Space acquisition strategies of *Zostera marina*

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Abstract of the Dissertation

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Seagrasses are a diverse group of clonal marine macrophytes. Their decline in recent decades has been an alarming component of estuarine urbanization, with large portions of historical coverage lost or degraded. Understanding patterns of dispersal and recruitment within and among the remaining populations is now critical to predicting the form and pace of recovery. Working in a developing eelgrass (*Zostera marina*) meadow in Shinnecock Bay, we combined field manipulations, genotypic surveys and correlative analyses to understand how vegetative growth and seedling recruitment contribute to space acquisition. First, we explored clonal foraging behavior and its effect on patch growth using subterranean nutrient additions over a two-year period, finding that enriched patch edges spread faster, were characterized by higher shoot densities, and followed a different seasonal growth pattern than un-amended controls. Second, we used high resolution, decade-long seagrass mapping with polymorphic microsatellite analysis to examine the interactive effects of pollination and seed dispersal distance on sexual reproduction and seedling recruitment. Pollination distances exceeded previously published reports by a factor of 5, with a maximum of 73.91 m. Seed dispersal varied systematically below 6 m for naked seeds, and randomly throughout the 56,250-m^2 study site for seeds deposited by floating reproductive shoots. Pedigree analysis showed that seedlings readily recruited to natal beds, with full sibling groups clustering neatly within larger half-sibling kinships at scales of 2 - 6 m. Finally, after establishing the importance of seedlings to patch expansion and meadow development, we sought to relate inter-annual variation in recruitment success to environmental variables, including wave energy, wind speed and direction, rainfall, and bottom-water temperature. Two multiple regression models were developed, one appropriate for the dispersal of naked seeds, and another for rafted flowers; both highlighted the roles of physiological stress and physical disturbance in controlling the colonization process. This dissertation illustrates how traditional modes of observation (i.e., the quadrat and transect) can augment molecular and GIS approaches in exploring patterns at the largest temporal and spatial scales, providing strong correlative evidence for formative processes and tangible targets for future manipulative work.

Dedication Page

To my wife, Carla, whose love and support make all things possible.

Frontispiece

Table of Contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter 3

Chapter 4

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Chapter 1

Introduction

INTRODUCTION

Seagrasses represent a phyletically diverse group of marine angiosperms comprising seventy-two species from four families (Alismatidae, Cymodoceaceae, Posidoniaceae and Hydrocharitaceae) within the superorder Alismatiflorae (Subclass Monocotyledonae; Les et al. 1997, den Hartog & Kuo 2006, Waycott et al. 2006). Since their first appearance in the Tethys Sea during the Cretaceous, these terrestrially derived macrophytes have colonized nearshore environments on every continent save present-day Antarctica (Vermaat 2009). At present, seagrasses cover an estimated 150,000 - 600,000 km^2 of benthic space (Kendrick et al. 2005, Waycott 2009) and can be found intertidally to depths exceeding 100 m (Lipkin 1975, Lipkin et al. 2003). Throughout this range, they inhabit topographically simple soft sediments and, by virtue of an upright growth-form and rooted habit, provide biogenic structure and oxygenation both above ('phyllosphere') and below ('rhizosphere') the sediment surface. In this way, seagrasses act as 'ecosystem engineers', generating autogenic as well as allogenic habitat for a host of taxa spanning many orders of magnitude in size (Jones et al. 1994).

Globally, seagrasses are thought to contribute between 20.73 and 101.39 teragrams of carbon per year to coastal ecosystems (Duarte et al. 2010). During the 20th century, most of this organic material (roughly, 90%) was thought to enter microbially-mediated foodwebs without contributing directly to secondary production (reviewed in Valentine & Heck 1999, Mateo et al. 2006). Argument for this came from a lack of large vertebrate grazers (*Chelonia mydas*, Sirenia and the marine Anatidae had all been depleted in the modern era), the recalcitrant nature of the leaf material (Mcmillan et al. 1980, Mcmillan 1984), a paucity of examples demonstrating grazer control of aboveground biomass (but see Rose et al. 1999, Valentine et al. 2000, Alcoverro & Mariani 2002), and its contribution to shoreline wrack and deep-sea detritus (Suchanek et al. 1985). While true in scope, we now recognize a far greater role for seagrass herbivory, finding that (1) episodic bouts of consumption, (2) 'cultivated' feeding strategies, (3) high rates of leaf turnover, (4) the effect of basal meristems, and (5) variation in leaf nitrogen can mitigate the above issues, affecting grazer abundance and identity while masking consumer effects on seagrass biomass (Valentine & Heck 1999, 2001, Goecker 2002, Mateo et al. 2006, Tomas et al. 2011).

Regardless of the fate of seagrass-derived carbon, the plants themselves form structural foundations for highly productive and palatable epiphytic communities that, in turn, fuel intense secondary production (Kitting et al. 1984, Heck et al. 2008). Invertebrate and finfish abundance and diversity within seagrass beds are all routinely higher than in adjacent un-vegetated habitat types, and often contain disproportionate numbers of larval and juvenile forms (Heck et al. 1995, Heck et al. 2003). This led to the consensus view that seagrasses were the pivotal nursery habitat within coastal estuaries. A recent review of these claims highlighted the salient role of structure rather than seagrass presence *per se* (Heck et al. 2003); however, the ratio of seagrass coverage to alternative structure (e.g., molluscan reefs and mangroves) validates the relative importance of seagrass to the early life histories of many nearshore species. Further, as rooted plants, seagrasses attenuate wave energy and tidal currents, trap suspended solids, and stabilize sediments, thereby contributing to water clarity while reducing shoreline erosion (Waycott 2009, Barbier et al. 2011). They also mitigate coastal pollution directly, through the cycling of nitrogen and phosphorus, and globally through the uptake and sequestration of carbon (Short & Neckles 1999, Waycott 2009, Kennedy et al. 2010).

Despite their recognized value, seagrass systems have been under assault in recent decades, suffering declines at the hands of anthropogenic stress (Waycott 2009). Among the varied ecological insults, reductions in the coastal light environment are believed to drive worldwide contractions in depth distribution and areal coverage (Ralph et al. 2007, Thom et al. 2008). These have resulted from: (1) increased sediment input related to changes in land-use patterns and runoff regimes (Kemp et al. 2004, Biber et al. 2009), (2) direct modification of watershed hydrology (Onuf 1996), (3) increased sediment re-suspension events caused by extensive dredging, shoreline hardening, and boat activity (Backman & Barilotti 1976), (4) increased aquaculture effluent (Ralph et al. 2007), (5) increased shading by bridges, docks and recreational moorings, and (6) episodic to chronic increases in water column chlorophyll content induced by coastal eutrophication and overfishing (Onuf 1996, Gobler et al. 2005, Lee et al. 2007, Ralph et al. 2007). Together with several notable die-offs (Durako 1994) and a pandemic wasting disease (Renn 1936, Short et al. 1987), an estimated 29% of global cover has now been lost, reducing the habitat quality and trophic connectivity of the remaining populations (Valentine et al. 2007, Waycott 2009, Kallen et al. 2012). Such losses have prompted the monetization of their ecosystem services (Costanza et al. 1997, Barbier et al. 2011) and fueled countless efforts, at all levels of government and many non-governmental organizations, to monitor, conserve and restore seagrass systems (Fonseca et al. 1995, Short & Burdick 2006).

Of the 5 species of seagrass that inhabit the temperate North Atlantic, only two are found in New York waters, *Zostera marina* Linnaeus and *Ruppia maritima* Linnaeus (Short et al. 2007). Here, autecology generally favors the cold-adapted *Z. marina*, displacing *R. maritima* (a predominantly sub-tropical species) to shallower depths and annual life cycles. Although warming temperatures may signal an expansion for *R. maritima* in the (Paul Bologna, personal communication), *Z. marina* presently dominates the seagrass landscape, existing almost entirely as dense monoculture (Moore & Short 2006, Short et al. 2007). Like most seagrasses, *Z. marina* can be found in shallow nearshore and estuarine waters from the intertidal through 10 m; water clarity, wave exposure, current velocity, sediment texture, and surficial organic content all further define local habitat (Bradley & Stolt 2006, Moore & Short 2006).

Arising from the Potomogetonaceae during the mid-Cretaceous (Kato et al. 2003), *Z. marina* is thought to have colonized the western Atlantic via the Arctic some 3.5 million years ago (Waycott et al. 2006, Vermaat 2009). One of perhaps four species in the family Zosteraceae, *Z. marina* is typical of the group's morphology (den Hartog & Kuo 2006). Phytomers consist of a node, inter-node, tubular leaf sheath, a reduced ligule, a strap-like blade and an axillary bud (Gibson 2009). Vegetative growth proceeds vertically by repetition of the phytomer (forming a shoot) and horizontally via clonal repetition along a lignified rhizome (den Hartog & Kuo 2006). Two groups of adventitious roots subtend each shoot and all rhizome nodes (den Hartog & Kuo 2006). Together, the shoot and rhizome form the fundamental unit of clonal proliferation or the 'ramet' (sensu Harper 1977). Monopodial branching proceeds under the control of a terminal or dominant apical meristem (Moore & Short 2006) while trailing rhizome internodes degrade over time (Burkholder & Doheny 1968, Reusch et al. 1998). Within this simple architectural plan, enormous morphological plasticity has been observed. Shoot length and width, leaf number, rhizome diameter, above- to below-ground biomass ratio, leaf turnover and shoot appearance rates can all vary up to an order of magnitude, and have been shown to be sensitive to environmental parameters, including temperature, nutrient availability, light and hydrodynamic regime (Short 1983, Larkum et al. 2006).

Sexual reproduction in perennial *Z. marina* occurs annually after the second year of life (Granger et al. 2003, Plus et al. 2003, Moore & Short 2006). Inflorescences are monoecious, branching sympodially to form a panicle of rhipidia (Churchill & Riner 1978). Each rhipidium contains a variable number of spathes, within which a flattened spadix, analogous to the terrestrial grass spike, encloses an alternating sequence of male and female flowers in a 2:1 ratio (Ackerman 2006). Flowering phenology in *Z. marina* varies latitudinally and is strongly regulated by temperature; in New York, floral induction occurs mid- to late-fall, primordial inflorescences appear in January at 0.5-3˚C, anthesis occurs in mid-May at 15˚C and fruit maturation is completed by the end of June at temperatures above 21˚C (Churchill & Riner 1978, Silberhorn et al. 1983). Pollen is filamentous and hydrophilic, delivered by water currents to receptive stigma (Cox et al. 1992, Ackerman 2006). *Z. marina* is self-compatible (Ackerman 2006). Inbreeding is regulated at the spadix level through protogynous floral development; however, selfing via inter-ramet geitonogamy can be quite high in monoclonal patches (Reusch 2001, Rhode & Duffy 2004, Waycott et al. 2006). Annual seed production contributes to transient seed banks (1000s of seeds m^{-2}) that germinate in the fall at temperatures below 20˚C, although yearlong (i.e., over winter) dormancy has been suggested (Orth & Moore 1983, Olesen & Sandjensen 1994, Orth et al. 2000). Issues of seed viability, seedling safe-site availability, bioturbation and seed predation (granivory) have all received scant attention in the literature, but are likely to be important determinants of seed bank size and annual recruitment patterns (but see Churchill 1983, Fishman & Orth 1996, Orth et al. 2000, Giba et al. 2003, Valdemarsen et al. 2011). Dispersal of seeds and propagules via tidal and oceanic currents have been shown to vary over 3 orders of magnitude (1 – 1000 m) depending on the form of the diaspora (i.e., negatively buoyant seeds or positively buoyant rhipidia), although long distance events probably occur at low frequencies (Kendrick et al. 2012). Recently, examples of vertebrate dispersal have been found, with several turtle, fish and water-fowl species forwarded as possible vectors (Sumoski & Orth 2012). Clearly, much more work is needed to understand the efficacy and dynamics of sexual reproduction and dispersal within and among *Z. marina* populations; however, the importance of sexual recruitment to bed establishment has long been appreciated. Numerous studies have highlighted seedling recruitment in the invasion and re-growth of denuded areas (Orth & Moore 1986, Plus et al. 2003, Lee et al. 2007), as well as in the recurrence of annual populations (Poumian-Tapia & Ibarra-Obando 1999, Orth et al. 2000). What remains uncertain is its role in the maintenance and growth of existing meadows.

Empirical estimates of seedling survival are routinely low (i.e., <10%), with the asymmetric competition (primarily for light) between larger, subsidized clonal recruits and smaller, newly established germinants resulting in high mortality rates for seedlings recruiting to established beds (Harrison 1993, Olesen 1999, Kim et al. 2014). Formal treatment of these issues requires true demographic study; however, until recently, this was limited by the challenges of identifying clone membership among visually identical ramets (Orth & Moore 1983). Modern genetic approaches using polymorphic microsatellites have just recently allowed investigators genet-level observation, and are beginning to show that elements of the mating system (i.e., selfing and outcrossing rates, multiple paternities, seed and pollen dispersal kernels) are capable of yielding genotypic structure at hierarchical scales within seagrass landscapes (Hammerli & Reusch 2003, Becheler et al. 2010, Zipperle et al. 2011). This has re-invigorated debate over the role of sexual recruitment in the maintenance of clone diversity over time (Reusch 2006, Becheler et al. 2010). That is, whether or not seedling recruitment merely catalyzes patch growth, after which competition winnows genotypic diversity and excludes further seedling recruitment, or whether repeated bouts of sexual recruitment can shape clone structure and contribute to patch growth and coalescence, a dichotomy often referred to as initial seedling recruitment (ISR) vs. repeated seedling recruitment (RSR) in the terrestrial literature (Eriksson 1993).

In coastal New York, seagrasses have undergone a steady decline punctuated by massive contractions and limited recoveries. Seagrasses once dominated all three estuarine systems on Long Island: the Long Island Sound (LIS), the Peconic Bays (PB) and the South Shore Estuaries (SSE; including Great South Bay, Moriches Bay and Shinnecock Bay). Despite a lack of historical mapping data (c. 1930's) and the limited existence of seagrasses in the SSE prior to inlet formation in 1931 (Dennison et al. 1989), it has been estimated that as much as 80,937 hectares may have once been found within the state (NYS Seagrass Taskforce 2009). Today, only 8,823 hectares are thought to remain, a loss of nearly 90% over the last 80 years (NYS Seagrass Taskforce 2009). Affecting this change has been a dramatic mix of anthropogenic stress and ecological perturbation. Two major events have received the bulk of critical attention, the pan-Atlantic wasting disease of the 1930's and a series of 'brown tides' during the mid-1980s.

Between 1931 and 1933, a northward spreading fungal infection plagued *Z. marina* populations on both sides of the Atlantic, destroying 90% of the known distribution and locally extirpating the species for as long as 30 years (Tutin 1938, Short et al. 1987). Transmitted by contact, infected plants presented black necrotic lesions that spread quickly (0.8 mm h^{-1}), depressing photosynthetic activity and cellular integrity, eventually killing the hosts (Ralph & Short 2002). Muehlstein et al. (1991) confirmed a protistan slime-mold, *Labyrinthula Zosterae* Porter et Muehlstein (Labyrinthulomycota) as the principal causative agent, and others have suggested that heat stress was a contributing factor to its virulence (Tutin 1938, NYS Seagrass Taskforce 2009). Today, *Z. marina* populations on both sides of the Atlantic suffer from intermittent to chronic infection, although no significant mass mortalities have since been recorded (NYS Seagrass Taskforce 2009).

Then, in the spring and early summer of 1985, the first of what are now annual 'brown tides' occurred in the northeastern United States (Sieburth et al. 1988, Milligan & Cosper 1997). Blooms of the pelagophyte, *Aureococcus anophagefferens* Hargraves et Sieburth (Sieburth et al. 1988) had immediate effects upon local shellfish and seagrass communities (Gobler et al. 2005). In Long Island (NY), bay scallop (*Argopecten irradians*) and hard clam (*Mercenaria mecenaria*) fisheries reported near complete failures with monetary losses in excess of \$3.3 million yr^{-1} (Gobler et al. 2005). Initial studies by the Suffolk County Department of Health Services, Office of Ecology were soon augmented by numerous divisions from within the National Oceanic and Atmospheric Administration, NOAA (Gobler et al. 2005). After two significant symposia (1988 and 1995), the formation and funding of several research initiatives, and more than 20 intervening years of study, a number of top-down and bottom-up regulatory pathways have been forwarded to explain brown tide occurrence (Gobler & Sanudo-Wilhelmy 2001, Nuzzi & Waters 2004, Gobler et al. 2005, Deonarine et al. 2006). These include (1) a shift in the nutrient environment toward reduced DIN:DON and increased DOC caused by the sewering of municipal wastes and the breakdown of non-brown tide blooms (Gobler & Sanudo-Wilhelmy 2001, Gobler et al. 2005), (2) the elimination of top-down control by meta- and protozooplankton due to prey selectivity and even-number (2 or 4) trophodynamics (Deonarine et al. 2006) and (3) the loss of non-selective suspension feeding bivalves (*Mercenaria mercenaria* and *Argopecten irradians*) to both overfishing and bloom-related demise (Milligan & Cosper 1997, Gobler et al. 2005).

Regardless of the proximate causes, the effect of the initial blooms on *Z. marina* coverage in Great South Bay was dramatic. Millegan and Cosper (1997) reported the shoaling of 1% light levels to less than one meter, resulting in more than a 50% reduction in the ecological compensation depth of seagrasses; Dennison (1988) observed the loss of 112 km^2 of submerged aquatic vegetation (SAV); and Cosper et al. (1987) estimated that as much as 55% of pre-bloom seagrass habitat in GSB and PB was functionally eliminated (GSB, 50 km^2; PB, 65 km^2). Similarly, Dennison et al. (1989) found significant declines in 3 of 5 sub-estuaries for which pre- and post-bloom distributions could be amalgamated. Today, brown tide blooms are a recurring event in the shallow estuaries of Long Island and are widely regarded as a significant threat to the establishment and persistence of seagrasses in New York waters (NYS Seagrass Taskforce 2009).

The preceding history of brown tides on Long Island was provided in some detail because it clearly illustrates a common paradigm in catastrophic seagrass loss. Prior to a massive die-off, managers and academicians generally lack quantitative knowledge regarding inter-annual variability in seagrass distribution. Existing data are too often restricted to *in situ* sampling and aerial mapping conducted at insufficient and arbitrary temporal and spatial scales, leading to a poor understanding not only of the total area of coverage but also of the trends and forcing functions that pre-date the catastrophic event. For example, Dennison et al. (1989) were forced to draw inferences on the effects of brown tide from historical seagrass data gleaned from transect/quadrat sampling and aerial photography from 1967, 1977-78 and 1988, conceding that as much as half of the losses had occurred 8 years prior to the first brown tide. In fact, many of the bays showed near linear declines across the examined period (Dennison et al. 1989). Further, bay-wide estimates of habitat loss by Cosper et al. (1987) appear to have been extrapolated using spot measures of deep edge of bed depth and bathymetry without direct measurement of meadow area, and at least two of the bays surveyed by Dennison et al. (1989) lacked comparable historical data altogether (i.e., the Peconic Bays and Gardiners Bay).

In many, if not all cases, multiple anthropogenic stressors (see above) associated with human population growth have enacted ecological change well before the collapse of seagrass meadows (van Katwijk et al. 2010). On Long Island, overfishing and habitat degradation eroded historically important top-down controls on water column production and algal growth decades before the first brown tide (NYS Seagrass Taskforce 2009). In some cases, data on these impacts are available to managers, particularly when the grazers are of economical importance, but this is not often the case when indirect effects of fisheries release trophic cascades that negatively impact invertebrate grazer guilds (Heck et al. 2000) or enhance mesopredator control of molluskan prey; e.g., mud crab predation on post-set bivalves (Carroll 2012). Then, in as much a symptom of ecosystem malfunction as a cause of decline, an algal bloom, disease or environmental fluctuation precipitates the loss of already stressed seagrasses in quantities measurable by coastal observers, sparking concern among varied stakeholders and prompting an academic and governmental response. Frequently, as was the case for the SSE during the 1980's, the proximate cause is articulated as a reduction in light transmittance and the fatal shading of seagrasses resulting from nutrient-replete water column production. The central problem with this conceptual model is that it follows from an overly simplistic view of the ecosystem that, although generally palatable to the lay public (i.e., human sewage causes problems when released into the local environment) and easily championed by local officials, misses something fundamental about how these coastal ecosystems function (Heck & Valentine 2007) and fails to promise a return of seagrasses once nutrient inputs are curtailed (NYS Seagrass Taskforce 2009). For example, the brown tides of 1980's undoubtedly contributed to the loss of significant portions of pre-bloom *Z. marina* distributions; however, in the most comprehensive analysis of coverage trends during that time, Dennison et al. (1989) found marked increases in only 2 out of 5 sub-estuaries afflicted with brown tide (Moriches Bay and Shinnecock Bay increased 80% and 83%, respectively). And, in 2009, a year following the densest brown tide recorded on Long Island (Gobler 2008), bi-annual surveys of *Z. marina* coverage in the Fire Island National Seashore (FIIS) revealed widespread gains relative to 2007 (Bradley Peterson, unpublished data).

These findings support the notion that seagrass responses to nutrient loading at the landscape scale are non-linear, sensitive to initial conditions of meadow stress (e.g., sulfide toxicity, epiphyte load, temperature), influenced by trends in historical coverage (i.e., system memory and inertia), and heavily dependent upon top-down controls on water column production. They also emphasize our inability to predict seagrass responses at meaningful spatial scales, both in terms of decline and re-growth. In fact, despite numerous efforts to model depth limits (Lathrop et al. 2001, Greve & Krause-Jensen 2004), assess habitat suitability (Kemp et al. 2004, Short & Burdick 2006), predict species distribution patterns (Lanyon & Marsh 1995, Tomasko et al. 2005, Bekkby et al. 2008, Grech & Coles 2010, Vacchi et al. 2012, Downie et al. 2013, Rubegni et al. 2013) and describe emergent properties of vegetative growth (Kendrick et al. 2005, Brun et al. 2006, Sintes et al. 2006, Wong et al. 2011), very little is known about the mechanisms of spatial change at the ramet, genet or patch levels beyond the overarching influence of light availability, nor have the dynamics of recovery been sufficiently constrained so as to offer predictive or forecast capabilities (Morris & Virnstein 2004, Costello & Kenworthy 2009, Cunha & Santos 2009).

The modern era of coastal management has seen marked improvement in the allocation of resources for spatially explicit inventories of aquatic resources, yet managers routinely operate with poor knowledge of seasonal distributions or reliable estimates of coverage variance at the sub-estuary scale. Mapping efforts remain the product of systematic site visitation, transect/quadrat sampling and aerial overflights conducted at multi-year scales. At present, the available history for any given site is less than 10 years (but see Morris et al. 2000, Bell et al. 2008, Costello & Kenworthy 2009), and often does not include sufficient environmental data to correlate with temporal change (Kendrick et al. 2005). This leaves anecdotal linkages rather than empirical ones to guide the interpretation of interannual trends, and erodes the recursive nature of prediction and response. Open questions remain regarding (1) the necessary scales of observation, (2) the environmental drivers or correlates that need to or can be measured, (3) the ecological parameters capable of influencing landscape scale distribution and (4) the best ways to model bed establishment and expansion.

This dissertation will seek to address these questions, adding to our knowledge of process-pattern interaction across a range of spatiotemporal scales and relating that dynamism to climatic condition. I carried out a series of remote sensing analyses, repetitive field surveys and *in situ* manipulations to advance the following objectives:

(1) To add more to our understanding of areal change than simple models of nutrient loading and inferences of light limitation, and to derive more from high resolution mapping than statistics of gain and loss. Specifically, I will examine how physical forces, sediment chemistry, temperature and plant architecture shape distribution patterns and constrain plant performance (e.g., persistence, vegetative growth and reproductive success/output).

(2) To explore the role of resource heterogeneity in the plastic responses of rhizome architecture (clonal foraging theory), biomass allocation (optimal partitioning theory) and physiological integration (division of labor theory), and to assess how these adaptive strategies interact at the patch level to drive differential edge growth.

(3) To combine high resolution, decade-long seagrass mapping with polymorphic microsatellite analysis to examine the interactive effects of pollination and seed dispersal distance on the dynamics of sexual recruitment across a range of spatial scales (centimeters to decameters)

(4) To develop a statistical forecasting model associating annual patch emergence rate (i.e., seed-borne recruitment) with time-lagged estimates of relative wave energy, atmospheric condition and water temperature.

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Chapter 2

Experimental evidence for clonal foraging in *Zostera marina* (Linnaeus)

Statement of Author Contribution

Study conception and design:	Furman, Jackson and Peterson
Acquisition of data:	Furman, Jackson and Peterson
Analysis and interpretation of data:	Furman
Drafting of manuscript:	Furman
Critical revision:	Furman, Jackson and Peterson

ABSTRACT

Clonal foraging in response to heterogeneously distributed water, light or mineral resources has been demonstrated for a number of terrestrial plant species. The existence of similar behavior in seagrasses and the cross-scale effects of clonal foraging on patch development, however, have not fully been explored. Our objective was to establish that spatial exploration by independent ramet clusters could generate emergent patch behavior consistent with clonal foraging theory. We also examined the effect of nutrient amendment on reproductive effort and seedling recruitment. Working in Shinnecock Bay, New York, USA, with the clonal marine angiosperm, *Zostera marina*, we attempted to stimulate directional growth along ten patch edges over a two-year period using subterranean fertilizer. Changes in ramet demography, patch expansion, seedling emergence and reproductive effort were quantified through repeated shoot censusing. We found that nutrient addition accelerated patch expansion. Enriched edges also exhibited significantly higher shoot densities, indicating that a ramet proliferation, selective ramet placement or a combination of the two responses had occurred. The seasonality of this effect was different between treatments and consistent among years, suggesting that a fundamental shift in the pattern and phenology of seagrass growth had occurred. Within patches, no detectable differences in per capita branching rates, demographic stability or reproductive effort were observed. This study provides the first evidence of foraging behavior in *Z. marina*, and offers new insight into its seasonal growth patterns during the as yet poorly understood colonization period.

INTRODUCTION

The modular growth of clonal plants provides an observable framework from which to explore the genet-environment interaction in both space and time. As an iterative record of plant responses, the spatial structure of genet growth (termed` 'ramet architecture') is a complex product of anatomical constraints, ontogeny and phenotypic plasticity (Huber et al. 1999). For many species, natural variation in key parameters, such as branching angle, branching frequency and spacer length, can lead to large differences in ramet placement and distribution (de Kroon and Hutchings 1995). Simulation models of clonal growth for a number of terrestrial (Cain 1990; Cain and Damman 1997; Cook 1985; Wong et al. 2011) and marine (Brun et al. 2007; Marba and Duarte 1998, 2003; Sintes et al. 2006) species have shown that this plasticity can explain emergent patch behavior, including: (1) nonlinear edge growth, (2) effective sweeping of un-vegetated space, (3) self-thinning and (4) central die-back or ring-like growth patterns.

Clonal plants, however, typically inhabit heterogeneous environments. In fact, spatiotemporal variability in the distribution of mineral resources is likely to be a universal features of terrestrial (de Kroon and Mommer 2006; Jackson and Caldwell 1993; Rajaniemi and Reynolds 2004) and marine (Jensen and Bell 2001) ecosystems, with patchiness driven by plant-soil (Jackson and Caldwell 1993; Stuefer 1996) and animal-sediment (Peterson and Heck 2001; Peterson and Heck 1999) interactions, respectively. Clonal plants have shown the capacity to exploit these landscapes by asexually reproducing at rates differential to resource availability (i.e, ramet proliferation) and/or adjusting their architecture to maximize ramet placement within high quality zones (i.e., clonal foraging) (Birch and Hutchings 1994; Humphrey and Pyke 1997; Ikegami et al. 2007; Oborny and Englert 2012). Together with similar processes acting on individual ramets (i.e., root proliferation and root foraging; de Kroon and Mommer 2006), elements of clonal growth have been implicated in controlling resource acquisition (Oborny and Hubai 2014; Sutherland and Stillman 1988), invasiveness (Keser et al. 2014; Song et al. 2013) and competitive dominance (Grime 2007; but see Kembel et al. 2008).

For many clonal plants, neighboring ramets remain vascularly connected, existing as integrated physiological units ('IPU'; Watson 1986) or ramet hierarchies (Briske and Derner 1998). For each IPU, translocation of photosynthate, water and nutrients (Price and Marshall 1999) can subsidize the growth of impoverished ramets (Oborny and Hubai 2014), often resulting in uptake specialization among IPU members (i.e., 'division of labor'; Stuefer 1996).

Modeling (Oborny and Hubai 2014) and empirical (Humphrey and Pyke 1997; Roiloa and Hutchings 2013) studies, conducted primarily on terrestrial species, have demonstrated that this sort of integration greatly influences foraging behavior, enhancing foraging efficiency and increasing competitive advantage, particularly when coupled with resource storage and a root or ramet proliferation response. However, because IPUs respond to resource availability collectively, integrated clones often exhibit phenotypic responses counter to those predicted for isolated individuals (Roiloa and Hutchings 2013); for example, root biomass of unitary plants lessens in nutrient replete soils, while IPUs tend to increase the root biomass of ramets within resource rich zones.

Further complicating the relationship between morphology and clonal trait expression are the effects of physiological plasticity. Localized changes in root biomass and architecture are effective strategies for temporally stable or predictable resources, but time lags and construction costs ill-adapt them to transient nutrient pulses. Resources of this type are often acquired by elevating nutrient uptake rates at the point of contact (Kembel et al. 2008; Keser et al. 2014; Roiloa and Hutchings 2013). Kinetic adjustments have been shown in terrestrial species to satisfy whole-plant demand from extremely small fractions of the root system (Caldwell 1994). Similar malleability in uptake performance underpins the ‘sit and wait’ strategy (i.e., ‘high scale’ root foraging) of some unitary, as well as clonal, plants (Grime 2007; Stuefer 1996). Given the rich interplay between phenotypic and physiological plasticity, visually assessing the relative contribution of clonal traits and generating a priori predictions regarding ramet dynamics can be quite challenging even for single genet systems (Brun et al. 2006; Humphrey and Pyke 1997), let alone for patches composed of multiple genets or species.

Architectural responses to variations in mineral resources have not been sufficiently investigated for seagrasses; however, the effects of nutrient addition on plant and meadow performance have been well studied. In a recent review of 28 studies involving 14 seagrass species, Cabaco et al. (2013) found that short-term fertilization resulted in nonlinear biomass-density relationships – with increasing shoot biomass and density occurring below species-specific thresholds and declines, indicative of self-thinning, above. Others have found reduced belowground biomass investment with increased nutrient loading, consistent with optimal partitioning theory (Lee and Dunton 2000; Statton et al. 2014 and references within; Wicks et al. 2009). Translocation of resources among physiologically connected individuals has been confirmed for many species, including *Cymodocea serrulata, Cymodocea nodosa, Halophila stipulacea, Halodule uninervis, Posidonia oceanica, Thalassodendron ciliatum, Thalassia hemprichii* and *Zostera noltii* (Duarte and SandJensen 1996; Marba et al. 2002; Vermaat 2009), but has not yet been demonstrated for *Zostera marina* or *Zostera novazelandica* (Duarte et al. 2006). As with terrestrial species, subsidies to patch edges are more common for species with relatively high growth rates than among k-selected taxa (Vermaat 2009). To date, only a single study (Jensen and Bell 2001) has looked at the effect of sediment nutrient heterogeneity on the shape of seagrass patch development, despite the clear importance of vegetative growth to patch expansion and coalescence (Duarte et al. 1994; Duarte and Sandjensen 1990). Jensen and Bell (2001) used subterranean fertilizers to examine ramet architectural responses by *Halodule wrightii* along a tidally influenced patch edge over a 5-week period. They reported significant reductions in rhizome internode distance and increased shoot biomass for phosphorus (P), but not for nitrogen (N) or N and P treatments (branching frequency was unchanged). Neither were they able to match natural variation in these parameters to in situ soil conditions.

