Context-Dependent Species Interactions in Seagrass Ecosystems

By

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A unifying observation in ecology is that environmental context can play a key role in regulating the sign, strength, and mechanisms of species interactions. Estuaries and coastal seas are ideal ecosystems for exploring the impacts of environmental variability on ecological interactions because they are characterized by strong, dynamic gradients of abiotic conditions, such as physical disturbance and biogeochemical stress. Estuaries also are heavily impacted by biotic disruptions, including overexploitation of top predators and invasion by non-native species. For my dissertation, I explored the effects of abiotic and biotic environmental context on species interactions in subtidal and intertidal meadows of eelgrass Zostera marina, a marine angiosperm and key habitat-forming species in temperate estuaries worldwide, in three unique chapters.

First, I tested how disturbance mediates competition between eelgrass and the burrowing ghost shrimp Neotrypaea californiensis in several California estuaries by conducting a series of transplantation and disturbance experiments. I found that both live eelgrass and structural mimics of belowground eelgrass tissues cause rapid declines in local ghost shrimp abundances, indicating that plant structure is an important mechanism in determining ghost shrimp distributions. However, ghost shrimp quickly colonize disturbances to eelgrass patch edges, which are common in shallow estuaries. Thus, coexistence is maintained by disturbance and a competition-colonization trade-off: eelgrass is competitively superior, but physical disturbances permit ghost shrimp coexistence at the landscape scale by modulating the availability of space.

Second, I explored how light availability influences the sign, strength, and mechanisms of interactions between eelgrass and blue mussels, Mytilus edulis. I hypothesized that light
availability regulates bivalve habitat modification and subsequent impacts on seagrasses by shifting net effects between alleviation of nutrient stress and intensification of sulfide stress. I manipulated light availability and blue mussel abundance in eelgrass mesocosms and measured biogeochemical and plant responses. I found that light modifies the effect of mussels on porewater ammonium, but eelgrass was not nutrient limited and, therefore, mussels did not enhance growth. Mussels increase sediment sulfides irrespective of light availability and, by reducing net oxygen flux (production and respiration), mussels and low light availability exacerbate sulfide intrusion of eelgrass tissues. Surprisingly, I found that sulfide stress did not affect plant growth, survival, or energy stores. Thus, habitat modification by mussels represents a risk to eelgrass, especially during low productivity conditions, but eelgrass can resist harm from short-term stress, even during light limitation. These findings suggest that while bivalve impacts on seagrasses may be variable in oligotrophic estuaries, they are likely negative in eutrophic systems, which are increasing globally.

Third, I examined how invasive mussels impact their native bivalve competitors through both direct effects and indirect, predator-mediated interactions. Competition is a major process by which introduced species impact native populations, communities, and ecosystems, but ecological theory predicts that invading prey can also interact with their native competitors indirectly through shared native predators. Nevertheless, indirect effects are largely overlooked in invasive species science and management. In southern California estuaries, introduction of the Asian mussel *Arcuatula senhousia* has been linked to profound changes in native bivalve assemblages. I performed three field experiments to assess the mechanisms of competition between *Arcuatula* and native bivalves, and evaluate the potential for *Arcuatula* to indirectly mediate native predator-prey dynamics. I found that *Arcuatula* reduces the diversity, abundance,
and size of native bivalve recruits by preemptively exploiting sediment surface space. When paired with native shallow-dwelling clams, *Arcuatula* reduces adult survival through overgrowth competition. However, *Arcuatula* also attracts native predators, indirectly increasing predation of native clams, especially poorly-defended species. Therefore, the vulnerability of invasive prey to predation can greatly exacerbate impacts on their native competitors.

Overall, my dissertation improves our basic understanding of the causes and consequences of variation in species interactions in shallow estuaries and coastal lagoons. Furthermore, this research enhances our ability to predict the impacts on species interactions caused by human-induced ecosystem disruptions, such as habitat destruction, eutrophication, and non-native species introductions, which are widespread concerns for estuaries and coastal seas.
INTRODUCTION

One of the most fundamental and persistent challenges in ecology is understanding the causes and consequences of variation in interactions among species. Across a wide range of communities and ecosystems, the sign, strength, and mechanisms of species interactions often vary under different abiotic and biotic conditions. For example, many environmental factors can mediate competition for limited resources by changing resource abundance and replenishment in time or space (Gause 1932, Hardin 1960, Hutchinson 1961). Physical disturbance can reduce the severity of competition and facilitate coexistence among strong competitors by creating spatial or temporal variation in resource availability (Connell 1978, Sousa 1979, Chesson and Huntly 1997, Roxburgh et al. 2004). Similarly, abiotic stress can regulate the strength of competition (Connell 1961, Grime 1973, Connolly and Roughgarden 1999) or facilitation (Clements et al. 1926, Odum 1969, Bruno et al. 2003). In many cases, changes in environmental stress can shift species interactions between competition and facilitation (Menge and Sutherland 1987, He et al. 2013), as in the case of competing salt-marsh plants that buffer one another from salinity or oxygen stress (Bertness and Callaway 1994, Bertness and Hacker 1994, Bertness and Leonard 1997). Abiotic context can also mediate the strength of predation by, for example, changing the foraging abilities of predators (Weissburg and Zimmer-Faust 1993) or the perceptive abilities of prey (Smee and Weissburg 2006).

Biotic factors can play equally important roles in mediating species interactions. Variation in the presence or abundance of consumers (e.g., herbivores, predators, parasites) is particularly well-known for altering the sign, strength, and mechanisms of species interactions (Hairston et al. 1960, Hatcher et al. 2006, Chesson and Kuang 2008). For example, keystone
predators can reduce competitive exclusion and increase biodiversity in the rocky intertidal by consuming dominant competitors for benthic space (Paine 1966, 1974). In other cases, higher trophic levels can regulate interactions among lower trophic levels by changing the abundance of intermediate consumers through trophic cascades (Estes and Palmisano 1974). The mere presence of a consumer can alter species relationships through trait-mediated indirect interactions (Werner and Peacor 2003) by changing the behavior of intermediate consumers (Trussell et al. 2002, 2003, Schmitz et al. 2004) or defenses in basal species (Leonard et al. 1999). The presence of basal species can also indirectly mediate herbivory or predation by changing consumer feeding preferences (Bracken et al. 2014).

In some cases, biotic context changes species interactions through complex, and sometimes unexpected, indirect pathways. Apparent competition occurs when one prey species indirectly harms another prey species by changing the abundance or foraging behavior of a shared predator (Holt 1977, Holt and Kotler 1987), as in the case of an invasive orchid that increases herbivory on co-occurring native orchids by attracting native flower-eating weevils (Recart et al. 2012). In cases of refuge-mediated apparent competition, habitat modification by plants provides a refuge from predation to herbivores and simultaneously increases herbivory on themselves or other nearby plants (Orrock et al. 2008, 2010, Dangremond et al. 2010). Habitat modification by basal species can also modify predation by increasing abiotic stress, as in the case of an invasive alga that induces hypoxia in native clams, causing them to emerge from the sediment (Wright et al. 2007, 2010), face a higher risk of predation (Wright et al. 2007, Byers et al. 2010), and indirect facilitate a community of hard-substrate epibionts (Gribben et al. 2009). Invasion by non-native ecosystem engineers (Jones et al. 1994, 1997) may be particularly likely to catalyze novel interactions among native species (Crooks 2002).
Shallow marine and estuarine ecosystems are excellent systems for exploring the impacts of environmental context on species interactions. These coastal margins are characterized by highly-variable disturbance regimes (e.g., swell and wind-waves, aerial exposure, erosion, sedimentation) and strong, dynamic abiotic gradients (e.g., light, salinity, dissolved oxygen, nutrients) that can produce stressful conditions (Denny 1988, Lapointe et al. 1994, Mullan et al. 2004, Reed et al. 2011). Estuaries and coastal seas are also heavily affected by biotic changes, such as frequent invasions by non-native species (Ruiz et al. 1997, 1999, Williams 2007, Williams and Grosholz 2008) and depletion of top predators and habitat-forming species (e.g., oysters) through overexploitation (Jackson et al. 2001, Wilberg et al. 2011, Baden et al. 2012).

Sessile or sedentary species, such as seagrasses (rooted marine angiosperms) and infauna, are particularly excellent study organisms because of their inability to evade varying environmental conditions and propensity to respond quickly to changes in resources, stress, or disturbance (Williams 1988, Williams and Ruckelshaus 1993, Edwards and Estes 2006, Reed and Hovel 2006, Walker et al. 2006, Byers et al. 2010).

Basic research on how environmental context affects species interactions is more than simply an academic pursuit because it can enhance our ability to understand and predict the impacts of human-induced ecosystem changes, such as physical disturbances (e.g., dredging, trawling, shoreline construction), abiotic stressors (e.g., climate change, eutrophication, hypoxia, wastewater and desalination discharge), and direct alteration of community structure (e.g., species introductions, overexploitation, aquaculture). These anthropogenic environmental impacts are widespread concerns for coastal marine and estuarine ecosystems and have considerable ecological and economic consequences (e.g., Cloern 2001, Orth et al. 2006, Waycott et al. 2009, Howarth et al. 2011).
For my dissertation, I explored the effects of three major types of environmental disruptions on species interactions: (1) physical disturbance, (2) abiotic resource stress, and (3) species introduction. All three studies were conducted in temperate shallow estuaries and coastal lagoons dominated by eelgrass, *Zostera marina* L., which is among the most abundant, broadly-distributed, and well-studied seagrass worldwide (Green and Short 2003, Moore and Short 2006). In the first chapter, I examined the ability of disturbance to mediate coexistence and competitive exclusion between two strong benthic ecosystem engineers: eelgrass and the burrowing ghost shrimp, *Neotrypaea californiensis*. In the second chapter, I evaluated the hypothesis that light availability regulates habitat modification by blue mussels, *Mytilus edulis*, and subsequent impacts on eelgrass by shifting net effects between alleviation of nutrient stress and intensification of sulfide stress. In the third chapter, I tested the potential for an invasive mussel, *Arcuatula senhousia*, to indirectly affect native bivalve competitors through shared native predators.

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Disturbance facilitates the coexistence of antagonistic ecosystem engineers in California estuaries

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Abstract

Ecological theory predicts that interactions between antagonistic ecosystem engineers can lead to local competitive exclusion, but disturbance can facilitate broader coexistence. However, few empirical studies have tested the potential for disturbance to mediate competition between engineers. We examined the capacity for disturbance and habitat modification to explain the disjunct distributions of two benthic ecosystem engineers, eelgrass Zostera marina and the burrowing ghost shrimp Neotrypaea californiensis, in two California estuaries. Sediment sampling in eelgrass and ghost shrimp patches revealed that ghost shrimp change benthic biogeochemistry over small scales (centimeters) but not patch scales (meters to tens-of-meters),
suggesting a limited capacity for sediment modification to explain species distributions. To
determine the relative competitive abilities of engineers, we conducted reciprocal
transplantations of ghost shrimp and eelgrass. Local ghost shrimp densities declined rapidly
following the addition of eelgrass and transplanted eelgrass expanded laterally into the
surrounding ghost shrimp-dominated areas. When transplanted into eelgrass patches, ghost
shrimp failed to persist. Ghost shrimp were also displaced from plots with structural mimics of
eelgrass rhizomes and roots, suggesting that autogenic habitat modification by eelgrass is an
important mechanism determining ghost shrimp distributions. However, ghost shrimp were able
to rapidly colonize experimental disturbances to eelgrass patch edges, which are common in
shallow estuaries. We conclude that coexistence in this system is maintained by
spatiotemporally-asynchronous disturbances and a competition-colonization trade-off: eelgrass is
a competitively-superior ecosystem engineer, but benthic disturbances permit the coexistence of
ghost shrimp at the landscape scale by modulating the availability of space.

Keywords: antagonism; bioturbation; competition; disturbance; ecosystem engineering;
eelgrass; estuary; ghost shrimp; habitat modification; seagrass; sediment biogeochemistry

Introduction

A fundamental challenge in ecology is to explain the coexistence of species competing
for limited resources (Gause 1932, Hutchinson 1961). Diverse assemblages of primary producers
and consumers may coexist despite intense competition for space (Paine 1966, Connell 1978),
even when strong competitors are organisms that create or modify habitat (ecosystem engineers,
Jones et al. 1994). For ecosystem engineers that modify the same abiotic resource in contrasting
ways, ecological theory predicts that antagonism can arise and result in local exclusion of inferior competitors (Hastings et al. 2007). However, general competition models show that inferior competitors may persist at the landscape scale when disturbances remove competitively-dominant species or change the availability of limiting resources (Sousa 1979, Roxburgh et al. 2004). Disturbances that are asynchronous in space and time, or disproportionately affect certain species (Chesson and Huntly 1997), should have a particular capacity for facilitating coexistence, such as in competition-colonization trade-off models (e.g., Levin and Paine 1974). Despite these theoretical advances, few studies have examined the causes and consequences of antagonism between ecosystem engineers or tested the potential for disturbance to mediate such competition (Jones et al. 2010).

Estuaries are ideal systems for testing how disturbance influences competition between ecosystem engineers because they are home to many different types of habitat-modifying species living within or emerging from the sediment, such as rooted plants, sessile invertebrates, and burrowing infauna (Jones et al. 1994). Shallow estuaries are also characterized by numerous natural (e.g., waves, erosion, sedimentation) and anthropogenic (e.g., dredging, vessel impacts, fishing, aquaculture) benthic disturbances that vary greatly in their frequency, spatial extent, and magnitude of impact (Walker et al. 2006).

We explored the potential for antagonistic ecosystem engineering and disturbance to explain disjunct distributions of two benthic species, eelgrass *Zostera marina* L. and the ghost shrimp *Neotrypaea californiensis* Dana 1854 (see photo in Appendix 1.A), which are conspicuous soft-sediment engineers that co-occur in estuaries along the west coast of North America from southern Alaska, USA to Baja California Sur, Mexico (MacGinitie 1934, McRoy 1968). Eelgrass produces a dense canopy of leaves and a thick mat of rhizomes and roots within
surface sediments. These biogenic structures alter hydrodynamics (Abdelrhman 2003), sediment accretion (Bos et al. 2007), belowground architecture (Marbà and Duarte 1998), and biogeochemical fluxes (Marbà et al. 2006). Ghost shrimp are highly-mobile burrowers, constructing complex networks of tunnels and chambers up to 90 cm deep (Dumbauld et al. 1996) and living in dense aggregations up to 500 individuals m$^{-2}$ (Posey 1986a). Continuous bioturbation by burrowing shrimps changes sediment resuspension (Siebert and Branch 2006), granulometry (Ziebis et al. 1996), and biogeochemistry (Webb and Eyre 2004). Many seagrasses are sensitive to sediment burial (Cabaçoa et al. 2008) and eelgrass growth can depend on sediment nitrogen (Williams and Ruckelshaus 1993). However, it is also possible that seagrass rhizomes and roots inhibit burrowing infauna (Orth et al. 1984). Thus, antagonistic modification of the benthic environment by eelgrass and ghost shrimp may result in competition for space, but disturbance may foster their apparent landscape-scale coexistence within California estuaries.

We empirically assessed the potential for disturbance to mediate the coexistence of antagonistic ecosystem engineers by addressing three sequential questions in this system: (1) Are eelgrass and ghost shrimp antagonistic ecosystem engineers? (2) What mechanisms explain their inverse spatial relationship? (3) Does disturbance facilitate coexistence? We first quantified observations of species distributions and measured habitat modification. Next, we assessed relative competitive abilities through reciprocal transplantation experiments. Lastly, we evaluated the capacity for benthic perturbations to mediate landscape-scale coexistence by conducting two disturbance experiments.
Methods

Study regions

Our study took place from June 2010 to June 2012 at Tomales Bay (38°10' N, 122°54' W) and Mission Bay (32°46' N, 117°14' W), located in northern and southern California, USA, respectively (Figs. 1.1A and 1.1B). Although separated by about 800 km, both estuaries exhibit typical Mediterranean seasonality in temperature and salinity, with winter freshwater inflow and hypersalinity during long, dry summers (Largier et al. 1997). Eelgrass and ghost shrimp are abundant throughout both bays in intertidal and shallow-subtidal areas where sandy beaches or mudflats are present.

Spatial relationship and sediment modification

To quantify the spatial relationship between eelgrass and ghost shrimp, we conducted intertidal surveys (wading at low tide) at Tomales Bay in summer 2010 and intertidal/subtidal surveys (scuba diving at high tide) at Mission Bay in summer 2012 (subtidal areas at Tomales Bay could not be accessed due to logistical constraints). At Tomales Bay, we surveyed 3 sites along the eastern shore: Hamlet, MacDonald, and Cypress Grove (Fig. 1.1A); at Mission Bay, we surveyed 1 site: Mariner’s Cove (Fig. 1.1B). At each site, we haphazardly chose 3 alongshore locations and conducted 50 m transects from the first co-occurrence of eelgrass and ghost shrimp along a depth gradient towards deeper water. Every 3 m along the transect line, we counted eelgrass shoots and ghost shrimp burrow mounds (i.e., burrow surface openings) in a 625 cm$^2$ quadrat. Using burrow surface openings as a proxy for ghost shrimp abundance is a rapid and nondestructive method that has been experimentally validated (Posey 1986b, Dumbauld and Wyllie-Echeverria 2003, Butler and Bird 2007). Burrow mounds scale linearly with ghost shrimp...
abundance because each ghost shrimp excavates a separate Y-shaped burrow, with 2 surface openings converging to a vertical network of tunnels and chambers (MacGinitie 1934, Griffis and Chavez 1988). If abandoned, burrows and their surface openings soon collapse (Swinbanks and Luternauer 1987, Dumbauld et al. 1996).

