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Effects of microtopographic variation and macroalgal cover on morphometrics and survival of the annual form of eelgrass (*Zostera marina*)

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Abstract

A disjunct population of the annual form of the seagrass *Zostera marina* that occurred in the upper intertidal zone of Yaquina Bay, Oregon was sampled to determine whether there were differences in recruitment, growth, survivorship and morphology associated with microtopographic location on the sand flat. Population responses of seagrass found in areas differing by only a few cm in vertical elevation were compared. There was higher plant abundance and higher number of shoots per plant in microtopographic low areas. Plants in lower areas also had significantly longer shoots, greater total above ground biomass, greater biomass per shoot, and greater biomass of reproductive spathes than plants growing in immediately adjacent, microtopographic high areas. Cover of green macroalgae was higher and accumulated more rapidly in microtopographic high areas as compared to low areas, and both spatially and temporally was correlated with decreased recruitment and increased plant loss in these slightly elevated areas. While impacts of desiccation may have played some role in determining the influence of microtopographic variation on the annual *Z. marina* population, macroalgal effects appear to be predominant.

Keywords

Annual *Zostera marina*; Annual eelgrass; Microtopography; Macroalgae impact; Seagrass; Recruitment

1.0 Introduction

As a result of large seasonal inputs of upwelled nutrients from the adjacent ocean, intertidal flats within the Yaquina estuary (Oregon, USA) experience summer blooms of green macroalgae (Hessing-Lewis et al. 2015). Observations of the sequence of blooms of green macroalgae on an estuarine sandflat had suggested that macroalgae became established first on patches of substrate that were slightly higher than surrounding areas (microtopographic highs), while establishment was delayed in shallow pools (microtopographic lows) that were immediately adjacent. Also occurring in this area of the intertidal sandflat is a spatially disjunct population of the eelgrass *Zostera marina*, with a life cycle distinct from the lower intertidal form (Bayer, 1979). The upper intertidal form of *Z. marina* has an annual life

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history (Keddy and Patriquin, 1978; Gagnon et al., 1980; Phillips et al., 1983; Keddy, 1987; Backman, 1991) while the lower intertidal form has a perennial life history. The annual life history is most typically found in intertidal areas and appears to be an adaptation to deal with extremes of environmental stress (Olesen and Sand-Jensen, 1994; van Lent and Verschuure, 1994). However, examples have been described of subtidal annual populations. One population from the Gulf of California occurs only in winter (McMillan, 1983; Phillips and Backman, 1983; Meling-Lopez and Ibarra-Obando, 1999; Santamaria-Gallegos et al., 2000), in apparent response to high summer water temperature near the southern limit of distribution. Another annual form from Korea is found near the maximum depth limit, where it experiences light limitation (Kim et al., 2014).

In response to large-scale losses of seagrass from human activity (Short and Wylie-Echeverria, 1996; Orth et al. 2006; Waycott et al., 2009), study of the conditions leading to success of eelgrass restoration, including intertidal populations in macrotidal systems, has become increasingly important (e.g. van Katwijk et al. 2000). A better understanding of factors influencing the recruitment success (e.g. van Katwijk and Wijgertang, 2004) is critical to the design and implementation of restoration projects that may rely on seeds (Orth et al., 1994; Pickerell et al., 2005; Marion and Orth, 2010) rather than shoot transplants. The effects of hydrodynamic exposure, sediment type, water cover, seed source, burial depth, and germination temperature have been described for seeds of *Z. marina* (van Katwijk and Wijgertang, 2004; Morita et al., 2010; Jarvis and Moore, 2015). The influence of microtopography and the interaction of small-scale physical relief with recruitment of macroalgae and the impacts on annual *Z. marina* recruitment and survival have not been previously described. Therefore, the objectives of the present study were to determine how the distribution patterns of green macroalgae and the annual form of *Z. marina* relate to microtopographic variation in the upper intertidal zone, and to determine whether there were differences in the population response patterns of annual eelgrass associated with microtopographic location. The null hypothesis was no effect of sandflat microtopography on *Z. marina* plant response metrics.