As a first step toward understanding the potential role of clonal foraging and ramet proliferation in the pace and shape of *Z. marina* colonization, we attempted to stimulate directional growth at the patch level using asymmetric nutrient additions within ten radially expanding, sub-tidal patches of perennial *Z. marina*. In the absence of clear a priori expectations regarding the form of this response, we simply asked: (1) can heterogeneously distributed mineral resources elicit a passively observable response in ramet configuration or demography, (2) at what spatial scale does this pattern manifest within a multi-genet environment, (3) what effect does clonal foraging and/or small-scale nutrient amendment have on reproductive effort, and (4) what role does seedling recruitment play in patch-scale foraging? In this study, we aimed to establish that spatial exploration by independent IPU clusters could generate emergent patch behavior consistent with clonal foraging theory.

MATERIALS AND METHODS

Study Site

All fieldwork was conducted in Shinnecock Bay, a backbarrier lagoon in southeastern Long Island, New York, USA. Tides are semi-diurnal with a range of 0.8 meters (USACE 2004). In the southeast portion of the bay, roughly 400 m from shore and 2.5 km east of the Inlet (40.857237° N, 72.450289° W), we selected ten mono-specific *Z. marina* patches (five in October 2011, increased to ten in July 2012). All were less than 4 m^2 and at least 0.5 m from adjacent seagrass at the time of choosing. Depths ranged from 0.33 - 0.39 m MLLW (mean ± 1 s.d.: 0.36 ± 0.02 m). Surficial sediments consisted of siliceous sands and were uniformly low in organic content (< 1% by loss on ignition at 500 ˚C for 5 h, B. T. Furman unpubl.).

Experimental Design

At each patch, permanent markers were installed to allow for consistent placement of a 4-m^2 quadrat with 100 equally sized cells. Counts of vegetative and generative shoots were conducted once in 2011 and 4x per annum, 2012 thru 2014. Periodicity corresponded to seasonal shoot growth and flowering cycles, with sampling at the onset of growth, during the time of maximal flowering, at peak aboveground biomass, and at or near the end of the growing season (i.e., March/April, May, July and October, respectively). During the spring, mid-summer and fall periods, one 100-g 15:3:3 (N:P:K) Jobe's Tree and Shrub® fertilizer spike was added to the center of every cell in each of two nonconsecutive quadrants; i.e., 25 per quadrant in an upper-left/lower-right or lower-left/upper-right arrangement, randomly selected at the start of the experiment. A pilot study, conducted in sub-tidal mesocosms using similar beach sand, confirmed the presence of a stable nutrient signal out to 20 cm. This pattern was persistent in quiescent, tidal conditions over the course of several days to weeks. Therefore, we anticipated a spatially limited, triannual pulse of N and P availability.

Physical confirmation of nutrient uptake (by *Z. marina*) and treatment contrast was prevented by *Mytilus edulis* recruitment during the spring of 2014. Settlement of mussel spat occurs annually in this portion of the bay, with individuals remaining confined to the leaves of *Z. marina*, and perishing as juveniles (approx. 5-10 mm, total length) to heat stress and predation by mid to late summer (B. J. Peterson, person. obs.). In October of 2013, however, recently settled juveniles were beginning to migrate to the sediment-water interface. In 2014, many had recruited to form adult aggregations. This had the dual effect of (1) inhibiting effective vegetative shoot censusing, as byssal threads tended to mangle and bind leaf material, and (2) contributing to sediment nutrient pools within ambient quadrants (Vinther et al. 2012). Unexpectedly, generative shoots were unaffected by mussel presence, arising from among even the densest mussels. Because floral induction occurs in mid to late fall (Churchill and Riner 1978), concurrent with initial mussel migration – but prior to mussel maturation – and because no impact on floral condition was observed, we chose to terminate vegetative growth analysis in October 2013 and reproductive analysis in May 2014. By early summer of 2014, mussel biomass and density had severely eroded patch condition, barring any material assessment of *Z. marina* nutrient concentration or description of rhizome architecture.

As a proxy, vegetative leaf tissue was sampled from twenty, unaffected, similarly sized patches located within the same meadow but unaffected by mussels. These patches were amended (10 ambient and 10 enriched) at the same periodicity (beginning in 2012), using the same fertilizer, but at a quarter of the application density. Eight weeks following nutrient addition, three replicate groups of 5 haphazardly selected shoots were collected from each patch. The two youngest leaves from each shoot were retained, gently cleaned of epiphytic growth, and dried at 60°C for >72 h. Samples (N = 60) were then ground to a fine homogeneous powder with a mortar and pestle, and total carbon and nitrogen contents were determined by oxidation in a Thermo EA1112 elemental analyzer following Fourqurean et al. (1992). The single effect of treatment status on the C:N ratio of aboveground vegetative biomass was compared using a Student's t-test.

The Experimental Unit

The use of gridded quadrat data to track sub-patch, ramet demography presents a number of challenging statistical questions. Namely, (1) at what spatiotemporal grain does sample independence permit unbiased inference testing, (2) how does the presence of active growth margins affect cellular estimates of shoot density, and (3) how does similarity in growth phase (i.e., recently colonized, unencumbered growth) and seasonal growth patterns influence spatiotemporal covariance structure?

The patches under study can be thought to exist in two dimensions as expanding masses of interdigitated IPUs, each of unknown density, genet composition and age. More precisely, the plagiotropic apical meristems responsible for ramet dynamics are, themselves, of unknown density, distribution and growth orientation (i.e., direction), receiving energetic subsidies from an unknown number of physiologically connected neighbors, while maintaining horizontal movement at seasonal rhizome elongation rates. Given that the underlying architecture controlling rhizome placement, and the precise demographic fate of these apicals, remain opaque to the passive observer, no remotely sensed measurement of sub-quadrat sample (i.e., at the cell or quadrant level) independence can be made. We can, however, deduce from available ramet spacer length (2 - 8 cm; Sintes et al. 2006), rhizome elongation rate (16 - 45 cm per yr; Duarte et al. 2006; Greve et al. 2005; Olesen and Sandjensen 1994), plastochrone interval (8.2 d; Uzeta et al. 2008), and nominal IPU size (growth axis length: <10 cm; B.T. Furman, pers. obs.) estimates that *Z. marina* growth could well be statistically independent at cellular grain sizes (20 cm x 20 cm), especially when sampled trimonthly, as a strong seasonality in shoot density has been found for this temperate species (Guidetti et al. 2002; Olesen 1999; Poumian-Tapia and Ibarra-Obando 1999). Such a view of patch growth likens cell-based measurements of ramet abundance to fixed-point observations of more mobile organisms, with turnover and movement of individuals occurring at much faster rates than the temporal grain of observation. Spatially, of course, the probability of seagrass appearance at patch edges and some proportion of demographic change between adjacent cells during successive time-steps must be the direct result of apical transfer among a subset of IPUs in neighboring cells. However, we argue that this influence is distributed unevenly across all 8 neighbors and may not be proportional to cell shoot density, as individual ramet location and growth orientation are not captured by gridded data. This is particularly true at active growth margins, where random cell placement grossly underestimates shoot density.

To properly quantify the spatiotemporal covariance structure, semivariance and autocorrelation analyses are required. These methods could not be used with our data, as the small size of our patches and a limited number of sampling events prevented sufficient spatial or temporal lag sizes. Alternatively, we investigated the influence of spatiotemporal covariance on cellular shoot density patterns using two complimentary simulation approaches. The percentage of cells with at least one significant Pearson correlation with a neighboring cell using an 8-neighbor rule (PCS), the median proportion of neighbors exhibiting a significant Pearson correlation (MPN) and the median of median significant Pearson correlation coefficients (MMP;

i.e., the median of medians derived from each set of neighboring cells) was calculated using vegetative time-series drawn from cells containing seagrass at the start of the experiment (i.e., 'baseline' cells). These values were tested against simulated distributions of the same metrics generated by random re-sampling within censusing periods (re-sampled with replacement, iterated 3,000 times). This approach was designed to remove any spatial and temporal autocorrelation, while preserving seasonal or annual trends that might have been conserved among patches. In a second simulation, we maintained baseline cell time-series while re-assigning them random positions within baseline patches (re-sampled with replacement, iterated 3,000 times). This eliminated spatial autocorrelation without affecting temporal dependence. Distributions from the two simulations were then compared using successive t-tests to examine the relative magnitude of spatial and temporal covariance. Finding no strong evidence for non-independence (see Results), we proceeded to examine the influence of asymmetric nutrient addition on patch development, variously defining the experimental unit at the cell, quadrant or patch level, depending on the scale of the question being asked and the resultant observational balance.

Cell-Level Dynamics

Consistent quadrat placement allowed for fine-scale accounting of vegetative growth dynamics over a 2-year period. At the cell level, we evaluated patterns in ramet-specific recruitment rate (RSR) and the coefficient of variation (CV) of ramet density. Calculation of RSR (d^{-1}) was adapted from Marba et al. (2005), taking the form:

$$RSR = \frac{\left(N_{t_1} - N_t\right)}{\left[\left(t_1 - t\right) \times N_t\right]} \tag{1}$$

where N_t and N_{t1} are the number of ramets at time t and t+1 , respectively. RSR was estimated for baseline cells and converted to seasonal anomalies by subtracting season means; balanced nutrient treatment representation was achieved by re-sampling with replacement. The mean seasonal anomaly was then calculated for each cell over the available observation period, eliminating the effect of repeated measurement. The main effect of nutrient addition was assessed using a one-way ANOVA on ranked data. To explore possible density-dependent effects of season and nutrient condition on cellular RSR, we simulated vegetative shoot recruitment over a 1-yr period for both nutrient enriched and ambient treatments. RSR estimates were calculated for all seagrass-containing cells. Seasonal durations were constructed using mean seasonal sampling intervals, yielding a model year of 362.73 d. At the start of a randomly selected season, a single ramet was placed within an isolated cell (one cell per treatment) and allowed to propagate for one year at rates re-sampled from appropriate season, treatment and cell density pools. To delineate initial cell density groups, empirical RSR distributions were binned into 5-ramet units (0 - 70 ramets per cell) based on the cell densities observed at the start of each season. Any time-step that produced a season-density-nutrient combination for which an estimate of RSR was unavailable was allowed to carry forward with no change in ramet abundance. All modeled cells falling to or below zero remained at that value for the balance of the model year. Final ramet densities (10,000 per nutrient treatment, N = 20,000) were compared using a one-way ANOVA. The coefficient of variation of ramet densities (shoots cell^{-1}) was calculated for all grass-containing time-series, beginning with either the zero preceding ramet recruitment or the initial baseline density. A two-way, Type III ANOVA on ranks was used to test for the single and interactive effects of nutrient enrichment and baseline condition (i.e., baseline or new growth) on cell-level shoot count CV.

Patch-Level Dynamics

Edge growth rate (EGR; cells per edge cell d^{-1}) was calculated for each quadrant at each sampling period, as

$$EGR = \frac{\frac{\sum D_{t_2}}{E_{t_1}}}{t_2 - t_1} \qquad (2)$$

where D is the distance score in number of cells at t_2 and E is the number of edge cells at t_1. A distance field, expressed as the number of quadrat cells from seagrass present in the previous time-step, was determined for each patch at each sampling period using an 8-neighbor rule; distance scores (D) represent the distance value achieved by each newly acquired cell during the following time-step. The number of edge cells (E) was determined using a 4-neighbor rule (as this encompasses the entire patch perimeter) and included an extra row on either side of the quadrant boundary to account for cross-border growth. EGR values are sensitive to un-mapped seagrass entering at the edges of the sampling quadrat. Such encroachment occurred in only 2 patches, and affected only 1 and 3 quadrants, respectively (10 cells total). These cells were disqualified upon first appearance, but allowed to factor into EGR calculation thereafter. A one-way ANOVA was used to test for the main effect of nutrient enrichment on EGR; data were converted to seasonal anomalies and ranked prior to analysis.

We also simulated annualized edge growth using EGR estimates for both nutrient enriched and ambient edges. EGR values were randomly selected by season and treatment (re-sampled with replacement, 10,000 iterations per treatment), and edge growth was simulated using mean seasonal durations to produce annual distance values, measured in distance score per edge cell. Modeled distributions for ambient and enriched treatments were compared using one-way ANOVA.

To quantify patterns in ramet density along the active growth margin (i.e., 'edge growth habit' or EGH), we calculated the sum of all ramets at time t_1 within the eight cells neighboring any central cell that had acquired seagrass during the following time-step (t_2). Neighborhood densities were then pooled by nutrient treatment. As with the EGR analysis, encroaching seagrass was removed upon first appearance. A one-way ANOVA on ranked data was used to evaluate the effect of nutrient enrichment on EGH.

Seedling Recruitment

Assuming that (1) local seed production varied positively with floral abundance, and (2) local seed retention exceeded trapping of exogenous seeds, then seedling recruitment should manifest as a spike in patch-level RSR during the season of maximum seedling emergence. This spike should also be proportional to the magnitude of the previous flowering event. To investigate this possibility, patch-level RSR values were calculated, converted to seasonal anomalies, and compared to whole-patch floral abundances using Pearson and Spearman correlations.

Reproductive Effort

Differential impact of nutrient treatment on reproductive investment was evaluated using (1) per capita flowering effort, (2) flowering probability by quadrat cell and (3) proportional flowering by quadrant as metrics of comparison. Because flowering is induced in the late fall, all flowers recorded in May were descendent from IPUs present during the previous October. To compare ramet-specific flowering intensity at the cell level, we standardized flowering abundance for all flowering cells in May to the vegetative shoot density observed within an 8-cell neighborhood (9 cells, total) during the previous October. These data were expressed as reproductive to vegetative shoot ratios (R:V) and analyzed using a two-way, interactive, Type III ANOVA on ranked data, with nutrient treatment and sampling period as fixed factors. The probability that seagrass-containing cells would undergo flowering was evaluated using logistic regression and ANOVA, with nutrient condition as a fixed factor. Cell-specific states (i.e., flowered or not flowered) from all three floral censuses were pooled by nutrient treatment and transformed via a binomial logit function prior to analysis. We used a one-way generalized linear model with a quasi-binomial error distribution to investigate the impact of fertilization on the proportion of flowering cells per quadrant, i.e., standardized to the number of seagrass-containing cells. The resultant model was evaluated for data dispersion, goodness-of-fit and cooks distance prior to interpretation.

Statistical Analysis

Parametric and non-parametric univariate analysis and data simulations were performed using the statistical software, R version 2.14.1 (R Development Core Team 2012). In all cases, response data were appropriately transformed to meet the assumptions of ANOVA. Effect patterns obtained using ranked data and/or unbalanced ANOVA designs were corroborated via permutation tests of main- and interaction-effect F-statistics; confirmatory results are not presented. Statistical significance was assessed at an alpha of 0.05.

RESULTS

Treatment Diagnostics

During the 720-day (n = 5; 444 d, n = 10) manipulative experiment, cell-level estimates of shoot density fell between 25 and 1,650 shoots m^{-2} (404 ± 305 shoots m^{-2}), exhibiting no clear long-term temporal trend. Mean density by sampling period ranged from 255 ± 161 to 566 ± 331 shoots m^{-2} with substantial seasonal and intra-patch variability. Percent coverage by patch (i.e., percent of sampling quadrat within which seagrass was present) began reasonably low at between 5 and 57% (33.1 ± 18.2%), reaching 58 to 92% (72.6 ± 12.7%) by October 2013. The ambient and enriched sections of each patch underwent similar changes: starting at 32.6 ± 19.8% and 33.6 ± 19.3% and ending at 69.0 ± 14.0% and 76.2 ± 16.4%, respectively. Only a single quadrant was filled during the observation period (an enriched quadrant within patch no. 6, July and October 2013), although quadrat-level treatment space was never exhausted. Maximum values were 90% and 94% for ambient and enriched halves, respectively. Patch growth did, however, commonly reach or exceed quadrat boundaries, continuing beyond our monitoring design to distances of no more than 40 cm.

Leaf tissue C:N analysis confirmed that nitrogen released by subterranean fertilizer spikes was (1) available to *Z. marina* roots and rhizomes, (2) still being incorporated into new growth 8 weeks post-addition, and (3) in excess of surrounding ambient conditions (square transformed, $t_{33} = 3.57$, $p = 0.001$). Mean enriched foliar C:N was 11% lower than ambient (20.67 ± 2.61, enriched; 23.22 ± 1.73 ambient). This supports the nutrient-limited status of *Z. marina* growth in Shinnecock Bay, particularly for populations inhabiting well-sorted, sandy sediments (Carroll et al. 2008; Wicks et al. 2009). Although direct measurement of treatment contrast and integrity could not be made, we maintain that pore-water transfer among adjacent quadrants was likely limited and could only have served to diminish treatment effects.

The Experimental Unit

A total of 331 quadrat cells contained seagrass at the start of the experiment. Of these cells, 85% had at least one significant correlation with a neighboring cell. Median PCS ranged from 0 - 1 with a median value of 0.40 (0.41 ± 0.28). Because only baseline cells were included in the analysis, the number of actual neighbors containing seagrass varied as a function of patch shape and cell position. The proportion of correlated neighbors was slightly influenced by this artifact, with mean numbers of significant neighbors rising linearly between 1 to 3.2 cells for 1- and 8-cell neighbor groups, respectively. The median correlation coefficient for significant neighbor relationships varied from 0.67 to 0.99 with a median of 0.88 (0.87 ± 0.07).

Both simulations yielded metric distributions with equivalent median and mean values. All three (PCS, MPN and MMP) were significantly different from random when re-sampled within sampling period (i.e., simulation one; $p<0.001$). Far fewer cells had a significant relationship with at least one neighbor, 0.39 ± 0.04 (range: 0.27 - 0.54). MPN was also lower, ranging from 0.00 to 0.125 (0.00 ± 0.002). Median MMP, however, was qualitatively similar with a mean of 0.85 ± 0.01 (range: 0.82 - 0.89). When cell-specific time-series were held constant, and only cell locations were permutated (i.e., simulation two), metric values more closely approximated actual data. PCS and MPN, but not MMP were significantly different from simulated distributions, 0.58 ± 0.03 (range: 0.45 – 0.68), 0.14 ± 0.02 (range: 0 - 0.25), and 0.89 ± 0.01 ($p = 0.868$; range: 0.87 - 0.91), respectively. T-test comparisons indicated that both simulations differed from each other by all three metrics (each significant at $p<0.001$).

By simply preserving cellular time-series, simulated PCS increased 47% over spatiotemporally random assignment, achieving a mean PCS that was 68% of the experiment value. This indicated that meadow-scale similarity in seagrass growth, particularly at the active growth margins, was responsible for the majority of the spatial covariance signal present in our data. That is, over bracketing of seagrass edges by gridded quadrat cells, resulting in artifactually low density estimates, combined with similar rates of ramet proliferation, led to correlated growth time-series even among spatially disjunct cells; to a lesser extent, this was probably true for all baseline cells, as the ten experimental patches were of roughly the same age and experienced identical environmental conditions. This assertion was corroborated by MMP patterns. Because the spatiotemporally random simulation yielded an MMP value that was 97% of the actual experimental value, and since the two simulations differed by only 4%, we concluded that true temporal autocorrelation was negligible at the cell level, appearing largely as product of limited cell density ranges and comparable rates of ramet proliferation among patches. As a result, we argue that ramet censuses were reasonably independent at the cell level, allowing for statistical inference testing at sub-quadrat scales; nevertheless, we favored quantitative metrics that limited repeated measurement and interpreted the results of cellular comparisons with caution, as the effect of neighbor correlation on Type I error could not be discounted.

Cell-Level Dynamics

Ramet-specific recruitment rates, calculated for baseline cells, ranged from -0.016 to 0.122 d^{-1} (enriched: n = 168, 0.007 ± 0.014 d^{-1}; ambient: n = 163 0.008 ± 0.018 d^{-1}). Seasonally-adjusted mean RSR values were unresponsive to nutrient addition ($F_{1,329}$ = 0.407, p = 0.524). Coefficient of variation of ramet density (1.016 ± 0.451 and 1.015 ± 0.435 for ambient and enriched cells, respectively) was equally unaffected by nutrient addition. Two-way interactive Type III ANOVA on ranked data revealed anticipated effects of baseline condition (i.e., established cells exhibited lower CV values than cells that had begun with 0 ramets; $F_{1,766}$ = 197.296, p<0.001); however, the main effect of nutrient addition was not significant in the full model ($F_{1,766}$ = 0.204, p = 0.651) nor in a one-way ANOVA on only new growth cells ($F_{1,437}$ = 0.252, p = 0.616).

Visual inspection of ramet recruitment as a function of initial cell density (Figure 1) revealed patterns consistent with cell crowding, as per capita recruitment fell to zero at between 5 and 35 shoots per cell (125 – 875 m^{-2}) depending on the season. Nutrient-enriched cells generally outperformed ambient at the lower end of the density spectrum, particularly in the spring; however, this pattern was reversed in the fall, when enriched RSR mirrored winter profiles, while ambient cells maintained higher recruitment rates. Simulated on an annual basis, these differences resulted in a statistically significant, 31% increase in recruitment within enriched cells ($F_{1,19998}$ = 943.5, p < 0.001). Final ramet densities ranged from 0 to 44.59 $cell^{-1}$; ambient and enriched means were 8.12 ± 6.00 and 10.64 ± 5.58 $cell^{-1}$, respectively.

Patch-Level Dynamics

Monthly edge growth rate ranged from 0.00 to 1.36 cells per edge cell. The upper limit was on par with patch expansion as visually assessed in quadrat time-series; however, because seagrass expansion occurs at scales smaller than the quadrat cell, often incompletely filling newly acquired cell-space, there is no direct method to convert EGR to sensible distance units. Mean monthly EGR was 0.153 ± 0.171 and 0.206 ± 0.224 cells per edge cell for ambient and enriched quadrants, respectively. Results of the one-way ranked ANOVA on seasonally adjusted EGR confirmed a significant nutrient response ($F_{1,\ 257}$ = 9.492, p = 0.002) with 35% faster growth for fertilized seagrass edges. Simulations based on empirical EGR distributions found

38% further annual spreading relative to ambient patches ($F_{1,19,998}$ = 3,428, p<0.001). On a seasonal basis, the effect of nutrient addition was not uniform (nutrient x season: $F_{1,3,251}$ = 4.0903, p = 0.007). A visual assessment of annual EGR patterns revealed distinctly different annual cycles in edge growth, with fertilized quadrants out-performing ambient ones during all seasons except autumn, during which ambient growth continued at near-summer pace, while enriched edges decelerated to winter levels (Figure 2). This pattern was consistent among years.

Edge growth habit analysis identified a total of 586 examples of edge expansion. In 2.7% of these cases, gains greater than one cell from the edge were recorded. This happened 3 times more often in enriched (n=12) than ambient (n=4) quadrants; however, because neighboring cell densities were not quantified prior to edge advancement, these data were removed from our EGH analysis. Among the 264 ambient and 306 enriched instances of single-cell expansion, neighboring ramet abundance varied from 1 to 152 vegetative shoots (equating to 3 and 475 shoots m^{-2}). Fertilization resulted in a significant 12% increase in neighboring ramet density preceding lateral growth ($F_{1, 568}$=7.548, p=0.006), representing a shift in ramification toward a more phalanx-like pattern of advance.

Seedling Recruitment

Experimental patches varied in vegetative shoot abundance from 44 to 2,444 (748.1 ± 556.1) shoots per patch over the course of the experiment with nearly all patches exhibiting seasonal oscillation amid net-positive inter-annual growth. Generative shoot abundance was equally variable, 0 to 473 (167.6 ± 146.9) per patch, with increasing floral abundance and R:V across the observation period. Successive Pearson and Spearman correlations between patch-level floral abundance and RSR seasonal anomalies provided a crude estimate of seedling recruitment (Figure 3). Of the 8 time periods we examined, spanning > 1.5 years post-flowering, we found significant evidence of seedling contribution during the second summer only, that is, between May and July of the year following seed dispersal. The relationship between flowering magnitude and RSR at the patch level was relatively strong, with a Pearson correlation coefficient of 0.90 (p = 0.039). This supports previous genetic work that showed germinants readily compete with established genets at this site (Furman et al. in press).

Intriguingly, both Pearson and Spearman correlation tests identified a significant, albeit small, reduction in per capita ramification during the first winter following flowering (Pearson:

r=-0.59, p=0.022; Spearman: r=-0.65, p=0.008). Given that genets begin flowering in their second spring (Granger et al. 2003), RSR reductions in the winter reflect a two-year lifespan for a portion of vegetative growth associated with a flowering IPU. Two-year longevity of *Z. marina* shoots was first proposed by Pedersen in 1913, and later reviewed by Duarte et al. (1994). Alternatively, this signal might represent the shunting of resources away from younger IPU members (Olesen 1999), resulting in their loss, or an overall reduction in branching rates for sexually active IPUs.

Reproductive Effort

A total of 985 flower-containing cells were censused during the course of the experiment (ambient: 448, enriched: 537). Conventional R:V ratios incorporate vegetative information collected only at the time of flowering. For comparison, our cell-level data, calculated in this way, varied from 0 to 0.72 with an overall mean of 0.23 ± 0.20. Annual means were 0.13 ± 0.18, 0.14 ± 0.11 and 0.37 ± 0.20 for 2012, 2013 and 2014, respectively. However, because our assessments were made at comparatively small spatial scales, encroachment of flowering ramets from adjacent cells could not be ignored; indeed, 7 to 16% of flower-bearing cells were unoccupied during the previous October, indicating that cross-cell transfer of flowers was a measurable component of cellular R:V. Furthermore, the magnitude of correlation between reproductive and vegetative shoot abundance was consistently higher for October than for any other time period, regardless of whether the target cell or an 8-cell neighborhood was used. Despite diagnostic evidence that our revised R:V metric more accurately depicted cell-level dynamics, no significant response to fertilization was found in either the interactive model ($F_{1,971}$ = 0.1101, p = 0.740) or a one-way model with nutrient condition as a fixed factor ($F_{1,975}$ = 0.0232, p = 0.879). Similarly, no effect on the probability that grass-containing cells would flower [df = 1/1352, deviance = 1.1729, $p(\chi^2)$ = 0.279] or on the proportion of flowering cells per quadrant [$F_{1,96}$ = 0.2597, p(>F) = 0.612] was observed.

DISCUSSION

Within the scope of phenotypic plasticity lies the potential for resource foraging by clonal plant modules ranging from the roots and rhizomes of single ramets to the emergent architecture of competing genets. In this study, we provide experimental evidence for the existence of edaphic resource foraging by *Z. marina* patches. Differential ramet proliferation, along patch margins, led to accelerated growth within resource-rich zones. Meanwhile, within patches, no detectable differences in per capita branching rates, demographic stability or reproductive effort were observed. Elicitation of patch-scale foraging by experimental nutrient addition occurred while under otherwise natural growth and competitive conditions, making the 2-year experiment a realistic demonstration of foraging potential, and a logical first step toward understanding its role in space acquisition by *Z. marina*.

Marine and terrestrial studies on clonal plant demography have reported that ramet emergence rates are often matched by density-dependent mortality along resource gradients or following nutrient enrichment (Cook 1985). Investigators have cited competitive stress brought on by above- and belowground crowding, as well as strategic ramet senescence as potential causes for resource-mediated mortality (Duarte et al. 2006; Duarte and Sandjensen 1990; Herbert and Fourqurean 2009). Our data support this view, as no cell-level changes in RSR or standing crop CV were found. Instead, ramet demography appears to have become de-coupled only at patch margins, where crowding effects are minimized. Because individual ramets were not marked during this study, no direct measurement of ramet turnover could be made; however, we posit that ramet birth rates may have been uniform throughout the treated space. If true, nutrient amendment would have altered shoot age distributions (Herbert and Fourqurean 2009), driving median age downward, and ultimately affecting patch-level attributes such as reproductive potential, carbon sequestration and sediment stabilization – all potentially enhanced by increased ramet turnover.

The study of clonal foraging has built upon four decades of terrestrial work on root proliferation (de Kroon and Mommer 2006) and recognizes similar distinctions between (1) biomass allocation responses, where structural components of individual ramets are modified (Birch and Hutchings 1994), (2) selective ramet placement, where elements of clonal architecture control ramet location (Ikegami et al. 2007), and (3) ramet proliferation, where branching probability becomes differential to resource availability (Sutherland and Stillman 1988). Of these, only the second element meets the strictest definition of clonal foraging. However, a lack of sufficient plasticity, interaction among the three elements, and the countervailing effects of clonal integration bring into question its prevalence in natural systems (Birch and Hutchings 1994; de Kroon and Hutchings 1995; Humphrey and Pyke 1997; Sutherland and Stillman 1988). For example, ramet spacer lengths have been found to be relatively insensitive to nutrient condition (Birch and Hutchings 1994) and manipulative studies of terrestrial species report greater responses of branching frequency relative to internode length or branching angle (Sutherland and Stillman 1988). Nevertheless, modeling studies notionally support the idea of selective ramet placement, particularly for resource patches greater than 5 times mean internode length (Sutherland and Stillman 1988) and those persistent enough for successive ramet generations to curtail directional inertia imposed by monopodial growth (Oborny and Englert 2012).

For *Z. marina*, spacer lengths tend to be on the order of 5 cm (Sintes et al. 2006), equating to an optimal resource grain of no less than 25 cm. In the present study, nutrient patches were 100 cm (diagonally, 141 cm), providing ample space for architectural modification to operate, while the 2-year observation period should have allowed for between 6 and 24 branching events per baseline genet (Greve et al. 2005; Harrison 1993). Resolving the influence of selective ramet placement and opportunistic ramet proliferation, however, requires physical examination of the rhizome architecture, which was prohibited by mussel recruitment in the last months of the experiment. Even with this information the two processes are often confounded, as adjustments to spacer length or angle also result in a concentration of rhizome buds, thereby increasing branching potential (Oborny and Englert 2012). We can, however, assert that selective ramet placement, if it did occur by means of architectural adjustment, did not impede patch expansion, as this was higher for enriched margins.

Annually, the effect of nutrient addition resulted in different seasonal patterns of patch elongation, with fertilized edges exhibiting a biphasic response, growing vigorously spring through summer, while ambient edges followed a more unimodal pattern. Seasonal effects on shoot length, biomass and density are all well understood for temperate seagrasses, with *Z. marina* increasing in all three measures upon onset of vernal growth (Duarte et al. 1994; Guidetti et al. 2002; Olesen 1999). Asexual recruitment of an annual cohort continues through late summer (Duarte and Sandjensen 1990; Poumian-Tapia and Ibarra-Obando 1999), during which time ramet populations experience their highest mortality rates (Olesen 1999), due to heat stress and the resultant carbon imbalance (Jarvis et al. 2014; Zharova et al. 2001). These effects are mitigated by rhizome storage of nonstructural carbohydrates, typically taking the form of sucrose (Burke et al. 1996; Vermaat 2009). Reserves have been shown to provide roughly 3-4 weeks of foliar support under adverse growing conditions and appear to be accumulated primarily during the spring (Burke et al. 1996). The shift in patch growth phenology, then, could alter the magnitude and mobilization of carbohydrate reserves with important downstream impacts on heat and shade tolerance. For example, if ramet proliferation draws resources away from typical storage patterns then the reduced fall growth rates we observed could have been the result of insufficient buffering capacity. If so, this might expose ramet populations to greater seasonal losses during anomalously harsh years (i.e., in terms of shade or heat stressors). Concomitant monitoring of rhizome carbohydrate patterns along active growth margins, therefore, represents a valuable research objective, with implications for patch stability during the critical colonization phase.

