Effects of habitat fragmentation on *Zostera marina* seed distribution

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A R T I C L E   I N F O

A B S T R A C T

Habitat fragmentation is a process which can alter the spatial configuration and reduce the overall area of a habitat. This generally results in a degradation of habitat functioning. Fragmentation of seagrass (*Zostera marina*) beds has become increasingly common, and it may threaten the valuable ecosystem services they provide. Sexual reproduction through flowering and seed dispersal could contribute to the species' potential resiliency by reducing its vulnerability to fragmentation. We investigated whether the proportion and density of flowering *Z. marina* shoots, and subsequently the density and distribution of seeds, differed between fragmented and continuous beds. Our results revealed that while flowering effort did not differ between the two bed types, seed density was significantly reduced in fragmented versus continuous beds. Further, seed distributions were altered in fragmented beds when compared to continuous beds, both within and directly outside the bed's boundaries. Seagrass patch size positively influenced seed density, with lower seed densities in small patches. Fragmented beds consistently contained fewer seeds per-unit-area than continuous beds, regardless of bed seagrass area and flowering effort. Collectively, these results emphasize the vulnerability of *Z. marina* to habitat fragmentation by demonstrating a negative effect on seed density and an impact on seed distribution, which likely reduces the potential advantages of sexual reproduction for bed growth and resiliency to perturbations.

1. Introduction

Habitat fragmentation is the process by which a continuous habitat is transformed into increasingly smaller, more numerous, and more isolated patches, resulting in a mixed landscape of structured habitat and unstructured matrix area that functions differently than the original continuous habitat (Wilcove et al., 1986). Fragmentation, which often occurs concomitantly with habitat loss, generally has negative effects on biodiversity, genetic diversity and population growth (Fahrig, 2003; Haddad et al., 2015). This process occurs in terrestrial, freshwater and marine communities. A marine species that is particularly vulnerable to fragmentation is the widespread submerged marine angiosperm, *Zostera marina* (eelgrass), commonly found in coastal regions and in estuaries ranging from temperate to near arctic waters in both the Atlantic and Pacific.

The productive and structurally complex habitat created by *Z. marina* offers a multitude of ecosystem services, such as providing nursery and foraging grounds, promoting nutrient cycling, increasing local sedimentation rates, and protecting against coastal erosion (Thayer et al., 1978; Thayer and Phillips, 1977). Though fragmentation of *Z. marina* can occur through natural disturbances such as wave energy, grazing, and wasting disease, anthropogenic forces have caused an estimated decline of 29% of seagrass area globally via fragmentation and outright bed loss (Waycott et al., 2009). Through reduced water quality, mechanical damage, and other indirect impacts to coastal waters, human disturbances have resulted in a degradation of the critical ecosystem services seagrasses provide (Short and Willy-Echeverria, 1996; Orth et al., 2006).

Fragmentation of *Z. marina* beds results in bare, unvegetated spaces that must be recolonized via vegetative or sexual reproduction. *Z. marina* reproduces through vegetative growth by rhizome elongation, as well as sexually by the dispersal of seeds for germination of new seedlings (Orth et al., 1994). Though beds rely heavily on vegetative reproduction for maintenance and survival, sexual reproduction through seed dispersal is thought to contribute substantially to genetic diversity, patch development, and recovery of disturbed areas (Greve
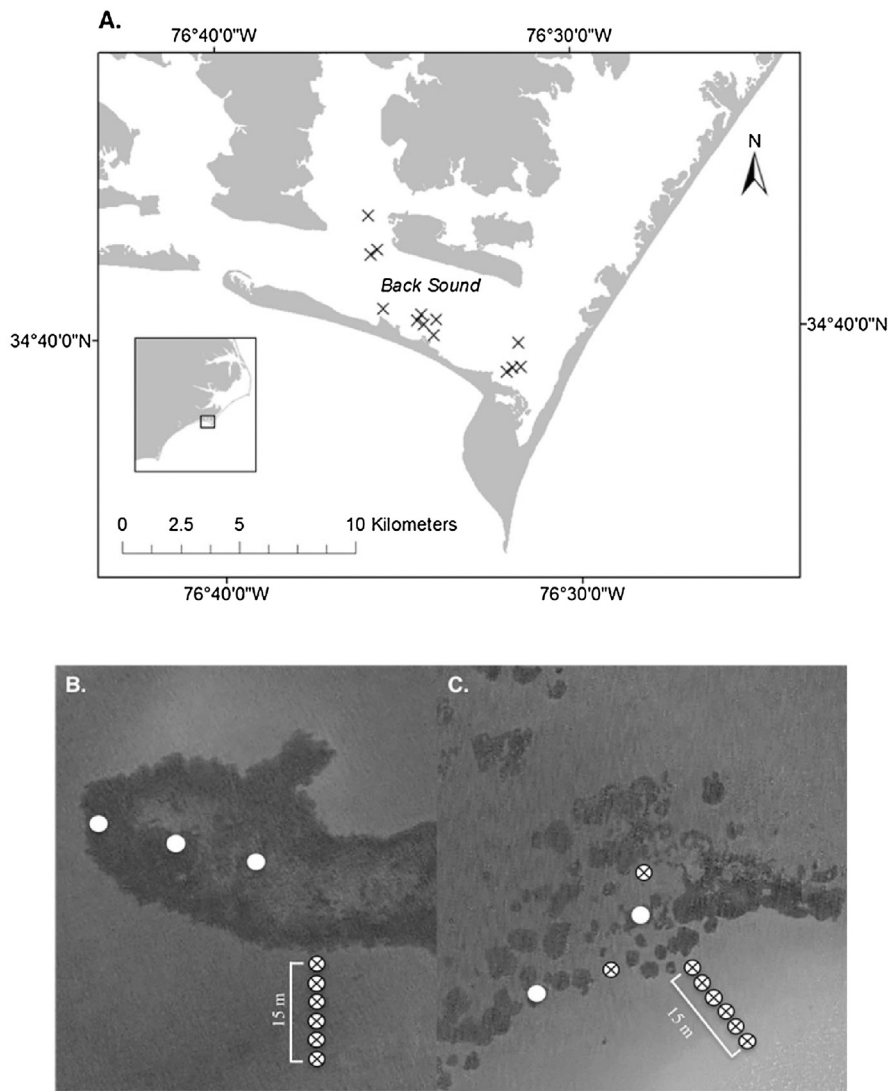
Fig. 1. Description of study area and sediment coring methods. (A.) Map of study area, Back Sound, North Carolina, with individual study sites marked. Depiction of sediment coring methods in continuous (B.) and fragmented (C.) beds, with example core positions represented. Within-grass cores are depicted as solid white circles, while within-sand cores are depicted as circle-crosses.

et al., 2005; Orth et al., 2006). Monoecious flowering shoots emerge in the late winter, and develop until ambient water temperatures reach approximately 20 °C in the summer, signaling a release of the seeds (Moore and Short, 2006). The seeds have a hard outer coating, exhibit a distinct dormancy period, and remain viable in the transient seed bank for approximately 12 months (Orth et al., 2000).

