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Eelgrass-Bivalve Interactions in a Changing Ocean

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Table of Contents

Chapter 2: Does Environmental Context Alter Bivalve and Snail Effects on Eelgrass Growth and Survival?.. 50

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Abstract

Foundation species consist of plants (e.g. seagrasses), algae (e.g. kelps) or animals (e.g. oysters, corals) that create habitat for a wide diversity of fauna. While foundation species support resident fauna, these residents also can impact foundation species, either positively by facilitation or negatively through competition or predation. Human-induced changes such as species introductions, warming, and eutrophication can alter these interactions. Using eelgrass (*Zostera marina*) as a study species, I explored how species introductions, warming, eutrophication, and habitat disturbance affected interactions between eelgrass and bivalves, which are common residents of eelgrass habitat.

My first chapter focused on the effects of a non-native seagrass species on Manila clams in Puget Sound, WA. Aquaculture of Manila clams is an important industry in Washington State. Non-native *Zostera japonica* has colonized mudflats in Puget Sound often used by Manila clam growers, which has created concerns about their effects on Manila clams and the industry they support. I analyzed a data set from a field experiment conducted by personnel from NOAA that tested for effects of habitat type (native eelgrass *Zostera marina*, non-native eelgrass *Zostera japonica*, and unvegetated mudflats) on Manila clam growth, survival, reproductive status, and condition. I found no evidence that *Zostera japonica* had negative effects on Manila clams; rather, differences in clam growth, survival, reproductive status, and condition were primarily driven by site-level differences such as temperature. My results do not indicate that Manila clam aquaculture will be negatively affected by *Zostera japonica* expansion, although my research did not address whether the process of Manila clam

harvesting may be affected by the transformation of mudflats into vegetated habitat by *Z. japonica*.

In Chapter 2 I focused on the effects of temperature and nutrients on clam-eelgrass interactions. Bivalves and eelgrass coexist in a variety of environmental conditions: while some studies find that bivalves have positive effects on eelgrass, by (e.g.) improving water clarity or depositing nutrients via pseudofeces, other studies have found negative effects due to competition for space or altered sediment chemistry via biodeposition. Eelgrass also coexists with a variety of crustacean and gastropod mesograzers, which benefit eelgrass by consuming epiphytic algae growing on its surface. I postulated that eelgrass-bivalve interactions may vary with environmental context; specifically, bivalves might be more likely to facilitate eelgrass under stressful conditions. I also postulated that mesograzer effects on eelgrass would be consistently positive across different conditions. My laboratory experiments indicated that clam effects on eelgrass growth or survival did not vary with temperature or nutrient treatments. However, I found that gastropod mesograzers may confer resilience to temperature stress for eelgrass, indicating that these epifauna may be important in maintaining eelgrass as temperatures rise due to climate change.

In chapter 3 I tested for joint effects of two types of disturbance on eelgrass infauna: habitat alteration by non-native Asian mussels, and small-scale eelgrass removal that commonly occurs from human use of estuaries. In shallow estuaries that support eelgrass, small-scale eelgrass removal via anchor scarring, propellor scarring and other physical mechanisms commonly co-occurs with the proliferation of space-occupying invasive species such as Asian mussels. I conducted a field experiment

testing the effects of eelgrass removal and addition of Asian mussels on eelgrass infauna, and hypothesized that when eelgrass was removed, Asian mussels would compensate for eelgrass loss by providing habitat for infauna, thereby sustaining a high diversity and abundance of organisms. I found limited effects of eelgrass disturbance on infauna, and overall higher infaunal abundance when Asian mussels were present. However, I did not find evidence that Asian mussels could compensate for eelgrass loss. Infauna may not have responded to disturbances and Asian mussels due to the regular occurrence of disturbances and high densities of mussels already present in heavily used estuaries like those in Southern California.

Overall, results of my three studies indicate that despite potentially strong effects of environmental factors such as temperature on eelgrass fauna, the native species that I studied exhibited resilience to species invasions and habitat alteration. The ecological roles of eelgrass fauna should be the focus of future research and considered in seagrass conservation and restoration efforts.

Introduction

Foundation species create habitat that reduces physical and biotic stressors, with positive effects on species diversity and abundance (Altieri and van de Koppel 2014; Bruno et al. 2003). In marine ecosystems, foundation species include canopy-forming kelps, seagrasses, reef-forming bivalves, corals, and mangroves. Foundation species can extend the range of other species, alter the physical environment, and modify species interactions (Altieri and van de Koppel 2014). For example, on rocky shores, ascidian beds extend the range of species usually found in the lower intertidal into the more stressful mid intertidal zone (Castilla et al. 2004). In salt marsh communities, cordgrass promotes growth of other plants by aerating substrate, stabilizing sediments, and reducing temperature and desiccation stress (Altieri et al. 2007).

While foundation species have strong effects on resident fauna, these animals can reciprocally facilitate foundation species success. For example, herbivorous fishes that use coral as shelter graze on competing algae growing on the surface of reef-building corals, thereby facilitating coral success (Hay 1991, Lirman 2001). When overfishing removes herbivorous fishes, algae overgrows corals and, in severe cases, can cause a phase shift from corals to macroalgae as the dominant space-holding organisms (Smith et al. 2010). Similarly, in seagrass beds, competitively dominant epiphytic algae can overgrow and smother seagrass (Sand-Jensen 1977). However, invertebrate mesograzers that benefit from habitat provided by seagrass consume epiphytes and prevent overgrowth, thereby facilitating seagrass persistence (Hughes et al. 2004, Whalen et al. 2013, Reynolds et al. 2014). Additionally, foundation species may be facilitated by co-occurring foundation species, known as secondary foundation species (Angelini et al. 2011, Thomsen et al. 2018). For example, bivalves living among seagrass beds can facilitate seagrass by enriching sediments with their pseudofeces and through increasing water column clarity via filter feeding (Reusch et al. 1994, Wall et al. 2008). Alternatively, foundation species may be harmed by the species they facilitate. For example, some mesograzers consume the seagrass itself in addition to epiphytic algae, and can cause seagrass decline (Zimmerman et al. 2001). Grazing by the introduced amphipod *Ampithoe valida* on seagrass led to widespread damage to San Francisco Bay seagrass beds (Reynolds et al. 2012).

Human-induced changes such as species introductions, climate change, and habitat loss can alter interactions between foundation species and their residents. For example, increased urchin herbivory in warmer waters can drive changes in kelp distribution (Franco et al. 2015). In seagrass systems, eutrophication can alter the interactions between seagrass, epiphytes and grazers by tipping the scales in favor of epiphyte growth, making grazing by epifauna crucial for seagrass survival (Reynolds et al. 2014). Thus, many human-induced changes affect ecosystems in ways that are hard to predict because they alter how species interact.

My study system to examine these questions was eelgrass (*Zostera marina*). Eelgrass is a species of seagrass which grows in shallow coastal waters throughout the Northern hemisphere. *Zostera marina* is a foundation species which supports a rich community of algae, invertebrates, fishes, birds, and marine mammals (Duffy 2006). Eelgrass beds are also considered nursery habitats due to the large number of organisms that inhabit them during early life stages (Duffy et al. 2014). Additionally, eelgrass acts as an ecosystem engineer, providing important ecological functions like sediment stabilization, carbon and contaminant sequestration, and nutrient cycling (Orth et al. 2006).

Eelgrass and other seagrasses coexist with a wide variety of bivalves, including mussels, clams, scallops and oysters. The sign and strength of eelgrass-bivalve interactions is highly context-dependent. For example, bivalves may facilitate eelgrass growth by filtering particles out of the water column, increasing water clarity and photosynthetic capacity for eelgrass (Wall et al. 2008). Additionally, bivalves may benefit seagrass through enriching the sediment with their pseudofeces (Reusch et al. 1994). Lucinid bivalves harbor sulfur-oxidizing bacteria in their gills, which allow them to alleviate sulphide stress for seagrasses when they co-exist (Chin et al. 2020). However, bivalves may also reduce seagrass growth and density by competing with seagrass directly for substrate space and, for some non-lucinid bivalves, by excreting toxic sulfides into the sediment (Reusch and Williams 1998; Vinther and Holmer 2008). Eelgrass effects on bivalves also vary among studies. Eelgrass can benefit bivalves by obscuring them from predators (Peterson and Heck 2001), or can harm bivalves by reducing water flow and delivery of food to bivalves (Allen and Williams 2003).

My dissertation focused on how eelgrass-bivalve interactions are affected by introduced species, temperature, eutrophication, and habitat loss. My first chapter focused on the effects of non-native eelgrass on Manila clams in Puget Sound. My second chapter focused on the effects of warming and eutrophication on interactions between clams and eelgrass. Finally, my third chapter examined whether interactions between eelgrass habitat loss and introduction of non-native, habitat-forming Asian mussels affect eelgrass infauna. Collectively, my results have implications for how eelgrass communities may respond to the growing influence of human-induced changes.

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Chapter 1

Limited Effects of Non-Native Japanese Eelgrass on Manila Clams in Puget Sound, WA

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Abstract

Non-native species have wide-ranging effects on local ecosystems, but their effects may be particularly strong when they are ecosystem engineers that alter habitat structure. Japanese eelgrass (*Zostera japonica*) is a non-native marine plant that has become established in Puget Sound (Washington, USA), where it coexists with native eelgrass (*Zostera marina*) but has replaced large swaths of mudflat habitat. Previous studies suggest that eelgrass (native or non-native) may have positive or negative effects on populations of the Manila clam (*Ruditapes philippinarum*), an important aquaculture species. However, previous studies have generally focused on examining the response of clams to *Z. japonica* removal, rather than quantifying differences in how clams fare between different habitat types. In this study we determined the effect of habitat type (native *Z. marina*, non-native *Z. japonica*, and mudflat) and site on Manila clam survival, growth, reproductive status, and condition. In the summers of 2011 and 2012 we outplanted Manila clams in each habitat type at each of three sites in Puget Sound and assessed them after three months, at which point we also measured bivalve recruitment to our experimental plots. Though habitat type did not influence Manila clam growth, reproductive status, or condition, the best-fitting model for survival included habitat, indicating lower survival in *Z. marina* than in either mudflats or *Z. japonica*. However, effects were relatively weak and varied in strength among our three sites in Puget Sound. We observed site-specific effects that were likely related to water temperature, as clam growth, survival and reproductive status were highest at Viewpoint Park where mean water temperature was highest. Bivalve community composition differed between *Z. japonica* and mudflat habitat, but both had significant overlap with *Z. marina*, which had lower overall species richness and diversity. Our results provide no evidence that *Z. japonica* has a consistent negative effect on clams. Instead, our data suggest that site-specific factors may be more important for clam populations than habitat type. This suggests that site selection is more important than habitat type when considering conditions for Manila clam aquaculture.

Keywords: aquaculture, eelgrass, invasive species, Manila clam, *Zostera*

Introduction

Ecosystem engineers are species that create biotic habitat for other organisms or modify abiotic habitat. By adding habitat structure to the seafloor, they are critical for maintaining marine biodiversity in coastal marine ecosystems such as coral reefs, kelp forests, and mangroves (Bell et al. 1991; Kovalenko et al. 2012). The fact that coral reefs cover less than 1% of the ocean floor but support 25% of marine biodiversity underscores the importance of structured biotic habitat in maintaining the populations of many other species (Plaisance et al. 2011; Sobha et al. 2023). Invasive species that are ecosystem engineers (Barbier et al. 2011) change the physical structure of marine habitats, which can alter species interactions, food webs, and the services that ecosystems provide. For example, invasive Asian mussels (*Arcuatula senhousia*) in southern California estuaries form dense mats, which replace local vegetation (Reusch and Williams 1998), alter bivalve recruitment (Castorani and Hovel 2015), and provide novel habitat in the interstitial spaces of their mats (Crooks and Khim 1999). Many marine plants and algae are ecosystem engineers and may have ecosystem-altering effects when they invade new habitats. For instance, in central California an invasive hybrid cordgrass (*Spartina alterniflora x S. foliosa*) has proliferated in tidal flats and has led to lower faunal species richness and abundance compared to uninvaded habitat (Neira et al. 2005).

Seagrasses are ecosystem engineers that occur along shallow coastlines of every continent except Antarctica (Orth et al. 2006). Seagrasses provide important habitat for fishes and invertebrates, and provide ecosystem services such as carbon sequestration, sediment stabilization, and reduction in storm surge (Orth et al. 2006).

Eelgrass (*Zostera marina*), one of about 60 seagrass species worldwide, forms extensive subtidal beds in shallow, temperate marine waters in the Northern Hemisphere (Hemminga & Duarte 2000), including Puget Sound (Washington, USA), where its geographical extent is used as a metric for evaluating the health of the sound (Washington State Department of Natural Resources). Habitat structure in this embayment and others from the Pacific Northwest to Humboldt Bay, CA, have been altered by the introduction of the non-native *Zostera japonica*, a species of eelgrass native to Japan that has expanded into previously unvegetated mudflats (Baldwin and Lovvorn 1994) and now covers thousands of hectares of shallow seafloor. *Zostera japonica* grows at higher tidal elevation than the native *Z. marina*, allowing the two habitat-forming species to co-occur. However, competition with *Z. japonica* has led to declines in *Z. marina,* and habitat disturbance tends to favor success of *Z. japonica* over *Z. marina* (Bando 2006). In this study, we addressed how alteration of mudflat habitat by the introduction of *Z. japonica* affects the growth, survival, reproductive status and condition of infaunal bivalves in Puget Sound.

Introduced species such as *Z. japonica* may have effects that are positive, negative, or neutral for native communities. Non-native species can lead to further invasions in a process known as an invasional meltdown (Simberloff and Von Holle 1999). In other cases, an introduced species may facilitate native species by providing habitat or forage (Rodriguez 2006) or have minimal interactions with native species. Effects may vary across taxa; for instance, an invader that is a primary producer may benefit some fauna by providing structured habitat (Dijkstra et al. 2017) or food (Cheng and Hovel 2010) while competing with local primary producers (Tsai et al. 2010). The addition of an introduced bryozoan (*Watersipora subtorquata*) led to increases in species richness and diversity of mobile invertebrates compared to unstructured habitat, mussel beds and ascidians (Sellheim et al. 2010). Because *Z. japonica* colonizes unvegetated mudflats, it might provide additional vegetated habitat for animals that use *Z. marina*, including waterfowl that normally consume native eelgrass (Baldwin and Lovvorn 1994). However, many shorebirds depend on unvegetated mudflats for foraging and may be negatively affected by conversion to *Z. japonica* beds (Page et al. 1999). Additionally, the addition of structure to mudflats occupied by infaunal filter-feeding bivalves may affect food availability (Allen and Williams 2003) and refuge potential for these animals.

Bivalves such as clams, mussels and oysters frequently co-occur with seagrasses, and studies have found both positive and negative effects of seagrasses on bivalves. Though reduced water flow in seagrass beds may decrease the amount of food that bivalves receive (Allen and Williams 2003; Fales et al. 2020), seagrass also may make bivalves less vulnerable to predators (Irlandi et al. 1995; Peterson and Heck 2001). In Puget Sound, the Manila clam (*Ruditapes philippinarum*) is an introduced species that is now widely distributed with naturally recruiting populations and is important for aquaculture (Anderson 1982; Ruesink et al. 2006). Manila clam aquaculture is a major industry in Washington, where yearly production is 4,082 metric tons (Saurel et al. 2014), with many farms located in Puget Sound. Bivalves such as Manila clams play an important ecological role as filter feeders that remove algae and other particles from the water column, which helps link benthic and pelagic food webs and ameliorate eutrophication (Peterson and Heck 2001). Aquaculture operations and

Z. japonica may be in conflict for space in Puget Sound, as intertidal mudflats are habitats commonly used for shellfish aquaculture operations (Beninger and Shumway 2018). How shifting habitat types affect clams is, therefore, of interest to aquaculture operations in Puget Sound.

We conducted a field experiment to test how introduced *Z. japonica* affects bivalves in Puget Sound, focusing primarily on the Manila clam due to its ecological and economic importance. Specifically, we tested how Manila clam growth, survival, reproductive status, and condition differed among three habitat types: native *Z. marina*, non-native *Z. japonica*, and mudflat. We hypothesized that Manila clams would fare best in mudflat habitat, where aquaculture operations are already successful, and worse in the vegetated non-native *Z. japonica* and native *Z. marina* habitats. We additionally measured settlement of native bivalve species in our experimental plots and hypothesized that bivalve assemblages would be less abundant and diverse in *Z. japonica* habitat than in the other habitat types.

Methods

Study sites

Puget Sound is an urbanized inland estuary in northwestern Washington, U.S.A., approximately 153 km in length and varying in width from 1.6 - 8 km. Puget Sound contains 23,000 hectares of subtidal and intertidal *Zostera marina* beds (Gaeckle et al. 2011). *Zostera japonica* has been found at more than 60 sites around Puget Sound since 2000 (Gaeckle et al. 2011). Our experiment took place at three sites in the South Central Basin of Puget Sound: Viewpoint Park, Tramp Harbor, and Dumas Bay (Figure

1.1). All three sites contain *Z. marina* and *Z. japonica* populations and have similar ranges of water temperature and salinity. The distance between sites was 7 - 10 km. All field experiments took place between August - November 2011 and July – November 2012.

Field experiment

In the summers of 2011 and 2012, we outplanted hatchery-raised Manila clams into native *Z. marina* beds, non-native *Z. japonica* beds, and mudflats at each of our three sites. Manila clams were donated by Taylor Shellfish (701 Broadspit Rd, Quilcene, WA 98376) and reared at the Quilcene National Fish Hatchery (Quilcene, WA). The mean initial length of outplanted clams was 13.9 mm. We held clams for up to a week in mesh bags in a flow-through seawater system before outplanting them for our experiment. Clams were marked uniquely with nail polish before outplanting so that they could be identified as individuals at collection (Supplemental Figure 1.5b). Before the experiment began, we measured the length, width, depth and weight of each clam. We deposited clams in individually-marked kitchen colanders, with 5 clams in each colander, and 5 colanders per habitat type at each site, for a total of 45 colanders and 225 clams in each year (Supplemental Figure 1.5a). The colanders were filled with sediment, and buried until the top of the colander was flush with the surrounding sediment. Clam growers generally plant clams directly into mudflats and cover with mesh screens (Zhang and Yan 2006), without colanders; however, some growers use bags or other containers. We positioned colanders radially around a central point in each habitat type. The distance between each colander and the central point varied between 1 m and 4 m. The colanders were covered with a mesh screen to protect clams from predators but still allow water flow. In the second year of the experiment, we implemented a second treatment within each habitat at each site to measure the effects of predation on clams in the different habitat types. This involved installing a second, uncovered colander adjacent to each covered colander, for a total of 10 colanders per habitat per site (*N*= 90 colanders and 450 clams total). In each year, we deployed nine HOBO loggers (one in each habitat at each site) for the duration of the experiment, which recorded temperatures every 20 minutes in 2011 and every 30 minutes in 2012.

We attempted to place colanders at approximately equal tidal elevation across all sites, but we also recorded the GPS coordinates at each habitat-site location to calculate tidal elevation. We used R packages 'raster', 'sp', and 'sf' to overlay the GPS points for each site/habitat/year combination on a topobathy raster obtained from NOAA's Digital Coast: Data Access Viewer tool. From this, we obtained a raster grid cell for each site/habitat/year combination and used these to compare the relative tidal elevation of all locations. The vertical resolution of this data was 22 cm. Tidal elevation ranged from -2.1 m to 0.7 m relative to MLLW (Supplemental Figure 1.1).

We characterized the surrounding environment at each site using transect surveys. In both 2011 and 2012, we laid a 30 m transect on the sediment and visually estimated percent cover of *Z. marina*, *Z. japonica*, macroalgae (primarily *Ulva* spp.), and unvegetated sediment in 0.25 m^2 quadrats at each meter mark. For quadrats that contained eelgrass, we counted shoots to quantify shoot density for *Z. marina* and *Z. japonica* (*Z. japonica* shoots were counted within 0.01 m^2 quadrats due to higher average shoot density of this species). We also recorded *Z. marina* and *Z. japonica* shoot heights and widths for three randomly chosen shoots of each species per quadrat (Hogrefe et al. 2014).

Statistical analyses

Our response variables were (i) individual growth rate, (ii) clam proportional survival, (iii) reproductive status, and (iv) clam condition. We quantified the growth rate of each clam by subtracting clam initial length from clam final length and dividing by days of exposure. Proportional survival was the proportion of clams that survived out of the original 5 in each colander. For reproductive status, we dissected the gonads of each clam and classified each clam as reproductively mature or not reproductively mature based on the appearance and mass of the gonads. We then used the proportion of reproductively mature clams per colander as a response variable in our analyses. Clam condition was quantified as the shell-free biomass of each clam at the end of the experiment divided by clam total biomass (Zeng and Yang 2020). At the end of the experiment, we recorded the number and size classes of unmarked, juvenile bivalves of any species that had recruited to the colanders during the experiment. In 2012, we also identified the recruited bivalves to the genus, or when possible, species level.

We performed all analyses in R studio, R version 4.2.2 (R Core Team 2023). Our predictor variables were year (2011 and 2012), habitat type (*Z. marina*, *Z. japonica*, mudflat), site, and interaction between habitat type and site. For the growth analysis, we included initial length of clams as a covariate to account for variation in growth rate due to clam size (Beal and Kraus 2002). Additionally, we conducted analyses including covariates (mean temperature, mean eelgrass shoot density, tidal elevation, and density of recruited bivalves) separately due to missing data in each of these variables for some sites and habitats. Mean eelgrass density was calculated for each site, and we excluded mudflats for analyses that included shoot density as a covariate. For growth rate and condition, we also included colander as a random effect to account for nonindependence between clams in the same colander. We removed uncaged colanders deployed in 2012 from analyses because almost all clams in uncaged colanders were eaten by predators. End-of-experiment data were missing for some clams: 11 clams in 2011 and 73 in 2012. We excluded these clams from final analyses.

We fit a series of linear mixed-effects models using the ‘nlme’ and ‘dredge’ packages in R. Candidate models including the habitat x site effect were constrained to also contain both main effects. We compared AIC values between models and reported on all models with dAIC <2.

For our growth and condition models, we fit a standard general linear model to the data (Gaussian errors, identity link). We visually inspected data for normality and linearity. Additionally, we repeated this process while allowing the variance in habitat and site to vary, to investigate whether random sources of variation were important. For our survival and reproductive status models, we converted data to a win/loss format, with one column each for alive and dead clams (or reproductively mature/immature), and each row a colander. We then fit a general linear model with a binomial distribution and logit link.

For our bivalve recruitment data, we used univariate and multivariate analyses to assess community composition and diversity across treatments. We quantified diversity using the Shannon Diversity Index. We performed nMDS and PERMANOVA analyses to examine the degree of similarity among communities in different treatments. We used a Bray-Curtis distance matrix to make the nMDS plot.

Results

Growth rate

The mean initial length of outplanted clams did not vary significantly between habitats or sites. The best model for clam growth rate included only site (Table 1.1). Mean growth rate was highest at Viewpoint Park and lowest at Dumas Bay (Table 1.2, Figure 1.2a). Growth rate increased with mean temperature ($p < 0.001$, $R^2 = 0.15$, Figure 1.3a, Supplemental Table 1.1). We found no effect of tidal elevation, number of recruited bivalves or shoot density on growth rate.

Survival

Mean proportional survival across all clams was 81% (± 2.1% SE). The best model for survival included habitat, site and year, but no interactive effect of habitat x site ($R^2 = 0.36$; Table 1.1). Survival was lowest in *Z. marina*, and did not vary significantly between *Z. japonica* and mudflats (Figure 1.2b). Survival was highest at Viewpoint Park, lower in Tramp Harbor, and lowest in Dumas Bay (Figure 1.2b). Survival also increased with mean temperature (Figure 1.3c, Supplemental Table 1.1) and tidal elevation (Supplemental Figure 1.1b, Supplemental Table 1.3), and decreased with eelgrass shoot density (Figure 1.4a, Supplemental Table 1.2). Survival increased with number of recruited bivalves (Supplemental Figure 1.2a, Supplemental Table 1.4).

Reproductive Status

The best model for reproductive status included site only (Table 1.1). The proportion of reproductively mature clams was significantly lower at Dumas Bay than at Viewpoint Park and Tramp Harbor ($p < 0.001$, Table 1.2, Figure 1.2c). Reproductive maturity was slightly negatively correlated with eelgrass density (Figure 1.4c) and in warmer water (Figure 1.3d, Supplemental Table 1.1). Neither the number of recruited bivalves nor tidal elevation affected reproductive status.

Condition

The best model for condition included only site ($R^2 = 0.530$, Table 1.1). Clam condition was significantly higher in Dumas Bay than in Viewpoint Park or Tramp Harbor ($p < 0.001$, Table 1.2, Figure 1.2d). We did not find an effect of mean temperature, tidal elevation, shoot density or number of recruited bivalves on clam condition.