2.0 Methods

2.1 Transect measurements

The study site was an annual *Zostera marina* bed located on the sand flat known locally as Idaho Flat, adjacent to the Hatfield Marine Science Center, Newport, OR, USA (44.62 N, -124.037 W). The Idaho Flat and Yaquina estuary is described in D'Andrea and DeWitt (2009). The surface of the sand flat has ripples and sand waves presumably generated by tidal flow and wave action with the bottom (Dingler and Clifton, 1984). Three parallel, fixed transects (60 m), separated by approximately 20 m and marked with PVC stakes, were established in mid-April, 2000, with a field surveyor's tape across the sand flat. Transects were established to insure that multiple patches of both microtopographic highs and lows were present on each transect. Operationally, microtopographic lows were distinguished from highs by the presence of either standing water or a visible sheen of moisture at low tide. The entire length of each transect was classified as either a microtopographic high or low area, typically resulting in 5-7 patches of each elevation. The first five high areas and

five low areas with sufficient area for the quadrat were selected for sampling. Within each of the 10 patches, two 1-m² quadrats were sampled at a randomly determined point within the patch by sequentially placing the quadrat frame to the right and left of the transect line ($n = 10 \text{ quadrats} * 2 \text{ microtopographic classes} * 3 \text{ transects} = 60 \text{ per sample date}$). Locations were re-randomized on each sample date. When patch extent was asymmetric to the transect, both quadrats were taken from one side. Disturbance from sampling both to the wider study site and to the areas within quadrats was minimal. Firm, sandy substrates coupled with rapid tidal currents meant that any surface disturbance had largely disappeared in 24 hours.

The sample frame was gridded with nylon line into twenty-five 20 × 20 cm squares. Percent coverage of macroalgal cover was estimated by counting the number of squares with greater than 50% coverage of algae. Algal cover was then moved if necessary to count and measure seagrass plants. The macroalgal cover was predominately (>99%) composed of multiple species of *Ulva*, both sheet and more filamentous forms, which initially attached to larger sediment particles but subsequently formed loosely attached mats (D. Young, U.S. EPA unpublished data).

Measurements made within each transect quadrat were: total number of *Z. marina* plants, total number of shoots, total length of the longest shoot (in mm, measured from sediment surface) from up to 5 plants per quadrat spread across the quadrat. As plants matured after the initial production of a single shoot from seed, additional lateral shoots were produced in some plants. Due to plant establishment near the sediment surface, attachment of multiple shoots to a plant could be readily discerned during census. The sediment surface was used as a reference point since the plants grew from seeds and did not form rhizomes. There was no evidence that presence of *Zostera* plants caused either deposition or erosion of the sediment surface in the study area, particularly as macroalgal cover increased, and any such variation was likely a small source of variance in the study.

The first visible seedlings of *Z. marina* within the study area emerged from sediments during March (see also Boese and Robbins, 2008), and thereafter the sampling of transects took place on 5 occasions: April 21-24, May 22-24, July 11-24, August 14-15, and October 11, 2000. By October, the annual plants were senescent and disappearing from the flat. Transects were also sampled in 2001 on 5 occasions, May 24, June 21, July 19, August 13, and October 20. However, *Z. marina* plants were so scarce in 2001 as to render data analysis for many parameters unfeasible. July, which represents a reasonable mid-point in the population cycle for the annual plants, was selected as a period to compare the transect data for number of plants and algal cover for the two years.

2.2. Fixed quadrats

Two square quadrats, 50 cm on a side, were established in patches representing either a topographic high or low near each of the three transects, with corners marked with short sections of plastic coated garden stakes. Quadrats were nonrandom, and selected to insure presence of sufficient *Zostera* plants such that individual plants could be tracked over time. These fixed quadrats were sampled using a 50 × 50 cm frame gridded with nylon twine into twenty-five 10 × 10 cm squares. The location of each seagrass plant in the quadrat was mapped to a grid in a field notebook so that survivorship of individuals could be followed.