Z. marina seeds are likely limited to primarily short-distance dispersal strategies (Orth et al., 1994), contributing to the maintenance and development of the parent bed. When seeds are released from the flower, they are negatively buoyant and drop quickly to the sediment surface (Harwell and Orth, 2002). Seeds have been shown to disperse < 14 m on the bare sediment surface after release (Orth et al., 1994), but this distance is highly variable and can be dependent on local micro-topography (Luckenbach and Orth, 1999). Approximately 5–13% of seeds are released with a small air bubble, and can float in the water column for at least 40 min with the potential to travel up to 200 m before falling to the benthos (Churchill et al., 1985). Long-distance dispersal by 'rafting' of detached flowering shoots is thought to account for only a small proportion, < 6%, of the seeds produced by a single meadow (Hosokowa et al., 2015). Therefore, most seeds likely remain within or near their parental source bed. Abiotic factors such as wind and currents generally drive the dispersal of seeds after their initial release, but biotic mechanisms such as predation and excretion by crabs and larger grazers such as waterfowl can also affect the distribution and density of seeds within meadows (Fishman and Orth,

1996; Sumoski and Orth, 2012; Infantes et al., 2016).

Throughout much of its range, the dominant life history strategy of *Z. marina* follows a perennial flowering pattern. However, some beds in particularly stressful environments (i.e., high summer temperatures, ice scour, heavy storms) exhibit an annual mode of increased flowering followed by extensive die-off after the flowering season (Jarvis et al., 2012). In fully annual populations, the standing crop is produced almost entirely from seeds each year, requiring the production of an extensive seed bank to ensure persistence (Jarvis and Moore, 2010; Jarvis et al., 2014). Both perennial and annual cycles have been observed in *Z. marina* beds in North Carolina, near the southernmost limit of the species' geographic distribution, where summer water temperatures reaching over 30 °C surpass the species' thermal tolerance (Jarvis et al., 2012). Understanding the response of sexual reproduction and seed bank dynamics to fragmentation in North Carolina would be particularly informative considering the stressful summer temperatures and seasonal above ground die-backs the species endures in this region. Prolonged exposure to high temperatures has been shown to result in losses of *Z. marina* shoot density and increased mortality (Bintz et al., 2003; Nejrup and Pedersen, 2008), which could result in fragmentation of a continuous meadow over time. Sexual reproduction may mitigate these seasonal losses of biomass, as well as provide critical genetic diversity that enhances the species' ability to respond to disturbances (Hughes and Stachowicz, 2004; Ehlers et al., 2008). However, Reusch (2003) found that seed production and pollination potential of Z.

marina was negatively affected by fragmentation and manipulation of shoot densities in the parent population.

The purpose of this study was to determine whether sexual reproduction differs between continuous and fragmented beds at the lower latitudinal limit of *Z. marina*'s range. Field surveys during and after the flowering season were designed to quantify and compare flowering effort and subsequent seed bank composition across both landscape types. We hypothesized that with the stress of high temperatures in this region exacerbating seasonal diebacks, potentially increased flowering effort in fragmented beds may allow them to expand existing patches and colonize bare spaces through the production and retention of seeds. Additionally, we expected to record differences in the density and distribution of seeds between fragmented and continuous landscapes. This study explores the potential vulnerability or resiliency of *Z. marina* to habitat fragmentation, and describes the impacts of bed-wide characteristics on seed bank dynamics.

2. Methods

2.1. Study area

The seagrass beds included in this study were located in Back Sound, North Carolina, a shallow coastal estuarine system with an average water depth of 2 m and a semi-diurnal tidal range of 0.7 m (Fig. 1A.). Salinities fluctuate from 24 to 36‰, and yearly water temperatures range from approximately 4–30 °C (Kenworthy et al., 1982; NOAA Weather Station BFTN7, 2014). Seagrass beds in Back Sound are often composed of mixed stands of *Z. marina* and *Halodule wrightii* (shoalgrass). *H. wrightii* is abundant in coastal regions along the southeastern U.S. Atlantic coast, with its northern range limit in North Carolina, and its presence potentially influences *Z. marina* seed bank dynamics. A total of 12 beds were selected based on a priori visual inspection of landscape configuration, 5 of which appeared continuous and 7 of which were fragmented, (See Table 1 for site descriptions). Separate landscapes were defined as a bed of seagrass separated from other seagrass habitat by an unvegetated distance of at least 25 m.

2.2. Aerial photograph analysis

In order to quantify landscape characteristics (area, landscape-scale percent cover, etc.) at each site, orthorectified aerial photographs of the study area were obtained and imported into ArcGIS for analysis. The photos were taken by the North Carolina Department of Transportation on May 26, 2013, and were organized by the Albemarle-Pamlico National Estuary Partnership (APNEP). Each individual site was digitized by manually outlining visible seagrass within the extent of the bed, excluding any bare spaces between or within patches. Seagrass

area was calculated as the additive areas of all seagrass polygons present within the landscape. The locations of all sediment cores collected for seed quantification were recorded from a handheld GPS at the time of sampling, the coordinates of which were later entered into ArcGIS and overlaid atop the aerial photographs using the same coordinate system. Excluding cores from bare sand, the area (m²) of the seagrass patch each core was collected from was quantified by manually outlining the extent of the patch.

Landscape size ranged from 10,918 to 108,841 m² for continuous beds and from 4336 to 31,465 m² for fragmented beds. Bed types were defined as continuous if the percentage of seagrass area contained within the bed's largest patch (largest patch index, LPI) was greater than 80%, or fragmented if the LPI was less than 75%. The average LPI was 93.85% ± 2.89% (mean ± SE) for continuous beds and 44.72% ± 7.38% for fragmented beds. On average, continuous and fragmented beds contained 19.8 ± 15.15 and 52.29 ± 12.30 discrete seagrass patches, respectively. Fragmentation metrics such as LPI and patch number were determined using the program FRAGSTATS v.4 (McGarigal et al., 2012). As a proxy for potential wave energy, average fetch (from the cardinal directions) was calculated as 2.78 ± 0.24 km for continuous beds and 2.48 ± 0.32 km for fragmented beds and compared via a two-sample *t*-test (t(10) = 0.687, P = 0.51).

