphology, spatio-temporal variability, temperature, eelgrass

Posted Date: September 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10743839/v1

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Title

Flowering effort, phenology, and morphology in intertidal seagrass (*Zostera marina*) vary along a temperature gradient within a Northeast Pacific estuary

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Submission Date

8/17/2026

Acknowledgements

For field and laboratory assistance, we deeply thank South Slough National Estuarine Research Reserve volunteers Janelle Kiewert, Kenny Kapler, and Misty White, South Slough staff Adam DeMarzo, Shon Schooler, and Jaime Belanger, and Oregon State University affiliates Zahra Vogel, Amber Newman, MK English, Michael Davison, Irie Cheng, and Adrian Puga, IMEDEA affiliate Xesca Reynes, and South Slough interns Chloe Cummins and Shreyann Seth. This study was made possible by funding from the National Oceanic and Atmospheric Administration 2022 – 2024 Margaret A. Davidson Fellowship to RSM and FT, the Mamie Markham Hatfield Scholarship 2023 – 2025 to LB, and the project PID2024-160458OB-I00, funded by the MICIU /AEI /10.13039/501100011033 / FEDER, UE) to FT.

Abstract

As seagrass populations face increasing environmental change, understanding fine-scale variation in sexual reproduction, which underpins recovery from disturbance, dispersal, and adaptive potential, is critical to successful conservation and restoration strategies. Temperature is both a driver of growth and reproduction and a primary facet of global change, making its relationship to sexual reproduction especially important to resolve. We investigated flowering in eelgrass (*Zostera marina*), a globally distributed seagrass facing widespread declines, across five sites (spanning 7 km) and two tidal heights within an estuary and assessed relationships to thermal conditions. Sites, but not tidal heights, were differentiated by temperature metrics describing warming trends (average daily mean and maxima) and temperature variability (average daily range). Eelgrass in the lower intertidal consistently exhibited greater abundance, flowering effort, and flowering shoot length, but rhipidia and spadices (with developing seeds) per shoot showed no clear vertical pattern. Instead, these morphological traits were positively associated with daily temperature variability, after accounting for spatial structure (i.e., site and tidal height) and time. Phenological variation in flowering effort was observed between meadows; the warmest site had a distinct signal of earlier flowering with predicted peaks between eight and ten weeks earlier than other sites. Our results point to important relationships between fine-scale environmental variation and the sexual reproductive dynamics of eelgrass populations, underscoring the need to consider small-scale and site-specific factors in eelgrass conservation and restoration strategies.

Key words

Seagrass flowering, phenology, morphology, spatio-temporal variability, temperature, eelgrass

Statements and Declarations

The authors declare no competing interests.

Introduction

Seagrasses are globally distributed nearshore foundation species (Hemminga & Duarte, 2000) that provide essential ecosystem services (e.g., fisheries, water quality, climate change attenuation; Barbier et al., 2011), but are now facing the cumulative effects of anthropogenic stressors, including a rapidly changing climate (Orth et al., 2006; Short & Wyllie-Echeverria, 1996). As a result, seagrass losses are occurring worldwide (Marbà & Duarte, 2010; Strydom et al., 2020; Thomson et al., 2015, Dunic et al. 2021), creating urgency around understanding how seagrass meadows can recover from mortality events.

The recovery for these marine flowering plants (i.e., marine angiosperms) may be lengthy, uneven or nonexistent (O'Brien et al., 2018) and is biologically driven by two main

reproductive mechanisms: vegetative propagation (i.e. asexual) via lateral expansion of ramets (clones), and sexual reproduction through flowering, fertilization, seed production, and seedling recruitment. While clonal propagation is often the primary means for the maintenance and expansion of perennial seagrass beds (Marbà & Duarte, 1998), sexual reproduction can become critical for initial recovery from large mortality events through seed germination and seedling recruitment after a disturbance or stressor has subsided (Greve et al., 2005; Jarvis & Moore, 2010; Lee et al., 2007; Plus et al., 2003; Ruesink et al., 2025). The products of sexual reproduction (e.g., flowers, fruits, seeds) also play a key role in long-distance dispersal, potentially allowing expansion of populations to other habitable locations (Harwell & Orth, 2002; Källström et al., 2008). Furthermore, and in contrast to clonal propagation, sexual reproduction significantly contributes to increases in standing genetic diversity (Johnson et al., 2020; Reusch, 2006), which provides a mechanism for adaptation to future environmental change through natural selection (Ackerman, 2006) and increases the resistance to and recovery from disturbances (Jahnke et al., 2015; Hughes & Stachowicz, 2004; Reusch et al., 2005).

Importantly, identifying fine-scale variation in flowering is essential, not only due to its influence on population health and resilience, but also for successful restoration efforts. Seagrass restoration is generally costly, laborious, and variably successful (Van Katwijk et al., 2009), and seed-based restoration methods are gaining traction (e.g., Golden et al., 2010; Govers et al., 2022; Orth et al., 2009; Zhao et al., 2016) as a scalable restoration option that potentially minimizes impact on donor beds while preserving genetic diversity and adaptive capacity at the restored site (Williams & Davis, 1996). Successful seed-based restoration plans hinge on accurate estimates of flowering magnitude and timing to inform harvesting efforts (Infantes & Moksnes, 2018).

In seagrasses, variation in flowering effort, phenology, and morphology is observed across environmental gradients, such as temperature, light, hydrodynamics, and nutrient availability (Cabaço & Santos, 2012; Lekammudiyanse et al., 2024; McDonald et al., 2016), with temperature regarded as one of the primary factors in controlling the timing and development of flowering (Bernier et al., 1993). Eelgrass (*Zostera marina* L.), found across the northern hemisphere (Short & Green, 2003) and occupying intertidal to shallow subtidal waters (Moore & Short, 2006), generally flowers annually, beginning in late spring following the seasonal increase in photoperiod and temperature (Hemminga & Duarte, 2000). Across its range, flowering in eelgrass is generally delayed as photoperiod and temperature decrease with increasing latitude (Blok et al., 2018; Silberhorn et al., 1983). In addition, regional environmental variation can override the latitudinal effect on phenology, observed as earlier flowering at relatively warmer locations (Vercaemer et al., 2021). Flowering effort (i.e., the proportion of total shoots that flower), can also vary across sites at a regional scale, with warmer, protected sites having both higher (Krumhansl et al., 2021; Vercaemer et al., 2021) and lower (Qin et al., 2020b; Vercaemer et al., 2021) flowering effort compared to cooler, exposed sites.

Furthermore, phenology and flowering effort also vary along the vertical depth gradient within meadows. Flowering timing is typically delayed with increasing depth (Infantes &

Moksnes, 2018; Vercaemer et al., 2021; Von Staats et al., 2021), while flowering effort in subtidal populations follows a unimodal distribution with depth (Olesen et al., 2017), peaking at intermediate depths and declining towards shallow and deep margins as exposure or light limitation increase. Yet, flowering effort can also increase at the upper margins of a meadow (Jacobs & Pierson, 1981; Ruesink et al., 2012, 2022, 2024). Greater environmental stress has been proposed to cue flowering, and in some populations, to drive a shift toward an annual life history characterized by significantly higher flowering effort (Boese & Robbins, 2008; Harrison, 1993).