To characterize how eelgrass and ghost shrimp modify the sediments they occupy, we measured biogeochemical parameters over both small and large spatial scales (i.e., centimeters vs. meters to tens-of-meters; see diagram in Appendix 1.B) at Hamlet during summer 2010. To determine small-scale impacts of bioturbation, we collected sediment cores (1.5 cm diameter × 5 cm deep) from paired burrow mounds and adjacent non-mound areas (i.e., coring 0–1.5 cm and 3–4.5 cm from the burrow entrance, respectively; n = 12 pairs). To determine larger, patch-scale effects, we also collected sediment samples from within 12 haphazardly-selected (unpaired) eelgrass and ghost shrimp patches (defined here as discrete areas dominated by eelgrass or ghost shrimp, generally on the range of 400 cm² to > 100 m²; see photo in Appendix 1.A). Sediments were frozen and later analyzed for the percentage of fine sediments (wet-sieving at 63 µm), organic matter (loss-on-ignition at 550 °C for 24 h), and sediment ammonium (porewater + adsorbed) concentration (spectrophotometrically following extraction in KCl (Koroleff 1976)).

We tested for differences in sediment parameters over small scales using paired-samples t-tests and over patch scales using independent-samples t-tests. Prior to performing t-tests, as well as all statistical analyses used in other experiments (see below), we tested for homogeneity of variances using Cochran’s test and used normal probability plots to test for normality. When necessary, we log-transformed (ln $x + 1$) data to meet the assumptions of parametric analyses.
Reciprocal transplantation experiments

In June 2010, we transplanted eelgrass into ghost shrimp patches to determine (1) if eelgrass could persist in ghost shrimp patches, (2) whether the introduction of eelgrass affected the abundance of ghost shrimp, and (3) how sediment biogeochemistry changed following the introduction of eelgrass. This experiment and all others (see below) were conducted at one site, Hamlet, in Tomales Bay. We transplanted eelgrass as intact rhizomes into square plots of 354 leaf shoots m$^{-2}$ (the mean density at Tomales Bay) and approximately 100% cover. To prevent dislodgment by hydrodynamic forces, we anchored each rhizome using V-shaped bamboo staples (Davis and Short 1997). To determine whether the effect of eelgrass on ghost shrimp and sediment biogeochemistry depends on patch size, we conducted transplantations at 4 spatial scales: 0.02 m$^2$, 0.09 m$^2$, 0.25 m$^2$, and 1 m$^2$ (n = 5, except for the loss of one 0.09 m$^2$ plot). Small eelgrass patches such as these are common at the areas in which we worked, and although eelgrass exists in larger patches as well, these were not feasible to create. We also designated eelgrass and ghost shrimp control plots (1 m$^2$; n = 5), which were unmanipulated except for the addition of bamboo staples. Plots were located in haphazardly-selected ghost shrimp patches 25–30 m from shore (about 0.3 m below mean lower low water), spaced 2–3 m apart, and randomly assigned treatments.

We quantified eelgrass and ghost shrimp persistence through time by sampling plots during low tides over the course of 7 months (3, 7, and 30 weeks after transplantation). Eelgrass abundance was visually estimated as percent cover (to the nearest 100 cm$^2$). At 30 weeks, we also quantified lateral eelgrass expansion into the surrounding ghost shrimp patches by measuring the maximum distance of rhizome growth from the original transplantation edge. Ghost shrimp density was estimated by counting surface burrow openings within a haphazardly
placed 400 cm$^2$ quadrat (all burrows were counted in 0.02 m$^2$ plots). To determine if sediment biogeochemistry was affected by transplantation, we collected 5 sediment cores from haphazard locations in each plot after 3 and 30 weeks, and processed sediment samples as described above. We tested for differences in response variables using separate analyses of variance (ANOVAs; one per sampling period). For this experiment and others (see below) where ANOVAs revealed strong evidence of a treatment effect on dependent variables ($P < 0.05$), we made post-hoc pairwise comparisons using Tukey’s honestly significant difference test and adjusted post-hoc comparisons for unbalanced data using the Tukey-Kramer method (Kramer 1956).

For our second transplantation experiment, we tested (1) whether adult ghost shrimp could establish burrows in eelgrass patches, penetrating past rhizomes and roots, and if so, (2) how they persist over time in eelgrass patches relative to unvegetated sediments. Therefore, in Apr 2012, we haphazardly chose 8 separate eelgrass patches with adjacent unvegetated sediment that lacked burrows (all patches located about 20 m from shore and separated by > 10 m). At each separate patch ecotone, we established 2 plots (25 cm diameter): 1 on the eelgrass side and 1 on the unvegetated side, with all plots > 1 m from the edge. Then, elsewhere at the site, we extracted 16 ghost shrimp from their burrows using a hand pump and transplanted 2 ghost shrimp to each plot (n = 8 pairs of plots). To allow ghost shrimp to burrow in the absence of predation (e.g., from shorebirds), we protected each pair of ghost shrimp for 24 h with a galvanized-wire cage (25 cm diameter × 30 cm high; mesh size = 1.3 cm), buried to 15 cm depth. All ghost shrimp began burrowing immediately upon release. We assessed ghost shrimp persistence by counting surface burrow openings within each plot 24 h and 1 month after transplantation. We tested for differences in surface burrow mound density using separate paired-samples $t$-tests (one per sampling period).
Findings from reciprocal transplantation experiments suggested that eelgrass has strong and rapid negative effects on local ghost shrimp abundance. We hypothesized eelgrass rhizomes and roots exclude ghost shrimp by impeding the formation or maintenance of surface openings or other burrow structures (Brenchley 1982), or otherwise interfering with shrimp behavior. To evaluate this possibility, it was necessary to isolate the effects of belowground biogenic structure from other potential eelgrass impacts, such as changes to hydrodynamics (Abdelrhman 2003). To accomplish this, we created structural mimics of eelgrass rhizomes (see photo in Appendix 1.C) collected from high and low density areas (0.25 m$^2$ with 884 and 294 shoots m$^{-2}$, respectively) by tracing rhizomes on plywood and carving these out using a palm router with 3.175 mm hemispherical bit. We created castings within the separate high-density and low-density molds using marine-grade polyurethane adhesive (3M Marine Fast Cure Adhesive Sealant 5200, 3M Company, St. Paul, Minnesota, USA), which dries to a durable, flexible, negatively-buoyant solid. We simulated roots using nylon fibers (2–5 cm long) attached to rhizome castings with water-resistant spray adhesive (3M Hi-Strength 90 Spray Adhesive). We allowed generous curing times and soaked mimics in flowing seawater for 72 h prior to field deployment.

Next, in Apr 2012, we established circular plots (0.25 m$^2$; located 15–20 m from shore and spaced > 2 m apart) within haphazardly-selected ghost shrimp patches and randomly applied one of four treatments: true (unmanipulated) control, procedural control, low-density mimic, and high-density mimic (n = 6, except for the loss of 2 high-density mimics). We planted rhizome-root mimics 1–3 cm deep and secured them to the sediment with 5 metal garden stakes. For procedural control plots, we planted a high-density mimic, removed it, and added 5 stakes.
determine treatment impacts on ghost shrimp abundance, we counted surface burrow openings within plots before the manipulation, and one and two months post-manipulation. We tested for differences in ghost shrimp density using separate ANOVAs (one per sampling period).

_Eelgrass disturbance experiments_

To determine the effect of benthic perturbations on the coexistence of eelgrass and ghost shrimp, we conducted two eelgrass disturbance experiments. Ghost shrimp migrate laterally into new habitat by burrowing or by crawling along the sediment surface (Posey 1986b, Harrison 1987). Additionally, ghost shrimp colonization can occur through the recruitment of planktonic postlarvae (Feldman _et al._ 1997). In an attempt to distinguish among these mechanisms and test the importance of the spatial pattern of disturbance, we undertook (1) an eelgrass patch-interior disturbance experiment to test for ghost shrimp colonization via postlarval settlement, and (2) an eelgrass patch-edge disturbance experiment to test for lateral ghost shrimp colonization.

For the patch-interior disturbance experiment, we removed eelgrass from the center of moderately-sized patches ($\geq 5.25$ m diameter, i.e., with at least 3 m of eelgrass surrounding disturbances). We excavated all vegetation (shoots, rhizomes, and roots) by hand and were careful not to remove sediments or modify sediment elevation. We created patch-interior disturbances in July 2010 to provide the greatest potential for recruitment of postlarvae, because ghost shrimp settlement peaks in late-summer through early-fall (Dumbauld _et al._ 1996). We hypothesized ghost shrimp would be more likely to colonize larger disturbances than smaller ones because of the greater area of unoccupied substrate and longer duration for which that area was unvegetated (Petraitis and Latham 1999). Therefore, we also included the spatial scale of disturbance as a factor, producing square removals of 0.02 m$^2$, 0.09 m$^2$, 0.25 m$^2$, 1 m$^2$, and 2.25
m² (n = 5, except for the loss of one 2.25 m² plot due to damage from drifting oyster aquaculture bags), which correspond to the size of common eelgrass disturbances (Walker et al. 2006). We selected eelgrass patches haphazardly (20–30 m from shore and separated by > 3 m at their nearest point) and assigned treatments randomly, including undisturbed eelgrass and ghost shrimp control plots (1 m²; n = 5). We assessed eelgrass recovery and ghost shrimp colonization through time by sampling plots during low tides over the course of 9 months (3, 7, 30, and 43 weeks after disturbance), measuring eelgrass abundance, ghost shrimp density, and sediment biogeochemistry as described above. We tested for differences in all response variables using separate ANOVAs (one per sampling period).

For the eelgrass patch-edge disturbance experiment (Apr–June 2012) we utilized a randomized complete block design. We haphazardly chose 12 eelgrass patches (all located 20–30 m from shore and > 10 m apart) and established a block at each patch (n = 12). Each block (1 m × 1 m) straddled the ecotone and contained 4 square plots (0.25 m²), each with a separate treatment (see diagram in Appendix 1.D). On the eelgrass side of the block, we randomly designated one plot as an eelgrass control and the other plot as an eelgrass disturbance, removing eelgrass as described above. On the ghost shrimp side of the block, both plots served as controls: one adjacent to the eelgrass control plot (‘ghost shrimp control 1’) and the other bordering the eelgrass disturbance plot (‘ghost shrimp control 2’). To evaluate ghost shrimp colonization, we measured burrow surface openings within each plot before the disturbance, and four and eight weeks post-disturbance. We tested for differences in ghost shrimp density using separate two-way ANOVAs (treatment and blocking factors as main effects) for each sampling period.
Results

Spatial relationship and sediment modification

Confirming our early observations (see photo in Appendix 1.A), surveys at Tomales Bay and Mission Bay revealed a strong inverse relationship between ghost shrimp and eelgrass densities (Figs. 1.1C and 1.1D). Ghost shrimp density declined precipitously between about 50–100 shoots m\(^{-2}\) and few ghost shrimp burrows were present beyond approximately 300 shoots m\(^{-2}\).

Sediment biogeochemical parameters displayed consistent differences over small spatial scales but not patch scales (Appendix 1.B). Sediments collected from burrow mounds contained less fine sediment \((t_{10} = 2.413, P = 0.036)\), organic matter \((t_{9} = 3.131, P = 0.012)\), and ammonium \((t_{11} = 8.616, P < 0.001)\) than adjacent non-burrow-mound sediments. In contrast, there were no differences for any of these parameters in sediments collected from haphazardly-selected ghost shrimp and eelgrass patches (Appendix 1.E).

Reciprocal transplantation experiments

Within the first 3 weeks transplanted eelgrass declined slightly from 100% cover (likely due to transplantation shock; Zimmerman et al. 1995) but nearly all transplantations persisted in ghost shrimp patches throughout the experiment except for the smallest size \((0.02 \text{ m}^2)\), which died out between 7 and 30 weeks (Fig. 1.2B; Table 1.1; Appendix 1.F). Eelgrass cover in transplantations was generally less than eelgrass control plots but more than ghost shrimp control plots. At the end of the experiment, surviving eelgrass transplantations had expanded into the surrounding ghost shrimp patches at an average rate of 39.3 cm \(y^{-1}\) \((± 25.6 \text{ SD})\).
Transplanted eelgrass had rapid negative impacts on local ghost shrimp abundances (Fig. 1.2A; Table 1.1; Appendix 1.F). Prior to manipulation, ghost shrimp density was apparently uniform among all eelgrass transplantation plots. Three weeks following transplantation, ghost shrimp density in smaller transplantations (0.09 m² and 0.02 m²) was no different than in ghost shrimp control plots ($P = 0.794$ and $P = 0.108$, respectively), but ghost shrimp density in larger transplantations (1.00 m² and 0.25 m²) was lower than that of ghost shrimp control plots ($P = 0.003$ and $P = 0.019$, respectively). However, after 7 weeks all transplantation plots, regardless of size, had lower ghost shrimp densities than ghost shrimp control plots ($P < 0.001$). This pattern persisted through 30 weeks. In contrast, sediment parameters did not differ among treatments at 3 or 30 weeks (Appendix 1.F), suggesting that temporal patterns of ghost shrimp density were not caused by changes to sediment biogeochemistry.

In the ghost shrimp transplantation experiment, nearly all transplanted ghost shrimp successfully burrowed within 24 h (Fig. 1.2C) and there was no difference in the density of burrow surface openings between plots in eelgrass and bare-sediment patches ($t_7 = 2.049, P = 0.080$). However, after 1 month eelgrass plots had fewer burrow mounds than bare-sediment plots ($t_7 = 3.969, P = 0.005$).

**Rhizome-root structural mimic experiment**

Rhizome-root structural mimics caused decreases in ghost shrimp density over time (Fig. 1.3; Table 1.1; Appendix 1.G). Ghost shrimp density did not vary among treatments before manipulation ($P = 0.546$). However, after 4 weeks ghost shrimp density was lower in high-density rhizome-root mimic plots than either true control or procedural control plots ($P = 0.025$ and $P = 0.006$, respectively). After 8 weeks, both high-density and low-density rhizome-root
mimic plots had lower ghost shrimp density than true control and procedural control plots ($P < 0.001$), but they did not differ from one another ($P = 0.664$). Ghost shrimp density did not differ between true control and procedural control plots after 4 or 8 weeks ($P = 0.859$ and $P = 0.843$, respectively), indicating no effect of the planting procedure.

*Eelgrass disturbance experiments*

In the eelgrass patch-interior disturbance experiment, ghost shrimp generally failed to colonize disturbances of any size (Fig. 1.4A; Table 1.1; Appendix 1.H). For all time periods, ghost shrimp density was higher in ghost shrimp control plots than in all other treatments. Eelgrass largely recovered from patch-interior disturbances within 43 weeks (Fig. 1.4B; Table 1.1; Appendix 1.H). During the majority of the experiment, eelgrass recovery was uniform: there was no difference in eelgrass cover among different-sized disturbance plots at 3, 7, and 30 weeks following disturbance ($P \geq 0.092$). By 43 weeks, however, eelgrass cover in smaller disturbances ($0.02 \text{ m}^2$, $0.09 \text{ m}^2$, and $0.25 \text{ m}^2$) was equivalent to that in eelgrass control plots ($P \geq 0.552$) while larger disturbance plots ($1.00 \text{ m}^2$ and $2.25 \text{ m}^2$) showed slightly less recovery ($P < 0.001$).

Sediment parameters in the eelgrass patch-interior disturbance experiment did not differ among treatments at 3, 30, or 43 weeks (Appendix 1.H), agreeing with results from the eelgrass transplantation experiment and from eelgrass and ghost shrimp patch-scale sediment sampling.

In contrast to the patch-interior disturbance experiment, ghost shrimp rapidly colonized disturbances to eelgrass in the patch-edge disturbance experiment (Fig. 1.4C; Table 1.1; Appendix 1.I). Before we created disturbances, eelgrass control plots and eelgrass disturbance plots had lower ghost shrimp densities than both adjacent ghost shrimp control plots ($P < 0.001$). Four weeks following disturbance, ghost shrimp density in disturbance plots was higher than
eelgrass control plots but lower than both ghost shrimp control plots \((P \leq 0.002)\). Eight weeks following disturbance, ghost shrimp density in disturbance plots was still higher than eelgrass control plots \((P < 0.001)\), but no different than either of the ghost shrimp control plots \((P = 0.299\) and \(P = 0.660\), for ghost shrimp control 1 and ghost shrimp control 2 plots, respectively).

**Discussion**

We found that disturbance and a competition-colonization trade-off facilitate the landscape-scale coexistence of two antagonistic ecosystem engineers in California estuaries. Eelgrass and ghost shrimp modify their surroundings in contrasting ways, resulting in indirect interspecific competition. In the absence of disturbance, eelgrass outcompetes ghost shrimp for space through physical alteration of the benthic environment. Ghost shrimp densities declined rapidly following the addition of eelgrass (Fig. 1.2A) and transplanted eelgrass expanded laterally into the surrounding ghost shrimp-dominated areas. Ghost shrimp transplanted to eelgrass patches failed to persist (Fig. 1.2C) and rhizome-root mimics quickly displaced ghost shrimp (Fig. 1.3). Although eelgrass outcompetes ghost shrimp for space via the production of belowground structure, disturbances to eelgrass patch edges are rapidly colonized by ghost shrimp living in adjacent habitat (Fig. 1.4C) and provide a spatiotemporal refuge for this inferior competitor.

_Ecosystem engineering_

Few studies have attempted to tease apart multiple mechanisms of habitat modification by ecosystem engineers. Our findings support the view that community-level impacts in coastal and estuarine sediments are often stronger for autogenic than allogenic mechanisms (Wilson
1990). For example, structural mimics of polychaete tubes have equivalent impacts on faunal abundance and diversity as live animals (Woodin 1978, Zühlke et al. 1998). Similarly, invasive mussels change native infaunal communities primarily by creating dense mats of byssal threads, not through allogenic changes (Crooks and Khim 1999). These patterns may be partly explained by the greater physical complexity and superior durability of autogenic constructs in unstructured soft-sediment habitats (Jones et al. 1994).