Covariates

Temperature differed between sites, and mean temperature was significantly higher at Viewpoint Park than other sites ($p < 0.001$, Figure 1.3b). Mean temperature also varied among habitats. Mean temperature was highest in mudflats, lower in *Z. japonica*, and lowest in *Z. marina* (Figure 1.3b). In Dumas Bay and Tramp Harbor, *Z. marina* shoot density was lower and less variable than *Z. japonica* shoot density, but shoot densities were similar between the two species in Viewpoint Park. Mean tidal elevation was -0.30 m, and ranged from -2.12 to 0.72 m. Tidal elevation was highest in mudflats and lowest in *Zostera marina* and was higher in Tramp Harbor than the other sites (*$p = 0.013$*, Supplemental Figure 1.1a and c).

Bivalve recruitment

In 2011, 82 bivalves recruited to our colanders (we did not record species identities in 2011). Recruitment was ten-fold higher in 2012: 891 individual bivalves consisting of nine taxa settled in our colanders (*Mytilus sp., Macoma sp., Mya arenaria, Tellina modesta, Tresus sp., Clinocardium nuttallii, Saxodomus sp., Protothaca sp.,* and *Tellina nuculoides*). Diversity and species richness were slightly lower and more variable in *Z. marina* than in *Z. japonica* and mudflats, although this difference was not statistically significant (Supplemental Figure 1.4). In 2012, community composition was significantly different across habitats and sites (Supplemental Figure 1.3 both $p = 0.001$, $R^2 = 0.208$, PERMANOVA). Community composition was significantly different between mudflat and *Z. japonica* communities ($p = 0.003$), but both had major overlap with *Z. marina* community composition (Mudflat - *Z. marina:* $p = 0.075$; *Z. japonica - Z. marina:* $p = 0.261$; Supplemental Figure 1.3a). Communities also varied across sites; Dumas Bay and Tramp Harbor had very little overlap in community composition (Supplemental Figure 1.3b), but both had significant overlap with communities in Viewpoint Park.

The most commonly found species in our samples were *Macoma sp.*, *Tellina modesta,* and *Saxodomus sp. Macoma sp.* occurred most commonly in *Z. japonica* in Tramp Harbor and Dumas Bay, and both *Tellina modesta* and *Saxodomus sp.* occurred primarily in *Z. japonica* and mudflats. *Saxodomus* densities were higher in mudflats than in *Z. japonica* beds.

Discussion

In this study, we tested for effects of a non-native ecosystem engineer on the Manila clam, an important aquaculture species in Puget Sound, Washington, USA. Using a field experiment, we found no effect of habitat type on clam growth, reproductive status, or condition. Though we found lower clam survival (in the absence of predation) in native *Zostera marina* habitat than in mudflat and non-native *Z. japonica* habitat, effects were relatively mild and varied in strength among our three sites in Puget Sound. Clams were primarily impacted by site level effects, and clam growth, survival, and reproductive status were positively correlated with temperature, which varied among our three sites. Native bivalve communities differed between *Z. japonica* and mudflats, while communities in both habitats had significant overlap with *Z. marina* communities. Overall, our results indicate that non-native *Z. japonica* does not have a consistent negative effect on Manila clams compared to *Z. marina* and mudflats, and that site-specific factors may be stronger drivers of clam growth and reproduction than habitat type. This suggests that increases in non-native *Z. japonica* habitat in Puget Sound are likely to have small effects on Manila clam populations and aquaculture operations. However, differences among habitats were significant for the native bivalve community and seem to be stronger than effects on Manila clams. This may indicate that *Z. japonica* is changing local bivalve community composition.

Non-native ecosystem engineers alter many marine ecosystems, and in the process may change the native communities supported by those systems. A notable example is the invasion of hybrid cordgrass (*Spartina alterniflora x S. foliosa*) into U.S. West coast estuaries (Neira et al. 2005). Cordgrass has changed benthic communities by shading sediment, reducing water flow, and occupying belowground habitat via rhizomes. These changes led to lower densities of surface-feeders and increases in detritivore density. Changes to communities tend to be more extreme when unvegetated mudflats are invaded compared with vegetated habitats (Grosholz et al. 2009). For example, proliferation of hybrid *Spartina* and associated attenuation of water flow led to a significant decrease in the recruitment of barnacle *Balanus glandula* to mussel shells compared to mudflats (Neira et al. 2006), and to a decrease in growth of clam *Macoma petalum* (Brusati and Grosholz 2007; Grosholz et al. 2009). A similar phenomenon occurs in estuaries invaded by the Asian mussel *Arcuatula senhousia*. Asian mussels have become common in Southern California estuaries, where they form dense mats of thousands of individuals. These mats alter sediment deposition and water flow, affecting the communities of organisms that live in these areas. Increases in Asian mussel populations have been associated with decreases in native bivalves with which they compete for space (Crooks 2001) and increases in species that can live among mussel mats, like polychaetes (Crooks and Khim 1999). In the Caribbean Sea, invasive eelgrass *Halophila stipulacea* is replacing native seagrass *Syringodium filiforme*. *Halophila stipulacea* is associated with larger fish and more epibionts than *S. filiforme* but fewer juvenile fish, potentially threatening the function of local seagrass beds as nursery habitats (Willette and Ambrose 2012).

Mudflats are highly susceptible to alteration by invasive species compared to other habitats. Mudflats lack aboveground structure and are often located in sheltered bays (Byers and Grabowski 2014), which are likely to have a higher volume of boat traffic introducing species and high levels of anthropogenic disturbance from pollution and development. These habitats are also used extensively for aquaculture of bivalves (Beninger and Shumway 2018), so the conversion of habitat from mudflat to vegetated habitat is of concern to clam growers. Seagrasses have extensive belowground rhizome systems, which may exclude invertebrates that burrow into the sediment and need a clear path to the water column for respiration, such as burrowing shrimp (Harrison 1987, Castorani et al. 2014). Seagrasses also change the sediment structure and composition, which can lead to changes in faunal communities compared to mudflats (Orth et al. 1984). Finally, many shorebirds rely on mudflats for foraging, and conversion to seagrass may lead to a loss of these foraging grounds for many shorebirds (Page et al. 1999).

It is surprising that outcomes for clams in our experiment were not different in the mudflat habitat compared to vegetated habitats. Impacts of vegetation on habitat quality for benthic invertebrates are well documented; for example, vegetative structure may provide protection from visual predators (Peterson and Heck 2001). However, we caged colanders to prevent predation from affecting results. If this is the main way that bivalves experience habitats differently, then our experimental design may explain the result. In 2012, we left half of the colanders uncovered to test predation, and almost all clams either died or disappeared, and were likely consumed by predators, demonstrating that predation pressure at our study sites is strong. Unfortunately, we were not able to assess whether predation differed among habitat types, since almost all clams across all habitat types had been eliminated by the end of the experiment.

Our results contrast with other studies focusing on interactions between *Z. japonica* and Manila clams (Tsai et al. 2010, Patten 2014). Tsai et al. 2010 found improved clam condition when *Z. japonica* was removed, but, similar to our study, found no effect on clam growth. Patten (2014) found that clam growth increased with the removal of *Z. japonica*. Their experiments involved quantifying how clams responded to the experimental removal of *Z. japonica* from mudflats, which may have contributed to differences in results compared to our study. Our study involved planting clams in *Z. japonica* and other habitats, and comparing how clams fared in each habitat, and did not involve manipulating *Z. japonica* presence or density. Additionally, both studies took place at a higher tidal elevation than ours. They found that *Z. japonica* retains water during low tide, potentially extending the period clams spend submerged. Given this, it is surprising that Tsai et al. found negative effects of *Z. japonica* on clams. Clams may have fared worse in general at these stressful, high tidal elevations compared to our experiment.

Zostera japonica in Washington has been treated at various times as a pest and a protected species. Before 2010, *Z. japonica* was a protected species in Washington, because it was categorized with the native *Z. marina,* which has been in decline and which is difficult to distinguish visually from *Z. japonica* (Mach et al. 2014). However, growing concerns about the effects of *Z. japonica* on shellfish aquaculture prompted a new approach, and it was then classified as a noxious weed (Washington State Noxious Control Weed Board 2012). Few studies have tested the effects of *Z. japonica* on native species, and more research is needed, particularly regarding the potential effects on shellfish aquaculture (Mach et al. 2014). In spite of the classification of *Z. japonica* as a nuisance species and impediment to clam aquaculture in Washington, our results do not indicate a consistent negative effect of this invasive ecosystem engineer on Manila clams. Additionally, studies have suggested that Manila clam aquaculture can be successful in *Z. japonica* beds in Korea (Park et al. 2011) and in *Z. marina* beds in China (Qin et al. 2016).

Zostera japonica and *Z. marina* are similar in aboveground appearance to the point that it can be difficult to visually differentiate between them in the field, which may explain why we did not observe strong differences in their effects on Manila clams. Since these congeneric seagrasses are structurally similar, the growth of *Z. japonica* may provide a habitat subsidy supplementing existing *Z. marina*, albeit at the expense of mudflat habitat. However, some differences in structure among *Z. marina* and *Z. japonica* may lead to differences in habitat quality. *Zostera marina* tends to have longer and wider leaves, while *Z. japonica* tends to grow in higher densities (Arasaki 1950, Sugimoto et al. 2017). These morphological differences may affect delivery of planktonic food to clams, as denser eelgrass slows water flow, potentially changing rate of food delivery to clams in the denser *Z. japonica* (Lacy and Wyllie-Echeverria 2011). Studies differ on whether slower currents would lead to less or more food delivery; while Lacy and Wyllie-Echeverria 2011 suggest that slower water will lead to more deposition of food particles for filter feeders, Allen and Williams 2003 found that reduction of water flow by eelgrass limited food delivery to bivalves.

In our study, trends in clam condition were opposite of those for growth rate, survival and reproductive status. Condition was higher in Dumas Bay than at other sites, which was untrue for other response variables. Condition patterns differing from survival may be a result of selection for the most robust individuals. In places with high mortality rates but also high condition, like Dumas Bay, clams that survived may have been healthier and more robust than those which died, and have a higher condition (Figure 1.3b and d).

Interestingly, density of recruited bivalves did not affect growth rate, reproduction or condition of our outplanted clams and was slightly correlated with survival. This indicates that competition was not strong enough to affect outplanted clams. This is surprising given that recruits settled at relatively high densities in some colanders (up to 50 bivalves per colander). The newly settled recruits may have been too small compared to the outplanted Manila clams to exert competitive effects.

Site-level differences

Site-level differences were evident across all response variables. These differences may have been due to temperature variation. Viewpoint Park had both the highest mean temperature of 13.6 °C and the highest clam growth rate, survival and reproductive status. Clam growth rate, survival and reproductive status increased with mean temperature. Our results indicate that even small changes of temperature, on the order of 0.5 °C, may affect biological processes. Previous studies found that Manila clam meat growth was maximal at 12 °C but was reduced at temperatures equal to and higher than 14 °C (Mann 1979), but another study found no differences in shell growth (Mann and Glomb 1978). However, other studies have found that the optimum temperature is 20 °C based on clam metabolism and energy budget (Han et al. 2008). Given these mixed results, it may be that other factors occurring in the field interact with temperature to influence growth rate and other response variables. For example, Dumas Bay, which had lower outcomes for clam growth, survival and reproductive status, is also the most urbanized site. While Viewpoint Park and Tramp Harbor are both located on the less-developed Vashon Island in Puget Sound, Dumas Bay is located in the highly developed corridor between Seattle, WA and Tacoma, WA. Long 1982 found that the second highest level of PCB pollution in the Puget Sound region occurred in Commencement Bay, which is adjacent to Dumas Bay.

Shoot density varied among sites. Shoot density was highest at Dumas Bay and lowest at Viewpoint Park (Figure 1.4b). Survival and reproductive status were negatively correlated with shoot density (Figure 1.4). Growth, survival and reproductive status were lowest in Dumas Bay and highest in Viewpoint Park, indicating that shoot density may have been partly responsible for site-level effects. Previous studies found that dense eelgrass slows water flow, leading to less food delivery to bivalves and therefore lower growth rates (Allen and Williams 2003).

Clam survival was slightly correlated with tidal elevation (Supplemental Figure 1.1). Tidal elevation affects how often intertidal clams are submerged. Clams at lower elevations spend more time underwater with access to planktonic food and less time enduring desiccation stress, and are therefore more likely to grow and survive. Thus, it is surprising that clams should have higher survival at a higher tidal elevation. However, plots in mudflats tended to be at a higher elevation compared to vegetated plots, so it is difficult to determine whether this difference was due to elevation or habitat type.

Bivalve recruitment

The bivalves that recruited to our colanders indicated different communities in *Z. japonica* as compared to mudflat habitat. Both communities had major overlap with *Z. marina* communities. Surprisingly, non-native *Z. japonica* habitat had the highest diversity among the three habitat types. Despite the differences between *Z. japonica* and mudflat communities, one of the most abundant species in our samples, *Tellina modesta,* was common in both *Z. japonica* and mudflats but not in *Z marina*. This may indicate that bivalve species that live in local mudflats can adapt to living in expanding non-native *Z. japonica* beds. *Zostera japonica* may have the highest diversity of habitats due to creating conditions in which bivalves adapted both to mudflats and to subtidal *Z. marina* can survive. Additionally, many bivalves are long lived, with potential lifespans of 10 years or more. Mudflat-adapted bivalves may have persisted through the transition to *Z. japonica* habitat.

Macoma sp. were found in high densities in *Z. japonica* but not in mudflats or *Z. marina*. *Macoma* sp. are commonly found in both mudflats and eelgrass beds (Hiebert 2015a), and may be an example of a generalist that will not be negatively affected by *Z. japonica* intrusion. However, *Saxidomus sp.* are commonly associated with sandy intertidal flats (Hiebert 2015b), and we found that they were less common in *Z. japonica* beds than in mudflats. *Zostera japonica* may lead to decreases in *Saxidomus gigantea,* (butter clams), which form an important recreational fishery in the Pacific Northwest (Hiebert 2015b), and are classified by Washington Department of Fish and Wildlife as a Priority Species (WDFW 2024).

Zostera japonica has been a focus for clam growers who are concerned it will affect their harvests. Our study used methods like those used in aquaculture operations, which involve growing clams in mudflats using plots covered with mesh to exclude predators. However, we used colanders instead of plots without containers, which may have affected the applicability of our results for aquaculture operations. Additionally, while our results do not indicate that *Z. japonica* will negatively affect clams, we did not directly test how *Z. japonica* would affect the act of harvesting. *Zostera japonica* may make it more difficult for clam growers to collect clams compared to a mudflat, due to the vegetation obscuring clams and tangling equipment. Growers may also be concerned about regulatory obstacles, as in the past *Z. japonica* was grouped with *Z. marina* as protected species (Mach et al. 2014). Future research should consider how *Z. japonica* expansion may affect clam harvesting techniques or the regulatory environment.

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Tables and Figures

Table 1.1. Top models for each response variable: growth rate, survival, reproductive status and condition with df, AIC_c, ∆AIC, model weights, adj R^2. Models shown are best models or ∆AIC < 2.

Response variable	Model	df	AIC_c	∆AIC	Model weight	R^2
Growth rate	Site only	13	-1518.9	0	0.96	0.26
Survival	Habitat, site and interaction	9	232.43	0	0.35	0.62
	Full model	10	233.06	0.63	0.25	0.63
	Habitat and Site	5	233.39	0.96	0.21	0.57
	Habitat, site and year	6	233.94	1.50	0.16	0.58
Reproductive status	Site and year	4	182.39	0	0.86	0.62
Condition	Site only	7	-577.98	0	0.85	0.50

Table 1.2. Summary results from linear mixed-effects models. The table includes the best model for each response variable (growth rate, survival, reproductive status and condition). ‘Zj’ = *Zostera japonica*, and ‘Zm’ = *Zostera marina*.

Response variable	Parameter	Estimates	SE	z-value	p-value
Growth rate	Intercept	0.043	0.003	14.6	0.000
	Site.TrampHarbor	0.0068	0.003	1.98	0.049
	Site.ViewpointPark	0.020	0.004	5.64	0.000
Survival	Intercept	-0.13	0.37	-0.36	0.72
	Habitat.Zj	0.24	0.45	0.53	0.59
	Habitat.Zm	-0.27	0.50	-0.54	0.59
	Site.TrampHarbor	1.52	0.51	2.99	**0.003**
	Site.ViewpointPark	3.20	0.81	3.95	**<0.001**
	Habitat.Zj:Site.TrampHarbor	0.19	0.71	0.26	0.79
	Habitat.Zm:Site.TrampHarbor	-1.11	0.69	-1.61	0.11
	Habitat.Zj:Site.ViewpointPark	-1.65	0.94	-1.76	0.08
	Habitat.Zm:Site.ViewpointPark	-0.72	1.00	-0.72	0.47
Reproductive Status	Intercept	1918.12	701.9	2.73	0.01
	Site.TrampHarbor	2.48	0.75	3.30	**0.001**
	Site.ViewpointPark	3.30	0.75	4.42	**<0.001**
	Year	-0.96	0.35	-2.74	**0.01**
Condition	Intercept	0.43	0.01	34.26	<0.001
	Site.TrampHarbor	-0.13	0.02	-8.20	<0.001
	Site.ViewpointPark	-0.12	0.02	-7.40	<0.001

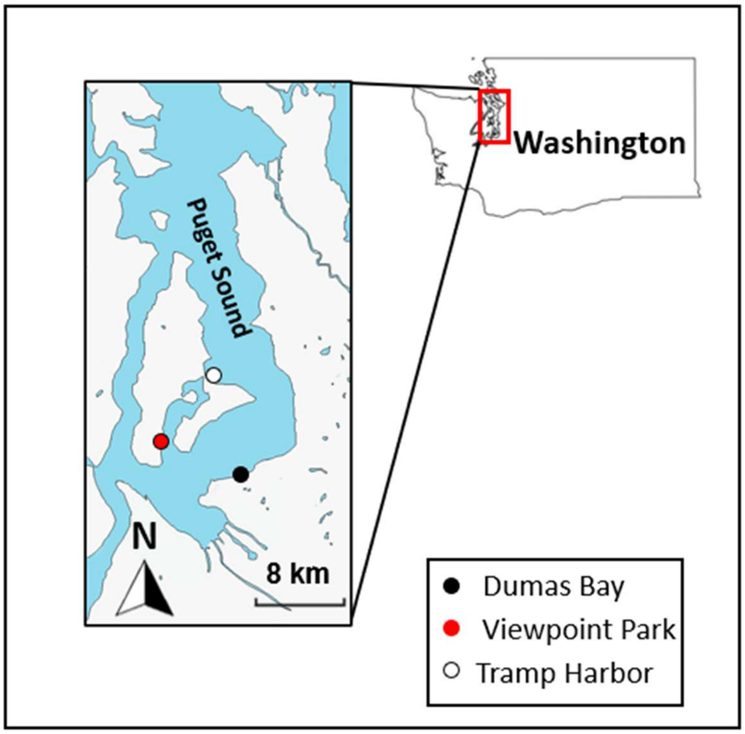


Figure 1.1. Map of study sites in Puget Sound, Washington, USA.

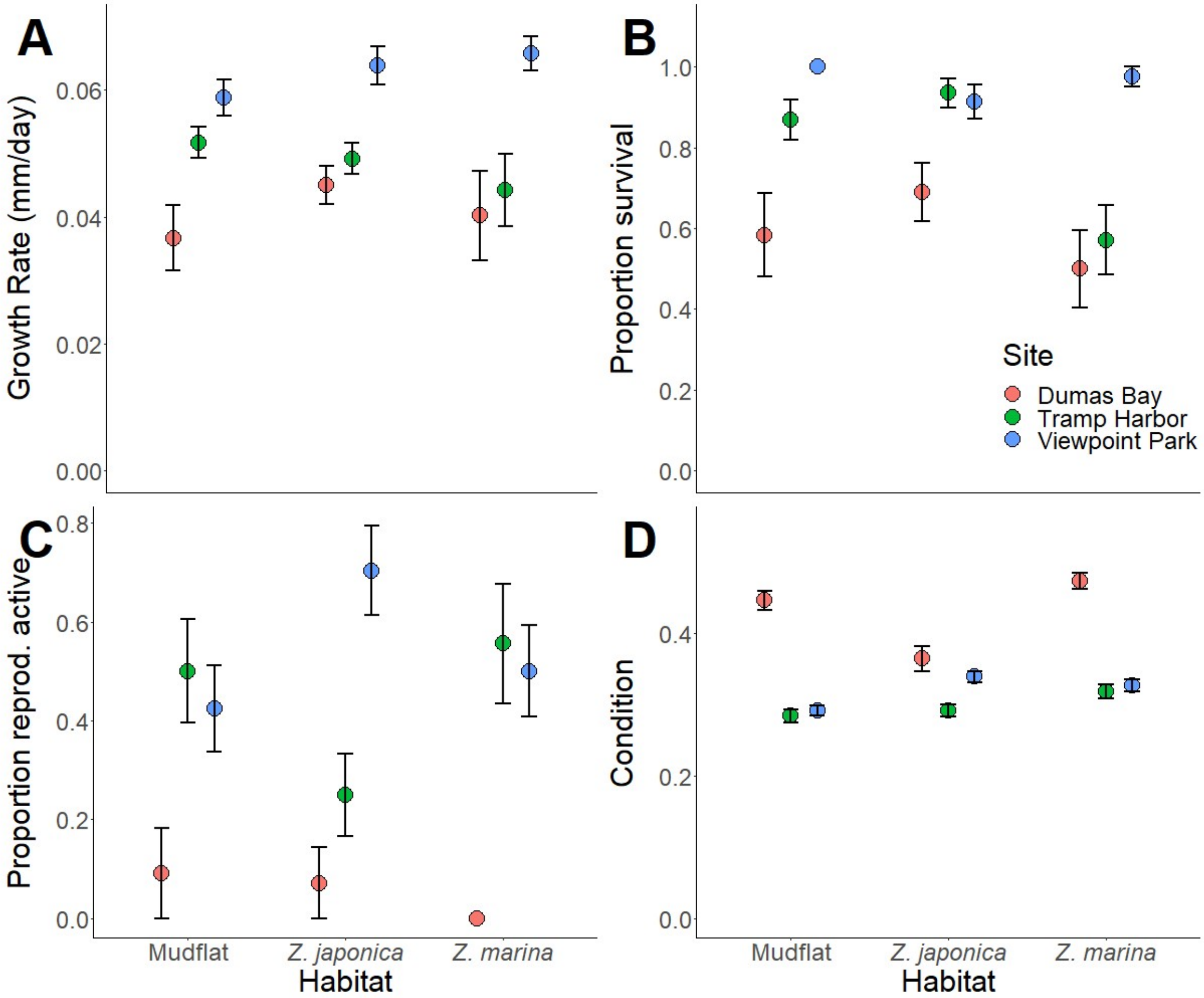


Figure 1.2. Clam response variables by habitat type and site. Data from 2011 and 2012 are pooled. Points represent mean value for each habitat and site, and are colored by site. Error bars represent +/- SE. **A.** Growth rate (mm/day), **B.** Proportion survival, **C.** Proportion reproductively mature, **D.** Condition.

