THE UNIVERSITY OF CHICAGO

SPATIAL PATTERN AND DYNAMICS IN ALTERNATIVE ECOLOGICAL STATES: AN EXAMINATION OF COMPETITION FOR SPACE BETWEEN TWO ECOSYSTEM ENGINEERS, THE SEAGRASS ZOSTERA JAPONICA AND THE SAND DOLLAR DENDRASTER EXCENTRICUS

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES
AND THE PRITZKER SCHOOL OF MEDICINE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

COMMITTEE ON EVOLUTIONARY BIOLOGY

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CHICAGO, ILLINOIS
AUGUST 2017

DEDICATION

For my father, who spoke for the trees.

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ACKNOWLEDGEMENTS

Thank you to my advisor, Tim Wootton, for your patience and support of me and my project, for giving me my independence, and for your faith in me across long distances. I am so grateful for your generosity with your time and your always-open office door, for both small concerns and for developing ideas in wide-ranging discussions and ideas so smart it takes me months to figure them out myself and realize you were right all along. Thank you for opening the doors for this natural historian and invertebrate nerd to become a quantitative ecologist.

I would like to thank my committee for their encouragement and faith in me and my project as it went through so many evolutions from its proposed form. Your feedback and helpful comments on research design, analysis, and the writing process of this project has been crucial, and committee meetings were far more fun than I would have ever expected. To Sue Kidwell, thank you for adopting me as the biologist on your paleontology field trips, and for expanding the time horizons over which I think about ecology. To Cathy Pfister, thank you for your mentorship throughout this whole process, for your support for my development as a teacher, and for always asking the hard questions I was afraid someone might ask. To Greg Dwyer, thank you for your overwhelming enthusiasm, for exhortations that this project, this one would be a PNAS paper for sure, and for the best math pedagogy I've ever experienced.

I would like to thank Dr. Sandy Wyllie-Echeverria at Friday Harbor Laboratories for his mentorship, friendship, and for opening up his lab and teaching me everything I know about seagrass. I am indebted also to Stefano Allesina, Mercedes Pascual, Michael LaBarbera, Megan

Dethier, and Richard Emlet, and Richard Strathmann for advice, assistance with code and finding papers, and for excellent conversations.

The administrators in the Committee on Evolutionary Biology, Carolyn Johnson and Libby Eakin, and Audrey Aronofsky, Bonnie Brown, and Connie Homan in Ecology and Evolution have been a tremendous resource and source of support for me, as well as the administration of Friday Harbor Laboratories, Bern Holthuis, Vicky Dauciunas, particularly Aimee Urata, a guardian angel who cared for my truck in Washington between summers.

I thank the San Juan County Land Bank and Ruthie Dougherty and Eliza Habeggar for access to Crescent Beach and for their support and opportunities to share my research with the community, and to the University of Washington Friday Harbor Marine Laboratories for support and resources. I thank the town of Eastsound, WA for welcoming this weird marine biologist into their community two weeks per month for three summers, and sharing their beautiful Crescent Beach with me.

This work would not have been possible without my sources of funding. The University of Chicago Evolutionary Biology Hinds Fund made my first field season possible. I was incredibly fortunate to be supported every summer at Friday Harbor Laboratories through small graduate research grants, including the FHL Discretionary Fund for Excellence and the Patricia Dudley Fellowship. The Lerner-Gray Grant for Marine Research from the American Museum of Natural History supported the initial studies on sand dollar movement and aggregation, and research funds from the Ecology of Infectious Marine Disease Research Coordination Network also

supported my fieldwork. Through most of my thesis I have been supported on a Department of Education Graduate Assistantship in Areas of National Need (GAANN) Grant, for which I am grateful for the financial support as well as the enriching activities that came as a result of being on this grant, especially the EcoShake reading group and paper clubs, and opportunities to present at retreats.

This work could not have been done without my field assistants, Maia Kreis, Natalie Rivlin, Annie Thomson, and Sara Bear-Magallanes, who became my friends and partners in the absurdly tedious, cold and wet work of literally counting blades of grass. Many other field volunteers, including Maya Groner, Mo Turner, Morgan Eisenlord, Tawm Perkowski, Kari Eckdahl, Patrick Landback, Karl Biederbeck, and many others, including the entire FHL Invertebrate Zoology cohort of 2014. The movement tracking image analysis was courageously tackled by Maia Kreis and by two University of Chicago undergraduates, Irene Zhang and Khashiff Miranda, who put in many tedious hours clicking on pictures of sand dollars and came up with wonderful observations.

I thank my lab, because being a part of the Wootton-Pfister lab these last several years has been an incredible community in which I've learned to ask for help, for hugs, for midnight comments on grant applications and have helped in return. I am so grateful to Orissa Moulton, who got me through my field seasons and more, to Courtney Stepien, who got me through my winters and more, and to Sophie McCoy, the big sister I never had. Thank you to Sara Jackrel, Kristen Voorhies, Aaron Kandur, Will Tyburczy, Liz Sander, Katherine Silliman, John Park, Mark Bitter, and Brooke Weigel for everything, and for being the best lab anyone could ask for.

To my dear friends in the graduate department here at University of Chicago, to my SGS ladies' night, to Darwin's Revenge Broomball and the wonderful friends at Friday Harbor Laboratories, thank you for the friendship and communities we built together. I would not have made it through without all of you. To my soulmate, Kari Eckdahl, who taught me how to have a best friend, and with whom I am so blessed to share our adventures in graduate school together and through life, thank you for the care packages and many, many phone calls.

Finally, I am so grateful for the support of my family. My mother, Dillon Henry, raised me to be independent and smart, to be brave and strong and stand up for myself, what I want, and what is right, and to communicate and listen. Your love for me and my dreams knows no end, and your support and sacrifices have buoyed and sustained me, from helping with fieldwork (in spite of seasickness and a hatred of cold and wet), to marching for science, to a million other less picturesque but no less meaningful moments. I am thankful to my sister, Lauren Henry, from whom I stole marine biology and with whom I share everything. You have been there for and with me through everything, through the road trips and late-night conversations and the difficult times, and in these last few weeks of writing, I can't tell you how much your pep talks have meant to me. Finally, I thank my father, Evan Henry, who passed away in September 2016, for raising me to be a scientist, to have heart, enthusiasm and always a positive attitude. Your care and voice for the natural environment inspired me on this career path, and your unconditional faith in me, glowing pride in my accomplishments, and total support of my dreams even when I had my doubts, I will have for my whole life. The memory of your love and pride has carried me through these last few months without you, and I know I have made you proud.

CHAPTER I

INTRODUCTION

Introduction and Ecological Context

Ecosystems are patchy, even within very similar environmental conditions. What determines spatial patterning of organisms in ecosystems? One important force is competition for space (Paine 1984). Many factors can affect which species and communities win in this competition for space, but for species with positive feedbacks, it may be who gets there first and excludes others (Sutherland 1974; Suding, Gross, and Houseman 2004). Once a species is established, it takes a disturbance or perturbation to give another species a chance to invade. Understanding these thresholds for switches is crucial for management as anthropogenic influences stress ecosystems in new ways.

Ecosystem engineers are one type of organism that may demonstrate such positive feedbacks. By physically changing biotic and abiotic materials or by altering resource availability, they alter their environment in a way that facilitates their own persistence and growth, and that enables or discourages other species in the community (C. G. Jones, Lawton, and Shachak 1994; C. G. Jones, Lawton, and Shachak 1997). When two species engineer the same environment in opposing ways, competition results and a system of alternative stable states may emerge. With the introduction of non-native species, new species interactions also emerge.

One challenge of studying ecosystem engineers is separating environmental factors that are inherent and enable a species, from those which the species generates themselves. In a system where species occupancy is patchy and has concomitant environmental differences, it is important to determine whether those environmental differences are engineered by the species or if there is some unmeasured environmental factor that determines occupancy. Ecosystem engineering also has the potential to develop “scale-dependent feedbacks”, meaning that different positive or negative effects work at different spatial scales. These effects have been shown to generate interesting, complex, and regular spatial patterns, particularly in seagrasses (Kefi, Holmgren, and Scheffer 2016).

As with other keystone species, the removal of ecosystem engineers can have large repercussions on an ecosystem (Paine 1969; Paine 1995). Keystone species are species that have a disproportionate effect on an ecosystem relative to their biomass. While the terms ecosystem engineer and keystone species are not mutually exclusive, there are some significant differences. Keystone species are usually referred to with regard to trophic interactions, such as how a predator controls herbivore population size, whereas ecosystem engineers alter the physical environment and habitat (C. G. Jones, Lawton, and Shachak 1994). Additionally, ecosystem engineers are defined without reference to biomass, and may even comprise the majority of biomass in a system.

When a species and their competitors are antagonistic ecosystem engineers, like bioturbating species and sediment-stabilizing species, this can completely restructure communities. This particular interaction among competitors has been shown empirically in the Netherlands, where high densities of bioturbating lugworms reduce the threshold disturbance (experimental removal) necessary to convert a *Zostera noltii* seagrass patch to muddy hollow (Eklöf et al. 2011).

One framework in which ecological systems may be examined is that of alternative states. The idea that there can be multiple stable configurations of populations and communities in a given habitat has been recurrent in ecology since the late 1960s (Sutherland 1974; Lewontin 1969; Beisner, Haydon, and Cuddington 2003). Theory suggests that ecosystems can exist in multiple stable configurations for a given habitat, determined simply by initial conditions (Lewontin 1969; Beisner, Haydon, and Cuddington 2003). For example, temperate rocky intertidal shores may be dominated by mussels or by diverse algae, depending on whether mussels have the opportunity to reach a size too large to be eaten by sea stars (Paine, Castillo, and Cancino 1985). For example, rocky subtidal coasts may be dominated by large kelp forests, which host high species diversity, or by urchin barrens, in which the only species that persist are low encrusting algae and the urchins that eat it (Paine 1995). In plant-dominated systems, the stability of these states may be reinforced through positive feedbacks, such as plants that stabilize sediment stabilization, prevent erosion, and enhanced soil conditions (van der Heide, van Nes, van Katwijk, Olff, and Smolders 2011) for more plant growth.

Alternative stable states have been demonstrated theoretically (Holling 1973; May 1977; Lewontin 1969; Beisner, Haydon, and Cuddington 2003), and empirically (Paine, Castillo, and Cancino 1985; van de Koppel et al. 2001; Petraitis and Dudgeon 2004; Paine and Trimble 2004; van der Heide, van Nes, van Katwijk, Olff, and Smolders 2011; Holling 1973). Given a pulsed perturbation that reaches a certain threshold size, an ecosystem may dramatically shift to a new equilibrium. The resistance of a community is the scale of disturbance it can tolerate and remain in the same state, while the resilience is the capacity for an ecosystem to recover from disturbance (Holling 1973; Grimm and Wissel 1997; Lewontin 1969; van de Koppel et al. 2001).

How can we test for alternative states? Connell and Sousa (Connell and Sousa 1983) proposed three components that must be present in any experiment that tests for alternative states.

1. The alternative states must be shown to exist in the same environment/habitat.
2. The experiment must be a pulse perturbation. If the pulse perturbation is small, the expectation is that the ecosystem will return to the pre-disturbance state. If the pulse perturbation is sufficiently large, it may switch to a new state that is locally stable.
3. The experiment must be done over a sufficient spatial and temporal scale to establish that the new state is persistent.

That third point, persistence, is tricky because it is undefined. What is sufficient? Petersen proposed (Peterson 1984) that alternative states might be shown by demonstrating that the same site may be occupied by different self-replacing communities. By utilizing the exact same location, it controls for the possibility that environmental conditions vary in undetected ways which might drive separation. A self-replacing community is one in which all individuals have been replaced by ones of a later generation. This is complicated and unfeasible to prove in this system for both species. The sand dollar *Dendraster* lives an estimated 13 years and individuals may migrate out of the study area. Seagrass is a clonal plant in which genetic clones in beds may live for millennia (Arnaud-Haond et al. 2012), but in species like *Zostera japonica* biomass dies back in winter and regenerates most above-ground biomass seasonally. Therefore, depending on your definition of an individual, it either turns over once a year, or never. The intractability of the self-replacing community requires that the ecologist interested in studying alternative states experimentally must either select a short-lived system, or incorporate methods of extrapolating over longer timescales.

Another basic idea in ecology and evolution is that of competitive exclusion(Gause 1934): if two species occupy the same niche, one will eventually outcompete the other. However, there are many mechanisms by which species competing for the same resource are have been shown to coexist.

One theory of how species may coexist in spite of competitive exclusion is the theory of disturbance-mediated coexistence. An extension of the Intermediate Disturbance Hypothesis, in which the greatest number of species is present in an ecosystem at moderate levels of disturbance. If one species is the greater competitor, but the other is more responsive following disturbance, coexistence under conditions of occasional disturbance may be possible. This dynamic has been shown in *Z. marina* beds interacting with bioturbating callianassid shrimps in California estuaries(Castorani, Hovel, Williams, and Baskett 2014a).

The inclusion of spatial information to competitive scenarios, even without environmental heterogeneity, holds the promise of coexistence. In a case where each species has positive feedbacks and there is little potential for change at boundaries, priority effects may be very important. One example experiment showed that three bacterial species with a rock-paper-scissors style of competition, in which each could out-compete one other species, could coexist when grown spatially segregated (Kerr et al. 2002). Spatial pattern however, does not guarantee coexistence of course, and may be more an indicator of feedbacks between organisms and their environment, such as trees on a savanna or hummocks and hollows in a mud flat (Rietkerk and van de Koppel 2008). These interactions are often the result of different scales of interactions – short-distance positive feedbacks, such as those between shoots in a seagrass bed or sand dollars in an aggregation, and long-range negative feedbacks(Rietkerk and van de Koppel 2008). Self-organized spatial patterns and threshold-like dynamics have been observed in seagrass beds as a result of interactions with grazers (van der Heide, Eklöf, van Nes, van der Zee, Donadi, Weerman, Olff, and Eriksson 2012), in seagrass-sediment interactions (de Boer 2007), and in interactions between ecosystem engineering and wave forces at depths (van der Heide et al. 2007; van der Heide, Bouma, van Nes, van de Koppel, Scheffer, Roelofs, van Katwijk, and Smolders 2010).

Spatial pattern, especially self-organized regular patterning in ecosystems, also can be associated with systems thought to be alternative stable states, with spatial patterning signaling shifts or transition zones between states (Rietkerk et al. 2004).

In the system examined in the studies in this dissertation, two states occupy flat sandy beaches - seagrass and sand dollars. The critical resource which these two seemingly disparate species compete for is space. Locally, the habitat available to these two species is overlapping and limited, a narrow band of sand between high and low tide. Both communities are locally stable and reinforced by positive feedbacks that maintain the dominant species.

In addition, both species are mutually exclusive occupiers of space. *Dendraster excentricus,* the Pacific sand dollar, is a motile, benthic suspension-feeder which forms dense aggregations that turn over sediment rapidly. Seagrass roots exclude *D. excentricus* and other hard-bodied burrowing species by inhibiting their mobility through the sediment (Brenchley 1982). Conversely, bioturbators like *D. excentricus* can also directly impact plants by uprooting rhizomes and burying new shoots and seedlings (Nelson 2009). Both species live in soft-sediment, shallow, coastal habitats and have mutually exclusive uses for that sediment. Given that these two species occupy this same stretch of habitat with seemingly homogeneous conditions, will one ultimately outcompete the other?

Bioturbation may not be enough to remove an established seagrass bed, but in combination with additional disturbance, such as a storm or heavy grazing, colonization by bioturbators may prevent re-establishment of seagrass or other benthic plants (Eklöf et al. 2011). In the Netherlands, bioturbating lugworms may colonize and convert *Zostera noltii* beds to a muddy hollow state where there has been a disturbance of a large enough size, preventing the recolonization of the seagrass (van der Heide, Eklöf, van Nes, van der Zee, Donadi, Weerman, Olff, and Eriksson 2012). A similar experiment with *D. excentricus* and *Zostera marina* (Backman 1984) found that along the boundary between the two species, each excluded the other from areas with established populations, but was able to invade and establish if a disturbance removed and excluded the original species near the edge of their boundaries. This provides motivation to examine macrophyte-bioturbator interactions as mediators for transitions between alternative states.

When both species occupy space on the same beaches, priority effects and density dependence seem to both be important - species that occupy a patch first and reach high density are likely to keep it, as the other species is prevented from invading, but at lower density for each species (for example, disturbed rhizomes) there may be opportunities for invasion. This experimentally tractable system provides an opportunity for studying spatial patterns and dynamics in alternative stable states in a natural context.

Study System

The intertidal zone is a narrow band of habitat where species must be adapted to life intermittently in both air and sea. Though species are exposed to huge variability in environmental conditions, adaptation to this harsh niche enables success in other ways, such as escape from predators or access to resources from both habitats. Therefore, space is a fiercely contested resource in rocky shores as well as soft-sediment habitats. On Crescent Beach, Orcas Island, Washington, as well as other numerous beaches in the Pacific Northwest, two species that compete for space are the Pacific sand dollar (*Dendraster excentricus)* and seagrasses (genus *Zostera*). Both species live in high-density monocultures, facilitated by positive feedbacks in their reproduction, behavior, and effective ecosystem engineering. This leads to a patchy landscape of mutually exclusive beds with strictly delineated edges.

Seagrasses in the genus *Zostera* are angiosperms adapted to exclusively marine lifestyles. They reproduce sexually, flowering and producing seeds that may overwinter in the seedbank, and asexually in two ways. They can regrow from a broken segment of rhizome, but the most common form of reproduction for seagrasses is clonal rhizomatous growth. The rhizome extends horizontally under the sediment and grows a new shoot, branching and sending out runners to make genetically identical beds many meters to kilometers wide. In this way, new seagrass growth is largely spatially contiguous with existing seagrass beds. Ecologically, in the Pacific Northwest, *Zostera* primarily functions as habitat and is rarely consumed by marine herbivores except as wrack. Waterfowl are known consumers of seagrass, disrupting rhizomes and uprooting whole plants patchily in a behavior called “dabbling” (van der Heide, Eklöf, van Nes, van der Zee, Donadi, Weerman, Olff, and Eriksson 2012) that can influence spatial pattern. Seagrasses generally have many ecosystem engineering effects. The dense rhizomes anchor sediment into place, facilitating infaunal communities of worms and clams. Blades slow water flow, accumulating particulate matter and sediment, increasing organic content and reducing erosion. Seagrass blades also create sheltered habitat for many species of fish, gastropods, arthropods and other epifauna. Seagrasses modify sediment conditions, influencing organic content with buried wrack that depletes oxygen in decomposition, but also increasing oxygen as a product of photosynthesis (Green and Short 2003; Larkum, Orth, and Duarte 2006b).

There are two species of *Zostera* present in the Pacific Northwest: *Zostera japonica* and *Zostera marina*. *Zostera japonica*, or Japanese eelgrass, is a non-native species thought to have been introduced to the Pacific Northwest in the 1930s with the oyster trade. *Zostera japonica* is a temperate shallow water marine species native to the western Pacific and non-native in the Pacific Northwest (Harrison and Bigley 1982), likely introduced in the 1930's with the oyster trade, which grew to dominate many shallow soft-sediment bays after the 1980's(Posey 1988; Mach, Wyllie-Echeverria, and Chan 2014). It is closely related to the native *Zostera marina* but has several major ecological differences. Non-native *Zostera japonica* has narrow, flat blades up to 60 cm long. Plants grow densely, with as many as 800 *Z. japonica* shoots in a 0.0625 m^2 area, and thin rhizomes forming a dense mat within the sediment. *Z. japonica* is relatively tolerant of desiccation and fairly higher in the intertidal, up to +2.5 ft MLLW, colonizing habitat previously free of vegetation. In competition experiments, *Z. marina* is able to outcompete *Z. japonica*, through shading or interference by rhizomes (Nomme and Harrison 1991), or possibly through community dynamics with epifauna and epiphytes. This interaction may set the lower boundary on where *Z. japonica* can live in the intertidal zone. Where they co-occur, they have been shown to differentiate habitat slightly, with *Z. japonica* on slightly higher (< 1 cm difference) and drier hummocks, and *Z. marina* occupying pools (Hannam and Wyllie-Echeverria 2015).

Seagrasses are one of the most widespread shallow marine ecosystems in the world, with the genus *Zostera* covering much of the shallow habitat in the northern hemisphere. Seagrasses around the world are both critical to ecosystem processes and, in many locations, threatened. These marine angiosperms stabilize soft sediment bottoms, create sheltered, complex habitat for both infaunal and pelagic species, and are highly productive (Larkum, Orth, and Duarte 2006; Green and Short 2003). In the Pacific Northwest, seagrass beds provide nursery habitat for salmonids, herring, dungeness crabs, and other fisheries species, and food for migratory and non-migratory waterfowl. Other ecosystem services include nutrient and contamination filtering from water, oxygen production in water and belowground, carbon sequestration, nutrient regeneration, high primary productivity, wave and current damping, and complex canopy and belowground structured habitat for many species (Green and Short 2003). They exhibit density-dependent positive feedbacks through ecosystem engineering activities and clonal growth(van der Heide, van Nes, van Katwijk, Olff, and Smolders 2011; van der Heide et al. 2007). Seagrasses increases the organic content of sediment both by facilitating the settlement of particulate organic matter (POM) from the water column and by leaching dissolved organic matter (DOM) through excretion and decomposition of tissues(Larkum, Orth, and Duarte 2006). Seagrass bed sediments have more structured oxygenation profiles (Greve, Borum, and Pedersen 2003) than bare, bioturbated sediments, which can also affect nutrient transformations.

Many factors threaten seagrasses, including eutrophication, turbid conditions from runoff and land use, total destruction through coastal construction, and destruction from shellfishing (Green and Short 2003). *Zostera* has also been subject to dramatic disease events, such as a die-off of 90% of the *Zostera marina* in the north Atlantic in the 1930s, from which the seagrass beds never recovered, and chronic disease in the pacific Northwest due to a labyrinthulid protozoan parasite (Hartog 1987; Short, Ibelings, and Hartog 1988; Wyllie-Echeverria et al. 2003; Muehlstein 1992), which have been associated in some cases with warm water events. While some of these processes kill whole beds, others leave seagrass beds fragmented and potentially vulnerable to further disruption. Although *Z. japonica* in the Pacific Northwest is not of conservation concern, many seagrasses worldwide share growth patterns and spatial patterns, and live in less easily accessed water depths. Understanding the growth habits, spatial patterns, and dynamics of competition for space with other species contributes to our knowledge of strategies for seagrass conservation worldwide.

The Pacific sand dollar (*Dendraster excentricus*) is a common species along the west coast of North America in several habitats, including tidal inlets, outer coast beaches below the wave base, tidal channels, and intertidally in protected bays (Merrill and Hobson 1970). In sheltered locations in the Salish Sea, such as the site for this research study on Orcas Island, Washington, this species is found in life position at low tide. Although intertidal populations are exposed to greater heat, dessication, and salinity stress, and predation by birds, living above the tide line provides an escape from subtidal predators like sea stars and crabs (Birkeland and Chia 1971). Intertidal *Dendraster* find refuge from adverse conditions, such as temperature and dessication stress during low tides or large waves, by burying into the sand, usually 1-10 cm deep. Mass death of sand dollars has been observed following hard freezes during low tide (pers. comm., R. Emlet) or if they are stranded in a pool at low tide in extreme heat. Blooms of ephemeral algae like *Ulva* can cause mass death as well, as *Dendraster* can become tangled in algae and prevented from burying.

Dendraster beds reach extremely high densities of individuals, from several hundred to thousands of sand dollars per square meter, in beds tens of meters long and wide. These high densities are maintained through several mechanisms. Chemical cues induce larvae to selectively settle in existing sand dollar beds(Highsmith 1982b) and may act as a cue for aggregating behavior in adults. Aggregation is thought to facilitate hydrodynamically efficient suspension feeding, as well as potentially protection from dislodgement and predation (O'Neill 1978; Timko 1976; Francisco and Herzka 2010; Merrill and Hobson 1970). While most species of sand dollar are deposit feeders, *Dendraster* assumes an inclined posture relative to the sand and uses tube feet to collect particulate organic material (POM) from the water column, passing it down the food grooves on its oral surface to the mouth (Chia 1969; Timko 1976). This inclined posture allows access to more of the water column, and in aggregations with other sand dollars, the camber of the shape of the sand dollar has been found to facilitate feeding for nearby sand dollars by directing the flow around the body (O'Neill 1978). The inclined posture has also been proposed as a mechanism against washout under high flow conditions(Telford 1981). This tuning of spatial position, and by extension, intraspecific density, to an “optimal gap” can depend on many factors. For example, they have been found to vary their feeding behavior in response to sediment organic content and intraspecific density, with less suspension feeding when there is greater organic material in their substrate (Francisco and Herzka 2010; Fodrie et al. 2007).

If population density is low, sand dollars can create “artificial” high density areas by clumping with one another, or aggregating, and therefore optimizing their feeding strategy. Disturbed sand dollars have been observed to re-aggregate in several days(Birkeland and Chia 1971). An extreme observation of this was described by Richard Emlet (pers. comm.) when after a mass death of 90% of a population of sand dollars in a hard freeze, population densities in survey plots the following season had not declined, indicating that the bed had contracted in size and retained its density. What behavioral cues, movement patterns, and other mechanisms regulate the formation of patches and the behavior at the outer limits and edge boundaries of sand dollar beds is not known.

Intertidal populations frequently vacillate between inclined and prone positions when submerged at high tide and exposed at low tide, respectively. This consistent fluctuation in test position disturbs the sediment, causing a much more bioturbated and unstable top layer of sand (Voss 2002; Smith 1981). In subtidal populations, these activities have been observed to promote a low-diversity, inconsistent assemblage of species (Smith 1981; Voss 2002).

There have been multiple studies on the benefits of *D. excentricus* aggregation. Larval metamorphosis and settlement near adults is induced by a chemical cue found (Emlet 1986, Highsmith 1982, Voss 2002), and it has been speculated that this same chemical cue induces adults to migrate toward one another (Highsmith 1982). Aggregating likely increases the efficiency of gamete mixing and fertilization (O'Neill 1978; Merrill and Hobson 1970). Aggregations may help protect from predation; for example, the burrowing of sand dollars excludes tube-building predators of larvae (Smith 1981).

At the focal site of this study, Crescent Beach, Orcas Island, WA, USA (48.6967° N, 122.9061° W), *Dendraster* and *Z. japonica* co-occur between approximately 0 ft. MLLW and -1.5 ft MLLW. Small patches (1-2 m diameter) of *Z. marina* occur in this zone, generally in pools, but deeper than -3.0 ft is exclusively *Z. marina* beds. *Z. japonica* grows nearly to the high tide line. *Dendraster* form large beds, tens of meters across, with jagged and discrete edges separating them from seagrass beds, equally large and patchy. It is unknown whether *Dendraster* previously lived further up on this beach, as the high zone was occupied by an oyster aquaculture operation from the 1990s to mid-2000s. It is clear from aerial images that by 1980, *Z. japonica* dominated the high zone of Crescent Beach (USGS). Although the beach is generally flat, microtopographic variations (0-1 cm) form slightly raised areas and pools, with *Z. japonica* generally on hummocks and *Dendraster* in pools. There are some demographic patterns – large, mature sand dollars (5-9 cm diameter) tend to dominate populations in the sand dollar beds, while areas marginal to the exclusive sand dollar beds (bare sand near the *Z. marina* zone, sparse *Z. japonica* areas) will have low densities of *Dendraster* less than 3 cm in diameter. It appears that small sand dollars are able to shelter in seagrass rhizomes until they reach a certain size, but it is unknown whether these individuals migrate away or die when they can no longer bury themselves completely. *Z. japonica* at Crescent Beach is heavily “dabbled” by Canada geese (*Branta canadensis*), resulting in seasonal patterns, with sparse blades in early spring, thick and lush grass by mid-July, and widespread, patchy dabbled “hollows” or “divots” by late August (pers. obs.). Brant geese, pintails, widgeons and other ducks are also known to graze heavily on *Z. japonica* (Baldwin and Lovvorn 1994; Thom, Miller, and Kennedy 1995; Thom et al. 2003; Thom et al. 1990) in other locations in the Pacific Northwest. Sand dollars in shallow habitats escape predation by sea stars, rays, and crabs during low tides, burying themselves to minimize exposure to heat, low salinity, and dessication.

At Crescent Beach, these factors combine to form a largely environmentally homogeneous area of nearly 8 hectares populated with dense, competing patches of *Dendraster* and *Z. japonica*. Mutually exclusive ecosystem engineering – seagrass through anchoring sediment, sand dollars through loosening and freeing it, appears to drive a zone with two discrete states, competing in a complex spatial mosaic. Our study is not the first to point out these positive feedbacks or the potential for alternative state dynamics in these organisms. *Dendraster* have been observed in patchy competition with tanaid shrimps, which prey on *Dendraster* larvae and are excluded by bioturbation (Sousa and Connell 1985; Highsmith 1982b). Seagrass species around the world have been examined as alternative states because shoot density often follows threshold patterns and can crash catastrophically (Scheffer et al. 2001; van der Heide et al. 2007). Seagrass can also function as an alternative state when in competition with other species such as macrophyte algae (Viaroli et al. 2008) or bioturbating callianassid shrimp(Castorani, Hovel, Williams, and Baskett 2014a). An unpublished thesis study by Backman (Backman 1984) examined *Dendraster* in competition for space with *Z. marina* in Puget Sound through experimental transplants, removals, and exclusions, and provided some of the motivation for our study. That study found that when *Dendraster* was excluded from the boundary with *Z. marina*, *Z. marina* expanded into the area previously occupied by *Dendraster,* and after the exclusion was lifted, *Dendraster* did not reclaim the space. Conversely, where *Z. marina* was removed and excluded, *Dendraster* took over and was not ousted once *Z. marina* was no longer excluded (Backman 1984). These dynamics, though in a different seagrass species, are suggestive of threshold dynamics between alternative states.

A final consideration in this system is that *Z. japonica* is a recent introduction, with less than 40 years as a dominant species at Crescent Beach. *Z. japonica* is a non-native species, and has been managed alternately as a protected species or a noxious weed by the state of Washington at different times since its introduction(Shafer, Kaldy, and Gaeckle 2013). An eradication effort of the *Z. japonica* at Crescent Beach was made by the land managers during the removal of beach-wide oyster farms in the mid-2000s, which reduced cover for several years. The *Dendraster* bed on the other hand, has been present for many decades, noted in anecdotal reports from Friday Harbor Laboratories in the early 1900s. It is possible that the dynamics at this site are not near an equilibrium and that *Dendraster* is acting as a resistor against the spread of *Z. japonica*, and may be eventually outcompeted. These factors suggest an ecosystem that is not at equilibrium and may experience transitions.

I therefore propose that this ecosystem is an opportunity to demonstrate a case of alternative states in nature. In addition, it is a strong candidate model system for examining the functioning and dynamics of alternative stable states through experiments and modeling. With a complex spatial mosaic, I was able to investigate the dynamics of spatial pattern between the two species. In this thesis, I used field experiments and the application of empirical data to a Markov model to examine sand dollars and seagrass as alternative states of the same ecosystem and the role that spatial pattern plays as a facilitating mechanism for coexistence, or as a bellwether of change.

DISSERTATION OVERVIEW

In this dissertation, I use a combination of observational field surveys across spatial and temporal scales, manipulative field experiments, experimental mesocosms and probabilistic and mechanistic models to study the dynamics of competition and coexistence between *Zostera japonica* and *Dendraster excentricus* and the resulting landscape spatial patterns between them. By studying the interactions between individuals and small patches of these two species, I have found insights about the landscape-scale patchy mosaic spatial patterns.

In the first chapter (Chapter II: Alternative states in space and time: an examination of spatially dependent competitive interactions between ecosystem engineers), I tested the hypothesis that sand dollars and seagrass behave as alternative stable states of the same ecosystem. Using field experiments I demonstrated that either species could invade the other and persist in the habitat. I used Markov models to extend these results to a timespan in which we can establish persistence of states and examine the dependencies of state changes on the spatial context of patches, showing that identity of surrounding areas is important to state changes. Finally, we use eigenvector analysis and clustering algorithms to support our assertion that these two species behave as alternative states and that disturbance may mediate the transitions between them.

In the second chapter (Chapter III: Cellular automata models and edge effects in competition: an examination of spatially dependent competitive interactions between ecosystem engineers), I examine the origin of the unique and patchy spatial pattern of seagrass and sand dollars in the interaction zone. I extend this conclusion that spatial context matters when predicting state transitions, to hypothesize that information about the identity of neighboring patches will generate

Plate 1.1: Tracks, traces, and GPS evidence of movement behaviors of sand dollars (top) and ecologists (bottom) at Crescent Beach, Orcas Island, Washington.

models that can predict large-scale pattern from small-scale dynamics. Through a paired experiment examining transition rates at boundaries between seagrass and sand dollars and a cellular automata model generated from that data, we found that the ratio of neighbors of different identities strongly determines the probability of transition. These rates generate temporary spatial structure on small scales, but did not capture the large-scale patterns, leading us to hypothesize alternative mechanisms or fates for the spatial pattern of patches in the future.

