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The Influence of Environmental Factors and Resource Availability on *Zostera marina* Flowering Intensity

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The Influence of Environmental Factors and Resource Availability on *Zostera marina* Flowering Intensity

A Thesis Presented to

The Faculty of the School of Marine Science

The College of William and Mary

In Partial Fulfillment of

The Requirements for the Degree of

Master of Science

By

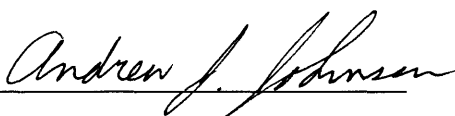
Andrew J. Johnson

2015

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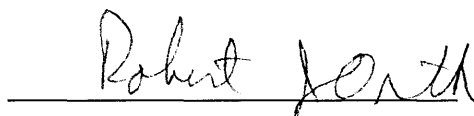
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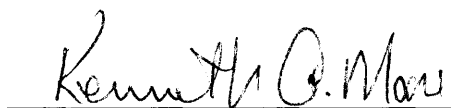


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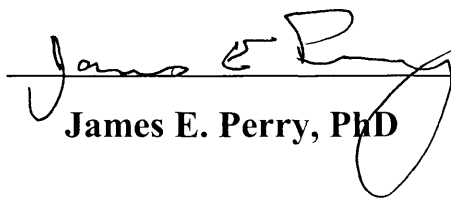
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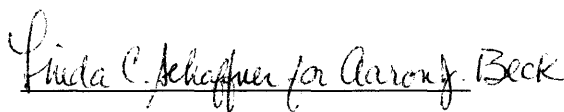
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Abstract

Sexual reproduction and the production of seeds are important for the resilience of all angiosperm species. For clonal species, such as the seagrass *Zostera marina*, resource allocation is complicated because these species reproduce both asexually and sexually, and the factors contributing to allocation to these two processes remains unknown. The goal of this study was, therefore, to investigate the importance of critical light, nutrient, and rhizome resources on *Z. marina* sexual reproduction and flowering intensity.

To evaluate the importance of sediment nutrients on *Z. marina* flowering intensity two distinct field manipulative experiments and one field survey were initiated: 1. Sediments within established *Z. marina* were fertilized during two periods of active growth (spring and fall) at two contrasting (estuarine and coastal lagoon) locations and the subsequent development of flowering was recorded, 2. Rhizome segments were transplanted to adjacent unvegetated environments to investigate the effects of sediment nutrients on individual shoots, 3. Surveys within and between three locations with apparent differences in sediment structure were designed to investigate the relationship between sediment conditions and flowering intensity. Rhizome segments were also transplanted to adjacent unvegetated environments to test the effect of shoot availability (with each segment having one, two, or three shoots) on sexual reproduction. To evaluate the importance of light and rhizome resources to flowering, two experiments and one field survey were also initiated: 1. In situ light availability was reduced using neutral density shading to test if light availability affected flowering intensity, 2. A field survey using fixed piers as shade structures was also used to determine if long-term shading influenced *Z. marina* flowering, 3. Cutting of rhizome connections of in situ plants was used to test if acute stress to belowground tissue prior to the development of flowering shoots would influence investment in sexual reproduction.

The addition of supplemental nutrients to the sediment during the fall growth period increased the number of spathes per flowering shoot the following spring relative to control plots at both estuarine and coastal lagoon locations. Similarly, field surveys across three locations demonstrated a direct relationship between ammonium availability and the percentage of flowering shoots. Although short term in situ shading did not significantly affect flowering intensity, measurements of flowering intensity around piers revealed lower percentages of flowering shoots directly beneath piers than areas one or three meters perpendicular to the pier. Eleven percent of transplants also produced more flowering shoots than the initial shoots planted indicating the development of flowering shoots can occur on shoots less than three months old.

Combined, these results indicate resource availability can influence sexual reproduction. Increasing belowground plant and nutrient resources increased investment in both vegetative and sexual reproduction, whereas, long term reductions in light resources were found to only decrease sexual reproduction. This suggests that the duration and magnitude of resource availability are both important in determining the

allocation of resources towards sexual reproduction, and through this investment the degree of resiliency in seagrass populations in an increasingly stressed coastal environment.

**The Influence of Environmental Factors and Resource Availability on
Zostera marina Flowering Intensity**

Introduction

Plants allocate resources towards major functions, including growth, maintenance, defense, and reproduction (Harper, 1967). All plant resources are divided between these mutually exclusive functions (Bazzaz et al., 2000), and the balance between reproduction (increased fecundity) and growth or maintenance (increased life span) can have direct impacts on fitness (Bazzaz et al., 1987). In clonal species, this allocation is further complicated as species can either invest in either sexual or asexual reproduction. Competing theories argue sexual reproduction will vary depending on the physiological or environmental state of the individual (Loehle, 1987; Sakai, 1995; Gardner and Mangel, 1999). Environmental stress and disturbance have been cited in several cases as cause for increased allocation for sexual reproduction. For example, osmotic stress (Van Zandt et al, 2003), water limitation (Abrahamson, 1975), excessive flow (Puijalon et al., 2008), and nutrient limitation (Short, 1983) are hypothesized to increase sexual reproduction in various terrestrial and aquatic plant species. Conversely, nutrient levels (Van Lent et al., 1995; Kettenring et al., 2011; Burkle & Irwin, 2009) and critical sizes (Weppeler and Stocklin, 2005; Aarsen et al., 1992) have also been found to increase investment in sexual reproduction.

Historical studies of seagrass distribution and reproduction have largely focused on vegetative growth and clonal reproduction. Increasingly the role of sexual reproduction and seed dispersal processes have emerged as important regulators of seagrass distributions and survival (Orth et al., 2006; Kendrick et al., 2012). With seeds, *Zostera marina* has the potential to colonize remote habitat hundreds of kilometers from the donor bed (Harwell et al., 2002; Källström et al., 2008). This dispersal is apparent in

genetic analyses demonstrating connectivity between spatially distant beds (Olsen et al., 2004; Reusch, 2002; Reynolds et al., 2013). Other studies have indicated the potential for seeds to enhance re-colonization of disturbed areas and thus aid in the resilience and recovery of existing beds (Inglis, 2000). Reusch et al. (2005) described increased resilience of genetically diverse *Z. marina* plots to elevated temperature stresses compared to monogenetic plots. These findings display the potential importance of sexual reproduction to seagrass population stability and expansion.

Zostera marina is a clonal, marine angiosperm with a wide range throughout the Northern Hemisphere (den Hartog, 2006). Flowering shoot production results in meristematic death for *Z. marina*. Thus, sexual reproduction in *Z. marina* shunts resources towards flowering and fruiting structures and constricts future clonal expansion (Hemminga and Duarte, 2000). In a survey of reproductive intensity along the Pacific coast of the United States, Phillips (1983) found higher flowering rates in populations of *Z. marina* at the boundary of the species' distribution than in *Z. marina* populations more central within its distribution. Across *Z. marina*'s geographic range this variable investment in sexual reproduction can be so profound two *Z. marina* life history strategies have been classified entirely upon flowering shoot production and the persistence of vegetative biomass (Keddy and Patriquin, 1978). This variation across geographic space suggests environmental and physical factors, such as temperature or nutrients, may increase or decrease flowering intensity in *Z. marina* as well as other seagrasses (Diaz-Almela et al., 2007; Short, 1983).

Z. marina growth in the Chesapeake Region follows a seasonal cycle: with periods of active growth during the spring and fall and times of senescence in the winter and summer periods due to cold (<13.2°C) or extreme warm (>22.7°C) water temperatures (Orth and Moore, 1986; Moore, 1992). Recent findings suggest chronic light stress, in combination with other factors like temperature, drives the health of *Z. marina* in the Chesapeake Bay (Moore and Jarvis, 2008). Critical nutrient resources have also been shown to influence *Z. marina* biomass and vegetative shoot number within Chesapeake Bay (Orth, 1977) and the Netherlands (Van lent, 1995). Interestingly, interstitial ammonium concentration has also been inversely correlated to vegetative and flowering shoot density (Short, 1983). Therefore a study of the effect of these essential resources during growth periods directly before flowering is potentially important for understanding investment in critical life history pathways for *Z. marina*.

The goal of this study was, therefore, to investigate the importance of critical light, nutrient, and rhizome resources on *Zostera marina* sexual reproduction and flowering intensity. Two experiments and one field survey were conducted to evaluate the importance of sediment nutrients to flowering: 1. Sediments with established *Z. marina* were fertilized during two periods of active growth (spring and fall) at two contrasting (estuarine and coastal lagoon) locations; 2. Individual shoots with attached rhizome segments were transplanted to adjacent unvegetated environments, to investigate the effects of sediment nutrients and belowground plant resources on sexual reproduction; 3. Surveys within and between three locations with apparent differences in sediment structure were designed to investigate the relationship between sediment

conditions and flowering intensity. To evaluate the importance of light and rhizome resources to flowering three experiments and one field survey were initiated: 1. In situ light availability was reduced using neutral density shading to test if light availability influenced flowering intensity; 2. A field survey using fixed piers as shade structures was also used to determine if long-term shading influenced *Z. marina*; 3. Cutting of rhizome connections of in-situ plants was used to test if acute stress to belowground tissue prior to the development of inflorescence would influence investment in sexual reproduction; and 4. Transplants with varying shoot numbers (1,2, or 3) were planted to determine if the number of shoots on a transplanted affected flowering intensity the following spring.

Methods

Study Sites

All manipulations and surveys were conducted in either seagrass meadows at Allens Island (-76.422W, 37.257N), Gloucester Point (-76.506W, 37.249N), or Sandy Point (76°23'53.908"W, 37°15'47.1"N) in the York River Estuary, Virginia, or within South Bay (-75.813W, 37.263N), Virginia, a coastal lagoon on the Delmarva Peninsula (Figure 1).

Manipulation of sediment nutrient availability

To assess the effect of sediment nutrient concentration on flower production *Zostera marina* beds in two locations (Allens Island vs. South Bay) and during two time periods (May and September 2013) were enriched with nutrients.

At each location, a single 100g 15N:3P:3K fertilizer spike was inserted into the sediment inside five 0.02 m² plots in May 2013 and in five additional plots in September 2013. Before placement of the nutrient spike within each fertilized plot, a 7 cm diameter sediment core was taken to a depth of 5 cm directly adjacent to the plot to establish baseline sediment nutrient concentrations. Five additional plots served as no fertilizer controls for a total of 15 plots at each location.

In May 2014, a 15 cm diameter, PVC core was taken from the center of each plot directly over the remnants of the nutrient spike in the sediment. Samples were rinsed with a sieve to removed sediment and placed on ice until frozen for later analysis. The numbers of vegetative and flowering shoots for each sample were subsequently counted. If intact flowering shoots were present, the height and number of spathes per flowering shoot were recorded for each shoot. The number of seedlings was recorded by identifying the root tip. Material was then separated into aboveground (vegetative and flowering material separate) and belowground constituents and dried at 65°C for five days.

Four additional nutrient plots were constructed just outside the manipulated plot areas within the same *Z. marina* meadow at both locations in September 2013 determine the duration and distance over which the fertilizer elevated sediment ammonium concentrations. Each of these plots received the same 100g nutrient spike each of the experimental plots received. Sediment cores were taken within these nutrient treatment plots after two and four weeks at 0, 10, and 20 cm away from the nutrient spike.

The top 5cm of each core was removed and extracted in 160 ml of 2M KCL in pre-weighed bags for one hour on a shaker table. After this period, 50 ml was centrifuged for six minutes at 3500 RPM and then filtered through 25mm syringe filters before being frozen. Frozen samples were then thawed and analyzed for DIN using a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002).

Once thawed, the remainders of the sediment cores were mixed until homogenous and an organic matter sample (at least 50g) was removed and dried for five days at 65°C. This dried sample was then pulverized and a 10-20g subsample was burned at 500°C for five hours. The remainder of the mixed core was refrozen and later thawed for sediment grain size analysis. This procedure was followed for all sediment cores taken unless otherwise specified.

General Survey of Reproductive Intensity along Gradients in Sediment Type

To explore the effect of spatial patterns in sediment characteristics within *Z. marina* beds on investment in sexual reproduction, surveys were conducted in May 2014 along potential sediment gradients at Allens Island and Sandy Point within the York River and within South Bay. At both locations in the York River, a “sandy” area was identified from which a 105 meter transect was run into a “muddy” area. These qualitative classifications at both sites were utilized to maximize the potential sediment differences within each location in addition to differences across all three locations. Every 15 meters two 1-m² quadrats were placed on either side of the transect tape. Within this 2m² area, three ring counts (0.03m²), three cores for vegetative and reproductive characteristics (0.02m²), and three sediment cores (20 cm², 5cm depth) were taken. In addition, three, 15 ml sediment pore water samples were collected from the center of each ring count to a depth of 7 cm.

Cores, sediment cores, and pore water samples were placed into labelled bags on ice and then frozen until analyzed. Because *Z. marina* grows extensively over larger distances in South Bay and sediment characteristics over these distances are more

difficult to discretely define the transect design highlighted above was not suitable to capture potential differences in sediment characteristics within the expansive *Z. marina* meadow. Instead, an initial qualitative exploration of sediment structure throughout the meadow was used to identify a “sandy” location and a “muddy” location. Within both of these locations, four random sites were sampled within ~50m² of each other with the same 2m² quadrats design described above. Only four sediment cores were collected per site, however, because sediment structure appeared more consistent at sites in South Bay than at the transect sites within the York River.