In our study, we found eelgrass to be an effective autogenic ecosystem engineer. The structure of macrophyte rhizomes and roots has been suggested as a major control on benthic marine and estuarine assemblages (Ringold 1979, Orth et al. 1984), but to our knowledge this study represents the first rigorous experimental test of this mechanism free from potentially-confounding factors. We posit that the eelgrass rhizome-root matrix, or concomitant changes to sediment structure, interferes with the ability of ghost shrimp to form or maintain burrow surface openings or other burrow structures (e.g., turnaround chambers). In the laboratory, ghost shrimp burial time increases six-fold in the presence of eelgrass rhizomes and roots (Brenchley 1982). In our field study, transplanted ghost shrimp succeeded in forming burrows within eelgrass patches, but they did not maintain them (Fig. 1.2C). Furthermore, rhizome-root mimics were equally effective in displacing ghost shrimp as live eelgrass (Figs. 1.2A and 1.3), offering strong evidence for this mechanism. Because ghost shrimp burrows are vertically-oriented and relatively narrow (11.9 cm ± 4.6 SD horizontal extent; Griffis and Chavez 1988), we speculate that ghost shrimp displaced in our experiments migrated laterally, away from eelgrass, and established burrows in unvegetated habitat free of biogenic obstacles. Our findings support early observations of an inverse spatial relationship between these species (Harrison 1987, Swinbanks and Luternauer 1987) and echo patterns from South African estuaries, where sediment
stabilization by seagrass reduces penetrability by burrowing shrimp (Siebert and Branch 2006, 2007).

Burrowing shrimps and other marine bioturbators have been described as archetypes of allogenic ecosystem engineering (Levinton 1995). Ghost shrimp in our study modified sediments over small scales, excavating coarse, organically-poor, low-nutrient sands and depositing them at the surface (Appendix 1.B). However, they failed to have detectable engineering effects at the patch scale. These results are consistent with findings from other regions, which show no correlation between grain size and ghost shrimp abundance (Harrison 1987, Swinbanks and Luternauer 1987).

We conclude that eelgrass is, in general, resistant to habitat modification by ghost shrimp. This finding is in contrast to benthic species that are sensitive to ghost shrimp bioturbation, such as clams (Peterson 1977), oysters (Dumbauld et al. 1997), and several deposit-feeding invertebrates (Posey 1986a). Small-scale habitat modification by ghost shrimp may have had stronger impacts if eelgrass sexual reproduction played a larger role in our study system. In Washington, USA, ghost shrimp burial reduces the survival and growth of seeds in the congener Zostera japonica (Dumbauld and Wyllie-Echeverria 2003). In our study, however, eelgrass spread was dominated by asexual growth in both the patch-interior disturbance experiment and the eelgrass transplantation experiment; only rarely did we observe seedlings.

The outcome of competition between autogenic and allogenic engineers may be mediated by differences in the relative rates of habitat construction and decay. For example, sediment excavation by an invasive isopod outpaces lateral spread and sediment accretion by saltmarsh plants, leading to bank erosion (Davidson and de Rivera 2010). In our system, ghost shrimp exhibit low rates of sediment turnover relative to some tropical and subtropical burrowing
shrimps, which are able to smother or shade adjacent seagrass (Suchanek 1983, Siebert and Branch 2006, 2007). In Indonesia, several burrowing shrimp species harvest seagrass leaves (Kneer et al. 2008), creating circular gaps in otherwise-contiguous meadows (S.L.W. pers. obs.). In other systems, such as New Zealand estuaries, co-occurring seagrass and burrowing shrimp have no measurable effects on each other (Berkenbusch et al. 2007). Thus the sign and strength of interactions between seagrasses and burrowing shrimps appear to vary by region and species.

Disturbance and coexistence

At equilibrium, interspecific competition is expected to lead to deterministic local extinction of the inferior competitor (Connell 1961). However, disturbance can increase the potential for coexistence by creating spatial and temporal variation in niche availability (Connell 1978, Sousa 1979). Based on ecological theory, we identify three processes to increase the potential for landscape-scale coexistence in our system: (1) environmental heterogeneity caused by spatiotemporally-asynchronous disturbances (Pacala and Tilman 1994), (2) differential resistance to certain disturbances (Hastings 1980), and (3) a trade-off between competitiveness and colonization ability (Levin and Paine 1974).

Synthesizing the results from our disturbance experiments, we found that loss of eelgrass creates spatiotemporal refugia for competitively-inferior ghost shrimp, but that the spatial pattern of disturbance matters tremendously (Sousa 1984). In our study, ghost shrimp rapidly colonized disturbances to eelgrass patch edges (Fig. 1.4C). All burrows found in this experiment were relatively large, indicating they belonged to adult ghost shrimp and strongly suggesting colonization was the result of lateral movement from adjacent ghost shrimp patches. By contrast, ghost shrimp failed to colonize disturbances to eelgrass patch interiors, even after 43 weeks (Fig.
1.4A). We suspect that adult ghost shrimp are unlikely to migrate past the eelgrass ecotone and thus they never encountered these areas of unvegetated habitat. Our results also indicate a failure of planktonic recruitment to the experimental disturbances over the ten months during which we observed these plots. Recruitment of ghost shrimp postlarvae is highly variable, both within and among years (Dumbauld et al. 1996), and it is possible that our experiment was conducted during a low-recruitment period.

Asynchronous benthic disturbances are common at both Tomales Bay and Mission Bay. Disturbances to eelgrass that we have observed at our study sites include swell, storm surge, desiccation, and grazing by migratory geese. Additional disturbances at our sites that primarily harm eelgrass but have the potential to affect ghost shrimp include boating impacts, trampling from foot traffic, and blooms of macroalgae (Ulva sp. and Gracilaria sp.) that shade the benthos and induce anoxia (Olyarnik and Stachowicz 2012). Differential resistance of eelgrass and ghost shrimp to this variety of disturbance agents provides an additional mechanism for coexistence (Hastings 1980).

Although an inferior competitor, ghost shrimp have a colonization advantage relative to eelgrass due to both a faster rate of lateral spread and a greater potential for long-distance dispersal. Our transplanted eelgrass expanded at a rate typical for this species (~26 cm y⁻¹; Marbà and Duarte 1998). Eelgrass took about 10 months to completely recover from 0.25 m² disturbances to patch interiors. By contrast, migrating ghost shrimp fully colonized disturbances of this size to eelgrass patch edges in no more than two months. In addition to rapid proximate colonization by adults, ghost shrimp can colonize distant habitats by the recruitment of planktonic postlarvae (Dumbauld et al. 1996). The large dispersal potential of ghost shrimp is evident from the high population connectivity measured for several estuaries distributed over 300
km of coastline in the US Pacific Northwest (Kozuka 2008). Eelgrass also is capable of sexual reproduction, but the vast majority of seeds disperse only a few meters from parent plants (Orth et al. 1994). Thus, although both species have the capacity for local and distant dispersal, ghost shrimp have a greater potential for dispersal to new habitats both within and among estuaries.

Minor differences in tidal distributions for eelgrass and ghost shrimp also seem important to the competition-colonization trade-off. In the California estuaries we studied, ghost shrimp achieve high densities (> 100 burrow surface openings m\(^{-2}\)) slightly above (several vertical centimeters) and below (1–2 vertical meters) eelgrass tidal limits (M.C.N.C. unpub. data). Eelgrass is likely restricted by temperature (Marsh. et al. 1986) or desiccation stress (Boese et al. 2005) in the upper-intertidal and light limitation at depth (Dennison 1987). These limited high-intertidal and low-subtidal zones, which appear marginal for eelgrass but suitable for ghost shrimp, may promote ghost shrimp persistence at the landscape scale by providing a spatial refuge from competition and a source of colonizers to nearby eelgrass disturbances.

Conclusions

Operating in isolation, neither disturbance nor a competition-colonization trade-off necessarily fosters coexistence (Chesson and Huntly 1997). The California estuaries we studied are characterized by (1) spatially-asynchronous benthic disturbances that modulate the availability of unoccupied space and predominantly impact eelgrass, and (2) clear differences in competitiveness and colonization abilities between two ecosystem engineers. Together, these result in a mosaic landscape with discrete patches dominated by eelgrass or ghost shrimp. Although eelgrass is sensitive to disturbance and slow to recover, once established it physically excludes and inhibits ghost shrimp through autogenic habitat modification. In spite of their
inability to coexist with or outcompete eelgrass, ghost shrimp persist at the landscape scale by rapidly colonizing disturbances to the edges of eelgrass patches and maintaining source populations above and below the eelgrass depth range. Theory demonstrates that antagonistic habitat modification can lead to local competitive exclusion, yet disturbance can facilitate broader coexistence. Our findings lend empirical support to these predictions and highlight the key role disturbance can play in structuring ecological communities.

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References


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Tables and Figures

Table 1.1. Results of ANOVAs testing for effects of the eelgrass transplantation experiment, rhizome-root structural mimic experiment, and eelgrass disturbance experiments on the density of ghost shrimp density (burrow surface openings m\(^{-2}\)) and eelgrass percent cover. \(P\) values < 0.05 are shown in bold.

<table>
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<th>Experiment and source of variation</th>
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Figure 1.1. Top: Study sites at Tomales Bay (A) and Mission Bay (B). Numbers indicate sites:
(1) Hamlet, (2) MacDonald, (3) Cypress Grove, and (4) Mariner’s Cove. Bottom: Relationship
between densities of eelgrass (leaf shoots m$^{-2}$) and ghost shrimp (burrow surface openings m$^{-2}$)
at Tomales Bay (C) and Mission Bay (D). Surveys were conducted at all sites; measurements of
sediment modification and all experiments took place at Hamlet.
Figure 1.2. Results from the reciprocal transplantation experiments. Top: Time courses are shown for mean (± 1 SE) ghost shrimp density (A; burrow surface openings m$^{-2}$) and eelgrass percent cover (B) following transplantation of eelgrass into ghost shrimp patches. Bottom: Bars show mean (+ 1 SE) ghost shrimp density (burrow surface openings plot$^{-1}$) following transplantation of ghost shrimp into bare sediment or adjacent eelgrass patches after 24 h and 1 month. In bottom figure (C), asterisks indicate significant differences ($P < 0.05$) between treatments within a sampling period.
Figure 1.3. Results from the rhizome-root structural mimic experiment. Time courses are shown for mean (± 1 SE) ghost shrimp density (burrow surface openings m$^{-2}$) following manipulation.
Figure 1.4. Results from the eelgrass disturbance experiments. Top: Time courses for mean ($\pm$ 1 SE) ghost shrimp density (A; burrow surface openings m$^{-2}$) and eelgrass percent cover (B) following removal of eelgrass from patch interiors. Bottom (C): Time courses for mean ($\pm$ 1 SE) ghost shrimp density (burrow surface openings m$^{-2}$) following removal of eelgrass from patch edges.
Appendices

Appendix 1.A. Photographs of a shallow subtidal area at Mission Bay (San Diego, California, USA) showing (A) eelgrass and ghost shrimp are patchily distributed with (B) abrupt ecotone transitions. Photo credit: K.A. Hovel.
Appendix 1.B. Mean (+ 1 SE) percent fine sediment, percent organic matter, and ammonium concentration for sediments collected from ghost shrimp burrow mounds, adjacent to burrow mounds, ghost shrimp patches, and eelgrass patches. See bottom for diagram of ghost shrimp burrow in cross-section, left, and intertidal landscape, right (not to scale). P values are indicated in the upper-right corner of each panel from paired-samples t-tests (A, C, E) and independent-samples t-tests (B, D, F). Asterisks indicate significant (P < 0.05) differences between samples.
Appendix 1.C. A photograph of high-density and low-density rhizome-root structural mimics on an intertidal ghost shrimp patch in Tomales Bay at low tide. Ghost shrimp burrow surface openings are visible. Photo credit: M.C.N. Castorani.
Appendix 1.D. A conceptual diagram of the randomized complete block design used in the eelgrass patch-edge disturbance experiment (not to scale). Replicates (n = 12) are 1 m × 1 m blocks located at the ecotone of separate eelgrass patches (> 10 m apart), with 4 treatments per block (0.25 m² square plots): eelgrass control (EC), eelgrass disturbance (ED), ghost shrimp control 1 (GSC 1), and ghost shrimp control 2 (GSC 2).
Appendix 1.E. A table of independent-samples $t$-test (two-tailed) results for sediment parameters surveyed over patch scales (eelgrass patches vs. ghost shrimp patches). Within each response variable, $n = 12$ for each patch type.

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Appendix 1.F. An ANOVA table for results from the eelgrass transplantation experiment. Within each response variable and sampling period, n = 5 for all treatments, except for 0.09 m² transplants due to the loss of 1 plot (n = 4). Ghost shrimp density data (burrow surface openings m⁻²) have been log-transformed (ln x + 1). P values < 0.05 are shown in bold.

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46
### Response variable: Percent sediment organic matter

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### Response variable: Sediment ammonium concentration

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Appendix 1.G. An ANOVA table for ghost shrimp density (burrow surface openings m$^{-2}$) results from the rhizome-root structural mimic experiment. Within each sampling period, n = 6 for all treatments, except for high-density rhizome-root structural mimics due to the loss of 2 plots (n = 4). P values < 0.05 are shown in bold.

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**Appendix 1.H.** An ANOVA table for results from the eelgrass patch-interior disturbance experiment. Within each response variable and sampling period, n = 5 for all treatments, except for 2.25 m² disturbances due to the loss of 1 plot (n = 4). Ghost shrimp density data (burrow surface openings m⁻²) have been log-transformed (ln x + 1). P values < 0.05 are shown in bold.

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### Response variable: Percent sediment organic matter

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### Response variable: Sediment ammonium concentration

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**Appendix 1.I.** An ANOVA table for ghost shrimp density (burrow surface openings m\(^{-2}\)) results from the eelgrass patch-edge disturbance experiment. Within each sampling period, \(n = 12\) blocks with 4 treatments per block. All data have been log-transformed (\(\ln x + 1\)). \(P\) values < 0.05 are shown in bold.

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CHAPTER 2

Light mediates bivalve habitat modification and impacts on seagrass

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\textsuperscript{2}Coastal and Marine Institute Laboratory and Department of Biology, San Diego State University, San Diego, California 92182 USA

\textsuperscript{3}Department of Biology, University of Southern Denmark, 5230 Odense M, Denmark.

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Abstract

Environmental context may influence the sign, strength, and mechanisms of species interactions but few studies have tested the potential for abiotic conditions to mediate interactions through multiple co-occurring stress pathways. Abiotic conditions may indirectly mediate species interactions by habitat-modifying organisms that are capable of simultaneously ameliorating and exacerbating multiple stressors. We hypothesized that light availability regulates bivalve habitat modification and subsequent impacts on seagrasses by shifting net effects between alleviation of nutrient stress and intensification of sulfide stress. To test this hypothesis, we manipulated light availability and blue mussel (\textit{Mytilus edulis}) abundance in eelgrass (\textit{Zostera marina}) mesocosms and measured biogeochemical and plant responses. Light
modified the effect of mussels on porewater ammonium, but eelgrass was not nutrient limited and, therefore, mussels did not enhance growth. Mussels increased sediment sulfides irrespective of light availability and, by reducing net oxygen flux (production and respiration), mussels and low light availability exacerbated sulfide intrusion of eelgrass tissues. Surprisingly, sulfide stress did not affect plant growth, survival, or energy stores. Thus, habitat modification by mussels represents a risk to eelgrass, especially during low productivity conditions, but eelgrass can resist harm from short-term stress, even during light limitation. Our findings suggest that while bivalve impacts on seagrasses may be variable in oligotrophic estuaries, they are likely negative in eutrophic systems, which are increasing globally.

**Keywords:** blue mussel *Mytilus edulis*; coastal eutrophication; context dependency; eelgrass *Zostera marina*; estuary; habitat modification; light; oxygen flux; stress gradient; sulfides

**Introduction**

The sign, strength, and mechanisms of interactions among species can depend on environmental context (Connolly and Roughgarden 1999, Burkepile and Hay 2006, Menge and Sutherland 1987). Abiotic stress is an important class of environmental conditions that can shift the direction of species interactions, as predicted by the stress gradient hypothesis (e.g., Bertness and Hacker 1994). This hypothesis has been especially applicable to species that physically or chemically ameliorate a single environmental stressor or increase the availability of a limiting resource (Norkko *et al.* 2006). Habitat-modifying organisms often simultaneously alleviate and exacerbate several stressors, creating the potential for highly complex species interactions (Kawai and Tokeshi 2007). However, few studies have experimentally assessed the potential for
environmental context to mediate the sign or strength of species interactions through multiple co-occurring stress pathways.

Estuaries are excellent ecosystems for testing the effects of environmental conditions on habitat modification and species interactions by virtue of their dynamic abiotic gradients (e.g., salinity, nutrients, light) and numerous species that modify physical and biogeochemical conditions of the seafloor, such as suspension-feeding bivalves and seagrasses. Bivalves are common, often abundant inhabitants of temperate seagrass meadows and may have positive, negative, or no effect on these plants. For instance, clams and mussels may facilitate seagrass growth by increasing the availability of sediment nutrients through biodeposition of feces and pseudofeces (e.g., Reusch et al. 1994, Carroll et al. 2008). However, other studies have shown that sediment enrichment by mussels can inhibit the growth of seagrasses by increasing concentrations of toxic sulfides (Vinther and Holmer 2008, Vinther et al. 2012). In other instances, bivalves may have mixed (Reusch and Williams 1998) or no (e.g., Worm and Reusch 2000, Wagner et al. 2012) effects on seagrass growth. These variable and inconsistent effects cannot be satisfactorily explained by nutrient availability, suggesting more complex interactions. We speculated that variation in light availability—the primary determinant of seagrass productivity (Dennison and Alberte 1985, Zimmerman 2006)—might help reconcile these disparate findings. Here we present the first experimental test of light as a moderator of bivalve-seagrass interactions.

We tested the hypothesis that bivalve modification of benthic biogeochemical conditions and the sign and strength of impacts on seagrasses are mediated by light availability. We predicted that bivalve biodeposition relieves seagrass nutrient stress by increasing sediment nitrogen, but only when light is not limiting. We also predicted that bivalve enhancement of
sediment sulfides inhibits seagrasses under light limitation, when low productivity diminishes the ability of seagrasses to maintain an oxygenated rhizosphere and resist sulfide intrusion (Holmer et al. 2005, Frederiksen and Glud 2006). Thus, we hypothesized that light availability mediates bivalve impacts on seagrasses by shifting the net effect between alleviation of nutrient stress and exacerbation of sulfide stress. To test our predictions, we manipulated bivalve abundance and light availability in seagrass mesocosms and measured biogeochemical and seagrass responses.