Grid mapping was selected in preference to photography because the presence of drift algae obscured seagrass and manual searching of quadrats was often required. As shoot size increased and reproductive structures began forming, from June-October, shoot lengths were separated into stem length and leaf length, presence/absence of reproductive structures (spathes) was recorded for all shoots, and length of spathes was measured. However, all shoot length measurements presented here are total length to be compatible with the transect measurements. Measurements and methods were otherwise the same as for the transect quadrats. The sampling of quadrats took place at approximately monthly intervals on six occasions from April through October, 2000.

Relative elevation measurements were obtained for each fixed quadrat and along each transect using a stadia (leveling) rod and transit on June 1, 2000, as sampling predated ready availability of techniques such as differential GPS and LIDAR. The transit tripod was placed immediately adjacent to the study area near the middle transect, the transit was leveled, and reading were taken from the rod (in decimal feet, to 0.01 ft) placed within high and low topographic areas. Readings were converted to cm.- Bathymetric measurements obtained in 2002 in the area of the sand flat in the study found an elevation of approximately + 1.2 m relative to Mean Lower Low Water (MLLW).

2.3 Supplemental sampling

In order to obtain detailed information on morphometrics associated with annual plants that were developing seeds, random sampling was conducted on August 22, 2000 to obtain plants from elevational microhabitats on the sand flat. As with transect and quadrats, high microtopographic areas had no standing water. The low microtopography category was split to determine if the depth of ponded water had any influence on morphometrics of annual *Z. marina*, such that low microtopographic areas were characterized by >1 cm water depth, while intermediate microtopographic areas had water <1 cm with deeper water only in small depressions. Totals of 11, 10, and 10 plants (containing multiple shoots) were collected from low, intermediate, and high microhabitats, respectively. Data on maximum shoot length, number of shoots, number of spathes, biomass of shoots, roots, spathes, and stage of spathe development (de Cock, 1980) were obtained.

2.4 Statistical analysis

Statistical analysis of the transect data consisted of Two-way Analysis of Variance (ANOVA), with factors Microtopographic Class (a=2) and Sample Month (b=5), with n=30 replicates in each combination of Class and Month. Significant differences among treatment levels were tested with the All Pairwise Multiple Comparison Procedure (Tukey test). Transect was not analyzed as an additional random factor since multiple transects were used only because the limited extent of the annual *Zostera* bed required multiple transects in order to obtain adequate sample size for detection of microtopographic differences. Correlation between number of plants and algal cover were computed from the transect data for comparison of mean values for July of 2000 and 2001, and for all sample data from all sample dates in both years, using the Pearson Product Moment Correlation.

Data from the fixed quadrats were analyzed using Two-way Repeated Measures ANOVA, with the factors Microtopographic Class ($a=2$) and Sample Month ($b=6$), with $n=3$ quadrats within each combination of Class and Month, with repeated measures within the factor Month. Morphometric comparisons for data collected from the random samples were analyzed using One-way ANOVA, using the factor Microtopographic Class ($a=3$) with $n=11$ replicate plants for the low areas, and $n=10$ plants for the intermediate and high areas. Multiple comparisons were conducted with the Holm-Sidak method. Where assumptions of ANOVA were not met, transformations were applied to the data. The arcsin square root transformation was applied to metrics expressed as percentages. Mortality rates for *Z. marina* plants were estimated by linear regression of number of plants versus Julian day. Regression equations for mortality rates in high and low microtopographic areas were compared with Analysis of Covariance (ANCOVA), with multiple comparisons conducted with the Holm-Sidak method. All statistical analyses were performed with Sigmaplot 13 software.