Increased flowering effort has been suggested as a warming-induced response in another seagrass (*Posidonia oceanica*; Astruch et al. 2026; Diaz-Almela et al., 2007), while evidence for this relationship in eelgrass is inconsistent. Increasing sea surface temperatures are associated with both increased (Potouroglou et al., 2014) and decreased eelgrass flowering (Qin et al., 2020a), while cooler temperatures associated with La Niña conditions in the Northeast Pacific have, conversely, been linked to greater flowering and overall abundance (Thom et al., 2003). Separately, experimental evidence supports earlier eelgrass flowering under warmer temperatures (Nolan et al., 2026; Sawall et al., 2021), independent of a latitudinal effect. Given this limited and often conflicting understanding of how temperature influences eelgrass flowering, and in the context of rapid climate-change driven warming (IPCC, 2023), it is increasingly urgent to investigate spatial patterns of flowering effort and phenology alongside their relationship with temperature.

The aim of this study is to characterize spatial and temporal variation in flowering of intertidal *Z. marina* populations and relationships with water temperature using lower Coos Bay, Oregon, as a study case. The southern (South Slough) arm of this estuary experienced substantial eelgrass loss following an unprecedented Northeast Pacific marine heatwave from 2014 to 2016 (Magel et al., 2022; Marin Jarrin et al., 2022), with subsequent recovery trajectories varying considerably across four sites (Marin Jarrin et al., 2022). These differences in recovery suggest that underlying mechanisms of recovery (e.g., sexual reproduction) may differ among locations in close proximity, highlighting the need to characterize local variation in flowering. We conducted our study at two tidal heights at multiple sites within the estuary to investigate the spatial scale and temporal patterns of variation in eelgrass flowering metrics (e.g., flowering effort, peak timing, shoot characteristics). We then examined relationships between flowering and temperature metrics to identify potential drivers of spatiotemporal variation across the estuary. Our study provides a framework for other nearshore and estuarine waters where eelgrass inhabits a range of environmental conditions that may influence sexual reproductive patterns.

Methods

Study Sites

Along the northern Pacific coast of North America, perennial eelgrass (i.e., *Zostera marina*) populations generally inhabit soft sediments from intertidal to shallow subtidal zones in

nearshore and estuarine waters (+ 1 to - 2 m relative to mean lower low water; Hannam et al., 2015). Flowering in this region typically begins in spring and continues through summer, representing a small fraction of shoots in a meadow (generally < 20%, Yang et al., 2013; Ruesink et al., 2022). Coos Bay is located in Southern Oregon, USA, where perennial intertidal eelgrass beds historically exist along an estuarine gradient (Everett et al., 1995; Hayduk et al., 2019). During the dry, summer months, upwelling brings colder, nutrient-rich ocean waters into the bay (typical of many U.S. Pacific Northwest estuaries), and in the winter months, downwelling prevails, accompanied by higher precipitation and increased freshwater inputs (Conroy et al., 2020; Hayduk et al., 2019; Hessing-Lewis & Hacker, 2013). We studied five sites spanning a geographic range of ~ 7 km (Fig. 1, Table S1) where eelgrass presently exists. Two of these sites – Valino Island and Collver Point – have been monitored quarterly to annually by the South Slough Estuarine Research Reserve, and total shoot density data after the occurrence of seagrass loss associated with the 2014-2016 marine heatwave (Marin Jarrin et al., 2022) reveal differing patterns of recovery. Eelgrass densities at Collver Point steadily returned to pre-decline abundances by 2020 (from 40 shoots m^{-2} in 2010, 10 shoots m^{-2} in 2016, to 44 shoots m^{-2} in 2022, to 55 shoots m^{-2} in 2025), while densities at Valino Island remained low until 2025 (106 shoots m^{-2} in 2010, 14 shoots m^{-2} in 2016, 8 shoots m^{-2} in 2022, and 66 shoots per m^{-2} in 2025) (South Slough NERR 2025, Marin Jarrin et al., 2022).

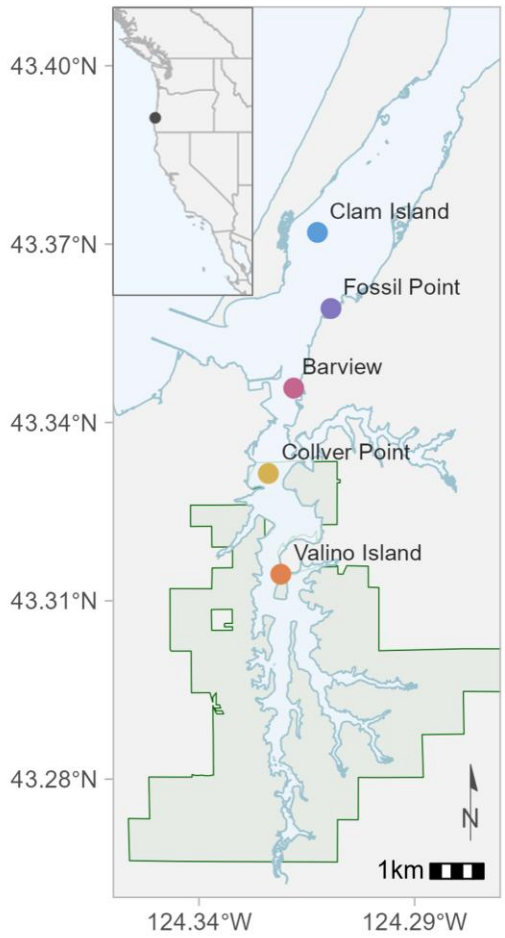


Fig. 1 Map depicting survey locations. Study sites are in lower Coos Bay and South Slough estuary, Oregon, USA, and span a geographic range of approximately 7 km. South Slough National Estuarine Research Reserve (SSNERR) is denoted in green. Site coordinates and estimated depths are found in Table S1

Eelgrass abundance and flowering

At each site, we established 100-meter long transects oriented parallel to the shoreline at two tidal heights; one established close to the upper limit of the intertidal bed, and another established at the lower intertidal margin of the bed. Horizontal distance between upper and lower transects of a site ranged between 10 and 60 m (Table S1). We conducted field surveys from January to December 2023 to characterize eelgrass meadow and shoot traits through time. We surveyed every two weeks during July and August and monthly during other months. Not all sites or tidal heights could be sampled each month due to limited accessibility during evenings or stormier weather, with higher low tides in winter.

