Antagonistic ecosystem engineering influences habitat mosaics: The role of burrowing shrimp in shaping estuarine foundation species

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A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2025

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Program Authorized to Offer Degree:
Biology

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Abstract

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Understanding the extent to which ecosystem engineers modify the physical environment is challenging, particularly in dynamic and heterogeneous systems like estuaries, where both organism distributions and abiotic conditions vary spatially and temporally. While bioturbating shrimp can influence sediment properties through reworking, disentangling their pathways of effects on foundational species such as seagrass and bivalves remains complex. Shrimp density-driven changes in sediment composition and penetrability may not fully explain their negative impacts on structure forming species, highlighting the difficulty of linking biotic and abiotic interactions in variable environments. Furthermore, the emerging issue of antagonistic ecosystem engineering—

where one engineer's activity prevents another from persisting at functionally relevant densities—compounds this uncertainty. This insight is crucial for predicting ecosystem resilience, guiding restoration efforts, and managing species interactions in dynamic coastal environments.

In Chapter 1, I explore the effects of bioturbating shrimp on seagrass. Because ecosystem engineers shape environmental conditions, interactions between ecosystem engineers can depend not only on the external environment but on “which species arrives when” within habitats. Yet, while endpoint outcomes for adults at high density have often been investigated, few studies have examined how these interactions change across density and life history stages. We tested for antagonistic engineering effects of the burrowing shrimp *Neotrypaea californiensis* (Dana, 1852) at a range of densities on eelgrass *Zostera marina* L., 1753, including seedlings as well as vegetative shoots. In an observational study, abrupt borders of eelgrass beds were not mirrored by shrimp, and shrimp were never excluded across the full range of observed eelgrass densities, patterns that are inconsistent with alternative stable states. However, eelgrass density declined with increasing shrimp density, and no eelgrass occurred at >336 shrimp m^{-2}. Survival of eelgrass transplants also declined with increasing shrimp density, and in a manipulative experiment, seedlings declined more rapidly than vegetative shoots within a shrimp bed. Thus, shrimp have strong antagonistic engineering effects on eelgrass that increase with shrimp density and can preclude successful seedling establishment and persistence of vegetative shoots.

In Chapter 2, I explore the effects of bioturbating shrimp on oysters. Epibenthic organisms on intertidal flats can be affected by underlying sediments and by the activities of bioturbating species that live there. Therefore, bioturbating shrimp have two potential pathways to affect small clusters of juvenile oysters (seeded cultch): directly by moving sediment to the surface, or indirectly by affecting sediment properties (grain size, organic content, penetrability). We examined how oyster (*Magallana gigas*) survival and size responded to a) shrimp (*Neotrypaea californiensis*) density, b) mud content and penetrability of sediment, and c) shrimp density due to their effects on sediment properties (indirect pathway). Seeded cultch were deployed from spring through summer at 31 intertidal sites varying in both shrimp density and sediment properties within Willapa Bay, Washington (USA). Shrimp density was negatively associated with mud and organic content but positively with sediment penetrability, as expected from known ecosystem engineering effects of shrimp. However, neither mud content nor penetrability contributed statistically to the negative impact of shrimp density on oyster survival and size. No oysters survived the summer above 50 – 100 shrimp m^{-2}, and remaining oysters were smaller with increasing shrimp density. Overall, negative effects of shrimp on benthic oysters likely occur through the deposition of sediment (28.9 ml $burrow^{-1}$ day^{-1}) rather than alteration of sediment properties. Our study highlights how the antagonistic ecosystem engineering effect of shrimp on oysters occurs independently of sediment responses to bioturbation and deposit-feeding and quantifies the conditions ensuring the persistence of ecologically- and commercially important foundation species.

In Chapter 3, I explore how transplants of seagrass into an area where it was previously excluded by burrowing shrimp can inform seagrass restoration strategies. Seagrass restoration has shown mixed results, even in environments that appear suitable, indicating on-going needs for improved restoration techniques. This study tracked eelgrass (*Zostera marina*) dynamics at two donor sites and one transplant site over multiple years, using resilience at donor sites and transplant establishment and expansion as key success measures. Despite finer sediments, higher shoot densities, and lower flowering frequencies at lower elevations, eelgrass morphology was similar at both high- and low-elevation donor sites. Recovery times increased with collection intensity, taking up to two years when large plots were completely cleared. Collected shoots were transplanted into plots of four sizes (0.0625–4 m^2) and three densities (25–125 m^2). Although larger, denser plots were expected to aid establishment in bioturbated areas, the highest initial establishment occurred in small, sparse plots. Over time, sparse and medium-density plots filled in, eventually converging with denser plots within a year. After two years, proportional shoot count changes were inversely related to plot size and density: small, sparse plots saw an 83-fold increase in shoot counts, while large, dense plots saw only a two-fold increase. Large, dense plots initially accumulated fine sediment and organic matter but lagged behind unvegetated areas after one year, likely due to bioturbator loss. Neither donor nor transplant sites showed facilitative effects, potentially due to intraspecific competition. Hydrodynamics around eelgrass shoots may have temporarily delayed sediment accumulation, a key ecosystem function. These findings contribute to improved eelgrass restoration strategies for fringe and upper margin intertidal areas.

Overall, I have addressed key interactions that shape the structure and function of intertidal soft sediments, showing how the outcomes among engineering species can become more predictable through incorporating factors such as density and per capita impacts. Borders between engineered habitats provide insight into species interactions, revealing how antagonistic ecosystem engineering shapes community structure. Our findings demonstrate that the effects of these interactions can vary across life history stages, with seedlings often more vulnerable than established individuals. For oysters, burrowing shrimp density serves as a strong predictor of performance, with sediment burial acting as the primary mechanism driving declines in survival and size. In eelgrass restoration, lower shoot collection intensity promotes faster donor site recovery, particularly in fringe and upper boundary habitats, ensuring resilience of natural beds. However, little evidence of self-facilitation in transplanted eelgrass suggests that intraspecific competition, rather than effects of positive density-dependence, constrains expansion, underscoring the complexity of habitat formation in bioturbated environments. Together, this research advances the understanding of ecosystem engineer interactions by demonstrating how antagonistic engineering can disrupt habitat formation and species persistence, emphasizing the importance of considering engineer density, life stage vulnerability, and sediment dynamics in conservation and restoration efforts.

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Acknowledgements

First and foremost, I would like to express my deepest gratitude to my advisor, Dr. Jennifer Ruesink, who has been an unwavering source of guidance and inspiration. Day in and day out, she taught me not only how to be a great scientist but also a compassionate mentor and a genuinely kind person. Whether it was during our countless sessions brainstorming work plans and study designs at the kitchen table or out in the field when things didn't always go as expected, Jen always demonstrated what it means to be a well-rounded leader. She truly is the full package, and her example is one I will always strive to follow. I would also like to extend my sincere thanks to those who have served on my committee. Dr. Andrea Ogston, thank you for encouraging me to "get my head in the mud" and helping me grasp how the physical environment plays a pivotal role in my research. Dr. Emily Carrington, your guidance has deepened my understanding of species and their interactions with the physical environment. Dr. Berry Brosi, thank you for helping me adopt a more holistic view of the interconnectedness between species and their communities.

It truly takes an army to navigate an academic journey, and mine includes many friends, family, and mentors. To my fellow Ruesink lab members, past and present—Fiona, Mo, Christine, Bryan, Maria, Jennifer, Haleh, Elena, Chris, Aspen, Milly, Mariah, and James—thank you for your support; I couldn’t have completed this work without you. I also want to Alan Trimble for providing scientific, but also professional guidance and mentorship as I navigated the last 5 years. To my friends near and far, thank you for your patience and encouragement. I promise I’ll be more social soon. To my mom, thank you for being the ultimate example of work ethic and perseverance, always supporting and loving Kids. This is just one small way I can show my gratitude. To my fiancé, Meg, thank you for your unwavering love and support; you’ve been my teammate throughout this process, and I promise no more shrimp talk! Lastly, to my dad, thank you for inspiring my love and respect for marine life and science. I dedicate this work to your memory.

Chapter 1: Antagonistic ecosystem engineering effects differ by seagrass life history stage and density of bioturbating shrimp

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Published in Journal of Experimental Marine Biology and Ecology

Hull, W. W., and J. L. Ruesink. 2024. Antagonistic ecosystem engineering effects differ by seagrass life stage and density of bioturbating shrimp. *Journal of Experimental Marine Biology and Ecology* 576:152016. https://doi.org/10.1016/j.jembe.2024.152016

Abstract

Because ecosystem engineers shape environmental conditions, interactions between ecosystem engineers can depend not only on the external environment but on “which species arrives when” within habitats. Yet, while endpoint outcomes for adults at high density have often been investigated, few studies have examined how these interactions change across density and life history stages. We tested for antagonistic engineering effects of the burrowing shrimp *Neotrypaea californiensis* (Dana, 1852) at a range of densities on eelgrass *Zostera marina* L., 1753, including seedlings as well as vegetative shoots. In an observational study, abrupt borders of eelgrass beds were not mirrored by shrimp, and shrimp were never excluded across the full range of observed eelgrass densities, patterns that are inconsistent with alternative stable states. However, eelgrass density declined with increasing shrimp density, and no eelgrass occurred at >336 shrimp m^{-2}. Survival of eelgrass transplants also declined with increasing shrimp density, and in a manipulative experiment, seedlings declined more rapidly than vegetative shoots within a shrimp bed. Thus, shrimp have strong antagonistic engineering effects on eelgrass that increase with shrimp density and can preclude successful seedling establishment and persistence of vegetative shoots.

Introduction

A challenge in ecology is explaining how the outcome of interactions depends on environmental context (Chamberlain et al., 2014). Resolving this challenge becomes increasingly important for ecosystem engineers that contribute to shaping environmental context (Jones et al., 1994), since feedbacks from the organisms themselves need to be incorporated. In extreme cases where organisms improve conditions for themselves and exclude others, alternative stable states can result (Knowlton, 1992; van de Koppel et al., 2001; Baskett & Salomon, 2010; Weerman et al., 2012; Hölker et al., 2015). Estuarine soft-sediments provide an ideal study system for examining the context-dependence of interactions, as land-water interfaces can be prone to geomorphic engineering. Their sediments may be either held in place or turned over by organisms (Fei et al., 2014), and the communities that contribute to habitat modifications of sediment structure and stability are often simple (Reise, 2002). Because engineering effects may increase with density, it appears essential to include density as a biological context in any scheme for antagonistic ecosystem engineering (previously also called biomechanical warfare; van Wesenbeeck et al. 2007).

In estuarine soft sediments, two common ecosystem engineers are burrowing callianassid shrimp, which *destabilize* sediment, and seagrasses, which *stabilize* sediment. Both can occur as single-taxon dominants and can generate positive feedbacks where the modification of their environment in turn benefits the engineers (shrimp: Tamaki & Ingole, 1993; seagrass: de Boer, 2007). The actions of each engineer further influence community composition and ecosystem processes in distinct ways (Fonseca & Fisher, 1986; Berkenbusch et al. 2000; Thomas et al. 2000; Siebert and Branch 2007).

Seagrasses are sensitive to changes in both light and sediment conditions, performing better in habitats where light intensity is high and turbidity is low (Duarte, 1991; Lee et al., 2007). By reducing water flow through the creation of above-ground structure, seagrasses can improve sediment stability and trap fine organic and inorganic particles (Fonseca and Fisher, 1986; Fonseca and Cahalan, 1992; Koch and Gust, 1999; Hemminga and Duarte, 2000; Madsen et al., 2001), creating positive feedbacks that enhance seagrass performance by reducing erosion and stimulating growth (Cardoso et al., 2004); However, in calmer conditions where shoot densities are high, negative feedbacks may occur via intraspecific competition (Ruesink et al., 2012, Yang et al., 2016).

Burrowing-shrimp modify their environment by reworking and burrowing in the substrate, altering the biogeochemical properties of sediments (Koike and Mukai, 1983; Waslenchuk et al., 1983; Ford et al., 1999; Webb and Eyre, 2004; D'Andrea and DeWitt, 2009; Jordan et al., 2009). Different lineages of burrowing shrimp feed by filter-feeding or deposit-feeding, and these feeding modes are linked to the structure and function of their burrows. Filter-feeding species pull water into their burrows to filter out suspended organic material while deposit-feeding species consume organic material within sediments or present along the walls of their burrow (Coelho, 2004). Since filter-feeding burrowing shrimp rely on stable sediments to construct their burrows, cohabitation with sediment-stabilizing species such as seagrass can occur (Siebert and Branch, 2006). The habitats of deposit-feeding shrimp are void of above-ground structure and are composed of sandy sediments with little organic matter. Additionally, sediment destabilization caused by sediment reworking influences macro-invertebrate communities by reducing the abundance of surface fauna either directly via sediment smothering or by eliminating sediment stabilizers (i.e. biofilms) and biochemical cues for recruitment (Branch and Pringle, 1987; Pillay et al., 2007, Pillay and Branch, 2011) and by facilitating burrowing species (Pillay et al., 2010). Some evidence indicates that adults facilitate intraspecific recruitment by allowing access to deeper sediment layers through bioturbation and the presence of established burrows, furthering the persistence and expansion of shrimp populations (Tamaki & Ingole, 1993).

Although the engineering effects of seagrass and deposit-feeding shrimp are wellunderstood, variable outcomes are reported for interactions between these taxa. If an established engineer can resist colonization by the other engineer, then such a result is consistent with either a deterministic but context-dependent winner, or mosaics maintained by priority, where a species persists once it reaches a threshold density where engineering effects exclude the other. Accordingly, transplants of seagrass into areas with deposit-feeding shrimp can be unsuccessful, likely resulting from reduced light availability through particle resuspension or smothering by sediment ejected from burrows (Roberts, 1981; Suchanek, 1983; Angel, et al., 2006; Siebert and Branch, 2006, 2007; Watson et al. 2023). Transplants of deposit-feeding shrimp into seagrass can also result in no alteration of the seagrass state (Harrison 1987; Berkenbusch et al., 2007; Castorani et al. 2014), likely arising from the ability of seagrass rhizomes to maintain sediment stability and act as a barrier that impedes the movement of deposit-feeding shrimp and prevents them from displacing seagrass (Brenchley 1982, Harrison 1987, Castorani et al. 2014). However, in systems where seagrasses typically dominate, frequent disturbance to seagrass beds can allow deposit-feeding shrimp to rapidly colonize areas where seagrass is lost (Castorani et al., 2014; Castorani & Baskett, 2020).

Most past work on seagrass-burrowing shrimp interactions has not addressed a range of densities, but rather the performance of one engineer in the presence of the other at either high density or in its absence. However, co-occurrence of engineers, typically at lower densities, suggests a threshold level below which both ecosystem engineers can coexist (Harrison, 1987; Siebert and Branch, 2006).

Which engineering species becomes dominant may depend on the life history stage of participating species. For example, burrowing species can gain priority when sediment reworking and destabilization cause the rapid burial of seeds and seedlings (van Wesenbeeck et al., 2007; Valdemarsen et al., 2011; Jarvis and Moore, 2015). Likewise, large burrowers can be displaced by the presence of dense roots and rhizomes created by seagrass and cordgrass (Pillay et al., 2010), but smaller individuals are able to occupy the interstitial spaces within the matrix of roots and rhizomes (Reise, 1985; van Wesenbeeck et al., 2007; Goerlitz et al., 2015). To our knowledge, only one study has examined the effects of deposit-feeding shrimp on early life history stages of seagrass, which found lower seed viability, higher mortality, and slower growth of introduced *Zostera japonica* seedlings in the presence of shrimp compared to seedlings in sediments where shrimp were chemically removed (Dumbauld & Wyllie-Echeverria, 2003). Currently, it is unknown whether the outcomes of seagrass-burrowing shrimp interactions are affected by the life history stage at which interactions occur.

Neotrypaea californiensis is a deposit-feeding shrimp (total length up to 70 mm, WDNR, 2020) that can reach densities of 500 m^{-2} on sandy estuarine tidal flats along the west coast of North America (MacGinitie, 1934; DeWitt et al., 2004; Dumbauld et al. 2021). Their complex burrows usually contain one or two surface openings and can extend >1 m into the sediment (Griffis and Suchanek, 1991; Dumbauld et al., 1996). Their extensive burrowing activities (deposit-feeding and burrow ventilation) cause the resuspension of sediments (Posey, 1986; Berkenbusch and Rowden, 2003; Ferraro and Cole, 2004; Bosley and Dumbauld, 2011) and increase the cycling of porewater within the upper portion of sediments (Volkenborn et al., 2012), creating a liquified and unstable tidal flat (Roberts, 1981; Bird, 1982; Posey, 1986). The average sediment turnover rate per shrimp is estimated at 49.1 g day^{-1}, which is high when compared to 4.2 g day^{-1} measured for *Upogebia pugettensis*, a filter-feeding species of shrimp found within the same estuaries (Dumbauld et al., 2004).

Our goal was to assess the antagonistic engineering effects of *Neoptrypaea californiensis* (shrimp) on the seagrass *Zostera marina* (eelgrass) and the contributions of shrimp density and eelgrass life stage to the outcome. We used three approaches: 1) an observational approach to determine whether shrimp densities could explain abrupt habitat borders of eelgrass that were not a function of tidal elevation; 2) an across-site approach of eelgrass transplants into different shrimp densities, at tidal elevations expected to be suitable for eelgrass, and 3) a within-site experiment in which vegetative and seedling eelgrass shoots were transplanted in and out of shrimp exclosures. Because antagonistic engineering is expected to occur through biogeomorphological changes, we concurrently sampled sediment grain size and organic content. We hypothesized that eelgrass would be excluded by shrimp only above a threshold density, and this threshold would be lower for seedlings than for vegetative shoots. We expected sediment to have reduced organic and mud content as shrimp density increased, although across sites this engineering effect could be overwhelmed by overlying hydrodynamics.

Methods

We used three approaches to determine the effects of shrimp on eelgrass. First, we used field surveys to quantify the distributions of engineers across borders between their associated habitats. Second, we implemented transplanting experiments to quantify the effects of shrimp density on eelgrass survival. Finally, we examined effects of shrimp density on the survival of different eelgrass life history stages by transplanting eelgrass seedlings and vegetative shoots into tidal flats with high and low shrimp densities.

2.1 Study locations

Willapa Bay and Grays Harbor, Washington, are relatively large estuaries on the US west coast. Willapa Bay covers 35,000 ha at mean high water, and Grays Harbor covers 23,500 ha (Hickey and Banas 2003). Freshwater enters these estuaries primarily in winter, whereas summer alternates between wind-driven upwelling and downwelling in the nearshore ocean, and these conditions propagate into the bay (Banas et al., 2004; Borde et al., 2003; Hickey and Banas, 2003; Ruesink et al., 2018). Both bays have shallow mean water depth (Willapa Bay 3.2 m; Grays Harbor 4.3 m), and therefore mesotidal water exchange of 2 m leads to the emergence of extensive intertidal mudflats at low tide (Dumbauld and McCoy, 2015; Hickey and Banas, 2003). Three major engineering taxa occupy these tidal flats in a mosaic that can appear as discrete patches: seagrasses, bivalve shellfish, and burrowing shrimp. Beds of seagrass (native *Zostera marina* and non-native *Zostera japonica*) cover roughly 32% and 8 – 13% of Willapa Bay's intertidal area respectively (Dumbauld and McCoy, 2015; Ruesink et al., 2006). *Zostera marina* occurs as a large morphotype, generally reaching 1 m shoot length in summer but restricted to densities < 100 m^{-2} (Thom et al. 2003). Shellfish aquaculture in Willapa Bay, which accounts for nearly 10 – 20% of the USA's total oyster harvest (mostly non-native *Crassostrea (Magallana) gigas*), has converted about 23% of the intertidal area for aquaculture use (Dumbauld and McCoy, 2015; Feldman et al., 2000; Ruesink et al., 2006). Oyster production from Grays Harbor is about one-fifth that of Willapa Bay (Decker, 2015), and Grays Harbor has roughly half the potential eelgrass habitat as Willapa Bay (Borde et al., 2003). Shrimp (*N. californiensis*) are most abundant in the mid to upper intertidal zone (Swinbanks and Murray, 1981; Dumbauld et al., 2011) and were mapped as occupying 14% of Willapa Bay's intertidal area in 2006 (Dumbauld et al., 2021).

2.2 Distribution of ecosystem engineers across habitat borders

Six eelgrass beds were selected in Willapa Bay during summer 2020 and two in summer 2021, each containing an abrupt border from high eelgrass cover to bare sediment lacking eelgrass along a tidal elevation contour (Fig. 1). With the border at the center of the transect, and the transect running parallel to shore, we sampled at 2, 4, 8, 16, and 32 paces on either side of the border, creating an approximately 70-m total transect in which sampling was denser towards the border where we had the greatest expectation of non-linear change. All samples were georeferenced (Garmin Geko) for later calculation of distance along transect in m.

At each sample position, we counted eelgrass shoots within a 0.25 m^2 area and then cored for shrimp. Multiple small-diameter cores were used to generate a single sample of shrimp density ("5-core sample"), which was physically easier than one large core. At each sample position, we cored 5 points within 1 m^2. The core was constructed of stainless steel (13 cm diameter, 36 cm long) with an attached handle, so several pulls were required per point to reach the target depth of 70 cm into the sediment. The sediment removed from each core was manually spread out to search for shrimp, which provide a vibrant yellow-pink contrast to sediment. Each shrimp was categorized by carapace length into five size classes (cut-offs at 4.14, 8.28, 12.49, and 17.42 mm; WDNR, 2020), each of which was associated with a wet biomass estimate (Supplemental Table 1). Shrimp densities were scaled to 1 m^2 by multiplying the total count in each 5-core sample by 16. While this hand-sorting may under sample shrimp in the smallest size class, these were rarely found when we added a step of sieving the top layer of sediment, and the larger shrimp are likely most important for bioturbation.

We collected a surface sediment sample at three distances on either side of the border (2, 16, 32 paces). These sediment samples were dried (50° C for at least 72 h), ashed (500° C for 3 hrs) for organic content via loss of mass on ignition and sieved for grain size analysis (Ro-Tap). Sieves followed a Wentworth scale (1000, 500, 250, 125, and 63 µm).

2.3 Eelgrass transplants

Eelgrass vegetative shoots were transplanted at 16 sites across three regions, including Willapa Bay and two regions within Grays Harbor, which varied in shrimp densities and did not currently support eelgrass (Table 1, Fig 1). In cases of current low shrimp density, they were known to be present at higher densities within the past two years and had either naturally declined or had been reduced through sediment compaction across large (2 ha) areas. Sites were approximately 100 meters apart within each region, with regions being separated by a minimum of 10 km. Rhizomes of at least 7 cm in length were buried several cm beneath the surface by hand, providing a natural anchor (Ruesink et al., 2018). Sites each contained three to five transplant plots in which ~20 shoots were placed within 0.25 m^2. These transplants occurred in spring or summer of 2022-2023. At each transplant site, shrimp densities were quantified by the 5-core sample method described above. Given some variation in the number of 5-core samples per site (N = 1-5), total shrimp counts from each 5-core sample were averaged for each transplant site to determine average shrimp densities. Shoot densities were resampled 25-40 days after transplantation, and survival was calculated as the fraction of shoots remaining across all plots per site. Shoots that survived and branched could contribute to apparent survival >1.

2.4 Life stage experiment

In May 2023 we transplanted vegetative eelgrass shoots and seedlings (length: 10.8 cm ± 0.8 SE) on two adjacent tidal flats on Long Island (Fig. 1). One area contained dense shrimp (374 shrimp m^{-2} ± 71 SE, N = 3), and the other had previously been an active shrimp bed in summer of 2021 (280 shrimp m^{-2} ± 82 SE, N = 20), but no longer contained dense shrimp after spring 2022 (16 shrimp m^{-2} ± 0 SE, N = 6). A total of twenty 1 m^2 plots were established in both areas with half having the top 10 cm of sediment excavated from them at random. We then anchored a double layer of 1 m^2 sheets of tightly woven burlap inside each excavated plot using PVC pipes at the corners. Shrimp were removed from excavated sediments before being replaced, creating a 10 cm layer void of shrimp, and preventing any deeper shrimp from reaching the surface within the plot. To ensure that sediment disturbance was consistent across plots, the surface sediments of plots without burlap were also excavated and replaced (but shrimp were not removed) and marked at two corners using PVC pipe, thus creating shrimp “exclusion” and “control” plots. We returned 24 hours later, after sediments within plots had settled, to assign eelgrass treatments. Half of all exclusion and control plots were transplanted with either 15 eelgrass vegetative shoots or 25 seedlings within the center of 0.25 m^2 areas to create a fully crossed design (Fig. 2). We returned 2 weeks later to determine the effects of shrimp presence (comparing two tidal flats) and exclusion treatment (comparing plot-level treatments) on vegetative shoots and seedlings (comparing life stage). To determine if shrimp re-entered exclusion plots from the side of adjacent sediments we counted surface holes within plots. Because some plots had already lost all transplants at 2 weeks, analyses of later time points were not carried out, although we continued to monitor at 6 and 12 weeks.

2.5 Data analyses

2.5.1 Habitat borders

Observational data of shrimp and eelgrass were analyzed for distribution along transects, and for the relationship between densities of the two species. Sediment conditions were compared between either side of transects and to shrimp density. Because we specifically placed eelgrass borders at the center of each 70-m transect, we expected a decline in eelgrass density along transects. Shrimp, in contrast, might be constant, increase (antagonistic to eelgrass), or decline (facilitative with eelgrass). Any shift in density in either species along the transect could occur gradually or abruptly (non-linearly with a sharp threshold). To characterize potential shifts in density we used the Poisson power-exponential function, which accommodates distinct curve shapes allowing all three possibilities (Wagner et al., 2012): constant density, exponentially declining (or increasing), or regions of different density separated by a threshold. This function requires that the positions where samples were taken along transects lie between 0 and 1, so we used proportional distance along each 70-meter transect, initiated from the eelgrass bed for both eelgrass and shrimp. More detailed methods are provided in the supplemental methods section. We then compared among the three models by small sample size corrected Akaike Information Criterion (AICc), which accounts for model fit and complexity. We selected the simpler model where the difference between AICc (Δ AICc) was less than 2.0.

We first checked for threshold distributions of eelgrass and shrimp using the Poisson power-exponential function at each site separately since we lacked methods to include site as either a fixed or random effect, given the optimization approach required (Supplemental Methods). As expected from situating transects so the center was at a habitat border, the threshold model typically fitted best for eelgrass, and we subsequently carried out a single analysis for data from all sites. When we discovered that shrimp only followed a threshold model at one of our eight sites, we instead applied a linear mixed model to test shrimp density as a function of proportional distance along transect, specifying site as a random intercept.

Eelgrass counts were tested for a relationship with shrimp density. Due to the occurrence of excess zeros and overdispersion, we fitted a generalized mixed model specifying the Tweedie probability distribution with a log link (*glmmTMB package*; Brooks et al. 2017). Since samples were recorded across 8 sites, to account for site-specific variability we included site as a random intercept in our model.

Silt ($\leq 63 \mu m$) and organic content were tested for effects of habitat (eelgrass or bare) and shrimp density, by a generalized linear mixed model specifying a beta distribution with a random intercept of site. Due to missing samples at two sites, total sediment sample size was three samples from each side of the eelgrass border at 6 sites (total 34).

2.5.2 Eelgrass transplants

For eelgrass transplants, we examined eelgrass survival as a response variable in relation to shrimp density, days since transplantation, and region. Eelgrass survival was calculated as the change in shoots counted relative to the number of shoots transplanted, thus survival greater than 1 can occur as eelgrass is clonal and able to increase shoots by branching. Gaussian data distribution was appropriate for this linear regression, based on inspection of residuals.

2.5.3 Life stage experiment.

We tested for the effects of tidal flat (high vs. low background densities of shrimp), exclusion treatment (control, exclusion), life history stage (seedlings, vegetative) and their two- and three-way interaction on square-root transformed eelgrass survival by a linear model. A square-root transformation was applied to eelgrass survival to better fit assumptions of normality and homogeneity of variance. If a significant three-way interaction occurred, we examined interactions between exclusion treatment and life history within the two tidal flats. If significant interactions between exclusion treatments and life history stage occurred at either tidal flat, we tested for Tukey’s pairwise comparisons (Hothorn et al., 2008) for all exclusion treatment by life history stage combinations. The analysis consisted of the first 16-days of the experiment. All statistical analyses were carried out in R version 4.2.3 (R Core Team 2022).

Results

3.1 Habitat borders

The eight sites spanned a wide range of both eelgrass and shrimp densities, with average densities of 31.6 (± 13.2 SE) – 426.3 (± 27.8 SE) shrimp m^{-2} in bare habitats and 8 (± 3.1 SE) – 69.6 (± 23.9 SE) shoots m^{-2} in eelgrass habitats (supplemental Fig. 1A, 1B). As expected from selecting apparent edges of eelgrass beds, eelgrass shoot density declined along transects at all sites, with the threshold model being the most common best model at describing declines across habitat borders, followed by the gradient model and then the constant model (Supplemental Table 2, Supplemental Fig. 2). In all cases, shoot density declined to zero at the end of the transect, as the transect had been positioned to have few shoots beyond the habitat border. Shrimp densities were opposite to eelgrass densities at 4 of our 8 sites but were not mirror distributions to eelgrass. The gradient model was the most common and best at describing changes in shrimp density across transects, followed by the constant model and then the threshold model (Supplemental Table 2, Supplemental Fig. 2).

When combining observations across sites, the threshold model was still the best model at describing declines in eelgrass density along transects (Table 2, Fig. 3A) whereas the best model for describing shrimp density along transects was the constant model (Table

2). However, since shrimp densities did not change abruptly along transects, a linear mixed model approach was preferred to account for transects at multiple sites. Shrimp densities gradually increased along transects (*est* = 150.85 95% CI [88.4, 213.3], $t_{1,82}$ = 4.8, P < 0.001; Fig. 3B), consistent with site-specific patterns revealed at most sites with the Poisson power-exponential method (Supplemental Table 2, Supplemental Fig. 2). The inclusion of the random effect of site was also significant ($\chi^2 = 32.1$, $P < 0.001$) and accounted for 53% of total variation in shrimp density.

Eelgrass density was negatively related to shrimp density (*est* = -0.01, 95% CI [-0.02, -0.001], $Z_{1,82}$ = -2.178, $P < 0.03$). The inclusion of a random intercept of site was significant ($\chi^2 = 135.07$, $P < 0.001$) and accounted for 28% of the total variation in eelgrass density. Eelgrass shoots were absent above 336 shrimp m^{-2} (Fig. 4). In contrast, eelgrass appeared unable to exclude shrimp, since shrimp were only absent from 1 of 83 samples and were present at low densities in the densest eelgrass.

Silt and organic content were highly correlated ($r = 0.96$, *d.f.* = 34, $P = < 0.001$), so we continued by examining the effects of habitat on silt content. We found a significant random intercept of site ($\chi^2 = 29.6$, $P < 0.001$) with 94% of the variation in silt content explained by site. We did not find a significant effect of habitat (*est* = 0.27, 95% CI [-0.10, 0.64], $Z_{2,42}$ = 1.43, $P = 0.20$) or shrimp density (*est* = -0.001, 95% CI [-0.004, 0.001], $Z_{2,42}$ = -0.97, $P = 0.33$) on silt content.

3.2 Eelgrass transplants

For eelgrass transplants, survival declined with increasing shrimp density (*est* = -0.002, $t_{5,11}$ = -3.1, *P* = 0.01; Fig. 5). No more than half of transplanted shoots survived a month when transplanted into shrimp at densities above 300 m^{-2}, whereas the intercept for survival without shrimp was >80%. There was no significant effect of either the Long Island (*est* = 0.34, $t_{5,11}$ = 1.2, *P* = 0.25) or Westport (*est* = 0.23, $t_{5,11}$ = 1.3, *P* = 0.22) region on transplant survival when compared to Damon Point, thus transplant survival did not differ regionally. Additionally, there was no effect of days since transplantation on transplant survival (*est* = 0.04, $t_{5,11}$ = 1.6, *P* = 0.13).

3.3 Life stage experiment

Surface holes were noticeably absent in the sediment layer between the burlap and the surface of exclusion plots in either the low- or high-density areas over the course of the experiment (Supplemental table 3). Only a single surface hole was observed in one control plot located within the low shrimp density area whereas control plots in the high-density shrimp area contained many.

Eelgrass seedlings survived poorly when transplanted into dense shrimp, but otherwise persisted well, whereas vegetative shoots were less negatively affected by dense shrimp. This overall result contributed to the significant three-way interaction in the experiment (*est* = -0.42, 95% CI [-0.72, -0.12], $t_{3,39}$ = -2.88, *P* = 0.007), that is the interaction between shrimp exclusion treatment and life history stage differed on the two tidal flats (high vs. low background densities of shrimp).

On the tidal flat with low-density shrimp, the interaction between exclusion treatment and life history stage was not significant (*est* = -0.15, 95% CI [-0.36, 0.05], $t_{2,19}$ = -1.63, *P* = 0.12) and was removed from the model. Overall, vegetative shoot survival was higher than seedlings after 16 days (*est* = 0.21, 95 % CI [0.08, 0.36], $t_{2,19}$ = 3.25, *P* = 0.01) and there was no effect of exclusion treatment on eelgrass survival (*est* = 0.11, 95% CI [-0.02, 0.26], $t_{2,19}$ = 1.75, *P* = 0.10). Both eelgrass vegetative shoot and seedling survival remained relatively similar after 16 days and until our last observation in August (Fig. 6A).

On the tidal flat with high-density shrimp, there was a significant life stage by exclusion treatment interaction (*est* = -57, 95% CI [-0.81, -0.34], $t_{3,39}$ = -5.19, *P* < 0.001). Seedling survival in control plots was significantly lower than all other life stage by exclusion treatment combinations (Table 3A), being the only treatment to decline to zero shoots after 16 days (Fig. 6B). Similarly, vegetative shoot survival in control plots was significantly lower than vegetative shoot survival in exclusion plots (Table 3C, Fig. 8B), but was similar to seedling survival in exclusion plots (Table 3B, Fig. 8B). Finally, seedling survival in exclusion plots was similar to vegetative shoot survival in exclusion plots (Table 3A, 3B).