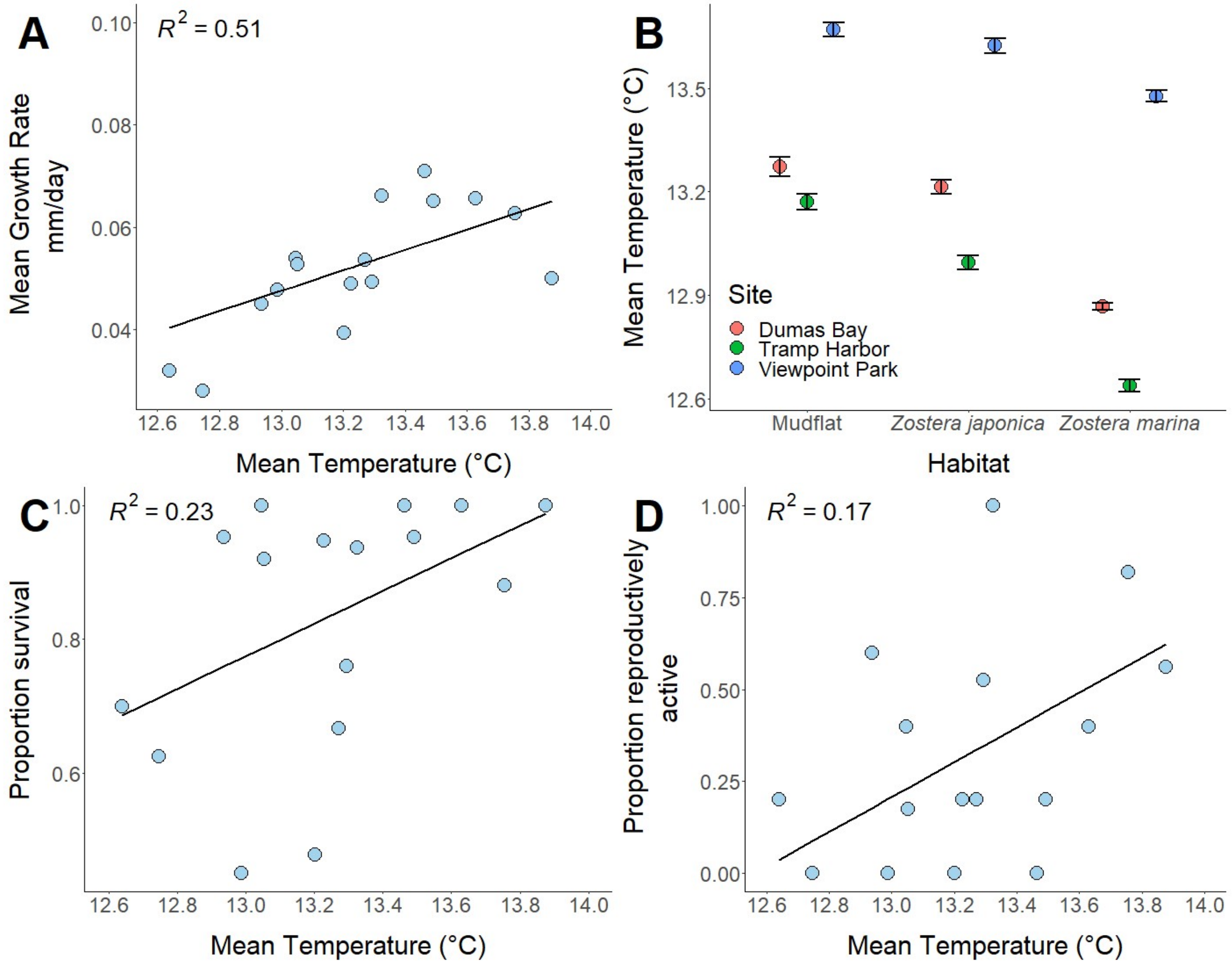


Figure 1.3. Temperature as it relates to site, habitat, growth rate, survival and reproductive status. Condition was not included because there was no interaction between temperature and condition. **A.** Mean temperature (°C) vs. growth rate (mm/day). Blue points are actual data, and the black line represents the modeled relationship between the two variables. **B.** Mean temperature (°C) by site and habitat. Points are colored by site and represent means, and error bars are +/- SE. **C.** Mean temperature (°C) vs survival. **D.** Mean temperature (°C) vs. reproductive status.

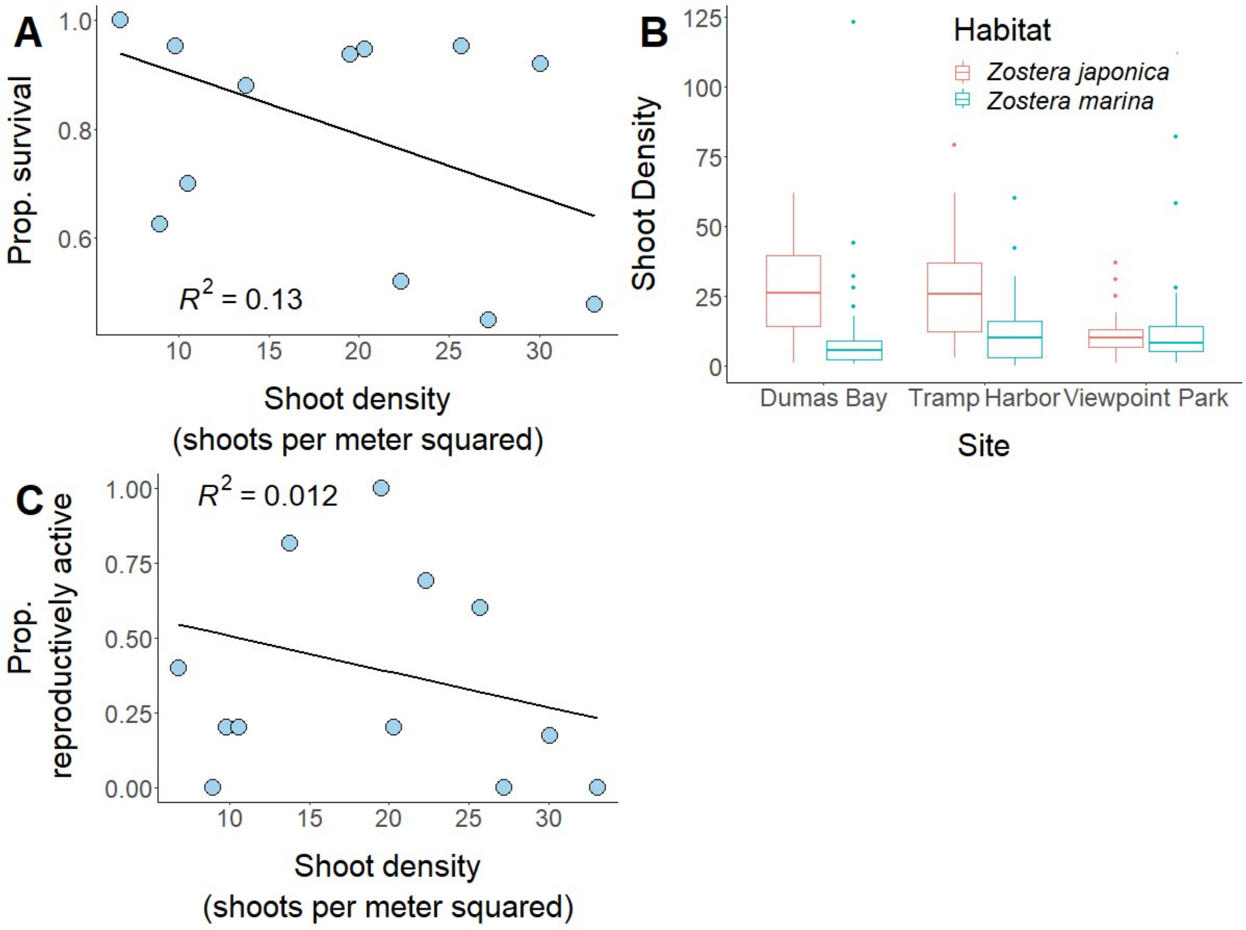


Figure 1.4. Mean shoot density as it relates to habitat, site, survival and reproductive status. Other response variables were not included because there was not a correlation between them and shoot density. **A.** Mean shoot density vs survival **B.** Mean shoot density vs. habitat and site. **C.** Mean shoot density vs reproductive status.

Supplemental Materials

Supplemental Table 1.1. Top models for analyses including mean temperature as a predictor with df, AIC$_c$, ∆AIC, model weights, R^2.

Response variable	Model	df	AIC$_c$	∆AIC	Model weight	R^2
Growth rate	Site only	13	-1404	0	0.36	0.24
	MeanTemp only	12	-1403.8	0.1	0.34	0.21
	Intercept only	11	-1402.8	1.16	0.20	0.17
Survival	MeanTemp and site	4	199.73	0	0.57	0.61
Reproductive status	Habitat, site, MeanTemp and year	7	158.54	0	0.47	0.73
	MeanTemp and site	4	159.46	0.92	0.30	0.67
Condition	Site only	7	-545	0	0.92	0.55

Supplemental Table 1.2. Top models for analyses including mean shoot density as a predictor with df, AICc, ∆AIC, model weights, R^2.

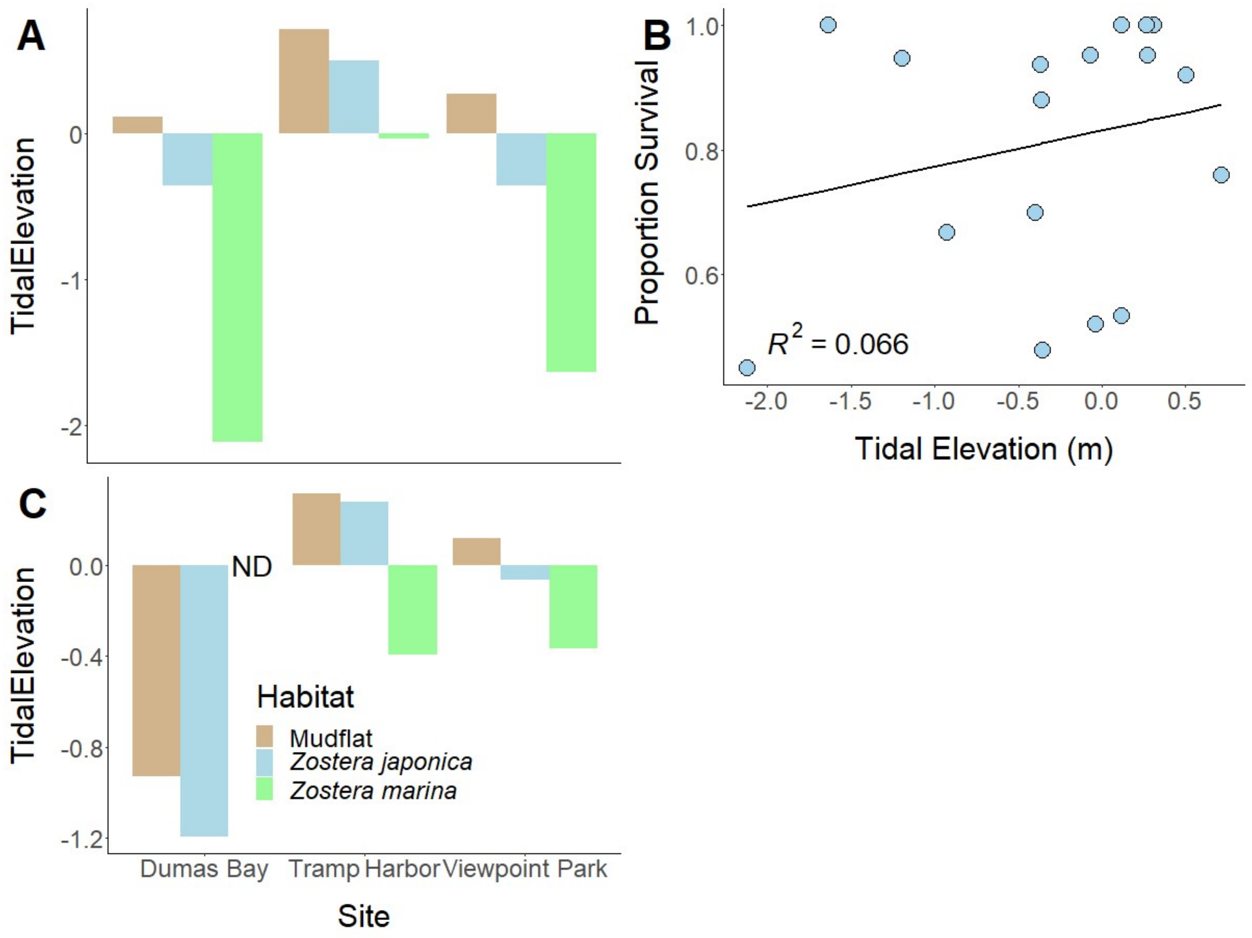
Response variable	Model	df	AIC_c	∆AIC	Model weight	R^2
Growth rate	Site only	10	-983	0	0.92	0.30
Survival	Full model	8	161.07	0	0.62	0.64
Reproductive status	Site and year	4	122.50	0	0.26	0.66
	Site only	3	122.52	0.02	0.25	0.64
	Site and shoot density	4	124.02	1.53	0.12	0.65
	Site, year and shoot density	5	124.37	1.87	0.10	0.67
Condition	Site only	5	-393	0	0.70	0.56

Supplemental Table 1.3. Top models for analyses including tidal elevation as a predictor with df, AICc, ∆AIC, model weights, *R^2*.

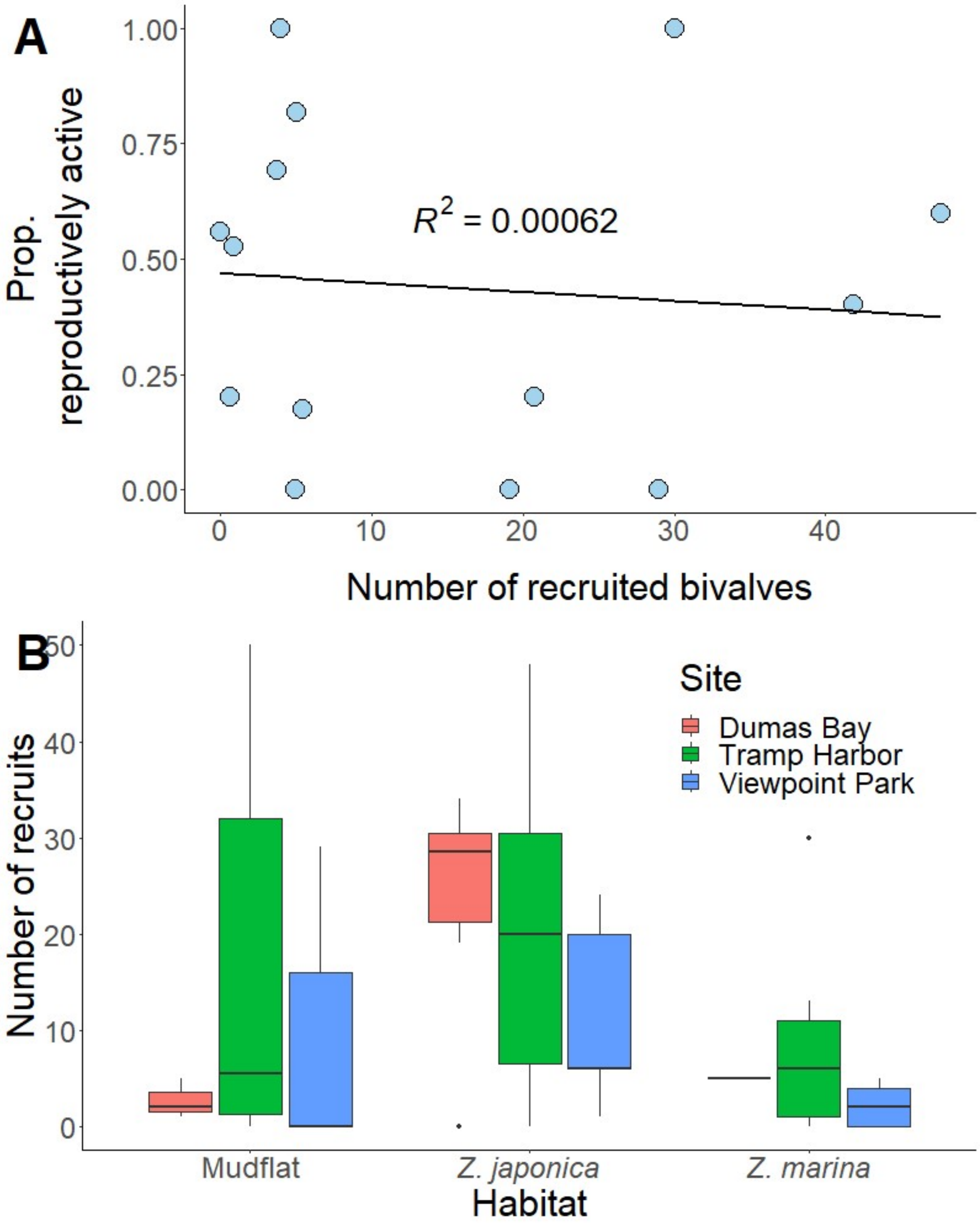
Response variable	Model	df	AIC_c	∆AIC	Model weight	R^2
Growth rate	Site only	13	-1484	0	0.88	0.25
Survival	Full model excluding habitat x site interaction	7	220.10	0	0.40	0.58
	Full model	11	220.72	0.63	0.29	0.63
Reproductive status	Site and year	4	182.10	0	0.64	0.59
Condition	Site only	5	-567	0	0.53	0.48

Supplemental Table 1.4. Top models for analyses including number of recruited bivalves as a predictor with df, AICc, ΔAIC, model weights, R^2.

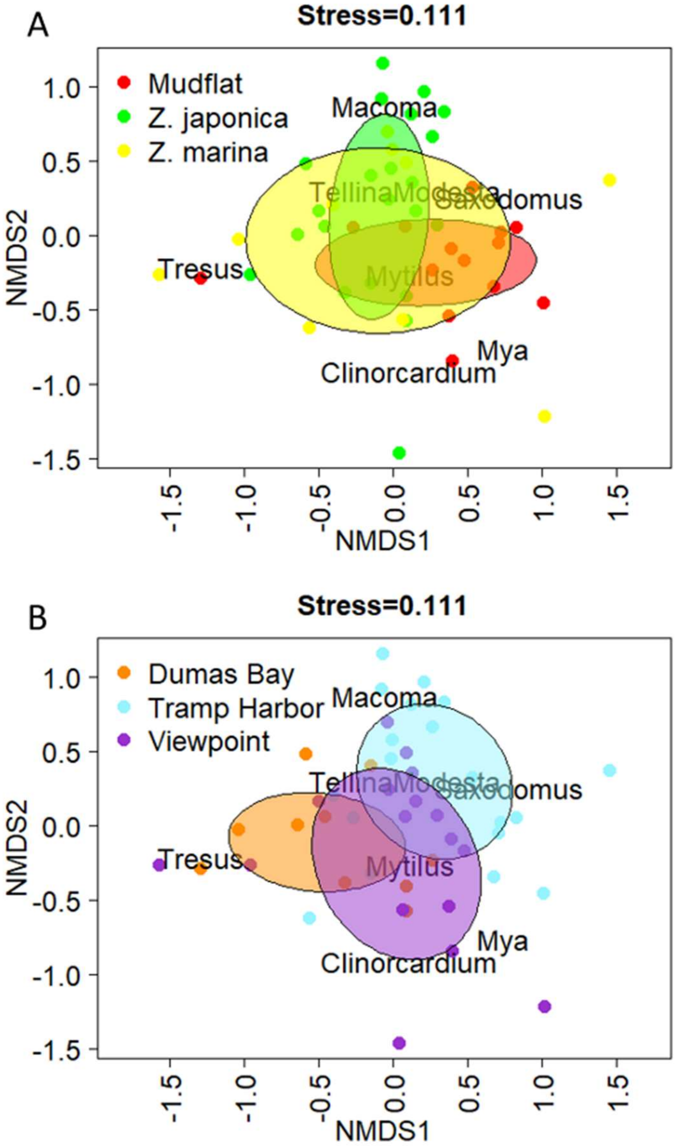
Response variable	Model	df	AIC_c	ΔAIC	Model weight	R^2
Growth rate	Intercept only	11	-1178	0	0.97	0.19
Survival	Habitat, site and interaction	9	157.69	0	0.60	0.71
Reproductive status	Site and year	4	155.64	0	0.53	0.45
	Site, year and recruits	5	157.09	1.45	0.26	0.47
Condition	Intercept only	5	-481	0	0.98	0.26



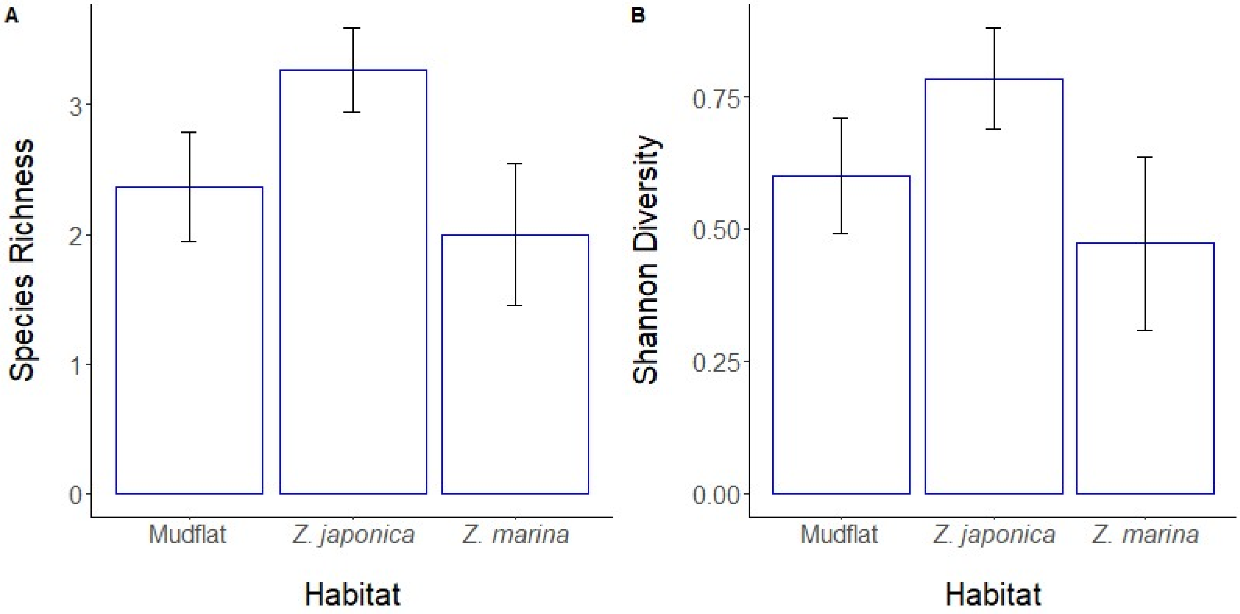
Supplemental Figure 1.1. Tidal elevation as it relates to habitat, site and survival. Other response variables are not included because there was not a correlation between them and tidal elevation. Tidal elevations are relative to local Mean Lower Low Water. **A.** Tidal elevation vs habitat and site for 2011. Bars are colored by habitat. **B.** Tidal elevation vs. survival, **C.** Tidal elevation vs habitat and site for 2012. Data for tidal elevation in *Z. marina* at Dumas Bay in 2012 was lost.



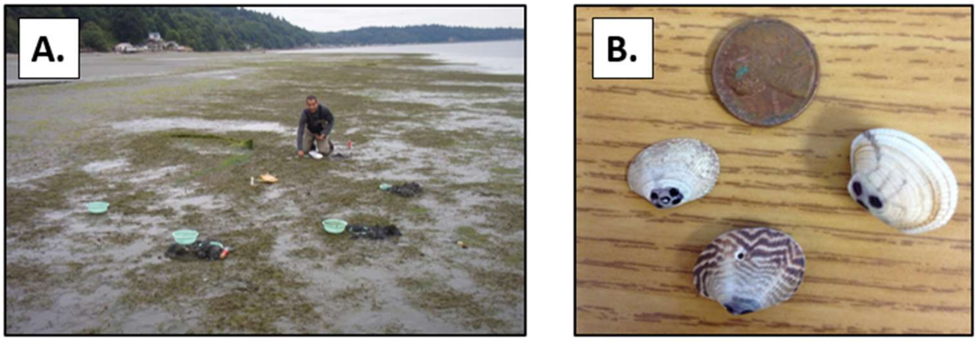
Supplemental Figure 1.2. Number of recruits bivalves as it relates to habitat, site and survival. Other response variables are not included because there was not a correlation between them and number of recruits. **A.** Number of recruits vs survival, **B.** Number of recruits vs habitat and site.



Supplemental Figure 1.3. **A.** nMDS plot of clam recruits by habitat, which is represented by color. **B.** nMDS plot of clam recruits by site, represented by color.



Supplemental Figure 1.4. Univariate metrics of diversity for recruited clams. **A.** Plot of species richness by habitat. **B.** Plot of Shannon diversity by habitat.



Supplemental Figure 1.5. A. Photo of outplanting clams at Viewpoint Park, WA. **B.** Examples of Manila clams used in the experiment, demonstrating how the clams were marked so that they could later be identified as individuals.

Chapter 2

Does Environmental Context Alter Bivalve and Snail Effects on Eelgrass Growth and Survival?

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Abstract

Seagrasses and bivalves are foundation species which often coexist, but studies are divided on whether bivalves tend to have positive or negative effects on seagrasses. Bivalves may negatively affect seagrasses through space competition and biodeposition leading to increases in sulfides, and may affect seagrasses positively through deposition of nutrients and increasing water clarity via filter feeding. Effects are likely to vary by bivalve species and abiotic context, such as temperature. My objective was to understand how eelgrass may respond to clam presence under different temperature and nutrient conditions. I ran a series of laboratory mesocosm experiments to test the effects of Manila clams (*Ruditapes philippinarum*) on eelgrass (*Zostera marina*) under different abiotic conditions, and to determine the comparative effects of clams and the snail *Alia carinata* on eelgrass growth under varying temperature conditions. I found limited evidence of clams impacting eelgrass under any conditions. Contrary to expectations, clams did not affect epiphyte buildup on eelgrass blades or the sides of the tank. Eelgrass growth and survival was generally lower under elevated temperatures and eutrophic conditions. Eelgrass grown without *Alia carinata* experienced decreased growth under higher temperatures, while eelgrass grown with *Alia* had the same growth rate at ambient and elevated temperatures. My results contrast other studies that have demonstrated effects of clams on algal buildup via filter feeding, and experimental design may have played a role in the lack of bivalve effects on eelgrass. However, my results indicate that *Alia* may help eelgrass be resilient to changes in temperature, and suggest that efforts to restore eelgrass may benefit from including *Alia* and other grazers.