In my third chapter (Chapter IV: Movement patterns and dynamics of aggregation in the Pacific sand dollar, *Dendraster excentricus*), I examine a proposed mechanism for sand dollar persistence and resistance to seagrass spread – aggregation. By examining the movement patterns and aggregating behavior of sand dollars in the field, in mesocosms, and in simulations, we built a framework for the study of sand dollar spatial pattern generation. I found that sand dollars alter their behavior when near conspecifics, slowing their velocity and reducing the frequency of movement, simple behavior changes that result in congregations. In my conclusions, I present how these results inform our understanding of alternative stable states and spatial patterns in nature; what they tell us about the interactions and competition between these two species in a specific place and what it may mean for management of the two species and for seagrass conservation writ broadly; and what future extensions of these models may be able to tell us, including integration of the models from Chapters III and IV.

CHAPTER II

ALTERNATIVE STATES IN SPACE AND TIME: AN EXAMINATION OF SPATIALLY DEPENDENT COMPETITIVE INTERACTIONS BETWEEN ECOSYSTEM ENGINEERS

ABSTRACT

The concept of alternative stable states in ecosystems has been demonstrated theoretically and is often invoked for understanding the behavior of ecosystems. However, experimentally tested empirical examples of alternative ecological states are rare, because few ecosystems may be observed and manipulated at the spatial and temporal scale necessary to establish a system as plausibly “stable” or persistent. We suggest that integrating experimental manipulations probing key properties that lead to alternative stable states with parameterized models of ecosystem dynamics exploring the implications of these properties offers a useful substitute for strictly empirical long-term study, which overcomes several problematic features of the traditional approach. We used reciprocal transplant experiments and context-dependent state-transition Markov models in a system of intertidal seagrass (*Zostera japonica)* and sand dollars (*Dendraster excentricus*) to test for the plausibility of alternative states and examine the relative contributions of biotic and abiotic interactions to the predicted stable distribution of these states. We found spatial pattern [and seasonality] to be driving factors in the dynamics between these two species, leading to different long-term dominance patterns among these ecosystem engineers that depended on both initial conditions and positive reinforcement of neighbors in the surrounding spatial matrix. Such spatial dependencies and sensitivity to initial conditions are key features of alternative stable states.

INTRODUCTION

The idea that there can be multiple stable configurations of populations and communities in a given habitat has been recurrent in ecology and can have important implications for understanding how to manage or conserve ecosystems (Lewontin 1969; Beisner, Haydon, and Cuddington 2003; Sutherland 1974). Given a pulsed perturbation that reaches a certain threshold size, an ecosystem may dramatically shift to a new basin of attraction. Alternative stable states have been predicted theoretically (Holling 1973; May 1977; Lewontin 1969; Beisner, Haydon, and Cuddington 2003), but empirical evidence is more limited and often controversial (Petraitis and Dudgeon 2004)(Petraitis 2013). For example, temperate rocky intertidal shores may be dominated by mussels or by diverse algae, depending on whether mussels reach a size escape from sea star predation (Paine, Castillo, and Cancino 1985), but theory suggests that size-dependence alone may create a persistent, but not stable, alternative state (Chase 1999). In plant-dominated systems, the stability of these states may be reinforced through positive feedbacks, such as plants that stabilize sediment, prevent erosion, and enhance soil conditions (van der Heide, van Nes, van Katwijk, Olff, and Smolders 2011; Suding, Gross, and Houseman 2004).

Competition for space has been long recognized as one of the most important forces in ecology(Connell 1961). Many factors can affect which species and communities outcompete others, but for species with positive feedbacks, dominance may depend on space preemption (Sutherland 1974; Suding, Gross, and Houseman 2004). Once a species is established, it takes a disturbance or perturbation to give another species a chance to invade in some systems positive feedbacks may initiate a rapid transition to a different stable state, a scenario for alternative stable states. These states may be characterized as communities with different sets or abundances of species, or in some cases, may be largely driven by only a few dominant species.

Ecosystem engineers are one type of organism that may mediate such positive feedbacks. By physically changing biotic and abiotic materials or by altering resource availability, ecosystem engineers shift their environment in ways that facilitate their persistence and growth, and enable or discourage other species in the community (C. G. Jones, Lawton, and Shachak 1994; C. G. Jones, Lawton, and Shachak 1997). When two ecosystem engineers modify the same environment in opposing ways, competition results and a system of alternative stable states may emerge.

How can we empirically test for alternative states? Connell and Sousa (Connell and Sousa 1983) proposed that three components could test for alternative states. First, the alternative states must be shown to exist in the same environment/habitat. Then there are two components for experiments. The experiment must be a pulse perturbation. If the pulse perturbation is small, the expectation is that the ecosystem will return to the pre-disturbance state. If the pulse perturbation is sufficiently large, it may switch to a new state that is locally stable. The experiment must be done over a sufficient spatial and temporal scale to establish that the new state is persistent.

While these tests are valuable conceptually, they are rarely achieved experimentally (Petraitis and Dudgeon 2004; Petraitis 2013). First, two habitats are never fully the "same"; there are infinite unmeasured environmental variations between and within sites which can never be disproven to drive states. In the case of ecosystem engineers, separating inherent environmental factors from those that the species generate themselves also presents a challenge. Petersen (1984) proposed an amendment: demonstrating that if the exact same location were sequentially occupied by different self-replacing communities, evidence for alternative states would exist. A self-replacing community is one in which all individuals have been replaced by ones of a later generation. However, this still does not help with many communities, especially ones with long-lived, large-bodied, or clonal species, for which carrying out an experiment over sufficiently long time may be impossible. Persistence will always be difficult to define and experimentally hard to prove. Even if it can be demonstrated, observing persistence after a generation does not prove that a system is actually in a stable state. Finally, the time frame to carry out experiments may be so long that the underlying environmental context changes, which may create conditions under which the possibility of alternative stable states changes, or may shift equilibrium states such that the system does not appear to be persistent, yet is in a stable state.

A different approach for studying alternative stable states integrates ecological models with empirical process studies focused on conditions that lead to such outcomes (e.g. Chase 1999, 2003). Theories of alternative stable states exhibit several general properties, including sensitivity to starting conditions, and positive feedbacks that promote alternative states. Ecological models that incorporate these properties and experimentally estimate parameters, can be explored for long-term behavior, which may indicate whether the mechanisms are strong enough to generate alternative states, and can probe the stability of these states. Here we illustrate this approach by integrating 3-year manipulative experiments varying the abundance of ecosystem engineers in an apparently homogeneous habitat with context-dependent Markov Chain models to probe the plausibly of alternative stable states.

Study System

In the intertidal zone of low-energy beaches along the northeastern Pacific coast, two organisms that coexist within a homogeneous habitat are the Pacific sand dollar (*Dendraster excentricus)* (hereafter *Dendraster* or sand dollar) and sea grass (primarily *Zostera japonica,* but *Zostera marina* in some locations). Both species existing in an interspersed patchy distribution of mutually exclusive, high-density beds with strictly delineated edges (Figure 2.1).

Figure 2.1: Aerial view of Crescent Beach, July 2013 (top), in which gray areas within the red zone are sand dollar beds and (bottom) underwater close view of boundary between seagrass and sand dollars during high tide.

Dendraster excentricus is a common species along the west coast of North America in coastal subtidal and intertidal soft-sediment habitats (Merrill and Hobson 1970). Intertidal *Dendraster* beds can have high densities of individuals (200-1000/m^2) (Chia 1969; Merrill and Hobson 1970), in beds tens to hundreds of meters long and wide. These high densities appear to be maintained through several positive feedback mechanisms. Chemical cues induce larvae to preferentially settle in existing sand dollar beds (Highsmith 1982a; Voss 2002) and may act as a cue for aggregating behavior in adults (Timko 1976; Celmer 1975). High densities may facilitate more hydrodynamically efficient suspension feeding, and protect sand dollars from dislodgement and predation (O'Neill 1978; Timko 1976; Francisco and Herzka 2010; Merrill and Hobson 1970).

Seagrasses in the genus *Zostera* are angiosperms adapted to exclusively marine lifestyles. They reproduce both sexually and asexually; most biomass is generated from clonal rhizomatous growth. The rhizome extends horizontally under the sediment and grows a new shoot nearby, making genetically identical, spatially contiguous beds many meters to kilometers wide. Ecologically, *Zostera* in the Pacific Northwest primarily functions as habitat and is rarely consumed by marine herbivores in situ. Waterfowl are known consumers of seagrass, clipping blades, disrupting rhizomes and uprooting whole plants patchily in a behavior called “grubbing” (van der Heide, Eklöf, van Nes, van der Zee, Donadi, Weerman, Olff, and Eriksson 2012) that can influence spatial pattern. Like sand dollars, sea grasses are also ecosystem engineers. Rhizomes anchor sediment into place, facilitating infaunal communities of worms and clams, and blades slow water flow, accumulating particulate matter and sediment, which increases organic content and reduces erosion, causing topographic variation.

Zostera japonica is a non-native species likely introduced to the Pacific Northwest in the 1930s with the oyster trade (Shafer, Kaldy, and Gaeckle 2013; Harrison and Bigley 1982). It is closely related to the native *Zostera marina,* but has several major ecological differences. *Z. japonica* is relatively tolerant of desiccation and lives higher in the intertidal, up to +2.5 ft MLLW, colonizing habitat previously free of vegetation (Harrison and Bigley 1982). *Z. japonica* has short, thinner blades up to 60 cm long and grows tangled mats of thin but tough rhizomes.

At our focal study site, Crescent Beach, Orcas Island, Washington, USA, *Dendraster* and *Z. japonica* co-occur between approximately 0 ft. MLLW and -1.5 ft MLLW (Figure 2.1). Small patches (1-2 m diameter) of *Z. marina* occur in this zone, generally in pools, but deeper than -3.0 ft is exclusively *Z. marina*. Where they co-occur, they have been shown to differentiate (and engineer) microtopography slightly, with *Z. japonica* on higher (<1 cm difference) and drier hummocks, and *Z. marina* occupying pools (Hannam and Wyllie-Echeverria 2015). *Z. japonica* grows nearly to the high tide line, but is thought to be limited in the low zone by access to light (Britton-Simmons et al. 2010). *Dendraster* form large beds, tens of meters across, with jagged, discrete edges separating them from seagrass beds, equally large and patchy (Figure 2.1). *Zostera japonica* is a recent introduction, with less than 40 years as a dominant species at Crescent Beach (images from USGS, NHAP aerial image archives). The *Dendraster* bed has been present for many decades.

These factors combine to form a largely environmentally homogenous area of nearly 8 hectares populated with dense, competing patches of *Dendraster* and *Z. japonica*. Mutually exclusive ecosystem engineering – seagrass through anchoring sediment, sand dollars through bioturbation – appears to drive a zone with two discrete states, competing in a complex spatial mosaic.

In this chapter, I hypothesize that the sand dollar *D. excentricus* and the seagrass *Z. japonica* can be described and examined as a system of alternative stable states. In a field experiment, I tested the susceptibility to invasion and colonization rates of each species through a series of reciprocal transplants and removals at varying degrees of disturbance, demonstrating that both species can successfully occupy the same environment. In addition, I show that outcomes of competition for space between these two species depends both on surrounding matrix and contingency. Using a Markov model I project the stable stage distributions of both species in the future under different conditions and find that although seagrass is favored overall, sand dollars persist when they have dense conspecifics nearby. Finally, using clustering and eigenvector analyses, we show that experimental plots can be divided into two clusters, and that disturbance-treated plots may exist in the middle and have the potential to converge with either cluster, a classic indicator of alternative states.

MATERIALS AND METHODS

Methods I: Field Experiment

We estimated rates of transition between states and conducted reciprocal transplants and removals of seagrass and sand dollars, monitored these over three years. The treatments were designed to mimic disturbance events such as goose grubbing or abrasion by floating logs, which may initiate a change between ecological states, and were placed in different starting contexts and with different initial conditions to probe for evidence of divergent trajectories and facilitation within species. Sand dollars and seagrass shoots were removed and transplanted manually into the opposite matrix in 1 m^2 plots (Figure 2.2). Treatments in the sand dollar matrix were: A) Sand dollar control, B) Seagrass transplant with sand dollar removal and exclusion by a mesh fence, C) Seagrass transplant, and D) Sand dollar removal. Treatments set in a seagrass matrix were: E) Seagrass control, F) Seagrass removal with sand dollar transplant, G) Seagrass removal, and H) Sand dollar transplant. Response was measured in density of sand dollars and density of seagrass shoots. Full sets of all eight treatments were replicated at three locations on Crescent Beach.

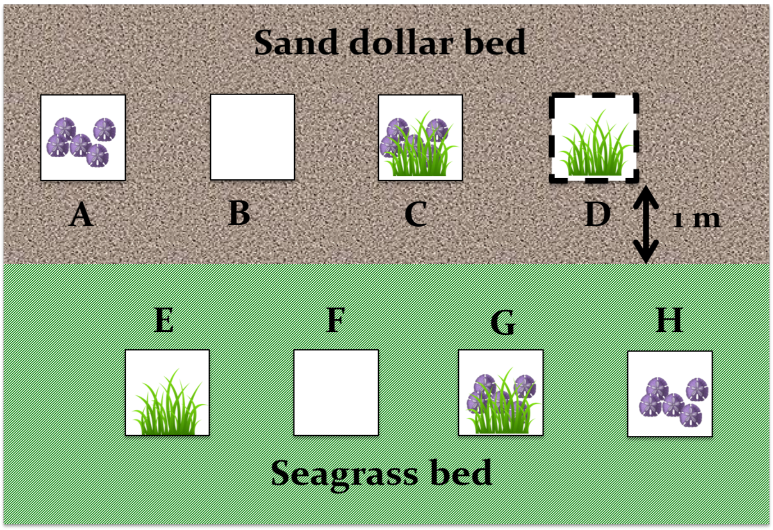


Figure 2.2: Experimental treatments, as seen from above. The horizontal line indicates a natural boundary between states existing prior to experimental treatment.

For seagrass transplants (C, D), shoots and rhizome mats were removed in 20-30 cm clods with a small shovel. Plants were rinsed with seawater over a rough sieve to return sediment to the removal hole and remove infaunal invertebrates. At the transplant site, rhizome mats were laid into the sand and secured with turf staples, which were removed two months post-transplant. This process was repeated to remove and transplant 1 m x 1 m square sections of grass per treatment, creating both the removal (F, H) and transplant treatments (C, D).

For treatments involving sand dollars, the starting number of sand dollars was normalized between treatments within a replicate (n = 3). In sand dollar additions (G), sand dollars were placed on top of seagrass, evenly spaced. Sand dollars were placed around the edges of the seagrass transplant without exclusion (C) treatment. In the exclusion treatment (D), 1 cm plastic mesh was buried 12 cm deep along the plot perimeter, with 2-4 cm above the sediment surface, to create a porous "sandbox", which would exclude sand dollars but not seagrass shoot growth. This plastic mesh was removed after one year of treatment in May 2014.

Plots were subdivided for censuses into a 4x4 grid of sixteen 0.0625 m^2 quadrats. Within each quadrat, individual seagrass ramets and sand dollars were counted. Sand dollars were removed manually from as deep as 10 cm, and replaced, with care to not disrupt seagrass rhizomes. If more than half of the body of an individual was within the quadrat, it was counted. Sand dollars below 1 cm in diameter were not counted. A small area of ramets was fully counted, then used to visually estimate density in 0.125 m^2 areas to the nearest 25 shoots.

Perturbations were applied in June 2013, and censuses conducted in June, July and August 2013, January, May, June, July, and August 2014, May, June, and July of 2015, and June of 2016. To test for general treatment effects, we applied a repeated measures multivariate analysis of variance (RM-MANOVA) with treatment as a fixed effect, followed by examination of species-specific responses with univariate ANOVAs.

Methods II: Predictions of stable-stage distributions using a context-dependent Markov chain model

We used state data measured in experiments in a nine-state Markov chain model to calculate transition rates and estimate stable stage distributions of sea grass and sand dollar abundance under different conditions, using the initial condition and surrounding matrix as treatments. Markov models are stochastic models in which future states depend only on the state during the preceding time point, and are easily parameterized with experimental and observational data. They and their extensions have been used in ecology to predict equilibrium proportions of species under both naturally occurring and experimental conditions (Wootton 2004; Nelis and Wootton 2010), to project community shifts in response to changing environments (Wootton et al. 2008), and to recreate landscape patch dynamics (Wright, Gurney, and Jones 2004). These models are parameterized with transition rates between states over some span of time, then each iteration of the model estimates the ending distribution of species over each census interval of time by multiplying these transitions rates with starting conditions. Long term behavior and stability can also be evaluated with eigenvector analysis (Caswell 2001)

Census data were taken in the 4x4 grid squares within each plot area; therefore, our data tracks the fate of individual patches of sand. Sea grass density in patches was converted to three states:

Absent, sparse sea grass (<100 shoots per square; median 50 shoots) and dense sea grass (>100 shoots per square, or approximately full occupancy; median 200 shoots). Sand dollar density was also divided into three states: absent, sparse sand dollars (<15 individuals per square; median 4), and dense sand dollars (>15 individuals per square; median 26). These species abundance categories were combined to create nine possible sand dollar-sea grass states. A transition rate was calculated based on the proportion of patches in each starting state that transitioned to a new state by the following annual census. Data were used from the four censuses taken annually in late June 2013-2016, which offered the longest series of annual time steps. These probabilities defined transition matrices for each plot in each treatment, allowing the dominant eigenvector to be calculated for each transition matrix to predict the long-term stable stage distribution of each plot. In a few cases, transitions into new states occurred in the final census of 2016, which precluded estimates of transitions out of that state for that plot. In these cases, we used the observed proportion of transitions into each state across the entire plot history as an estimate of transition rates from these unobserved starting states.

We evaluated treatment effects by testing whether models of treatment-specific transitions fit better than models without treatments using goodness of fit tests (Caswell 2001). We then explored the implications of any treatment differences by analyzing the stable state distributions of Markov Chain models for each plot, parameterized with the transition data collected over the three-year span of the experiment. We tested for alternative states by applying two-stage cluster analysis (SPSS 2016) to the predicted sand dollar and sea grass abundance derived from by multiplying the stable state distributions by the median abundances of each species in each state class. Because this clustering analysis assumes independence of variables, which was neither expected based on natural history, nor supported by the data, we analyzed the dominant axis of variation from a principle components analysis on the predicted stable stage abundance of sand dollars and sea grass. To test for alternative states, we compared AIC values derived from a single cluster model to those from multi-cluster models. We also inspected the relationship between cluster membership and treatment replicates to determine whether initial conditions and the species dominating the surrounding matrix were always associated with membership in the same stable state distribution cluster.

We also explored the role of temporal variability in relation to long-term stable stage distributions of each quadrat by examining the subdominant eigenvalues of each Markov Chain model. Subdominant eigenvalues of Markov Chain models provide information on the dynamics of the model and convergence to a stable state distribution. Models with real eigenvalues converge directly to the stable state distribution, whereas those with eigenvalues that have imaginary parts exhibit fluctuations before converging to a stable state distribution. Rates of convergence in the absence of disturbance are inversely proportional to the magnitude of the largest subdominant eigenvalue. We further explored the behavior of the subdominant eigenvalues in the context of plot responses to seasonal variation in environmental conditions such as light availability and storm surge. To do this, we created summer and winter Markov Chain models for each experimental plot from transitions between earliest and latest census within years (excluding the one January census), and between the latest census in one year and the earliest the following year, respectively. We then analyzed these models for their predicted long-term stable state distribution, and examined whether the predicted differences in winter versus summer models were related to annual model behavior.

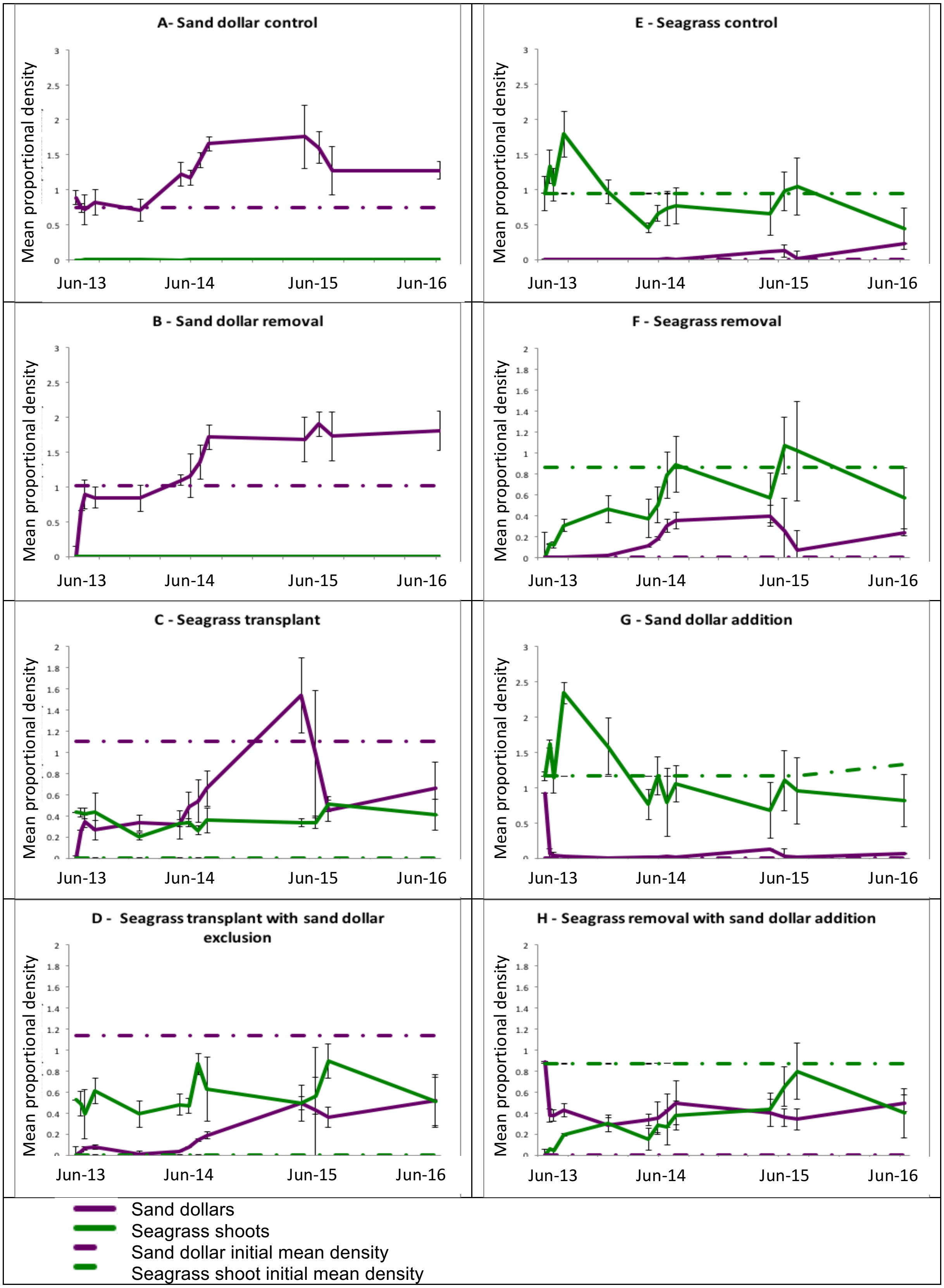


Figure 2.3: Trends over time of seagrass and sand dollar shoot density

RESULTS

Treatments created significant differences in sand dollar and sea grass abundance through the 3-year course of the experiment (Figure 2.3, Tables 2.1 - 2.3; Repeated Measures MANOVA, P <0.0001). Variability in treatment effects was affected both by the experimental conditions within each plot and by the dominant species matrix in which the plot was located (Tables 2.1 - 2.3). The effect of treatment interacted with sampling time for both species as many plots converged from initial experimental states to their former natural states (Figure 2.3, Tables 2.1 – 2.3; Repeated Measures MANOVA $P < 0.0001$). In general, habitat matrix and initial sea grass treatment affected both species, whereas the effect of manipulating sand dollars had relatively weak effects on sea grass.

The treatment effects of the experiment were reflected in transition patterns among sea grass-sand dollar states across each treatment. A Markovian model with transitions that depended upon starting state fit better than a purely random matrix (equal probabilities; $G_{71} = 591$, $P < 0.0001$) or a transition matrix with a single distribution of outcomes regardless of starting state ($G_{64} = 352$, $P < 0.0001$). A model that accounted for differences in treatments fit better than a model with transitions pooled across all plots ($G_{568} = 607$, $P < 0.014$).

Projection of long-term stable stage distributions for Markov Chain models from each plot provided evidence of two alternative states, one dominated by sand dollars, the other by seagrass (Figure 2.4). Two-step cluster analysis better supported a model with two clusters rather than one cluster ($\Delta AIC = 8.238$). In general, cluster membership was associated with the identity of the species that dominated the area surrounding each plot (Figure 2.4), suggesting that the outcome of perturbed states was context dependent, and positively influenced by the local density of each ecosystem engineer.

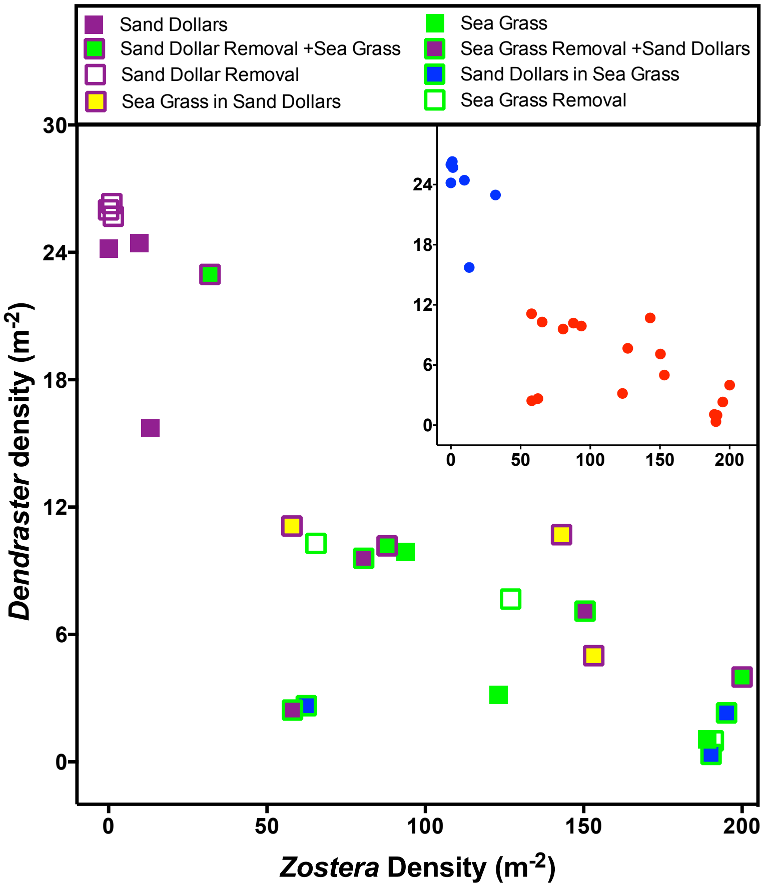


Figure 2.4: Long-term projections of dominant species abundance in each plot on experimental sand flats manipulating sand dollars and sea grass, coded by experimental treatment. Colors of point borders reflect the dominant species comprising the habitat the plot was in (green-sea grass, purple-sand dollar), the colors of the point interiors reflect treatments within each plot. Projections derived from stable state distributions of plot-specific Markov Chain Models, parameterized by observed transitions among abundance classes (absent, low, high) of sand dollars and sea grass collected over 3 years. Inset: Membership assigned by two-step cluster analysis based on stable state distributions ($\Delta AIC = 8.24$). Overlapping points are slightly jittered to visually identify treatments.

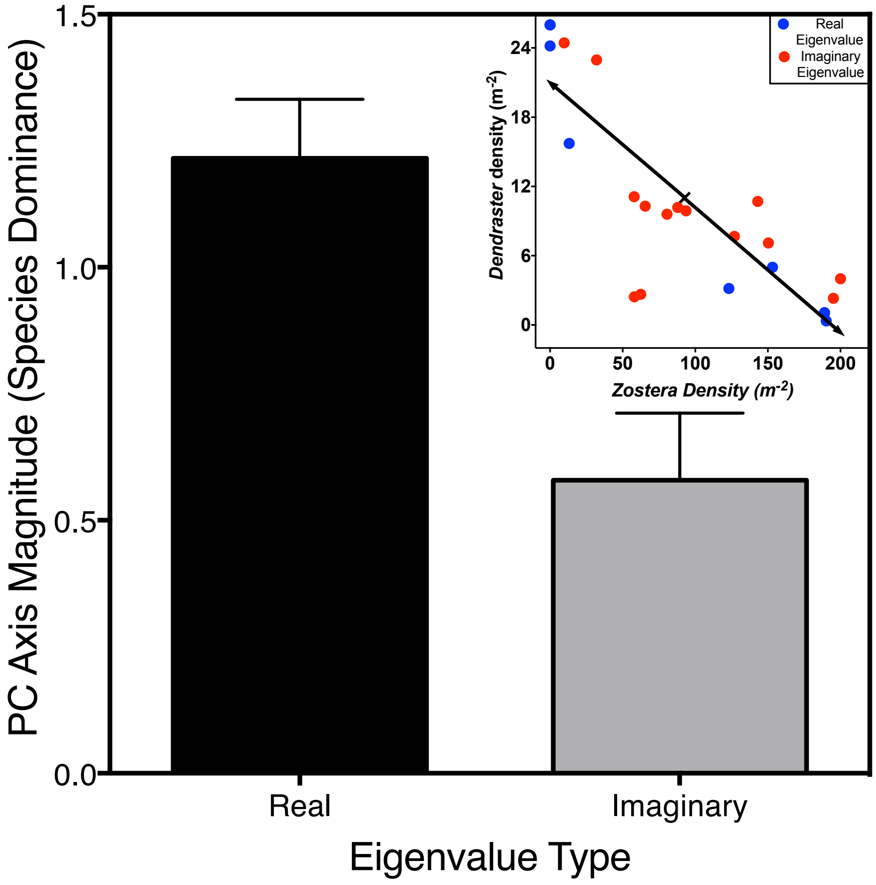


Figure 2.5: Relationship between the type of eigenvalues exhibited by Markov Chain models based on annual transitions for each experimental plot and the magnitude of the score from the dominant principle component summarizing projected stable state abundances of sea grass and sand dollars (mean ± 1. s.e). Models with real eigenvalues exhibit direct convergence to the stable state distribution, those with imaginary parts exhibit fluctuating convergence. Inset: relationship between stable stage distributions of sea grass and sand dollars projected for each experimental plot, with points color coded by whether eigenvalues are all real (blue) or have imaginary parts (red). The black line shows the dominant principle component axis, with a value of zero indicated by the cross-hatch. Higher-magnitude values, corresponding to higher dominance by one species, are attained at the arrow heads.