2.3. Flowering effort

To quantify the average shoot density of *Z. marina* in each bed as well as the ratio of flowering to vegetative shoots, six 0.063 m² quadrats were haphazardly placed over vegetated substrate, and all seagrass shoots present within each quadrat were removed by the roots. *Z. marina* flowering and vegetative shoots were separated and counted in the lab. Sampling occurred in May 2014, when the flowering season was determined to be at its approximate peak based on observations of flowers in the region, and when water temperatures reached the optimal range for flowering, 20–21 °C (Moore and Short, 2006).

2.4. Sediment coring for seeds

To sample the distribution and density of seeds at specified positions within each bed, 10-cm diameter sediment cores were taken to a depth of approximately 10 cm, as *Z. marina* seeds are generally buried no deeper below the sediment surface (Morita et al., 2007). Sediment core samples were collected in July 2014, after the flowering season had ended and sufficient time had passed for all seeds to settle. In continuous beds, two transects ran from the center of the bed to the edge. The first transect direction was selected haphazardly, with the second being approximately perpendicular to the first. In each transect, one core sample was taken at the starting point, located at the approximate

Table 1

Descriptions of individual sites, including site name, type (fragmented or continuous), overall bed area (a polygon encompassing all vegetated and bare space within the bed (m²)), area of seagrass within the site (m²), landscape-scale seagrass percent cover (%), average vegetative *Z. marina* per 0.25 m² (± 1 SE), average flowering *Z. marina* per 0.25 m² (± 1 SE), average vegetative *H. wrightii* per 0.25 m² (± 1 SE), average *Z. marina* seeds per 0.25 m² (within vegetated substrate only (± 1 SE)), average fetch (meters, calculated from N, S, E, and W orientations), number of discrete seagrass patches, and largest patch index (% of area contained within the largest patch).

Site	Type	Bed Area	Seagrass Area	Percent Cover	Vegetative *Z. marina*	Flowering *Z. marina*	*H. wrightii*	*Z. marina* Seeds	Fetch	# of Patches	LPI
C1	Cont	14566.66	13899.38	95.42	292 ± 15	20 ± 5	1093 ± 246	319 ± 58	3071.24	2	90.58
C2	Cont	59802.79	44362.67	74.18	471 ± 79	33 ± 16	978 ± 143	329 ± 92	2873.62	5	98.77
C3	Cont	11401.35	11323.11	99.31	463 ± 31	22 ± 5	886 ± 160	138 ± 100	2945.17	1	100
C4	Cont	10918.31	8353.58	76.51	601 ± 51	121 ± 13	217 ± 66	504 ± 106	1729.66	11	95.61
C5	Cont	108840.81	60801.03	55.86	565 ± 69	61 ± 9	701 ± 132	319 ± 56	3298.18	80	84.28
F1	Frag	23718.44	6260.04	26.39	427 ± 68	45 ± 3	523 ± 133	104 ± 68	3036.36	119	38.46
F2	Frag	31464.99	13007.67	41.34	326 ± 72	13 ± 3	590 ± 100	106 ± 28	1999.12	43	61.48
F3	Frag	27810.62	11676.76	41.99	409 ± 69	28 ± 8	28 ± 8	88 ± 27	3095.24	39	48.56
F4	Frag	12652.67	5231.86	41.35	621 ± 85	136 ± 26	243 ± 67	358 ± 87	1914.57	49	24.25
F5	Frag	22157.81	9900.56	44.68	330 ± 99	37 ± 13	971 ± 212	191 ± 84	3128.94	58	19.35
F6	Frag	4335.63	1994.40	46.00	312 ± 58	20 ± 5	894 ± 286	48 ± 16	3162.26	13	46.43
F7	Frag	17012.03	8938.02	52.54	645 ± 104	148 ± 41	113 ± 48	279 ± 53	1007.47	45	74.50

center of the bed; a second core sample was collected halfway between the center and the edge of the bed, the location of which differed for each bed based on its size; and a third core sample was taken at the edge of the bed (Fig. 1B.).

In fragmented beds, one core sample was taken within each of two different vegetated patches near the center of the bed; within each of two vegetated patches along the edge of the bed; within each of two bare, unvegetated areas in the interior region of the bed; and in each of two bare areas along the edge of the bed (i.e., 8 cores per bed; Fig. 1C.). In both continuous and fragmented beds, two additional transects were used to sample directly outside of the bed. These transects ran perpendicular to the edge of the bed, and one core sample in each transect was collected at the following distances away from the edge of the bed: 0, 2.5, 5, 7.5, 10, and 15 m (Fig. 1B.,C.).

Each core was wet-sieved in the field in 400-μm mesh bags to wash away sediment. Remaining coring contents were taken to the lab where they were frozen until processed, which involved individually examining them under a dissecting microscope. Any seeds, whether they were fully intact or the casing of an already germinated or dead seed, were identified and counted. *Z. marina* and *H. wrightii* shoots in each core were also counted.

2.5. Data analysis

2.5.1. Site characteristics

To determine whether the bed types contained similar overall area and landscape-scale percent cover of seagrass, two-sample *t*-tests were utilized to compare those variables between fragmented and continuous beds. Bed areas were log-transformed to avoid violating the assumption of normality; no transformations were necessary for percent cover. A generalized linear mixed effects model (Bolker et al., 2009) with a poisson error distribution was used to determine whether the density of vegetative *Z. marina* shoots differed between fragmented and continuous beds. In this analysis, bed type (fragmented or continuous) was treated as a fixed effect and site (bed) as a random effect. This test was repeated to compare the density of *H. wrightii* shoots between bed types. For these and all subsequent generalized linear models, statistical significance was assessed via Wald chi-squared tests using type II sum of squares to account for the slightly unbalanced nature of the data.

2.5.2. Flowering effort

The proportion of flowering *Z. marina* shoots was determined by dividing the number of flowering shoots by the total number of *Z. marina* shoots in each quadrat. We used a generalized linear mixed effects model with a binomial error distribution to determine whether flowering proportion differed between bed types. In this analysis, bed type was treated as a fixed effect and site (bed) as a random effect. Similarly, we used a generalized linear mixed effects model with a poisson error distribution to determine whether density of flowering *Z. marina* shoots (number of shoots per 0.25 m²) differed between bed types, with bed type serving as the fixed effect and site as a random effect.