At the landscape level, the foraging response we observed could allow developing *Z. marina* patches to exploit sediment nutrient signals generated by seagrass-associated fauna, including tube-building amphipods (e.g., *Ampelisca* spp. and *Corophium* spp.) or polychaetes (e.g., *Clymenella* spp.), as well as larger bivalves such as *Mercenaria mercenaria*. Because these species commonly occur among seagrass patches, but are not obligate seagrass residents (Bostrom et al. 2006), their nutrient footprints might generate resource-rich conduits for seagrass expansion. This could potentially speed patch coalescence, providing faster access to the disturbance protection conferred by greater patch size (Olesen and Sandjensen 1994), jointly adding to meadow coverage and stability during periods of colonization. Over time, differential rates of lateral spreading might also reveal landscape patterns in edaphic condition, such as nutrient heterogeneity left by perished seagrasses, exposed glacial peat deposits or subtidal groundwater outflows.

Surprisingly, fertilization did not enhance flowering rates, regardless of the scale of observation. We know from previous work conducted at this site (Jackson et al. unpubl.) that nutrient addition can have significant effects on the pace of floral development, the size of generative shoots, and the number of seeds per inflorescence. Vegetative proliferation within nutrient hot spots could therefore still have broad indirect effects on reproductive performance.

Increasing overall ramet abundance, decreasing ramet age and reducing inter-patch distances would all enhance reproductive potential and success within developing meadows, providing vital dispersal capability and seed bank density at precisely the time of greatest space availability (Greve et al. 2005; Lee et al. 2007).

In conclusion, transient sub-patch resource heterogeneity unevenly distributed across the active growing margin of *Z. marina* patches potentiated vegetative growth and patch expansion. Incremental edge advances were characterized by significantly greater ramet numbers, indicating that a ramet proliferation, selective ramet placement or a combination of the two responses had occurred. The seasonality of this effect was consistent among experiment years and suggestive of a fundamental shift in the growth pattern and phenology of seagrass edges exposed to nutrient replete conditions. Direct effects on reproductive intensity were not recorded; however, patch-level foraging may function indirectly in sexual performance at the meadow scale. Although the precise contribution of clonal architecture could not be examined empirically, their interpretation is often difficult under field conditions, regardless of available physicochemical data. This study establishes, for the first time, the role of foraging behavior in the space acquisition strategies of *Z. marina*, providing vital information regarding vegetative growth patterns during the poorly understood colonization period.

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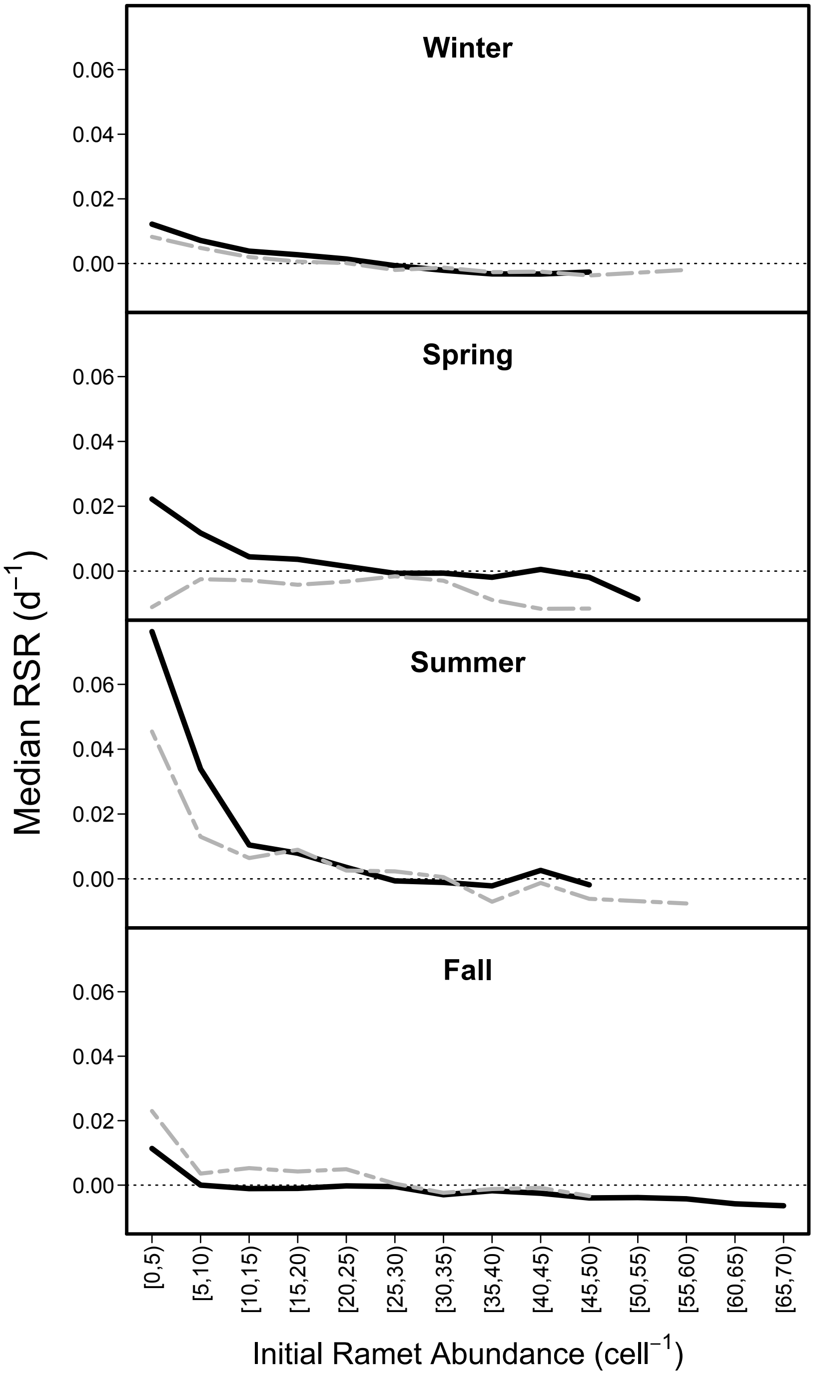

Figure 1. Seasonal patterns of median ramet-specific recruitment rate (RSR; ramets d^{-1}) for enriched (solid black line) and ambient (gray dashed line) cells displayed as a function of initial cell densities (ramets quadrat cell^{-1}; binned at 5-ramet intervals prior to median calculation).

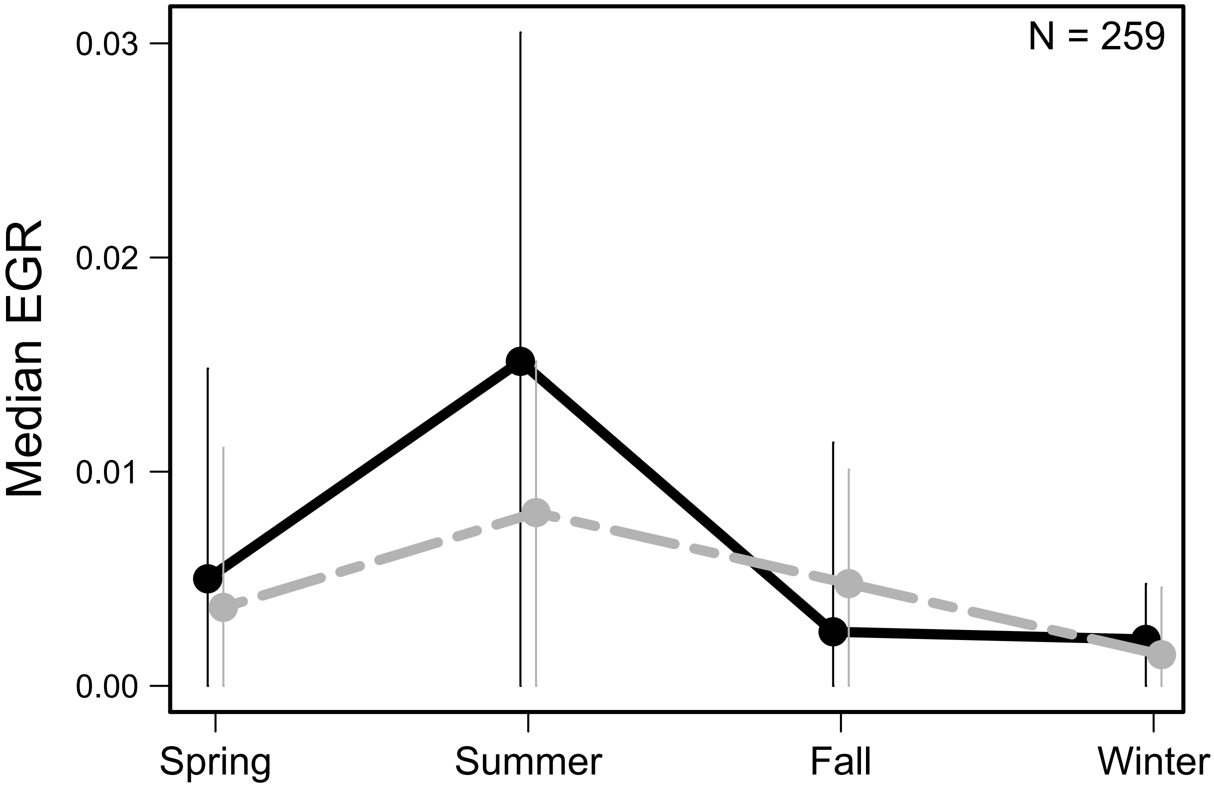

Figure 2. Seasonal patterns in median edge growth rate (EGR; cells per edge cell d^{-1}) for enriched (solid black line) and ambient (gray dashed line) quadrants over the 2-year observation period. Error bars represent +/-1.5 interquartile ranges or 'Tukey Whiskers'.

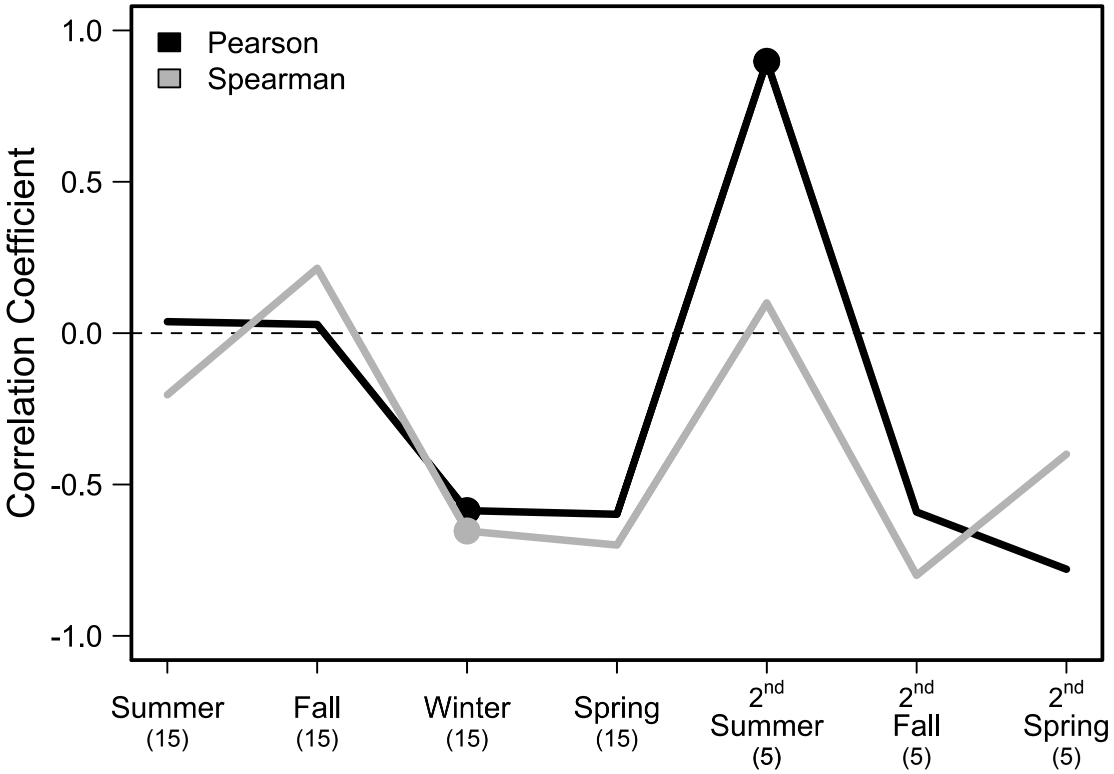

Figure 3. Temporal patterns in Pearson (solid black line) and Spearman (solid gray line) correlation coefficients obtained between the magnitude of floral abundance (flowers per patch) and patch-level, seasonal RSR anomalies for successive seasons following flowering. Filled circles denote a statistically significant relationship. Parenthetical values on the abscissa represent the number of observation per test.

Chapter 2

Sexual recruitment in *Zostera marina*: a patch to landscape-scale investigation

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Statement of Author Contribution

Study conception and design:	Furman, Jackson, Bricker and Peterson
Acquisition of data:	Furman, Jackson, Bricker and Peterson
Analysis and interpretation of data:	Furman
Drafting of manuscript:	Furman
Critical revision:	Furman, Jackson, Bricker and Peterson

ABSTRACT

Seagrasses are a diverse group of clonal marine macrophytes. Their disappearance in recent decades has been an alarming component of estuarine urbanization, effectively transitioning vast portions of global coverage to disturbed or recovering states. Understanding dispersal and recruitment patterns within and among extant populations is now vitally important to predicting both the form and pace of recovery. Working with a perennial ecotype of *Z. marina* within a shallow lagoon in Long Island, New York, USA, we combined high resolution, decade-long seagrass mapping with polymorphic microsatellite analysis to examine the interactive effects of pollination and seed dispersal distance on the dynamics of sexual recruitment across a range of spatial scales (centimeters to decameters). We found clone structure to be restricted to less than 3 m across a 56,250-m^2 study site. Pollination distances ranged from 0.57 to 73.91 m, while seed dispersal varied systematically from 1.85 and 5.31 m for naked seeds, and randomly throughout the study site (0.17 to 34.54 m) for seeds deposited by floating reproductive shoots. Pedigree analyses corroborated these findings, with full sibling groups clustering neatly within larger half-sibling kinships at spatial scales of 2-6 m. We successfully demonstrate that over a four-year period sexual reproduction and seedling recruitment played appreciable roles in the colonizing process of *Z. marina*, configuring the landscape through the deposition of rafted seeds, and contributing to patch expansion via the limited dispersal of naked seeds.

INTRODUCTION

Seagrasses are terrestrial-derived, marine angiosperms that provide important ecosystem services in shallow and intertidal habitats throughout the world ocean (Barbier et al. 2011; Costanza et al. 1997). They have recently undergone significant population declines (Waycott 2009), resulting from a myriad of factors affecting urbanized coastal systems (Backman and Barilotti 1976; Biber et al. 2009; Ralph et al. 2007). These losses have been drastic, publicly visible and are feared to be accelerating (Durako 1994; Renn 1936; Short et al. 1987).

Owing to a lack of historical distribution data and the need to document coverage trends over large spatial scales, current coastal management paradigms often include landscape-scale mapping of seagrass meadows (Bell et al. 2008; Costello and Kenworthy 2009; Morris et al. 2000). These maps have now become commonplace in both the management and academic literature; however, they only rarely contribute to predictive capability within the systems they document. Investigators using these data have had some success in modeling static patterns of coverage, particularly meadow contractions subsequent to declining water quality (Downie et al. 2013; Lathrop et al. 2001; Short and Burdick 2006). But, these efforts have usually been limited to post-hoc reconstructions (i.e., 'hindcasting') by a lack of real-time environmental data (Kendrick et al. 2005). Further, as statistical projections of environmental or physiological space, they cannot describe spatiotemporal coverage patterns within habitable zones; that is, they are unable to track distributions within shallow systems or to forecast recovery dynamics following perturbations.

These sorts of questions are most directly addressed through spatially explicit, discrete (stage-based) or continuous (differential) population growth models; however, obtaining the empirical data needed to parameterize these models has been a significant challenge. First, seagrasses are clonal plants with subterranean, impermanent rhizome connections, making the repetitive, non-destructive field-identification of clones (i.e., genets) notoriously difficult without sophisticated and costly genetic approaches (Cook 1985; Orth and Moore 1983). Second, the genet itself may be functionally immortal (Reusch et al. 1999a), as there appears to be no evidence for programmed senescence in any species, complicating traditional interpretations of population growth (Eriksson 1993). Finally, there exists considerable species and regional variability in reproductive effort, varying from highly reproductive annual ecotypes to populations maintained entirely through vegetative growth and fragmentation (Orth et al. 2000).

As a result, we generally understand inter-annual patterns in seagrass distribution through the lens of vegetative recruitment – measured in the field using traditional quadrat and transect sampling of ramet density and percent cover [(Morris et al. 2000; Neckles et al. 2012; Olesen and Sandjensen 1994); but see (Brun et al. 2007; Marba and Duarte 2003; Sintes et al. 2006) for models of rhizome architecture]. Mechanisms of dispersal and seedling recruitment are, therefore, only evoked for annual species (Orth et al. 2000), those with significant seed banks (Fonseca et al. 2008) or for denuded and disturbed areas (Lee et al. 2007; Peterson et al. 2002; Plus et al. 2003).

Recently, this view has been challenged, as a spate of work using polymorphic microsatellites suggests that elements of mating system effectiveness (i.e., selfing and outcrossing rates, multiple paternities, seed and pollen dispersal) combine to generate genotypic structure at hierarchical scales within seagrass landscapes (Becheler et al. 2010; Hammerli and Reusch 2003; Zipperle et al. 2011). These findings have re-invigorated a debate over the role of sexual recruitment, not only in meadow development and small-scale disturbance recovery (Macreadie et al. 2014), but also in maintaining clone co-existence through time (Becheler et al. 2010; Reusch 2006). That is, whether or not seedling recruitment merely catalyzes patch growth, after which competition winnows genotypic diversity and excludes further seedling recruitment, or whether repeated bouts of sexual recruitment shape clone structure and contribute to patch growth and coalescence (Eriksson 1993).

At present, only a handful of pioneering studies have assessed the size and spatial arrangement of genets, and fewer still have attempted to quantify the mechanisms and consequences of contemporary gene flow, including pollen or seed dispersal, near neighbor relatedness and bi-parental inbreeding (Reusch 2001; Ruckelshaus 1996; Zipperle et al. 2011). In fact, for most seagrasses, basic information on how mating system effectiveness and inter-genet competition regulate recruitment dynamics remains unknown. Replicate studies exist for very few species. Those that have been conducted represent only small portions of a species' geographic range, and so it is not surprising that broad patterns of reproduction and recruitment have yet to emerge. Methodologically, the majority of available work has been conducted at hectare-scales within continuous meadows for which detailed site histories are not known (Billingham et al. 2007; Kamel et al. 2012; Reusch 2000). Estimates of genetic neighborhoods and the spatial continuity of some relatedness measures indicate that hectare-scale investigations may be sufficient to capture relevant dynamics (Billingham et al. 2007; Hammerli and Reusch 2003; Ruckelshaus 1996); however, measures of effective pollen transport continue to scale with sampled distance, suggesting that larger landscapes are needed to fully understand seagrass mating systems. Further, the long-term role these processes play in meadow development cannot be inferred from instantaneous measures of genet distribution (Becheler et al. 2014). Working in Brittany, France, Becheler et al. (2014) provided the first explicit treatment of temporal variation in genotypic diversity, finding evidence for continued seedling recruitment over a three-year period within more or less continuous meadows of *Zostera marina* Linnaeus.

Repeated genet surveys, such as Becheler et al. (2014), represent a significant advance in the study of genet recruitment and competition; yet, there is risk in interpreting spatiotemporal patterns of genet structure relative to a constant baseline, as the developmental state of contiguous meadows cannot be assumed homogenous. Temporally uneven patch recruitment and subsequent coalescence frequently characterize meadow formation, and physical disturbance thereafter, acts to generate and maintain mosaics of developmental stage and age. Integrating the results of genet surveys across such meadows undoubtedly obscures the temporal evolution of the processes under study. We therefore maintain that studies examining the recursive roles of mating system effectiveness and genet dynamics in structuring seagrass landscapes must do so with spatially explicit knowledge of site history, preferably matching the developmental state of interest with the system being studied.

Working with a sub-tidal, perennial ecotype of *Z. marina* in a shallow, backbarrier lagoon in eastern Long Island, New York, USA, we provide the first multigenerational assessment of mating system effectiveness and seedling recruitment patterns for a colonization phase meadow of known site history (2001-2013). Specifically, we explored (1) clone structure at two, nested spatial scales – decimeter to decameter grain, hectometer-scale extent, (2) seed dispersal distance derived from landscape-scale mapping of recently recruited patches, (3) pollen dispersal distance from parentage assignments of fertilized seeds, and (4) reconstructed pedigrees for a series of recently coalesced patches within a developing meadow. In this study, we aimed to integrate measures of contemporary gene flow and high-resolution aerial photography, thereby placing sexual recruitment dynamics squarely within the context of landscape scale distributional patterns.

MATERIALS AND METHODS

Study Organism

Eelgrass (*Z. marina*) morphology and modular architecture is typical of the Zosteraceae (Den Hartog and Kuo 2006). Phytomers consist of a node, inter-node, tubular leaf sheath, a reduced ligule, a strap-like blade and an axillary bud (Gibson 2009). Vegetative growth proceeds vertically by repetition of the phytomer and horizontally via clonal repetition along a lignified rhizome (Den Hartog and Kuo 2006). Monopodial branching is controlled by the terminal apical (Moore and Short 2006), while trailing rhizome internodes degrade over time (Burkholder and Doheny 1968; Reusch and Bostrom 2011). Sexual reproduction in perennial *Z. marina* occurs annually after the second year of life (Granger et al. 2003; Moore and Short 2006; Plus et al. 2003). Inflorescences are monoecious, branching sympodially to form a panicle of rhipidia (Churchill and Riner 1978). Each rhipidium contains a variable number of spathes, within which a flattened spadix, analogous to the terrestrial grass spike, encloses an alternating sequence of male and female flowers in a 2:1 ratio (Ackerman 2006). Flowering phenology in *Z. marina* varies latitudinally and is strongly regulated by temperature; in New York, primordial inflorescences first appear at 0.5-3˚C in January, anthesis occurs mid-May at 15˚C and fruit maturation is completed by the end of June, after temperatures have reached 21˚C (Churchill and Riner 1978; Silberhorn et al. 1983). Pollen is filamentous and hydrophilic, delivered by water currents to receptive stigma (Ackerman 2006; Cox et al. 1992). *Z. marina* is self-compatible (Ackerman 2006). Inbreeding is regulated at the spadix level through protogynous floral development; however, selfing via inter-ramet geitonogamy can be quite high in monoclonal patches (Reusch 2001; Rhode and Duffy 2004; Waycott et al. 2006). Annual seed production contributes to transient seed banks (1000s of seeds m^{-2}) that germinate in the fall at temperatures below 20˚C, although yearlong (i.e., over winter) dormancy has been suggested (Olesen and Sandjensen 1994; Orth et al. 2000; Orth and Moore 1983).

Study Site

All fieldwork was conducted in Shinnecock Bay, Long Island, New York, USA. This backbarrier lagoon comprises a variety of marine habitat types, including: salt marshes, inter- and sub-tidal sand and mud flats, *Crepidula* spp. pavement and shell-hash, dredge spoil islands, tidally-influenced creeks/rivers and *Z. marina* meadows (Carroll 2012; Usace 2004). Depths are relatively shallow, varying from 0-4 m with a bay-wide mean of 2 m (MLLW); tides are semi-diurnal with a range of 0.8 meters (Usace 2004).

Z. marina colonized the bay during the 1930s and 1940s following a series of storm-related breaches to the barrier beach system (Dennison et al. 1989). Subsequent meadow development and patterns of persistence over the intervening 80+ years have been poorly documented. Spatial distributions do not exist for any year, and the only quantitative estimates of areal coverage remain those provided by Dennison et al. for 1967 and 1988 (1989). To better understand recent bay-wide trends, we gathered aerial photography via online access and personal communication from local, state and federal agencies. Geospatial images of varying quality were obtained for 1941, 1994, 2001-2002, 2004 and 2006-2010. In 2011, we used this information to select a rectangular site measuring 250 m (parallel to shore) x 225 m (total area, 56,250 m^2) in the southeastern portion of the bay, roughly 400 m from shore and 2.5 km east of the Inlet. Depths at this location ranged from 0.25-1.25 m MLLW. Surficial sediments consisted of siliceous sands and were uniformly low in organic content (< 1% by loss on ignition at 500 ˚C for 5 h, B. T. Furman unpubl.). Site orientation and boundaries were chosen: (1) to encompass the full cross-section of seagrasses in this portion of the bay, (2) to minimize border contact with contiguous seagrass patches, (3) to eliminate the influence of light availability on distribution patterns and (3) to capture dynamics for a meadow undergoing the initial phases of colonization (i.e., sexual recruitment followed by patch growth and coalescence).

Site Mapping

In June of 2011, we began bi-monthly mapping of the study site by means of a custom-built, balloon-mounted camera. During each flight, a tethered 1.83-m diameter, helium-filled, advertising balloon carrying a Canon PowerShot® A495 digital camera mounted with a self-leveling Picavet suspension rig was towed systematically across the site at an altitude of ~100 m. Images were taken automatically every 10 seconds using the freely available firmware modification, Canon Hack Development Kit (CHDK). For each mapping, a subset of images depicting level, clearly focused scenes were mosaicked in Hugin 2011.4.0 and georeferenced using Environmental Systems Research Institute (ESRI) ArcGIS software with the aid of subsurface control points, including 15 anti-foul painted 45-cm paver stones stabilized with a reinforcing bar and visual landmarks identified in the 2010 NYSDOP (and subsequent) imagery.

Balloon mosaics (ground pixel resolution = 10-15 cm) were combined with the only comparable agency data, those from the New York Statewide Digital Orthoimagery Program (NYSDOP; ground pixel resolution = 15 cm), to generate a sequence of high-resolution thematic habitat maps spanning 13 years: 2001, 2007, 2010-2013. For all time-points, seagrass presence was photo-interpreted at an absolute resolution of 1:100 or greater using the ESRI ArcGIS software, ArcMap 9.2, recorded manually as polygonal feature classes, and corrected using standard topological rules.

Seed Dispersal Distance

Discontinuous seagrass patches can arise from a number of pathways: (1) an existing patch can become fragmented, (2) dislodged ramets can take root after being transported via tidal or wind-driven currents, (3) a recently denuded area can be in the processes of recovery from seed bank or surviving rhizomes and (4) a single or set of seeds can disperse to new locations. Of these, the fragmentation and transport of extant ramets does not appear to operate at our study site, as wind-waves and tidal currents prevent the successful re-establishment of rhizomes. The remaining processes were discriminated using our knowledge of site history, with the goal of isolating sexually recruited patches within the 2010 landscape. This year was chosen because it represented the single largest recruitment event observed during the 13-year observation period. To mitigate the risk of falsely identifying seedling recruited patches due to fragmentation and recovery we restricted our analysis to patches outside of an amalgamated distribution of the previous 9 years (2001, 2006, 2007 and 2009), including lower-resolution 2009 imagery from the USDA - Farm Service Agency's National Agriculture Imagery Program (ground pixel resolution = 1 m). Placing a 20-m buffer in the east-west direction reduced the confounding effects of seagrasses outside of the mapped area acting as a seed source. This was not necessary in the north-south orientation because the original site boundaries fully enclosed the historical coverage. The only remaining patches were small, isolated, sexually recruited patches. Straight-line distances from the centroids of these patches to the edge of the amalgamated history were calculated using the ArcGIS software extension, 'Spatial Analyst' (cell edge = 0.1 m, distance values extracted to centroid points). We then used an iterative random resampling of the distance grid (N=4,865; 20,000 iterations) to investigate the contribution of seed dispersal via rafting of buoyant reproductive shoots (a spatially random process at the scale of the study site) relative to stationary release of negatively buoyant seeds (a diffusive process).

Sampling Design

Seagrass genotypic structure and mating system effectiveness were evaluated across the study site at three, nested spatial scales (decameter, meter and centimeter grain sizes; Fig. 1A-C, respectively) using polymorphic microsatellite analysis. Collection of an initial round of genetic samples was conducted on 7 October 2011. A search radius of 3 meters was established around each point in a 20-m alternating grid (156 locations; Fig. 1A). Within each circle containing seagrass (N=137), the youngest leaf from a single shoot was collected, cleaned of epiphytic growth and stored on desiccant in a 20-ml scintillation vial. On 10 June 2013, we collected a second set of samples from a circular sub-site containing 138 small patches (1.42 ± 4.31 m^2; hereafter, mean ± 1 s.d.) within a 20-m radius of a 'central' (hereafter, 'focal') patch (Fig. 1B). The sub-site was monitored during the previous 4 weeks to track floral development. Once sufficient pollination and embryo growth had occurred, we used a combination of haphazard and gridded quadrat (2 x 2 m quadrat divided into 20 x 20 cm cells) sampling to collect genetic samples from all 138 patches. Gridded sampling was conducted at both the focal patch (N=62) and a larger chain of recently coalesced patches (hereafter, 'expansion' patch) immediately to the west, wherein one sample was drawn from each cell (N=285; Fig. 1C). The remaining patches were sampled haphazardly, one sample per patch (N=104; Fig. 1B). In all cases, a 'sample' consisted of a single reproductive shoot, from which a leaf (providing the maternal genotype) and a fertilized ovary from the tallest rhipidium were excised. To maximize the likelihood of successful paternity assignment, only 23 seeds from or near the focal patch were considered further. Paired samples were stored together in 20-ml scintillation vials containing silica desiccant. The locations of all samples were mapped using a Trimble Geo-XT handheld DGPS unit, running ArcPad 8.0.

Microsatellite Genotyping

DNA extraction for the 20-m vegetative samples followed the methods of Peterson et al. (2013). DNA from the finer-scale (cm to dm), reproductive samples was extracted in bulk at the University of Iowa. Seeds and leaf tissue were stabilized to prevent DNA loss. Biomass was placed into a series of 96 well extraction plates. The extraction plates were shipped to Iowa, where an Autogenprep 740tm extracted the DNA. DNA quantity yield, and quality analysis was performed at the University of Virginia Seagass Genetics Lab. Aliquots of extracted DNA were then used in two separate multiplex PCR amplifications on an MJ Research™ PTC-200 thermocycler using *Z. marina* specific, 5'-fluorochrome (standard labels Hex, Tet, Fam) labeled polymorphic microsatellite markers. Polymerase chain reactions (PCR) were performed in 20 µL reactions containing approximately 30 ng of template DNA, 0.5 U of Bioline Immolase DNA Taq (Bioline Pty. Ltd. CA), 1.5 µL 10x Bioline Immobuffer (160 mM $(NH_4)_2$ SO_4, 670 mMTris-HCl pH 8.3, 0.1% Tween-20), 2.5 mM $MgCl_2$, 0.133 mM each dNTP, BSA at a concentration of 0.1 µg/µL, and 0.33 mM fluorescently labeled forward (FAM, HEX, or TET) and reverse microsatellite primers. Thermal cycling protocols consisted of a 7-minute 94°C denaturing step followed by 30 cycles of at annealing temperature of 50°C. For all cycles denaturing steps were conducted at 94°C and extension temperature was 72°C. PCR products were analyzed using a MEgaBACE™ 1000 DNA analysis system (GE Healthcare). MEgaBACE Genetic Profiler 2.2 (GE Healthcare) was used to assign alleles for each sample at each locus relative to an internal size standard (MEgaBACE™ ET400-R size standard, GE Healthcare, Buckinghamshire UK).