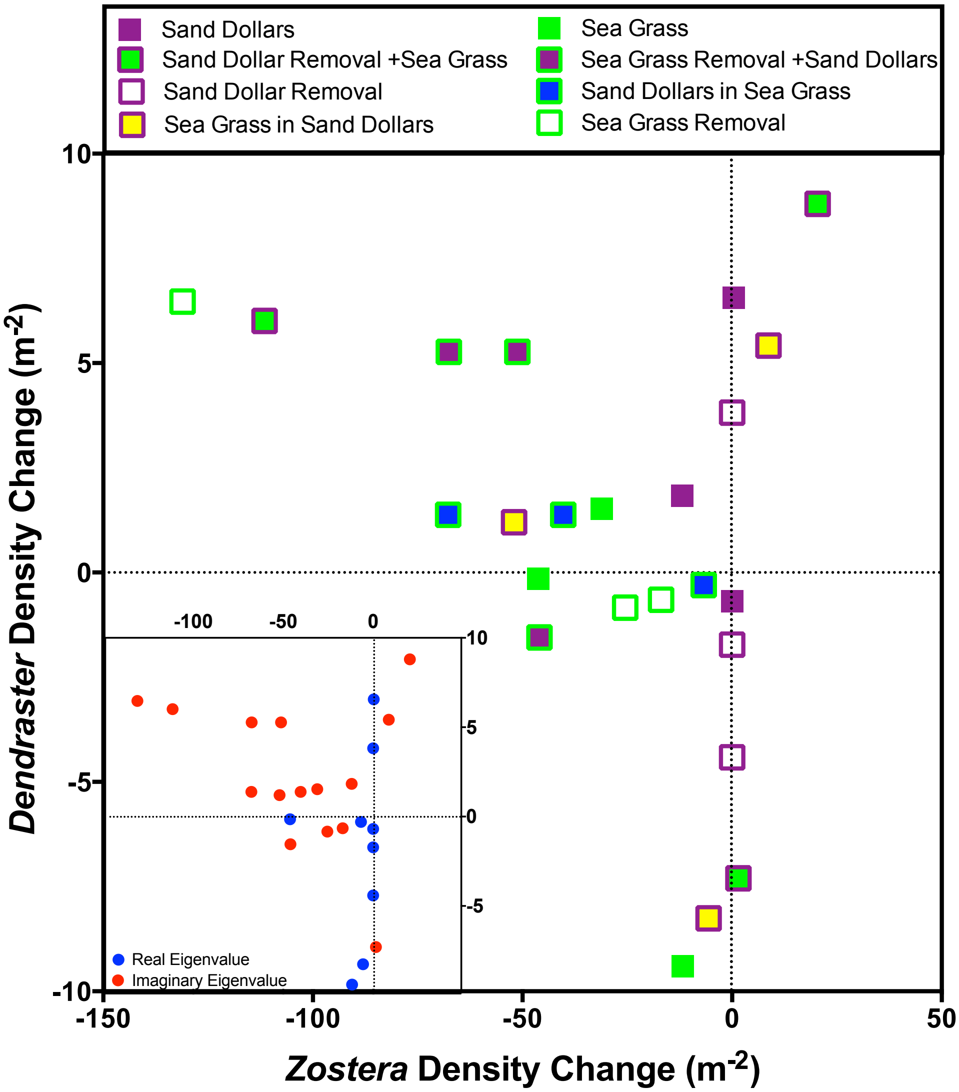


Figure 2.6: Relationship between differences in the projected long-term stable state abundances of sea grass and sand dollars between seasons, the experimental treatments of each plot, and the predicted fluctuations in states based on the existence of imaginary parts of eigenvalues for plot specific Markov Chain models (inset). Stronger seasonal sensitivity in sea grass corresponds to fluctuations during convergence to the stable state over annual time steps, which may facilitate context-dependent switches in transitions under environmental variability.

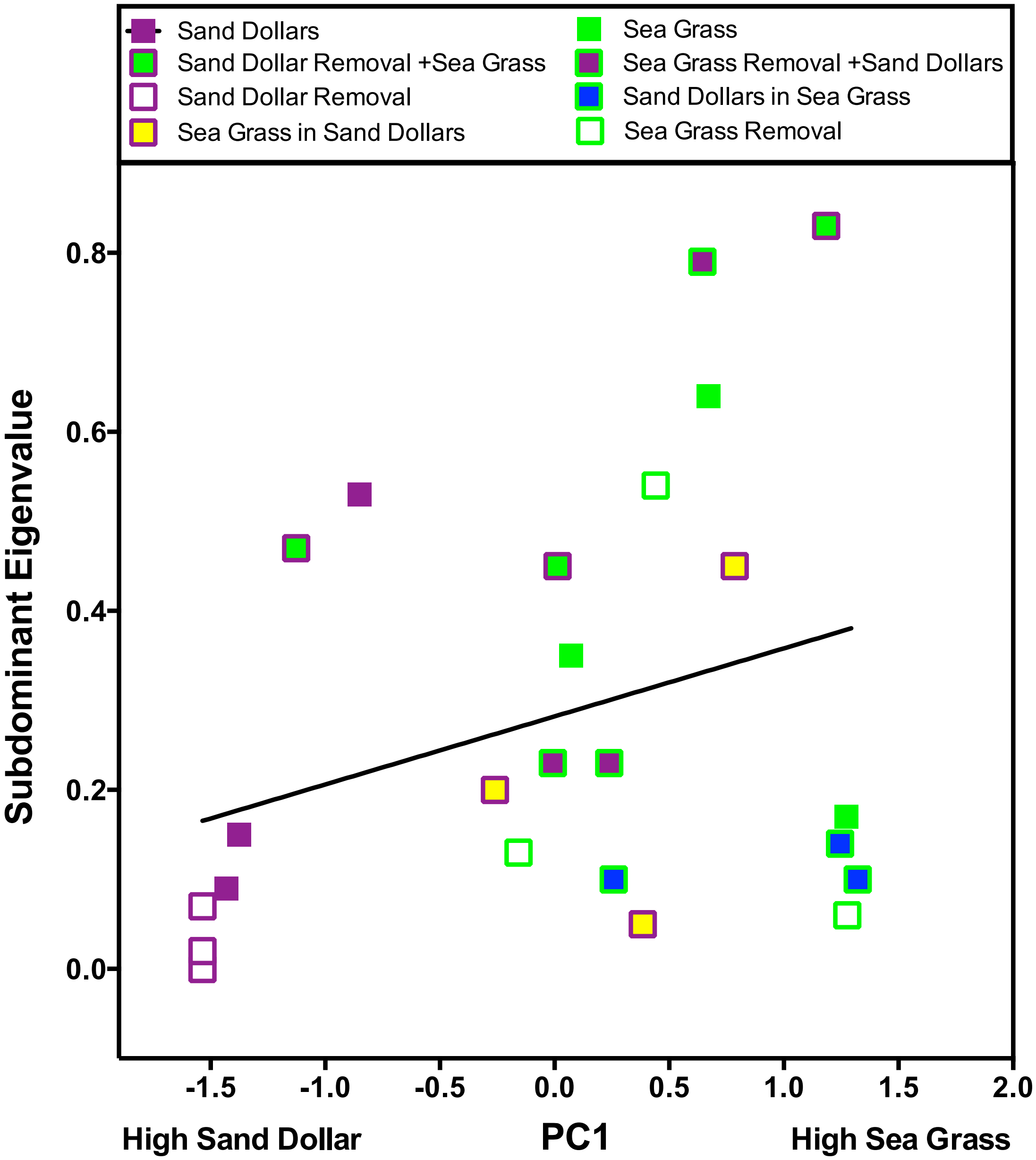


Figure 2.7: Relationship between projected stable stage composition summarized by the dominant principle components describing joint abundance of sand dollar and sea grass and the subdominant eigenvalue, which corresponds to the rate of return to stable stage composition. Linear regression: $r^2 = 0.22, P = 0.02$.

Cluster membership was not completely determined by the context of the surrounding area (Figure 2.4), indicating sensitivity to initial conditions within the plot. Switching from the surrounding matrix generally occurred when sea grass was present in a sand dollar-dominated matrix, although such switching was not entirely predictable. Patterns of switching were associated with the subdominant eigenvalue of the transition matrix of the plot (Figure 2.5). The magnitudes of the PC scores summarizing the relative abundance of sand dollars to sea grass tended to be higher (more dominated by one species) for plot models with real subdominant eigenvectors (1.22 ± 0.35, $n=9$) compared to plot models with imaginary subdominant eigenvector parts (0.58 ± 0.51, $n=15$, t-test, $P = 0.003$). The effects of seasonal fluctuations on transitions corresponded to the eigenvalue type of the annual matrix (Figure 2.6): Plots which exhibited seasonal transitions, such as seagrass controls with summer growth and overwintering that projected large differences in stable state abundances (for example, Figure 2.3, panel F), were generally the plots that exhibited eigenvalues with imaginary parts (Figure 5, *Zostera*: $t_{19.1} = 2.828$, $P = 0.011$; *Dendraster*: $t_{22} = -1.934$, $p = 0.071$). The real part of the subdominant eigenvalue varied with treatment (Figure 2.7, ANOVA on log(1/eigenvalue), $F^{7}_{16} = 4.405$, $p = 0.007$), but not with surrounding matrix type (a priori contrast, $t_{16} = 1.291$, $P = 0.22$). Post-hoc testing showed sand dollar removal treatments within a sand dollar matrix had unusually low subdominant eigenvalues (Figure 2.7, $P < 0.05$), indicating extremely rapid return to stable state distributions.

Table 2.1: Repeated measures MANOVA of treatment effects on ln(x+1)-transformed sand dollars and sea grass.

Source	*Wilk's L*	*df*[*]	*F*	*P*
Between Subjects				
Intercept	*0.006*	*2,15*	*1189.9*	*<0.0001*
Treatment	*0.006*	*14,30*	*24.7*	*<0.0001*
Matrix	*0.042*	*2,15*	*170.6*	*<0.001*
Plot Treatment	*0.021*	*6,30*	*29.8*	*<0.001*
*Matrix*Plot Trt.*	*0.120*	*6,30*	*9.4*	*<0.001*
Within Subjects				
Date	*0.385*	*26,414*	*9.7*	*<0.001*
*Date*Treatment*	*0.091*	*182,141*	*5.3*	*<0.001*
*Date*Matrix*	*0.343*	*26,414*	*11.2*	*<0.001*
*Date*Plot Treatment*	*0.239*	*78,414*	*5.6*	*<0.001*
*Date*Matrix*Plot Trt.*	*0.453*	*78,414*	*2.6*	*<0.001*

Table 2.2: Results of repeated measures ANOVA of treatment effects on ln(x+1)-transformed sea grass abundance following analysis with repeated measures MANOVA.

Source	*SS*	*df*[*]	*MS*	*F*	*P*
Between Subjects					
Intercept	*3750.0*	*1*	*3750.0*	*1801.4*	*<0.0001*
Treatment	*1185.4*	*7*	*169.4*	*81.4*	*<0.0001*
Matrix	*459.2*	*1*	*459.2*	*220.6*	*<0.001*
Plot Treatment	*589.4*	*3*	*196.5*	*94.4*	*<0.001*
*Matrix*Plot Trt.*	*136.8*	*3*	*45.6*	*21.9*	*<0.001*
Error	*33.3*	*16*	*2.1*		
Within Subjects					
Date	*37.3*	*4.20*	*8.9*	*6.1*	*<0.001*
*Date*Treatment*	*243.6*	*29.39*	*8.3*	*5.7*	*<0.001*
*Date*Matrix*	*88.4*	*4.20*	*21.1*	*14.5*	*<0.001*
*Date*Plot Treatment*	*106.2*	*12.60*	*8.4*	*5.8*	*<0.001*
*Date*Matrix*Plot Trt*	*49.0*	*12.60*	*3.9*	*2.7*	*0.005*
Error(Date)	*97.8*	*67.18*	*1.5*		

[*] Within-subjects d.f. adjusted using the Greenhouse-Geisser method to account for lack of sphericity

Table 2.3: Results of repeated measures ANOVA of treatment effects on ln(x+1)-transformed sand dollars following analysis with repeated measures MANOVA.

	SS	*df*[*]	*MS*	*F*	*P*
Between Subjects					
Intercept	*994.0*	*1*	*994.0*	*754.6*	*<0.0001*
Treatment	*348.0*	*7*	*49.7*	*37.7*	*<0.0001*
Matrix	*185.3*	*1*	*185.3*	*140.7*	*<0.001*
Plot Treatment	*145.1*	*3*	*48.4*	*36.7*	*<0.001*
*Matrix*Plot Trt*	*17.5*	*3*	*5.9*	*4.4*	*0.019*
Error	*21.1*	*16*	*1.3*		
Within Subjects					
Date	*44.7*	*3.73*	*12.0*	*13.8*	*<0.001*
*Date*Treatment*	*127.3*	*26.12*	*4.9*	*5.6*	*<0.001*
*Date*Matrix*	*50.5*	*3.73*	*13.4*	*15.4*	*<0.001*
*Date*Plot Treatment*	*54.1*	*11.19*	*4.8*	*5.6*	*<0.001*
*Date*Matrix*Plot Trt*	*23.1*	*11.19*	*2.1*	*2.4*	*0.016*
Error(Date)	*51.9*	*49.7*	*0.87*		

[*] Within-subjects d.f. adjusted using the Greenhouse-Geisser method to account for lack of sphericity

DISCUSSION

Our experiments provide three lines of evidence for several key signatures associated with the development of alternative stable states. First, the study provided evidence of context-dependence in the form of local positive density dependence on the outcome of experimental treatments. Specifically, when a species numerically dominated the area surrounding the experimental plots, those plots often moved to dominance by that species (Figure 2.3, Tables 2.1 - 2.3). This observation of context-dependence by itself does not demonstrate positive feedback with local density, however; an alternative hypothesis is that areas exhibiting dominance by different species differ in subtle undetected ways (Petraitis and Dudgeon 2004).

Second, the dynamics implied by the transitions within different plots led to stable state distributions occupying two statistically distinct community patterns: dominance by sea grass and dominance by sand dollars (Figure 2.4, inset). This result matches the observations of local patches dominated by the two species that originally motivated the experiment, and supports the hypothesis of the existence of local alternative states. The abundance patterns of the clusters derived from the plot-specific model projections did exhibit some variability, however (Figure 2.4). We suspect that this variability arose from data limits to estimating parameters on a plot-specific basis, but it is possible that subtle context-dependence leads to one or more mixed species states that our cluster analysis was not able to detect.

Third, the dynamics implied by the transitions in each plot indicate sensitivity to initial conditions derived from the pulsed experimental manipulations within the plots (Tables 2.1 - 2.3). Specifically, the transition dynamics within a subset of plots led to different dominance states than those characterized by the surrounding area (Figure 2.4). Furthermore, in some cases replicate plots within the same experimental treatment diverged toward different dominance states (Figure 2.4). By showing that plots are not constrained to converge back to their initial dominance state, these results contradict the hypothesis that dominance differences arise because of subtle, spatial differences driven by the external environment (Petraitis and Dudgeon 2004), and better support the notion that local abundance tends to facilitate dominance through positive feedback. Hence, by taking these three lines of evidence together, we suggest that the observed patch distributions of our study species are the result of alternative stable states at a local scale within this sand flat ecosystem.

Natural history observations and prior work involving these and related species provides mechanistic insight and further support for the existence of alternative stable states. *Dendraster* have been observed in patchy competition with tanaid shrimps, which prey on *Dendraster* larvae and are excluded by bioturbation (Highsmith 1982a; Sousa and Connell 1985). Seagrass species may be predisposed to exhibit alternative states because shoot density often follows threshold patterns and can crash catastrophically (Scheffer et al. 2001; van der Heide et al. 2007). Seagrass may also function as an alternative state when in competition with other species such as macrophyte algae (Viaroli et al. 2008) or bioturbating callianassid shrimp (Castorani, Hovel, Williams, and Baskett 2014b). Backman (1984) examined *Dendraster* in competition for space with *Z. marina* in Puget Sound through experimental transplants, removals, and exclusions, and found that when *Dendraster* was excluded from the boundary with *Z. marina*, *Z. marina* expanded into the area previously occupied by *Dendraster.* Several months after the exclusion was removed, *Dendraster* did not reclaim the space. Conversely, where *Z. marina* was removed and excluded, *Dendraster* took over and was not ousted once *Z. marina* was no longer excluded (Backman 1984). These dynamics, though in a different seagrass species than our study, are suggestive of threshold dynamics between alternative states.

Our analysis of the context-dependent Markov Chain models derived for each plot also suggest features that may predispose particular configurations to deviate from each other and the surrounding area. Plots with the strongest projected dominance of a species generally exhibited real eigenvalues, indicating systems that return directly to a stable state following perturbations. This pattern was particularly true of plots lacking sea grass within a sand dollar matrix, which tended to return both directly and rapidly to their stable stage distribution, probably because the mobility of sand dollars allows them to quickly invade empty space from the surrounding matrix. In contrast plots that were projected to have more mixed composition tended to have some eigenvalues with non-real parts, indicating a tendency for populations to fluctuate, which offers more opportunity to flip dominance states following a perturbation. Analysis of season-specific transitions suggest that seasonal fluctuations contribute to these fluctuating patterns over annual time scales. In particular, plots with moderate sea grass abundance appeared to flourish during summer but suffer during winter, perhaps because production under reduced light conditions could not counteract effects of higher wave wash when fewer neighbors were present to stabilize sediments and more room was available for sand dollars to bioturbate the sand with reduced plant interference.

Integrating mathematical models with experimental data allowed us to uniquely gain insight into the likelihood of alternative stable states in our system of competing ecosystem engineers without the limitations of long-term empirical observation necessary to document complete community turnover. The context-dependent Markov Chain model approach we chose was useful for several reasons, including its relatively direct analogy to interference competition by characterizing transitions among states in space, the availability of analytical tools to determine stable state distributions and characteristics of convergence to that state, and the relative accessibility of the approach to readily-collected experimental data. Other approaches may be more effective in other scenarios, although the alternative stable state concept is usually applied to characterize ecosystems based on the dominant species in a defined area. We note that employing a simple Markov Chain model is not an ideal approach for assessing alternative stable states, as these models tend to converge on a single equilibrium except under extremely unusual (modular) patterns of transitions. By exploring the behavior of these models under different experimental contexts relevant to the development of alternative stable states, such as local density and variation in initial starting conditions, application of Markov Chain models became a useful tool for probing this phenomenon.

CONCLUSIONS

Our study found evidence for alternative stable states over the relatively small scale of our experimental plots and the local dominance matrix. We were not able to address how these dynamics would play out at larger scales, such as the entirety of Crescent Bay. Doing so will require more extensive data on disturbance regime, spatially explicit modeling of collections of plots, and development of functions describing the transition among contexts. Nevertheless, we believe that the integrated experimental and modeling approach outlined here will facilitate a greater understanding of the existence of alternative states in nature.

CHAPTER III

CELLULAR AUTOMATA MODELS AND EDGE EFFECTS IN COMPETITION: AN EXAMINATION OF SPATIALLY DEPENDENT COMPETITIVE INTERACTIONS BETWEEN ECOSYSTEM ENGINEERS

ABSTRACT

The spatial patterning of organisms in environments has long been of interest to ecologists, and particularly how it operates on varying spatial scales, between competing species, and as a function of transitions between alternative ecological states. In some cases, spatial pattern at large scales has been shown to be the result of emergent properties of small-scale, measurable dynamics, such as local positive feedbacks. On flat sandy beaches in Washington state, the seagrass *Zostera japonica* and the sand dollar *Dendraster excentricus* occupy mutually exclusive patches in the intertidal zone. By measuring growth and transition rates at boundaries between the two species, we parameterized a spatially explicit model to test what drives the changes between patches, and whether the small-scale changes drive large scale patterns. Our models predicted some spatial clustering at small scales that did not match the expected field size, but led us to new hypotheses.

INTRODUCTION

The dynamics of the spatial arrangement of organisms on landscapes has long been a subject of interest to ecologists. Organisms are often patchily distributed in the environment, driven by a wide range of mechanisms, both abiotic and biotic. Abiotic conditions are patchily distributed, so therefore, many organisms follow favorable conditions and are correspondingly patchily distributed. However, even under homogeneous or otherwise universally favorable environmental conditions, a species rarely occupies all of the possible suitable space. One hypothesis to explain this observation is that interactions with other species, such as competition, limit where an organism may live.

Regular patterns across seemingly homogeneous space are a feature of wetlands and savannas, coral reefs and mudflats, forming regular patterns of vegetation or organisms. A combination of short-range positive feedbacks and long-range negative feedbacks (Rietkerk and van de Koppel 2008), an extension of the activator-inhibitor principle describing autocatalytic chemical reactions (Turing 1990). Spatial pattern also gives clues to criticality, or a sudden change in ecological state when parameters change slightly (such as with alternative states), and catastrophic shifts. Spatial patchiness may develop at the transition between the two states as an alternative to homogeneity as edge effects and patch size become important to state transitions (Pascual and Guichard 2005; Rietkerk 2004).

Fewer empirical studies have approached how interspecific interactions can structure space and entire communities. Wootton (2001) examined the question of whether species interactions occurring at a local scale can generate landscape-scale patterning of space-occupying organisms.

Using empirical observations of transitions occurring in mussel beds following disturbances, he parameterized a cellular automata model that successfully predicted both the gap sizes and community composition observed in nature. This model predicted spatial pattern on scales four orders of magnitude larger than those observed on small spatial scales, lending hope to the possibility of predicting large-scale spatial pattern using small-scale interaction information in some systems. A key result was that realistic spatial pattern only arose when disturbance was transmitted locally on rapid time scales.

Spatial patterning in seagrass beds can also be driven by forces like wave scour or seed bank transportation (Fonseca et al. 2008; Fonseca et al. 2002), and self-organizing effects of positive feedback via sediment stabilization by rhizomes. Rhizomes anchor seagrass shoots while long blades may cause longer-range sediment scouring (van der Heide, Bouma, van Nes, van de Koppel, Scheffer, Roelofs, van Katwijk, and Smolders 2010). Clonal growth, in which seagrass grows new shoots laterally from older shoots, also contributes to positive feedbacks (Fonseca et al. 2008).

Sand dollars and seagrasses exhibit positive feedbacks through opposing activities: sediment disruption and stabilization, respectively. Consequently, they exhibit competitive interactions with each other, which play out through a patchy distribution of the species across nearly homogeneous sand-flat habitats (Chapter II, Appendix A-F). I hypothesized that the interaction of the two species could drive the complex spatial pattern between them, and that the resulting spatial patchiness could facilitate larger scale coexistence. In previous studies, I integrated experimental manipulations and dynamic models, which provided support for alternative stable states at local scales, and evidence that such outcomes were dependent on the spatial context of species dominance in the surrounding area (Chapter II). These results indicate that spatially-localized interactions may play a key role in determining spatial distributions and the mechanisms underlying species coexistence. Examining spatially explicit dynamics at the boundaries between patches of dominant space occupiers offers a powerful window to understanding the development of spatial patterning. Here I integrate experimental studies exploring spatially localized interactions at patch boundaries, and use these data to parameterize a cellular automaton of the sand flat system to explore the large-scale consequences of local interactions on landscape scale pattering and species coexistence.

Study System

On flat sandy beaches in Washington state, non-native seagrass (*Zostera japonica*) competes for space with sand dollars (*Dendraster excentricus*) in a manner well-described as alternative stable states. This interaction is spatially dependent, (Chapter II), such that the identity of the matrix surrounding an experimental plot was a strong predictor of final state. I therefore examined how the spatial pattern, in terms of the arrangement of patches and patch boundaries, affects the results of competition between seagrass and sand dollars.

Because of the clonal, sessile nature of seagrass and the benthic, motile lifestyle of sand dollars, shifts between these two ecological states likely depend on invasion dynamics at territory edges. Here I ask, how does this spatial component affect state shifts? Our previous experiments and non-spatial models predicted different outcomes depending on the state of the surrounding matrix, emphasizing the importance of spatially local interactions.

The spatial pattern of occupancy between sand dollars and seagrass shows sharp boundaries between patches, with few sand dollars found in seagrass beds, and sparse seagrass rare in sand dollar beds. Patches range in spatial scale from meters to tens of meters wide (Figure 3.1). These patches likely result with a combination of history, hydrogeography, and antagonistic interactions between the two species.

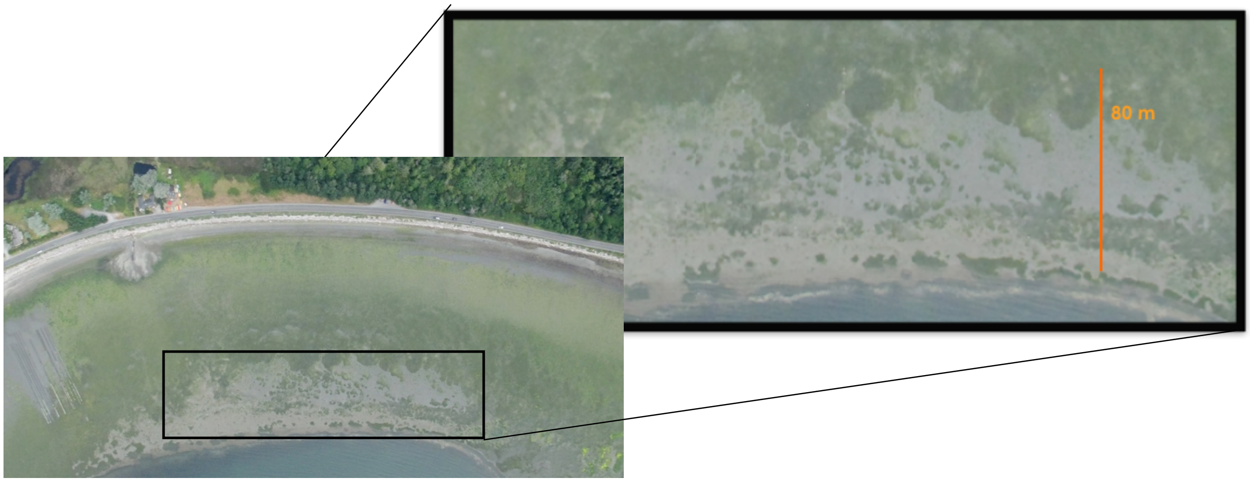


Figure 3.1: Aerial view of Crescent Beach, Eastsound, Orcas Island, WA. Green areas are seagrass, primarily Z. japonica, and gray zones are bare sand and sand dollar beds.

I examined spatial dynamics at boundaries between sand dollars and seagrass at Crescent Beach, on Orcas Island, Washington in plots with and without experimental disturbance to determine the strength of small-scale antagonistic interactions at boundaries between seagrass and sand dollars. Using data derived from my experiments, I parameterized a cellular automaton to model spatial pattern on a beach-wide scale, and I used the model to test whether small-scale interactions between neighboring patches were sufficient to explain the large-scale spatial patterns seen in the study area.

MATERIALS AND METHODS

Field experiment

I used a pulse-removal experiment at pre-existing boundaries between seagrass and sand dollar beds to determine rates of invasion and resistance at edges. Experimental plots were 1-m x 4-m areas spanning a pre-existing boundary between sand dollars and seagrass at the 2-meter midline (Figure 3.2). Fences on long sides (plastic 12.7 cm terrace board, Master Mark Plastics), were sunk into sand until flush with the sediment surface, prevented movement of sand dollars and the growth of seagrass rhizomes between treatments. Open ends allowed invasion and migration.

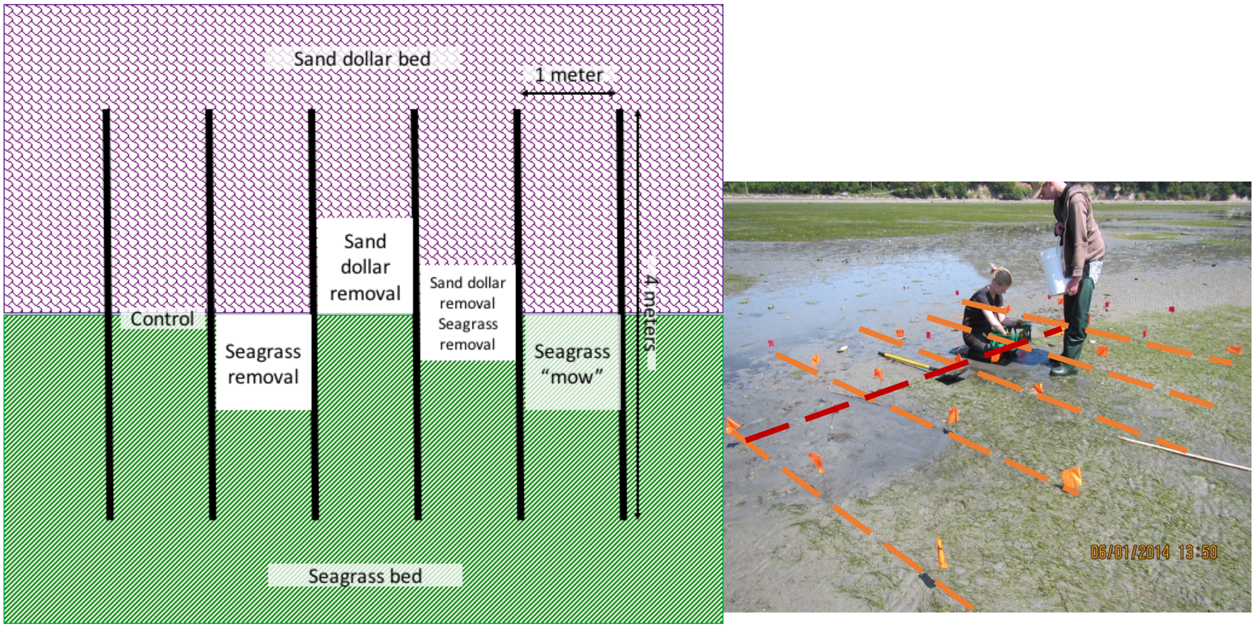


Figure 3.2: (left) Experimental design. Vertical lines represent enclosure edging, separating treatments. (right) One replicate set in the field. The red dashed line indicates the pre-existing boundary of seagrass and sand dollars, and the left-most row in the foreground shows an area of recently removed seagrass.

Treatments were:

1. Control (n=5);
2. Sand dollar removal in the meter adjacent to the midline boundary on the sand dollar side (n=5);
3. Full seagrass removal (blades and rhizomes) 1-m2 of the midline on the seagrass side (n=5);
4. Dual species removal (Half & Half), with both species fully removed from 0.5m on each side of the midline (n=3);
5. “Mowed” seagrass, with blades removed within the meter near the midline (n= 3).

Seagrass was removed by shovel to 30 cm depth, and was separated from the sediment by rinsing through a rough sieve. Sediment was returned to the removal hole. Sand dollars were removed manually to a 20 cm depth and then transported out of the study area. “Mowed” plots were cut manually with scissors to less than 1-cm blade height, leaving rhizomes intact, and biomass of blades was removed from study area. Grass was “mowed” every two weeks in June 2014 & June 2015.

Presence/absence data were taken for a grid of points within plots in June and August 2014 & 2015, and June 2016. Points surveyed using a 1-m^2 quadrat with 64 marks at 11.1-cm intervals in an 8 x 8 square, with a landscape flagging pin inserted into the sand at each point. Points where a seagrass shoot was less than 2-cm from the pin location were counted as grass present, and those in which the pin contacted a sand dollar upon insertion (confirmed manually) were counted as sand dollar present. Data on presence of *Zostera marina* and clams (*Clinocardium* sp. and *Tresus* sp.)

were also taken, but were excluded for this analysis. Areas where herbivory by geese (*Branta canadensis*, Canada goose) removed seagrass were also noted.

Invasion Speed Analysis

Point-contact data were subdivided into eight parallel presence/absence transects within each treatment replicate, and the distribution of first contacts, mean position of points, and other comparisons of spatial change from the start line of each plot was compared across treatments.

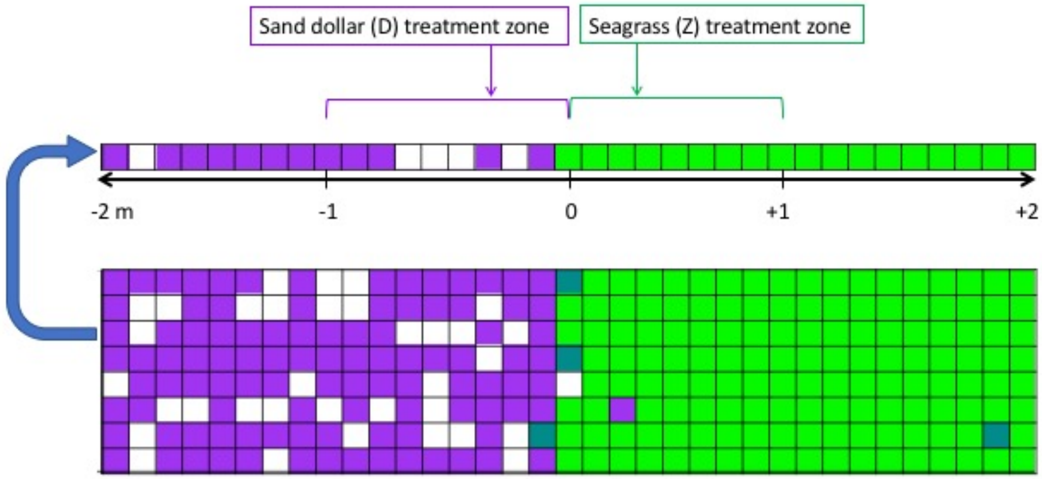


Figure 3.3: Method of isolating individual rows of census as replicates for analysis of rates of spread. Zone between -1 and 0 is where sand dollar removals are done, while 0 to +1 is where seagrass manipulations occurred.

Experimental plots were analyzed as replicated one-dimensional strips (Figure 3.3) to describe the invasion front of the plots. These transects were analyzed in four ways:

1. The mean furthest excursion of sand dollars into seagrass territory, and of seagrass into the sand dollar zone. This is the distance past the start line (0 m) to the furthest excursion, with the mean distance taken among all one-dimensional strip replicates.

2. “Invasion front”, or mean weighted distances of occurrences within the invasion zone. This is the mean distance of all occurrences of sand dollars within the 0 m to +1 m zone, and vice versa.
3. “Vote counting” or counts of occurrences of each species within the full zone, from -1 to +1.
4. “Vote counting”, only within the invasion zone, (0 m to -1 for seagrass, and 0 m to +1 for sand dollars).