2.5.3. Sediment coring for seeds

Next, a generalized linear mixed effects model (poisson distribution) was performed using data from only vegetated areas within all sites to compare seed counts from distinct positions between both bed types. This model incorporated seed count per core sample as the response variable, bed type and position within the bed (center or edge) as fixed explanatory effects, and position nested within site as the random explanatory effects. A separate generalized linear mixed effects model (poisson distribution) was performed using data from fragmented sites only to compare seed counts from different positions within the bed, as well as among core types, vegetated patch or bare sand. This model incorporated seed count as the response, position within the bed (center or edge) and type of core (patch or sand) as fixed explanatory effects, as

well as site and position nested within site as the random explanatory effects.

To determine the relationship between seed density and distance away from the bed edge, we fit separate generalized linear models for each bed type. The quasipoisson error distribution was utilized to account for overdispersion of the count data. Similar analyses were performed to identify the relationship between *Z. marina* and *H. wrightii* shoot densities within each sediment core, as well as between each of those species and the associated number of *Z. marina* seeds.

We used a generalized linear model (quasipoisson distribution) to characterize the relationship between *Z. marina* seed count per core and the size (area) of the seagrass patch the core was taken from. Patch areas were log-transformed due to non-normality and high variability, as the sizes ranged from < 5 m² to > 60000 m².

We used generalized linear models (quasipoisson distribution) to quantify the relationship between flowering *Z. marina* shoots per 0.25 m² and seagrass bed area, as well as the number of *Z. marina* seeds per 0.25 m² and bed area. Bed areas were log-transformed due to skew and high variability. To investigate the relationship between the average density of flowering *Z. marina* shoots to the average density of *Z. marina* seeds within both fragmented and continuous beds, a linear regression was performed. The residuals were then extracted for each data point (each site), representing the difference between the observed values and those predicted by the linear regression. Next, the residuals of fragmented and continuous beds were compared via a two-sample *t*-test.

For all statistical analyses, the open-source statistical software R was utilized (R Development Core Team, 2008), and analyses were conducted using the 'lme4' and 'car' packages (Bates et al., 2015; Fox and Weisberg, 2011).

3. Results

3.1. Site characteristics

Overall area of seagrass in fragmented beds (8144 ± 1461 m², mean ± SE) was lower than continuous beds (27747 ± 10502 m², P = 0.04). Landscape-scale percent cover of seagrass was higher in continuous beds (80% ± 7.9%) than fragmented beds (42% ± 3%, P < 0.01). The density of vegetative *Z. marina* shoots did not differ significantly between fragmented (438.4 ± 34.8 shoots per 0.25 m²) and continuous beds (478.1 ± 30.1 shoots per 0.25 m², P = 0.53). Similarly, *H. wrightii* shoot density was not significantly different between fragmented (480.2 ± 75.3 shoots per 0.25 m²) and continuous beds (775.2 ± 87.6 shoots per 0.25 m², P = 0.15).

3.2. Flowering effort

Flowering effort did not differ between the two bed types. The average proportion of *Z. marina* flowering shoots to total *Z. marina* shoots was not significantly different between continuous (< 0.1 ± 0.1) and fragmented beds (0.1 ± 0.1; Fig. 2A., P = 0.65). The average density of *Z. marina* flowering shoots was also similar between bed types, with continuous beds containing 51.5 ± 8.2 flowering shoots per 0.25 m² and fragmented beds containing 60.9 ± 10.6 flowering shoots per 0.25 m², on average (Fig. 2B., P = 0.92).

3.3. Sediment coring for seeds

Differences in seed density and distribution were observed between fragmented and continuous beds. There were fewer seeds in vegetated areas within fragmented beds (166.1 ± 28.2 per 0.25 m²) than in continuous beds (336.0 ± 41.4 per 0.25 m²) on average (Fig. 3A., P = 0.01). There was no significant effect of position within the bed, center or edge, on seed density in vegetated areas in both bed types
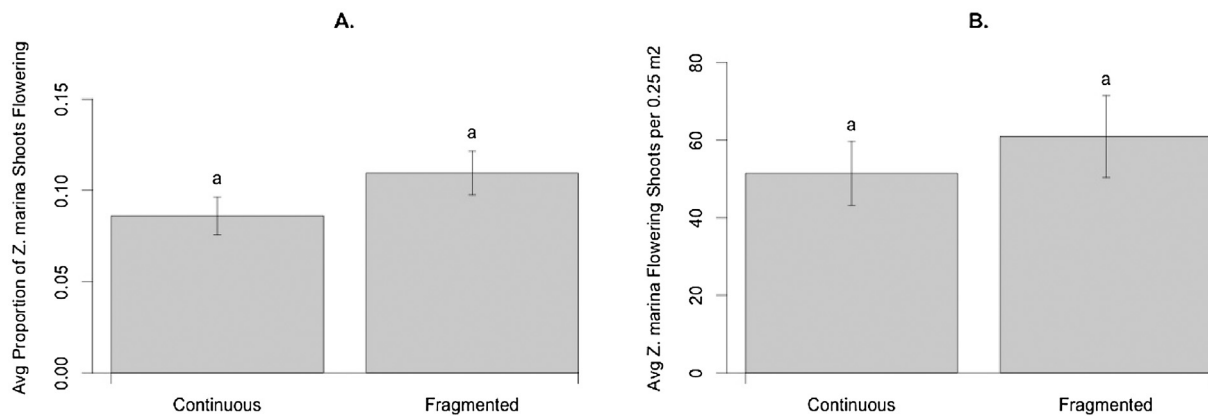
Fig. 2. Flowering effort of *Z. marina* within continuous and fragmented beds. (A.) Comparison of the average proportion of flowering *Z. marina* shoots to vegetative *Z. marina* shoots between continuous and fragmented beds (P = 0.65). (B). Comparison of the average density of *Z. marina* shoots per 0.25 m² between continuous and fragmented beds (P = 0.92). Error bars represent ± 1 SE.

(Fig. 3A., P = 0.16). Within fragmented beds, there were fewer seeds in bare sand between seagrass patches (63.7 ± 16.1 per 0.25 m²) than in vegetated areas (166.1 ± 28.2 per 0.25 m²) on average (Fig. 3B., P < 0.01).