Methods

Study system

The blue mussel, *Mytilus edulis* L., is a suspension-feeding epibenthic bivalve that commonly co-occurs with eelgrass, *Zostera marina* L., in mixed or patchy distributions in intertidal and shallow-subtidal zones of the temperate North Atlantic Ocean, North Sea, and Baltic Sea (e.g., Reusch et al. 1994). Studies of the effects of *M. edulis* on *Z. marina* in the Baltic Sea have been particularly conflicting (e.g., Reusch et al. 1994, Worm and Reusch 2000, Vinther et al. 2012). We conducted our study in Denmark, where coastal waters are often eutrophic (median total nitrogen = 550 µg N L\(^{-1}\); median chlorophyll \(a\) = 5.1 µg L\(^{-1}\)) and turbid (median turbidity = 10.0 mg dry L\(^{-1}\)), resulting in highly-variable benthic light availability (Secchi depth range = 0.3–17.0 m) (Nielsen et al. 2002).

Experimental design

To determine the role of light availability in mediating blue mussel habitat modification and impacts on eelgrass, we factorially manipulated mussel abundance (present vs. absent) and light availability (high vs. low) in an indoor mesocosm experiment at the University of Southern
Denmark (Odense, Denmark). Mesocosms consisted of transplanted sediments and eelgrass in 19 L plastic buckets (20 cm diameter × 17 cm tall; n = 6 buckets per treatment). We housed eelgrass mesocosms in two aquaria (each 1.1 m length × 0.7 m width × 0.6 m height) that were respectively illuminated at high and low light availability. We illuminated both aquaria on a 12 h:12 h day:night cycle using Philips SGR 140/400 watt lamps (three HQT-BT/D bulbs in high-light aquarium vs. one bulb in low-light aquarium; Royal Philips, Amsterdam, The Netherlands). To further reduce light in the low-light aquarium, we shaded the surface with neutral-density black plastic netting. Aquaria shared flowing seawater (total vol. ≈ 1200 L; connected through a sump with filtration media) that we collected unfiltered from the Marine Biological Research Centre (Kerteminde, Denmark) and partially (15% = 175 L) replaced weekly to prevent nutrient accumulation. To maintain 100% air-saturation of the water, we distributed 14 air stones evenly across each aquarium.

To characterize abiotic conditions, we measured temperature and light availability every 15 minutes using a data-logging pendant sensor (HOBO UA-002-64, Onset Computer Company, Bourne, Massachusetts, United States of America (USA)) in each aquarium, attached to stands at eelgrass canopy height (30 cm above the sediment (see below) and 13 cm below the air-water interface). We calibrated illuminance readings to photosynthetically-active radiation (PAR; 400–700 nm) measured with a PAR sensor (LI-COR LI-250A light meter with LI-193 Underwater Spherical Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA). During daytime, mean canopy-height irradiance in high-light and low-light aquaria was 574 ± 289 (SD) and 97 ± 70 μmol photons s⁻¹ m⁻², respectively (high variability in measured irradiance was due to air bubbles periodically crossing the sensor and occasional fouling of the sensor surface). We chose these irradiance levels to represent conditions at which light availability would or would not limit
eelgrass growth (i.e., above and below the light-saturation point, $P_{\text{max}}$), respectively (Dennison and Alberte 1985). At night, both aquaria were completely dark. Aquaria had similar flow (1,200 L h$^{-1}$), temperature (14.4 °C), salinity (13.4 ± 0.8 PSU), water-column air saturation (100%), and water-column nutrients (18.5 ± 1.7 µg NO$_3$ L$^{-1}$; 11.5 ± 7.7 µmol NH$_4^+$ L$^{-1}$).

**Field collections**

In February and March 2013, we collected sediments and eelgrass from haphazardly-selected patches at a shallow (0–2 m depth) subtidal site at Svenstrup Beach in western Funen, Denmark (55°28’7” N, 9°45’17” E). We excavated sediments from the upper 15 cm by shovel, sieved them to remove fauna and detritus > 1 mm, and stored them in the recirculating seawater system until planting (3 d). We carefully uprooted eelgrass, rinsed them of sediments, and transported them in coolers to the laboratory (< 2 h). To reduce thermal shock, we kept eelgrass in a temperature-controlled room, with aerated seawater and intermediate light (150–250 µmol photons s$^{-1}$ m$^{-2}$), and increased the temperature gradually (+1 °C d$^{-1}$) from 1 °C to 14 °C. Next, we selected undamaged terminal shoots (leaf length > 10 cm and rhizome length ≥ 2 cm, with 3–5 internodes, intact roots, and no lateral shoots) and carefully removed senescent tissues. We then filled each mesocosm with homogenized sediment (10 cm layer) and transplanted 28 shoots in haphazard arrangement (= 891 leaf shoots m$^{-2}$, corresponding to eelgrass densities typically observed in mixed eelgrass-mussel patches (Reusch *et al.* 1994, Vinther *et al.* 2012)). We allowed two weeks for transplanted eelgrass to establish under intermediate light (150–250 µmol photons s$^{-1}$ m$^{-2}$) and then randomly assigned light and mussel treatments to each mesocosm.

In March 2013, we gathered mussels by hand from haphazardly-selected floating docks at the Marine Biological Research Centre and retained medium-sized mussels (51.9 ± 5.1 mm
length, a typical size for mussels from local mixed eelgrass-mussel beds; H. F. Vinther *unpublished data*). We transported mussels to the laboratory and acclimated them using the same procedure as for eelgrass. To half of the mesocosms, we carefully added 28 mussels (= 891 mussels m\(^{-2}\) or 27.6 ± 3.6 g dry soft tissue per mesocosm), creating an unhummocked epibenthic layer of approximately 100% mussel cover. This mussel density corresponds to densities commonly found in mixed eelgrass-mussel patches in the Baltic Sea (Reusch *et al.* 1994, Vinther *et al.* 2012). Mussels that died during the experiment (< 5%) were replaced. We fed mussels a microalgal diet of resuspended dried *Spirulina* sp. (2 g dry d\(^{-1}\) ≈ 5 µg chl. a L\(^{-1}\) recirculated through the integrated seawater system), which supports the growth of *M. edulis* (Alunno-Bruscia *et al.* 2001).

After applying light and mussel treatments, we randomized the arrangement of mesocosms within each aquarium and, to control for within-aquarium heterogeneity in water flow or light availability, randomly re-arranged mesocosms twice per week. We also created control mesocosms (n = 4), filled with sediments but not eelgrass or mussels, which were used to quantify initial benthic biogeochemical conditions without disturbing the sediment in experimental mesocosms.

*Benthic biogeochemical measurements*

To characterize treatment effects on benthic biogeochemical conditions, we collected porewaters and sediments at the start and end of the experiment. To determine sediment nutrient pools, we sampled porewaters (0–5 cm depth) from experimental mesocosms using porous (0.15 µm) “sippers” (Rhizon SMS, Rhizosphere Research Products, Wageningen, The Netherlands) arranged haphazardly (3 per mesocosm). We filtered (0.45 µm) and froze samples for analysis of
porewater ammonium, the preferred nitrogen source for eelgrass (Short and McRoy 1984), and later analyzed them colorimetrically by the salicylate-hypochlorite method (Bower and Holm-Hansen 1980) on a flow-injection autoanalyzer (Lachat Quick Chem 8500, Lachat Instruments, Loveland, Colorado, USA). From these porewater samples we also measured concentrations of dissolved organic carbon (DOC) using a total organic carbon analyzer (Shimadzu TOC-5000, Shimadzu Corporation, Kyoto, Japan).

To determine how light and mussels affected sediment sulfur pools, we collected sediment cores (18 mm diameter × 3 cm depth; 3 homogenized cores per mesocosm) haphazardly within each mesocosm (avoiding roots and rhizomes in experimental mesocosms). We preserved sediments in 20% zinc acetate, froze them, and later distilled them to quantify acid-volatile sulfides (AVS = porewater H₂S + iron-monosulfides) by colorimetric concentration analysis (Fossing and Jørgensen 1989). Under short-term organic enrichment (e.g., biodeposition), AVS is the main form of sediment sulfide accumulation (Holmer and Frederiksen 2007).

To aid in determining sulfide intrusion of eelgrass tissues (see below), we collected and distilled separate sediment cores (as above) to measure sulfur isotopic composition (δ³⁴S). We quantified seawater (sulfate) δ³⁴S by collecting water-column samples (n = 3) and precipitating sulfate using hot barium chloride. We determined sediment and seawater δ³⁴S using a continuous-flow isotope ratio mass spectrometer plus elemental analyzer (Frederiksen et al. 2008; Thermo Scientific Delta V Advantage plus Flash EA 1112, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). We report stable isotope signatures in standard delta notation (units per mil, ‰):

\[ \delta^{34}S = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000 \]
where \( R = \frac{^{34}S}{^{32}S} \).

**Production and respiration measurements**

To estimate community-scale primary production and respiration, each week we measured fluxes of oxygen in submerged mesocosm enclosures for a subset of experimental mesocosms (\( n = 3 \) per treatment, randomly-selected each week; it was not practical to measure all mesocosms weekly). The enclosures were clear cylindrical plastic bags (6 L) that were sealed over each bucket and contained a small pump (Ehiem AS1000, Eheim GmbH & Co. KG, Deizisau, Germany) that circulated water (5 L min\(^{-1}\)). Affixed to each enclosure was an oxygen optode patch that we used to measure oxygen concentration every 10–15 min using a fiber-optic oxygen optode setup (PreSens Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany). Incubations were conducted for 3–4 h across the end of the light cycle and beginning of the subsequent dark cycle. We calculated hourly rates of oxygen change using linear regressions. We then estimated net community production (NCP) as oxygen flux during the light incubation, community respiration (CR) as oxygen flux during the dark period, and gross primary production (GPP) as NCP + CR (Glud *et al.* 2009).

**Eelgrass survival, growth, and condition measurements**

To quantify changes to eelgrass survival over time, we counted shoots weekly during the first 3 weeks and twice per week during the final 2.5 weeks (changes to eelgrass density accelerated during this time). We used the needle-punch method to measure leaf growth by puncturing all shoots 10 days before the experiment concluded and, at the end of the experiment, haphazardly-selecting 10 terminal shoots from each mesocosm and measuring the width and
linear elongation of all leaves. We converted rates of leaf areal production (mm$^2$ shoot$^{-1}$ d$^{-1}$) to leaf mass production (g dry shoot$^{-1}$ d$^{-1}$) by linear regression of leaf area (estimated as leaf length $\times$ width) and leaf dry mass ($p < 0.0001$, $R^2 = 93.8\%$).

At the end of the experiment (37 days after applying treatments), we collected eelgrass, separated tissues (shoots + leaves, rhizomes, and roots), and measured biomass after freeze-drying for 48 h. We measured total carbon and nitrogen content of young leaves (youngest leaf pair), rhizomes (youngest two internodes), and roots (attached to young rhizomes) by elemental analysis (Carlo Erba EA1108 CHN analyzer, Carlo Erba Reagents, Milan, Italy). To determine eelgrass energy stores (i.e., non-structural carbohydrates), we sequentially extracted soluble sugars and starches from young rhizomes and measured concentrations colorimetrically using an anthrone assay standardized to sucrose (Zimmerman et al. 1995). We focused on young tissues because we could be confident these grew under our experimental manipulations (as opposed to older tissues, which might reflect responses to prior field conditions).

To assess sulfide intrusion of young eelgrass tissues, we determined the fraction of tissue total sulfur (TS) derived from sediment sulfides ($F_{\text{sulfide}}$) as:

$$F_{\text{sulfide}} = \frac{\delta^{34}S_{\text{tissue}} - \delta^{34}S_{\text{sulfate}}}{\delta^{34}S_{\text{sulfide}} - \delta^{34}S_{\text{sulfate}}}$$

where $\delta^{34}S_{\text{tissue}}$ is the value measured in the leaves, rhizomes, or roots, $\delta^{34}S_{\text{sulfate}}$ is the value measured in seawater, and $\delta^{34}S_{\text{sulfide}}$ is the value measured in sediment AVS pools (Frederiksen et al. 2008). We measured eelgrass tissue TS and $\delta^{34}S$ using methods identical to sediments (see above). If sulfides invade eelgrass roots, oxygen delivery via aerenchyma precipitates sulfides as elemental sulfur ($S^0$) and other oxidized compounds (Holmer et al. 2005). Thus, to provide ancillary evidence of sulfide intrusion, we measured $S^0$ concentrations in young roots by high-performance liquid chromatography (Agilent 1100 Series HPLC with UV detector (265 nm),
Agilent Technologies Inc., Santa Clara, California, USA) following methanol extraction (Zopfi et al. 2001).

**Statistical analyses**

We tested the effects of mussels, light, and the interaction between these factors on benthic biogeochemical and eelgrass conditions with separate fully-factorial analyses of variance (ANOVAs) using R 3.0.2 (R Development Core Team 2013). We tested the effects of light and mussel treatments, time (i.e., week of sampling), and interactions between all factors on NCP and CR with repeated-measures ANOVAs using JMP 10.0 (SAS Institute Inc., Cary, North Carolina, USA). We hypothesized that porewater ammonium might explain variation in leaf C:N. We also predicted that porewater DOC might explain variation in sediment sulfides (AVS). Lastly, we hypothesized that sediment AVS or oxygen flux during day (NCP) or night (CR) might explain variation in sulfide invasion of plant tissues ($F_{\text{sulfide}}$ or root S$^0$). Therefore, we tested for relationships between these *a priori*-selected response variables with linear regressions using R 3.0.2.

Before performing ANOVAs or linear regressions, we determined normality of the residuals using normal probability plots and homoscedasticity of the residuals by visually examining the relationship between residuals and fitted values, and by using Cochran’s test for equality of variances. For linear regressions, we also determined data linearity by visually examining the relationship between response and predictor variables. Before performing repeated-measures ANOVAs, we tested for sphericity using Mauchly’s test. When necessary, we log-transformed [ln (x + 1)] data to meet the assumptions of these parametric analyses.
Results

Benthic biogeochemical conditions

Mussel and light treatments had an interactive effect on porewater ammonium concentrations (Table 2.1). At high light availability, mussels increased ammonium concentrations, as we predicted, whereas at low light availability ammonium concentrations were high regardless of mussel presence (Fig. 2.1A). In contrast, neither mussels nor light affected porewater DOC (Table 2.1). Mussels increased sediment AVS pools irrespective of light treatment (Table 2.1; Fig. 2.1B). Porewater DOC was positively correlated with sediment AVS pools \((P = 0.0099, R^2 = 27.7\%)\).

Production and respiration

Mussel and light treatments had an interactive effect on net community production (Table 2.2), as both low light and mussels suppressed NCP, but mussels had a relatively greater effect in high light than in low light (Fig. 2.2A). Low light and mussels enhanced community respiration without interaction (Fig. 2.2B). Neither NCP nor CR varied through time. Combining NCP and CR data revealed that high-light mesocosms lacking mussels were, generally, net autotrophic \((GPP > 0)\), whereas all other treatments were net heterotrophic \((GPP < 0)\) (Fig. 2.2C).

Eelgrass condition: nutrient content and sulfide intrusion

High light reduced leaf nitrogen (Table 2.1; Fig. 2.3A), increased leaf carbon (but this effect was slight: \(36.05 \pm 0.36 \) vs. \(35.39 \pm 0.74\) percent by weight for high light and low light, respectively), and increased leaf C:N (Fig. 2.3B), while mussels had no effects on these variables. Porewater ammonium was positively correlated with leaf nitrogen \((P = 0.0205, R^2 = \)
22.1% but not leaf growth, regardless of light treatment ($P = 0.4996$ and $P = 0.3453$ for high light and low light, respectively).

Mussels and light both caused changes in sulfide intrusion of eelgrass (evidenced by $F_{\text{sulfide}}$, root $S^0$ (Table 2.1), $\delta^{34}\text{S}$, and TS (Tables 2.3 and 2.4)). $F_{\text{sulfide}}$ was greatest for roots (range = 23–85%), followed by rhizomes (13–45%) and leaves (4–23%). In high light, mussels increased sulfide intrusion of leaves ($F_{\text{sulfide}}$ (Fig. 2.4A) and $\delta^{34}\text{S}$), while in low light leaf sulfide intrusion was high regardless of mussel treatment (low light increased leaf TS irrespective of mussel treatment). Low light and mussels increased sulfide intrusion of rhizomes ($F_{\text{sulfide}}$ (Fig. 2.4C), $\delta^{34}\text{S}$, and TS). Root $F_{\text{sulfide}}$ (Fig. 2.4E) and $\delta^{34}\text{S}$ were highly variable, with effects of light but not mussels (neither affected root TS). However, low light and mussels both increased root $S^0$ concentrations (Fig. 2.5A).

Sulfide intrusion of eelgrass tissues was driven partly by sediment sulfides. AVS was positively correlated with leaf $F_{\text{sulfide}}$ ($P = 0.0019$, $R^2 = 37.6\%$) but not rhizome $F_{\text{sulfide}}$ ($P = 0.0619$) or root $F_{\text{sulfide}}$ ($P = 0.0837$). Sulfide intrusion was primarily influenced by oxygen flux during day (NCP), but not night (CR). NCP explained substantial $F_{\text{sulfide}}$ variation for all tissue types (leaves: $P = 0.0004$, $R^2 = 45.8\%$, Fig. 2.4B; rhizomes: $p < 0.0001$, $R^2 = 56.4\%$, Fig. 2.4D; roots: $P = 0.0418$, $R^2 = 18.3\%$, Fig. 2.4F). In contrast, CR was not correlated with leaf $F_{\text{sulfide}}$ ($P = 0.9967$) or rhizome $F_{\text{sulfide}}$ ($P = 0.2797$), and loosely correlated with root $F_{\text{sulfide}}$ ($P = 0.0443$, $R^2 = 17.9\%$). Root $S^0$ was not correlated with sediment AVS ($P = 0.9088$), but strongly negatively correlated with NCP ($P < 0.0001$, $R^2 = 59.6\%$, Fig. 2.5B).
**Eelgrass survival, growth, and energy stores**

Despite mussel impacts on benthic biogeochemical conditions, oxygen consumption, and sulfide intrusion of eelgrass tissues, light drove all differences in eelgrass survival, growth, and energy stores (Table 2.1). Shoot density had a stable-to-increasing trend under high light, while eelgrass deteriorated under low light, particularly after about 3 weeks (Fig. 2.6A). Declines in shoot density in low light were driven by mortality of (transplanted) terminal shoots (Fig. 2.6B) and near-absence of (new) lateral shoot propagation (Fig. 2.6C). By contrast, nearly all eelgrass survived in high light and propagated an average of 10 ± 4.3 new shoots per mesocosm. Similarly, leaf growth (Fig. 2.6D) and rhizome soluble sugars (Fig. 2.6E) were over six times greater in high light than low light, but were unaffected by mussels. Rhizome starch concentrations were unaffected by treatments (Table 2.1).