Aerial image analysis

Aerial images of the field site were taken from a small aircraft with a wing-mounted point-and-shoot camera from an altitude of 2500 feet in July 2012, 2013, and 2014 at extreme low tide. Images were cropped to include study transect areas from 0’ to -3.0’ tidal elevation, utilizing replicable landmarks in images. Images were analyzed for seagrass cover using FIJI (Schindelin et al., 2012) by converting images to binary (present-absent) data (percentile method) and applying a particle analysis, which identified contiguous patches of seagrass and calculated their area. This method was also applied to model outputs for predicted seagrass, and distributions of patch areas between empirical photos and model simulations were compared and analyzed using R.

Spatially-Explicit Modeling

To carry out spatially-explicit analyses, each data point was characterized both by its own state (occupied by sand dollar (D), seagrass (Z) or empty (0)) and by the states of the neighboring 8 sampling points. Resurveying the points annually allowed the estimation of transitions among states that were dependent on starting condition of the point and its neighbors (Figure 3.3).

To estimate the probabilities of transition under different conditions, we fit a multinomial logistic regression model (multinom function in package nnet, R) with the following parameters: number of sand dollar neighbors (out of eight), number of seagrass neighbors, number of empty neighbors, ratio of seagrass neighbors to sand dollar neighbors, year, and state at previous time point. The best fitting model was determined using AIC and used to compute transitions that were used as parameters in the cellular automaton.

A 2-D cellular automaton was constructed in the R programming language on a 1000 x 1000 grid, representing 111 m x 111 m of space in 11.1 cm grid squares. Initial abundances were set to 33% seagrass, 33% sand dollars, and 34% empty, which were distributed in different simulation runs according to different initial spatial patterns:

1. Random,
    a. Using best-fit model transitions.
    b. Using best-fit model transitions with a simulated disturbance to mimic the effect of goose herbivory, removing seagrass in half of the locations with 8 seagrass neighbors at each time step (removing 4x4 pixels, or 0.44 m x 0.44m).
2. Clustered sand dollars, (a field of 67% seagrass/33% empty with a 200 x 200 pixel square of 67% sand dollar/33% empty in the center)
3. Clustered seagrass, (a field of 67% sand dollar/33% empty with a 200 x 200 pixel square of 67% seagrass/33% empty in the center)
4. Banded pattern. (5 stripes of 67% sand dollars/33% empty, alternated with 5 stripes of 67% seagrass/33% empty)

Each cell effectively transitioned instantaneously, assessing neighbor identities and drawing from the probabilities predicted by the best-fitting multinomial logistic regression model at one time step, and shifting to a new state for the next time step with all of the other cells. This generated a spatial matrix for each of 100 generations, which spanned a time period over which the pattern and proportion of states appeared to stabilize. To isolate the effects of spatially-local interactions, results from the cellular automaton were also compared to a non-spatial Markov chain model (e.g. Chapter 1) using transition rates that did not take neighbor states into account. In addition, we generated several simulations using hypothetical transition probabilities designed to generate maximum spatial patchiness.

We analyzed results of simulations for spatial autocorrelation using Ripley's K, and compared distributions of the areas of patches measured using particle analysis in FIJI (ImageJ). These model distribution results were compared to those of original empirical data and aerial images, from which seagrass patch sizes could be extracted (but not sand dollars).

RESULTS

Invasion Rates

Qualitatively, control treatments showed slow, steady encroachment of seagrass into sand dollar territory, with gains up to 30 cm in two months. Sand dollars recolonized sand dollar removal treatments immediately, with comparable long-term results to the control. Seagrass removal treatments reinvaded removal areas slowly, but "mowed" treatments grew back more quickly. Seagrass removal and "mowed" treatments were invaded by sand dollars at modest rates, gaining as much as 50 cm, compared to controls in which sand dollars lost ground. These results suggest that seagrass can outcompete sand dollars under low disturbance conditions, but sand dollars are quicker to colonize following disturbances like clam harvest and grazing by migratory waterfowl.

Statistical results for measures of invasion are shown in Tables 1-3. Seagrass follows a growth pattern in which initial colonization is by sparse shoots, followed by thickening of the bed. In all metrics, neither sand dollars nor seagrass in the dollar removal treatment differed from controls. This is likely due to the fact that sand dollars rapidly recolonized (~48 hours) areas they were removed from, and as this was a pulse perturbation, then the remaining two years of the experiment were indistinguishable from the control. Controls and dollar removals both showed modest, steady growth of seagrass into sand dollar zones (Figure 3.4).

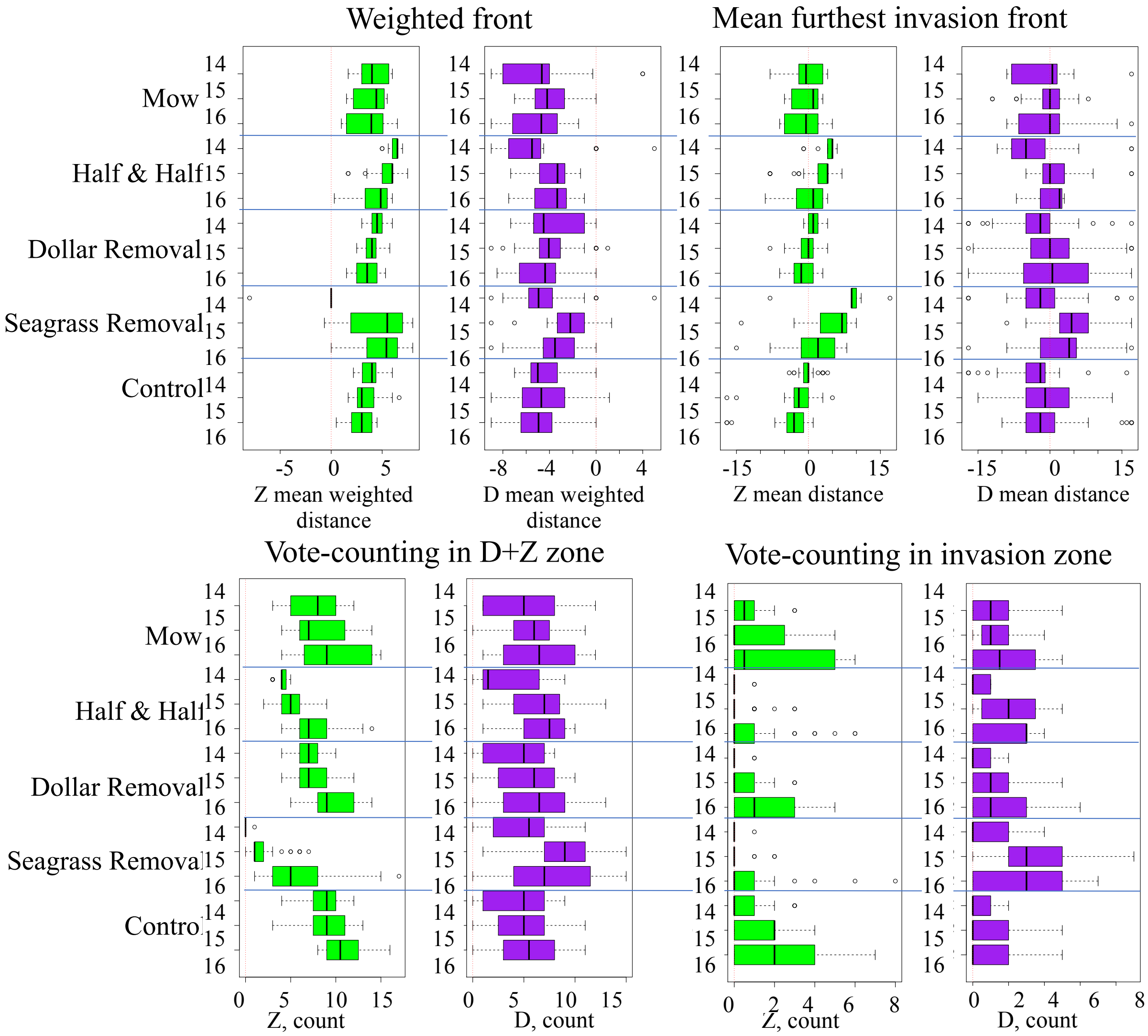


Figure 3.4: Summary of metrics of invasion by treatment. In each plot, the useful information is in comparing to the controls. In the weighted front and mean furthest invasion front, the bar plot represents the mean distance that either Z or D reached in the invasion zones. In the vote-counting analyses, the value is the number of occurrences in the invasion zones. Within each treatment there are three rows – the top is the earliest value, when the treatment was applied in 2014, middle is 2015, and bottom is 2016.

Tables 3.1 - 3.3: T-tests of whether treatments were significantly different under different methods of measuring spread.

1. Did change occur within controls between Year 1 and Year 3?

Metric	Result	Significance
Furthest excursion	Both D and Z advanced significantly into the other's territory	D: $p < 0.05$ Z: $p < 0.05$
Weighted mean position	Not different	NS
Vote-counting	Not different	NS

2. Did sand dollar removal treatments differ from controls?

Metric	Result	Significance
Furthest excursion	No difference in D, but Z grew more than in the control.	D: NS Z: $p < 0.05$
Weighted mean position	Not different	NS
Vote-counting	No difference in D, but Z grew more than in the control.	D: NS Z: $p < 0.05$

3. Did seagrass removal treatments differ from controls?

Metric	Result	Significance
Furthest excursion	Aligned with control: Did not go back to looking like controls	D: $p < 0.0001$ Z: $p < 0.0001$
	With control boundary aligned to disturbance boundary: Same amount of territory gain for D and Z as in controls.	D: NS Z: NS
Weighted mean position	Not different	NS
Vote-counting	More D in removal, more Z in the control.	D: $p < 0.0001$ Z: $p < 0.0001$

In all four metrics the seagrass removal treatment showed significant growth and recovery in the two years following removal, especially by 2016. The weighted front did not change between 2015 and 2016, however, both vote-counting metrics showed a dramatic increase, reflecting an increase in shoot density. The furthest invasion also increased in the seagrass removal treatment. Sand dollars in the seagrass removal treatment made gains into the territory that seagrass was removed from too, reflected especially in the vote-counting metrics, which showed much higher presence of sand dollars than in controls, and an increased invasion front (mean up to +5).

The mow treatment did not change the weighted front metric relative to the controls in seagrass, but slightly depressed seagrass density as measured by the vote-counting and furthest excursion. Although most metrics for sand dollars did not differ in the mow treatment, the vote-counting in the invasion zone did show sand dollars gaining in seagrass territory relative to controls.

The half removal treatment behaved as a smaller seagrass removal for the seagrass metrics. However, the sand dollars made as many gains into seagrass territory by most metrics as the full seagrass removal, except when measured by mean furthest excursion front. This suggests that sand dollars invaded at effectively the same rate and density in both exclusions, but that the furthest invasion was limited by the unremoved grass.

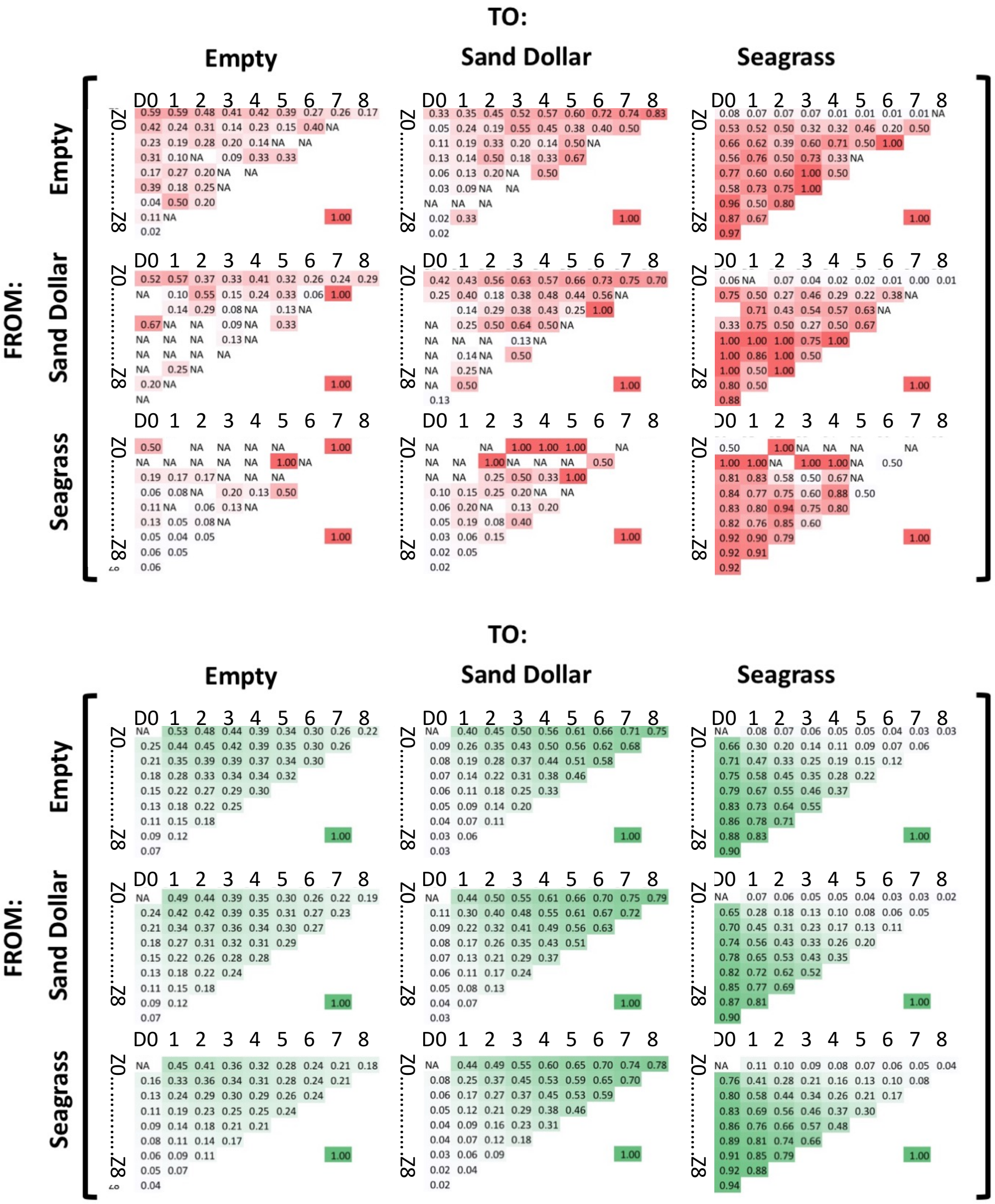


Figure 3.5: Transition probabilities by identity of neighbors, for empirical data observations (red, top) and predicted using multinomial regression (green, bottom, best-fit model from Table 3.1). Darker red color indicates higher rate of transition. Horizontal axis is increasing sand dollar neighbors from left to right, vertical axis is increasing seagrass neighbors. Not all transitions were observed and are left either blank or NA. The nine grids represent the nine possible Markovian

transitions between the three states, for example, describing all cells which started as seagrass (Z) and remaining seagrass in the following time step (Z), in the lower right corner. Within each of the 9 grids is represented a matrix of neighbor combinations, with number of sand dollar neighbors increasing along the x-axis and the number of seagrass neighbors increasing along the y-axis. Since the total number of neighbors can never be greater than eight, the probabilities along the diagonal for the lower half of the grid are undefined.

Spatially-Dependent Transitions

Several patterns can be interpreted from the spatially-explicit transition grid (Figure 3.5, red). First, transitions to seagrass (the right column of matrices) have higher probabilities than transitions to either empty or sand dollar. Within that, the lower left corner of the grid is darker, indicating that higher numbers of seagrass neighbors increase the probability of transitioning to seagrass in the next time step. This is true in sand dollars as well; the high values in the upper right of the grids in the middle column show that with higher numbers of sand dollar neighbors, transitions to sand dollars are more likely. Transitions to empty are also more likely with fewer neighbors, indicated by the fact that the highest transitions to empty occur in the upper left corner. However, transitions to empty occur with relatively high frequency with all numbers of sand dollar neighbors.

The best fit multinomial model (Table 3.4) included all the parameters and predicted 57% of the empirical transitions correctly, a marked improvement over random (33%). Year (2014-2015 vs. 2015-2016) exhibited statistically significant explanatory ability, but we did not explore time-varying models in this study, and therefore used the model which included all the parameters except for year for simulations and figures. This model captured the basic transition behavior of the empirical data (Figure 3.6, green).

Table 3.4: Model formulations and AIC scores for multinomial logistic regression models fitted to the data. % Data correct reflects the proportion of the empirical data successfully predicted by the model.

Formula	AIC	ΔAIC	% Data correct
~ S_{t-1} + E + D + Z + (D / (D+Z))	**9215.516**	**0**	**0.575**
~ S_{t-1} + (D / (D+Z))	9387.654	172.138	0.569
~ S_{t-1} + E + D + Z	10486.65	1271.134	0.533
~ E + D + Z	10502.72	1287.204	0.532
~ Z	11027.13	1811.614	0.516
~ S_{t-1}	12127.24	2911.72	0.483
~ D	12974.37	3758.854	0.459
~ 1 (Null)	17733.89	8518.374	0.344

Key to parameters:

D : number of sand dollar neighbors, out of 8

Z : number of seagrass neighbors, out of 8

E : number of empty neighbors, out of 8

S_{t-1} : State at previous time point

Y : Year

D / (D+Z) : proportion of neighbors that are sand dollars out of non-empty neighbors

The models including the parameter D/(D+Z), or the ratio of sand dollar to seagrass neighbors, produced the greatest ΔAIC, improving the predictive power of the model by almost 4%. In examining the behavior of this parameter further (Figure 3.6), we found that a greater proportion of seagrass neighbors predicts switches to seagrass, and respectively for sand dollars, similar to prior results (Chapter II). Transitions to empty occur most frequently when there are starting states of sand dollar or with many empty neighbors. The starting state, delineated by point color, doesn't change the trend, only the magnitude of transitions. Therefore, neighbor identity ratio has a much stronger effect on the end state than the identity of the state at the prior time point, as shown by the similar trends of all three starting states.

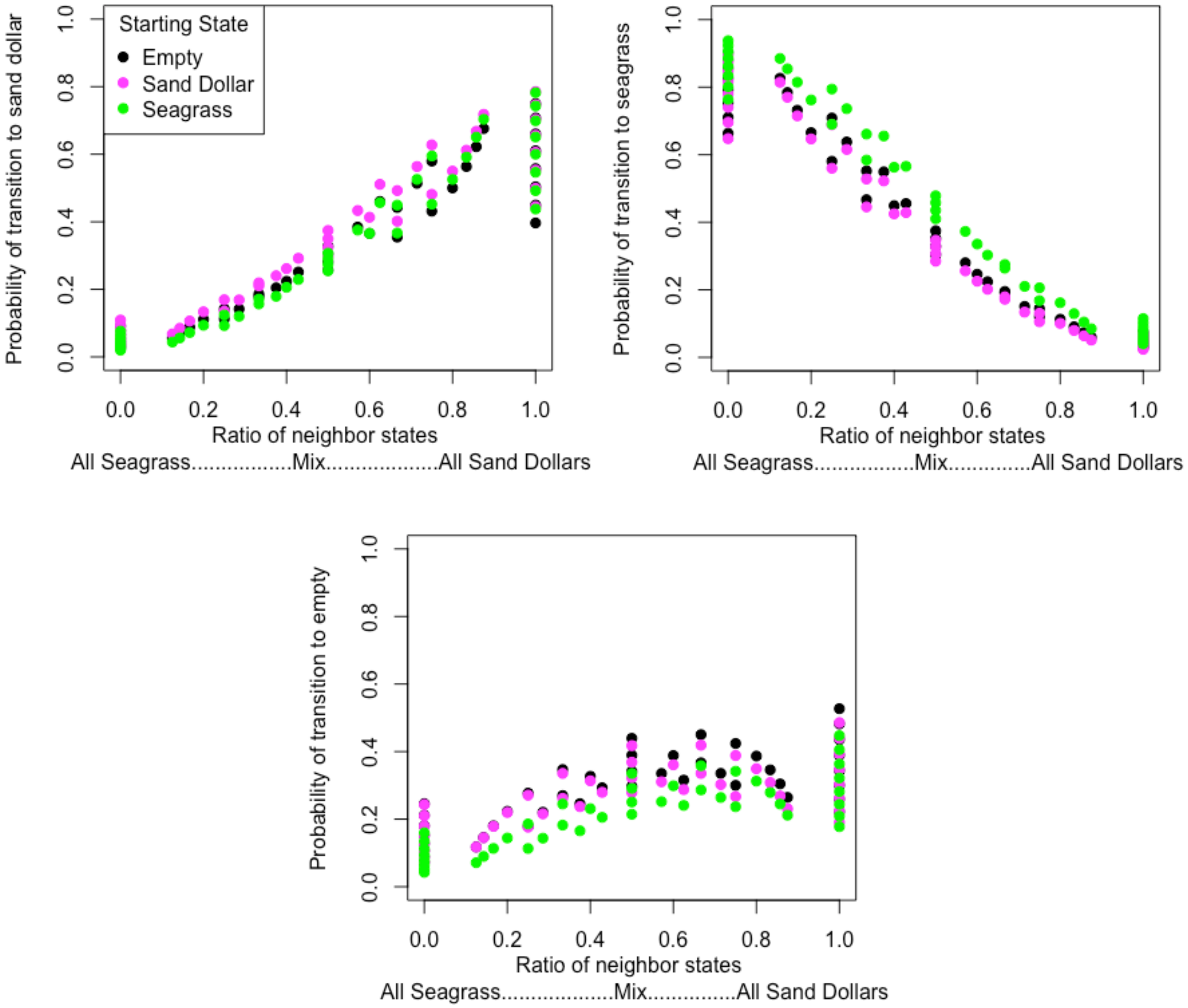


Figure 3.6: Multinomial logistic regression model best fit probabilities of transition to dollar (D, left), to seagrass (Z, right), and empty (0, bottom) against the ratio of D:Z. Left side of x-axis indicates greater proportion of seagrass neighbors, while the right is a greater proportion of dollar neighbors. Color indicates starting state – black for empty, green for seagrass, and purple for sand dollar.

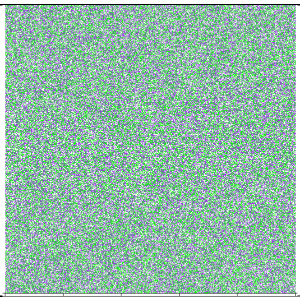
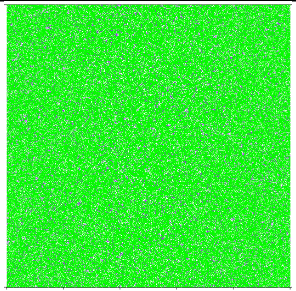
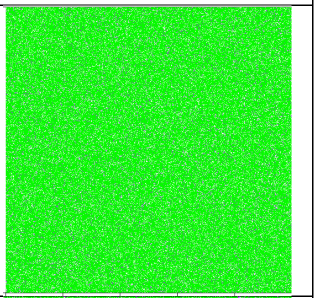
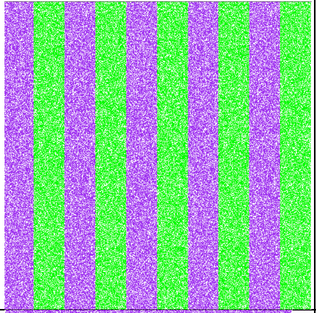
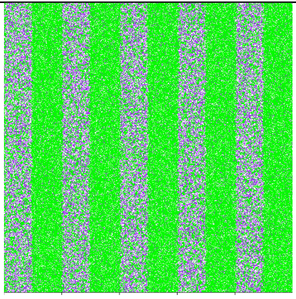
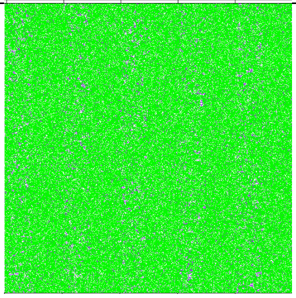
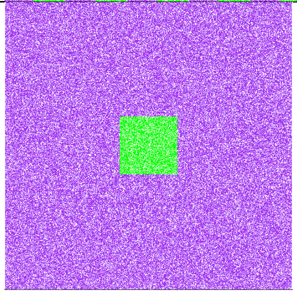
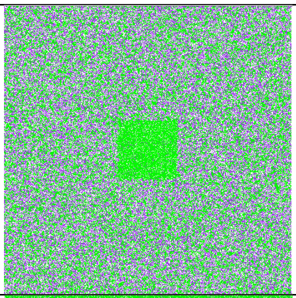
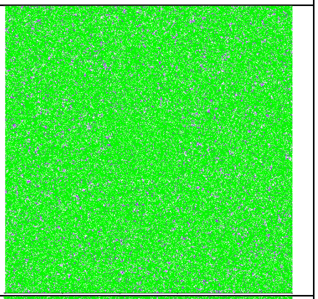
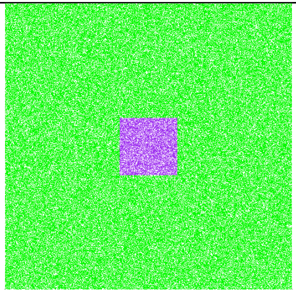
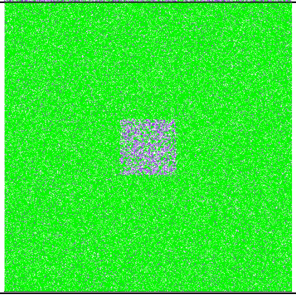
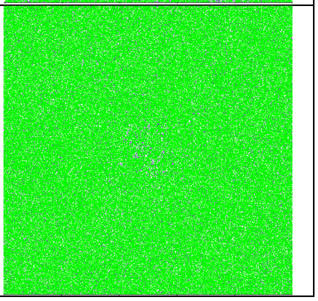
Treatment Description	Starting Conditions T = 0	T = 15	T = 30
Random 0.34 empty 0.33 sand dollar seagrass			
Banded rows 0.67 seagrass, 0.33 empty and 0.67 sand dollar, 0.33 empty			
Seagrass Patch 0.67 seagrass, 0.33 empty, in the middle of 0.67 sand dollar, 0.33 empty			
Sand Dollar Patch 0.67 sand dollar, 0.33 empty in the middle of 0.67 seagrass 0.33 empty			

Figure 3.7: Spatially-explicit modeling results, using transition rates based on neighbor identities fitted with multinomial logistic regression (Model 9). All plots represent 111m x 111 m.

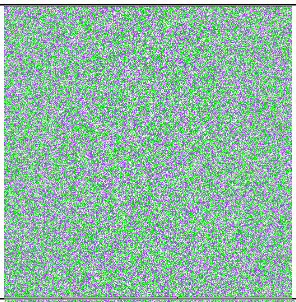
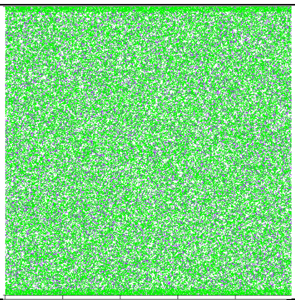
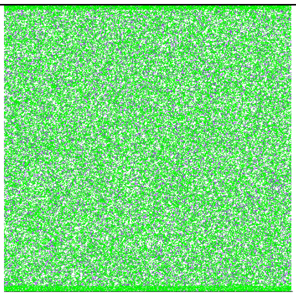
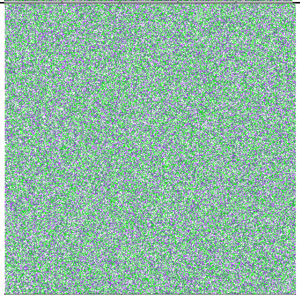
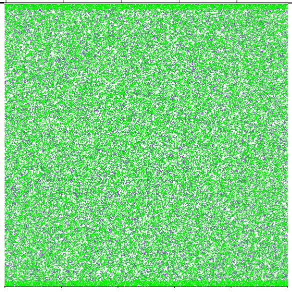
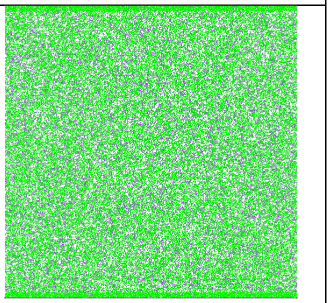
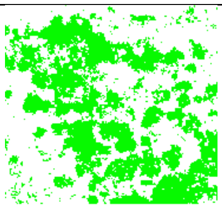
Treatment Description	Starting Conditions T = 0	T = 15	T = 30
Random, with goose disturbances to grass (0.44 x 0.44 m removals)			
Grass Block, with goose disturbance			
Scaled comparison with field seagrass patchiness	 80 m x 80 m		

Figure 3.8: Spatially explicit modeling results with modified probabilities and experimental conditions. All plots are 111m x 111 m unless otherwise specified.

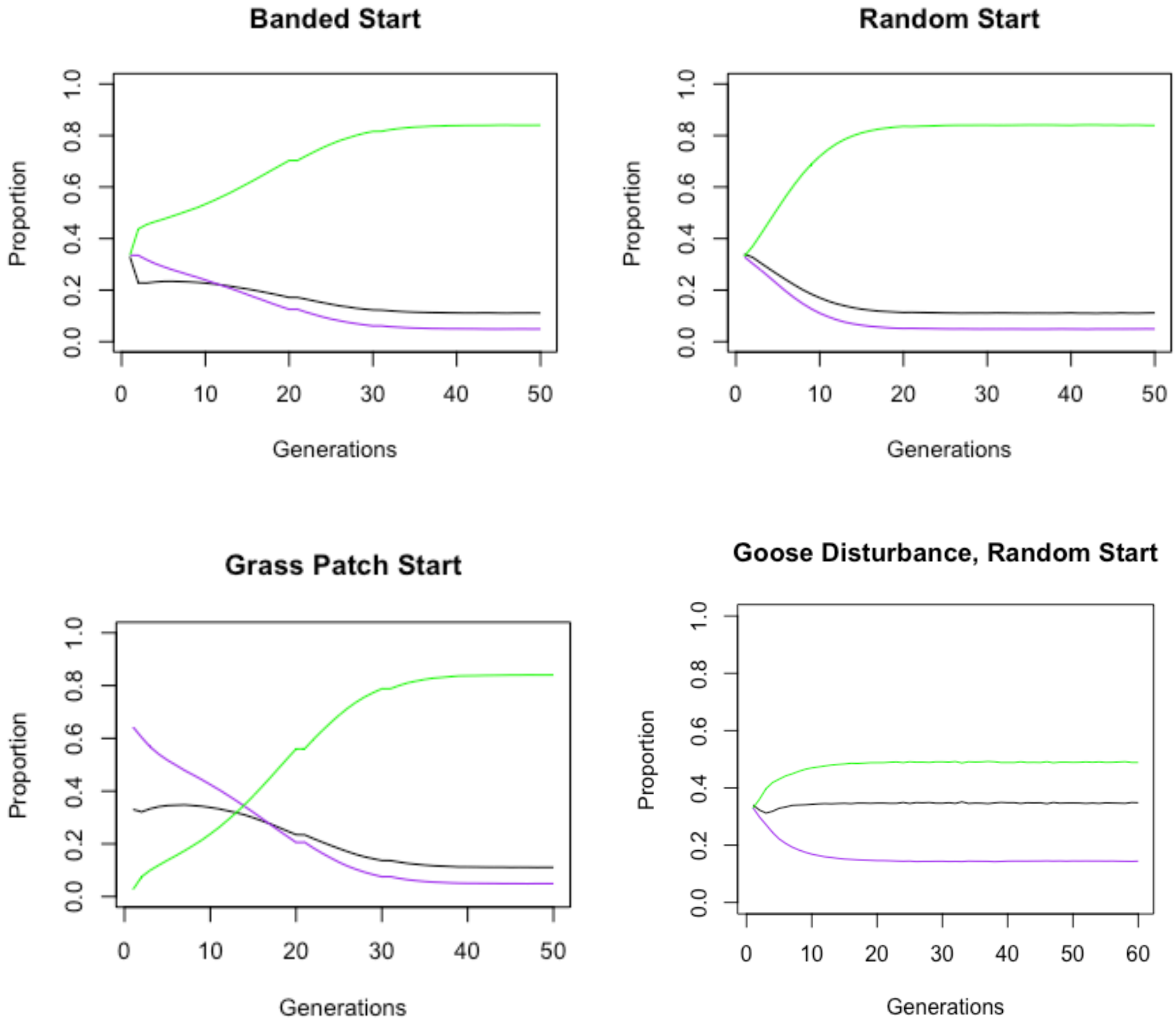


Figure 3.9: Trajectories of proportions of sand dollars, seagrass, and empty states at each time point in simulations.