Transplant Study

To investigate the effect of sediment nutrients on the allocation pathways of a single meristem, segments of *Z. marina* shoots with attached rhizome lengths of at least 5 cm were removed from populations at Allen’s Island in the York River and were transplanted in October 2013 in areas historically vegetated upstream and downstream of Gloucester Point, VA. Both of these locations historically maintained *Z. marina* populations which are hypothesized to have declined due to decreasing water quality and high summer water temperatures (Moore and Jarvis, 2008; Moore et al., 2012). Because these transplants were planted in historically vegetated but currently bare sediment in the fall of 2013, the fate of individual meristems could be tracked through the fall and winter until flowering in spring 2014 without jeopardizing their survival under elevated summer water temperatures. At each location, 24 single-shoot rhizome segments were planted. Alongside 12 of these transplants, a 100g, 15N:3P:3K nutrient spike was inserted beneath the transplant to test if increased nutrients during a peak growth period influenced sexual reproduction. In addition, 36 rhizome segments with either one, two, or three individual

shoots (n=12), were transplanted to evaluate if meristem availability or the presence of multiple meristems altered resource allocation towards sexual reproduction the following spring. These meristems must also share resources stored within each rhizome segment and thus the shoot number treatments also altered the internal rhizome resources available to each individual shoot on a transplant. Transplants were arranged in 4 m² plots containing every treatment in a known order to aid in recovery and identification of transplants and their treatment and were separated by at least one meter.

In May 2014 each surviving shoot-rhizome transplant was gently excavated, placed into a labelled bag on ice, and frozen until later analysis. Transplants were thawed and analyzed for shoot count, growth, and reproductive effort metrics outlined above (see *Manipulation of Sediment Nutrient Availability*).

Manipulation of Available Light

To test if the amount of available light may alter investment in sexual reproduction, five floating buoys elevated ~15cm above the water surface at the same depth were placed over a *Z. marina* meadow on the northern shore of the York River. These buoys were covered in 30 percent shade cloth to shade an area of ~2.31 m². Thirty percent shade cloth was chosen to lower photosynthetic rates of manipulated individuals without influencing their survival through the manipulation period (Moore et al., 1997). These buoys were placed after water temperatures dropped consistently below 25°C in September 2013 and left floating until May 2014. Buoys were checked and adjusted weekly to ensure shading was as consistent as possible. For one period in January-

February 2014 shade buoys were removed for 10 days to prevent significant structural damage from ice accumulations

The flowering intensity and reproductive effort of shaded areas were compared to neighboring, non-shaded *Z. marina* plots of the same area and depth in May, 2014. Six ring (0.03m²) counts along the periphery of the experimental regions and three core (0.02m²) subsamples within the center of the shaded and unshaded areas were taken from every plot for plant characteristics.

Photosynthetically active radiation (PAR) reaching the experimental plots and control plots was measured three times over a four hour period in July 2014 to directly estimate differences in PAR between the shaded and non-shaded areas in the experiment with a LI-COR[®] LI-1400 data logger. Measurements were taken 17cm from the sediment surface directly beneath two buoys and in an adjacent area exposed to ambient sunlight for use as a control. All PAR measurements were taken from approximately the same depth (<5cm difference).

In situ Light Gradient Study

In the spring of 2014 flowering intensity was surveyed around piers constructed over *Z. marina* meadows on the North shore of the York River near Allens Island. At five North-South facing piers, flowering intensity was recorded along twelve piling intervals. At each piling interval three ring counts recorded the number of flowering and vegetative shoots directly beneath, one meter and three meters perpendicular to each pier. Counts alternated from the east to west side of the pier with each piling interval. This procedure was performed over a similar depth range (<10cm difference between piers). Pier heights

were also recorded at the first piling sampled, the middle of the sampled zone, and at the last piling sampled along each pier extending out from shore. These values were then averaged to form a singular, comparable pier height.

To quantify the degree to which these piers shade *Z. marina* a LI-COR[®] LI-1400 sensor was utilized to measure PAR beneath one of the sampled piers and one meter from the edge of the pier. At both of these locations 12 PAR measurements were recorded 17 cm from the sediment surface every five seconds at noon and two in the afternoon.

Rhizome Disturbance Study

To evaluate the effect of acute stress on sexual reproduction for *Z. marina*, twenty 0.02m² plots were constructed in September 2014 with the same PVC stake design as described for the nutrient enrichment experiments at Allens Island and in South Bay. Ten plots in each location received six uniform shovel stabs (three cuts in two perpendicular directions). Each stab was separated by ~5 cm. Other than these shovel stabs, other construction disturbances, e.g. excessive walking and tearing at standing vegetation, was minimized to the extent possible. Ten additional plots at each location functioned as control plots.

Statistical Analysis

Generalized linear mixed models were used to model experimental responses from all experiments, and to assess differences among treatments within each experiment. Count data, such as counts of vegetative shoots and flowering shoots were expressed as shoots per meter square and then fit to a Poisson distribution. Continuous data, such as biomass and shoot height measurements were fit to a Gaussian distribution.

For the nutrient enrichment plots each respective block was treated as a random factor. In the transplant experiments, the location of the transplants around Gloucester Point was treated as a random factor, as the location of the transplants were not of particular interest but a precaution to ensure sufficient transplants would survive the winter. The particular shade buoy for a given sample was also treated as a random factor as inherent, random differences in the location or construction of the shade buoys existed. Similarly, the particular pier sampled, the piling interval sampled, and the distance of a sample from the pier were also treated as nested, random variables as once again the intent of the experimental design was geared toward testing pier shading overall. In all cases, the intercept but not the slope was allowed to vary by the random factor.

Effective nutrient data were natural log transformed and analyzed with Analysis of Deviance to determine differences between the effective treatment of nutrient enriched plots at Allens Island and South Bay. Kruskal-Wallis Rank Sum Tests were used to evaluate the effective shading level beneath shade buoys and sampled piers relative to unshaded areas.

For the 2014 sediment transect survey, vegetative and flowering shoot count data, pore water ammonium concentration, and sediment organic matter were averaged across the eight sampled areas at each location. These twenty-four areas were then analyzed with generalized linear models to determine the effect of pore water ammonium concentration and/or sediment organic matter on investment in sexual reproduction. Models containing percent sediment organic matter, sediment pore water ammonium concentration, or both were compared using Akaike's information criterion (AIC). If the difference between models (ΔAIC) was <2 the most parsimonious model was selected.

Because ammonium concentrations were an order of magnitude different between the three locations sampled, all concentrations were \log_{10} transformed.

All statistics were performed in R statistical analysis software (R Development Core Team, 2008). A Type I Error rate of 0.05 was established for all statistical tests. Linear mixed effect models were constructed using the *lmer* function and generalized linear mixed effect models were conducted using the *glmer* function from the *lmerTest* and *lme4* packages (Bates et al., 2013). Analysis of deviance with Type III Wald Chi-square values were calculated using the *Anova* function within the *car* package (Fox and Wiesberg, 2011).

Results

Manipulation of Sediment Nutrient Availability

Effective Nutrient Enrichment Assay

Nutrient enrichment significantly elevated sediment ammonium at Allens Island and South Bay ($p=0.01$, $p<0.0001$ respectively) (Figure 2). South Bay plots exhibited higher sediment ammonium ten ($p<0.0001$) and twenty ($p=0.002$) centimeters from the nutrient spike relative to control cores. Sediment ammonium was only significantly enriched at Allens Island within ten centimeters of the nutrient spike ($p=0.01$). Sampling period (two and four weeks from nutrient application) did not drastically affect sediment ammonium levels. Pore water samples collected in May 2014 indicate pore water ammonium concentrations at Allens Island were elevated relative to South Bay.

Shoot Densities

No significant differences in flowering shoot density were observed between locations or any nutrient enrichment treatments. The effect of sediment nutrient enrichment in fall 2013 on the number of vegetative shoots varied significantly with location ($\beta=2.4\pm 1.2$, $p<0.001$; Figure 3). Similarly, the fall nutrient treatment interacted significantly with location on the percentage of flowering shoots per plot ($p=0.07$). Lastly, *Z. marina* collected at Allens Island exhibited elevated vegetative shoot numbers ($\beta=0.77\pm 1.1$, $p=0.001$) relative to samples collected at South Bay.

Reproductive Effort

The number of spathes per flowering shoot were elevated in fall nutrient enriched plots relative to control plots ($\beta=1.3\pm 1.1$, $p=0.008$; Figure 4). Similarly, fall nutrient enrichment significantly increased flowering shoot heights relative to control plots ($\beta=23\pm 6.4$, $p<0.001$). Spathes per flowering shoot and flowering shoot height were not different between spring fertilized and control plots. Allens Island also exhibited marginally more spathes per flowering shoot ($p=0.07$) and significantly larger flowering shoots ($\beta=16.3\pm 7.1$, $p=0.02$) than flowering shoots collected within South Bay. Flowering shoots within plots fertilized in the spring ($\beta=0.21\pm 0.1$, $p=0.5$) and fall ($\beta=0.4\pm 0.9$, $p<0.001$) were significantly different from control plots.

General Survey of Reproductive Intensity along Gradients in Sediment

Shoot Densities

AIC model comparison indicated a model incorporating both sediment pore water ammonium concentrations and sediment organic matter the most appropriate for evaluation of the number of vegetative shoots in an area (Table 1). A significant interaction existed between pore water ammonium concentrations and sediment organic matter on mean vegetative shoot number per sampled area ($\beta=0.41\pm 0.03$, $p<0.001$). This interaction may exist because locations with moderate ammonium concentrations exhibited the lowest sediment organic matter and the highest density of vegetative shoots. Flowering shoot density and percentage models were not clearly distinguishable and as such the most parsimonious model with the lowest AIC score was selected (sediment pore water ammonium alone). The number ($\beta=3.3\pm 0.5$, $p<0.001$) and percentage of

flowering shoots increased ($\beta=0.01\pm 0.003$, $p=0.002$) with mean sediment pore water ammonium across locations (Figure 5). No trends were discernible between the muddy and sandy classifications within each location.

Reproductive Effort

Comparison of models predicting spathes per reproductive shoot demonstrated the strongest relationship between percent sediment organic matter and spathe number of flowering shoots rather than pore water ammonium. Percent sediment organic matter ($\beta=-5.1\pm 1.6$, $p=0.004$) significantly predicted spathe number (Figure 6). AIC scores for models of reproductive shoot height were indistinguishable from one another. Both sediment organic matter ($\beta=-9.9\pm 4.5$, $p=0.04$) and pore water ammonium concentrations ($\beta=0.06\pm 0.02$, $p=0.05$) contributed significantly to their models. For all metrics of sexual reproduction across locations sampled, the estimated linear coefficients of sediment organic matter were negative while those of pore water ammonium were positive.

Transplant Study

Transplant Survival

Transplant survival varied significantly by locations within the York River. Only 36 of 60 total transplants were recovered at the downstream location. Of the 24 nutrient enrichment treatment transplants at this location, only 10 transplants survived until spring sampling. The upstream location exhibited significantly higher transplant survival as 57 of 60 total transplants survived.

Shoot Densities

Neither the nutrient nor shoot number treatment significantly affected either the number or percentage of flowering shoots per transplant. Addition of nutrients significantly increased the number of vegetative shoots per transplant relative to control transplants at the end of the study period ($\beta=3.4\pm 1.1$, $p<0.001$; Figure 8). As expected, the final number of vegetative shoots per transplant was also significantly higher in the three shoot transplants relative to single shoot transplants ($\beta=1.3\pm 1.1$, $p<0.001$; Figure 9). This difference in final vegetative shoot number did not, however, exist between the two-shoot initial transplants and the single-shoot initial transplants.

Across both the nutrient enrichment and initial shoot number treatments the majority of transplants either did not produce flowering shoots (42%) or produced one flowering shoot (38%). However, 20% of all transplants produced more than one flowering shoot. If the expected number of flowering shoots is limited to the number of vegetative shoots initially planted (#of flowering shoots-# of initial shoots planted), 66% of transplants produced fewer flowering shoots than expected, 23% of shoots produced the expected number of flowering shoots, and 11% produced more flowering shoots than expected (Figure 10).

Reproductive Effort

Nutrient enriched transplants exhibited marginally more spathes per flowering shoot ($\beta=1.2\pm 1.1$, $p=0.06$) and heights of flowering shoots ($\beta=8.1\pm 4.3$, $p=0.08$) relative to control transplants (Figure 11). The number of spathes per flowering shoot, the

height of flowering shoots, and flowering shoot biomass did not significantly differ between the shoot number and control treatments.

Manipulation of Available Light

Effective Treatment Survey

The shade buoys maintained significantly lower PAR levels in shaded regions relative to adjacent unshaded regions ($\beta=-178\pm 18$, $p < 0.001$; Figure 12). Mean PAR levels recorded beneath shade buoys indicate $\sim 388 \pm 14$ $\mu\text{mols photons m}^{-2}\text{s}^{-1}$ reached experimental plots relative to $\sim 572 \pm 24$ $\mu\text{mols photons m}^{-2}\text{s}^{-1}$ within control plots. Over the limited time period sampled with the LI-COR, shade buoys reduced 30-40% of PAR reaching the *Z. marina* canopy relative to unshaded areas.

Shoot Densities

Shading lowered the number of vegetative shoots per square meter relative to adjacent unshaded areas ($\beta=0.85\pm 1.1$, $p=0.02$) but did not significantly impact either the number of flowering shoots ($p=0.24$) or the percentage of flowering shoots ($p=0.8$; Figure 13)).