During each field survey, we recorded the number of flowering and vegetative shoots at five-meter intervals along the transect (n = 20) using a 0.25 m x 0.25 m (0.0625 m^{-2}) quadrat and calculated flowering effort as the ratio of flowering to total shoots (sum of flowering and

vegetative). In addition, ten flowering shoots were randomly collected along each transect. For each flowering shoot, we 1) measured shoot length from the base of the stem to the distal tip of the leaf from the youngest apical rhipidium, 2) counted the total number of rhipidia and spathes, and 3) recorded the developmental stage of each spadix (the seed-bearing organ enclosed in each spathe) (Fig. S1). We defined spadix developmental stages according to DeCock (1980): style erection (Growth stage 1), back-bending of the styles after pollination (GS2), erection of anthers and release of pollen (GS3), seed maturation after anthers dehisce (GS4), seed release (GS5), and empty and senescing spadix (GS6). For the purposes of this study, we define the "shoot reproductive output" for a flowering shoot as the number of growth stage 4 (GS4) spadices (fertilized and developing seeds) per shoot, and transect-level reproductive output as GS4 spadices per m^{-2}.

Temperature regimes

Temperatures in each eelgrass bed and at each tidal height were recorded semi-continuously (i.e. every 15 minutes) at 5 cm below the water-sediment interface using HOBO© temperature data loggers (Onset © Corporation) situated at the midpoint of each transect. We calculated a suite of daily temperature metrics (Fig. S2) to investigate differences in patterns of warming and thermal variability among and within sites, summarized across three-week window periods prior to each sampling date at a given site and tidal height, as in Wong and Dowd (2023). To minimize multicollinearity prior to multivariate analyses, we calculated pairwise Pearson correlation coefficients among temperature metrics within each season. The retained metrics (pairwise coefficients ≤ 0.75; Dormann et al., 2013) included mean, minimum, maximum, and daily range calculated from the 10th to 90th percentile (Fig. S2). Daily values for means, minima, and maxima were calculated from raw data and averaged across the three-week window, and range (10th to 90th percentile) was calculated as the difference between daily minima (10th percentile) and maxima (90th percentile) and averaged across the three-week window.

Statistical Analyses

We used generalized linear models (GLMs; Zuur et al., 2009) to compare eelgrass shoot density (negative binomial with log link), flowering effort (quasibinomial with logit link), shoot length (Gaussian), GS4 spadices per shoot (negative binomial with log link), and number of rhipidia per shoot (Gaussian) during the 2023 flowering season (May through August) between sites and tidal heights (unordered factors), and through time (tide series as an ordered factor). While we observed early stage flowering shoots across sites in April, we did not include this tide series in our statistical analysis because one site was not sampled (these sampled data remain in our visualizations). From these GLMs, we selected a best-fitting model by comparing Akaike Information Criterion (AIC) values and akaike weights (quasi-AIC and weights for the quasibinomial model) between (1) a model of main effects only, (2) three models of main effects

with one of three two-way interactions, and (3) a model with main effects and all two-way interactions. The contribution of individual variables was assessed using analysis of deviance via the "anova()" function in the "stats" package in R. We computed estimated marginal means for post-hoc comparisons between factor levels of site and tidal height using the "emmeans" package in R (Lenth et al., 2025). Gaussian GLMs were conducted using the "stats" package in R and negative binomial and binomial GLMs were conducted using the "MASS" package in R (Venables & Ripley, 2002). In addition, for each site and tidal height at each sampling time point, an estimate of transect-level reproductive output was calculated as the product of a) mean flowering shoot density (m^{-2}) and b) mean number of GS4 spadices per shoot, with the SE of the product estimated via standard error propagation (Goodman, 1960).

We used generalized additive models (GAMs; Zuur et al., 2009) to examine variation in the phenology of flowering effort across sites and tidal heights over twelve months. GAMs are appropriate for modeling complex nonlinear relationships between response and explanatory variables (Wood, 2017), as observed with the seasonality of eelgrass flowering. We fit the GAM with a global temporal smooth term to model an *a priori* overall seasonal pattern, separate smooth functions estimated for data from each site and tidal height for any deviations from this global pattern, and site and tidal height as additional fixed parametric predictors. Smooth functions were created with cyclic smoother regression splines to model the seasonality in the response variables by constraining the beginning and end points of our time component (day-of-year, [0-365]) (Pedersen et al., 2019). We extracted the predicted timing of peaks across sites and tidal heights; predictions were made on a dataset of 365 days so that predicted peaks were not constrained to our sampling dates.

To characterize temperature variation across sites and tidal heights, we performed unconstrained ordination (principal component analysis, PCA) on temperature metrics (daily mean, minima, maxima, and 10th to 90th percentile range) that were standardized (mean = 0, SD = 1) prior to analysis. We assessed PC axis significance and temperature metric contributions using "PCAtest" in R, which runs 1,000 random permutations and uses bootstrap resampling to compute 95% confidence intervals around explained variance percentages and mean loadings (Camargo, 2022). To test whether temperature metrics were significantly structured spatially, we conducted permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) on PC scores using site and tidal height as explanatory variables.

We conducted partial redundancy analysis (pRDA) to test whether thermal conditions explained significant variation in eelgrass flowering traits after removing spatial effects (i.e., site and tidal height). Trait and temperature metrics were standardized (mean = 0, SD = 1) separately. Out of the four temperature metrics used in the earlier PCA, we dropped minimum temperature in order to ensure acceptable values for variance inflation factors of < 5 (Kim, 2019). We assessed model significance using permutation tests and tested individual RDA axes and terms (temperature metrics). Trait and temperature variable loadings on significant RDA axes were used to identify candidate trait-temperature relationships for subsequent analysis at the transect level using generalized linear mixed models (GLMMs), with day-of-year as a covariate. We used

AIC to identify the best-fitting model, indicative of the strongest temperature predictor for each assessed trait. For these best-fitting models, we compared a fixed-effects-only model against an identical model with nested random intercepts for site and within-site tidal height differences (site:tidal height) using a likelihood ratio test (LRT). Multivariate analyses were performed using the "vegan" package (Oksanen et al., 2022) and GLMMs using the "glmmTMB" package in R (Brooks et al., 2017).

Results

Variation in flowering effort, plant abundance, and flowering shoot traits

Between April and early September, flowering effort measured at a given tidal height and sampling day ranged from 1% to 27% (± 6%) (Fig. 2a). Flowering effort was consistently greater at lower tidal heights (log odds = 1.059, $p < 0.001$), corresponding to a 30% relative increase (9.1% vs. 11.8% of flowering shoots, Tukey contrasts, $p = 0.006$; Fig. 2b). While flowering effort also varied significantly among sites ($p < 0.001$, Table S2), the only significant pairwise comparison was between the site with the greatest flowering effort (Barview lower, peaking at 27%) and the site with the least (Fossil Point upper, peaking at 10%) (Fig. 2b).

