

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

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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 *Zostera marina* within a shallow lagoon in Long Island, New York, U.S.A., 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 three meter across a 56,250-m² study site. Pollination distances ranged from 0.57 m to 73.91 m, while seed dispersal varied systematically from 1.85 m to 5.31 m for naked seeds, and randomly throughout the study site (0.17 m 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.

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

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 (Morris et al. 2000; Bell et al. 2008; Costello and Kenworthy 2011). 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 (Lathrop et al. 2001; Short and Burdick 2006; Downie et al.

2013). 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, nondestructive field-identification of clones (i.e., genets) notoriously difficult without sophisticated and costly genetic approaches (Orth and Moore 1983; Cook 1985). 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,

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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 interannual 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 [(Olesen and Sandjensen 1994; Morris et al. 2000; Neckles et al. 2012); but see (Marba and Duarte 2003; Sintes et al. 2006; Brun et al. 2007) 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 (Peterson et al. 2002; Plus et al. 2003; Lee et al. 2007).

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 (Hammerli and Reusch 2003; Becheler et al. 2010; Zipperle et al. 2011). These findings have reinvigorated 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 coexistence through 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 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 biparental inbreeding (Ruckelshaus 1996; Reusch 2001; Zipperle et al. 2011). In fact, for most seagrasses, basic information on how mating system effectiveness and intergenet 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 (Reusch 2000; Billingham et al. 2007; Kamel et al. 2012). Estimates of genetic neighborhoods and the spatial continuity of some relatedness measures indicate that hectare-scale investigations may be sufficient to capture relevant dynamics (Ruckelshaus 1996; Hammerli and Reusch 2003; Billingham et al. 2007); however, measures of effective pollen transport continue to scale with sampled distance, suggesting that larger land-

scapes 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 subtidal, perennial ecotype of *Z. marina* in a shallow, backbarrier lagoon in eastern Long Island, New York, U.S.A., 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, internode, 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; Plus et al. 2003; Moore and Short 2006). 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 (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 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 (Orth and Moore 1983; Olesen and Sandjensen 1994; Orth et al. 2000).

Study site

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

uring 250 m (parallel to shore) × 225 m (total area, 56,250 m²) 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 m to 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 five hours, 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 (4) 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 bimonthly 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 s using the freely available firmware modification, Canon Hack Development Kit. 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 sub-surface control points, including 15 antifoul painted 45-cm paver stones stabilized with a reinforcing bar and visual landmarks identified in the 2010 New York Statewide Digital Orthoimagery Program (NYSODP) (and subsequent) imagery. Balloon mosaics (ground pixel resolution = 10–15 cm) were combined with the only comparable agency data, those from the NYSODP (ground pixel resolution = 15 cm), to generate a sequence of high-resolution thematic habitat maps spanning 13 yr: 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 nine 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 ($N = 4865$; 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 three 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 subsite containing 138 small patches ($1.42 \pm 4.31 \text{ m}^2$; hereafter, mean $\pm 1 \text{ s.d.}$) within a 20-m radius of a “central” (hereafter, “focal”) patch (Fig. 1B). The subsite was monitored during the previous four weeks to track floral development. Once sufficient pollination and embryo growth had occurred, we used a combination of haphazard and gridded quadrat ($2 \times 2 \text{ m}$ quadrat divided into $20 \times 20 \text{ cm}$ 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