Keywords: *Zostera marina*, *Ruditapes philippinarum*, *Alia carinata*, seagrass-bivalve interactions, foundation species, eutrophication, warming

Introduction

Marine ecosystems such as coral reefs, kelp forests, and seagrass beds support high levels of faunal diversity (Altieri and van de Koppel 2014). Abiotic stressors including warming (Hughes et al. 2018; Gauzens et al. 2020; Venegas et al. 2023), ocean acidification (Guinotte and Fabry 2008; Kroeker et al. 2013; Vizzini et al. 2017), and eutrophication (Diaz and Rosenberg 1995; Smith 2003; Mvungi and Pillay 2019) are increasingly impacting these coastal ecosystems and the habitat they provide, yet our understanding of anthropogenic stressor effects on key species interactions is limited (but see Vergés et al. 2016). Interactions between abiotic stressors and their consequences for marine ecosystems can be complex and context-dependent. For example, the combined impacts of warming and ocean acidification on coral may be synergistically negative in some cases (Anthony et al. 2008), or they may act in opposite directions in other cases (McCulloch et al. 2012; Kroeker et al. 2013).

Seagrasses are widespread foundation species that create biotic habitat for epifaunal and infaunal organisms in coastal marine ecosystems (Duffy et al. 2014). Seagrasses coexist with a wide variety of bivalves, including mussels, clams, scallops and oysters. The direction and strength of seagrass-bivalve interactions varies by bivalve species and context. For example, oysters, mussels, and clams facilitate growth of eelgrass (*Zostera marina*) by filtering particles out of the water column, increasing water clarity and photosynthetic capacity for eelgrass (Wall et al. 2008). Additionally, blue mussels (*Mytilus edulis*) benefit eelgrass through enriching the sediment with their pseudofeces (Reusch et al. 1994). Lucinid clams harbor sulfur-oxidizing bacteria in their gills, which allow them to alleviate sulfide stress for seagrasses when they co-exist (Chin et al. 2020). However, bivalves also reduce seagrass growth and density by competing with seagrass directly for space (Reusch and Williams 1998) or by excreting pseudofeces into sediments resulting in increases of toxic sulfides due to bacterial activity (Vinther and Holmer 2008).

The magnitude and direction of species interactions also depend on abiotic context, which varies widely across local abiotic gradients and the extensive geographic range of seagrasses. For example, light alters the effect of *Mytilus edulis* on *Z. marina*: under low light conditions, mussels cause sulfide stress for eelgrass, but not under high light conditions (Castorani et al. 2015). One theory related to these interactions is the stress gradient hypothesis, which postulates that positive interactions are more likely to occur under stressful conditions (Bertness and Callaway 1994).

Low light is a stressor with the potential to limit seagrass photosynthesis and growth (Bintz and Nixon 2001, Lee et al. 2007). Light attenuation can be caused by increases in water column nutrient concentrations driven by eutrophication, which stimulates growth of phytoplankton and epiphytic algae. It is well established that invertebrate crustacean and gastropod mesograzers can control algal epiphytes on seagrass leaves (Hughes et al. 2004, Whalen et al. 2013, Reynolds et al. 2014). However, bivalves have the capacity to filter phytoplankton from the water column, and thus may reduce light attenuation (Peterson and Heck 2001a, 2001b) and alleviate light stress under eutrophic conditions (Wall et al. 2008). Additionally, clams may reduce epiphyte load on eelgrass by consuming algal spores and reducing algal settlement rate on eelgrass (Peterson and Heck 2001b).

In addition to low light, other stressors like high water temperature can also decrease eelgrass growth (Lee et al. 2007). Temperature and light may have interactive effects on eelgrass. High temperatures increase respiration rates, such that in warmer waters seagrasses require more light to maintain growth (Bintz et al. 2003, Lee et al. 2007). In fact, Krumhansl et al. 2021 found that in warmer waters eelgrass was less resilient to light limitation than in cooler waters. And, while under eutrophic conditions bivalves may be facilitative, when light is not limiting, bivalves may provide little facilitation or, by (e.g.) increasing concentrations of toxic sulfides in seagrass sediments via biodeposition, may have negative effects on seagrass (Vinther and Holmer 2008).

To address the knowledge gap of how interactions will vary under multiple stressors, I conducted a mesocosm experiment to quantify the effect of bivalves on eelgrass growth and survival under different combinations of nutrient and temperature stress. My objective was to understand the source of variation in the direction and strength of bivalve effects on eelgrass, and how bivalve effects compare to mesograzer effects. I manipulated bivalve presence, nutrient conditions (as a mechanism of causing eutrophication), and temperature. Additionally, I compared bivalve effects on eelgrass to those of an abundant local epifaunal gastropod, *Alia carinata* (hereafter *Alia*), that consumes epiphytic algae on eelgrass leaves (Voigt and Hovel 2019). I hypothesized that under high-stress (eutrophic, high temperature) conditions, bivalves would facilitate seagrass by drawing down nutrient concentrations, thereby reducing epiphyte load. Under low-stress (oligotrophic, low temperature) conditions, I hypothesized that bivalve effects on nutrients would be less important, and bivalves would have little effect on seagrass growth. However, temperature effects may interact with light effects such that under high temperature, higher stress on seagrass would render the alleviation of light stress by bivalves more important. Finally, I hypothesized that *Alia* effects on eelgrass growth and survival would be positive under all conditions, but more important under stressful conditions, given that their ability to consume epiphytes which could hinder eelgrass growth may help eelgrass persist under stressful conditions.

Methods

Study system and taxa

I conducted laboratory experiments at San Diego State University's Coastal and Marine Institute Laboratory (CMIL) in San Diego, CA. For my experiments, I used eelgrass *Zostera marina*, the Manila clam *Ruditapes philippinarum* and the snail *Alia carinata*. *Zostera* is a genus of marine angiosperms that grow in coastal wave-protected areas of every continent except Antarctica, and provides habitat for a variety of fishes, mammals and invertebrates (Orth et al. 2006). Manila clams are commonly found in eelgrass beds (Tsai et al. 2010) and harvesting of Manila clams often occurs nearby or within eelgrass beds (Qin et al. 2016). Manila clams are non-native in southern California but have been found there since the 1990s (Talley et al. 2015). They have an established population in San Diego and are an important species for aquaculture globally (Cordero et al. 2017). Manila clam densities in San Diego are ~4 clams m^{-2} (Talley et al. 2015). *Alia carinata* (hereafter *Alia*) is a small gastropod and a very common epifaunal resident of San Diego eelgrass beds, with densities in the range of 0.5 – 1 individual per shoot (Sirota and Hovel 2006; authors' unpublished data).

Experiment I: Effects of Manila clams, temperature and eutrophication on eelgrass

I conducted two trials of a fully-factorial experiment examining effects of temperature, eutrophication, and Manila clam presence on eelgrass growth and survival. My trials took place from 21 February – 17 March, and 5 September – 4 October, 2023. I collected eelgrass from Shelter Island in San Diego Bay (32°42′48.34″N, − 117°13′32.43″W) 2 – 4 days before beginning experiments. I obtained

Manila clams from the Hog Island Oyster Company (Marshall, CA). Clams and eelgrass were held in flow-through seawater at CMIL. Clams were held for at least 3-4 days before experimentation to acclimate them to warmer temperatures than would be found in the Northern California aquaculture facility in which they were raised, which is a difference of ~ 7 – 9 °C. Clams were periodically fed LPB Frozen Shellfish Diet (Reed Mariculture) prior to experiments.

At the beginning of the experiment, I planted eelgrass shoots in indoor mesocosms (5.4 L). Each experimental mesocosm was filled with ~3-6 cm of a mixture of beach sand and sediment from the eelgrass bed to introduce beneficial microbes for eelgrass growth (Tarquinio et al. 2019). Eelgrass shoots were planted by gently burying the rhizomes in this mixture. In the first trial, each mesocosm received 6 shoots, while in the second trial each mesocosm received 8 shoots. Before planting eelgrass, each shoot was rinsed, scraped to remove any epifauna or epiphytes, and trimmed to 15 cm to standardize initial length, leaving ~ 2 – 5 cm of the sheath. Rhizomes were trimmed to ~ 2.5 cm. After planting, I allowed eelgrass to acclimate to laboratory conditions for 7-9 days before implementing treatments.

Eight experimental mesocosms were arranged in each of 4 large troughs (*N= 32*, Figure 2.1a). The 4 troughs contained water baths to regulate the temperatures of the mesocosms. There was no water flow between the trough water bath and the mesocosms. Each mesocosm contained standing water, with some flow created by airstones. Approximately half of the volume of water was replaced every 2-3 days in each mesocosm. To prevent any mesocosm from receiving different light or temperature conditions, I rotated the mesocosms clockwise every 2-3 days. I suspended full-spectrum 80 W ANTLUX LED Plant Grow Lights above the tanks to provide light for eelgrass growth.

I placed aquarium heaters in the troughs to manipulate temperature. Two of the troughs contained aquarium heaters, while the other two did not. I changed which troughs were heated between the trials to minimize bias due to trough-specific differences. The temperature treatments were ambient (unheated) vs. water heated to 2-3°C above ambient conditions. I monitored temperature and light using data loggers (Onset Brand HOBO Data Loggers) that were rotated among different mesocosms of the same treatment during the experiment. In the first trial, the mean temperature was 18.2 °C (± 0.01°C SE) in ambient mesocosms and 21.6 °C (± 0.03 °C SE) in heated mesocosms (Supplemental Figure 2.2a). In the second trial, mean temperature was 22.8 °C (± 0.01°C SE) in ambient mesocosms and 25.4 °C (± 0.01°C SE) in heated mesocosms (Supplemental Figure 2.2b). Water temperatures in San Diego Bay and Mission Bay are between 13 – 24 °C (Largier et al. 1997), so my experiment represents the upper range of what eelgrass would experience in the field.

For each mesocosm receiving a bivalve treatment, two Manila clams were weighed and measured lengthwise, before gently placing them, siphon upwards, in the sediment of the tank. The mean initial shell length (SL) of clams was 36.3 mm (± 2.06 SD) in trial 1 and 36.4 mm (± 1.23 SD) in trial 2. Mean initial clam length did not vary significantly among treatments. I checked clams in mesocosms every 2-3 days and when necessary, replaced any dead clams. I also replaced water in tanks containing dead clams, since they may have been contaminated with bacteria associated with clam necrosis. Over the course of trial I, three clams died, one in a control mesocosm and two in a heated, eutrophic mesocosm. During trial II, eleven clams died. Three were in unheated, eutrophic mesocosms and eight were in heated, eutrophic mesocosm.

In trial 1, I simulated eutrophication by adding Miracle Gro liquid fertilizer (N-P-K = 12-4-8) every 2 - 3 d. I added 1 ml of liquid fertilizer at the beginning of the experiment, and added 0.2 ml every 2 - 3 days thereafter. I added an equal volume of filtered seawater to all other tanks as a control. This level of eutrophication is high compared to other experiments testing eutrophication on eelgrass-bivalve interactions in a mesocosm setting (Wall et al. 2008). However, this treatment did not affect light levels as anticipated, so for trial 2, the nutrient treatments received the same 1 ml of liquid fertilizer initially, but I increased later additions to 0.3 ml every 2 - 3 days thereafter. In the second trial, the eutrophication treatments also received locally collected mixture of epiphytic algae and macroalgae every 2 - 3 days. Algae was mixed by hand to homogenize it into a solution and filtered with mesh to remove any small animals. I added 30 ml of algae solution initially, and 20 ml every 2 - 3 days thereafter. I added equivalent volumes of filtered to seawater to all control tanks.

The response variables were eelgrass shoot growth rate, final eelgrass biomass, and eelgrass shoot survival. I measured shoot growth rate using the hole punch method (Dennison 1987). For trial 1, I punched shoots 7 days before starting treatments, and shoots grew for 16-17 days after treatments were administered. For trial 2, I allowed eelgrass to acclimate to treatments for 8 days before hole punching shoots and each shoot was allowed to grow for approximately 11-12 days after hole punching. I changed the timing of hole punching relative to onset of treatments for the second trial to allow eelgrass to acclimate to experimental conditions before growth was measured, rather than including acclimation in the growth measurement period.

I calculated shoot growth rate by summing the new growth (in centimeters) of all leaves on each shoot and dividing by the number of days between hole punching and measuring. For each mesocosm, I also recorded the number of surviving shoots at the end of the experiment. In trial 2, I also measured the number of surviving shoots when treatments were initiated and midway through the experiment. For the second trial, I excluded from analyses shoots already found to have died when treatments were initiated.

I also measured the biomass (dry weight) of epiphytic algae scraped from eelgrass leaves. Epiphyte biomass was standardized by dry weight of eelgrass. However, I was unable to measure epiphytic biomass in trial 2, because eutrophic tanks had high shoot mortality, and eelgrass that was heavily fouled in these tanks may have died before the end of the experiment. I therefore visually assessed algae buildup on tank walls and created a qualitative scale to rank algae biomass between tanks. I posited that algae buildup on walls would be correlated with levels of eutrophication and algae growth in the water column, as demonstrated by Blumenshine et al. 1997, who found increased algae growth on plastic strips of mesocosm materials with enrichment of the water column. I used this scale as a proxy of stress caused by epiphytic algae on eelgrass. I visually assessed and scored tanks on a scale of 1-6. Scoring criteria for each category was as follows: 1 = no algae, 2 = scattered patches of algae, 3 = less than 1/3 of surface covered in algae, 4 = about half covered, 5 = mostly covered, and 6 = completely covered, with a thick layer of algae in some parts (Supplemental Figure

2.1). Pictures of mesocosms were scored independently by two people and I used the mean of these values to assign a score to each mesocosm.

Experiment II: Clam and Alia *effects under different temperature conditions*

The second experiment focused on the effects of *Alia* and clams on eelgrass growth and survival under differing temperature conditions. I collected eelgrass and *Alia* from Shelter Island, San Diego, CA on 22 June 2023 and ran a fully factorial experiment from 22 June – 20 July 2023 testing how eelgrass growth responded to temperature (ambient/high) and presence of animals. I established the following four treatments both in ambient and high-temperature conditions: 1) Manila clams only, 2) *Alia* only, 3) both Manila clams and Alia, or 4) no animals. Each mesocosm received 8 shoots. The average initial length of clams was 36.41 mm (± 3.53 SD), and I added two clams to each tank in treatment groups 1 and 3. I weighed and added 10 *Alia* to each tank in treatment groups 2 and 3. The average weight of all *Alia* in the mesocosms at the end of the experiment was 1.14 g per mesocosm (± 0.26g SD). Each treatment was replicated 4 times (*N* = 32 experimental units), and used the same general set-up and protocol as experiment I (Figure 2.1b), except that I hole punched shoots on the same day as beginning treatments, and I did not collect epiphyte data. The mean temperature in ambient mesocosms was 22.1 °C (± 0.01 °C SE) and was 22.9 °C (± 0.014 °C SE) in heated mesocosms (Supplemental Figure 2.2c). The temperature difference is smaller in this trial than other trials due to difficulties regulating temperature during this trial. Despite this modest change, temperature did affect eelgrass growth and survival (Figure 2.3).

I checked the *Alia* every 2-3 days and removed any *Alia* I found in non-snail mesocosms. I did not measure how many escaped, but due to observing *Alia* in non-snail mesocosms, I also added an additional 5 *Alia* to each snail mesocosm about halfway through the experiment to compensate for escapees.

Statistical analyses

I used a series of linear mixed effects models with a Gaussian distribution to examine how my treatments affected shoot growth rate. I included temperature, eutrophication, and bivalve/animal treatments as categorical fixed effects. I included trough and mesocosm as random effects to account for nonindependence among shoots in the same mesocosm and mesocosms in the same trough. I nested mesocosm within trough in my analyses. I also conducted separate analyses that included mean light levels recorded by HOBO loggers. For survival analyses, I used a generalized linear model with a binomial error structure and logit link, since shoots survival is a binomial response variable. All analyses were performed in R studio, R version 4.2.2 (R Core Team 2023). I used packages “lmerTest” and “nlme” to run models and ggplot2 to create plots.

Results

Contrary to my hypothesis, I did not find that clams mediated temperature or eutrophication effects for eelgrass. Mean light levels did not affect eelgrass growth rate or survival in any experiment (Supplemental Table 2.1). Epiphyte build-up on eelgrass (observed in experiment I, first trial), and the mesocosm walls (observed in experiment I, second trial) were not reduced by clams (Figures 2.4 and 2.5), and in some cases higher algae loads were associated with clams. For both trials of experiment I, clams had no effect on light levels (Supplemental Figure 2.3).

Experiment I

In my first trial, no treatments had significant effects on eelgrass growth rate or survival (Tables 2.1 and 2.2). Mean epiphyte biomass per gram of eelgrass dry biomass was 0.13 g (± 0.02 SE) in eutrophic mesocosms and 0.09 g (± 0.02 SE) in control mesocosms (Figure 2.4). For heated tanks only, light levels were lower in eutrophic tanks.

In my second trial, clams did not affect eelgrass growth rate regardless of nutrient or temperature treatment (Figure 2.2b). Eelgrass growth rate was not significantly different between temperature and nutrient treatments (Table 2.1). Eelgrass survival was lower in heated tanks than ambient tanks ($p = 0.001$), lower in tanks with nutrients added compared with control tanks ($p < 0.001$), and lower in tanks with clams than those without clams ($p = 0.01$, Table 2.2, Figure 2.2d). Mesocosms with clams and nutrients added had lower eelgrass survival than control mesocosms (Table 2.2, Figure 2.2d). Contrary to my hypotheses, clam and eutrophication treatments did not affect light levels (Supplemental Figure 2.3). Mean algae score was 4.7 (± 0.28 SE) in eutrophic mesocosms, and 2.3 (± 0.35 SE) in control mesocosms (Figure 2.5 and Supplemental Figure 2.1).

Experiment II

Eelgrass growth rate was not significantly different by temperature or animal treatment (Table 2.2, Figure 2.3a). Although effects were not statistically significant, I observed that for mesocosms with no animals or with both clams and *Alia*, growth rate was lower in the high temperature treatment (Figure 2.3a). This was not the case for mesocosms with just clams or just *Alia*, in which growth rate was the same across the two temperature treatments. Positive effects of *Alia* on growth rate were slightly stronger than those of clams, although not statistically significant. Additionally, *Alia* had positive effects on eelgrass survival: eelgrass survival was lower in heated than in control mesocosms ($p = 0.03$, Table 2.2), except for mesocosms with *Alia* only, in which survival was the same in heated and control mesocosms (Figure 2.3b). Clams did not affect light levels (Supplemental Figure 2.3).

Discussion

I investigated how interactions between clams and eelgrass change under different abiotic conditions, and how clam and epifaunal snail effects on eelgrass may differ. I did not find consistent effects of clams on eelgrass growth, but one experiment indicated that clams may be associated with lower survival for eelgrass shoots. Eelgrass growth and survival was generally lower under higher temperatures and eutrophic conditions. My results did not support my hypothesis that clams would mediate temperature and eutrophication effects for eelgrass. Unexpectedly, I also found that *Alia* may buffer eelgrass against the negative effects of warming.

Epifaunal grazers including *Alia* aid eelgrass growth by consuming competing epiphytes that grow on eelgrass leaves (Reynolds et al. 2014). Several studies have documented the positive effects that epifauna including *Alia* have on eelgrass (e.g. Voigt and Hovel 2019). However, to my knowledge, no other study has demonstrated that eelgrass may be buffered from negative effects of warming by epifaunal gastropods. Increased temperatures may lead to increases in epiphytic algae growth (Lawrence and Bolton 2023; Mvungi and Pillay 2019), such that in warm water, the presence of *Alia* may ameliorate competitive stress for eelgrass. These results have implications for eelgrass response to climate change, indicating that eelgrass growing with *Alia* may be more resilient to warming waters. However, Lawrence and Bolton (2023) found that algal growth under extreme temperatures was too rapid to be ameliorated by grazers. My results may have differed because my heated treatment was ~ 23 °C, while Lawrence and Bolton 2023 implemented a heated treatment of 30 °C. Grazers may only be able to regulate increasing in epiphytic growth up to a threshold temperature. Additionally, at extreme temperatures grazers may be at their own thermal limits, as would eelgrass even without epiphyte stress.

Climate change and herbivory interactions have been found in other systems. In kelp forests, Franco et al. 2015 found that urchin and fish herbivory of kelp was higher in warmer areas and led to kelp being found less often in open areas and more often in protected crevices. In intertidal systems, the presence of herbivorous limpets reduced the impact of climate change on the ecological community by enabling barnacles to persist, which support several other species (Kordas et al. 2017). In corals reefs, herbivores facilitate corals by consuming algae (Hay 1991), and studies show that herbivorous tropical fish are migrating poleward with warming (Vergés et al. 2016). The loss of these herbivorous fish due to warming may therefore affect the dominance of corals over algae. My study adds to a body of literature finding that interactions between climate change and herbivory may have consequences for marine foundation species.

The results of my first experiment did not support my hypothesis that clams would affect eelgrass via reduction of light stress. I may have found limited effects of bivalves because I used a bivalve that grows in low densities. Effects may vary by bivalve density; while clams and scallops tend to grow individually (Wells 1957), oysters and mussels often form dense aggregations. These differences in density will strongly affect how they interact with local seagrasses. My study used a density of clams (86 m^{-2}) higher than local abundances (4 m^{-2}), which are low enough to be difficult to replicate in small containers (I placed two clams in each 5.4L container). Even with these elevated densities, using a bivalve with low natural densities may be the reason that I did not see an effect compared to studies using organisms that grow in aggregate, like oysters or mussels. For example, Agnew et al. 2022 examined the effects of Pacific oysters on eelgrass wasting disease prevalence and severity in *Zostera marina*, based on the idea that oyster filtration would remove disease microbes from the water column. They used 15 oysters in a 4 L container, compared to my 2 clams in a 5.4 L container, because oysters tend to grow in large reefs while clams are solitary and grow at low densities. However, Wall et al. 2008 used clams at a similar density to my experiment and demonstrated an increase in leaf area productivity, and reduction in chlorophyll *a* in the water column for tanks with clams.

Although I did not find that light was limiting for eelgrass in my experiment, many studies have documented that light is a strong determiner of eelgrass distribution and growth (Bintz and Nixon 2001, Lee et al. 2007, Wall et al. 2008) and that decreases in light caused by declining water quality and increasing phytoplankton concentrations do affect eelgrass (Short et al. 1995; Carroll et al. 2008). Therefore, it is likely that my experimental design contributed to this result. Wall et al. 2008 used much larger mesocosms than my study, which may have affected the dynamics between clams and algae buildup. Since Manila clams often grow at low densities, using larger containers may have allowed us to replicate this density more accurately. Additionally, eutrophication and light limitation happen at large scales and may have been easier to test in a larger mesocosm with a greater depth for light limitation to occur.

I found that elevated temperature and eutrophication had a negative effect on eelgrass. Previous studies have documented the reduction in eelgrass growth under high temperatures (Nejrup and Pedersen 2008) and eutrophication (Short et al. 1995). Temperatures of 10 – 20 °C are ideal conditions for eelgrass, and temperatures 25 °C and above are associated with decreased survival (Nejrup and Pedersen 2008). My study indicates that even small changes in temperature, on the order of less than 1 °C can affect eelgrass growth and survival. Short et al. (1995) demonstrated that additions of ammonium and phosphate increased epiphytic algae on eelgrass blades and reduced eelgrass growth, which I also found in my study. Mvungi and Pillay (2019) found that seagrass *Zostera capensis* shoot density, length and biomass responded negatively to both warming and eutrophication, but that eutrophication had a stronger negative effect. They did not find interactive effects of warming and eutrophication on *Z.*

capensis. Similarly, I did not find interactive effects of temperature and eutrophication. My results add to literature demonstrating negative but noninteractive effects of warming and eutrophication on eelgrass.

During the two trials of experiment I, a total of 14 clams died and were replaced: 10 in a heated, eutrophic mesocosm, three in an unheated, eutrophic mesocosm, and one in a control mesocosm. Clams died in all of the heated, eutrophic mesocosms and three of four eutrophic mesocosms in trial II. This indicates that clams fared worse in warm, eutrophic conditions, and while I replaced clams that died, these deaths may indicate clams were nearing their thermal thresholds and explain why clams had limited effects on eelgrass. They also may have been experiencing sulfide stress caused by high temperatures and eutrophication (Liu et al. 2023). However, at higher temperatures clam clearance rate should also be higher, along with benefits to eelgrass, if they are within the range of thermal tolerance. Results from previous studies about clam thermal tolerances have been mixed. While Tamayo et al. 2013 found that clearance rates of Manila clams increases with temperature between 10 and 24 °C, Mann 1979 found that clam meat growth was optimal at 12 °C, and Han et al. 2008 found that clam metabolism was optimized at 20 °C. Clams may not have fared well partly because I got them from Hog Island Oyster Co., which operates in colder waters in Northern California.