Average eelgrass shoot density spanned 45 (± 13) to 292 (± 51) shoots m^{-2} (Fig. 2c), and increased at lower tidal heights (log mean = 0.271, $p < 0.001$), with densities 33% higher than at upper tidal heights while holding other variables constant (Tukey contrasts, $p < 0.001$; Fig. 2d), although this pattern disappeared at Clam Island (Tukey contrasts, n.s.). Across sites, shoot density tended to be highest in spring (April and May), and decreased gradually throughout summer until early August (Fig. 2c).

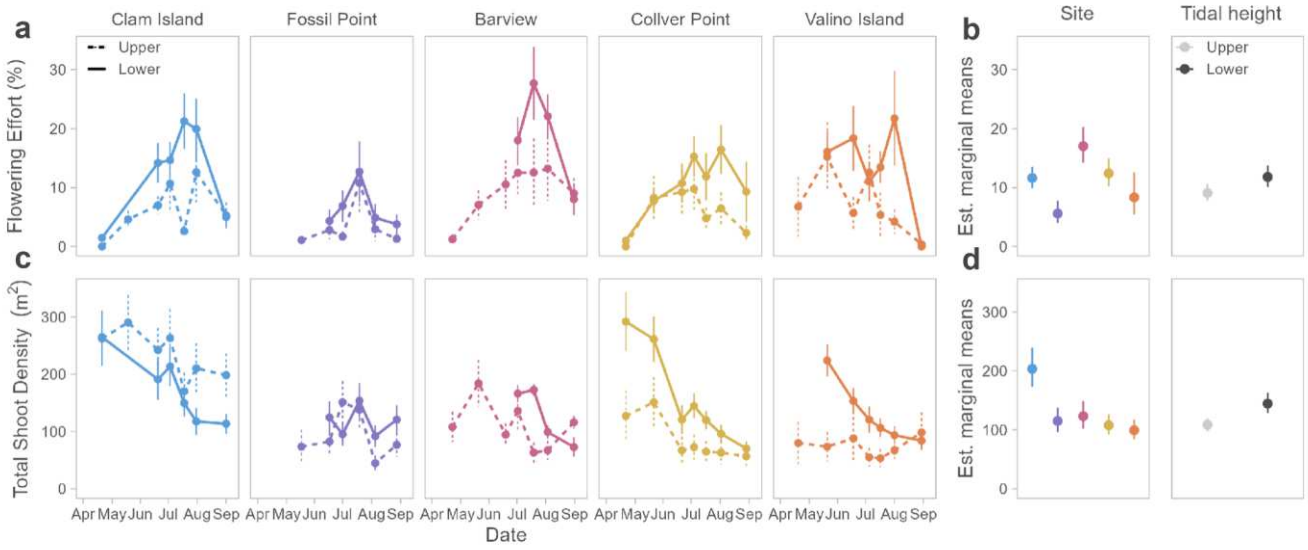


Fig. 2 Mean (± SE) and estimated marginal mean (± SE) of *Zostera marina* flowering effort (a, b) and total shoot density (c, d) across sites and tidal heights from April through early September of the 2023 flowering season. Sites are denoted by colors and tidal heights by line type (in line graphs) and shade (in est. marginal means plots). Total shoot density (m^{-2}) was estimated from 0.25 x 0.25 m (0.0625 m^{-2}) quadrats (n = 20 for each site and tidal height on a given sampling date)

Across the entire flowering season, flowering shoots at the lower tidal height (averaging 181 ± 12 cm) were almost twice as long as flowering shoots from the upper tidal height (averaging 108 ± 9 cm; Tukey contrasts, $p < 0.001$; Fig. 3a-b). There was also a significant effect of site ($p < 0.001$), and between site and tidal height ($p < 0.001$, Table S2) on shoot length; across the upper tidal height, significantly shorter shoots were found at Clam Island (Tukey contrasts, $p < 0.001$).

Both the number of rhipidia per shoot and GS4 spadices per shoot differed significantly across sites ($p < 0.001$, Fig. 3c-f; Table S2), but not tidal heights. Valino Island had the highest average number of rhipidia per shoot (peak of 8 ± 1 rhipidia in early August; Fig. 3d, Tukey contrasts, $p < 0.05$). The number of GS4 spadices per shoot was lowest at the site with the longest shoots (Barview, averaging 6 ± 1 GS4 spadices per shoot, Fig. 3e-f), and significantly lower than that of Clam and Valino Island (Fig. 3f; Tukey contrasts, $p < 0.001$).

Peak reproductive output at the transect level ranged from 131 (± 80, Valino upper) to 515 (± 145, Barview lower) GS4 spadices m^{-2} (Table S3). Average output across the flowering season ranged from 45 (± 25, at Fossil Point upper) to 265 (± 51, Clam Island lower) GS4 spadices m^{-2} (Fig. S3), and these patterns resembled more closely those of flowering effort (Fig. 2a) than of GS4 spadices per shoot (Fig. 3c). Reproductive output was greatest at all lower tidal heights except for that of Fossil Point (Fig. S3, Table S3).