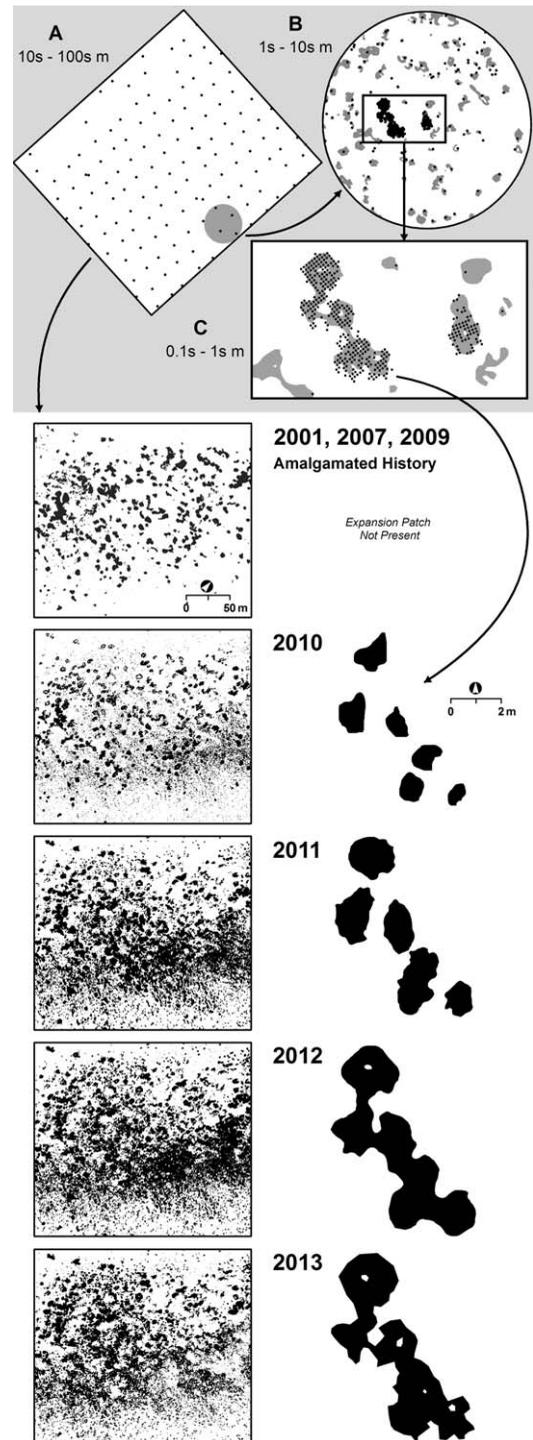


Fig. 1. Upper panel: (A) study site boundaries ($225 \times 250 \text{ m}$; $56,250 \text{ m}^2$) and nested-scale sampling array with decameter (A), meter (B), and centimeter (C) spatial grain. Black dots denote sampling locations. Lower panel: sequence of meadow and patch development for the entire 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.

rhizidium 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 polymerase chain reactions (PCR) amplifications on an MJ Research™ PTC-200 thermocycler using *Z. marina* specific, 5'-fluorochrome (standard labels Hex, Tet, Fam) labeled polymorphic microsatellite markers. PCR were performed in 20 μ L reactions containing approximately 30 ng of template DNA, 0.5 U of Bioline Immolase DNA Taq (Bioline Pty.), 1.5 μ L 10 \times Bioline Immobilizer (160 mM (NH₄)₂SO₄, 670 mM Tris-HCl pH 8.3, 0.1% Tween-20), 2.5 mM MgCl₂, 0.133 mM each dNTP, BSA at a concentration of 0.1 μ g/ μ L, and 0.33 mM fluorescently labeled forward (FAM, HEX, or TET) and reverse microsatellite primers. Thermal cycling protocols consisted of a seven-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; Silander 1985; Reusch and Bostrom 2011; Becheler et al. 2014). 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. (2007) and Arnaud-Haond and Belkhir (2007). All MLGs differentiated at a seventh locus by a genetic distance less than 6 bp were evaluated for lineage assignment using a $P_{\text{sex(FIS)}}$ 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 multiramet 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- vs. 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

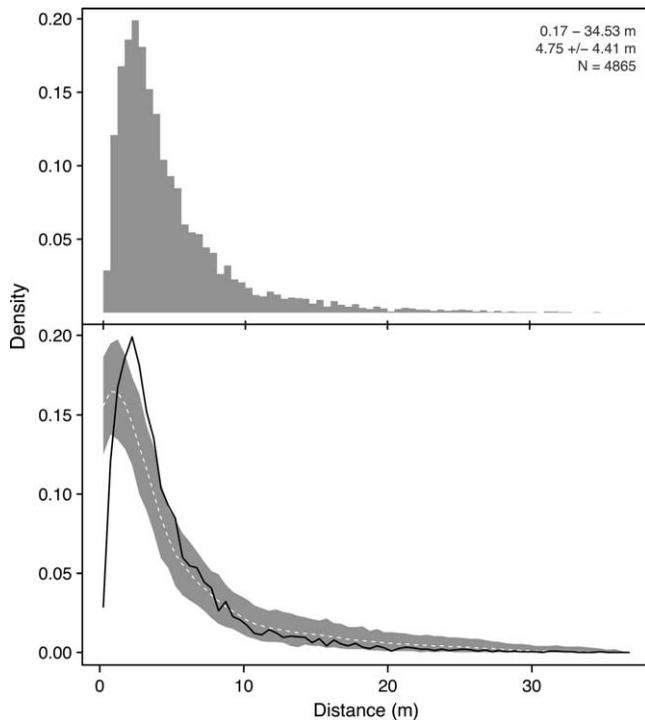


Fig. 2. Upper panel: density histogram of seed dispersal distance ($N = 4865$), as estimated from patches recruited during the 2010 mapping relative to an amalgamated historical distribution (2001, 2007, and 2009). Lower panel: results of an iterative random sampling of all potential dispersal distances 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.