Table 3.5: Stable proportions of states for spatial vs. non-spatial model

	Empty	**Sand Dollar**	**Seagrass**
Non-spatial, using Chapter 2 data	17.9%	24.5 %	57.5%
Spatial, Model 9, (all starting conditions)	11.1 %	5.0%	83.9 %
Simulated goose disturbance	34.8 %	14.4 %	48.9 %

Spatially-Explicit Modeling

The spatially-explicit cellular automaton models generally converged on nearly-homogeneous seagrass dominance, regardless of starting condition, in 15-30 time steps (Table 3.5, Figure 3.7, Figure 3.9). My previous empirical work suggests that seagrass outcompetes sand dollars under low disturbance conditions, but sand dollars are quicker to colonize following disturbance (Chapter II). Initial spatial pattern for simulations did not alter the final stable proportions of states, but the spatial model resulted in stronger seagrass dominance than the non-spatial model, which was determined from the dominant eigenvector (Table 3.5).

Analysis of aerial images from 2012, 2013, and 2014 using particle analysis (FIJI, Fiji Is Just ImageJ) provided an expected distribution of patch sizes (Figure 3.10). The analysis software picked up on patches as small as 1 pixel, corresponding to about 20 cm. Although patch sizes were skewed by the high sensitivity, the mean patch area was 1.2-1.9 m^2, with standard deviation of 16-19 m^2. The largest patch was 678.9 m^2, but patches of 1-10 m^2 are common. Applying this same particle analysis to the results of cellular automata models from the time steps exhibiting the most clustering found a very different pattern, of many more small patches (Figure 3.10).

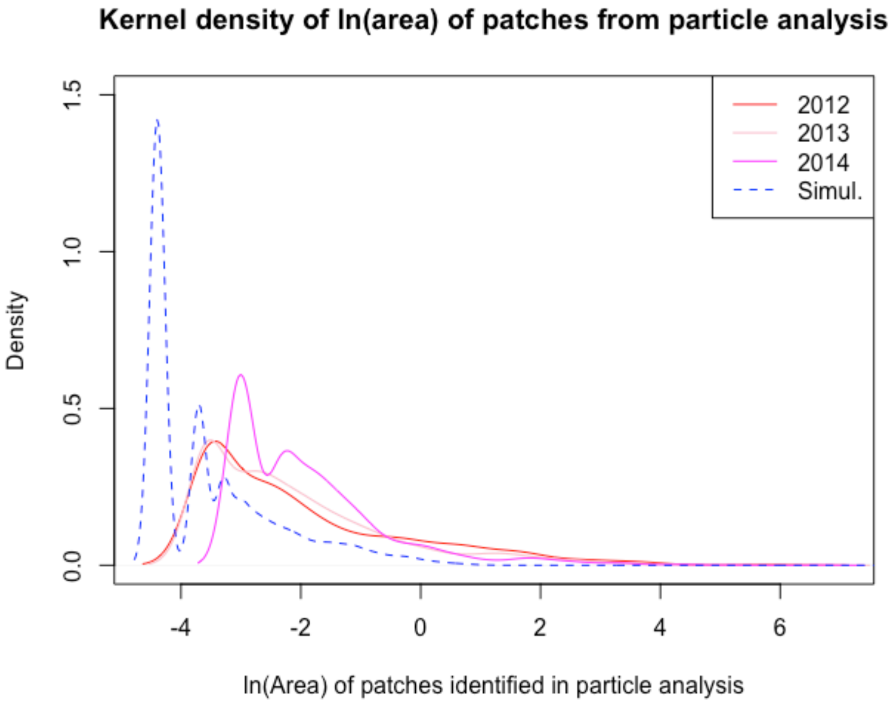


Figure 3.10: Distribution of seagrass patch area (log scale) from aerial imagery compared to particle analysis on stable stage distribution of random simulation.

DISCUSSION

Previously, we established that spatial context mattered to the fate of patches, in which a neighborhood of one state influenced the likelihood of converging on that state. In this chapter, I found that that the interaction at edges and the small-scale interactions could reveal insights into the spatial patterns of seagrass and sand dollar beds.

In the field manipulations, I found that, as in Chapter 2, the removal of seagrass did allow some invasion by sand dollars, and that sand dollars recolonize rapidly following removal. The underwhelming effects of the mow treatment indicate that rhizomes or their effects play a large role in inhibiting sand dollar invasion, more so than blades. Seagrass made small gains in the control, indicating some competitive dominance, however sand dollars successfully colonized areas of removal. This was possible in this experiment and not in Chapter 2 because of the allowance of migration from spatially contiguous areas.

Transition rates differed between the two years of observations, as indicated by regression models which included year as a parameter. There are two hypotheses to explain this effect: environmental conditions differed between the two years, or there were slightly different dynamics in the successional stage of recolonizing the seagrass removal areas. In the first year, seagrass seemed to grow sparsely but covered a wide area, whereas in the second year it filled in those areas with higher densities of shoots. Seagrass therefore may be most susceptible to damage when it is expanding its range, but also take over areas more quickly than expected.

The patterns of the transition probabilities (Figure 3.6) and the comparisons between models (Table 3.4) reveal that nearest neighbor identity ratios are a strong predictor of state in the following time step, more than the counts of individual neighbor states. In addition, the state at the previous time step is surprisingly unimportant – the model based solely on previous time step performs more poorly than one based solely on the number of seagrass neighbors. This may reflect the fact that sand dollars and empty are much more likely to switch back and forth, a function of the motility of sand dollars. These results indicate that spatial pattern, or at least, identity of neighboring patches, has a strong influence on the future identity of cells in a spatial matrix.

Though sand dollars do not go completely extinct in the spatially-explicit models, what we know about the life history and population biology of sand dollars is such that we do not expect that sand dollars would survive in the low densities predicted. In this case, the spatial model did not seem to support a future coexistence of the two species. The over-representation of empty space within sand dollar beds within our models, or at least the treatment of empty space within a seagrass bed and empty space within a sand dollar bed as equivalent, may have disadvantaged sand dollars.

Although the final states did not look very spatially structured, there were stages in between states, particularly in the Seagrass-Patch-in-Sand-Dollar-Bed treatment, which did appear to be spatially structured, with large blobs of resilient sand dollars eventually surrounded by grass. It is possible that the spatial pattern at Crescent Beach is an unstable one – as the interaction between the introduced *Zostera japonica* and the native sand dollar has only been occurring for ~40 years, it's possible, and suggested by the model, that we are not observing a stable state. Rather, the patchy pattern may be indicative of an ecosystem in criticality, switching between alternative states, as described by Pascual and Guichard (2005).

The mismatch in scale of spatial patterning and patch sizes (Figure 3.10) between simulation and observation suggests that additional mechanisms may play important roles in the system. Several mechanisms may not be captured within our experiments and models. There may be longer-range influences of neighbors, affecting transition probabilities from two or three patches away, especially if those patches create a larger buffer. Aggregating behavior of sand dollars to high density may result in more dense clumping of sand dollars, both leaving some space open for seagrass to invade, while also mustering a greater defense against lateral shoot growth in some locations. This aggregative behavior occurs on the individual scale (5-7 cm), and may not be picked up with the survey method used to gather our data (11.1 cm). *Z. japonica* grows in a seasonal cycle, with high growth in the summer and dormancy over winters, and at Crescent Beach, has been heavily grazed by geese in recent years in late July and August. These disturbances may contribute to the persistence of sand dollars and the sub-dominance of the seagrass.

While the simulations created some spatial pattern, it did not replicate the meters-scale patchiness observed in higher views. There are several hypotheses for what may drive this pattern mismatch. First, historical contingency in the face of an invading species. The sand dollar bed predates the seagrass, and the two have interacted for only about 40 years. The spatial pattern could be a relict from the shape of sand dollar beds prior to seagrass invasion, or a pattern of invasion by "leap-frogging" seagrass recruits, either from seeds or settled and rooted wrack. There are potential hydrogeographic explanations, drawing from the observation that seagrass tends to be on hummocks and sand dollars in hollows, a microtopographic difference of less than a centimeter, but potentially a driver of segregation between the two species or a function of ecosystem engineering. It is possible we are not capturing the spatial pattern in appropriate detail in sand dollar beds, which may be indicated by the frequent switches between empty and dollar states. An examination of second order effects, or examining the transition probability effects of looking back two time steps, could ameliorate this effect.

One factor in which this study differed from previous studies was the absence of locally positive effects over short timescales. Our study used annual transitions, however, this elides many dynamics, including those captured in Chapter II, like seasonal oscillations. In Wootton's previous study of mussel bed patch dynamics, a key result was that realistic spatial pattern only arose when disturbance was transmitted locally on rapid time scales. We did not identify or track any mechanisms that led to this kind of fast-scale positive local effect that seem to be critical to generating spatial pattern. Other examples of this effect include forest fire models, in which spatially patchy areas are cleared and there is a lag between disturbances (Pascual and Guichard, 2005). We incorporated regular disturbance into one model (Figure 3.7), mimicking disturbances due to goose grubbing or clamming. This model made the seagrass bed sparser and also facilitated a slightly higher proportion of sand dollar states, which indicates that this type of disturbance is worthy of further study (Table 3.5).

CONCLUSIONS

Based on theory and the results from Chapter II, I predicted that incorporating information about the state of near neighbors would generate a more accurate model, would replicate spatial patterns seen in the field, and would promote coexistence between two competitors. If we take the results at face value, the experiments and simulations suggest that Crescent Beach is in a transitional ecological configuration and unlikely to retain its patchy patterning as the non-native seagrass *Zostera japonica* appears poised to slowly but surely achieve dominance. There remains the possibility of a number of dynamics not captured, however. There may be larger scale drivers of spatial pattern, such as hydrodynamic wave influences, seasonal oscillations as identified in Chapter II, or similar to the mussel bed model, small-scale rapid response dynamics with positive feedbacks, such as the population dynamics and aggregating behavior within sand dollar beds.

CHAPTER IV

MOVEMENT PATTERNS AND DYNAMICS OF AGGREGATION IN THE PACIFIC SAND DOLLAR, *DENDRASTER EXCENTRICUS*

ABSTRACT

The sand dollar *Dendraster excentricus* forms large, spatially patchy but dense beds in multiple habitats in coastal waters along the Pacific coast. Several mechanisms create advantages to aggregation, including feeding, protection, and reproductive success, but the behavior and movement patterns that lead to the creation of these dense aggregations are less well studied, and the consequences for landscape level spatial pattern even less so. Using a combination of field surveys, behavioral observations, and individual-based models, we found that sand dollars modify their behavior when conspecifics are nearby, decreasing velocity and adjusting turning angles. We tested whether these behavioral mechanisms, iterated over many sand dollars in space and time, could generate the type of spatial patterns and clumping observed in the field. We found that our models did generate aggregation, but that mechanisms for the maintenance of large patches are still unknown.

INTRODUCTION

Organisms can occur patchily and in clusters for many reasons. They can be clustered around resources or favorable environmental conditions. Aggregation can provide protection from predators by diluting predation rates, for example in aphids fed on by ladybugs (Turchin and Kareiva 1989). In many species, especially in marine systems with broadcast spawning, aggregating increases reproductive success, as sexual reproducers may be more likely to find a mate, gametes may have higher fertilization success (Levitan, Sewell, and Chia 1992; Denny, Dairiki, and Distefano 1992, Keasar et al. 2010), and juveniles developing near adults may have greater survival (Highsmith 1982, Slattery 1992, Cameron and Schroeter, 1980). While these are all likely reasons *why* an organism would find aggregating beneficial, a more open-ended and broad question is *how.* What are the behavioral choices that organisms make that lead to aggregations? What cues from their conspecifics or the environment do they respond to? And finally, how do choices by individuals result in the spatial pattern of organisms in clusters and over landscapes? Given the myriad environments, means of motility, and sensory capabilities of organisms, it would be impossible to characterize any universal rules. Matching scale of process to scale of pattern is a challenge. However, with some basic information, it is possible using a range of models to describe and predict the dynamics of aggregations for a range of case studies.

For this study, we define aggregation as the clustering of individuals found together more closely than expected at random. This differs from a grouping, in which a gathering of organisms may move together as a unit, such as a flock of swallows or a school of fish (Okubo 1986). Models of groupings and aggregation are concerned primarily with the behavior and dynamics within a group, rather than the movements of the group as a whole, such as migration. Most models of aggregation begin with a null model of a random walk (Skellam 1951; Okubo 1986). To induce aggregation, this random walk may incorporate attractive or repulsive effects relating to the conditions around or within the organism.

Turchin (1998) laid out a framework for building empirically-based quantitative models of movement involving estimating a relatively simple suite of model parameters, and iteratively testing the model with field data, revising parameter estimates, and re-measuring fit until model predictions fail to improve, and then inspecting the results for any persistent deviations, which may aid in the identifying additional important variables. One example of an individual-based movement model applied to an empirical system and question used a simulated flight path of butterflies on a grid to predict the spatial distribution of eggs (R. E. Jones 1977; R. E. Jones et al. 1980). The model incorporated a decision tree-like flow chart in which the simulated butterfly would make choices about movement and egg-laying stochastically, but with rates based on factors such as whether a plant was encountered or nearby. This model was pioneering in that it observed movements at a small experimental scale (20-90 m) which then were able to replicate egg distributions at a larger scale (1 km).

On intertidal beaches and bays in the Pacific Northwest, seagrass and sand dollars act as ecosystem engineers that counteract each other via sediment stabilization through rhizome anchoring and destabilization through bioturbation. The sand dollar *Dendraster excentricus* lives on soft sediment coasts in dense beds in the hundreds of sand dollars per square meter (Merrill and Hobson 1970). These aggregations are thought to facilitate more efficient feeding and gamete fertilization (O'Neill 1978; Timko 1976; Highsmith 1982). Seagrasses such as those in the genus *Zostera* are marine angiosperms that anchor soft sediment into place with ropy rhizomes, creating complex habitat for many species. These two species occur in a patchy mosaic that may facilitate coexistence; however, the mechanisms generating spatial pattern is unknown.

I hypothesize that the aggregating behavior of sand dollars and tendency of the population to cluster, rather than to diffuse to fill the entire range of possible habitat, creates openings in space for seagrass to invade. The dynamic between seagrass invasion and sand dollar motility following disturbance may facilitate coexistence between these two species. Studying aggregation may bring a more mechanistic understanding of the dynamics between these two species than our phenomenological cellular automaton models.

I also sought to test whether small-scale aggregation interactions over large population and spatial scales develops emergent spatial patterns similar to those observed on full-beach scales. If this model fails to predict the scale of spatial patterning in sand dollars, results would point to alternative mechanisms involving interspecific interactions and disturbance-colonization mechanisms to explain spatial distributions and coexistence.

Background

The Pacific sand dollar, *Dendraster excentricus* occurs in dense aggregated beds along the Eastern Pacific from southern Alaska to Baja California, Mexico (O'Neill 1978; Emlet 1986; Francisco and Herzka 2010; Voss 2002; Merrill and Hobson 1970) both subtidally and intertidally. They inhabit four main types of locations along the coast; coastal inlets, which include bays and tidal channels and the outer coast, including protected and exposed outer coastal waters (Merrill and

Hobson 1970). *D. excentricus* are facultative suspension feeders that burrow the anterior portion of their body (test) into the sand, leaving the posterior region up in the flow to collect particulate organic material (POM) (Nakamura 1995; Smith 1981; Highsmith 1982; O'Neill 1978; Chia 1969; Merrill and Hobson 1970; Fodrie et al. 2007; Francisco and Herzka 2010; Timko 1976). Subtidal populations exposed to high current speeds are uniformly distributed, do not frequently move from the inclined position, and are therefore noted to stabilize the sediment (Merrill and Hobson 1970; Voss 2002). Intertidal populations frequently move between inclined and prone positions when submerged at high tide and exposed at low tide, respectively, and regularly change location. This consistent movement disturbs the sediment, maintaining an unstable top layer of sand (Voss 2002; Smith 1981). Additionally, intertidal populations of *D. excentricus* sometimes aggregate in smaller clumps of individuals throughout their range on a given beach.

Many of the potential adaptive benefits of aggregation discussed in the introduction have been studied in *D. excentricus*. A chemical cue associated with adult sand dollars induces larval metamorphosis and settlement (Emlet 1986; Highsmith 1982; Voss 2002), facilitating reproductive success and survival. Sediment engineering by adults can protect larvae by excluding predators of larvae such as tube-building organisms (Smith 1981) and tanaid shrimp (Highsmith 1982) from the sand dollar beds, increasing successful recruitment. Fertilization of gametes is higher in high-density aggregations (Merrill and Hobson 1970; Timko 1976). It has been speculated that this same chemical cue induces adults to migrate toward one another (Highsmith 1982). Although the chemical structure is not known, it remains in the sediment several days after sand dollars have not been present.

Aggregation may serve an anti-predatory function, particularly for juvenile sand dollars sheltered by adults; sand dollars are preyed on by crabs, fish, sea stars and gulls. For sand dollars in protected bays, there are fewer predators intertidally than subtidally (Birkeland and Chia 1971) and predation pressure by sea stars is sufficiently strong to limit sand dollar beds to intertidal zones in Puget Sound, WA.

Facilitation of efficient feeding also contributes sand dollars aggregation. Under laminar high flow conditions, such as those experienced by subtidal, open coast populations of *D. excentricus*, there is a hydrodynamically “optimal gap” (O'Neill 1978) between individuals that facilitates high feeding efficiency. At low densities, individual sand dollars act as solitary units and feed as if they were alone on the beach, whereas at high densities intraspecific competition plays a large role in reducing feeding rate. At the optimal gap, individuals engaged in suspension feeding create just the right amount of turbulence to facilitate the highest efficiency of catching food for all in the aggregation (O'Neill 1978). If density is low on the beach, individuals can create locally higher density areas by clumping with one another, thereby optimizing their spacing to maximize food capture.

This study focuses on the behavioral cues and patterns that result in sand dollars creating clumps of individuals within a larger intertidal population. This study tests the question of what combination of population density, behavior, and other factors influence the aggregating behavior of sand dollars, and then probes the large-scale consequences of these behaviors in creating patterns of spatial distribution

This study was conducted by observing *D. excentricus* from Crescent Beach, Eastsound, Orcas Island, WA in the field and in mesocosms at Friday Harbor Laboratories, Friday Harbor, WA. Considering Merrill and Hobson's beach classifications, Crescent Beach might be classified somewhere between a protected outer coast because of the nutrient levels in the waters, and a bay due to its geology. The sand dollar population on this beach is positioned between 0.0 m MLLW and -2.0m MLLW. The population stretches approximately 700 m along the beach, and densities occur between 200-400 adult individuals per square meter.

To our knowledge, this is the first examination of spatial pattern and aggregation in densely aggregated sand dollar beds. Theoretical examinations of aggregation using empirical systems and data have studied insects, particularly in ephemeral aggregations for breeding or reproduction (Turchin and Kareiva 1989; Turchin and Omland 1999), or on landscape ecology to study gap replacement of trees in forests (Botkin and Miller 1974). Field data to parameterize these observations can be laborious to collect, and few species exhibit congruent enough behavior in a lab setting to be applicable to models. Our sand dollar aggregation model tackles several of those challenges, combining field and mesocosm data to parameterize an individual based model of sand dollar aggregation to understand what drives sand dollar behavior, how field spatial patterns form, and how that may influence competition for space with other species, notably, seagrass.

MATERIALS AND METHODS

Methods I: Field Experiment

We tested the range and movement patterns of sand dollars within a dense bed over several weeks in the field using mark-recapture methods. Sand dollars were removed from a 1 m^2 circle (0.52 m radius) surrounding a rebar "start" stake. Sand dollars were tagged and replaced into the same area within 3 hours of removal. Sand dollars were tagged with two methods: 1) for 6-8 cm sand dollars, Internal passive integrated transponder (PIT) tags (N = 98), and 2) for 4-7 cm sand dollars, loop tags (N = 172).

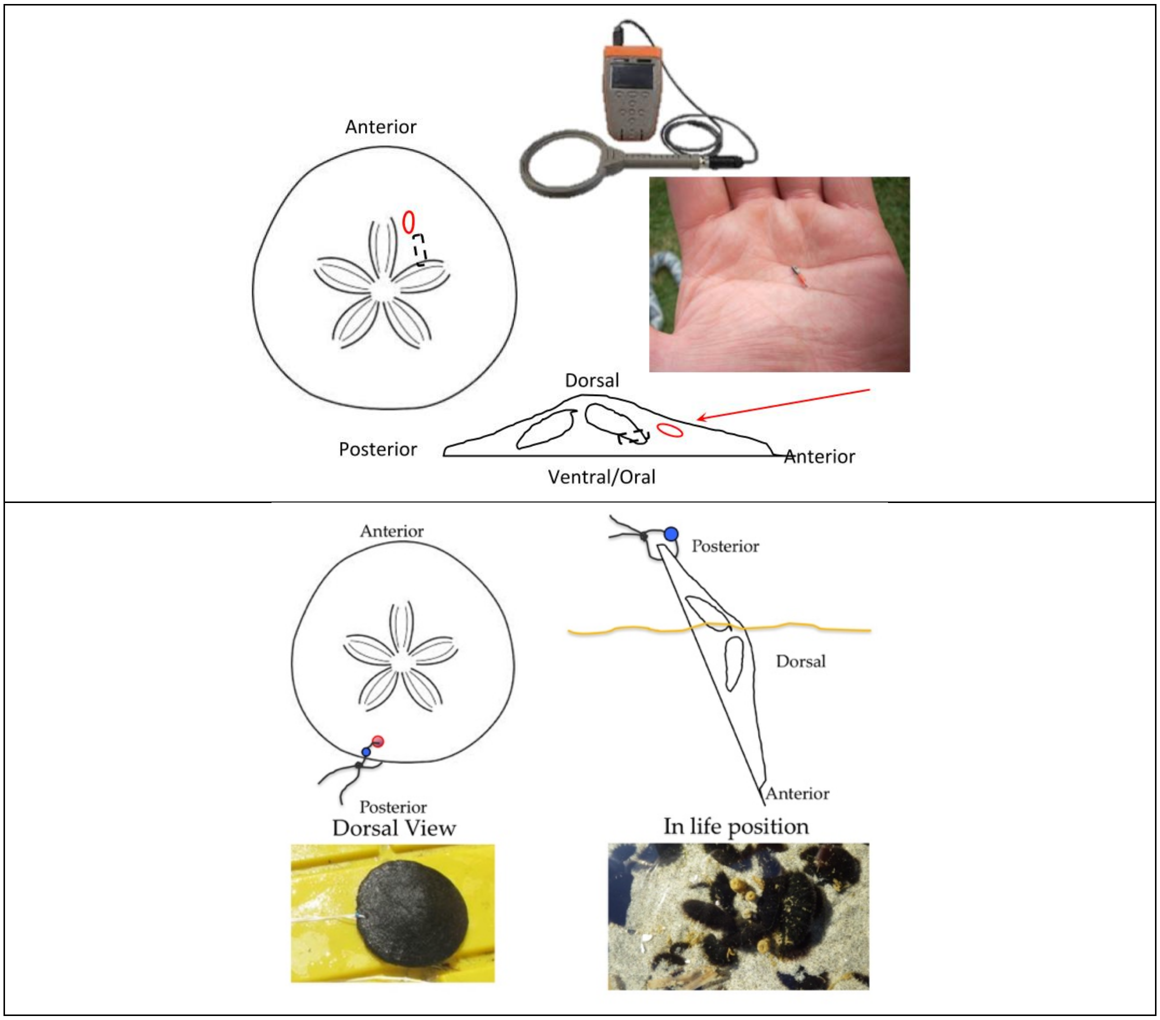


Figure 4.1: PIT tag insertion method (top) and floss tag method (bottom).

PIT tags (Figure 4.1, top) were read for codes prior to insertion, sterilized in 70% EtOH and placed in individual labeled vials. A Dremel tool was used to drill a small hole in the test. Drilling was done at an angle parallel to the major plane of the organism, just off center from the largest petalloid, inserting the drill in a posterior direction. This location was intended to avoid damaging organs. The tag was inserted into this hole using forceps, and the sand dollar was then placed in a bucket until return to the beach.

Loop tags (Figure 4.1, bottom) were made by gluing bee tags (tiny discs with unique color and number combinations) with cyanoacrylate or wood glue to short lengths of plain waxed dental floss. Using a Dremel, a hole was drilled through the organism like a piercing, just off-center at the posterior end, avoiding the anus and the body cavity and remaining in the reinforced calcium carbonate ring (Figure 4.1, bottom). The loop was tied through the hole with sufficient slack to allow spine movement, and floss was trimmed to 0.5 – 1 cm length past the knot. This location did not puncture the body cavity and did not appear to affect mortality or movement. Tags were removed from individuals at the end of the experiment.

Figure 4.2: Location of mark-recapture study, including census method with protractor and meter tape.

Distance from the “start” stake/zone and angle of movement were measured using a protractor indicating angles from a second reference stake from 0 to 360 degrees to the nearest 5 degrees and a transect tape placed at the central rebar stake (Figure 4.2) to the nearest cm to the center of the sand dollar test. PIT tagged individuals were detected as the tide left the beach using a BioMark reader, while floss-tagged individuals were found visually/manually. For the first census, two persons searched an area 3-m in diameter from the "start" stake in a one-hour timed search. For the second census, two persons searched an area 5 m in diameter for two hours.

Methods II: Mesocosm Time-Lapse Movements

We performed a series of time-lapse photography observations of movement patterns in sand dollars in flow-through seawater mesocosms to examine behavior under varying conditions of population density. A GoPro Hero 2 camera equipped with a time lapse intervalometer (CamDo Solutions, model T-004) was mounted above the water surface and set to record a photograph of the tank every 10 minutes. Photographs were then analyzed as an image sequence using the plug-in MTrackJ (Meijering, Dzyubachyk, and Smal 2012) within the software FIJI (Schindelin et al. 2012). Scale was calibrated for each trial using a 10-cm scale bar placed in the tank for the first image. The center of the petalloids of the aboral surface was used as the landmark for tracking individual sand dollars within the tank in MTrackJ. Output of computer track logs were comprised of data about position, travel distance, and angle of movement in pixels.

Data on sand dollar movement in mesocosms was taken in two separate experiments, both conducted at Friday Harbor Laboratories, Friday Harbor, WA. In the first experiment, conducted in August and September 2014, a 1-m diameter round clear plexiglass outdoor mesocosm was filled with ~15 cm of autoclaved Quikrete^{TM} play sand and water to ~35 cm depth. Seawater is pumped in from the entrance to Friday Harbor through the general lab seawater supply, while outflow drainage exited through a standpipe in the center of the tank. The inflow tubing was set ~15 cm above the sediment, directed away from the standpipe to facilitate flow in a circular fashion around the tank. There was a metal fence cover above the surface of the tank to exclude debris and disruptions. Sand dollars in this experiment experienced natural light conditions, but temperatures were consistently cold and flow rate was consistently high. Forty sand dollars 4-6 cm in length were placed in the tank in a uniform pattern, then photographed at 10 minute (or fewer) intervals for three days at a time. Two replicates of this three-day trial were taken.

In the second experiment, conducted in 2015 and 2016, three round 15-gallon plastic utility tubs (rope handles) were filled with Quikrete^{TM} play sand to a depth of 10 cm, with a tub diameter of 42 cm at the sediment-water interface. These tubs were set indoors in sea tables, which were connected to a flow-through seawater system. Inflow to tanks came through a "soaker hose", a loop of 5/8th inch PVC tubing perforated with 1/8th inch pores at close intervals along the length of the loop, to disperse flow evenly in the tank. Flow into all tanks was calibrated daily to a rate of ~58 mL/second throughout the course of the experiments by disconnecting flow and measuring the time to fill a standardized container. Soaker hoses were cleaned between each trial of the experiment, every three days, to ensure even flow. Flow out of tanks was through holes in the tubs above the inflow tube. To eliminate variability in lighting conditions, and thus diurnal cues, and to minimize algal growth, natural light was blocked from windows using black plastic sheeting, and fluorescent lights were left on for the duration of the experiment.

Before the first trial, sand was "conditioned" by placing a dozen or so sand dollars in the tank. Before each trial, sand was stirred and allowed to settle, then 1, 2, 3, 4, 5, 10, or 20 sand dollars (4-6 cm diameter) were placed in the tank. Sand dollars were acclimated for 24 hours, then the camera took pictures every 10 minutes for three days. No sand dollar was used for a trial twice, and sand dollars were returned to the collection site after experiments were completed.

Data was correlated with date, time, and tide height at the time of the photograph. By looking at how often and under what conditions greater or lesser movement occurred, we sought insights about what drove sand dollar movement.

Mean and standard deviation of distances to nearest neighbors for sand dollars under different moving conditions were computed by log-transforming the data. A two-step cluster analysis (SPSS) on the log-transformed velocity data was used to examine whether a natural threshold of speed between "movers" and "stayers" existed.

Methods III: Aggregation model

We constructed an individual-based movement model parameterized with data from the laboratory experiments to determine whether observed movement behaviors as functions of neighbors were sufficient to replicate large-scale spatial pattern in sand dollar beds, independent of external factors such as disturbance or local interactions with competitors.

Each simulated sand dollar (Q) had a location and facing angle (x_Q, y_Q, θ) for each time point (t), within a toroidal arena (dimensions 200 cm x 200 cm) with initial positions (x_{Q0}, $y_{Q0,}$ θ_{Q0}). These positions and initial facing directions (θ_{Q0}) were started in two ways: either randomly spread through the whole arena, or in a small cluster in the larger arena to test the persistence of clumps. Each sand dollar also assessed the distance to each of its neighbors and stored the values of distance to nearest neighbor (M) and number of neighbors within 7 cm (A) for each time point. For each time step, the simulated sand dollar made several choices. First, it decided whether to move or not move, based on a movement probability distribution. The probability of moving, P(move), reflects the likelihood that a sand dollar will move or not in a given time step as a function of distance to nearest neighbor, based on analysis of a bimodal pattern observed in the data (e.g., Figure 4.8). If $M < 7$ cm, the probability of moving was 0.05, while if $M > 7$ cm the probability of moving was determined by a fitted logistic function, modified to approach an asymptote of 0.568. Hence this function increases P(move) as M increases.

If the choice was to move, then a travel distance (V) and an angle of direction relative to an individual's prior orientation direction ($\Delta\theta$) were chosen. If the choice was not to move, then only an angle of direction was chosen. These values determined the position and facing direction at the next time point ($x_{Q1}, y_{Q1}, \theta_{Q1}$). The probabilities with which each of these choices were made depended on draws from the distributions of empirically measured parameters M and A. These choices were made for each sand dollar in the simulation ($N = 100$, 1000, or 4800) at each time point, and all individuals moved at once. The model was iterated over 1000 10-minute time steps, or 200 time steps for $N = 4800$ (t).

The distance traveled (V) for each moving individual was drawn randomly from neighbor-dependent probability density functions based on all the velocities observed for a given M. Angle change was determined using fitted values for a von Mises distribution (R, CircStats package), which approximates the normal distribution wrapped around a circle. To control for artifacts of tank size, distributions were fit only to observations which were > 6 cm from a tank wall. If moving, then $\Delta\theta \sim \text{vonMises}(\eta = 0, \kappa = 0.047)$; if not moving, $\Delta\theta \sim \text{vonMises}(\eta = 0, \kappa = 1.027)$.

Combinations of experimental conditions for sand dollar simulations are detailed in Table 4.1.

Table 4.1: Starting conditions for simulations of sand dollar movement and aggregation

N	**Arena size/ density per square meter**	**Starting configuration**
100	400 cm x 400 cm 6.25 sand dollars / m^2 (low density)	Random
1000	400 cm x 400 cm 62.5 sand dollars / m^2 (low density)	Random
10000	500 cm x 500 cm 400 sand dollars/ m^2 (high density)	Random

In addition, a field census of a 3 m x 3 m area of dense sand dollar bed was taken, assessing presence/absence of sand dollars within each 6.25 cm square within the grid to create a map that then was analyzed for common spatial pattern metrics. This fine-scale field spatial pattern of sand dollar beds was compared to the model spatial outputs, as well as compared to landscape level spatial patterns generated using aerial imagery using common spatial pattern analysis metrics that measure clustering and overdispersion. These included F(r), the empty space function, G(r), the nearest neighbor distance distribution, J(r), the function $\frac{1-G}{1-F}$, which relates F(r) and G(r) in a ratio with an expected value of 1 for random data. K(r), also known as Ripley's K or the reduced second moment function, and g(r), the pair correlation function, $g(r) = \left[\frac{d}{dx}K(r)\right]/(2\pi r)$. All functions were run in R using the package "spatstat", and are based on descriptions in Ripley (2005), and the J-function in Lieshout and Baddeley (1996).