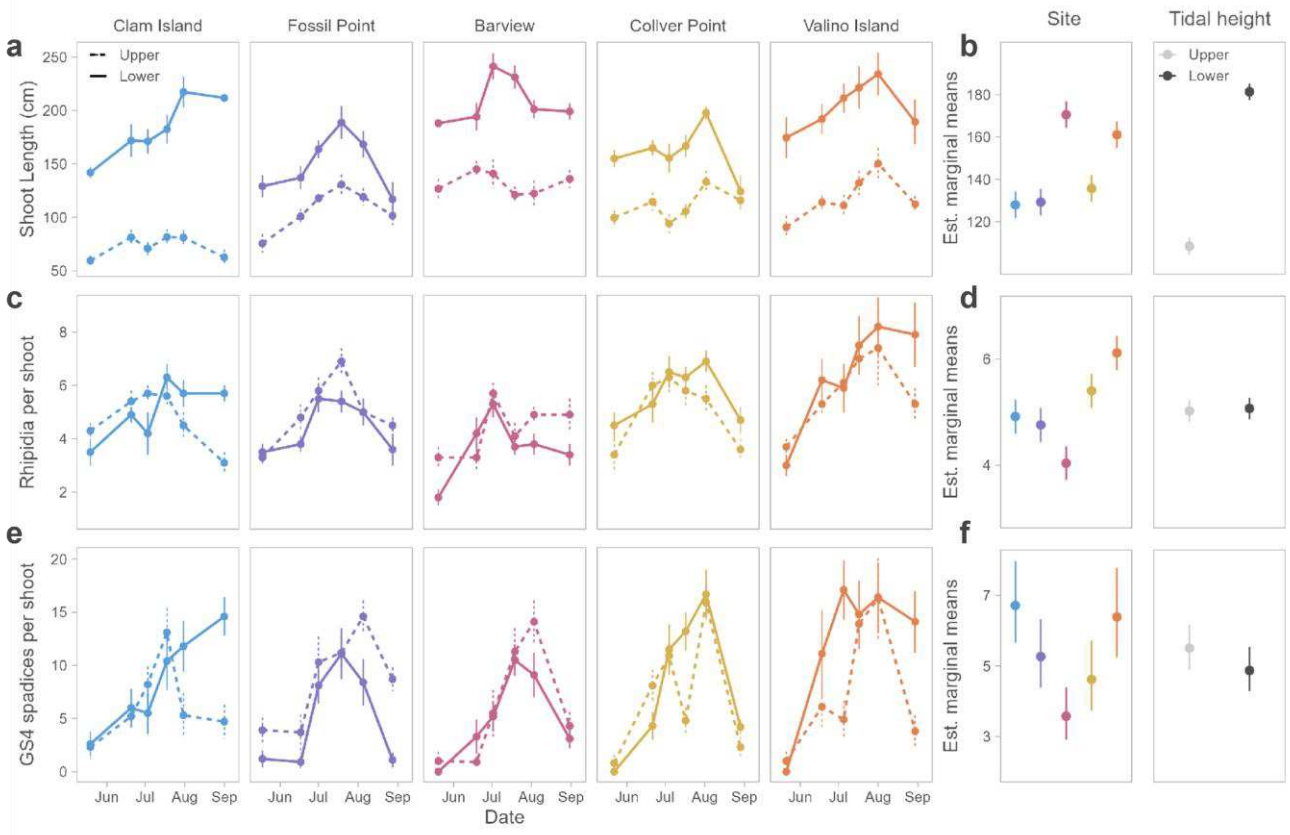
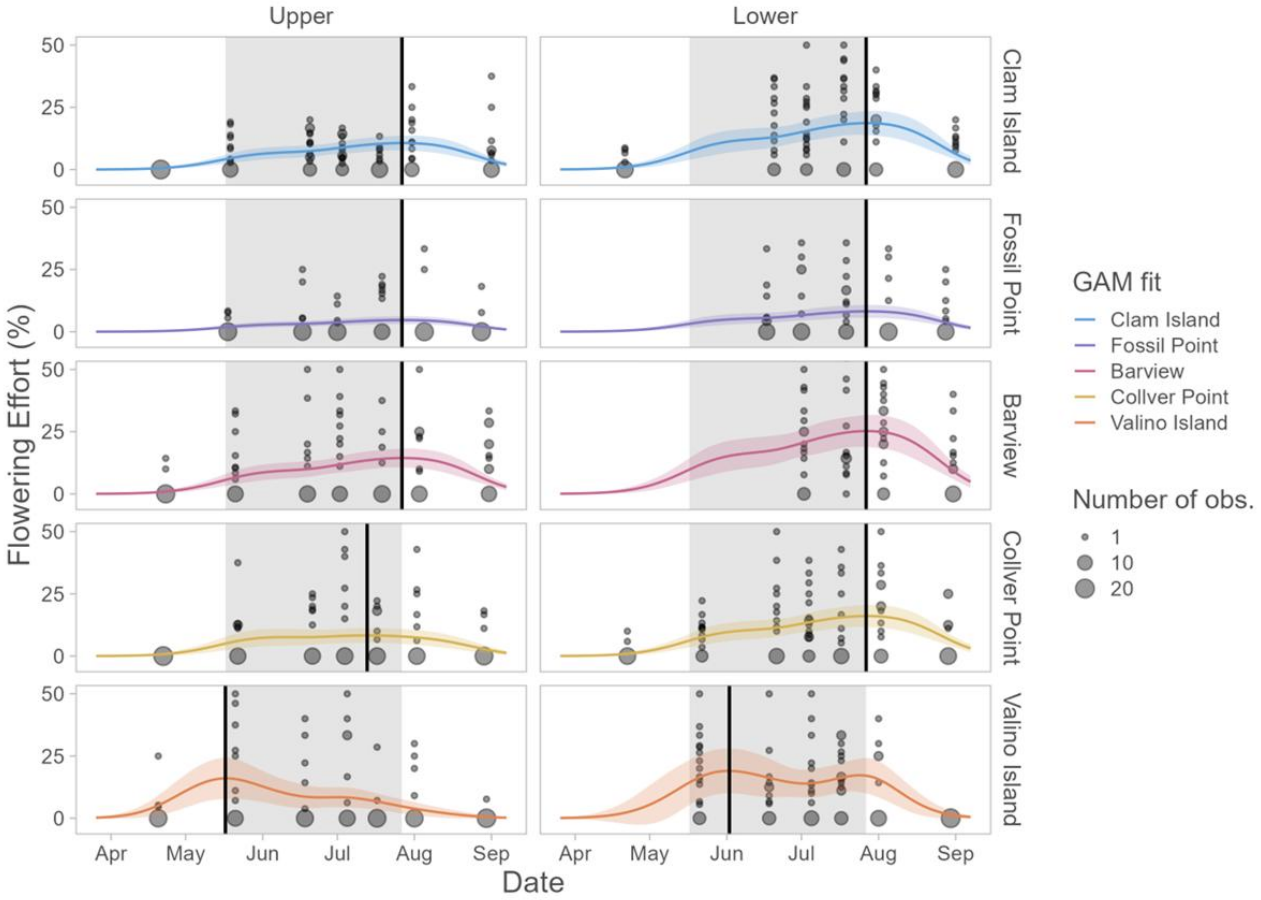


Fig. 3 Mean (± SE) and estimated marginal mean (± SE) for flowering shoot length (cm) (a, b), number of rhipidia (c, d), and GS4 spadices per shoot (e, f) across sites and tidal heights from May through late August of the 2023 flowering season. Sites are denoted by colors and tidal heights by line type (in line graphs) and shade (in est. marginal means plots)

Variation in timing of flowering

The GAM for flowering effort explained 43.5% of total variance in flowering effort (Fig. 4). As expected, a significant general seasonal pattern of flowering effort across sites was observed (*s(day of year);* $p < 0.001$, Table S4); however, the mid-estuary South Slough site (i.e., Valino Island) showed a significant deviation from this general temporal pattern for both tidal heights ($p < 0.001$, Table S4). This site had predicted peaks approximately eight and ten weeks earlier than other sites, in the lower and upper tidal height, respectively (Fig. 4). In accordance with this prediction, the observed peak at this mid-estuary site's upper tidal height was during May, while all other observed peaks across sites were between early July and early August (Table S5).