Clonal Membership

Somatic mutation is a common, but underappreciated consequence of vegetative reproduction in clonal plants, often resulting in small departures from the natal multilocus genotype (MLG; Becheler et al. 2014; Reusch and Bostrom 2011; Silander 1985). To account for these effects, we used the program GENCLONE 2.1 to assign MLGs to statistically derived multilocus lineages (MLLs) following the methods of Arnaud-Haond et al. (Arnaud-Haond and Belkhir 2007; 2007). All MLGs differentiated at a 7th locus by a genetic distance less than 6 bp were evaluated for lineage assignment using a $P_{sex(FIS)}$ threshold of 0.01.

Paternity Analysis and Pollen Dispersal Distance

Paternity analysis was conducted with CERVUS 3.0 following the methods of Kalinowski et al. (2007) and Marshall et al. (1998). Duplicate MLGs were removed from the dataset prior to analysis. Maternal genotypes were retained as potential fathers to account for geitonogamous pollination, bringing the total number of candidate fathers for this study to 522. The genotyping rate was set to 1% based on previous work at this location (Peterson et al. 2013). As discussed in Zipperle et al. (2011) the significance of any given paternity assignment is constrained by three elements: (1) the frequency of genotyping error, (2) the number of fathers included in the analysis and (3) the proportion of parents sampled from the actual population. Among these, the proportion of sampled parents is often the most difficult to estimate accurately. Past investigators working with clonal plants have extrapolated genet density or size over the sampled area to calculate the magnitude of the parental pool; however, the spatial scale of our investigation and the genet density observed within the expansion patch (~18 MLL m^{-2}, see Results) prevented reasonable scaling of genet structure, yielding percentages prohibitive to confidence testing (values ranged from 0.1 to 12.9%). We therefore agree with Sork et al. (1999), who argued against "unambiguous paternity assignment", and chose to interpret the 'most likely candidate' parents as simply 'viable' parents within the landscape. Pollen dispersal distances (PDDs) were then calculated as straight-line distances between seed locations and their respective candidate fathers. Random sampling of the potential distance matrix was simulated in the statistical package, R to confirm that sampling density had no undue effect on the recovered PDD (n=20, iterated 10,000 times with replacement). For paternal MLGs that were part of a multi-ramet MLL, additional pollination vectors were created from the seed location to each member of the MLL.

Pedigree Reconstruction

To investigate the role of sexual recruitment in patch coalescence, genetic samples (reproductive leaf material) from the expansion patch were analyzed for full (FS) and half (HS) sibling relationships using PEDIGREE 2.2 (Herbinger 2005). PEDIGREE uses a simulated annealing algorithm (a Markov Chain Monte Carlo process or MCMC) to search the space of potential pedigree relationships to find group partitions that maximize difference in the aggregate pairwise likelihood ratio (i.e., full-sibling to unrelated) between within- versus among- family groupings. Annealing parameters, 'temperature' and 'weight' control MCMC behavior during the sampling process, increasing sampling volatility and reducing group splitting, respectively. We investigated sibship partitions using a fully orthogonal mixture of temperature (10, 30 and 50) and weight (0, 5 and 10) settings, replicated 10 times, and evaluated potential solutions using the number of reoccurring partitions, as well as the overall partition score. The global partition and group cohesion scores for the best FS and kin group (FS+HS or KG) configurations were assessed for statistical significance by permutation using 1,000,000 iterations at an alpha of 0.05. Only those FS groups that were fully nested within KG groups of greater than 2 MLGs were retained for further study. These results were then tested for continuity with MLL assignments and expanded to include all unassigned MLL members. To place the pedigree information within the context of meadow development, some estimate of annual genet-specific reproductive effort must be made. Specifically, how many generations were likely sampled by selection of reproductive leaf material within the expansion patch? And, how likely were the PEDIGREE relationships to have been strictly collateral?

Empirical data on the influence of genet or ramet age on reproductive effort were unavailable, so we used shoot count data from an ongoing experiment to independently examine the effects of patch age on reproductive effort. Briefly, ten patches (five in October 2011, expanded to ten in July 2012) were selected within the study site. All patches were less than 4 m^2 at the time of choosing. At each patch, permanent markers were installed to allow for consistent placement of a 4-m^2 quadrat with 100 equally sized cells. Manual counts of vegetative and generative shoots were conducted once in 2011 and 4x per annum in 2012 and 2013. Periodicity corresponded to seasonal shoot growth and flowering phenology.

Temporal effects on floral density were investigated for two phases of patch development: recruitment and radial expansion. The first, elapsed-time from recruitment to first flowering, was assessed using reproductive shoot counts in cells from the five initial patches (N=45) and the aggregate density of the entire patch as replicates (N=5). Comparisons were made regarding floral densities and reproductive to vegetative shoot ratios (R:V) recorded in the initial 2012 survey relative to when each patch was first observed in either the NYSDOP or balloon aerials. Because all patches were clearly visible in the 2011 imagery, values were assigned to each of three treatments: (1) not present in 2010 (2) partially present in 2010 and (3) present in 2010. Here, 'partial' refers to patches that were visible in 2010 but not in the same location or of the shape as sampled in 2012, suggesting ramet mortality and patch migration. To examine how expanding patches allocate reproductive effort over time, we first retained only cells (full ten-patch, 2-year dataset) that were not occupied during the baseline sampling (i.e., only those cells for which accurate ages could be calculated), and then queried the dataset for cells occupied over a continuous time-period including a reproductive shoot survey. These data were then pooled and examined using polynomial regression.

RESULTS

Seed Dispersal Distance

A total of 4,865 patches were identified as sexual recruits within the 2010 mapping. Dispersal distances, calculated as the straight-line distance to amalgamated historical coverage, varied from 0.17 to 34.54 m, with a mean of 4.75 ± 4.40 m and median of 3.40 m (Fig. 2). The sample distribution was positively skewed (skewness = 2.4) and strongly leptokurtic (k = 7.5), closely matching that of the potential distance grid: range = 0.10-36.24 m, mean = 5.38 ± 5.75 m and median = 3.35 m. Iterative resampling of the distance grid identified two departures from random behavior, lower than expected recruitment between 0 and 0.92 m and higher than random recruitment between 1.85 and 5.31 m (Fig. 2). We speculate that short-distance recruitment (i.e., less than the peak value of 2.25 m) may have been limited by seed predation proximate to existing seagrass, resulting in predation halos. Such effects have been observed for urchin grazing in coral reef systems (Ogden et al. 1973), and may approximate the foraging behavior of granivorous invertebrates in patchily distributed seagrass systems; however, very little is currently known regarding either the pattern or intensity of seed predation in *Z. marina* meadows (Orth et al. 2000). Also, the lack of recruitment, particularly below 1 m, could have been a methodological artifact of using recently recruited patches to infer seed dispersal. Because this approach integrated germination success, seedling mortality, and a short window for vegetative growth, there was the potential for neighboring recruits to have coalesced with adjacent seagrasses prior to mapping. Such cases would have been excluded from our analysis. Between 1.85 and 5.31 m, recruitment exceeded predictions of a spatially random process, offering clear evidence for the contribution of locally produced seeds via diffusion from nearby patches (i.e., through the rolling and saltational jumping of individual seeds). Interestingly, the signal terminated at 5.31 m, a distance consistent with the upper range suggested by previous studies of seed dispersal in *Z. marina* (Billingham et al. 2007; Ruckelshaus 1996).

Clonal Structure

Genet diversity, measured as either the number of MLGs or MLLs per sampled ramet, was extremely high at the largest spatial scales. Of the vegetative material collected at 20-m intervals (N=137), only a single multi-ramet MLG, consisting of two ramets, was recovered (MLGs ramet^{-}

^{1} = 0.99). Closer examination of this isolated pair revealed allelic differences at an 8th locus; however, we retained it in our dataset for consistency with subsequent samples that were resolved to only 7 loci. MLL diversity was marginally lower at 0.98 MLLs ramet^{-1}. Here we found three MLLs, each with two ramets. Surprisingly, both additional MLLs spanned extremely long distances (167.5 and 188.4 m, respectively) and so were likely either further discriminatory artifacts of 7-loci genotyping or examples of long distance dispersal by selfed seeds. Historical distributions were inconsistent with vegetative growth as a mechanism linking the two pairs of clones; however, in both cases, the southerly ramets were situated in younger portions of the meadow, supporting the selfing hypothesis. Including data from all spatial scales, the appearance of MLLs separated by more than 3 m (exceeding reasonable expectations of vegetative growth at the site) was extremely rare, occurring only 12 times across the landscape, ranging in distance from 4.8 to 188.4 m (41.13 ± 64.39 m).

Of the 588 samples, GENCLONE identified 521 distinct MLGs, comprised of 1 to 9 ramets per clone (1.129 ± 0.737 ramets MLG^{-1}), and 461 MLLs, with 1-24 ramets per lineage (1.275 ± 1.49 ramets MLL^{-1}). Although a more thorough sampling of contiguous patches would have been necessary to confirm the upper limit of vegetative growth across the study site (Arnaud-Haond et al. 2007), multi-ramet MLLs (>2 ramets) were generally found within contiguous seagrass patches and tended to aggregate at maximum separation distances of less than 3 m. Nevertheless, many of these clusters appeared to be in rough accordance with patch history, with higher concentrations of clones centered on the older portions of the patch; however, some degree of intermingling was observed (Fig. 3). Within the expansion and focal patches, the combined MLL density was found to be 18.84 MLLs m^{-2} (total area sampled: 15 m^{2}).

Paternity Analysis

Viable paternal contributors were identified for 20 of 23 (87%) seed-leaf pairs. Three of the fathers were part of multi-ramet MLLs, yielding 27 PDD estimates. Pollination distances ranged from 0.57 to 73.91 m (Fig. 4), and were not significantly influenced by sampling density. The maximum exceeded previous reports for *Z. marina* using pollen recapture methods (15 m) by nearly 5 fold (Ruckelshaus 1996) and for microsatellite-based paternity assignments for *Z. noltii* (9.62 m) by a factor of 7 (Zipperle et al. 2011). As in other studies, PDD continued to scale with study size, suggesting that the full extent of pollen transport may not have been found.

Given that our sampling densities beyond 20 m from the focal patch were quite low, it is somewhat surprising that viable parents at these distances were found at all. This suggests that pollen transport of greater than 20 m may be a common feature of the *Z. marina* mating system. The median PDD was 9.11 m (13.90 ± 15.19 m), consistent with pollination events documented or inferred by others working at the scale of 1s to 10s of meters (Reusch 2003; Zipperle et al. 2011).

Interestingly, none of the sampled seeds were the product of geitonogamous selfing. In fact, none of the viable parents were within 0.57 m of each other, despite the minimum sampling distance of 0.2 m. Further, only 15% of fathers were found within the same patch as their putative offspring. We infer from this that the developmental asynchrony controlling self-pollination at the spathe level must extend in some way to nearest-neighbor pollination. Potential mechanisms include gradients in temperature regime and small-scale heterogeneity in porewater nutrient concentrations; in fact, recent manipulative work conducted at our study site (Jackson et al. unpubl.) supports the role of nutrient supply in controlling both the onset and vigor of reproductive effort and development.

Of the 23 paternal genotypes, 3 (13%) were viable parents for multiple seeds (3, 2 and 2 offspring, respectively). PDDs for these cases ranged from 6.20 to 15.08 m. In 86% of these events (6/7) pollen was transported between adjacent patches, and two of the three fathers contributed pollen to adjacent mothers (20-cm separation). Pollination distances for these matches ranged from 9.51-9.66 and 14.89-15.08 m, respectively. While not dispositive, these data support the notion that pollen clouds produced during anthesis remain cohesive over intermediate distances.

Pedigree Reconstruction

PEDIGREE successfully assigned 112 of the 188 MLLs within the expansion patch to 12, spatially coherent, full sibships nested within 5 kin groups (73% of ramets; Fig. 5). We propose local seed retention as the most parsimonious explanation for these patterns, meaning that 60% of MLLs were produced within the expansion patch and survived long enough to reach reproductive age. This suggests that close relative competition within expanding patches may be quite common and that these seedlings can compete effectively with vegetative recruits for both space and resources. Despite having run PEDIGREE on MLG data, all of the KG groups were consistent with clonal lineages and only a single MLL was divided by any FS partition (MLL #387, with 24 ramets; Table 1). Ten of the twelve significant FS groups were nested within a KG; both unassigned FSs were in close proximity to the largest group of unassigned MLLs in the youngest portion of the patch. Both the FS and KG groups formed spatially coherent aggregations at two scales of organization. Full sibships generally covered 1-2 m in asymmetrical clusters, while KGs spanned 2-6 m. The largest KGs (#'s 1 and 2; 23 and 41 MLLs, respectively) occupied discontinuous distributions, indicating a possible crosspollination between two parental genets.

Using simple Mendelian inheritance rules, we could not find a viable parental genotype within any of the FS or KG groups. Because we sampled only reproductive shoots at this scale, we cannot infer the distribution or presence of older genets (i.e., whether or not the sampled MLLs were imbedded within a matrix of vegetatively expanding parental clones); however, the lack of potential parents in the pedigree data implies that genet flowering intensity may have diminished with time. This interpretation was consistent with two independent analyses of patch growth and reproductive effort, conducted on similarly sized patches imbedded within the same landscape at the same time as the present study. Regardless of which metric was considered [reproductive shoot density (m^{-2}) or R:V] or which experimental unit was assessed (quadrat cell or whole patch), a step-wise relationship between the time of first appearance and reproductive effort was found (Fig. 6). The only patch not observed in the 2010 aerials yielded zero reproductive shoots when sampled in 2012, while the 'partial' treatment was of intermediate intensity. We interpret this pattern as evidence that newly recruited patches might require an additional growing season prior to flowering. Similar delays were not observed, however, for laterally expanding ramets from patches in the same landscape. Both floral density and R:V measures exhibited a unimodal response to age, with peaks occurring the following growing season (298 and 232 d, respectively), followed by virtually no reproductive effort at 433 days (Fig. 6). The limited flowering observed in the same season as lateral invasion, presumably through vegetative growth, most likely represented small positional errors in quadrat replacement and the lateral spreading of reproductive ramets from adjacent, older quadrat cells. As neither method of assessment definitively characterized the influence of genet or ramet age on reproductive effort, both scenarios were tentatively incorporated into a working model of pedigree reconstruction (Fig. 7).

Genet-level delays in post-recruitment reproduction were reconciled with second growth season patterns found for vegetatively propagated ramets by placing the recruitment year for the expansion patch (i.e., the 6 constituent patches) in the spring of 2009. Recruited patches likely consisted of an extremely small number of initial genets per patch (Waycott et al. 2006). We speculate that the first, and potentially the primary, flowering of this cohort (the parental or 'P' generation) occurred during the spring of 2011. Many of the offspring from this event (the first filial or 'F_1' generation) were retained within or proximate to their natal patches, settling either adjacent to or (more likely) among the adult ramets of the P cohort. These genets then expanded clonally during 2012 and were in bloom during the 2013 sampling event. Limited flowering by the P generation likely occurred in 2012, producing a second cohort (F_1'); however, these genets would not have flowered until 2014, and so were excluded from or were otherwise under-sampled by our survey. The resultant chronology, the first of its kind for *Z. marina* in New York waters, corroborates the apparent collateral nature of genet relationships obtained in 2013, and successfully integrates information obtained through aerial mapping, traditional ramet censuses, microsatellite-based clonal lineage assignment, and statistical pedigree reconstruction.

DISCUSSION

It is well established that seagrass distributions can vary across a range of spatiotemporal scales within otherwise suitable habitat (Bell et al. 1997; Kendrick et al. 2005; Olesen and Sandjensen 1994). Yet, the task of ascribing quantitative values to this dynamism (i.e., the form or rate of change) remains the province of anecdote. Indeed, understanding how sexual and vegetative reproduction translates into the acquisition and maintenance of space remains a significant challenge for seagrass ecologists and managers alike.

Recent parallel advances in the cost and availability of mapping resources (e.g., GIS, DGPS, digital orthophotography, etc.) and polymorphic microsatellite-based genotyping have made these issues tractable for the first time. Recent work on *Z. marina* and its congeners has begun: (1) to document clone structure, size and age (Billingham et al. 2007; Reusch et al. 1999b), (2) to examine mating system effectiveness and its influence on the size of genetic neighborhoods (Reusch 2000; Reusch 2003; Zipperle et al. 2011) and (3) to investigate how near-neighbor interactions contribute to disturbance recovery, seed set and genet fitness (Billingham et al. 2007; Macreadie et al. 2014; Reusch 2006). The nascent genetic literature, however, is replete with contradictory evidence, and few biogeographic patterns have yet to emerge. To wit: genetic erosion is wide-spread in New York waters: Campanella et al. (2010), but see Peterson et al. (2013); heterozygosity predicts genet vigor: Reusch (2006), but see Billingham et al. (2007); dispersal balances genetic drift: Ruckelshaus (1996), but see Becheler et al. (2010); local populations are founder-controlled: Reusch et al. (1999) and Olsen et al. (2004), but see Reusch (2006) and Becheler et al. (2014); and vegetative growth exceeds seedling recruitment as a mechanism of disturbance recovery: reviewed in Macreadie et al. (2014).

Reproductive variation has been a well-recognized attribute of the widespread and phenotypically plastic Zosteraceae – with reproductive effort, flowering phenology, certain patterns of clone diversity and clone size often exhibiting significant relationships with hydrodynamic regime, depth, physiological stress, geographic isolation and time since colonization (Becheler et al. 2010; Kamel et al. 2012; Olsen et al. 2004). However, even as we seek to better understand the interaction of local conditions on sexual reproduction and recruitment, the diversity of plant responses offers a tremendous opportunity to link process to pattern within individual meadows. That is, in addition to the controlling influence of physiochemical and hydrodynamic condition on meadow shape and volatility, the spatiotemporal dynamics of seagrass distributions may, in fact, have additional underpinnings in the varied expression and success of its mating system. Understanding these connections does not require geographic consensus within the literature, but rather only that studies be conducted in a spatially explicit manner within seagrass landscapes of known distributional histories. Our goal in this study, therefore, was to test this thesis by linking mating system effectiveness, seed dispersal and sexual recruitment to landscape-scale patterns of meadow development.

In our system, evidence of clone structure, investigated across three nested scales (decimeters to decameters), was limited to less than 3 m and was consistent with areal coverage trends. Both indicated that the site contained an establishment phase meadow (Cheplick 1998) undergoing a minimum 13-year colonization process, characterized by episodes of sexual recruitment followed by patch growth and coalescence. Throughout the field component of the study, reproductive effort was high by published standards (39±34.5 flowers m^{-2} in 2012; as measured by 64, 0.0625 m^2 quadrats, unpublished data) and, although variable, no apparent site-wide or center-to-edge gradients were detected following a systematic deflowering of patches ranging from 2.5 to 20 m^2 (N=58; Lisa Jackson, pers. comm.). We suspect that sexual reproduction may have been prevalent at the site throughout the colonization period. This, along with a lack of large-scale physical disturbances over the same timeframe, allowed us to explore a series of questions relating sexual recruitment to meadow physiognomy.

How does seed dispersal contribute to space acquisition?

Dispersal of seeds and propagules can span three orders of magnitude (1s – 1000s m) depending on the form of diaspora (i.e., negatively buoyant seeds or positively buoyant shoots and spathes) (Kendrick et al. 2012; Vermaat 2009; Waycott et al. 2006). Despite the clear importance of long distance transport in realizing a cosmopolitan distribution (Olsen et al. 2004) and maintaining subpopulation connectivity (Peterson et al. 2013), most seed bank, seed release and genotypic surveys have found dispersal distances of less than 5 meters (Billingham et al. 2007; Ruckelshaus 1996), suggesting a leptokurtic, fat-tailed dispersal kernel.

Consistent with this view, evidence for the diffusive flux of naked seeds terminated at 5.31 m, beyond which unoccupied habitat space was sampled in a spatially random fashion. Dispersal beyond that threshold was far from limited, however, since all investigated distance classes (0 -

40 m) experienced some level of sexual recruitment. We interpret this to mean that seeds distributed by rafted reproductive shoots play an important role, not just in long distance gene exchange, but also in expanding the spatial footprint of existing seagrass meadows. The median distance for this mode of dispersal could not be reliably estimated from our data, as it exceeded our mapped area, but it seems probable that, once aloft, buoyant shoots would transport developing seeds well beyond their natal patch. Given a two-week maturation process (Billingham et al. 2007), combined with repeated bouts of tidal mixing, it would follow that recently colonized meadows begin with very little genetic structure and periodically receive recruits from distant sources. This mode of dispersal, then, can play a significant role in the spatial structuring of *Z. marina* meadows, providing access to unoccupied substrate at contact rates beyond the capacity of diffusive kernels and, in the case of our study site, setting the stage for small-scale competition among expanding kinship groups.

Using disjunct patches to infer characteristics of seedling recruitment within 5 m of parental sources was confounded by the time lag between settlement and recruitment, as well as the unmeasured influence of mobile granivores. The calculated distances also represent conservative estimates of seed dispersal, as the maternal origin of a dispersed seed was certainly not, in all cases, the edge of the most proximate patch. Nevertheless, statistical patterns of local retention were found between 1.85 and 5.31 m. This was consistent with independent pedigree analyses conducted within the expansion patch, as FS groups tended to cluster within larger HS kinships at scales of 2-6 m. Aggregations of this type have been found by others, using similar microsatellite-based survey methods (Hammerli and Reusch 2003; Zipperle et al. 2011), suggesting limited dispersal for at least some portion of the annual seed set. But what role do these locally retained seeds play in centrifugal growth and patch maintenance? Specifically, are seeds trapped by existing vegetative shoots, whereafter they intermingle with existing clones, or does competition with established genets force recruitment to the margins or internal gaps of seagrass beds, resulting in clone mosaics? For the purposes of discussion, we will refer to the former pathway as ‘admixed’ and the latter as ‘agglomerative’ models of patch development.

Although agglomerative models have been proposed in the past (Billingham et al. 2007), at least three lines of evidence point toward the admixed model as explaining centrifugal growth within our study site. First, we recovered no parental genotypes from our 2013 sampling of the expansion patch. We explain this as a third-season drop in reproductive effort, supported by genet- and ramet-level reductions in flowering intensity observed elsewhere within the same meadow over the same time period using patches of similar size and age. By selecting reproductive shoots during our fine-scale survey, we failed to sample parental clones; however, their lack of presence does not indicate genet senescence, as bi-monthly aerials showed no signs of such loss. We, therefore, argue that vegetative growth by the P generation continued as the primary means of patch expansion, within which F_1 clones were retained as seeds. The close spatial agreement between the F_1 sibships and the founding P generation supports this idea. Second, reproductive shoots from the F_1 generation were, themselves, intermingled. Finally, shoot emergence rates for the MLLs surveyed within the expansion patch were within the upper range reported for annual (Reusch 2000) and perennial (Olesen and Sandjensen 1994) populations of *Z. marina*, indicating unencumbered vegetative growth. We interpret this to mean that seedling recruits effectively competed both with adjacent seedling kin and with established genets for space and resources. Age-structured, phalanx growth does not appear to function in patch growth and coalescence, the dominant mode of large patch formation, at our study site.

How does pollen dispersal mitigate kinship interactions and constrain genetic neighborhoods?

In contrast to terrestrial systems, where pollen transport tends to exceed seed dispersal in dictating the spatial extent of gene flow (Sork et al. 1999), most of the work conducted in seagrass systems has reported limited pollen availability and dispersal (<10 m), citing the importance of floral asynchrony, cryptic self-incompatibility and inbreeding depression in controlling self-pollination and bi-parental inbreeding within spatially restricted genetic neighborhoods (Hammerli and Reusch 2003; Reusch 2001; Reusch 2003). These studies, however, were conducted at hectare scales in continuous meadows with low floral densities and so their findings may not be directly transferable to our system. In fact, we estimate that the median pollen dispersal distance over the 2011 to 2013 period was nearly three times that of seed dispersal, and found no evidence for pollen limitation (additional morphological surveys confirmed this; Jackson et al. in prep). While we do not provide a quantitative measure for the size of the genetic neighborhood, we reason that, annually, pollen transport may be the single most important determinant of gene flow, with seed input via rafted shoots playing a less frequent role.

Selfed seeds were only rarely found, and could only be weakly inferred, by our genotypic survey, while none of the paternity samples appeared to be the product of self-pollination. This was not surprising, given the prevalence of inter-patch – and presumably inter-clone – pollination (85%) and a minimum PDD (0.57 m) that approached the scale of clone structure. It therefore appears that pollen transport readily exceeds clonal growth during the early stages of colonization and that nearest neighbor pollination, even among adjacent clones, may be an infrequent occurrence for ovaries of the tallest rhipidium. The arrangement of KGs within the expansion patch supports this view, as crosspollination among neighboring clones occurred in only one of five KGs.

It is still unclear how much spathe height controls pollen accessibility and seed dispersal within *Z. marina* canopies (but see Ackerman 2002); however, our results are consistent with taller flowers receiving pollen from distant sources. Interestingly, two lines of evidence suggest that pollen clouds remain cohesive during transit. Parentage assignments of paired seed-leaf samples identified a subset of fathers contributing genetic material to adjacent mothers with PDDs of 10-15 m, while the pedigree data yielded 17 multi-MLL FSs (i.e., multiple offspring from a single genet pairing) within the expansion patch. Clearly, the diffusion of sticky pollen chains need not be isotropic, but the occurrence of spatially aggregated pollen at considerable distances from source flowers was particularly surprising. Based on the frequency of multi-MLL FSs, and their close-proximity SDD, we speculate that the effectiveness of pollen clouds, in terms of multiple contact rates, may be greatest at lower portions of the pollen-receiving inflorescence, as seeds produced there would have the highest probability of local retention.

In conclusion, over at least a four-year period, sexual reproduction and seedling recruitment played appreciable roles in the colonizing process of *Z. marina*, configuring the landscape through the deposition of rafted seeds, and contributing to patch expansion via the limited dispersal of naked seeds. Molecular evidence supports an admixed model for this process, as genet competition among vegetatively and sexually produced individuals did not appear restrictive to seedling recruitment or vegetative propagation. As patches mature and expand, the potential for bi-parental inbreeding will undoubtedly increase; however, our PDD estimates indicate that at least the tallest rhipidium will have access to pollen originating from outside of the KG footprint. Nevertheless, cohesive pollen clouds fertilizing multiple ovaries per receiving genet, and limited SDDs, frequently result in the aggregation of collateral relatives, and so competition among close kin may in fact be a regular feature of *Z. marina* meadows. That these processes were still contributing to meadow development after more than 13 years suggests that seedling safe site availability, and not mating system effectiveness, may be most limiting to meadow establishment over longer spatiotemporal scales.

Conclusions

As a growing proportion of global seagrass coverage transitions to disturbed or recovering states, understanding how remaining populations exploit under-saturated habitat space will be vitally important to managers and academics alike. The application of polymorphic microsatellite analysis toward the study of mating system effectiveness, meta-population connectivity and recruitment dynamics has been invaluable to this effort; however, there remains a need to link recruitment mechanisms to coverage changes occurring at landscape scales. To accomplish this, high-resolution spatial data obtained over multi-year periods will be required; that is, detailed histories of colonization and disturbance events must be known. As a test case for integrating traditional genotypic surveys with distributional time-series data, our study provides the first, clear evidence that mating system performance and seedling recruitment can dictate both the form and pace of space acquisition by *Z. marina* in the northeastern United States. Importantly, we would not have been able to correctly interpret genet size and configuration as seedling recruitment and cohort overlap, nor would we have been able to place our chronological pedigree within the context of patch expansion and coalescence without the use of high-resolution site history data. We feel that this underscores the need to map genet and areal coverage distributions in tandem, and urge others to incorporate similar approaches into their current inference structure, either by targeting study sites that have previously been mapped or by augmenting repeated genotypic surveys with larger-scale coverage mapping.

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Kingroup KG	Full-Sibship FS	Number of MLLs	Number of Ramets	MLL Designation (Number of Ramets)
1	1	4	26	387 [20] 447 (1) 512 (4) 515 (1)
1	14	4	5	413 (1) 414 (1) 416 (1) 517 (2)
1	3	6	18	352 (2) 353 (8) 387 [4] 502 (1) 503 (1) 505 (2)
1	9	5	11	91 (1) 93 (1) 196 (7) 436 (1) 453 (1)
1	UA	4	4	356 (1) 474 (1) 481 (1) 490 (1)
2	2	13	25	11 (3) 30 (2) 218 (1) 248 (1) 249 (1) 253 (1) 254 (1) 279 (1) 306 (1) 307 (1) 308 (1) 310 (10) 312 (1)
2	6	11	15	115 (2) 116 (1) 118 (1) 123 (1) 328 (1) 331 (1) 332 (1) 333 (1) 339 (1) 397 (4) 412 (1)
2	7	5	12	108 (1) 300 (6) 341 (2) 342 (2) 347 (1)
2	UA	12	12	109 (1) 130 (1) 284 (1) 285 (1) 326 (1) 327 (1) 345 (1) 348 (1) 411 (1) 489 (1) 491 (1) 521 (1)
3	4	11	21	41 (3) 47 (8) 51 (2) 59 (1) 66 (1) 78 (1) 80 (1) 156 (1) 181 (1) 273 (1) 277 (1)
3	UA	14	19	43 (1) 44 (1) 45 (1) 46 (1) 50 (4) 74 (1) 75 (3) 77 (1) 82 (1) 367 (1) 372 (1) 373 (1) 374 (1) 439 (1)
4	34	2	2	419 (1) 516 (1)
4	5	2	17	366 (9) 479 (8)
4	UA	2	2	493 (1) 518 (1)
9	UA	4	4	15 (1) 31 (1) 169 (1) 495 (1)
UA	17	4	4	122 (1) 349 (1) 359 (1) 360 (1)
UA	8	9	11	39 (1) 52 (1) 81 (1) 146 (1) 162 (3) 272 (1) 281 (1) 430 (1) 437 (1)

[ ] - MLL divided by FS partition   UA - Unassigned

Table 1. Summary statistics for the reconstructed pedigree of unique MLL genotypes within the ‘expansion patch’. Numeric values identify group designation at the kingroup, full-sibship and MLL levels of organization; number of MLLs and ramets per kingroup, as well as number of ramets per MLL, are also provided. Brackets denote an MLL divided by a full sibling partition, and ‘UA’ refers to groups unassigned either a kin or full-sibling group.