The simulation spatial patterns were also compared to the field survey spatial patterns. The spatial data at the end of simulations were mapped to a 64 x 64 grid, with each square corresponding to 6.25 cm x 6.25 cm. Any quantity of points was noted as a binary presence or absence, essentially increasing the pixel grain size, mapping simulation occurrences into a presence-absence pixel map on a spatial scale similar to the field survey. This analysis was done for the N = 1000 (Figure 4.16, right) and the N = 4800 simulation treatments. The N= 1000 treatment equals about 62 individuals/m^2, while N = 4800 treatment corresponds roughly to 300 sand dollars per square meter, which is a realistic estimate for a dense bed of sand dollars.

RESULTS

Results I

The field tagging experiment demonstrated long-term movement distances of sand dollars diffusing through a population, and established that they did, in fact, move. PIT-tagged sand dollars suffered high mortality (69.4%), and surviving PIT-tagged sand dollars traveled significantly less distance than loop-tagged sand dollars, suggesting the tagging procedure compromised sand dollar health. In contrast, loop-tagged sand dollars however, were recaptured at high rates (42% at T1, 63% at T2) without observable impairment. Therefore, we focused on results from loop-tagged sand dollars only. Although many of the glued bee-tag IDs did not stay attached to the floss, the floss usually still remained and was visible. Therefore, sand dollars were individually identified when possible. Fourteen individually-identifiable tagged sand dollars were recaptured at both time points (Figure 4.3), while 72 total sand dollars were recaptured on day 17, and 109 total on day 44 (Figure 4.4).

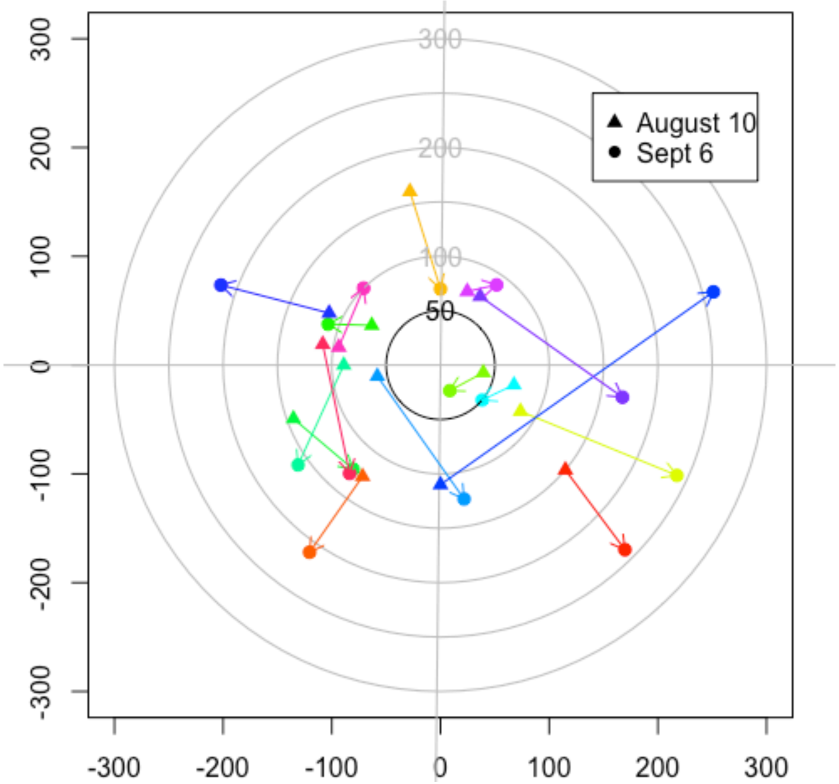


Figure 4.3: Circular plot showing the positions of individual sand dollars between August 10 (circles) and Sept 6 (triangles). Points of the same color denote the same individual. The black circle at 50 denotes the release zone of sand dollars on July 24, 2014.

Sand dollars traveled a net mean distance from the start area of the release circle of 2.46 cm/day in the first 17 days and 2.33 cm/day in the first 44 days. Sand dollars showed no indication of preferred movement in any direction relative to the beach boundaries, based on Watson's test for circular uniformity (test statistic = 0.0869, $p > 0.10$).

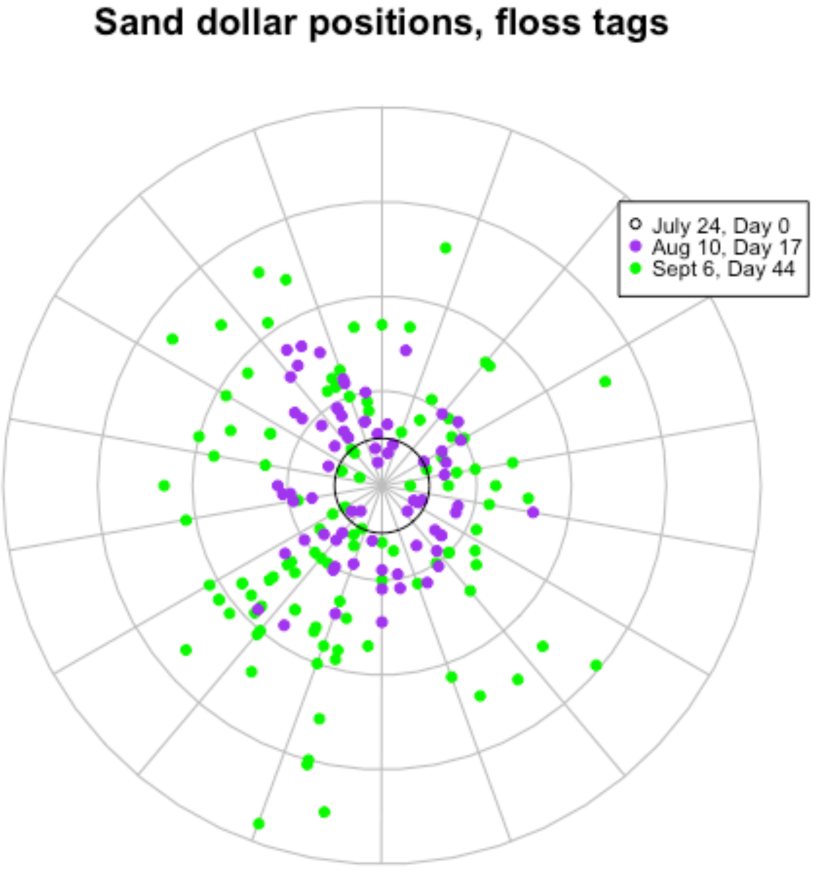


Figure 4.4: Positions of all sand dollars (not individually identified) recaptured on August 10 (Day 17, purple) and Sept. 6 (Day 44, green). Concentric circles indicate 100 cm intervals, while black circle indicates the release area on July 24 (Day 0)

Results II

We examined the results from the mesocosm studies for relationships among the following variables: velocity/distance traveled, distance to nearest neighbor, number of neighbors in the interaction zone, angle changes from prior movement direction, density in treatment (i.e., how many sand dollars in the mesocosm), and proportion of time steps spent moving vs. not moving. Due to greater replication, only results from the 42 cm mesocosms are reported below.

The majority of time steps in any given track have zero, or very close to zero velocity. A cluster analysis of the velocity data favored two clusters of velocity types over one cluster, with the log-transformed velocities ($log(Velocity + .001)$) with a better model fit (ΔAIC = 21663, and ΔAIC = 29314 for log-transformed). The threshold velocity between clusters was 0.0022 cm/sec, which we used for the remaining analyses as the cutoff between "movers" and "stayers". In addition, sand dollars with fewer numbers of neighbors (1 or 2) showed a greater proportion of time steps spent moving than any of the treatments with higher density. An inverse relationship (movepercent ~ 1/treatment density) was fitted and explained the data with an $R^2 = 0.81$.

While treatment density demonstrated strong trends, behavioral modeling and parameterization focus on the degree to which sand dollars alter their behavior relative to their neighbors. In time steps in which sand dollars moved (> 0.0022 cm/sec, "movers"), their nearest neighbor was more frequently further away (M > 10), compared to time steps in which sand dollars did not move (V < 0.0022 cm/sec, "stayers"). The mean distance to the nearest neighbor (M) for "movers" was 7.30 cm (sd = 2.43), while for movers it was significantly lower at 3.91 cm (sd = 2.30) (Figure 4.5, Student's T-test, log-transformed, p >> 0.001). As the body length of all sand dollars used in these trials was between 5-7 cm, a sand dollar whose center is 7 cm from another's central track point is likely to be in contact. Inverting this relationship and plotting the ratio of movers to stayers against distance to nearest neighbor (Figure 4.6) indicates a threshold behavior, in which sand dollars > 7 cm from each other are about 6 times more likely to move (Probability of moving if M < 7 is 0.06, if M > 7 = 0.31).

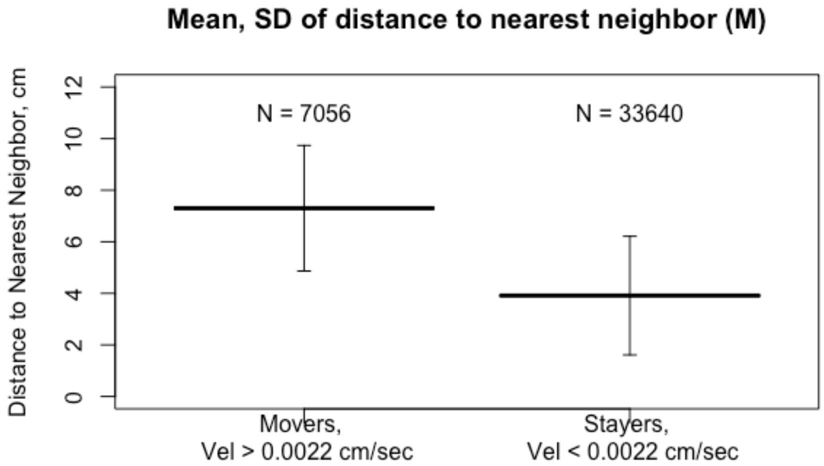


Figure 4.5: Mean nearest neighbor distance for "movers" and "stayers". When individual sand dollars are moving, neighbors tend to be further away than in timesteps when sand dollars are staying put. Error bars indicate standard error.

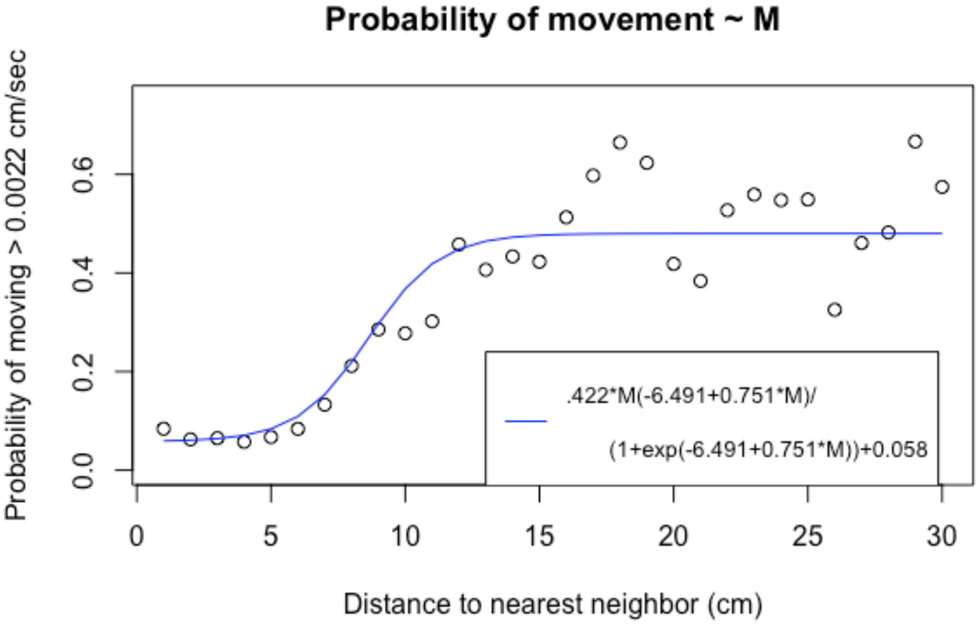
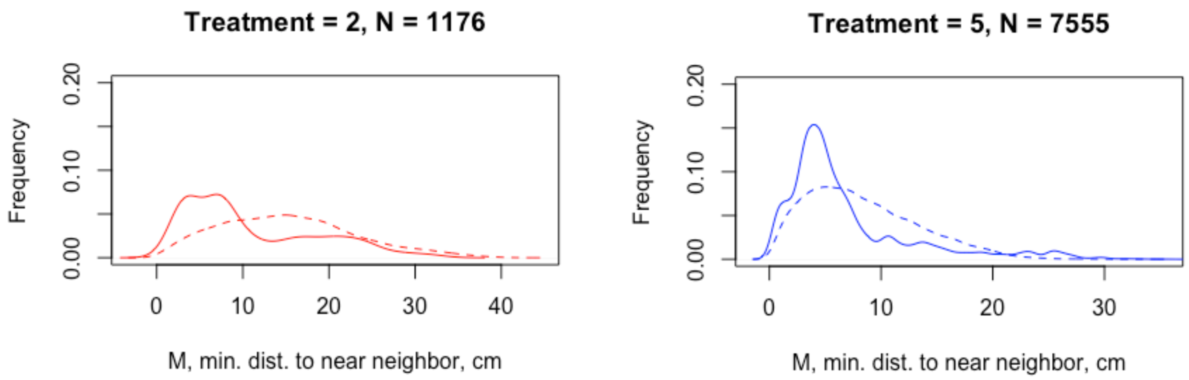
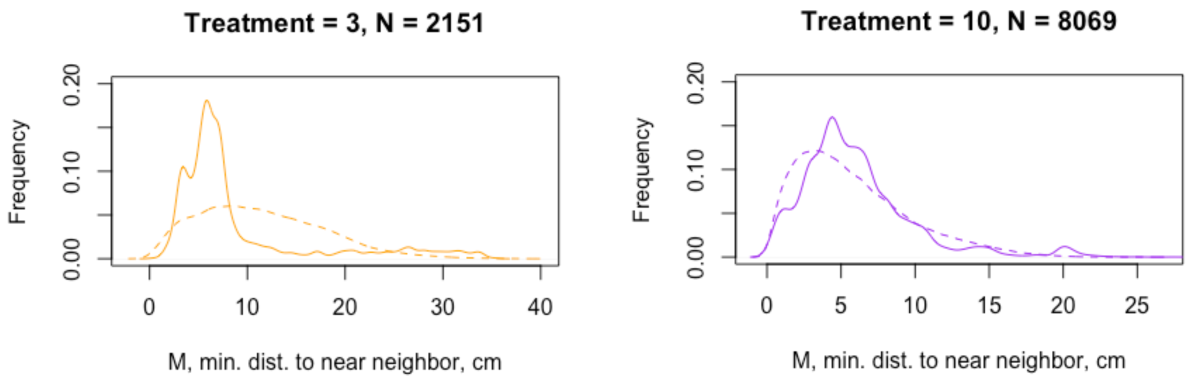


Figure 4.6: Probability of moving by distance to nearest neighbor. When sand dollars are further than 7 cm from their nearest neighbor, probability of movement increases.



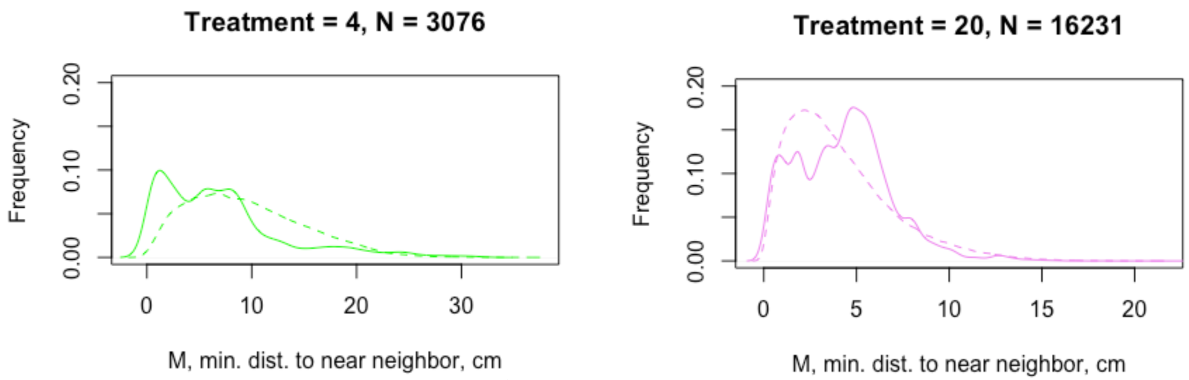
a. K-S test, D = 0.3383, p-value < $2.2e^{-16}$

b. K-S test, D = 0.2424, p-value < $2.2e^{-16}$



c. K-S test, D = 0.3848, p-value < $2.2e^{-16}$

d. K-S test, D = 0.0931, p-value < $2.2e^{-16}$



e. K-S test, D = 0.2353, p-value < $2.2e^{-16}$

f. K-S test, D = 0.1258, p-value < $2.2e^{-16}$

Figure 4.7: Expected distributions of minimum distances based on random placement of points in a 42 cm circle (dashed lines) compared to observed distributions (solid lines) with different numbers of sand dollars in the tanks. Significance values for Kolmogorov-Smirnov tests are reported under each plot.

To ensure that these minimum distances were not just a function of a small tank at varying densities of individuals, and just clustered by chance, we simulated a basic null model of minimum distances based on 1000 iterations of 1-20 points randomly generated within a 42-cm diameter circle and calculated the expected distribution of nearest neighbor distances for each treatment. A Kolmogorov-Smirnov test was used to test whether empirical neighbor distances fit the null distribution. The empirical distributions differed significantly from null distributions in all treatments (Figure 4.7, K-S test statistics reported in figure). For tanks with 1, 2, 3, 4, and 5 sand dollars, minimum distances in the empirical distributions were lower than expected by chance, suggesting strong clustering (Figure 4.7, a-d). For the 10 and 20 treatments (Figure 4.7, e-f), however, neighbor distances were higher than expected by chance, suggesting over-dispersion. Therefore, there is a relationship between nearest neighbor distance and probability of significant movement.

The velocity of moving sand dollars was affected by distance to neighbors (Figure 4.8). Once a sand dollar was moving, its speed was determined by the distance to its nearest neighbor. Sand dollars near other sand dollars moved less far (black peak), while those with greater distances tended to move further (blue, purple).

In addition to nearest neighbor distance, we tested whether the number of neighbors within the threshold range for predominantly "stayers" ($M < 7$ cm) affected the velocities and probabilities of sand dollar movement. As with distance from nearest neighbor, sand dollars move most often when they have fewer neighbors (Figure 4.9). This relationship increasing slightly with 6 neighbors, suggesting crowding.

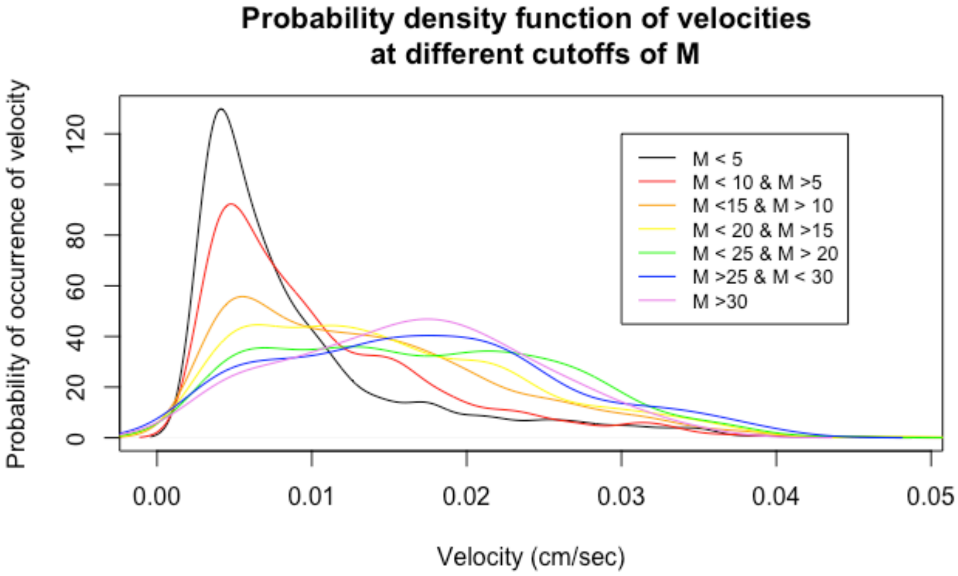


Figure 4.8: Probability density function of velocities under different minimum distances.

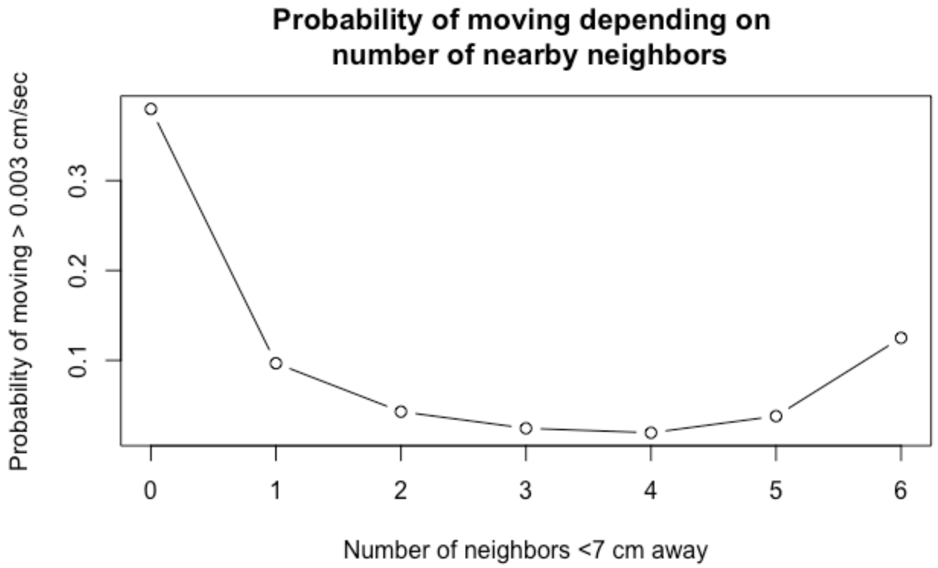


Figure 4.9: Probability of movement depending on number of neighbors within a radius of 7 cm.

The turning angle, or the angle change from one time step to the next, differed by velocity, minimum distance to nearest neighbor, and number of neighbors nearby. The distribution of turn angles was fitted to a von Mises distribution, an approximation of the normal distribution wrapped around a circle, which is described by the coefficients η (direction) and κ (concentration parameter). These coefficients were estimated separately for movers and stayers using maximum likelihood with the vm.ml function in the CircStats package in R (see Figure 4.11).

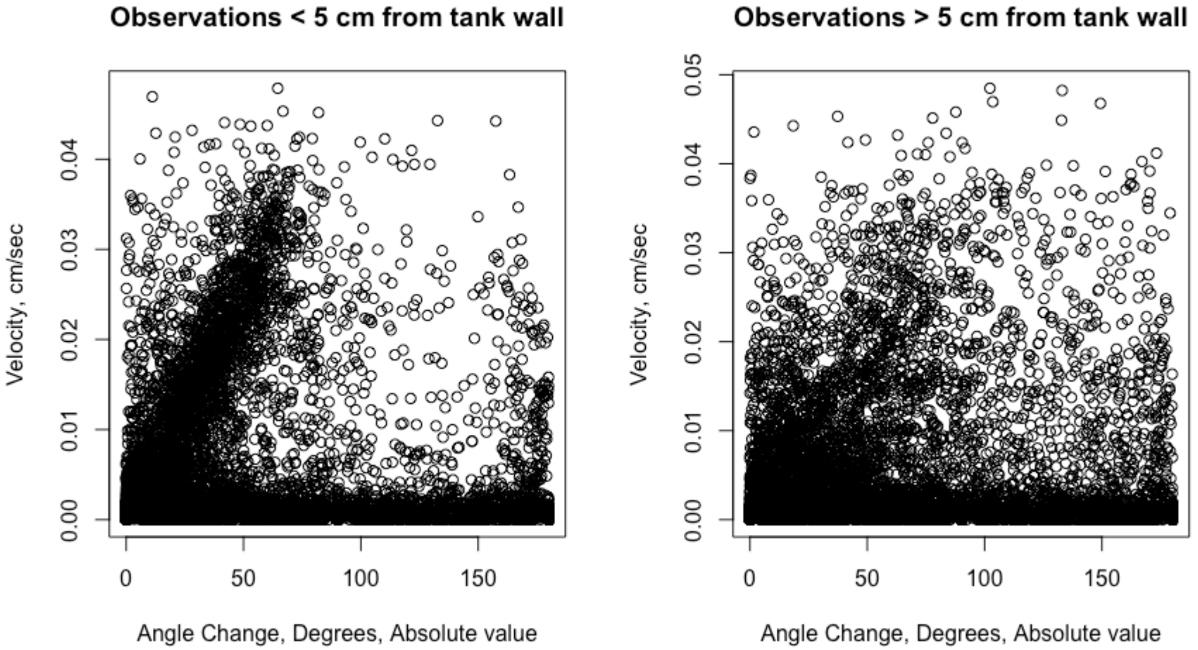


Figure 4.10: Velocity in cm/sec (y-axis), by angle change, separated by sand dollars within 6 cm of a tank wall and further than 6 cm from a tank wall. The strong trend in the near-wall observations (left) reflects the movements in which sand dollars changed angle more strongly than expected because they tracked the wall of the mesocosm. This trend is not invisible, but much weaker in observations away from walls (right).

There was a strong artifact of the experimental set-up evident in the relationship of velocity to turn angle (Figure 10, left). A commonly observed behavior of sand dollars in treatments with few individuals was that a sand dollar would follow the outside perimeter of the tank in circles. This resulted in a strong band of observations in which velocity strongly positively correlated with angle change, an intuitive result in which the further one travels along the perimeter of a circle, the greater the angle change will be, as chords of the circle. We controlled for this artifact by excluding sand dollars which were less than 6 cm from a wall, identified analytically (Figure 10, right), and used the subsetted data for remaining analyses and parameterizations relating to angle change.

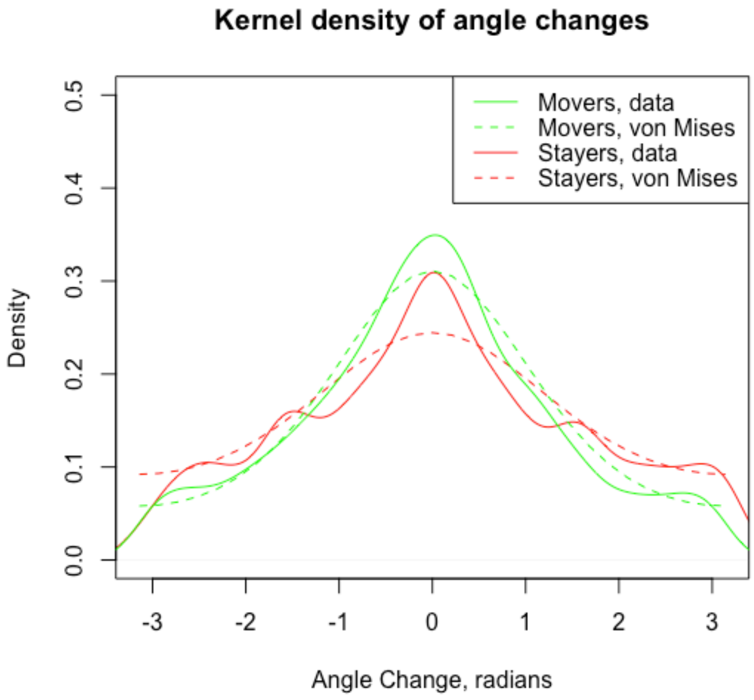


Figure 4.11: Kernel density of turning angles (solid lines), depending on velocity in the same timestep, and fitted von Mises distributions (dashed) for movers (green) and stayers (red).

Once the tank artifact was removed, on average the movers and stayers had very similar distributions of angle changes, shown in Figure 4.11. von Mises distributions fitted to movers (η = -0.06218 (0.03404), κ = 0.8386 (0.03102)) and stayers (η = -0.0285 (0.04109), κ = 0.4877 (0.02063) showed surprisingly few differences. The fitted von Mises values for movers and stayers were used in the simulations.

Results III

Using spatial metrics such as the F, G, J, and K functions to examine the simulation spatial patterns, the field survey patterns, and the aerial image landscape patterns gave different results when measuring clustering. Beginning simulations in a clustered arrangement did not produce different results than the random start, therefore, only random start will be reported here. In all simulations by the end of 1000 timesteps, there were more sand dollars within an aggregated distance ($M < 7$ cm) to another ($N = 100$, 8% at $t= 1$, 43% at $t = 1000$; $N = 1000$, 62.8% at $t = 1$, 80.4% at $t = 1000$; $N = 4800$, 99.9% at $t = 1$, 99.9% at $t = 100$), illustrated in Figure 4.12. The F(r) function, or empty space function, measure the distribution of all distances from an arbitrary point to other points, and is lower empirically than predicted by the poisson distribution (blue), supporting clustering. The G function for both $N = 100$ and $N = 1000$ is also elevated from the null, supporting clustering. IN figures I have depicted the J function, which combines the F and G functions and is compared against a constant of 1. Deviations in which the data falls below 1 indicate clustering, while deviations larger indicate dispersion. Finally, the K-function indicates the scale at which points are clustered, with r representing an increasing radius from the point, and the K-value is the number of neighboring points within that radius compared to an expected null of a random distribution. Where the empirical line is above the null line, at the spatial scale of r the data is clustered. The $N = 100$ treatment generated this clustering (Figure 4.13, top), while the simulation did not generate relatively as much clustering in the $N = 1000$ treatment (Figure 4.13, middle), showing modest deviations in J, and no deviation from the null in the Ripley's K. In the highest density treatment, $N= 4800$ (Figure 4.13, bottom), the K function did not signal clustering, while J indicated clustering at somewhat larger spatial scales.

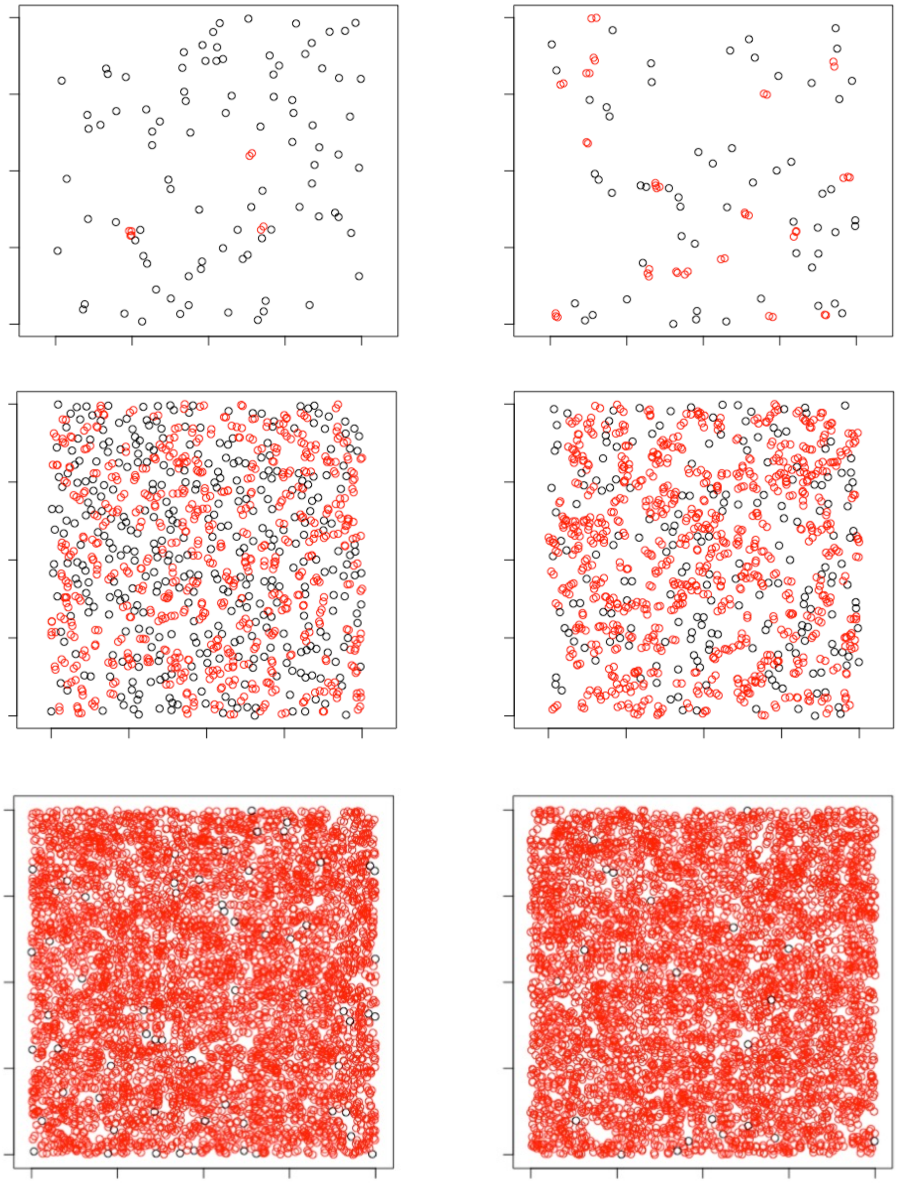


Figure 4.12: Spatial positions of simulated sand dollars, with minimum distances less than 7 cm (red) and greater than 7 cm (black). Starting random distribution (t = 1, top left) of 100 sand dollars (top left) and ending (t = 1000, top right), starting random distribution of 1000 sand dollars (t = 1, middle left) and ending (t = 1000, middle right, and starting random distribution of 4,800 sand dollars (bottom left) and ending (t = 100, bottom right), in a 400cm x 400 cm toroidal arena.