325 **Fig. 4** Predicted phenology of *Zostera marina* flowering effort at each site and tidal height
326 estimated by the GAM, colored by site as a function of observed values (gray circles). Size of
327 circles indicates number of observations (n = 20 quadrats in total for each site and tidal height
328 pair) on a given date. Colored shading indicates confidence intervals of 2 SEs. Vertical lines
329 indicate the predicted day-of-year of peaks by the model, with time between earliest and latest
330 predicted peak shaded in gray

331 *Thermal conditions*

332 The PCA differentiated sites based on temperature metrics describing warming and
333 thermal variability (Fig. 5). PC1 was significant ($p < 0.001$), accounting for 59.2% (47.8 –
334 71.5%) of the variance and defined by significant loadings from mean temperature, maximum
335 temperature, and range (Table S6). PC2, accounting for 33.2% (23.5 – 41.8%) of the variance,
336 was also significant ($p = 0.003$), and statistically associated with minimum temperatures. Group
337 centroids differed significantly between sites ($p < 0.001$), but not by tidal height. Valino Island, a
338 mid-estuary site, experienced the highest mean temperature (17.4 °C) and maximum temperature
339 (23.1 °C) in late July. In contrast, the site nearest the estuary mouth, Barview, and a mid-estuary

site near the main channel of Coos estuary, Clam Island, were positioned opposite Valino Island along PC1 and had lower mean temperatures (12.7 and 13.3 °C, respectively) and lower maxima (17.8 and 15.8 °C) during late July. Their positioning along PC1 reflects fewer acute warming events and less overall warming throughout the flowering season.

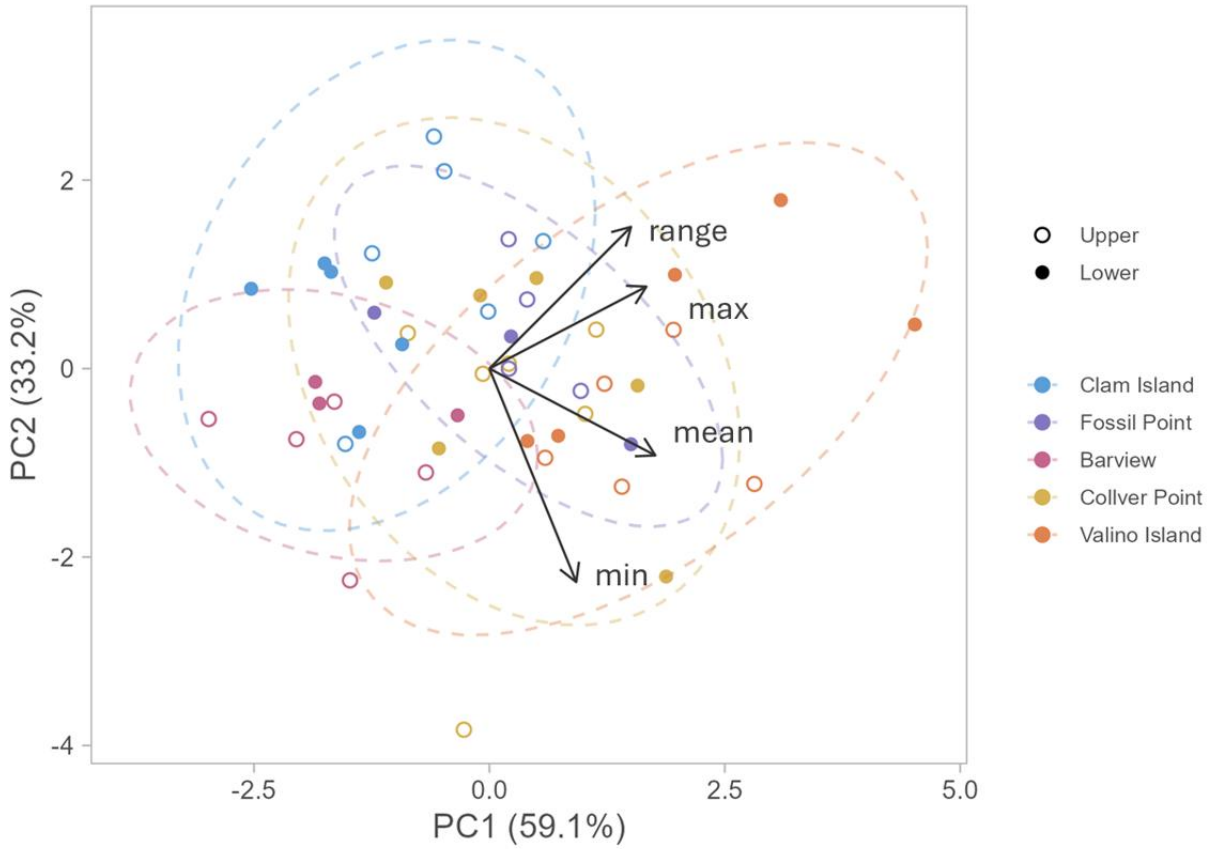


Fig. 5 PCA biplot of temperature metrics (daily range, mean, maxima, and minima) summarized across three weeks prior to each sampling time point for each site and tidal height. Loadings by each temperature metric are indicated by the direction and length of the associated vector. Sites are denoted by color and tidal heights by fill. Dashed ellipses represent the 95% confidence regions for each site

Relating patterns of flowering to temperature

The pRDA (Fig. 6a, Table S7) provided evidence that thermal variation was associated with variation in eelgrass flowering traits (adj. $R^2 = 0.23$, $p < 0.001$). RDA1 was statistically significant ($p < 0.001$), explaining 29.8% of constrained variance. All three temperature metrics (daily mean, maximum, and range) were individually significant (Table S7), with daily temperature range showing the strongest loading on RDA1. On RDA1, GS4 spadices and rhipidia per shoot showed the highest positive loadings, while total shoot density showed the highest negative loading, indicating that these three traits were most strongly associated with thermal variation after accounting for spatial structure.

GLMMs using all three temperature metrics as candidate predictors indicated that daily temperature range was the best predictor of GS4 spadices per shoot and rhipidia per shoot (Fig. 6b-c), and the LRT showed no benefit from random effects in either model ($p = 1$, Table S8). With each 1 °C increase in range, rhipidia per shoot increased by about 16% and GS4 spadices per shoot increased by 33.5% ($p < 0.001$, Table S9). Both shoot traits also increased with day-of-year, but this temporal effect was smaller (0.3% to 1.3% per day, Table S9) than that of temperature range, even across the full flowering season. In contrast, total shoot density exhibited no significant response to temperature range or mean temperature. Instead, density declined significantly with day-of-year in both models ($p < 0.01$, Table S9). LRTs supported random effects for both shoot density models ($p < 0.001$, Table S8), consistent with earlier results showing strong tidal height effects on shoot density.