Discussion

When overlying physical conditions of the environment are similar, discrete habitat borders may nevertheless emerge due to positive feedbacks, in which the species themselves generate environmental conditions that exclude the other. However, the habitat borders observed in our study of intertidal mudflats did not emerge from reciprocal antagonistic ecosystem engineering. Shrimp distributions were not a mirror-image of eelgrass, which would be expected if positive feedbacks by each taxa excluded the other, and eelgrass across the full range of densities never excluded shrimp. Further, this co-existence of eelgrass and shrimp occurred across sites with variable sediment type, suggesting that hydrodynamic conditions does not alter the interaction outcome for eelgrass and shrimp. Nevertheless, one aspect of antagonistic ecosystem engineering was supported by our results, since we documented shrimp densities above which eelgrass could not persist, and this density was more effective at excluding eelgrass at earlier life stages.

Higher densities of bioturbators resulted in reduced performance and survival of eelgrass, a sediment-stabilizing species. This antagonism within soft sediments has long been recognized across a wide variety of taxa (Suchanek, 1983; Harrison, 1987; Philippart, 1994; Dumbauld and Wyllie-Echeverria, 2003; Valdemarsen et al., 2011; Kneer et al., 2013; Castorani et al., 2014; Goerlitz, 2015). In our observational study, no eelgrass occurred in sediments exceeding 336 shrimp m^{-2} (Fig. 4). In the experiment, both eelgrass life history stages declined in the presence of shrimp densities near 300 m^{-2}, with seedlings experiencing the greatest declines and almost complete elimination after a few weeks (Fig. 6). The capacity of eelgrass to establish within a shrimp bed is impaired at still lower densities, given the pattern in transplants across a gradient of shrimp densities (Fig. 5).

Distributions of seagrasses and burrowing shrimp are mutually exclusive in many parts of the world (Brenchley, 1982; Suchanek, 1983; Posey 1986; Townsend and Fonseca, 1998; Berkenbusch et al., 2000, 2007; Berkenbusch and Rowden, 2007), so the pervasive presence of shrimp in nearly all cores of our observational study was unexpected. Within Willapa Bay, shrimp appear to be the more dominant engineer due to sediment reworking by shrimp either uprooting or burying seedlings and adult shoots. Studies in South African estuaries have similarly shown that burrowing-shrimp can reduce seagrass transplant survival (Watson et al., 2023) and can be locally eliminated to improve seagrass survival (Angel et al., 2006). However, the return of shrimp eliminates seagrass (Siebert and Branch, 2007). In previous work examining interactions between shrimp and seagrass in eastern north Pacific estuaries, eelgrass has been suggested as the competitive dominant. In central and southern California, eelgrass is resistant to the effects of shrimp, outcompeting them for space. Only when disturbances to eelgrass patch edges remove shoots from sediments are shrimp able to colonize from adjacent habitats (Castorani et al., 2014). Likewise, in British Columbia, Canada, shrimp populations were shown to decline when native (*Z. marina*) and non-native (*Z. japonica*) eelgrass encroached but were able to recolonize lost habitats when vegetation was removed (Harrison, 1987). As eelgrass branches and grows, the shoots create a matrix of roots and rhizomes below the surface. These matrices can act as a barrier, impeding the mobility of shrimp and their ability to maintain their burrows (Brenchley, 1982; Siebert and Branch, 2006; Castorani et al., 2014), as well as negatively affect other burrowing estuarine species (Brenchley, 1982; Orth et al., 1984). However, the effectiveness of rhizome mats at impeding shrimp likely depends on shoot density and, for other species of burrowing shrimp, the attributes of their burrows (Siebert and Branch, 2007). Where eelgrass is the competitively dominant engineer, shoot densities average roughly 350 shoots m^{-2} (Castorani et al., 2014). Eelgrass densities in Willapa Bay average roughly 100 shoots m^{-2} (Ruesink et al., 2012), which is still above our recorded shoot densities near eelgrass habitat borders (Fig. 4, Supplemental Fig. 1B). Therefore, if the presence of rhizome mats hinders shrimp, it is likely that eelgrass densities in Willapa Bay are too low to reduce or eliminate shrimp from sediments, explaining their occurrence on either side of eelgrass habitat borders.

Bioturbation by burrowing-shrimp is a suggested cause of seagrass declines by reducing available light for photosynthesis, smothering shoots and seedlings with sediments, or uprooting them (Suchanek, 1983; Dumbauld and Wyllie-Echeverria, 2003; Siebert and Branch, 2006; Berkenbusch et al., 2007). In the Wadden Sea and Danish fjords, similar effects are produced by bioturbating lugworms (*Arenicola spp*.), which can unbury seeds and uproot seedlings, or move seeds and seedlings down in the sediment to a depth where they are not viable (Philippart, 1994; van Wesenbeeck et al., 2007; Valdemarsen et al., 2010, 2011). In our life stage experiment, the disappearance of transplants was due to uprooting or complete burial of individuals by shrimp. Only when shrimp were in very low abundance (Fig. 6A) or when shrimp were excluded using burlap membranes (Fig. 6B) did seedlings and vegetative shoots persist.

The engineering effects of burrowing shrimp and seagrass alter the sediment characteristics of their environments. Seagrass shoots obstruct water flow, causing silts and organic particles to settle out, helping to stabilize sediments (Fonseca and Fisher, 1986; Fonseca and Cahalan, 1992; Koch and Gust, 1999; Hemminga and Duarte, 2000; Madsen et al., 2001), whereas sediment reworking by burrowing shrimp reduces organic content and resuspends silts and fine particles into the water column where they are swept away (Kioke and Mukai, 1983; Waslenchuk et al, 1983; Ford et al., 1999; Webb and Eyre, 2004; D’Andrea and DeWitt, 2009; Jordan, 2009).

Given the strong biogeomorphological impacts of each species observed in other published studies, nonsignificant relationships for silt content in this study likely reflect that transects were positioned across a broad range of hydrodynamic and depositional environments. Specifically, the strong site-level differences in sediment properties may be caused by distinct overlaying hydrodynamic conditions at each site. It remains to be determined how much shrimp can alter sediment properties in Washington coastal estuaries. Eelgrass was not consistently associated with finer, more organic sediments in our study, which aligns with results from Richardson et al. (2008) in Willapa Bay and some eelgrass transplant results (Amone-Mabuto et al. 2022).

Observations of habitat borders and experimental manipulations of density provide novel insight into how eelgrass responds to antagonistic ecosystem engineers across a range of densities. Key areas warrant future exploration. We did not experimentally test if high eelgrass densities could reduce shrimp, which may contribute to contrasts here (shrimp dominant) compared to other work (eelgrass dominant). Certain levels of antagonistic ecosystem engineering may be necessary before shrimp are able to occupy and encroach on eelgrass habitats, or eelgrass may outcompete shrimp. Based on our findings, eelgrass and shrimp habitats are not alternative to one another in Willapa Bay, but rather shrimp are capable of existing within a range of eelgrass densities; when at sufficiently high densities, shrimp can eliminate seedlings and likely prevent eelgrass from returning. The ensuing mosaic of seagrass and sand flats of dense bioturbators also influences the community of associated taxa, abundance, and biodiversity (Dittmann, 1996; Berkenbusch et al., 2000, Angel et al., 2006; Siebert and Branch, 2005, 2007; Gross et al., 2019).

Conclusion

While discrete habitat borders may emerge from self-promoting positive feedback created by engineering species, eelgrass meadow edges in Willapa Bay cannot be attributed to this mechanism, because shrimp did not change abruptly at the same positions. Nevertheless, increasing shrimp density negatively impacts eelgrass. Eelgrass could not persist above shrimp densities near 300 m^{-2}, and this density more rapidly excluded earlier eelgrass life history stages. While previous studies suggest mutually exclusive distributions of seagrasses and deposit-feeding shrimp, our observations in Willapa Bay document coexistence of antagonistic ecosystem engineers at low densities. These findings provide further insight into the interactions between seagrass and burrowing shrimp and highlight the need for further research to characterize the mechanisms shaping their interactions and habitat dynamics.

Acknowledgements

We thank Zachary Forster from the Washington Department of Fish and Wildlife for logistic support and Alan Trimble for boat support. We also thank Haleh Mawson, James Fitzpatrick, Aspen Katla, and Milly Marriot-Green for help in the field and Fiona Boardman who provided helpful edits. This manuscript is dedicated to the memory of Lynn Hull.

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Tables and Figures

Table 1. Total eelgrass (*Zostera marina*) shoots transplanted in 0.25 m^2 and average shrimp densities (*Neotrypaea californiensis*) ± 1 SE (number of plots for shoot totals and samples for shrimp density in parentheses). Final shoot counts were made at the number of Days shown following transplant. Ditto marks mean that the information is the same as the line above.

Lat	Lon	Transplant Date	Days	Initial & Final total shoots	Average shrimp m^{-2}
46.99510	-124.13196	8/22/2022	28	77 (3), 55 (3)	32 ± 0 (2)
46.99550	-124.13070	″	″	47 (3), 56 (3)	40 ± 8 (2)
46.99644	-124.13223	″	″	58 (3), 31 (3)	32 ± 16 (2)
46.99750	-124.13120	″	″	50 (3), 48 (3)	96 ± 0 (2)
46.99777	-124.13235	″	″	52 (3), 45 (3)	16 ± 16 (2)
46.99747	-124.13123	″	″	45 (3), 47 (3)	40 ± 24 (2)
46.99791	-124.13252	″	″	66 (3), 20 (3)	88 ± 24 (2)
46.99797	-124.13154	″	″	73 (3), 62 (3)	88 ± 40 (2)
46.89532	-124.08759	8/18/2023	25	60 (3), 46 (3)	112 (1)
46.89350	-124.08768	″	″	60 (3), 50 (3)	80 (1)
46.89218	-124.08822	″	″	60 (3), 31 (3)	368 (1)
46.89080	-124.08878	″	″	60 (3), 64 (3)	32 (1)
46.88954	-124.08989	″	″	60 (3), 21 (3)	240 (1)
46.51402	-123.97892	4/21/2022	29	150 (5), 110 (5)	280 ± 38 (6)
46.51483	-123.97970	5/06/2023	40	75 (5), 0 (5)	379 ± 72 (3)
46.51398	-123.97739	″	″	75 (5), 61 (5)	16 ± 0 (3)

Table 2. Summary of parameters and ΔAICc comparisons between models for eelgrass and shrimp densities. NegLL is the negative log-likelihood. Models favored by differences in ΔAICc for a given species are shown in bold.

Species	Model	α	β	θ	NegLL	ΔAICc
Eelgrass	**Threshold**	9.35	239.99	8.55	252.81	0
	Gradient	15.78	3.36	1	313.97	111.31
	Constant	4.30	0		454.94	385.26
Shrimp	Threshold	6.35	-0.85	0.74	357.53	22.74
	Gradient	6.99	-0.79	1	358.07	32.65
	Constant	10.69	0		378.40	0

Table 3. Tukey’s pairwise comparisons of treatment by life stage combinations in the presence of shrimp. Ditto marks mean that the information is the same as the line above.

Comparisons			Est.	95 % CI	$T_{2,19}$	*P*
A) Exclusion seedlings	-	Control seedlings	0.82	[0.59, 1.04]	10.5	< 0.001
Exclusion vegetative	-	″	0.89	[0.67, 1.12]	8.32	< 0.001
Control vegetative	-	″	0.65	[0.43, 0.87]	11.43	< 0.001
B) Exclusion vegetative	-	Exclusion seedling	0.08	[-0.15 0.30]	0.98	0.76
Control vegetative	-	″	-0.16	[-0.39, 0.06]	-2.14	0.18
C) Exclusion vegetative	-	Control vegetative	0.24	[0.02, 0.47]	3.12	0.03

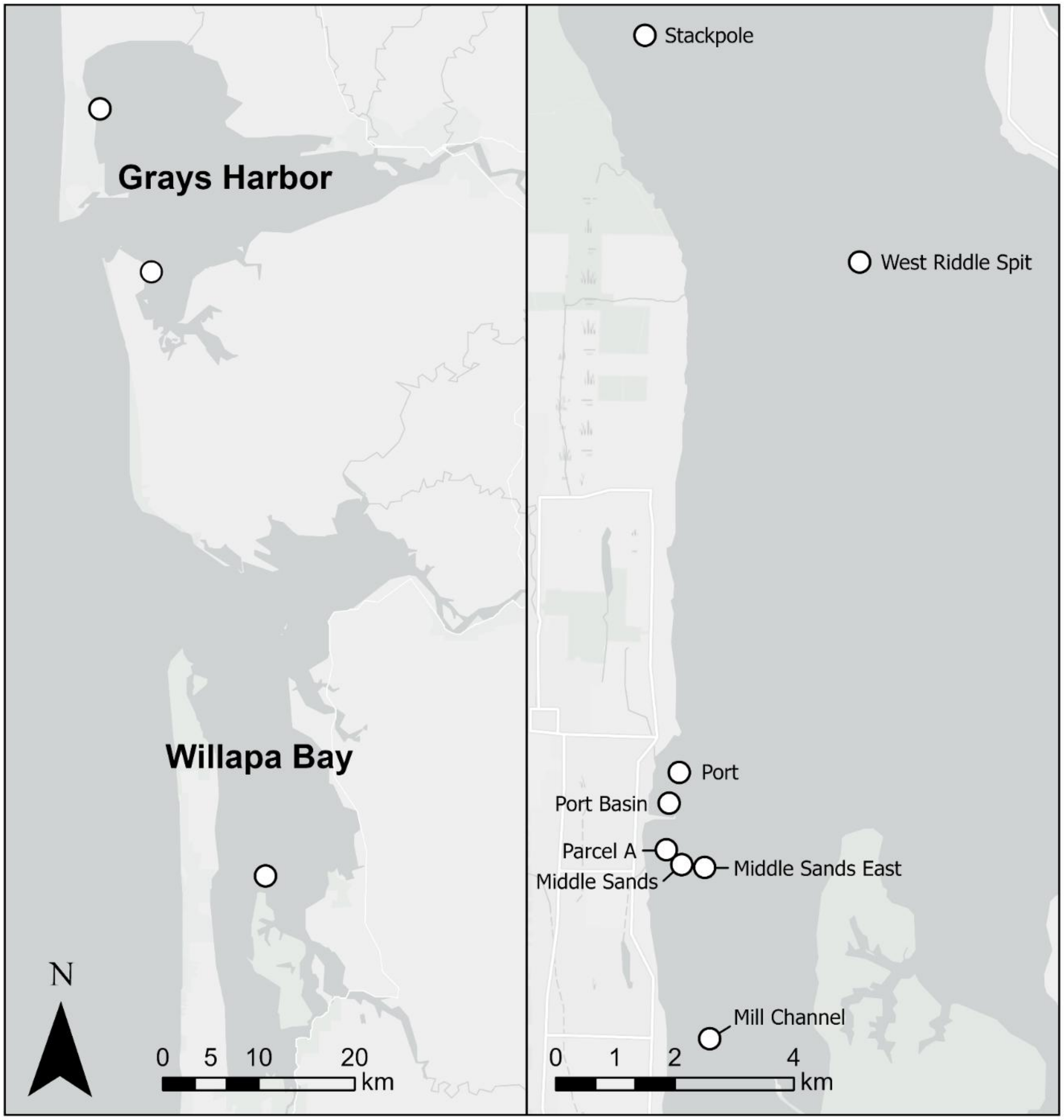


Figure 1. Locations of eelgrass transplants in Willapa Bay, WA and Grays Harbor, WA (left) and the 8 eelgrass habitat borders in Willapa Bay (right). The Long Island marker includes the location for one of our across-bay eelgrass transplant sites as well as our eelgrass life stage experiment.

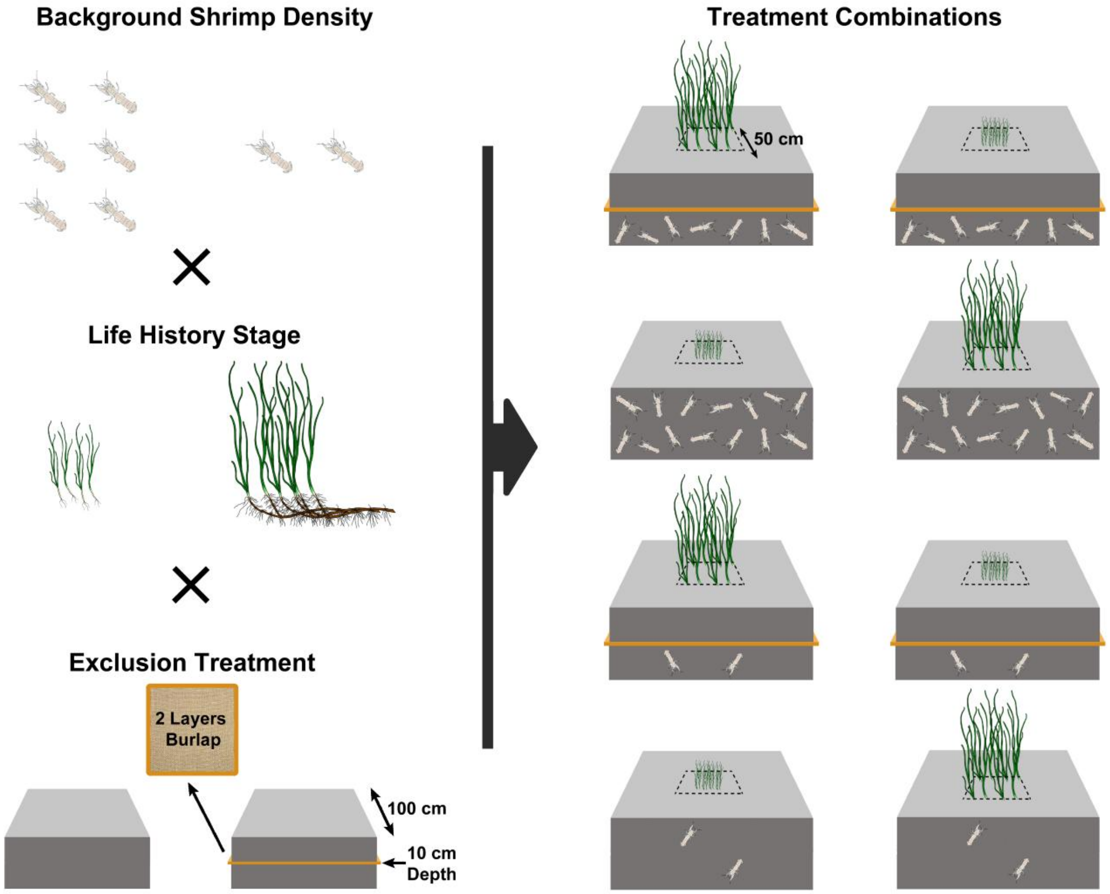


Figure 2. Diagrammatic depiction of our different treatments and their associated levels (left) and their combinations (right). Dashed lines represent 0.25 m^2 plots in the center of 1 m^2 plots.

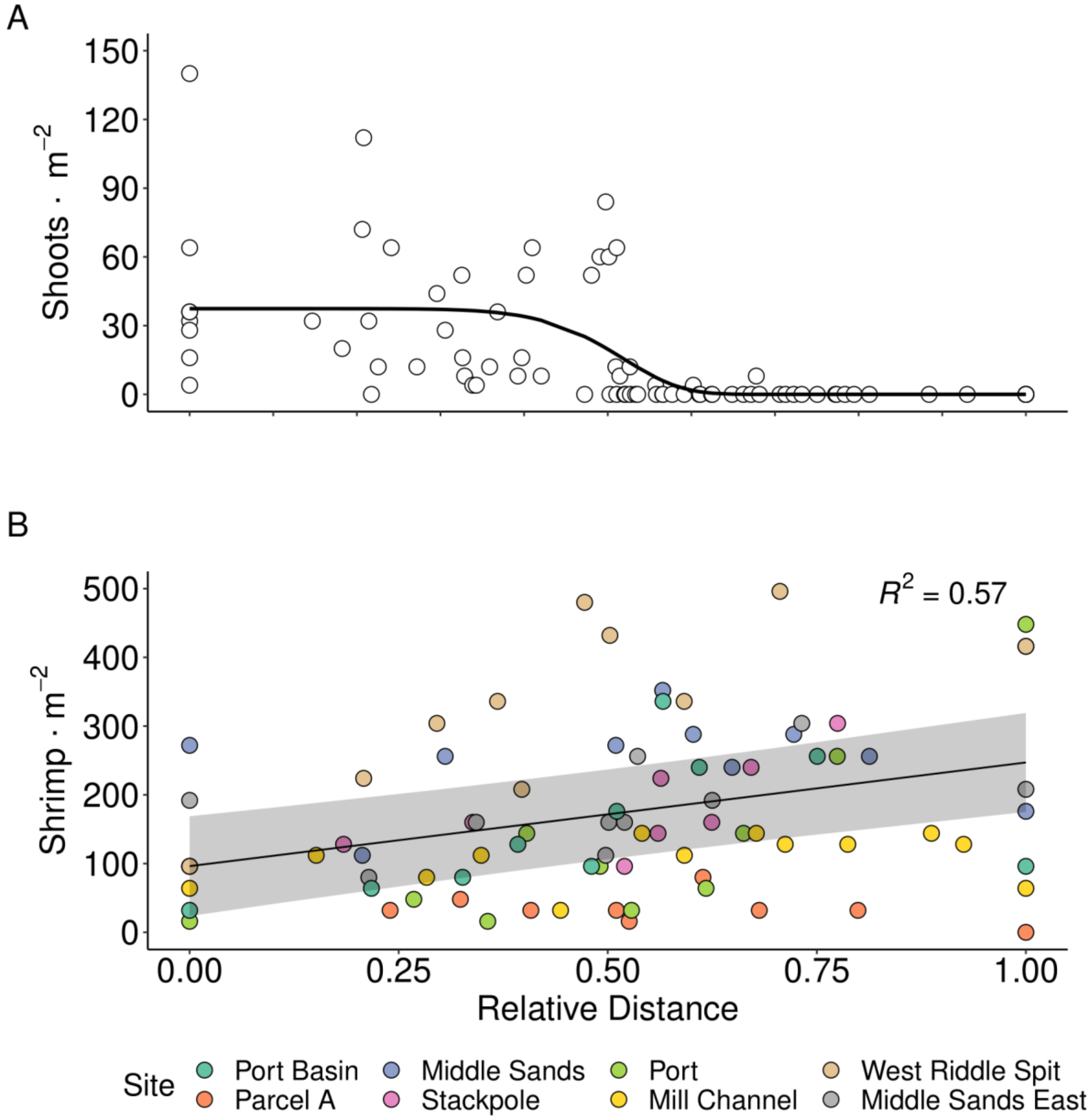


Figure 3. Models describing A) changes in eelgrass density across transects and B) the overall relationship between shrimp density and relative distance along transects. Relative distance is the distance across transects starting furthest in the eelgrass habitat on the left (0) and ending furthest in the bare habitat on the right (1) with the habitat border in the middle (0.50). Points represent observed counts for eelgrass and shrimp. Lines represent estimated values and in panel B, the shaded area represents 95% confidence intervals. R^2 value represents the conditional R^2.

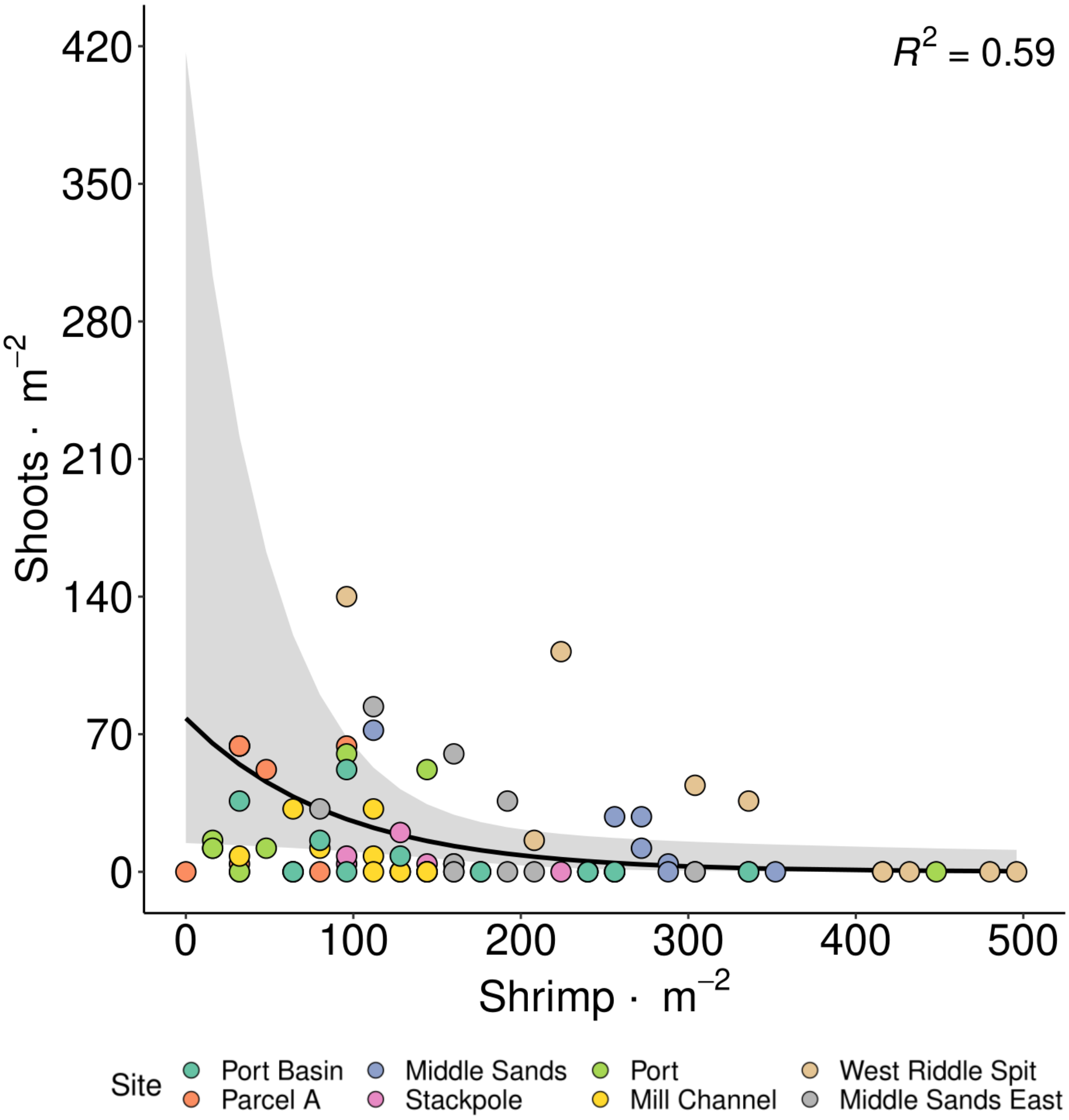


Figure 4. Relationship between shrimp density and eelgrass density from habitat borders. Line represents predicted values with 95% confidence intervals. The R^2 value represents the conditional R^2.

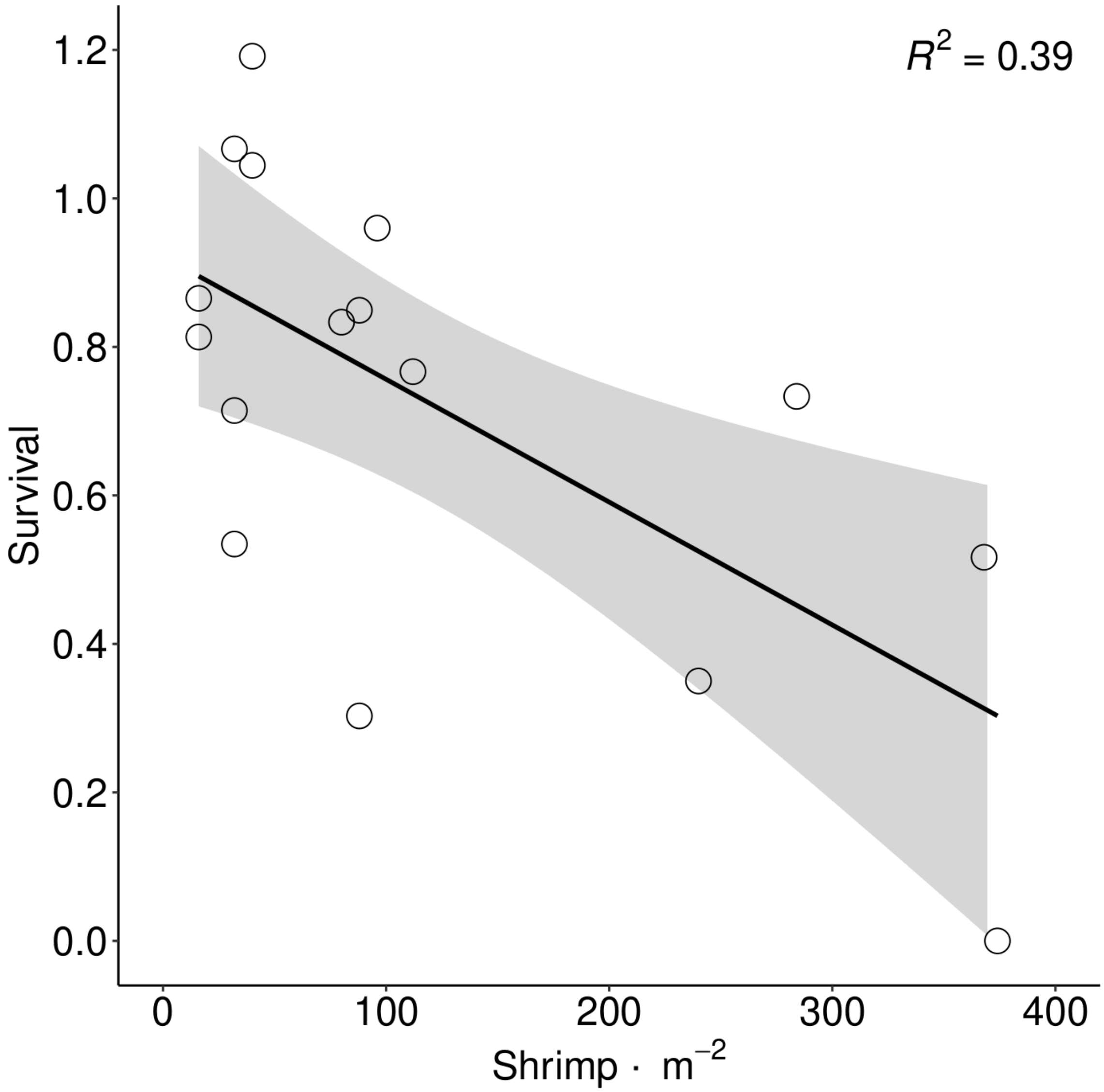


Figure 5. Effects of shrimp density on transplanted eelgrass survival. Black line represents the line of best fit and the grey error surrounding the line represents 95 % CI intervals. The R^2 value represents the marginal R^2.

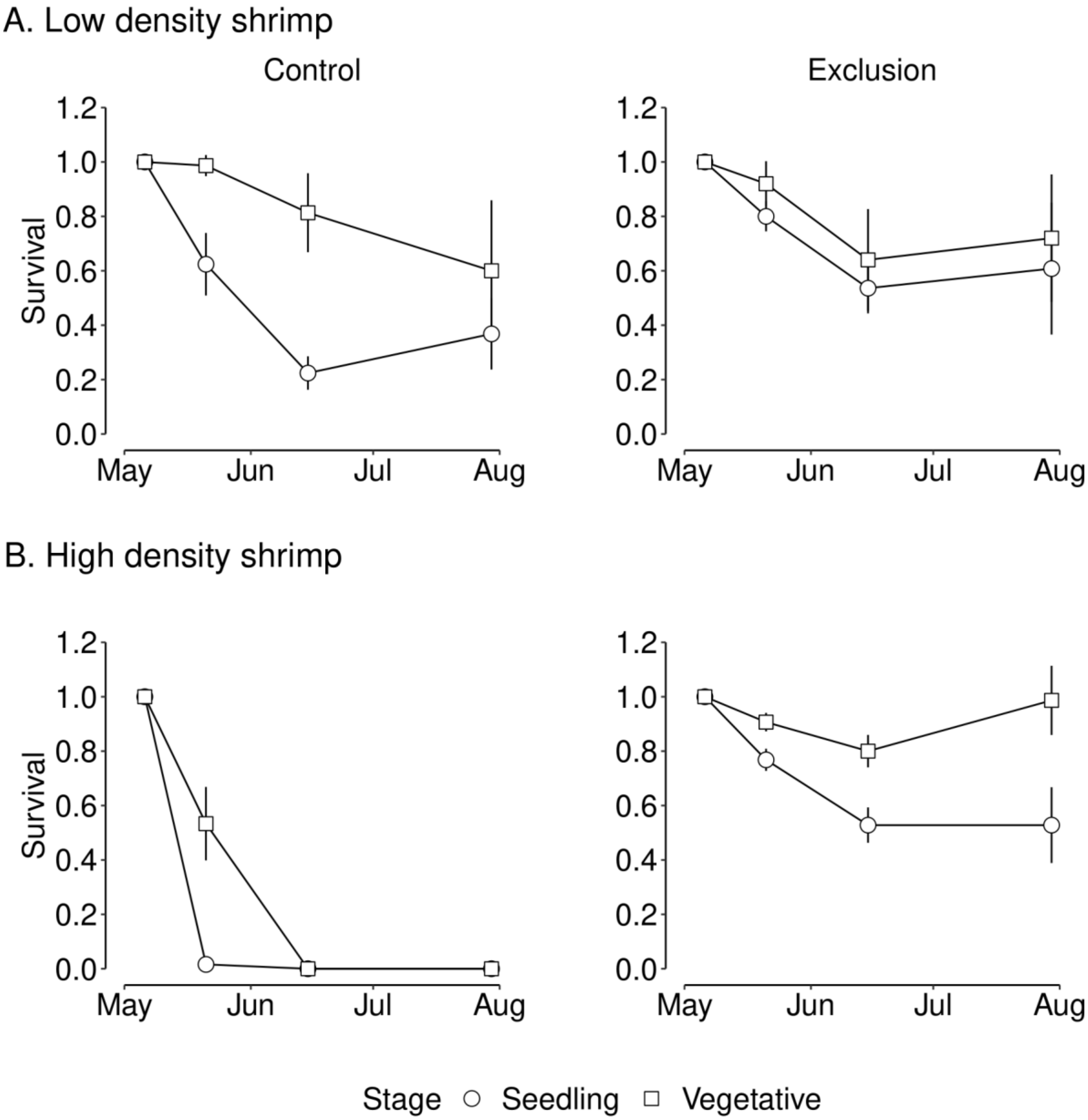


Figure 6. Effects of exclusion treatments on *Z. marina* vegetative shoots and seedling survival within A) a tide flat with a low background shrimp density and B) a tide flat with a high background shrimp density. Error bars represent ± 1 standard error (N = 5). Some error bars are small and hidden behind points. Points and error bars that are greater than and extend beyond 1 are due to eelgrass densities increasing past their initial transplant densities.