A caveat of the experiment is that I had strong trough-level effects, although I changed which troughs received which temperature treatments between trials. I included trough as a random effect in my models to account for this; nonetheless, the strong temperature trends I observed often were not significant. I had a relatively small number of troughs, which made it difficult to separate trough effects from heating treatment.

Another caveat of this experiment is that I did not measure effects of clams on sediment chemistry. Bivalves can also impact eelgrass growth by biodeposition. Bivalve pseudofeces can provide nutrients for eelgrass growth (Reusch et al. 1994), but they can also introduce chemicals that are deleterious for eelgrass growth, like sulfides (Vinther and Holmer 2008). Future studies should consider quantifying porewater nutrients to assess bivalve effects, and should assess levels of chemicals that may affect bivalve filtering capacity, such as sulfides.

My study has potential applications for eelgrass restoration. My results indicate that epifaunal grazers may help seagrass be resilient to warmer temperatures, and therefore should be included in restoration plans. Restoring epifauna such as *Alia* alongside eelgrass may increase chances for success and would be relatively easy to collect and distribute. However, special attention should be given to which epifauna species are chosen for restoration. While positive effects of *Alia* (Voigt and Hovel 2019) and some other species of epifauna on eelgrass are well-established (Hughes et al. 2004, Reynolds et al. 2014), other epifauna species have negative effects on eelgrass. In general, eelgrass restoration practitioners should consider including fauna in their plans to potentially boost the resilience of the system.

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Tables and Figures

Table 2.1. Analysis of variance table for linear mixed effects models. The models are testing for effects of treatments on eelgrass growth rate for experiments I and II. The combined analysis excludes the nutrient treatment. Results from random effects are reported separately in Supplemental Table 2.2.

Trial	Parameter	Sum sq	NumDF	DenDF	F value	p-value
First	Temp	82.83	1	2.15	3.61	0.19
	Nutrient	0.01	1	16.48	0.00	0.98
	Clam	67.51	1	16.48	2.94	0.11
	Temp:Nutrient	9.01	1	16.48	0.39	0.54
	Nutrient:Clam	2.08	1	16.66	0.09	0.77
	Temp:Clam	2.93	1	16.48	0.13	0.73
	Temp:Nutrient:Clam	14.53	1	16.66	0.63	0.44
	Residuals		56			
Second	Temp	17.96	1	5.66	1.35	0.29
	Nutrient	27.46	1	23.33	2.06	0.16
	Clam	0.61	1	25.06	0.05	0.83
	Temp:Nutrient	16.15	1	23.33	1.21	0.28
	Nutrient:Clam	0.22	1	24.34	0.02	0.90
	Temp:Clam	8.95	1	25.06	0.67	0.42
	Temp:Nutrient:Clam	0.71	1	24.34	0.05	0.82
	Residuals		49			
Experiment II	Temp	112.04	1	1.46	4.73	0.21
	Animal	44.69	3	23.00	0.63	0.60
	Temp:Animal	77.60	3	23.00	1.09	0.37
	Residuals		104			

Table 2.2. Results from linear mixed effects model testing for effects of temperature, clams, and nutrients on eelgrass survival. P-values represent the results of t-tests for each variable. Each variable is compared to the control. H = heated treatment, L = nutrients added treatment, B = clams added treatment.

Trial	Parameter	Estimates	SE	t-value	p-value
First	Intercept	0.18	0.64	0.28	0.78
	Temp	-1.27	0.95	-1.34	0.18
	Nutrient	-0.72	0.69	-1.04	0.30
	Clam	-0.36	0.68	-0.52	0.60
	Temp(H):Nutrient(L)	0.48	1.04	0.46	0.64
	Nutrient(L):Clam(B)	1.50	1.01	1.49	0.14
	Temp(H):Clam(B)	1.08	0.97	1.11	0.27
	Temp(H):Nutrient(L):Clam(B)	-2.43	1.47	-1.66	0.10
Second	Intercept	2.87	0.85	3.38	0.001
	Temp	-3.29	1.01	-3.26	**0.001**
	Nutrient	-4.40	1.05	-4.20	**<0.001**
	Clam	-2.42	0.97	-2.49	**0.013**
	Temp(H)Nutrient(L)	2.35	1.35	1.75	0.080
	Nutrient(L):Clam(B)	2.29	1.21	1.89	**0.058**
	Temp(H):Clam(B)	1.92	1.31	1.47	0.141
	Temp(H):Nutrient(L):Clam(B)	-1.71	1.79	-0.95	0.340
Experiment II	Intercept	0.68	0.44	1.55	0.12
	TempH	-1.35	0.62	-2.18	**0.03**
	AnimalB	0.14	0.62	0.23	0.82
	AnimalB+S	0.30	0.63	0.48	0.63
	AnimalS	0.47	0.64	0.73	0.46
	TempH:AnimalB	0.14	0.87	0.16	0.87
	TempH:AnimalB+S	-0.45	0.88	-0.51	0.61
	TempH:AnimalS	1.18	0.90	1.31	0.19

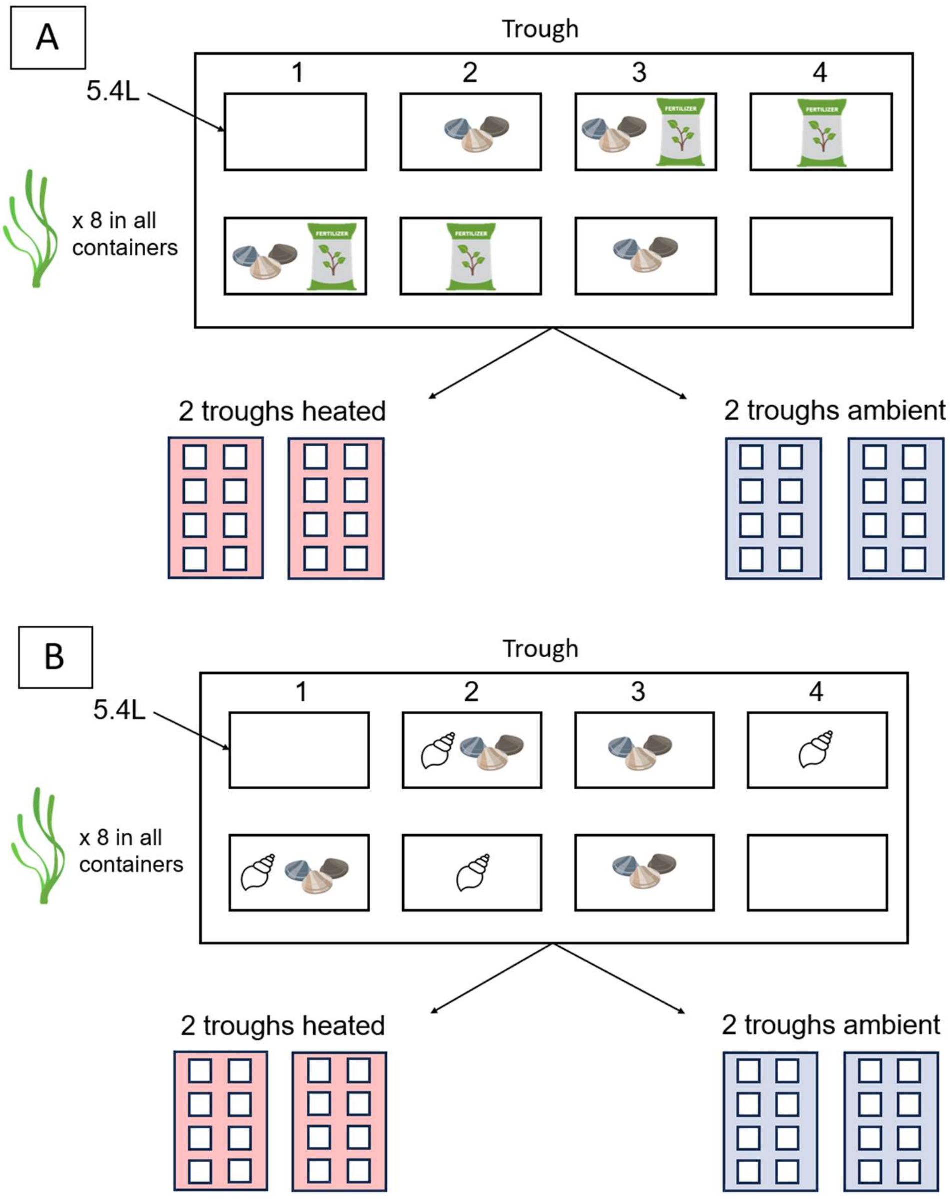


Figure 2.1. A. Experimental schematic for experiment I. Treatments were 1. Control, 2. Two Manila clams, 3. Manila clams and fertilizer/algae, 4. Only fertilizer/algae. **B.** Schematic for experiment II. Treatments were 1. Control, 2. *Alia* and two Manila clams, 3. Manila clams only, 4. *Alia* only. All treatments were replicated in 2 heated troughs and 2 troughs at ambient temperature. Modeled after Figure 1 in Agnew et al. 2022.

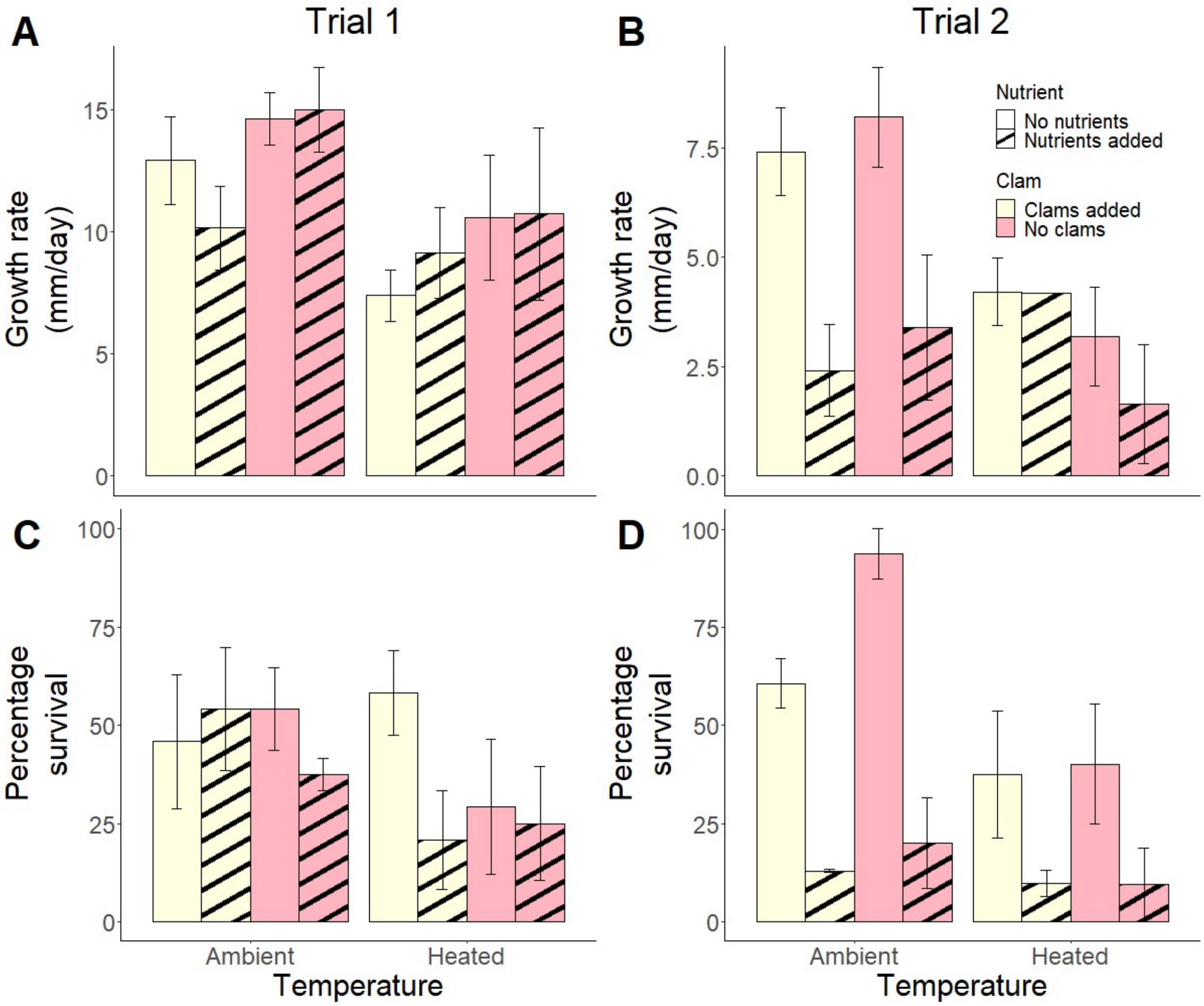


Figure 2.2. Eelgrass growth rate and survival from two trials of experiment I. **A.** Mean growth rate (mm/day) of eelgrass in trial I. **B.** Mean growth rate of eelgrass in trial II. **C.** Mean percentage survival of eelgrass shoots in trial I. **D.** Mean percentage survival of eelgrass shoots in trial II. Colors represent clam treatment and pattern represents nutrient treatment. Error bars represent +/- SE.

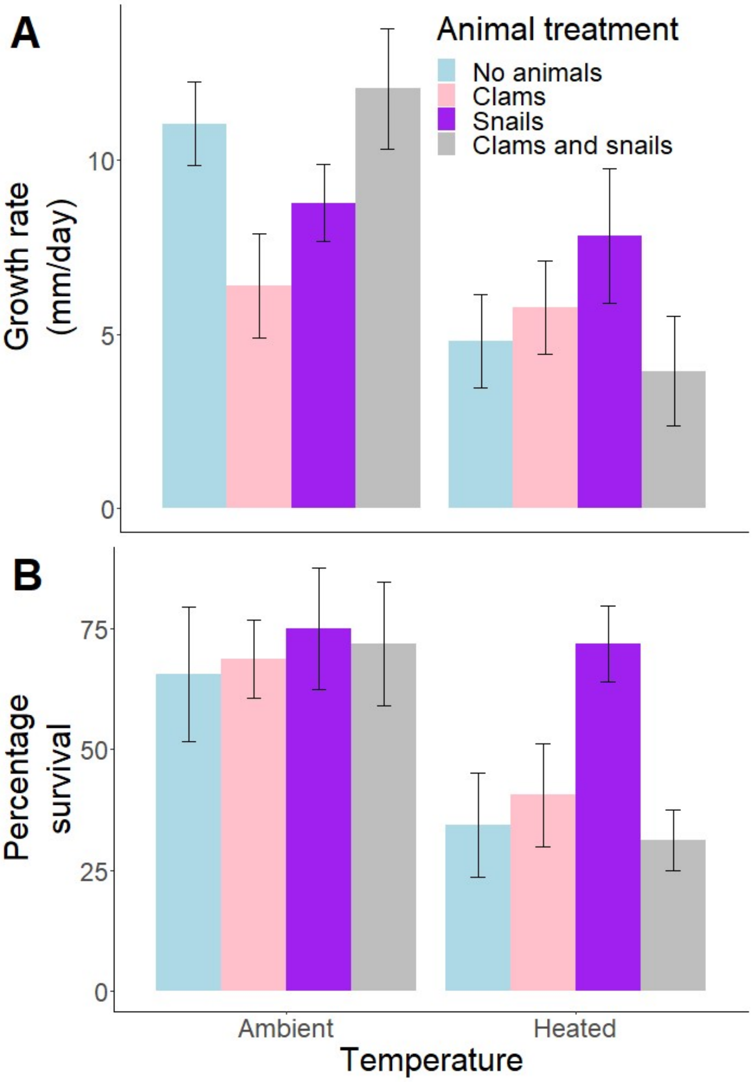


Figure 2.3. Eelgrass growth rate and survival from experiment II. **A.** Mean growth rate (mm/day) of eelgrass. **B.** Mean percentage survival of eelgrass shoots. Colors represent animal treatment. Error bars represent +/- SE.

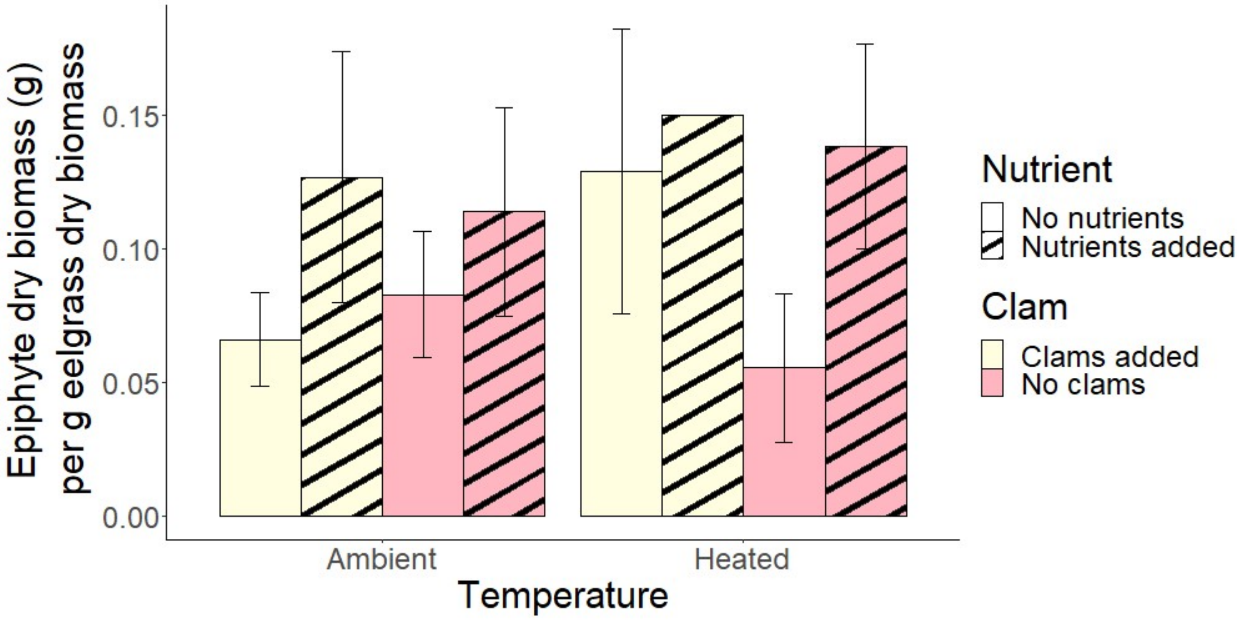


Figure 2.4. Mean epiphyte biomass per gram eelgrass for each treatment combination. Color corresponds to clam treatment and pattern corresponds to nutrient treatment. Error bars represent +/- SE.

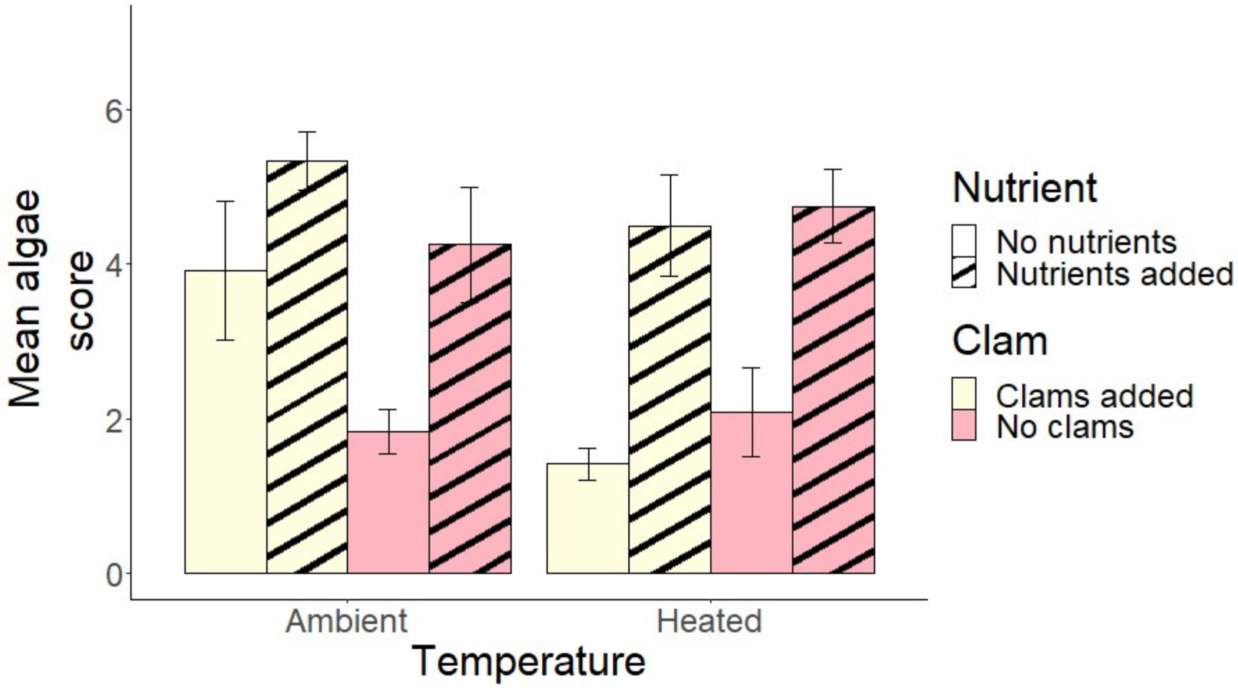


Figure 2.5. Mean algae score for tanks in each treatment. Color corresponds to clam treatment and pattern corresponds to nutrient treatment. Error bars represent +/- SE.

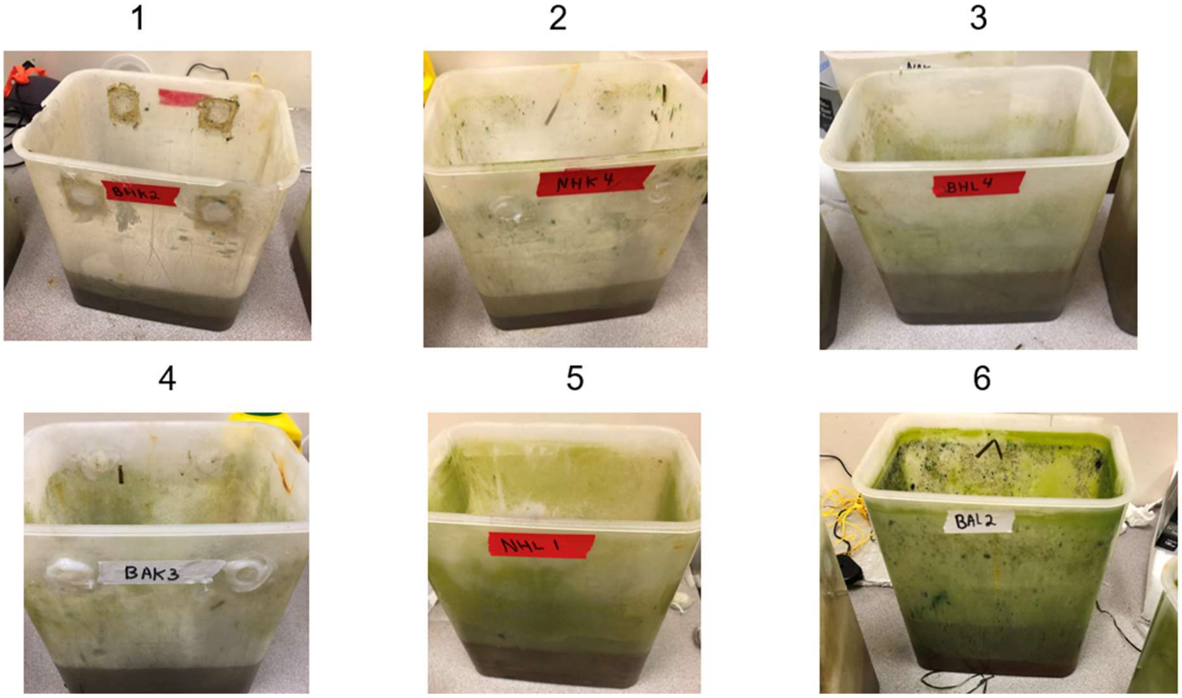
Supplemental Materials

Supplemental Table 2.1. Results from linear mixed effects model testing the effect of light levels on eelgrass growth and survival in experiments I and II.