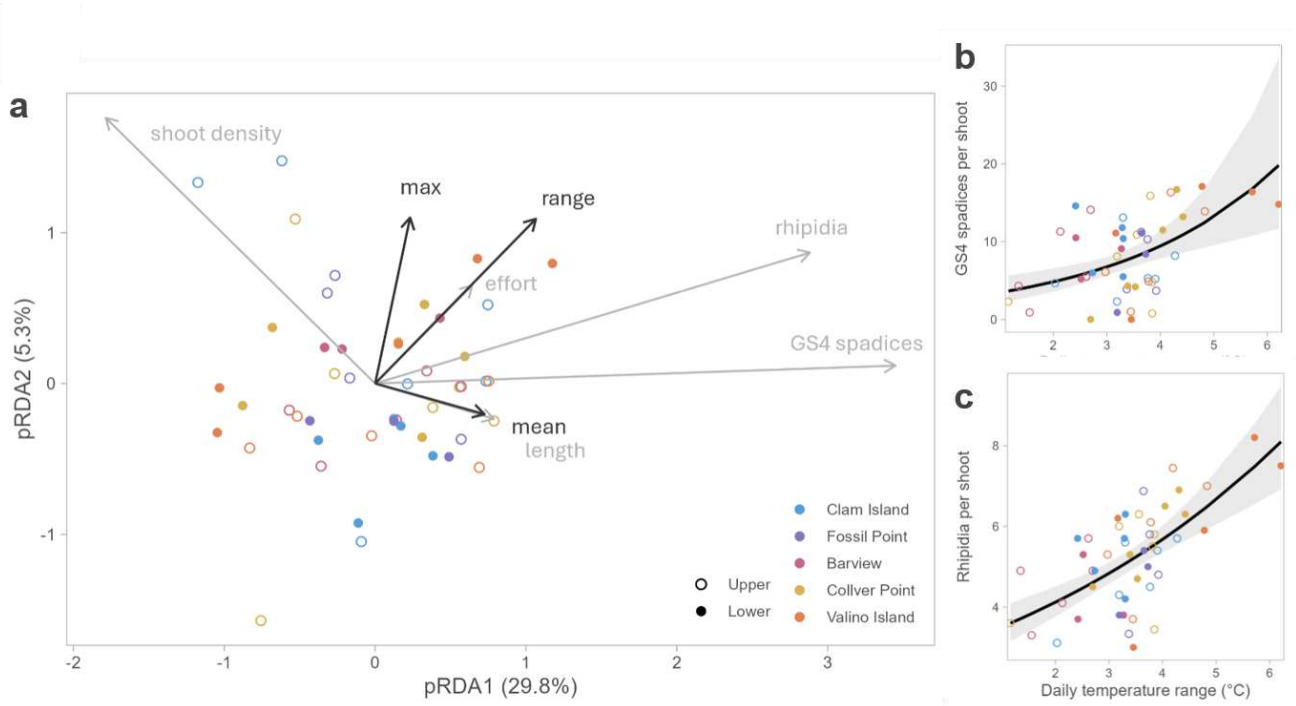


Fig. 6 (a) Partial redundancy analysis (pRDA) triplot of flowering shoot and meadow characteristics (grey) constrained by temperature metrics (black) after accounting for spatial structure (site and tidal height). Loadings of each temperature metric are indicated by vector direction and length, reflecting direction and strength of association, respectively. Axes represent the first two constrained ordination axes, with percentages indicating the proportion of constrained variation explained by each. (b - c) Partial effects of temperature range ($p < 0.001$) from best-fitting GLMs for (b) GS4 spadices per shoot and (c) rhipidia per shoot, marginalizing over sampling date and spatial structure, 95% prediction intervals are in gray, and full model summaries are found in Table S9. Sites are denoted by color and tidal height by fill

Discussion

Our study highlights that substantial variations exist in the magnitude and timing of flowering effort at two distinct spatial scales: within (i.e., tens of meters) and across (i.e., less than 10 km apart) intertidal eelgrass meadows within a single estuary. Across the vertical intertidal gradient where plant abundance and flowering effort increase with depth, temperature did not vary. Instead, temperature metrics diverged across sites in the estuary, and the mid-estuary site in South Slough, which has the warmest and most variable daily temperatures, had a distinct signature of an earlier flowering peak. Daily temperature variability was positively associated with metrics of shoot reproductive output (GS4 spadices and rhipidia per shoot). Our findings also indicate that flowering shoot density, rather than shoot reproductive output (GS4 spadices per shoot), shapes sexual reproductive output within meadows.

Flowering in plants is an energetically costly process that competes with vegetative growth for limited internal resources (Reznick 1985). Intertidal eelgrass meadows in this study exhibited greater densities and flowering effort at lower tidal heights, suggesting that upper intertidal stressors may be influencing energy allocation. For example, eelgrass at the upper margins of a meadow can experience episodic desiccation stress during prolonged periods of exposure (Boese et al., 2005; Leuschner et al., 1998), during which carbon assimilation is reduced (Leuschner et al., 1998). Likewise, high irradiance associated with low tide exposure can act as a stressor, triggering photoprotective mechanisms that limit physiological damage but reduce photosynthetic efficiency (Park et al., 2016; Petrou et al., 2013, for *Zostera muelleri*). While temperature can vary across the intertidal gradient within an eelgrass meadow (Boese et al., 2005), we did not find evidence of significant temperature differences between tidal heights, although this may be partly due to the placement of our loggers within the sediment, which might have hampered the detection of any potential thermal differences that may arise from air-temperature differences during low tide exposure.

Our finding of greater flowering effort at lower tidal heights contrasts with many studies documenting greater flowering at the upper margins of intertidal eelgrass meadows (Boese & Robbins, 2008; Jacobs & Pierson, 1981; Ruesink et al., 2012, 2022, 2024). In these cases, investment in flowering may be a stress response to unfavorable environmental conditions (Cabaço & Santos, 2012, Marín-Guirao et al., 2019) that would enable recovery from seed in the event of shoot mortality. In contrast, other observations both in intertidal and subtidal eelgrass meadows suggest the existence of an intermediate elevation zone for flowering resulting from optimal environmental conditions that minimize stressful conditions within a gradient of light availability and physical (e.g., wave, dessication) exposure (Lekammudiyanse et al., 2023, Olesen et al., 2017). In fact, an intertidal eelgrass growth model (Wang et al., 2024) predicted that an optimal elevation for eelgrass presence and growth lies where upper-intertidal desiccation stress and lower-intertidal light limitation are minimized, and this may also apply to flowering effort. Evidence supports both inhibiting and promoting effects of stress on eelgrass flowering, underscoring the need to identify the physiological mechanisms involved and the timescales over which they act.

Higher flowering effort at lower intertidal heights may provide greater capacity for recovery and resilience relative to the upper intertidal through increased seed production, burial (i.e., larger seed bank), and seedling establishment at this depth. Seed dispersal in *Z. marina* is highly localized, typically limited from ones to tens of meters (Furman et al., 2015; Orth et al., 1994). In this study, distances between intertidal height transects within a given site ranged from 10 to 60 meters (Table 1). At sites with wider, gentler sloping meadows, greater distance between the tidal heights may prevent seeds from homogenizing across the meadow, creating a discrepancy in recovery capacity between the intertidal heights. In contrast, at sites with narrow fringing meadows where our transects were closely spaced (ca. 10 - 20 m; Valino Island and Collver Point), greater seed production in the lower intertidal may contribute to the seed bank of the entire meadow.