3.0 Results

The mean elevation differences between the microtopographic highs and lows were highly significant for both the transects (difference = 4.4 cm; t-test, $P<0.0001$; mean (s.d.) relative elevations, High = 129.92 (1.58) cm, Low = 125.50 (2.11) cm) and the fixed quadrats (3.2 cm, t-test, $P<0.01$; mean (s.d.) relative elevations, High = 127.76 (1.18) cm, Low = 124.56 (0.84) cm) on the sampling date.

3.1 Transect results

For the analysis of mean number of plants m^{-2} , there was a significant interaction term between elevation and month, and low areas had significantly greater number of plants than high areas from July through October, but not earlier in the summer (Fig. 1A, see Supplementary Material Statistical Tables for all ANOVA tables and results of multiple comparison tests). Mean number of shoots m^{-2} was significantly greater in the low compared to high areas, while neither the month nor interaction term were significant (Fig. 1B). Mean number of plants m^{-2} was a maximum in May and mean number of shoots m^{-2} was a maximum in July, and both declined subsequently (Figs. 1A, B). Plant mortality rates for the period May through October, although slightly greater in high as compared to low microtopographic areas, were not significantly different between the two areas (Fig. 2A; Supplementary Material Statistical Results - ANCOVA table).

There was a significant interaction term between elevation and month for maximum shoot length, and low areas had significantly longer shoots than high areas from July through October, but not earlier in the summer (Fig. 1C). Mean maximum shoot length peaked in both microtopographic areas in August. The proportion of algal cover was significantly greater in high compared to low areas, and differed significantly among months, with all months significantly different from each other except April and May. The interaction term was not significant. Algal cover increased in both areas over the entire period of study (Fig. 1D), although cover in low areas lagged several months behind high areas until August, at which point it was similar.

3.2 Quadrat results

The fixed quadrats generally confirmed the patterns observed with the transect samples. There were significant differences over time in the mean number of plants m^{-2} (Fig. 3A), but there was no statistically significant difference for elevation and there was no significant interaction (see Supplementary Material Statistical Tables for all ANOVA tables and results of multiple comparison tests). However, in contrast to the Transect data, plant mortality rates in the fixed quadrats for the period April through October, where individual plants were tracked, were significantly greater in the high microtopographic areas (Fig. 2B; Supplementary Material Statistical Results - ANCOVA table). As a result, while number of plants was initially relatively similar, it was reduced by two thirds in the high quadrats by October (Fig. 3A).

For mean number of shoots m^{-2} (Fig. 3B) there was a significant interaction between elevation and month, with low areas having significantly greater number of shoots than high areas from August through October, but not earlier in the summer. Number of shoots per plant was initially similar for the first two months, then more than doubled for plants within low areas while slightly decreasing over time within high areas (Fig. 3B).

There was also a significant interaction between elevation and month for maximum shoot length, with low areas having significantly longer shoots than high areas from August through October, but not earlier in the summer (Fig. 3C). Shoot length increased in both areas from April through July, then changed little within high areas while continuing to increase in low areas (Fig. 3C). Again, there was a significant interaction between elevation and month for the proportion of algal cover, which was significantly greater in high as compared to low areas in May through August, but not other months (Fig. 3D). Algal coverage increased in both microtopographic areas over the summer, but accumulation was more rapid in high areas, with low areas lagging accumulations in high areas from May through August. The response pattern for number of reproductive shoots was similar to that for number of shoots, with a significant interaction between elevation and month, with low areas having significantly greater number of reproductive shoots than high areas from August through October, but not earlier in the summer (Supplementary Figures, Fig. 1).