**Discussion**

Our study highlights the value of comprehensive, mechanistic approaches for testing the role of environmental context in mediating species interactions. Environmental context can alter the degree of habitat modification and the sign and strength of subsequent species interactions in plant and animal communities (e.g., Bertness and Hacker 1994, Connolly and Roughgarden 1999, Norkko *et al.* 2006), but complex interactions can arise when abiotic conditions simultaneously affect multiple stress pathways (Kawai and Tokeshi 2007). We found that light availability indirectly mediates blue mussel habitat modification by altering plant metabolism, and these biogeochemical changes (i.e., sulfide and oxygen concentrations) feed back to influence eelgrass vulnerability to sulfide stress. Light modified the effect of mussels on porewater ammonium, but eelgrass was not nutrient limited and, therefore, mussels did not
enhance growth (Figs. 2.1A and 2.3). Mussels increased sediment sulfides irrespective of light availability (Fig. 2.1B). Light, mussels, and their interaction strongly affected oxygen fluxes (Fig. 2.2) and these in turn regulated sulfide intrusion of eelgrass tissues (Figs. 2.4 and 2.5). Light mediated the effect of mussels on sulfide intrusion of leaves, but not rhizomes or roots, which were governed by light and mussels without an interaction of these factors (Figs. 2.4 and 2.5). Surprisingly, sulfide stress did not affect plant survival, growth, or energy stores, which were categorically impaired by low light but unaffected by mussels (Fig. 2.6). Thus, although low light and mussels exacerbate sulfide stress, eelgrass is capable of resisting harm, at least over short time scales (e.g., weeks). Our findings suggest environmental context plays a limited role in mediating eelgrass-mussel interactions. Mussels may particularly enhance the risk of sulfide stress to eelgrass in eutrophic estuaries, which are typically characterized by reduced light availability, impaired benthic productivity, and frequent occurrences of benthic hypoxia.

*Nutrient dynamics in sediments and eelgrass*

We found the potential for blue mussels to enhance sediment nutrients depends on light availability. Mussels increased porewater ammonium in high light, whereas concentrations were high regardless of mussel presence in low light (Fig. 2.1A). This result was likely due to reduced root uptake of ammonium (Dennison *et al.* 1987) and/or eelgrass mortality and decomposition of leaf litter (Pedersen *et al.* 1999). As expected, leaf nitrogen was positively correlated with porewater ammonium, but changes to nutrient availability among treatments did not translate into differences in eelgrass nutrient condition (Fig. 2.3), probably because ammonium supply exceeded plant demand (Dennison *et al.* 1987). Instead, light availability drove patterns of leaf nitrogen. Due to impaired photosynthesis and reduced carbon demand, low-light plants likely
stored nitrogen in their tissues instead of assimilating it into new growth, resulting in leaves with
greater nitrogen and lower C:N (Moore and Wetzel 2000). Furthermore, porewater nutrients
were not correlated with leaf growth, regardless of light treatment. Mussels probably did not
enhance eelgrass growth because ammonium concentrations were above levels at which eelgrass
growth saturates (~100 µmol NH$_4^+$ [L porewater]$^{-1}$; Dennison et al. 1987). Water-column and
porewater nutrient concentrations in our experiment were similar to those found in eelgrass beds
of eutrophic regions, such as the Baltic Sea (Nielsen et al. 2002), suggesting that similar
dynamics may occur in the field.

*Sulfur dynamics, oxygen fluxes, and sulfide intrusion of eelgrass*

We found that blue mussels enhance sediment sulfide concentrations irrespective of light
conditions (Fig. 2.1B). Mussels likely stimulated sulfate reduction by depositing organic matter
(Kautsky and Evans 1987), consuming oxygen (Fig 2; Carlsson et al. 2010), and/or reducing
oxygen flux across the sediment-water interface with the physical structure of their shells
(Jørgensen 1982).

Interestingly, however, sediment sulfides were not the primary driver of sulfide intrusion
into eelgrass tissues. AVS pools explained $F_{\text{sulfide}}$ patterns in leaves, but not rhizomes or roots.
Instead, NCP (i.e., daytime oxygen flux; Fig. 2.2A) was the best predictor of sulfide intrusion
(Figs. 2.4B, 2.4D, 2.4F, and 2.5B). Because estuarine sediments are mostly anoxic and reduced,
seagrass roots and rhizomes experience periods of oxygen deprivation and rely on oxygen
supplied internally by leaves via photosynthesis or externally by passive diffusion from the water
column via aerenchyma. In the absence of sufficient oxygenation of belowground tissues, the
oxidative barrier surrounding eelgrass roots deteriorates (Frederiksen and Glud 2006), allowing
the invasion of toxic sulfides through root tips and eventually leading to meristem necrosis. In our experiment, eelgrass grown under low light produced less oxygen (Fig. 2.2A) and mussel respiration enhanced near-bottom oxygen consumption (Fig. 2.2B). Therefore, we infer that sulfide intrusion was primarily driven by a combination of reduced eelgrass photosynthesis and mussel respiration.

Corroborating previous studies (e.g., Frederiksen et al. 2006), we found the risk of tissue intrusion by sediment sulfides is greatest for roots, intermediate for rhizomes, and lowest for leaves (Fig. 2.4). Mussels increased sulfide intrusion of leaves in high light, whereas intrusion was relatively high regardless of mussel presence in low light (Fig. 2.4A). For rhizomes, both low light and mussels exacerbated sulfide stress (Fig. 2.4B). For roots, we expected the fraction of eelgrass tissue sulfur derived from sediment sulfides ($F_{\text{sulfide}}$) might be highly variable (Fig. 2.4E); eelgrass roots often have highly dynamic $\delta^{34}$S due to the heterogeneous nature of porewater dissolved sulfide pools (Oakes and Connolly 2004). However, despite highly variable root $F_{\text{sulfide}}$, the concentrations of elemental sulfur ($S^0$) in root tissues clearly indicate both low light and mussels enhanced sulfide intrusion (Fig. 2.5A). Both indicators of sulfide intrusion that we measured in our mesocosm study ($F_{\text{sulfide}}$ and root $S^0$) are similar to values observed in situ for Zostera marina (in Denmark; Frederiksen et al. 2006), suggesting that similar sulfur dynamics may occur in the field.

These findings suggest that mussel impacts on eelgrass depend on environmental context. Mussel-driven enhancement of sediment sulfides is likely to enhance the risk of sulfide intrusion only when eelgrass productivity is reduced (e.g., under low light availability) or water-column dissolved oxygen concentrations are diminished (e.g., during hypoxic events). Eelgrass living among mussels may be at greatest risk of sulfide stress in low light environments, such as in
turbid estuaries or near the lower depth range for eelgrass, where mussels might limit colonization. Low water clarity and benthic hypoxia commonly accompany eutrophication (Cloern 2001, Howarth et al. 2011), suggesting coastal nutrient pollution may mediate mussel effects on eelgrass sulfide stress. This possibility is particularly concerning because eutrophication has increased in temperate estuaries worldwide, including regions where eelgrass and mussels frequently co-occur in the North Atlantic Ocean, North Sea, and Baltic Sea (Howarth et al. 2011 and references therein).

_Eelgrass survival, growth, and energy stores_

Sulfide intrusion of eelgrass is typically accompanied by a reduction in photosynthetic activity (Goodman et al. 1995), growth (Holmer et al. 2005), and rhizome sugar reserves (Holmer and Bondgaard 2001). While estimates of sulfide toxicity vary widely (Lamers et al. 2013), the levels of sulfide intrusion we measured in our study are indicative of harm (Frederiksen et al. 2008). Nevertheless, while light and mussels both influenced sulfide intrusion of eelgrass, mussels did not affect eelgrass nutrient content, growth, rhizome energy stores, or survival (Figs. 2.3 and 2.6). Instead, light availability drove all changes in these responses irrespective of mussel presence. Not surprisingly, eelgrass thrived under high light, growing quickly, propagating new lateral shoots, and accumulating excess sugars in their rhizomes. Conversely, plants under low light grew slowly, suffered high shoot mortality, propagated few lateral shoots, and nearly exhausted their energy stores.

Our findings suggest that during short periods (i.e., days to weeks), eelgrass is resistant to sulfide stress caused by mussels, even under low-light conditions. Critically, however, this conclusion may be contingent on the temporal scale of our study. While sulfide intrusion was
relatively substantial, even the highest sediment sulfide concentrations in our mesocosms were modest when compared to eelgrass sediments in many field settings (e.g., Frederiksen et al. 2006, Holmer and Nielsen 2007). Therefore, had our experiment been longer in duration or higher in temperature, it is possible that mussel biodeposition could have caused an accumulation of sediment sulfides and cumulative harm to eelgrass. In the field, mussels may also have stronger negative effects on eelgrass seedlings, which are more sensitive to sulfide stress than adults (Dooley et al. 2013).

**Context-dependent effects of bivalves on seagrasses**

Despite intensive research efforts, generalizations about the effects of suspension-feeding bivalves on seagrasses remain elusive. Although bivalve habitat modification and impacts on seagrasses appear to vary based on environmental context, they are not deterministically governed by light or ambient nutrient concentrations.

In oligotrophic waters (\(< \sim 150 \mu\text{mol NH}_4^+ [\text{L porewater}]^{-1}\)), bivalves can facilitate seagrasses. For example, blue mussels and hard clams (*Mercenaria mercenaria*) can relieve eelgrass nutrient limitation (Reusch et al. 1994, Carroll et al. 2008). Similarly, by fertilizing porewaters, tulip mussels (*Modiolus americanus*) can improve turtlegrass (*Thalassia testudinum*) leaf nutrient condition (Peterson and Heck 1999) and growth (Peterson and Heck 2001a, b). However, other studies have shown that enhancement of porewater ammonium by blue mussels, geoduck clams (*Panopea generosa*), and oysters (*Crassostrea gigas*) does not affect eelgrass leaf nitrogen or growth (Worm and Reusch 2000, Ruesink and Rowell 2012, Wagner et al. 2012), despite low ambient nutrient concentrations (\(< 30, < 100, \text{ and } < 20 \mu\text{mol NH}_4^+ [\text{L porewater}]^{-1}\), respectively). Impacts on eelgrass by non-native Asian nest mussels (*Arcuatula senhousia*) in an
oligotrophic bay (< 100 µmol NH$_4^+$ [L porewater]$^{-1}$) vary from facilitative to inhibitive depending on the physiological response considered (i.e., leaf vs. rhizome growth; Reusch and Williams 1998). In fact, at least two studies have shown negative impacts of oysters (C. virginica and C. gigas) on seagrasses (shoalgrass, Halodule wrightii, and eelgrass) in low nutrient (< ~150 µmol NH$_4^+$ [L porewater]$^{-1}$) estuaries, possibly by stimulating sediment sulfides or physically competing for space (Dumbauld et al. 2009, Booth and Heck 2009).

In contrast to studies in oligotrophic systems, experiments under eutrophic conditions (> ~500 µmol NH$_4^+$ [L porewater]$^{-1}$) have found exclusively that blue mussels negatively impact eelgrass by enhancing sulfate reduction and inducing sulfide stress (Vinther and Holmer 2008, Vinther et al. 2008, 2012). In eutrophic regions of the Baltic Sea, blue mussels and eelgrass have an inverse spatial relationship that may be explained in part by mussel-induced increases in sediment sulfide concentrations (Vinther et al. 2012). Our experiment, which mimicked eutrophic conditions, builds upon these and other studies by improving understanding of the biogeochemical mechanisms underpinning bivalve-seagrass interactions in eutrophic systems and the role of light in governing some of these dynamics. We speculate that bivalve effects on seagrasses may be variable in oligotrophic ecosystems, where light availability is usually high and nutrients can limit productivity, but may be categorically negative in high-nutrient estuaries, where the potential for fertilization is minimal but the risk for sulfide stress is high due to reduced light availability and oxygen concentration that typically accompany eutrophication (Howarth et al. 2011).
Caveats and future directions

It is important to consider two major caveats to our conclusions. First, mesocosm experiments are, by design, controlled approximations of real ecosystems. Several factors excluded in our study, such as tides, strong hydrodynamic mixing, continuous diel changes in light availability, bioturbation by infauna, and predator effects on bivalve populations and feeding behavior, may play important roles in mediating bivalve habitat modification and impacts on seagrasses. Second, our experiment tested seagrass-bivalve interactions on small spatial and temporal scales. Bivalves may inhibit seagrasses in eutrophic systems on small scales (e.g., cm\(^2\) to 10s of m\(^2\)) by causing sulfide stress, but at landscape to ecosystem scales (e.g., hectares to 1,000s of km\(^2\)) bivalves may facilitate seagrasses by improving light penetration through filtration of the water-column (Wall et al. 2008). In fact, suspension-feeding bivalves are capable of reducing phytoplankton populations on the scales of entire estuaries (Officer et al. 1982, but see Pomeroy et al. 2006). Due to the increasing eutrophy of temperate estuaries worldwide (Howarth et al. 2011), it is possible that bivalve-seagrass interactions may be commonly represented by simultaneous small-scale inhibition (via increased sulfide stress) and large-scale facilitation (via decreased light stress).

Seagrass populations (including eelgrass) are rapidly declining worldwide due in large part to accelerating coastal eutrophication (Waycott et al. 2009; Howarth et al. 2011). Concurrently, human activities have dramatically changed the abundance and composition of bivalve assemblages in and near seagrass meadows through commercial aquaculture (Pawiro 2010), overharvesting of wild populations (Newell 1998, Cloern 2001), restoration or mitigation of water quality using bivalves (Stadmark and Conley 2011), restoration or mitigation of water quality using bivalves, and introduction of non-native bivalves, which sometimes outcompete
natives (M. C. N. Castorani and K. A. Hovel unpublished manuscript (see Chapter 3)). Thus, understanding how bivalves and eutrophication interactively impact seagrasses is important to ecosystem-based management and conservation of estuaries. To our knowledge, studies of bivalve-seagrass interactions under eutrophic conditions are largely limited to a single pair of species (Z. marina and M. edulis) within one region (the western Baltic Sea). Therefore, to determine the generalizability of our hypotheses, future studies should assess bivalve-seagrass interactions in other eutrophic estuaries. Future work should also aim to disentangle the conflated physical and chemical effects of eutrophication (e.g., light availability, nutrient concentration, and dissolved oxygen saturation) in mediating bivalve impacts on seagrasses, as well as the potential for dependency on the spatial scale of bivalve-mediated changes.

Acknowledgments

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References


Tables and Figures

*Table 2.1.* Results of fully-factorial ANOVAs testing for effects of light treatment, mussel treatment, and the interaction of light and mussel treatments on benthic biogeochemical conditions, eelgrass nutrient condition, sulfide intrusion of eelgrass, and eelgrass survival, growth, and energy stores. *P* values < 0.05 are shown in bold.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Treatment:</th>
<th>Light</th>
<th>Mussels</th>
<th>Light × Mussels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Benthic biogeochemical conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater NH$_4$†</td>
<td>1, 20</td>
<td>8.542</td>
<td>0.008</td>
<td>1, 20</td>
</tr>
<tr>
<td>Porewater DOC</td>
<td>1, 19</td>
<td>2.469</td>
<td>0.133</td>
<td>1, 19</td>
</tr>
<tr>
<td>Sediment AVS</td>
<td>1, 19</td>
<td>1.097</td>
<td>0.308</td>
<td>1, 19</td>
</tr>
<tr>
<td>Eelgrass nutrient condition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf percent carbon</td>
<td>1, 20</td>
<td>7.693</td>
<td>0.012</td>
<td>1, 20</td>
</tr>
<tr>
<td>Leaf percent nitrogen</td>
<td>1, 20</td>
<td>119.71</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Leaf carbon:nitrogen</td>
<td>1, 20</td>
<td>201.834</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Sulfide intrusion of eelgrass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf $F_{sulfide}$</td>
<td>1, 19</td>
<td>5.290</td>
<td>0.033</td>
<td>1, 19</td>
</tr>
<tr>
<td>Rhizome $F_{sulfide}$</td>
<td>1, 19</td>
<td>14.182</td>
<td>0.001</td>
<td>1, 19</td>
</tr>
<tr>
<td>Root $F_{sulfide}$</td>
<td>1, 19</td>
<td>6.528</td>
<td>0.019</td>
<td>1, 19</td>
</tr>
<tr>
<td>Root elemental sulfur †</td>
<td>1, 20</td>
<td>18.853</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Eelgrass survival, growth, and energy stores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total shoot density</td>
<td>1, 20</td>
<td>139.401</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Terminal shoot density</td>
<td>1, 20</td>
<td>99.011</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Lateral shoot density †</td>
<td>1, 20</td>
<td>198.416</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Leaf growth rate</td>
<td>1, 20</td>
<td>211.888</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Rhizome soluble sugars</td>
<td>1, 20</td>
<td>218.559</td>
<td>&lt; 0.001</td>
<td>1, 20</td>
</tr>
<tr>
<td>Rhizome starches</td>
<td>1, 20</td>
<td>0.391</td>
<td>0.539</td>
<td>1, 20</td>
</tr>
</tbody>
</table>

† = variables log-transformed [\(\ln (x + 1)\)] prior to analysis.
Table 2.2. Results of fully-factorial repeated-measures ANOVAs testing for effects of light treatment, mussel treatment, time (i.e., week of sampling), and interactions between all factors on net community production (NCP) and community respiration (CR). \( P \) values < 0.05 are shown in bold.