Of the plant morphological traits measured, only shoot length varied across the vertical gradient. Shoots were longer in the lower intertidal, likely reflecting a common photo-adaptive response to light limitation in deeper water (Dennison & Alberte, 1986; Krause-Jensen et al., 2000). While reproductive output of a shoot can be positively correlated with shoot size (Waller, 1988), we did not observe greater rhipidia or spadices per shoot at our lower tidal heights, in contrast to many other *Zostera* spp. studies (Jacobs & Pierson, 1981; Vercaemer et al., 2021; Von Staats et al., 2021, but see Phillips et al., 1983b). In our study, plants at depth may possess enough energy to invest in the production of larger shoots for sufficient light capture, but may not be able to afford the additional metabolic cost of proportionally increasing their number of rhipidium or spadices per shoot, since light availability may be relatively low in this nutrient rich estuary with high algal abundance (Hayduk et al. 2019, Hessing-Lewis & Hacker 2013).

A distinct signal of earlier flowering occurred at the warmest site in our study, Valino Island, where temperatures during the flowering period averaged 15.5 ± 2.1 °C (compared to averages between 12.1 ± 1.5 °C and 13.8 ± 1.8 °C at other sites). Earlier flowering under warming conditions in *Z. marina* (Sawall et al., 2021; Nolan et al., 2026) has been interpreted as a stress response to depleted energy reserves from the greater respiratory demands induced by higher temperatures (Sawall et al., 2021). This phenological shift, however, can also be accompanied by an increase in shoot reproductive output (Nolan et al., 2026), suggesting that warming does not always act as a physiological stressor. Indeed, because temperature regulates metabolic rate (Brown et al. 2004), natural spatial and temporal variations in temperature likely drive differences in seagrass flowering timing, for instance, across larger (hundreds of km) / latitudinal geographic scales (Blok et al., 2018; Ito et al., 2021; Lekammudiyanse et al., 2024). Across sites (i.e. few km apart) in this study, daily mean temperatures accounted for most of the variation in thermal conditions. Higher temperatures observed at the mid-estuary site of Valino Island are likely driven by decreased river discharge into shallower channels during warmer months, while sites closer to the mouth of the estuary (Barview) or in the main channel (Clam Island) sit adjacent to deeper, dredged waterways and are more strongly influenced by cooler, upwelled oceanic waters (Hayduk et al., 2019; Marin Jarrin et al., 2022). Although we did not measure plant carbon reserves, because the among-site temperature variation we observed over

one growing season aligns with the estuary's characteristic thermal regime (Marin Jarrin et al., 2022), the earlier flowering peak at Valino likely reflects natural variation in the temperature-dependent reproductive process (Blok et al., 2018; Lekammudiyanse et al., 2024; Phillips et al., 1983a) rather than a stress response to anomalous warming.

Two traits characterizing shoot reproductive output, the number of rhipidia and GS4 spadices per shoot, were positively associated with daily temperature variability after accounting for the effect of time. Daily temperature variations broaden with increasing distance up-estuary, likely reflecting the influence of warmer air temperatures and decreased river discharge on water temperatures during summer months (Marin Jarrin et al., 2022). However, such variations in daily temperatures may not be the only components of environmental variability influencing shoot reproductive output. For instance, Valino Island, the mid-estuary site which exhibits both the greatest daily temperature range and highest shoot reproductive output, sits at South Slough's turbidity maximum, where turbidity and sediment delivery peak (Keogh et al. 2025), suggesting that additional components of environmental variability may also contribute to increased allocation to sexual reproduction (Cabaço and Santos 2012). Furthermore, at this site of highest shoot reproductive investment, eelgrass recovery has lagged behind Collver Point, a nearby (i.e. < 2 km away) site which recovered fully, following declines associated with the 2014-2016 heatwave (Marin Jarrin et al., 2022; Helms unpublished data). These diverging recovery trajectories, despite similarly low shoot densities after the declines, underscores the need to link covarying estuarine conditions (including thermal and sediment dynamics) not only to shoot reproductive investment, but also to its outcomes, including seed bank formation and seedling establishment.

Small-scale differences in flowering effort, timing, and traits also have important practical implications for restoration, particularly in guiding the choice of source sites and tidal heights when collecting plant material for shoot transplantation and seeding. Seed-based methods in *Zostera marina* rely on collecting flowering shoots with fertilized seeds (Infantes & Moksnes, 2018; Marion & Orth, 2010). This requires reliable estimates of flowering effort and shoot-level reproductive output to identify sites productive enough to support harvesting goals while minimizing impact to the site. Accordingly, flowering shoot density and spathe (or seed) density per shoot are both needed to accurately estimate meadow output, i.e., seed production normalized for a given tidal height or site (m^{-2}). While meadow output can be highest at sites with less flowering shoots but greater shoot reproductive output (Infantes & Moksnes, 2018; Olesen et al., 2017), we observed that flowering shoot densities and effort (correlated meadow traits) may instead be the largest determinant in seed production, consistent with findings in Verceamer et al. (2021). Thus estimating shoot reproductive output, using the number of viable seeds per shoot as the most direct measure, will be important to determine the number of flowering shoots needed to meet a target seed collection goal. Beyond flowering effort and shoot output, which inform harvesting locations, estimates of peak timing are needed for selecting optimal harvesting windows. The large temporal variation detected in flowering timing (e.g., peak flowering differing by up to 70 days) across only 7 kilometers in a single estuary is

substantial and important to consider, given that anthesis, seed development (GS4), and seed dehiscence occur over 30 to 40 days within a flowering shoot (De Cock, 1980). Therefore, there could be little to no overlap in optimal harvest windows among nearby sites, meaning harvest would require multiple site visits rather than a single collection, limiting the efficiency of large-scale seed sourcing.

Our study advances our understanding of patterns and drivers of *Zostera marina* reproduction and emphasizes how they may change dramatically at local scales, which is key to predicting recovery mechanisms and to guiding restoration planning in the midst of documented declines worldwide (Orth et al. 2006, Dunic et al., 2021). Variability in flowering effort, timing, and traits offers a starting point for understanding how sexual reproduction shapes recovery trajectories; however, this link requires additional direct testing of seed-based recruitment and establishment. Further, clarifying other environmental correlates of sexual reproduction alongside temperature is increasingly urgent under projected environmental change and ongoing anthropogenic disturbances to nearshore ecosystems, given the often additive, synergistic, or antagonistic effects of multiple stressors on seagrass health and persistence (Lefcheck et al., 2017; Stockbridge et al., 2020). Our results can guide study designs in other coastal and estuarine environments where eelgrass spans environmental gradients, particularly where baseline flowering data are scarce but recovery and restoration planning is a priority.

CRediT authorship contribution statement

Lara Breitkreutz: Writing – original draft, Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Funding acquisition. Alicia Helms: Writing – review & editing, Conceptualization, Methodology, Investigation, Resources. Ryan Mueller: Writing – original draft, Conceptualization, Methodology, Investigation, Resources, Funding acquisition. Fiona Tomas: Writing – original draft, Conceptualization, Methodology, Investigation, Resources, Funding Acquisition.

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