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 two 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, 10 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 $4\times$ 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 10-patch, two-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 4865 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 m 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 m and 0.92 m and higher than random recruitment between 1.85 m 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 one meter, could have been a methodological artifact of

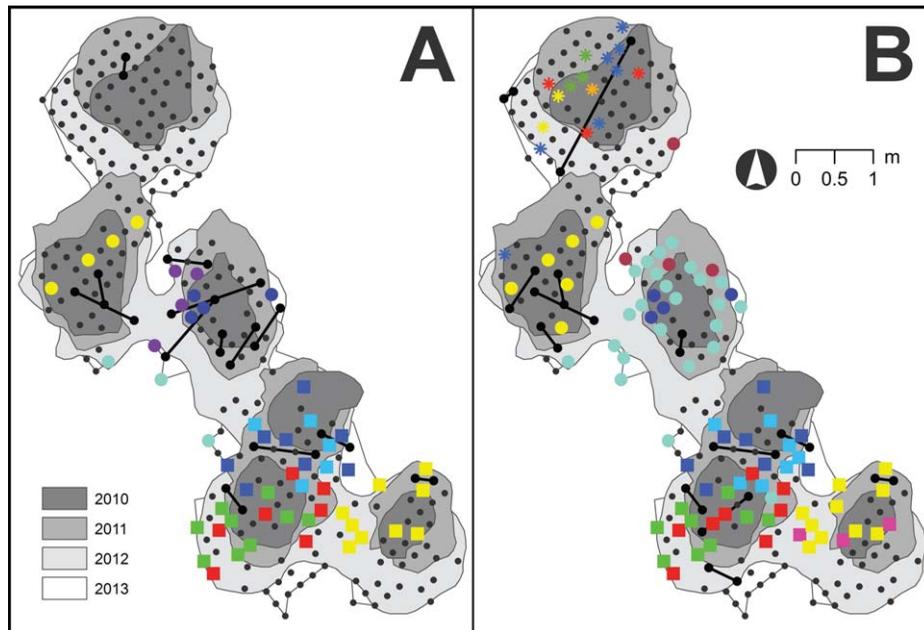


Fig. 3. Distribution of MLG (A) and MLL (B) designations within the “expansion” patch. Solitary clones are depicted as black dots, those with two ramets as barbells, and those with three or more ramets as unique combinations of shape and color.

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 m 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* (Ruckelshaus 1996; Billingham et al. 2007).

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 multiramet MLG, consisting of two ramets, was recovered ($MLGs\ ramet^{-1} = 0.99$). Closer examination of this isolated pair revealed allelic differences at an eighth locus; however, we retained it in our dataset for consistency with subsequent samples that were

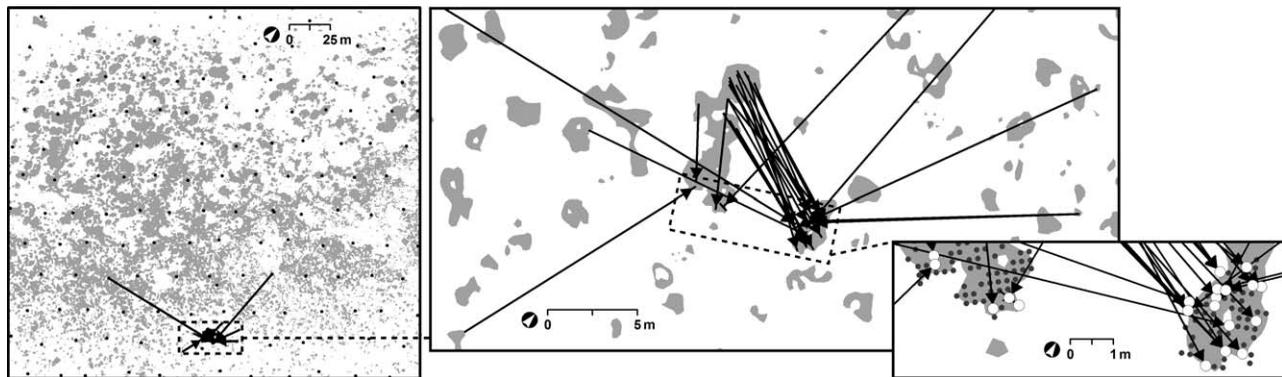


Fig. 4. Multiscale map of pollen dispersal events. Black arrows denote pollen transfer from putative fathers to mother/seed locations; the 2013 seagrass distribution is shown in gray. Left panel: full study site. Middle panel: intermediate-scale enlargement of the dashed extent rectangle. Right panel: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