Reproductive Effort

The number of spathes per flowering shoot ($p=0.3$) and height of flowering shoots ($p=0.1$) from shaded plots was not significantly different from flowering shoots from unshaded areas.

In situ Light Gradient Study

Pier Light Effective Treatment

PAR measured beneath the piers was significantly lower than PAR measured in the open *Z. marina* meadow ($p < 0.001$). Overall PAR was reduced ~30% in measurements beneath piers than measurements taken in an open *Z. marina* meadow (Figure 14).

Vegetative Shoots

Vegetative shoot densities were significantly lower directly underneath sampled piers ($\beta = 0.90 \pm 1.0$, $p = 0.02$; Figure 15) and in areas one meter outside the piers ($\beta = 0.91 \pm 1.0$, $p = 0.04$) relative to areas three meters from sampled piers.

Flowering Shoots

Flowering shoot densities were significantly lower in counts taken directly beneath sampled piers ($\beta = 0.65 \pm 1.1$, $p < 0.001$) than three meters outside sampled piers. This difference in flowering shoot density was not apparent one meter from sampled piers ($p = 0.7$). The percentage of shoots one ($\beta = 0.8 \pm 0.05$, $p < 0.001$) and three meters from the pier ($\beta = 0.5 \pm 0.05$, $p = 0.003$) was also significantly higher than the percentage of shoots flowering beneath sampled piers (Figure 16).

Rhizome Disturbance Study

Shoot Density

Flowering shoot densities ($p = 0.3$) and the percentage of shoots flowering ($p = 0.6$) were not significantly different in disturbed plots relative to control plots (Figure 17). Vegetative shoot densities, however, were significantly lower in disturbed plots than

control plots ($\beta=0.74\pm 1.1$, $p<0.006$; Figure 18). In addition, vegetative shoot density ($\beta=0.61\pm 1.1$, $p<0.006$) was significantly higher at Allens Island than in South Bay.

Reproductive Effort

The flowering shoots produced within these disturbed plots did not exhibit significantly different numbers of spathes ($p=0.4$; Figure 19), different heights ($p=0.6$), or exhibit different biomass ($p=0.2$) than flowering shoots within control plots either. Once again, however, flowering shoots sampled at Allens Island were significantly taller than flowering shoots collected within South Bay ($\beta=-9.2\pm 3.5$, $p=0.009$). The number of spathes per flowering shoot ($p=0.1$) and mean biomass per flowering shoot ($p=0.8$) were not different between either location within the study.

Discussion

Role of Sediment Nutrients on Flowering

Overall this study suggests that there is a positive relationship between resource availability and plant investment in sexual reproduction. At Allens Island and South Bay the addition of nutrients into the sediments during the fall, when plants initiate growth following summer senescence in this region, but prior to the following spring's flowering season, increased spathe numbers 30% and flowering shoot heights ~20cm relative to the control. Nutrient enriched transplants produced 10-20% more spathes and flowering shoots 8cm taller than control transplants (although this increase was not statistically significant). Other fertilized plants have also been reported to increase investment in flowering and sexual reproduction (Kettenring et al., 2011; Van Lent et al., 1995; Dormann and Woodin, 2002). Critical size and allometric relationships have been shown to influence sexual reproduction in some species and may be important components with *Z. marina* flowering as well. (Aarsen et al., 1992; Weppler and Stocklin, 2005; Wiener, 2004). Interestingly some terrestrial species have shown little or no response in flower production with increased nutrient resources (Bia et al, 2009; Niu et al, 2008).

Surveys within and between locations with expected differences in sediment structure and nutrient regimes revealed the density and percentage of flowering shoots increased across locations with increasing pore water ammonium concentrations.

Although AIC scores indicated a model of percent sediment organic matter alone had the strongest support for predicting spathes per flowering shoot across locations, a nearly comparable model ($\Delta AIC=3$ from optimal model) of just pore water ammonium concentrations also suggested spathes per flowering shoots increased with pore water ammonium across locations ($\beta=.06\pm 0.01$, $p=0.03$; Figure 7). For all measured metrics of clonal and sexual reproduction the relationship with percent sediment organic matter was negative and pore water ammonium concentration positive. Interestingly, all significant trends in flowering shoot density, the percentage of flowering shoots, and the number of spathes per flowering shoot are driven by differences in pore water ammonium and percent organic matter between the three locations sampled rather than across any of the qualitative categorizations sampled at all three locations. Other important factors may explain some of the strong location differences. For example, depth differences between locations were $\sim 10\text{-}20\text{cm}$. Using the Beer-Lambert Law ($I_z=I_0e^{-k_d z}$) and, assuming uniform initial light conditions (I_0) and light attenuation coefficients (k_d) across locations, these depth differences could result in a 10-25% reduction in light between locations. Similarly, the significance and strength of differences in percent organic matter across the three locations indicates other sediment characteristics, such as sulfide concentration, could also explain differences in flowering production between locations and have an impact on seagrass metabolism (Goodman et al., 1995; Homer & Bondgaard, 2001). Samples of sediment grain size indicate a link ($R^2= 0.52$, $p<0.001$) between the percent sediment organic matter and the percentage of silt and clay in sediments at a sampled location, however, many factors, such as the porosity and adsorption of ammonium, could still vary across the locations sampled. These additional potential differences

between locations might also explain the significant interaction of sediment pore water ammonium concentrations and percent sediment organic matter on the density of vegetative shoots within a location as both light availability and sulfide concentration could also influence vegetative growth and reproduction. On the whole, however, the higher densities of flowering shoots and increased number spathes and heights of flowering shoots within areas of higher pore water ammonium concentration suggests a positive relationship between an essential resource (nitrogen) and investment in sexual reproduction.

Role of Light and Rhizome Resources for Flowering

Light availability appears to be an important regulator of investment in sexual reproduction. Long-term shading associated with five, North-South facing piers lowered the density and percentage of flowering shoots beneath piers. Measurements of PAR beneath one sampled pier demonstrated significantly diminished light levels (~30%) under the pier from areas open to ambient sunlight. Burdick and Short (1999) reported lower *Z. marina* vegetative shoot densities and diminished growth at similar and higher levels of pier shading in Massachusetts. Results presented here suggest not only will vegetative growth decrease with lower light availability but so will investment in sexual reproduction. The height and orientation of the pier can substantially alter the shading of a particular pier as lower piers and piers with an East-West orientation would shade more intensely than this estimate. Similarly, the effect of shading on growth likely alters with season within the Chesapeake Region as the light compensation point will change with water temperature (Dennison, 1987; Moore and Jarvis, 2008). As such, the pier related reduction of light may impact resource acquisition and metabolic state of *Z. marina* more

severely than the estimated reduction in available light observed in the current study. Short-term manipulation of light availability with floating shade buoys did not significantly impact flowering intensity in shaded areas relative to controls. Van lent et al. (1995), reduced ~70% of ambient light in their experimental plots and effectively below $\sim 100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ for less than the 6 hours per day, the proposed light saturation point and compensation photoperiod needed for *Z. marina* growth and recorded significant reductions in flowering production (Dennison, 1987; Dennison & Alberte, 1985). Mean PAR levels recorded beneath shade buoys indicate $\sim 388 \pm 14 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ reached experimental plots relative to $\sim 572 \pm 24 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ within control plots under ideal shading conditions, both of which are well above the $\sim 100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ proposed light saturation point (Dennison, 1987). As a result, a lack of response in sexual reproduction to experimental shading can be attributed to either a lack of a true connection between shading and sexual reproduction or to an insufficient alteration of available light intensity or the duration of the reduction to illicit a response in sexual reproduction.

Duration and Magnitude of Resource Availability

Results in this and other studies suggest resource availability increases the percentage and effort of flowering in an area and lower resource availability may lower the percentage of flowering shoots in an area. Van Lent et al. (1995) reported higher densities of *Z. marina* flowering shoots and higher spathes per flowering shoot within fertilized plots and lower densities of flowering shoots and spathes per flowering shoot within shaded plots. Backman and Barilotti (1976) also recorded no *Z. marina* flowering

shoots within extensively shaded plots adjacent to flowering areas in ambient sunlight. However, nutrient enrichment in the spring or fall of 2013 did not increase the density or percentage of reproductive shoots for either treatment in 2014. Similarly, seven months of shading did not lower the density or effort of flowering shoots directly beneath experimental shade buoys relative to control flowering shoots. This suggests the relationships between resource availability and flowering intensity is complex.

The duration and magnitude of resource availability appear important in determining the allocation of resources towards sexual reproduction. In the two examples from this study cited above the treatments used to change resource availability may have been insufficient in intensity or duration to illicit a measurable response in reproductive investment relative to control samples. Increased nutrient resources have been shown to have varying effects on sexual reproduction depending on the species manipulated (Grainger & Turkington, 2013; Burkle & Irwin, 2009) or the level of nutrient enrichment (Liu et al., 2008). Both the spring and fall 2013 nutrient enrichments involved only a single fertilization event during a period of active *Z. marina* growth in the Chesapeake Region. Given the months long spring *Z. marina* growth period (Moore et al, 2000), the duration over which nutrients were elevated was quite short.

Similarly, although effective treatment trials demonstrated the shade buoys were capable of shading 30% of ambient light from manipulated plots, the effectiveness of this shading under varying environmental conditions was never evaluated, nor were more rigorous shading treatments tested. Sustained reductions in light availability from fixed piers, however, may explain significant reductions in the percentages of flowering shoots relative to areas open to ambient sunlight. More intense shading has also been reported to

lower the percentage of *Z. marina* flowering shoots relative to controls (Van Lent et al., 1995).

Lastly, cutting *Z. marina* rhizome networks did not significantly impact flowering in the cut areas relative to control areas despite reductions in both vegetative and flowering shoot number. Transplants with different initial shoot numbers did not differ in proportional investment to sexual reproduction. Non-structural carbohydrates are known to peak during periods of active *Z. marina* growth and sustain populations through stressful time periods (Burke et al., 1996). Despite attempts to manipulate the non-structural carbohydrate reserves available per manipulated shoot in both experiments in this study, neither altered flowering intensity between treatments. Although short-term manipulations of rhizome resources and shoot densities did not measurably alter investment to flowering, a more long-term reduction in carbohydrate reserves or sustained periods of high shoot densities might have a more profound effect on flowering investment. In general, our findings concur increased belowground resources will increase sexual reproduction and decreased light resources will negatively impact sexual reproduction, but this effect may depend on the magnitude or time over which the resource is available or reduced.

Transplant experiments conducted here indicate fall growth may also contribute substantially to the density of flowering shoots in an area. Temperature and light are hypothesized to be cues inducing meristematic shifts from vegetative to flowering shoot production in *Z. marina* (Setchell, 1929; Backman and Barilotti, 1976). Both temperature and photoperiod also likely drive flowering shoot phenology and may drive key reproductive effort metrics by constraining flowering shoot growth and development

within key temperature windows (Silberhorn et al., 1983). Floral induction for *Z. marina* is hypothesized to occur around February within the Chesapeake Region (Silberhorn et al., 1983). The first floral primordia in 2014 was recorded upon dissection on January, 29 (*unpublished data*). All transplants in this study were anchored and assessed for survival in October 2013. When sampled in May 2014, ~11% of transplants produced more flowering shoots in May 2014 than vegetative shoots planted in October 2013 (Figure 10). This difference between the original number of shoots per transplant and the flowering shoots produced per transplant implies shoot production occurring between October 2013 and January 2014 contributed to sexual investment the ensuing flowering season, May 2014. This result suggests flowering potential for *Z. marina* within the Chesapeake Region may to some extent be a function of population recovery from stressful summer conditions through the fall before floral induction at the onset of winter. If only the terminal shoots surviving the summer stress period could flower, only the initial number of shoots per transplant could be induced to flowering shoots. The results here clearly demonstrate this pattern is not always the case and indicate many shoots produced in the fall growth period are capable of shifting to flowering shoots that winter. This additional flowering potential may contribute to the resiliency of a population after a period of dieback or disturbance.

Evaluating Other Factors Affecting *Zostera marina* Sexual Investment

While differences in nutrient or light availability to *Z. marina* as tested here can be related to differences in plant investment in sexual reproduction, many other genetic or environmental factors (such as temperature or CO₂ limitation), untested here, likely contribute to observed reproductive variability (Potouroglou et al., 2014; Zimmeran,

2015 *personal communication*). Selection for sexual reproductive investment could also be occurring over longer temporal scales and larger spatial scales than tested here. Annual populations of *Z. marina* exhibit high flowering intensity levels regardless of resource availability at the time of flowering as extreme local selection dictates investment in critical life history pathways, i.e. seed production, which allows the population to recruit after periods of acute stress (Keddy and Patriquin, 1978; Santamaria-Gallegos et al., 2000; Jarvis et al., 2012). To further explore factors contributing to local variability in sexual reproduction, studies should focus on the different phases in the phenology of sexual reproduction in *Z. marina* (Silberhorn et al., 1983). For example, analyses of flowering shoot densities should be closely and directly tied to the floral induction period and the physiological or environmental criteria dictating the receptivity of individuals to environmental cues for meristematic shifts from vegetative to flowering shoot production at the time this meristem identity shift occurs. Similarly, more specific experiments should investigate the role of resource availability and environmental characteristics and their interaction on post-floral induction flower production and the successful production of seeds. Both meristem identity shifts, from vegetative to flowering shoot production, and reproductive effort, the number and quality of flowers, are important factors in evaluating investment and success in sexual reproduction.