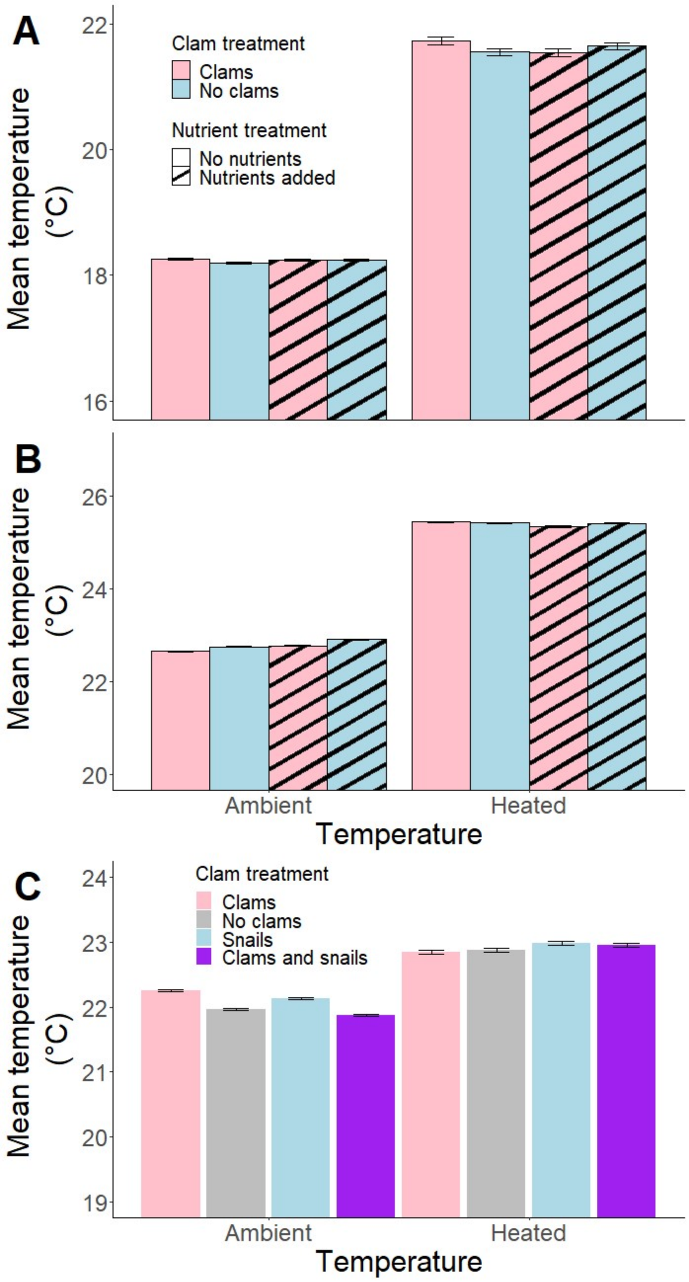
Experiment	Response variable	Parameter	Estimates	SE	t-value	p-value
I, Trial I	Growth	Intercept	12.77	2.81	4.55	<0.001
		MeanLight	-0.14	0.21	-0.69	0.50
I, Trial II	Growth	Intercept	7.03	2.65	2.66	0.02
		MeanLight	-0.18	0.26	-0.68	0.50
I, Trial I	Survival	Intercept	-0.01	0.74	-0.02	0.99
		MeanLight	-0.05	0.05	-0.93	0.35
I, Trial II	Survival	Intercept	0.87	1.26	0.69	0.49
		MeanLight	-0.18	0.12	-1.55	0.12
II	Growth	Intercept	6.65	3.07	2.17	0.04
		MeanLight	0.08	0.28	0.28	0.79
II	Survival	Intercept	0.41	0.77	0.53	0.59
		MeanLight	-0.01	0.07	-0.07	0.94

Supplemental Table 2.2. Results of F-tests for random effects from eelgrass growth models described in Tables 1. Npar = number of model parameters, logLik = log likelihood, LRT = likelihood ratio test statistic.

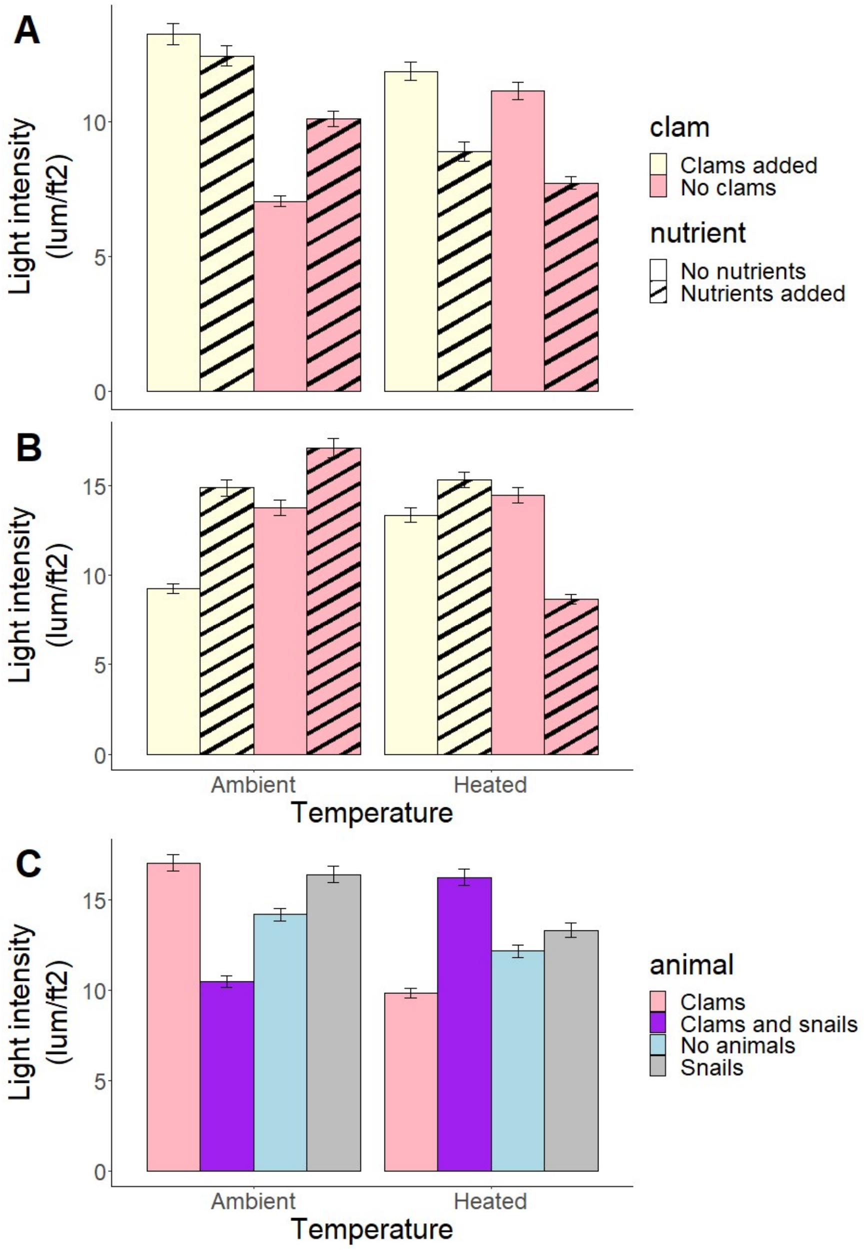
Experiment/Trial	Random effect	npar	logLik	LRT	DF	p value
Experiment I, Trial I	Trough	10	-186.95	0.27	1	0.60
	Trough:Tank	10	-187.12	0.61	1	0.43
Experiment I, Trial II	Trough	10	-151.21	0.005	1	0.94
	Trough:Tank	10	-152.72	3.02	1	0.08
Experiment II	Trough	10	-342.8	0.14	1	0.71
	Trough:Tank	10	-348.5	11.39	1	**<0.001**


1 2 3 4 5 6

Supplemental Figure 2.1. Algae scoring examples, from each category 1 – 6.



Supplemental Figure 2.2. Temperature by treatment in experiments I and II. **A.** Temperature by treatment in trial I of experiment I. **B.** Temperature in trial II of experiment I. **C.** Temperature in experiment II. For all experiments, color corresponds to clam/animal treatment and for experiment I, pattern corresponds to nutrient treatment. Error bars represent +/- SE.



Supplemental Figure 2.3. Light intensity (lum/ft^2) by treatment**. A.** Light intensity by treatment in trial I of experiment I. **B.** Light intensity in trial II of experiment I. **C.** Light intensity in experiment II. For all experiments, color corresponds to clam/animal treatment and for experiment I (panels A and B), pattern corresponds to nutrient treatment. Light shown is only between 5am and 10pm each day. Error bars represent +/- SE.

Chapter 3

Effects of Invasive Asian Mussels and Eelgrass Removal on Infauna Communities in San Diego, CA

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Abstract

Habitat loss is a common problem for nearshore marine ecosystems, but it often co-occurs with other disturbances such as the proliferation of non-native species, some of which form novel habitats. Though non-native foundation species may have negative effects on some local fauna, they may facilitate others by providing structure that compensates for loss of local habitat. The non-native Asian mussel *Arcuatula senhousia* is an ecosystem engineer that has been established in Southern California eelgrass (*Zostera marina*) beds for decades. In San Diego, eelgrass beds often experience physical disturbance, such as anchor and propellor scarring, leading to small-scale habitat loss. I conducted two field experiments to examine how Asian mussels and small-scale eelgrass removal singly and interactively affect the abundance, diversity, and community composition of eelgrass infauna. I hypothesized that Asian mussel mats may compensate for eelgrass loss via the creation of structure to support infaunal communities. In Southern California eelgrass habitat, I factorially combined treatments of addition/absence of Asian mussels and eelgrass removed/undisturbed, and examined their effects on native communities after nine weeks. In a subsequent experiment, I added a mussel mimic treatment to examine whether changes associated with Asian mussels are primarily attributed to structural changes to the benthos. I found variable effects of Asian mussels and eelgrass removal on infauna community composition, species diversity, and organismal abundance. Total infaunal abundance and taxon richness were higher in plots with Asian mussels compared to plots without mussels added, regardless of whether eelgrass was removed. I also found higher infaunal abundance in disturbed plots with Asian mussels than in undisturbed plots without Asian mussels. Our results suggest that non-native Asian mussels may provide habitat for organisms such as polychaetes and small gastropods that may not otherwise exist in eelgrass beds, increasing overall diversity.

Key words: invasive species, habitat loss, Zostera marina, multiple stressors, infauna

Introduction

Habitat loss is a widespread and commonly occurring threat to the biodiversity supported by those habitats (Tilman et al. 1994; Worm and Lenihan 2014). In marine ecosystems, multiple mechanisms can cause habitat loss, such as climate change-driven increases in storm frequency, which can destroy kelp forests (Castorani et al. 2018), overfishing leading to cascades which negatively affect coral reefs (Mumby et al.

2006), and eutrophication causing seagrass decline (Orth et al. 2006). In addition to these large-scale events leading to major habitat loss, smaller scale, episodic disturbances within existing matrices of habitat can also affect ecological communities. Smaller-scale disturbances can take the form of boulders flipped by waves disturbing intertidal algal populations (Sousa 1979), trampled patches of mussel beds on rocky shores (Mendez et al. 2018) or canopy gaps in mangrove forests caused by lightning strikes (Sherman et al. 2000). Although each incidence of these disturbances may be small in scale, they can be high in frequency and extent, and drive changes in faunal communities. For example, in seagrasses abundance and species richness of fauna are lower in propeller scars compared to the surrounding undisturbed habitat (Uhrin and Holmquist 2003).

Habitat loss does not occur in isolation; it occurs alongside many other stressors with which it may have interactive effects. Studies have documented interactive effects between habitat loss and climate change on biodiversity (Mantyka-pringle et al. 2012), between habitat degradation and fishing on coral reef fish communities (Wilson et al. 2010), and between small-scale habitat disturbance and species invasions in fouling communities (Altman and Whitlatch 2007). For example, Altman and Whitlatch (2007) found that in fouling communities, regular disturbances led to an increase in space occupied by non-native species and a decrease in space occupied by native species. These examples demonstrate the importance of understanding how effects of habitat loss may be compounded by other stressors.

Some non-native species form novel habitats, altering physical conditions and other organisms’ access to food, shelter and other resources needed for survival

(Jones et al. 1994) and potentially competing with native ecosystem engineers (Crooks 2009). For example, *Spartina alterniflora* is a salt marsh grass native to the western Atlantic which has hybridized with *S. foliosa* and become an invasive species on the eastern Pacific coast. In its invaded habitat, *S. alterniflora x S. foliosa* spreads rapidly, changing water flow and sedimentation patterns of former mudflats, which may both increase its capacity for continued spread and increase negative effects on diversity and biomass of local fauna (Callaway and Josselyn 1992; Neira et al. 2005; Neira et al. 2006). Non-native ecosystem engineers may facilitate native communities by providing alternative habitat to compensate for the loss of native habitat, or may create a novel habitat that facilitates an alternative community, which may include other non-native species (i.e. 'invasional meltdown': Simberloff and Von Holle 1999).

Which of these options occur may depend on the invader's structural complexity, the condition of the native habitat and whether the habitat provided by the non-native species fills a niche whose availability currently limits local fauna (Sellheim et al. 2010). For example, a non-native reef-forming polychaete, *Ficopomatus enigmaticus*, creates substrate for settlement of native macroalgae *Polysiphonia subtilissima*, while it did not grow in adjacent sedimented areas (Bazterrica et al. 2012). Similarly, Sellheim et al. 2010 found that habitat provided by non-native bryozoan *Watersipora subtorquata* facilitated mobile animals, leading to more diverse assemblages compared with other habitat types. Thus, it is possible for non-native species to have positive effects on the abundance of local fauna, particularly when compared to bare sediment or degraded native foundation species.

The non-native Asian mussel (*Arcuatula senhousia*) is a small marine mussel (≤ 30 mm shell height) that lives in intertidal and shallow subtidal soft sediment habitats, including within seagrass beds. *Arcuatula senhousia* was introduced to Southern California eelgrass (*Zostera marina*) beds in the 1960s (Macdonald 1969), where it has become numerically dominant with densities that can be orders of magnitude higher than those of native bivalves such as *Solen rostriformis* (Crooks 2001). Asian mussels are ecosystem engineers, capable of changing the physical, chemical, and ecological properties of an invaded habitat, and at high densities can inhibit seagrass growth (Reusch and Williams 1998). Mussels form extensive byssal mats on top of soft substrate, which trap sediments and lead to increased quantities of fine sediments and organic matter. Native species richness and total faunal abundance are often higher inside byssal mats as compared to bare tidal mudflats (Crooks 1998, Crooks and Khim 1999), likely due to the structure providing habitat for small invertebrates.

Seagrass beds harbor a high diversity of infaunal invertebrates such as polychaetes, bivalves, and gastropods, which are facilitated by the structure provided by seagrass rhizomes (Knowles and Bell 1998), protection from predators (Irlandi 1994) and food delivery (Irlandi and Peterson 1991). In seagrass beds, Asian mussels may affect native bivalves by limiting seagrass growth, competing directly for resources, or through apparent competition in which Asian mussels attract predators which then prey on native infauna (Castorani and Hovel 2015). However, other fauna may respond positively to the physical modifications that result from Asian mussel proliferation. For example, densities of the tanaid *Leptochelia dubia* and gastropod *Barleeia subtenuis* are higher in Asian mussel mats than bare tidal mudflats (Crooks 1998). Asian mussels may also serve as alternative prey resource for predators, reducing predation on native infauna. Asian mussels have thin shells which are easy for predators to drill or crush, and their populations are often controlled by native predators (Cheng and Hovel 2010), indicating that they are highly susceptible to predation by gastropods, crustaceans, fishes, and birds (Kushner and Hovel 2006). However, attracting predators can also result in increased predation on all species. Thus, Asian mussels likely have positive effects on some species and negative effects on others.

Asian mussels and habitat loss may have interactive effects. Degradation of seagrass habitat leads to declines in epifaunal and infaunal species (Frost et al. 1999). If seagrass habitat quality is degraded, the structure provided by Asian mussels may be more important for maintaining abundant assemblages. However, sites with more predators may exert greater control over mussels, lessening their impact on native assemblages (Cheng and Hovel 2010). Additionally, mussels are more likely to successfully establish in degraded eelgrass (Reusch and Williams 1998).

My objective was to understand how small-scale eelgrass removal affects infauna communities, and how this effect may be altered by a non-native mussel. Specifically, I asked: 1) how are eelgrass infauna communities affected by Asian mussels and eelgrass removal, and do these stressors have interactive effects? 2) What is the effect of Asian mussels on eelgrass? and 3) How does eelgrass affect Asian mussel survival? I hypothesized that when seagrass is disturbed via removal of shoots and rhizomes, mussels will have a facilitative effect on seagrass infauna through the creation of habitat structure in the form of byssal mats. Asian mussels will act as a foundation species when the necessary structure usually provided by the primary foundation species, seagrass, is reduced via removal. Thus, when seagrass is removed, infauna diversity and total abundance will be higher when mussels are present than when they are absent. However, when seagrass is intact, I predict that mussels will have little effect on seagrass infauna. I further hypothesized that Asian mussels would have a negative effect on eelgrass biomass, as previous studies have demonstrated limited eelgrass rhizome expansion when mussels were added (Reusch and Williams 1998). Finally, I hypothesized that eelgrass would inhibit establishment of Asian mussels, due to previous findings that Asian mussels transplanted to dense eelgrass beds experienced high mortality (Reusch and Williams 1998).

Methods

Study site

I conducted my study at Hidden Anchorage, a small (0.11 km^2) embayment adjacent to Fiesta Island near the back of Mission Bay, San Diego, CA (32.77, -117.22, Figure 3.1). Mission Bay covers 1861 ha and is a highly modified, low-flow estuary that contains intertidal and subtidal eelgrass meadows and dense populations of Asian mussels (Crooks 1998). Mission Bay is the largest aquatic park in the United States, and is heavily used by the public for fishing, boating, swimming, and other forms of recreation (City of San Diego 2024). Many unvegetated “scars” caused by anchors and propellers are found throughout the eelgrass beds in the bay. Asian mussel densities are highest in the muddy and silty sediment found in the back portion of Mission Bay, which can experience salinities elevated by as much as 2 PSU compared to the bay mouth (Largier et al. 1997), and are often found in eelgrass beds (Kushner and Hovel

2006). Asian mussel density at Hidden Anchorage is temporally variable but reaches values as high as 1000 mussels/m^2 (Dexter and Crooks 2000). Eelgrass is found from the intertidal to approximately 3 m below MLLW in Mission Bay, including in the silty back bay area (Merkel & Associates 1999). The average percent cover of eelgrass at my study site was 82.3% (± 26.5 SD), with bare sediment making up the remainder (unpublished data).

Mussel collection

I collected Asian mussels at Fiesta Island in Mission Bay on 29 June, 2021 and on 15 July, 2022. Asian mussels were taken back to the San Diego State University Coastal and Marine Institute Laboratory, where they were gently removed from their byssal mats, counted, and held in outdoor flow-through mesocosms until the experiment began. The average length of Asian mussels at my study site was 9.28 mm (± 3.8 SD), based on 100 mussels collected after the experiments began.

Experiment I: Effects of Asian mussels and eelgrass removal on infauna

In experiment I, I established 32 small (0.05 m^2) circular plots at my study site and factorially varied eelgrass removal (removed vs. undisturbed) and mussel addition (mussels added vs. no mussels added; Figure 3.2a). I marked plots by anchoring 25 cm diameter x 2 cm high PVC rings into the sediment. For eelgrass removal treatments, I used SCUBA to remove all eelgrass shoots and rhizomes from inside the plot, to simulate the scarring that anchors or propellers may cause. For Asian mussel treatments, I added 50 mussels per plot (1,000 mussels m^{-2}) to represent average Asian mussel densities in this area (Castorani and Hovel 2015). All plots were placed 2 m apart along a single subtidal (approximately 1.5 m below MLLW) transect running parallel to shore, with treatments in random order. After adding mussels, I covered plots with cages (30.5 cm height, 94 cm circumference) made of 0.6 cm galvanized steel mesh to deter predators from consuming mussels. This mesh size is small enough to exclude most predators, although small drills such as *Pteropurpura festiva* (Cheng and Hovel 2010), which has a shell length of 20 – 70 mm, may have been able to enter cages, particularly juveniles. To determine the effect of mesh cages on infauna and eelgrass, I established eight cage-free control plots in which I did not disturb eelgrass or add mussels, in addition to my 32 caged experimental plots. I monitored temperature during the experiment using a HOBO Pendant temperature logger (Onset HOBO Data Loggers).

I deployed all plots on 30 June – 1 July 2021. I checked cages and removed fouling organisms on cages using a stiff bristle brush at least once per week for the duration of the experiment, which ran until 2 September 2021 (9 weeks). Any cages that were toppled were either righted or replaced if damaged. At the conclusion of the experiment, I collected a 10 – 20 cm deep core sample from each plot using a 20 cm diameter PVC corer. I filtered the samples through a 1000-micron box sieve and separated eelgrass and fauna, which included infaunal bivalves and annelids, fauna at the sediment surface, and some epifauna captured incidentally. I froze eelgrass and preserved fauna in 95% ethanol for later processing.

Experiment II: Inclusion of mussel mimics

I repeated the experiment in the summer of 2022 but added a mussel mimic treatment to assess the effects of mussel structure vs. other potential effects of mussels. Thus, in experiment II, eelgrass removal plots and undisturbed plots had either (1) live Asian mussels, (2) mussel mimics, or (3) no added mussels or mimics (*n* = 6 for each treatment; Figure 3.2b). As in Experiment I, I also included cage-free control plots with no cages (*n* = 6). Mussel mimics consisted of black polyethylene shade cloth/mesh to recreate the structure of the byssal mats formed by mussels. Six artificial mussels consisting of silicone were attached to the mat, applied in the approximate size and shape of local Asian mussels. For plots with live mussels, I added 75 Asian mussels when the experiment began on 18 July 2022, and I then added an additional 16 mussels to each mussel plot on 16 August, to replace any that had died. The experiment lasted 8.5 weeks and ended on 16 September 2022 at which time plots were sampled as in 2021.

Sample processing

In the laboratory, I counted and identified to the lowest possible taxonomic level all invertebrates including amphipods, crustaceans, molluscs, polychaetes and oligochaetes. For annelids (including mobile polychaetes and oligochaetes, but not tube-dwelling polychaetes), I recorded abundance but did not identify with greater taxonomic resolution. I only counted Asian mussels when I could clearly tell the animal was alive when collected. To determine whether the animal was alive when collected, I looked for tissue between the valves, since the tissues of dead mussels tend to decompose very quickly (author’s personal observation). For eelgrass, I separated above-ground from below-ground material, dried material in a drying oven at 60 °C for 48 h, and obtained dry mass for each plot. For 10 (out of 42) samples, I only had data on wet weight, and calculated dry weight using a ratio of dry weight = 0.095 * wet weight. I obtained this ratio by finding the relationship between wet and dry weights for samples for which I had all data (Wickham et al. 2019).

Statistical analyses

I hypothesized that mussels would have a facilitative effect on eelgrass infauna when eelgrass was removed, but not when eelgrass was undisturbed. My hypothesis will be supported if infauna diversity and abundance are higher in plots that had Asian mussels added and eelgrass removed compared with plots that had eelgrass removed but no Asian mussels added. In this case, I would also expect no difference in infauna communities in plots with Asian mussels added and undisturbed eelgrass compared with no mussels and undisturbed eelgrass. I also hypothesized that Asian mussels would reduce eelgrass biomass and that eelgrass would inhibit Asian mussel establishment. These hypotheses will be supported if eelgrass biomass is lower in plots with Asian mussels compared with plots that had no mussels, and if Asian mussel survival is reduced in plots with undisturbed eelgrass compared with eelgrass removal plots.

I conducted separate analyses for Experiment I and Experiment II. For each experiment, my dependent variables were total organismal abundance (total number of individuals), taxon richness, Shannon diversity, and community composition. I excluded

Asian mussels from community diversity and abundance analyses. I assessed organismal abundance, taxon richness and Shannon diversity using general linear models. My fixed effects were Asian mussel treatment, eelgrass removal treatment, their interaction, cage treatment and position of the plot on the transect. Although I randomly allocated treatments along my transect, I observed a potential gradient along the transect and therefore decided to include it to account for variation unrelated to treatments. Organismal abundance was low in 2022, preventing me from evaluating Shannon diversity in that year. For my linear models, I examined data visually using quantile plots, and I log or square root transformed data as necessary to meet the assumptions of general linear models. I used Tukey’s tests for post-hoc analyses for linear models. I considered $p \leq 0.05$ to constitute evidence of a difference among treatments, and when $0.10 \geq p > 0.05$, I used Cohen’s *d* to assess effect size and determine whether an effect was detected. I used nMDS plots based on Bray-Curtis dissimilarities followed by PERMANOVA and post-hoc pairwise comparisons when appropriate to determine whether infaunal community composition varied by treatment. Finally, I used linear models to examine whether final eelgrass biomass differed by Asian mussel treatment and whether final number of Asian mussels in plots differed by eelgrass removal treatment. All analyses were conducted in R version 4.2.2 (R Core Team 2024). I conducted community analyses using the R package ‘vegan’.