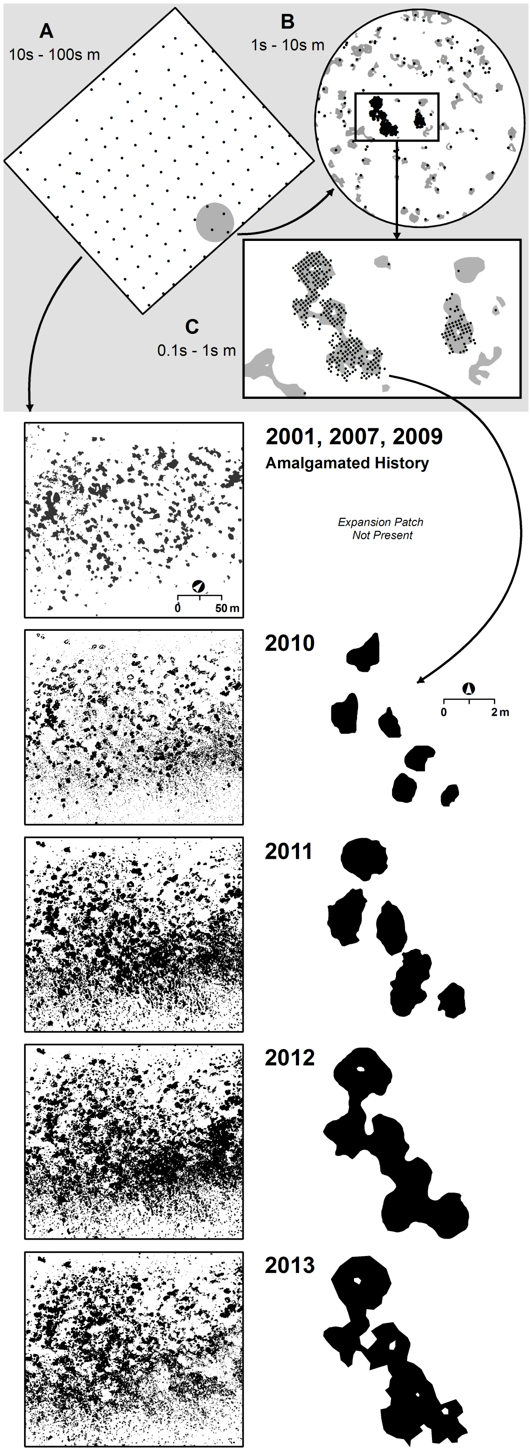

Figure 1. Upper panel: (A) study site boundaries (225 x 250 m; 56,250 m^2) and nested-scale sampling array with decameter (A), meter (B) and centimeter (C) spatial grain. Black dots denote sampling locations. Lower panel: Sequence of meadow and patch development for the entire study site (left) and the 'expansion patch' (right) during the 2001 to 2013 observation period. The amalgamated history represents a merged overlay of coverage distributions from the 2001, 2007 and 2009 time-points. Note that the 'expansion patch' did not exist during 2001-2009.

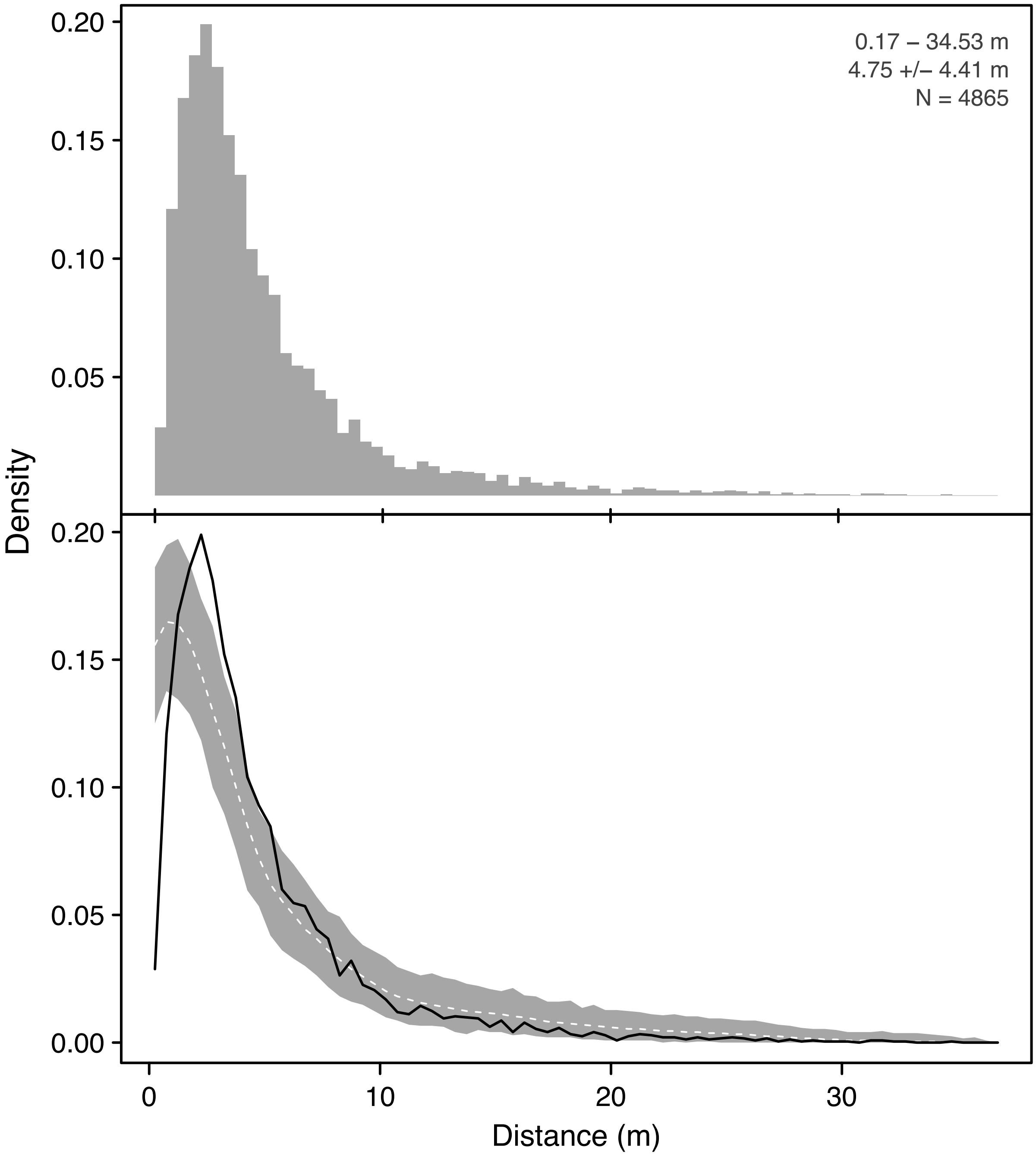

Figure 2. Upper panel: density histogram of seed dispersal distance (N=4,865), as estimated from patches recruited during the 2010 mapping relative to an amalgamated historical distribution (2001, 2007 and 2009). Lower panel: results of an iterative random sampling of all potential dispersal distances within the study site during the transition from 2009 to 2010 (lower panel; 20,000 iterations). 100% confidence intervals shaded in gray. Median values depicted as a white dashed line; empirical distribution shown in black.

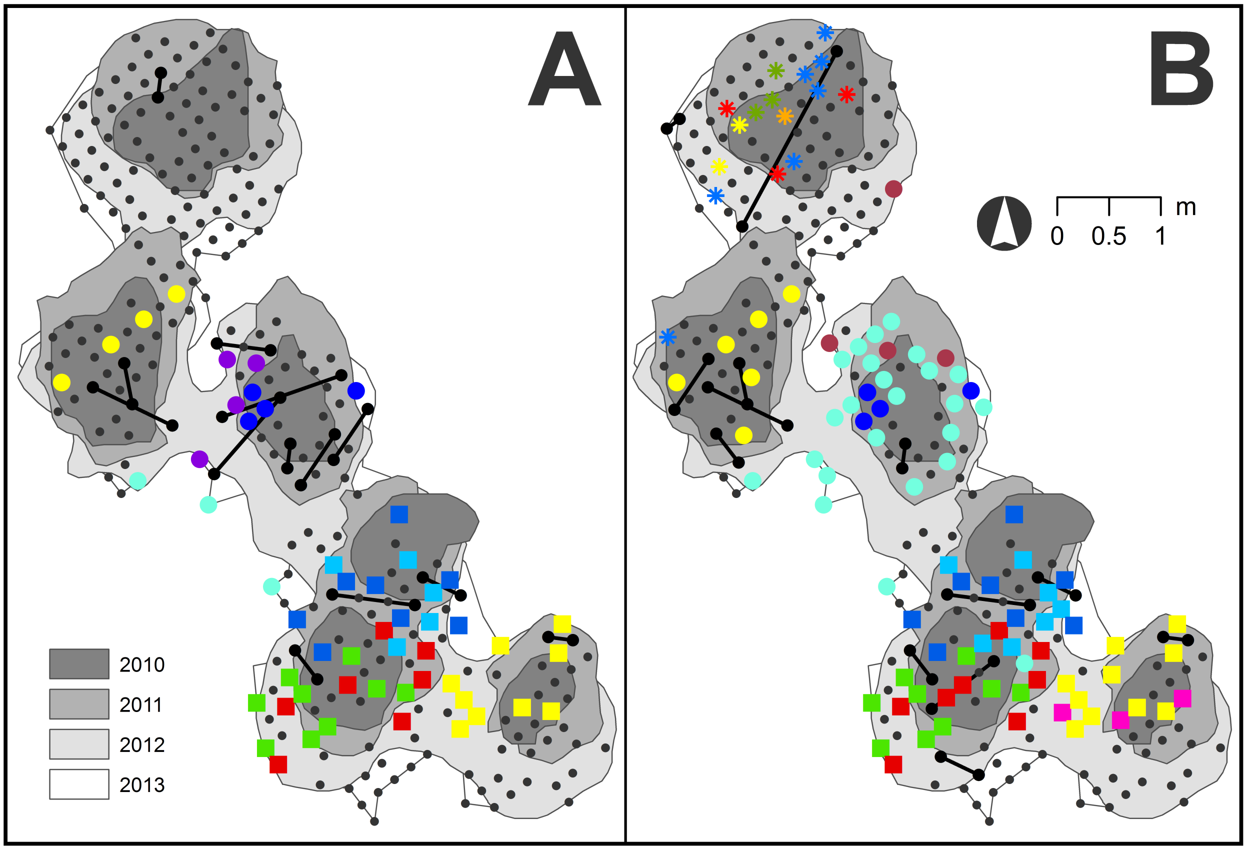

Figure 3. Distribution of MLG (A) and MLL (B) designations within the 'expansion' patch. Solitary clones are depicted as black dots, those with two ramets as barbells, and those with three or more ramets as unique combinations of shape and color.

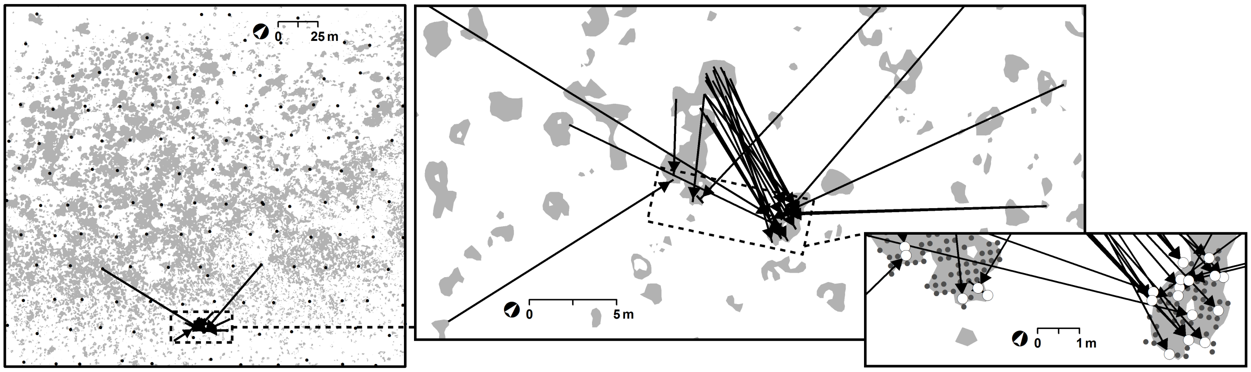

Figure 4. Multi-scale map of pollen dispersal events. Black arrows denote pollen transfer from putative fathers to mother/seed locations; the 2013 seagrass distribution is shown in gray. Left panel: full study site. Middle panel: intermediate-scale enlargement of the dashed extent rectangle. Right panel: further enlargement of the 'expansion' and 'focal' patches showing the locations of the leaf/seed (white circles) and leaf only sample locations (black circles).

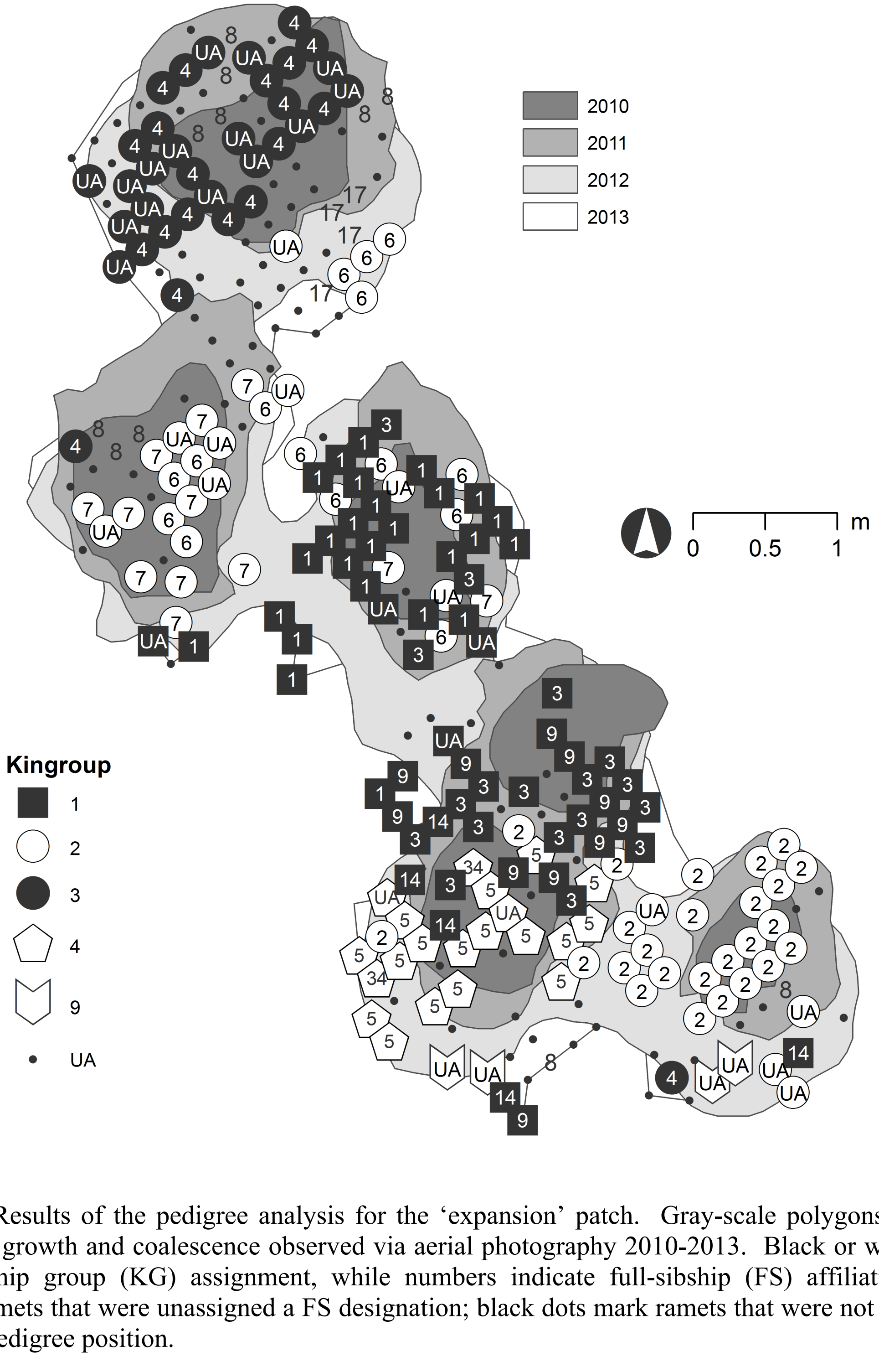

Figure 5. Results of the pedigree analysis for the ‘expansion’ patch. Gray-scale polygons depict the sequence of growth and coalescence observed via aerial photography 2010-2013. Black or white shapes denote kinship group (KG) assignment, while numbers indicate full-sibship (FS) affiliation. ‘UA’ identifies ramets that were unassigned a FS designation; black dots mark ramets that were not statistically assigned a pedigree position.

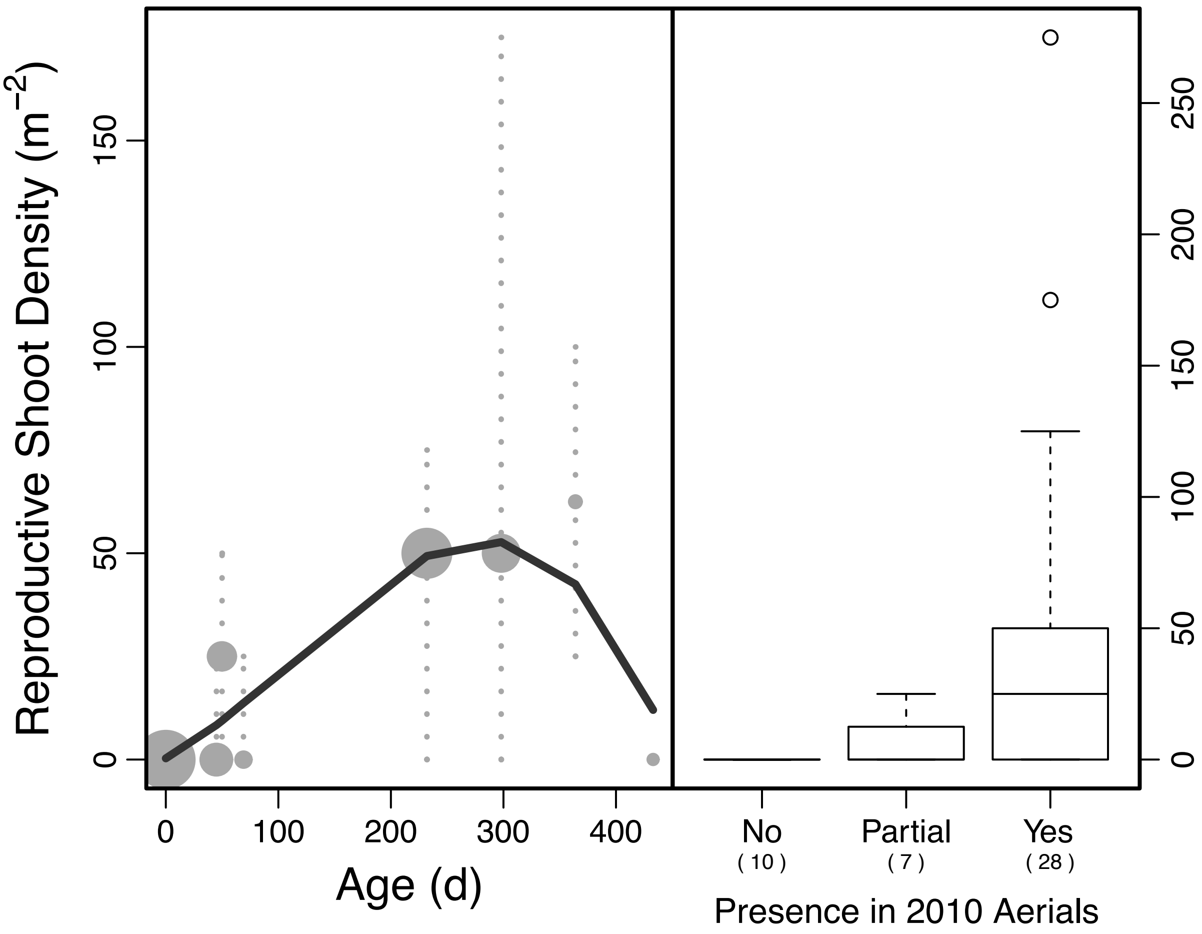

Figure 6. Selected results of the independent assessment of reproductive effort with age. Left panel: reproductive shoot density (m^{-2}) as a function of age since vegetative colonization (d). Gray circles have been scaled to sample density (replicate cells) and fit with a 3rd order polynomial. Right panel: reproductive shoot density (m^{-2}) as a function of status in the 2010 aerials (not present, partially present or fully present). Boxplots mark median values with a central bar, the 1st and 3rd quartiles with a box, the +/- 1.5 interquartile ranges with 'Tukey whiskers' and outliers with open circles.

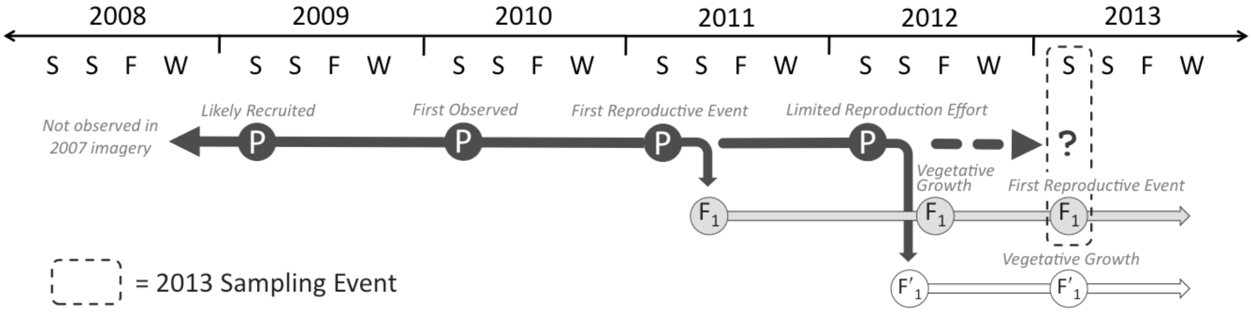

Figure 7. Reconstructed chronological pedigree for genets recruiting to the 'expansion' patch. 'P' denotes parental generation, 'F_1' the first filial generation, and 'F'_1' the second cohort of offspring from the P generation. The dashed line indicates the cohorts likely sampled during the 2013 genotypic survey. 'S S F W' identifies spring, summer, fall and winter by year (2008-2013).

Chapter 3

Sexual recruitment in *Zostera marina*: progress toward a predictive model

Statement of Author Contribution

Study conception and design:	Furman and Peterson
Acquisition of data:	Furman and Peterson
Analysis and interpretation of data:	Furman
Drafting of manuscript:	Furman
Critical revision:	Furman and Peterson

ABSTRACT

Ecophysiological stress and physical disturbance are capable of structuring meadows through a combination of direct biomass removal and recruitment limitation; however, predicting these effects at landscape scales has rarely been successful. To model environmental influence on sexual recruitment in perennial *Zostera marina*, we selected a sub-tidal, light-replete study site with seasonal extremes in temperature and wave energy. During an 8-year observation period, areal coverage increased from 4.8 to 42.7%. Gains were stepwise in pattern, attributable to annual recruitment of patches followed by centrifugal growth and coalescence. Recruitment varied from 13 to 4,894 patches per year. Using a multiple linear regression approach, we examined the association between patch appearance and relative wave energy, atmospheric condition and water temperature. Two successful models were developed, one appropriate for the dispersal of naked seeds, and another for rafted flowers. Results indicated that both modes of sexual recruitment varied as functions of wind, temperature, rainfall and wave energy, with a regime shift in wind-wave energy corresponding to periods of rapid colonization within our site. Temporal correlations between sexual recruitment and time-lagged climatic summaries highlighted floral induction, seed bank and small patch development as periods of vulnerability. Given global losses in seagrass coverage, regions of recovery and re-colonization will become increasingly important. Lacking landscape-scale process models for seagrass recruitment, temporally explicit statistical approaches presented here, could be used to forecast colonization trajectories and to provide managers with real-time estimates of future meadow performance; i.e., when to expect a good year in terms of seagrass expansion. To facilitate use as forecasting tools, we did not use statistical composites or normalized variables as our predictors. This study, therefore, represents a first step toward linking remotely acquired environmental data to sexual recruitment, an important measure of seagrass performance that translates directly into landscape-scale coverage change.

INTRODUCTION

Seagrass landscapes are spatiotemporally dynamic (Olesen & Sandjensen 1994, Bell et al. 1997, Kendrick et al. 2005). Metrics of floral composition (Lanyon & Marsh 1995), shoot density (Guidetti et al. 2002), areal coverage (Morris & Virnstein 2004) and landscape configuration (Olesen & Sandjensen 1994) have all been shown to vary widely over seasonal to inter-annual times-scales. Unfortunately, as in other ecologies, natural rates and patterns of variability have been eclipsed by modern-era declines (Waycott 2009). An alarming component of estuarine urbanization, the loss of seagrasses has spurred considerable effort to document and understand changing coverage patterns (Fonseca et al. 1995, Short & Burdick 2006). Initial work focused on the intuitive relationship between meadow contraction and water quality, and the resultant bio-optical and secchi-depth models explain a large proportion of the spatiotemporal variance (Gallegos & Kenworthy 1996, Duarte et al. 2007, Biber et al. 2008). Although paradigmatically instructive, the univariate role of light limitation on deep edge of occurrence is, however, insufficient to predict distributions for specific landscapes over annual timescales; that is, while true for regions or time-series spanning large ranges in depth or light penetration, light limitation models are insensitive to typical gradients in light stress (Greve & Krause-Jensen 2004). Aimed at more precise forecasting, and fueled by the growing availability of GIS data, contemporary investigators have employed a host of sophisticated numerical and statistical approaches to describe seagrass coverages, viz., habitat suitability (Kemp et al. 2004, Short & Burdick 2006) and species distribution models (Bekkby et al. 2008, Grech & Coles 2010, Downie et al. 2013, March et al. 2013), ecological process models (Verhagen & Nienhuis 1983, Zharova et al. 2001, Jarvis et al. 2014), and vegetative growth models (Marba & Duarte 2003, Brun et al. 2006, Sintes et al. 2006, Wong et al. 2011). Together, these efforts have broadened the range of predictor variables (e.g., salinity, slope, wave energy, current flow, tidal range, sediment characteristics, temperature, etc.) and offer potential advantages over traditional mapping techniques (Fourqurean et al. 2003, Infantes et al. 2009, Grech & Coles 2010).

The modeling of seagrass landscapes is often less expensive than physical surveys (Grech & Coles 2010). Statistical and process models are less subject to the artifacts and limitations of photo-interpretation, and tend to integrate longer time-scales – blurring some of the spatial dynamism confounding static seagrass maps (Verhagen & Nienhuis 1983, Bekkby et al. 2008). Models also allow for the evaluation of change, and the assessment of alternate scenarios of climate and human impact (Infantes et al. 2009). However, nearly all of these approaches implicitly assume that seagrass distributions remain in equilibrium with the local environment (Fourqurean et al. 2003, Greve & Krause-Jensen 2004). There are a number of reasons why this may not be the case.

First, although seagrass loss can be quite fast (physical disturbances are instantaneous and carbohydrate reserves last mere weeks), the mechanisms of spatial expansion operate on much slower time-scales (Burke et al. 1996, Vermaat 2009, but see van Katwijk et al. 2010). Viewed largely as a vegetative process, patch growth has been found to match rhizome elongation rates at centimeters to meters per year with strong seasonal cycles in production (Duarte & Sandjensen 1990, Zharova et al. 2001, Duarte et al. 2006). Therefore, at landscape scales, environmental forcing seagrass coverage may not be apparent for some time. A number of investigators have addressed these offsets by exploring time-lagged predictor variables (Greve & Krause-Jensen 2004, Bekkby et al. 2008, Rasheed & Unsworth 2011); however, no evidence for systematic delays has yet been found (Greve & Krause-Jensen 2004), and up to a decade may in fact be necessary for adequate meadow response (Fourqurean et al. 2003). Second, we know very little about the mechanisms and relative importance of sexual recruitment in seagrasses (Orth et al. 2000, Jarvis et al. 2014). Recent genetic surveys of *Zostera marina* have begun to highlight the role of pollen and seed dispersal in meadow development (Hammerli & Reusch 2003, Becheler et al. 2010, Zipperle et al. 2011); however, very few quantitative estimates of contemporary gene flow exist, and dispersal kernels for seeds, rafted flowers and vegetative fragments remain poorly constrained for any seagrass species (Ruckelshaus 1996, Kendrick et al. 2012). Nowhere is this more evident than for regions undergoing recovery (Morris & Virnstein 2004, Cunha & Santos 2009, Costello & Kenworthy 2011), where the pace and shape of re-colonization can only be described as enigmatic (Duarte et al. 2006). Without a quantitative understanding of recruitment dynamics, errors of commission will continue to undermine the development and interpretation of spatially explicit distribution models, and to force anecdotal explanations of recovery.

Toward filling this gap, we sought to relate time-lagged climatic variables to recruitment success within a sub-tidal, colonization phase, *Z. marina* meadow in Shinnecock Bay, New York, USA. A previous study of the same location revealed that sexual recruitment was much more important than vegetative growth with respect to space acquisition over a 13-year period

(2001-2014), and that the rate of recruitment varied widely by year. Concurrent estimates of floral densities and seed production made within the meadow over 3 years (2012-2014) indicated that no substantive changes in reproductive effort or mating system effectiveness had occurred (L. Jackson, unpubl.). Because the system was (1) uniformly shallow and therefore light-replete, (2) unaffected by drift or epiphytic algal growth, (3) mono-specific and binary in composition – i.e., *Z. marina* embedded within a sandy matrix, (4) wave exposed and (5) subject to temperature fluctuation, we hypothesized that autecological stress would control seedling survival, and that variability in the physical environment would lead to inter-annual patterns in sexual recruitment.

Using a multiple linear regression (MLR) approach, we examined the association between isolated patch emergence (i.e., seed-borne recruitment) and estimates of relative wave energy, atmospheric condition and water temperature over an 8-year period (2007-2014). Two models were developed, one appropriate for the dispersal of naked seeds, and another for seeds delivered by rafted flowers. To facilitate their use as forecasting tools, we did not use statistical composites (i.e., principal components) or normalized variables (i.e., Z-scores) as our predictors. This study therefore represents a first step toward providing managers with a baseline forecasting tool linking easily acquired environmental data to sexual recruitment, an important measure of seagrass performance that translates directly into landscape-scale coverage change. Although conducted on a relatively small spatial scale (56,250 m^2), this study applies directly to similar locations within Shinnecock Bay (i.e., sandy, wave-exposed shoals) and stands as a proof of concept, adaptable to other landscapes wherever sufficient seagrass coverage and environmental data exist.

MATERIALS AND METHODS

Study Site

All fieldwork was conducted in Shinnecock Bay, a backbarrier lagoon system in southeastern Long Island, New York, USA. Depths throughout the bay are relatively shallow, varying from 0-4 m with a mean of 2 m (MLLW); tides are semi-diurnal with a range of 0.8 meters (USACE 2004). In 2011, in the southeast portion of the bay, roughly 400 m from shore and 2.5 km east of the only oceanic inlet (40.857237° N, 72.450289° W), we selected a rectangular site measuring 250 m (parallel to shore) x 225 m (total area, 56,250 m^2). Depths here ranged from 0.25-1.25 m MLLW. Surficial sediments were siliceous sands uniformly low in organic content (< 1% by loss on ignition at 500˚C for 5 h, B. T. Furman unpubl.). Site orientation and boundaries were chosen: (1) to encompass the full meadow cross-section, (2) to minimize border contact with contiguous seagrass patches, (3) to eliminate the influence of light availability on seagrass distribution and (4) to capture sexual recruitment dynamics within a colonization phase meadow.