3.3 Random samples, morphometric comparison

The total biomass of individual plants collected from microtopographic low areas on the sand flat were significantly larger than that for both intermediate ($p < 0.001$) and high ($p < 0.003$) areas, which did not differ from each other (Table 1, see Supplementary Material Statistical Tables for all ANOVA tables and results of multiple comparison tests). ANOVA results for spathe biomass ($p < 0.001$), g spathe⁻¹ ($p < 0.001$), g shoot⁻¹ ($p = 0.001$), and maximum shoot length ($p < 0.001$) all had similar results to total biomass, with low areas having significantly greater values than either intermediate or high areas, which did not differ from each other (Table 1). Number of shoots per plant ($\log_{10}$ transformed) were significantly different among groups ($p = 0.036$), with low areas greater than high areas. For number of spathes, both low and intermediate areas had significantly greater number of spathes than did high areas ($p < 0.001$). There was a significantly higher percentage of pollinated spathes ($p < 0.001$) in the low area compared to both the intermediate and high

areas. Because there were high correlations between number of spathes and numbers of spathes in various stages of development (e.g. flowering, mature seeds, senescent), statistical comparisons among elevations followed the same pattern as for percentage of pollinated spathes, and statistical comparisons are not provided here. There was a highly significant positive, curvilinear relationship of number of spathes to total biomass ($y = 110.1 * (1 - e^{-0.373x})$); $r^2 = 0.84$, $p < 0.0001$; Supplementary Figures, Fig. 2).

3.4 Between year comparison

Both mean percentage algal cover and number of plants were compared for July transect samples in 2000 versus 2001. July was selected because differences in algal cover narrowed after this month as algae continued to accumulate across the sand flat. Percentage algal cover in high areas was significantly greater than for low areas in both years, and algal cover was significantly higher in 2001 than in 2000 (Fig. 4A; Two-way ANOVA, Supplementary Material Statistical Results). Conversely, mean number of *Z. marina* plants was significantly greater in 2000 than in 2001, and greater in low than in high areas (Fig. 4B; Two-way ANOVA, Supplementary Material Statistical Results).

3.5 Plant loss rates and algal cover

The relationship of plant number to macroalgal cover was examined by combining data for all dates sampled in both 2000 and 2001, excluding October, 2001, during which both parameters were near zero. There were significant negative linear regressions of mean number of plants m^{-2} versus mean percentage algal cover for both low and high microtopographic areas (Fig. 5). The regression slopes of high and low microtopographic areas were significantly different (Supplementary Material Statistical Results - ANCOVA table). The greatly lowered abundance of plants in low areas in 2001 that was associated with the presence of high algal cover from the very start of seedling recruitment (Section 3.4) generated a much steeper plant loss rate in low areas in the two-year data set compared to that seen in the 2000 data alone (Fig. 2A).

4.0 Discussion

Microtopographic relief resulting from water movement across intertidal sandflats and subtidal sand bottoms has been found to influence the composition and abundance of components of the benthic infaunal community (Hogue and Miller, 1981; Fenwick, 1984; Grant, 1984). For seagrasses, previous studies have suggested a relationship between distribution of intertidal seagrasses and microtopographic features (Harrison, 1982; Shafer, 2007; Hannam and Wyllie-Echeverria, 2015; Hannam and Moskal, 2015). More recent experimental manipulations have explored consequences of sediment microtopography to multiple seagrass species. Bos et al. (2007) transplanted seedlings of annual *Z. marina* and demonstrated that the seagrass caused sediment accretion of from 0.4 - 0.7 cm over the 3-month period of observation at a site in the Wadden Sea, Netherlands. Also from the Wadden Sea, Van der Heide et al. (2012) determined that a localized topographic mosaic of *Zostera noltii* was the result of the interaction of sediment accretion forming elevated areas (~ 6 cm) with persistent seagrass while hollows that retained water were heavily grazed by birds, resulting in bare areas in topographic lows. These studies focused on the effects of

seagrass on microtopography, and did not examine the influence of microtopography on characteristics of the seagrasses. However, relatively recently Hannam and Wyllie-Echeverria (2015) reported on a transplant study of the interactions of native *Z. marina* and introduced *Z. japonica* as influenced by microtopographic variation of intertidal habitat in Padilla Bay, WA. Over the 14-month study period, microtopographic highs, had 40% fewer shoots and 95% lower biomass than the shoots placed in pools in topographic lows (Hannam and Wyllie-Echeverria, 2015).