Supplemental Material

Supplemental Methods

The Poisson power-exponential function accommodates distinct curve shapes allowing all three possibilities (Wagner et al. 2012): constant density, exponentially declining (or increasing), or regions of different density separated by a threshold. The Poisson power-exponential function takes the following form:

(1) $$f(x) = ae^{-bx^{\theta}}$$

where x is the proportional distance along the transect, a is a scale parameter for overall abundance, b is a scale parameter for steepness of change, and θ is a shape parameter incorporating a threshold between high and low density ($\theta > 1$ for threshold). This function requires that the value of x (distance along transect) lies between 0 and 1, so we used proportional distance along each 70-meter transect, initiated from the eelgrass bed for both eelgrass and shrimp. The log-likelihood (L) for the Poisson power-exponential was calculated as:

(2) $$L = \sum_{i-1}^{n} y_i \log(\hat{f}(\mathrm{x_i})) - \hat{f}(\mathrm{x_i}) - \log(\mathrm{y}_i!)$$

where n is the number of observations, $\hat{f}(\mathrm{x_i})$ is the fitted mean given by Eq. (1) and y is the response variable (i.e., eelgrass or shrimp density). We estimated parameters by minimizing the negative log-likelihood (*optim* function) for the three cases: 1) threshold model where a, b, and θ were allowed to vary; 2) exponential model where $\theta = 1$, and 3) constant model where $\theta = 1$ and $b = 0$. Then, we compared among the three models by small sample sized corrected Akaike Information Criterion (AICc), which accounts for model fit and complexity. We considered models where the difference between AICc (Δ AICc) was less than 2.0 to indicate equivalent explanatory power.

Supplemental Table 1. *Neotrypaea californiensis* size classes and average wet mass. Values within parentheses represent standard error and n is the sample size of shrimp per size class.

	Carapace Length (mm)	Wet Mass (g)	n
Large	> 17.42	6.70 (± 1.68)	10
Medium	12.49 – 17.42	4.01 (± 1.04)	50
Small	8.28 – 12.49	1.53 (± 1.03)	75
Extra Small	4.14 – 8.28	0.36 (± 0.60)	120
Recruit	< 4.14	0.05 (± 0.02)	3

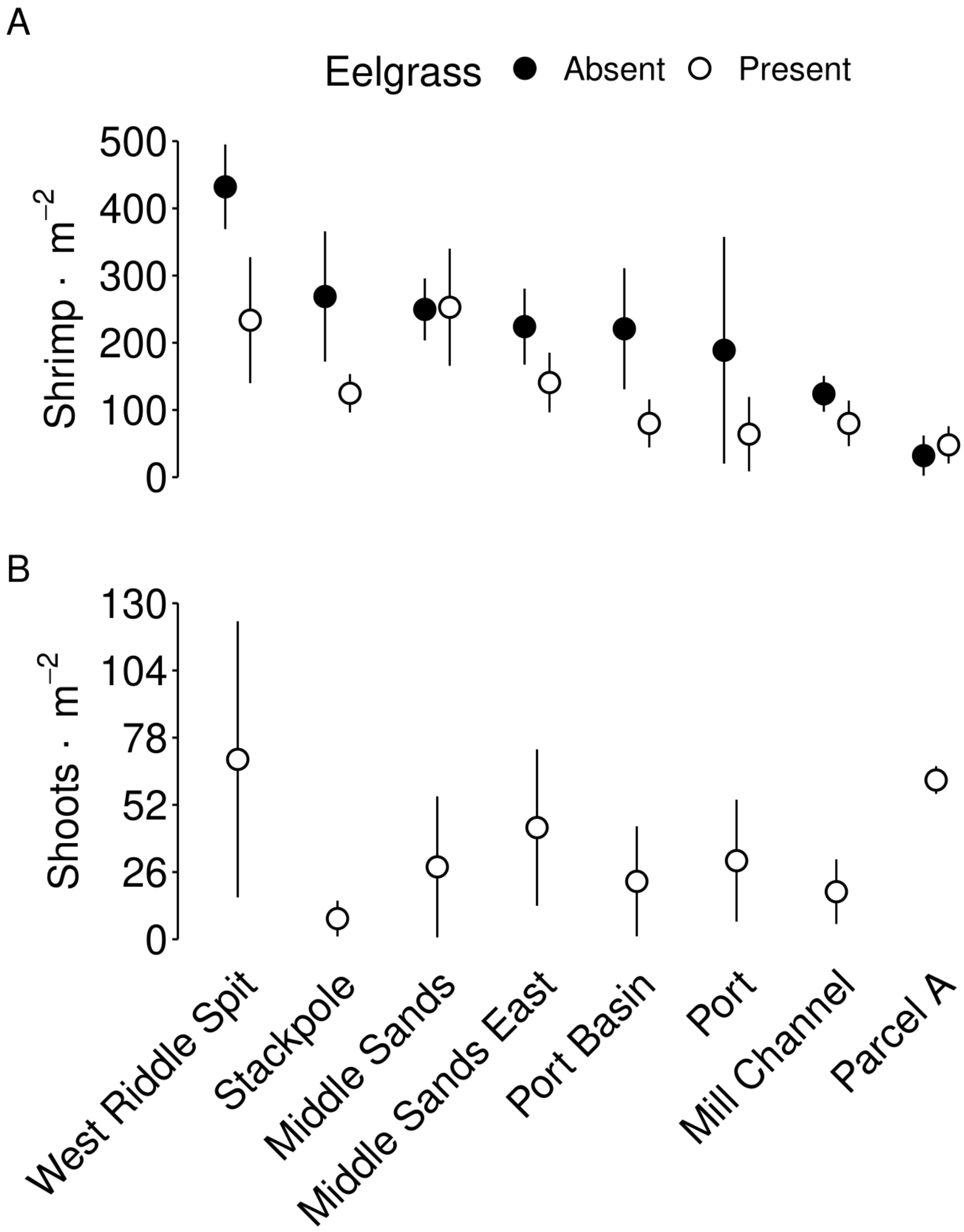
Supplemental Table 2. Summary of parameters and ΔAICc comparisons between models for eelgrass density and shrimp abundance. NegLL is the negative log-likelihood. Models favored by differences in ΔAICc for a given species are shown in bold.

Site	Species	Model	α	β	θ	NegLL	ΔAICc
Middle Sands	Eelgrass	**Threshold**	11.44	32.35	4.43	13.28	0
		Gradient	16.05	4.02	1	23.23	8.90
		Constant	3.6	0		45.12	44.68
	Shrimp	Threshold	16.23	0.39	22.91	27.35	17.27
		Gradient	15.26	-0.05	1	28.20	7.97
		Constant	15.7	0		28.22	0
Middle Sands East	Eelgrass	**Threshold**	9.47	745.13	11.34	39.42	0
		Gradient	12.07	1.85	1	53.31	20.21
		Constant	5.4	0		59.62	26.50
	Shrimp	Threshold	8.92	-0.58	1.43	26.70	7.31
		Gradient	8.41	-0.59	1	26.40	0
		Constant	11.4	0		27.73	3.67
Mill Channel	Eelgrass	Threshold	8.20	17.35	2.53	9.11	2.26
		Gradient	11.24	5.49	1	11.77	0
		Constant	1.77	0		34.26	38.66
	Shrimp	Threshold	4.01	-0.63	0.27	28.36	12.12
		Gradient	5.25	-0.42	1	28.60	5.04
		Constant	6.69	0		29.25	0
Parcel A	Eelgrass	**Threshold**	16.22	53.37	6.64	21.21	0
		Gradient	25.20	2.78	1	36.24	19.07
		Constant	8.1	0		58.59	55.76
	Shrimp	Threshold	5.85	1.44	0.54	16.33	14.24
		Gradient	5.09	1.57	1	16.44	3.44
		Constant	2.5	0		18.72	0
Port	Eelgrass	**Threshold**	7.16	745.13	11.16	24.81	0
		Gradient	8.63	1.84	1	39.97	19.32
		Constant	3.8	0		44.48	20.34
	Shrimp	Threshold	1.29	-3.10	1.16	24.00	10.89
		Gradient	0.99	-3.34	1	24.05	0
		Constant	7.9	0		52.13	48.15
Port Basin	Eelgrass	**Threshold**	5.23	745.13	10.48	25.44	0
		Gradient	8.49	2.85	1	31.10	3.74
		Constant	8.7	0		92.30	12.00

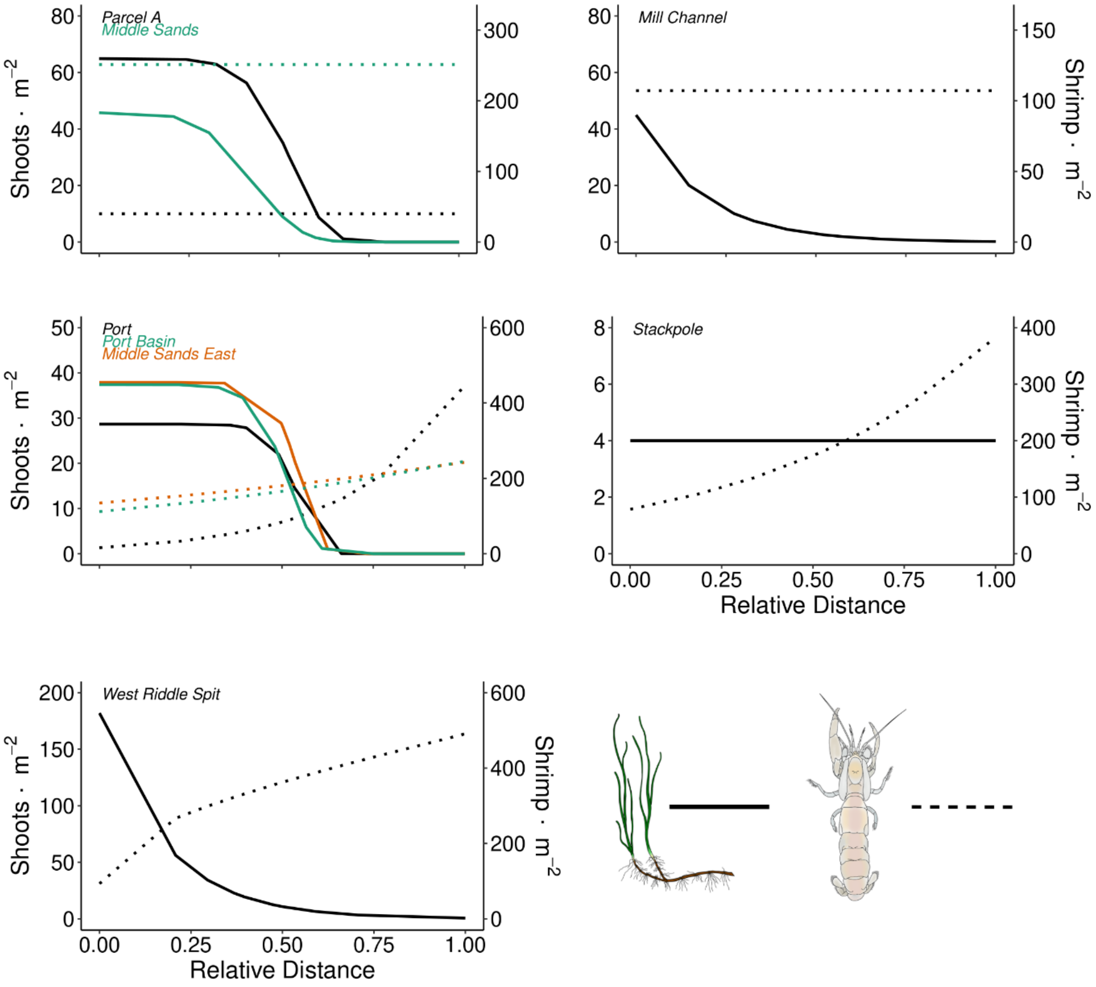
	Shrimp	Threshold	9.78	0.49	51.80	36.47	14.64
		Gradient	5.17	-1.14	1	31.10	0
		Constant	9.4	0		37.25	2.28
West Riddle Spit	Eelgrass	**Threshold**	33.80	64.84	3.63	13.16	0
		Gradient	45.49	5.65	1	27.40	0.49
		Constant	5.4	0		59.62	8.47
	Shrimp	**Threshold**	5.83	-1.66	0.29	28.53	0
		Gradient	12.72	-1.00	1	32.07	7.31
		Constant	20.8	0		39.72	10.98
Stackpole	Eelgrass	Threshold	2.48	745.13	10.87	9.00	6.04
		Gradient	3.45	3.06	1	11.86	0.76
		Constant	1	0		15.48	0
	Shrimp	Threshold	6.63	-1.40	1.76	23.74	9.77
		Gradient	4.91	-1.58	1	24.36	0
		Constant	12.3	0		34.79	12.85

Supplemental Table 3. Average surface hole counts within plots control and exclusion plots in the low- and high-density shrimp areas. SD = standard deviation and SE = standard error.

Site	Treatment	Date	Holes m^{-2}	SD	SE
Low density shrimp	Control	5/21/2023	0.4	1.26	0.4
		6/15/2023	0	0	0
		7/30/2023	0	0	0
	Exclusion	5/21/2023	0	0	0
		6/15/2023	0	0	0
		7/30/2023	0	0	0
High density shrimp	Control	5/21/2023	102.4	19.4	6.1
		6/15/2023	91.6	19.0	6.0
		7/30/2023	131.6	54.8	17.3
	Exclusion	5/21/2023	0	0	0
		6/15/2023	0	0	0
		7/30/2023	0	0	0



Supplemental Figure 1. Average A) shrimp density in the presence and absence of eelgrass along transects, B) eelgrass density on the eelgrass side of transects at each site. Error bars represent standard deviation and when small, are hidden behind the dots.



Supplemental Figure 2. The best models describing the distribution of eelgrass and shrimp densities across transects at each site. Relative distance represents the distance across transects starting furthest in the eelgrass habitat on the left (0) and ending furthest in the bare habitat on the right (1) with the habitat border in the middle (0.50).

Chapter 2: Sediment burial caused by bioturbating shrimp negatively affects juvenile oyster survival and size.

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Published in Marine Ecological Progress Series

Hull, WW, Subbotin, EL, and Ruesink, LJ (2025) Sediment burial by biotubating shrimp negatively affects juvenile oyster survival and size. Marine *Ecological Progress Series.*

https://doi.org/10.3354/meps14831

Abstract

Epibenthic organisms on intertidal flats can be affected by underlying sediments and by the activities of bioturbating species that live there. Therefore, bioturbating shrimp have two potential pathways to affect small clusters of juvenile oysters (seeded cultch): directly by moving sediment to the surface, or indirectly by affecting sediment properties (grain size, organic content, penetrability). We examined how oyster (*Magallana gigas*) survival and size responded to a) shrimp (*Neotrypaea californiensis*) density, b) mud content and penetrability of sediment, and c) shrimp density due to their effects on sediment properties (indirect pathway). Seeded cultch were deployed from spring through summer at 31 intertidal sites varying in both shrimp density and sediment properties within Willapa Bay, Washington (USA). Shrimp density was negatively associated with mud and organic content but positively with sediment penetrability, as expected from known ecosystem engineering effects of shrimp. However, neither mud content nor penetrability contributed statistically to the negative impact of shrimp density on oyster survival and size. No oysters survived the summer above 50 – 100 shrimp m^{-2}, and remaining oysters were smaller with increasing shrimp density. Overall, negative effects of shrimp on benthic oysters likely occur through the deposition of sediment (28.9 ml burrow^{-1} day^{-1}) rather than alteration of sediment properties. Our study highlights how the antagonistic ecosystem engineering effect of shrimp on oysters occurs independently of sediment responses to bioturbation and deposit-feeding and quantifies the conditions ensuring the persistence of ecologically- and commercially important foundation species.

Introduction

Ecosystems are composed of many habitats, some of which are created by ecosystem engineers, which modify the physical environment and thus the availability of resources to other species (Jones et al. 1994). In estuaries, tidal flats comprise a mosaic of different habitats developed and dominated by different engineers manipulating their habitats in characteristic ways. Engineers that create biogenic structure (autogenic) reduce sediment resuspension (Fonseca & Cahalan 1992, Meyer et al. 1997, Hemminga & Duarte 2000) ultimately stabilizing sediments and preventing erosion (Gregalis et al. 2008, Powers et al. 2009, Marin-Diaz et al. 2020, Infantes et al. 2022). In contrast, engineers that modify tidal flats through mechanical activity (e.g., physically mediated interactions) tend to do so through the process of bioturbation, which can eliminate sediment stabilizers (i.e. biofilms) and mud (particles < 63 μm) causing increased sediment resuspension and destabilization (Reise 2002, Pillay & Branch 2011, Kristensen et al. 2012). Interactions between these different engineering taxa can be antagonistic, with bioturbation resulting in the exclusion or elimination of structure-forming engineers through either burial or ejection from the sediment (Siebert & Branch 2006, Valdemarsen et al. 2011, Suykerbuyk et al. 2012, Hull & Ruesink 2024). These interactions may also play out in a management context where bioturbators are pests, preventing other engineers, such as oysters, from growing on tidal flats (Dumbauld et al. 2001, 2006).

Sediments are known to influence oysters in mechanistically distinct ways across their life cycle. The presence of mud and sediments on hard substrates can render otherwise suitable habitat unfit for the settlement of oyster spat (McKinney et al. 1976, MacKenzie 1983). Settled oysters can discern between inert and organic particles during feeding (Haven & Morales-Alamo 1970), while sedimentation can reduce valve opening and filtration times, and even clog and abrade gill tissue, causing metabolic stress and reduced growth (Grant et al. 1990, Suedel et al. 2015, Poirier et al. 2021). In cases where complete burial occurs, oysters are unable to effectively feed and respire, resulting in death (Essink 1999, Colden & Lipcius 2015). Overall, sedimentation has been considered a main cause of failure for intertidal and subtidal reefs (Bahr & Lanier 1981, Taylor & Bushek

2008, Powers et al. 2009), and on aquaculture beds where oysters are grown on the bottom, survival is impaired as mud content increases (Ruesink et al. 2023).

Bioturbation involves the transport of sediment by organisms within the sediment column (DeWitt 2009). Bioturbation can result from burrowing or feeding (Kristensen et al. 2012) and includes deposition at the sediment-water interface. In estuarine systems, thalassinidean shrimp rank among the most influential bioturbators. During burrowing and sediment reworking, they reduce mud and organic content around burrows (Flach & Tamaki 2001, Pillay and Branch 2011, Pillay 2019). This increases the average grain size and destabilizes habitats, leading to greater sediment penetrability and erodibility (MacGinitie 1934, Swinbanks & Luternauer 1987, Rowden et al. 1998, Contessa & Bird 2004). Surface-dwelling fauna tend to be negatively affected either through direct smothering and burial or by eliminating stabilizing biofilms and biochemical cues for recruitment (Branch & Pringle 1987, Pillay et al. 2007a, D’Andrea & DeWitt 2009, Pillay & Branch 2011). The creation of these coarse-grained, destabilized habitats can even facilitate other burrowing species (Pillay et al. 2010) further preventing establishment of autogenic engineers. Yet, the effects of thallassinid shrimp vary globally. Key considerations are rates of sediment movement by shrimp, the overlying sediment response to abiotic drivers (water energy, sediment supply), and the traits of other taxa that make them susceptible to destabilized sediments (Pillay 2019).

Because the engineering effects of thalassinids span granulometry, biochemistry, stability, and turnover of sediments, multiple mechanisms may underlie antagonistic effects on suspension-feeders or seagrasses. Burial by sediment ejected from burrows would be a direct effect (Fig. 1), whereas turbidity and increased susceptibility to erosion are indirect (DeWitt 2009). Both pathways could be at play in U.S. estuaries of the Pacific Northwest where *Neotrypaea californiensis* (Dana 1854), a thalassinid known as ghost, sand, or burrowing shrimp, is a pest in intertidal culture of Pacific oysters (*Magallana gigas* Thunberg 1793). *Neotrypaea californiensis* reach lengths of 7 cm at densities exceeding 500 m^{-2} and burrow to 1 m depth, creating unstable, fluidized sediment (MacGinitie 1934, Bird 1982, Posey 1986). Sediment ejection rates have been estimated at 18-50 ml $shrimp^{-1}$ day^{-1} (MacGinitie 1934, Swinbanks & Luternaur 1987). While shrimp are unable to burrow through an oyster reef (Stevens 1929), farmers do not plant oysters at high densities to avoid conspecific competition (Cognie et al. 2006, Dumbauld et al. 2021). Ground culture of oysters does not provide a sufficient barrier against shrimp, and relatively low densities of shrimp (20 – 40 burrows · m^{-2}) can cause widespread oyster mortality on a ground-culture bed (Dumbauld et al. 2006). Juvenile oysters settled on cultch are considered particularly vulnerable (Dumbauld et al. 1996, 1997, 2001). A management threshold for control has been set at 10 burrows m^{-2} (Dumbauld 2006), although growers currently lack a cost-effective way of reducing shrimp densities on oyster beds (Ruesink et al. 2025).

The objective of this study was to explore relationships among juvenile oyster performance, sediment type, and shrimp density across an existing habitat mosaic, since experimental removal of shrimp confounds effects of the disturbance and the shrimp themselves (Pillay and Branch 2011). Shrimp activity may directly affect oysters through ejection and deposition of sediment (Fig. 1) or indirectly create habitats unsuitable for oysters. Separately, we compared sediment deposition with and without burrows using a common and reliable method of entrapment in tubes (Maire et al. 2008). The overall design addressed the relative importance of direct and indirect pathways affecting juvenile oysters and better-defined shrimp densities that prevent successful oyster culture.

In our study we outplanted juvenile oysters across intertidal sites differing in shrimp densities and sediment properties to answer the following questions:

1) How much of the total variation in sediment properties across estuarine sites is related to shrimp density?
2) Is oyster survival better predicted by shrimp density directly, or via an indirect pathway through sediment properties?
3) Where oysters survive, is oyster size better predicted by shrimp density or by sediment properties?

A short-term sediment capture study was carried out to determine deposition rate of sediment by shrimp in the field. This work was contextualized by measurements on two classes of aquaculture beds, those consistently used for growing shellfish, and those recently or soon abandoned due to the increased presence of shrimp. Our aim was to determine if the sediment levels identified to impair the performance of outplanted juvenile oysters also occurred at the commercial scale in aquaculture beds.

Materials & Methods

We used three approaches to determine the effects of shrimp on oysters. First, we implemented an oyster outplant study to quantify the effects of burrowing shrimp on sediment properties and their effects on oyster performance through direct and indirect pathways. Second, we quantified the relationship between mud content and shrimp density on abandoned and persistent shellfish aquaculture beds. Finally, we examined how much sediment is ejected from shrimp burrows by comparing the volume of entrapped sediment ejected from shrimp burrows, from within a shrimp bed but not over burrows, and in areas where shrimp were removed.

2.1 Study Location

Willapa Bay and Grays Harbor, Washington are large estuaries located on the west coast of the United States (Fig. 2). Willapa Bay covers 35,000 ha at mean high water, whereas Grays Harbor covers 23,500 ha (Hickey & Banas 2003). Both have a shallow mean water depth (Willapa Bay 3.2 m; Grays Harbor 4.3 m), that lead to extensive intertidal mudflats at low tide (Hickey & Banas 2003, Dumbauld & McCoy 2015). Both estuaries experience influxes of freshwater in winter and influences of offshore wind-driven upwelling in summer (Borde et al. 2003, Hickey & Banas 2003, Banas et al. 2004, Ruesink et al. 2018).

Three functionally dominant taxa are common on tide flats in Willapa Bay and Grays Harbor: seagrasses (native *Zostera marina* and non-native *Zostera japonica*), bivalve shellfish (mainly non-native *Magallana gigas* [Pacific oysters]), and shrimp (*Neotrypaea californiensis*). Shellfish aquaculture is conducted on 23% of Willapa Bay's intertidal area and as such, accounts for 10 – 20% of the USA's total oyster harvest (Feldman et al. 2000, Ruesink et al. 2006, Dumbauld & McCoy 2015). Oyster reserves occupy another 11.2% of intertidal area within Willapa Bay, 18% of which is occupied by feral oyster reefs (Dumbauld et al. 2011). Since their introduction from Japan in 1928 (Sayce 1976), Pacific oyster have consistently established reliable wild set in Willapa Bay since 1934 (Trimble et al. 2009, Dumbauld et al. 2011). Shrimp are distributed broadly throughout the estuary, occupying approximately 14% of Willapa Bay's intertidal area (Swinbanks & Murray 1981, Dumbauld et al. 2011, 2021). However, their densities tend to be higher towards the mouth of Willapa Bay (Dumbauld et al., 2021). Oyster production from Grays Harbor is roughly one-fifth of Willapa Bays (Decker, 2015) and while the distribution of shrimp has not been mapped as in Willapa Bay, shrimp are known to be abundant in the mid to upper intertidal zone (Swinbanks and Murray 1981, Dumbauld et al. 2011).

2.2 Seeded cultch outplants

To determine the effects of shrimp and sediment characteristics on oyster survival and size, we outplanted juvenile oysters in 31 low intertidal sites (0 to + 0.3 m relative to mean lower low water, MLLW) across Willapa Bay (Table S2, Fig. S1A). Sites were chosen based on surface characteristics to span a range of sediment and shrimp conditions without confoundment by tidal elevation (similar for all sites) or distance along an estuarine gradient. In relation to distance along the estuary, although the mud content of sediment tends to increase into the estuary (Ruesink et al. 2015) and shrimp are more common towards the mouth (Dumbauld et al., 2021), we found sites with a range of these characteristics throughout the bay. Our prior experience working in these areas, combined with local ecological knowledge from farmers, made us aware of heterogeneity in sediment properties, shrimp densities, and tidal elevations, which informed our site selection.

At each outplant site, shrimp, sediment, and penetrability were measured at three positions (replicates) spaced at least 2 m apart, at the same time as oysters were outplanted. Surface sediment (30 cm^3) was collected by inserting a syringe corer (3 cm diameter opening) 4.5 cm into the sediment. Sediment samples were then dried in a drying oven at 60°C to a constant weight (~ 72 h) and ashed (500°C for 3 h) for organic content via loss of mass on ignition (LOI) and sieved (Wentworth scale: 1000, 500, 250, 125, and 63 µm) for grain size analysis (Subbotin & Ruesink 2021, Hull & Ruesink 2024). Sediments smaller than 63 µm were labeled as mud (silt + clay) and both LOI and sediment fractions were weighed (nearest 0.0001 g). Sediment penetrability was based on how far a free-weight cone penetrometer descended into the sediment. The penetrometer consisted of a 159 cm long stainless-steel rod (1.2 cm diameter) with a 2.3 kg sliding weight. This weight dropped freely from 52.5 cm above the top of the rod, landing on the bottom base plate and driving the rod into the sediment. The sliding weight was dropped 3 consecutive times onto the base plate, and penetration depth (cm) of the rod into the sediment was determined after the third drop (WADNR 2020).

Shrimp were sampled at each outplant site through a manual 5-core method. This method involves coring five points within a 1 m^2 area, each of which has sediment extracted to a depth of 70 cm. The core was a stainless steel 5” clam gun (13 cm diameter, 36 cm deep), so several pulls were required per sampling point to reach the target depth. The sediment removed from each core was manually spread out to search for shrimp, which provide a vibrant yellow-pink contrast to sediment. We then scaled shrimp densities to 1 m^2 by multiplying the total count in each 5-core sample by 16. Although hand-sorting may under-sample very small shrimp (< 4.1 mm carapace length), we rarely found them when we included an additional step of sieving the top layer of sediment. In the few cases where we did encounter such individuals, they were excluded from density measurements as the larger shrimp are likely most important for bioturbation. This manual core method has a distinct advantage due to ease of use in the field, and in cases where the 5-core method was used next to a larger-diameter core, counts were similar or higher by a factor of two (Ruesink et al. 2024).

Both naturally and in aquaculture, oysters recruit onto pieces of shell (cultch). On-ground oyster culture practices in Willapa Bay and Grays Harbor typically result in roughly 5–20% cover (10-40 pieces of cultch m^{-2}) on aquaculture beds (Wagner et al. 2012). We deployed 10 pieces of cultch with hatchery-set oysters (10.8 ± 4.6 SD oysters $shell^{-1}$, N = 40 shells, 10.9 ± 3.7 SD mm shell height, N = 384 oysters) at each site. A hole was drilled into each piece of cultch and two pieces of cultch were secured to a single PVC pipe with approximately 40 cm of braided fishing line. Cultch were then tethered in place by driving 5 pieces of pipe with attached cultch (30 cm tethers) into the sediment until the top of each pipe was flush with the surface of the tidal flat (Fig. S3). All cultch were deployed in late April 2022 and collected in late August 2022 (Table S2), representing a typical summer growing season. Collected cultch were cleaned and counted for the number of living and dead oysters on each shell. We then measured the shell height (mm) of the first 10 live individuals per shell and shucked them to measure dry tissue mass (g). Tissue was dried in a drying oven at 50°C for 48 h and 100°C for another 48 h. Both shell height and tissue mass (metrics of size) were used as proxies for growth as it is extremely difficult, if not impossible to accurately measure shell mass of oysters from cultch, a required component for calculating condition index, without damaging the shells of individuals.

2.3 Conditions on persistent and abandoned shellfish aquaculture beds

Based on conversations with shellfish growers from both estuaries, we sampled 10 large (~ 2 ha) intertidal beds in Willapa Bay and three in Grays Harbor. All beds were sampled because of recent abandonment or farmer concerns regarding the potential abandonment as shrimp densities increase. Within a year, nine of the 13 beds ceased production, while four continued on with productive shellfish cultivation (Table S1, Fig. S1B). As detailed above, shrimp densities were sampled by deep manual cores (5-core sample method) and a surface sediment sample was simultaneously collected for later processing for organic content and grain size. In each bed, a grid was established using four transects that ran parallel to the nearest channel and extended toward the shore. Five points were sampled along each transect, resulting in a total of 10 to 20 replicate samples, depending on the duration of the tide, weather conditions, and the size of the field crew. The distances between sample points along the transects, as well as the overall size of the grid, were adjusted according to the bed size to ensure full coverage of the area. These data were visualized on axes of shrimp density and sediment mud content, distinguishing persistent shellfish culture from abandoned beds.

2.4 Sediment Ejection

Shrimp excavate and expel sediment from their burrows to the sediment-water interface. Methods of sediment entrapment involve collecting sediments deposited at this interface by placing a trap around the site of expulsion and returning after a known period to measure the amount of sediment collected (Maire et al. 2008). This approach has been commonly used for collecting sediment expelled from the burrows of bioturbating shrimp (Dumbauld et al. 1997, 2004, Berkenbusch & Rowden 1999, Fritz 2002) and allows a better account of sediment reworking. At one site in Willapa Bay (46.4920°N, -124.0216°W) with dense shrimp (209.6 ± 25.0 shrimp m^{-2}, N = 10), we used an entrapment method to determine daily sediment ejection from burrows. Sediment expelled from burrows was captured by placing circular pieces of cotton fabric (120 µm, ~7.5 cm diameter) with small central holes (~8 mm) over burrow openings (> 3 mm diameter, Dumbauld et al. 2021). To ensure expelled sediments were captured on top of the fabric, we placed PVC tubes (30 cm x 7.6 cm) with twenty 2-mm holes in the side and mesh-covered tops (~ 7 mm openings) around each burrow, pressing them halfway into the sediment (Fig. S2A). To determine baseline sediment deposition, we had two reference conditions: 1) entrapment within the shrimp bed but not centered on a burrow, and 2) entrapment within plots without shrimp. For the first reference condition, the fabric and tubes were situated to avoid burrow openings. For the second reference condition, we placed fabric and tubes within plots (2.5 m x 4 m) where no burrows were present because shrimp had previously been removed via sediment consolidation in June 2024. This was done by collapsing sediment galleries to a depth of 1 m via mechanical vibration, causing shrimp to become immobilized and unable to rework sediments before dying a few days later (Ruesink, pers. obs.).