Results

Experiment I: Asian mussels and eelgrass removal

I found a total of 821 organisms across 16 taxa, and the mean Shannon diversity was 1.04. The most common animals in my plots were the brittle star *Ophiothrix spiculata* (8.2 (± 1.5 SE) animals per 0.07 m^2 plot), the isopod *Paracerceis sculpta* (3.3 ± 0.8 SE), the amphipod *Ampithoe* sp (1.2 ± 0.4)., the clam *Macoma* sp (1.5 ± 0.2) and polychaetes and oligochaetes (4.8 ± 2.4) (Table 3.1). Mean water temperature during experiment I was 23.3 °C (± 0.95 SD).

Organismal abundance per plot differed significantly between mussel treatments, with total organismal abundance (excluding Asian mussels) higher in plots containing Asian mussels regardless of eelgrass removal treatment ($p = 0.04$, Tables 3.2 and 3.3). Neither taxon richness nor Shannon diversity differed significantly across mussel or eelgrass removal treatments or their interaction (Table 3.3). There was a trend towards more polychaetes and oligochaetes in eelgrass removal plots, although the effect was not significant ($p = 0.09$, Cohen's $d = 0.52$). The community composition of native infauna did not vary significantly by Asian mussel treatment but were marginally different between removal treatments (PERMANOVA, $p = 0.14$ and 0.06 respectively, Table 3.5). These results did not support my hypothesis that Asian mussel addition and eelgrass removal would interactively affect native fauna.

Despite cages that deterred predation on mussels, Asian mussel mortality was high. Mean Asian mussel mortality was 77.6%, ranging from 42 – 98% (Supplemental Figure 3.1a). Asian mussel mortality did not affect any of my response variables. Eelgrass removal did not affect Asian mussel survival; there was no significant difference in Asian mussel survival in removal vs. undisturbed plots (Supplemental Table 3.1). Additionally, Asian mussels did not affect eelgrass biomass; there was no significant difference in eelgrass biomass in plots containing Asian mussels and control plots (Supplemental Figure 3.2).

Position along the transect influenced Asian mussel abundance and eelgrass biomass: the number of Asian mussels at the end of the experiment ($p < 0.001$, Supplemental Table 3.1) and eelgrass biomass at the end of the experiment ($p = 0.004$, Supplemental Table 3.2) both increased from the southeast end of the transect to the northwest end of the transect.

Experiment II: Inclusion of mussel mimics

Organismal abundance, taxon richness and Shannon diversity of native fauna were much lower in 2022 than in 2021 (Table 3.2, Figure 3.3). I found a total of 123 organisms across six taxa in experiment 2, and the mean Shannon diversity in 2022 was 0.20. There were five bivalves in my plots: one *Mytilus* sp., two *Chione undatella*, and two *Macoma* sp. I found the gastropod *Volvarina taeniolata* in 14 of my total 42 plots, polychaetes and oligochaetes in 22 plots, and brittlestar *Ophiothrix spiculata* in 4 plots. Three plots were lost in Experiment 2. Mean water temperature during experiment II was 24.8 °C (+ 1.09 SD)).

I found a significantly higher organismal abundance in caged plots than in cage-free plots (*ANOVA,* $p = 0.03$), and a higher abundance in plots with Asian mussels than those with no mussels ($p = 0.03$, Table 3.4). I found no effect of mussel mimics on any response variable. There was some evidence that Asian mussel presence and eelgrass removal had an interactive effect on organismal abundance ($p = 0.07$, Table 3.4).

Removal plots with mussels had more organisms than undisturbed plots with no mussels (Tukey's HSD, *p* = 0.07, Cohen's *d* = 1.81). Plots with Asian mussels also had higher taxon richness than plots with no mussels (*p* = 0.05, Tukey's HSD). Communities of native infauna did not vary across removal treatments but did vary by Asian mussel treatment (PERMANOVA, *p* = 0.04, Table 3.5). Communities in plots with Asian mussels differed significantly from those with no mussels but did not differ compared with mussel mimics (*p* = 0.03, Table 3.5).

Asian mussel mortality was high and variable, ranging from 58 – 100% mortality (Supplemental Figure 3.1b), and did not vary significantly between eelgrass removal treatments (Supplemental Table 3.1). Eelgrass mortality was also high during the experiment and was not affected by Asian mussel treatment (Supplemental Figure 3.2b). Mean eelgrass biomass at the end of the 2022 experiment was 0.26 g (± 0.07 SE) per 0.07 m^2 plot compared to 0.39 g (± 0.1 SE) in 2021, indicating less eelgrass was left at the end of the experiment in 2022 than in 2021, although this difference is not statistically significant (t-test, *p = 0.28*). In 2022, the highest biomass plot had 2.1 g of eelgrass and 12 plots had no eelgrass by the end of the experiment, while in 2021 the highest biomass plot had 3.6 g and 11 plots had no eelgrass by the end of the experiment. Mussel mortality and eelgrass mortality were not correlated.

Mean water temperature was 1.5 °C higher in 2022 than 2021, and the minimum temperature recorded was 3.5 °C higher in 2022 than 2021.

Discussion

I conducted a field experiment testing the interactive effects of a non-native ecosystem engineer and loss of native habitat on native infauna in Southern California eelgrass habitat. I found some impacts of Asian mussels and eelgrass removal on native communities, although effects were not consistent. Generally, removal of eelgrass had little effect on infauna, while Asian mussels were associated with higher abundance of organisms. I found no effect of mussel mimics, potentially indicating that living mussels created some effect separate from structural effects.

I found interactive effects of eelgrass removal and Asian mussels in only one case: total abundance of organisms in experiment II was greater in removal plots with mussels than in undisturbed plots with no mussels. I expected to find that Asian mussels may provide structure to increase biodiversity primarily in case of eelgrass removal, and that this would not occur when undisturbed eelgrass provided sufficient structure. However, due to the already patchy nature of the eelgrass at my site and the die-off I observed in 2022 likely related to extreme temperatures (mean water temperature during experiment II was 24.8 °C (+ 1.09 SD), compared to 23.3 °C (+ 0.95 SD) in experiment I), a disturbance in eelgrass may have been provided naturally in addition to my treatment (Supplemental Figure 3.2). In such conditions, the mussel may have been providing structure for organisms that was otherwise lacking. However, I observed high mussel mortality and did not find effects of my structural mimic. Asian mussels that died during the experiment may have provided a food source that attracted animals, increasing overall abundance in my Asian mussel plots.

I found higher total abundance of organisms and taxon richness in plots with Asian mussels. Previous studies have found that Asian mussel beds can support a higher diversity of organisms than bare mudflats due to the added structure (Crooks 1998), but have not investigated their effects compared to vegetated habitats. Asian mussel plots may have harbored greater numbers of organisms even compared to plots with eelgrass due to creating a different kind of structure than eelgrass beds. Asian mussels create a hard substrate for organisms to attach to, which is otherwise absent in soft-bottomed eelgrass beds. However, many of the species I found are mobile, not sessile species attaching to hard substrate. In this case, small mobile invertebrates may inhabit the interstitial spaces among mussel mats (Crooks and Khim 1999) or have arrived to prey on mussels.

These results contribute to a growing body of literature suggesting that some non-native species may have positive effects on some species in their new ecosystem (eg. Crooks 2002; Wonham et al. 2005). In this case, I found that higher abundances of native fauna were associated with Asian mussels, which may help to increase diversity in a bay experiencing heavy recreational use. However, whether this means mussels are a ‘good’ addition to the ecosystem is a matter of perspective. If the management goal is to reduce non-native species as much as possible, any non-native species would not be ideal. However, if a non-native species increases diversity or populations of a desired native species, they may be a positive addition to the ecosystem.

I did not find an effect of my structural mimic treatment in 2022. Previous studies, such as Crooks and Khim 1999, found that mussel mat mimics increased overall abundance and species richness of fauna. Differing results may have been linked to differences in design of the mussel mimic, as my mussel mimic was constructed of black mesh with silicone "mussels" attached, and Crooks and Khim 1999 used furnace filter material. The mimic made by Crooks and Khim 1999 was thicker and likely allowed for more interstitial space for fauna to inhabit compared to my mimic. Contrasting results between our study and previous studies that found strong effects of mussels also could be due to changes in the community caused by Asian mussels in the intervening years. Additionally, strong effects of Asian mussels on native bivalves can occur via apparent competition, when mussels attract shared predators that then prey on native bivalves (Castorani and Hovel 2015), which we did not test in our study.

Asian mussel populations have been established in Mission Bay for several decades (Crooks 1998), so species able to survive in this environment may be those that are hearty enough to withstand competition from Asian mussels. Previous studies have shown *Macoma* sp. to be robust to Asian mussel invasion, as compared to *Chione sp.* and other native species (Crooks 2001). In my study, I found that *Macoma sp.* was the most abundant native bivalve, and that others were relatively rare. Crooks 2001 found declines in the native bivalve *Solens rostriformis*, which I did not find in any of my plots in either year of the experiment. Crooks 2001 also found declines in *Chione sp*. and not *Macoma nasuta*, which they attributed to the feeding mode of each species. While *Chione* is a surface suspension feeder, and faced inhibition by dense Asian mussel mats, *M. nasuta* lives deeper in the sediment and is a deposit feeder, which may have made it less vulnerable to changes brought on by invasion by Asian mussels. Unlike previous studies (Castorani and Hovel 2015) I did not observe declines in native bivalves in my Asian mussel plots.

Anchor and propeller scarring from boats are common in Mission Bay, which may explain why I did not see strong effects of my removal treatment on communities of native fauna; fauna surviving there may be those well adapted to this type of frequent, small-scale habitat loss. I found relatively low density and diversity of native fauna in all plots. This may indicate that frequent instances of small-scale habitat loss in Mission Bay had previously driven down populations of native species, with only hardy species persisting. Alternatively, my small-scale disturbances may have had little effect in a patchy seagrass bed with high edge effects. In an experiment clearing patches of intertidal mussel beds, Sousa 1984 found that clearing size significantly affected the community that recruited there, with small patches dominated by invertebrate grazers and algae avoided by grazers, while larger patches had fewer grazers and more competitively-dominant but grazer-preferred algae. In my experiment, those species better adapted to small patches of cleared eelgrass may have recruited there, such as infauna organisms that are usually inhibited by eelgrass rhizomes.

I also found that Asian mussels did not reduce eelgrass biomass, while previous studies have found the opposite. Reusch and Williams 1998 found that Asian mussels reduced eelgrass growth, particularly in sparse and patchy beds. The eelgrass in my study site was patchy, particularly during the summer months when temperatures regularly exceeded 25 °C, which is warm enough to stress eelgrass (Nejrup and Pedersen 2008). Thus, it is surprising that I did not find that Asian mussels negatively affected the patchy, stressed eelgrass at my study site. It is reasonably likely that growth of Asian mussel populations needs to be observed over a longer period to result in changes in eelgrass biomass. Additionally, in my experiment mussels may not have had a strong effect on eelgrass due to high mussel mortality.

My experiment occurred over a relatively short period (8 – 9 weeks), and I may have observed more changes over a longer period. For example, animals may have migrated to my experiments on different timetables. Mobile worms may be able to access my plots relatively quickly, while bivalves, which are less mobile, may have had lower migration rates to the plots. However, previous experiments have used similar timetables to evaluate Asian mussel effects on bivalve recruitment, such as Castorani and Hovel 2015, which lasted 11 weeks. And, in experiments testing recolonization of polychaetes after defaunation in Mission Bay, a few polychaete species began to return within 2 weeks of defaunation (Levin 1984). In my experiment, likely adult bivalves colonized my plots, instead of juveniles, as I did not find very small life stages of bivalves.

In 2022, much of the eelgrass at my site died off considerably over the course of the summer, and overall abundance and diversity of native fauna was lower. Additionally, only five native bivalves were found across all plots in 2022, and none in control plots, which represent the unmanipulated eelgrass bed. This is surprising given that eelgrass beds are generally rich habitats for many types of bivalves including mussels, oysters, clams, and scallops. In my study area of Mission Bay, San Diego, CA, previous studies have documented many bivalve species living in the eelgrass beds, such as *Tresus nuttallii, Donax gouldii,* and *Leukoma staminea* (Castorani and Hovel 2015). The eelgrass die-off in 2022 may have reduced the population of native infauna, or they may have been reduced by the same factors that led to eelgrass declines. Likely temperature, which was higher in 2022 than average, contributed to the declines of both eelgrass and associated fauna. Eelgrass die-offs begin when water temperatures are above 25 °C (Nejrup and Pedersen 2008), and the mean temperature in 2022 was 24.8 °C. Additionally, I may have missed some of the infauna species if they were dwelling deeper in the sediment than my core sampler (10 – 20 cm) reached.

Asian mussel mortality was high in both years of my experiment even though I used cages to exclude predators. In fact, I did not see a significant difference in Asian mussel numbers between caged and uncaged control plots, indicating that at my site predation may not be a major driver of mortality (Supplemental Figure 3.1). However, there was a significantly higher organismal abundance in caged plots than in my cage-free plots in experiment II, potentially indicating predators being attracted to mussels. Despite the successful invasion of Asian mussels into Southern California eelgrass beds, other studies have found that transported Asian mussels experience high mortality. Reusch and Williams 1998 found high mortality of mussels transported into established eelgrass beds. However, they found higher survival when mussels were transported into sparser eelgrass. In my experiment, mortality was not affected by eelgrass removal treatment, even though I expected my removal treatment to create an opportunity for the Asian mussels to establish where they may otherwise have faced competition from eelgrass. Castorani and Hovel 2015 found 42% ± 23% mortality of Asian mussels in an experiment similar to mine where Asian mussels were transported to caged plots in Mission Bay. The high mortality across treatments in my experiment may have been a result of stress during harvest and transport to the experimental site, or due to removal from the mats they naturally grow in. Stress and mortality associated with mussel removal and transport has been observed in other systems (Nguyen et al. 2020). Additionally, although previous studies have postulated that eelgrass and Asian mussels compete directly for space in Mission Bay (Reusch and Williams 1998), this might not be the case. I frequently observed mats of Asian mussels growing among dense eelgrass rhizomes. The species often coexist, as demonstrated by the robust populations of both species in Mission Bay for many years.

Because so many Asian mussels were lost over the course of the experiment, my mussel treatment may not have been consistent across plots. This may be why my mussel treatment did not have as strong effects on fauna communities as I would have expected. However, even with this mortality I did see some mussel effects on my response variables. Future studies examining faunal communities in transported mussel beds may need to use very high quantities mussels and assume that significant mortality will occur, as it did in my and previous experiments. A final caveat of my experiment is that during the experiment, some cages were toppled and later righted when I checked on them, potentially giving predators an opportunity to enter cages and affect the mussel treatment.

In 2021, many plots had high densities of brittle stars, up to 38 per plot. High densities of brittle stars may lead to increases in eelgrass mortality (Altstatt 2005). Brittlestars in large numbers can lead to eelgrass mortality by smothering and scraping eelgrass shoots and altering sediment dynamics and rhizome growth (Altstatt 2005). Additionally, while *O. spiculata* is primarily a filter feeder, it also scavenges and may have consumed other fauna in my experiment (Carlton 2007).

I found that annelids tended to appear in higher abundances in disturbed plots. Annelids may have been inhibited by eelgrass rhizomes and therefore colonized gaps caused by my removal treatment. Annelids are a diverse group, and effects of eelgrass may also vary across taxa. For example, Omena and Creed 2004 found that for seagrass *Halodule wrightii*, surface-feeding polychaetes were associated with longer shoots, while suspension feeders were associated with more extensive rhizomes. The annelids I examined were all mobile polychaetes or oligochaetes, which have different life histories and feeding modes than tube building polychaetes; while tube-dwellers are primarily sedentary filter feeders (Levin 1982), mobile polychaetes and oligochaetes can move around to capture food as a predator or grazer (Rosa et al. 2008). Future studies should examine annelids in greater taxonomic resolution to understand how non-native species and habitat disturbance may affect their diversity and abundance.

As coastal marine ecosystems change under stressors like habitat loss and non-native species, it is important to consider how these stressors may interact, and how non-native ecosystem engineers may change ecosystems experiencing habitat loss. My results indicate limited interactive effects, but that Asian mussels may affect diversity and abundance of local species. Future studies should consider how other stressors like warming, ocean acidification and coastal pollution may interact with Asian mussel and habitat loss effects.

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Tables and Figures

Table 3.1. List of taxa identified in each experiment, and associated abundance

Taxa	Year	Abundance across all plots	Mean abundance per plot (+/- SE)
Ophiothrix spiculata	2021	329	8.2 (+/- 1.5)
Mobile polychaetes and oligochaetes		192	4.8 (+/- 2.4)
Paracerceis sculpta		133	3.3 (+/- 0.8)
Macoma sp.		58	1.5 (+/- 0.2)
Ampithoe sp.		46	1.2 (+/- 0.4)
Volvarina taeniolata		34	0.9 (+/- 0.5)
Bulla gouldiana		13	0.3 (+/- 0.2)
Ostrea lurida		4	0.1 (+/- 0.05)
Amphipoda		2	0.05 (+/- 0.03)
Bivalvia		2	0.05 (+/- 0.05)
Chione sp.		2	0.05 (+/- 0.03)
Laevicardium substriatum		2	0.05 (+/- 0.03)
Fish		2	0.05 (+/- 0.03)
Crustacea		1	0.025 (+/- 0.025)
Gastropoda		1	0.025 (+/- 0.025)
	2022		
Mobile polychaetes and oligochaetes		87	2.3 (+/- 0.5)
Volvarina taeniolata		24	0.6 (+/- 0.2)
Ophiothrix spiculata		7	0.2 (+/- 0.1)
Chione sp.		2	0.05 (+/- 0.04)
Macoma sp.		2	0.05 (+/- 0.04)
Mytilus sp.		1	0.03 (+/-0.03)

Table 3.2. Mean abundance of infauna occurring per plot, organized by treatment. These numbers do not include Asian mussels.

Year	Asian mussel treatment	Disturbance treatment	Mean	SE
2021	Mussels added	Disturbed	25.1	5.4
	Mussels added	Undisturbed	21.4	6.2
	No mussels	Disturbed	33.3	21.5
	No mussels	Undisturbed	11.4	2.1
2022	Mussels added	Disturbed	6.2	1.5
	Mussels added	Undisturbed	7.3	3.7
	Mussel mimic	Disturbed	5.5	0.5
	Mussel mimic	Undisturbed	4.5	1.5
	No mussels	Disturbed	1.3	0.3
	No mussels	Undisturbed	2.1	0.9

Table 3.3. Results of linear models describing the relationship between experimental treatments and response variables (organismal abundance, taxon richness, and Shannon diversity) for experiment I.

Variable	Treatment	Df	MS	F value	p value
Organismal abundance	Mussels	1	4.81	4.69	**0.04**
	Disturbance	1	0.79	0.77	0.39
	Mussels * Disturbance	1	0.02	0.02	0.90
	Cage	1	0.45	0.44	0.51
	Transect Position	1	0.10	0.10	0.76
	Residuals	34	1.03		
Taxon Richness	Mussels	1	9.20	3.13	**0.09**
	Disturbance	1	10.36	3.52	**0.07**
	Mussels * Disturbance	1	4.17	1.42	0.24
	Cage	1	1.00	0.34	0.56
	Transect Position	1	2.18	0.74	0.40
	Residuals	34	2.94		
Shannon diversity	Mussels	1	0.32	1.55	0.22
	Disturbance	1	0.34	1.66	0.21
	Mussels * Disturbance	1	0.05	0.24	0.63
	Cage	1	0.05	0.27	0.61
	Transect Position	1	0.21	1.04	0.31
	Residuals	34	0.21		

Table 3.4. Results of linear models describing the relationship between experimental treatments and response variables (organismal abundance and taxon richness) for experiment II.

Variable	Treatment	Df	MS	F value	p value
Organismal abundance	Mussels	2	4.30	4.16	**0.03**
	Disturbance	1	0.02	0.01	0.90
	Mussels * Disturbance	2	2.94	2.84	**0.07**
	Cage	1	1.44	1.40	0.25
	Transect Position	1	1.70	1.64	0.21
	Residuals	30	1.03		
Taxon Richness	Mussels	2	2.96	2.96	**0.07**
	Disturbance	1	0.19	0.19	0.66
	Mussels * Disturbance	2	0.96	0.96	0.40
	Cage	1	0.81	0.81	0.37
	Transect Position	1	0.78	0.78	0.38
	Residuals	30	1.00		

Table 3.5. Results of PERMANOVAs and pairwise posthoc test to test for differences among communities by treatment.

Year	*Parameter*	*Df*	*Sum sq*	*R^2*	*F value*	*p-value*
2021	Mussels	1	530.38	0.04	1.50	0.14
	Disturbance	1	742.66	0.05	2.10	**0.06**
	Mussels*Disturbance	1	209.17	0.01	0.59	0.79
	Cage	1	142.00	0.01	0.40	0.65
	Transect Position	1	434.46	0.03	1.23	0.32
	Residual	34	12013.46	0.85		
2022	Mussels	2	75.64	0.21	2.92	**0.04**
	Disturbance	1	2.42	0.01	0.19	0.84
	Mussels*Disturbance	2	12.89	0.04	0.50	0.67
	Cage	1	1.99	0.01	0.15	0.85
	Transect Position	1	11.89	0.03	0.92	0.36
	Residual	20	259.20	0.71		
			Posthoc test			
2022	Mussels vs mussel mimic	1	0.29	0.07	1.28	0.27
	Mussels vs no mussels	1	0.79	0.16	3.34	**0.03**
	Mussel mimic vs. no mussels	1	0.55	0.13	2.33	0.07

Figure 3.1. Map of study site at Fiesta Island in Mission Bay, San Diego, CA. Map created by C. Bickley. The red point on map marks Hidden Anchorage, my study site.

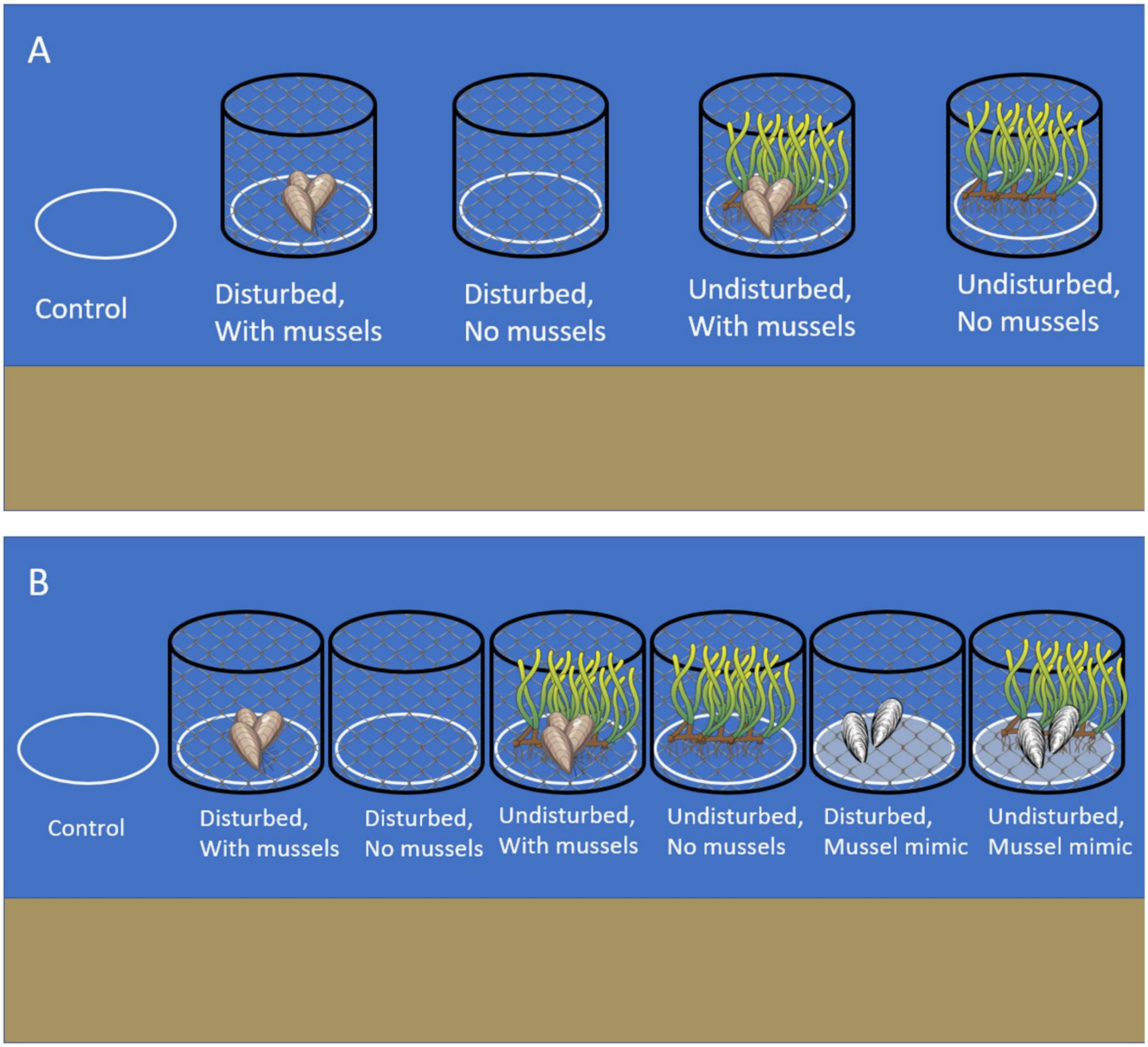


Figure 3.2. A. Experimental set-up in the first year, summer 2021. Treatments included control, disturbed/undisturbed, mussels/no mussels. **B.** Experimental set-up in the second year, 2022. Treatments included: control, disturbed/undisturbed, and no mussels/mussels added/mussel mimic. All factors in both years were crossed factorially.