Seagrass Reproduction and Resilience

Eutrophication and light limitation threaten seagrasses worldwide (Orth, et al., 2006). Populations experiencing stress from light limitation could produce fewer flowers and subsequently fewer viable seeds. Thus, the resilience of an already strained system

could be dampened. Likewise, systems in a state of recovery could potentially face seed limitation scenarios as individuals may not provide sufficient seed source for expansion of initial recruits colonizing a potentially substandard or stressful environment or dispersal of potential recruits from individuals in more favorable environments may be less likely. If recruits into an area are unable to produce sufficient seed and to accrue population size, positive feedbacks, such as lower water velocity and increased clarity, associated with critical densities (Moore, 2004), may also be limited further lowering the chances of successful population recruitment. For *Z. marina* and most plant species sexual reproduction provides important genetic variation and serves as the sole portion of their life history in which movement over large spatial scales is possible (Reusch et al., 2005; Harwell & Orth, 1982). Especially within stressful environments, the production of seeds also provides a secure seed bank capable of recolonizing bare patches or entire meadows in the event of a total dieback (Inglis, 2000; Plus et al., 2003; Moore & Jarvis, 2008).

Conclusions

Z. marina growing with high resource availability in the Chesapeake Region would be more likely to invest in sexual reproduction than individuals with lower resource availability. Fluctuations in critical resources, such as light, could alter investment in life history strategies in these populations, however, the magnitude and timing of these fluctuations and the duration over which these fluctuations occur may dictate the extent to which investment between life history strategies changes, as regular and acute stresses have selected for high investment in sexual reproduction by annual and mixed-annual *Z. marina* populations around the world (Keddy and Patriquin, 1978;

Santamaria-Gallegos et al, 2000; Jarvis et al, 2012). The population level effects of chronic and irregular stress, such as low light availability, on investment in *Z. marina* sexual reproduction is still unclear. As such, further research is needed to determine the factors most affecting investment in the different stages of flowering shoot production and to explore the effect of resource availability at larger, ecologically relevant scales. Given the increasing stresses human populations and climate change are imposing on coastal environments, understanding key life history pathways of vital seagrass species' will be instrumental in ensuring the sustainability and resiliency of these valuable populations and the services they provide.

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Figures

Figure 1

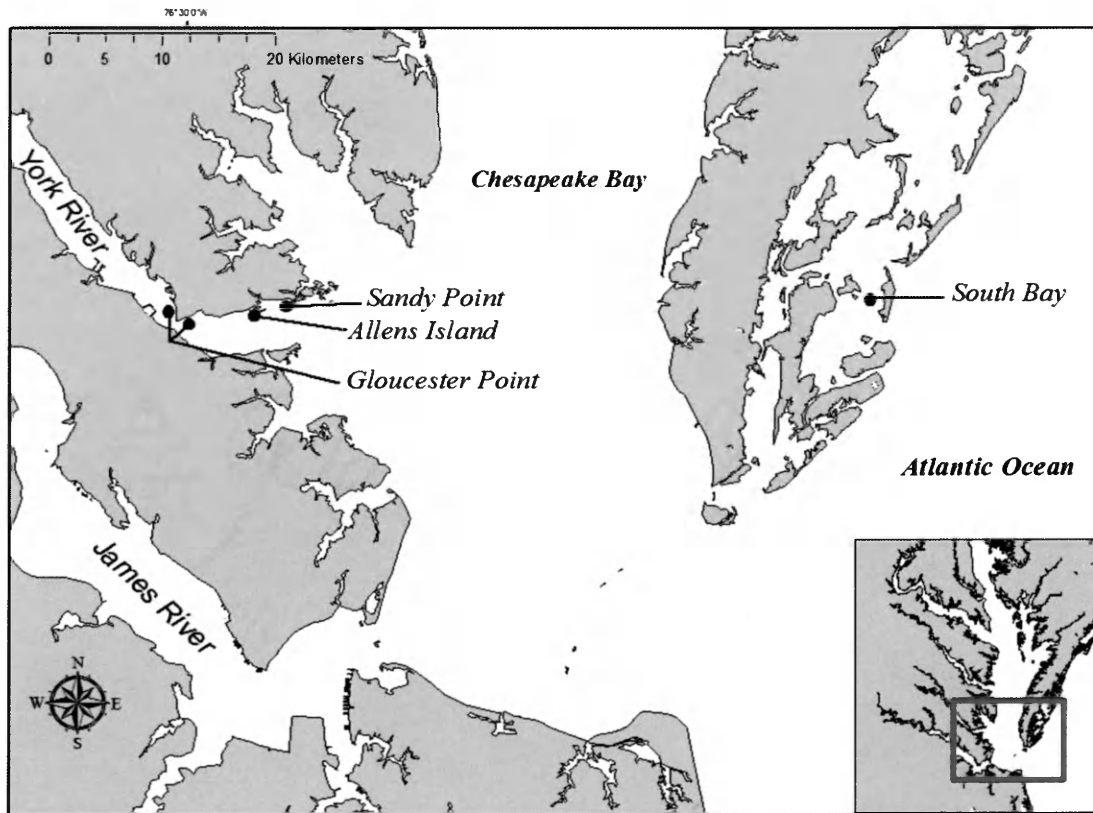


Figure 1 shows the locations at which studies occurred. Study sites in the York River, a tributary of Chesapeake Bay, occurred within an estuarine setting while studies conducted within South Bay occurred in a coastal lagoon setting along the eastern shore of the Delmarva Peninsula.

Figure 2

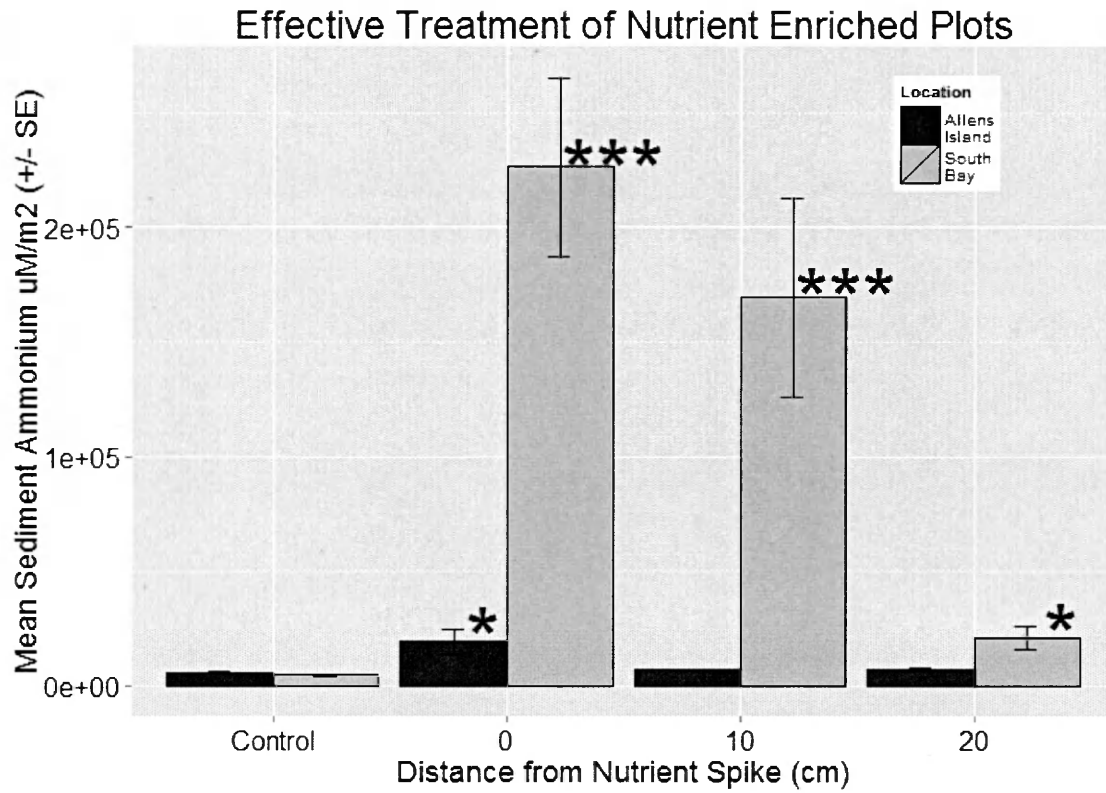


Figure 2. Sediment ammonium concentrations were significantly higher at both locations within 10 cm of the nutrient spike relative to estimates of ambient sediment ammonium concentrations ($p=0.01$ & $p<0.001$). The increase in sediment ammonium relative to controls was larger and over a greater distance at South Bay than Allens Island.

Figure 3

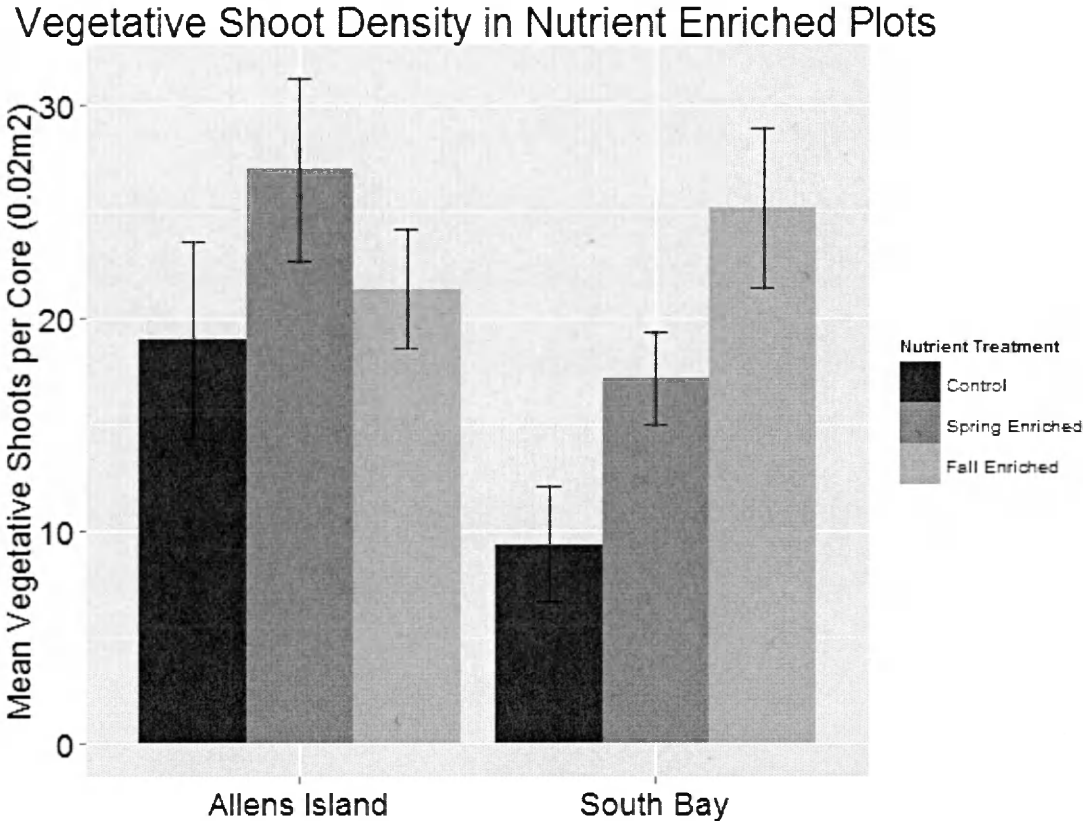


Figure 3. The density of vegetative shoots within fall nutrient enriched plots interacted significantly with the location of the plot ($\beta=2.4\pm 1.2$, $p<0.001$). This interaction stems from a more substantial increase in vegetative shoot densities in South Bay than at Allens Island.