We expected that the deployment of tubes over burrow openings would damage some shrimp burrows and prevent sediment ejection at the surface by shrimp. To account for the potential sample loss due to the absence of burrows inside the tubes, we doubled the number of tubes over burrow openings. A total of 24 replicates were placed over burrow openings, 12 within the shrimp bed but avoiding burrows, and 12 within plots where shrimp were removed (4 tubes in 3 plots, Fig. S2B). Pieces of fabric that were successfully placed over shrimp burrows generally had small mounds of sediment deposited on top of the fabric (Fig. S2C). We deployed the tubes on 6 Aug 2024, at low tide, leaving the tubes for 24 h, after which the fabric and their accumulated sediments were collected. Collected sediments were then air–dried for 48 h, followed by pouring the sediment into a volumetric tube to determine the volume of collected sediment. While mass and volume are both reported in the literature following sediment capture, volume was selected here as most consistent with past studies and relevant to the determination of burial depth (MacGinitie 1934, Swinbanks & Luternauer 1987).

2.5 Statistical Analysis

Prior to any analysis, oyster, shrimp, and sediment data from one Long Island outplant site were excluded because the site, initially on a tidal flat, became part of a drainage channel because of channel formation and slough meandering that occurred during the study. Additionally, three sediment samples, one from Bay Center, Bay Center Cut-off, and Long Island, were also dropped as they were spilled during processing. This left 87 samples of sediment properties, corresponding to a local shrimp density, across 30 sites. At one site on Middle Sands, we were unable to find either cultch or PVC pipes at the end of the deployment due to deep burial beneath the sediment. This site was incorporated as zero survival and excluded from size analysis. All statistical analyses were carried out in R version 4.2.1 (R Core Team 2022).

2.5.1 Relationship between sediment properties and shrimp density

The first question in this study was how much of the variation in sediment properties was explained by shrimp density. We visualized multivariate patterns in sediment (proportion 1000, 500, 250, 125, and 63 µm, mud, and organic content) by Principal Component Analysis ordination (PCA), using all 87 samples. Prior to visualization, data were normalized by subtracting the mean and dividing by the standard deviation of each proportion category. The optimum number of principal components (PC) for analysis was determined using a scree plot where eigenvalues for PCs greater than the average eigenvalue were retained. We then examined how each retained PC and penetrability were related to shrimp density via linear regression. Additionally, in an explicitly multivariate approach, the effect of shrimp density on the set of seven sediment variables was analyzed via permutational analysis of variance (PERMANOVA) specifying a Euclidean distance matrix.

2.5.2 Relationship of oyster survival to direct and indirect pathways from shrimp density

The second question addressed the relationship of oyster survival to shrimp density, including sediment properties that could be intermediate drivers. We used structural equation modeling (SEM) to identify the overall response of oyster survival to three environmental conditions: shrimp density, sediment penetrability, and sediment muddiness (mud content of sediment samples). The SEM output was evaluated with a maximum likelihood estimator with robust standard errors. Replication was at the level of site, which meant averaging samples collected within a site. Three samples were averaged for environmental data, and 10 cultch were averaged for survival measurements (living oysters/total oysters).

2.5.3 Relationship of oyster shell height and tissue mass to sediment and shrimp variables

The third question addressed whether the size of surviving oysters was better predicted by sediment or shrimp variables. Among 30 outplant sites on tidal flats, only 17 had surviving oysters. This number was not sufficient to build a structural equation model for oyster size in response to environmental variables. Instead, we compared the three environmental variables as predictors of oyster size. The response variable was the average shell height or tissue mass of living individuals per site. We constructed four generalized linear models, three that included each fixed effect alone (shrimp density, mud content, penetrability) and a null model with no predictors. Response variables (tissue mass or shell height) required log-transformation to meet gaussian error assumptions. We then compared among the four models by small sample size corrected Akaike Information Criterion (AICc), which accounts for model fit and complexity. We selected the simpler model where the difference between AICc (Δ AICc) was less than 2.0.

2.5.4 Sediment Ejection Rates

Burial as a mechanism of impact hinges on the amount of sediment expelled from shrimp burrows relative to sediment deposition caused by other means within the shrimp beds and in areas where they have been removed. To do this we used analysis of variance (ANOVA) to examine differences in average volume of sediment captured within tubes with burrow openings, without burrow openings, and without surrounding shrimp. We censored 5 (of 24) samples that we had attempted to center on a shrimp burrow but did not contain an opening at the end of the trial. Upon detecting a significant effect of treatment, we used Tukey’s Honest Significant Difference (HSD) to determine differences in sediment volume between groups. Visual inspection of residual diagnostic plots from the

ANOVA indicated heteroscedasticity and deviation from normality. To meet model assumptions, we log-transformed sediment volume and re-fitted the model.

Results

3.1 Seeded cultch outplants

Considering the first question regarding the amount of variation in sediment properties related to shrimp density, we effectively sampled a wide range of conditions within Willapa Bay. Across the 87 samples from 30 outplant sites, the proportion of organic material ranged from 0.6% – 6.0%, mud content ranged from 0.1% – 25.0%, penetrability ranged from 37.3 – 151.0 cm, and maximum shrimp density was 496 m^{-2}. Of the 30 outplant sites, 5 had zero shrimp. Based on R^2 values, shrimp density explained 28% of the variation in the multivariate sediment data (PERMANOVA; pseudo-$F_{1,85} = 32.9$, $p = 0.001$) and 25% of the variability in penetrability (Linear regression; $t = -3.0$, *d.f.* = 28, $p < 0.005$). The multivariate sediment data were well described by two principal component axes. PC1 (59.0% of variation) gave roughly equal weight to all response variables except the proportion of 250 µm and 63 µm grain sizes and was interpreted as a measure of sediment muddiness (< 63 µm and higher organic content), whereas PC2 (18.7%) gave opposing weights to the proportion of 250 µm and 63 µm grain sizes and was interpreted as sand grain size (very fine to medium). Shrimp density explained 37% of the variation in Sediment muddiness (PC1) and was reduced at higher shrimp densities (Linear regression;

t = -7.1, *d.f.* = 85, $p < 0.001$, Fig. 3). However, shrimp density only explained 1.4% of the variation within the sand component (PC2) and was not related to shrimp density (Linear regression; *t* = -1.1, *d.f.* = 85, p = 0.27). Overall, shrimp explained about one-third of the variation in sediment properties, especially mud and organic content.

Regarding the second question of whether sediment properties contributed an indirect pathway by which shrimp affected oyster survival, they did not. A direct negative link from shrimp density was sufficient to explain the bulk of the variation in oyster survival. The structural equation model relating oyster survival directly and indirectly to shrimp showed a good fit to data (*d.f.* = 1, $\chi^2 = 0.65$, *CFI* = 0.99, *TLI* = 0.94, *SRMR* < 0.04, *RMSEA* = 0.09). Oyster survival declined due to a direct negative effect of shrimp density (Table S3, Fig. 4) with total mortality occurring when exposed to shrimp densities above 50 – 100 shrimp m^{-2} (Fig. 5A). Several other direct pathways were significant, specifically because increasing shrimp densities were associated with increased sediment penetrability and reduced sediment muddiness (Table S3, Fig. 4), consistent with the regression results relevant to question 1. Greater sediment penetrability corresponded to a decrease in oyster survival, while sediment muddiness had no effect (Table S3, Fig. 4). Despite two sequential significant direct links (shrimp to penetrability, and penetrability to oyster survival), this indirect pathway was not statistically significant (Table S4, Fig. 4).

For our third question regarding predictors of oyster size, shrimp density provided a significantly better predictor of final shell height and tissue mass compared to sediment muddiness or penetrability (Table S5A, S5B). Shell height declined with increasing shrimp density (Generalized linear model; $z = -2.20$, $p = 0.03$, Fig. 5B), such that oysters grew to 54.2 ± 13.4 mm SD at low shrimp densities but only to 26.6 ± 10.0 mm SD at shrimp densities that allowed some survival over the summer (Fig. 6). Oyster tissue mass and shell height were strongly correlated (log-transformed variables on a per-oyster basis, $r = 0.77$, $t = 14.3$, $p < 0.001$), and the negative effects of shrimp density extended to tissue mass (Generalized linear model; $z = -3.17$, $p = 0.002$).

3.2 Persistent and abandoned aquaculture beds

As context for the performance of outplanted oysters, shrimp densities of 50–100 m^{-2} were present on most shellfish aquaculture beds that had recently been abandoned or were abandoned soon after sampling (Fig. 7). Two abandoned beds had >150 shrimp m^{-2}, and two had fewer than 50 shrimp m^{-2} (Fig. 7). These last two included one that was likely abandoned for reasons other than shell sinking and one that has since been replanted. Notably, the abandoned beds spanned a wide range of sediment mud content, supporting the idea that shrimp effects can be distinguished from sediment effects. Only one persistent aquaculture bed overlapped with the densities of abandoned beds (78 shrimp m^{-2}), and these oysters were grown off-bottom, while shrimp had become rare in the bed by 2024 (Ruesink, pers. obs.).

3.3 Sediment ejection

The volume of sediment captured differed among the three groups: tubes with burrows, without burrows in the shrimp bed, and in plots where shrimp were removed (ANOVA; *F* = 205.1, *d.f.* = 2, $p < 0.001$). Tubes with burrows captured an average of 28.9 ml of sediment burrow^{-1} day^{-1} (± 3.4 SE), which was significantly greater than the 3.0 ml tube^{-1} day^{-1} (± 0.4 SE) captured in tubes without burrows ($p_{adj} < 0.001$, Fig. 8). Similarly, sediment capture in tubes with burrows was significantly greater compared to the 0.95 ml tube^{-1} day^{-1} (± 0.11 SE) captured in tubes from plots where shrimp were removed ($p_{adj} < 0.001$, Fig. 8). Additionally, the average sediment captured in tubes without burrows was significantly greater than that in tubes from plots where shrimp were removed ($p_{adj} < 0.001$, Fig. 8).

Discussion

Sediment properties within estuarine ecosystems are influenced by overlying physical conditions and exert strong influences over the performance of species (Lenihan 1999, Green & Coco 2014, Pratt et al. 2014). Bioturbation is an additional biological process that can affect sediment and other organisms (Jones & Jago 1993, Berkenbusch et al. 2000, Mermillod-Blondin et al. 2004, Volkenborn et al. 2007, Pillay & Branch 2011). An antagonistic role of bioturbation on epibenthic organisms on tidal flats was a major outcome of our research, in which *Neotrypaea californiensis* reduced oyster performance and altered sediment properties. However, although sediment penetrability increased and mud content declined with increasing shrimp density, neither of these factors contributed statistically to the negative impact of shrimp on oyster survival and size. Instead, deposition of ejected sediments from shrimp burrows at the sediment-water interface constituted a likely direct pathway that buries oysters. Excavated and resuspended sediment could also interfere with the feeding and respiration of juvenile oysters, as inorganic suspended matter can restrict the feeding and respiratory efficiency of bivalves (Murphy 1985, Grant & Thorpe 1991, Ellis et al. 2002, Pillay et al. 2007b,c). Due to the challenges in distinguishing target burrowing species from other burrowers and the uncertainty associated with using burrow counts to estimate organism density, obtaining direct measurements of burrower density are c (Schlacher et al. 2016). Direct measurements of shrimp density provide a reliable metric for predicting the impact of shrimp on oyster survival in tidal flats. Oyster performance declined with increasing shrimp density, and no oysters survived above 50 – 100 shrimp m^{-2}, which is just 10% of maximum densities (Dumbauld et al. 2021). Densities at or above this level of shrimp were also a common feature of shellfish beds that were abandoned for farming. Abandoned beds reflect the lack of effective shrimp control methods following a restriction on pesticide application in 2018 (Ruesink et al. 2025).

Neotrypaea californiensis is often called sand shrimp because it tends to be associated with sandy sediments, but this association could arise from either habitat selection by shrimp or ecosystem engineering. A summary of studies detailing the relationship between burrowing shrimp and sediments revealed that bioturbation by burrowing shrimp can influence sediment composition (Pillay & Branch 2011). Sediment reworking by the burrowing activities of shrimp can reduce mud content surrounding burrows, so that increases in shrimp population size and range result in temporal and spatial reductions in mud content (Flach & Tamaki 2001). Smaller particle sizes ejected from burrows are primarily unconsolidated and very erodible, even at low current speeds (Pillay et al. 2007c) making them susceptible to resuspension, and deposition in adjacent areas (Rowden, et al., 1998). Additionally, shrimp can reduce organic material within sediments through deposit-feeding and by facilitating the remineralization of organic matter (MacGinitie 1934, Swinbanks & Luternauer 1987, Webb & Eyre 2004). Shrimp density explained some but not all the variation in muddiness (principal component loading mud and organic content), given that sediment properties range widely even in the absence of shrimp. Also, while declining muddiness may be explained by the feeding and bioturbating activities of shrimp, the negative association between shrimp density and muddiness could have causality in either direction, where shrimp make sediments sandier by reducing mud content or sandy sediments attract shrimp. Pillay et al. (2008) have described how the arrival of dense populations of the sand prawn *Kraussillichirus kraussi* in Durban Bay, South Africa, coincided with transformation of sediments from muddy sand to sand, and that bioturbation then had ripple effects on epifauna and infauna, as well as being correlated with a switch in the diets of fish. However, sand grain size distribution (63 µm – 1000 µm) in our study was not correlated with shrimp density. In estuarine systems, sediment transport depends not only on biological influences but also on grain size and hydrodynamics (Williamson & Ockenden 1996). Therefore, our results align with evidence that organisms affect mud content in sandy sediments, whereas larger-scale drivers such as water motion and sediment sources determine the general transport and deposition of mud and sand (Flach & Tamaki 2001, Volkenborn et al. 2007, Green & Coco 2014).

Activities such as feeding, burrow upkeep, and pumping increase the penetrability of sediments (Siebert & Branch 2005a, b, 2006). These activities, considered sediment reworking, hinder the consolidation process that otherwise allows sediment to become more compact (Jones & Jago 1993, Rowden et al. 1998, Botto & Iribarne 2000). Habitats hosting burrowing shrimp, where the sediments within beds are continually reworked and turned over, are highly penetrable and saturated with water (Posey 1986, Rowden et al. 1998). In our study, sediments likewise increased in penetrability with increasing shrimp densities, up to roughly three times more penetrable than those with few to no shrimp and were “soupy” with water covering much of the low-lying areas in and around burrow mounds.

Burrowing shrimp alter their sediment environments, impacting benthic communities in both positive and negative ways. While they can benefit other burrowing species (Brenchley 1982, Seibert & Branch 2005, 2006), their activities can also negatively impact various benthic organisms, including corals (Aller & Dodge 1974), tanaids and spionid worms (Posey 1986), gastropods (Flach & Tamaki 2001), clams and mussels (Peterson 1977, Murphy 1985, Pillay et al. 2007a, b, c, 2008), and seagrasses (DeWitt 2009, Hull & Ruesink 2024), and potentially the body condition of benthic foraging fish (Pillay et al. 2012). Similarly, many reports show how ground-cultured oysters are harmed by shrimp (Stevens 1929, Dumbauld et al. 1996, 2006). More generally, juvenile oysters buried for a week typically die (Widdows et al. 1989, Hinchey et al. 2006). In the tropics, shell fragments were rapidly buried beneath the surface due to the "conveyer-belt" style sediment turnover caused by *Neotrypaea harmandi* (Suchanek 1983). For adult oysters, survival significantly declined when 90% or more of an individual oyster's shell height was buried, and mortality reached 50% when oysters were buried by 108% of their shell height (Colden & Lipcius 2015). Effects of shrimp are more harmful to juvenile than adult oysters, due to differences in ability to outgrow deposition (Dumbauld et al. 1996, 1997, 2001). At 13 outplant sites in our study, cultch was not visible at the surface after four months, and sometimes the cultch was 30 cm deep, requiring excavation to be retrieved. These sites did not have any live oysters.

In our study, we observed a decline in oyster survival across a range of low shrimp densities. Additionally, we identified a maximum density (50-100 shrimp m^{-2}) beyond which short-term juvenile oyster survival is zero. While management might benefit from identification of a threshold, finding a range where problems for shellfish culture start occurring is to be expected. Other environmental factors also move sediment (Green & Coco 2014, Roca et al. 2016, Correia & Smee 2022), as well as modify the impacts of shrimp activity (Pillay 2019).

The declining trend in oyster survival with increased shrimp densities was mirrored by a reduction in the sizes achieved by oysters. Sediment deposition negatively impacts oyster growth (Lenihan 1999), for instance, due to feeding disruption caused by sediment in the water column or deposited on the oysters. Changes in abiotic conditions such as oxygen availability and sedimentation can create stressful conditions for shellfish growth (Peterson 1985, Lenihan 1999, Poirier et al. 2021).

Sediment expelled and deposited from shrimp burrows on tidal flats poses a significant risk to oysters, especially smaller ones attached to shells. Similar volumes of sediment ejection were estimated for *N. californiensis* in California (20-50 ml shrimp^{-1} day^{-1}, MacGinitie) and British Columbia (18 ± 9 ml shrimp^{-1} day^{-1}, Swinbanks) (MacGinitie 1934, Swinbanks & Luternauer 1987). However, the rate of sediment ejection is likely to increase with shrimp (and therefore burrow size). We selected burrows with diameters greater than 3 mm (Dumbauld et al. 2021), which were far enough from other burrows to have one burrow per tube. This choice likely underestimates ejection rates per shrimp, given they have multiple burrow entrances, although it may overestimate sediment falling immediately around burrows, since the tubes prevented off-site transport. Although the design clearly attributed increased sediment deposition to the presence of shrimp burrows, the quantitative value should be interpreted cautiously as it applies only to larger shrimp and in particular environmental conditions. Sediment ejection rates of *N. californiensis* decline during colder winter months and gradually increase as temperatures rise into summer (Fritz 2002). Past reviews have highlighted that sediment turnover is highly temporally variable (Rowden & Jones 1993). The one-time ejection rates that we measured were sufficient to generate observed cultch burial. In the shrimp bed where we collected sediments, shrimp burrows (>3 mm diameter) averaged 102.9 burrows m^{-2}. By multiplying the average ejection rate (28.9 ml burrow^{-1} day^{-1}) by burrow density, we estimate that approximately 3 liters m^{-2} day^{-1} is deposited at the sediment-water interface within the shrimp bed, or 3 mm of sediment deposition per day, aligning with the 30 cm burial depth of cultch after four months in our outplant study.

A paradigm in soft-sediment ecology is that benthic community composition and species-specific performances respond to sediment properties (Jones 1950, Thrush et al. 2003, 2005, Pillay et al. 2008, Colden & Lipcius 2015, Poirier et al. 2021). Here, any relationships of oyster performance to grain size and organic content were apparently overwhelmed by the vertical movement of sediment by shrimp. Thus, it appears to be the burial rate rather than the sediment properties per se that influence oysters. All ecosystem engineers would be expected to exert their effects through changes in environmental conditions and resource availability (Power 1990, Strauss 1991, Pringle et al. 1993), but we consider the conveyor belt of sediment, which is then deposited at the surface, to be more of a direct pathway by which shrimp affect oysters, relative to changing muddiness or penetrability. Although a direct negative effect of penetrability was detected for oyster survival, the effect of shrimp mediated through penetrability did not statistically improve the model fit for oyster survival, which was strictly a function of shrimp density. That is, we were unable to detect any effects of shrimp density mediated through either sediment penetrability or muddiness or in combination on oyster survival. For oyster survival the direct effect of shrimp density (which equates to rate of burial) is strong, and both indirect pathways do not account for additional variation in oyster survival.

Conclusion

Our research underscores the relationship between bioturbating species such as burrowing shrimp, sediment dynamics, and the performance of oysters within estuarine ecosystems. Burial during sediment deposition caused by the burrowing activities of shrimp emerged as a direct mechanism for oyster mortality. Likewise, decreases in the sizes (shell heights) achieved were associated with increasing shrimp density, suggesting that the sediment deposition resulting from shrimp activity hampers oyster size as well as increasing mortality. Despite the potential for shrimp effects to be mediated through changes in sediment penetrability and muddiness, additional variation in oyster survival was not explained by indirect pathways, further solidifying shrimp density as a strong predictor of oyster survival and performance. Shrimp densities near the levels that buried and killed outplanted oysters were also present at shellfish beds that were abandoned for farming. Our study underscores the need to explore biological and physical interactions in shaping the survival of benthic species such as oysters, which is crucial for understanding resilience strategies for key foundation species.

Acknowledgments

We thank Taylor shellfish, Pacific Seafoods, Markham Oyster, Jolly Roger Oysters, Bay Center Mariculture, John Heckes, and Pete Heckes, as well as the Washington State Oyster Reserves (Zachary Forster), for allowing site access. We would also like to thank Fiona Boardman, James Fitzpatrick, Mariah Joston, Milly Marriot-Green, Haleh Mawson, Chris Jendrey, Kyra McClelland and Christine Nolan for help in the field and sample processing and Andrea Ogston who provided helpful edits. Funding was provided by the Washington State Department of Agriculture (K3201, K4119) and the Pacific States Marine Fisheries Commission (Pacific Shellfish Institute grant [#20-31G] under Award #NA18NMF4720007). The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or the US Government. This manuscript is dedicated to the memory of Lynn Hull.

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Figures

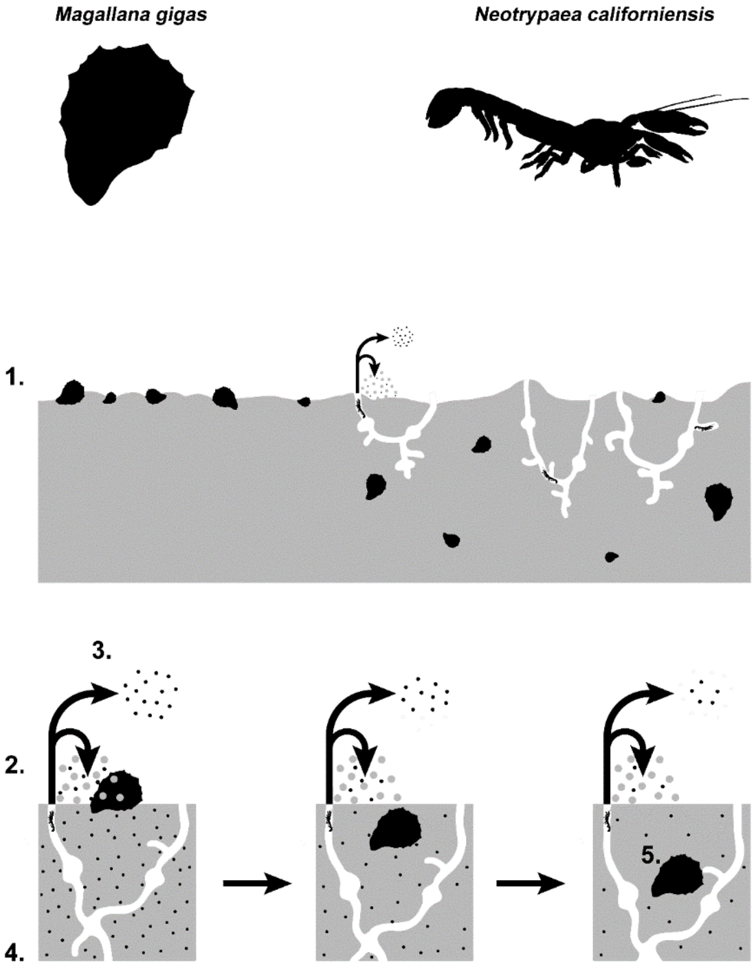

Figure 1. Influence of burrowing shrimp on oysters and the mechanism responsible for their mortality. In this ecosystem, shrimp excavate sediments both while deposit-feeding and maintaining their burrows (1). Sediments are carried to the surface by shrimp (2), which loosens sediments and allows particles to interact with overlying water. Some mud particles (smaller black dots) are deposited back on the surface while others are transported laterally to adjacent areas within the habitat due to water flow (3), whereas sands (larger grey dots) fall to the surface. This process destabilizes the tidal flat (4) and buries oysters in sediments where they eventually die (5). Eventually, continued reworking of sediments results in the net loss of smaller particles and the reduction of mud content.

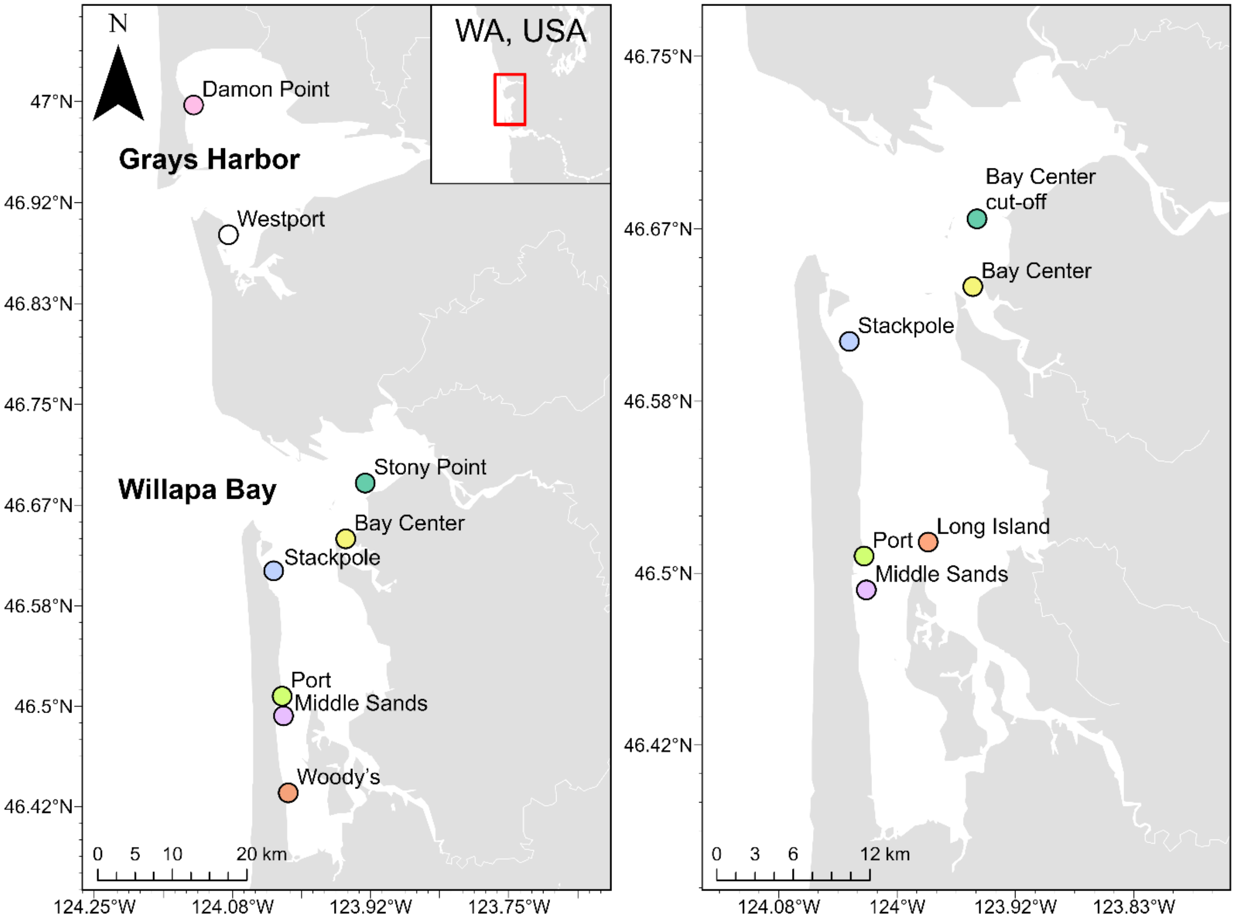

Figure 2. Maps displaying locations of sampled aquaculture beds in Gray Harbor and Willapa Bay, USA (left panel) and the locations of cultch outplant sites in Willapa Bay (right panel). Geolocations and descriptions of each site are in Supplemental tables 1 and 2 and Supplemental Figure 1.

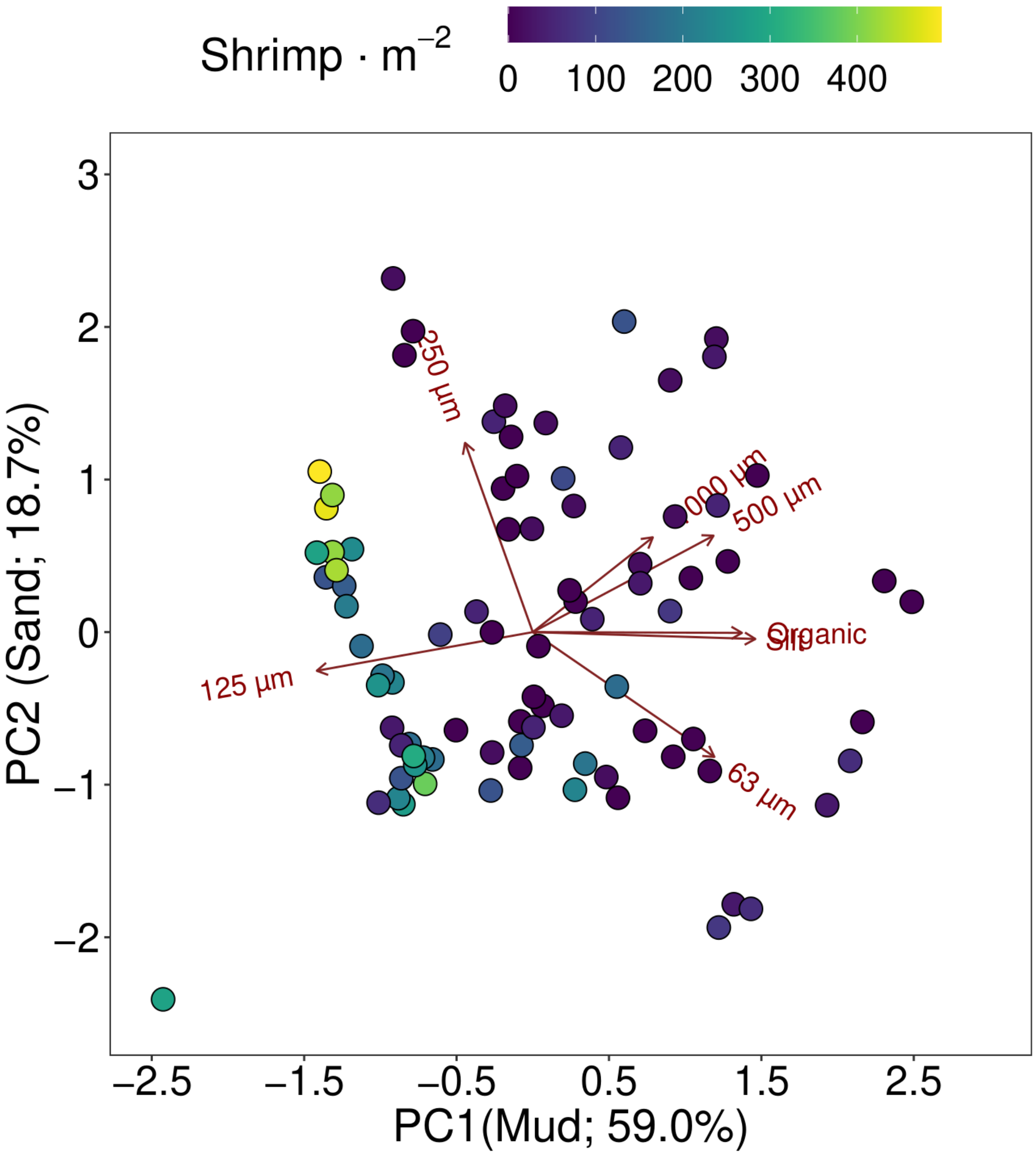

Figure 3. Principal component analysis diagram of the effects of burrowing shrimp density on sediment muddiness (PC1) and sand grain size (PC2).

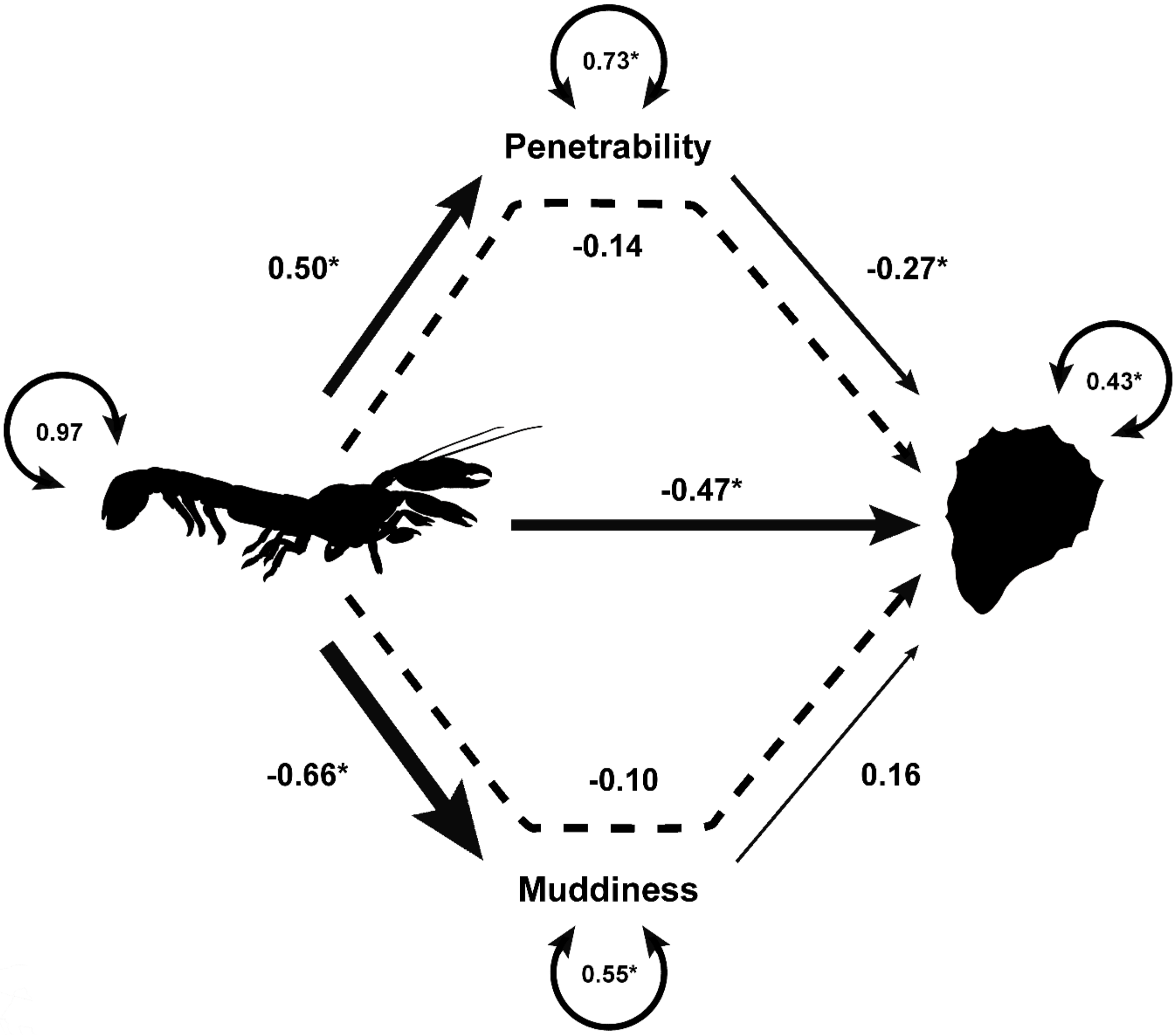

Figure 4. Conceptual model of relationships between burrowing shrimp density and sediment characteristics that influence oyster survival. Values represent standardized estimates and asterisk represent significance. Solid arrows are direct effects and dashed arrows are indirect effects. The illustration of the shrimp represents shrimp density, and the oyster represents oyster survival.