Patterns of seed density outside of the bed edge differed between fragmented and continuous beds. There was no significant difference in seed density among any positions along the 15-m transects outside of fragmented beds (Fig. 4B., (β (model parameter estimate) = −0.03) P = 0.37). Conversely, outside of continuous beds, there was a negative relationship between seed density and distance from the bed edge, indicating higher seed counts at the 0 m mark compared to the remainder of the transect (Fig. 4A., (β = −0.09) P < 0.01).

When considering the other species of seagrass in this system, *H. wrightii*, multiple patterns emerged. No significant relationship was found between *H. wrightii* shoot count per 0.25 m² and *Z. marina* shoot count per 0.25 m² (Fig. 5A., (β = −0.04) P = 0.08). There was, however, a positive relationship between *Z. marina* shoot count per 0.25 m² and the number of *Z. marina* seeds per 0.25 m² (Fig. 5B., (β = 0.05) P = 0.02). Conversely, the relationship between *H. wrightii* shoot count per 0.25 m² and *Z. marina* seed count per 0.25 m² was negative (Fig. 5C., (β = −0.02) P = 0.02).

A positive relationship was observed between *Z. marina* seed density per 0.25 m² and the size (m²) of the seagrass patch the cores were taken from (Fig. 6, (β = 0.23) P < 0.01). There was no significant relationship between seagrass bed area (m²) and *Z. marina* flowering

shoot density (Fig. 7A., (β = −0.31) P = 0.34). There was also no significant relationship between seagrass bed area (m²) and *Z. marina* seed density per 0.25 m² (Fig. 7B., (β = 0.47) P = 0.09). However, there was a difference between fragmented and continuous beds in the residual values extracted from the linear regression comparing average density of flowering *Z. marina* shoots to average density of *Z. marina* seeds. The residuals were positive on average for continuous beds, meaning they fell above the regression line, and the opposite was true for fragmented beds, indicating that fragmented beds contained fewer seeds than continuous beds with similar flowering shoot densities (Fig. 7C., P < 0.01).

4. Discussion

Our results indicate that although flowering effort did not differ between fragmented and continuous *Z. marina* meadows, seed distributions and densities were significantly affected by fragmentation. In beds where the eelgrass was fragmented, seed density in vegetated areas was lower than within continuous beds, and seeds were at their lowest densities in bare areas that were devoid of any adult plants. These results illustrate the vulnerability of *Z. marina* to fragmentation, as a reduced seed bank in fragmented beds could impede patch development and colonization of bare areas.

Habitat fragmentation did not have a detectable effect on the effort that *Z. marina* expended on flowering, with the average proportion and
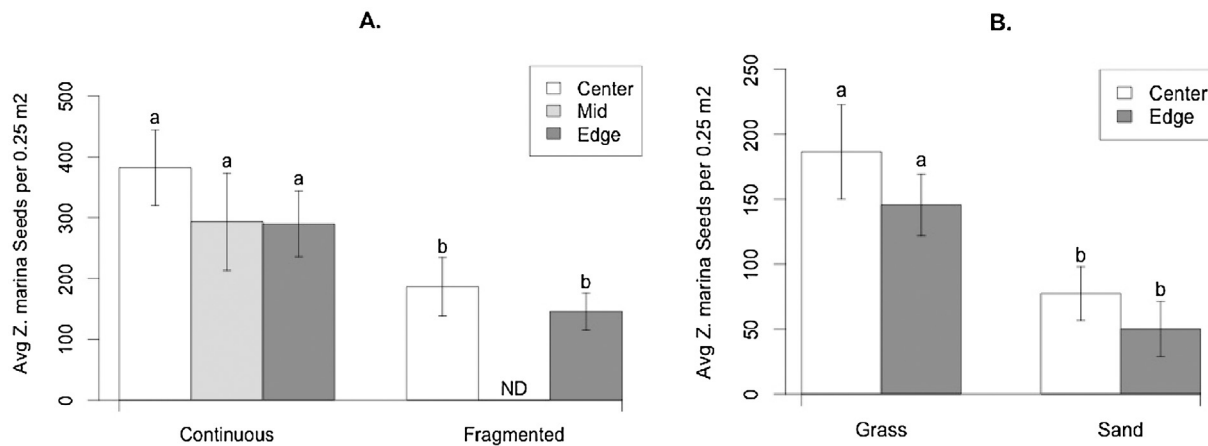

Fig. 3. Effect of habitat fragmentation on within-bed *Z. marina* seed densities. (A.) Average seed densities across fragmented and continuous beds, from cores in vegetated areas exclusively (P = 0.01). Average seed densities at specific positions within the bed, center or edge, compared across bed types (P = 0.16). (B.) Average seed density compared between vegetated and bare areas within fragmented beds (P < 0.01). Comparison of average seed densities at specific positions within the bed, center or edge, within fragmented beds (P = 0.27). Error bars represent ± 1 SE.
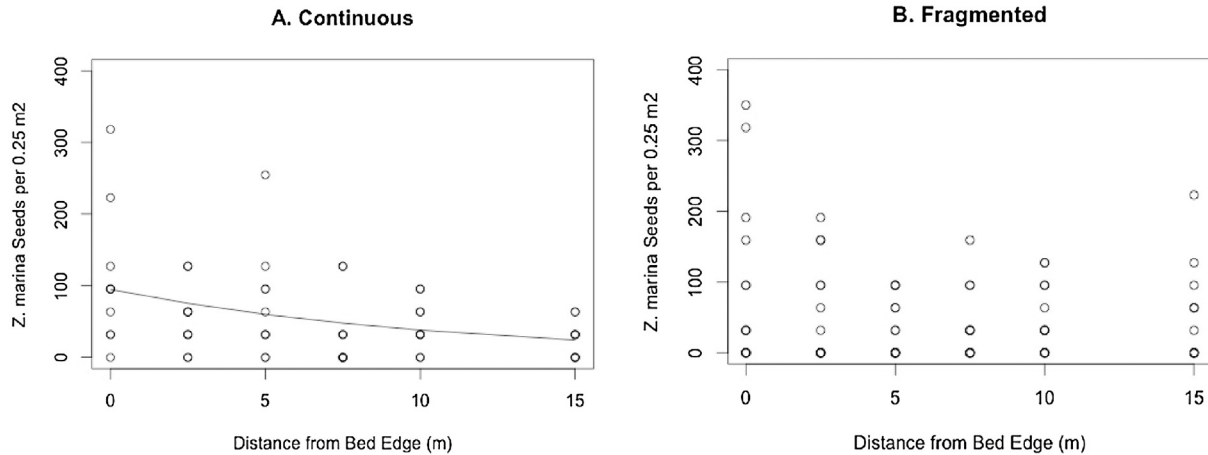
Fig. 4. Influence of habitat fragmentation on *Z. marina* seed density outside of the bed edge. Seed density along a transect beginning at the outer edge of the bed, with increasing distance from continuous beds (A., $P < 0.01$), and fragmented beds (B., $P = 0.37$). Generalized linear regression line (quasipoisson error distribution) represented in panel A.