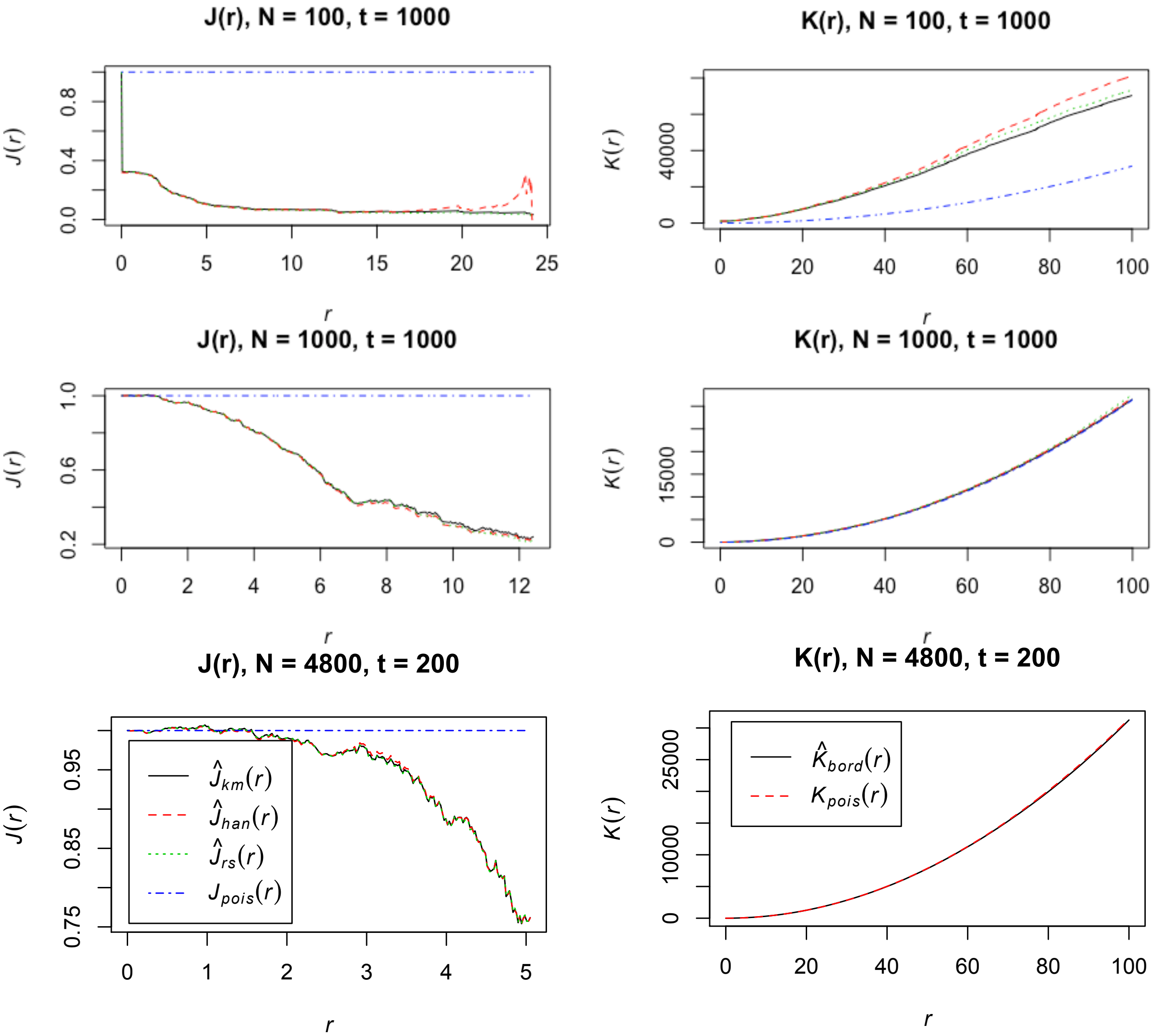


Figure 4.13: Spatial clustering statistics J and K for sand dollars in simulations, N = 100 (top left and top right) and N = 1000 (middle left and middle right) for 1000 timesteps. Analysis applied to points at t = 1000, and densest simulation treatment, N = 4800 for 200 timesteps (bottom left and bottom right). Analysis applied to points at t= 200. Blue line indicates the value expected for a random distribution, black is the raw data, and red and green indicate two additional fitting metrics that do not substantially differ from the raw data. For the J function, clustering is indicated where data falls below the poisson, indicated in all treatments, while for the K function clustering is indicated when the data is above, as in the N = 100 treatment.

When simulation final point distributions were binned into pixels to be comparable to field survey data, spatial clustering statistics showed different results. The field survey showed clear clustering trends in all four metrics (Figure 4.14, left), though the J function was unusual, indicating dispersion at small scales and clustering at larger scales. The pixelated simulation (Figure 4.14, right) lost all signal of spatial clustering, with the J and K functions (Figure 4.15) indicating no clustering at all, or dispersion at high scales from the J function.

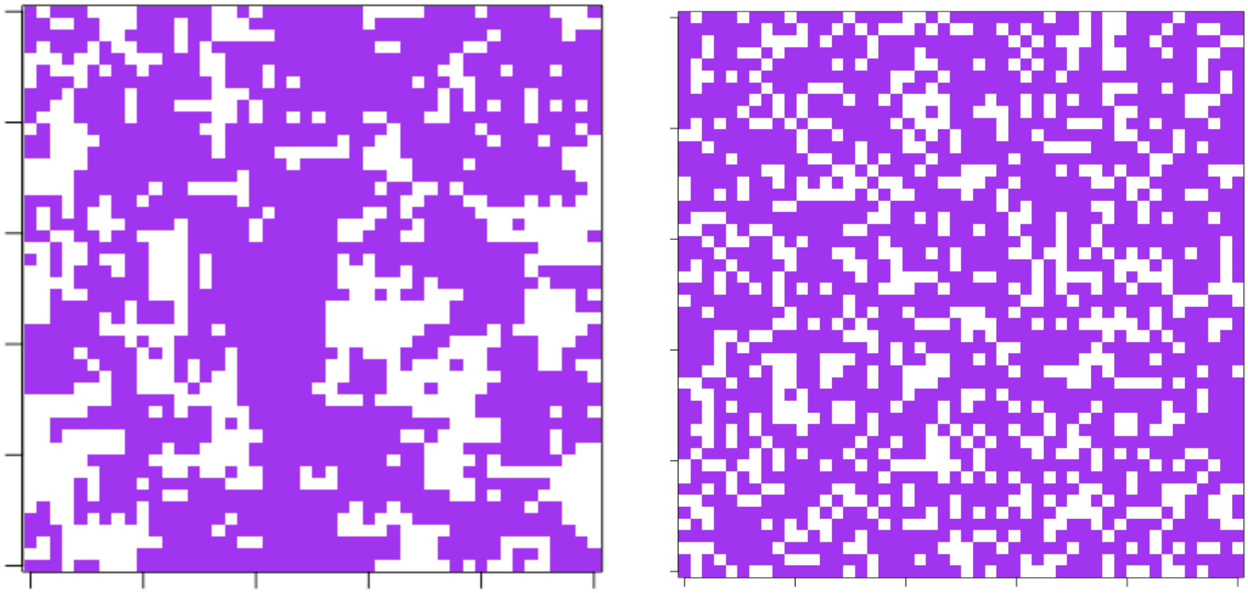


Figure 4.14: Pixel map of presence/absence of sand dollars in the field (left) in a 3 m x 3 m area, surveyed in 6.25 cm x 6.25 cm squares, each represented by one pixel, and a binned spatial pattern of simulated data, cropped to represent 3 m x 3 m (right, same data as Figure 4.14), and scaled to pixel size to match the field survey.

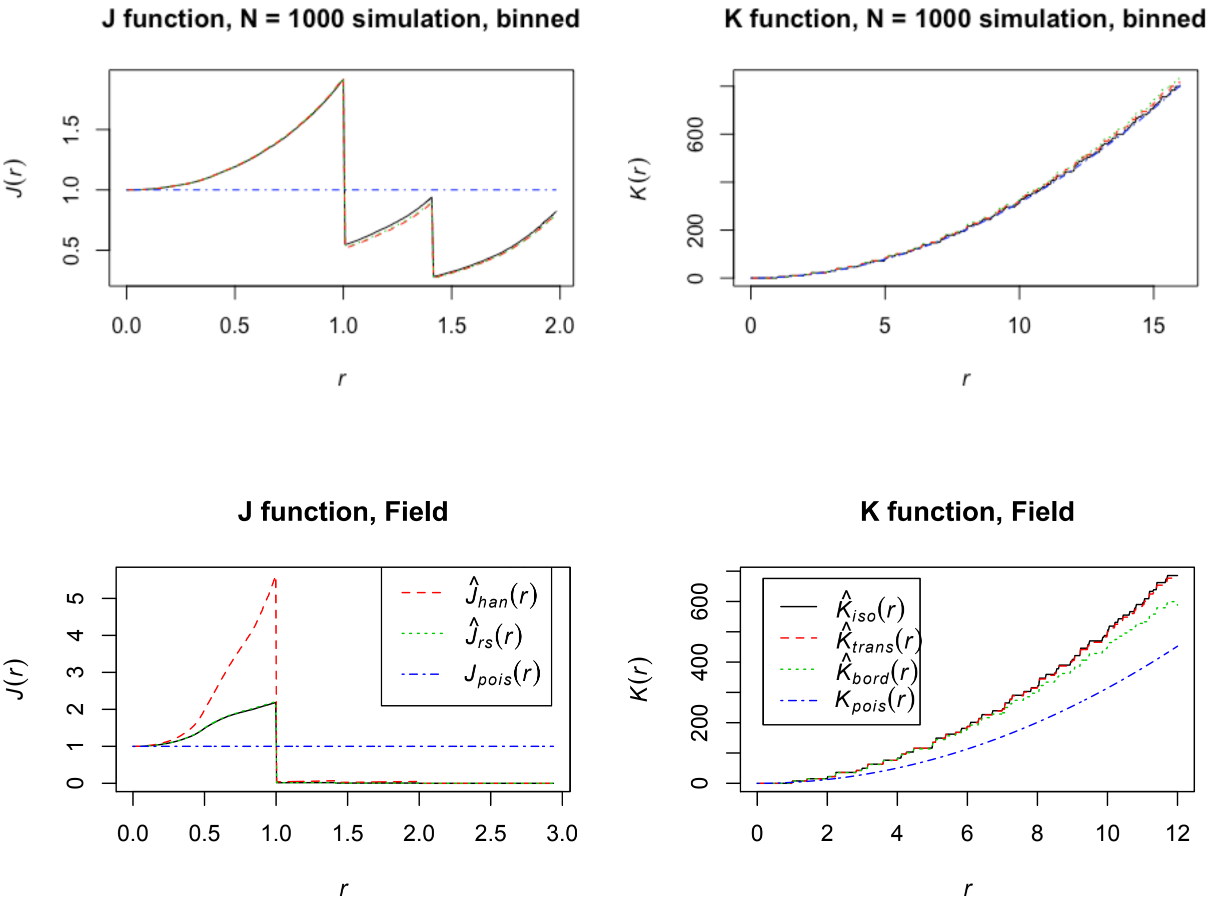


Figure 4.15: (top) Spatial statistics for simulations run with 1000 sand dollars for 1000 time steps in 400 cm x 400 cm of space, binned to 6.25 cm x 6.25 cm pixels for comparison to field survey (Figure 4.14, right). (bottom) Spatial statistics for field survey of spatial pattern, high intertidal sand dollar bed (Figure 4.14, left). The empirical data (black) and two fitting metrics (red and green) show the degree of clustering, as compared to a Poisson function (blue).

DISCUSSION

These experiments and observations have elucidated how sand dollars interact with one another in their environment and form aggregations through behavior relative to conspecifics. Sand dollars are an ideal study system in which to employ individual based random walk movement models. The response in which sand dollars reduce their movement when encountering conspecifics is a novel finding for this species, as might be expected from studies on clumping and particle delivery (Timko 1976; O'Neill 1978). Sand dollars also slightly increase their rate of movement when extremely close to conspecifics, observed as the slight rise at $M = 0$ (Figure 4.8), which suggests that sand dollars do not like to be stacked on top of one another, but also may be a part of tuning to an optimal distance for particle capture (Timko 1976; O'Neill 1978).

This study is one of the first to effectively tag and recapture sand dollars. Echinoderms are notoriously hard to tag, either sloughing off marks, pushing them out of the body cavity, or dying (Sonnenholzner, Montano-Moctezuma, and Searcy-Bernal 2010). We found similar effects of body cavity puncture, that is, high mortality, to other studies in urchins. However, the floss tag method, which avoided puncturing the main body cavity was minimally invasive and effective. Improved data collection might be attained by using a tag in which identification is fully integrated (e.g. spaghetti tag from Floy Tag & Manufacturing Inc.), thereby avoiding tag or identifier loss experienced when using bee tags, and to investigate anti-fouling tags. While it did not create problems over the course of this study, the floss tags did accumulate ephemeral algal growth that had the potential to affect movement.

The mark-recapture experiment illustrated movement under conditions of high density (~400 sand dollars/m^2) in the field. All of the sand dollars in this area would have been in contact with conspecifics in all locations, and therefore would have not had opportunity to demonstrate the aggregative behaviors that we observed in mesocosms. Furthermore, the movements into and out of aggregations at small scales may be more relevant in reducing rates of movement of individuals (apparent diffusion coefficient) over larger scales while still maintaining clumped patterns that are not expected in true diffusion arising from a random walk, as individuals move from aggregation to aggregation.

Qualitatively, in the mesocosm experiment we observed several other behaviors that were not easily measured with tracking. Sand dollars would form clusters that would be stable for long spans of time, but then in a single timestep all would scatter and move rapidly for several timesteps until reforming a new cluster. One possible explanation for this is that once a day the water inflow was disconnected to calibrate the flow rate, and disruptions relating to this measurement may have increased turbulence and stimulated movement. Sand dollars may also be responding to internal rhythms such as diurnal cycles or tide cycles. We also observed that sand dollars would display the strongest angle changes, near reversals of directions, upon leaving a cluster, to return.

The outdoor mesocosm had several challenges in maintaining constant flow, and we found that sand dollars clustered under the inflow and stopped moving. Since we were seeking to isolate movement patterns in response to conspecifics, this drove the experimental design for the smaller mesocosm experiment.

Although sand dollars have the capacity for fast travel (>10 cm/minute), they spend the vast majority of time in single locations. On an intertidal beach embayment like Crescent Beach, sand dollars are at the surface, in the upright posture, feeding during high tides, unless wave conditions are exceptionally strong. During low tides when sand dollar beds are out of water, sand dollars bury into the sand as deep as 15 cm to escape heat and desiccation. This daily movement may be in response to temperature, flow, or light changes, or may be an internal rhythm following tide cycles. This means that every time the tide comes back in, sand dollars reemerge from the sand and jockey for favorable positioning. We might not expect to see sand dollars collected from open coast populations, which live in deeper waters and are exposed to greater wave forces but more consistent currents, respond in the same ways.

Sensory ecology of sand dollars is not well-understood beyond the chemotactic responses described in the introduction. Several species of urchins have been shown to respond to light cues with photoreceptors distributed throughout tube feet (Ullrich-Lüter et al. 2011), and *D. excentricus* larvae use UV photoreception for diel migration throughout the water column (Pennington and Emlet 1986). Our behavioral results suggest that sand dollars respond to other sand dollar presence when in physical contact, in a way that they do not respond upon encountering other obstacles, like the walls of a tank.

While the models did generate clustering patterns, they did not achieve the within-bed clustering observed in the field survey, or the landscape level bed patterning seen in the aerial imagery. In our study we examined individual movement of sand dollars in tanks, which is something of a flawed proxy for the behaviors they may have in the field, but it allowed us to manipulate population density, which is known to be important and drive movement to fine-tune spacing (O'Neill 1978; Francisco and Herzka 2010). There may be other factors that drive the creation of open areas, such as movement away from predators, piles of drift algae or other debris. Other factors still may help define the edges of beds, such as competition with seagrass, increased mortality at bed edges due to predation or dislodgement by waves, or other hydrodynamic effects.

The analysis technique used to compare simulation spatial patterns to the field spatial pattern was flawed as well. The metrics were not intended for presence/absence observations in a grid, and an approach that utilizes windows or pixel maps may be preferable in the future. Data collection of spatial pattern of individual dollars in the field is challenging, and though grid data collection gave some insights, analysis of positions of individual sand dollars from photographs taken from above while the tide is high may be more useful for comparisons with individual based model outputs.

Other factors which may be incorporated into the models in the future based on the data already collected include whether turning angle orients toward nearest neighbor, whether number of neighbors aggregated nearby affects behavior, and whether less-random searching behavior (such as alternating left and right directions) occurs in sand dollars away from neighbors. The tendency of sand dollars to wander away from clumps is not a pattern seen in the field as often – boundaries can be relatively sharp, and sand dollars often are observed returning to clusters. One of the challenges of individual based modeling is computational – scaling up to population and landscape level processes is intensive, and we were not able to replicate the bed sizes observed at Crescent Beach at this time. Improvements to the speed of the model runs and utilization of servers may yield further results in the future.

This type of model is both intuitive and easy to tailor to empirical systems and data, and can be applied to a wide range of questions. It can also be computationally intensive, however, when applied to large scales with population-level patterns of organisms. Other approaches to aggregation may predict grouping size, invasion fronts, or diffusion speed using systems of partial differential equations or fractal analysis. There are hybrid approaches, such as that used by Kareiva and Shigesada (Kareiva and Shigesada 1983) in extending the work by Jones on cabbage butterflies to a formula for net squared displacement that bridges the gap between a diffusion model and the detailed empirical ones.

In addition, interactions with other species on the beach may play a role, particularly, the seagrass *Zostera japonica*, which is the other major space-occupying species at Crescent Beach. As seagrasses also exhibit spatial dynamics and positive feedbacks, seen in the aerial image (Figure 4.20), both species may be contributing to the patchy spatial pattern observed at Crescent Beach.

CONCLUSIONS

Our simulations did induce clustering, however they primarily explain and simulate patterns observed within clusters, rather than emergent patch formation on landscape scales. There are several extensions to this model that could be added to better capture this patterning. First, sand dollars could adjust their directionality in relation to the location of conspecifics rather than in relation to their prior movement, thereby preferentially moving in the direction of the conspecific. Introducing positive density-dependent mortality under the assumption that sand dollars outside clusters experience higher mortality due to predation or environmental stress. Finally, the model may need to be integrated with information on sea grass distributions and disturbance regimes, which may arise from competition. While movement behavior alone can create aggregation, key differences in modeled and observed behavior suggest that spatially-localized interactions among these species (Chapter III) indeed play a key role in determining the coexistence of these contrasting ecosystem engineers.

APPENDIX A

INTRODUCTION

I conducted a series of experiments to test environmental differences between sand dollar beds and seagrass beds. These experiments examined several hypotheses about how these factors related to the occupancy and ecosystem engineering activities of both species.

H0: There are no environmental differences between seagrass beds and sand dollar beds.
H1: There are environmental differences between seagrass beds and sand dollar beds which are inherent to the environment and function as drivers for the spatial pattern of where seagrass and sand dollars live.
H2: There are environmental differences between seagrass beds and sand dollar beds which come about as a result of occupancy by either seagrass or sand dollars, i.e., ecosystem engineering.

I examined these hypotheses through qualitative descriptions of sediment cores, experimental tests of relative flow through each habitat, observation of dissolved oxygen in porewaters at different sediment depths, and organic content between the two habitats. I also took observations of temperature conditions experienced at the upper and lower limits of the tidal range, not a comparison between habitats. Using a manipulative experiment, I examined whether similar environmental conditions developed with transplants and removals of these two bed types.

The results of these experiments show that while in some cases there are detectable differences in environmental conditions between the two habitats, they are generally small relative to the wide range in conditions experienced across the entire site. These experiments did not determine which differences are engineered rather than inherent (i.e., distinguish H1 and H2). On the whole, I conclude that at this time, the environmental parameters measured and reported in Appendices B-F showed few differences and are unlikely drivers of competition or spatial pattern between these two species.

APPENDIX B

SEDIMENT CORES

Four sediment cores were taken at low tide on September 4, 2014 and six on September 13, 2014 for a total of 9 cores, with four taken in sand dollar beds and five in seagrass beds. Cores were collected using a clear plexiglass core with a 4 inch diameter and were photographed, described, and measured in the field, with samples taken from each distinct layer and colors described using a Munsell standardized color chart.

Cores are described individually below and visually in Figure B.1:

1) Sand dollar bed. Fine sand of the same grain size all the way through. Some shell fragments in top 5 cm. Two thicker, darker layers of fine sand in middle.
    a. 0 – 5 cm. Color: 5Y 4/1 dark gray, fine sand, some shell fragments.
    b. 5-10 cm. Color 5Y 2.5/1 black, fine sand, dark color, no shells.
    c. 10-11 cm. Color 5Y 4/1 dark gray, fine sand, lighter color.
    d. 11-14.5 cm. Color 5Y 2.5/1 black, fine sand, dark color.
    e. 14.5- 16 cm. Color 5Y 4/1 dark gray, fine sand, light color, some shell fragments.
2) Sand dollar bed. Fine sand. Upper layer (0-8 cm) darker, with a gradient through to the bottom of the core. Shelly hash layer of bivalve shells at bottom (22-27.5 cm)
    a. 0- 8 cm. mixed layer of fine sand, dark (5Y 2.5/1 black) and lighter (5Y 4/1 dark gray)
    b. 8-25 cm. Gradient of color in fine sand from darker (5Y 4/1 dark gray) at 8 cm to light (5Y 6/1 gray) at 25 cm
    c. 25-27.5 cm. Bivalve hash layer. Shells are brittle and chalky.

3) Seagrass bed. Fine sand with slightly darker color at top, gradient of sand color through whole core with darker at top (5Y 4/1 dark gray) and lighter at the bottom (5Y 6/1 gray).
    a. 0-8 cm. Dense rhizomes.
    b. 8-18 cm. Sparse rhizomes, abundant polychaete worms.
    c. 18-24 cm. fine sand.
    d. 24-35 cm. increasing shell fragments with depth, all bivalve shells. Largest fragments 2-3 cm length.
4) Seagrass bed. Fine sand with seagrass rhizomes.
    a. 0- 8 cm. Dense rhizomes.
    b. 8-25. Sparse rhizomes. Sand color 5Y 5/1 gray.
    c. 25-30. Very few rhizomes, shelly hash in fine sand, color 5Y 6/1 gray. Large chalky bivalve shells.
5) Sand dollar bed. No shells, no particles, anoxic zone.
    a. 0-3 cm lighter gray, tan color. Color 5Y 5/1 gray.
    b. 3-4 cm dark gray fine sand. Color 5Y 2.5/1 black.
    c. 4-17 cm medium gray fine sand. Color 5Y 4/1 dark gray.
6) Sand dollar bed.
    a. 0-3.3 cm. tan, light gray, fine sand. Color 5Y 5/1 gray.
    b. 3.3-9.3 cm. Dark gray anoxic layer. Color 5Y 2.5/1 black.
    c. 9.3-19 cm. Lighter dark gray, less tan, no shells. Color 5Y 4/1 dark gray.
7) Seagrass bed (sparse) with occasional small sand dollars. Single layer of color.
    a. 0-1 cm. Clean sand, light gray, fine sand. Color 5Y 5/1 gray.
    b. 1-2 cm. Sparse thin thread-like rhizomes, light gray, fine sand. Color 5Y 5/1 gray.

    c. 2-20 cm. Clean fine sand, 5Y 4/1 dark gray. Some worms at 15-18 cm.

8) Seagrass bed.
    a. 0-6 cm. Visible seagrass, thick, uncuttable rhizomes. Color 5Y 4/1 dark gray.
    b. 6-23 cm. Consistent, fine gray sand, some rhizomes, some worms. Color 5Y 4/1 dark gray.

9) Seagrass bed. Consistent fine sand throughout depth, with slightly more tan color at surface and slightly more gray color at bottom, consistent with colors observed in other cores.
    a. 0-1 cm. Grass blades and diatom/ephiphyte “flock”. Orange-tan color.
    b. 1-7 cm. Dense seagrass rhizomes. Sand slightly more tan-gray. Color 5Y 4/1 dark gray.
    c. 7-11 cm. Sparse rhizomes. Though slightly less tan, still closest to Color 5Y 4/1 dark gray.
    d. 11-20.7 cm. No rhizomes, clean, fine gray sand. Color 5Y 4/1 dark gray.

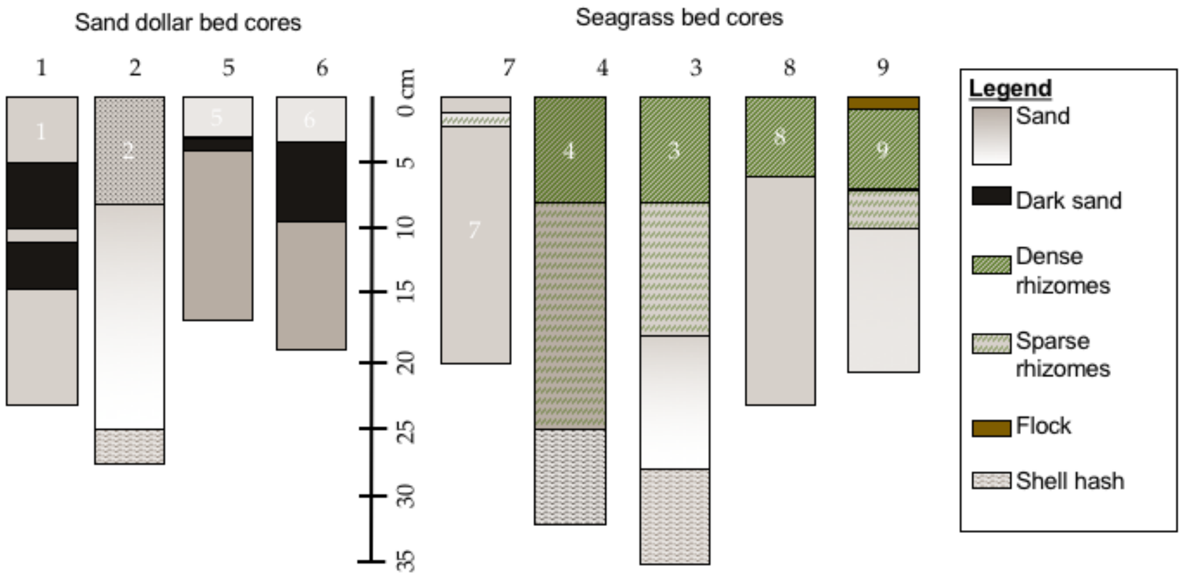
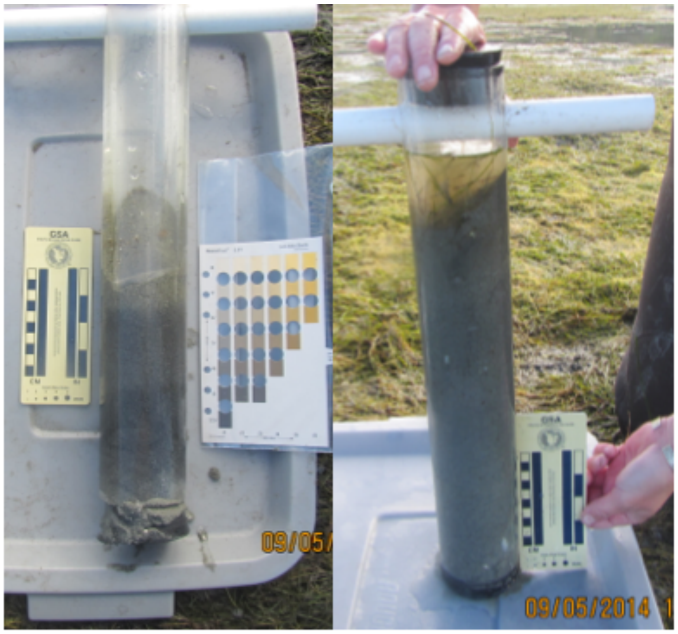


Figure B.1: Visualization of cores separated by those taken from sand dollar beds and those taken from seagrass beds. Colors exaggerated to show contrasts.



Core 1 Core 8

Figure B.2: Photographs of cores taken in sand dollar bed (left, Core 1) and in seagrass bed (right, Core 8) showing sand color and striped banding.

Within sand dollar beds exist some stratification of layers resulting in dark zones, either due to anoxia, or potentially deposition of fine pigment particles from decomposing sand dollars. It is likely that sand dollars only mix sediments to several centimeters of depth and prevent mixing further below, resulting in anoxic zones. Seagrass displayed surprisingly clean sand, and therefore may be oxygenating sediments more thoroughly. The shelly hash layer of bivalves likely results from a process of biogenic stratification by “conveyor belt” feeders such as *Arenicola* sp., which are abundant at Crescent Beach. These species advect finer particles to the sediment-water interface leaving a lag of coarse material at feeding depth.

APPENDIX C

RELATIVE FLOW RATES

An experiment was conducted to determine relative water movement, turbulence or flow forces experienced across a series of different soft-sediment habitats. Dissolution blocks (clod cards, methods from C. Couch, adapted from Jokiel and Morrissey 1992 and Burns et al. 2011) were made of Plaster of Paris (professional, DAP brand) prepared according to the directions and poured into a silicone mold, tapping the mold to remove bubbles. Plaster was allowed to set, then dried in a drying oven at 85° C until mass stabilized. In the first experiment in 2015, a muffin tray was used, and a bent galvanized steel wire (about 9 inches, 4.5 inches folded) was inserted into the center. Mean weight prior to deployment was 93.5 g, with a standard deviation of 6.4 g. Dissolution blocks were wired to bamboo garden stakes or rebar stakes 1-3 cm above the sediment and left for 24 hours. Stakes were placed in areas at least 0.5 m from another type of habitat.

In the second experiment in June 2016, plaster was set in a 1-inch cone dessert mold and a bamboo skewer was placed upright in the center of the mold, penetrating to the base of the mold. Mean weight prior to deployment was 32.7 g, with a standard deviation of 2.1 g. In the second experiment, several blocks were placed in a bucket of still sea water, changed gently daily, as a control for dissolution in still water. Blocks were placed in the field by inserting the bamboo skewers into the sediment to place the blocks 1 cm above the sediment surface.

A fully factorial experiment was performed with three treatments in three locations – sand dollar, seagrass, and bare sand in high, mid, and low intertidal zones. The high zone was located at approximately 0.00 m MLLW, the mid at -0.15 m MLLW, and the low at -0.30 m MLLW. As the high zone and mid zone do not have naturally occurring bare patches, 2 m diameter areas were cleared of sand dollars temporarily for the placement of “bare sand” areas. For the experiment in 2015, 45 blocks were deployed, with 5 per treatment and location. In 2016, 60 blocks were deployed, resulting in 6 blocks per treatment, including 6 in still water.

After either 24 or 72 hours, the blocks were collected and dried again at 85° C and masses were compared, with the assumption that blocks which lost more mass indicated greater flow.

I hypothesized that seagrass beds would exhibit slower flow and less dissolution than either sand dollar beds or bare sand. I also hypothesized that within some distance from the sediment, sand dollars might also slow flow and inhibit dissolution. I hypothesized that the low zone would experience the greatest dissolution because it is subject to the greatest amount of time immersed and may experience greater wave forces.

For the 2015 experiment in which blocks were deployed for 24 hours in calm conditions, very little mass was lost. There were no significant differences between treatments within the high or low zones, but within the mid zone, bare sand experienced greater dissolution (t-test, SD vs BS, $p > 0.02$; S vs BS, $p > 0.01$). The low zone had significantly higher dissolution than both the mid and high zones (t-test, low vs. mid, $p >> 0.001$, low vs. high, $p >> 0.001$).