Sexual Recruitment

Sexual reproduction in perennial *Z. marina* is thought to occur during the second season of growth and annually thereafter (Granger et al. 2003, Plus et al. 2003, Moore & Short 2006); however, genet-wide flowering intensity has been shown to decline after the initial reproductive event (Furman et al. 2015). In New York, flowering phenology is strongly correlated to temperature: floral induction occurs mid- to late-fall, primordial inflorescences appear in January at 0.5-3˚C, anthesis occurs in mid-May at 15˚C and fruit maturation is completed by the end of June at temperatures above 21˚C (Churchill & Riner 1978, Silberhorn et al. 1983). Seed dispersal covers three orders of magnitude (1s to 1,000s m), the distance and nature of which depend strongly on the form of the diaspora (Waycott et al. 2006, Vermaat 2009, Kendrick et al. 2012). Negatively buoyant seeds diffuse roughly 5 meters via current- and wave-mediated rolling and saltational jumping (Ruckelshaus 1996, Billingham et al. 2007, Furman et al. 2015), while the deposition of seeds from positively buoyant reproductive shoots and shoot fragments (i.e., spathes and rhapidia) can extend 10s to 1,000s of meters in a more or less spatially random fashion (Billingham et al. 2007, Kendrick et al. 2012, McMahon et al. 2014). Dehisced seeds contribute to transient seed banks (1000s of seeds m^{-2}) capable of germinating by their first fall, at temperatures below 20˚C; however, yearlong (i.e., over winter) dormancy has been suggested (Orth & Moore 1983, Harrison 1993, Olesen & Sandjensen 1994, Orth et al. 2000). Correlative shoot demography data and December field surveys conducted at our site support the latter, indicating that a majority of seedlings emerge late May through early August, consistent with a spring germination (Furman et al. unpubl.). Genetic surveys have shown that isolated patches can begin with a single clone (Waycott et al. 2006). If so, seedling branching rates of between 2 and 12 x during the initial growth season (Harrison 1993, Greve et al. 2005) and nominal patch spreading rates of between 13 and 46 cm y^{-1} (Olesen & Sandjensen 1994, Reusch et al. 1998, Greve et al. 2005) would delay detection of sexually recruited patches to, at minimum, the following spring. Based on these field and literature values, we have developed a working life history timeline (Fig. 1) that tracks the inference period for sexual recruitment from the spring of observation backward nearly three years to the fall of floral induction.

To map sexual recruitment at our site, we gathered aerial photography from local, state and federal agencies via online access and personal communication. Annual geospatial images were obtained from the New York Statewide Digital Orthoimagery Program (NYSDOP; ground pixel resolution = 15 cm) and the USDA - Farm Service Agency's National Agriculture Imagery Program (ground pixel resolution = 1 m; 2009 only) for the years 2006-2007, 2009-2010. In June of 2011, we began bi-monthly mapping of the study site by means of a custom-built, balloon-mounted camera (2011-2014, ground pixel resolution = 10-15 cm); see Furman et al. 2015 for a full description of methods and available data. The images were photo-interpreted at an absolute resolution of 1:100 or greater using the ESRI ArcGIS software, ArcMap 9.2, and all seagrass patches were delineated manually as polygonal feature classes. The final sequence of high-resolution thematic habitat maps spanned 8 years, 2006-2014.

Sexual recruits were operationally defined as isolated, discontinuous patches that first appeared in spring-acquired images (March – May, when available). Recruits were distinguished from recently fragmented patches and areas recovering via surviving rhizomes by masking all locations at which seagrasses were recorded as present, nominally 2001 through the preceding December. Because the site was tidally swept and wave-exposed, we assumed patch formation through the re-settlement of up-rooted ramets to not occur (B. J. Peterson, person. obs). To minimize the confounding effects of naked seed transport from un-mapped areas, an internal 20-m buffer was placed over the site in an east-west direction. This was not necessary in the north-south orientation because the original site boundaries fully bracketed seagrass coverage. Recruits were divided into two classes following Furman et al. (2015): those falling within 6 m of seagrass mapped two springs prior (i.e., 0-6 m or naked seed dispersal, NSD) and those beyond (i.e., > 6 m or rafted seed dispersal, RSD). The numbers of recruits per year were then standardized by the availability (m^2) of bare space within these distance classes at the time of seed dehiscence. Since NSD recruits were, by definition, produced locally, they were further normalized to the amount (m^2) of seagrass coverage present during seed production. This area was calculated after the application of a internal 14-m, east west buffer (to the full site boundaries) to account for a maximum 6-m dispersal from maternal sources. Years for which coverage was unavailable at a two-year offset (2007 and 2010) were processed using distributions from the previous year.

Relative Wave Energy

Relative wave energies (RWE; J/m) were estimated using NOAA's Center for Coastal Fisheries and Habitat Research Wave Exposure Model (WEMo v3.1). Hourly wind data were from Gabreski Airport in Westhampton Beach (USAF-WBAN station 744865-14719; Fig. 2A) and bathymetric data were from the NOAA's National Geophysical Data Center (Coastal Relief Model, 'shinneco_9331'). WEMo uses the top 5% of recorded wind speeds to propagate simple linear waves along each of 32 lines of fetch, providing a useful measure of wave energy condition at any number of user-defined positions (Fonseca & Bell 1998). RWE values were generated for monthly intervals (2000-2014) at two scales of observation, bay-wide using a modified 200-m alternating grid of 964 positions (Fig. 2B) and site-wide using a 12-m alternating grid of 369 positions (Fig. 2C).

To characterize multivariate spatiotemporal patterns in wave energy, we first calculated Euclidean distance similarity matrices for each recruitment period between 2003 and 2014. This period was defined as May through the third previous August (Fig. 1), encompassing all major life history stages for sexual recruits censused by our aerial surveys. We then generated a Spearman rank correlation matrix among years and conducted a second-stage analysis within each scale domain, both site and bay (Clarke et al. 2006). This approach had the unique benefit of circumventing issues of repeated measurement while still allowing for a full suite of multivariate comparison (Clarke et al. 2006). We used non-metric multidimensional scaling (nMDS) ordination, group-average agglomerative hierarchical clustering, and analysis of similarity (ANOSIM) routines to describe and quantify multi-year wave energy regimes at both the site and bay scales.

To reduce spatial complexity, wave energy data were summarized prior to MLR analysis. Based on the premise that rafted flower delivery would be a function of wave-mediated disturbance and distance from source, we binned bay-wide RWE measures into 2,000-m zones radiating from site boundaries (Fig. 2B). This interval was chosen to approximately bracket regions of known seagrass coverage within the bay, and to provide a fair sampling of wave energy along the bay's longest axis. To construct similar subdivision within our study site, group-average agglomerative hierarchical clustering and nMDS analysis of the full RWE time-series (January 2000 to April 2014) was used to delineate zones of statistically similar decadal behavior. During this process, spatially coherent clusters were favored. Mean, maximum and standard deviation estimates for site-wide, monthly wave energy conditions were also examined.

Water Temperature

On-site temperature records were unavailable for the required duration (2004-2014), and so an effort was made to estimate historical bottom-water temperatures from more accessible meteomarine and atmospheric variables (e.g., air temperature, wind chill, sea-surface temperature). Within our study site, we directly measured bottom-water temperatures for 2012-2014 at 15-min intervals using HOBO Onset light and temperature dataloggers anchored 10 cm from the sediment-water interface. In June of 2012, we placed sensors at the corners and center of the site, and in March of 2013, we expanded this to include 13 additional dataloggers. Daily site-wide means were calculated and, to dampen sensor fluctuations, the time-series was smoothed using a 7-pt running average. Comparable sea-surface temperatures were obtained from the two closest NOAA National Data Buoy Center stations, 44017 and 44025 (Fig. 2A). Oceanic datasets were averaged to produce a complete 2004-2014 daily mean time-series. Associated air temperature (°C) and wind speed (m/s) data were acquired from Westhampton Beach Gabreski Airport (Fig. 2A). Using MLR, we modeled the temperature offset between site and oceanic conditions using a fully saturated model with air-sea differential (°C), wind chill (W m^{-2}) and wind speed (m/s, mean conditions during the previous 3 days) as predictor variables (adj-R^2 = 0.7134, $P<0.001$). The relationship was then hind-cast to generate a 2004-2014 time-series of daily mean site temperatures. These data were crosschecked using monthly bay-wide (11 sites) temperature ranges recorded by the Suffolk County Department of Health Services. Model performance was acceptable, placing projected temperatures within 1 degree of observed values in 93% of cases (N=107).

Monthly summaries were calculated by dividing the number of days mean conditions fell within each of 6 temperature zones (<0° C, <5° C, <10° C, 10-20° C, >20° C and >25° C) by the number of days available within each month. We based these thresholds on literature values for cold- and heat-related temperature stress, a well-known control on *Z. marina* productivity and distribution (Burkholder & Doheny 1968, Short & Neckles 1999, Zharova et al. 2001, Lee et al. 2007b, Jarvis et al. 2014). Threshold exceedance values for three months (January 2009, September - October 2011) were linearly interpolated due to missingness.

Atmospheric Variables

Because of the shallow, enclosed nature of Shinnecock Bay, we chose to include monthly estimates of atmospheric condition as potential correlates with on-site disturbance. Mean monthly rainfall was calculated using precipitation data obtained from the Long Island MacArthur Airport in Islip, NY (USW00004781; Fig. 2A). Mean wind directions were derived from Gabreski Airport observations after extraction of continuous orthogonal components (i.e., 'northness' and 'eastness') following the methods of Zar 1999 (Zar 1999, Bergenius et al. 2005). Mean monthly wind speeds (m/s) from the same dataset were averaged after removal of 'variable' and 'calm' observations.

Multiple Linear Regression Analysis

To mitigate issues associated with data mining (i.e., ghost degrees of freedom and spurious temporal correlations) we employed a mixed approach to variable selection, integrating both site and biological knowledge with statistical information at each phase of the process. Because we began the investigation with few data regarding the critical time-period for sexual recruitment – that is, which life history stages were the most vulnerable to ecophysiological stress – we adopted an exhaustive time-lagging procedure. All potential predictor variables (N=27) were screened for linear relationships with each of the dependent variables (i.e., NSD and RSD recruitment) using Pearson correlations; (1) monthly offsets extending backward one year, (2) a sequence of expanding durations of up to one full previous year, (3) full 24- and 36-month means, and (4) 3-month means extending back 3 years were evaluated. Those exhibiting a significant relationship with either or both response variables ($P<0.05$) were visually assessed for outlier influence and dispersion along the abscissa. Multicollinearity among the remaining variables was quantified by hierarchical clustering of absolute transformed Pearson correlation matrices, one matrix per response variable. Co-linear groups were set at a threshold of 0.70 (Greve et al. 2005) and culled to a maximum of 4 per group based on ecological interpretability and statistical similarity (i.e., redundancy). All potential combinations of uncorrelated variable sets were then entered into the 'regression with empirical variable selection' or REVS procedure following the methods of Goodenough et al. 2012 (Goodenough et al. 2012). This method uses an all-subsets approach, implemented through the 'leaps' package for R, to investigate and rank predictor importance so as to empirically order a manual stepwise regression (Miller 2009, R Development Core Team 2012). The resulting candidate models were sorted by adj-R^2 and diagnostically tested for multicollinearity (variable inflation factor or VIF), model complexity (Akaike's Information Criterion or AIC), model bias (Mallows' C_p statistic), standardized residual normality (Shapiro-Wilk's test), homogeneity of variance (Breusch-Pagan test), residual independence (Durbin Watson statistic), and outlier influence (Cook's distance). To account for small sample size ($N=7$) and to address issues of over-fitting, we further evaluated candidate models using a combination of second-order AIC (AIC_C), AIC_C weights and leave-one-out cross-validation (LOOCV; Wagenmakers & Farrell 2004). The best model for each recruitment type (NSD and RSD) was selected based on (1) diagnostic performance, (2) parsimony of biological explanation, (3) future repeatability (i.e., independent variables that were statistics of location were preferred over those of dispersion) and (4) number of implicated life history stages. Response variables were log-transformed to meet assumptions of normality. All univariate analyses were conducted on the open-source R-Package (v2.14.1), and all multivariate analyses were performed using PRIMER (v6.1.15).

RESULTS AND DISCUSSION

Sexual Recruitment

Seven snapshots of meadow development were recorded during the 8-year observation period. During this time, *Z. marina* coverage increased from 4.8 to 42.7% of the mapped area. Gains were stepwise in pattern and attributable to annual recruitment of new patches – all less than 4 m^2 at the time of first observation – followed by slow centrifugal growth and coalescence. Recruitment was not constant over time, however, varying across 2 orders of magnitude from 13 to 4,894 patches yr^{-1} with a mean (± 1 s.d.) of 1,076 ± 1,716 patches yr^{-1}. Naked seed recruits (0-6 m) ranged 11 to 2,978 (722 ± 1,025) patches yr^{-1}, while rafted seed recruits (> 6 m) were less abundant at between 2 and 1,916 (353 ± 706) patches yr^{-1}. Minima and maxima for both recruitment types took place in 2009 and 2010, respectively, with higher numbers of recruits observed post-2009.

Wave Energy Regimes

nMDS ordination of bay-wide conditions suggested two distinct clusters of recruitment-relevant RWE, one covering 2004-2009 and another 2010-2014 (Fig. 3A). ANOSIM revealed these groups to be highly significantly different (global R = 0.978, P = 0.001) despite an among-group correlation coefficient of 0.96. Interestingly, both anomalous years (2009 and 2010) were somewhat isolated within their respective clusters, each along the same axis in the 2-d representation (stress = 0.01). Graphical overlays of NSD and RSD recruitment rates were consistent with the view that multivariate sequences of bay-wide RWE mirrored long-term patterns in sexual recruitment, and even appeared to structure gradients of recruitment success within each long-term regime (Figs. 3B and 3C). Similar patterns were found for wave energies estimated within the study site, as Spearman rank-based Mantel tests between bay- and site-wide RWE resemblance matrices (RELATE procedure in PRIMER) were highly correlated ($\rho = 0.858$, P = 0.001). Likewise, nMDS and hierarchical clustering analyses described identical clusters (2004-2009 and 2010-2014) that were also significantly different (global R = 1, P = 0.001; Fig. 4A). Not surprisingly, patterns at this small spatial grain were more correlated with each other ($\rho = 0.990$) and less suggestive of gradients when overlain with sexual recruitment rates (Figs. 4B and 4C). We interpreted this to be a possible artifact of WEMo model performance, whereby the resolution of bay-wide bathymetric data (cell-size = 83.2 x 83.2 m) was more suited to linear wave propagation at the landscape scale, providing a richer treatment of small differences in wind conditions when modeled over the same time period. For this reason, we favored RWE-based predictors drawn from the bay scale when selecting among potential independent variables (see ‘Variable Selection’).

Unfortunately, no specific test for group cohesion exists for second-stage analysis; however, successive similarity percentage (SIMPER) tests run on transposed component matrices (i.e., by recruitment period at the site-scale) and temporal patterns of median RWE values indicated that the nMDS clusters were a product of slightly relaxed wave energies during the 2010-2014 regime. We posit that such changes, which would amount to a reprieve for vulnerable seeds and seedlings, could be particularly impactful at our study site, as long-term (2000-2014) averages place it within the highest wave energy zone of the bay (Fig. 2B).

nMDS ordination and hierarchical clustering of long-term (January 2000 through April 2014) RWE patterns at the site level identified 6 roughly contiguous zones of wave energy (hereafter, a-f; Figs. 2C and 2D). One-way ANOSIM confirmed these to be statistically distinct (global R = 0.998, P = 0.001) with extremely high pair-wise R-values, all 0.993 to 1. Mean conditions within zones a-f over the 178-month period (January 2000 to October 2014) were 399.2 ± 2.93, 350.51 ± 11.57, 454.88 ± 3.71, 507.34 ± 8.25, 195.79 ± 3.42 and 24.62 ± 9.87 J/m, respectively, or d, c, a, b, e and f in descending order (Figs. 2C and 2D). As would be expected under scenarios of disturbance-control, seed-borne patches during the largest recruitment event, in 2010, were aggregated within the higher energy zones (i.e., a, c and d) (Fig. 2D), suggesting that quiescent conditions may have facilitated recruitment success.

Multiple Linear Regressions

Variable Selection

Significant Pearson correlations between environmental variables and log-transformed NSD and RSD recruitment estimates were found for 152 time-lagged summaries: N = 48 and 105, respectively. Outliers or biphasic groupings unduly influenced a large number of these relationships, leaving only 23 (NSD) and 30 (RSD) viable predictors for multicollinearity assessment. Both lists were further pared to eliminate statistical and temporal redundancy, which yielded 12 variables clustered into 4 groups for the NSD model, and 9 variables into 3 groups for the RSD model. Counter to expectations – we had anticipated disturbance of distant flowers to control RSD recruitment – both sets of significant correlates were composed of nearly equivalent proportions of wave energy (56-8%) and site-specific (33%) products. NSD predictors were also temporally clustered, corresponding to floral induction, seed bank and patch development life history stages (Fig. 1). Eight of these varied negatively with recruitment, with 3 of the 4 positive associations linked to seed bank conditions during the fall and winter. In slight contrast, (1) only a single RSD-associated variable (mean maximum RWE during the second previous August to October) was positively related to recruitment, (2) nearly all of the viable predictors (75%) implicated conditions during patch development, and (3) a fourth life history stage was added, flower growth and maturation (negatively correlated to 0° C exceedance during February to April).

While it is tempting to interpret these patterns mechanistically, it is important to recognize that independent variable associations are purely descriptive and, as such, are unable to define causality within the system (Fourqurean et al. 2003). Nevertheless, the correspondence of time-lagged disturbance measures to particularly vulnerable life history stages, such as seed banks and initial clonal growth, is encouraging, and provides specific targets for future manipulative work. For example, understanding the physiological controls of floral induction (Churchill & Riner 1978) and seed germination (Orth et al. 2000), as well as the precise mechanisms and seasonality of small patch mortality (Olesen & Sandjensen 1994, Mills & Fonseca 2003), are all tractable research questions.

Model Selection

After duplicate removal, the modified REVS procedure yielded 31 candidate NSD models; 6 of these failed diagnostic testing due to multicollinearity, model bias and residual non-normality. The remaining models explained between 77 and 99.8% of the variation using 2 to 4 predictors. Although we recognize that ensemble predictions (i.e., the use of multiple models) often provide important insights into the scale and nature of forecast uncertainty, and have been used successfully to describe *Z. marina* coverage in the past (Downie et al. 2013), we chose to select a single, or best, model for each recruitment type in order to simplify initial predictions and interpretation. Future implementation of these methods, however, may wish to query multiple models as some portion of the underlying statistical patterns may have proven falsely positive.

Based on statistical diagnostics and ecological considerations, the most promising NSD model additively used (1) mean 10° C exceedance during the previous September to November ('10C_p1yNOS'), (2) mean rainfall during the second previous November to January ('rain_p2yJDN'), and (3) mean wind eastness during the preceding 3-yr period ('eastness_p36mo') – taking the form: log(NSD) = -8.72710 * 10C_p1yNOS + 0.00139 * rain_p2yJDN + 8.48952 * eastness_p36mo - 4.37839 (adj-$R^2 = 0.998$, $P < 0.001$; Fig. 5A). This model was ranked 8th based on AIC_C weights; however, it out-performed all other candidates in LOOCV and had the lowest un-adjusted AIC value. Interestingly, despite the large number of available inverse relationships, two of the three independent variables were positively correlated with NSD recruitment, and none of them were estimates of wave energy. The latter suggests that once seagrass patches become established, subsequent NSD recruitment may not be strongly limited by wind waves. Whether this is a function of greater densities of NSD seed banks (Ruckelshaus 1996, Furman et al. 2015) or some hydrodynamic modification afforded by proximate seagrass coverage (Fonseca & Bell 1998) remains unclear.

Cold-induced torpor (< 10°) during early patch development was indicated as a negative control on NSD recruitment. This could be a product of clone mortality or merely a reduction in vegetative propagation, leaving patches too small to identify in spring-acquired imagery. As the balloon-mapping program continues, fall and winter maps will help to resolve this issue through the identification of late-arriving recruits, especially during anomalously cold years. Future, manipulative work should examine the physiological mechanism behind this relationship. Our expectation is that metabolic depression acts directly to limit branching frequency and carbon storage; however, likely covariates, such as reduced solar irradiance and bioturbation rates cannot be discounted.

We interpret the influence of rainfall as a source of freshwater to extant seed banks, with direct effects on germination rates. Similar effects of precipitation and terrestrial runoff have been suggested for Pacific populations of *Z. marina* {Phillips, 1983 #1337}. If not a direct germination cue, rainfall rates can also co-vary with meteomarine conditions promoting temperature stratification in surficial sediment layers or the scarification of seed-coats (i.e., intermittent storm activity not captured by WEMo), as both are known to be drivers of seed germination (Orth et al. 2000).

For RSD recruitment, the REVS procedure generated 14 models. Poor fit disqualified 3 of them from further consideration, leaving 11 candidate models explaining 80 to 98% of the variance. The majority of predictors comprising these models were wind or RWE-related (78%), as were all three independent variables of the best model: (1) mean RWE within the 14,000- to 16,000-m distance class during the previous November to April (‘d16000_pAprToNov’), (2) mean wind speed during the previous May (‘mWindSpd_pMay’), and (3) mean RWE in the 0- to 2,000-m distance class during the previous February (‘d2000_pFeb’). The model: log(RSD) = -0.23381 * d16000_pAprToNov - 0.54899 * mWindSpd_pMay - 0.00107 * d2000_pFeb + 8.07992, was ranked 2nd in AIC_C weights, 1st in LOOCV performance and explained 98.1% of the variation ($P = 0.002$). Importantly, all three of these terms referenced wave energy characteristics experienced during early patch formation, all negative correlates with recruitment. This is consistent with the notion that isolated patches remain vulnerable to hydrodynamic forces for some time after initial formation (Olesen & Sandjensen 1994, Greve et al. 2005). Whether this was due to burial (Mills & Fonseca 2003), scour (Fonseca & Bell 1998) or breakage of ramets is unclear from these findings; however, some stability appears to be conferred by the presence of adjacent individuals (Cheplick 1998, Kendrick et al. 2005), as the loss of established patches (~ 1 m^2) was not regularly observed. Similar findings have been reported for small patch dynamics in Danish waters, where a size threshold of 32 ramets was identified as a necessary buffer against physicochemical disturbance (Olesen & Sandjensen 1994).

Model Predictions

Limited by the available time-series, we were unable to validate either model on new coverage data. However, projections for the un-measured 2008 recruitment event fit *a priori* expectations based on known changes in landscape configuration, and together provide a sensible picture of sexual recruitment dynamics over the modeled period (Figs. 5A and 5B). Both the NSD and RSD models yielded values intermediate to 2009 and 2010 estimates. Admittedly, this approach was slightly confounded by the duplication of seagrass and distance class information, although we feel model success here reflects positively on our capability to process future data.

The relative contributions of NSD and RSD recruitment to our study site were assessed using the fitted data by standardizing model predictions to fixed seagrass coverage and available space values (Fig. 5C). Temporal patterns of NSD:RSD recruitment and their propagated errors showed that NSD recruitment or the dispersal of naked seeds from source plants was comparatively more important than RSD or the dispersal of rafted seeds during the 2007 to 2011 period. This reversed after 2011, in rough parallel with rising seagrass coverage (Fig. 5C). One interpretation of this pattern is that long distance dispersal of rafted flowers was relatively unimportant to space acquisition at dispersal distances of 2,000 to 4,000 m, or roughly the distance from the study site to the next largest meadow prior to 2010, and only after seagrasses began to densely colonize the southeastern shoreline did RSD recruitment contribute significantly to meadow expansion. If true, this means that RSD kernels operate largely below 2,000 m, a hypothesis that is testable using current genetic methods. Alternatively, this could have been an artifact of sharp reductions in available space in RSD distance class, particularly the loss of distances greater than 20 m. By increasing the relative contribution of more proximate recruits, RSD estimates could have been conflated with limited numbers of NSD recruits beyond 6 m – a possibility supported by empirical estimates of seed dispersal distance (Ruckelshaus 1996, Furman et al. 2015).

CONCLUSIONS

Ecophysiological stress and physical disturbance are known agents of change in seagrass systems, capable of spatially structuring meadows through a combination of direct biomass removal and recruitment limitation (Gallegos & Kenworthy 1996, Fonseca & Bell 1998, Jarvis et al. 2014). In the present study, we selected a light-replete study site with seasonal extremes of temperature and wave energy to model environmental effects on sexual recruitment over an 8-year period. Two successful multiple linear regression models were developed, one described the annual recruitment of seed-borne patches arising from the diffusive flux of naked seeds (NSD) and, another, from seeds deposited by rafted flowers (RSD).

Results indicated that sexual recruitment varied as a predictable function of wind, temperature and wave energy, with long term multivariate patterns in wave energy corresponding to periods of rapid colonization within our site. Notably, no comparable multivariate patterns were found for combinations of atmospheric condition (i.e., rainfall, wind speed, wind direction or air temperature). Univariate correlational patterns with time-lagged climatic summaries consistently showed floral induction, seed bank and small patch developmental periods to be most vulnerable to disturbance. Of these, seedling survival was probably the most important, as seedling safe-site availability has been shown to control aspects of space acquisition during periods of rapid expansion and recovery (Plus et al. 2003, Lee et al. 2007a), and physical forces are known to play significant roles in seedling survival and resultant bed morphology (Fonseca & Bell 1998, Infantes et al. 2009, Infantes et al. 2011).

For this modeling approach to have broader spatiotemporal applicability (i.e., forecasting power), sufficient environmental variation must have been sampled during its initialization. That is, meadow responses to a full range of climatic conditions need to have been incorporated into the model for it to then accurately predict recruitment when presented with new data. At present, there is no way to know if this has occurred; however, (1) normal temperate-latitude cycles of temperature and wave energy were observed, (2) the time-series included at least one anomalously warm year, 2012, (3) a strong hurricane impacted the system, Hurricane Sandy, in 2012, and (4) a wide range of sexual recruitment was documented, including the largest event recorded since 2001. Future application of the models developed in this study should apply caution, however, if input data fall outside the ranges reported in Table 1, as the underlying correlational structure may no longer be valid.

Although spatially explicit, discrete (stage-based) or continuous (differential) population growth models are best suited to describing landscape dynamics, it may be sometime before working process models of seagrass recruitment become available (Fourqurean et al. 2003). Until then, statistical forecasting tools such as the ones described above could be used in conjunction with more common habitat distribution models to better understand the drivers and temporal patterns of coverage change. This is particularly true for areas of commission error, where habitat models predict seagrass presence but none is currently found. With so much of the global habitat space lost or degraded, these regions are increasingly common. Methods such as ours could be used to predict colonization trajectories for areas of commission error proximate to extant seagrasses, and to augment success criteria by quantifying coverage expectations for management zones and restoration projects that lack appropriate reference locations, allowing coastal managers to track conservation and mitigation progress in real-time. To our knowledge, this study represents the first attempt to relate relative wave energy to recruitment success in a temporally explicit manner, at a scale of action necessary for effective coastal management.

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Table 1: Observed monthly ranges for environmental variables, 2004 to 2014.

Abbrev.	Description	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
SAV	pct. of site covered by seagrass (%)	-	-	-	-	4.83-42.70	-	-	-	-	-	-	-
25C	prop. of days mean daily temp. exceeded 25° C	0.00	0.00	0.00	0.00	0.00	0.00	0.00 - 0.52	0.00 - 0.52	0.00 - 0.13	0.00 - 0.07	0.00	0.00
20C	prop. of days mean daily temp. exceeded 20° C	0.00	0.00	0.00	0.00	0.00 - 0.10	0.10 - 0.60	0.90 - 1.00	0.87 - 1.00	0.18 - 0.90	0.00 - 0.33	0.00	0.00
optimal	prop. of days mean daily temp. was optimal	0.00 - 0.16	0.00	0.00 - 0.19	0.03 - 0.60	0.77 - 1.00	0.40 - 0.90	0.00 - 0.10	0.00 - 0.13	0.10 - 0.82	0.53 - 1.00	0.50 - 0.93	0.03 - 0.35
10C	prop. of days mean daily temp. was less than 10° C	0.84 - 1.00	1.00 - 1.00	0.81 - 1.00	0.40 - 0.97	0.00 - 0.23	0.00	0.00	0.00	0.00 - 0.07	0.00 - 0.14	0.07 - 0.50	0.65 - 0.97
5C	prop. of days mean daily temp. was less than 5° C	0.32 - 0.92	0.48 - 0.96	0.16 - 0.90	0.00 - 0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00 - 0.07	0.06 - 0.68
0C	prop. of days mean daily temp. was less than 0° C	0.00 - 0.32	0.00 - 0.18	0.00 - 0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
rain	monthly precipitation (tenths of mm)	553 - 1065	320 - 1580	253 - 2391	389 - 1852	380 - 1494	314 - 2241	532 - 1655	67 - 2939	333 - 1896	56 - 3574	486 - 1160	545 - 2242
eastness	magnitude of the easterly wind component (unitless)	-0.51 - -0.11	-0.59 - -0.14	-0.43 - 0.11	-0.37 - 0.17	-0.29 - 0.21	-0.33 - 0.19	-0.44 - -0.00	-0.24 - 0.02	-0.26 - 0.18	-0.34 - 0.04	-0.40 - 0.05	-0.51 - -0.17
northness	magnitude of the northerly wind component (unitless)	0.02 - 0.46	0.04 - 0.39	-0.04 - 0.45	-0.25 - 0.20	-0.35 - 0.16	-0.51 - -0.01	-0.54 - -0.13	-0.40 - 0.01	-0.19 - 0.14	-0.18 - 0.31	-0.18 - 0.48	-0.03 - 0.37
mWindSpd	mean wind speed (m/s)	4 - 5	5 - 6	4 - 6	4 - 5	4 - 5	3 - 4	3 - 4	3 - 4	4	4 - 5	4 - 5	4 - 6
mRWE	mean RWE (J/m) at the site (N=369)	318 - 603	428 - 1057	292 - 967	232 - 635	165 - 763	119 - 424	51 - 224	49 - 195	69 - 262	120 - 652	174 - 677	255 - 794
sdRWE	standard deviation of RWE (J/m) at the site (N=369)	93 - 227	131 - 465	99 - 383	55 - 208	42 - 278	23 - 125	13 - 58	13 - 54	13 - 69	28 - 243	44 - 249	65 - 296
mxRWE	maximum RWE (J/m) at the site (N=369)	425 - 853	605 - 1556	402 - 1376	283 - 846	220 - 1065	141 - 549	74 - 283	69 - 290	88 - 367	146 - 924	252 - 941	321 - 1115
a_mRWE	mean RWE (J/m) in Zone A of the site (N=48)	309 - 624	381 - 1126	279 - 1023	233 - 653	141 - 792	119 - 424	43 - 221	43 - 195	62 - 231	120 - 689	152 - 704	251 - 837
b_mRWE	mean RWE (J/m) in Zone B of the site (N=75)	280 - 520	405 - 847	268 - 779	221 - 581	149 - 663	123 - 400	44 - 207	42 - 189	69 - 254	113 - 541	175 - 586	237 - 655
c_mRWE	mean RWE (J/m) in Zone C of the site (N=30)	355 - 697	451 - 1291	333 - 1169	253 - 733	176 - 905	122 - 471	59 - 252	57 - 208	73 - 262	133 - 785	168 - 795	283 - 952
d_mRWE	mean RWE (J/m) in Zone D of the site (N=153)	397 - 793	543 - 1450	377 - 1294	272 - 802	209 - 994	131 - 523	63 - 269	60 - 236	78 - 319	140 - 856	209 - 885	305 - 1044
e_mRWE	mean RWE (J/m) in Zone E of the site (N=46)	195 - 257	235 - 384	134 - 350	135 - 313	113 - 304	86 - 248	38 - 160	36 - 171	59 - 203	95 - 268	139 - 290	190 - 340
f_mRWE	mean RWE (J/m) in Zone F of the site (N=17)	18 - 28	22 - 34	21 - 32	23 - 32	20 - 31	18 - 28	10 - 24	16 - 27	18 - 25	16 - 31	20 - 31	23 - 33
d2000	mean RWE (J/m) 0 - 2000 m from site (N=228)	266 - 488	411 - 876	333 - 758	186 - 495	143 - 590	92 - 328	70 - 198	90 - 203	95 - 287	101 - 567	181 - 502	225 - 631
d4000	mean RWE (J/m) 2000-4000 m from site (N=263)	147 - 270	175 - 458	159 - 435	126 - 293	96 - 376	62 - 190	37 - 198	52 - 261	87 - 352	44 - 634	121 - 477	115 - 345
d6000	mean RWE (J/m) 4000-6000 m from site (N=107)	67 - 106	78 - 195	60 - 148	52 - 124	51 - 145	32 - 88	17 - 80	23 - 113	34 - 117	24 - 204	61 - 174	55 - 135
d8000	mean RWE (J/m) 6000-8000 m from site (N=165)	119 - 171	134 - 253	119 - 226	78 - 167	62 - 177	47 - 117	28 - 87	38 - 94	47 - 133	37 - 218	88 - 186	88 - 199
d10000	mean RWE (J/m) 8000-10000 m from site (N=113)	88 - 138	101 - 242	85 - 209	67 - 144	59 - 189	36 - 105	22 - 95	27 - 125	44 - 172	29 - 277	77 - 226	65 - 175
d12000	mean RWE (J/m) 10000-12000 m from site (N=70)	43 - 79	54 - 131	48 - 137	41 - 77	31 - 126	18 - 61	12 - 60	16 - 82	27 - 123	15 - 177	36 - 138	35 - 111
d14000	mean RWE (J/m) 12000-14000 m from site (N=9)	7 - 11	8 - 20	6 - 19	8 - 19	6 - 15	3 - 9	2 - 9	3 - 13	5 - 15	3 - 18	7 - 15	7 - 16
d16000	mean RWE (J/m) 14000-16000 m from site (N=9)	17 - 28	24 - 62	20 - 50	15 - 35	11 - 40	7 - 21	4 - 14	7 - 13	7 - 17	7 - 44	11 - 32	16 - 43

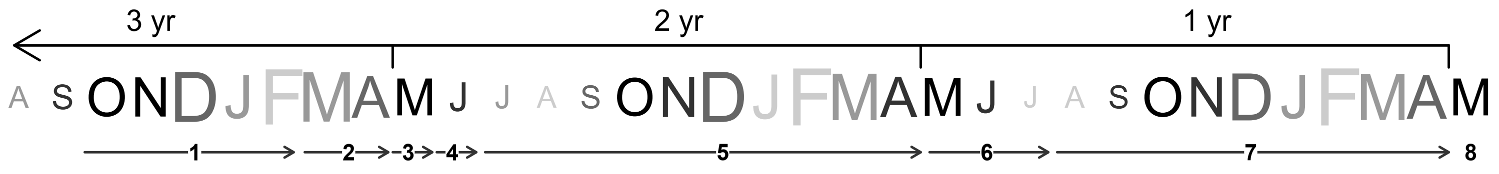

Figure 1. A life history timeline for sexual recruits censused via aerial photography. Monthly abbreviations are positively scaled to mean relative wave energy (RWE; J/m) and color graded to mean bottom-water temperature (proportion of days within optimal range). All means were calculated using the appropriate offset relative to spring census; annual marks describe the direction of offset. Bottom arrows denote significant life history stages during the inference period for sexual recruitment: i.e., (1) floral induction, (2) flower development, (3) anthesis, (4) embryo development and seed dehiscence, (5) seed bank, (6) seedling emergence, (7) patch development, and (8) photographic census.