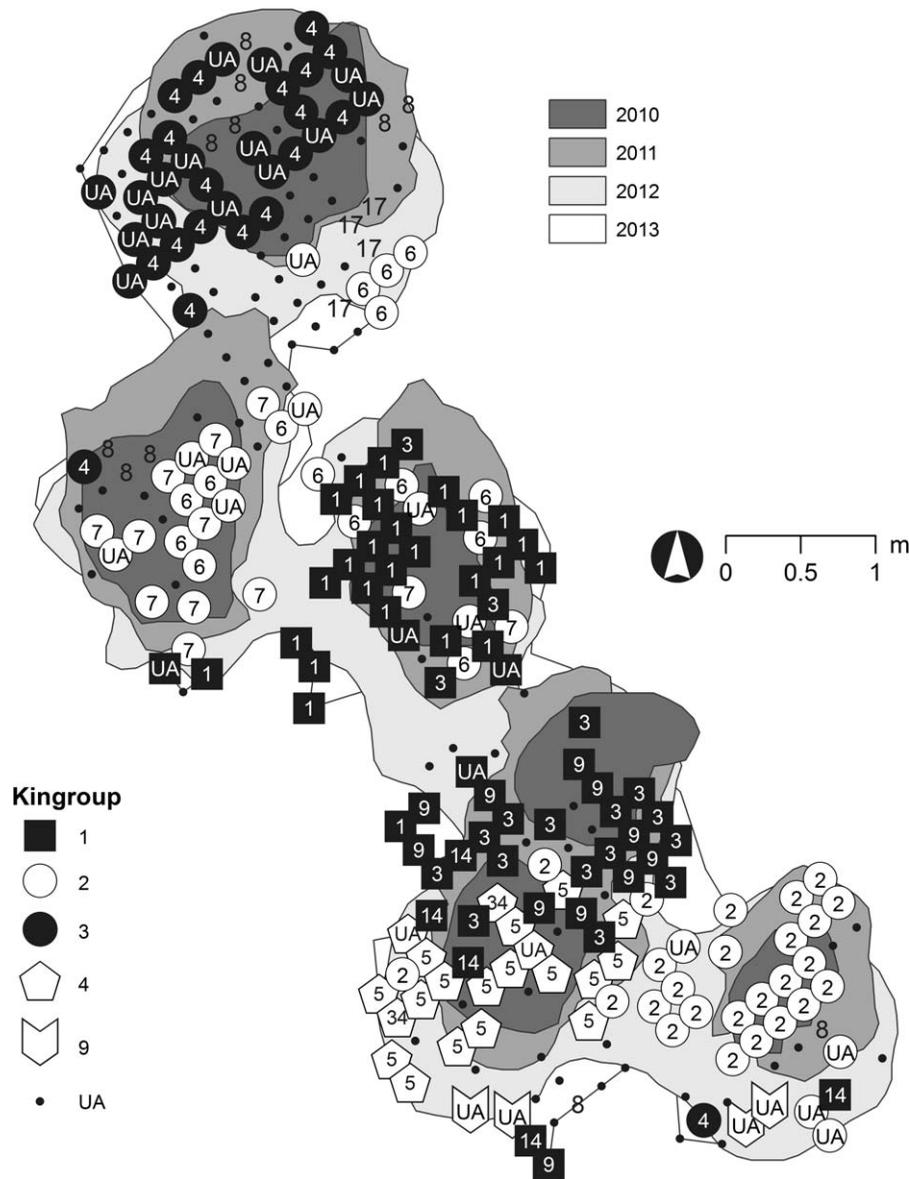


Fig. 5. Results of the pedigree analysis for the “expansion” patch. Gray-scale polygons depict the sequence of growth and coalescence observed via aerial photography 2010-2013. Black or white shapes denote kinship group (KG) assignment while numbers indicate full-sibship (FS) affiliation. “UA” identifies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

resolved to only seven 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 m and 188.4 m, respectively) and so were likely either further discriminatory artifacts of seven-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 three meter (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 m to 188.4 m (41.13 ± 64.39 m).

Of the 588 samples, GENCLONE identified 521 distinct MLGs, comprises of one to nine 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), multiramet MLLs (>2 ramets) were generally found within

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.