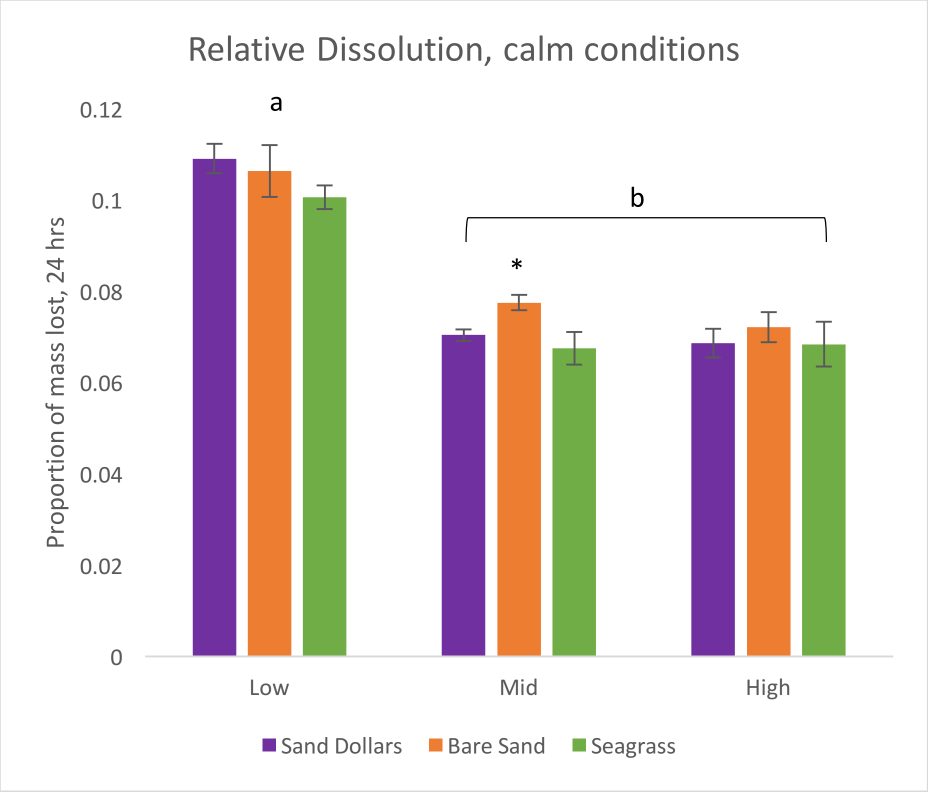


Figure C.1: Experimental results showing mass lost from dissolution blocks, in which greater mass lost corresponds to higher flow conditions, in blocks deployed for 24 hours in calm conditions. Mid and High zones do not differ significantly from each other (b), but both differed from Low (a). Within-zone differences were only detected in the Mid zone (*).

In the 2016 experiment in which blocks were deployed for 72 hours in moderately turbid conditions, a greater proportion of mass was lost, and all treatments had greater loss than the still water control. All three zones differed significantly in mass lost, with the low zone experiencing the greatest loss and the high experiencing the least (t-test, $p >> 0.0001$). Within zones there were occasional differences, mostly barely significant. In the low zone, sand dollar and bare sand areas lost more mass than seagrass areas (t-test, SD vs. S, $p >> 0.0001$, BS vs S, $p > 0.05$).

Seagrass and sand dollar barely significantly differed in the mid zone (t-test, SD vs. S, $p > 0.05$), but neither differed from bare sand.

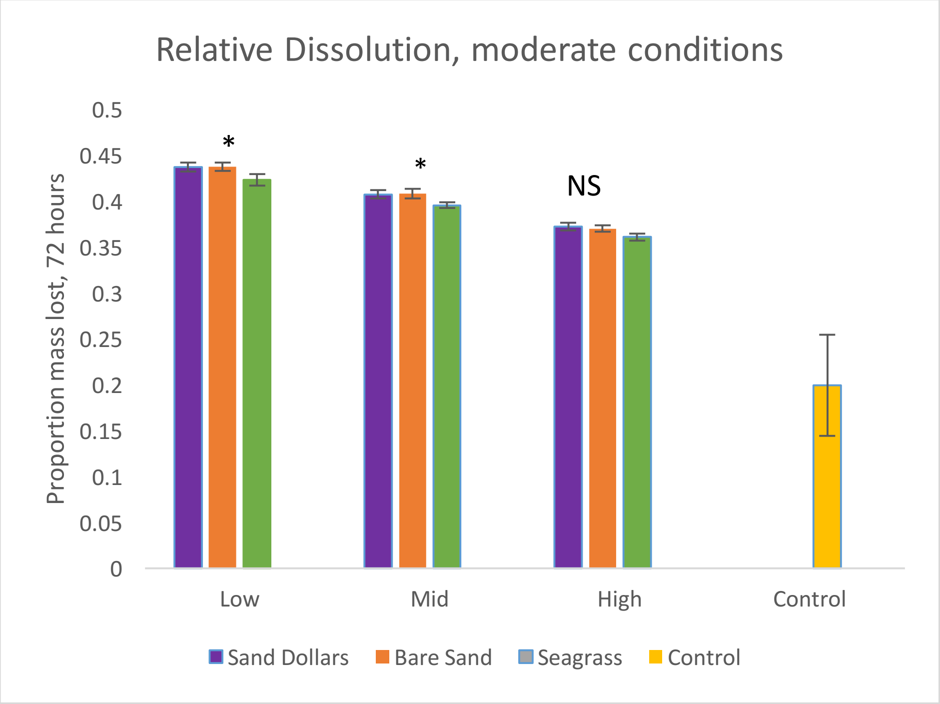


Figure C.2: Experimental results showing mass lost from dissolution blocks, in which greater mass lost corresponds to higher flow conditions, in blocks deployed for 72 hours in moderately calm conditions. Low, Mid, and High zones all differ significantly from each other (not denoted in chart). Within-zone differences were detected in the Low zone, in which seagrass had lower flow, and the Mid zone, where seagrass differed from sand dollar but neither differed from bare sand.

From this experiment, I conclude that while seagrasses, and under some conditions, sand dollar beds, do occasionally reduce flow across the benthos, the effect is negligible enough to be difficult to detect, and is far less significant than the difference between the high, mid and low tide heights.

APPENDIX D

TEMPERATURE

The thermal extremes of sand surface temperatures during summer months were examined for the upper limit and lower limits of the sand dollar bed at Crescent Beach, Orcas Island, WA. The only year data was collected in this fashion was 2013, which was the coolest and rainiest summer of the three years of the experiments. Therefore, these temperatures are representative of a low range of possible temperatures experienced during summer by benthic organisms in the intertidal zone of Crescent Beach during the experiments conducted in this thesis.

Data were collected using two HOBO TidbiT data loggers (ONSET Corp.) secured with zip ties to rebar stakes placed at 0.00 m MLLW and -0.30 m MLLW, measured using a laser level relative to predicted tide heights. Pendants were placed partially buried in sand, with LED sensors flush with sediment surface. Pendants were deployed from June 20, 2013 to August 22, 2013. Data were then compared with tidal heights predicted using the Rtide package in R and compared between high and low zones.

The difference in tide height between the upper limit and lower limit of the sand dollar bed resulted in a statistically significant difference in mean temperature (Paired t-test, df = 17820, $p << 0.001$, see bar plot), with a mean difference of 1.09° C. The high zone also experienced dramatically higher excursions into high temperatures during extreme low tides. However, these temperatures are unlikely to have been experienced by living sand dollars, which burrow into the sediment to avoid exposure. The temperature at the sediment surface of the bed is generally higher than the open waters of the surrounding straits, which is usually no higher than 10.5° C in

July, likely due to low levels of fluid mixing on the broad, flat beach. The cause of the cold water event between July 14-17 is unknown, but those days experienced rain and some gusty winds, which may have contributed to both shading and mixing.

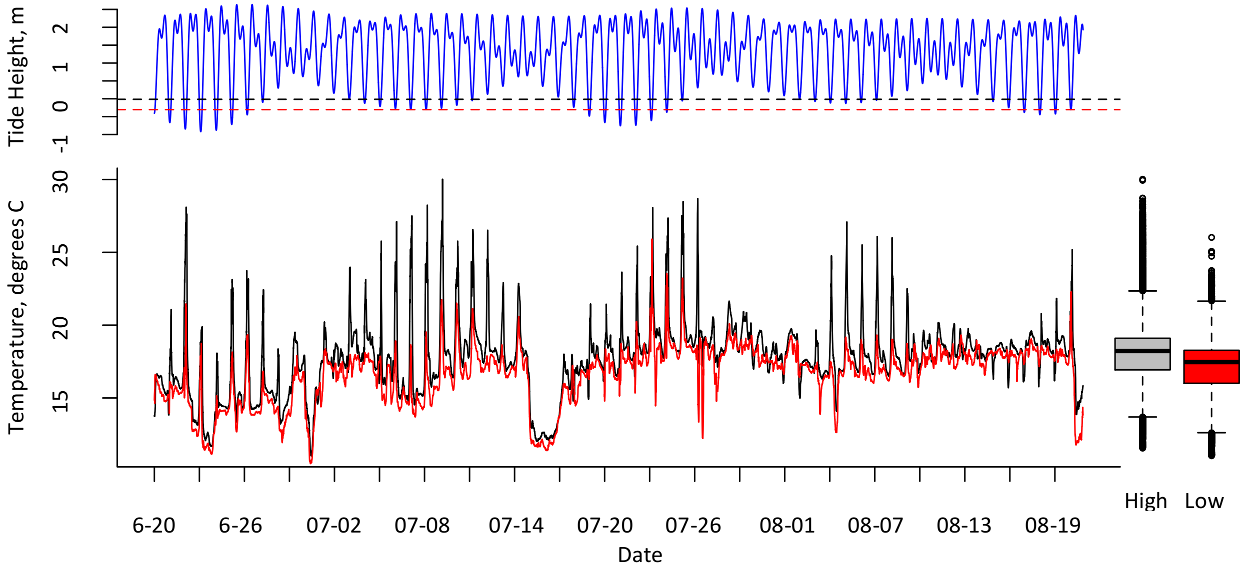


Figure D.1: Measured temperatures at the high limit of the sand dollar bed (High, black/gray), and the low limit of the sand dollar and seagrass beds (Low, red), over the summer of 2013 from June 20 to August 20. The High zone experienced significantly greater thermal stress during extreme low tides (blue), which occur in midday in summer, and on average was about 1°C warmer at all times, suggesting low water mixing during high tides and a strong thermal gradient that could alter interactions at different tide heights.

APPENDIX E

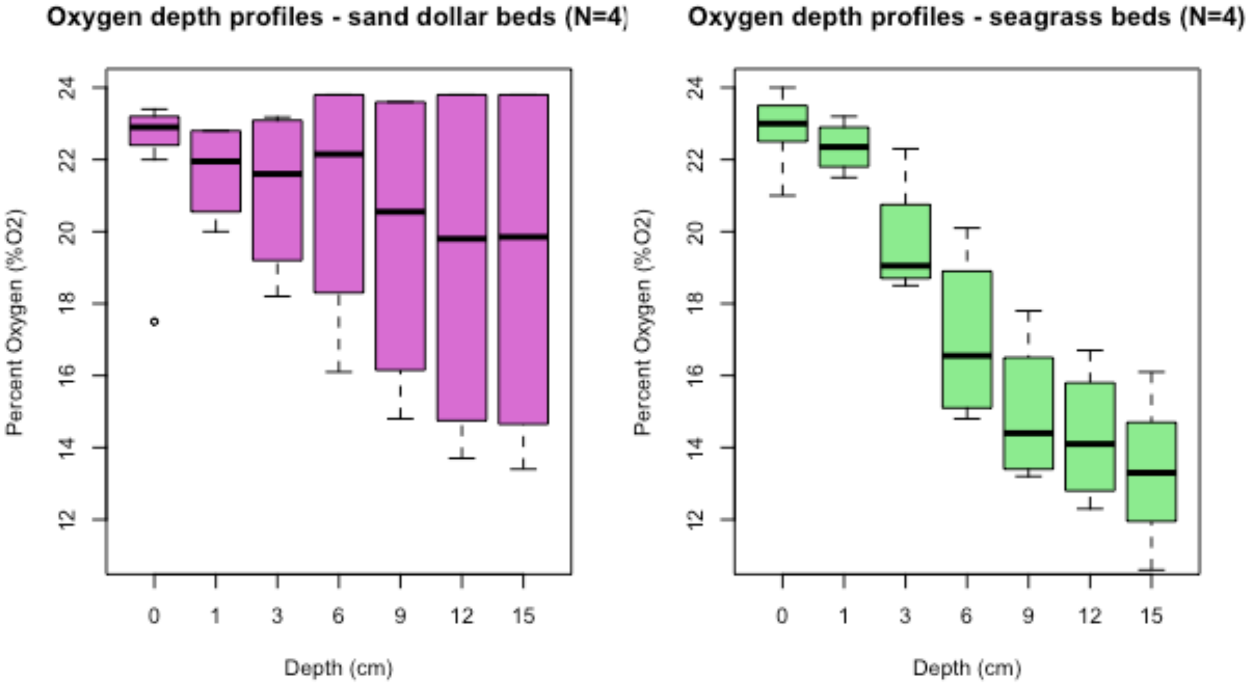
DISSOLVED OXYGEN

I examined whether oxygen availability within sediments varied along a sediment depth gradient, and whether these patterns varied between seagrass and sand dollar beds.

A NeoFox optical oxygen transmitter equipped with a FOSPOR fiber optic oxygen probe with a stainless steel sheath and concurrent temperature probe was used to measure dissolved oxygen in sediments by inserting the probe directly into the sediment at prescribed depths. A modified 1 mL plastic serological pipette with a mesh tip was used to shield the sensitive chemical coating from damage, resulting in some data smear at different depths. The device was controlled through an Armor X7 Tablet running Windows 7 and the NeoFox Ocean Optics software in the field, and housed in a Pelican case to keep it waterproof. A NaturePower Elite power pack battery with AC outlet was used to run the devices in the field.

A ring stand was set adjacent to area to be sampled, and probes are mounted on ring stand above the sample area. Calibration was done in air, and temperature and percent oxygen in air was sampled. The first measurement is taken in the overlying water (0 cm). The probes are inserted into the sand at gradually increasing depth, to 1, 3, 6, 9, 12, and 15 cm. Oxygen concentration was allowed to stabilize to within 0.2 percent points, which could take up to several minutes.

Observations were taken in twelve locations over a single low tide in August 2015. Four locations in seagrass, four in a sand dollar bed, and four in border zone areas, with some seagrass and some sand dollars. Overlying water had between 22-24% O_2.



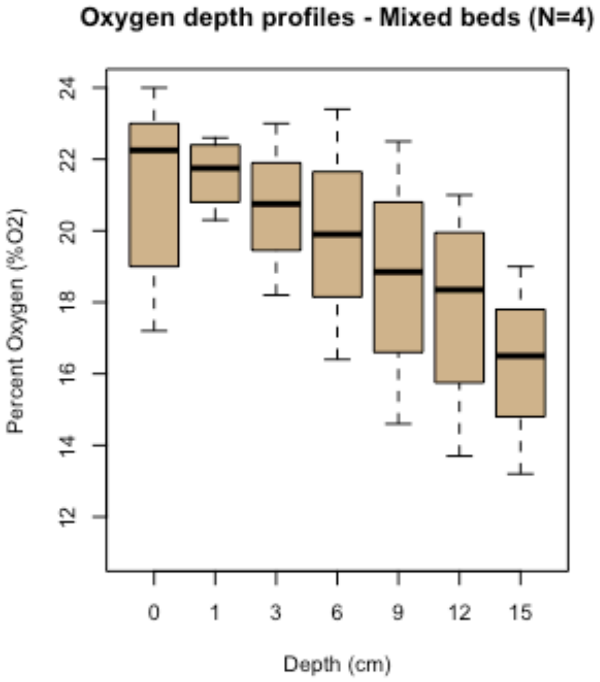


Figure E.1: Percent oxygen saturation detected at different sediment depths. Observation at 0 cm indicates overlying water. Box plot shows mean of four observations in each habitat type, and show a decline in dissolved oxygen with increasing depth.

There were no significant differences in oxygen profiles and oxygen concentration at any depth between treatments. Although the figure for sand dollar beds suggests that oxygen patterns differed, the trend is driven by one location in which all depths matched the overlying water, and those observations were taken last, as the tide came in. These observations suggest that while there is a depth gradient in oxygen availability during low tide, this gradient disappears when the tide comes back in because overall these sands are well-drained and readily oxygenated to 15 cm by overlying water. Therefore, I found no differences in infaunal oxygen availability.

APPENDIX F

SEDIMENT ORGANIC CONTENT

I examined organic content in sediments in seagrass beds, sand dollar beds, and experimental manipulations, including seagrass transplants and removals from 1, 2, or 3 years prior to sampling, and sand dollar exclusions.

Five replicate areas of sand dollar bed were manipulated to examine sediment organic content, with six treatments. Mesh fencing was used to create an exclusion area for each replicate, with individual treatments isolated using plastic rounds created by halving 5-gal buckets into 13 cm deep rings, which were sunk to be flush with the sediment surface and create an inclusion.

Table F.1: Experimental design of controlled sand dollar and seagrass transplants for measurement of sediment organics.

	Sand dollars	Seagrass	Bare sand
Excluded sand dollars, plastic inclusion	1 – D tmt	2 – J tmt	3 – X tmt
Excluded sand dollars, no plastic inclusion	NA	NA	4 – X outside
No exclusion, plastic inclusion	5 – D outside	6 – J outside	NA

1) Half bucket contains 30 sand dollars in sand, with sand dollars excluded up to 27 cm away from tube with mesh fence
2) Half bucket contains a transplanted seagrass plug, with sand dollars excluded up to 27 cm away from tube with mesh fence

3) Bare sand contained within a half bucket, with sand dollars excluded up to 27 cm away from tube with mesh fence
4) A bare patch of sand, with sand dollars excluded up to 27 cm away from tube with mesh fence
5) Sand dollars isolated in a half bucket, in an existing sand dollar bed.
6) Seagrass isolated in a half bucket, in an existing seagrass bed

To create removal areas, we installed a 1.5 x 1.5 m square perimeter of 1-cm plastic mesh to a depth of 10 cm, with 1 cm of mesh above the surface of the sediment. At corners we installed 2-foot steel rebar stakes with about 4 inches above the surface to secure the mesh. Sand dollars were removed by hand, then all sand in the plot to a depth of ~ 8 inches was sieved with a 0.125 cm mesh to homogenize the structure and oxygen, and remove large clams, worms, and shells.

All five exclusion replicates were placed in large open sand dollar beds greater than 30 cm distance from a seagrass bed, around -0.5 ft tide height. Replicates 1 and 2 are in the same bed, diagonally about 1 m apart, Rep 3 is by itself in the middle, slightly higher on a hummock, and Reps 4 and 5 are right and left in the same bed. About 1500 sand dollars were removed from each exclusion replicate.

Sediment was collected using a modified 60 mL syringe with a diameter of 26.7 mm, for a total volume of 30-40 mL. Sediment samples are extruded into a small labeled snack-sized Ziploc bag, then frozen upon return to labs. First sample was left unfrozen for one month. For analysis, samples were defrosted in a warm water bath and manually homogenized. Approximately half the sample was deposited into an aluminum weigh dish, while the other half was refrozen. Samples were dehydrated in a drying oven at 70 degrees C for 24 hours until a stable weight was achieved. Samples were weighed to the nearest 0.0001 g, covered with foil, then ashed in a muffle furnace at 550° C for one hour (after reaching 550 degrees - total time in oven was about 1 hour, 10 minutes). Within one hour of removal samples were weighed again to the nearest 0.0001 g. The dishes were washed and weighed after ashing, though it's recommended dishes be weighed prior to addition of samples in the future.

Sediment was collected from all treatments three total times in 2015. In addition, in 2016, samples were collected from various other locations at the beach which had been disturbed in prior experiments, such as where seagrass had been removed 2 and 3 years prior or transplanted 1, 2, or 3 years prior. Samples collected in 2016 were ashed for 24 hours at 550° C, with sample dishes weighed prior to ashing.

Figures F.1 and F.2 show the amount and change over time in % of mass lost in ash-free dry mass of all treatments, or the percent organic content of sediment. Sediments at this site have sediment organic content that ranges from 0.4% - 1%, which is typical of relatively clean sand. The organic content declines in all treatments over the course of the summer. There are no significant differences between any treatments at any time point (paired t-tests, $p > 0.05$). The general trend however, suggests that unmanipulated seagrass (J outside) and unmanipulated sand dollar (D outside) started with the highest organic content.

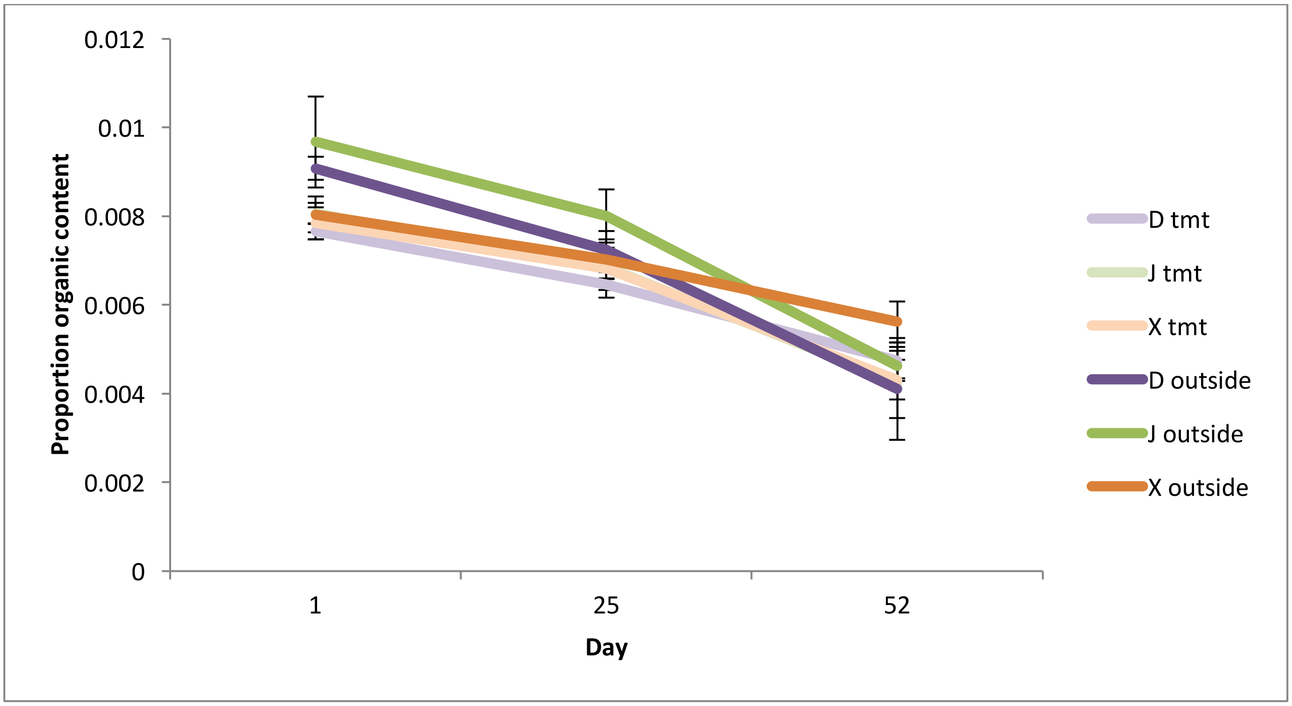


Figure F.1: Change in sediment organic content over time, June-August 2015.

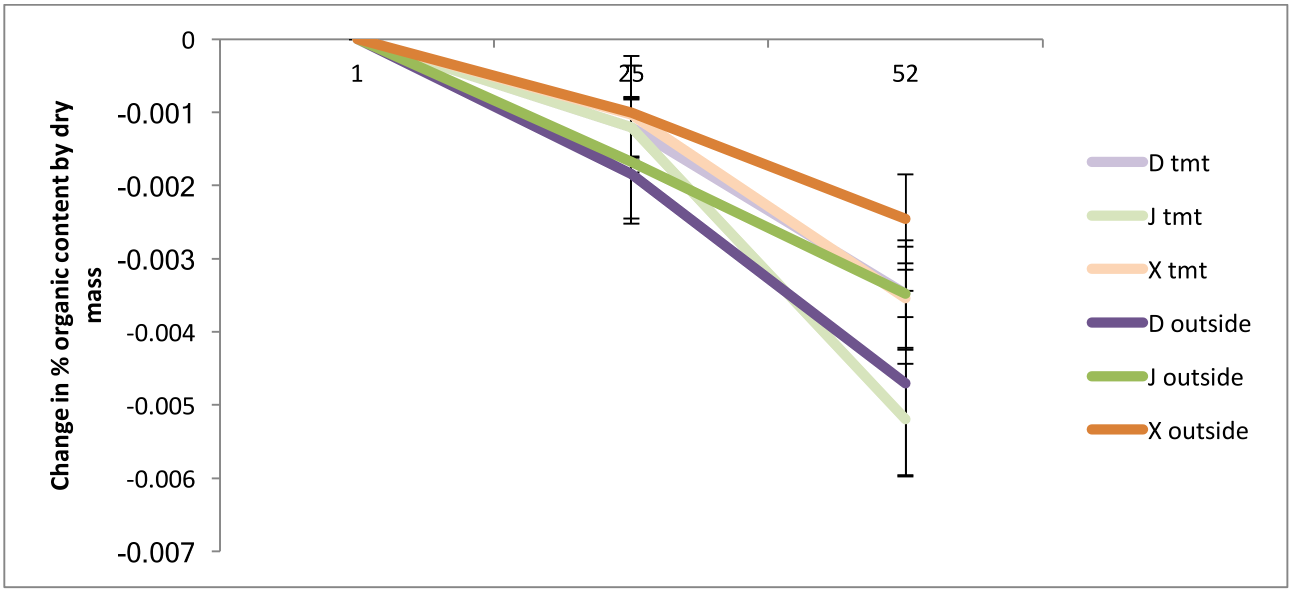


Figure F.2: Change in percent organic content over time relative to initial conditions at T = 1

However, not all treatments declined equally. Though no results were significant, seagrass treatments declined more steeply than sand dollar treatments.

Finally, when comparisons were done between manipulations and their controls (outside seagrass vs. 52 days transplanted seagrass, outside sand dollars vs. sand dollars in inclusions, and sand dollar excluded bare sand within an inclusion vs. not), some trends emerged, which again, were not significant, but it appeared that bare sand areas decreased in organic content slightly less relative to the declines in sand dollar and seagrass treatments.

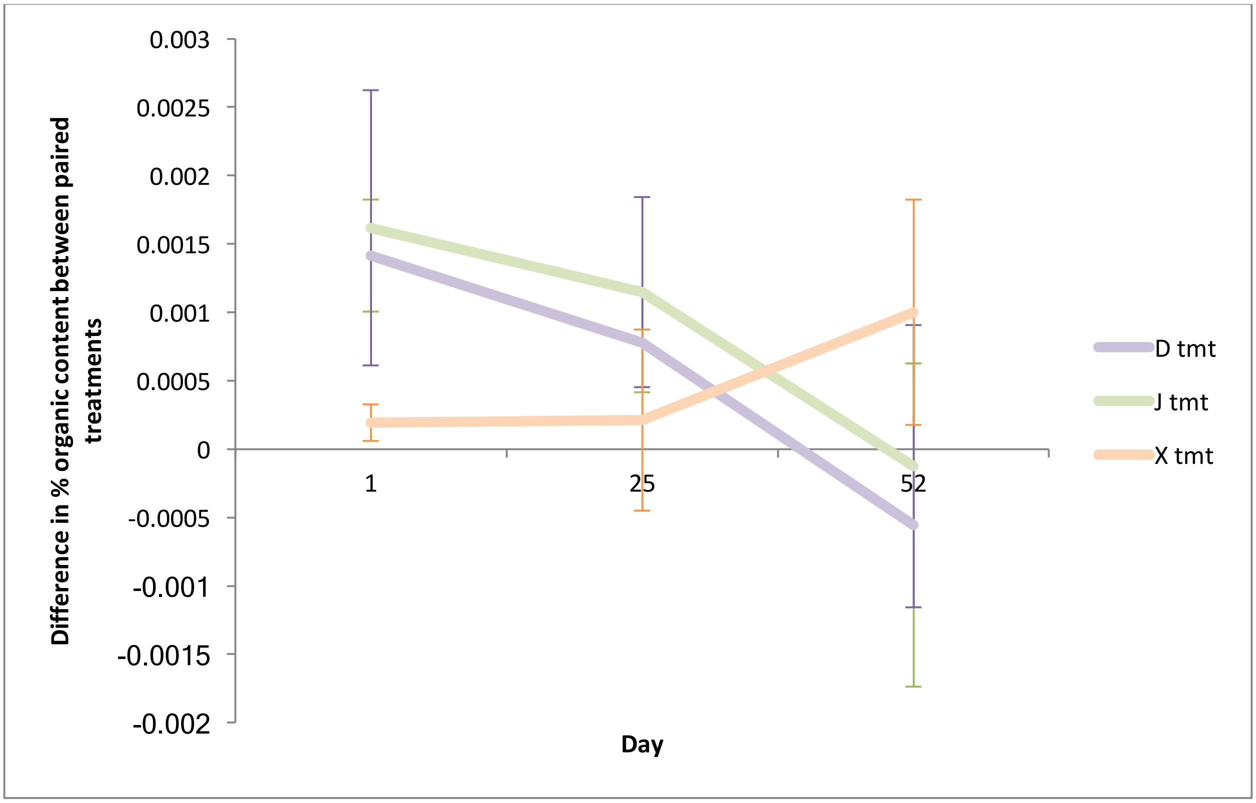


Figure F.3: Difference in percent organic content between treatments and their corresponding unmanipulated controls.

In the more observational part of the survey, samples were collected in 2016 from locations with varying conditions corresponding to experimental removals and transplants done in Chapter II and III:

1) Undisturbed seagrass beds (n = 16)
2) Undisturbed sand dollar beds (n = 19)
3) 2 – year prior seagrass removal (n = 8)
    a. Current state seagrass (n = 5)
    b. Current state sand dollar (n = 3)
4) 3 – year prior seagrass removal (n = 7)
    a. current state seagrass (n = 5)
    b. current state sand dollar (n = 2)
5) 3 – year prior seagrass transplants (n = 6)
6) Disturbed sand dollar beds (n = 23)

Three outlier locations were excluded from analyses because of inaccuracy, such as weight gain with ashing or order of magnitude difference. The overall average organic content of sediments from all locations (without outliers) was 0.00839, with a standard deviation of 0.0014.

ANOVA tests of variance (Tables F.2, F.3, F.4) found no significant effect of starting state, ending state, treatment (i.e., transplant or removal), or years since disturbance on the organic content of sediments. When separated by end state to test for interactions, interactions of seagrass endstate with pre-treatment state and years post-treatment were significant. Seagrass one year post disturbance (that is, very recently grown, following disturbance or 1-year transplant) appeared to have higher organic content than two or three years post-disturbance. Since the previous experiment only showed significant differences in change over time, with no significant differences within a date, and all samples in 2016 were collected on one date, I conclude that I cannot reject the null hypothesis that there are no between habitat differences in organic content in sediments at Crescent Beach.

Table F.2: Analysis of Variance Table, Response = Proportion Mass Lost, all data

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
State.Pre.treatment	1	0.00000691	6.9144e-06	1.3682	0.2454
Treatment	4	0.00001920	4.7999e-06	0.9498	0.4395
Endstate	2	0.00000349	1.7434e-06	0.3450	0.7092
Years.post.tmt	1	0.00000469	4.6902e-06	0.9281	0.3381
Residuals	85	0.00042956	5.0536e-06		

Table F.3: Analysis of Variance Table, Response = Proportion Mass Lost, Endstate sand dollars

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
State.Pre.treatment	1	1.1000e-07	1.0960e-07	0.0397	0.8431
Treatment	3	1.0579e-05	3.5263e-06	1.2759	0.2950
Years.post.tmt	1	2.9490e-06	2.9486e-06	1.0669	0.3076
Residuals	42	1.1608e-04	2.7638e-06		

Table F.4: Analysis of Variance Table, Response = Proportion Mass Lost, Endstate seagrass

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
State.Pre.treatment	1	2.9912e-05	2.9912e-05	4.1970	0.04745*
Treatment	4	4.2700e-07	1.0700e-07	0.0150	0.99954
Years.post.tmt	1	3.1628e-05	3.1628e-05	4.4378	0.04181 *
Residuals	38	2.7082e-04	7.1270e-06		

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