Figure 4

Spathes per Flowering Shoot Across Nutrient Treatments

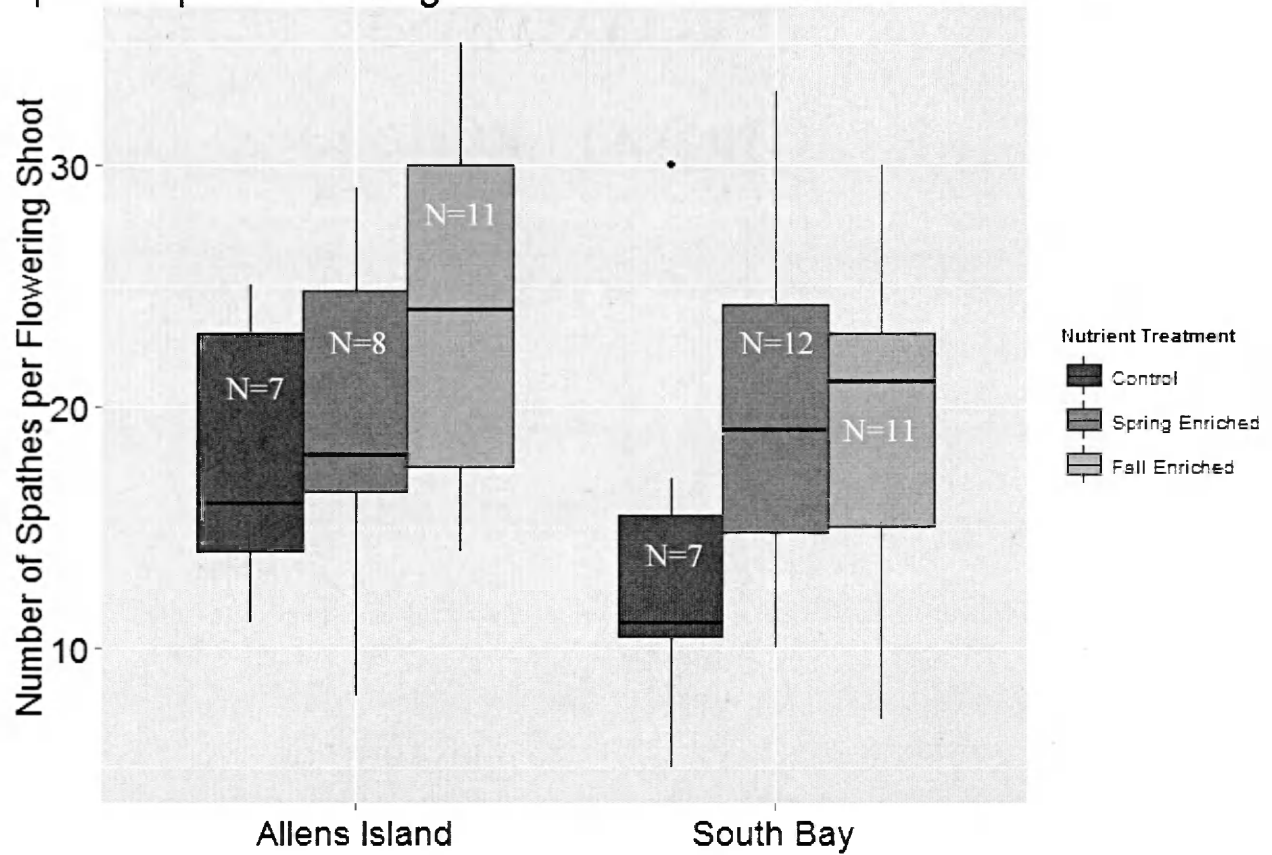


Figure 4 shows the distribution of spathes per flowering shoot for all nutrient enrichment treatments at both locations of the study. Nutrient enrichment during both time periods elevated the number of spathes per flowering shoot. The increase in spathes was only determined to be significantly different within the fall nutrient treatments ($\beta=1.3\pm 1.1$, $p=0.008$).

Figure 5

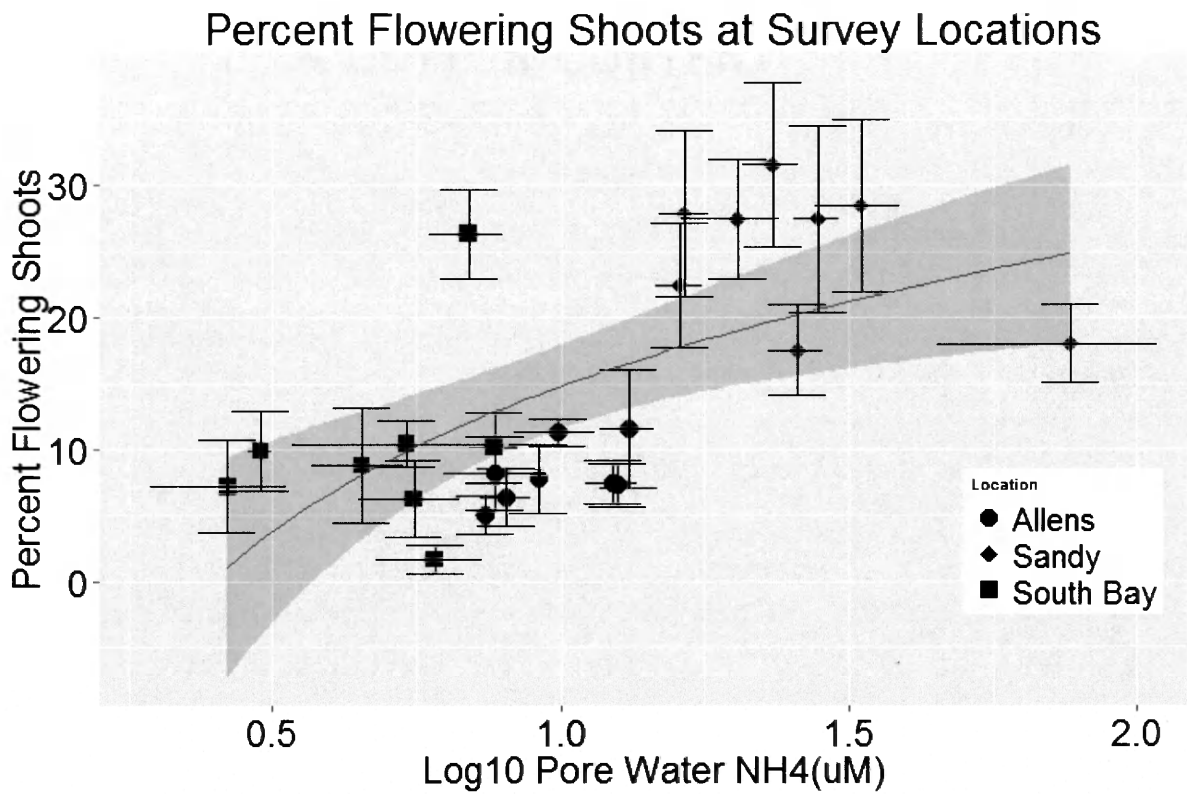


Figure 5. The percent of flowering shoots in samples increased with pore water ammonium concentrations between locations ($\beta=0.01\pm 0.003$, $p=0.002$). Although different sediment structure was identified and sampled within each location this effect was minimal relative to the differences between the locations sampled.

Figure 6

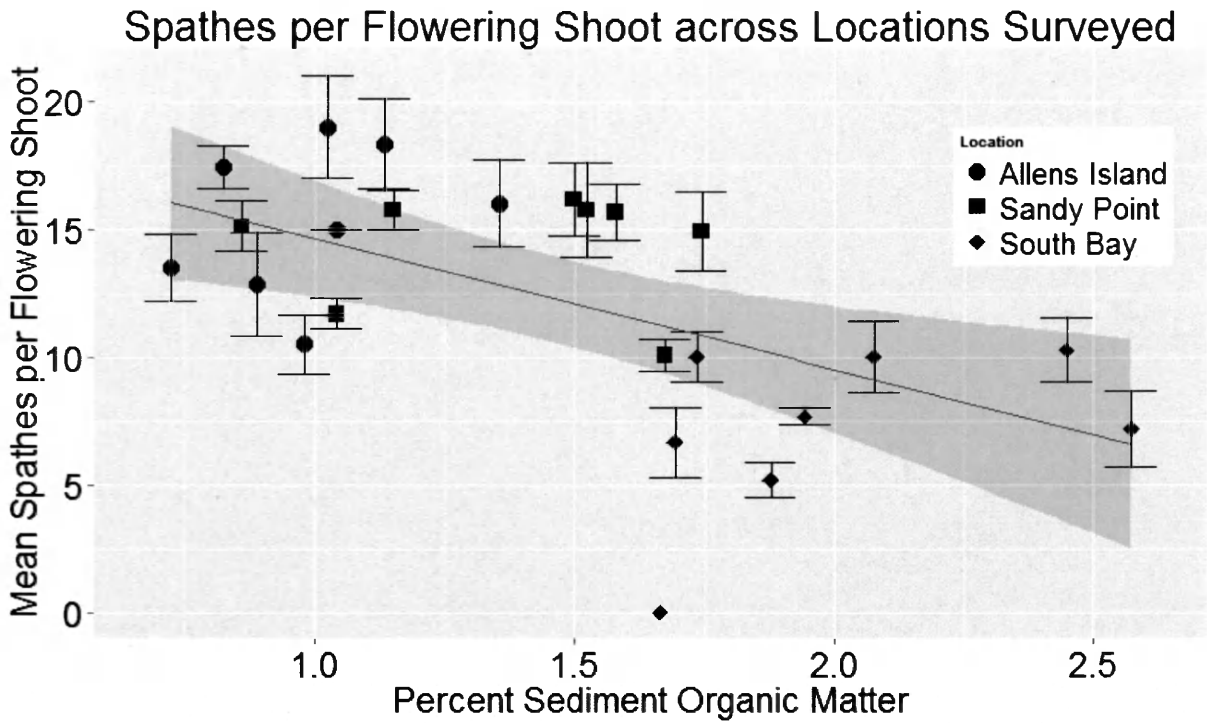


Figure 6. The number of spathes per flowering shoots decreased with the percent sediment organic matter across all three locations sampled in May 2014 ($\beta = -5.1 \pm 1.6$, $p = 0.004$). Metrics of sexual reproduction generally increased with pore water ammonium concentrations across locations and decreased with percent organic matter across locations.

Figure 7

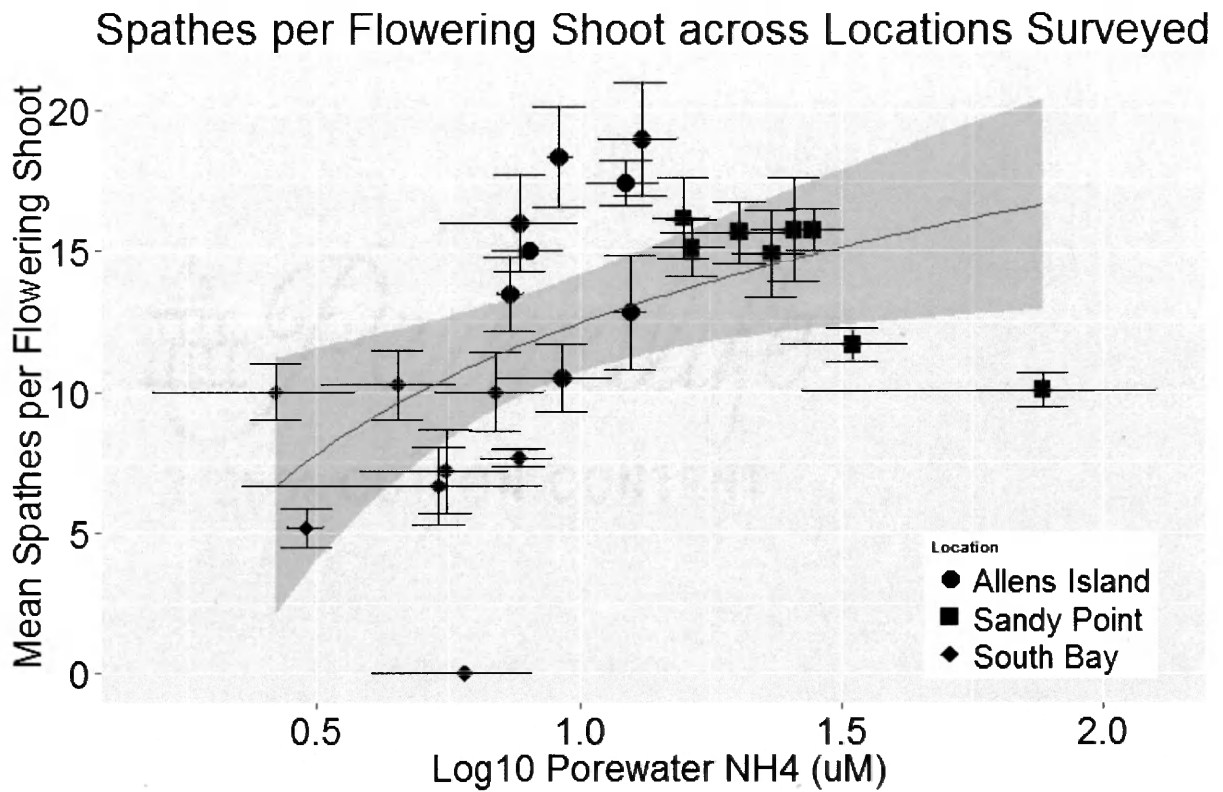


Figure 7. The number of spathes per flowering shoots increased with sediment pore water ammonium concentrations across the locations sampled ($\beta=.06\pm 0.01$, $p=0.03$).

Figure 8

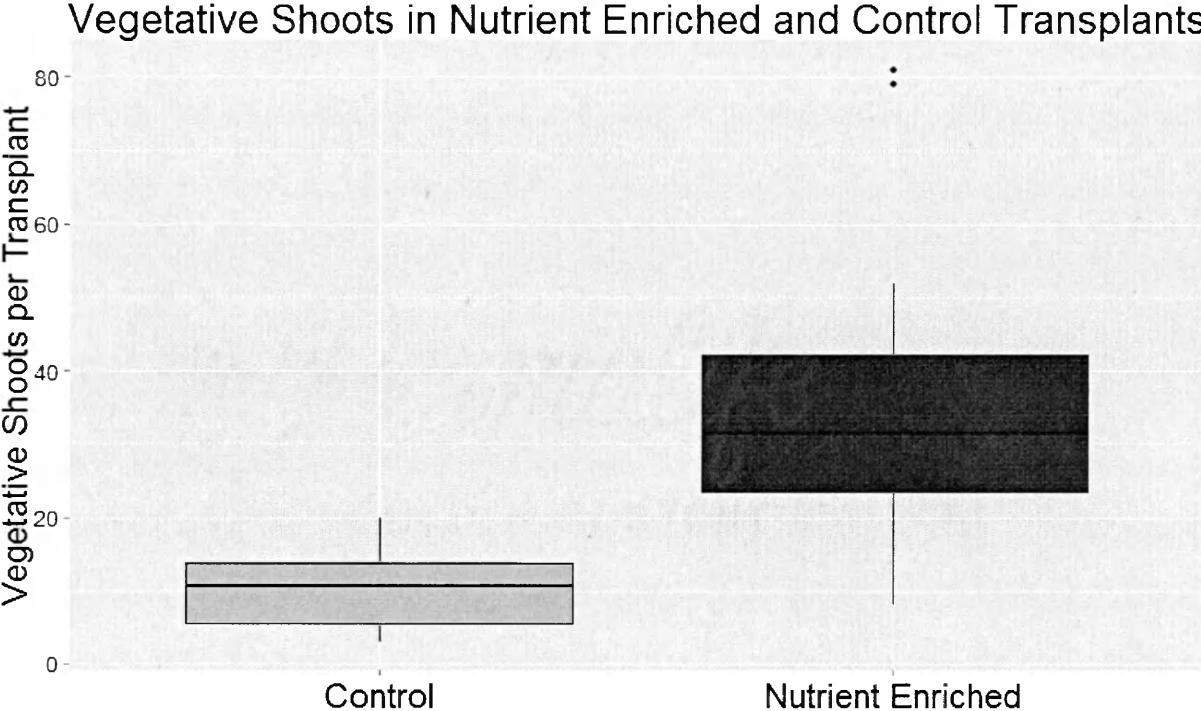


Figure 8. Nutrient enriched transplants (n=14) had significantly more vegetative shoots than control transplants (n=18) ($\beta=3.4\pm 1.1$, $p<0.001$). Nutrient enriched transplants did not have significantly different numbers of flowering shoots relative to control transplants.