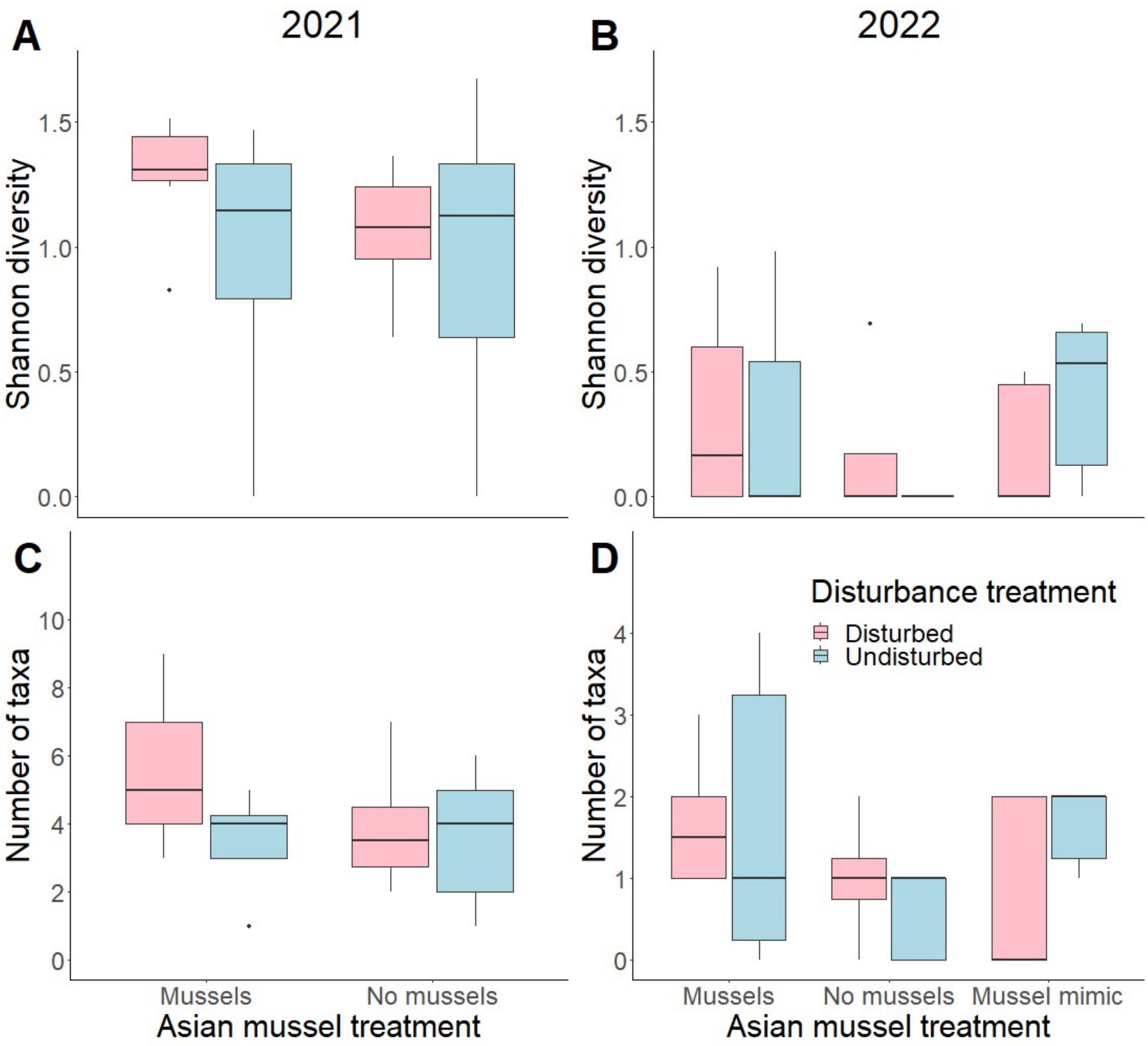


Figure 3.3. A. Shannon diversity of infauna in 2021, **B.** Shannon diversity of infauna in 2022, **C.** Taxon richness by treatment in 2021 **D.** Taxon richness by treatment in 2022. Boxplots are color coded by disturbance treatment. The midline of each boxplot represents the median, while the box represents the middle two quartiles of data, and the vertical lines represent the upper and lower quartile of data.

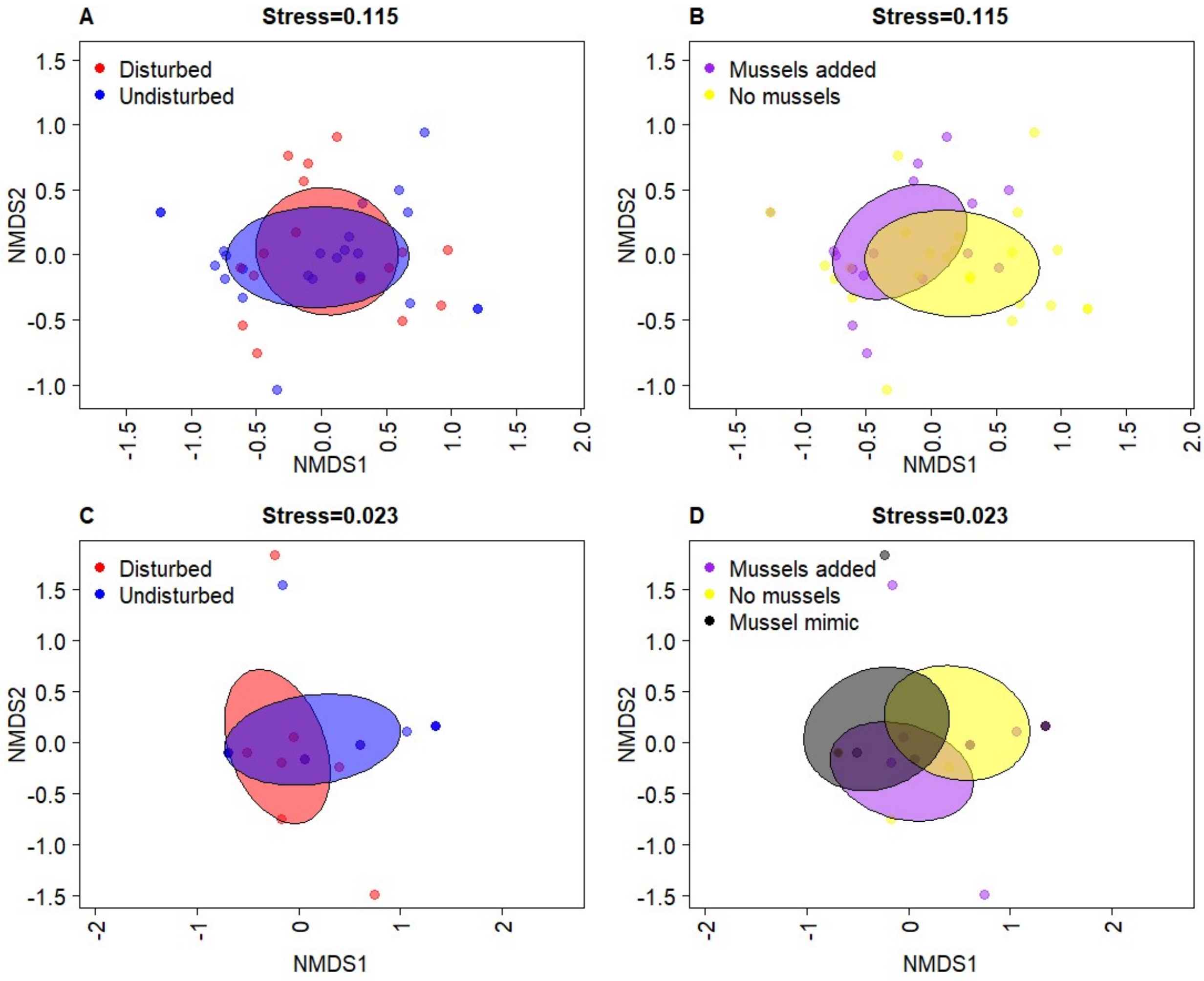


Figure 3.4. A. nMDS plots for all fauna in 2021. Color corresponds to disturbance treatment. **B.** nMDS for 2021, color corresponding to mussel treatment. **C.** nMDS for 2022, color corresponding to disturbance treatment. **D.** nMDS for 2022, color corresponding to mussel treatment, including added mussel mimic treatment in 2022.

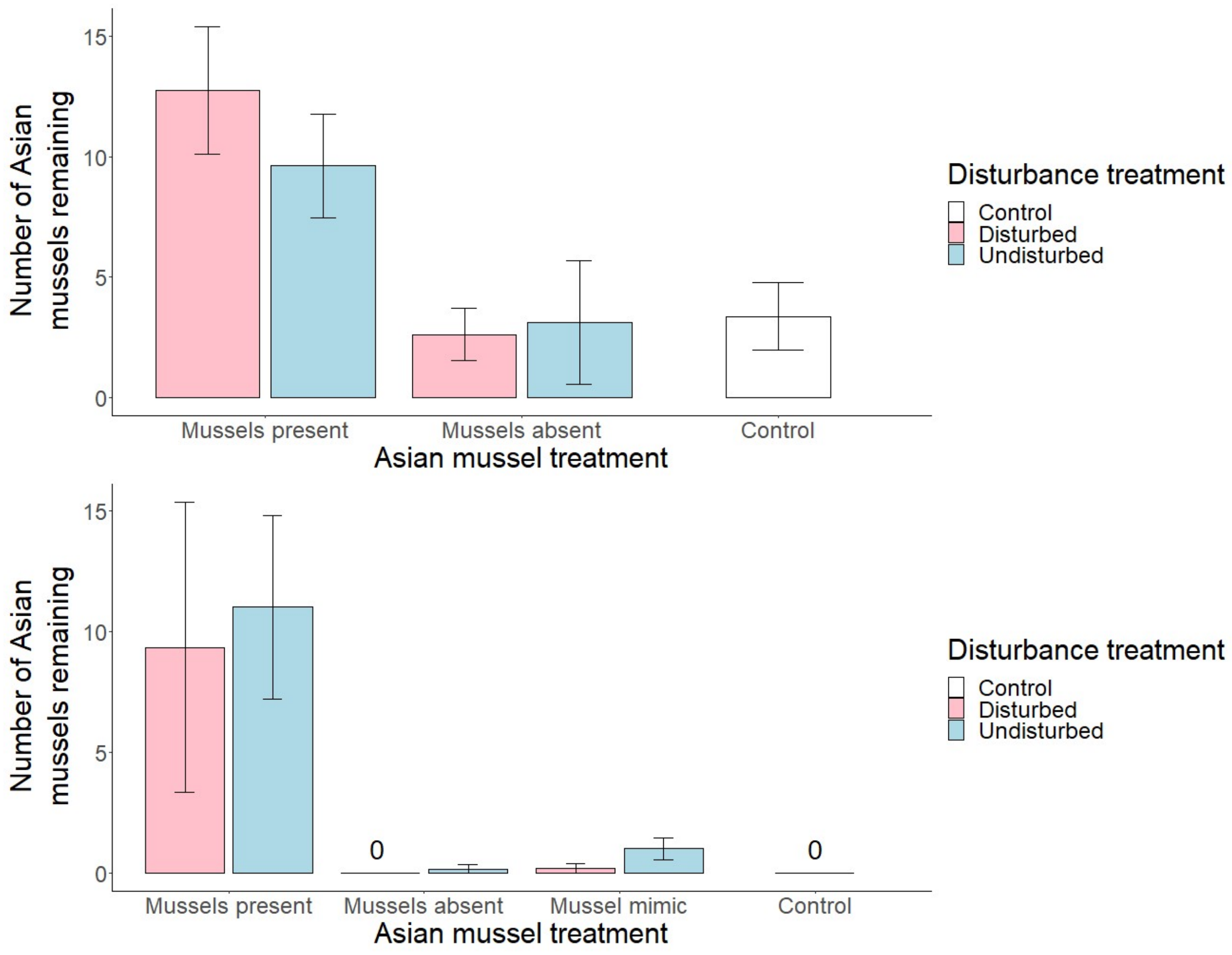
Supplemental Materials

Supplemental Table 3.1. Results of linear models testing Asian mussels remaining at the end of the experiment by disturbance, Asian mussel and cage treatments, along with transect position.

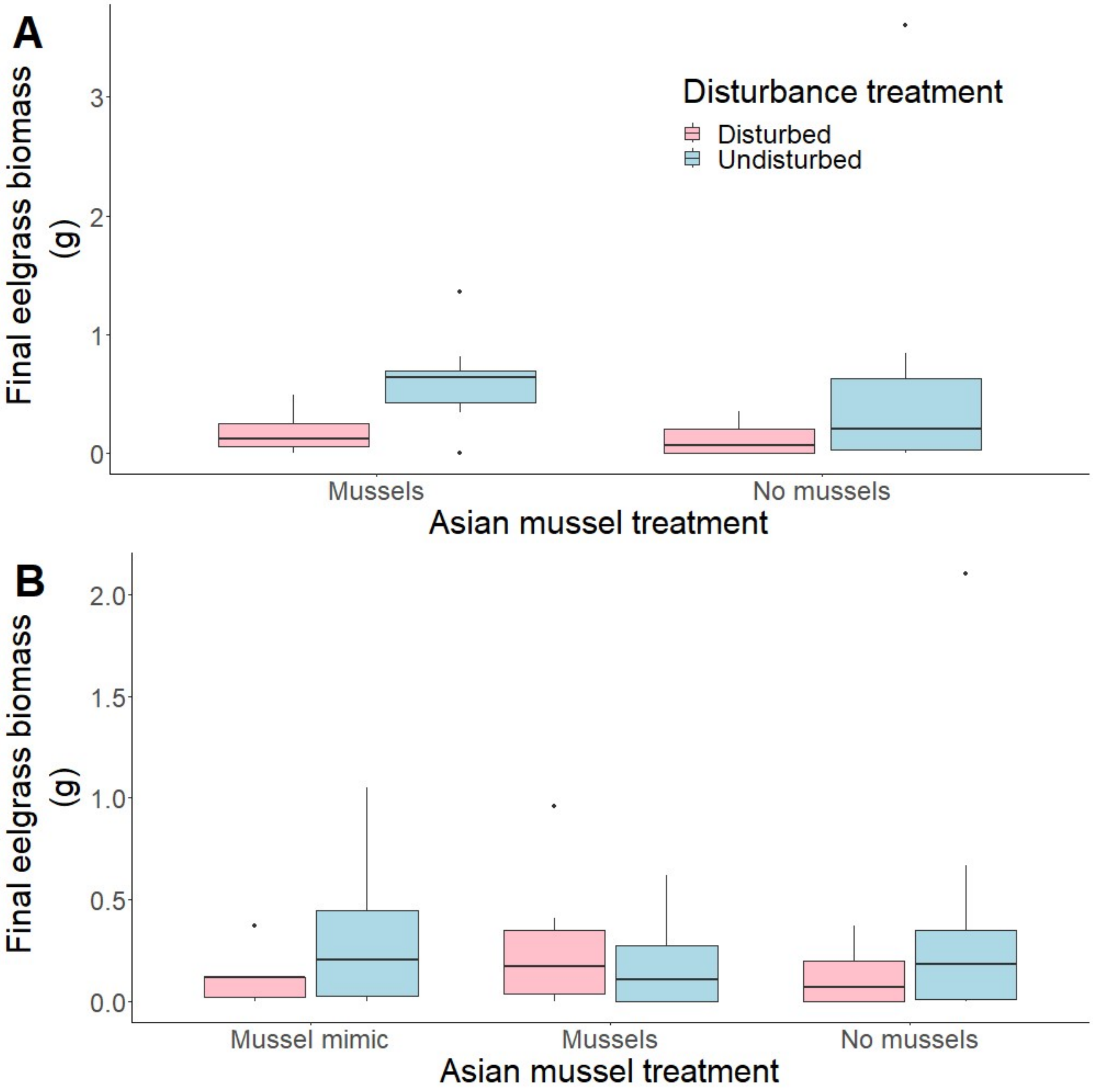
Year	Parameter	Df	MS	F value	p-value
2021	Mussels	1	594.67	23.91	**<0.001**
	Disturbance	1	51.34	2.06	0.16
	Mussels * Disturbance	1	39.66	1.59	0.22
	Cage	1	6.11	0.25	0.62
	Transect Position	1	335.11	13.48	**<0.001**
	Residuals	34	24.87		
2022	Mussels	2	406.27	8.29	**0.001**
	Disturbance	1	5.31	0.11	0.74
	Mussels * Disturbance	2	1.57	0.03	0.97
	Cage	1	0.78	0.02	0.90
	Transect Position	1	0.17	0.00	0.95
	Residuals	32	46.03		

Supplemental Table 3.2. Results of linear models testing final eelgrass dry biomass by disturbance, Asian mussel and caging treatments, as well as transect position.

Year	Parameter	Df	MS	F value	p-value
2021	Mussels	1	0.06	0.20	0.66
	Disturbance	1	1.57	5.74	**0.02**
	Mussels * Disturbance	1	0.16	0.58	0.45
	Cage	1	1.05	3.85	**0.06**
	Transect Position	1	2.53	9.26	**0.004**
	Residuals	34	0.27		
2022	Mussels	2	0.02	0.09	0.92
	Disturbance	1	0.13	0.70	0.41
	Mussels * Disturbance	2	0.07	0.39	0.68
	Cage	1	0.38	2.11	0.16
	Transect Position	1	0.00	0.00	0.95
	Residuals	30	0.18		



Supplemental Figure 3.1. A. Number of Asian mussels in all plots at the end of the experiment in year 1, organized by treatment. Color indicates eelgrass disturbance treatment. The initial number of Asian mussels in each Asian mussel treatment plot was 50. **B.** Number of Asian mussels surviving at the end of the experiment in year 2. Initial number of mussels per treatment plot was 75, with 16 added during the experiment. Error bars represent ± SE.



Supplemental Figure 3.2. Final dry eelgrass biomass by treatment for **A.** 2021 and **B.** 2022. Color indicates eelgrass disturbance treatment. The midline of each boxplot represents the median, while the box represents the middle two quartiles of data, and the vertical lines represent the upper and lower quartile of data.

Conclusions

My dissertation focused on how interactions between eelgrass (*Zostera marina*), an important foundation species, and bivalves are affected by introduced species, temperature, eutrophication and habitat loss. My findings generally indicate strong responses by eelgrass fauna to temperature and resilience to species invasions, and have implications for future research, conservation and restoration efforts.

In chapter 1, I investigated the effects of a non-native eelgrass on Manila clams in Puget Sound, WA. I found that compared with unvegetated mudflats and native eelgrass, non-native eelgrass did not have any effect on Manila clam growth, survival, reproductive status or condition. I concluded that non-native eelgrass is unlikely to have negative effects on local Manila clam growing operations. However, future studies should consider how non-native eelgrass may affect the act of harvesting, such as entanglement of equipment or difficulty locating clams among eelgrass beds (Ferriss et al. 2019). These aspects were not part of my study. Additionally, the experiment revealed strong effects of predation on Manila clam survival, but I was not able to test whether predation varied by habitat type, which could be a salient direction of new research.

In chapter 2, I conducted laboratory mesocosm experiments to examine how temperature and eutrophication altered interactions between eelgrass and clams. Previous studies have varied in finding negative (Vinther and Holmer 2008) vs. positive (Peterson and Heck 2001) effects of bivalves on eelgrass, and I posited that these effects may be context-dependent. I also conducted experiments to compare effects of infaunal clams and an epifaunal mesograzer common to local seagrass beds, snail *Alia* *carinata*, which is known to benefit eelgrass by grazing on epiphytes (Voigt and Hovel 2019). I did not find an effect of clams on eelgrass under any conditions, contrary to my hypothesis that clams would have positive effects on eelgrass under high temperature, eutrophic conditions. However, this result may not hold true if eutrophication causes greater light limitation than I was able to implement in my experiments. I did find evidence that snails may alleviate warming stress for eelgrass, indicating that they could help eelgrass persist under climate change. Future studies should examine the mechanisms behind interactions between eelgrass, clams and grazers, such as by measuring water clarity and sediment composition.

In chapter 3, I examined how the loss of a native foundation species combined with the addition of a non-native foundation species affected infauna in a San Diego eelgrass bed. I hypothesized that the addition of Asian mussels, a non-native species that adds structure by forming mats (Crooks 1998), would compensate for eelgrass loss by creating new habitat for eelgrass infauna. I also tested the effect of a mussel mimic to inspect structural effects of mussels. I did not find an interactive effect between eelgrass loss and Asian mussels on infauna, and in general found limited evidence for an effect of habitat loss. I found higher abundance of native fauna associated with Asian mussels, potentially indicating that mussels can create habitat, although this effect did not interact with habitat loss to indicate that mussels compensate for eelgrass loss. I experienced high mortality of mussels in my experiments, which may have affected the strength of the mussel treatment.

My findings contribute to a significant body of literature indicating that temperature is an important factor affecting marine species (Venegas et al. 2023). In chapter 1, I found that Manila clam growth responded more strongly to temperature than to habitat type. Clam growth was positively associated with temperature, likely because the higher temperatures in the range we observed were closer to optimal growth temperatures for Manila clams (Han et al. 2008). In chapter 2 I found that eelgrass growth and survival declined with temperature stress, and that clam survival was reduced under high temperatures. In chapter 3, I witnessed a major die-off of eelgrass due to extreme temperatures. As our oceans continue to warm under the influence of climate change, my results indicate that eelgrass and bivalves at the edge of their temperature range will likely continue to suffer declines.

In my studies, native species were generally resilient to effects of introduced species. In chapter 1, I observed that Manila clam growth, survival, reproductive status and condition were not negatively affected by an introduced eelgrass, despite opposite prevailing opinions among clam growers (Washington State Noxious Control Weed Board 2012). In chapter 3, I found that introduced Asian mussels were generally associated with higher abundances of native fauna. Given that the species tested in both experiments are ecosystem engineers, I expected them to have major impacts when invading a new system (Jones et al. 1994). Asian mussels form mats that trap sediments and alter water flow dynamics (Crooks 1998), while the spread of *Z. japonica* converted unvegetated habitat to vegetated, creating an entirely different ecosystem. The fact that I did not observe effects of these invaders while previous studies have (Reusch and Williams 1998; Tsai et al. 2010) may be due to experimental design, or resilience of local fauna to changes wrought by new species. These results join a growing body of literature suggesting that some introduced species have positive effects on native fauna within their new ecosystem (eg. Crooks 2002; Wonham et al. 2005). Future research should investigate whether there are certain traits or environmental conditions which could be used to predict the effects of an introduced species in a given setting.

My results have potential implications for eelgrass restoration and conservation and bivalve aquaculture. Eelgrass restoration efforts may benefit from including epifaunal grazers such as snails, shrimp, and amphipods in their plans, which may boost eelgrass resistance to warming temperatures. Additionally, collecting and adding these epifaunal grazers would be relatively easy to do, given their abundance and small body size (Hughes et al. 2004). Future researchers should consider testing the use of epifaunal grazers in eelgrass restoration in a field experiment. In terms of aquaculture, my results indicate that bivalve aquaculture and eelgrass may be able to coexist, despite concerns that eelgrass may negatively affect bivalves (Washington State Noxious Weed Control Board 2012). While my study (chapter 1) involved a non-native eelgrass, this finding has implications for interactions between native eelgrass and bivalve aquaculture, with the possibility that the two could coexist to the mutual benefit of both.

As human-induced changes continue to worsen in coastal marine ecosystems, research examining how these changes impact foundation species and species interactions will be ever more important. Like my third chapter on interactions between habitat loss and species invasions, future studies should also examine how human-induced impacts interact with each other, and the consequences of these interactions for marine ecosystems. The insights gained from this research will be crucial to informing successful restoration and conservation of marine ecosystems in the Anthropocene.

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