<table>
<thead>
<tr>
<th>Response variable:</th>
<th>NCP</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>df</td>
<td>( F )</td>
</tr>
<tr>
<td>Light</td>
<td>1, 8</td>
<td>359.389</td>
</tr>
<tr>
<td>Mussels</td>
<td>1, 8</td>
<td>158.986</td>
</tr>
<tr>
<td>Light × Mussels</td>
<td>1, 8</td>
<td>6.085</td>
</tr>
<tr>
<td>Time</td>
<td>3, 6</td>
<td>2.343</td>
</tr>
<tr>
<td>Time × Light</td>
<td>3, 6</td>
<td>1.152</td>
</tr>
<tr>
<td>Time × Mussels</td>
<td>3, 6</td>
<td>0.749</td>
</tr>
<tr>
<td>Time × Light × Mussels</td>
<td>3, 6</td>
<td>0.289</td>
</tr>
</tbody>
</table>

Table 2.3. Results of fully-factorial ANOVAs testing for effects of light treatment, mussel treatment, and the interaction of light and mussel treatments on \( \delta^{34} \)S and total sulfur (TS) for leaf, rhizome, and root tissues. \( P \) values < 0.05 are shown in bold.

<table>
<thead>
<tr>
<th>Treatment:</th>
<th>( \delta^{34} )S</th>
<th>Light</th>
<th>Mussels</th>
<th>Light × Mussels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response variable</td>
<td>df</td>
<td>( F )</td>
<td>( P )</td>
<td>df</td>
</tr>
<tr>
<td>( \delta^{34} )S</td>
<td>Leaf</td>
<td>1, 20</td>
<td>2.412</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td>Rhizome</td>
<td>1, 20</td>
<td>18.629</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>1, 20</td>
<td>5.536</td>
<td><strong>0.029</strong></td>
</tr>
</tbody>
</table>

| TS | Leaf  | 1, 20 | 20.262 | < 0.001 | 1, 20 | 2.427 | 0.135 | 1, 20 | 0.153 | 0.700 |
| | Rhizome | 1, 20 | 11.595 | **0.003** | 1, 20 | 7.608 | **0.012** | 1, 20 | 1.249 | 0.277 |
| | Root   | 1, 20 | 2.111 | 0.162 | 1, 20 | 0.076 | 0.786 | 1, 20 | 1.918 | 0.181 |
Table 2.4. Summary of $\delta^{34}$S (‰) for sediment AVS and leaf, rhizome, and root tissues, and total sulfur (TS; µmol [g dry tissue]⁻¹) for leaf, rhizome, and root tissues as a function of light and mussel treatments. Values are reported as mean ± SD.

<table>
<thead>
<tr>
<th>Light treatment:</th>
<th>High light</th>
<th>Low light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mussel treatment:</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>$\delta^{34}$S (‰)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sediment AVS</td>
<td>$-24.41 \pm 3.97$</td>
<td>$-20.56 \pm 3.28$</td>
</tr>
<tr>
<td>Leaf</td>
<td>$16.59 \pm 0.63$</td>
<td>$12.18 \pm 2.03$</td>
</tr>
<tr>
<td>Rhizome</td>
<td>$13.08 \pm 0.48$</td>
<td>$8.79 \pm 2.30$</td>
</tr>
<tr>
<td>Root</td>
<td>$1.93 \pm 5.86$</td>
<td>$0.90 \pm 5.04$</td>
</tr>
</tbody>
</table>

| TS (µmol [g dry tissue]⁻¹) | | | | |
| Leaf | $121.90 \pm 7.05$ | $137.06 \pm 18.04$ | $159.97 \pm 24.65$ | $169.05 \pm 21.69$ |
| Rhizome | $84.06 \pm 11.06$ | $123.41 \pm 35.53$ | $129.98 \pm 22.48$ | $146.63 \pm 24.14$ |
| Root | $148.51 \pm 22.01$ | $196.61 \pm 51.17$ | $230.72 \pm 102.41$ | $198.58 \pm 80.89$ |
Figure 2.1. Effects of light and mussels on benthic biogeochemical conditions. Mean (+ 1 SE) concentrations of (A) porewater ammonium (µmol L\(^{-1}\)) and (B) sediment acid-volatile sulfides (µmol cm\(^{-3}\)). Horizontal solid and dashed lines indicate mean ± SE, respectively, for sediments and porewaters collected at the beginning of the experiment from control mesocosms.
Figure 2.2. Time series of (A) net community production, (B) community respiration, and (C) gross primary production, expressed as mean oxygen flux ± SE. Symbols indicate separate treatments. Note differences in the scale of y-axes.
Figure 2.3. Effects of light and mussels on eelgrass nutrient condition. Mean (+ 1 SE) leaf tissue (A) nitrogen content (percent by weight) and (B) carbon-to-nitrogen ratio (mol:mol).
Figure 2.4. Effects of light and mussels on sulfide intrusion of eelgrass tissues, measured as $F_{\text{sulfide}}$. Figs. 2.4A, 2.4C, and 2.4E show mean (+ 1 SE) $F_{\text{sulfide}}$ (%) in leaves, rhizomes, and roots, respectively. Figs. 2.4B, 2.4D, and 2.4F show linear regressions between net community production (NCP; mmol $O_2$ m$^{-2}$ 12 h$^{-1}$) and $F_{\text{sulfide}}$ in leaves, rhizomes, and roots, respectively. Symbols indicate separate treatments and gray shading indicates 95% confidence interval. Note differences in the scale of y-axes.
Figure 2.5. Effects of light and mussels on sulfide intrusion of eelgrass roots, measured as root elemental sulfur (S\(^0\)). Fig. 2.5A shows mean (+ 1 SE) root S\(^0\) (\(\mu\)mol [g dry tissue\(^{-1}\)). Fig. 2.5B shows linear regression between NCP and root S\(^0\) (log-transformed, ln (x + 1)). Symbols indicate separate treatments and gray shading indicates 95% confidence interval.
Figure 2.6. Effects of light and mussels on eelgrass density, growth, and energy stores. (A) Time series of mean (± SE) eelgrass density (shoots m$^{-2}$), where symbols indicate separate treatments. Figs. 2.6B–E show mean (+ 1 SE) terminal (B) and lateral (C) shoot density (no. mesocosm$^{-1}$), (D) leaf growth rate (mg dry shoot$^{-1}$ d$^{-1}$), and (E) soluble sugar concentration in rhizome tissues (mg per g dry tissue).
CHAPTER 3

Invasive prey indirectly increase predation on their native competitors

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Abstract

Invasive prey can interact with native prey directly by competing for shared resources or indirectly by changing the abundance or behavior of shared predators. However, both the study and management of invasive prey have historically overlooked indirect effects. In southern California estuaries, introduction of the mussel \textit{Arcuatula senhousia} has been linked to profound changes in native bivalve assemblages. We performed three field experiments to assess the mechanisms of competition between \textit{Arcuatula} and native bivalves, and evaluate the potential for \textit{Arcuatula} to indirectly mediate native predator-prey dynamics. We found that \textit{Arcuatula} reduces the diversity, abundance, and size of native bivalve recruits by preemptively exploiting sediment surface space. When paired with native shallow-dwelling clams, \textit{Arcuatula} reduces adult survival through overgrowth competition. However, \textit{Arcuatula} also attracts native predators, indirectly increasing predation of native clams, especially poorly-defended species.
Therefore, the vulnerability of invasive prey to predation can greatly exacerbate impacts on their native competitors.

**Keywords:** apparent competition; Asian mussel Arcuatula (Musculista) senhousia; bivalves; competition; estuary; indirect interactions; invasive; non-native; predation; recruitment

**Introduction**

Competition for limited resources is a major process by which introduced species impact native populations, communities, and ecosystems (Ruiz *et al.* 1999, Shea and Chesson 2002, Byers 2009), but ecological theory predicts that invading prey can also interact with their native competitors indirectly through shared native predators (Noonburg and Byers 2005). Nevertheless, indirect effects are largely overlooked in invasive species science and management, even though they appear to be common and significant mechanisms of impact (White *et al.* 2006, Ricciardi *et al.* 2013) and can complicate eradication or restoration efforts (Cory and Myers 2000, Zavaleta *et al.* 2001). Introduced prey have the potential to indirectly affect native predator-prey systems in two primary ways: diminishing mortality of native prey by shifting predation pressure away from natives (Fairweather 1985) or enhancing mortality on natives by increasing predator abundance or foraging success (i.e., apparent competition; Holt 1977, Holt and Kotler 1987). However, few studies have tested the potential for invasive prey to indirectly mediate predation on their native competitors.

Suspension-feeding bivalves are ubiquitous invaders of marine, estuarine, and freshwater ecosystems and excellent organisms for testing the direct and indirect effects of invasive prey. Once established, introduced bivalves many achieve numerical dominance relative to natives
Invasive bivalves are commonly prey for a wide range of native predators (Robinson and Wellborn 1988, Molloy et al. 1997). Despite substantial ecological and economic impacts (Sousa et al. 2009), introduced bivalves are understudied relative to other taxonomic groups, with a few notable exceptions (e.g., *Corbicula fluminea*, *Crassostrea* spp., *Dreissena* spp.). Furthermore, most examinations of indirect interactions between introduced and native bivalves have been anecdotal or correlative (Ruesink et al. 2005).

In southern California estuaries, introduction of the Asian nest mussel, *Arcuatula* (= *Musculista* senhousia) (hereafter referred to as *Arcuatula*), has been implicated in profound changes to native bivalve assemblages over the past 50 years (Crooks 1998, 2001, Dexter and Crooks 2000). *Arcuatula* is a small (≤ 28 mm shell length, SL), fast growing (Crooks 1996), and prolific shallow-burrowing mytilid (Fig. 3.1A), commonly found in densities of thousands of adults m$^{-2}$ and sometimes exceeding 10,000 m$^{-2}$ (Dexter and Crooks 2000, Williams et al. 2005). At high densities, *Arcuatula* transforms the structure of surface sediments by producing dense, tangled webs of byssal ‘cocoons’ (Crooks and Khim 1999). Evidence suggests that *Arcuatula* may reduce the growth and survival of clams in native (Uchida 1965) and introduced (Crooks 2001) ranges, but the mechanisms of this interaction remain obscure. It is possible that *Arcuatula* competes with native bivalves by preempting or competitively exploiting benthic space (Peterson and Andre 1980). However, *Arcuatula* is highly susceptible to predation by a suite of native predators (Reusch 1998, Crooks 2002b, Cheng and Hovel 2010) and, therefore, might influence
native bivalve assemblages indirectly by changing the abundance or behavior of shared consumers.

We tested the hypothesis that *Arcuatula* indirectly mediates predation on their native bivalve competitors (Fig. 3.1B). We first evaluated the strength and mechanisms of competition between *Arcuatula* and native bivalves by performing two experiments to quantifying interactions with early and adult life-history stages. Then, in a third experiment, we assessed the effects of *Arcuatula* on native predators and the consequences for native bivalve survival.

**Methods**

*Study region and fauna*

Our study took place in two estuaries in San Diego, California, USA (Fig. 3.1C): Mission Bay (32°46' N, 117°14' W) and San Diego Bay (32°43' N, 117°11' W). Both bays have typical Mediterranean seasonality in temperature (~14–25 °C) and salinity (~32–36 PSU), with winter freshwater inflow and hypersalinity during long, dry summers (Largier *et al.* 1997).

*Arcuatula* was first observed in San Diego in the 1960s (MacDonald 1969) and is now the most abundant bivalve in intertidal and shallow-subtidal soft-sediments (Crooks 1998, 2001, Dexter and Crooks 2000). Less abundant, but relatively common native soft-sediment clams and cockles include *Chione* spp., *Laevicardium substriatum*, *Leukoma* (=*Protothaca*) *staminea*, *Macoma nasuta*, and *Tagelus* spp. (Crooks 1998, 2001). Native bivalve predators are numerous and diverse, and include drilling gastropods, lobsters, crabs, fishes, and wading shorebirds (Reusch 1998, Crooks 2002b, Cheng and Hovel 2010, MCNC unpub. data).
Experiment 1: Competition effects on native bivalve recruitment

To evaluate the potential for preemptive competition by Arcuatula and quantify impacts on early life-history stages of native bivalves, we manipulated Arcuatula density and measured bivalve recruitment. We first collected adult Arcuatula (SL = 27.1 ± 2.7 (SD) mm) from several locations in Mission Bay and kept them in flow-through seawater aquaria at the San Diego State University Coastal and Marine Institute Laboratory until the experiment commenced (< 3 weeks). In Feb. 2012 at an intertidal sandflat in Mission Bay (Mariner’s Cove, Fig. 3.1C), we excavated sediments (top 15 cm) by shovel, sieved them to remove fauna > 1 mm, and filled them into experimental plots, which consisted of PVC rings (15 cm diameter × 5 cm depth) with plastic mesh bottoms (nominal mesh size = 1 mm). Next, in an adjacent intertidal seagrass (eelgrass, Zostera marina) meadow, we cored and removed vegetation (25 cm diameter × 10 cm depth), filled in each gap with sieved sediment, and installed a plot flush with the sediment surface. All plots were separated by > 1 m and at 0 m elevation relative to mean lower low water (MLLW). We set up this experiment near the mouth of Mission Bay because bivalve richness and abundance is greater there than the relatively depauperate back-bay (Crooks 1998, Dexter and Crooks 2000). For this experiment and others, we placed plots within eelgrass habitat (or artificial eelgrass habitat, see below) because density and survival for many native bivalves are higher in seagrass meadows than unvegetated sediments (Peterson et al. 1984).

After establishing plots, we randomly applied a density treatment to each and carefully transplanted Arcuatula as they are found in nature (posterior end slightly protruding above the sediment surface) to create plots with 0, 250, 500, 1000, 2000, 4000, or 8000 mussels m⁻² (n = 6), corresponding to densities commonly observed in the region (Dexter and Crooks 2000, Williams et al. 2005). Pilot studies showed that even at high density, transplanted mussels do not
migrate out of PVC rings and rapidly (< 1 h) reattach their byssal threads and resume feeding. Pilot studies also indicated that predation of *Arcuatula* would greatly compromise density manipulations. Therefore, we covered each plot with a galvanized-wire cage (25 cm diameter × 30 cm high; mesh size = 1.3 cm), buried to 15 cm depth. Once to twice per week, we gently scrubbed cages and checked them for predators (none were found). To control for artifacts of our design, we also created ‘true control’ plots, consisting of eelgrass gaps (as described previously) filled directly with sieved sediment (in lieu of PVC rings), uncaged, and otherwise treated exactly as experimental plots (n = 5 due to the loss of one true control plot from anchor damage).

After 11 weeks, we collected and froze all plots (we used corers to collect sediments from true control plots). We sieved (1 mm) and preserved the contents of each plot in 95% ethanol and later identified, counted, and measured (SL to nearest mm) all bivalves. To correct for occasional *Arcuatula* mortality, we calculated *Arcuatula* density as the average between the start and end of the experiment. To generate conservative estimates of recruitment, we excluded unidentifiable specimens and did not measure the size of damaged individuals. We characterized bivalve recruit composition as species richness, species evenness, and Simpson’s index of diversity.

To determine the effect of caging on all response variables, we compared caged and uncaged control plots (zero *Arcuatula* m⁻²) using two-tailed *t* tests in R 3.0.2 (R Development Core Team 2013). To determine normality of the residuals for this test and other parametric analyses (in this experiment and others, see below), we used normal probability plots. To assess data linearity and homoscedasticity of the residuals, we visually examined the relationship between residuals and fitted values, and used the Brown–Forsythe test for equality of variances. We tested for the effect of *Arcuatula* density on all response variables using separate ordinary least squares (OLS) linear regressions using R 3.0.2. When necessary to meet the assumptions of
OLS regression, we log-transformed [\ln (y + 1)] recruitment data. When nonlinearity or heteroscedasticity could not be resolved by transformations, we performed nonlinear regressions using JMP 10.0 (SAS Institute Inc., Cary, North Carolina, USA). We selected nonlinear models based on comparisons of corrected Akaike information criterion (AICc) values to optimize goodness-of-fit but avoid model overfitting (Hurvich and Tsai 1989). We report nonlinear model fit as AICc and pseudo-R² \[= 1 – (\text{residual sum-of-squares} / \text{corrected total sum-of-squares})\].

**Experiment 2: Competition effects on adult native bivalves**

To determine the potential for exploitative competition between *Arcuatula* and adult native bivalves, we tested the effect of *Arcuatula* density on the growth and survival of two native clam species—the egg cockle, *Laevicardium substriatum*, and the wavy venus, *Chione undatella*—in the absence of predators. We suspected these two native species might be particularly likely to compete with *Arcuatula* because they share similar distributions, feeding behaviors, and burrowing depths. Both *L. substriatum* and *C. undatella* inhabit surface sediments in intertidal and shallow-subtidal estuarine areas at densities generally < 10–20 clams m⁻² (MacDonald 1969, Reusch and Williams 1999).

We sought to manipulate *Arcuatula* and bivalve abundance in eelgrass habitat without destroying habitat structure during the transplantation process. Therefore, we recreated eelgrass canopy structure by creating square plots (170 cm²) using artificial seagrass units (ASUs; see Appendix 3.A for construction methods), which are commonly used as experimental surrogates of seagrass leaf structure (Virnstein and Curran 1986). In Feb. 2005, we filled 120 ASUs with sieved (1 mm) sediment collected from a subtidal site in San Diego Bay (Coronado, Fig. 3.1C).
and installed ASU plots flush with the sediment surface adjacent to a nearby subtidal eelgrass meadow (0.5–1.0 m below MLLW; plots separated by > 1 m).