Figure 9

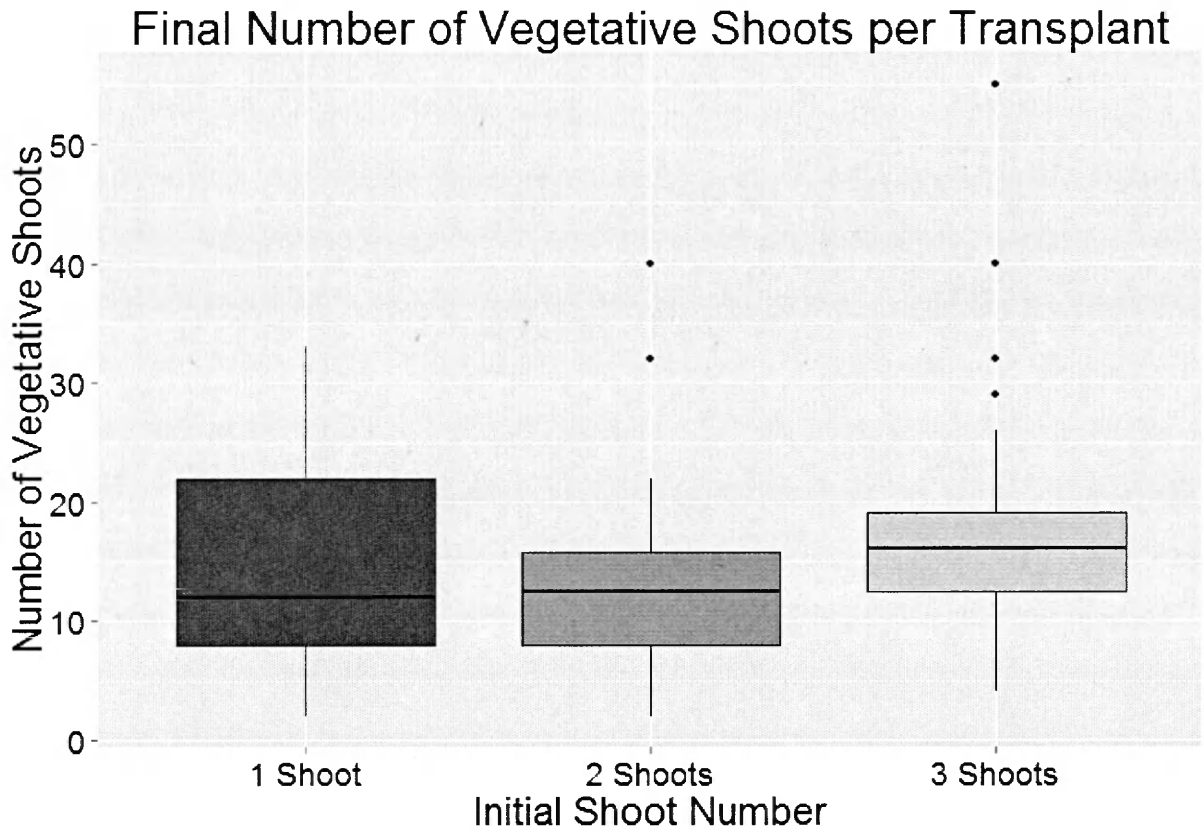


Figure 9. The final number of vegetative shoots was predictably higher within the three shoot transplants ($n=23$) than in the one shoot transplants ($n=21$) ($\beta=1.3 \pm 1.1$, $p < 0.001$). Two shoot transplants ($n=20$) exhibited no significant difference from one shoot transplants. Like the nutrient enriched transplants, no differences in sexual reproduction existed between the shoot number treatment transplants.

Figure 10

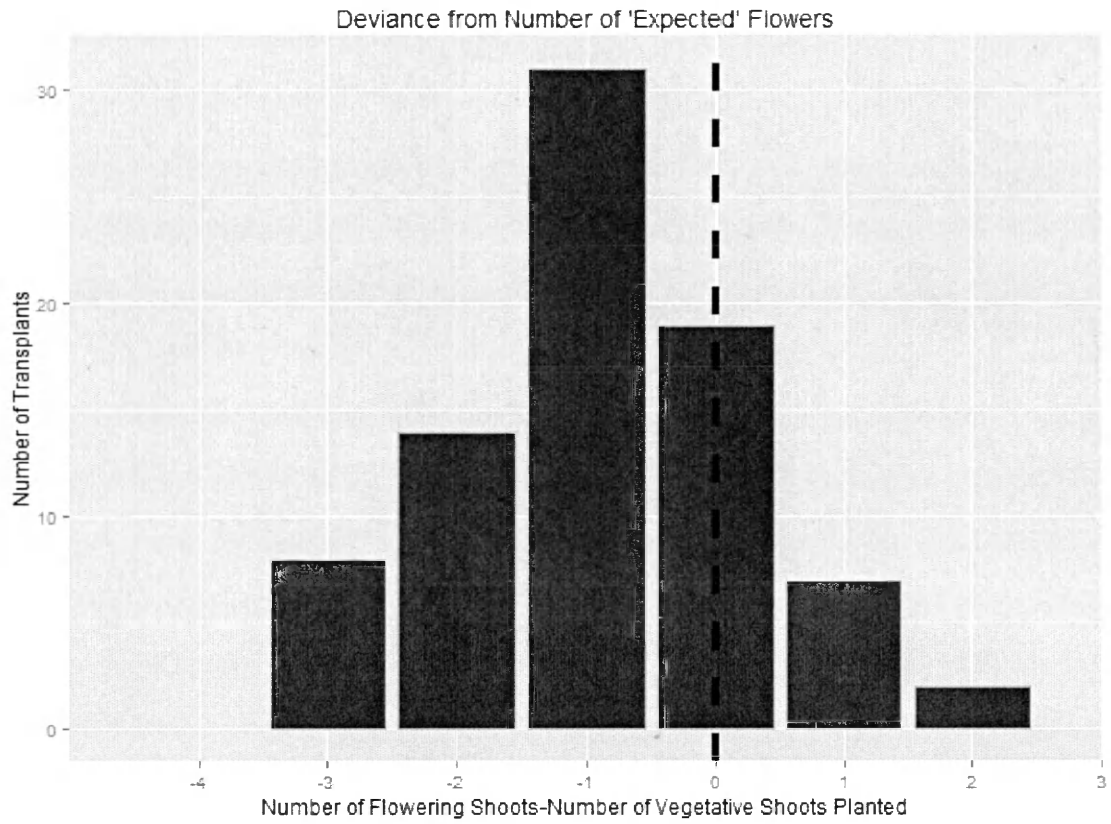


Figure 10. The distribution of flowering shoots per transplant minus the number of shoots originally planted demonstrates approximately 11 percent of transplants produced more flowering shoots than vegetative shoots initially planted. This flower production implies shoots produced after transplantation in the fall were “induced” to shift meristem identity in a matter of a few months’ growth.

Figure 11

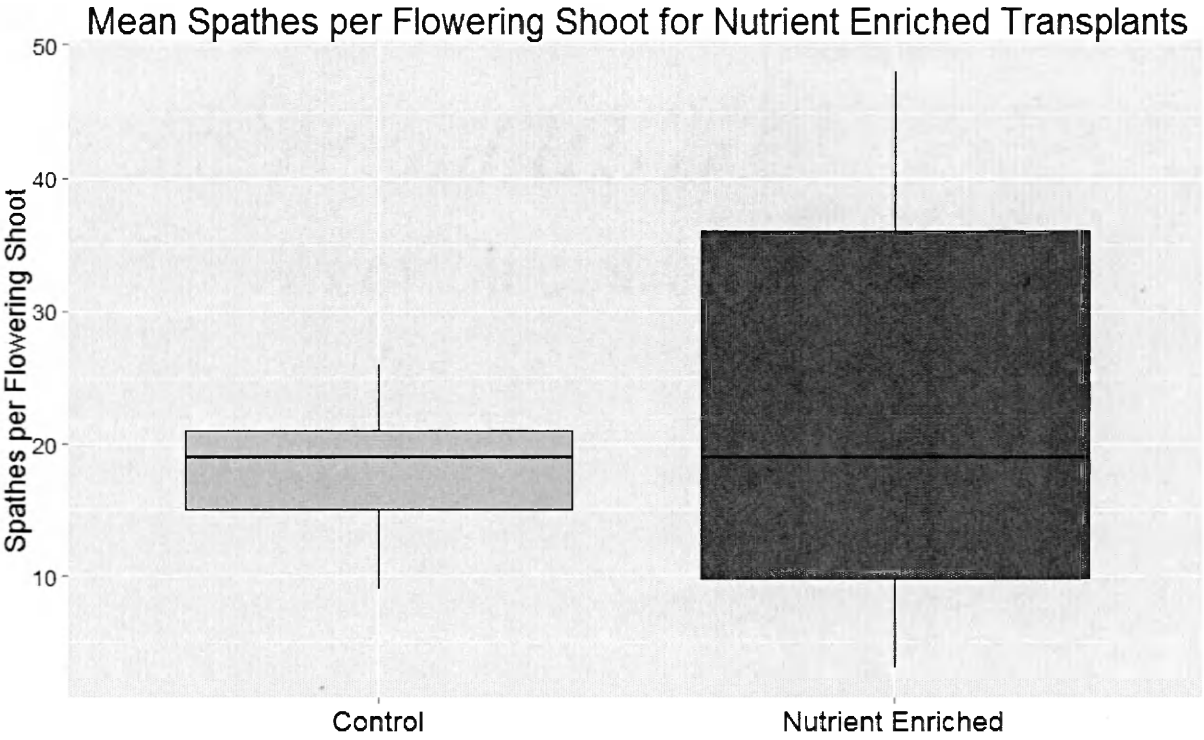


Figure 11. The mean number of spathes per flowering shoot were higher within nutrient enriched transplants (n=9) relative to control transplants (n=12), however, this difference was not statistically significant ($\beta=1.2\pm 1.1$, $p=0.06$).

Figure 12

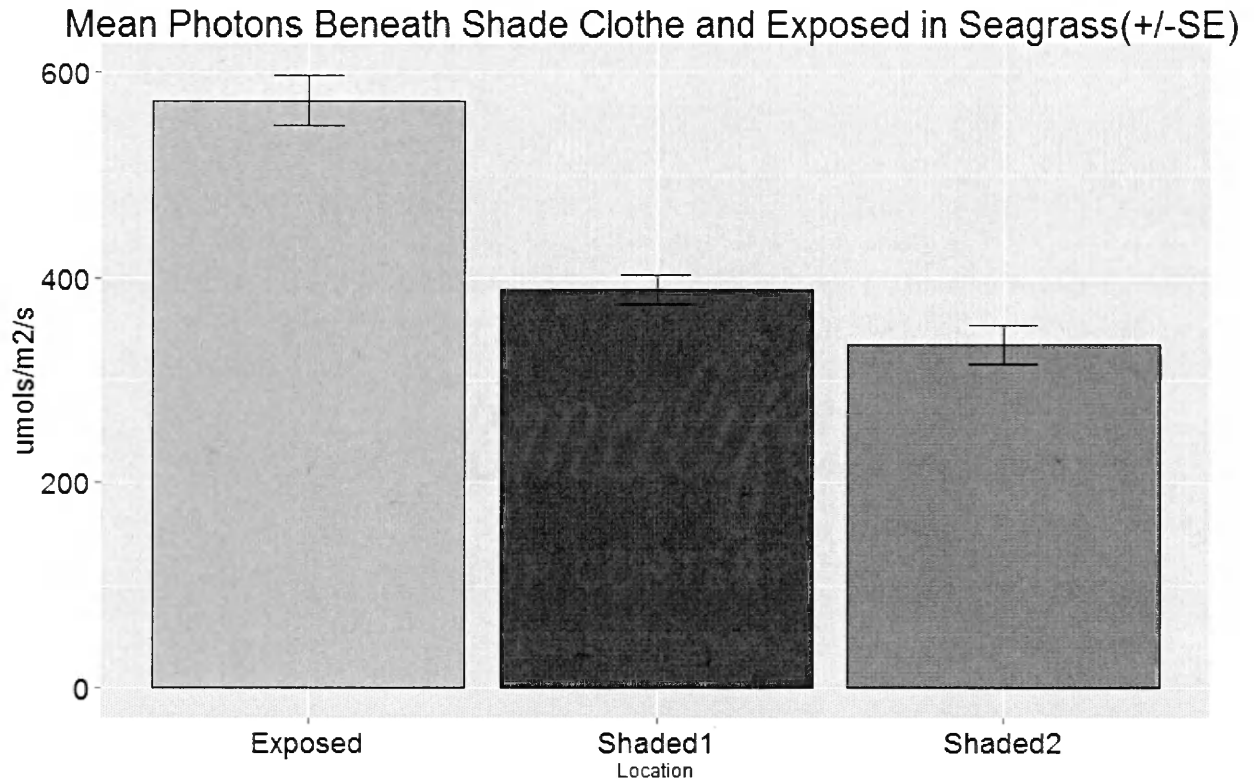


Figure 12. PAR measurements beneath two constructed shaded buoys were significantly lower than PAR measurements taken from an unshaded adjacent area of similar depth ($\beta=-178\pm 18$, $p < 0.001$). Dennison et al (1987) reported PAR saturation levels of $\sim 100 \mu\text{mols/m}^2\text{s}$. Although the shade buoys lowered PAR levels significantly relative to background PAR levels, the mean PAR over the time period sampled were never below this estimated saturation level.