For the annual form of *Z. marina* studied here, plants in topographic lows had significantly higher plant abundance, higher number of shoots per plant, longer shoots, greater total aboveground biomass, greater biomass per shoot, greater number and biomass of reproductive spathes, and a higher percentage of pollinated shoots. The total number of spathes produced was predicted by the biomass of *Z. marina*, regardless of the microtopographic location. The extent of difference in these plant parameters is rather surprising given that the average vertical difference in elevations measured was only on the order of 3-4 cm. However, a similar response was seen for transplanted, perennial *Z. marina* plants, which had fewer branches, shorter rhizomes, and shorter, narrower leaves on microtopographic highs compared to lows, where maximum elevation differences were on the order of 10 cm (Hannam and Wyllie-Echeverria, 2015). Which potential microhabitat difference (e.g. desiccation, temperature, degree of algal shading) leads to the morphological differences is not known. In the laboratory, Morita et al. (2010) have shown that seeds of the annual form *Zostera marina* exposed to cold can produce differences in morphology versus unexposed seeds, but it is unlikely seeds in the field in the study area would experience temperature differentials of the experimental magnitude used (14-18 °C).

Given the variation in seedling survival, growth and reproductive output (Van Katwijk and Wijgengangs, 2004; Hannam and Wyllie-Echeverria, 2015: this study) associated with microtopographic differences, selective rather than random seedling placement during seedling based restoration would appear to increase the chances of success. Orth et al. (2006) have encouraged the use of seed-based seagrass restoration, and the greatly increased seed production of annual *Z. marina* relative to the perennial form (Kim et al., 2014) would appear to offer an attractive source for seed harvest. Van Katwijk and Wijgengangs (2004) demonstrated that seed source may affect the survival rate of annual form *Z. marina* seedlings, and Jarvis and Moore (2015) found differences in germination times and better success of perennial seeds compared to those from a mixed-annual population. Better understanding of the multiple factors affecting *Z. marina* seed success, including microtopographic variation in seed source, appear to still be required.

In general, the upper intertidal zone appears to be a marginal habitat for *Z. marina*. Plants that occupy the zone tend to have an annual strategy, tend not to form extensive rhizomes, and instead use available energy to produce large numbers of reproductive shoots and seeds (Keddy and Patriquin, 1978; Silberhorn et al., 1983, Keddy, 1987; Boese et al., 2005; Jarvis and Moore, 2015). Boese and Robbins (2008) found that above +1 m MLLW in the Yaquina estuary, *Z. marina* is reestablished annually from seedlings recruited from the seed bank, and Boese et al. (2005) proposed that desiccation was a primary factor setting the upper limit of *Z. marina* in the intertidal of the Pacific Northwest. Within this intertidal zone, one possible

effect of microtopographic differences may be through varied desiccation stress, which may be greater in the microtopographic high areas that do not retain water during low tides. However, evidence for the role of desiccation is mixed. Kentula and McIntire (1986) found that sand deposition at the edges of intertidal seagrass beds created shallow basins that retained water, and that perennial *Z. marina* growing in these tide flat depressions was in better condition than plants that were completely exposed. However, Shafer et al. (2007) found that the leaf-scale response of photosynthesis to desiccation was insufficient to explain distribution patterns of *Z. marina* in the region, and suggested that a more complex, whole plant response was required. Van Katwijk and Wijgergangs (2004) experimentally determined that the presence of a permanent layer of water at a site significantly increased the number of shoots produced by annual *Z. marina* seedlings, a result consistent with those from the present study. However, there was no evidence for a desiccation effect in an experimental transplant of perennial *Z. marina* shoots to microtopographic highs, since shoot density was stable over the initial 2-month summer period (June -August; Hannam and Wyllie-Echeverria, 2015). Whether annual and perennial forms of *Z. marina* have differential responses to desiccation is not known.