density of flowering shoots differing between bed types by only 0.02 flowering shoots/total shoots and 9.5 flowering shoots per 0.25 m², respectively. Though not observed in this study, flowering effort can increase in response to high temperature stress (De Cock, 1981; Potouroglou et al., 2014), heightened wave activity, and increasing water depth (Fonseca and Bell, 1998), three major sources of stress or disturbance that can drive fragmentation. Fonseca and Bell (1998) found that seagrass bed landscape characteristics such as bed coverage and shape (perimeter to area ratio) were strongly correlated with wave exposure and current speed in Back Sound, indicating the fragmented beds in our study may exist in regions experiencing stronger hydrodynamic forcing than the continuous beds (i.e. stronger channelized flow versus sheltered conditions). However, the flowering effort of both bed types, though they exist in the highest range of the species' tolerable temperatures and in a variable estuarine system, did not appear to be affected differently by these environmental factors.

Eelgrass meadows have the capacity to significantly reduce current velocities, and patch edges are especially important in controlling current flow (Fonseca et al., 1982) For example, Peterson et al. (2004) found current velocities in high density continuous eelgrass can be reduced up to 60% at 0.25 m into the bed. This influence of eelgrass beds on local hydrodynamics may be a critical factor in controlling the dispersal of seeds after their release, as the density of seeds found in vegetated areas within our fragmented beds was significantly lower than within the continuous beds. This suggests that seeds produced in fragmented beds may experience stronger or more variable currents that remove them from vegetated areas more readily than those released within continuous beds, where current velocities may be lower or more consistent. Therefore, the slowing and directing of currents by the eelgrass itself, in addition to the bed's location within the estuary (as discussed previously, Fonseca and Bell, 1998), may drive differences in hydrodynamics, and thus seed distribution, between fragmented and continuous beds.

Higher seed densities were found at the outer edge of continuous beds as compared to farther away from the bed. Thus, continuous beds appear to be retaining a significant amount of the seeds produced near the bed edge. In contrast, seeds were found at statistically equal densities from 0 to 15 m away from the edge of fragmented beds. The even distribution of seeds outside of these fragmented beds lends support to the hypothesis that they may be experiencing more dynamic and stronger flow regimes than they would at the continuous sites. Since short-distance dispersal accounts for a large proportion (i.e., > 90%) of the settled seeds within and near a bed (Hosokawa et al., 2015), the majority of these seeds found outside of each bed were likely produced there, not from a different bed in the region.

The lowest seed densities across both meadow types occurred in the

bare spaces within fragmented beds. While it is unclear what density of seeds is required to successfully colonize a bare region, it is possible that the observed densities are low enough to be limiting eelgrass survival in those areas. For example, Jarvis and Moore (2010) determined that following a 2005 large-scale mortality event of *Z. marina* in the York River (VA, USA), seed germination accounted for the majority of recovery the following year. However, the available seed bank density in that region was reduced greatly to a maximum density of 12.5 ± 6 seeds per 0.25 m² in 2006. A subsequent die-off in 2006 resulted in an almost complete lack of recovery the following year, suggesting that seed availability was perhaps not high enough to support recolonization. Therefore, the reduced density of seeds in inter-patch bare spaces (63.7 ± 16.1 per 0.25 m²) compared to vegetated areas within fragmented beds observed in this study may be similarly limiting the colonization potential of seedlings. Although that density is nearly 5-times higher than the density reported in the York River, germination success of *Z. marina* seeds is variable, and can be as low as < 5% in natural systems (Orth et al., 2003), suggesting that generally low seed densities may result in minimal to no seedling production. Environmental conditions in those sandy, exposed areas may not have been conducive to germination, perhaps explaining why adult plants were not already present. Any seedlings produced in bare spaces would likely exist as single shoots, or in very small low-density patches, which may not survive to the following year (Ramage and Schiel, 1999; Worm and Reusch, 2000). For instance, *Z. marina* patches containing < 32 shoots have been found to experience intense mortality and rapid turnover (Olesen and Sand-Jensen, 1994), so the colonization potential of a few seedlings in inter-patch bare spaces is likely quite low.

The size of a seagrass patch positively influenced seed density, with larger patches generally containing higher densities of seeds. However, there was high variability in seed densities within large patches, while small patches generally contained low seed densities. This "wedge-shaped" pattern in the data indicates that seed density is partially influenced by patch size, but there are other unmeasured factors controlling the distribution and density of seeds in fragmented landscapes, especially in large patches (Cade et al., 1999). This result highlights the importance of investigating habitat fragmentation from both a patch and landscape scale. The effects of habitat patch size may be influential on variables such as seed density and distribution, but other large-scale factors such as hydrodynamics and habitat configuration are also critically important in understanding those patterns.