We collected adult *Arcuatula* and native clams from several sites in San Diego Bay and held them in captivity as described previously. To determine native clam growth rates (change in SL), we labeled shells by exposing them to a solution of fluorescent dye (calcein; 500 mg L\(^{-1}\)) for 24 h and measured initial SL (nearest 0.1 mm; mean initial SL for *C. undatella* and *L. substratum* were 23.0 ± 1.0 mm and 12.6 ± 0.2 mm, respectively). After tagging clams, we factorially manipulated native clam species (*C. undatella* or *L. substratum*; 1 clam plot\(^{-1}\)) and *Arcuatula* density (10 treatments: 0, 125, 250, 500, 750, 1000, 1500, 2000, 3500, or 5000 mussels m\(^{-2}\)) in each ASU by transplanting *Arcuatula* and native clams as described previously (n = 6, except for the loss of 1 *C. undatella* plot and 3 *L. substratum* plots due to storm damage; treatments assigned randomly). To prevent predation, we covered each plot with a galvanized-wire cage (13 cm length × 13 cm width × 35 cm tall; mesh size = 1.0 cm), buried to 10 cm depth. We gently scrubbed cages and checked them for predators weekly. Despite cages, small predatory crabs were discovered within a few plots.

After 61–78 days (it was not feasible to sample all plots concurrently), we collected plots and identified each clam as (1) alive, (2) dead and not consumed (i.e., valves intact), or (3) preyed upon (i.e., dead with valve broken, crushed, or drilled and flesh absent, or missing). We excluded from our calculations plots in which cages failed or otherwise exhibited evidence of predation. We used binomial logistic regressions in JMP 10.0 to test the effect of *Arcuatula* density on native clam survival (alive vs. dead; species analyzed separately). We rejected models not meeting the Hosmer and Lemeshow (1989) goodness-of-fit criteria. For ease of interpreting
the ecological relevance of model results, we report the effects of Arcuatula density on parameter estimates and odds ratios in units of 1000 mussels m$^{-2}$.

For living clams, we measured SL (nearest 0.1 mm) and estimated the daily growth rate [\(= (\text{final SL} - \text{initial SL}) / \text{no. days in the field}\)]. Smaller bivalves grow faster than larger ones (Peterson 1982) and OLS regression revealed that native clam initial SL was highly negatively correlated with growth for both species (\(L.\ substriatum\): \(n = 23, P = 0.0017, R^2 = 38.0\%\); \(C.\ undatella\): \(n = 46, P < 0.0001, R^2 = 29.9\%\)). Therefore, we used the residuals from these models as a measure of relative (i.e., size-specific) growth rate. We tested the effects of Arcuatula density on the relative growth rate of each native clam species separately using OLS regression in JMP 10.0. To correct for occasional Arcuatula mortality, we calculated Arcuatula density as the average between the start and end of the experiment.

**Experiment 3: Indirect effects on native predator-prey dynamics**

To test the potential for Arcuatula to indirectly affect native bivalves through shared native predators, we measured the effect of Arcuatula density on predator abundance and the proportional mortality of two native clam species. Although they occupy similar niches, \(L.\ substriatum\) and \(C.\ undatella\) differ greatly in their size and shell thickness. We hypothesized that the larger, thicker-shelled, and more inflated \(C.\ undatella\) is less susceptible to predation than the smaller, thinner-shelled \(L.\ substriatum\) and, therefore, more resistant to predator-mediated impacts by Arcuatula.

We created uncaged ASU plots (22 cm length \(\times\) 22 cm width \(\times\) 7 cm depth and separated by 2 m, but otherwise as described previously) in a shallow subtidal eelgrass meadow in Mission Bay (Ventura Cove, Fig. 3.1C; approximately 1 m below MLLW). Next, in June 2004, we
collected bivalves from Mission Bay (as described previously) and factorially manipulated native clam identity (*L. substriatum* or *C. undatella*; 8 clams plot\(^{-1}\) = 160 clams m\(^{-2}\)) and *Arcuatula* density (0, 160, or 640 mussels m\(^{-2}\)) in each plot during two separate 5-day trials (for each trial, n = 3 plots per clam species per *Arcuatula* density treatment; treatments assigned randomly). To avoid confounding our experiment with competition effects between *Arcuatula* and native clams, we chose our highest *Arcuatula* density treatment to be modest (640 mussels m\(^{-2}\)) relative to observed field densities because results from *Experiment 2* suggested that competition would be minor at this density. To control for the effects of transplantation stress on native clam survival, for each trial we also created plots for two control treatments (one per native clam species) consisting of a caged ASU plot (cage similar to *Experiment 2*) containing *L. substriatum* or *C. undatella* (8 clams plot\(^{-1}\)) and a ‘high’ density of *Arcuatula* (640 mussels m\(^{-2}\)).

After each trial, we collected all plots and counted the abundance of predators. We identified clams and mussels as alive, dead and not consumed, or preyed upon (as described previously). We presumed missing clams were consumed by predators (e.g., fishes, octopuses) because we recovered 91–100% of clams in caged control plots. We used separate two-way analyses of variance (ANOVAs) in JMP 10.0 to test the effects of native species identity and *Arcuatula* density on (1) native clam total proportional mortality, (2) proportion of native clams consumed, and (3) the density of predators observed in plots at the end of the experiment. Where ANOVAs showed evidence of treatment effects (*P* < 0.05), we made post-hoc pairwise comparisons using Fisher’s Least Significant Difference test.
Results

Experiment 1: Competition effects on native bivalve recruitment

Preemptive competition for space by *Arcuatula* reduced the diversity and evenness of native bivalve recruits and, for a few abundant taxa, altered recruit density and size. Recruits from 22 native bivalve taxa colonized our plots. *Arcuatula* density was uncorrelated with species richness ($P = 0.696$), inversely correlated with species evenness ($P = 0.00658$, $R^2 = 17.1\%$), and marginally inversely correlated with Simpson’s diversity ($P = 0.0910$, $R^2 = 7.0\%$) (Fig. 3.2).

The majority of taxa (17) were rare, having $\leq 19$ individuals among all plots (Appendix 3.B). We performed separate regression analyses of density and size for the 6 species that represented 95% of all bivalves (Fig. 3.3; Appendix 3.C). Increasing *Arcuatula* density was matched by a saturating (logistic) increase in *Leukoma staminea* density ($y = 436/(1 + e^{-0.00311(x-283)})$; AICc = 564.5, pseudo-$R^2 = 30.6\%$), an exponential decrease in *Tresus nuttallii* density ($y = 262e^{-0.000228x}$; AICc = 495.2, pseudo-$R^2 = 32.8\%$), a linear decrease in *Donax gouldii* density ($P = 0.034$, $R^2 = 10.8\%$), and no change in the density of *Chione* spp., *Cooperella subdiaphana*, or *Saxidomus nuttalli* (all $P > 0.126$). Density of the most abundant native species, *L. staminea*, was strongly inversely correlated with species evenness ($P < 0.0001$, $R^2 = 68.8\%$), while the next most-abundant taxa showed much weaker associations (*T. nuttallii*: $P = 0.013$, $R^2 = 14.5\%$; *D. gouldii*: $P = 0.150$).

Increasing *Arcuatula* density was correlated with a marginal linear decrease in the mean size (SL) of *Leukoma staminea* recruits ($P = 0.098$, $R^2 = 6.9\%$) and an exponential decrease in the size of *Tresus nuttallii* recruits ($y = 8.51 + 6.49e^{-0.00102x}$; AICc = 197.4, pseudo-$R^2 = 51.2\%$), while the size of the other 4 species were unaffected (all $P > 0.311$).
Comparisons of caged and uncaged control plots showed that cages had no effects on species richness \((P = 0.517)\) or Simpson’s diversity \((P = 0.142)\), but caused a marginal decrease in evenness \((P = 0.070)\). Recruitment was higher in caged than uncaged plots for \textit{Donax gouldii} \((71 \pm 21 \text{ vs. } 24 \pm 22 \text{ clams m}^{-2}; t_9 = 3.55, P = 0.006)\) and \textit{Tresus nuttallii} \((231 \pm 49 \text{ vs. } 53 \pm 45 \text{ clams m}^{-2}; t_9 = 6.21, P < 0.001)\), marginally lower for \textit{Cooperella subdiaphana} \((0 \text{ vs. } 12 \pm 11 \text{ clams m}^{-2}; t_9 = 2.45, P = 0.071)\), and no different for other species \((\text{all } P > 0.294)\).

\textbf{Experiment 2: Competition effects on adult native bivalves}

When paired with shallow-dwelling native clams, \textit{Arcuatula} strongly competed for surface sediment space, reducing adult native bivalve survival but not growth. In control plots \(\text{without Arcuatula}\), 100\% of \textit{Chione undatella} and 84\% of \textit{Laevicardium substriatum} survived. In experimental plots, survival of both native clams was inversely correlated with \textit{Arcuatula} density \((C. undatella: P = 0.0309; L. substriatum: P = .0186; \text{Fig. 3.4 A–B}; \text{see Appendix 3.D} \text{ for parameter estimates})\). For each increase of 1000 \textit{Arcuatula} m\(^{-2}\), the estimated odds of survival decreased by 1.64\(\times\) (95\% CI: 1.05–2.63) for \textit{C. undatella} and 1.91\(\times\) (95\% CI: 1.18–3.54) for \textit{L. substriatum}.

\textit{Arcuatula} density was not correlated with the relative growth rate for either native clam species \((C. undatella: P = 0.216; L. substriatum: P = 0.813; \text{Fig. 3.4C–D})\). However, only one \textit{L. substriatum} survived \textit{Arcuatula} densities > 2500 mussel m\(^{-2}\), limiting the range of mussel densities available for growth analysis.
Experiment 3: Indirect effects on native predator-prey dynamics

Arcuatula indirectly increased the mortality of native clams, especially for poorly-defended species, by changing the behavior of native predators. There was no effect of trial number on response variables ($P > 0.2$) and, therefore, we pooled trials. In caged control plots, native clam survival was high (100% for C. undatella and 91% for L. substriatum). In uncaged plots, both total proportional mortality of native clams and the proportion of clams consumed were higher in plots with greater Arcuatula densities, and higher for L. substriatum than for C. undatella (Table 1; Fig. 3.5A–B). There was a trend ($P = 0.075$) for an interaction of clam species and Arcuatula density in explaining proportional consumption, with relatively greater predation of L. substriatum in the densest Arcuatula treatment (640 mussels m$^{-2}$). Our measures of proportional consumption are likely conservative because we did not include dead clams with undamaged shells, even though these might reflect predation.

Arcuatula density and native clam species had an interactive effect on the density of predators found within plots (Table 1; Fig. 3.5C). Predators aggregated in plots with highest densities of Arcuatula (640 mussels m$^{-2}$). However, predators were more abundant in plots with L. substriatum than C. undatella at the highest density but not at lower densities. Several predators colonized uncaged plots: Lophopenopeus spp. (mud crabs), Hypsoblennius gentilis (blennies), and gastropods (Pteropurpura festiva and Conus californicus). Pteropurpura festiva was the most abundant and responsible for the majority of bivalve consumption, as evidenced by conspicuous drill holes in bivalve shells.
Discussion

We have demonstrated that invasive prey can harm their native competitors through both direct and indirect interactions. Our findings highlight the important role that indirect effects play in non-native species impacts (White et al. 2006, Ricciardi et al. 2013). In our study, exploitative competition by an invasive mussel affected both juvenile and adult stages of native southern California bivalves. By preempting space, *Arcuatula* reduced the diversity and evenness of bivalve recruits (Fig. 3.2) and, for a few abundant taxa, suppressed recruit density and size (Fig. 3.3). Competition between *Arcuatula* and adults of two native clams resulted in decreased survival of both native species, suggesting overgrowth competition (Fig. 3.4). At very modest densities, *Arcuatula* also attracted native predators, indirectly increasing mortality of native clams, especially the poorly-defended *Laevicardium substriatum* (Fig. 3.5). Our study illustrates that the vulnerability of invasive prey to predation can exacerbate impacts on their native competitors.

Preemptive competition and larval recruitment

Results from our first experiment add to a growing body of literature suggesting that introduced species can affect native communities by altering recruitment dynamics (e.g., Gribben and Wright 2006). Spatial and temporal variability in propagule recruitment of is a major determinant of community structure (Tilman 1994), especially for sessile assemblages in benthic marine and estuarine ecosystems (Gaines and Roughgarden 1985). We found that preemption of space by *Arcuatula* decreases the diversity and evenness (but not richness) of native bivalve recruits, and alters recruit density and size for a few abundant taxa. Analogously, an introduced polychaete alters the recruitment of native sessile invertebrates by changing benthic habitat
structure (Holloway and Keough 2002) and an invasive brown alga reduces recruitment of native giant kelp by reducing light availability (Ambrose and Nelson 1982).

There are several pre- and post-settlement processes that could explain the alteration of native bivalve recruitment by *Arcuatula* (Connell 1985). First, *Arcuatula* may have changed the behavior of native larvae by altering settlement cues (Woodin 1991). Second, *Arcuatula* may have modified the availability or suitability of habitat. At moderate to high densities, *Arcuatula* monopolizes surface sediments and transforms benthic structure with a tangled web of byssal threads, thereby causing changes to infaunal communities (Crooks and Khim 1999). Third, it is possible that *Arcuatula* directly altered bivalve recruitment by filtering and ingesting native larvae (Davenport *et al.* 2000). Introduced oysters (*Crassostrea gigas*) more efficiently consume bivalve larvae of native species than of conspecifics (Troost *et al.* 2008); this is an interesting but unexplored possibility for *Arcuatula*.

Preemptive competition by *Arcuatula* altered recruitment density for the three most abundant native taxa. *Arcuatula* was correlated with a saturating increase in the density of Pacific littleneck clams, *Leukoma staminea*, possibly by creating suitable interstitial habitat for these very small recruits (SL = 2.2 ± 0.6 cm). *Arcuatula* was also correlated with decreases in the densities of Pacific gaper clams, *Tresus nuttallii*, and California bean clams, *Donax gouldii*. These two species were among the largest recruits and possibly competed with *Arcuatula* for space in surface sediments. *Donax gouldii* is a relatively mobile clam typically found in the swash zone of sandy beaches and it is possible that density reductions for this species were driven by larval settlement aversion or post-settlement emigration. *Arcuatula* also reduced the size of *L. staminea* and *T. nuttallii*, possibly by reducing near-bottom phytoplankton concentrations, interfering with feeding behavior, or confining interstitial space.
One caveat to our measurements of *Arcuatula* impacts on native recruitment is that densities of *T. nuttallii* and *D. gouldii* recruits were 3–4 times higher in caged than uncaged control plots. Pilot studies showed that cages were necessary to maintain *Arcuatula* density treatments. It is possible that predator presence or lower bivalve densities might weaken interactions between *Arcuatula* and native bivalve recruits. In addition, the effects of *Arcuatula* on native bivalve recruitment likely vary among locations or differing oceanographic conditions.

*Resource competition and habitat modification in soft-sediments*

Results from our second experiment support the idea that bivalves occupying similar depth zones compete for living space or access to the water column (Peterson and Andre 1980, Peterson 1982) and indicate that habitat modification is a major mechanism by which invasive species impact natives (Crooks 2002a). We found that for each increase of 1000 *Arcuatula* m⁻², the odds of mortality increased by 64% for *Chione undatella* and 91% for *Laevicardium substriatum*. We suspect that overgrowth by *Arcuatula* outcompetes shallow-burrowing native bivalves by modifying the habitat in ways that reduce surface sediment space, limit access to water-borne food, and restrict clam movement or feeding. While short-siphoned clams like *C. undatella* and *L. substriatum* may be unable to alleviate overgrowth competition with *Arcuatula* by burrowing deeper into the sediment, deep-dwelling species are probably unaffected as long as they can maintain siphon access to the water column, as has been shown for the bent-nose clam, *Macoma nasuta* (Crooks 2001). Still, *Arcuatula* may have significant impacts on early life-history stages of deep-burrowing bivalves, as our results indicate for *Tresus nuttallii* recruits.

Contrary to earlier work (Crooks 2001), we found no effect of *Arcuatula* on native clam growth. Competitive effects on growth are typically detected before effects on survival (Peterson
but rapid mortality can preclude the ability to measure growth effects. Furthermore, if
growth suppression occurs only at high densities (over 5000 mussels m$^{-2}$; Crooks 2001), low
survival of native bivalves in our densest *Arcuatula* treatment (5000 mussels m$^{-2}$) may have
limited our ability to detect changes in growth.

*Apparent competition between introduced and native prey*

Results from our third experiment illustrate that invasive prey can indirectly increase
predation rates on native competitors by changing the abundance or behavior of shared predators,
but that the magnitude of apparent competition strongly depends on the vulnerability of natives
to predation. Native predators can be important to reducing the success and impacts of invasive
prey (Robinson and Wellborn 1988, Molloy *et al.* 1997, Kimbro *et al.* 2013) and, in our study
system, a suite of native predators (crustaceans, fishes, gastropods, shorebirds) consume
*Arcuatula* and provide limited biotic resistance (Reusch 1998, Crooks 2002b, Cheng and Hovel
2010). However, our findings suggest that the wide palatability and high susceptibility of
*Arcuatula* may come at a cost to vulnerable native clams. Instead of being preferred by predators
and thereby reducing predation on native prey (Fairweather 1985), *Arcuatula* increases predation
on equally-susceptible native bivalves by attracting predators, even at very modest densities (640
mussels m$^{-2}$). Thus, invasive prey can strongly impact natives even in the absence of enemy
release. The most abundant and important predator found in our experiment was the festive
murex, *Pteropurpura festiva*, a drilling gastropod known to aggregate in dense *Arcuatula* patches
(Kushner and Hovel 2006). Supporting earlier theoretical work (Noonburg and Byers 2005), our
results indicate that the vulnerability of invasive prey to predation can exacerbate impacts on
their native competitors. In a terrestrial analog, palatable invasive orchids indirectly harm co-
occurring native orchids by attracting native flower-eating weevils (Recart et al. 2012). Our findings are distinct from most existing studies of apparent competition caused by non-native prey, which primarily involve refuge-mediated effects (e.g., Orrock et al. 2008).

Interestingly, we found that the ability of invasive prey to indirectly harm their native competitors depends on the vulnerability of native prey to predation. Native predators consumed a high proportion of smaller, thin-shelled *L. substriatum* but not larger, thick-shelled *C. undatella*. When competition among native bivalves is strong, we suspect that apparent competition between *Arcuatula* and poorly-defended native bivalves could have positive indirect effects on less vulnerable prey, such as well-defended (e.g., *Chione* spp.) or deep-dwelling species (Reusch 1998).