We suggest that an equally important factor affecting microtopographic distribution is the extent and timing of macroalgal cover experienced by *Z. marina*. Dense macroalgal cover may negatively impact seagrass by shading, alteration of the chemical environment through decreased oxygen, increased ammonium and sediment sulfides toxic to seagrass, and by physical damage and removal (van Katwijk et al., 1997; Hauxwell et al., 2001; Boese and Robbins, 2008; Huntington and Boyer, 2008; Olyarnik and Stachowitz, 2014; Hessing-Lewis et al., 2015). Valdemarsen et al. (2010) have also shown that drifting algal mats can result in considerable (40%) mortality to *Z. marina* seedlings due to physical disturbance. Rasmussen et al. (2012) experimentally demonstrated that algal mats can reduce the survival of *Z. marina* seedlings, primarily via shading rather than due to chemical changes under the mat. However, Dooley et al. (2013) used laboratory exposures and found that *Z. marina* seedlings were killed at hydrogen sulfide concentrations found at some field sites where loss of *Zostera* without recolonization had occurred. Thus, macroalgal mats may interact with annual *Zostera* plants in multiple ways at multiple life stages.

Dense macroalgal cover appeared to inhibit *Z. marina* seedlings in the study area from producing first leaves (Supplementary Material Fig. 3), and thus prevented the seedlings from establishing and growing in the first place. This mechanism is consistent with the observation of 3× greater seedling density in microtopographic lows together with a 14× greater algal coverage in high areas from Transects during April 2000. Also consistent is the observation that the dense macroalgal mats that became established in late summer 2000 persisted through the winter into 2001, such that algal coverage was much higher throughout the study area in May 2001 (99% high, 97% low) as compared to May 2000 (4.5% high, 0% low). Associated with the increased algal coverage were sharply reduced numbers of young plants (May 2000, 5.73 high, 9.97 low; May 2001, 0.3 high, 2.9 low). However, alternate hypotheses cannot be totally excluded. Microtopographic variation in seed density in the sediment was not measured, and a physical sorting of seeds affecting relative initial seedling densities is conceivable. Seeds of *Halodule uninervis* have been shown to accumulate in troughs of small sand waves (Inglis, 2000; Orth et al., 2007). Alternately, a difference in

seed germination rates due to desiccation might occur, although Van Katwijk and Wijgergangs (2004) found that the presence of permanent water in depressions on a tide flat had no effect on percentage of either seed germination or seed survival, suggesting that desiccation stress may not be a principal factor in initial seedling success.

Following seedling establishment, coverage of macroalgal mats tended to increase more rapidly in microtopographic high areas, but was eventually matched by that in lower areas. Macroalgal mats also decreased plant numbers in both microtopographic areas. From the transect measurements in 2000 which give the best estimate across the annual bed as a whole, plant loss rates were relatively similar in both topographic high and low areas (Fig. 2A). For the fixed quadrats which were established with similar initial plant densities (Fig. 2B), the loss rate was 3× greater in microtopographic high areas than in low areas, which is consistent with the much more rapid buildup of macroalgae in these quadrats (Fig. 2D.). The association of plant loss with algal cover is further emphasized by the data combined over 2 years (Fig. 5), which shows significant negative relationships in both high and low microtopographic areas. Above 80% macroalgal cover, there was little difference in the mean number of plants between microtopographic areas. By July, 2000 in the present study, blackened sediments and the smell of hydrogen sulfide were observed, so chemical toxicity may have contributed to mortality as well shading and physical disturbance effects. These observations on annual plants are consistent with those of Boese and Robbins (2008), that decreases of perennial shoot densities also corresponded to increased accumulations of macroalgae in the Yaquina estuary.