Despite similar patterns in flowering effort and seed density compared to their overall vegetated area, fragmented beds consistently contained fewer seeds than continuous beds. This finding indicates that flowering effort as described by the density and proportion of flowering
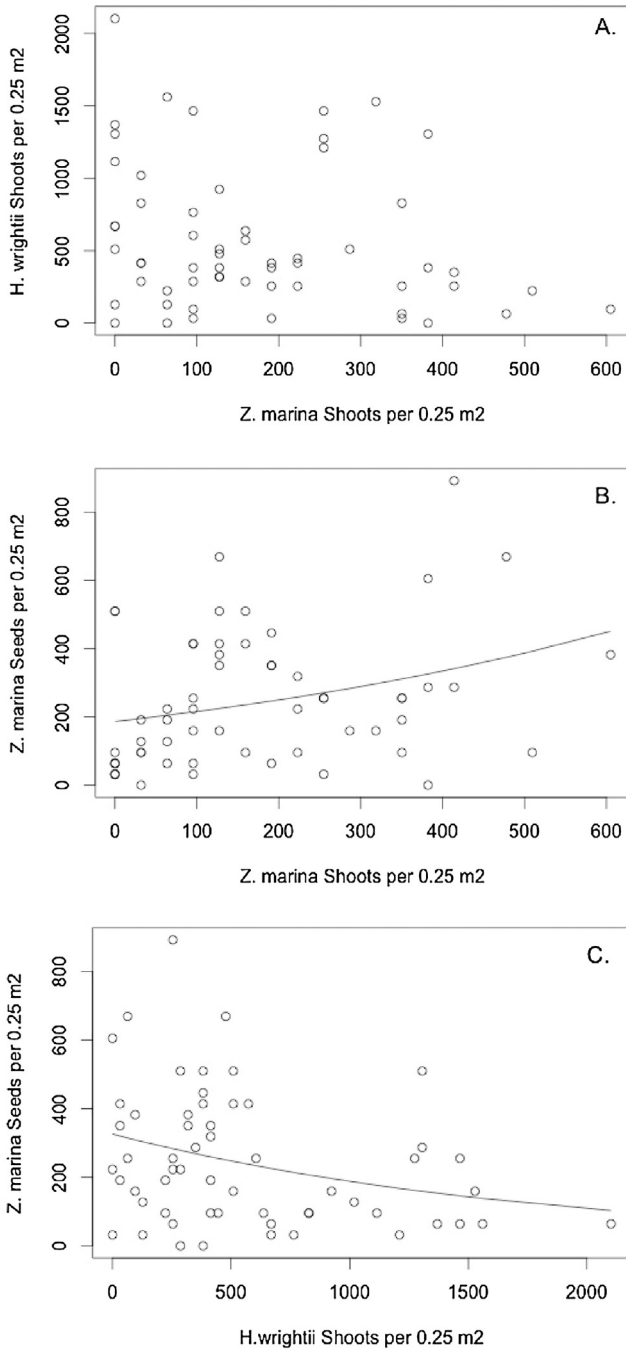
Fig. 6. *Z. marina* seed density across varying seagrass patch sizes. Positive relationship between *Z. marina* seed density per 0.25 m^2 and seagrass patch size (m^2 log transformed, $P < 0.01$), with the generalized linear regression line (quasipoisson error distribution) represented.

success, it is possible that flowering shoots in isolated patches within fragmented beds could be experiencing pollen limitation, and thus reduced seed production per shoot. This pattern has been observed in terrestrial plants (Cunningham, 2000; Knapp et al., 2001), and Reusch (2003) detected a similar negative effect of fragmentation on *Z. marina* reproductive output. Therefore, depressed pollination, separate from flowering effort, may have influenced the seed production of fragmented beds, followed by potential physical factors affecting the retention of those seeds after they were released.

Reduced pollination potential may negatively impact the viability of seeds produced in fragmented beds, due to limited access to genetically diverse pollen. However, Stubler et al. (2017) observed the highest density of viable seeds in small (2.5 m^2) patches of *Z. marina* as compared to larger patches (up to 20 m^2) within fragmented meadows. This indicates that small patches may produce high numbers of viable seeds, which could offset the suppressed seed density in highly fragmented beds. In addition to variability in pollination and seed viability, the number of inflorescences per flowering shoot can vary depending on environmental conditions during the flowering season (De Cock, 1980). Though not measured in the present study, it is possible that flowering shoots grown under stressful conditions in fragmented beds would produce fewer inflorescences, and thus fewer seeds, regardless of flowering shoot density and proportion. These metrics of flowering effort (inflorescences per flower) and success (number of viable versus non-viable seeds) may have influenced the observed differences in sediment seed bank densities between fragmented and continuous meadows.

A negative relationship emerged between *H. wrightii* shoot density and *Z. marina* seed density. Though we did not detect a relationship between *H. wrightii* and *Z. marina* shoot densities, environmental factors likely influence the distribution and densities of these two species in Back Sound, one of the few regions on the East coast where they coexist. For example, *H. wrightii* can survive in shallower waters than *Z. marina,* and can even tolerate aerial exposure at low tide (Thayer et al., 1984). In areas where environmental conditions are poor for *Z. marina*, *H. wrightii* may thrive, and vice versa. Additionally, while *Z. marina* is abundant in the cooler months through the winter and spring, it senesces in the summer heat while *H. wrightii* remains year-round. Our results suggest that if *Z. marina* seeds are dispersed to a region with high *H. wrightii* density, they do not get entangled and settle in the structure *H. wrightii* provides. This lack of a beneficial interaction between the two species may have influenced the low observed *Z. marina* seed densities in areas with high *H. wrightii* density, in addition to the abiotic factors driving the distribution of each species in the estuary. Further investigation is required to understand how the interaction of these

Fig. 5. Interaction between *H. wrightii* and *Z. marina*. (A.) Comparison of *H. wrightii* shoot count per 0.25 m^2 and *Z. marina* shoot count per 0.25 m^2 ($P = 0.08$). (B.) Positive relationship between *Z. marina* seed count per 0.25 m^2 and *Z. marina* shoot count per 0.25 m^2 ($P = 0.02$). (C.) Negative relationship between *Z. marina* seed count per 0.25 m^2 and *H. wrightii* shoot count per 0.25 m^2 ($P = 0.02$). Panels B and C include generalized linear regression line (quasipoisson error distribution).