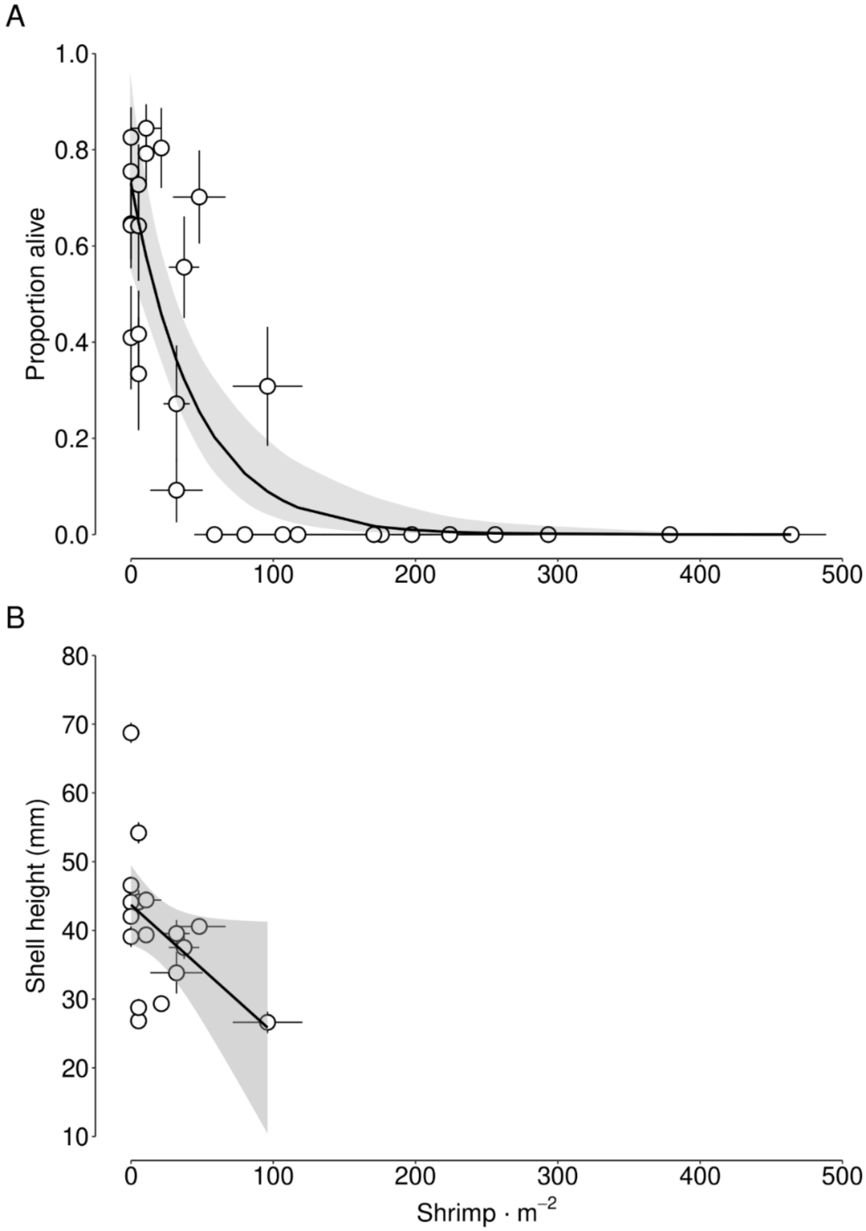

Figure 5. Performance of outplanted oysters (*Magallana gigas*) across sites differing in shrimp density (*Neotrypaea californiensis*). A) proportion of living oysters on cultch and B) shell height of living oysters in relation to burrowing shrimp density. Points are site averages (N = 3 shrimp samples, N = 10 cultch, variable numbers of living oysters), and error bars represent ± 1 standard error. Trendline represents the line of best fit and error around the line shows 95% confidence intervals.

Figure 6. Examples of seeded cultch collected from sites differing in burrowing shrimp densities.

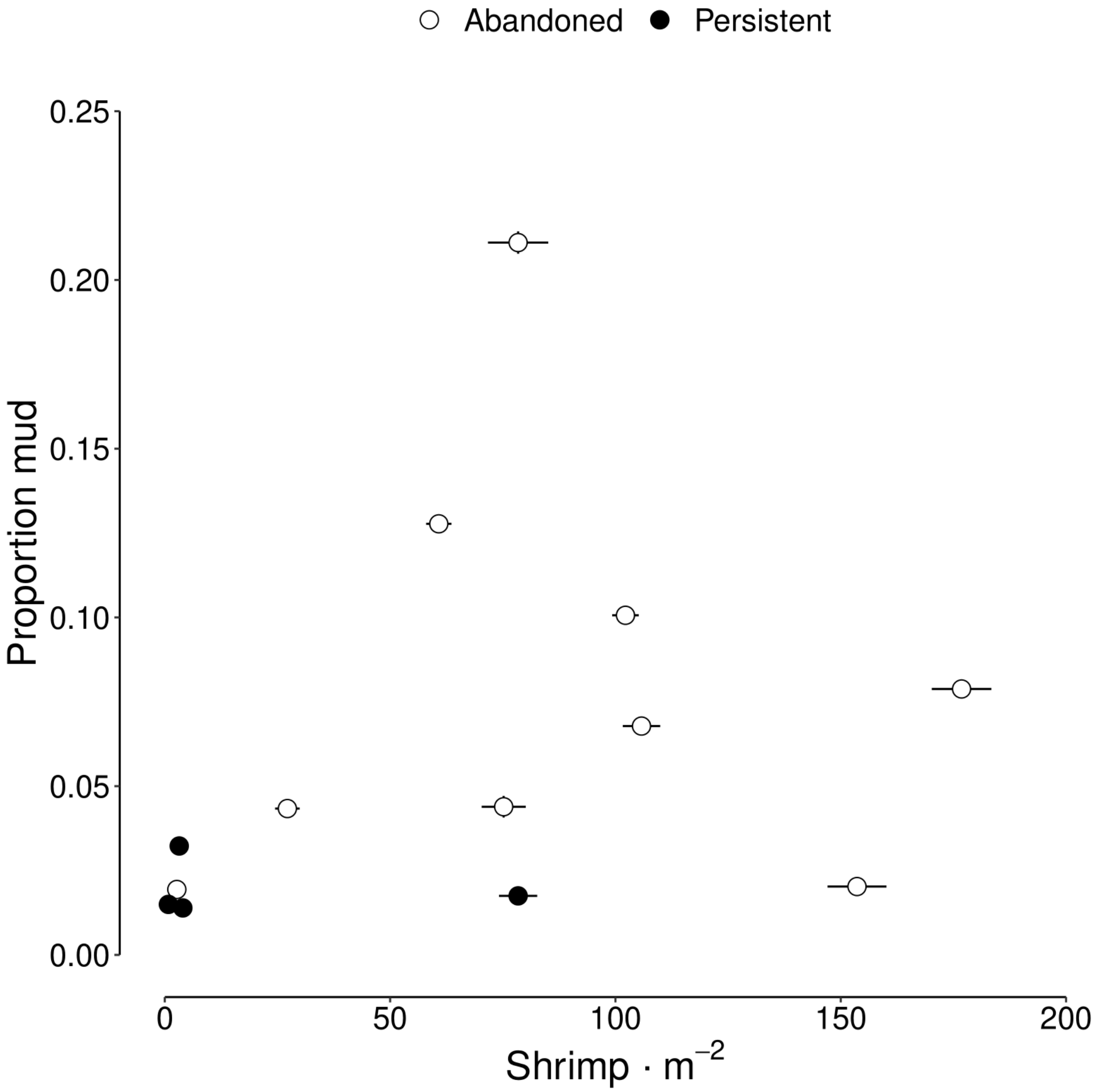

Figure 7. Mean shrimp densities and the proportion mud of sediment across 13 aquaculture beds, many of which were abandoned for farming prior to or shortly after sampling. Error bars represent ± 1 standard error (N = 10 – 20). All vertical and some horizontal error bars are small and are hidden behind points.

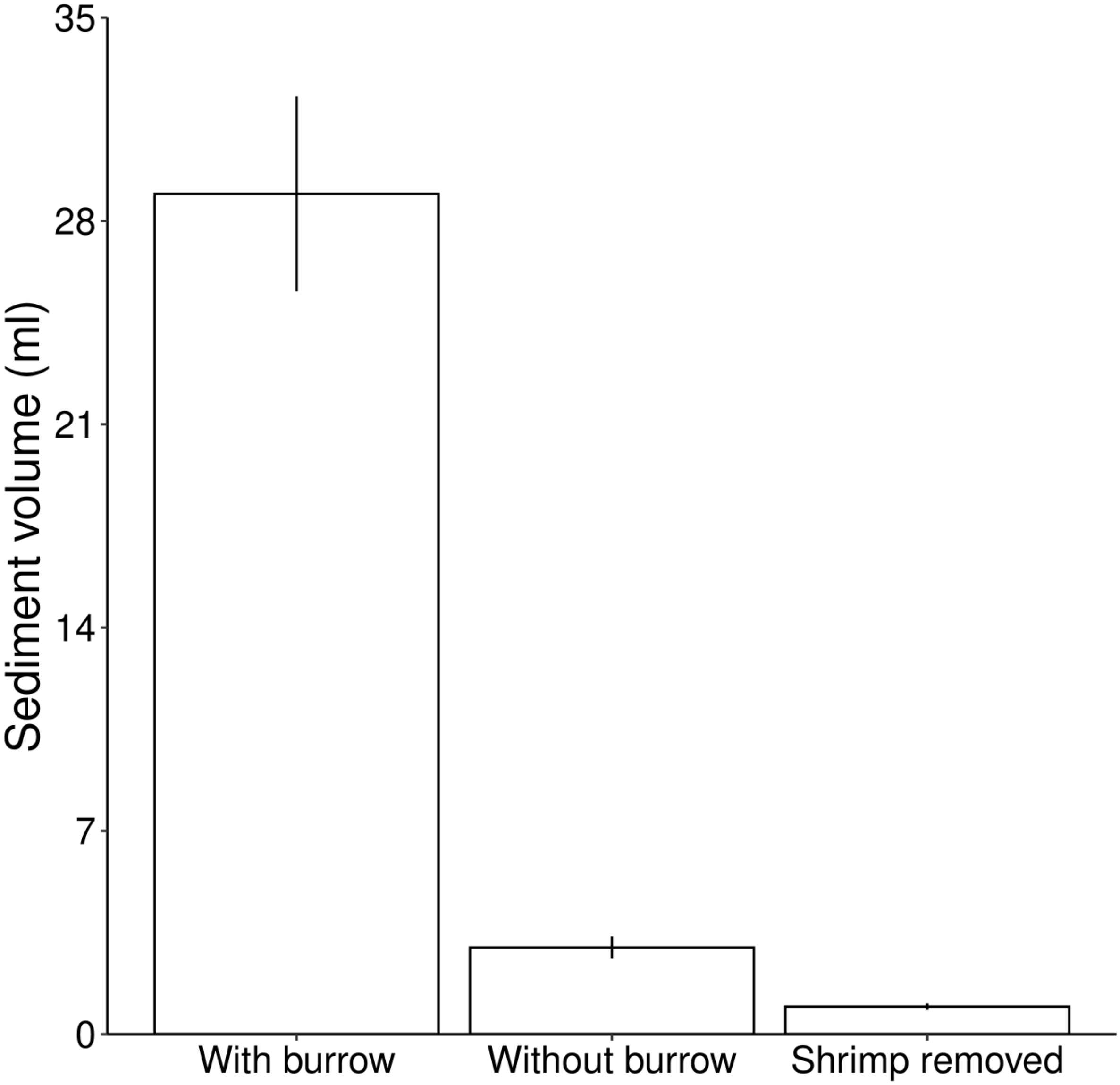

Figure 8. average volume of sediment captured within tubes with and without burrows within the shrimp bed and in plots where shrimp were removed. Error bars represent ± 1 standard error.

Supplemental Material

Table S1. Shellfish aquaculture beds and their operational status.

Site	Culture type	Latitude	Longitude	Date sampled	N	Status
GH Damon 7	Oyster ground culture	46.992	-124.130	7/29/2022	10	Abandoned 2019
GH Damon 11	Oyster ground culture	46.997	-124.130	7/29/2022	10	Abandoned 2019
BC flip	Off-bottom oysters	46.638	-123.947	4/30/2021	10	Abandoned 2022
BC LL	Off-bottom oysters	46.653	-123.947	7/1/2022, 7/4/2023	8, 10	Abandoned 2022
Stony Pt	Oyster ground culture	46.685	-123.923	11/14/2020	12	Abandoned 2020
Westport lower	Oyster ground culture	46.890	-124.088	3/29/2021	10	Abandoned 2019
MS B99	Oyster ground culture	46.491	-124.022	6/7/2021	10	Abandoned 2022
MS LL	Off-bottom oysters	46.492	-124.019	7/2/2022	18	Abandoned 2022
Port Clam	Clam culture	46.505	-124.027	5/28/2021	20	Abandoned 2020
Stackpole N	Oyster ground culture	46.611	-124.026	11/15/2020	15	Persistent
Stackpole S	Oyster ground culture	46.585	-124.020	10/16/2020	20	Persistent
Port flip	Off-bottom oysters	46.508	-124.022	11/1/2020	20	Persistent
Woody’s	Clam culture	46.428	-124.016	10/17/2020	20	Persistent

Table S2. Locations of cultch outplants in Willapa Bay, WA in 2022.

Site ID	Deployed	Collected	Days	Latitude	Longitude
BC.B	4/22	8/27	127	46.63546	-123.94246
BC.BOT	4/22	8/26	126	46.6407	-123.94373
BC.FB	4/22	8/26	126	46.63873	-123.94663
BC.M	4/22	8/27	127	46.63956	-123.94687
BC.P	4/22	8/26	126	46.63007	-123.94813
BCCO.BOT	4/22	8/26	126	46.67258	-123.94675
BCCO.LL	4/22	8/26	126	46.67145	-123.94366
BCCO.LL.N	4/22	8/26	126	46.67409	-123.94414
BCCO.LL.S	4/22	8/26	126	46.6682	-123.94572
BCCO.NO.BOT	4/22	8/26	126	46.67255	-123.94922
LI.H	4/23	8/27	126	46.51857	-123.97988
LI.N	4/23	8/27	126	46.51552	-123.98029
LI.N.NO	4/23	8/27	126	46.51507	-123.97807
LI.SS	4/23	8/27	126	46.51402	-123.97893
LI.SS.MUD	4/23	8/27	126	46.51414	-123.97972
LI.SS.NO	4/23	8/27	126	46.51398	-123.97741
MS.E	4/21	8/27	126	46.49233	-124.01966
MS.E.NO	4/21	8/24	125	46.49274	-124.0184
MS.W	4/21	8/24	125	46.49233	-124.0220
MS.W.LL	4/21	8/24	125	46.49187	-124.02157
MS.W.MUD	4/21	8/24	125	46.49129	-124.02143
PB	4/18	8/24	128	46.5019	-124.02735
PB.FB	4/18	8/24	128	46.50787	-124.0233
PB.FB.N	4/18	8/24	128	46.50829	-124.02321
PB.NO	4/18	8/24	128	46.50186	-124.02651
PBFB.NO	4/18	8/24	128	46.50669	-124.0240
SP.NE	4/20	8/25	127	46.63128	-124.03618
SP.NO	4/20	8/25	127	46.61571	-124.03465
SP.NO.CLAM	4/20	8/25	127	46.61222	-124.03365
SP.NO.MUD	4/20	8/25	127	46.60884	-124.04055
SP.NW	4/20	8/25	127	46.62028	-124.03654

Table S3. Regression results of direct pathways from the structural equation model shown in Fig. 6.

Response	Predictors	estimate.	*Z*	*P*	Std est.
Survival	Shrimp density	-0.47	-2.70	0.01	-0.47
	Penetrability	-0.27	-2.10	0.03	-0.27
	Muddiness	0.16	0.84	0.40	0.16
Penetrability	Shrimp density	0.50	3.27	0.001	0.50
Muddiness	Shrimp density	-0.66	-5.61	< 0.001	-0.66

Table S4. Regression results of indirect pathways from the structural equation model shown in Fig. 6. Predictors in parentheses represent the predictors an effect was mediated through. Pen = "penetrability" and mud = "muddiness".

Response	Predictors	estimate	*Z*	*P*	Std est.
Survival	Shrimp density (Pen)	-0.14	-1.80	0.08	-0.14
	Shrimp density (Mud)	-0.10	-0.84	0.40	-0.10
	Shrimp density (Pen & Mud)	0.01	0.80	0.43	0.01

Table S5. Summary of ΔAICc comparisons between single-factor models for oyster A) shell height and B) tissue mass.

Predictors	Log likelihood	AICc	ΔAICc	Weight
A) Burrowing shrimp	8855.1	-17712.3	0	1.000
Penetrability	8621.6	-17245.1	467.1	<0.001
Muddiness	8288.0	-16578.0	1134.3	<0.001
Null	8153.7	-16309.4	1402.9	<0.001
B) Burrowing shrimp	-2827.2	5652.4	0	1.000
Penetrability	-4193.0	8384.1	2731.6	<0.001
Muddiness	-4461.2	8920.5	3268.0	<0.001
Null	-4516.6	9031.1	3378.7	<0.001

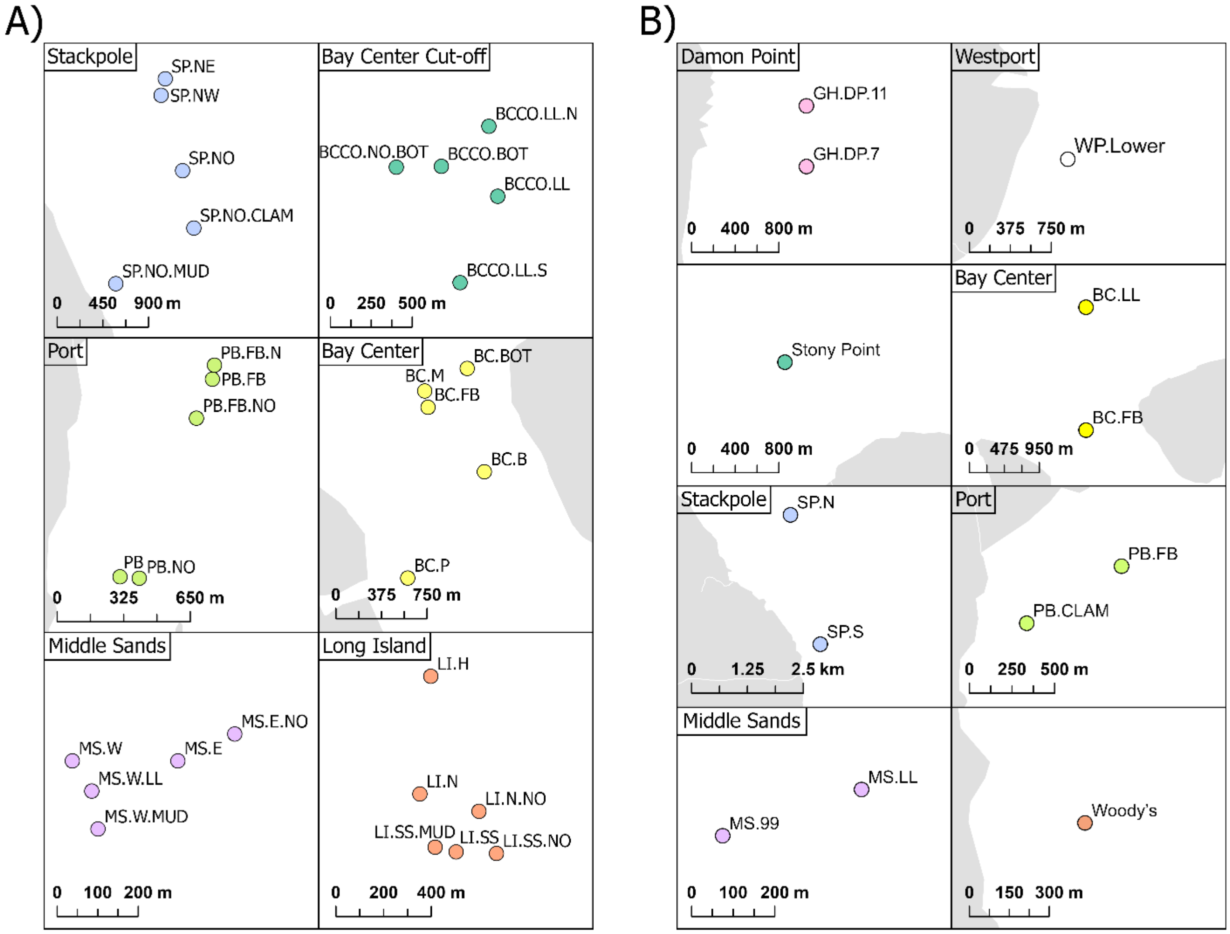

Figure S1. Locations of A) cultch outplant sites and B) sampled aquaculture beds cultch within Willapa Bay and Grays Harbor.

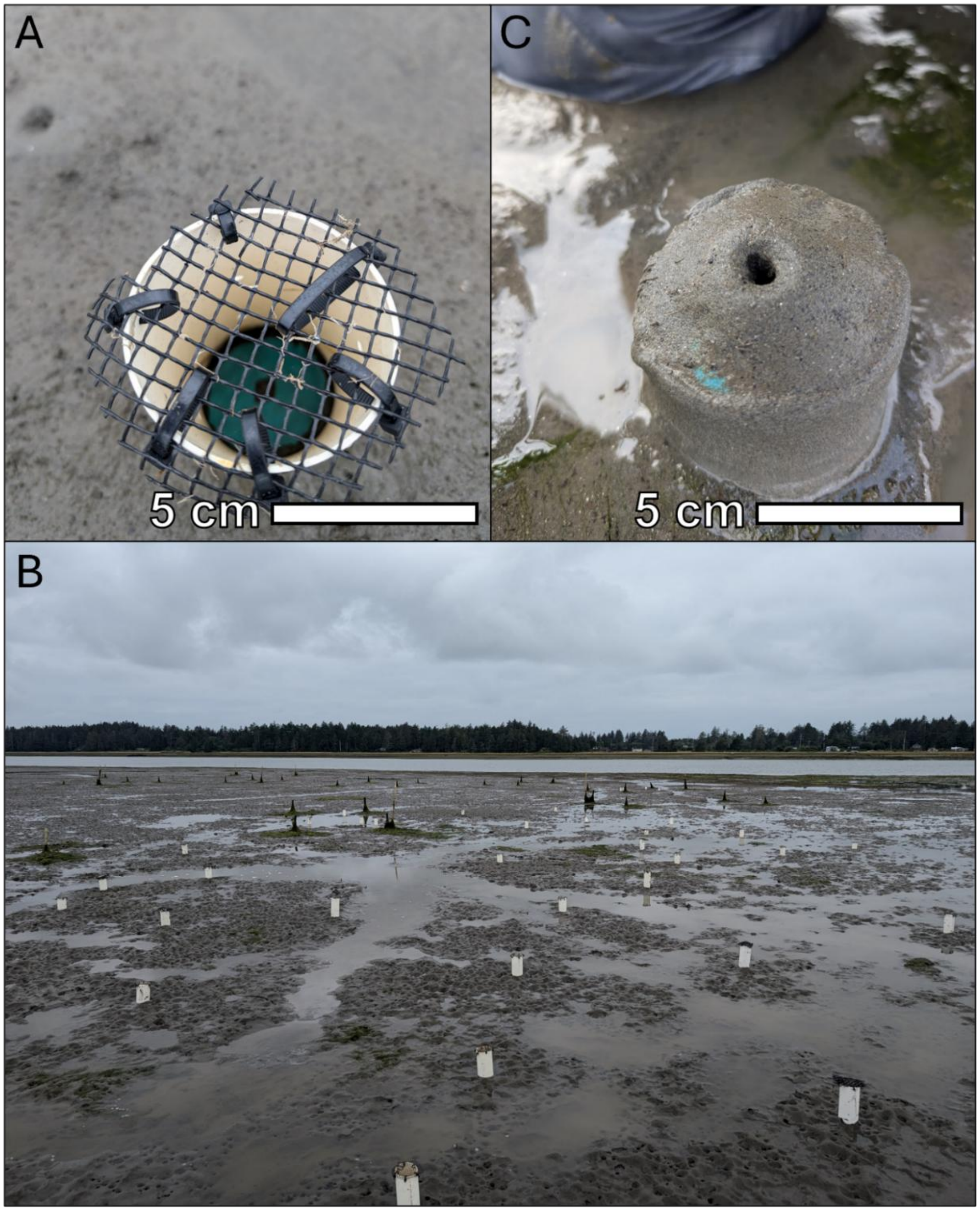

Figure S2. Images of A) fabric and PVC tube over a shrimp burrow, B) fabric and PVC tubes over shrimp burrows, in sediment within shrimp bed, and within vibration plots (marked at corners with taller PVC pipe), and C) ejected sediment that accumulated on top of fabric (blue) placed over a shrimp burrow.

Figure S3. Seeded cultch deployed at a site with high burrowing shrimp density, also showing the water content at low tide. Each PVC pipe (shown within black circles) has two pieces of seeded cultch for a total of 10 pieces per site.

Figure S4. Example of cultch buried roughly 30 cm deep by burrowing shrimp and requiring excavation to be retrieved. The average burrowing shrimp density at this site was 371.6 shrimp m^{-2} ± 44.7 SE.

Chapter 3: Shoot collection intensity and transplant configuration affect intertidal eelgrass (*Zostera marina* L.) restoration success

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Abstract

Seagrass restoration has shown mixed results, even in environments that appear suitable, indicating on-going needs for improved restoration techniques. This study tracked eelgrass (*Zostera marina*) dynamics at two donor sites and one transplant site over multiple years, using resilience at donor sites and transplant establishment and expansion as key success measures. Despite finer sediments, higher shoot densities, and lower flowering frequencies at lower elevations, eelgrass morphology was similar at both high- and low-elevation donor sites. Recovery times increased with collection intensity, taking up to two years when large plots were completely cleared. Collected shoots were transplanted into plots of four sizes (0.0625–4 m^2) and three densities (25–125 m^2). Although larger, denser plots were expected to aid establishment in bioturbated areas, the highest initial establishment occurred in small, sparse plots. Over time, sparse and medium-density plots filled in, eventually converging with denser plots within a year. After two years, proportional shoot count changes were inversely related to plot size and density: small, sparse plots saw an 83-fold increase in shoot counts, while large, dense plots saw only a two-fold increase. Large, dense plots initially accumulated fine sediment and organic matter but lagged behind unvegetated areas after one year, likely due to bioturbator loss. Neither donor nor transplant sites showed facilitative effects, potentially due to intraspecific competition. Hydrodynamics around eelgrass shoots may have temporarily delayed sediment accumulation, a key ecosystem function. These findings contribute to improved eelgrass restoration strategies for fringe and upper margin intertidal areas.

Introduction

Global seagrass losses are attributed to coastal development, destructive fishing practices, land-based nutrient and pollutant run-off, and climate change (He and Silliman, 2019; Hughes et al., 2013; Orth et al., 2006; Short and Wyllie-Echeverria, 1996), which can shift conditions outside the tolerance of seagrass and/or towards antagonistic species. While a clear first step in restoration is to redress degraded physical conditions (Fonseca et al., 1998; Ward and Beheshti, 2023), best practices for collecting and planting seagrass continue to be explored. A global meta-analysis on seagrass restoration highlights that, beyond suitable conditions, the success of restoration efforts depends on project scale, donor site proximity, and the method of planting (van Katwijk et al., 2016).

Critical factors for restoration success should be assessed at both the donor and restoration sites. At a donor site, success is defined based on the time to return to normal density, whereas success at the restoration site encompasses both initial establishment and subsequent infilling and spread. Higher transplant densities can enhance shoot survival and plot expansion through mutual protection among shoots (Fonseca et al., 1998). Larger transplant plots generally show greater long-term survival and expansion, while smaller plots often experience slower lateral growth and higher mortality until critical sizes are reached (Duarte & Sand-Jensen 1990a, 1990b; Olesen & Sand-Jensen 1994; Almela et al. 2008; Paulo et al. 2019). Such positive density-dependence within seagrasses is further supported by evidence that higher spathe densities in pollen-limited seagrasses lead to greater seed set, enhancing reproductive success (van Tussenbroek et al., 2016). However, excessively dense planting may result in self-shading and intraspecific competition, which can immediately stunt plant growth and also reduce population growth (Ralph et al., 2007; Ruesink et al., 2012; Yang et al., 2016). Therefore, the distribution of shoots by density and plot size may influence successful restoration.

When shoots are collected for restoration, small fractions of a bed are removed (i.e. <10% of total shoots, Fonseca et al. 1998). The impact of shoot collections on donor populations would be difficult to detect statistically and could in-fill rapidly, given that low-density shoots often enhance clonal branching (shoot emergence rates; Ruesink et al. 2012; Ruesink 2018; Boardman & Ruesink 2025). It is unclear what level of shoot collection from donor sites might exceed eelgrass' recovery capacity.

Donor site elevation is an understudied feature that could be optimized for shoot collection, as it influences shoot density, flowering, and overall resilience. Shallow donor sites are prone to temperature stress and desiccation, whereas light may be limiting at deeper sites, affecting both metabolic balance and reproductive success (Aoki et al., 2020; Lee et al., 2007; McGlathery et al., 2012). Water depth can also drive differences in flowering, with shallow populations typically maintaining higher flowering densities than deep populations, which can exhibit delayed flowering development (Infantes and Moksnes, 2018; Kamel et al., 2012; von Staats et al., 2021). Traits of eelgrass change with tidal elevation in ways that could either erode or enhance resilience (Ruesink et al. 2024).

Eelgrass (*Zostera marina* L.) is the most widely distributed species of seagrass in the northern hemisphere and has a long history of restoration efforts (Addy, 1947) with variable success (Orth et al., 2010; Ward and Beheshti, 2023). Restored eelgrass can trap fine particles and organic matter, contributing to Blue Carbon storage (Aoki et al., 2021). Documenting sediment change following transplants is essential to understanding the ecosystem services of eelgrass and potential for restoration to have co-benefits of carbon offsets. In a bay where eelgrass is overall widespread (Willapa Bay; Dumbauld & McCoy 2015), we experimentally tested different collection and planting configurations as we moved shoots from donor sites to an unvegetated area caused by bioturbation. The focal questions were:

1. How do shoots sourced from a low-intertidal site and from the upper margin of the eelgrass distribution in Willapa Bay compare morphologically, which could influence transplant success?

2. How rapidly do beds with different levels of shoot collection return to background densities at low and high elevation?
3. How do transplant density and plot size affect a) initial (2-month) shoot persistence, b) infilling within the plot, and c) population growth?
4. Do large dense eelgrass transplant plots accumulate fine particles and organic matter, consistent with sediment trapping and carbon sequestration?

First, we expected intertidal eelgrass from lower elevations to be larger than eelgrass from higher elevation donor sites. Second, we expected intertidal donor sites to be more resilient to shoot collection at higher elevations, given the upper margin of eelgrass has already exhibited resilience to natural disturbances. Third, we expected higher planting densities and larger plot sizes would enhance early survival and later, expansion of eelgrass (population growth). Finally, we expected particle and organic matter to be higher inside versus outside eelgrass.

Methods

2.1 *Study sites*

Willapa Bay, at 35,000 ha, is one of the largest estuaries on the US west coast (Fig. 1, Borde et al. 2003). Due to a shallow mean water depth (3.2 m) and moderate tidal exchange (2.3 m between mean low and high tide), extensive intertidal flats, representing about half the bay's area, emerge at low tide (Dumbauld and McCoy, 2015; Hickey and Banas, 2003). Beds of seagrass (*Zostera marina* and *Zostera japonica*) cover approximately 32% and 8-13% of the bay's intertidal area, with *Z. marina* occurring between +0.6 m and -1 m relative to mean lower low water (Ruesink et al. 2006, 2010, Dumbauld and McCoy 2015). Given their large morphotype (1 m shoot lengths in summer), shoot densities are typically < 100 per m^2 (Thom et al. 2003).