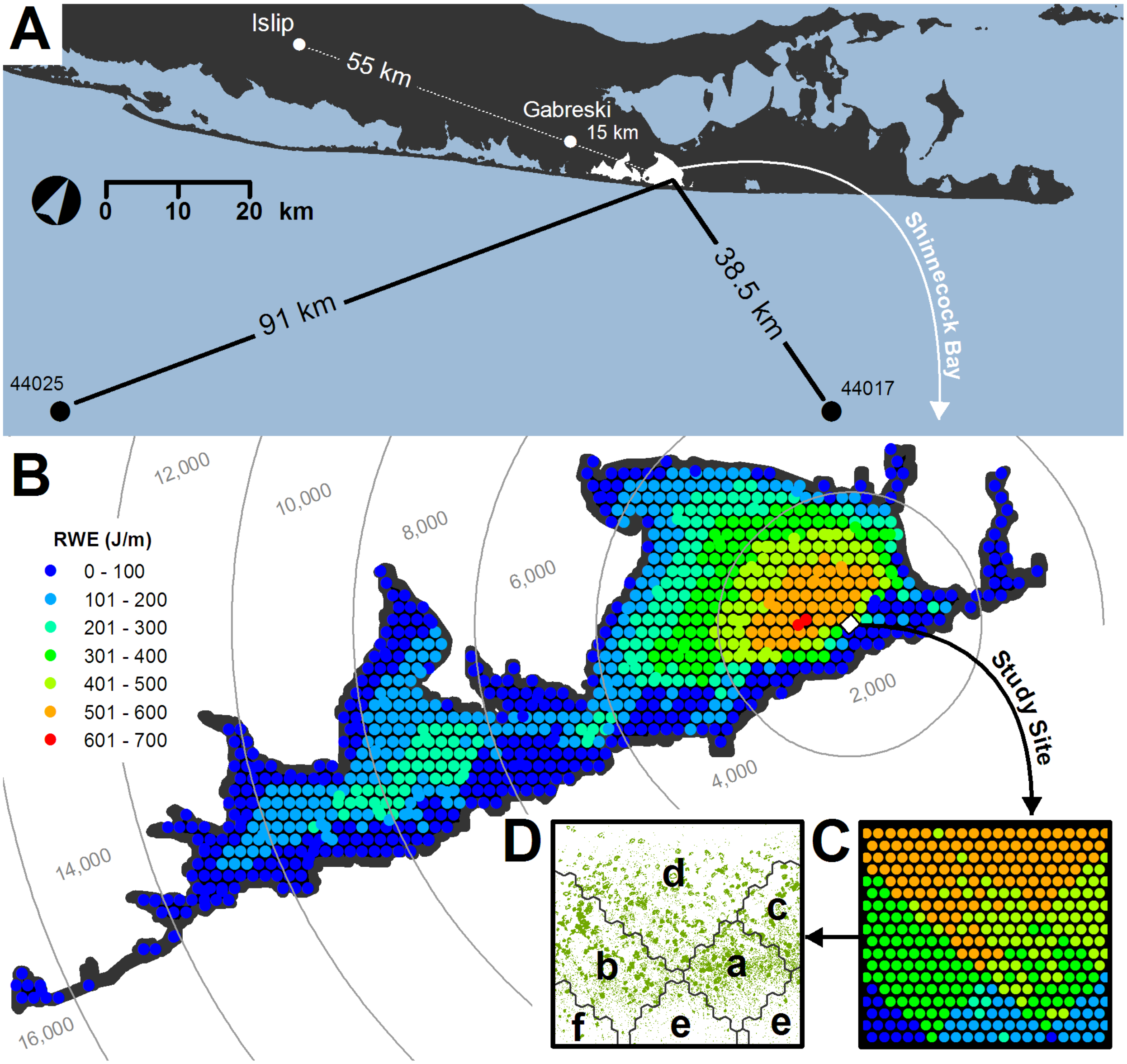

Figure 2. (A) Map of Shinnecock Bay, Long Island, New York in relation to sources of environmental data. (B) Mean wave energy conditions 2000 to 2014. Study site (56,250 m^2) depicted as a white box. Gray lines delineate distance radii extending from site borders. (C) Mean relative wave energy (RWE) within the study site, 2000 to 2014. (D) Statistically significant multivariate zones (a-f) of RWE behavior, 2000 to 2014. Green polygons depict 2010 seagrass coverage.

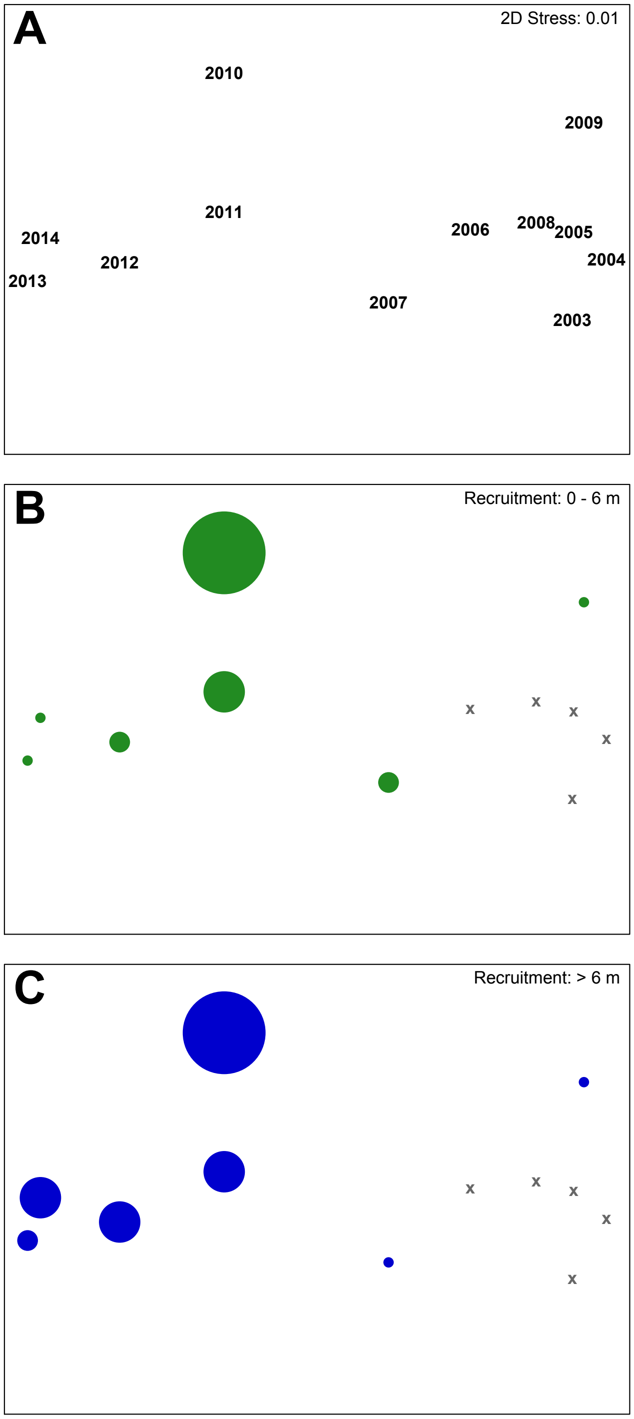

Figure 3. Second-stage nMDS of monthly relative wave energy (RWE) within Shinnecock Bay, 2003 to 2014. Each symbol represents an RWE sequence during the recruitment period, defined as May of observation through the third previous August. Symbols are shown by (A) yearly recruitment event and superimposed with (B) NSD and (C) RSD recruitment. X's reference years for which no recruitment estimates were made.

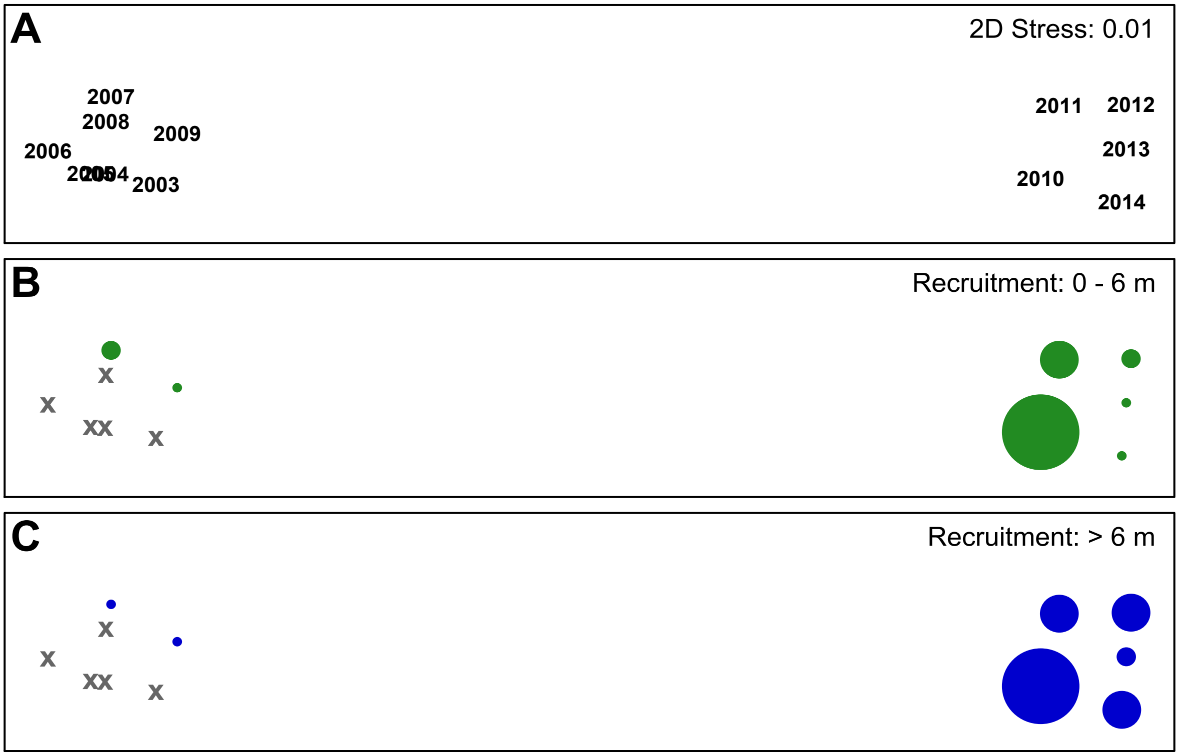

Figure 4. Second-stage nMDS of monthly relative wave energy (RWE) within the study site, 2003 to 2014. Each symbol represents an RWE sequence during the recruitment period, defined as May of observation through the third previous August. Symbols are shown by (A) yearly recruitment event and superimposed with (B) naked seed dispersal (NSD) and (C) rafted seed dispersal (RSD) recruitment. X's reference years for which no recruitment estimates were made.

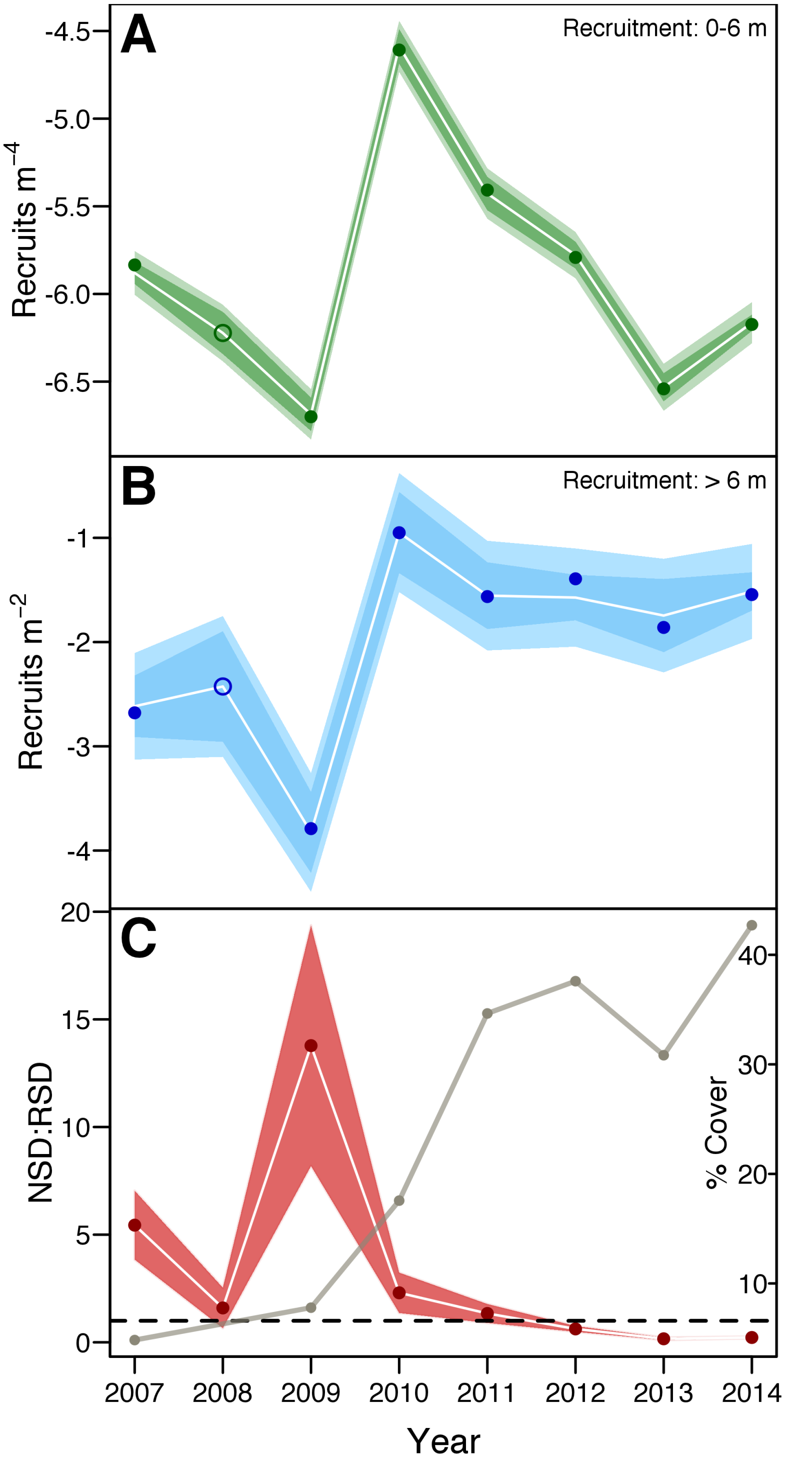

Figure 5. Sequence of modeled (A) naked seed dispersal (NSD) and (B) rafted seed dispersal (RSD) recruitment. Dark shaded regions depict 95% confidence intervals, lighter regions 95% prediction intervals. White lines follow model predictions. Filled circles locate actual values and open circles model predictions. The relative importance of NSD to RSD recruitment (C) as calculated using standardized, fitted data (filled, red circles) compared to the proportion of the study site covered by seagrass (tan line). Red shaded region shows the propagated error for each NSD:RSD estimate.

Chapter 4

Summary

SUMMARY

Overview

In the preceding chapters, we document clonal foraging, mating system effectiveness and sexual recruitment by *Zostera marina* within a shallow, subtidal landscape. The inferred action scales for these processes matched coverage changes over a 13-year period, during which time colonization proceeded via patch recruitment, followed by centrifugal growth and frequent coalescent events. Molecular evidence supported unrestricted gene flow, with effective pollination distances exceeding 50 meters and seed dispersal mechanisms (1) aggregating collateral relatives at less than 10 meters and (2) reaching un-vegetated space at 10s - 100s of meters. Within established patches, we confirmed seedling growth and sexual maturity, and reasoned that sexual recruits were major contributors to patch growth, persistence and genet diversity. Beyond these patches and despite unchanging floral densities, we observed large fluctuations in the annual rate of seed-borne patch recruitment. We attributed this variability to natural oscillations in climatic condition, finding statistically significant relationships between correlates of physicochemical disturbance (i.e., wind, temperature and wave energy) and meadow performance, particularly during vulnerable life history stages.

Our approach to understanding space acquisition by *Z. marina* combined manipulative and mensurative approaches, operated across a range of spatial scales (i.e., ramet to meadow), and brought together two emerging disciplines within seagrass ecology: GIS and genetics. We contend that this approach strengthened our findings by providing historical context to present-day patterns and corroborating mechanisms suggested by snap-shot genotypic and floral surveys. The manuscripts presented in this dissertation benefitted greatly from preliminary surveys of site bathymetry, sediment chemistry and granulometry, sediment height change, and seagrass scour. These investigations were conducted to null result, and so were not presented in manuscript form; however, they refined our knowledge of the disturbance regime, and so we briefly review them below.

Site History and Characterization

All field studies were conducted within Shinnecock Bay, Suffolk County, New York. This bar-built estuary drains the eastern-most watershed of Long Island's South Shore Estuarine

System (SSE) and extends ~15 km from the Quogue canal in the west to Taylor Creek in the east (USACE 2004). The backbarrier lagoon covers approximately 39 km^2 and comprises a variety of marine habitat types including salt marshes, inter- and sub-tidal sand and mud flats, *Crepidula* spp. pavement and shell-hash, dredge spoil islands, tidally-influenced creeks/rivers, and seagrass meadows (USACE 2004, Carroll 2012). Depths vary from 0 - 4 m with a bay-wide mean of 2 m (MLLW); tides are semi-diurnal with a range of 0.8 meters (USACE 2004). Landlocked to the east, Shinnecock Bay maintains hydrologic connections to Moriches Bay in the west via the Quantuck and Quogue canals, the Atlantic Ocean to the south though the Shinnecock Inlet (c. 1938), and the Peconic Bay system to the north, where the Shinnecock Canal (c. 1919) controls water movement through a series of tidal gates and locks (USACE 2004, Carroll 2012).

The first major appearance of seagrasses within the bay likely occurred during the 1930's, after storm breaches to the barrier beach system began to salinize the estuary (Dennison et al. 1989); however, very little is known regarding the full sequence of colonization or subsequent occupation during the past 75 years. In 2004, the United States Army Corps of Engineers, New York District (USACE-NYD), seeking to compile submerged aquatic vegetation (SAV) data for a south shore reformation study, concluded that insufficient mapping data existed for their purposes, finding nothing for Shinnecock Bay (USACE 2004). To date, the only quantitative estimates available for seagrass coverage in the bay remain those provided by Dennison et al. (1989) for 1967 and 1988, and unpublished, incomplete geospatial data photo-interpreted by Steve Schott of Cornell University for 2007 (pers. comm.). We know from Renn (1937) that early *Z. marina* colonists quickly achieved distributions similar to today (i.e., patchy coverage predominantly on the sandy shoals of the south shore and limited beds in Tiana and northern Shinnecock Bay), and that these populations were resistant to wasting disease, exhibiting minimal dieback despite clear signs of infection. Similar resilience was demonstrated during the first major brown tide blooms of the 1980's, when Dennison et al. (1989) reported an 83% increase in seagrass coverage spanning the bloom years.

Unfortunately, without sufficient historical data, we remain unable to comment on long-term coverage trends, particularly at the basin scale. To reconstruct contemporary trajectories at the scale of our study site, we compiled a rough sequence of aerial and/or satellite photographs via online access and personal communication from a number of State and Federal agencies, including: the National Oceanic and Atmospheric Administration's Coastal Services Center

(NOAA CSC), the United States Army Corps of Engineers' Coastal and Hydraulics Laboratory (USACE CHL), the United States Department of Agriculture - Farm Service Agency's National Agriculture Imagery Program (USDA FSA NAIP), the New York Statewide Digital Orthoimagery Program (NYSDOP) and the New York State Department of State - Division of Coastal Resources (NYSDOCR). Geospatial images, of varied quality and coverage, were obtained for 1941, 1994, 2001 - 2002, 2004 and 2006 - 2010, and have been added to high-resolution mapping data collected at bi-monthly intervals using our balloon imaging technique (see Chapter 2).

From these resources, it was clear that our study site, which measured 56,250 m^2 in the southeast corner of Shinnecock Bay, had undergone dramatic changes during the previous 13 years. Between 1994 and 2009, our aerials show that seagrasses persisted at low levels (<10% areal coverage), then, beginning with an explosion of sexual recruitment in 2010, coverage rose steadily to 40% (Figure 1). Extrapolating ramet densities garnered from shoot count data (collected 2011 - 2014), we estimate that site-wide numbers of ramets rose from 300,000 (c. 2001) to 6.7 million (c. 2014), yielding conservative estimates of 22,212 and 432,360 genets, respectively.

To place this expansion into ecological context, we first needed to identify the principal agents of disturbance. Specifically, we were interested in (1) whether physical disturbance had controlled meadow expansion or was constraining meadow shape [e.g., spatial self-organization (van Wesenbeeck et al. 2008, van der Heide et al. 2010)], (2) whether vegetative growth had tracked mineral resource heterogeneity or site geomorphology (itself a product of tidal currents and wind generated wave activity) and (3) whether large-scale bedforms were impinging on the site. To approach these issues, several large-scale field efforts were undertaken to characterize the site in terms of bathymetry (depth, aspect and slope), sediment height change, wave energy regime, sediment texture and chemistry, bottom-water temperature, and seagrass scour. Wave energy and bottom-water temperatures were presented in Chapter 3.

Bathymetric data were collected on September 9, 2013 using a Simrad ES60 split-beam echosounder with a 7-degree beamwidth, operating at 120 kHz. Depth estimates were interpolated to GPS timestamps, linearly corrected for tide change using points separated by < 0.5 m, and standardized to mean lower low water (MLLW) using NOAA's tidal predictions for station No. 8512671. Point data were then converted to raster format (1 x 1 m cell size), block averaged (mean) using a search radius of 5 m, resampled to a 5 x 5 m cell size, returned to point format, and investigated for semivariance structure using the statistical software, R (R Development Core Team 2012). An exponential model was therein selected and fit to the sample data using the R package 'gstat' (Pebesma 2004). Coefficients were applied to an isotropic Kriging Model (ordinary) in ESRI's ArcGIS Geostatistical Analyst's Wizard and the resulting interpolation was exported to raster format (2 x 2 m cell size). Aspect and slope datasets were then derived using the ArcGIS software extension, 'Spatial Analyst'. These data revealed coherent spatial structure consistent with our personal observation of the site (Figure 2). Depths ranged from 0.25 to 1.25 m (MLLW) with a mean of 0.54 ± 0.21 m (mean ± 1 s.d.). Bowl-shaped depressions of roughly 10-15 m in diameter and 0.25 m excavation depth were found in some abundance (~ 20) throughout the site; in deeper portions the pattern transitioned to a spur-and-groove morphology, with 1 prominent spur and 3-4 lesser features.

Sediment movement (accretion and/or erosion) driven by wind-generated waves and tidal currents were monitored across the site with a nested array of sediment height tiles. Tile design was modeled after that of Bell et al. (1999) and used buried acrylic plates (10 x 10 x 0.012 cm; insertion depth, ~15 cm) with 1 m of green ribbon strung through center-drilled holes to provide subterranean platforms from which the height of overlying sediments can be repeatedly measured by metal probe. Later versions had buoyant polypropylene rope in place of ribbon to increase visibility and durability during long-term deployments. Initial site-wide sampling involved the placement of 369 tiles arranged in a 12-m alternating grid. The array was measured relative to baseline conditions (June 22-24, 2011) on three occasions: July 28-29, 2011, September 21-22, 2011 and March 16-17, 2012, providing intervals of 35.7, 53.2 and 204.7 days. Inspection of these data failed to yield evidence of spatial continuity or the existence of migrating bedforms at or above the 12-m scale; however, deposition events as high as 8.9 cm and erosion as deep as -13.0 cm were observed. Mean sediment movement (absolute value) was 1.7 ± 1.6 cm with slightly positive net accretion (0.9 ± 2.2 cm). The latter observation supports long-term trends in shoal development, which seems to have extended northward during the last 75 years, based on aerial imagery.

Sediment porosity, C:N ratio and percent organic content were quantified in June of 2011 for each location in the same 12-m alternating grid used for sediment height sampling. Undisturbed surficial sediments from within 25 cm of each sediment tile were collected using duplicate syringe-cores (5 cm^3, 2 cm injection depth). All samples were transferred to 20-mL scintillation vials, returned to the laboratory and dried to a constant mass at 70 ˚C. The first core was evaluated for percent organic content (as % dry weight) as determined by loss on ignition at 500 ˚C for 5 h. Porosity was estimated for the second core using the methodology of Carroll et al. (2008), and the equations and parameter values of Berner (1971). These samples were then manually homogenized by mortar and pestle and analyzed for carbon and nitrogen content using a CE Instruments Flash EA 1112 Elemental Analyzer (Atkinson & Smith 1983). The resulting point data layers were then assessed for semivariance structure and degree of anisotropy, fit to ordinary kriging models and interpolated using R and ArcGIS; in all three cases spherical models were selected following repeated leave-one-out cross validations. Data were re-sampled using 6 x 6 m block averaging with 6-point (3 horizontal x 3 vertical) estimation per cell and converted to ESRI GRID files. These spatial data layers revealed largely homogenous sediment condition (Figure 3), falling within natural variation supporting *Z. marina* growth in other portions of the South Shore Estuary (SSE) and Peconic Bay system (B. Peterson, per. comm.). Porosity ranged from 0.43 to 0.48 (0.45 ± 0.01) in a minor shoreward gradient suggesting depth and tidal effects on sediment sorting. Organic content (%) and elemental C:N exhibited little to no site-wide patterning and were consistent with nutrient-limited sandy sediment (% organic, 0.27 ± 0.07; C:N, 7.45 ± 1.84). Considered together, sediment chemistry and texture did not appear to drive meadow development; these metrics were not responsive to seagrass presence nor were they restrictive to lateral growth. However, overall low values indicate that nutrient limitation could moderate productivity, potentially affecting rhizome architecture and vegetative recruitment (i.e., branching) rates (see Chapter 1 for a full discussion of these issues).

We initially hypothesized that physical disturbance, arising from sediment movement and mechanical stresses imposed by wind waves and tidal currents were limiting bed expansion, generating internal gaps and removing smaller patches. To investigate this, we mapped scoured patch edges throughout the site during a synoptic survey conducted in July of 2013. During this effort, groups of 10 or more individuals systematically traversed the site by foot and snorkel, locating and mapping evidence of recent disturbance, i.e., exposed and upturned rhizomes (Figure 4). These data were inconsistent with size-dependent or directional effects and so the interesting propagation-inhibition dynamics observed by van Wesenbeeck et al. (2008) for *Spartina alterniflora* and van der Heide et al. (2010) for *Zostera noltii* were discounted for the whole of our site; however, we acknowledge that much more detailed monitoring of shoot dynamics, over longer periods of time, would be needed to investigate this mechanism at sub-site scales. Our conclusion, based on visual assessment of the scour patterns recorded in July, and considering the full time-series of meadow development, was that (1) physical disturbance matched large-scale bathymetric features (particularly N- and NE-facing slopes), (2) scour was concentrated in zones of high wave energy, and (3) did not appear to constrain patch growth in any consistent (i.e., spatially patterned) way. Interestingly, once edges began to recede they often continued for some time (Figure 4); the direction of which suggested tidal currents to be among the causal mechanisms.

Our synthesis of these disparate data highlights the role of wave energy and temperature in controlling some aspects of vegetative growth and persistence, but having major influence on the form and pace of sexual recruitment (Chapter 3). WEMo data locate the site within the highest wave energy zone of the bay (Chapter 3). RWE estimates were found to vary seasonally (highest in the fall and spring) with strong intra-site patterns (i.e., yielding distinct multivariate statistical zones, Chapter 3). While not linearly related to sediment height change, as we could find no correspondence between WEMo-estimated wave energy and measured sediment height change, our scour data were consistent with frequent, albeit episodic disturbance to surface sediments. Wave-induced sediment movement likely restricts the storage of organic material, as well as contributes to burial and redistribution of annual seed banks – particularly for un-vegetated locations. The results of our sexual recruitment model (Chapter 3) intimated susceptibility of isolated seedlings and small patches to wave-mediated disturbance during the winter and spring. Sediment tile and scour data support this as a direct effect on seedling mortality through burial or erosion, occurring with some regularity throughout the year.