Figure 13

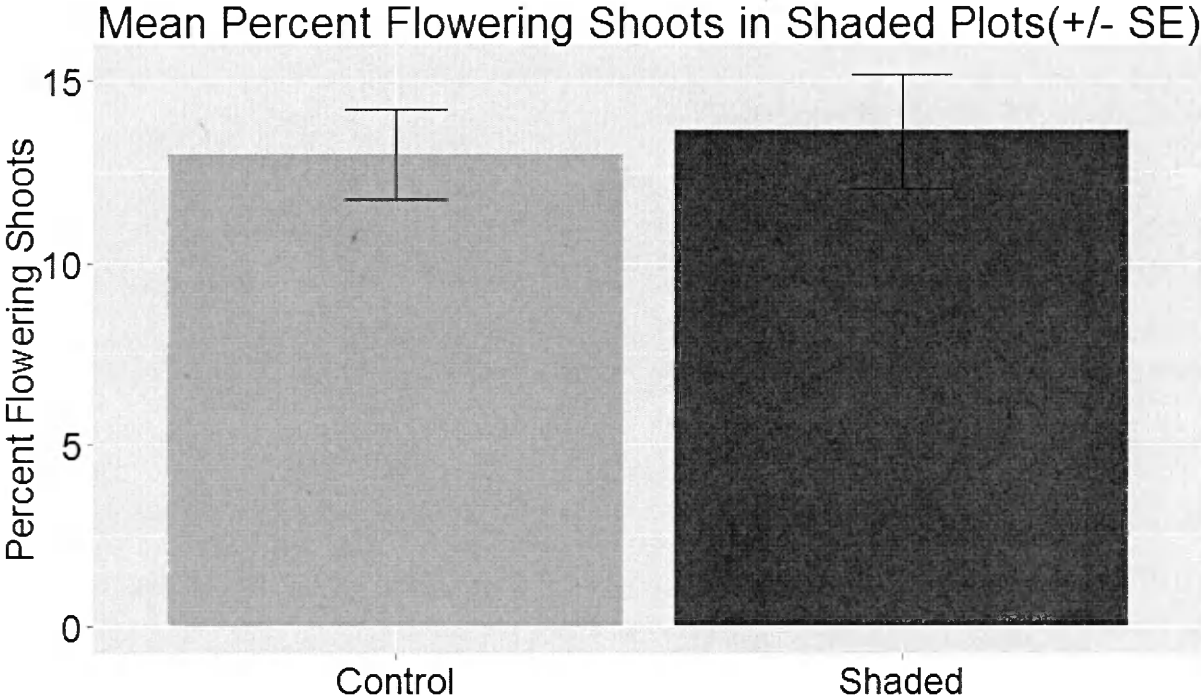


Figure 13. The percentage of flowering shoots beneath shade buoys was not significantly different from the percentage of flowering shoots within adjacent control plots.

Figure 14

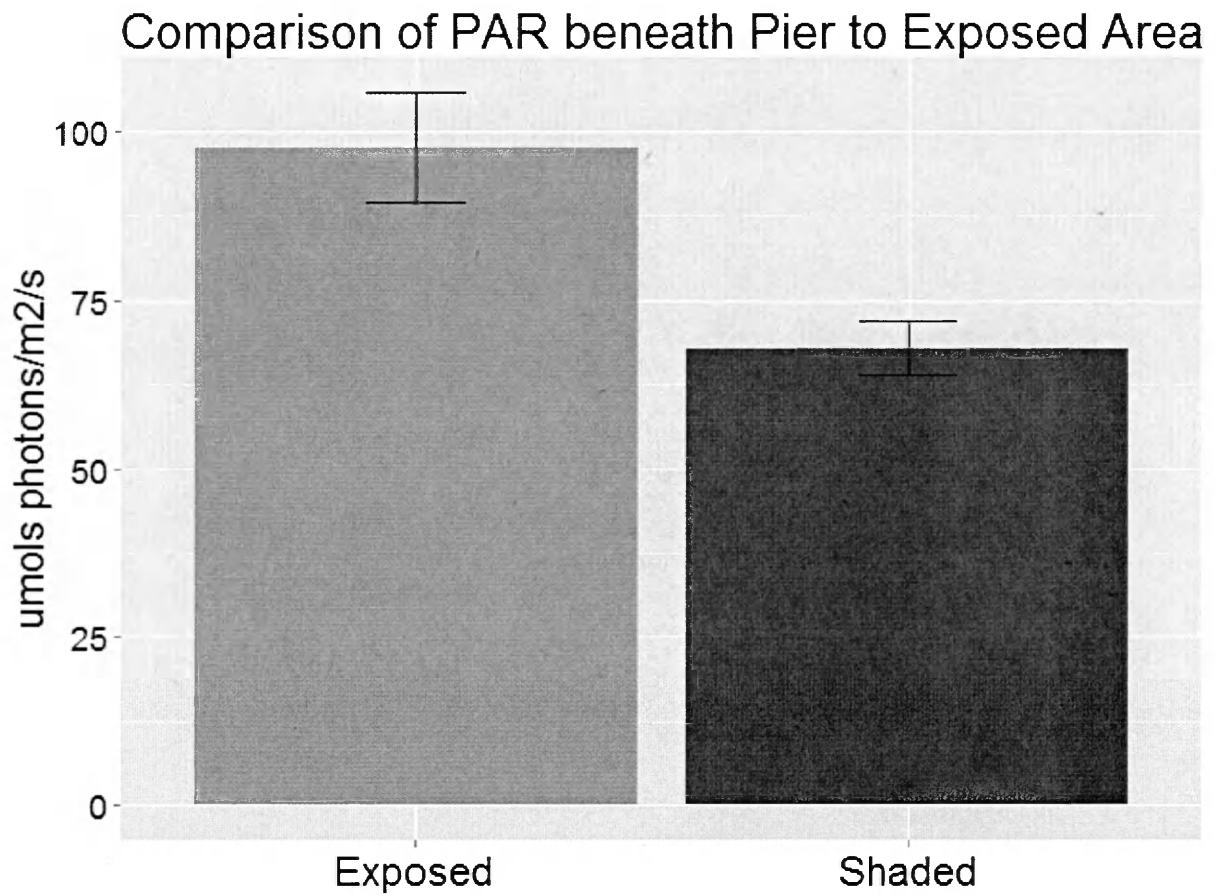


Figure 14 demonstrates the mean PAR beneath the sampled pier was significantly lower than PAR measurements taken outside the pier area ($p < 0.001$). Cloud cover at the time of sampling lowered overall light levels, but the relative difference between the pier shaded and exposed areas was still significant.

Figure 15

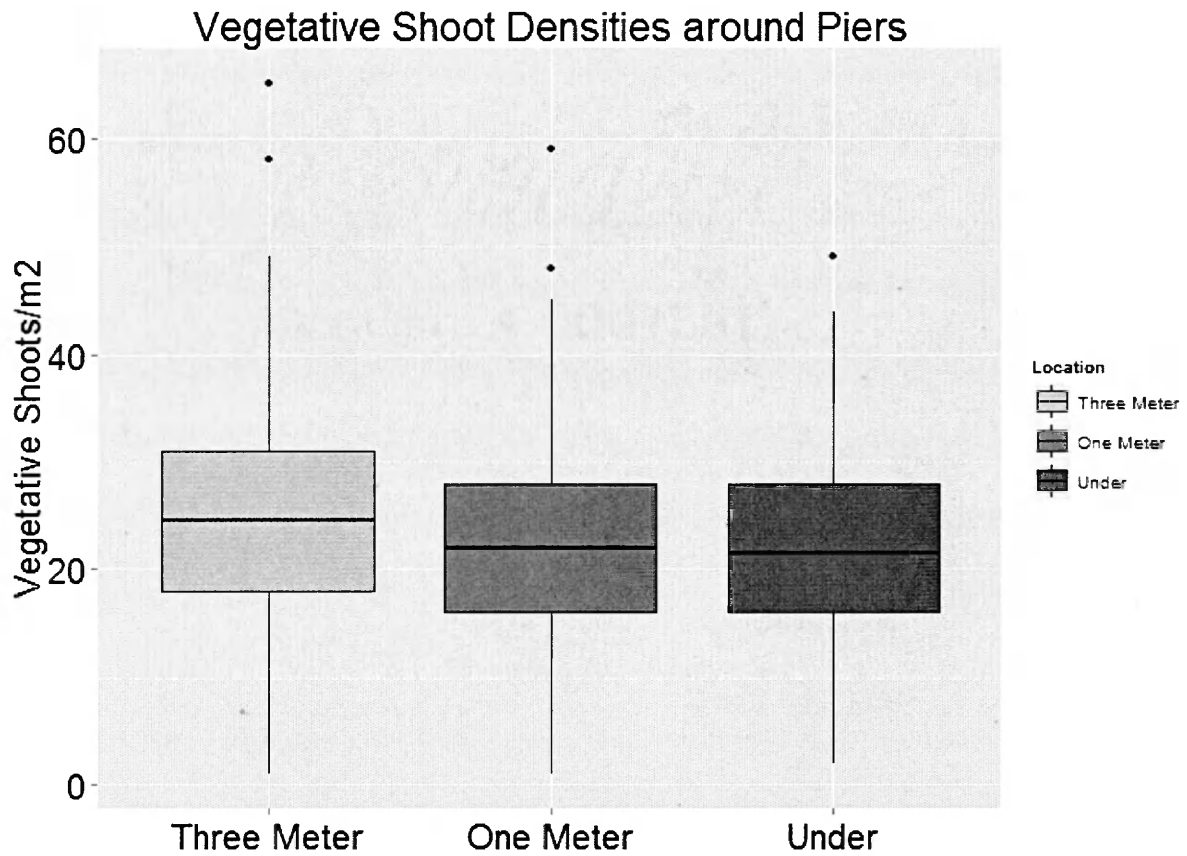


Figure 15. The density of vegetative shoots underneath the pier was significantly lower than the number of vegetative shoots three meters from the pier ($n=180$, $\beta=1.1\pm 1.0$, $p=0.02$). No difference between vegetative shoot densities beneath sampled piers and one meter from sampled piers was detected.

Figure 16

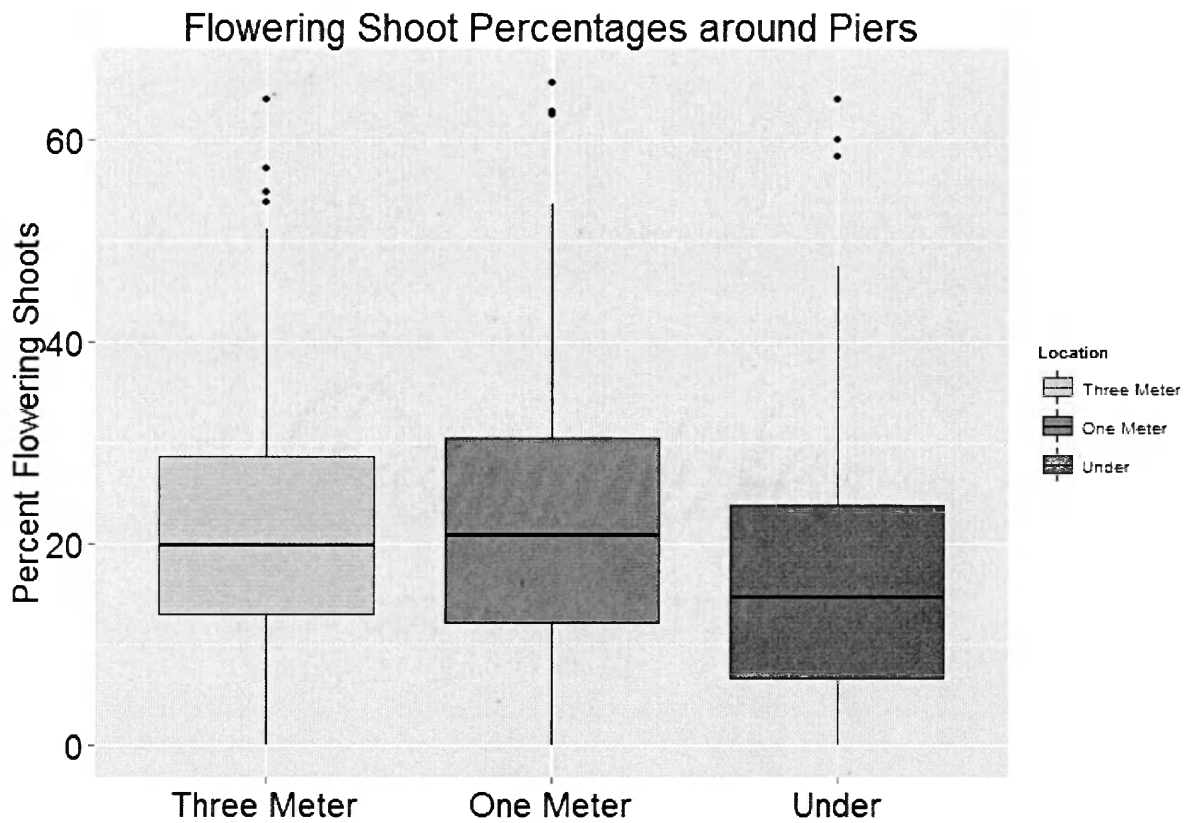


Figure 16 shows the distributions of 180 flowering shoot percentages at three distances from 5 North-South facing piers in the York River. The number of flowering ($n=180$, $\beta=1.5\pm 1.1$, $p<0.001$; $\beta=1.5\pm 1.1$, $p<0.001$) and vegetative shoots ($\beta=1.1\pm 1.0$, $p=0.02$) as well as the percentage of flowering shoots ($\beta=0.8\pm 0.05$, $p<0.001$; $\beta=0.5\pm 0.05$, $p=0.003$) is significantly higher outside the pier than directly beneath the pier.