The transplant area was located on the Long Island Oyster Reserve (46.515, -123.977) at an elevation of ~ 0.3 MLLW on an intertidal flat without shellfish aquaculture (Fig. 1). Eelgrass was initially absent from the area due to high densities of bioturbating infaunal shrimp (*Neotrypaea californiensis*; Hull and Ruesink 2024), which occurred at 347 m^{-2} (38 SE, N = 10) when the study was initiated. By the following spring, shrimp were confined to the southwest corner of the study area and eventually left altogether. We do not think that our transplants caused this shift or die-off because transplant plots occupied less than a quarter of the area, and the high-density shrimp distribution continued to be dynamic on this tidal flat over multiple years.

Donor sites were on either side of the transplant area and differed in elevation and sediment properties. The lower-elevation donor site (0 m MLLW) occurred below Pacific oyster (*Magallana gigas*) reefs along the edge of a channel, at moderate shoot densities (42.2 shoots m^{-2} ± 6.3 SE, N = 6) and harbored shrimp (*Neotrypaea californiensis*) at low densities (96.0 m^{-2} ± 25.3 SE, N = 5). The higher-elevation donor site (+ 0.6 m MLLW) harbored shrimp at densities that are challenging for eelgrass (198.0 m^{-2} ± 34.0 SE, N = 5), so eelgrass was sparse (26.2 m^{-2} ± 2.8 SE, N = 6). The lower-elevation site was muddier with sediments containing a greater percentage of fines (17.9% ± 0.7 SE, N = 7) compared to the higher-elevation site (5.6% ± 0.5 SE, N = 10). Complete recovery of 2 × 2 m gaps in eelgrass meadows occurs in two years in Willapa Bay, specifically gaps created in summer are no longer detectable two summers later (Ruesink et al., 2012). The donor sites in the current study were not within meadows, but rather in fringing eelgrass along a channel and near the upper-elevation limit of the distribution, intermixed with bioturbators.

2.2 *Multivariate eelgrass morphology at donor sites*

To assess potential differences in eelgrass between the two donor sites, we measured a suite of morphological traits from shoots at both sites. A total of 126 terminal shoots (N = 61 low elevation, N = 65 from the high elevation) were collected by hand, ensuring as much of the rhizome was collected intact as possible. Shoots were measured for sheath width (mm), sheath length (cm), maximum blade length (cm), recent internode length (cm), and the number of branches and the number of internodes per standardized 7-cm length of rhizome (fewer internodes indicates faster extension). We then separated the above- and below-ground (7-cm) portions of each shoot (cut at the first visible node), cleaned them, and dried them in a drying oven at 60°C for 72 h. Afterward, the dry weights of the above- and below-ground portions were recorded. In total, seven morphological traits contributed to describing above-ground size and shape and below-ground investment in branching, storage, and extension.

2.3 *Collection intensity experiment at donor sites*

In July 2020, we systematically removed eelgrass shoots from 4 m^2 plots at both donor sites to create four levels of collection intensity treatment, aimed at assessing eelgrass resilience to disturbance (physical removal). Specifically, we removed 50%, 80%, and 100% of shoots from within the plots, alongside control plots with no removal (0%). Plots were spaced at 3-m intervals in a grid. Each removal treatment was replicated six times at each site, with treatment assigned randomly, resulting in 24 plots in total at each donor site. Shoot densities and the proportion of flowering shoots within the treatments were monitored by counting shoots within a strip of 2 × 0.5 m running through the center of each plot during spring and summer from July 2020 to August 2024.

2.4 *Eelgrass transplant experiment*

To assess the effect of plot size and shoot density on transplant success, we transplanted eelgrass from the two donor sites into the transplant area at three densities and four plot sizes (3-factor design). We implemented a fully crossed design with three shoot density levels (25, 64, and 125 m^2) and four plot sizes (0.0625, 0.25, 1, and 4 m^2), resulting in 2 to 500 shoots per plot. The experiment was arranged in a grid of ten blocks (7 m × 21 m), with half randomly populated with shoots from each donor site (Fig. S2). Four of the five blocks populated with shoots from each donor site contained all 12 size-density combinations, while the fifth excluded the 4 m^2 plots since we expected the most variability in outcome among the smaller plots. The experimental setup began on July 7 and was completed by July 24, 2020. Two blocks in the southwest corner were in the path of new sloughs that formed about a year after experiment set-up, and these were excluded from analysis because the sediment eroded and removed shoots.

All transplant plots were completely counted for shoots two weeks after set-up (3 – 4 Aug 2020), and sheath lengths of 1-16 randomly selected shoots per plot were measured in the field from the start of the experiment (24 July 2020). The number of eelgrass sheaths measured increased with plot size. Thus, sixteen shoots were measured in 4 m^2 plot, twelve in 1 m^2 plots, three in 0.25 m^2 plots, and at least one, but no more than three in 0.0625 m^2 plot. At multiple timepoints throughout spring and summer from 2020–2022, all plots were resampled to measure eelgrass shoot counts and sheath length, with shoot counts recorded separately for shoots within the original transplant plot and those extending beyond the plot boundaries. To examine changes in sediment characteristics due to transplanted eelgrass, we collected surface sediment samples (3 cm deep scoop) from inside and outside 4 m^2 high density treatments, which provided the most extreme contrast, at six timepoints during the two-year experiment. Collected sediments were dried until constant mass (50 °C for at least 72 h), ashed (500 °C for 3 h) for percent organic content via loss of mass on ignition

(LOI) and sieved to determine percent fine particle content (silt + clay: < 63 µm).

2.5 *Data analysis*

All analyses were done in R v.4.2.1 (R core team 2022). For analysis of both the shoot collection and transplant experiment we used similar approaches to examine effects of predictors on response variables separately at one timepoint in each year and used model selection to identify the best combination of predictors influencing donor site recovery and transplant success.

2.5.1 *Eelgrass morphology*

We visualized multivariate patterns in eelgrass morphology between the two donor sites by Principal Components Analysis ordination (PCA) using 119 samples (low elevation donor site N = 61, high elevation donor site N = 58). Seven individuals were excluded from the analysis due to incomplete or damaged above- or below-ground material. Prior to visualization, we normalized data by subtracting the mean and dividing it by the standard deviation of each morphological measurement. The optimum number of principal components (PC) for analysis was determined using a scree plot where eigenvalues for PCs greater than the average eigenvalue were retained. We then examined whether the set of morphological measurements differed by donor site via permutational analysis of variance (PERMANOVA) specifying a Euclidean distance matrix (*adonis2* function, vegan package, Oksanen et al. 2022).

2.5.2 *Collection intensity experiment*

We examined the effects of collection intensity and elevation on the resilience of eelgrass to shoot collection intensity by analyzing differences in shoot densities two months after experimental setup and one-, two-, three-, and four-years post-collection. We treated each timepoint independently to assess when shoot densities across treatment levels converged ("recovery"). To determine whether recovery had occurred, we compared a full model that included fixed effects of collection intensity (continuous), elevation (two levels, categorical), and their interaction to all reduced models, as well as a null model, using AICc (Akaike's Information Criterion, corrected for small sample sizes). For all models, we specified a Tweedie family distribution to account for the occurrence of zeros and positive numbers resulting from complete shoot removal in some plots. We selected simpler models where the difference in AICc (Δ AICc) was < 2.0. At timepoints when collection intensity remained significant, we analyzed differences in shoot densities between reference plots and each level of collection intensity using linear models. Since three comparisons were made within each donor site at each timepoint, a Bonferroni corrected acceptance value of 0.017 was used.

The proportion of flowering shoots within plots was analyzed for the effects of collection intensity, donor site elevation, and their interaction. The proportion flowering was calculated as the number of flowering shoots divided by the total number of shoots per plot. We compared full and all reduced models and a null model via AICc, choosing simpler models where difference in ΔAICc was < 2.0. For all models we specified a beta family distribution and included a dispersion term to address expected variations in the proportion of flowering shoots between treatment combinations. Proportion flowering data were bound between 0 and 1. To meet assumptions of the beta family distribution, values of 0 were adjusted to 0.001, and values of 1 were adjusted to 0.999. Separate tests were done at the timepoint with peak flowering in 2021, 2022, and 2023; 2020 was excluded because plots were established that year, and 2024 data were collected too late in summer for flowering to be evident.

2.5.3 *Transplant success*

Initial success of transplants was measured at 2 months (September 2020) as the fraction of transplanted shoots remaining ("shoot retention"). We tested the effects of plot size (categorical), density (continuous), and their interaction on shoot retention using a linear mixed model. Donor site was not included since shoot morphologies were indistinguishable (see results) and exploratory analyses of transplant outcomes suggested no donor-site effect. We specified a Gaussian family distribution and included a random effect of block. In cases of significant plot size x density interactions, we first tested whether the slope of the relationship between plot density and shoot retention differed from zero within each level of plot size using the emmeans package (Lenth 2022). Subsequently, we compared slopes across plot size levels, with pairwise comparisons performed using Tukey-adjusted p-values. Finally, we compared the estimated marginal means (EMMs) for shoot retention at each level of plot size, while controlling for plot density, with pairwise comparisons of EMMs also using Tukey-adjusted p-values.

To assess plot infilling within the transplant experiment, we focused on counts within the original plot area in July 2021 and July 2022, examining each sampling date independently; that is, the response variable was the shoot density within the original plot area. To assess population growth, we used the total count of shoots, including those moving outside the perimeter of the original transplant plots. The difference between the initial number of transplanted shoots and the total shoots counted at a given timepoint, divided by the initial number of transplanted shoots, was used to calculate proportional shoot counts, serving as a metric of population growth, For infilling and population growth at each timepoint, we compared full models that included fixed effects of plot size (categorical), plot density (continuous), and their interaction, to all reduced models, as well as a null model, using AICc. We selected simpler models where Δ AICc was < 2.0. For all models examining infilling in 2021 and 2022, we employed a Gaussian family distribution but applied a square-root transformation for 2021 to improve normality and meet assumptions of residual variance. Similarly, for all models investigating population growth, we used a Gaussian family distribution but applied a log (+1) transformation to proportional shoot count for 2021 and 2022 to improve normality and meet assumptions of residual variance. All models included a random effect of block. For infilling and population growth analyses, the same statistical framework used in the shoot retention analysis was applied if a significant interaction was detected. Specifically, we first tested whether the slope of the relationship between plot density and the response differed from zero within each level of plot size, then compared slopes across plot size levels, and ending by comparing EMMS for the response variable at each plot size level, while controlling for plot density. Due to the difficulty of determining the precise location of the 0.0625 m^2 plots relative to a single corner post, these plots were excluded from the infilling analysis.

To examine fraction flowering in the transplant experiment, we followed the same statistical framework used in the collection intensity experiment, except that predictors were plot size, plot density, and donor site and their interactions. We decided to include donor site as a predictor as elevation is known to affect eelgrass flowering. Since we only transplanted adult vegetative shoots within the transplant area, flowering could only be examined in 2021 and 2022, and we elected to examine a single timepoint with the highest proportion of flowering shoots within each year.

We examined the effect of plot size, plot density, and their interaction on transplanted eelgrass sheath length. We compared a full model, which included fixed effects of plot size, density, and their interactions, to a series of reduced models using AICc, incorporating plot as a random effect in each model. As in analyses of transplant density, we focused on analyzing data collected during the summer of each year of the experiment.

The proportions of fine and organic material within sediments were tested for the effect of eelgrass (inside and outside of large high-density plots), timepoint (September 2020, July 2021, July 2022), and their interaction by a generalized linear mixed model specifying a beta error distribution with a random intercept of block. In case of a significant interaction term, we calculated EMMs and performed Tukey-adjusted pairwise comparisons to determine significant differences between habitats at each timepoint. We did not collect sediment samples before or immediately after transplant, starting only when eelgrass was established in plots.

Results

3.1 *Morphology*

Across the 119 terminal shoots collected from high and low elevation donor sites, maximum blade length ranged from 20.0 cm – 110.0 cm, sheath length from 8.4 cm – 31.5 cm, and sheath width from 4.8 mm – 14.9 mm. The number of branches ranged from 0 – 2, the number of internodes per 7 cm from 3 – 11, and longest internode from 0.4 cm – 3.7 cm. The above- and below-ground biomass ranged from 0.08 g – 6.74 g and 0.11 g – 0.48 g, respectively. In this multivariate analysis of morphology, 45.0% of the variation along PC1 was explained by metrics of shoots size and 15.8% of the variation along PC2 was explained by variation in below ground features of shoots. Donor sites were not fully separated, and most observations from either location overlapped in ordination space (Fig. S2). Based on R^2 values, donor site only explained 0.8% of the variation in the multivariate eelgrass morphology data ($F_{1,117} =$ 1.0, p = 0.33). Based on the results of PERMANOVA and PCA, we did not include donor site as a predictor in analyses of eelgrass transplants, except flowering.

3.2 *Collection intensity at donor sites*

Shrimp densities at the high elevation donor site varied significantly in the first two years. They increased from the start of the experiment to the next spring, then disappeared by the spring before the second year (Fig. 2). The low elevation donor site showed a similar decline in shrimp densities over the same period (Fig. 2).

Shoot densities in plots subjected to lower collection intensities recovered to reference plot densities more quickly than plots where complete collections (100% shoots removed) occurred, regardless of elevation (Fig. 2). At both high and low elevation donor sites, shoot densities in 50% collection treatments returned to reference levels within two months (Table S1, S2). For 80% collection treatments, recovery was also achieved within two months at high elevations, but at low elevations, it took nearly a year (Table S1, S2). Complete 100% collections required the longest recovery time, with densities resembling reference plots only after almost two years at both donor sites (Table S1, S2). Following this recovery period, shoot densities across collection treatments remained similar until the end of the experiment, except for the 50% collection intensity treatment at the low elevation site, which exhibited significantly higher shoot densities compared to reference plots in May 2023 and 2024 (Table S1, S2). By this point in the experiment, shoot densities had converged across collection treatments at both elevations (Fig. 2). The results of the statistical analysis examining effects of collection intensity and elevation on shoot density at each timepoint can be found in the supplemental results (Table S3).

The proportion of flowering shoots in the first spring after shoot collection was affected by collection intensity and elevation (Table S4A), where the proportion of flowering shoots declined with increasing collection intensity (est = -0.02, $z = -2.6$, $p < 0.01$) and was higher at the high elevation donor site (est = 2.1, $z = 4.5$, $p < 0.01$. Fig. S3). The following year we observed a lag in flowering at both donor sites, with peak frequency in July rather than May, and the best model describing difference in the proportion of flowering shoots was the null model (Table S4B). During the final year of the experiment, the proportion flowering was greater at higher elevation (Table S4C, est = 0.6, $z = 2.0$, $p = 0.04$, Fig. S3).

3.3 *Transplant experiment*

As previously mentioned, we observed notable changes in shrimp distributions within our transplant area, like those observed at donor sites. Initially, shrimp densities were high (347.2 m-2, 38.4 SE, N = 10) at the start of the experiment. However, by the following spring, these densities had significantly decreased (6.4 m^{-2}, 2.6 SE, N = 10). Shrimp densities remained comparably low before becoming completely absent by the summer of the final year.

There was a significant main effect of plot density ($\chi^2 = 6.4$, *d.f.* = 1, $p = 0.01$) and interactive effect of plot size and density on the proportion of transplanted shoots remaining after two months ($\chi^2 = 11.4$, *d.f.* = 3, $p < 0.01$). For all plot sizes, except for tiny plots, increasing shoot density led to lower shoot retention (Table S5A), and this relationship did not differ in strength across plot sizes (Table S6A, Fig. 3). Likewise, small plots had marginally greater shoot retention than large plots, but retention did not differ between all other plot sizes (Table S7A).

The best model explaining plot infilling in both 2021 and 2022 was the full model (Table S8A, S8B). In 2021, the effect of plot size was significant ($\chi 2 = 11.2$, *d.f.* = 2, $p < 0.01$), as well as the interaction between plot size and density ($\chi 2 = 22.6$, *d.f.* = 2, $p < 0.01$). In smaller plots, transplants still occurred at higher density a year after they had been transplanted at higher density; however medium and large plots didn’t show this effect, meaning that all densities had in-filled and converged (Table S5B, Fig. 4). Smaller plots had a stronger response to transplant density compared to other plot sizes, as well as overall lower densities than larger plots (Tables S6B, S7B). In 2022, densities in all plots had doubled relative to the prior year and were close to the original high-density treatments

(Fig. 5). Only plot size had a significant effect ($\chi2 = 7.8$, *d.f.* = 2, $p = 0.02$), and the interaction between size and density was marginal ($\chi2 = 5.5$, *d.f.* = 2, $p = 0.06$). The trend was for plots established with small, sparse treatments to remain lower in density within the original transplant area, but no post-hoc comparisons were significant (Table S7C).

For population growth, the best model was the full model for both 2021 (Table S9A) and 2022 (Table S9B). In 2021, plot density ($\chi2 = 9.9$, *d.f.* = 1, $p < 0.01$), plot size ($\chi2 = 9.2$, *d.f.* = 3, $p = 0.02$), and their interaction ($\chi2 = 14.9$, *d.f.* = 3, $p < 0.01$) all influenced population growth. In general, as plot density increased, population growth decreased, except in small plots, where there was no effect (Table S5C, Fig. S5). The tiny plots showed the strongest response, with lower densities leading to faster growth (Table S6C). These plots also showed higher growth than large plots, with only minor differences between other plot sizes (Table S7D). In 2022, plot density ($\chi2 = 12.7$, *d.f.* = 1, $p < 0.01$), plot size ($\chi2 = 17.0$, *d.f.* = 3, $p < 0.01$), and their interaction ($\chi2 = 28.1$, *d.f.* = 3, $p < 0.01$) affected population growth. The effect of plot density became stronger as plot size decreased, with tiny plots increasing most rapidly in shoot counts from lower initial densities (Table S5D, S6D, Fig. S5). Additionally, tiny plots outperformed the large plots, with only marginal or no differences between other plot sizes (Table S7E).

The proportion of flowering shoots in the transplant experiment was best explained by a model with a main effect of plot size in both 2021 (Table S10A) and 2022 (Table S10B). For both years, the effect of plot size was significant (2021: $\chi^2 = 11.4$, *d.f.* = 3, $p <$

0.01; 2022: $\chi^2 = 11.4$, $d.f. = 3$, $p < 0.01$). In 2021, tiny plots exhibited less flowering than all other plot sizes (Table S10A, Fig. S6). This trend persisted in 2022, although flowering in tiny plots was only marginally different from small plots (Table S10B, Fig. S6).

Sheath length was best explained by a null model in both 2020 and 2022 (Table S12A, S12C), and by a model only including the main effect of plot size in 2021 (Table S12B). Plot size had a significant effect on sheath length ($\chi^2 = 8.6$, $d.f. = 3$, $p < 0.03$), however tiny plots had longer shoots than medium plots and there were no differences between any other plot sizes (Table S13, Fig. S7). Shoots averaged 17.1 cm (± 0.4 SE) at transplant, 14.2 cm (± 3.1 SE) after 1 yr, and 27.5 cm (± 4.4 SE) after 2 yr as plots had nearly coalesced to form a meadow.

Fines and organic (LOI) content in sediment were highly correlated (Pearson's r = 0.85, $p < 0.001$, N = 43) and followed a similar shift from lower to higher values during the transplant experiment. Eelgrass (presence/absence), time, and their interaction significantly affected the percent fine particles within sediments (Table S14A). Two months after the large, dense plots were established, they contained finer sediments than outside the plots but thereafter changes occurred more gradually within the eelgrass plots compared to the unvegetated areas. By summer of 2021, percentage fines were significantly higher outside of plots, and by summer of 2022, no difference was observed between inside and outside plots (Table S15A, S15B, Fig. 10). Similar patterns were observed for percent organics (Table S14B, Fig. 6).

Discussion

While positive interactions are known to support seagrass restoration, our experimental test of transplant density and plot size showed little evidence of self-facilitation, even initially with dense bioturbators. Instead, the plot sizes and densities we chose were strongly negatively-density-dependent, with the sparse smallest plots showing population growth that was 83 times greater than in the large, high-density plots by the second year. Initially, this experimental restoration altered the sediment as expected by accumulating fines and organics. Over the following year, however, sediment changed more rapidly outside than within high-density eelgrass plots, which we interpret to mean that eelgrass was less important than bioturbating shrimp, which vacated most of the transplant site within the first year. Donor sites recovered more rapidly from lower collection intensity, and 4 m^2 gaps in both fringing and upper-margin eelgrass recovered within 2 years, a rate similar to meadows (Ruesink, 2018).

Plots with lower collection intensity matched reference plots more quickly, likely due to higher residual meristem densities that facilitated faster recovery. Likewise, gap recovery (100% removal) at both donor sites occurred within two years, aligning with previously documented recovery rates for eelgrass meadows (Boese et al., 2005; Ruesink et al., 2012). Both donor sites exhibited substantial fluctuation in shoot densities (Fig. 4), consistent with upper-margin and fringing habitats that may experience natural disturbances (Robertson and Mann, 1984; Ruesink et al., 2024). These natural fluctuations did not evidently slow or prevent recovery. Nevertheless, consistent with best practices for sourcing eelgrass for restoration, our results highlight the importance of low-intensity shoot collection to ensure rapid recovery of donor sites (Fonseca et al., 1998).

Seagrass flowering is a plastic trait, responding to changes in environmental conditions (Keddy, 1987; Lekammudiyanse et al., 2024). Tidal elevation influences eelgrass life history traits, with greater investments in flowering typically observed at higher intertidal elevations (Backman, 1991; Harrison, 1993; Phillips et al., 1983). We observed a similar increase with elevation for flowering at donor sites, which did not persist when shoots from both sites were transplanted to an elevation in between. Higher elevations may therefore constitute stressful environments where shoots increase investment in seed production as a bet-hedging strategy. Collection intensity did not shift flowering frequency, which adds more evidence that disturbance such as thinning shoots does not cause an immediate response in flowering (Ruesink et al. 2012). The reduced flowering in the sparse smallest plots in the transplant experiment was a sole case where experimental manipulations altered sexual reproduction. We suspect this may be an indirect result of the rapid branching (clonal reproduction) in these treatments, as they encountered the least intraspecific competition, and these new shoots had not reached the age (>1 yr) where flowering is likely (Setchell 1929).

Positive density dependence has been observed in eelgrass and other seagrass meadows. In field observations, higher density plots can increase survival and plot expansion in eelgrass (Olsen and Sand-Jensen 1994) and *Posidonia oceanica* beds (Almela et al., 2008; Olesen and Sand-Jensen, 1994). Experiments manipulating shoot density in mesocosms and *in situ* showed that higher transplant densities supported survival and long-term density (Worm and Reusch, 2000; Zhang et al., 2022). In a review of eelgrass restoration approaches from the U.S. Pacific Northwest, higher transplant densities had positive effects on shoot densities 5-7 months later (Ward and Beheshti, 2023), and a meta-analysis of global seagrass bed restoration found that using higher numbers of shoots during transplantation increased shoot survival (van Katwijk et al., 2016). Given the dynamic and stressful coastal environment of seagrass habitats, it is not surprising that a large number of positive feedbacks in seagrass beds can exist (Cardoso et al., 2004; Nyström et al., 2012; Suykerbuyk et al., 2016; Valdez et al., 2020; van der Heide et al., 2007). Previous eelgrass transplantations have performed well in this bay (Ruesink 2018) and eelgrass is widespread throughout (Ruesink et al. 2006, 2010), thus it could be possible for small patches of eelgrass to grow and expand rapidly in this system.

Despite widespread evidence of self-facilitation in seagrasses, our transplants primarily demonstrated intraspecific competition. Early transplant success was greater in low-density treatments, and population growth occurred faster for smaller and sparser plots. Additionally, the presence of shrimp early in the experiment did not eliminate smaller plots as we might have anticipated. The density of shrimp early in the experiment was lower than what is known to fully eliminate eelgrass (Hull and Ruesink, 2024), but shrimp may have been responsible for re-setting the densest initial plots to a lower density. The comparison to the densities in the donor sites, where shrimp were also present with eelgrass at medium densities ($< 50\ m^{-2}$), further supports the notion that lower planting densities may have been more conducive to eelgrass establishment. Intraspecific competition is common in eelgrass meadows. Higher densities of adult shoots can negatively impact seedling survival in calmer environments and reduce shoot emergence rates (Ruesink, 2018; Yang et al., 2016). Further, increased self-shading can occur in dense seagrass beds (Ralph et al., 2007), potentially causing declines in the health and survival of shoots. Low-density planting may have allowed eelgrass to establish more successfully by reducing competition, allowing more access to resources, and avoiding the negative effects of overcrowding. This may indicate that for the initial stages of restoration, the lower-density planting we selected may have been sufficient for facilitation, while higher-density planting encountered resource competition.

We examined two metrics of longer-term success of transplanted eelgrass: ability to in-fill from low density, based only on the initial area transplanted, and population growth including all descendent shoots. The result for infilling was more rapid than gap recovery at the donor sites, with nearly all treatments converging to the same density within a year. Densities may have remained relatively low for initially low-density medium plots because these had the least “core” area where shoots would grow into already-occupied space and the most “edge” where shoots would leave the plot as rhizomes extended. Rapid infilling after successful establishment of eelgrass has occurred where initial transplant density no longer predicted shoot densities 1 and 2 years post transplantation, and shoot densities between reference meadows and restored plots converged around comparable densities (Ward and Beheshti, 2023).

The population growth of some of the transplant plots was remarkable and clearly related to the proximity of shoots to unvegetated areas, either within the plot (low density) or outside (smallest area). In a study evaluating the development of planted seagrass beds in Tampa Bay, Florida, recovery rates showed a positive linear relationship to planting spacing (Fonseca et al., 1996). These results suggest that both plot size and density play an important role in population growth during restoration, with smaller plots under lower density conditions promoting higher growth rates, potentially due to reduced competition or environmental factors that differ across plot size.

Seagrass, as ecosystem engineers, can influence sediment dynamics. Therefore, eelgrass restoration can also serve additional purposes such as promoting sediment deposition. By reducing water flow through the presence of shoots, seagrass beds facilitate the deposition of fine particles, enhance sediment stability, and capture organic and inorganic matter (Fonseca and Fisher, 1986; Fonseca and Cahalan, 1992; Hemminga and Duarte, 2000; Koch and Gust, 1999; Madsen et al., 2001). Although the percentage of fines and organic matter (LOI) were elevated in eelgrass plots within a few months of transplantation, these short-term differences reversed, and the eelgrass plots lagged behind the unvegetated areas in accumulating fine and organic matter until stabilizing after just more than a year. It is possible that this pattern could be attributed to the disappearance of burrowing shrimp as we assume that there were no long-term changes in fine-sediment supply to the site. While shrimp were not physically removed from our experimental area, their departure could have caused a reduction in sediment turnover and increased sediment compaction, resulting in faster accumulation of fine and organic material in areas without eelgrass (Contessa and Bird, 2004; Wynberg and Branch, 1994). Additionally, the flow around the base of shoots (where leaves are held within the sheath) may have caused the ascension or scouring and therefore limiting sedimentation (Bouma et al., 2007; Koch et al., 2006; Marin-Diaz et al., 2020). Regardless of the delay, the percentage of fine and organic particles stabilized, ultimately increasing since the start of the experiment, but with no difference in and out of eelgrass after two years. While this pattern may reflect that the entire tidal flat had nearly coalesced into a meadow, it is also the case that burrowing shrimp appear to be much stronger engineers of sediment conditions than transplanted eelgrass in promoting important ecosystem functions that eelgrass restoration is known to reestablish (McGlathery et al., 2012).

Conclusion

Low-density transplants provide an opportunity for each shoot to contribute substantially to restoration, unimpeded by competition from nearby shoots. In keeping with the two-year development of shoots before they can flower, these treatments where the most branching occurred also had lower flowering frequencies, which could influence other aspects of resilience. Yet clearly the outcome we observed was particular to this restoration site, since self-facilitating interactions are necessary for restoration success in many cases, though we lack sufficient information on self-facilitating mechanisms at this site (van Katwijk et al. 2016; Valdez et al. 2020). After two years, monitoring of the eelgrass transplant plots was no longer possible because the plots had coalesced, and shoots could not be attributed to their original treatment. The shoots developed a taller canopy [and higher density] by the second year of the experiment in keeping with this transition to eelgrass meadow, which coalesced across this new meadow 0.12 ha and has persisted at the site at least two additional years (pers.obs.). The transformation of this tidal flat from bioturbator-dominated to seagrass meadow shows the strong engineering effects of different parts of the habitat mosaic, while also pointing out the challenge of attribution of sediment change when such a shift occurs. Here, the timing of events highlights bioturbation over seagrass as the major mechanism underlying the observed sediment change. Future work can continue to evaluate the resilience of fringing habitats (along upper margins or channels) in comparison to meadows of eelgrass. These insights will help refine restoration strategies, optimize transplant densities, and improve long-term success for both restored meadows and donor site recovery.

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Figures

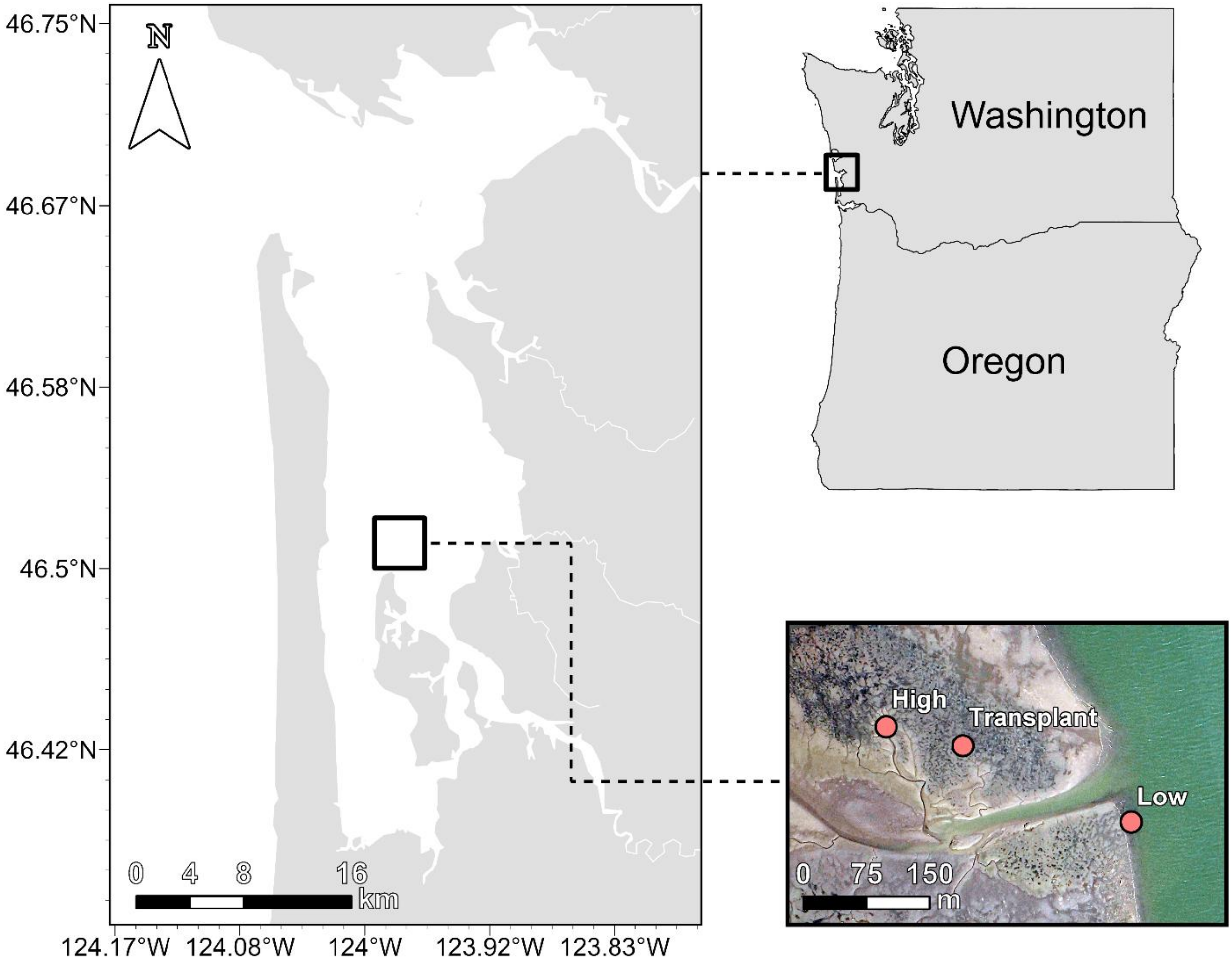

Figure 1. Location of the transplant site (Transplant) and two donor sites at high and low elevation within Willapa Bay, WA, USA. The aerial low tide image in the bottom right image is from summer of 2024 (attribution: image courtesy of Google Earth, accesses January 2024).