**Implications for long-term changes to native bivalve assemblages**

Introduction of *Arcuatula* to southern California in the 1960s was followed by several decades of change to native bivalve assemblages. In Mission Bay, populations of *Chione* spp., *Cryptomya californiensis* (softshell clams), *Saxidomus nuttalli* (butterclams), *Solen rostriformis* (jack-knife clams), and *Tresus nuttallii* have decreased over the past 50 years (Crooks 1998, Dexter and Crooks 2000). Although many factors may have contributed to these declines, including dramatic habitat modification and overharvesting, the results of this study and Crooks (2001) suggest that invasion by *Arcuatula* may have played an important role. Presently, *Arcuatula* is the most common and abundant bivalve in intertidal and shallow-subtidal soft-sediments in San Diego estuaries (Dexter and Crooks 2000, Crooks 2001, authors’ personal observations). Concomitant with declines in native bivalve populations and invasion of *Arcuatula*, eelgrass meadows have been heavily degraded and fragmented, resulting in up to
90% loss in San Diego Bay (Reusch and Williams 1998). This decrease in native habitat may enhance *Arcuatula* spread and impacts on native bivalves because expansive eelgrass meadows reduce *Arcuatula* growth, fecundity, and survival (Reusch and Williams 1999).

Indirect effects are increasingly being recognized as an important component of invasive species science (White et al. 2006, Ricciardi et al. 2013) and management (Cory and Myers 2000, Zavaleta et al. 2001). Consideration of the community and ecosystem-scale impacts of invasive species should lead to more effective management decisions with fewer unforeseen consequences. For example, without experimental data, one might have assumed that *Arcuatula* facilitates native bivalves by representing a preferred food source for native predators instead of causing harm through apparent competition. Efforts aimed at mitigation of *Arcuatula* and other invasive species should consider both biotic and abiotic indirect interactions, especially across trophic levels, as well as the potential indirect benefits of habitat (e.g., eelgrass) restoration. Conservation of native bivalves in southern California should consider the gradual, multidecadal changes in bivalves assemblages to avoid restoration goals based on shifting baselines.

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### Tables and Figures

**Table 3.1.** Results of factorial ANOVAs testing for effects of *Arcuatula senhousia* density, native clam species (*Chione undatella* and *Laevicardium substriatum*), and their interaction on total proportional mortality, proportional consumption (i.e., mortality due to predation), and the density of predators within plots (*Experiment 3*). *P* values < 0.05 are shown in bold.

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Figure 3.1. (A) Arcuatula senhousia transforms the physical structure of surface sediments. Photo shows a high density (8,000 mussels m$^{-2}$) plot from Experiment 1 exposed during low tide (photo credit: M.C.N. Castorani). (B) Hypothesized relationships between Arcuatula and native bivalves (photographs not to scale). Solid and dashed arrows indicate direct and indirect interactions, respectively. Introduced mussels and native bivalves may compete for surface-sediment space or water-borne food. In addition, a suite of native predators consumes both introduced and native mussels, creating the potential for indirect effects. (C) Map of study sites in San Diego, California, USA. Site numbers correspond to experiments (1 = Mariner’s Cove, 2 = Coronado, 3 = Ventura Cove).
Figure 3.2. Relationship between Arcuatula senhousia density and native bivalve (A) species richness, (B) Simpson’s index of diversity, and (C) species evenness (Experiment I).
Figure 3.3. Relationship between *Arcuatula senhousia* density and the density (A–C) and size (mean shell length; D–F) of native bivalve recruits for the three most abundant species (*Experiment 1*). See text for nonlinear regression functions. See Appendix 3.C for plots of less abundant species (*Saxidomus nuttalli, Chione* spp., and *Cooperella subdiaphana*). (Bivalve photographs not to scale.)
Figure 3.4. Relationship between *Arcuatula senhousia* density and the probability of survival (A–B) and relative growth rate (C–D) of adult native clams, *Chione undatella* (n = 46) and *Laevicardium substriatum* (n = 23) (Experiment 2). In A–B, points are actual survival data and lines represent predicted survival from binomial logistic regression models (see text for summaries of fit and odds ratios). In C–D, relative (i.e., size-corrected) growth rate represents the residuals from a regression of shell growth on initial clam shell length. (Bivalve photographs not to scale.)
Figure 3.5. Effects of Arcuatula senhousia density on mean (+ 1 SE) (A) total proportional mortality of adult native clams (Chione undatella and Laevicardium substriatum), (B) proportion of native clams consumed (i.e., proportional mortality due to predation), and (C) density of predators in plots (Experiment 3). (Bivalve photographs not to scale.)
Appendices

Appendix 3.A. Description of artificial seagrass unit (ASU) construction methods.

In Experiments 2 and 3, we recreated eelgrass canopy structure by creating artificial seagrass units (ASUs). ASUs were square PVC frames (13 cm length × 13 cm width × 7 cm depth) with plastic mesh bottoms (nominal mesh size = 1 mm). A grid (4 × 4) of monofilament provided attachment points for structural mimics of eelgrass shoots (16 plot$^{-1}$ = 1,000 shoots m$^{-2}$, representing a dense eelgrass patch for San Diego Bay (Reusch and Williams 1999)), which consisted of a pair of buoyant green polypropylene ribbon ‘leaves’ (5 mm width × 25 cm length).

Appendix 3.B. Bivalve recruit taxa found in Experiment 1, ranked by total abundance among all plots.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Taxa</th>
<th>Common name</th>
<th>Abundance (N)</th>
<th>Percentage of total N</th>
<th>No. plots with N &gt; 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leukoma staminea</td>
<td>Pacific littleneck clam</td>
<td>680</td>
<td>43.7%</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>Tresus nuttallii</td>
<td>Pacific gaper clam</td>
<td>434</td>
<td>27.9%</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>Donax gouldii</td>
<td>California bean clam</td>
<td>153</td>
<td>9.8%</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>Saxidomus nuttalli</td>
<td>California butterclam</td>
<td>109</td>
<td>7.0%</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>Chione spp.</td>
<td>California venus</td>
<td>71</td>
<td>4.6%</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>Cooperella subdiaphana</td>
<td>Shiny cooperclam</td>
<td>29</td>
<td>1.9%</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>Lyonsia nesiotes</td>
<td>California lyonsia</td>
<td>19</td>
<td>1.2%</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>Leptopexcten latiauratus</td>
<td>Kelp scallop</td>
<td>8</td>
<td>0.5%</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Arcuatula senhousia</td>
<td>Asian nest mussel</td>
<td>8</td>
<td>0.5%</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>Lasaea adansonii</td>
<td>Ruddy lasaea</td>
<td>7</td>
<td>0.5%</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>Sphenia fragilis</td>
<td>Atillean sphenia</td>
<td>7</td>
<td>0.5%</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>Modiolus spp.</td>
<td>Horsemussel</td>
<td>5</td>
<td>0.3%</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>Cryptomya californica</td>
<td>California softshell clam</td>
<td>4</td>
<td>0.3%</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>Tagelus spp.</td>
<td>Razor clam</td>
<td>4</td>
<td>0.3%</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>Laevicardium substriatum</td>
<td>Egg cockle</td>
<td>3</td>
<td>0.2%</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>Order Nuculoida</td>
<td>Nut clam</td>
<td>3</td>
<td>0.2%</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>Argopecten ventricosus</td>
<td>Pacific calico scallop</td>
<td>2</td>
<td>0.1%</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>Cyclocardia spp.</td>
<td></td>
<td>2</td>
<td>0.1%</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>Mytilus spp.</td>
<td></td>
<td>2</td>
<td>0.1%</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>Family Petricolidae</td>
<td></td>
<td>2</td>
<td>0.1%</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>Limopsis spp.</td>
<td></td>
<td>1</td>
<td>0.1%</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>Macoma spp.</td>
<td></td>
<td>1</td>
<td>0.1%</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>Family Ostreidae</td>
<td>Oyster</td>
<td>1</td>
<td>0.1%</td>
<td>1</td>
</tr>
</tbody>
</table>
Appendix 3.C. Relationship between *Arcuatula senhousia* density and the density (A–C) and size (mean shell length; D–F) of *Saxidomus nuttalli*, *Chione* spp., and *Cooperella subdiaphana* recruits (*Experiment 1*). All *P* > 0.126. (Bivalve photographs not to scale.)

Appendix 3.D. Parameter estimates from binomial logistic regression analysis testing the effects of *Arcuatula* density (in units of 1000 mussels m$^{-2}$) on the survival of native clams (*Experiment 2*). *P* values < 0.05 are shown in bold.

<table>
<thead>
<tr>
<th>Native clam species</th>
<th>Model parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>$\chi^2$</th>
<th>df</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chione undatella</em></td>
<td>Intercept</td>
<td>2.300</td>
<td>0.554</td>
<td>17.21</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td><em>Arcuatula</em> density</td>
<td>−0.493</td>
<td>0.228</td>
<td>4.66</td>
<td>1</td>
<td>0.0309</td>
</tr>
<tr>
<td><em>Laevicardium striatum</em></td>
<td>Intercept</td>
<td>1.100</td>
<td>0.494</td>
<td>4.96</td>
<td>1</td>
<td>0.0260</td>
</tr>
<tr>
<td></td>
<td><em>Arcuatula</em> density</td>
<td>−0.649</td>
<td>0.276</td>
<td>5.53</td>
<td>1</td>
<td>0.0186</td>
</tr>
</tbody>
</table>
CONCLUSIONS

The preceding dissertation chapters illustrate how variation in three major types of environmental disruptions—physical disturbance, abiotic resource stress, and species introduction—affect the sign, strength, and mechanisms of species interactions. My findings improve our understanding of the role of environmental context in mediating species interactions, particularly in shallow estuaries and coastal lagoons dominated by eelgrass (*Zostera marina*), and raise new questions about how environmental dependencies affect ecological interactions.

In Chapter 1, I determined the role of disturbance in mediating competition between eelgrass and burrowing ghost shrimp, two strong habitat-modifying species. I discovered that in the absence of disturbance, eelgrass outcompetes ghost shrimp locally by producing dense, obtrusive belowground structure, validating early hypotheses about this potential mechanism (Ringold 1979, Peterson 1982, Orth *et al.* 1984). However, physical disturbance increases the potential for landscape-scale coexistence by creating a spatiotemporal refuge from competition for ghost shrimp. Ghost shrimp have a colonization advantage to eelgrass, quickly capitalizing on disturbances to eelgrass patch edges, and maintain source populations above and below the eelgrass depth range. These findings demonstrate that antagonistic habitat modification can lead to local competitive exclusion, yet disturbance can facilitate broader coexistence.

Comparison of these findings to prior studies (Suchanek 1983, Siebert and Branch 2006, 2007, Berkenbusch *et al.* 2007, Kneer *et al.* 2008) suggests that the sign and strength of seagrass–burrowing shrimp interactions vary by species and region, and raises new questions about the role of environmental context. How does the strength of habitat modification (or other
mechanisms of competition) interact with disturbance regimes to determine the probability of landscape-scale coexistence? How would increases in disturbance of eelgrass change the probability of ghost shrimp persistence? What is the importance of refuges from competition for coexistence? How might differences in sediment organic matter influence rates of ghost shrimp bioturbation? Future empirical and theoretical studies should aim to resolve these questions. Spatially-explicit mathematical models may provide particular insight by simulating species interactions over spatial and temporal scales not feasible for field studies (Hastings 1980, Tilman 1994, Tilman et al. 1997, Balzter et al. 1998, Winkler et al. 1999, Chesson 2000, Sears and Chesson 2007).

In Chapter 2, I tested the hypothesis that light availability mediates modification of benthic biogeochemical conditions by blue mussels and the sign and strength of impacts on eelgrass. I found that light availability indirectly mediates blue mussel habitat modification (concentrations of nutrients, sulfides, and oxygen) by altering eelgrass metabolism, and that these changes feed back to influence eelgrass vulnerability to sulfide stress. Therefore, blue mussels may particularly enhance the risk of eelgrass sulfide stress in eutrophic estuaries, which are typically characterized by reduced light availability, impaired benthic productivity, and frequent occurrences of benthic hypoxia (Cloern 2001, Conley et al. 2009, Howarth et al. 2011). However, I also discovered that eelgrass has some capacity to resist harm due to sulfide stress, indicating that environmental context plays a limited role in mediating overall eelgrass-mussel interactions.

The direct implications of these results are largely limited to the effects of light on blue mussel–eelgrass interactions. Future work should expand upon this study by disentangling the conflated physical and chemical effects of eutrophication (e.g., light availability, nutrient
concentration, and dissolved oxygen saturation) in mediating the impacts of bivalves on seagrasses, ideally through careful field experiments. Modeling studies could also provide insight by exploring the potential for the sign of seagrass-bivalve interactions to depend on the spatial scale of bivalve-mediated changes. Suspension-feeding bivalves can reduce phytoplankton populations on the scales of entire estuaries (Officer et al. 1982, Newell et al. 2007), but there may be severe limits to bivalve mediation of eutrophication due to spatial or temporal mismatches between phytoplankton blooms and bivalve filtration (Pomeroy et al. 2006, 2007). Also, my work was limited to interactions in temperate estuaries. How might bivalve-seagrass interactions vary in tropical seas, which differ strongly in productivity, seagrass nutrient and light limitation, seagrass and bivalve taxonomy, and sediment biogeochemistry (e.g., rates of sulfide reduction, sulfide accumulation, and nutrient cycling)? Lastly, humans are changing the abundance and composition of bivalve assemblages in or near seagrass meadows through aquaculture (Pawiro 2010), overharvesting of wild populations (Newell 1998, Cloern 2001), restoration or mitigation of water quality using bivalves (Stadmark and Conley 2011), and introduction of non-native bivalves (Williams 2007). What will be the long-term effects of such changes on the abundance and distribution of seagrasses, which already face numerous stressors and are declining globally (Short et al. 1991, Orth et al. 2006, Waycott et al. 2009)?

In Chapter 3, I conducted a series of experiments to explore how an invasive mussel, Arcuatula senhousia, indirectly interacts with native bivalve competitors by mediating the abundance and behavior of shared predators. I found that Arcuatula reduces the diversity, abundance, and size of native bivalve recruits by preemptively exploiting sediment surface space. This finding supports a growing body of literature that suggests introduced species can affect native communities by altering recruitment dynamics (e.g., Gribben and Wright 2006).
Furthermore, *Arcuatula* decreases the survival of adult native clams in two ways: (1) by outcompeting natives for space or access to water-borne food and (2) by increasing predation on native clams, especially poorly-defended species, through apparent competition. These findings support earlier empirical (Recart et al. 2012) and theoretical work (Noonburg and Byers 2005), indicating that the vulnerability of invasive prey to predation can exacerbate impacts on their native competitors.

To determine the generalizability of these results, future research should seek to understand how *Arcuatula* affects native bivalve recruitment among locations, seasons, and years. Furthermore, it would be interesting to test the relative effects of invasive bivalves on early vs. late life-history stages. Native predators of bivalves differ between the subtidal (e.g., fishes, lobsters, crabs) and intertidal (e.g., shorebirds, gastropods) zones. How might predator-mediated effects, such as apparent competition, depend on the identity and foraging behavior of native predators? For example, different functional responses among native predators to invasive species might dictate the ability to provide ‘biotic resistance’ to invasion (Kushner and Hovel 2006, Cheng and Hovel 2010, Kimbro et al. 2013). Does *Arcuatula* change interactions (e.g., competition, predation) among native bivalve predators? One might hypothesize that complex interactions such as these would be likely if *Arcuatula* attracts both small, specialist predators (e.g., drilling gastropods) and large, generalist predators (e.g., lobsters, crabs, fishes).

From an applied perspective, restoration of native assemblages should consider the potential for interactions between native prey, introduced prey, and native predators. In southern California, eradication of *Arcuatula* is not likely to be feasible given the current abundances and distributions, as well as the cryptic nature of these small mussels. But, restoration of eelgrass meadows (Reusch and Williams 1999, Albentosa 2002) and populations of native predators
(Reusch 1998, Crooks 2002, Cheng and Hovel 2010) could probably reduce *Arcuatula* populations to levels characterized by relatively low impacts on native flora and fauna. However, my findings suggest that such mitigation strategies would probably have indirect impacts on the survival of native bivalves and competition among native bivalve species. Future research is needed to minimize the potential for ‘ecological surprises’ (Paine *et al*. 1998, Doak *et al*. 2008), especially when predicting the outcomes of mitigation or restoration scenarios (Cory and Myers 2000, Zavaleta *et al*. 2001).

Together, my findings support the idea that both abiotic and biotic environmental context are critical to characterizing the causes, consequences, and mechanisms of variation in species interactions. My results show that ecological responses to disturbance, stress, and introduced species can be complex and hard to predict *a priori*, indicating the need for incorporating environmental context in community-scale studies. Human-caused environmental changes are numerous and accelerating in estuaries and coastal seas broadly, but especially in seagrass meadows (Orth *et al*. 2006, Williams 2007, Waycott *et al*. 2009). Given these concerns and the complexities of species interactions, more research is needed to improve our ability to predict the effects of anthropogenic ecosystem changes. Future research should aim to resolve the causes of population resilience and recovery from environmental change and variability, as well as to determine the consequences of environmental context for species interactions and community dynamics, especially where there is high potential for interactions among multiple ecosystem stressors. Replication of experiments and observations among sites spanning broad environmental gradients, such across latitudes or oceanographic regions, offer great promise in addressing these persistent challenges. Long-term (e.g., decades) ecological studies also have great potential to help resolve these unanswered questions by capturing repeated temporal
variation in environmental conditions. Similarly, mathematical models can yield new insight by simulating dynamics over spatial and temporal scales that are challenging for empirical studies.

References


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Recart, W., J. D. Ackerman, and A. A. Cuevas. 2012. There goes the neighborhood: apparent competition between invasive and native orchids mediated by a specialist florivorous weevil. Biological Invasions 15:283–293.