King- roup KG	Full- sibship FS	Nmber 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)								

contiguous seagrass patches and tended to aggregate at maximum separation distances of less than three meter. 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 $18.84 \text{ MLLs m}^{-2}$ (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 m 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 fivefold (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 ($13.90 \pm 15.19 \text{ m}$), consistent with pollination events documented or inferred by others working at the scale of ones to tens 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, three (13%) were viable parents for multiple seeds (3, 2, and 2 offspring, respectively). PDDs for these cases ranged from 6.20 m 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

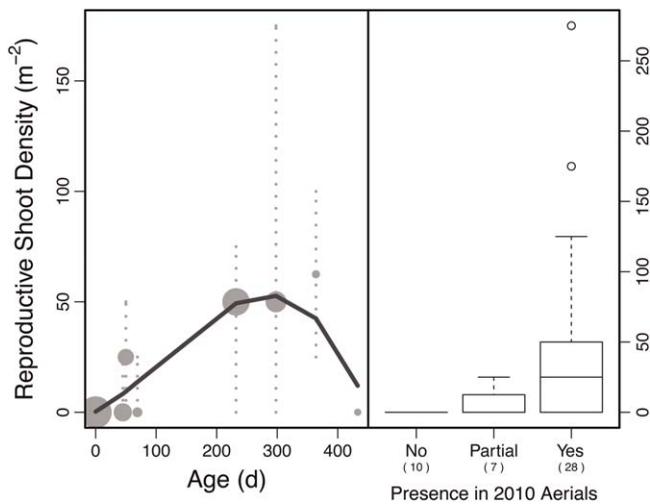


Fig. 6. Selected results of the independent assessment of reproductive effort with age. Left panel: reproductive shoot density (m^{-2}) as a function of age since vegetative colonization (d). Gray circles have been scaled to sample density (replicate cells) and fit with a third order polynomial. Right 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 first and third quartiles with a box, the ± 1.5 interquartile ranges with “Tukey whiskers” and outliers with open circles.

9.51–9.66 m to 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 five 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 d and 232 d, respectively), followed by virtually no reproductive effort at 433 d (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 postrecruitment 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 six 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

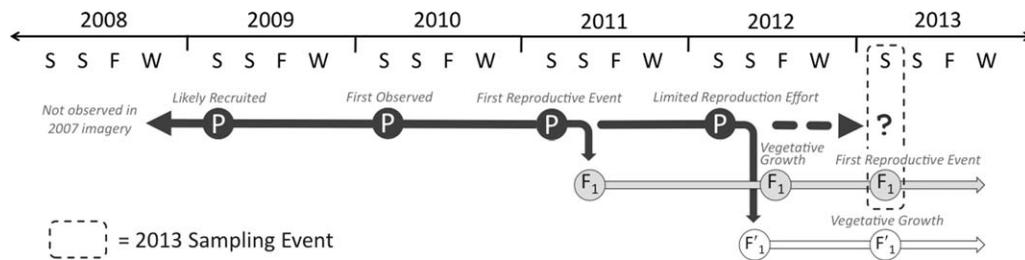


Fig. 7. Reconstructed chronological pedigree for genets recruiting to the “expansion” patch. “P” denotes parental generation, “ F_1 ” the first filial generation, and “ F'_1 ” the second cohort of offspring from the P generation. The dashed line indicates the cohorts likely sampled during the 2013 genotypic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

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 (Olesen and Sandjensen 1994; Bell et al. 1997; Kendrick et al. 2005). 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 (Reusch et al. 1999b; Billingham et al. 2007), (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 (Reusch 2006; Billingham et al. 2007; Macreadie et al. 2014). 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 *Zostera*-ceae—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 (Olsen et al. 2004; Becheler et al. 2010; Kamel et al. 2012). 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 three meters 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 m² to 20 m² ($N = 58$; 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) (Waycott et al. 2006; Vermaat 2009; Kendrick et al. 2012). 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 five meters (Ruckelshaus 1996; Billingham et al. 2007), 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, as 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 only 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 five meters 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 m and 5.31 m. This was consistent with independent pedigree analyses con-

ducted 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 bimonthly 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 biparental inbreeding within spatially restricted genetic neighborhoods (Reusch

2001; Hammerli and Reusch 2003; 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., unpubl). 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 interpatch—and presumably interclone—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 rhizodium. 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 seed dispersal, 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 biparental inbreeding will undoubtedly increase; however, our PDD estimates indicate that at least the tallest rhizodium will have access to pollen originating from outside of the KG 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 yr 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 multiyear 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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