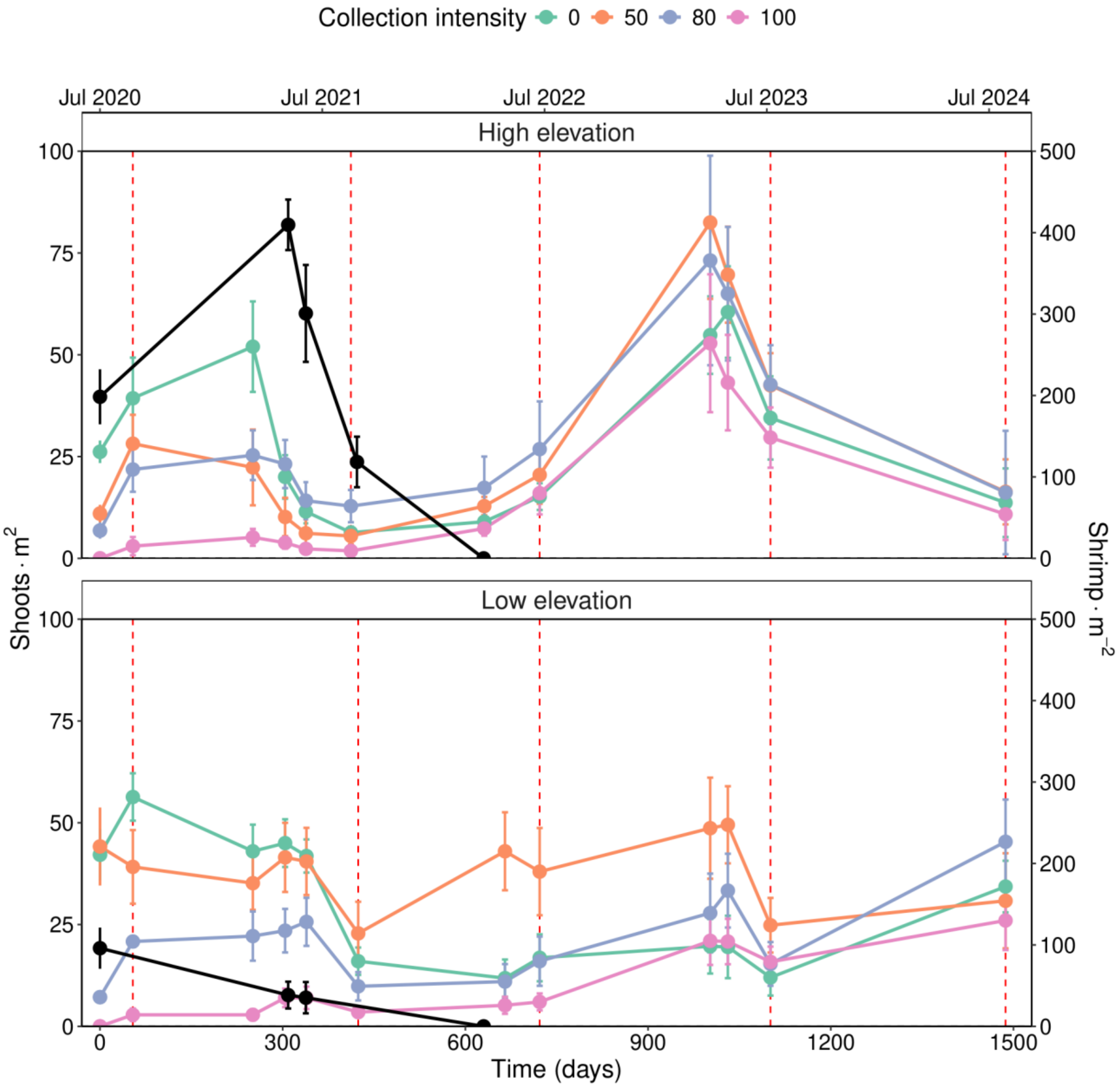

Figure 2. Changes in average shoot densities in plots with different shoot collection intensities for eelgrass (*Zostera marina*) at high and low elevation donor sites. Error bars represent ± 1 standard error (N = 6) and red dashed lines represent analyzed timepoints. Some error bars are small and are hidden behind points. Black points represent burrowing shrimp densities (N = 5).

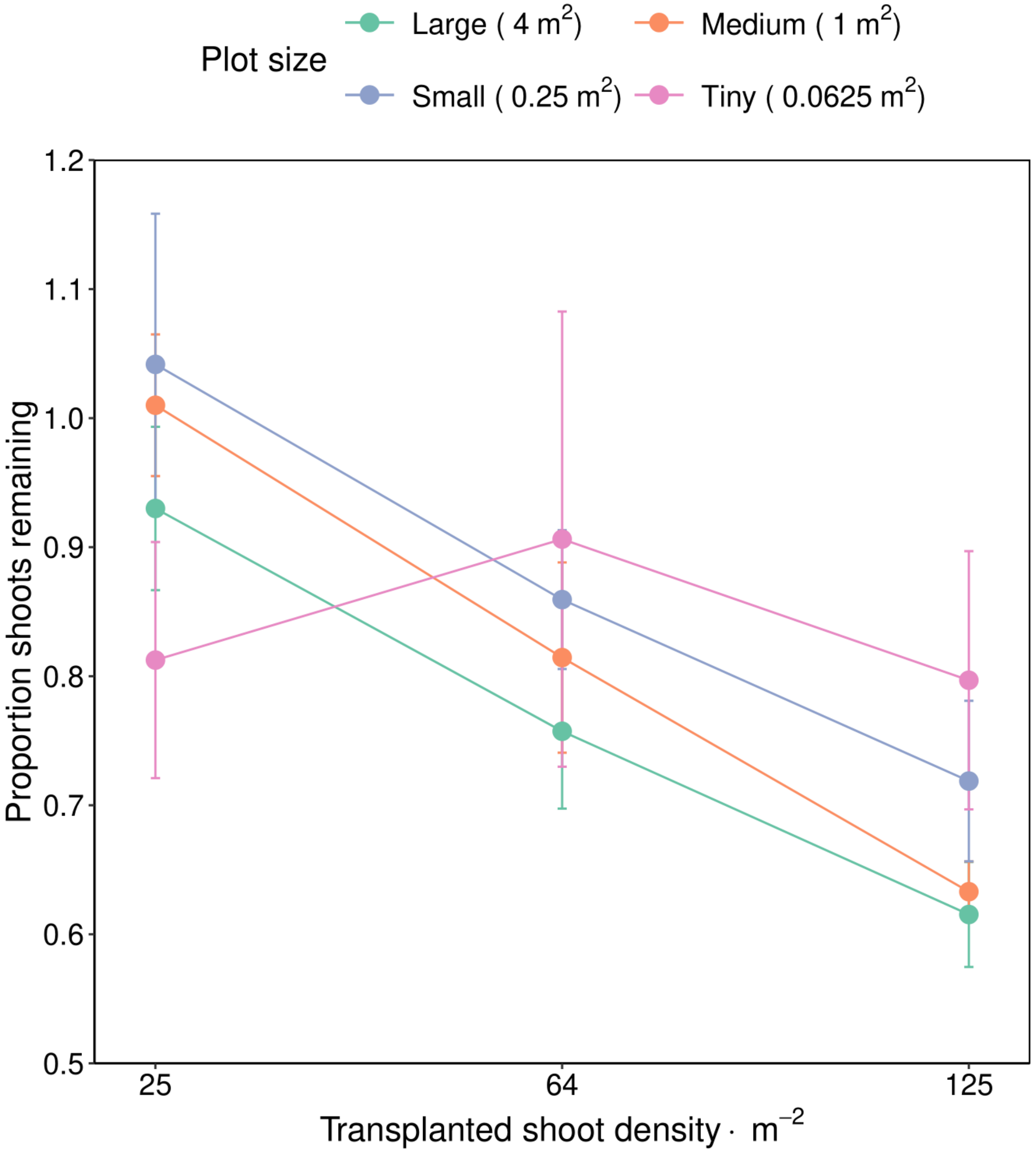
Plot size
Large (4 m²)
Medium (1 m²)
Small (0.25 m²)
Tiny (0.0625 m²)
Proportion shoots remaining
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
25
64
125
Transplanted shoot density · m⁻²

Figure 3. Eelgrass (*Zostera marina*) proportion of surviving shoots two months after transplant in July 2020, Willapa Bay, WA, USA. Treatments were established across three initial shoot densities and four plot sizes. Error bars represent ± 1standard error (N = 5, except N = 4 for 4 m^2 plots).

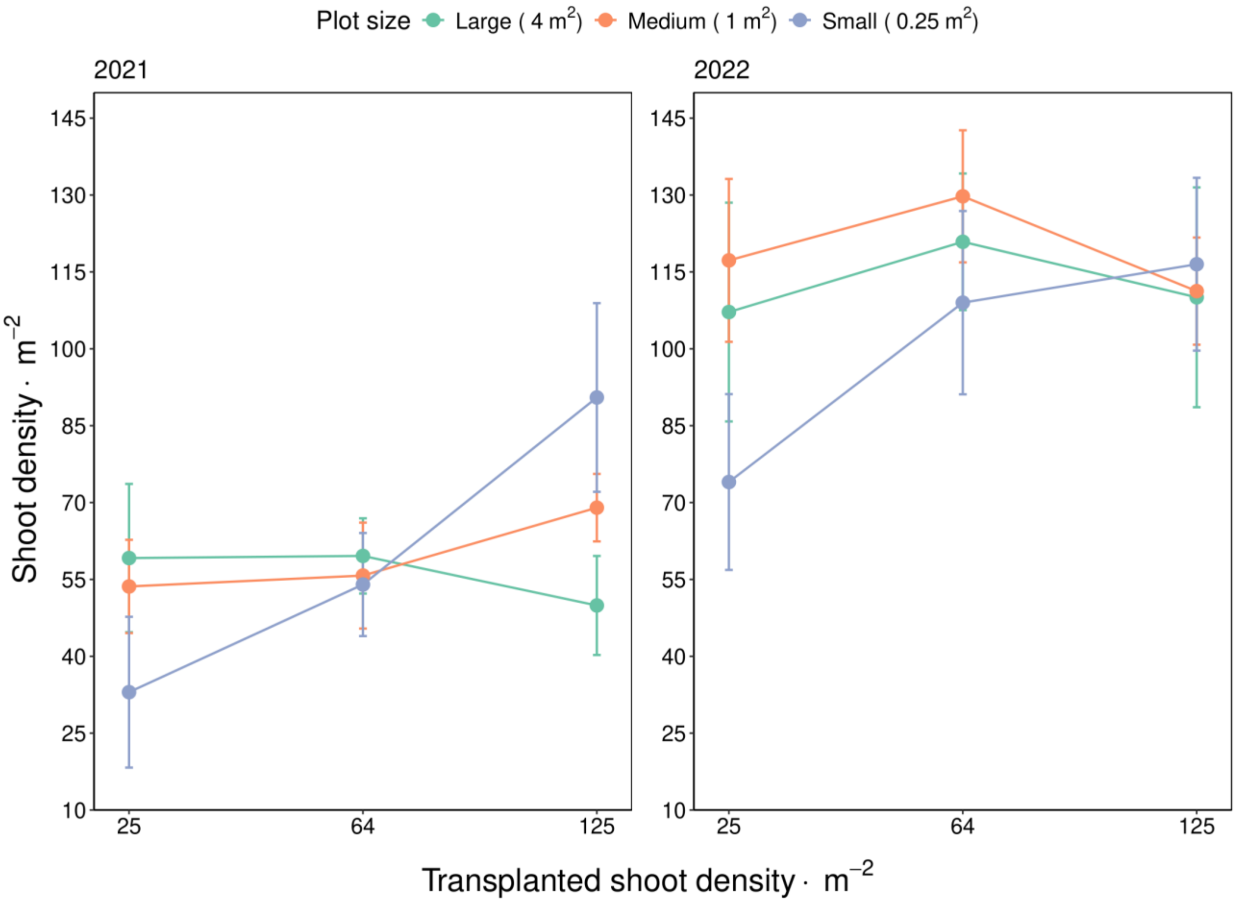

Figure 4. Mean eelgrass (*Zostera marina*) shoot density in transplant plots one (2021) and two years (2022) after transplant at three initial densities and three initial plot sizes, Willapa Bay, WA, USA. Error bars represent ± 1 standard error.

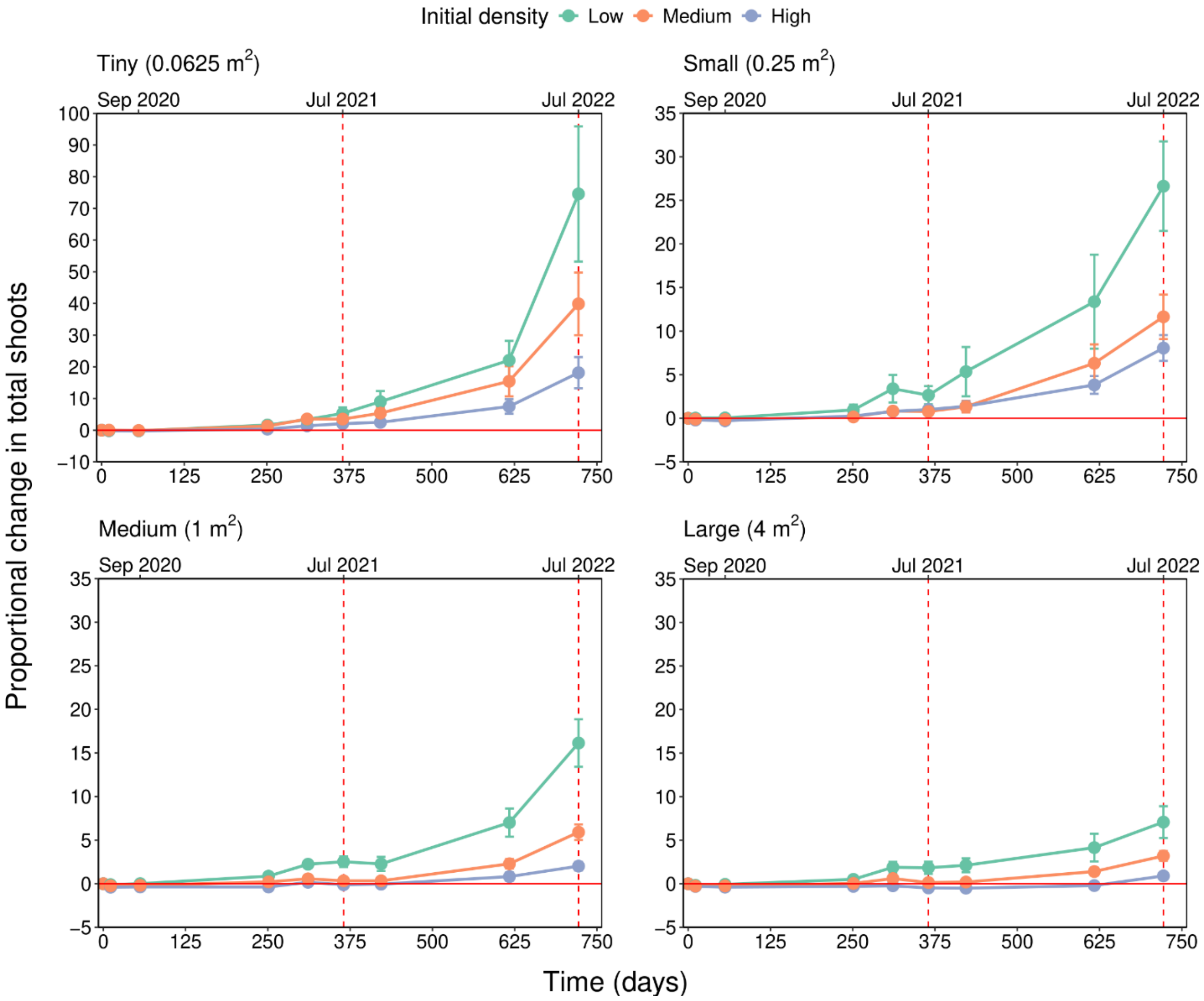

Figure 5. Eelgrass (*Zostera marina*) population increase over two years, standardized to initial counts in treatment plots, which were established at three densities and four plot sizes, Willapa Bay, WA, USA. Proportional change was calculated as (Nt-N0)/N0. Error bars represent ± 1 standard error and red dashed lines represent analyzed timepoints. Some error bars are small and are hidden behind points. The solid red horizontal line marks zero proportional change. Note the change in the y-axis scale for tiny plots.

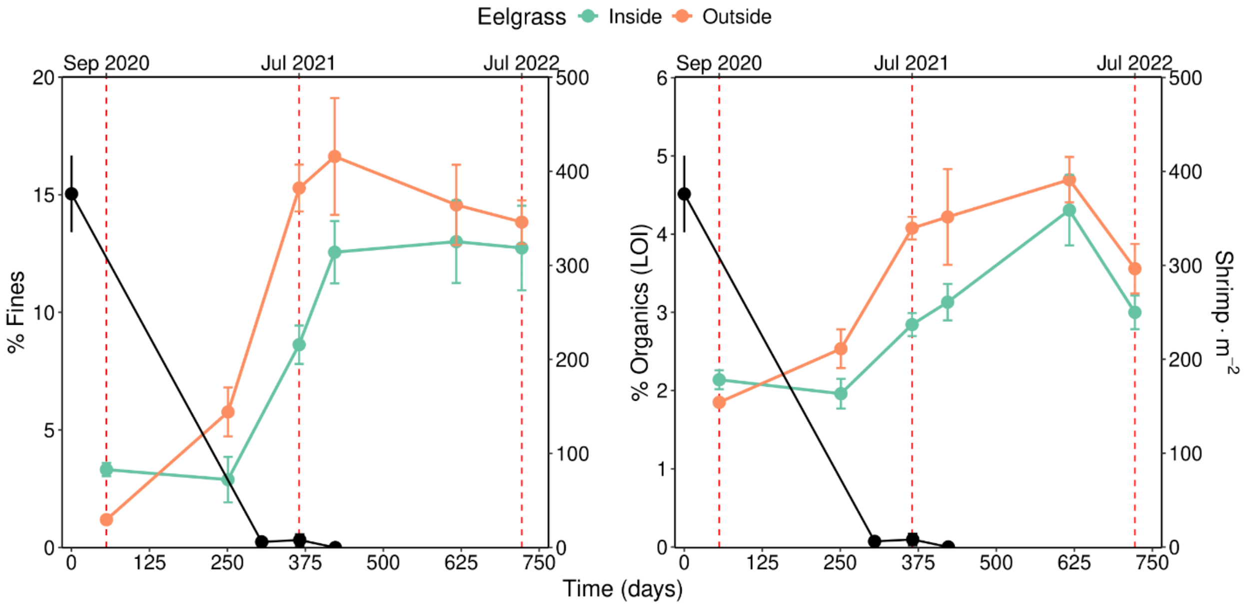

Figure 6. Changes in average percent fine and organic material (LOI) in sediment samples from inside and outside of 4 m^2 plots over the course of the transplant experiment, Willapa Bay, WA, USA. Error bars represent ± 1 standard error and red dashed lines represent analyzed timepoints. Some error bars are small and are hidden behind points. Black points represent average shrimp density (N = 10).

Supplemental Material

Results

Collection intensity alone was the best at describing differences in shoot densities during the first two months of the experiment (Table S3A). During this period, shoot densities decreased with increasing collection intensity (est = -0.02, z = -6.0, p <0.001). However, by the first elevation had become the only significant predictor of shoot density with lower elevations exhibiting higher densities than higher elevations (Table S3B, est = 0.7, z = 2.4, p = 0.01), In the second year the null model was the best predictor of shoot density (Table S3C), and by the third year, elevation again was the best at describing differences in shoot densities (Table S3D), with higher densities observed at the high elevation donor site (est = -0.79, z = -3.9, p < 0.001). In the final year, elevation alone was again the best at describing differences in shoot densities (Table S3E), and shoot densities were higher at the low elevation donor site (est = 0.9, z = -2.9, p < 0.01).

Table S1. Comparison of reference plots (0% shoot collections) to collection intensity treatments within the high elevation donor site over the course of the experiment. Bolding represents significant differences. Bonferroni corrected acceptance of 0.017.

Year	Month	comparison	estimate	*z*	*P*
2020	September	50%	-0.22	-1.00	0.317
		80%	-0.22	-1.69	0.092
		100%	**-0.36**	**-3.89**	**0.000**
2021	April	50%	-0.59	-2.24	0.025
		80%	-0.33	-2.30	0.021
		100%	**-0.47**	**-4.54**	**<0.001**
	May	50%	-0.20	-1.51	0.130
		80%	0.04	0.44	0.663
		100%	**-0.16**	**-3.20**	**0.001**
	June	50%	-0.11	-1.31	0.191
		80%	0.03	0.54	0.588
		100%	**-0.09**	**-3.28**	**0.001**
	September	50%	-0.02	-0.36	0.716
		80%	0.08	1.69	0.092
		100%	**-0.05**	**-2.91**	**0.004**
2022	April	50%	0.08	1.11	0.267
		80%	0.10	1.11	0.269
		100%	-0.02	-0.52	0.604
	July	50%	0.11	0.75	0.454
		80%	0.15	1.05	0.294
		100%	0.01	0.15	0.882
2023	April	50%	0.55	1.44	0.150
		80%	0.23	0.73	0.464
		100%	-0.02	-0.11	0.910
	May	50%	0.18	0.62	0.537
		80%	0.06	0.25	0.804
		100%	-0.17	-1.17	0.242
	July	50%	0.16	0.68	0.497
		80%	0.10	0.64	0.525
		100%	-0.05	-0.42	0.675

2024	August	50%	0.05	0.25	0.801
		80%	0.03	0.16	0.875
		100%	-0.03	-0.30	0.768

Table S2. Comparison of reference plots (0% shoot collections) to collection intensity treatments within the low elevation donor site over the course of the experiment. Bold represents significant differences. Bonferroni corrected acceptance of 0.017.

Year	Month	comparison	estimate	*z*	*P*
2020	September	50%	-0.34	-1.75	0.080
		80%	**-0.44**	**-6.51**	**<0.001**
		100%	**-0.53**	**-9.73**	**<0.001**
2021	April	50%	-0.16	-0.93	0.354
		80%	**-0.26**	**-2.56**	**0.010**
		100%	**-0.40**	**-6.58**	**<0.001**
	May	50%	-0.07	-0.37	0.710
		80%	**-0.27**	**-2.97**	**0.003**
		100%	**-0.38**	**-6.62**	**<0.001**
	June	50%	-0.03	-0.16	0.874
		80%	**-0.20**	**-2.47**	**0.014**
		100%	**-0.35**	**-7.76**	**<0.001**
	September	50%	0.14	0.88	0.376
		80%	-0.08	-1.40	0.162
		100%	**-0.12**	**-3.78**	**<0.001**
2022	**May**	**50%**	**0.62**	**3.22**	**0.001**
		80%	-0.01	-0.15	0.884
		100%	-0.07	-1.45	0.147
	July	50%	0.42	1.90	0.057
		80%	-0.01	-0.11	0.913
		100%	-0.11	-1.93	0.053
2023	April	50%	0.58	2.25	0.024
		80%	0.10	0.76	0.447
		100%	0.01	0.16	0.870
	May	**50%**	**0.60**	**2.70**	**0.007**
		80%	0.17	1.28	0.202

		100%	0.01	0.15	0.878
	July	50%	0.26	1.75	0.081
		80%	0.04	0.52	0.600
		100%	0.04	0.72	0.470
2024	August	50%	-0.07	-0.29	0.773
		80%	0.14	0.99	0.321
		100%	-0.08	-0.95	0.344

Table S3. Comparison of full and reduced generalized linear models explaining differences in eelgrass shoot density in the collection intensity experiment during A) 2020, B) 2021, C) 2022, D) 2023, and E) 2024. Bolded models represent the best models.

Year	Model	*d.f.*	Log likelihood	AICc	ΔAICc	Weight
A) 2020	Null	3	-200.8	408.1	25.4	<0.01
	Elevation	4	-200.3	409.5	26.8	<0.01
	Removal	**4**	**-186.2**	**382.7**	**0**	**0.53**
	Elevation + removal	5	-186.3	384.0	1.2	0.31
	Elevation × removal	6	-186.1	386.1	3.4	0.11
B) 2021	Null	3	-161.9	330.4	3.9	0.05
	Elevation	**4**	**-159.2**	**327.2**	**0.7**	**0.26**
	Removal	4	-160.1	329.2	2.7	0.10
	Elevation + removal	5	-157.6	326.5	0	0.37
	Elevation × removal	6	-156.7	327.5	1.0	0.23
C) 2022	**Null**	3	**-191.5**	**389.5**	**0**	**0.55**
	Elevation	4	-191.5	391.9	2.4	0.17
	Removal	4	-191.4	391.6	2.1	0.19
	Elevation + removal	5	-191.4	394.1	4.6	0.06
	Elevation × removal	6	-190.5	395.1	5.5	0.04
D) 2023	Null	3	-203.9	414.4	10.4	<0.01
	Elevation	**4**	**-197.5**	**404.0**	**0**	**0.72**
	Removal	4	-203.9	416.8	12.9	<0.01
	Elevation + removal	5	-197.5	406.4	2.5	0.21
	Elevation × removal	6	-197.4	408.9	4.9	0.06
E) 2024	Null	3	-197.5	401.6	5.6	0.04
	Elevation	**4**	**-193.6**	**396.1**	**0**	**0.69**
	Removal	4	-197.5	404.0	7.9	0.01

	Elevation + removal	5	-193.6	398.5	2.5	0.20
	Elevation × removal	6	-193.5	401.1	5.1	0.05

Table S4. Comparison of full and reduced generalized linear models explaining differences in the proportion of flowering shoots from the collection intensity experiment during A) May 2021, B) July 2022, C) May 2023. Bolded models represent the best models.

Year	Model	*d.f.*	Log likelihood	AICc	ΔAICc	Weight
A) 2021	Null	**2**	78.9	-153.5	11.3	<0.01
	Elevation	4	85.8	-162.7	2.1	0.16
	Removal	4	79.8	-150.7	14.1	<0.01
	Elevation + removal	6	**89.4**	**-164.8**	**0**	**0.48**
	Elevation × removal	8	91.9	-164.2	13.0	0.35
B) 2022	**Null**	**2**	**71.8**	**-139.4**	**0**	**0.74**
	Elevation	4	72.3	-135.6	3.8	0.11
	Removal	4	72.5	-136.0	3.4	0.13
	Elevation + removal	6	72.7	-131.4	8.0	0.01
	Elevation × removal	8	74.2	-128.8	10.6	<0.01
C) 2023	Null	**2**	34.7	-65.2	8.3	<0.01
	Elevation	4	**41.1**	**-73.3**	**0.3**	**0.36**
	Removal	4	35.0	-61.0	12.5	<0.01
	Elevation + removal	6	43.2	-72.3	1.3	0.22
	Elevation × removal	8	46.6	-73.6	0	0.42

Table S5A. Comparison of plot density slope to zero for A) Shoot retention, B) infilling in 2021, C) population growth in 2021, and D) population growth in 2022 within each level of plot size.

Year	Experiment	Plot size	*slope*	*d.f.*	*t*	*p*
A) 2020	Shoot retention	4 m^2	-0.0001	80	-2.34	0.01
		1 m^2	-0.0020	80	-3.31	<0.01
		0.25 m^2	-0.0058	80	-2.55	0.01
		0.0625 m^2	-0.0011	80	-0.11	0.91
B) 2021	Infilling	4 m^2	-0.002	58	-0.65	0.52
		1 m^2	0.012	58	1.34	0.18
		0.25 m^2	0.159	58	4.51	<0.01
C) 2021	Population growth	4 m^2	-0.002	80	-3.15	<0.01
		1 m^2	-0.008	80	-3.39	<0.01
		0.25 m^2	-0.013	80	-1.49	0.14
		0.0625 m^2	-0.115	80	-2.85	<0.01
D) 2022	Population growth	4 m^2	-0.002	80	-3.57	<0.01
		1 m^2	-0.007	80	-4.82	<0.01
		0.25 m^2	-0.017	80	-2.90	<0.01
		0.0625 m^2	-0.072	80	-2.96	<0.01

Table S6. Pairwise comparisons of density slopes for A) shoot retention, B) infilling in 2021, C) population growth in 2021, and D) population growth in 2022 within each level of plot size.

Experiment	Year	Plot size comparison	*estimate*	*d.f.*	*t*	*p*
A) Shoot retention	2020	4 m^2 – 1 m^2	0.002	80	2.47	0.07
		4 m^2 – 0.25 m^2	0.005	80	2.35	0.09
		4 m^2 – 0.0625 m^2	0.001	80	0.07	1.00
		1 m^2 – 0.25 m^2	0.004	80	1.64	0.36
		1 m^2 – 0.0625 m^2	-0.001	80	-0.09	1.00
		0.25 m^2 – 0.0625 m^2	-0.005	80	-0.47	0.97
B) Infilling	2021	4 m^2 – 1 m^2	-0.01	58	-1.47	0.31
		4 m^2 – 0.25 m^2	-0.16	58	-4.55	<0.01
		1 m^2 – 0.25 m^2	-0.15	58	-4.03	<0.01
C) Population growth	2021	4 m^2 – 1 m^2	0.01	80	2.38	0.09
		4 m^2 – 0.25 m^2	0.01	80	1.25	0.60
		4 m^2 – 0.0625 m^2	0.11	80	2.80	0.03
		1 m^2 – 0.25 m^2	0.01	80	0.59	0.94
		1 m^2 – 0.0625 m^2	0.11	80	2.64	0.05
		0.25 m^2 – 0.0625 m^2	0.10	80	2.44	0.08
D) Population growth	2022	4 m^2 – 1 m^2	0.01	80	3.64	<0.01
		4 m^2 – 0.25 m^2	0.02	80	2.63	0.05
		4 m^2 – 0.0625 m^2	0.07	80	2.90	0.02
		1 m^2 – 0.25 m^2	0.01	80	1.60	0.39
		1 m^2 – 0.0625 m^2	0.07	80	2.67	0.05
		0.25 m^2 – 0.0625 m^2	0.06	80	2.23	0.12

Table S7. Pairwise comparisons of estimated marginal means of A) shoot retention in 2020, B) infilling in 2021, C) infilling in 2022, D) population growth 2021, and E) population growth in 2022 between plot sizes

Experiment	Year	Plot size comparison	*estimate*	*d.f.*	*t*	*p*
A) Shoot retention	2020	$4\ m^2 – 1\ m^2$	0.1	80	1.4	0.53
		$4\ m^2 – 0.25\ m^2$	0.4	80	2.6	0.57
		$4\ m^2 – 0.0625\ m^2$	0.2	80	0.2	1.00
		$1\ m^2 – 0.25\ m^2$	0.3	80	2.2	0.14
		$1\ m^2 – 0.0625\ m^2$	0.1	80	0.1	1.00
		$0.25\ m^2 – 0.0625\ m^2$	-0.2	80	-0.3	0.99
B) Infilling	2021	$4\ m^2 – 0.25\ m^2$	-14.0	58	-4.2	<0.01
		$4\ m^2 – 1\ m^2$	-0.3	58	-0.4	0.89
		$1\ m^2 – 0.25\ m^2$	-13.6	58	-4.1	<0.01
C) Infilling	2022	$4\ m^2 – 0.25\ m^2$	-126.0	58	-2.0	0.12
		$4\ m^2 – 1\ m^2$	-2.0	58	-0.1	1.00
		$1\ m^2 – 0.25\ m^2$	-124.0	58	-2.0	0.12
D) Population growth	2021	$4\ m^2 – 1\ m^2$	0.4	80	1.7	0.33
		$4\ m^2 – 0.25\ m^2$	1.0	80	1.5	0.41
		$4\ m^2 – 0.0625\ m^2$	8.4	80	2.7	0.04
		$1\ m^2 – 0.25\ m^2$	0.6	80	1.0	0.74
		$1\ m^2 – 0.0625\ m^2$	8.1	80	2.6	0.05
		$0.25\ m^2 – 0.0625\ m^2$	7.5	80	2.4	0.09
E) Population growth	2022	$4\ m^2 – 1\ m^2$	0.1	80	0.9	0.82
		$4\ m^2 – 0.25\ m^2$	0.8	80	2.1	0.16
		$4\ m^2 – 0.0625\ m^2$	5.0	80	2.7	0.05
		$1\ m^2 – 0.25\ m^2$	0.7	80	1.9	0.25
		$1\ m^2 – 0.0625\ m^2$	4.9	80	2.6	0.05

	0.25 m^2 – 0.0625 m^2	4.2	80	2.2	0.14

Table S8. Comparison of full and reduced linear mixed models explaining changes in eelgrass infilling within plots in the transplant experiment during A) 2021, and C) 2022. Each model includes a random effect of block. Bolded models represent the best models.

	Model	*d.f.*	Log likelihood	AICc	ΔAICc	Weight
A)	Null	3	-149.9	297.7	19.7	<0.01
	Plot size	5	-149.5	297.0	19.1	<0.01
	Plot density	4	-149.8	297.7	19.7	<0.01
	Plot size + plot density	6	-149.5	297.0	19.1	<0.01
	Plot size × plot density	**8**	**-140.0**	**278.0**	**0**	**0.99**
B)	Null	3	-338.3	674.6	8.9	<0.01
	Plot size	5	-336.5	671.0	5.3	0.01
	Plot density	4	-3380	674.1	8.4	0.01
	Plot size + plot density	6	-336.5	671.0	5.3	0.10
	Plot size × plot density	**8**	**-333.8**	**665.7**	**0**	**0.87**

Table S9. Comparison of full and reduced linear mixed models explaining the proportional change in eelgrass shoot density in the transplant experiment during A) 2021, and B) 2022. Each model includes a random effect of block. Bolded models represent the best models.

	Model	*d.f.*	Log likelihood	AICc	ΔAICc	Weight
A)	Null	3	-94.1	186.3	50.2	<0.01
	Plot size	4	-82.9	163.8	27.7	<0.01
	Plot density	6	-82.5	162.9	26.8	<0.01
	Plot size + plot density	7	-75.9	149.7	13.7	<0.01
	Plot size × plot density	**10**	-69.0	136.1	**0**	**0.99**
B)	Null	3	-205.9	409.7	359.3	<0.01
	Plot size	4	-194.0	386.0	335.6	<0.01
	Plot density	6	-182.3	362.7	312.3	<0.01
	Plot size + plot density	7	-179.8	357.6	307.2	<0.01
	Plot size × plot density	**10**	**-26.2**	**50.4**	**0**	**0.99**

Table S10 Comparison of full and reduced generalized linear mixed models explaining the proportion of flowering shoots in the transplant experiment during A) 2021, and B) 2022. Each model includes a random effect of block. Bolded models represent the best models.