Figure 17

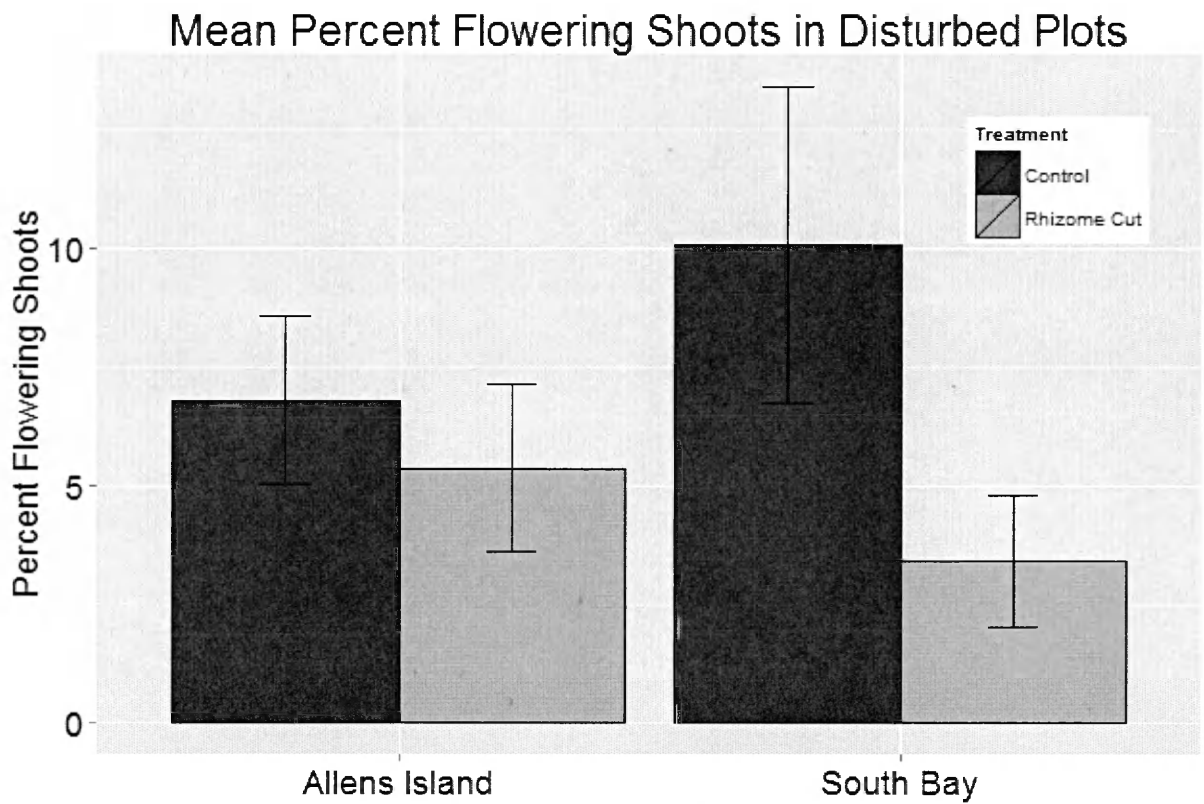


Figure 17. Although disturbed rhizome plots (0.02m²,n=5) at both locations exhibited lower percentages of flowering shoots relative to control plots, no statistically significant difference in the percentage of flowering shoots between plots was statistically significant.

Figure 18

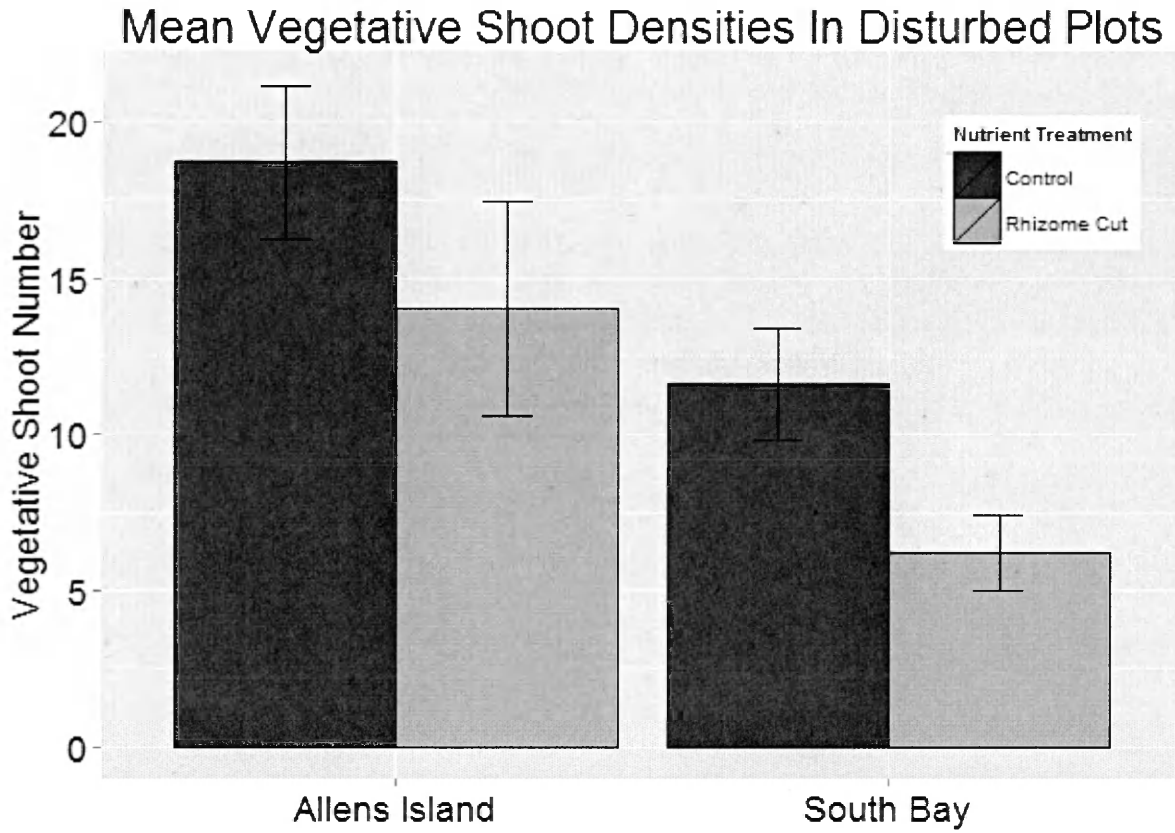


Figure 18. The density of vegetative shoots in disturbed plots (0.02m^2 , $n=5$) was significantly lower than the density of vegetative shoots in control plots ($\beta=0.74\pm 1.1$, $p<0.006$). In addition, Allens Island plots exhibited significantly more vegetative shoots than South Bay plots ($\beta=0.61\pm 1.1$, $p<0.006$).

Figure 19

Mean Number of Spathes per Flowering Shoot in Disturbed Plots

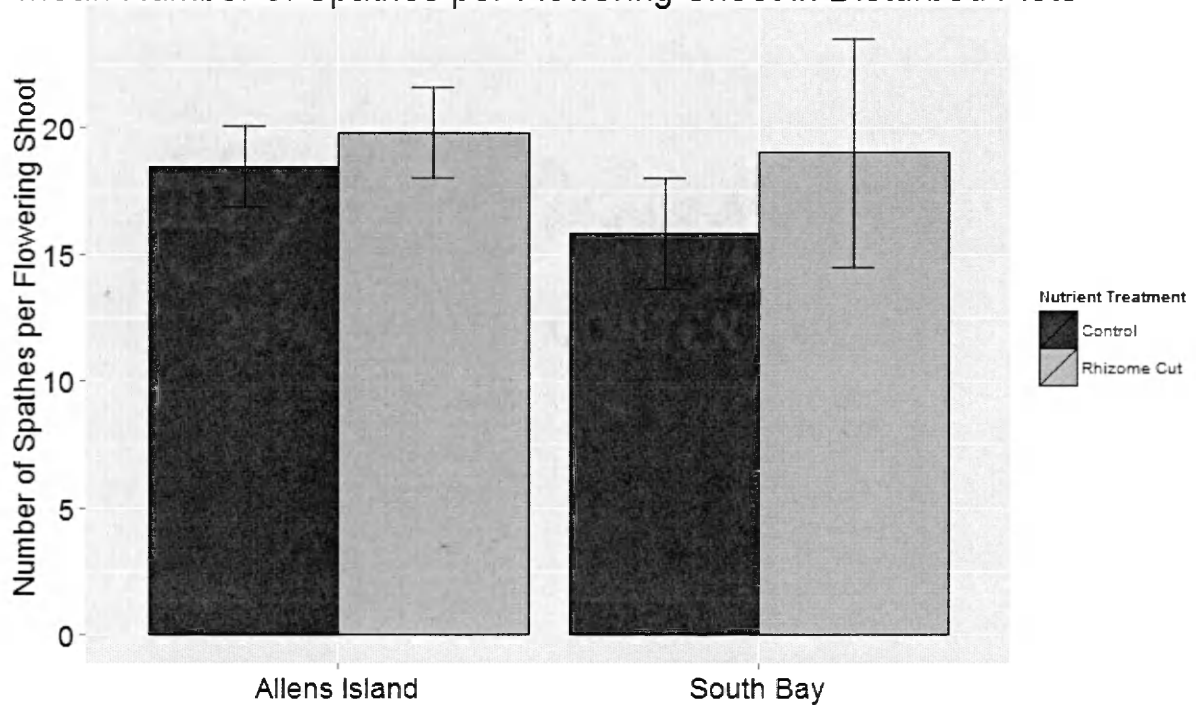


Figure 19. The number of spathes per flowering shoot within control (n=15; n=11, respectively) and disturbed plots (n=13; n=3, respectively) at Allens Island and South Bay were not statistically distinguishable from one another.

Table 1:

AIC Scores for Model Comparisons in Survey Across Sediment Gradients					
	Flowering Shoot Density	Vegetative Shoot Density	Percent Flowering Shoots	Spathes per Flowering Shoot	Flowering Shoot Height
Log₁₀(Pore water NH₄)	290.7	3319	45.9	141.7	187.3
Sediment OM	311.1	2077	57.0	138.1	186.9
Both	289.2	1888	46.3	139.3	187.7

Table 1. All models were evaluated based on AIC score for every dependent variable. The most parsimonious model with the lowest AIC score was selected as the best model for a given dependent variable.

Table 2:

Effect of Resources on Investment in Sexual Reproduction for <i>Zostera marina</i>					
Resource	Study	Quality	Duration	Effect on Flower Shoot Number	Effect on Reproductive Effort
Belowground Nutrients	Meadow Enrichment	Enhanced	Ephemeral	-	+1.3
	Transplant Enrichment		Ephemeral	-	+1.2*
	Survey Across Variable Locations		Historic	+3.34	+0.06
Light	Shade Buoy Manipulation	Diminished	Ephemeral	-	-
	Survey Around Piers		Historic	-2.5	N/A
Shoot Availability	Shoot Number Transplants	Enhanced	Ephemeral		

Table 2. Results from all studies indicate the addition of resources increases investment in sexual reproduction overall. Conversely, diminishing a critical resource was also shown to negatively impact investment in sexual reproduction. The time scale of resource availability may influence the effect of its presence or absence on *Z. marina* allocation pathways as only long term resource differences changed the relative density of flowering shoots, while short-term additions of nutrients altered reproductive effort.

VITA

Andrew J. Johnson was born in Richmond, Virginia in April 1988. He graduated from Monacan High School in 2007. Andrew then enrolled at the University of Virginia from which he graduated with a B.S. in Biology in 2011. He entered the School of Marine Science at the Virginia Institute of Marine Science in 2012.