While impacts of desiccation may play some role in determining the influence of microtopographic variation on the annual *Z. marina* population in the present study, macroalgal effects appear to be the dominant factor. As macroalgal cover increased, moisture would be trapped within and under the algal mats, which would tend to diminish the effects of desiccation on seagrass shoots within the mats, but this would be counteracted by increasing shading and chemical impacts. In the Yaquina estuary, the main impact of desiccation on *Z. marina* distribution in the upper intertidal appears to be through acute desiccation events (Boese et al., 2005; Boese and Robbins, 2008) rather than chronic effects. However, there was no indication from the data in the present study that such an acute desiccation event occurred. Thus, it appears that microtopographic relief can strongly interact with cover of algal mats to determine overall recruitment and survival of annual plants of *Z. marina* at the patch scale. As future summer air and water temperatures in the region continue to rise (Mote et al., 2014), stress associated with algal mats may increase, particularly via sulfide produced through algal decomposition. Occurrence of annual *Z. marina* in the upper intertidal habitat in estuaries of the U.S. west coast may become increasingly limited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Comparison (mean + s.d.) of microtopographic high and low areas for annual *Zostera marina* and algal cover measured along transects. A - mean number of plants m^{-2} , B – mean number of shoots m^{-2} , C – mean shoot length (mm), D – mean percent algal cover. * significant difference in elevations within months where the interaction is significant.

Figure 2.

Mean abundance over time for annual *Zostera marina* plants from high and low microtopographic areas from A) transects and B) fixed quadrats. Mortality rates are estimated by the slopes of the regression equations as decrease in number of plants d⁻¹. Error bars = ± 1 SE; regression analyses provided in Supplementary Statistical Results.

Figure 3.

Comparison (mean + s.d.) of microtopographic high and low areas for annual *Zostera marina* and algal cover measured from fixed 0.25 m² quadrats. A - mean number of plants 0.25 m², B – mean number of shoots 0.25 m², C – mean shoot length (mm), D – mean percent algal cover (p values from analysis of arcsin square root transformed data). * significant difference in elevations within months where the interaction is significant.

Figure 4.

Comparison of mean (+ s.d.) A) percentage algal cover and B) number of annual *Zostera marina* plants m^{-2} in high and low microtopographic areas between July 2000 and July 2001.

* significant difference between elevations, $p < 0.005$; ** significant difference between years, $p < 0.001$. ANOVA tables in Supplementary Material.

Figure 5.

Comparison of regression relationships in high and low microtopographic areas of mean monthly plant number versus mean monthly percentage algal cover for 2000 and 2001, excluding October 2001 for which both variables were zero. Error bars = ± 1 SE for both x and y variates; regression analyses provided in Supplementary Statistical Results.

Table 1

Comparisons of morphometric measurements (mean, s.d., 0.25 m⁻²) for annual *Zostera marina* obtained from random samples taken within low, intermediate and high microtopographic areas from randomly placed quadrat samples on August 20, 2000. All biomass as g DW.

Metric	Mean Low	S.D. Low	Mean Intermediate	S.D. Intermediate	Mean High	S.D. High
Shoot Biomass	2.61	1.68	0.89	0.60	0.27	0.37
Spathe Biomass	1.27	0.86	0.48	0.36	0.16	0.28
Root Biomass	0.31	0.29	0.12	0.08	0.04	0.04
Total Biomass	4.19	2.65	1.50	1.00	0.48	0.68
Root:Shoot Biomass Ratio	0.09	0.07	0.10	0.04	0.23	0.29
Plant Length (mm)	698	170	491	113	389	118
No. of Shoots	5.09	3.91	3.50	1.65	2.30	1.42
g shoot ⁻¹	0.64	0.48	0.26	0.14	0.11	0.11
No. of Spathes	73.45	31.63	45.60	26.20	18.60	26.87
g spathe ⁻¹	0.02	0.01	0.01	0.00	0.01	0.00
Pollinated Spathes	39.45	19.25	27.20	19.55	10.40	16.20