Year	Model	*d.f.*	Log likelihood	AICc	ΔAICc	Weight
A) 2021	Null	3	158.4	-310.6	15.4	<0.001
	Density	4	162.5	-316.5	9.5	0.004
	Size	**6**	**169.5**	**-326.1**	**0**	**0.510**
	Source	4	158.4	-308.4	17.6	<0.001
	Density + size	7	169.6	-323.8	2.2	0.170
	Density + source	5	162.5	-314.4	11.7	0.001
	Size + source	7	169.7	-324.0	2.1	0.180
	Desnity × size	10	171.4	-320.0	6.1	0.020
	Density × source	6	162.5	-312.1	14.0	<0.001
	Size × source	10	171.2	-319.6	6.5	0.020
	Density × size + source	11	171.5	-317.7	8.4	0.007
	Density × source + size	9	169.7	-319.2	6.9	0.020
	Size × source + density	11	171.2	-317.1	9.0	0.006
	Density × size + density × source	12	171.5	-315.0	11.0	0.002
	Density × size + size × source	14	173.1	-312.6	13.4	<0.001
	Density × source + size × source	12	171.5	-314.9	11.2	0.002
	Density × size + density × source + size × source	15	173.4	-310.3	15.8	<0.001
	Density × size × source	18	175.7	-305.7	20.3	<0.001
B) 2022	Null	3	90.4	-174.5	5.3	0.033
	Density	4	91.2	-173.9	5.9	0.025
	Size	**6**	**96.4**	**-179.8**	**0**	**0.466**
	Source	4	90.4	-172.3	7.5	0.011
	Density + size	7	96.5	-177.7	2.1	0.165
	Density + source	5	91.2	-171.7	8.1	0.008
	Size + source	7	96.4	-177.5	2.2	0.152
	Desnity × size	10	96.7	-170.5	9.2	0.005
	Density × source	6	91.3	-169.6	10.1	0.030
	Size × source	10	98.9	-175.0	4.8	0.043
	Density × size + source	11	96.7	-168.1	11.7	0.001
	Density × source + size	9	96.6	-173.0	6.7	0.016
	Size × source + density	11	99.1	-172.8	7.0	0.014
	Density × size + density × source	12	96.8	-165.5	14.3	<0.001
	Density × size + size × source	14	99.4	-165.2	14.6	<0.001
	Density × source + size × source	12	99.2	-170.4	9.4	0.004
	Density × size + density × source + size × source	15	99.5	-162.6	17.2	<0.001
	Density × size × source	18	103.0	-160.4	19.3	<0.001

Table S11 Pairwise comparisons of estimated marginal means of proportion flowering between plot sizes in A) 2021 and B) 2022.

Year	Plot size comparison	*estimate*	*d.f.*	*t*	*p*
A) 2021	4 m^2 – 1 m^2	0.44	80	1.7	0.33
	4 m^2 – 0.25 m^2	0.41	80	1.6	0.38
	4 m^2 – 0.0625 m^2	1.30	80	4.6	<0.01
	1 m^2 – 0.25 m^2	-0.02	80	-0.1	1.00
	1 m^2 – 0.0625 m^2	0.86	80	3.1	0.01
	0.25 m^2 – 0.0625 m^2	0.88	80	3.3	0.01
B) 2022	4 m^2 – 1 m^2	0.07	80	0.3	0.99
	4 m^2 – 0.25 m^2	0.14	80	0.6	0.92
	4 m^2 – 0.0625 m^2	0.70	80	3.0	0.02
	1 m^2 – 0.25 m^2	0.06	80	0.3	0.99
	1 m^2 – 0.0625 m^2	0.63	80	2.9	0.02
	0.25 m^2 – 0.0625 m^2	0.56	80	2.6	0.05

Table S12. Comparison of full and reduced linear mixed models explaining eelgrass sheath length within the transplant experiment in A) 2020, B) 2021, and C) 2022. Each model includes a random effect of plot. Bolded models represent the best models.

Year	Model	*d.f.*	Log likelihood	AIC	ΔAIC	Weight
A) 2020	**Null**	**3**	**-185.9**	**377.7**	**0.0**	**0.45**
	Plot density	4	-185.9	379.7	2.0	0.17
	Plot size	6	-183.4	378.9	1.2	0.25
	Plot density + plot size	7	-183.3	380.6	2.9	0.10
	Plot density × plot size	10	181.6	383.2	5.5	0.03
A) 2021	Null	3	-223.7	453.4	2.2	0.20
	Plot density	4	-223.7	455.3	4.2	0.18
	Plot size	**6**	**-219.6**	**451.2**	**0.0**	**0.32**
	Plot density + plot size	7	-219.0	552.0	0.8	0.12
	Plot density × plot size	10	-517.8	555.6	4.4	0.19
B) 2022	**Null**	**3**	**-256.3**	**518.7**	**0.9**	**0.20**
	Plot density	4	-255.5	518.9	1.2	0.18
	Plot size	6	-252.9	517.8	0.0	0.32
	Plot density + plot size	7	-252.9	519.8	2.0	0.12
	Plot density × plot size	10	-518.8	518.8	1.0	0.19

Table S13. Pairwise comparisons of estimated marginal means of sheath length between plot sizes in 2021.

Plot size comparison	*estimate*	*d.f.*	*t*	*p*
4 m^2 – 1 m^2	-0.64	84	-0.7	0.87
4 m^2 – 0.25 m^2	-0.11	84	-0.1	1.00
4 m^2 – 0.0625 m^2	1.46	84	1.8	0.29
1 m^2 – 0.25 m^2	0.52	84	0.7	0.90
1 m^2 – 0.0625 m^2	2.09	84	2.8	0.03
0.25 m^2 – 0.0625 m^2	1.57	84	2.1	0.16

Table S14. Analysis of deviance (type III Wald chi-square tests) testing for main and interactive effects of eelgrass (inside/outside) and time on A) silt content and B) organic content. Each model includes a random effect of sample within location.

Response		Model	*d.f.*	χ^2	*P*
A)	Silt	Eelgrass	1	24.5	<0.01
		Time	1	55.5	<0.01
		Eelgrass × Time	2	17.7	<0.01
B)	Organic content	Eelgrass	1	21.4	<0.01
		Time	1	21.3	<0.01
		Eelgrass × Time	2	13.4	<0.01

Table S15. Pairwise comparisons of estimated marginal means of silt content A) between inside and outside of 4 m^2 plots and B) at each sampling timepoint in the transplant experiment.

Treatment	Comparison	estimate	z-ratio	*P*
A) 4 m^2 plot*s*				
Inside	Sept 2020 – July 2021	-1.0	-5.2	<0.01
	– July 2022	-1.4	-7.5	<0.01
	July 2021 – July 2022	-4.0	-2.9	0.01
Outside	Sept 2020 – July 2021	-2.5	-6.8	<0.01
	– July 2022	-2.4	-6.5	<0.01
	July 2021 – July 2022	0.1	1.0	0.60
B) Time				
Sept 2020	Inside – Outside	0.9	2.3	0.02
July 2021	Inside – Outside	-0.7	-4.6	<0.01
July 2022	Inside – Outside	-0.1	-1.1	0.23

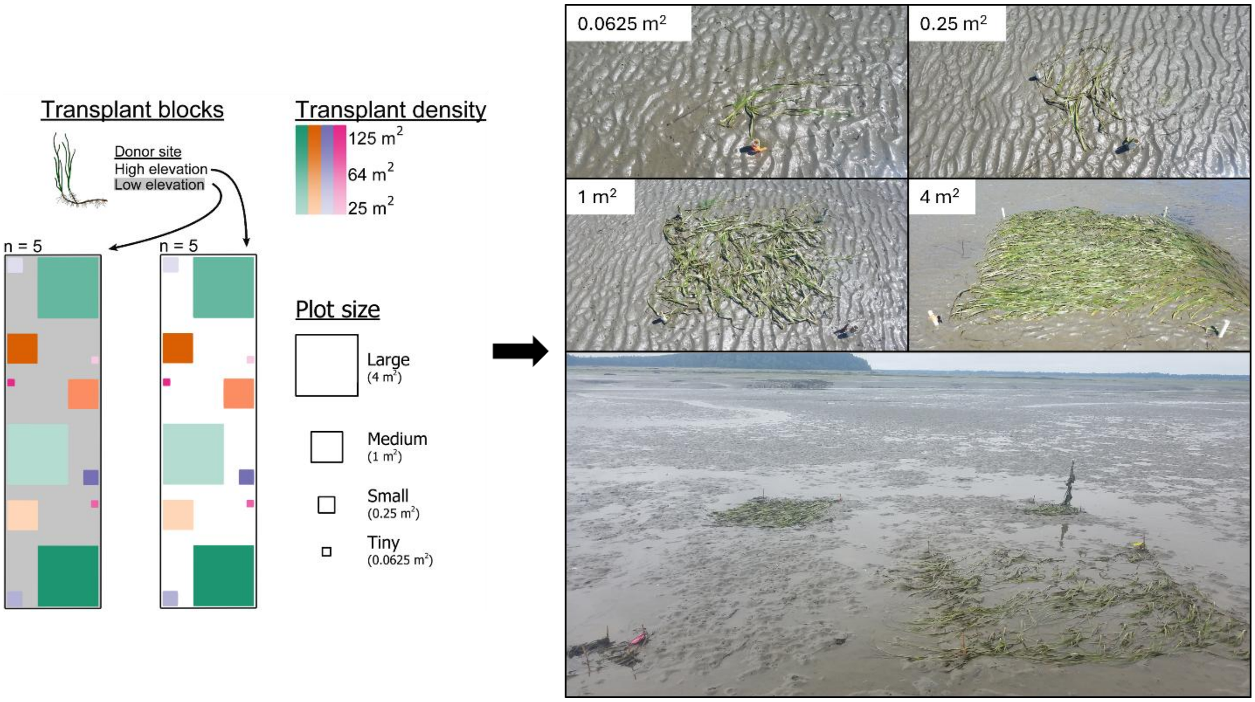

Figure S1. Illustration of eelgrass (*Zostera marina*) transplant plot design (left) and examples of transplant plots (right) on the Willapa Bay Long Island Oyster Reserve, Washington, USA.

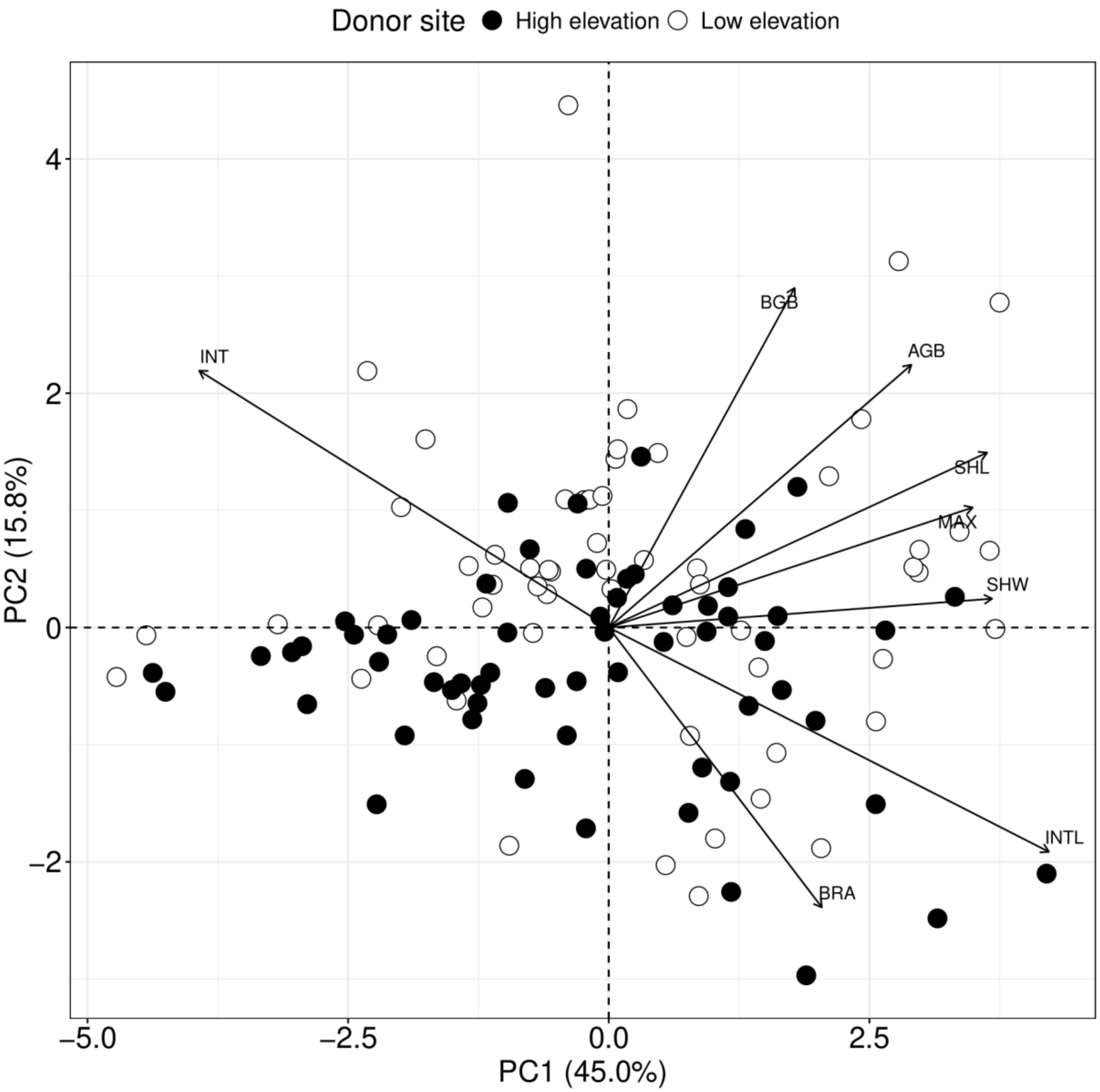

Figure S2. Principal components analysis ordination of eelgrass (*Zostera marina*) morphological traits between high and low elevation donor sites Willapa Bay, WA, USA. Above ground biomass = “AGB”, below ground biomass = “BGB, number of branches = “BRA”, internode count = “INT”, longest internode length = “INTL”, Maximum shoot length = “MAX”, sheath length = “SHL”, and sheath width = “SHW”.

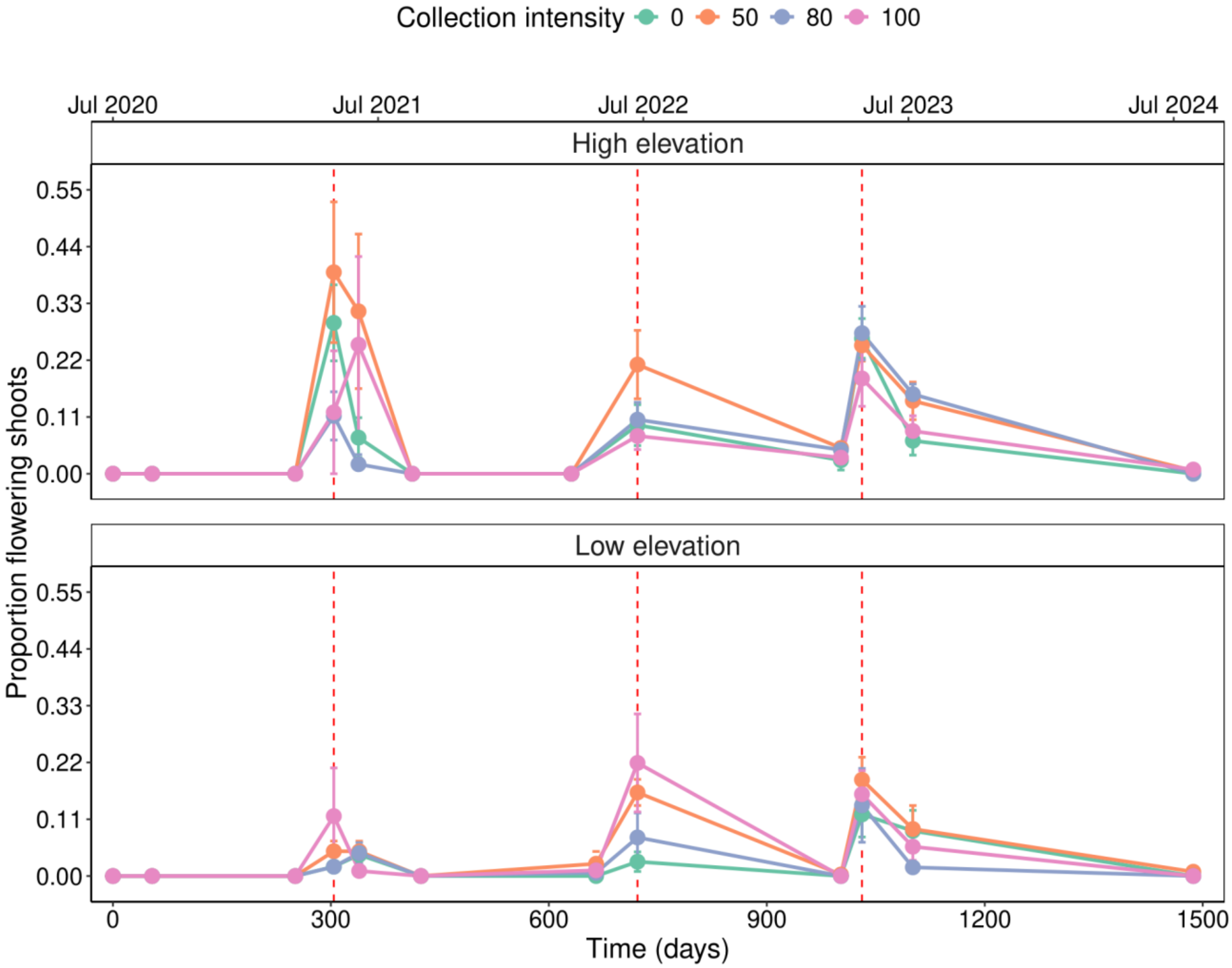

Figure S3. The average proportion of flowering shoots in plots with different shoot collection intensities for eelgrass (*Zostera marina*) at high and low elevation donor sites in Willapa Bay WA, USA. Error bars represent ± 1 standard error and red dashed lines represent analyzed timepoints. Some error bars are small and are hidden behind points.

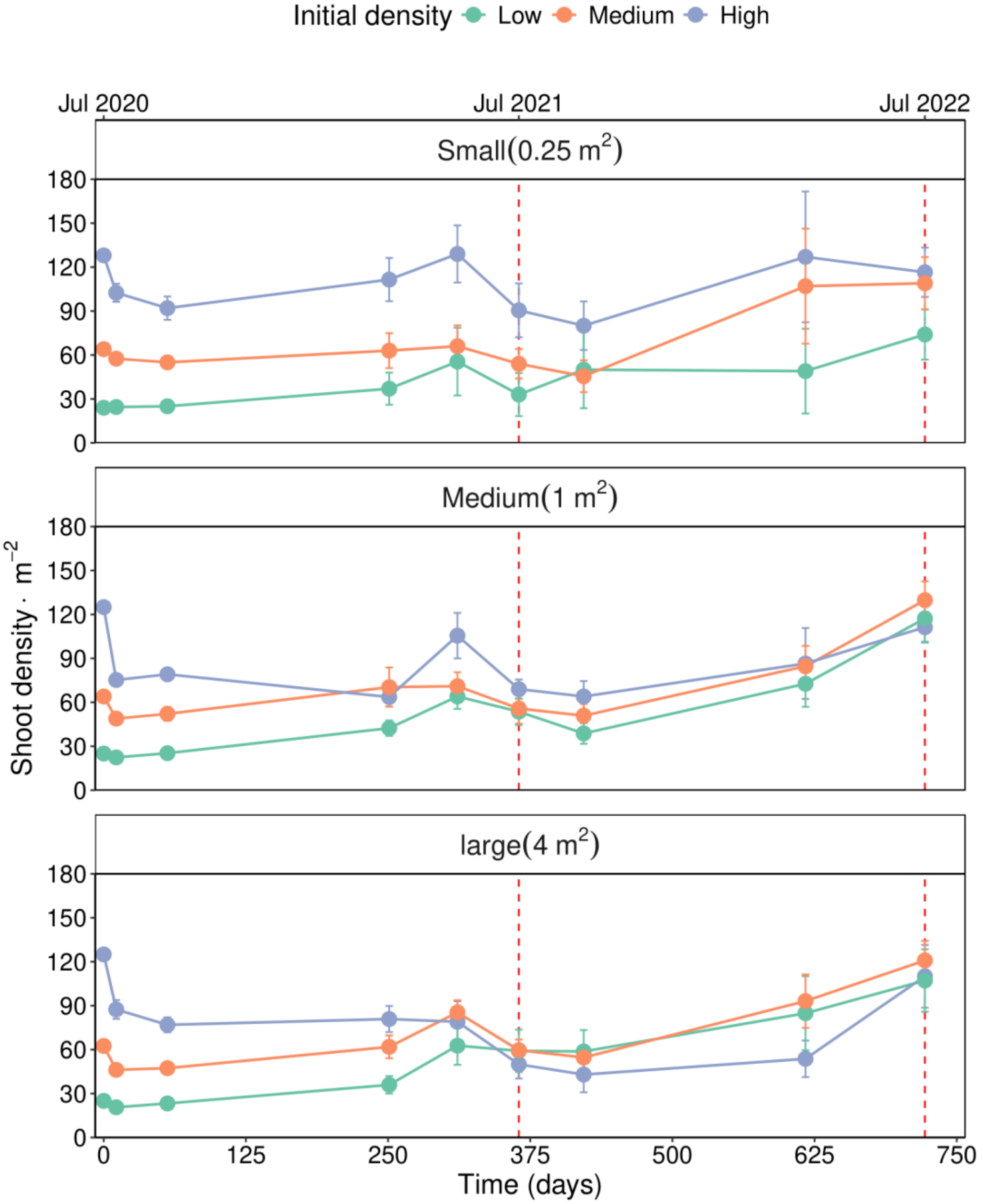

Figure S4. mean changes in eelgrass shoot densities within plot size and density treatment combinations over the course of the transplant experiment. Error bars represent ± 1 standard error and red dashed lines represent analyzed timepoints assessing eelgrass recovery. Some error bars are small and are hidden behind points.

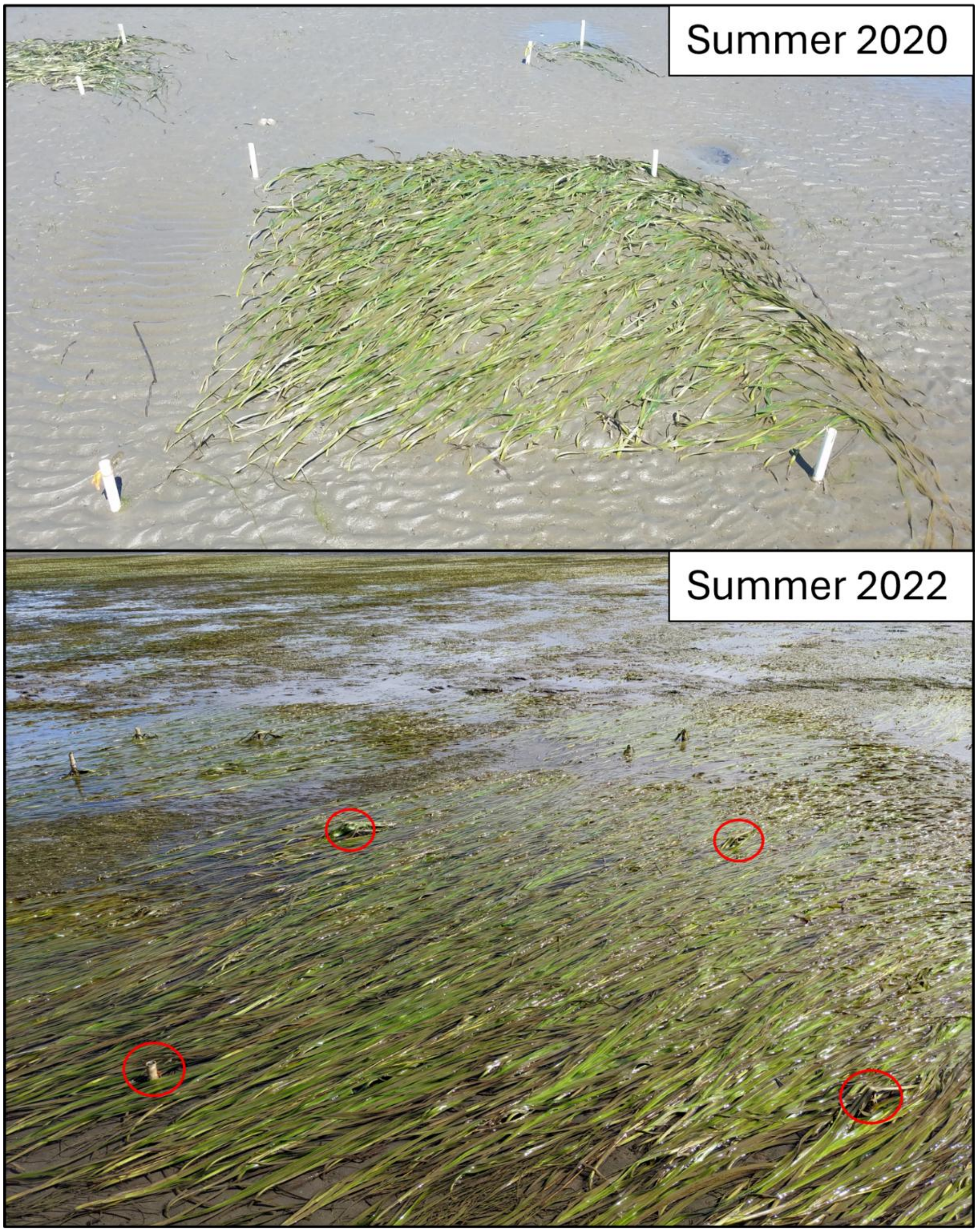

Figure S5. Example of transplant plot expansion from 2020 to 2022, Willapa Bay, WA, USA. By summer of 2022 most plots had expanded well beyond the bounds of their original area, merging with neighboring plots to form continuous plots of Eelgrass.

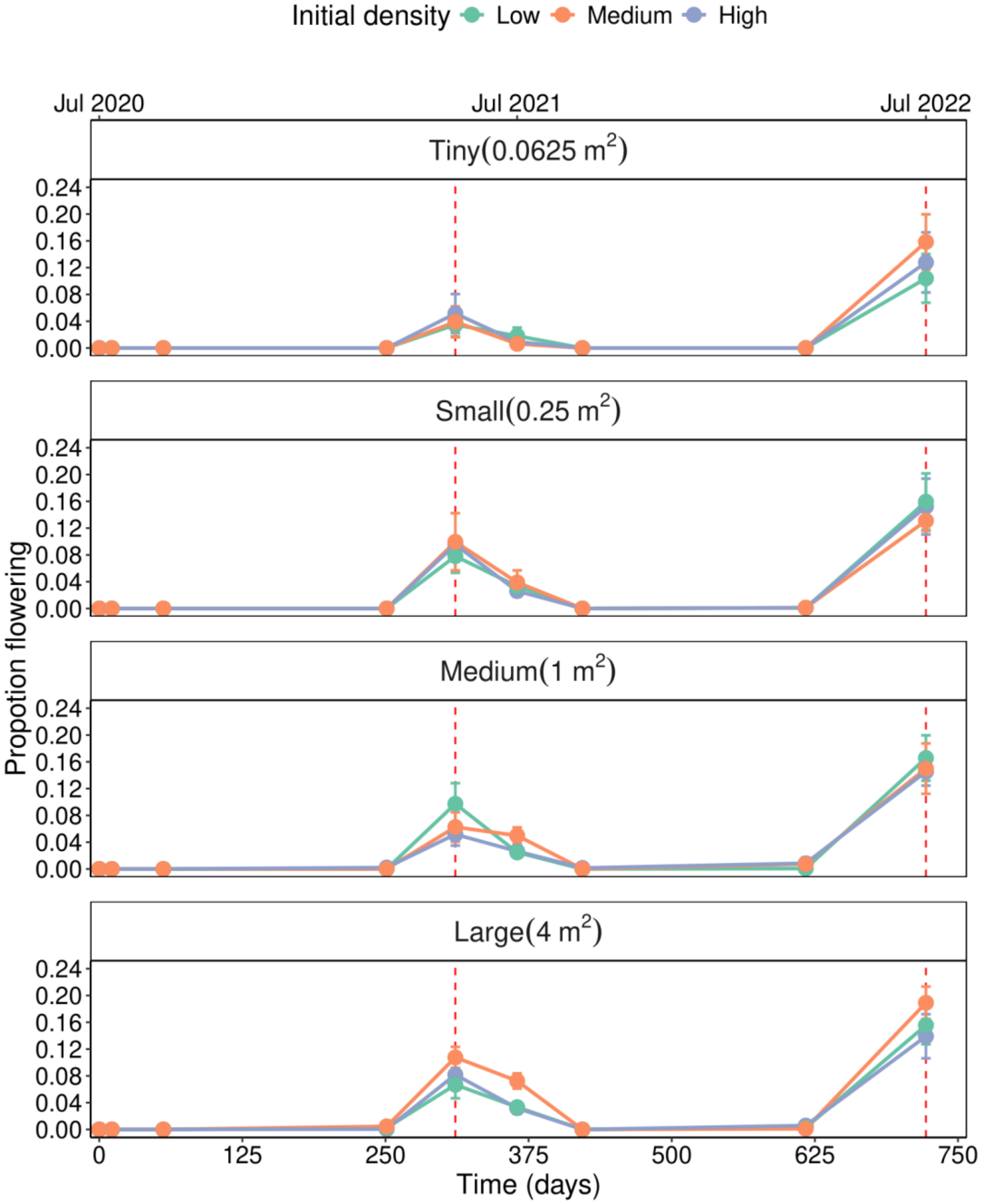

Figure S6. The average proportion of flowering shoots of eelgrass (*Zostera marina*) within transplant treatments in Willapa Bay WA, USA. Error bars represent ± 1 standard error and red dashed lines represent analyzed timepoints. Some error bars are small and are hidden behind points.

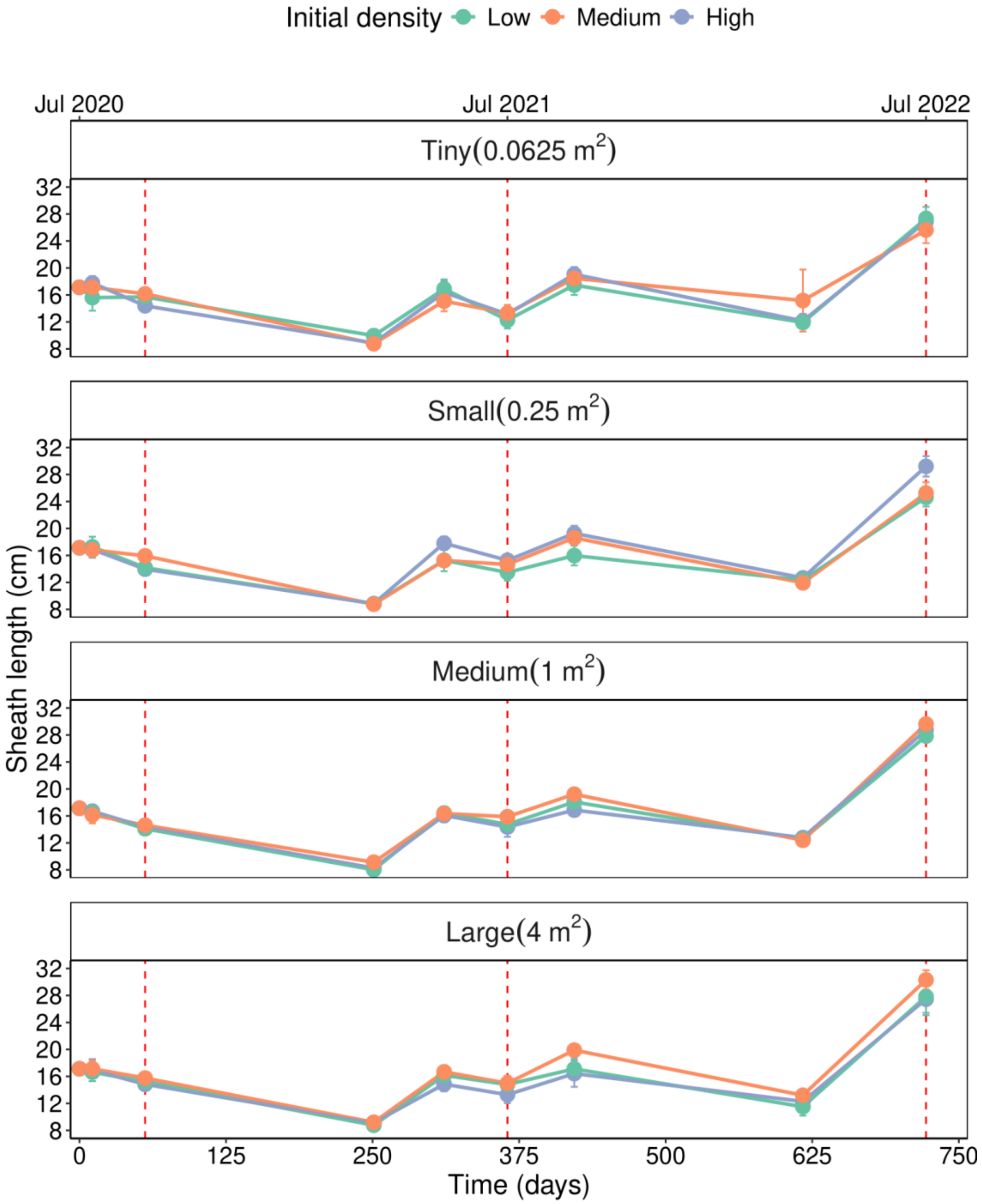

Figure S7. The average sheath length of shoots within transplant treatments plots in Willapa Bay WA, USA. Error bars represent ± 1 standard error. Some error bars are small and are hidden behind points. Red dashed lines represent analyzed timepoints.