shoots, regardless of seagrass bed area, is generally not a strong predictor of future seed-bank densities. Differences in total landscape seagrass area and cover did vary among fragmented and continuous seagrass beds, which likely influenced the total production of seeds. However, our results suggest that the overall availability of flowering shoots in a bed does not definitively determine the number of seeds retained in the sediment seed bank. This pattern has been observed elsewhere, with the number of reproductive shoots being weakly correlated to corresponding seed bank densities after the flowering season (Harwell and Orth, 2002). Though we did not examine pollination
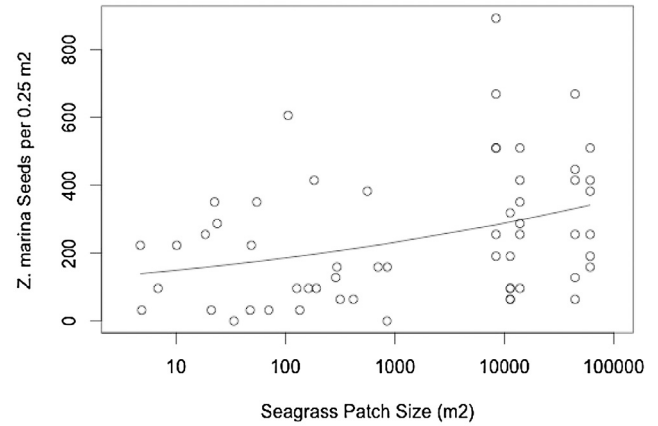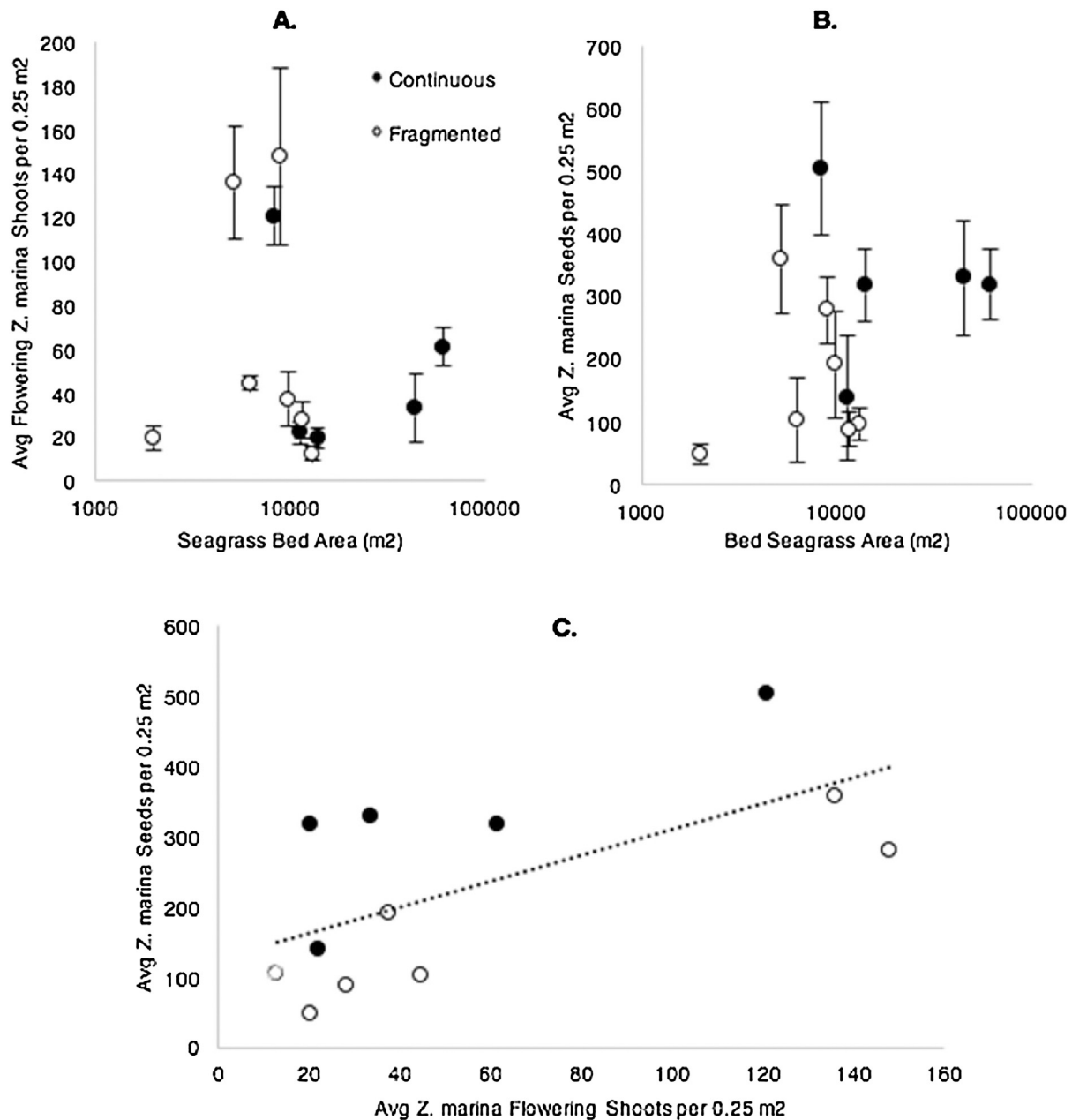
Fig. 7. Effect of seagrass area and *Z. marina* flowering effort on *Z. marina* seed densities. (A.) Flowering *Z. marina* shoot density across seagrass bed areas (m², log transformed, P = 0.34). (B.) Comparison of seagrass bed area (m², log transformed) and *Z. marina* seed density per 0.25 m² (P = 0.09). (C.) Linear regression comparing flowering *Z. marina* shoot density and seed density per 0.25 m², with a significant difference in the residuals of fragmented and continuous beds (P < 0.01).

species may affect *Z. marina* sexual reproduction across a range of environmental conditions.

The negative effect of habitat fragmentation on seed production and retention observed in this system aligns with many similar studies in terrestrial plant communities (Aguilar et al., 2006). Habitat fragmentation has far-reaching effects on not only the reproductive success of plant communities (Aizen and Feinsinger, 1994; Steffan-Dewenter and Tscharntke, 1999), but it can also result in a reduction of biodiversity and an alteration of interactions with associated fauna (Debinski and Holt, 2000; Yeager et al., 2016). Since certain members of the faunal community can consume and excrete seagrass seeds (Fishman and Orth, 1996; Sumoski and Orth, 2012; Infantes et al., 2016), the effects of habitat fragmentation on those communities may in turn influence the magnitude of seed predation and biotic dispersal pathways. However, these effects are highly variable in seagrass ecosystems, and the influence of habitat fragmentation on flowering dynamics and seed dispersal is poorly understood (Boström et al., 2006).

Reduced seed densities in fragmented *Z. marina* beds could impede patch development and colonization of barren areas. These findings have implications for the conservation and management of this critically valuable habitat. With an estimated 29% global loss in the historical abundance of seagrasses (Waycott et al., 2009), growth and maintenance of meadows is of utmost importance. In *Z. marina*'s southernmost limit in North Carolina, where summer water temperatures already reach the species' thermal tolerance, any future increases in temperature stress or wave activity may pose significant threats to the species' persistence (Carr et al., 2012). Sexual reproduction can provide an alternative to vegetative growth under these stressful conditions, but it is unclear if barren areas will be colonized by seedlings without active restoration efforts. Our results indicate how eelgrass seed production may be affected by habitat fragmentation, which has implications for not only the health and persistence of the species itself,

but also its role as a critical estuarine habitat that provides several valuable ecosystem services.

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