Seasonal and long-term temperatures were discussed in Chapter 3; however, when considered over a full one-year period, we discovered significant spatial patterns (Figure 5). Using 15-minute temperature data from our 12-sensor array, we calculated and spatially interpolated exceedance durations (in hours, Figure 5) based on known thresholds for *Z. marina* physiology (Chapter 3). Anecdotally, these temperature gradients were spatially concordant with late-summer die-back, mussel recruitment, and reproductive shoot height and developmental state, suggesting that temperature stress could be a major factor controlling annual carbon balance, with implications for reproductive effort/timing, vegetative growth and persistence.

In total, our preliminary surveys showed the study site to be (1) nutrient limited though unrestrictive to seagrass growth, (2) homogeneous in terms of sediment chemistry and granulometry, (3) temperature-stressed, particularly in the summer, and (4) frequently impacted by wind-generated waves, resulting in sediment movement. These conditions appeared to primarily affect recent seed-borne patches with longer-term vegetative growth and expansion tracking large-scale geomorphology (i.e., greater seagrass coverage on spur, rather than groove or depressional features). To some extent tidal currents and wind-waves responsible for eroding larger beds; however, these processes remain poorly understood as they occurred with much less frequency. Within this context, we investigated the role mineral resource heterogeneity plays in patch development (Chapter 1), the efficacy and consequences of sexual reproduction and seedling recruitment (Chapter 2), and the influence of climatic variation on sexual recruitment success (Chapter 3).

Clonal Foraging

Clonal foraging in response to heterogeneously distributed water, light or mineral resources has been demonstrated for a number of terrestrial plant species. The existence of similar behavior in seagrasses and the cross-scale effects of clonal foraging on patch development, however, have not fully been explored. Our objective was to test whether spatial exploration by independent ramet clusters could generate emergent patch behavior consistent with clonal foraging theory. Specifically, we attempted to stimulate directional growth along ten patch edges over a two-year period using subterranean fertilizer. Changes in ramet demography, patch expansion, seedling emergence and reproductive effort were quantified through repeated shoot censusing, using gridded quadrats common to seagrass monitoring programs. As a result of nutrient addition, we decreased foliar C:N by 11% (20.67 ± 2.61, enriched; 23.22 ± 1.73 ambient) and elicited 35% faster patch expansion. Enriched edges did so with significantly higher shoot densities, as there were 12% more ramets in the eight neighboring grid cells prior to lateral growth.

The seasonality of the nutrient effect differed between treatments and was consistent among years, suggesting that a fundamental shift in the pattern and seasonality of seagrass growth was elicited. Nutrient enriched cells generally outperformed ambient while at the lower end of the shoot density spectrum, particularly in the spring; however, this pattern reversed in the fall, when enriched ramet-specific recruitment mirrored winter profiles, while ambient cells maintained summer rates of recruitment. Visual inspection of this time-series revealed patterns consistent with cell crowding, as per capita recruitment fell to zero at between 5 and 35 shoots per cell (125 - 875 m^{-2}) depending on the season. Simulated on an annual basis, seasonal differences resulted in a statistically significant, 31% increase in enriched recruitment.

Within patches, no detectable differences in per capita branching rates, demographic stability or reproductive effort were observed. Marine and terrestrial studies on clonal plant demography have reported that ramet emergence rates are often matched by density-dependent mortality along resource gradients or following nutrient enrichment (Cook 1985). Investigators often cite competitive stress brought on by above- and belowground crowding, as well as strategic ramet senescence as potential causes for resource-mediated mortality (Duarte & Sandjensen 1990, Duarte et al. 2006). Our data support these views, as no cell-level changes in ramet-specific recruitment rate or ramet density coefficient of variation (CV) were found; ramet demography became de-coupled only at patch margins, where crowding effects would have been minimized. If nutrient-enhanced recruitment rates were consistent throughout the treated space (i.e., even where matched by density-dependent mortality rates), then similar nutrient hotspots could alter shoot age distributions (Herbert & Fourqurean 2009), driving median age downward, ultimately affecting patch-level attributes such as reproductive potential, carbon sequestration and sediment stabilization (all enhanced by increased ramet turnover). In conclusion, transient sub-patch resource heterogeneity unevenly distributed across the active growing margin of *Z. marina* patches potentiated vegetative growth and patch expansion. Incremental edge advances were characterized by significantly greater ramet numbers, indicating that ramet proliferation, selective ramet placement or a combination of the two responses had occurred.

Mating System Effectiveness

The disappearance of seagrasses in recent decades has transitioned vast portions of global coverage to disturbed or recovering states (i.e., underutilized habitat space). Understanding dispersal and recruitment patterns within and among extant populations is now vitally important to predicting both the form and pace of recovery. To examine the interactive effects of pollination and seed dispersal distance on the dynamics of sexual recruitment across a range of spatial scales (centimeters to decameters), we combined high resolution, decade-long seagrass mapping with polymorphic microsatellite analysis.

Although more intensive spatial sampling would have been needed to fully understand clone structure, our evidence for clonal growth was limited to less than 3 m. Indeed, among the 588 sampled ramets, the appearance of multi-locus lineages (MLLs) separated by more than 3 m was extremely rare, occurring only 12 times across the landscape. Peterson et al. (2013), while sampling *Z. marina* throughout the SSE, Peconic Bays and Long Island Sound systems, obtained identical results, reporting multi-locus genotypes (MLGs) to range 1-3 m. This suggests that large contiguous clones could be quite rare in New York waters. Whether this is a function of genet turnover or simply a sampling artifact of high genet densities remains unclear

Pollination distances ranged from 0.57 to 73.91 m (13.90 ± 15.19 m; median: 9.11 m) with no evidence of geitonogamous selfing. Seed dispersal varied systematically from 1.85 to 5.31 m for naked seeds, and randomly throughout the study site (0.17 to 34.54 m) for seeds deposited by floating reproductive shoots. Pedigree analyses corroborated these findings, as full sibling groups clustering neatly within larger half-sibling kinships at spatial scales of 2-6 m. This means that (1) selfing is rare, (2) pollen kernels regularly exceed the diffusive flux of seeds from local genets, (3) retention and recruitment of close kin is common, and (4) the long-distance dispersel via fragmented flowers efficiently samples available habitat space at scales of at least 10s of meters.

An important ancillary finding of this work was the role seedling recruitment can play in patch maintenance. Our shoot count data (Chapter 1) yielded some evidence for seedling contribution during the second summer, that is, between May and July of the year following seed dispersal. Genotypic surveys confirmed this for a different set of patches, and showed that these individuals were recruiting in intermingled clusters, potentially among existing (i.e., parental) clones. We referred to this process as ‘admixed’ rather than ‘agglomerative’, and adduced three patterns to support this view.

First, we recovered no parental genotypes from our 2013 sampling of the expansion patch. We explained this as a third-season drop in reproductive effort. Genet- and ramet-level reductions in flowering intensity were observed elsewhere within the same meadow over the same time period using patches of similar size and age. By selecting reproductive shoots during our fine-scale survey, we failed to sample parental clones; however, their lack of presence does not indicate genet senescence, as bi-monthly aerials showed no signs of such loss. We, therefore, argue that vegetative growth by the parental generation continued as the primary means of patch expansion, within which F_1 clones were retained as seeds. Second, reproductive shoots from the F_1 generation were, themselves, intermingled. And finally, shoot emergence rates for the MLLs we surveyed were within the upper range reported for annual (Reusch 2000) and perennial (Olesen & Sandjensen 1994) populations of *Z. marina*, indicating unencumbered vegetative growth. It therefore appears that seedling recruits effectively compete both with adjacent seedling kin and with established genets for space and resources.

In conclusion, over at least a four-year period, sexual reproduction and seedling recruitment played appreciable roles in the colonizing process of *Z. marina*, configuring the landscape through the deposition of rafted seeds, and contributing to patch expansion via the limited dispersal of naked seeds. Molecular evidence supports an admixed model for this process, as genet competition among vegetatively and sexually produced individuals did not appear restrictive to seedling recruitment or vegetative propagation. As patches mature and expand, the potential for bi-parental inbreeding should increase; however, our pollen dispersal distance estimates indicated that at least the tallest rhipidium will have access to pollen originating from outside of the kin-group footprint. Nevertheless, cohesive pollen clouds fertilizing multiple ovaries per receiving genet, and limited seed dispersal distances, frequently result in the aggregation of collateral relatives, and so competition among close kin may in fact be a regular feature of *Z. marina* meadows. That these processes were still contributing to meadow development after more than 13 years suggests that seedling safe site availability, and not mating system effectiveness, may be most limiting to meadow establishment over longer spatiotemporal scales (see Chapter 3).

Sexual Recruitment

Ecophysiological stress and physical disturbance are known agents of change in seagrass systems, capable of spatially structuring meadows through a combination of direct biomass removal and recruitment limitation (Gallegos & Kenworthy 1996, Fonseca & Bell 1998, Jarvis et al. 2014). Chapter 2 confirmed that sexual recruitment was much more important than vegetative growth to space acquisition over a 13-year period (2001-2014), and that the rate of seed-borne patch recruitment varied widely by year. Concurrent estimates of floral densities made within the meadow over 3 years (2012-2014) indicated that no substantive changes in reproductive effort or mating system effectiveness had occurred. Because the system was (1) uniformly shallow and therefore light-replete, (2) unaffected by drift or epiphytic algal growth, (3) mono-specific and binary in composition – i.e., *Z. marina* embedded within a sandy matrix, (4) wave exposed, and (5) temperature stressed, we hypothesized that inter-annual variability in sexual recruitment would be a predictable function of physical disturbance.

Using a multiple linear regression (MLR) approach, we examined the association between isolated patch emergence (i.e., seed-borne recruitment) and estimates of relative wave energy, atmospheric condition and water temperature over an 8-year period (2007-2014). Two successful models were developed, one appropriate for the dispersal of naked seeds, and another for rafted flowers. Both modes of sexual recruitment varied as predictable functions of wind, temperature and wave energy, with long term multivariate patterns in wave energy corresponding to periods of rapid colonization within our site. Notably, no comparable multivariate patterns were found for combinations of atmospheric condition; i.e., rainfall, wind speed, wind direction or air temperature.

Temporal correlations between sexual recruitment and time-lagged climatic summaries consistently showed floral induction, seed bank and small patch developmental periods to be the most vulnerable to disturbance. Of these, seedling safe-site availability appears to play a primary role. It has been previously shown to control aspects of space acquisition during colonization and recovery (Plus et al. 2003, Lee et al. 2007), and physical forces such as the ones investigated here are known to limit seedling survival and constrain bed morphology (Fonseca & Bell 1998, Infantes et al. 2009, Infantes et al. 2011). Our sediment tile and seagrass scour data confirmed that wave-induced sediment movement was a viable mechanism in the uprooting and burial of recent germinants and our temperature data showed considerable periods of tolerance exceedance. The MLR models quantified this to some extent, showing how environmental variation could have been responsible for episodic bouts of sexual recruitment within our site, at least for un-vegetated spaces. Importantly, these models illustrate how ramet-scale observations of reproductive effort (Chapter 1) and patch-scale estimates of mating system effectiveness (Chapter 2) translate to landscape-scale patterns of recruitment success and, ultimately, meadow development.

Conclusions

From its inception, seagrass ecology has struggled to extrapolate from the ramet to the patch, from the genet to the population, from the bed to the meadow (Marba et al. 2005). Scaling problems are not new to ecology (Levin 1992); however, since we tend to assess seagrass status and to value their ecosystem services at the largest spatial scales, the need to predict changes occurring within the landscape remains exceedingly important. Bridging this gap will mean incorporating traditional modes of observation (i.e., the quadrat and transect) with more advanced GIS and molecular approaches. This dissertation has demonstrated how combining these methods can advance our understanding of patterns occurring at the largest temporal and spatial scales, while providing strong correlative evidence for formative processes and tangible targets for future, manipulative work. The ability to identify genets and to map them in space proved invaluable to understanding the resurging seagrass beds of Shinnecock Bay, and will undoubtedly continue to yield new insight as seagrass ecologists work to link process to pattern across ever-expanding scales of time and space.

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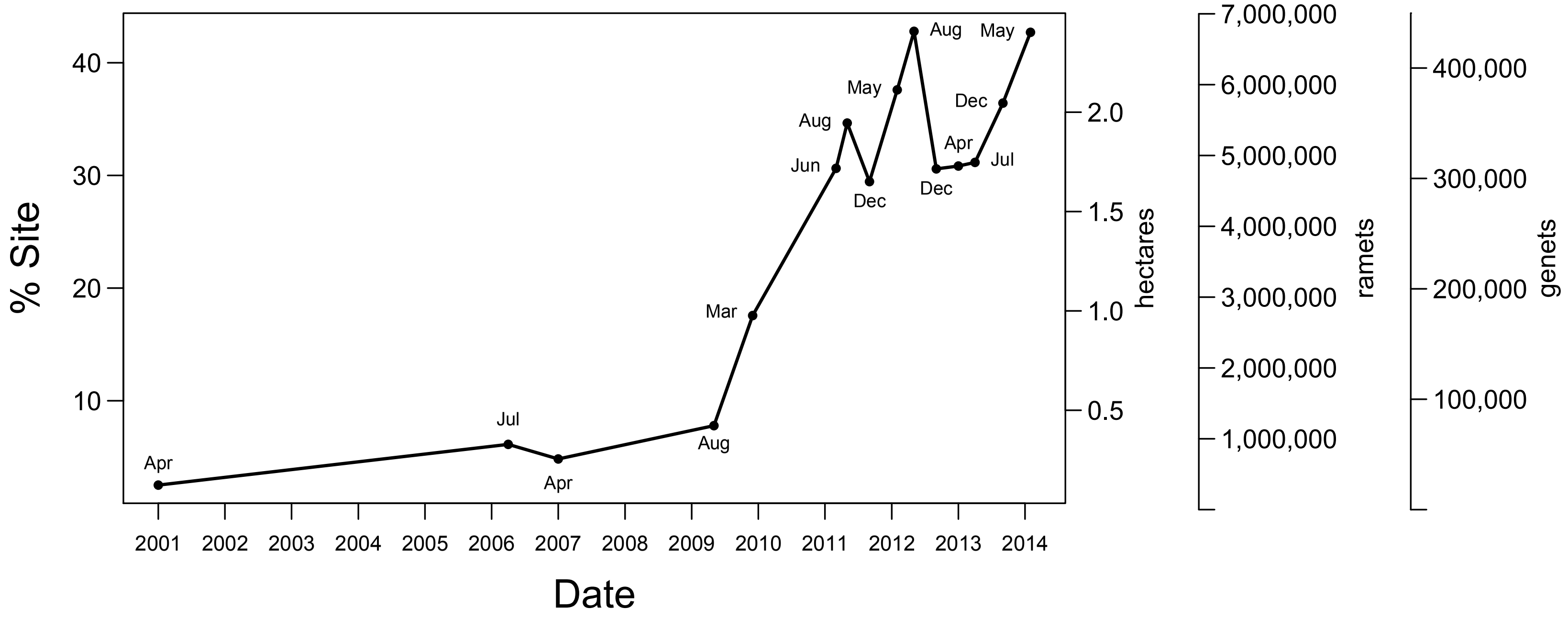

Figure 1. Time-series of meadow development (2001 – 2014) with progress represented as percent cover, areal coverage (ha), number of ramets, and number of genets. Ramet abundance was estimated by random re-sampling of shoot count data after summing grid-cell counts by sampling date (N=10). Genet abundance was extrapolated from 18 ind. m^{-2} based on multilocus genotype densities inferred from microsatellite data. Coverage, ramet and genet values were underestimated for 2001 and 2007 due to incomplete aerial photographic data. The unmapped area was small (15%) and sparsely populated during subsequent time points.

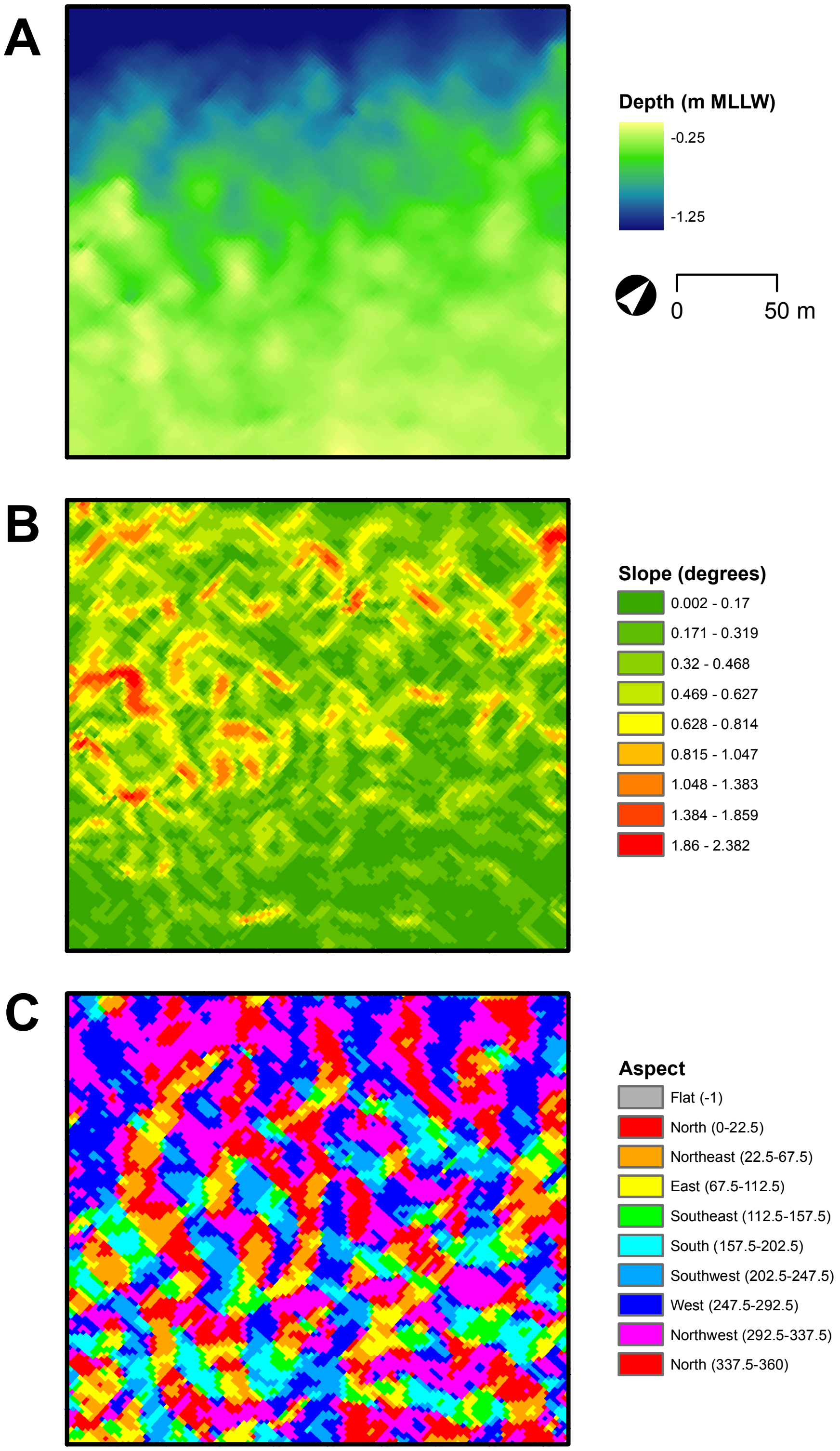

Figure 2. Depth (A), slope (B) and aspect (C) data for the study site. Bathymetric survey was conducted on September 9, 2013 using a split beam echosounder.

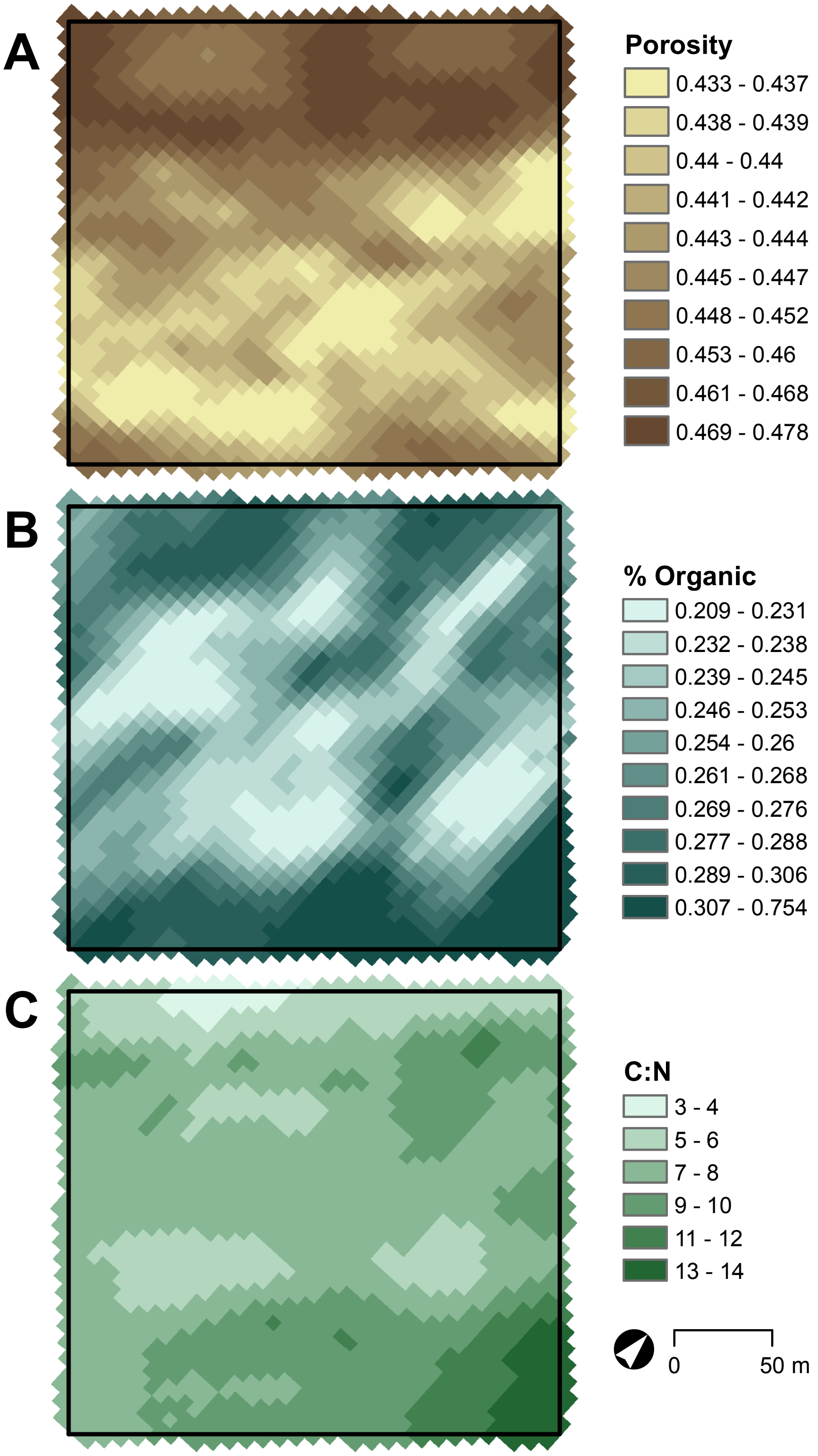

Figure 3. Site-wide sediment porosity (A), percent organic content (B) and C:N ratio (C). Interpolated surfaces were derived from surveys of undisturbed surficial sediments (upper 2 cm) in a 12-m alternating grid.

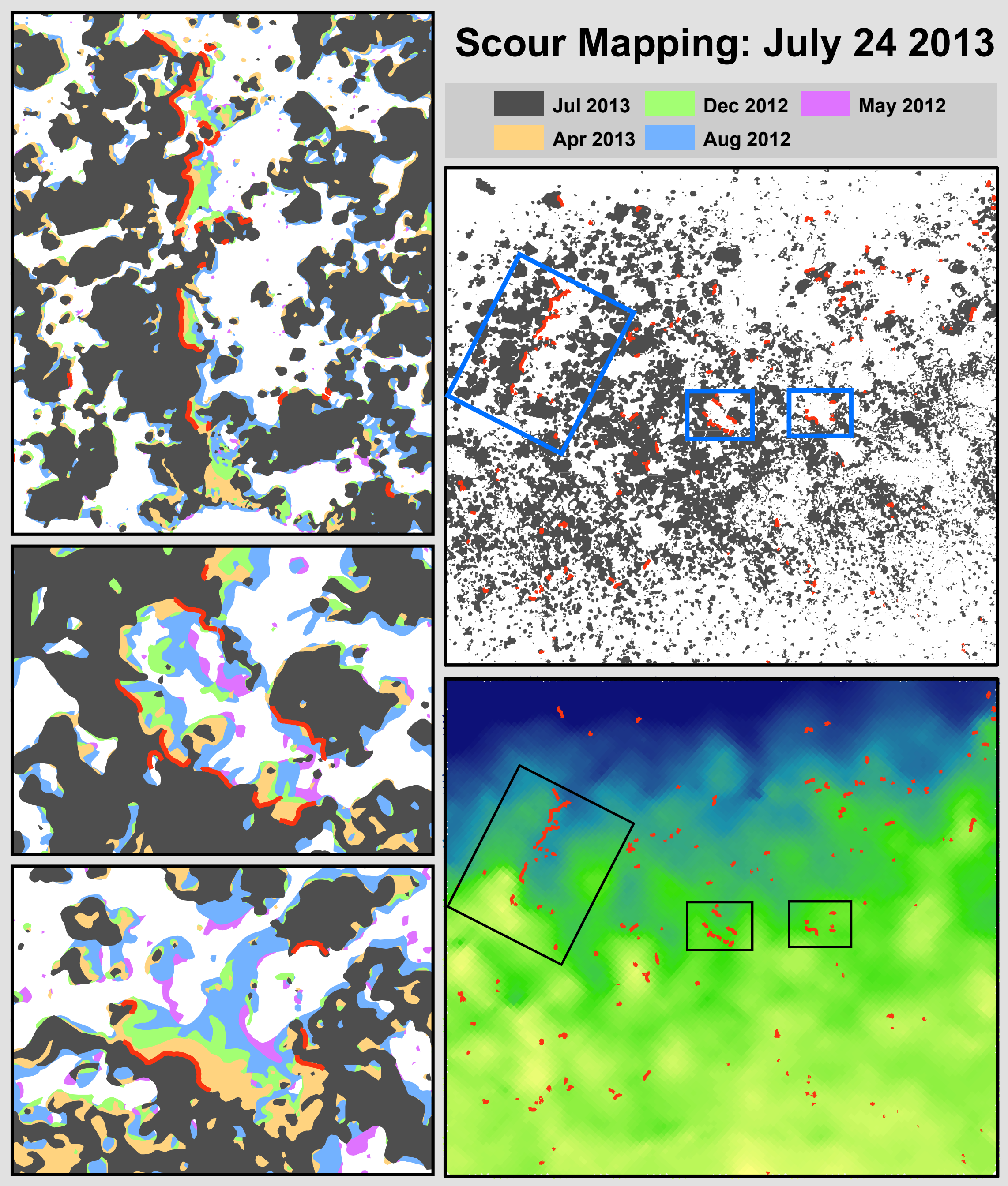

Figure 4. Results of site-wide seagrass scour surveys conducted on July 24, 2013. Scoured patch edges are depicted as red lines and displayed in reference to meadow condition at the time of sampling (seagrass represented as gray polygons) and site bathymetry (see Figure 2A). Expanded insets illustrate long-term recession of patch edges, colored polygons show previously mapped seagrasses.

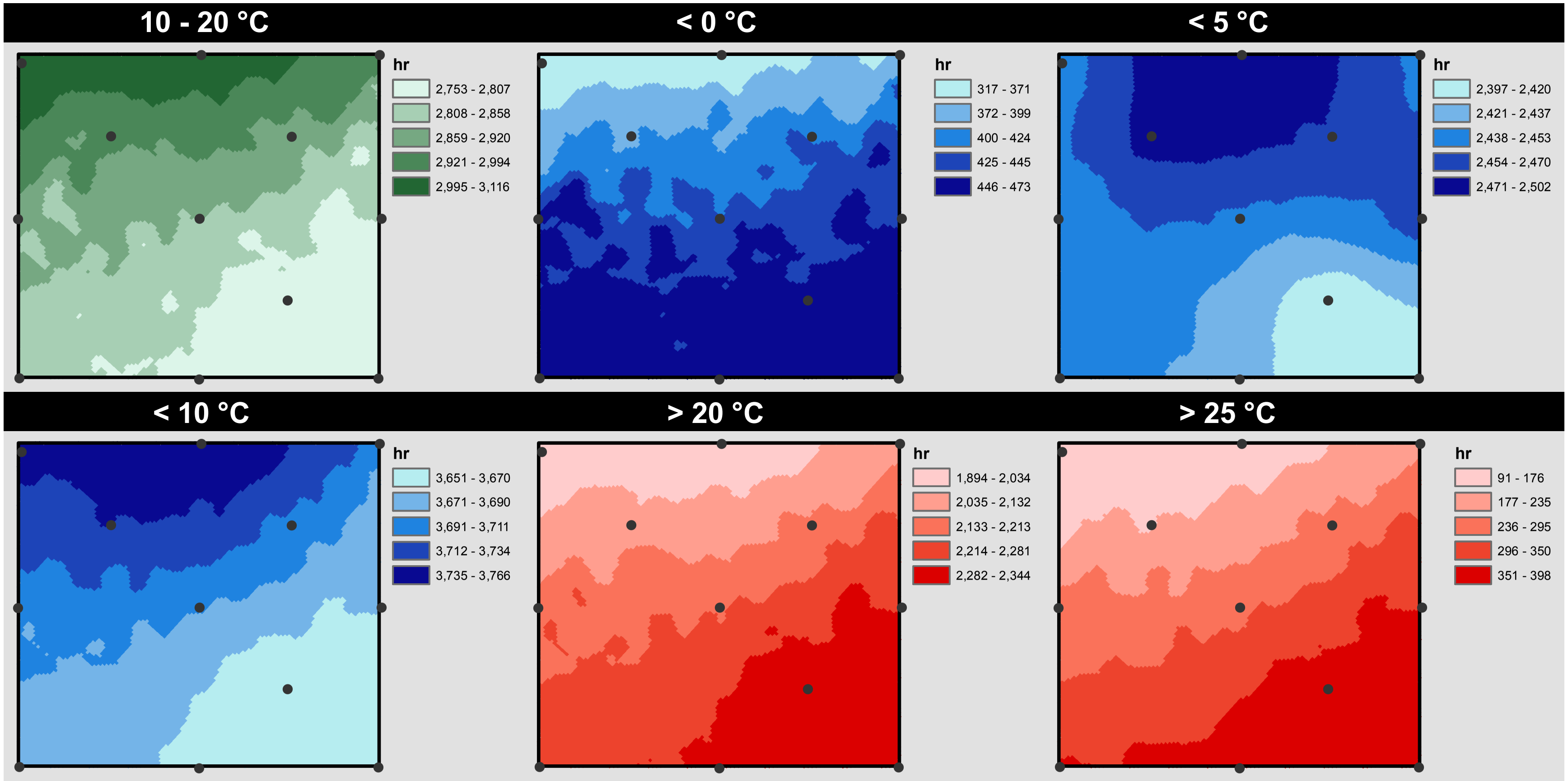

Figure 5. Optimal (*Z. marina*) temperature exceedance durations calculated for the study site using 1 year of 15-min bottom-water temperature observations at a 12-sensor array (black dots).

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